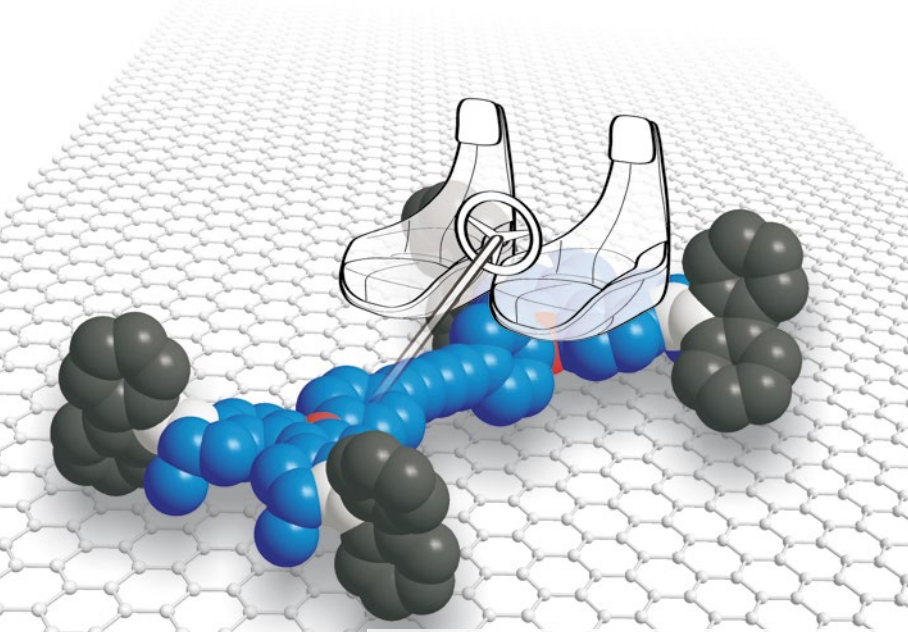
ALBERTO CREDI
VINCENZO BALZANI



MOLECULAR MACHINES

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CHAPTER I

THE WORLD OF ATOMS AND MOLECULES

Chemistry, around us and in us

It is difficult to imagine any manifestation of our daily life in which chemistry is not involved in any way (Balzani 2014). Chemistry is all around us and in us. Chemistry is *around us* in the natural phenomena indispensable for life, such as photosynthesis, and in artificial products that are important for civilization (pharmaceuticals, fertilizers, plastic materials, semiconductors and detergents); and it is *in us* because the functioning (or malfunctioning) of the human body is regulated by chemical reactions. Indeed, life is chemistry in action. Without over simplification, we can say that all the manifestations of life, including what we call cognitive categories (learning, memory, thought, experience and dreams), are ultimately the result of chemical reactions that are too complex to interpret, at least for now.

Atoms

There are about one hundred *elementary atomic species* in nature, each represented with one or two letters of the alphabet: H for hydrogen, C for carbon, N for nitrogen, O for oxygen, P for phosphorus, S for sulfur, Cl for

chlorine, Co for cobalt, and so on. These elementary species, according to the repetitiveness of their chemical and physical characteristics, are ordered in the Periodic Table or Periodic System, as coined by famous chemist and writer, Primo Levi, in his renowned book. The Periodic Table (Fig. 1) was created in 1869 by Dmitrij Mendeleev, a Russian chemist who first highlighted, even without understanding the reasons, the similarities between the properties of different elements. According to many scientists, Mendeleev's work was the most ingenious breakthrough of the last ten centuries. For many years the Periodic Table has been regarded as something extraordinary, as if divine. Although today the reasons for the similarities between the various elements are well known, the Periodic Table still maintains its unaltered charm as the clear order of the elements provides a glimpse into the intrinsic and profound order of Nature. The Periodic Table itself contains, in a concise and unitary way, a good part of chemistry: no other scientific discipline can boast such an iconographic table.

Atoms have a spherical shape with different dimensions, depending on the type of element, and they are very small. The largest atom, cesium (Cs), has a radius of 0.24 nm (nm is the abbreviation of *nanometer*, which measures one billionth of a meter). In general, atoms are not isolated; they spontaneously tend to combine – that is, to form bonds – with other atoms, according to precise laws, in order to form *molecules*.

Molecules

Names, formulas, models

In the chemical formulas used to indicate molecules, the number of atoms of the same type that are part of a molecule is indicated by a subscript. For example, the oxygen molecule, formed by two oxygen atoms, is represented by the formula O_2; the water molecule is made up of two hydrogen atoms and one oxygen atom, and is thus represented by the formula H_2O. Many molecules are composed of a greater number of atoms. For example, the molecule of acetic acid (Fig. 2a, left) consists of 8 atoms, of which

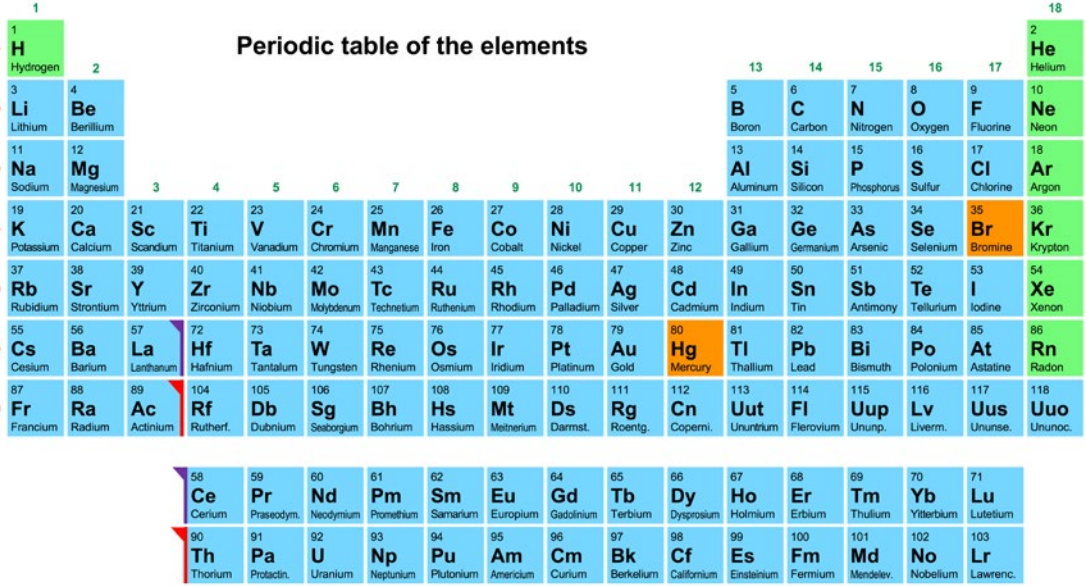


Fig. 1. The Periodic Table of the Elements, icon of Chemistry.

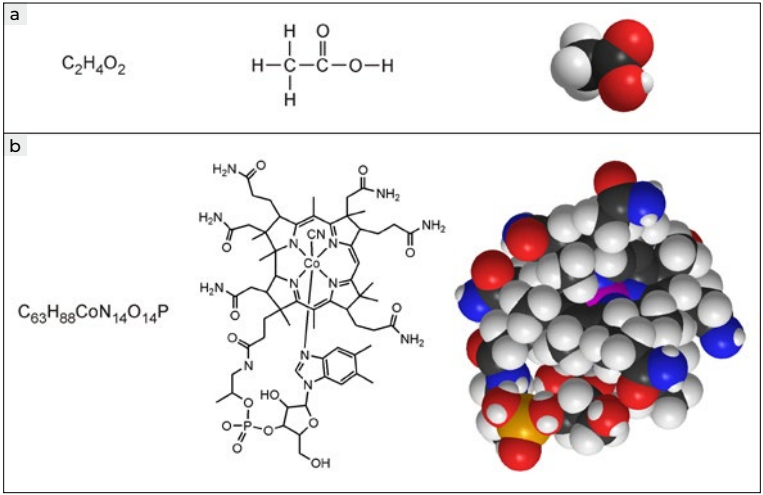


Fig. 2. The molecule of acetic acid (a) and vitamin B12 (b). On the left, the *brute formula* that indicates the composition of the molecule. At the center, the *structural formula* that shows how the various atoms are linked together. On the right, the *three-dimensional model* of the molecule, magnified millions of times compared to the real size, obtained by computer calculations.

two carbon, four hydrogen and two oxygen: $C_2H_4O_2$. There are, however, many more complex molecules, such as vitamin B12, which is composed of 181 atoms: $C_{63}H_{88}CoN_{14}O_{14}P$ (Fig. 2b, left).

Like animals and plants, molecules have common names (water, acetic acid, vitamin B12) and scientific names (in the case of water, dihydrogen monoxide). The scientific names of large molecules are extremely complex and therefore are hardly ever used. Since the names are not enough to get oriented in the enormous and variegated world of molecules, it is necessary to resort to another type of representation; chemical formulas. H_2O, $C_2H_4O_2$ and $C_{63}H_{88}CoN_{14}O_{14}P$ are called *brute formulas* and indicate only which and how many atoms the molecule is composed of. These formulas are not very useful because they do not specify which atoms

are bonded to which, nor do they specify their spatial arrangement. For example, 6 carbon atoms and 6 hydrogen atoms can be combined in 217 different ways, which means that the same formula, C_6H_6 corresponds to as many as 217 different molecules, of which the best known is benzene. This example also makes it clear that, with the hundreds of atomic species available, it is possible to obtain a huge number of molecules. Therefore, we often resort to *structural formulas*, that highlight how the various atoms are linked together.

In these formulas, the bonds are the "glue" that holds the atoms together, and are represented by dashes that unite the symbols of the connected atoms. For small molecules, the structural formulas are simple and, along with clearly indicating how the atoms are linked, they also give an idea of the shape of the molecule (Fig. 2a, center). For large molecules, the situation becomes increasingly complex and the structural formulas end up looking like an intricate web of signs. In these cases, we try to represent the molecule with simplified structural formulas (Fig. 2b, center): for example, carbon atoms, C, which are very common especially in the molecules of living organisms, are no longer explicitly indicated in the structural formula, but it is understood that they occupy the intersecting positions of the dashes that indicate the links. Likewise, the hydrogen atoms, H, bound to the carbon atoms, and the dash that represents their bond, are not indicated.

Although very useful to scientists, structural formulas are not known for their attractive qualities. The most realistic and significant way to represent the molecules is based on the use of three-dimensional models, enormously enlarged, but in scale, compared to reality. These models are built with an interlocking mechanism similar to the one used in the well-known Lego game, starting from rigid plastic spheres that represent the various types of atoms, with small cavities in which junctions representing the chemical bonds can be inserted. Each sphere representing an atom is one hundred million times larger than the real size of the corresponding atom, so that the model is to scale and therefore accurately represents the relative dimensions of the various molecules and the parts that constitute them. To

distinguish the various types of atoms, or rather the most recurring ones in important molecules, conventional colors are used: white for hydrogen (H), black for carbon (C), red for oxygen (O), blue for nitrogen (N), yellow for sulfur (S), orange for phosphorus (P), green for chlorine (Cl).
Represented with three-dimensional models (Fig. 2, a and b, on the right), the molecules appear as macroscopic objects and acquire part of the charm they would have if we could see them in their reality. In the case of very large molecules, however, molecular models are also difficult to decipher. As we will see further on, an ultimate way to represent the most complex molecules and the aggregates of molecules that make up molecular devices and machines is to use schemes of various types that help clarify the shape, properties and functions of these systems.

Invisible, but well-known

To understand chemistry, we need to have the following two concepts in mind: molecules are small, very small (nanometric), but they are three-dimensional *objects* that have their own specific dimension, composition, structure and shape; from these characteristics we can derive their specific properties, such as the effect on organisms.
To get a better idea of how small molecules are, just imagine that in a drop of water there are so many molecules that if we could distribute them among all the inhabitants on Earth, each would receive about 200 billion. Objects of such small dimensions escape our daily experience and the common experimental investigations, so that it is difficult not only to accept their usefulness, but also to believe in their real existence. Goethe said that science must be on a human scale and opposed the use of the microscope by stating that what cannot be seen with the naked eye should not be sought, because it is obviously hidden from the human eye for some good reason. This statement is contrary to the logic of science, which particularly in recent years, has pushed its investigations increasingly towards the microscopic world, not only to learn more about nature, but also to exploit the advantages that can derive from a technological point of view.

Taken individually, a molecule can neither be seen nor weighed or measured. Despite these limitations, molecules have no hidden secrets for chemists who have, for over a hundred years, learned to distinguish, build and use them by exploiting their collective properties. This concept is expressed admirably by Primo Levi whose book, *The Monkey's Wrench*, gives the definition of the profession of the chemist compared to that of an engineer:

> […] My profession, my real one, the profession I studied in school and that as kept me alive so far is the profession of chemist. I don't know if you have a clear idea of it, but it's a bit like yours; only we rig and dismantle very tiny constructions. We're divided into two main branches, those who rig and those who dismantle or break down, and both kinds are like blind people with sensitive fingers. I say blind because, actually, the things we handle are too small to be seen even with the most powerful microscopes: so we've invented various intelligent gadgets to recognize them without seeing them (Levi 1978).

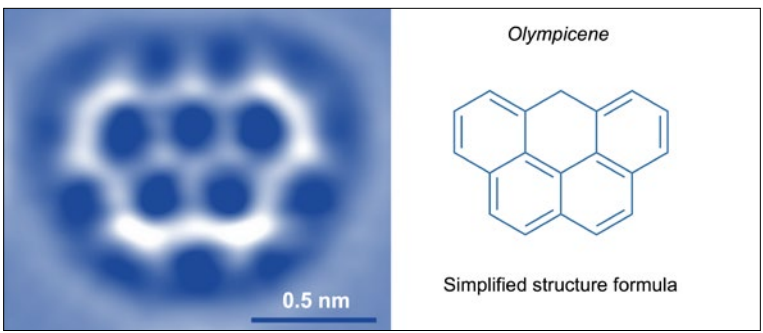


Fig. 3. Image of a complex molecule, 6H-benzo[*cd*]pyrene, obtained with the technique of atomic force microscopy (AFM). This molecule, conceived and built to celebrate the 2012 London Olympics, is known as *olympicene*. Credits: IBM Research - Zurich, University of Warwick, Royal Society of Chemistry.

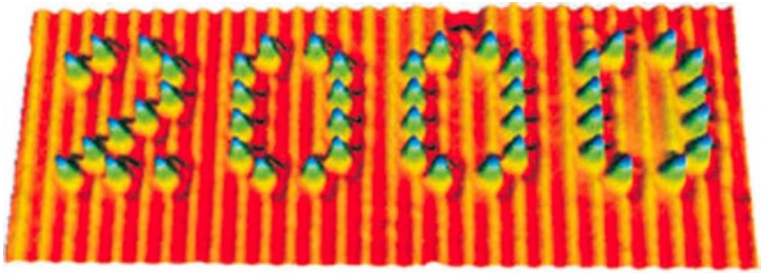


Fig. 4. The date of the new millennium, obtained by placing 47 molecules of carbon monoxide (CO) on a copper surface. The structure was obtained and visualized by scanning probe microscopy techniques. The actual length of this writing is 16.3 billionths of a meter. Figure reproduced courtesy Copyright © 2005 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

Although with the most recent advances in science it is possible to "see" (through images obtained with suitable instruments called scanning probe microscopes, Fig. 3) and even "touch" (with ultra-thin tips) single molecules to use them for "nano-writing" (Fig. 4), the world of molecules is essentially a mental representation. But it is a very objective and rational representation, as chemists have a wealth of information about molecules, such as composition, weight, size, shape, reactivity, ability to interact with light and electricity, and tendency to either remain rigidly associated with each other (solid state), slide on one another (liquid state) or move freely on their own (gaseous state).

Molecules in action

Molecules represent the starting point for interpreting the properties of matter and for understanding the intimate essence of the phenomena that occur around us and in us. For example, the phenomenon that allows a tree to use sunlight to produce flowers and fruits is the natural photosynthetic process. To understand how this process takes place (Fig. 5), we need to zoom in, shrinking down to the nanoscale, past the tree (meters), leaves (centimeters), cells (hundredths of a millimeter), chloro-

plasts (thousandths of a millimeter), grains (ten thousandths of a millimeter), to reach the molecules that, as we have seen, are one millionth of a millimeter (that is, a nanometer) in size.

In plants there are aggregates formed by a certain number of molecules, appropriately assembled and integrated so as to constitute real nanometer-sized devices. The process of converting solar energy into chemical energy is accomplished by these devices (called *reaction centers*) that are capable of using the energy of sunlight to cause a chemical reaction called *charge transfer*. This reaction is followed by many others that involve other molecular-based devices and machines and that, in the end, produce the fragrant and nutritious molecules that constitute the flowers and the fruits.

