Reconstitution of Sensor System Consisting Biomolecules, Cells and Their Mechanisms

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To achieve natural communications, we need adequate reproduction of the senses brought by molecules such as olfaction in addition to visual and acoustic senses. One reasonable way to achieve this issue would be constructing sensing system by using biomolecules and cells as they are, or by extracting their mechanisms. Here, we introduce the research activity of Bio ICT Laboratory concerning the reconstitution of sensor system consisting biomolecules, cells and their mechanisms.

1 Introduction

In regard to human sensory information, current information and communications technology focuses on communication of visual and auditory information. However, incorporating these natural forms of human communication into ICT is an important issue in human-friendly ICT. This will be made possible by making the characteristics of telecommunications interfaces similar to the natural senses of people, or in other words, by accurately capturing all sensory information and converting them into communicable electromagnetic signals, then recreating the sensory information.

A lot of research and development is currently being carried out into vision and hearing using light and sound as the information carrier, and research in this field of ICT has become quite mature. On the other hand, research and development into technology to convert the senses of taste, smell and touch into realistic electromagnetic signals are still at a developmental stage. We have not yet succeeded in accurately recreating the characteristics of these senses in the living body with their ability to selectively and flexibly detect a diverse range of chemical substances and mechanical parameters. Biomolecules and cells are responsible for this selective flexibility, and it is hoped that using them directly or building a sensory system based on such biological systems will lead to breakthroughs in ICT to further advance natural communication between people.

In this special issue, we reported on the Bio ICT Laboratory's search for the fundamental principles of life, and initiatives to establish applied biofunctional technology in Chapter 2, "Search for Basic Principle of Life" and Chapter 3, "Application Technology of Biological Function." This research is still at a fundamental stage, and it has been a long-winded process. However, we have so far gained state-of-the-art scientific knowledge, knowhow on how to handle materials, knowhow on assessment methods, etc., through our research, and making use of this knowledge has enabled examination of the principles behind new technology. In this section we will discuss our initiatives to build bio-based sensor systems that make use of elemental technology and knowledge in bio-ICT research.

2 Organisms recognize molecular information: Looking at olfaction as an example

Organisms not only make use of light and sound in communication, but the use of chemical substances as an information carrier is also extremely widespread. For example, the sense of smell and taste are used to recognize substances in the environment, or substances given off by food, enemies, etc. There are also hormones, which control the functions of internal organs, and chemicals needed for cells to function properly by communicating information within cells. In fact, chemical substances play vital roles throughout biological systems.

Let us take an overall view of molecular recognition in the living body by focusing on the olfactory system as an example (Fig. 1).

People are known to be able to differentiate between several hundred thousand odorous substances using the 350 or so types of receptors (protein sensor molecules involved in molecular recognition) found on the surface of

