Nanotechnology and Life: An Engineer's Perspective

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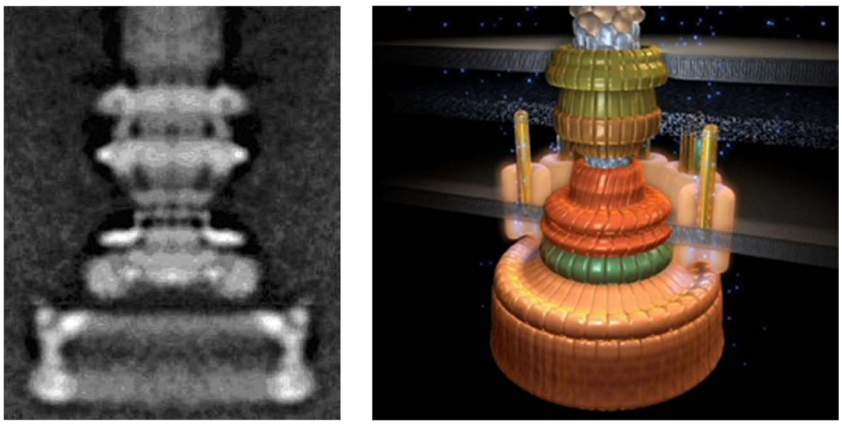

Fig. 1. ***Bacterial flagellum basal body. Left: electron microscope image. Right: 3-D rendering. Adapted from [13].***

I. INTRODUCTION

Nanotechnology is widely recognized as one of the 21st century's most promising technologies.

Nanotechnology deals with the design, development, and manipulation of materials and devices with at least one dimension sized on a nanometer scale. It involves fields of science as diverse as surface science, organic chemistry, molecular biology, semiconductor physics, microfabrication, with a vast range of applications, such as in medicine, electronics, biomaterials, and energy production.

Its ultimate goal is to be able to predictably design, construct, and control nanosystems, tailoring them to specified needs. This is the classical task of engineers, requiring a quantitative modeling of the problem of interest, detailed and accurate enough to make a reliable design possible.

Now, in the case of the applications to biology and medicine, living bodies are seen by nanotechnologists as the (essentially passive) environment in which the nanosystems perform their tasks. This modelization does not take into account the actual nature of living beings, which has been uncovered piece by piece in a long process, converging in the last 50 years to form a unified picture, which can be conveyed by a simple sentence: living organisms are hierarchically integrated sets of nanomachines.

On the other hand, while this may be clear to biologists, they lack reliable methodological and theoretical tools that allow the generalization of their findings into quantitative models, suited to a design procedure.

Filling this gap would allow the engineering of biosystems at the nanometric scale, which is the aim of "nanobiology" [1], a new field of research that has the potential of revolutionizing both nanotechnologies and biotechnologies.

Among the conceivable possibilities, there are medical applications, where damaged physiological functions are restored by replacing natural nanomachines with artificial ones, for example, the pancreatic cells with nanofactories able to restart the endogenous production of insulin in diabetics. A different set of possibilities would be the replacement of living organisms in biotech production, for example, the replacement of the current bacteria used for drugs and enzymes production with artificial

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highly versatile nanofactories, more efficient and less dangerous than their natural counterparts.

This article is an attempt to give an idea of this scenario from the engineering point of view, putting in perspective the problems and challenges, and giving an idea of the possible developments and their implications.

II. LIVING ORGANISMS AS INTEGRATED SETS OF MACHINES

To clarify why living organisms are to be viewed as a set of cooperative machines, however complex, let us follow the time flow of the scientific progress made in biological sciences. As a starting point, we can pinpoint one of the most revolutionary results of modern science, which was obtained some 180 years ago: living organisms are made of the same chemical matter as everything. One hundred and fifty years ago there was another important achievement: despite the chemical elements being the same in the living and not living worlds, only if you look to living organisms you find that they are bound together in very complicated molecules—appropriately defined biomolecules—whose prototypes are proteins.

About 80 years ago, scientists realized that each type of protein performs a given set of functions, so that a single protein can be described as a tiny molecular machine in the nanometer size range: a biological nanomachine.