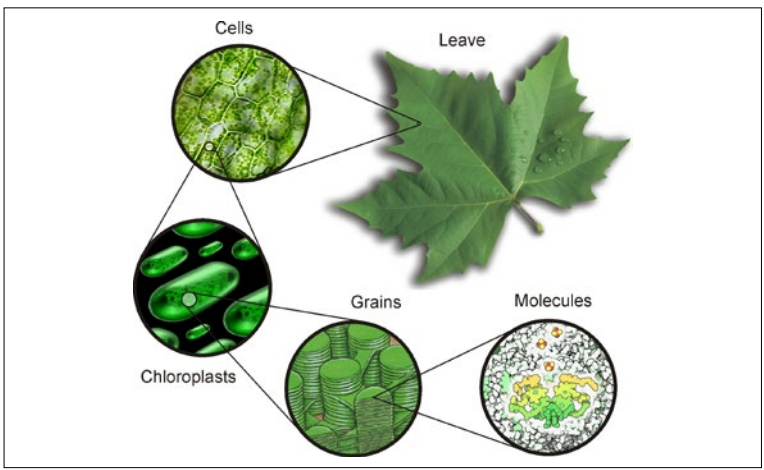


Fig. 5. To understand how photosynthesis takes place, it is necessary to enter with a zoom in the infinitely small world. A leaf (dimensions of a few centimeters) is made of cells (50 micrometers) inside which are the chloroplasts (5 micrometers), which in turn consist of grains (discoidal structures of 200 nanometers in diameter). The grains contain molecules (dimensions of a few nanometers) that are capable of absorbing sunlight and thus starting the photosynthetic process.

The invisible world of molecules also fills the human body. In our hands and arms, legions of small molecular motors allow us to pick up objects, to turn the pages of a book and to perform a myriad of other movements. In our eyes, light causes structural changes in certain molecules that send signals to the brain; the processing of these signals, carried out by armies of molecular messengers and switches, allows us to recognize the words we are reading and to grasp their meaning. In the meantime, without we even realize it, the invisible oxygen molecules that are contained in the air are captured one by one in the lungs and transported, in all parts of the body that need it, by other larger molecules capable of housing them, which are in the blood of the alveoli. In other words, all that we are and what we do is thanks to the action of an endless number of molecules, organized in nanoscale devices and machines that we cannot see individually in action, but are working with great efficiency, high speed and stunning precision (Goodsell, 2009).

Molecules made to order

As already mentioned, with hundred atomic species available, a vast number of molecules can be formed. The chemist, *explorer* of nature, has discovered tens of millions to date. Over the years, the chemist has also become an *inventor* and has begun to create artificial molecules, behaving exactly as Leonardo da Vinci said: «[…] where nature finishes producing its species there man begins with natural things to make with the aid of this nature an infinite number of species […]» (Richter 1977). The molecules synthesized by chemists in their laboratories are much more numerous than the natural ones discovered so far. Chemistry, therefore, is a "book" not only to "read" (exploration of molecules and natural processes), but also to "write" (development of molecules and artificial processes) and if the part not yet read is very large and complicated, the one to be written is practically infinite in extension and complexity.

Much of the creative activity of chemists has brought enormous benefits to humanity. Chemists have invented molecules capable of healing

diseases and alleviating pain, as well as molecules that protect us from cold and heat; molecules to dye textiles and to make food more pleasant; molecules that protect the eyes from excessive light and protect the skin when exposed to the sun; molecules that repel insects and fragrant molecules; *intelligent* molecules, capable of processing electrical or light signals, and of revealing the presence of other molecules. Chemists have also created molecules that, judging by models enlarged one hundred million times, have peculiar shapes, sometimes aesthetically valuable (tree-shaped molecules, knot, chain, bridge and dome), demonstrating that creativity and beauty can be combined even in the world of the infinitely small. Unfortunately, as it often happens in human activity, chemists have also created molecules without worrying about their danger and the side effects they may have on organisms and the environment; even worse, sometimes they intentionally program and synthesize new molecules capable of destroying, burning, poisoning and killing.

Supramolecular systems

The ability of chemistry to provide "custom-made" molecules opens up new perspectives in various fields of science and technology. Each molecule has intrinsic properties that can be seen as a set of information exploitable in the interaction with other molecules or with external electrical or light stimuli. When molecules meet, each "reads" the information elements contained in the other and, depending on the characteristics of these elements, the molecules can either ignore each other, or react with the formation of new species, or aggregate, giving rise to *supramolecular systems* (Lehn 1995). A supramolecular system is therefore obtained by the association of two or more molecules, which takes advantage of the so-called molecular recognition, based on specific interactions (the lock-and-key concept) such as, for example, hydrogen bonding (Fig. 6).
Moreover, using synthetic methods it is possible to join molecules that do not have elements of spontaneous recognition, but which can be in-

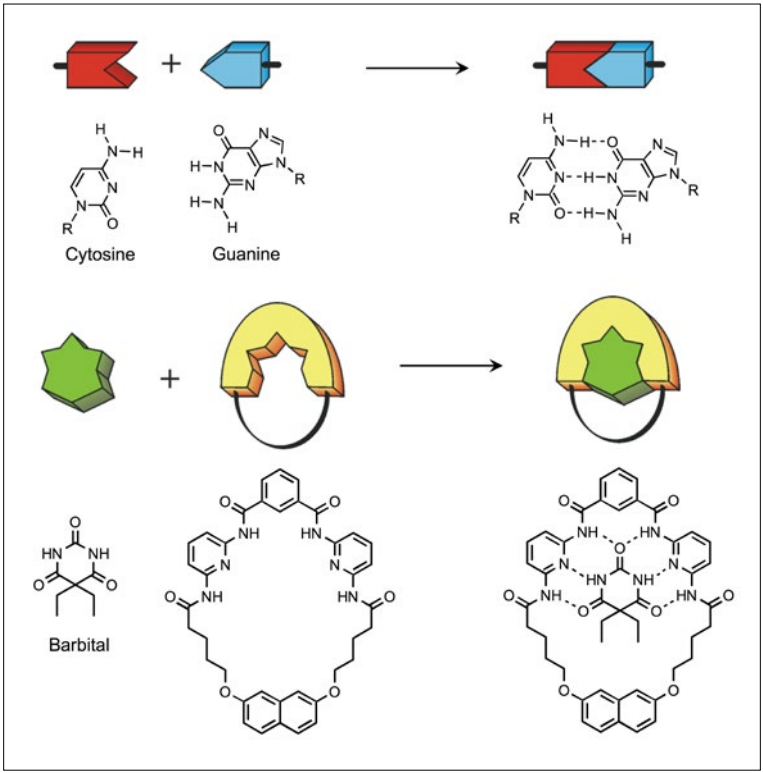


Fig. 6. Two examples of molecules that are able to recognize each other and associate. In both cases the interaction responsible for the recognition is based on the formation of N–H···O and N–H···N hydrogen bonds. For each example, both the chemical formulas and their cartoon representations are shown, from which the spatial complementarity (lock-and-key principle) can be clearly seen.

teresting to associate for specific purposes. Therefore, supramolecular systems can be formed in various ways. If these systems are to perform interesting functions, it is necessary that the molecular components have very precise chemical and physical properties so that in the supramo-

lecular system, thanks to the interaction between the single molecular components, new properties emerge. In this way, supramolecular chemistry becomes *engineering at the molecular level* and can contribute to the development of nanotechnology (Balzani 2008).

Miniaturization

Since the dawn of civilization, technology has dealt with materials of various kinds, processing and forging them with increasingly sophisticated tools to transform them into useful objects. The possibility of having molecules with desired characteristics now allows us to think of a technology that operates on a molecular level, that is, at the nanometer scale; this is *nanotechnology*. To better understand the essential terms of this topic, let us consider the issue of miniaturization.

The first electronic computer was built in 1946 by the University of Pennsylvania at the request of the United States Army. The computer was called ENIAC and occupied the space of an apartment (180 square meters), weighed 30 tons, consumed 200 kW, contained 18000 thermoionic valves, 1500 relays, wires, junctions, and broke down very frequently. Its computing power was negligible compared to that of today's smartphone. How did the revolution that led to current computers happen? The answer is in an increasingly enhanced miniaturization that caused a reduction of the size of various components and, more frequently, their replacement. In the race towards miniaturization (Fig. 7), a *top-down* approach, that consists of working macroscopic pieces of materials with special techniques, was followed. This approach, however, has intrinsic limitations; in practice one cannot easily reach below one hundred nanometers. Although it is already a very small size (about a thousandth of the thickness of a hair), «there's plenty of room at the bottom», as physicist Richard Feynman observed in his famous lecture (which we will talk more extensively about in Chapter III) alluding to the dimensions of atoms and molecules (Feynman 1960). To continue with the miniaturization process, alternatives to those used

so far needed to be found; among these, the so-called *bottom-up* approach is particularly promising. According to this approach, ultra-miniaturized systems are obtained from molecular components that are *programmed* to integrate from the structural point of view and interact from the functional point of view, according to the principles of supramolecular chemistry.

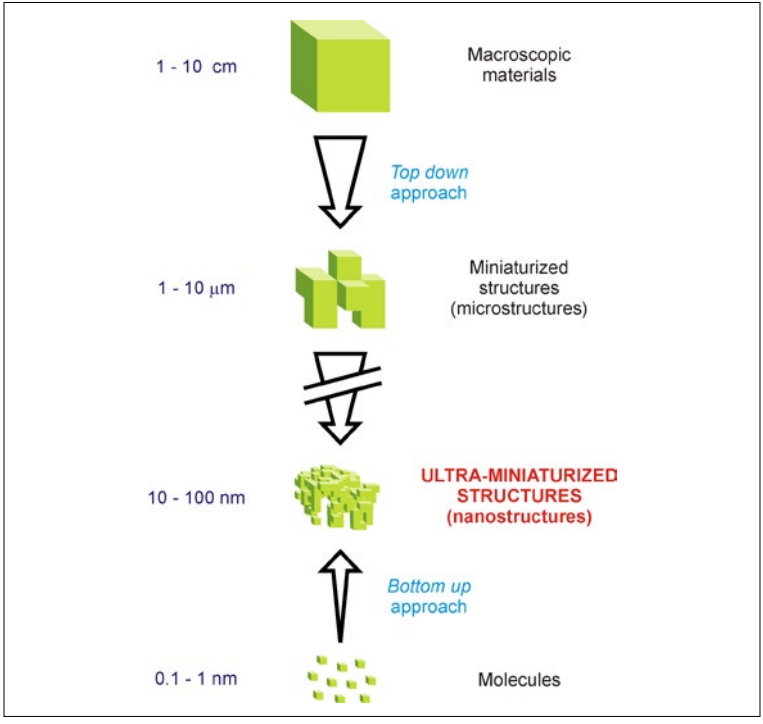


Fig. 7. To build miniaturized structures – that is, in the micrometer scale – a *top-down* approach can be used. Macroscopic pieces of the chosen material are worked with special techniques and transformed into a set of miniaturized units. However, to obtain ultra-miniaturized structures – that is, in the nanometer scale – the top-down approach cannot be followed. It is necessary to use a *bottom-up* approach starting from molecules – that is, nano-sized objects – suitably programmed to form the required structure.

Nature is an astounding master in the process of bottom-up construction. First, nature has prepared a great number of precisely programmed molecules, which in the course of evolution have given rise to all the molecular devices and machines that allow us to walk, eat, talk, see, think; in other words, live. All the biological devices and machines and – rising in the scale of complexity – cells, tissues, organs, systems, and finally the human body, are formed in nature by self-assembly (i.e. by spontaneous assembly) of simpler components which are appropriately programmed. Going up the ladder of complexity step by step, nature has thus reached that extreme wonder that is a human being.
In their laboratories, scientists are not able to climb the scale of complexity from the atom to man. They are capable of *manipulating* life quite profoundly, but they are not able to *construct* it, even in its most basic form. However, scientists have learned to create molecules programmed to construct nanostructures from the bottom up, that are capable of performing functions upon electrical, chemical or light stimulation. In other words, they have created devices and machines at the molecular level which, although much simpler than those found in living organisms, are equally interesting from a scientific point of view and potentially useful for many applications.

The chemist as an engineer

The bottom-up logic followed by chemists to construct nano-sized devices and machines is very simple and can be illustrated as shown in Fig. 8. To create a device of the macroscopic world (for example, a hair dryer), an engineer builds components (a switch, a fan, a resistance), each of which is able to perform a specific action, and then assembles them in an appropriate manner, thus obtaining an apparatus which, powered by energy, performs a useful function. The chemist proceeds in the same way, with the difference, however, being that the engineering process takes place at the molecular level. Once the function that the device has

to perform is established, the construction of the necessary components, which are molecules capable of carrying out specific tasks (programmed molecules), begins; then the various molecular components are assembled into organized supramolecular structures, so that the coordinated set of actions of the components can give rise to the required function (Balzani 2008).

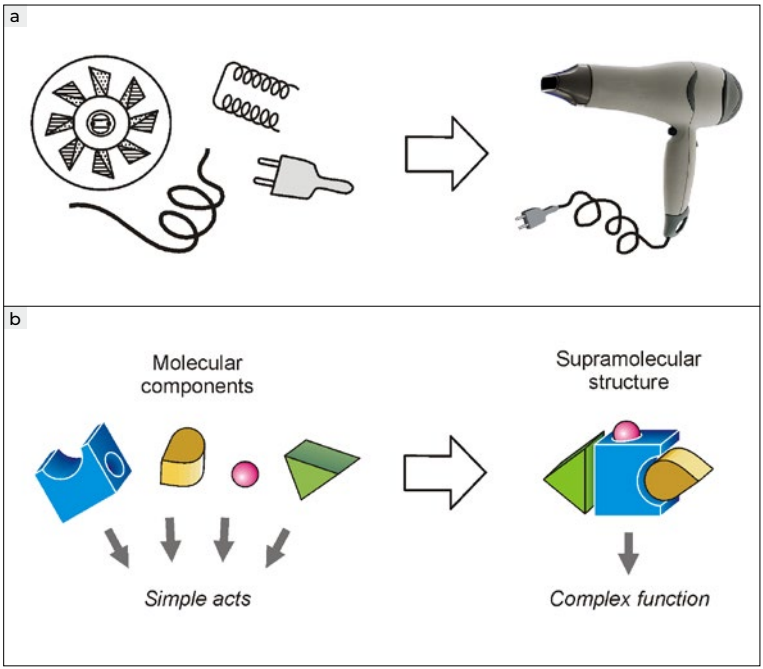


Fig. 8. A macroscopic device (a) is an ordered set of components constructed for the purpose of performing a function. This concept can also be applied to the construction of ultra-miniaturized devices, that is, on the nanometer scale (b). By appropriately assembling a certain number of selected molecules, a supramolecular structure capable of performing a more complex function than those performed by the individual components can be obtained.

Research in this field has already allowed to obtain a vast series of devices at a molecular level able to imitate the functions performed by the components of today's electronic equipment: wires capable of conducting electrons or energy, switches capable of allowing or prohibiting the passage of these flows, plug/socket and extension systems, current rectifiers, antennas for the collection of light energy, memory elements, logic gates, etc. In this book, we will describe a few most significant examples of artificial molecular machines; first, however, it is useful to briefly go over the operations of some natural molecular machines.

CHAPTER II

NATURAL MOLECULAR MACHINES

Movement: from cells to living organisms

Movement is one of the main attributes of life. Nature has equipped living organisms with very complex supramolecular aggregates that work inside cells as devices and mechanisms (for simplicity, we shall call them *machines*) able to satisfy the needs of the cells themselves. They promote chemical reactions that transform certain molecules into others which are necessary for cell life, transport molecular material, copy and transduce the genetic code into proteins, exchange information with other cells, and so on. Also, all the macroscopic movements of living organisms, from bacteria to whales, and the noblest functions of man, from talking to thinking, are consequences of myriad actions and movements at the molecular level. It is estimated that around ten thousand different types of molecular machines are at work in the human body (Goodsell 2009).