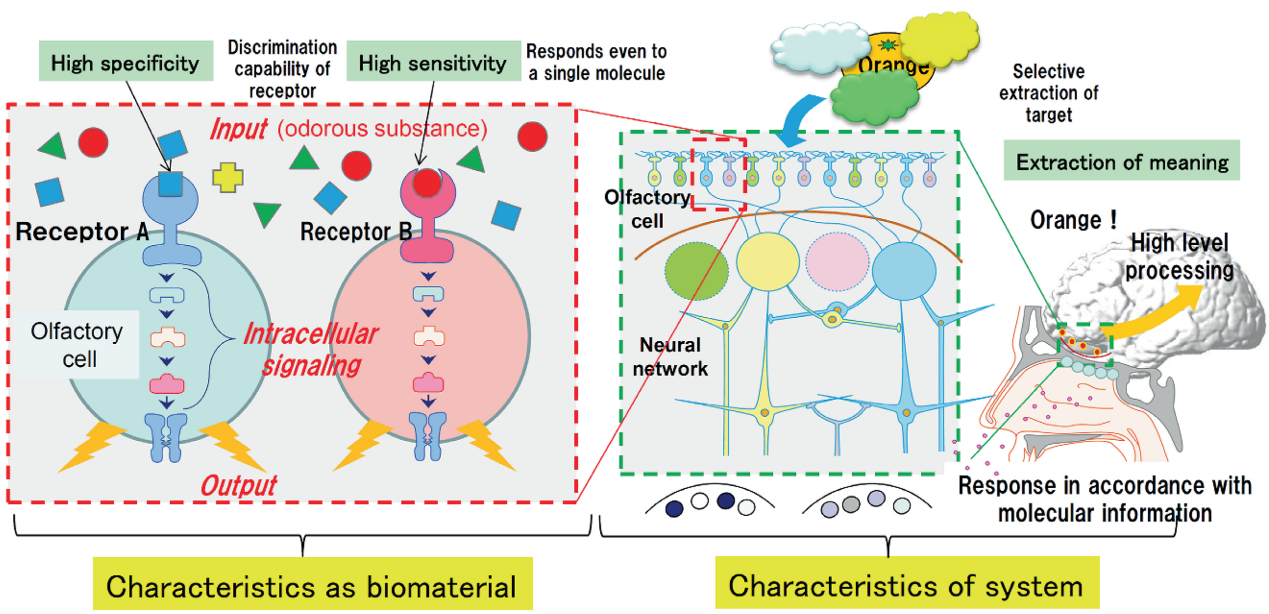

Fig. 1 Molecular recognition in a living organism. Example of an olfaction system

olfactory cells lining the back of the nasal cavity. They are sensitive enough to recognize smells on a level of 1 ppb (one part per billion). This is the equivalent of detecting a single drop of an odorous substance in a 25 m pool. They are also able to differentiate between the three-dimensional structures of molecules such as the difference between isomers or a difference of one functional group, which is a level of precision difficult to achieve in artificial sensors. This is thought to be due to the high level of specificity of receptors to substances, and the nonlinearity of the strength of response or time response characteristics in detecting substances. It is thought to be largely due to the specific characteristics of the biomaterial. As in the example of police dogs that have advanced capabilities to recognize the odor of a particular criminal and find that person within a sea of odors, the ability to recognize the target smell from among a mixture of substances comes from a characteristic unique to organisms, to extract necessary information from an environment with a lot of noise. A huge amount of data on odorous substances is attained from the sources of smells and the environment, but organisms are good at categorizing them by their overall characteristics such as whether they are dangerous or safe to handle, whether they are edible or rotten, etc. This system is believed to be based on the characteristics of networks that join cells together, or in other words the mechanisms behind biological systems.

Unlike the sensory systems of living organisms such as the example of olfaction given above, current artificial taste or olfaction sensors merely identify the presence or absence of a single chemical substance, or its concentration, and they do not enable the same kind of sensory perception that people actually experience. Recreating the information that the living body actually experiences requires the recognition of a variety of stimuli from chemical substances with the same level of specificity as found in the living body. It also requires the building of a sensor that like the living body is able to process noise and integrate detected signals with flexibility. Achieving this will require initiatives to examine the principles behind the building of sensory systems that make use of biomaterials and systems. Or speaking from the opposite perspective, our understanding can be deepened by recreating mechanisms for people to recognize information communicated by molecules using the materials that we have at hand.

3 Building a sensory system using biomaterials

Our goal in building sensory systems using biomaterials is to make use of biological sensory receptor mechanisms as they are, or enhance their functions before using them as sensors, to build a system that responds to a variety of sensory inputs. They will complement existing sensors that detect only one type of input, and they will take advantage of the merits of biofunctions. Achieving this will require the building of a method of (1) detecting molecular information using biomaterials, (2) assessing and evaluating the detected signal data using biomaterials, and (3) processing the obtained signals and extracting meaningful data.

