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Trends in Nanobiology

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7.1 Introduction

National governments have adopted policies for promoting nanotechnology since then President Bill Clinton announced the National Nanotechnology Initiative in January 2000. A goal of nanotechnology is to create what surpasses the semiconductor device, whose miniaturization will probably reach its limit in the near future. While there are some candidates of such products like molecular-electronic elements, people are trying to develop products to which brand-new principles are applied, such as quantum computers and DNA computers. Meanwhile, microprocessing and single-molecule measurement, which are fruits of nanotechnology, have come to be employed in the field of life science such as in the development of new medicines, gene function analysis and research on biological information transmission.

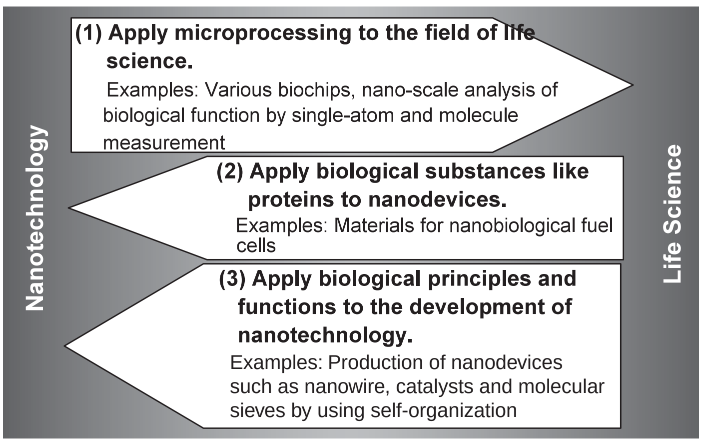
In this report, we introduce to you trends in nanobiology, which is widely seen as a boundary sphere between life science and nanotechnology.

7.2 What is nanobiology?

The Promotion Strategy of Prioritized Areas of the Council for Science and Technology Policy of the Cabinet Office recognizes nanobiology, in which medical micro systems and materials and biological mechanisms are applied and managed, as one of the five crucial domains in the field of nanotechnology and materials science. This strategy defines the primary goal in nanobiology as discovery of the basic principles for establishing a high-efficiency and ultra-integrated system with biomolecular structures and functions applied.

In the U.S., six educational and research institutions jointly established the Nanobiotechnology Center (NBTC). It employs the word "nanobiotechnology" with its definition as follows: "Nanobiotechnology applies the tools and processes of nano/microfabrication to build devices for studying biosystems. Researchers also

Figure 1: Three spheres extending across nanotechnology and life science



Source: Authors' own compilation

learn from biology how to create better micro-nanoscale devices."[1]

In the meantime, there is a term indicating a more limited sphere than nanobiotechnology does. The Asia-Pacific Economic Cooperation Center for Technology Foresight, which is estimating the future development of nanotechnology, reports on "nanobiosystems." According to its paper, nanobiosystem is "the use of biology to aid in the development of nanotechnology." In addition, this report says nanobiosystem has two levels.[2] First is to utilize biological materials as devices or functional materials. For example, enzymes with high specificity and catalytic powers may help to produce nano-fuel cells and bioelectronic elements. The second level of nanobiosystem is to scrutinize biological principles in order to grasp and use them. For instance, highly functional nanosensors have been produced by analyzing the function of ion channels, which detect chemicals, and imitating its structure and properties.

Nanobiology and nanobiotechnology are new ideas, so their definitions are still nebulous. Yet, we can deem that these fields include three spheres as follows, and as shown in Figure 1. They; (1) employ and apply nanotechnology such as microprocessing to the field of life science, (2) directly use biological materials in producing nanodevices, and (3) utilize biological functions and principles in developing nanotechnology.

7.3 Application of the fruits of life science to nanotechnology

In this chapter, we introduce to you instances of research and application regarding spheres (2) and (3) of Figure 1, which can be called nanobiosystem research. Table 1 shows research categories and examples on which the APEC Nanotechnology Position Paper reports.

We should also mention that Table 1 includes the development of new devices by imitating biological functions without using biological materials, as well as the direct use of biological materials like cells and enzymes.[2] Let us look at some examples in detail.