The existence of natural molecular machines has been known for a long time, but it was only in recent years that the mechanisms of their functioning began to be studied in detail (Schliwa 2003). We have seen that these systems operate, in the dimension of nanometers, through mechanical movements; often complex, but sometimes simple such as rotations and linear displacements of components of the supramolecular system. The surprising thing is that in many cases, these movements

are apparently similar to those performed by machines of the macroscopic world, even if in organisms everything happens as a response to chemical interactions, particularly intermolecular bonds that break or form. The formal similarity with the movements taking place in the world of macroscopic machines allows us to schematically represent the movements of molecular machines in graphic form. In these graph-

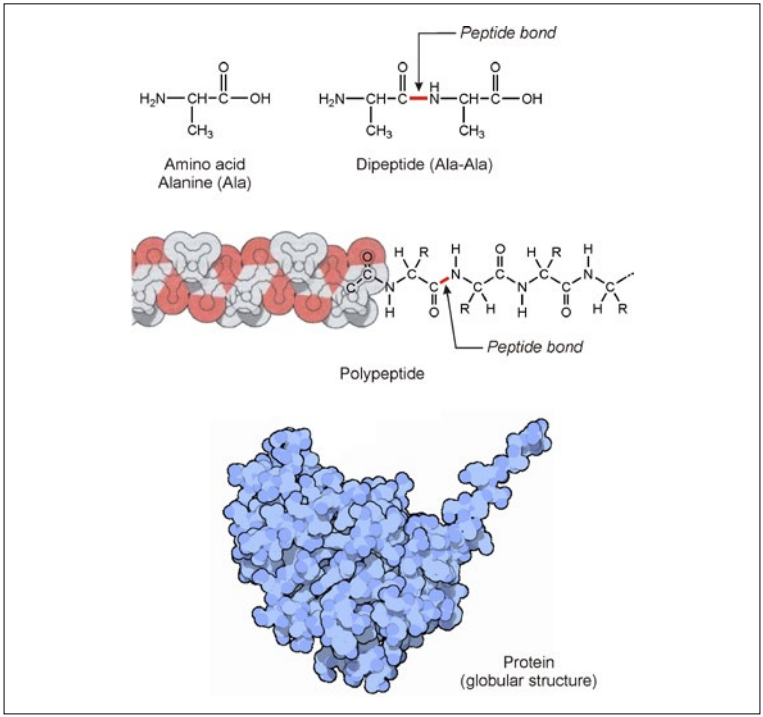


Fig. 9. Most natural molecular machines are made up of proteins, long molecules consisting of chains of amino acids joined together by peptide bonds. Protein amino acid chains tend to fold to give globular structures capable of performing very specific functions. Credits: RCSC PDB and David S. Goodsell, The Scripps Research Institute, La Jolla, USA.

ical representations, it is frequently impractical to report the formulas or models of the molecules involved. As we shall see, we often resort to using schemes of various types showing the shape of the large molecules involved, their mutual interactions, and the function that the supramolecular system performs.
In reality, natural nanomachines have very different shapes from those of the machines and objects in the macroscopic world. They look like large agglomerates of atoms, piled up without any apparent planning. In fact, the great majority of natural molecular machines are composed by proteins, molecules consisting of modular chains of amino acids (Fig. 9), which tend to coil up to provide globular structures. These chains can contain from a dozen to thousands of amino acids, depending on the function they have to perform.

Linear movements

All the voluntary and involuntary muscular movements of our body derive from natural nanomotors that develop linear movements. Very complex protein molecules called *myosins* are responsible for these movements. Such molecules consist of long tails to which two large heads are connected (Fig. 10a, top right). In muscle cells (Fig. 10a), many myosin molecules are assembled through the tails to give a filament from which the heads extend; these attach themselves to other filaments, called *actin*, parallel to those of myosin and acting as a guide. A chemical reaction involving the adenosine triphosphate species (ATP, Fig. 11) provides energy to the system that is used to radically change the shape of the myosin heads and to force it, as a consequence of this change of shape, to move along the actin filament (Fig. 10b).
In a rapidly contracting muscle, each unit of myosin moves five times per second, covering a distance of about 10 nm with each movement. It is estimated that two billion nanometric movements are needed to generate the force required to catch a baseball.

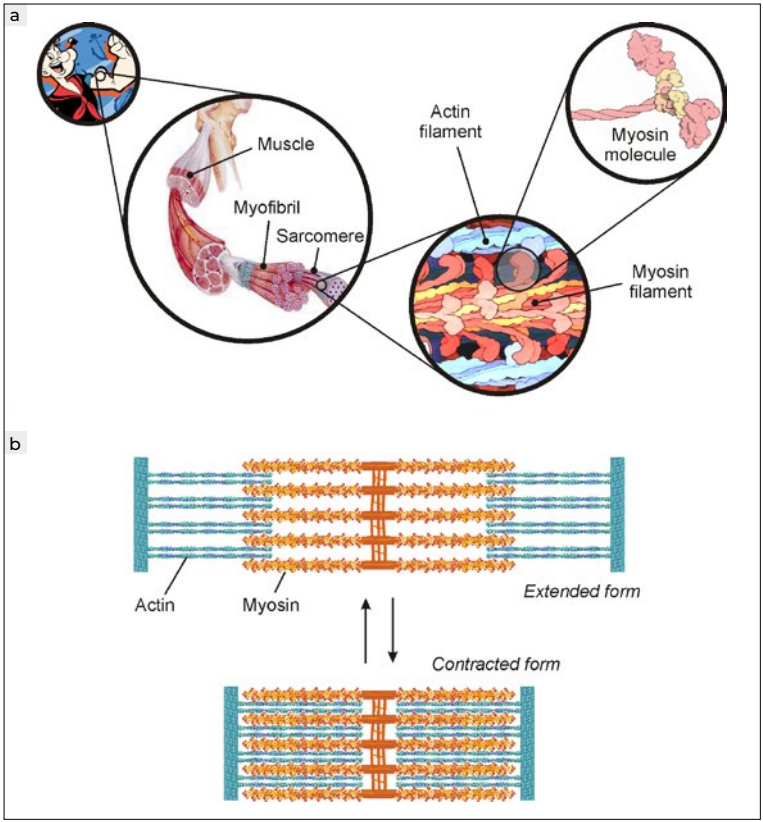
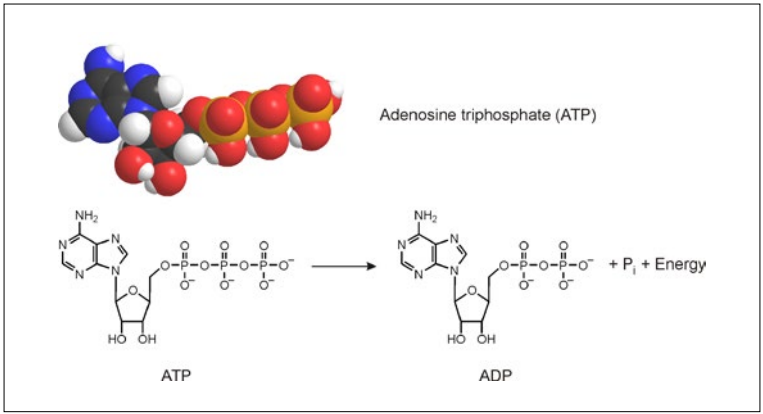


Fig. 10. Muscle function. (a) Schematic representation of the myosin molecule which has a long tail to which two large heads are attached. In muscle cells, many myosin molecules are intertwined by the tails to give a filament from which the heads extend. These attach themselves to other parallel filaments consisting of molecules of another protein, actin. These filaments, which are able to glide over one another, are contained in sarcomeres. (b) Muscle contraction is generated by the chemical reaction described in Fig. 11 which modifies the shape of the heads of the myosin molecules forcing them to glide over the actin filaments. The sarcomere thus passes from an expanded form to a contracted form. Credits: RCSC PDB and David S. Goodsell, The Scripps Research Institute, La Jolla, USA.



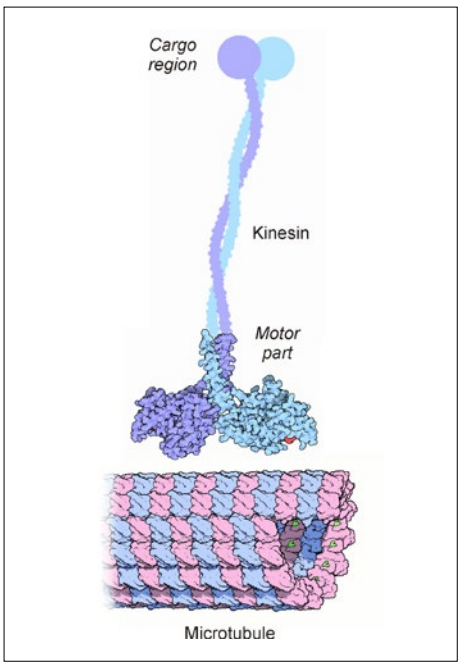


Fig. 11. The adenosine triphosphate (ATP) molecule is the *energy currency* of cells. By breaking the ATP molecule to give adenosine diphosphate (ADP) and phosphate (P_i) energy is obtained, used to feed cellular functions.

Fig. 12. Kinesin is a linear molecular motor capable of carrying a load along a track consisting of a microtubule. Also in this case the energy required to operate the motor part of the kinesin comes from the breakup of ATP molecules in particular sites located in the heads. It should be noted that the various parts of the figure are not to scale: the microtubule is much larger than the kinesin. Credits: RCSC PDB and David S. Goodsell, The Scripps Research Institute, La Jolla, USA.

The task of linear molecular motors is not limited to muscle contraction. A real railway network operates inside the cells to transport substances from one side of the cell to the other. These nanometric "freight trains" are driven by linear molecular motors such as *kinesin* and *dynein*.
Kinesin, for example, consists of a motor part, possessing two heads, and an area intended for the capture and release of the cargo to be transported (Fig. 12). The two heads of the motor part, due to chemical reactions involving ATP that they can accommodate, alternatively bind and dissociate from a microtubule (a protein filament 30 nm wide and 50000 nm long), practically *walking* on it in 72 nm steps, at a speed of 1000 nm per second.

Rotary movements

One of the most studied natural nanomachines is certainly the one tasked with the synthesis of adenosine triphosphate (ATP), a chemical species which, as we have seen (Fig. 11), provides the energy for all muscle movements and therefore also presides over vital functions. This machine is 10 nm in size and is very complex (Fig. 13). Schematically, it is constituted by a cylindrical unit *C*, formed by long protein molecules wrapped in a helix, which crosses the cell membrane (wall), and by a unit γ fixed to *C*. When the concentration of hydrogen ions (H^+) outside the membrane is higher than the concentration inside, a flow of hydrogen ions is generated through the *C* unit which thus begins to rotate as if it were a mill; the unit γ, which is integral to *C*, also rotates (Fig. 13a). The rotating γ unit acts as a mechanical camshaft that successively deforms three sites in the α and β molecules that surround it, causing the transformation of ADP (adenosine diphosphate) into ATP in each of them (Fig. 13b).
In short, this natural molecular machine is a genuine rotary motor which, powered by a flow of hydrogen ions, produces mechanical work which in turn allows a low energy content substance (ADP) to be con-

verted into a substance with a higher energy content (ATP). In organisms, therefore, cellular fuel (ATP) is totally regenerated from its own waste, thanks to a molecular machine. To get an idea of the amazing work done by these nanomachines, just think that one day an individual consumes about 70 kg of ATP, and that each ATP molecule is recycled on average 700 times.

However, the operation of this nanomachine is even more complex and stunning. In fact, it combines two molecular motors, shown in Fig. 13a with F_1 and F_O, joined to the same cam γ. These two engines are both able to rotate, but in opposite directions. The F_O motor, as mentioned above, exploits the flow of hydrogen ions to turn in one direction, while the engine F_1, using the energy produced by the conversion of ATP into ADP (i.e. the reverse of the reaction mentioned above), rotates in the opposite direction. Since the two motors are connected in an integral manner, the direction of rotation is imposed by the strongest one. Thus, when the concentration of hydrogen ions inside the membrane is lower than outside (as it happens in bacteria due to a photosynthetic process), F_O is the most powerful motor and F_1 is forced to turn backwards. In chemical terms, this means that a current of hydrogen ions is "consumed" and the valuable ATP that supplies energy to the organism is produced. On the other hand, when hydrogen ions abound inside the membrane, the F_1 motor prevails, forcing F_O to turn backwards. In chemical terms, this means that the body can use the energy of ATP to pump the hydrogen ions where they are needed.

Motors made of natural proteins can be used to operate artificial mechanical devices. An example of this type is shown in Fig. 14 (Soong 2000). F_1-ATP synthase units, taken from a bacterium, were chemically fixed on metal posts about 100 nm in diameter and 200 nm in height, manufactured by electron beam lithography. A nickel bar measuring 150 nm in diameter and about 1000 nm in length was attached to the γ cam of each F_1 unit. When energy was supplied to the system in the form of ATP, the F_1 nanomotors were found to rotate, dragging

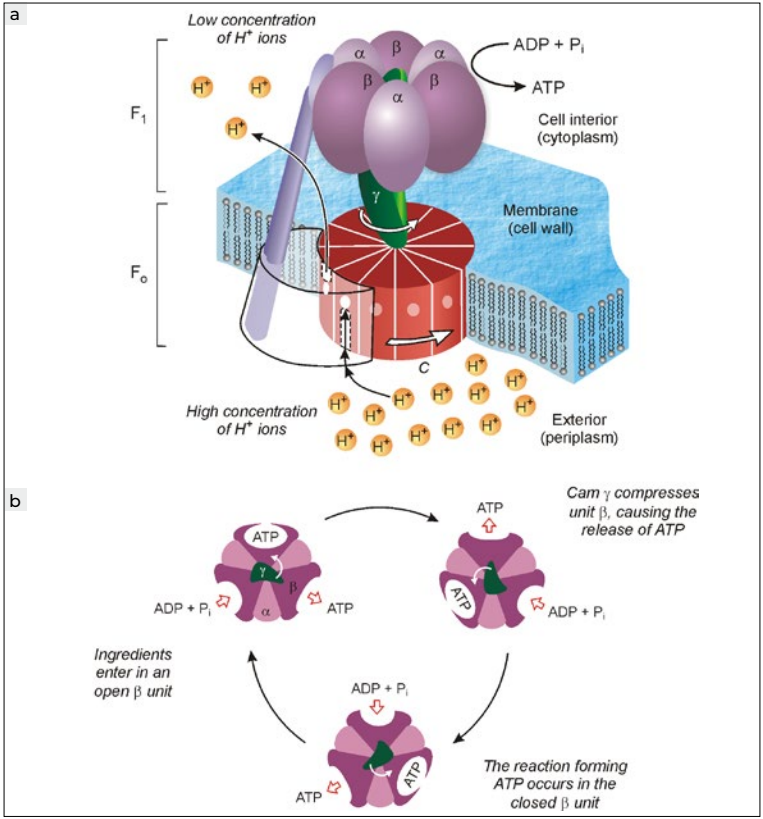


Fig. 13. Depiction of the enzyme ATP synthase tasked with the synthesis of adenosine triphosphate (ATP) starting from adenosine diphosphate, ADP, and inorganic phosphate (P_i). This enzyme, around 10 nm in size, consists of two rotating molecular motors, F_O and F_1, coupled together (a). In the normal functioning of the enzyme, a different concentration of hydrogen ions on both sides of the cell membrane causes a flow of the same ions through unit *C*. This flow rotates the unit *C* as if it were a mill. The γ cam, integral with *C*, presses successively on the catalytic units α and β of F_1, causing the formation of ATP from ADP and phosphate ingredients. The top view of the enzyme (b) shows how the γ cam, by rotating, deforms in sequence the three sites where the synthesis of ATP takes place.

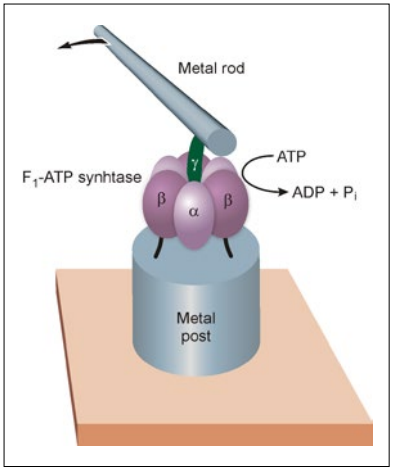


Fig. 14. The F_1 part of the enzyme ATP synthase was used as an engine of an artificial nanodevice. The catalytic units α and β of the enzyme (see also Fig. 13) are fixed on a metal post with a diameter of about 100 nanometers (see text), while a metal bar about one micrometer long is tied to the γ cam. By administering ATP to the system, the molecular motor starts the cam, which in turn rotates the metal rod; the latter is sufficiently large to be observed under an optical microscope. It should be emphasized that the different parts of the illustration are not to scale: the metal bar is in fact about 100 times larger than the molecular motor to which it is connected.

the metal bars in their motion. Since the latter are large enough to be observed with a normal optical microscope, and the metal supports are sufficiently distant from each other, this experiment permitted to "film" the functioning of single biomolecular motors.