In regard to the building of a method of detecting molecular information using biomaterials, we will need to develop technology for stably handling biomaterials (biofunctional molecules such as receptors, cells, etc.), which in general are unstable. We will also need to establish technology for modifying and adjusting biomaterials to build recognition elements for molecular information, then bond them to substrates and biological membranes, and control and manipulate them. In regard to the building of a method of assessing and evaluating the detected signal data using biomaterials, we will need to develop technology using biological systems to extract signals from among the noise and amplifying them. We will also need to develop technology to convert the responses of biofunctional molecules and cells into optical signals and electromagnetic signals such as potential changes and detect them. In building a method of processing the obtained signals and extracting meaningful data, signal data from multiple detectors will be processed, and the information that is targeted for detection will be identified, or an algorithm will need to be established to process the signals in a meaningful way upon creating a system for processing information.

In the process of carrying out the research discussed in Chapters 2 and 3, the Bio ICT Laboratory established technology for stably handling biomaterials based on advanced scientific knowledge of cells and biofunctional molecules. We also have advanced technology for manipulating and modifying biomaterials, and positioning them on substrates. In regard to modifying biofunctional molecules and cells for artificial use, the important elemental technologies involve our initiatives in building protein molecule systems using DNA (refer to *3-3* "Templated Self-Assembly of Biomolecules with High Spatial Precision") and building DNA molecular robots (TX Sensor) (refer to *3-5* "Nanotechnology for Future Molecular Systems using DNA"), as well as technology for modifying cellular functions by introducing artificial substances into them (refer to *3-2* "Artificial Induction of Organelle Formation in a Living Cell"). Using cutting-edge methods will be key to detecting signals, such as the single molecule assessment method and imaging method mentioned in *2-5* "Microscopies for the Biological Functions." Regarding the establishment of an algorithm for signal processing in the living body, knowledge on the