(1) Example of the use of biological materials: nanobiological fuel cell

Researchers are trying to make a miniature fuel cell using catalysts, or biological enzymes, instead of a platinum catalyst. Various enzymes exist in a living organism to help smooth redox reaction. Distinct biological catalysts are employed for hydrogen oxidation and oxygen reduction in developing a fuel cell comprising of hydrogen and oxygen. However, many technological hurdles lie in generating as much energy as possible, and it is necessary to establish a precise nano-scale structure in which active sites of enzymes, substrates and complementary elements are in

Table 1: Nanobiosystem research discussed in the APEC Nanotechnology Position Paper

Research spheres	Examples
Application of biological principles to existing fields of study	Enzyme transistors, whole-cell biocomputing, DNA computers
Biological and chemical sensors	Physical sensors using receptors, biomimetic sensors
Bioelectronics	Electronics elements and circuit parts using enzymes, antibodies, antigens and DNAs; nanobiological fuel cells
Opto-nanobiosystems	Biocomputer device elements, optobioelectronic device elements
Biological nanomachine	Parts of nanoelectromechanical systems (NEMS) and microelectromechanical systems (MEMS); chemical chip parts; drug-delivery systems (DDS)
Building of nanostructures of biomolecules by using self-organization	Molecular sieves, catalysts
Artificial organs and muscle with biological functions applied	Actuators, tissues for regenerative medicine

Source: Authors' compilation on the basis of a reference [2] ideal locations. Finding and making such an optimum structure are the immediate objectives in this field.

(2) Example of the use of self-organization: production of silver nanowire

Among various biological functions, it is hoped self-organization will be applied to the development of nanowire. In the method of making a nanostructure by building up each atom and molecule through the use of an atomic force microscope (AFM), though certain structures can be easily built, there are technological obstacles in making large structures and carrying out mass production. In contrast, mass production may be easily realized if self-organization is applied.[3]

Substances in a living organism that effectively employ self-organization are often used when devices are produced by self-organization. In this section, we introduce to you an example in which hydroquinone, a substance involved in biological energy conversion, is applied.

B. H. Hong *et al.* of Pohang University of Science and Technology, South Korea, made silver nanowire with a diameter of about 0.4 nanometer and a length of several micrometers using a template containing hydroquinones.[4] Metal nanowire attracts attention as a nanoelectronic material for its applicability in producing ultra-micro electronic circuits. Hong *et al.* made a template with an inner diameter of several nanometers by the self-organization of calix[4]hydroquinone, a compound including hydroquinones. This substrate consists of four hydroquinone subunits and has a calix-like shape. Hong *et al.* generated nanowire by letting this template absorb a silver nitrate solution and reducing silver ions by irradiating ultraviolet rays. In the cross section of this template, tubes form square-lattice-like arrays, and silver nanowire grows inside of these tubes. According to this paper, similar nanowire can be made with gold, palladium, platinum and mercury ions.

(3) Example of the imitation of biological functions: chemical sensors using artificial ion channels

An ion channel is a molecule converting stimuli into electric or chemical signals in the cell. It has a nano-scale tubular structure, penetrates the biomembrane, and comprises a pore in its center through which ions pass. This pore has an ion selectivity filter, so only specified ions can pass through it. This filter has a “door” with a sensor, and this door opens or closes in response to external stimuli. Researchers are striving to develop such products as a sophisticated ion sensor, and an amplifier of electric and chemical signals making good use of the function of ion channels. However, the functional principles of ion channels have not been fully revealed as yet.[5]

In the meantime, researchers are attempting to synthesize artificial ion channels with simple structures that can be easily made in order to understand the function of ion channels. There is a lot of research on artificial channels in which peptides are used as in biological ion channels. Meanwhile, Sokabe *et al.* of the Graduate School of Medicine, Nagoya University, synthesized artificial channels consisting of molecules with simpler structures without peptides, *i.e.*, comprising of hydrophilic and hydrophobic chain molecules, in order to understand the fundamental principles of ion channels.[5]

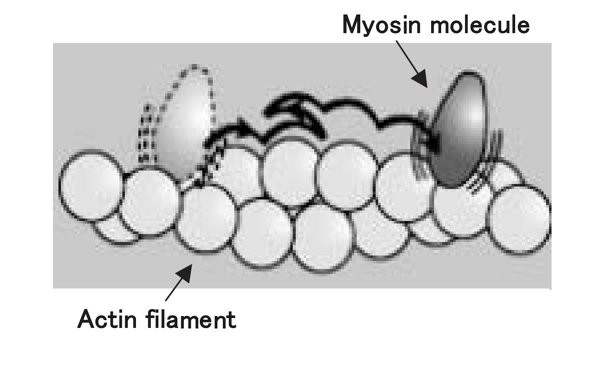
7.4 Examples of the application of nanotechnology to life science

In this chapter, we introduce to you examples of research regarding sphere (1) in Figure 1.