Shape changes

Enzymes are globular structures made up of wrapped protein chains. In the entangled cluster of molecular chains that constitute them, enzymes possess specific sites (Fig. 15), which are slits or cavities of very particular sizes and shapes, capable of rigorously selecting the access of external molecules by a lock-and-key mechanism. These active sites are where the chemical reactions promoted by the enzyme take place.

The accessibility to the active sites can be regulated by structural changes of the enzyme caused by interactions with external molecules. This is how the thousands of enzymes in our body can be activated or deactivated on demand, depending on the needs of the organism.

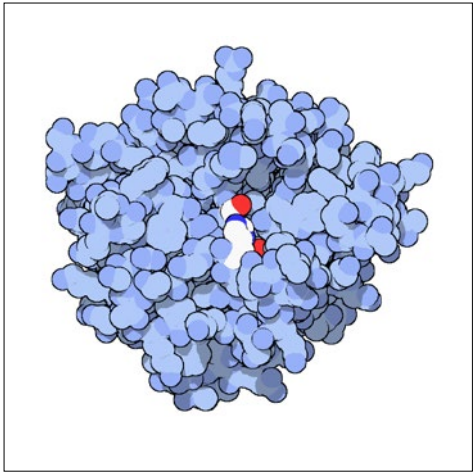


Fig. 15. *Chymotrypsin* is a very important enzyme for the functioning of our body. In this three-dimensional representation, the active site of the enzyme (in light color) can be seen at the center of the cluster of protein chains (in gray). Credits: RCSC PDB and David S. Goodsell, The Scripps Research Institute, La Jolla, USA.

A typical example of an enzyme whose active site can be activated and deactivated is *aspartate transcarbamylase*, also known as ATCase. This enzyme is present in bacterial cells, where is responsible for the synthesis of two very important molecules, thymine and cytosine. As shown schematically in Fig. 16, ATCase is composed of six large *catalytic units* (that is, tasked with promoting the reaction) whose position is controlled by six smaller units, which constitute the *regulatory system* of the enzyme. Note that the representation shown in the figure is neither a chemical formula nor a scaled molecular model, but only a schematic drawing that attempts to explain the way this complex enzyme works. The active site of the enzyme is located where two catalytic units face one another; if they are slightly separated, their active sites are free and functional, while if they are in close contact, they interact directly, preventing the access of the reagents. When the molecules produced by the enzyme over accumulate, their presence causes the regulatory units to change shape, forcing the catalytic units to get closer until the active site is shut down.

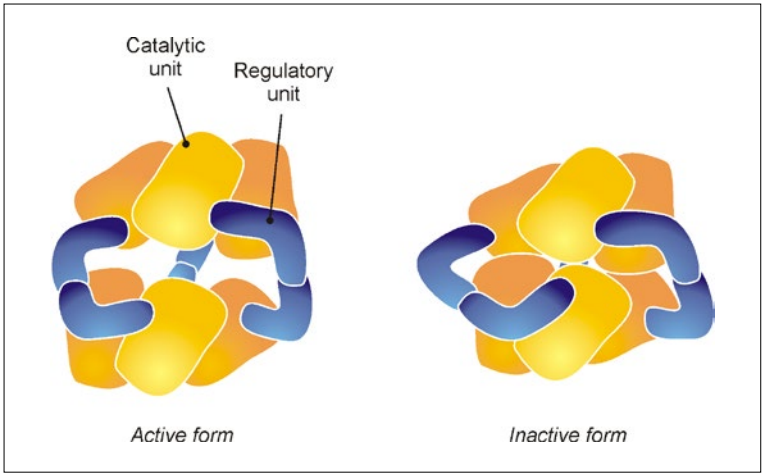


Fig. 16. The enzyme *aspartate transcarbamylase* is present in bacteria, where it is responsible for the synthesis of two important molecules, thymine and cytosine. This enzyme is able to self-regulate: an excess of synthesized molecules causes a change in shape (allosteric mechanism) that blocks the activity of the enzyme.

Enzymes such as the one just described, whose operation relies on a change of shape, are called *allosteric* (from the Greek ἄλλοσ, different, and στερεόσ, solid). Allosteric effects are a very effective way devised by nature to provide feedback to the system in order to keep a chemical process under control.

CHAPTER III

ARTIFICIAL MOLECULAR MACHINES

From Feynman's joke to the Nobel Prize in Chemistry

The idea of creating artificial molecular machines was first theorized by Richard Feynman, Nobel Laureate in Physics, in the famous lecture *There's plenty of room at the bottom* held on December 29, 1959 at a meeting of the American Physical Society. In the conference, which many consider to be the founding manifesto of nanotechnology, Feynman asks in an almost joking manner: «What are the possibilities of small but movable machines? They may or may not be useful, but they surely would be fun to make» (Feynman 1960). Feynman cites the possibility that ultra-miniaturized mechanical devices could be injected into a patient to diagnose or even repair malformations to internal organs. This idea would be resumed a few years later (1966) in the film *Fantastic Voyage*, from the novel by Isaac Asimov. In short, during the 1960s the construction of nanometric machines, albeit a scientifically valid idea, appears to be a topic closer to science fiction than to science. Be careful, though: history has shown on various occasions that today's science fiction can become the reality of tomorrow.

In order to further develop the discussion initiated by Feynman, it was necessary to wait until the 1980s, when another physicist, Eric Drexler, proposed the possibility of constructing a nanometer-sized robot capable

of manufacturing anything – including replicas of itself – using as a raw material the individual atoms (the so-called *universal assembler*) (Drexler 1986). In his 1959 lecture Feynman also noted that «The principles of physics, as far as I can see, do not speak against the possibility of maneuvering things atom by atom» (Feynman 1960). This idea, however, has never been realized and, according to chemists, it is not even feasible. They know very well that atoms are highly reactive species and that, therefore, they cannot be *taken* from one material and *carried* to another, as if they were simple Lego blocks. Even the eventual robotic arm, in fact, would be made of atoms, which would end up reacting with the atoms that it would like to manipulate. Feynman himself acknowledges:

> Ultimately, we can do chemical synthesis. […] The chemist does a mysterious thing when he wants to make a molecule. […] And, at the end of a difficult process, he usually does succeed in synthesizing what he wants. By the time I get my devices working, so that we can do it by physics, he will have figured out how to synthesize absolutely anything, so that this will really be useless (Feynman 1960).

The universal assembler seems therefore destined to remain an object of fantasy, the protagonist of stories such as the exhilarating *Order of the Cheap* by Primo Levi (Levi 1966) or the disturbing *Prey* by Michael Crichton (Crichton 2002).

In the last twenty years chemists have been able to obtain devices and machines at the nanometric level, starting from programmed molecules and following the criteria of supramolecular chemistry (Balzani 2000; Browne 2006; Balzani 2008; Erbas-Cakmak 2015). Although it is not possible to imitate in detail what happens in nature, where molecular machines of incredible complexity are formed spontaneously by self-assembly of programmed molecules, with the chemical bottom-up approach (Figs. 7 and 8) it has been possible to build quite sophisti-

cated artificial molecular machines and motors. The design phase is, of course, very delicate as the following aspects must be foreseen: 1) the type of energy to be used to make the machine work; 2) the type of movement that the machine must perform; 3) the way in which movements can be controlled; 4) the signals that highlight the movements themselves; 5) the need to operate in a cyclic and repetitive manner; 6) the time taken to complete a cycle; 7) the function that can derive from the movements performed.

As we have already seen for natural molecular machines, the mechanical motion implies substantial changes and this result can be achieved in artificial systems only if at least one of the molecular components of the machine is involved in a chemical reaction. Thus, one has to provide, in some form, the energy needed (point 1) to activate the chemical reaction at the basis of the movement, which can be (point 2) of various types (for example, rotary or linear), and whose control (point 3) can be performed with antagonist chemical reactions. The signals that report on the operation of the machine (point 4) arise from modification of the properties of the system (for example, color changes) that accompany the movements. The latter, in turn, must involve reversible reactions in order to enable cyclic operation (point 5). The time scale in which a cycle is completed (point 6) can range from picoseconds (10^{-12} s, or thousandths of a billionth of a second) to hours, depending on the chemical nature of the system. Finally, various functions can in principle be obtained from the work of the machine (point 7), as it will be shown later.

Some of these aspects – namely, those related to machine control, the signals to verify its functioning, the need to establish a cyclic behavior, and the assessment of operation times – are related to problems that chemists are able to deal with good mastery; the aspects concerning energy supply and the directional control of movements are more critical. Nowadays, numerous types of artificial molecular machines are relatively simple to obtain; the frontier of research in this area, as we will see in Chapter IV, has therefore moved towards the study of the problems asso-

ciated with the exploitation of nanometric movements for technological and medical applications.
Beyond the possible practical uses, many of which are currently only imaginable, research on artificial molecular machines certainly has the merit of having radically changed the relationship between molecules and scientists. In fact, the introduction of an engineering-type mindset has enormously stimulated the ingenuity and creativity of chemists. This has led to the development of new research lines, often of a highly multidisciplinary nature, which in turn have generated new challenges, thus creating that virtuous circle on which scientific progress is based. These are the reasons which led the Royal Swedish Academy of Sciences to award the 2016 Nobel Prize for Chemistry «for the design and synthesis of molecular machines» to Jean-Pierre Sauvage, Fraser Stoddart and Ben Feringa (Fig. 17). The recognition of these three distinguished scientists and pioneers in the field testifies to the scientific maturity of the idea of molecular machines, which was born almost as a joke in 1959 and subsequently developed and consolidated thanks to the decades-long commitment of many researchers all over the world.

Some things to know about the nanometric world

The observation of natural systems reveals that molecular machines are not simply miniaturized versions of macroscopic machines. In fact, the reasoning in which one claims to shrink the size of objects down to the nanometric scale, without considering the corresponding change in the properties and behavior of the matter, leads to totally wrong conclusions (Jones 2004).
Although the physical laws that regulate matter are always the same, their practical consequences depend on the dimensional scale of observation. For example, macroscopic machines are typically built with rigid materials and their operation can take advantage of temperature differences with the environment, as happens in thermal machines such as combustion

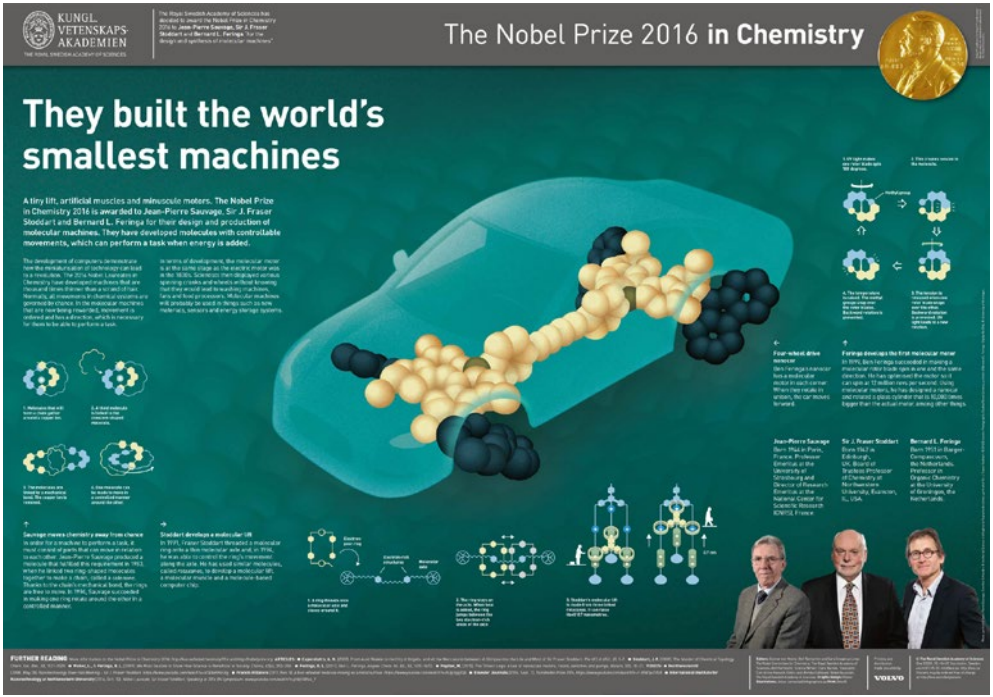


Fig. 17. The poster celebrating the 2016 Nobel Prize in Chemistry. Copyright © The Royal Swedish Academy of Sciences, https://www.nobelprize.org/.

engines. Conversely, molecular machines are made up of "soft" and flexible parts, and must operate at a constant temperature (determined by the environment in which they are located), because the heat flows very rapidly on the nanometer scale. Due to the tiny mass of molecules, the effects of gravity and inertia, which are so important in the mechanics of macroscopic bodies, are irrelevant in the nanometric world. This realm is dominated by intermolecular interactions, which instead are often negligible in the macroscopic world.

The main characteristic of movement in the nanometric world, however, is the fact that objects of this size are subjected to the random and incessant motion determined by thermal agitation – in other words, Brownian motion. The second law of thermodynamics states that it is not possible to extract work from Brownian motion. It cannot be eliminated, unless it is at absolute zero, and its intensity is proportional to temperature. At room temperature the Brownian motion has a disruptive effect on the movement of very small objects; it is estimated that the thermal agitation to which a molecule is subjected corresponds to a power tremendously higher than that supplied by ATP hydrolysis in a biomolecular machine (Astumian 2002). In short, for a molecule, using energy to move in a controlled manner following a precise direction is like trying to ride a bike during an intense earthquake. Since the latter cannot be seized, the only way to move forward is to take advantage of the shocks in the right direction. Natural molecular machines do exactly this: they use energy (ATP) to rectify the disordered thermal motion, so that the movement in a certain direction becomes more probable than that in the other directions. In other words, it is thermal agitation that drives the molecular machines; in order that this thrust not be limited to producing random effects (which cannot be used to perform work), an external energy source is required. Understandably, obtaining controlled and directional movements in a molecular system is very difficult, and the design must take into account very different aspects in comparison with the design of a macroscopic device.

The energy problem

We have just seen that ordered molecular movements cannot be obtained by exploiting the Brownian motion of a medium at a constant temperature. Molecular machines, like macroscopic ones, therefore need to be fed from an external source of energy.

For most machines in the macroscopic world, the necessary energy is obtained from reactions between oxygen and substances with high energy content (fuels) carried out in internal combustion engines. We have stated that processes of this type, which among other things occur at elevated temperatures and high pressures, cannot be used to power molecular machines. They too, however, can exploit chemical reactions that take place at a constant temperature and in mild conditions. In the famous 1959 speech, Feynman noted: «[…] an internal combustion engine is impossible. Other chemical reactions, liberating energy when cold, can be used» (Feynman 1960). This is exactly what happens in biological nanomachines, where the reactions that release the energy necessary for their functioning (typically, the hydrolysis of ATP) take place at ambient temperature and pressure and proceed through several successive stages, in each of which a small amount of energy is put at stake. Apart from these differences, the fact remains that both macroscopic and nanometric machines work by consuming a source of fuel. This inevitably leads to the formation of waste products, the elimination of which is a necessary condition for preserving the proper functioning of the machine. As we have seen (Fig. 13), nature has admirably solved this problem by recycling, through metabolism and the action of ATP synthase, ADP and phosphate in the production of new ATP (Goodsell 2009).

Research on artificial molecular machines has shown that it is possible to operate these systems not only by chemical energy, but also – and often more conveniently – by electrical or light energy (Ballardini 2001). These two forms of energy are particularly interesting as they allow suitably designed systems to operate without the formation of

waste products. In addition, both electrical energy and light can be administered to molecules in a precisely controlled manner and with much higher resolution over time and space than chemical fuels. A further advantage of these forms of energy is that the techniques used to transfer them to molecular machines also allow us to study their functioning.

In describing artificial molecular machines we will use simplified structural formulas to indicate the chemical compounds involved and schemes to illustrate the types of mechanical movements performed by the machine.