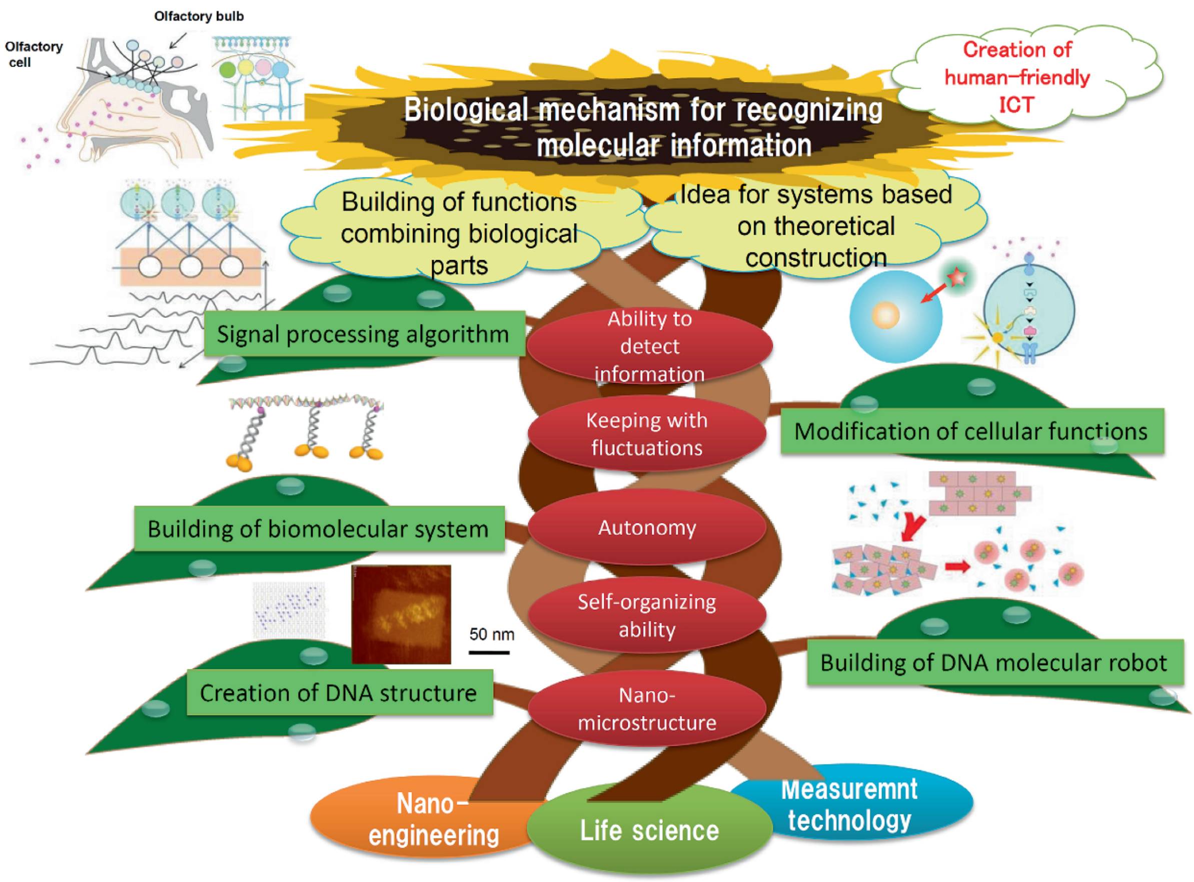

Fig. 2 Building a sensory system using biomaterials, and research into elemental technology

model of the mechanism of movement in a system consisting of random behavior, which plays a central role in biological systems, will no doubt be important, as it is mentioned in *3-4* "Functional Algorithm based on Noise Rectifying, Inspired by Bio Molecular Mechanism" (Fig. 2).

From here onward, we will focus on the building of systems currently being undertaken at the Bio ICT Laboratory (Fig. 3). In actually building a sensory system, there are many parts to choose from. The conventional method is to first use components that are easy to obtain, handle, and are well understood, and combine them with well-established technology. Based on this concept, we decided to use bacteria as the cellular system for implementing in the initial input stage. The sizes of cells differ depending on the species, but they are very small ranging in size from 1 micron to several dozen microns. These cells are packed with molecular communication networks consisting of several million to several hundred million molecules. The Blue Brain project being undertaken by EPFL and IBM has ascertained that recreating the behavior of one nerve cell requires the processing power of one portable PC. Using a cell in its original state means deploying a huge, natural integrated circuit at the point of entry into the system.

Bacterial cells detect concentration gradients of chemical substances (amino acids such as serine and aspartic acid, and sugars such as ribose and galactose) that they feed on in the environment around them. They then control the rotational state of their flagella to swim toward the areas of higher concentration (if the chemical substance is poisonous they swim away from it)[1].

The receptors of bacteria for detecting chemical substances (protein sensor molecules) are equivalent to the receptors of human beings for sensing smell and taste, but in *Escherichia coli* for example, there are only four types of receptors. Their structures, amino acid arrangements, and the basic properties of the molecules and other structures that they target have so far been thoroughly researched, so using them to build a system will ensure that uncertainties are kept to a minimum. Furthermore, the signaling pathway within the cell after a target molecule has been recognized has been thoroughly analyzed. And taking advantage of the fact that it is easy to modify the properties of cells through genetic engineering, it is possible to increase the number of receptor variations or artificially give them new properties such as making them glow in response to stimuli[2]–[4]. By nature, they are characterized by their ability to reveal the properties of detected molecules through the direction of flagellar rotation (clockwise or anticlockwise) and the frequency of change. Being able to