The list of all the proteins (and of some other biomolecules) sufficient to build a given living organism is transmitted from parents to kinships in a nucleic acid string (chiefly the DNA; we have known this for 70 years). This list, called genome, contains the description of the thousands of protein types which make up a specific living organism. Of course, a list of nanomachines is only the first step. You also need to map the spatial and functional relationships of every machine with all its neighbors, to get a complete, integrated, and fully working system.

If we look at a living organism, the different biological nanomachines are hierarchically integrated in functional apparatuses (organelles) within the cells, tissues, and organs. This hierarchical organization can be compared to the integration of production machineries (proteins) in a production line (a particular biochemical pathway in an organelle), part of an industrial plant (the cell), which in turn is part of a production district (the tissue) in a city (the organ), and eventually in a nation (the organism) which includes all the cities. This organization is not without consequences for the functional control of the overall system, requiring exactly tuning the activity of an astronomically high number of different nanomachines, which all together act to sustain life. The key resides in temporal and spatial separation of the hierarchically integrated production lines we have just mentioned.

To understand this, consider that the job of a single bionanomachine in a biochemical pathway consists in acting on other nanomachines, i.e., in mechanically actuating, assembling, dismantling, or conglobating another type of biological macromolecule. To put it simply, for a bionanomachine to do its work, the presence/absence of at least another nanomachine is a conditional switch. From this perspective (recalling our similitude), the production lines of the tiny farms which are the living cells work as if each labor/nanomachine passes an order/job to the next in line.

In a cell, any production line is spatially isolated from other, potentially interfering lines. Sometimes the production line is activated by a particular nanomachine—usually at the beginning of a pathway—which is switched on and off by chemical modifications or by an external stimulus such an hormone, but in most cases, the control of the production pipeline is not taken up at the level of the nanomachines composing it, which cannot be selectively switched on/off: it is actuated at a hierarchically superior level by building up or destroying the appropriate nanomachines at the appropriate time.

In other words, the hierarchical integration of the pathways in the cell, then in tissues and organs up to the full body of a living organism, allows the coordinated control of several different functions in a top–down approach. If you isolate a protein from this hierarchic system, it will retain its ability to perform a given job; however, you will lose the possibility to control it.

This is why current efforts of the biomedical scientists are aimed at mapping of the hierarchical organization of molecular networks and at the study of the way they work as a whole. After several decades dedicated to the study of the interactions of few proteins at a time, the modern system biology approach now looks at hierarchical networks of several thousands of different biomolecules from a topological and dynamical point of view. To continue using the parallel of the industrial organization, modern biologists are unraveling the design project of the production plant (the cell) and they are starting to also tackle the hierarchically superior levels.

III. ENGINEERING LIFE

Biotechnologists may insert any sort of DNA in a living organism to let it produce any protein, sometimes modifying its behavior. Such an approach, which produced most of the spectacular results achieved in the field of biological sciences, is far from an engineering one. It is more comparable to life-hacking, in the sense that you insert in a cell a new software (i.e., a genetic program) to modify its production lines so to get a desired product out of them. As such, it can be hardly exploited to design and build an arbitrary nanomachine, able to perform a specific task in a controlled way. In particular, your control over the production line will be limited to inserting the instructions for

assembling it; you will not be able to switch on and off the line at your will. Moreover, your production line will be immersed in the cellular environment, which, on the one hand, is resource consuming (an entire cell must be maintained to operate a single biochemical pathway) and, on the other hand, will interfere with the artificial production line and its products, in such a complicated way that detailed predictions of the actual effects are nearly impossible [2]. In some selected cases, biotechnologists have indeed resolved to isolate *in vitro* the biochemical pathway of interest, for example the DNA-synthesis pipeline, and to operate it outside the cell. The reaction kit to produce a DNA strand, which is used in every biology lab, is based on several biological macromolecules and other chemicals, mixed up in a vial and reacting together to produce the desired DNA; however, while the process has gained in terms of efficiency thanks to the decreased environment complexity, the control of the involved nanomachines, which is obtained through heating and cooling cycles, is still far from optimal. In fact, this is the equivalent of heating a city to activate a production line, instead of switching on the machines of interests, and it is obviously restricted to those applications where heat does not damage the operating environment and the thermal inertia is not a problem.