Shape changes

One of the first reported examples of an artificial molecular machine is system **1** shown in Fig. 18. It consists of two ring molecules, called crown ethers (A), connected to a central –N=N–unit (B) that can change its structure (in the chemical nomenclature, passing from the linear *trans* form to the bent *cis* and vice versa) due to the absorption of light of appropriate color. When a solution containing this system in the *trans* form is irradiated with ultraviolet light, the change in the structure of the

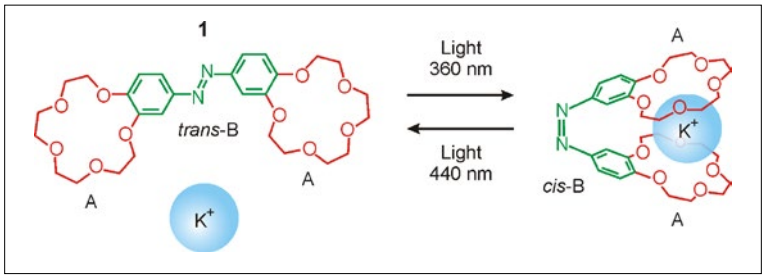


Fig. 18. Molecular tweezers operated by light. The tweezers, when closing due to the action of ultraviolet light (wavelength of 360 nm), can capture a potassium ion (K^+) which is then released when the tweezers open due to the action of blue light (wavelength of 440 nm).

central unit B causes the two side rings A to approach, which can thus capture a potassium ion (K^+). Using visible light, or leaving the system in the dark, the reverse process occurs with the consequent release of the K^+ ion. This mechanical action is comparable to that of nanometer-sized tweezers, which could form the basis for the construction of systems capable, within an organism, of transporting and releasing drugs, or eliminating harmful substances.

Linear movements

Most research in the field of artificial molecular machines capable of performing linear movements is currently focused on systems called rotaxanes (Fig. 19). A *rotaxane* is formed by a filiform molecule threaded in a ring-like molecule; the presence of bulky groups (called *stoppers*) at the ends of the filiform component prevents the slipping off of the ring (Sauvage 1999). Systems of this kind, if carefully designed, can perform

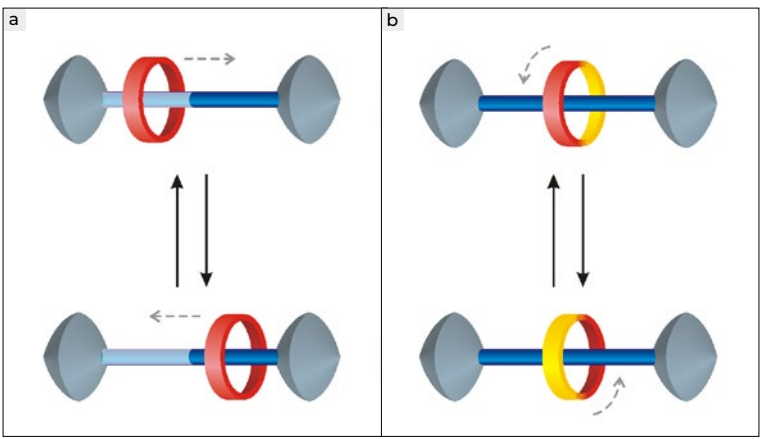


Fig. 19. Schematic representation of linear (a) and rotary (b) mechanical movements that can take place in a rotaxane.

mechanical movements such as those shown in the figure when they are appropriately stimulated. The peculiarity of a rotaxane is that its molecular components, although not chemically bound together, cannot be dissociated. There is a mechanical constraint between wire and ring that maintains the integrity of the nanostructure, while allowing a certain degree of freedom of movement of the components with respect to one another (Bruns 2017).
Below are some examples of molecular machines based on these systems, also chosen to show how light, chemical or electrical energy can be used to make mechanical movement happen.

Molecular shuttles

In a rotaxane, the movement of the ring along the thread (Fig. 19a) corresponds, at a molecular level, to the movement of a shuttle along a track. The first example of this type, developed in 1994 at the University of Birmingham (United Kingdom) by Fraser Stoddart's group, is represented by the rotaxane **2** shown in Fig. 20 (Bissell 1994). It is formed by ring A and the linear component B in which there are two distinct units, B1 and B2. The first one, called benzidine, can be charged positively either by adding two protons (H^+) or removing an electron. The second unit is called biphenol and, like benzidine, is an electron donor. These units represent two potential *stations* for the ring, which is instead an electron acceptor. It is attracted by both B1 and B2 by virtue of donor-acceptor interactions; however, since these interactions are stronger with B1 than with B2, the ring is initially on station B1. However, if an acid is added to the solution containing the rotaxane, the benzidine acquires two H^+ ions and loses the ability to interact with the ring; consequently, the latter moves to station B2. If at this point a basic substance is added to the solution, the benzidine is restored and the ring returns to station B1, completing the operating cycle (Fig. 20b).
The alternating movement of A between B1 and B2 can be repeated many times because the acid-base reaction that governs it is perfectly reversible.

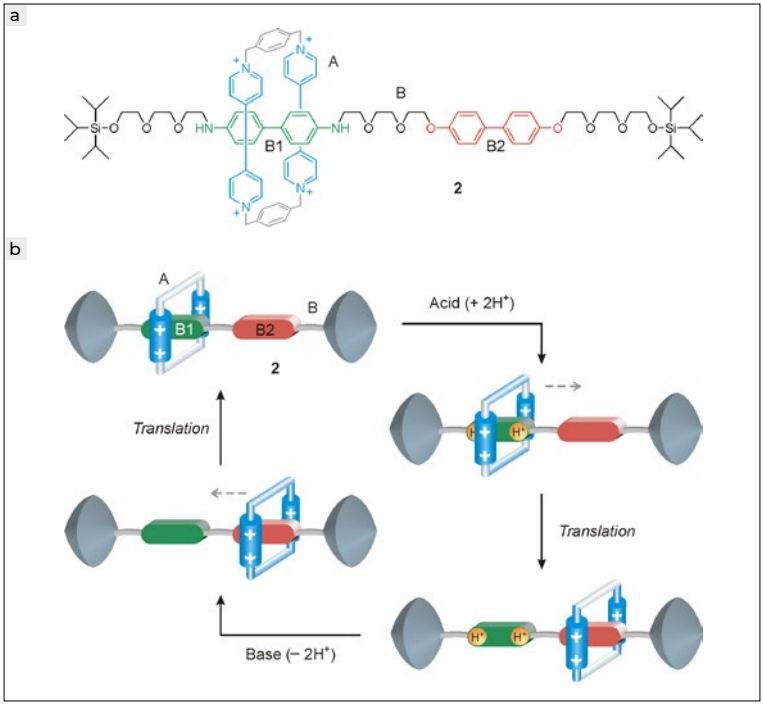


Fig. 20. A molecular shuttle operated by a chemical reaction. (a) Simplified structural formula of rotaxane **2**. (b) Schematic representation of shuttle operation. Ring A, initially positioned on station B1, moves along thread B towards station B2 when an acid is added, and returns to station B1 for subsequent addition of a base. The same result can be obtained with electrical stimuli, oxidizing and reducing the station B1.

The only limitation derives from the fact that subsequent additions of base and acid lead to the formation of waste products which, if not eliminated, after some time can compromise the functioning of the system.

The molecular shuttle shown in Fig. 20 can also be operated with electrical stimuli. In fact, removing an electron from the benzidine (chemists call this process *oxidation*) eliminates its ability to attract the ring, which

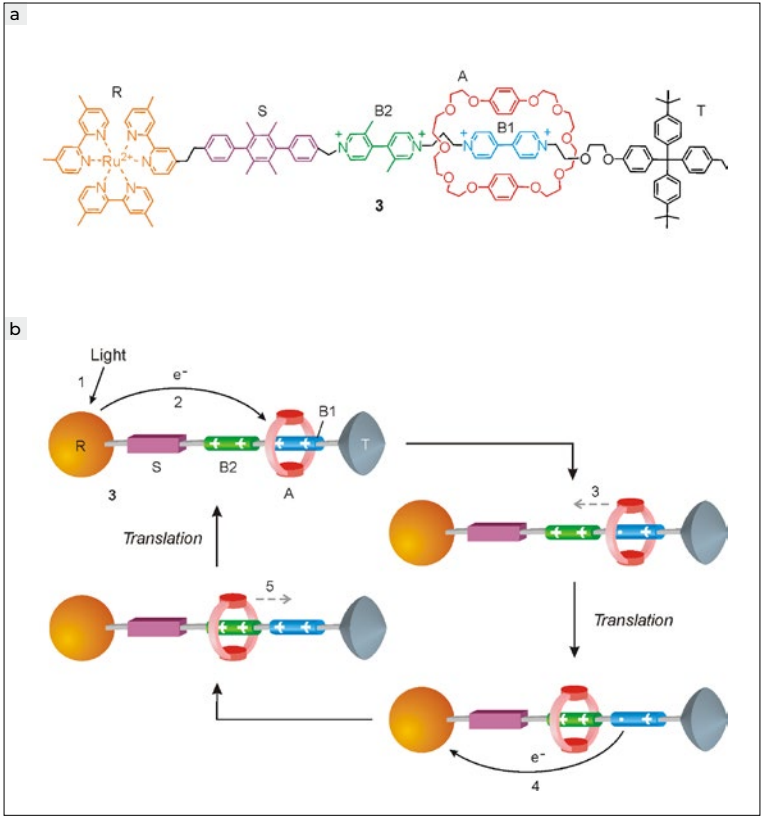


Fig. 21. A four-stroke linear nanomachine powered by light stimuli. Part (a) of the figure shows the simplified structure formula of rotaxane **3**. In part (b), the sequence of events caused by light excitation is illustrated using a cartoon representation of the rotaxane. Initially, ring A resides on station B1. The absorption of a photon of light (process 1) by the ruthenium complex (R) is followed by the transfer of an electron (process 2) from R to B1. The latter unit no longer interacts with ring A, which consequently moves to B2 (process 3). At this point an electron returns from B1 to the ruthenium complex (process 4): the primary station is regenerated and the A ring returns onto it (process 5). All processes are very fast and an entire cycle takes place in less than one thousandth of a second.

then moves to B2. Subsequently, by returning the electron to the oxidized benzidine (*reduction*) it is possible to restore the primary station B1, which will again be surrounded by the molecular ring (Bissell 1994).

After this first study, numerous prototypes of molecular shuttles driven by chemical or electrical stimuli were described. Examples of molecular light-driven shuttles are much rarer; one of them, developed in our laboratory in collaboration with the Stoddart group, is shown in Fig. 21 (Balzani 2006). The structural and functional complexity of this system gives an idea of the level of sophistication achieved in the design and construction of molecular machines. It is a rotaxane (**3**) comprising a ring component A, with characteristics of electron donor, and a linear component consisting of several modules: i) a ruthenium complex (R) which carries out, in addition to the function of stopper, also the fundamental one of absorbing the light used by the system; ii) two units, B1 and B2, having electron acceptor characteristics: these are the two stations on which ring A can stop; iii) a rigid spacer S and a second stopper T. The initial state of the system is that in which ring A surrounds unit B1, which is an electron acceptor more effective than B2. Following the light excitation of the ruthenium complex R, a series of movements take place in the system which can be described schematically as follows (Fig. 21).

a) Destabilization of the initial structure: following the absorption of light (process 1) an excited state of R is obtained which transfers an electron to the station B1 (process 2) surrounded by the ring A. Following this electron transfer, station B1 loses its electron acceptor characteristics and no longer interacts with A;
b) Displacement of the ring: as its interaction with B1 is lacking, ring A moves (process 3) and passes on station B2 with which it can interact;
c) Electronic reset: at this point a process opposite to that caused by light brings an electron from the disabled station B1 (no longer surrounded by A) back to the ruthenium complex that had initially transferred it (process 4), thereby restoring the electron acceptor character of station B1, which is thus reactivated;

d) Structural reset: following the electronic reset, ring A returns onto station B1 (process 5), restoring the initial structure.

In conclusion, a light pulse causes, through four stages, the alternate movement of the ring along the thread from right to left and then from left to right without generating waste products; this system can therefore be considered a four-stroke linear motor, operated by light.

The nanoelevator

The experience gained with the simplest prototypes of artificial nanomachines has allowed scientists to design and build systems of ever-increasing complexity. For example, the three-dimensional development of a shuttle powered by chemical energy has led our research group, again in collaboration with Stoddart's, to build what can be called a *nanometric elevator* (Badjic 2004). As shown in Fig. 22a, it consists of a three-branched frame (T), each of which contains two stations – an ammonium (T1) and a bipyridinium (T2) ion – and a molecular platform (P) obtained from the union of three ring compounds A. The three branches of T are inserted in the three rings of P, giving rise to a triple joint structure (**4**) in which the rings of the platform surround the ammonium stations of the frame, thanks to the presence of hydrogen bonds. However, if a base is added, the ammonium units T1 lose a hydrogen ion (H^+) and, as a consequence, the ability to interact with the rings of P; the latter are therefore free to move and encircle the bipyridinium units T2, with which they give a donor-acceptor interaction. By subsequent addition of an acid (H^+) the system returns to the initial structure. Schematically (Fig. 22b), the system can therefore be represented as a platform hinged on three columns that rises and falls between two levels, identified by the T1 and T2 stations, following commands (variations in acidity, or pH) coming from the exterior. The molecular elevator aroused great curiosity among the experts, and is depicted in the poster of the 2016 Nobel Prize in Chemistry (Fig. 17).

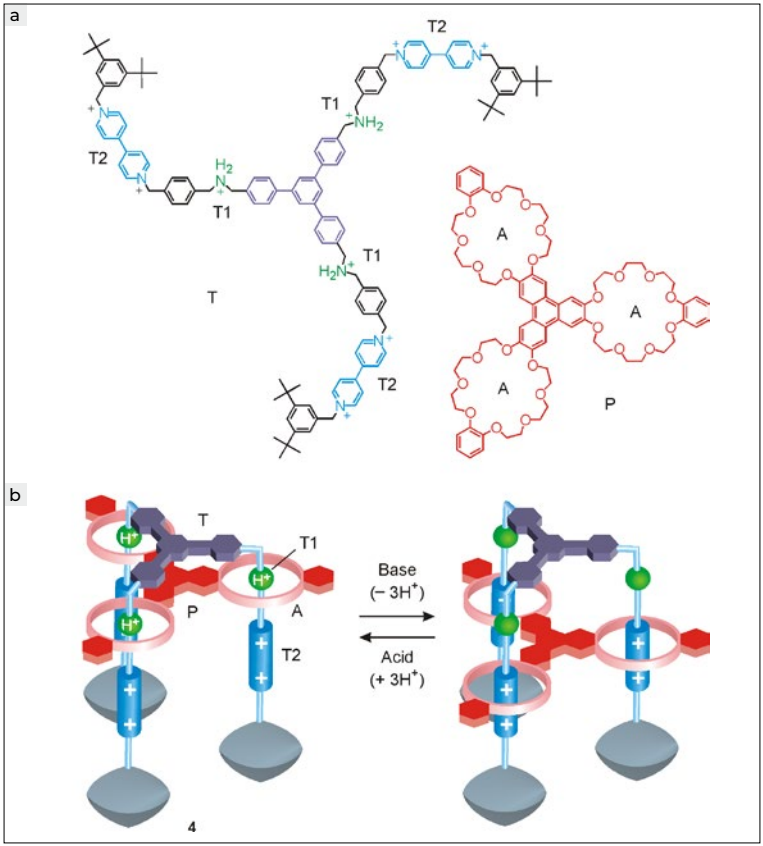


Fig. 22. A nanometric elevator. (a) Simplified structural formulas of the two components of the system, which can be considered a three-dimensional evolution of a molecular shuttle similar to the one illustrated in Fig. 20. A frame with three branches T, each of which contains two stations T1 and T2, is assembled with the three-ring P platform. In the supramolecular system thus obtained (**4**) the three branches of the frame are inserted into the three rings of the platform, as illustrated schematically in (b). The presence of the stoppers at the lower ends of the branches ensures the integrity of the structure. The position assumed by the three rings of P along the three branches of T can be modified by adding a base or an acid.