7.4.1 Analysis of motor molecules in the muscle by single-molecule measurement

Measurement and control of proteins, with their size of several tens of nanometers, are necessary to understand the function of biological machines like molecules involved in muscular movement. Researchers are promoting the development and application of high-accuracy measurement and molecular manipulation, making good use of the fruits of nanotechnology such as with an AFM and optical tweezers. This technique, which is called single-molecule measurement, is helping in the pursuit of research on motor molecules, kinds of molecular machines. This research has progressively revealed the unique mechanism of the molecular machine.[6]

Figure 2: Movement of a myosin molecule



Soruce: Authors' compilation on the basis of the reference [6] of Prof. Toshio Yanagida

First, let us look at the motor mechanism of myosin, a protein, as an example of a motor molecule. Myosins move along actin filaments, in which proteins called actin are bound together (Figure 2). This movement requires chemical energy generated by hydrolysis of ATP, a biological energy source. Recently, single-molecule measurement has clarified how myosins gain chemical energy from ATPs and move. Myosins move at random on actin filaments by the Brownian movement before receiving energy from ATPs. Directions of their movement are not set because they randomly move backward and forward with equal probability. Research with single-molecule measurement revealed that directional movement is efficiently produced from the combination between random and mechanical movements of system by the chemical energy of ATP. This is a discovery overturning the established theory. This research clarified a mechanism peculiar to a molecular machine and different from that of an artificial machine. It is hoped that this system will be applied to actuators of artificial muscles and robots.

7.4.2 Application of nanochip and microchip technology to life science

In this section, let us introduce to you the trends in nanochip and microchip technology, which will probably become practicable in the near future. In research after the completion of DNA nucleotide sequence analysis, it is necessary to accomplish mass processing and reduction of analysis and reaction time by highly integrating experimental systems and speeding up experimental procedures. The 30 to 40 thousand human genes are presumably producing about one hundred thousand to one million proteins.[7] We need not only to analyze this enormous amount of proteins but also to isolate and purify them. The high throughput screening explained below, to which nanochip and microchip technology is applied, may help such studies.

(1) Microchamber array

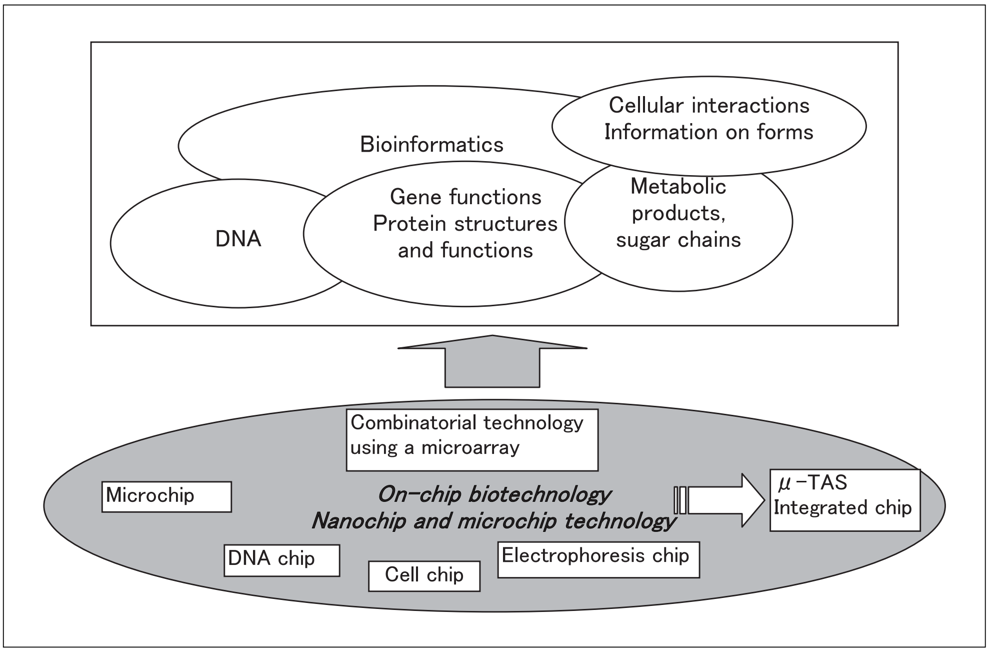
In this section, we introduce to you an example of the application of nanochip and microchip technology to the discovery of medicine working on proteins. Effectiveness of various synthesized compounds, which are medicine candidates, should be measured promptly in developing new medicine. A microchamber array with reactors miniaturized to the micro/nanometer scale is prepared using the technique of semiconductor microprocessing. Then, each compound is arranged in a distinct reactor, and the array is processed simultaneously by applying reagents and sampling materials. In this way, effective compounds are found. It is hoped that reduction of analysis time, saving of reagents and improvement of their sensitivity will be realized by highly integrating several thousand to tens of thousand reactors onto a single chip.[8]