For all these reasons, the current "bioengineering" or "synthetic biology" approaches cannot truly satisfy an engineer: the possibility to accurately figure out the interactions of a machine with its environment, the optimization in the number of components and energy used to get a process accomplished, and the possibility to fully control its activity cycles are all crucial elements still missing in the modern molecular biology field.

From this perspective, a DNA synthesizer, which does not imitate the corresponding biological machinery (the protein apparatus which replicates the DNA in every living cell), has all the blueprints of a truly engineered object, because, to build it, engineers started from a minimal set of chemicals, reacting in the most efficient way, some mechanical principles (to get a working, old-fashioned steel-and-plastic machine), and a task to be accomplished (the synthesis of a DNA strand); on the basis of these principles, they designed a machine, whose working is perfectly deterministic, without uncontrolled effects from the environment, and they powered it with a standard source of energy.

However, a DNA synthesizer is not a nanomachine. The advent of nanotechnologies, hence the availability of tools and procedures to build and manipulate nanoscopic objects, is eventually allowing engineers to break the macroscopic barrier, developing the ability of:

1) designing and building from scratch a machine made by biological macromolecules, assembling the proper pieces in a suitable way;
2) powering such a machine;
3) operating the machine in a predetermined way, possibly via a remote control device.

As for the first point, we want to stress that nature assembles the biological nanomachines by relying on the self-affinity of their molecular components. Self-affinity rules dictate both the final shape of a given macromolecule and how different macromolecules will join together in a precise 3-D conformation. In particular, by selecting an appropriate sequence of "molecular meeting" events, natural nanomachines are assembled piece by piece, obeying only the thermodynamics governing the chemical recognition among the components. This, of course, means that the exact sequence is of paramount importance, so that much of the regulatory effort of a cell is devoted to setting up the proper timing and spatial constraints which are apt to produce the right nanomachine from its molecular components.

The same way nature does, so do chemists when building complex macromolecules in a sequential way, by joining different reactants into a predefined sequence of chemical reactions piece by piece. Following this approach, there has been significant work investigating the possibility of realizing complex molecular architectures starting from a range of available components, such as carbon cage structures, DNA, and organometallic compounds. Remarkably, many of them are inspired by macroscopic counterparts such as molecular motors, cantilevers, valves, shuttles, scissors, barrows, elevators, and recently nanovehicles [3]–[6]. However, the simple reduction of macroscopic machines to their nanoscopic counterparts, while admirable *per se*, did not bring any significant application, despite the inspirational "artist views" of nanorobots entering human bodies or similar fictional anticipations.

This is not only due to the scale effects, but also, and to a greater extent, to the fact that the physics governing the nanoscopic world, though classical, is very different and strongly affected by short-range forces and thermal fluctuations. Unless this fact is explicitly taken into account at the design stage, any simple reduction of a macroscopic machine to the nanoscopic level will produce, in better instances, a nice chemical toy, not a reliable tool for operating at such scales.

Possibly, the simplest way to overcome this difficulty is to rely on (at least) 3.5 billion years of chemical evolution which were spent improving nanomachines composing all living beings. Some of these natural nanomachines are remarkably similar to what engineers were able to conceive—for example, the bacterial flagellum basal body (Fig. 1) is very similar to an engine, with a rotor, a stator, and so on—while most of others are completely different from anything we could ever dream of, such as the ribosome (Fig. 2).

In the last 100 years, we have developed technologies to look at them in detail, reproduce them in any number of copies, and even to assemble