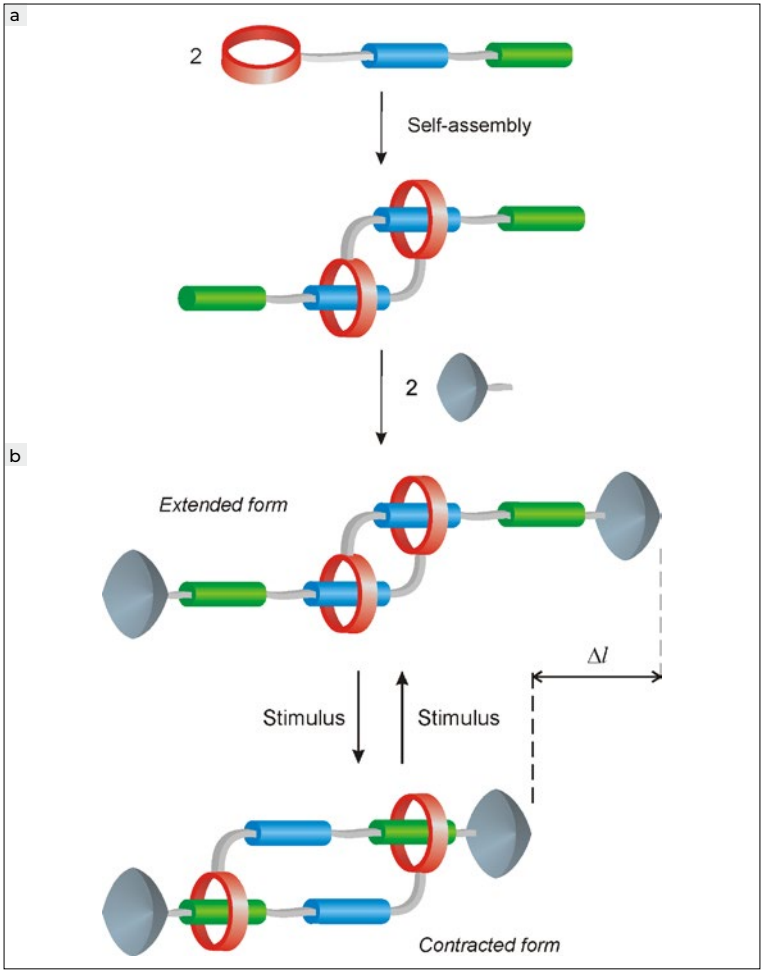


Fig. 23. A molecular muscle based on a double rotaxane. (a) Synthesis of the rotaxane by self-assembly and subsequent stoppering. (b) Schematic representation of extension and contraction movements. The variation in length Δl obtained in such artificial molecular muscles is about one nanometer.

Molecular muscles

Another interesting development of rotaxane-based molecular shuttles is the construction of nanomachines that can stretch and shorten on command. Since such movements recall the extension and contraction of the sarcomere, the functional unit of skeletal muscles (Fig. 10), these nanomachines are called molecular muscles. The first attempt to create a molecular muscle dates back to the year 2000, when Jean-Pierre Sauvage and his collaborators at the University of Strasbourg, in France, applied the molecular shuttle strategy to a double rotaxane with the topology represented in Fig. 23 (Jiménez 2000). The system is composed of two equal components, each consisting of a thread-like portion containing the two stations and a molecular ring at one end. Taking advantage of self-assembly, the two components are inserted one into the other; then attaching two stoppers to the free ends, the desired structure is obtained (Fig. 23a). Imagining that the starting conformation is the extended one (this implies that the primary station is the one closest to the ring of the same component, as in Fig. 23), the application of a stimulus capable of deactivating the primary station should cause displacement of the rings along the relative axes towards the secondary station, causing the system to contract. The subsequent extension would be activated by an opposite stimulus (Fig. 23b).

The experiments showed that the prototype built by Sauvage, designed to respond to electrical stimuli, does not work; probably because the molecule is overall too rigid to undergo such a profound structural change. Later, other research groups, including that of Fraser Stoddart, succeeded in developing functional molecular muscles, based on the scheme shown in Fig. 23, controlled by chemical or light stimuli (Bruns 2017). As we will see in Chapter IV, by integrating these molecular muscles into polymers, materials capable of performing macroscopic movements can be obtained.

Rotary movements

Catenanes

A catenane is a system formed by two (or more) ring molecules chained together; in it we find the mechanical constraint between the molecular components already seen for rotaxanes. The simplest catenane has only two rings (Fig. 24a). In specifically designed structures of this type, one ring can be made to rotate with respect to the other by means of appropriate stimulation. To highlight this movement, however, it is necessary that at least one of the two rings has different stations, as is the case of catenane **5** (Fig. 24b) developed by Sauvage and collaborators (Livoreil 1994).

This catenane is constituted by ring A, which contains a phenanthroline (A1) and a terpyridine (A2) site, and ring B, which contains only a phenanthroline site (B1). The system also comprises a copper ion (Cu^{+}), which binds strongly to the two phenanthroline sites (A1 and B1), forcing them to stay close together. To rotate the ring A with respect to B, this structure must be destabilized – a result that can be achieved with an electrochemical stimulus which, by removing an electron from the Cu^{+} ion, modifies its bonding properties. The Cu^{++} ion thus formed prefers to interact with the terpyridine site, causing a 180° rotation of ring A with respect to B.

If, at this point, again through an electrochemical stimulus, the electron that had previously been removed is returned to the copper ion, the latter regains its initial characteristics. Consequently, the ring A rotates again 180° with respect to B restoring the initial structure. Systems like the one just described, although very interesting, are limited by the fact that two successive 180° rotations will not necessarily take place in the same direction. In fact, assuming that the first rotation (induced by oxidation of Cu^{+} to Cu^{++}) occurs in a clockwise direction, the subsequent rotation (induced by the reduction of Cu^{++} to Cu^{+}) has the same probability of occurring clockwise or counterclockwise. In other

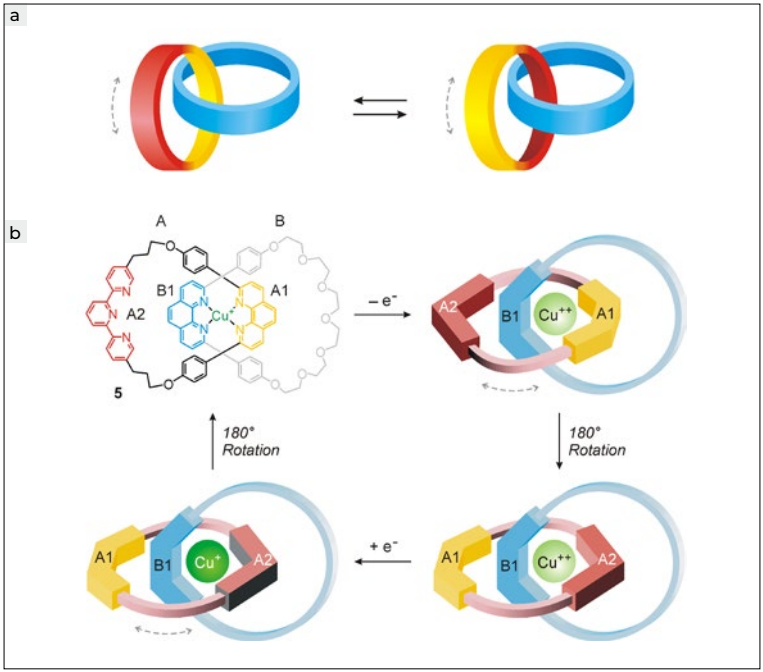


Fig. 24. (a) Schematic representation of the rotary movement of one molecular ring with respect to the other in a catenane. (b) Rotation of the rings of a catenane operated by electricity. Catenane **5** consists of two rings, A and B, which contain sites capable of binding a copper ion (Cu^{+}). Initially, the Cu^{+} ion is bound to phenanthroline-type sites A1 and B1. The electrochemical oxidation of the copper ion to Cu^{++} causes the destabilization of the initial structure and the 180° rotation of the A ring with the formation of a structure in which the Cu^{++} ion interacts with the A2 and B1 sites. An inverse stimulus (reduction, which converts the Cu^{++} ion to Cu^{+}) returns the system to the initial structure.

words, catenanes such as **5** cannot function as rotary motors, instead behave like random oscillators.

Catenanes such as the one shown in Fig. 24 constitute however the starting point for obtaining the unidirectional and repeated rotation of a ring

with respect to the other in response to external stimuli. A control element that allows to select the direction of rotation – clockwise or counterclockwise – in each movement of 180° needs to be included in the design. This was elegantly done at the University of Edinburgh by David Leigh and collaborators with the catenane **6** (Fig. 25) (Hernandez 2004). It consists

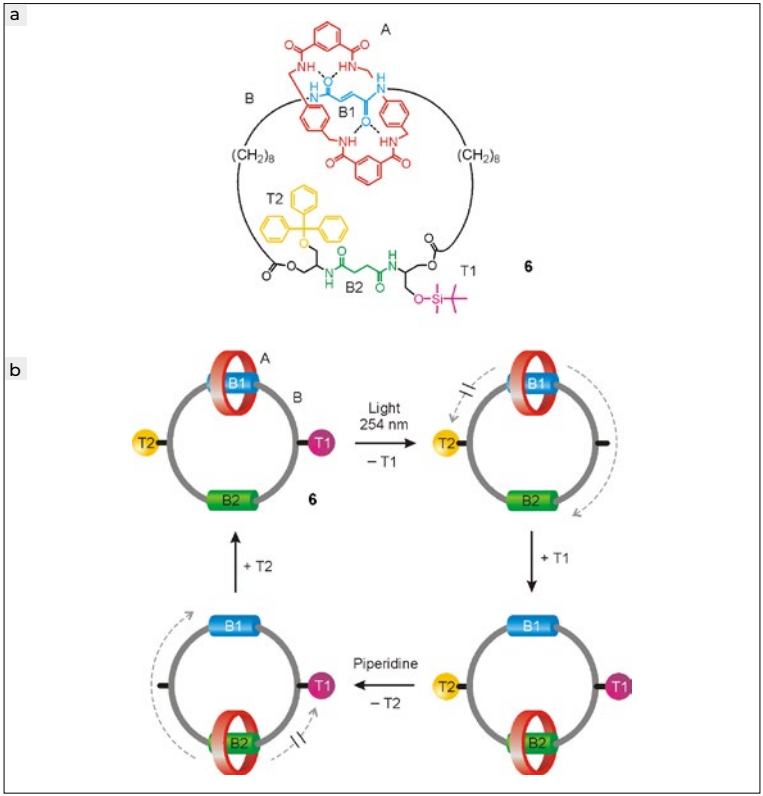


Fig. 25. (a) Structural formula of catenane **6** and (b) schematic representation of the sequence of processes at the base of the unidirectional rotation of the ring A around the ring B.

of a smaller ring A and a larger ring B; along the latter, the primary station B1, a bulky group T1, the secondary station B2 and a bulky group T2 are positioned in this order.
Initially, ring A surrounds station B1; when the latter is deactivated with a light stimulus, A tries to reach the B2 station. This movement, however, is only possible by removing one of the two bulky groups. For example, by selectively removing T1 as shown in the figure, ring A reaches B2 moving along B only in a clockwise direction, and there it remains trapped when T1 is repositioned. At this point, upon resetting station B1 by heating and selectively removing T2, ring A returns to its initial position moving along B in a clockwise direction. A system of this kind allows a total control of the relative motion of the two rings (unidirectional rotation in a clockwise or counterclockwise direction, or oscillation) depending on the order in which the stimuli that influence station B1 and bulky groups T1 and T2 are administered. The flip side of the coin is that the chemical transformations depicted in Fig. 25 are rather complex to carry out from a practical point of view. Subsequently, the same research group created a catenane in which the unidirectional rotation is driven by a single chemical reagent (Wilson 2016).

Light-driven rotary nanomotors

The first artificial rotary nanomotor was developed in 1999 by Ben Feringa's group at the University of Groningen in the Netherlands (Koumura 1999). The motor, illustrated in Fig. 26, is operated only by light. In the same way as compounds having a –N=N– group (Fig. 18), also those containing a –C=C– group (for example, stilbene, Fig. 26a) may exist as *trans* and *cis* isomers. In systems of this type, light excitation of one of the two isomers – for example, the *trans* one – can cause the 180° rotation of one of the two molecular units with respect to the other, with formation of the *cis* isomer. The excitation of the latter can then cause the return to the initial *trans* isomer, through a subsequent 180° rotation. In simple compounds such as stilbene, the direction of the rotary

motion is random, so it cannot be taken for granted that the *trans* → *cis* → *trans* transformation occurs through a complete rotation (i.e. of 360° in the same direction). The *cis* → *trans* transformation, in fact, can involve a rotation of 180° in the opposite direction to that of the *trans* → *cis* transformation, as already discussed in relation to the catenane shown in Fig. 24.

However, in appropriately designed compounds containing the –C=C– double bond, one can make both the *trans* and the *cis* form not planar, but rather distorted. This peculiarity facilitates rotation in one direction with respect to the other, thus making it possible to obtain a complete rotation of one unit with respect to the other with two successive light stimuli. An example of this type is compound **7** shown in Fig. 26b, whose operating mechanism is illustrated schematically in Fig. 26c. Following the absorption of light, the initial *trans* form turns into a *cis* structure that is unstable because it is distorted. This structure can relax (i.e., decrease its energy) spontaneously, maintaining the *cis* configuration, through a further rotation. The stable *cis* species thus obtained can absorb light, transforming itself into the distorted *trans* form, which subsequently evolves into the starting stable *trans* form, thus completing the 360° rotation.

In the last fifteen years the functioning of the molecular motor **7** and of other systems derived from it has been studied in great detail and in a wide variety of media (solutions, surfaces, liquid crystals, polymers, membranes) (Erbas-Cakmak 2015). As we will see in the next section, this class of rotary nanomotors has recently been used to build materials capable of contracting and expanding under the action of light.

Other nanomachines

As we have said before, for reasons of space (and also for not going into too much detail) we must limit ourselves to describing a few examples of nanomachines, chosen for their innovative value or because they are par-

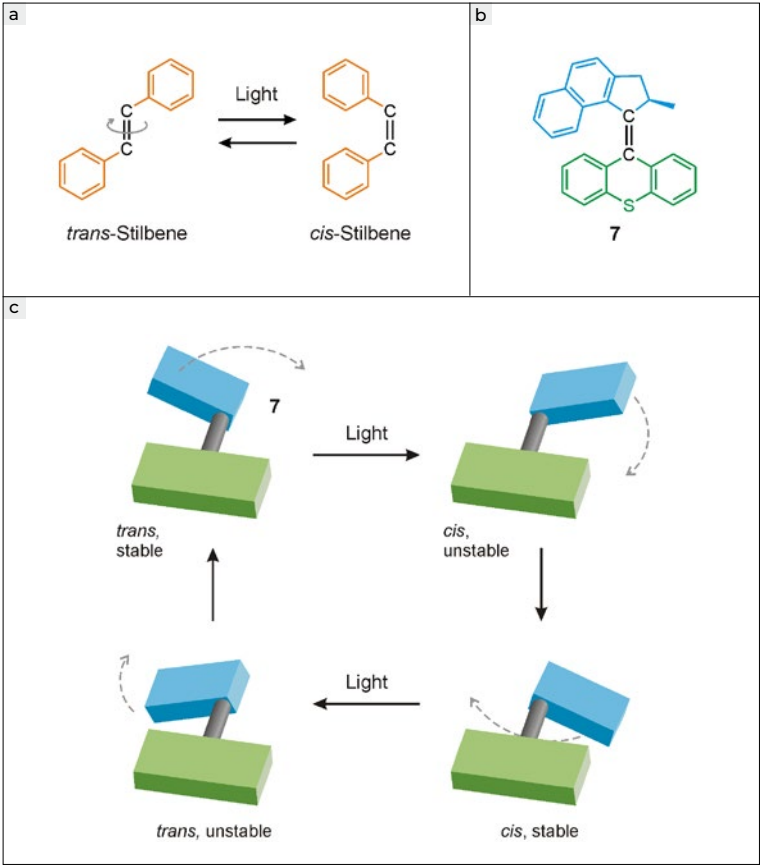


Fig. 26. Rotary nanomotors. (a) Molecules that possess the –C=C– group, like stilbene, can exist in two structurally different forms – *trans* and *cis* – interconvertible by light stimulation. (b) Molecule **7** is more complicated, but it is of the same type as stilbene; therefore, it can also exist in the *trans* and *cis* forms. The bold bond is projected above the plane of the page. (c) Thanks to an accurate design, in species **7** the absorption of two photons of light causes the rotation of 360° of one of the two units with respect to the other. Since unidirectional rotation can be repeated several times by the absorption of further photons, **7** functions as a light-driven rotary nanomotor.

ticularly representative. We must also remember, however, that since the 1990s many molecular and supramolecular systems capable of behaving like molecular machines or motors have been designed, constructed and studied. Among them it is worth mentioning those that use biomolecules – in particular DNA and RNA – as molecular components.

The development of this category of artificial nanomachines is closely linked to a rapidly expanding research sector: that of DNA nanotechnology. In the early 1980s, scientists (notably Ned Seeman at the University of New York) realized that DNA molecules could be used to build nanostructures in a controlled manner. The chemical properties of DNA molecules can be accurately programmed by choosing the sequence of bases (adenine, guanine, cytosine and thymine). DNA can exist as single molecules, or pair off to form the famous double helix and other different structures. There is a large number of enzymes capable of *cutting* and *sewing* DNA molecules with great precision. These and other structural and functional features of DNA have allowed the creation of very complex nanostructures, such as the now famous *origami*.