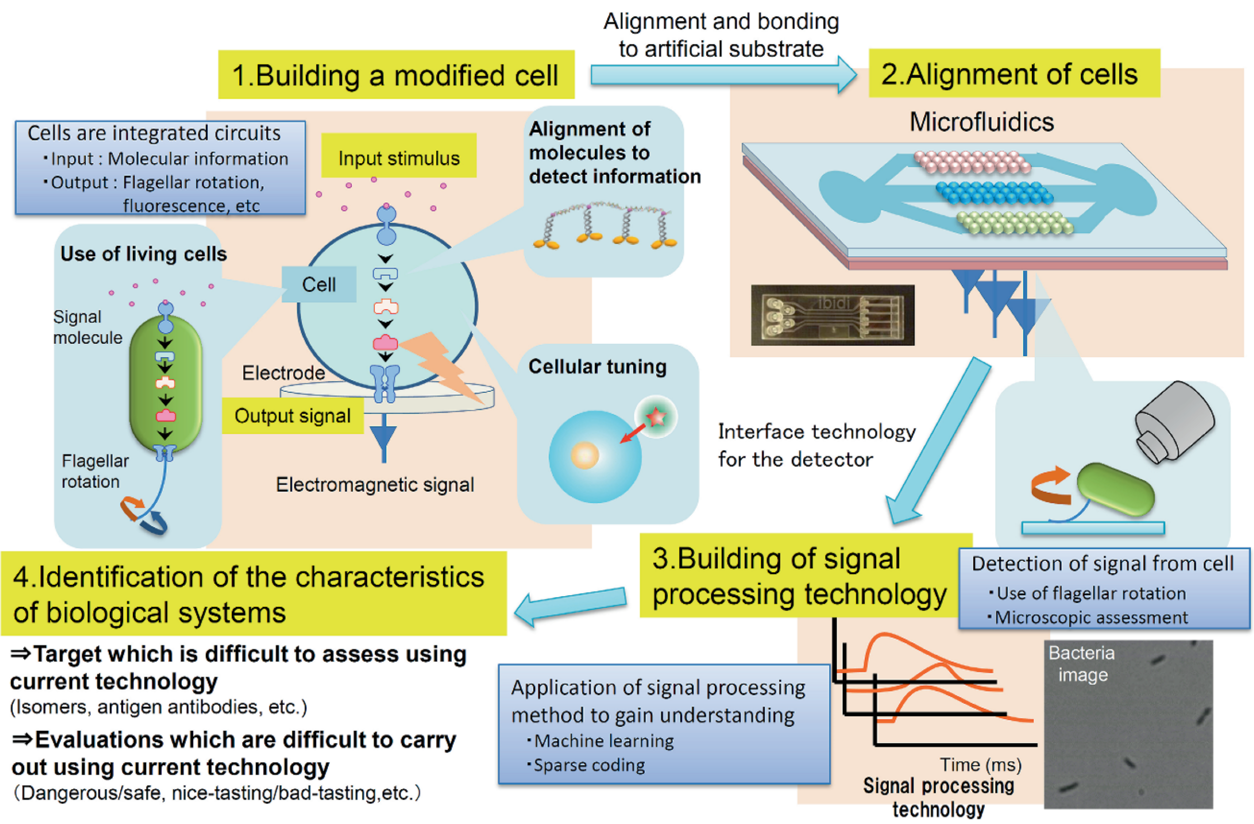

Fig. 3 Building a sensory system using cells and molecules

use this characteristic to assess molecular information is another major merit[5].

For signal detection, we used technology for positioning cells onto the surface of substrates based on microfluidics, which has made major advances in recent years[6]. As a sensory system we did not build a huge analyzer, but we positioned cells on a substrate of several centimeters squared. Microscopic assessment technology was used to detect the rotation of the flagella as mentioned earlier, as the response signal, and huge amounts of data were collected in real time from the group of cells.

For signal processing, it is necessary to extract meaningful data from the huge volume of data collected. To do this we not only implemented the machine learning method used in analyzing big data, but we also implemented cutting-edge knowhow used to analyze neural networks, such as the sparse coding method[7].

What we verified by building this sensory system was the method of building a system based on a device which detects the random behavior (fluctuation and failure) of organisms, the method of detecting information which takes the nonlinear properties of receptors into account, the tuning method of identifying a large variety of substances using only a limited number of receptor types, and the mechanism for drawing conclusions from data with numerous variables gathered from the detection target. It is hoped that this will lead to the development of sensory systems that are able to detect targets that are difficult to detect using current technology (the 3-dimensional structure of molecules, antigen antibody interactions, etc.), or make evaluations in ways similar to the sensory perceptions of people (determining whether the target substance is safe or dangerous, or nice-tasting or bad-tasting), which is also difficult to achieve using current technology.