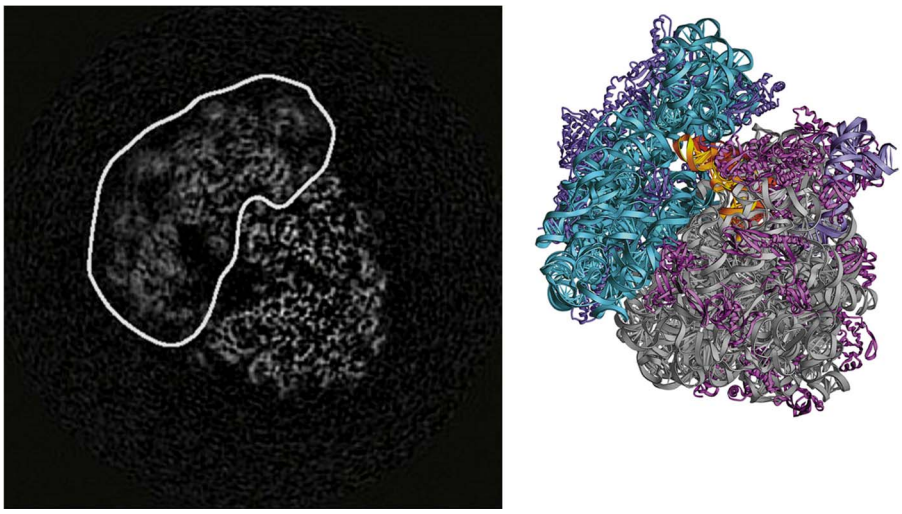

Fig. 2. *Ribosome. Left: electron microscope image. Right: 3-D rendering. Adapted from [14].*

them in a predetermined way. Hence, time has arrived to look at natural nanomachines and their components as a source of inspiration and building pieces for the realization of complex nanostructures, able to perform specific tasks in a controlled way. However, we should also quantitatively model the nanomachine operational environment, including the several other nanomachines which they will possibly encounter during their activity.

Concerning the operational environment of the nanomachine, thanks again to nanotechnologies, we are now at a point where it can be designed—and thus extremely simplified—and realized without the need to insert a nanomachine into a cell to let it operate. For example, recently, 200-nm-wide reaction chambers have been realized, filled with the necessary chemicals for the synthesis of a specific protein [7]. The protein synthesis was accurately verified, and it was found to be consistent with several parameters connected to the reaction volume and the physico–chemical characteristics of the nanometric reactors. This result basically means that we can manufacture nanometric versions of the biotechnologist reaction vials, so we do not need a cell to let our nanometric production line work. At this point, it should be clear that both building useful nanomachines and confining them to submicrometric size factories are feasible processes.

We can thus turn our attention to the problem of powering nanomachines; also in this case, it is still useful to recall how nature does it. All bionanomachines are powered by means of electrochemical potentials, which can be of geothermal origin, or may be created by breaking down high-energy compounds, or are originated exploiting interaction of light with some special pigments (in the photosynthetic process). Therefore, first attempts to propel man-made nanomachines have been focused on imitating the cell, powering them by means of electrochemical potentials in the form of pH or ionic strength gradients, or by high energy compounds (molecular fuels) or by light. However, current fueling approaches suffer from one or more drawbacks, namely:

1) the adverse effects of chemical fueling, which limit the applications involving living organisms;
2) the irreversible poisoning of the solution;
3) the necessity to provide fuel, preventing all those applications where fuel is not available directly in the solution and cannot be easily added to it;
4) in the case of optical powering, the limited penetration and damaging effects of the required high-energy UV radiation.

However, engineers are not limited to imitating nature for powering nanomachines. For instance, several approaches exploiting electrical fields have been proposed [3]. Apart from their scarce penetration, electrical fields have broad side effects though, for example currents caused by electric fields in ionic or polar solvents. On the contrary, as biological tissues are practically transparent to low-frequency magnetic fields, very recently an alternative approach has emerged, based on using such fields as driving agents [8]–[10]. Living organisms do not rely on this source of energy, possibly because high intensity fields did not exist before man, but this only means that we had to develop a proper actuator of the right size, something which we have now in the form of magnetic nanocrystals.

The use of such components to power nanomachines by extracting energy from a magnetic field allows also to approach the third point of our list, i.e., the control of operations of a nanomachine. As we already noted, in

this case, we cannot rely on the simple imitation of nature, if the control must affect a single nanomachine and must be remote, meaning not relying on local interactions with some other chemicals or macromolecule. This is because, as already stressed, the cell usually does not need to control the operation of a single nanomachine, but instead activates or turns off entire molecular production lines. The line starts working when it is fully in place, and each nanomachine does its job if there is a corresponding partner to work with. In our original production line similitude, every time a line needed to be operated, one had to build all the components, and every time, when the plant closed, one had to destroy all the components to switch the production off. From an engineering point of view, this is a very inefficient way to operate, because you need many other components, and you lack sufficient control over the activity of your specific nanomachines. For example, if you want to have a bionanomachine acting in the human body, you do not want to limit your actions to those rare districts (and timings) where a full range of biological nanomachines to partner with are present; you want to actuate it independently from the specific environment, and you want to decide when to switch it on and off.