(2) Protein chip

Draft sequence of the human genome was announced in February 2001, and the number of human gene products, or proteins, is thought to reach 30 to 40 thousand. Functions of 60% of these proteins have been revealed to some extent, while 40% are still unknown. Researchers are advancing analysis of conformation, production volume and interaction of these proteins with unrevealed functions.

A protein chip as well as a surface plasmon resonance sensor, which is a real-time analyzer of interactions between biological substances, and a mass spectroscope is used in the analysis of protein interaction. Ligands such as antibodies, enzymes and hormones are fixed on a chip, solutions including proteins are applied there, and then proteins interacting with the ligands are analyzed with a mass spectroscope. Currently, 20 to 30 materials on a single chip can be analyzed in about ten minutes. Researchers hope to develop a

Figure 3: Application of nanochip and microchip technology in life science



Source: Authors' compilation on the basis of references [8, 11]

chip with which thousands of materials can be analyzed in a short time.[9]

Chip technology is already used in gene function analysis, and application of nanochip and microchip technology will be crucial as well in analysis of proteins, metabolic products and cellular interactions. Moreover, researchers are promoting the development of on-chip technology, with which analyses that laboratories have conducted through several steps by using various analyzers can be carried out with a chip by making analyzers compact and automatized. The Micro Total Analysis System, or μ-TAS, is an example of such system (Figure 3).

Semiconductor microprocessing has been used in nanochip and microchip technology. Silicon technology, which has been developed for a long time, is applied to improve biochips and μ-TAS, and some of them have already come into practical use. Miniaturization of these chips will probably develop as in semiconductors for the time being.[10] Several enterprises have already launched R&D of biochips, expecting rapid growth of the life-science market.

7.5 Arguments over nanobiology

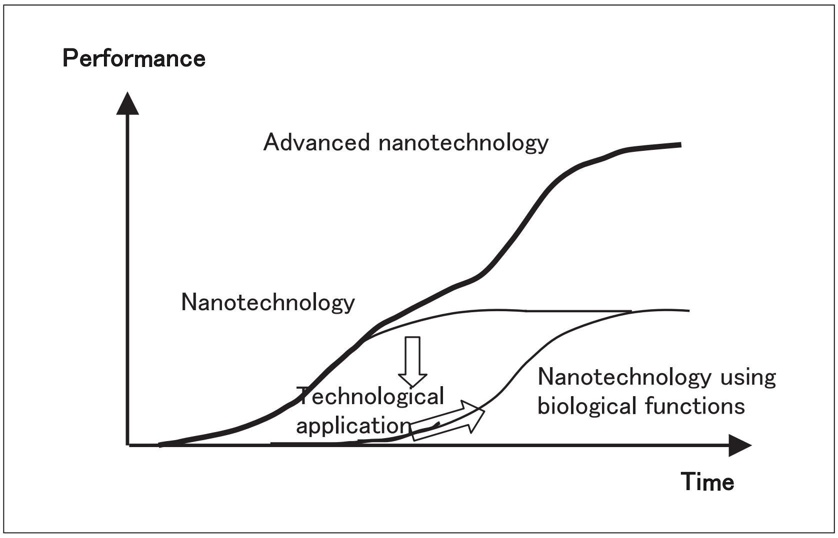
People hope that nanobiology will bring about qualitative changes in nanotechnology by incorporating biological functions and principles that artificial machines do not have. For example, people tend to think that nanotechnology requires an enormous sum of money considerably exceeding the level of private research as in R&D of semiconductor devices. However, if nanotechnology changes qualitatively along with the development of nanobiology, its research may become small in scale and inexpensive.[2, 3] Yet, prediction of the long-term technological and economic impact of nanobiology is impossible so far.