Scientists interested in artificial DNA nanostructures soon realized that they could be modified in response to external stimuli, that is, they could behave as molecular machines. In most cases also the stimuli employed are represented by DNA molecules, although ions, small molecules, proteins (enzymes), light or electrical stimuli can be used. The operation of these nanomachines is too complex to be described quickly here. Suffice it to say that, with DNA, nanoscale switches, logic gates, memories, tweezers, linear and rotary motors, up to real programmable robots capable of moving and manipulating nanometric objects by executing instructions given from the outside, have been realized (Thubagere 2017). Despite the scientific value of these results, the considerable complexity and very high cost of these systems has, for the time being, relegated them to laboratory curiosity. However, various experiments are underway for practical applications, mainly related to the development of systems for the targeted release of drugs.

CHAPTER IV

A GLIMPSE INTO THE FUTURE

Nanomachines: what can they offer?

We have seen that in living organisms biomolecular machines perform a wide variety of functions in a timely and incessant manner. The crucial role entrusted by nature to these tiny devices is a compelling demonstration of their usefulness. Many scientists predict that artificial molecular machines will lead to innovative applications in several fields of technology and medicine. One can think, for example, that with nanomachines we will be able to construct materials whose properties adapt to external conditions, plastics capable of bending on command, mechanical nanoactuators, ultra-miniaturized memories and processors, nanometric probes capable of diagnosing diseases, intelligent drugs that become active only in the right place at the right time. Why then, despite the considerable progress made in the construction of molecular machines, have they not yet entered our everyday life?

First of all it must be reminded that biomolecular machines are extremely sophisticated systems, the result of evolutionary processes lasting millions of years. At present it is not possible to reproduce in the laboratory nanomachines of structural and functional complexity comparable to natural ones. We must also consider, however, that scientific and technological research is progressing at great speed: for example, the molecular

machines that we know how to build today would have been practically unthinkable only thirty years ago.
After dealing with numerous fundamental questions, both conceptual and practical, research on nano-machinery has entered a phase of maturity, in which the attention of scientists is shifting from the demonstration of the validity of an idea (*proof of principle*) to the construction of useful devices, able to work in the real world. In this section we will describe some recent studies that show how artificial molecular machines, properly organized among themselves and/or interfaced with the surrounding environment, are capable of performing functions of various kinds. The results of these experiments give hope that in the not too distant future nanomachines could really trigger a new industrial revolution.

Molecular machines for making molecules

One of the main tasks of natural molecular machines is the production of other molecules. The most important examples of this type of nanomachines are DNA polymerases, which deal with the replication of DNA molecules, and ribosomes, in which proteins are manufactured (Goodsell 2009).
In 2013 David Leigh and his collaborators at the University of Manchester, in Great Britain, succeeded in building a molecular machine capable of synthesizing a chain of three amino acids (tripeptide), according to a predetermined sequence programmed in the machine itself (Lewandowski 2013). Although the system is very sophisticated and exploits a complex series of chemical reactions, it is possible, leaving out the details, to explain its operation in a rather simple manner as shown in Fig. 27.
Rotaxane **8** (Fig. 27a) is composed of a molecular ring A, on which there are a catalytic site (S) and a docking site (G) which can react with an amino acid, and of a rigid filiform component B, stoppered (T) at one end, along which there are three different amino acids (X, Y and Z) in a predetermined order. Since the amino acids are bulky, they act as stoppers

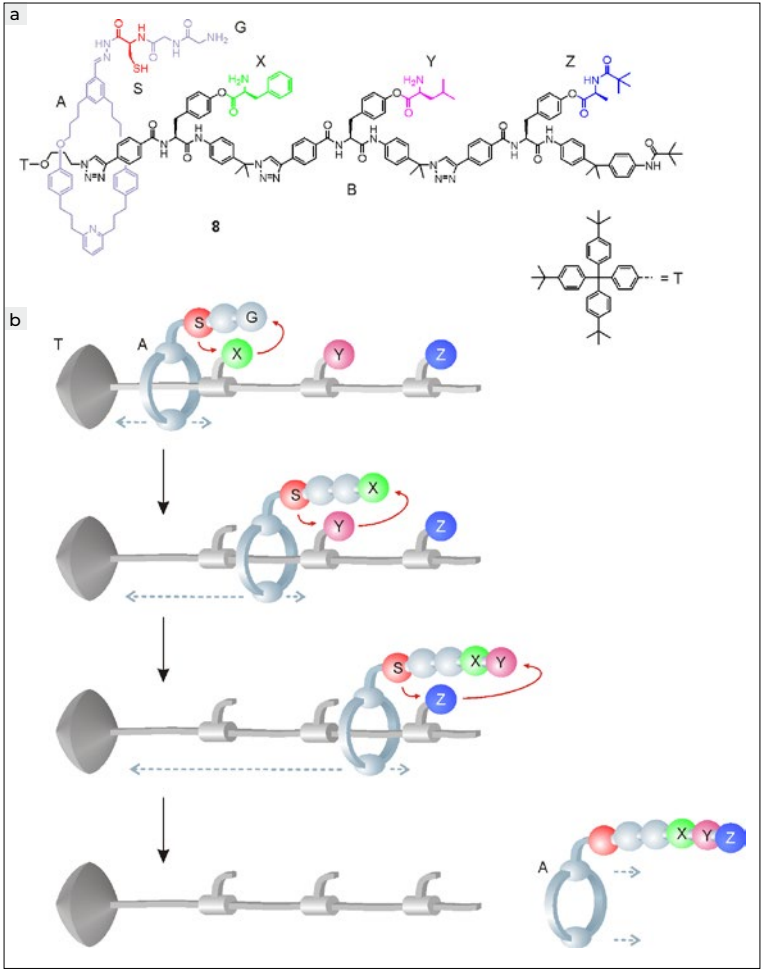


Fig. 27. An artificial molecular machine capable of building a chain of amino acids. (a) Simplified structural formula of rotaxane **8** and (b) schematic representation of the synthesis of a tripeptide starting from the sequence of three amino acids located along the molecular filament.

for the translational motion of the ring A along the filament B. Initially, therefore, the ring A can move along the filament due to the Brownian motion only between the stopper T and the amino acid X (Fig. 27b). When the ring arrives near X, the S site, breaking and forming chemical bonds, transfers the amino acid X from component B to the docking site G of the ring. Once X has been removed from the filament, ring A, moving along the filament, can reach the amino acid Y; when this happens, the process described above is repeated and the amino acid Y is transferred from the filament to the ring. Subsequently, when the third and last amino acid (Z) is transferred in the same way, the ring can separate from the filament and the tripeptide synthesized by the molecular machine can be isolated (Fig. 27b).

This system is less sophisticated and much less efficient than a ribosome, but it must be considered that it is a thousand times smaller and is completely synthetic. One day, perhaps, the chemical industries will use molecular machines to prepare drugs or other useful substances. It can also be hypothesized that nanomachines capable of preparing biomolecules directly inside the organism could be used to correct biochemical dysfunctions, such as vitamin or protein deficiencies, avoiding the onset of diseases.

Ultra-miniaturized binary memories

The artificial molecular machines discussed in most of the above examples are interesting not only for their mechanical aspect, but also from an information technology point of view. In fact, they can exist in two distinct states, interconvertible through external impulses of a luminous, chemical or electrical nature. For example, in the rotaxanes of Figures 20 and 21 the ring can reside along the thread either on the left or on the right station. Also the systems represented in Figures 22 to 24 are bistable. Information can therefore be *written* on them according to a binary logic. The state in which the system is, on the other hand, can be easily *read* since some

of its properties (for example, absorption or emission of light of specific wavelength) change drastically in the passage from one state to another.
Jim Heath of the California Institute of Technology, in collaboration with Fraser Stoddart, used bistable rotaxanes and catenanes to build solid state electronic memories (Green 2007). In this study the researchers exploited top down miniaturization methods to fabricate the electrical contacts of the memory elements; the latter, however, consist of molecular machines obtained through a bottom up approach. In short, the position of the ring along the filament of the rotaxane, which depends on the electrical potential difference applied to the contacts, determines the electrical conductivity of the junction. Therefore the memory can be written by varying the applied electrical potential, while the reading is entrusted to a conductivity measurement.
Thanks to the fabrication of electrical contacts with a thickness of less than 100 nm, memory elements containing no more than 100 rotaxane molecules could be constructed, reaching the incredible density of 10^{11} bits per cm^2 (Green 2007). Some scientists see in these and other related research the first steps towards the construction of a new generation of computers (chemical computers) which, based on nanometric components, could offer performances much higher than those of the computers in use today. The thing, perhaps, is not surprising that much, if we think of the capacity of that special, perhaps inimitable, chemical computer that is our brain.

Nanovalves: drugs become smart

To maximize therapeutic efficacy and minimize side effects, a drug should ideally react in a targeted manner within the body only where and when it is needed. Unfortunately, the reality is that drugs do not behave in this way as they often fail to reach the goal, possibly because they are degraded by the immune system or attack healthy tissues. In some cases, the active ingredient in the drug remains in the body for too long, causing adverse reactions. In other cases, drugs can remain in circulation for too short a time

to be effective. One way to overcome these drawbacks is to use controlled release systems (drug delivery). They are essentially compounds, or groups of compounds, able to host the drug molecule, transport it into the organism and release it in the right place and at the right rate. The development

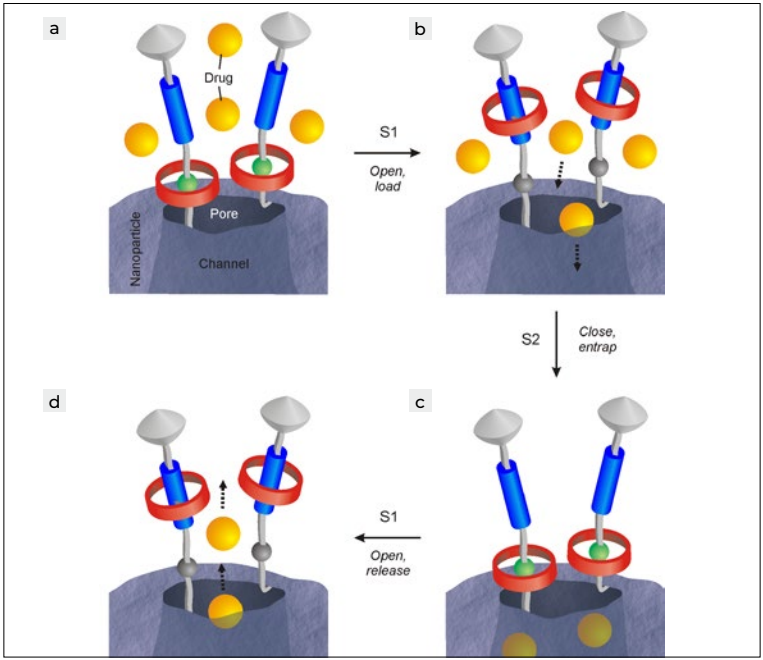


Fig. 28. Functional scheme of a system for molecular transport and controlled release based on nanovalves, i.e., porous silica nanoparticles modified with molecular shuttles. (a) In the initial situation the molecular rings of the shuttles are located on the primary station, which is close to the pore. The valve is therefore closed, and the access of any drug molecules to the empty channels is precluded. (b) The S1 stimulus deactivates the primary station of the shuttle, causing the rings to move away from the surface and opening the pores. Drug molecules can penetrate the nanoparticle channels. (c) The S2 stimulus resets the primary station, thereby closing the valve; the molecules remain trapped in the nanoparticle. (d) The subsequent application of the S1 stimulus causes the release of the drug.

of efficient, versatile and selective systems for controlled transport and release is undoubtedly one of the main topics of pharmacological research. Porous silica nanoparticles are interesting in this context because they possess channels in their interior that can host small molecules; they are also stable, biocompatible, non-toxic and easy to prepare. To carry out the controlled release, however, it is necessary to have a strategy that allows to trap the drug in the nanoparticle and to release it by an action triggered by a stimulus that can be endogenous (for example a tumor marker) or external (for example the light). This result has been obtained by exploiting the movement of molecular machines to open and close the entrance of the pores that connect the internal channels with the surface of the nanoparticle (Bruns 2017).
As shown in Fig. 28, molecular shuttles similar to those described in Fig. 20 have been chemically bonded to the surface of the nanoparticles, near the pores. Experiments have shown that the pores can be opened and closed by moving the rings of the rotaxanes far and near with respect to the entrance of the pores, respectively. The nanoparticle functionalized with molecular machines therefore behaves as a kind of nanometric valve (Fig. 28). The properties of these nanovalves can be adjusted by modifying structural parameters such as the length of the connector between the rotaxane and the surface, the distance between the stations of the shuttle, and the initial position of the movable ring. Through this approach, nanovalves have been constructed that are controlled by light, enzymatic or ionic stimuli (e.g. pH variations), capable of accommodating and releasing various types of molecules, including metal complexes, fluorescent species and anticancer drugs. Although we are still very far from Asimov's *Fantastic Voyage*, these results give an idea of the potential offered by molecular machines in the medical field.

Molecular machines to convert (solar) energy

In Chapter II we described the functioning of the ATP synthase (Fig. 13). This nanomachine exploits the difference in hydrogen ion concentration between two solutions separated by a membrane to produce ATP, a sub-

stance with a high energy content. In practice, therefore, it converts one form of chemical energy into another. The use of artificial molecular machines to transform and store energy is not only interesting from a conceptual point of view, but also potentially very important from the application point of view. Some significant steps in this direction were made in 2015 by Fraser Stoddart's group at Northwestern University (USA) and in our laboratory at the University of Bologna.

With a skillful design, Stoddart and collaborators were able to build a molecular strand B consisting of an end E capable of inserting and transferring molecular rings A in a unidirectional manner by exploiting a chemical reaction, a thread-like portion S without stations for the rings, and an end cap T (Fig. 29a). Following reduction and oxidation processes, caused by reagents added to the solution in which the molecular machine is dissolved, the rings are pushed along the filament and transferred to the thread-like portion S which acts as a "reservoir", where they remain trapped (Cheng 2015). Such a system behaves like a real molecular pump that converts the energy of the redox reaction into another form of chemical energy, represented by the high concentration of molecular rings in the reservoir.

At the same time in our laboratory we built a molecular pump that uses light energy (Ragazzon 2015). The system (Fig. 29b) consists of a solution containing molecular filaments X and molecular rings Y. The filament X contains a –N=N– unit (W), which can change shape (from *trans*, linear, to *cis*, bent; see Fig. 18) following the absorption of photons of light, a station V for the ring, and a bulky terminal group Z. When the component W is in the linear *trans* form, the ring passes over the filament crossing W to reach the V station. Following the absorption of a photon of blue light, the W unit passes to the much more cumbersome *cis* form; consequently, the ring must leave the filament crossing the end Z. The subsequent arrival of another photon, identical to the previous one, restores the *trans* form of the W unit, thus closing the cycle. This sequence of processes, and the consequent unidirec-

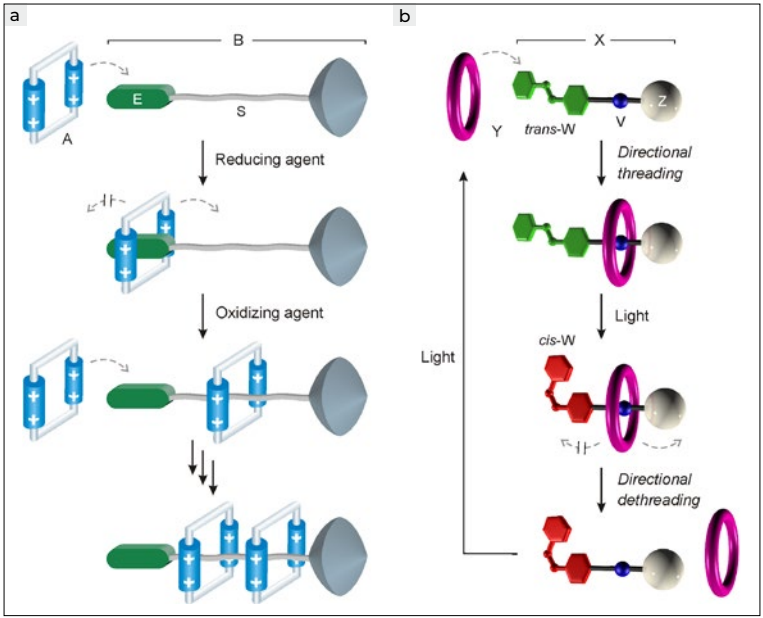


Fig. 29. Molecular pumps for energy conversion at the nanometer scale. (a) A system that uses the energy of a redox chemical reaction to "concentrate" molecular rings in a molecular "reservoir", thus obtaining a high-energy content situation. (b) A light-driven molecular pump. The molecular rings pass unidirectionally and continuously through the molecular filament under the action of a continuous light source, like a common lamp or the Sun.

tional transit of the ring with respect to the filament, is repeated until the solution is illuminated with blue light. It should be emphasized that the molecular device, at present, dissipates light energy without converting it into a useful form, because the rings are transferred in the same solution from which they are taken. However, it is potentially capable of transforming solar energy into chemical energy, if the rings are transferred between distinct compartments, as in the case of the system illustrated in Figure 29a.