First attempts to achieve this goal have already been proposed. For example, the use of heat to control the activation of temperature-sensitive nanomotors has been suggested [11]. However, it is virtually impossible to achieve a significant localized heating of micrometric or submicrometric regions [8], so we cannot control single nanomachines or avoid unwanted side effects due to the thermal motions and/or denaturation of biological macromolecules caused by heat.

On the other hand, magnetic nanoparticles coupling with external magnetic fields may be used to generate mechanical solicitations on an attached biological macromolecule exactly when needed. While it is likely that, given the magnetic properties of the currently available nanoparticles and achievable field gradients, we can only exploit torques to actuate nanomachines made of biological or synthetic macromolecules [9], the range of potential applications is wide because 1) torques may be used to actuate several different types of nanomachines; and 2) specific biological components can be used to transform a torque in a traction. As an example, applying a torque on a short DNA double helix with its extremities not free to rotate causes the double helix to vary in length, exerting a corresponding force along its axis.

To illustrate how torques may be used to control biological nanomachines, we refer the readers to the work reported in [10].

In this example, researchers attached magnetic nanocrystals to a peculiar type of proteins on the surface of a particular type of cell. These proteins, called epidermal growth factor receptors (EGFRs), in presence of another protein in the external environment [which is called epidermal growth factor (EGF)], group in discrete clusters on the cell surface. The formation of the cluster triggers a cellular process related to proliferation. The magnetically modified versions of EGFR exploit an external static magnetic field to align the magnetic moments of the nanoparticles, which induces their clustering by reciprocal magnetic attraction, thus activating the cell without the need of EGF. In such a way, scientists are able to switch on/off a complex cellular process by a remote command switching on/off of an appropriate field.

Summing up, it should be clear at this point that all the fundamental steps necessary for engineering biological processes are actually addressable.

IV. CONCLUSION

The merging of molecular biology with nanotechnologies has been often depicted as a truly revolutionary step in science; we feel that to fully realize the potential of this revolution, an engineering perspective must be adopted. This is a necessary step to leave the current "preindustrial" stage of biotechnology, where people skilled in the field rely on a trial-and-error approach, so as to reach an "industrial mature stage," where one must be able to optimally design, build, and control the nanoscopic production lines required to produce a given product or to perform a specific job. To this end, a new cultural paradigm should be spread among engineers to make them aware of the true nature of living organisms as hierarchically integrated sets of machines. These machines can be exploited and reused outside living organisms, and can be integrated in artificial factories together with unnatural nanocomponents. In such a way, not only can we optimize the productive process of interest, but also, by combining nanomachines of different origin, we can exponentially increase the types of production lines (and corresponding products) without being limited to processes and nanomachines compatible with the life of a cell or to designs emerged from natural evolution. Being able to control single biochemical pathways outside a cell would also overcome most of the ethical dilemma connected with the biotechnological exploitation of living organisms, restricting the use of living beings to those cases where this is actually unavoidable.

In the light of this perspective, a final remark is in order, concerning the peculiar risks connected to the possibility of being fully successful in achieving a complete and remote control of a molecular biosystem. To illustrate this point, let us refer to the results reported in [12], i.e., the remote control of the activity of an ionic channel (protein TRPV1) through magnetic nanoparticles, anchored to the cellular membrane of neurons in the brain of the humble roundworm *Caenorhabditis elegans*. Under stimulation by an alternating magnetic field, it has been proven without any doubt that TRPV1 is activated, causing an influx of calcium into the neuron.

This influx causes pain and burning sensations, so that this work can be considered a successful attempt at controlling an organism with a central nervous system by remotely inducing pain to cause a fugue behavior. Clearly, this entails risks and new ethical problems, which increase the responsibility of scientists and engineers and must not be overlooked.

Are engineers ready to accept the challenge? ■

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