Most research on the application of life science to nanotechnology as shown in Table 1 is still at the basic level, except for studies on biological and chemical sensors. Even the concept that these products will be environment-friendly is not fully understood yet.

How to organize nanobiological research is highly interesting. People point out the necessity of the creation of new spheres extending across various fields of study and of comprehensive efforts beyond borders between different disciplines.

Functions peculiar to biomolecular machines, which are different from artificial machines, are being clarified in the boundary sphere between nanotechnology and life science. Although much research has been conducted to apply such functions to nanotechnology, most of these studies are at the basic level. In the meantime, in the

Figure 4: Concept of the development of nanotechnology with biological functions applied.



Source: Authors' own compilation

nanochip and microchip technology, which is a field of application of existing nanotechnology, researchers have already made road maps for putting this technology to practical use.[12]

After biological function research with existing nanotechnology, nanobiology will probably grow into an applied research with biological functions used. It is greatly hoped that the appropriate use of unique biological functions will bring nanotechnology to a higher level (Figure 4).

7.6 Conclusion

Will biology be the science in the 21st century? The answer is yes and no, says Cees Dekker, professor of the Molecular Biophysics Group, Department of Applied Physics of Delft University of Technology, the Netherlands. He explains the reason as follows.[13] Science has ramified as well as deepened in the 20th century. In this process, each field of study has fostered its own system that may be even called a culture. However, in the 21st century, people from various fields must jointly pursue research for clarifying the function of the biological system. Unveiling the function of the biological system is difficult through the conventional domain of biology only, and cooperation with other fields such as physics is essential. Therefore, his answer is “no” from the viewpoint that the 21st century will not be the century of biology only. However, it is “yes” from the viewpoint that a primary research objective in science will be the clarification of the biological mechanism.

Nanotechnology has realized microscopic measurement and control of biomolecules. Nanotechnology is making researchers from different fields of study scrutinize the same object. For example, in a living organism, physicists study structure and movement of biomolecules, chemists study biochemical reactions, and biologists study phenomena peculiar to the organism. It is hoped that clarification of the biological function progresses further with nanotechnology, a novel framework where various fields are merged together.

In addition, understanding of, for example, soft matters is thought to be necessary to reveal the biological function. Soft matters include liquid crystal, polymers, gel and colloid, which have been studied in the field of materials science. However, physical understanding of soft matters is still poor and a mountain of tasks is left to be tackled.[14]

Moreover, the concept of self-organization, whose application to nanotechnology is thought to be crucial, has not obtained a scientific consensus as yet. In the International Symposium on Chaos and Order in Chemistry held in 1999, researchers from various fields of study from molecular design to chemical chaos reconsidered the concept of self-organization, but a solid consensus was not established on any one definition.[15]

In this way, numerous difficulties involving different fields exist in revealing the biological function, like comprehension of soft matters and understanding and reestablishment of the concept of self-organization. The twofold answer of Prof. Dekker may be based on such a background.

Attempts for merging various fields of study are carried out in the forefront of research such as international conferences and schooling where the latest research results are discussed. Recently, young researchers such as graduate students majoring in biology, physics and chemistry from Asian and other countries gathered, learned and discussed up-to-date life science like nanobiology at the Asian Winter School "New Trends of Biochemical Physics" hosted by the Institute for Molecular Science.

As understood from the above discussions, nanobiology is still a new sphere with many problems to be solved. However, we need to foster and develop nanobiology as a merged domain breaking down the borders between different academic disciplines in order to fulfill its great potential.

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