Of course, the construction of molecular machines that allow the exploitation of solar energy is not around the corner. There are many complex problems to solve; last but not least, how to use the chemical energy accumulated by nanomachines. The problem is really fascinating: it is about realizing an artificial photosynthesis, which however works in a substantially different way compared to photosynthesis in plants and bacteria. Since the future of our civilization depends on the solution of the energy problem, the challenge is too important not to be taken up.

A four-wheel drive nanovehicle

So far we have described artificial molecular machines whose functioning has been observed by measuring collective properties, i.e. determined by a very large number (many billions of billions) of molecules, typically contained in a solution. We have also seen, however, that nowadays, with appropriate techniques, single molecules can be imaged (Figs. 3 and 4). The question that arises is therefore: can we operate and observe the movement of *one single* molecular machine?

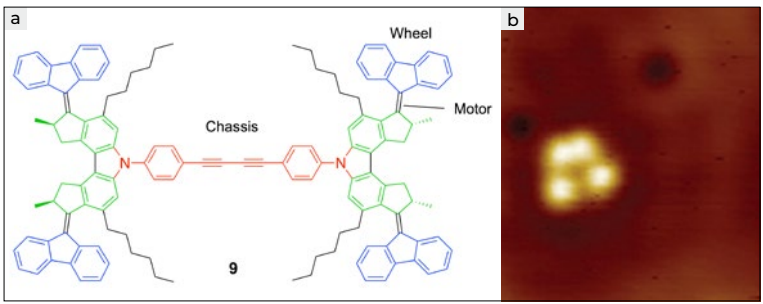


Fig. 30. (a) Simplified structural formula of the nanovehicle **9**. The bold bonds are projected above the plane of the page, while the dashed ones are below the plane. (b) Image of a single molecule of **9** deposited on a copper surface, obtained with a scanning tunneling microscope under high vacuum conditions at –266 °C. Figure reproduced with permission from *Nature*, vol. 479, pp. 208-211 © 2011 Springer Nature.

In this regard, a team of chemists (Ben Feringa and collaborators at the University of Groningen) and physicists (Karl-Heinz Ernst and collaborators at the Swiss Federal Laboratory for Materials Science and Technology) did a spectacular scientific enterprise. Chemists built a real nanovehicle (compound **9**, Fig. 30a), consisting of four rotary motors of the type shown in Fig. 26 mounted on a rigid frame (Kudernac 2011). Physicists then deposited single molecules of **9** onto a copper surface under conditions of high vacuum and at very low temperature (–266 °C), and observed them with a scanning tunneling microscope (STM).

Under the STM microscope, the nanovehicle appears as a four-lobed object; each of them represents a molecular motor (Fig. 30b). Using the microscope probe, the researchers applied an electric potential to the nanovehicle, such as to operate the rotary motors (which instead in solution are set in motion by the light; see Fig. 26); subsequently, they again recorded the image of the molecule. Some of the observed nanovehicles moved directionally on the surface, covering a distance of 6 nanometers after ten excitation pulses. This behavior can only be explained by admitting that the propulsion is due to the interaction between the metal surface and the wheels of the nanowire set in rotation by the electrical stimulus. Since each wheel is connected to an independent motor, we are faced with a four-wheel drive nanovehicle.

This study undoubtedly represents a milestone in the field of artificial molecular machines, not only for its scientific content, but also for its high symbolic value and the impact on people's imagination. It is no coincidence that the nanovehicle was highlighted in the poster of the 2016 Nobel Prize in Chemistry (Fig. 17).

Artificial muscles

In skeletal muscles a huge number of myosin molecular motors are organized according to a precise hierarchical structure (Fig. 10). Many myosin molecules form filaments, which, together with the actin filaments,

constitute the sarcomere. Many sarcomeres, connected one after the other, give rise to myofibrils which, in turn, are associated in bundles, and so on up to the macroscopic muscle. The concerted action of billions of billions of myosin molecules, each of which develops a force of a few thousandths of a billionth of a Newton, is more than enough to raise, for example, the book you are reading. This observation contains an important message: with nanomachines one can do mechanical work in our world. What needs to be done is to insert a very large number of nanomachines in an organized structure, such that they can be stimulated simultaneously and their individual mechanical effects are added together. In practice, we need to build a system capable of amplifying movement from the nanometric to the macroscopic scale.

A clever strategy to achieve this is to combine molecular machines with polymer chemistry. Polymers are molecules of high weight – typically from a few hundred to a few thousand times that of the water molecule – obtained by combining smaller molecules (monomers), usually all of the same type or very similar to each other. Among the polymers present in nature there are proteins (or polypeptides, obtained by combining amino acids, Fig. 9), nucleic acids such as DNA and RNA, and polysaccharides such as starch and cellulose. Synthetic polymers, more commonly known as plastics, are among the most important technological innovations of the last century and include materials we are constantly dealing with, such as polyethylene, polyvinyl chloride (PVC), polystyrene, polyesters (PET, polycarbonate) and polyamides (Nylon, Kevlar). From the repetition of many monomers (polymerization) it is possible to obtain linear macromolecules, consisting of a single chain of monomers, or branched macromolecules, in which different chains of monomers are connected to each other at various points.

In general terms we can think of using a molecular machine as a monomer for the construction of a polymer. For example, the research group led by Nicolas Giuseppone at the University of Strasbourg succeeded in obtaining a linear polymer (Fig. 31a) using artificial molecular muscles

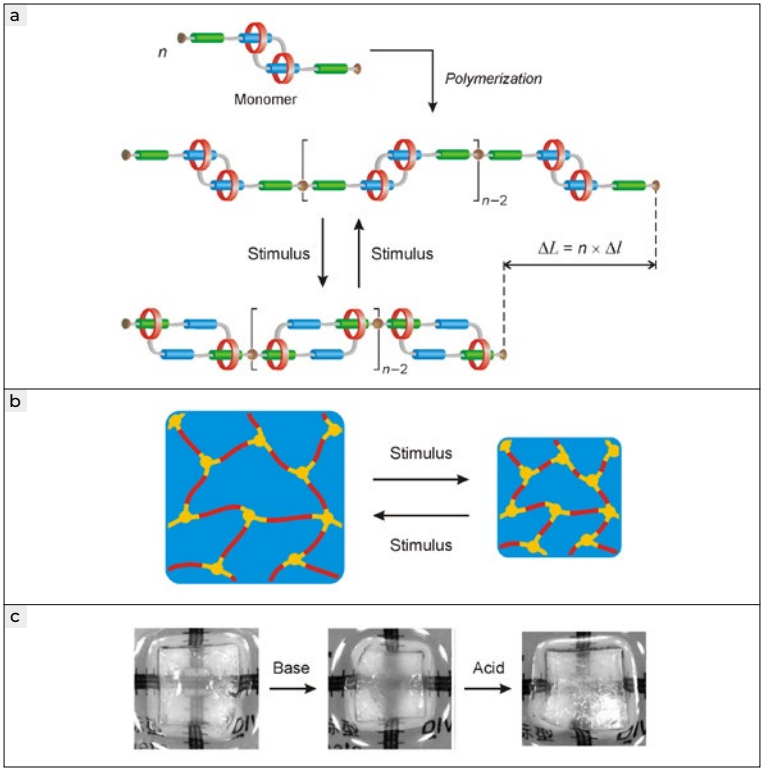


Fig. 31. (a) In a linear polymer constructed using molecular muscles based on double rotaxanes as monomers, the simultaneous contraction and extension of the monomers, caused by external stimuli, can produce an effect on a larger scale. If the macromolecule is sufficiently rigid, its variation in length (ΔL) is proportional to the number of monomers (n) and to the extent of their contraction/extension (Δl). (b) Schematic representation of the contraction and extension of a branched polymer fragment containing molecular muscles (in black) in the chains. (c) Photographs of a macroscopic fragment of a branched polymer of the type represented in (b), which show a decrease in the volume of the material when it is immersed in a basic solution; the initial volume is restored following treatment with an acid solution. Figure adapted with permission from the *Journal of the American Chemical Society* vol. 139, pp. 14825-14828 © 2017 American Chemical Society.

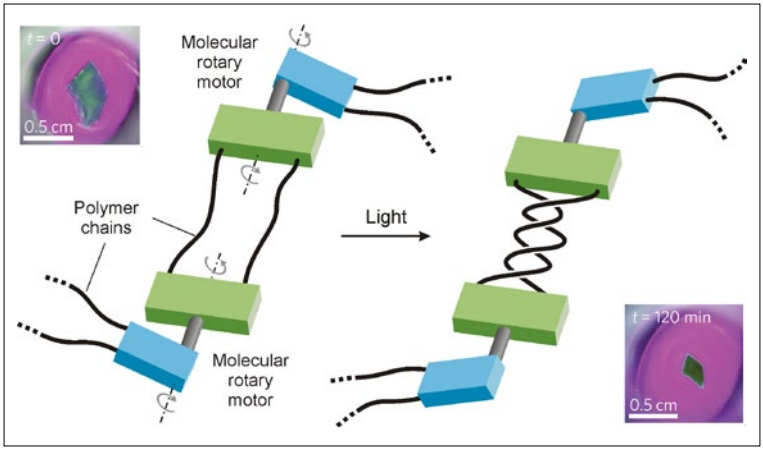


Fig. 32. In a branched polymer containing rotary molecular motors similar to **7** (Fig. 26), the rotation of the nanomotors induced by light causes the polymer chains to twist and the consequent macroscopic contraction of the material. The photographs show a polymer fragment at the beginning of the experiment (top left) and after two hours of irradiation with ultraviolet light (bottom right). Photographs reproduced with permission from *Nature Nanotechnology*, vol. 10, pp. 161-165 © 2015 Springer Nature.

based on double rotaxanes (Fig. 23) as repetitive units. Experiments have shown that the polymer chains change their length by a few micrometers in response to the stimulation of their monomeric nanomachine components with acids and bases, demonstrating the amplification of molecular motion (Fig. 31a). More recently, applying the same strategy, Giuseppone and collaborators have constructed a branched polymer in which the distance between the branching points can be varied on command thanks to the molecular muscles (Fig. 31b). As shown in Fig. 31c, a fragment of this polymer shrinks when exposed to a basic solution, which causes the molecular muscles to pass from the expanded to the contracted form. After the subsequent treatment with an acid solution, the fragment regains its original size (Goujon 2017). There are also systems of similar structure that use light as a source of energy for movement.

Equally fascinating and promising are the results obtained in branched polymers in which rotary nanomotors of the type shown in Fig. 26b and c have been inserted. As shown schematically in Fig. 32, by irradiating the material with ultraviolet light, the rotation of the molecular motors is activated and, consequently, the polymeric chains are twisted. This profound structural change, which affects the entire illuminated portion of the material, causes its contraction on a macroscopic scale (Li 2015). It is interesting to note that such a polymer is able to store part of the incident light energy in the form of chemical energy, associated with the entanglement of chains. The system represented in Fig. 32 has recently been modified by inserting in the ramifications, in addition to the rotary motors, also appropriate molecular dissipators that allow the unwinding of the tangled chains and the release of the accumulated energy. Plastics that work as mechanical actuators, based on molecular motors, are today a reality. The next step is to study possible practical applications in areas such as biomedical devices or robotics.

CONCLUSION

Thanks to millions of years of evolution, nature has built molecular devices and machines capable of performing complex and essential functions for the life of organisms. Instead, only one hundred and fifty years have passed since the birth of the Periodic Table of the Elements, an icon of chemistry. About sixty years ago Richard Feynman started talking (jokingly) about nanotechnology, and less than forty years ago the tunneling scanning microscope was invented, which allows one to see and manipulate single molecules.
Chemists have been designing, building and studying artificial nanomachines for about three decades. First they developed very simple and rudimentary systems; then, once the fundamental principles governing the movement of objects at the nanometer scale were understood, and the necessary modeling and experimental tools were acquired, they moved on to more sophisticated devices. Researchers are now learning to integrate molecular machines into organized structures and to make them interact appropriately with the environment in which they are located, so as to obtain useful functions.
Although the systems studied so far are enormously less complex and with very modest performances compared to natural nanomachines, research in recent years shows that with artificial molecular machines it is possible to process information, convert energy, synthesize other molecules, deliver drugs and build mechanical actuators. It is worth mentioning in this regard the concluding reflection contained in the motivation of the 2016 Nobel Prize for Chemistry:

> Compared with the machines that changed our world following the industrial revolution of the nineteenth century, molecular machinery is still in a phase of growth. However, just as the world stood perplexed before the early machines, such as the first electric motors and steam engines, there is the potential for a similar ex-

> plosive development of molecular machines. In a sense, we are at the dawn of a new industrial revolution of the twenty-first century, and the future will show how molecular machinery can become an integral part of our lives. The advances made have also led to the first steps towards creating truly programmable machines, and it can be envisaged that *molecular robotics* will be one of the next major scientific areas (Ramström 2016).

If at the moment we can hypothesize that in the near future molecular machines can be used in practice in some sectors of technology and medicine, perhaps the most innovative applications are still beyond the reach of our imagination. Apart from these certainly important aspects, research on molecular machines already has many scientific and cultural merits. First of all it has awakened curiosity, sharpened the ingenuity and stimulated the creativity of scientists (in particular chemists), many of whom have discovered to be, in fact, real molecular engineers.

Secondly, since the study of molecular machines involves areas of chemistry and biology, but also of physics, mathematics, engineering and medicine, scientists from different disciplines, even apparently distant from each other, have begun to talk to each other – something by no means automatic and banal – and to interact. These "unconventional" collaborations will allow us to face important challenges, both at the borders between the disciplines and within them. In solving open problems, new ones will also be identified, thus nurturing the virtuous circle at the base of scientific and cultural progress, in which curiosity, research and discovery follow each other without interruption. The scientist is a lucky person because he works in this cycle that nobody can interrupt: there will always be something new to discover, something unexpected will always happen, someone will always have a new idea. Precisely for this reason the scientist is also a humble person: he knows that the world is a mystery that dominates him. Joseph Priestley, the first scientist to investigate photosynthesis, expressed this condition in an admirable way:

> The greater is the circle of light, the greater is the boundary of the darkness by which it is confined. But, notwithstanding this, the more light we get, the more thankful we ought to be, for by this means we have the greater range for satisfactory contemplation. In time the bounds of light will be still farther extended; and from the infinity of the divine nature, and the divine works, we may promise ourselves and endless progress in our investigation of them: a prospect truly sublime and glorious (Priestley 1779).

Finally, it should be remembered that frontier research such as that on nanomachines is almost always carried out in collaboration between laboratories in different countries in various parts of the world. Among human activities, scientific research is among the longest and most widely globalized ones. In science, not only ideas but also people circulate with great freedom on a planetary scale. In our laboratories, for example, European, American, Indian, Iranian, Chinese, Japanese and Australian researchers have worked and are still working together. This is undoubtedly a positive globalization, which has a particular value in a historical period like the one we are going through, characterized by the closure of borders and the construction of walls.

Scientists, in virtue of their privileged positions, are called to work for the progress of humanity. Which means to develop science, but also to protect the environment and to fight social injustice. A scientist cannot hide behind the hypocrisy of neutral science. A responsible scientist must be concerned that his research is used for peaceful purposes and not for war, for alleviating poverty and not for increasing privileges, and for taking care of our fragile planet, the only place where we can live. As said by Albert Einstein: «Concern for man himself and his fate must always constitute the chief objective of all technological endeavors; never forget this in the midst of your diagrams and equations.»

This recommendation, of course, also applies to molecular machines.

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The design and construction of machines and motors of molecular size is a stimulating scientific challenge and a primary objective of nanotechnology. During the past thirty years, chemists have taken up this challenge and learned how to make and operate simple nanoscale machines. Although these tiny devices are not yet part of our everyday life, we are approaching a new industrial revolution that opens up radically new perspectives for applications in catalysis, smart materials, robotics, information technology, and medical diagnostics and therapy.

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