4 Heading toward the future

At the Bio ICT Laboratory we have been carrying out research in the hope of finding the seeds of innovation for the next generation of ICT, centered on the search for the mechanisms behind biomolecular and cellular systems, and technology to make use of them. In our first approach, we have so far succeeded in acquiring detailed information on the structure of biological systems, from the level of single biomolecules up to the level of cells. Furthermore, we have enabled an almost hands-on-like investigation of biomolecules, cell behavior, and the correlation between input and output through the method of reconstituting the functions of biomolecular systems and development of advanced assessment methods, to acquire a wealth of scientific knowledge on the underlying mechanisms. Next, we combined technology for building nanometer to micrometer sized structures with technology for handling biomolecules, to succeed in aligning protein molecules and developing methods for controlling functions, or developing new methods for modifying the functions of cells. At the same time we developed a behavioral model for biological systems that function as they move randomly, that can be used in engineering. We also put forth a concept for molecular communication using biomolecules and other substances as information carriers, and carried out research to develop elemental technology and to investigate the underlying principles. Our initiatives to try and sow the seeds for new technology of the future by gaining a detailed understanding of biological phenomena at molecular and cellular levels, are still at the developmental stage. However, we are steadily building up our fundamental knowledge on how to create intelligent functional complexes consisting of countless molecular devices that mimic biomolecules and cells. Moving forward we will continue steadily deepening our understanding of the structural principles governing biomolecular and cellular systems, to acquire technology on methods to gain free control over this knowledge, and recreate intelligent biological functions. We will create techniques to provide revolutionary interfaces that override the boundaries of systems, such as microsystems and macrosystems, low-energy operating systems and high-energy operating systems, biological systems and mechanical systems, etc. Through these initiatives we hope to contribute to the future of ICT, including the building of human-friendly ICT.

References

1 G. H. Wadhams and J. P. Armitage, "Making sense of it all: bacterial chemotaxis," Nat Rev Mol Cell Biol, Vol. 5, pp. 1024–37, Dec. 2004.

2 J. M. Kralj, D. R. Hochbaum, A. D. Douglass, and A. E. Cohen, "Electrical Spiking in Escherichia coli Probed with a Fluorescent Voltage-Indicating Protein," Science, Vol. 333, pp. 345–348, July 15 2011.

3 J. B. Stock, G. S. Lukat, and A. M. Stock, "Bacterial Chemotaxis and the Molecular Logic of Intracellular Signal Transduction Networks," Annual Review of Biophysics and Biophysical Chemistry, Vol. 20, pp. 109–136, 1991.

4 H. Tajima, K. Imada, M. Sakuma, F. Hattori, T. Nara, N. Kamo, et al., "Ligand Specificity Determined by Differentially Arranged Common Ligand-binding Residues in Bacterial Amino Acid Chemoreceptors Tsr and Tar," Journal of Biological Chemistry, Vol. 286, pp. 42200–42210, Dec. 9 2011.

5 S. Terasawa, H. Fukuoka, Y. Inoue, T. Sagawa, H. Takahashi, and A. Ishijima,

"Coordinated Reversal of Flagellar Motors on a Single Escherichia coli Cell," Biophysical Journal, Vol. 100, pp. 2193–2200, May 4 2011.

6 W. H. Tan and S. Takeuchi, "A trap-and-release integrated microfluidic system for dynamic microarray applications," Proceedings of the National Academy of Sciences of the United States of America, Vol. 104, pp. 1146–1151, Jan. 23 2007.

7 B. A. Olshausen and D. J. Field, "Emergence of simple-cell receptive field properties by learning a sparse code for natural images," Nature, Vol. 381, pp. 607–609, June 13 1996.

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