# Nanobiotechnology

## New Technologies Expanded by Interdisciplinary Integration for a Healthy Society

National Institute of
Advanced Industrial Science
and Technology
AIST

# New Technologies Expanded by Interdisciplinary Integration for a Healthy Society

Collaboration Promotion Department
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## Expectations for nanobiotechnology

We hope everyone can live long in a healthy state. This is thought to be one of the basic conditions to realize a safe and secure society. The importance of health is also shown in the New Health Frontier Strategy set up by the Japanese government, and the social interest in health has increased more than ever before.

In such a situation, various problems have arisen that cannot be solved in the conventional technical field alone. For example, for routine health care or predictive diagnosis to spread in society, a technology is needed that allows examinations to be done easily and quickly at hospital or home. This is the concept called POCT (point-of-care testing), which means easy and quick testing carried out easily and quickly at or near the site of patient care. An indicator to quantitatively show a fair or poor physical condition is called a biomarker. To realize POCT, it is necessary to search for a science-based biomarker and develop a small inexpensive chip device to measure it. For implementation of such a device, huge interest is being taken in nanobiotechnology, which is an integration of bioscience and nanotechnology. A wide range of applications of nanobiotechnology is also expected in various fields such as predictive diagnosis, medical care, drug discovery and support for it, and the environment.

In general, there are two approaches of nanobiotechnology: top-down and bottom-up. The top-down approach analyzes biological phenomena and biomolecules using nanotechnology such as microfabrication technology. The bottom-up approach builds a molecular assembly and enhances its functions making use of the properties of biomolecules. Technologies for observation, measurement and manipulation at the nanoscale are also important as fundamental technologies. Because biomolecules contain in themselves structures for self-assembling and/or recognizing other molecules, bottom-up nanobiotechnology can be said to be a sort of supramolecular chemistry using biomolecules. The top-down approach is artificial and hard technology, while the bottom-up approach is soft technology that uses principles of biology and supramolecular chemistry. Combination of these technologies opens up various possibilities, for example, to fabricate a diagnostic chip with higher selectivity.

## Nanobiotechnology research at AIST

A feature and advantage of AIST is that researchers from life science and other various fields can work together to exercise their collective power. Another strength is that the environment is prepared to be favorable and appropriate for conducting interdisciplinary research such as nanobiotechnology. The research underway at AIST includes basic research to

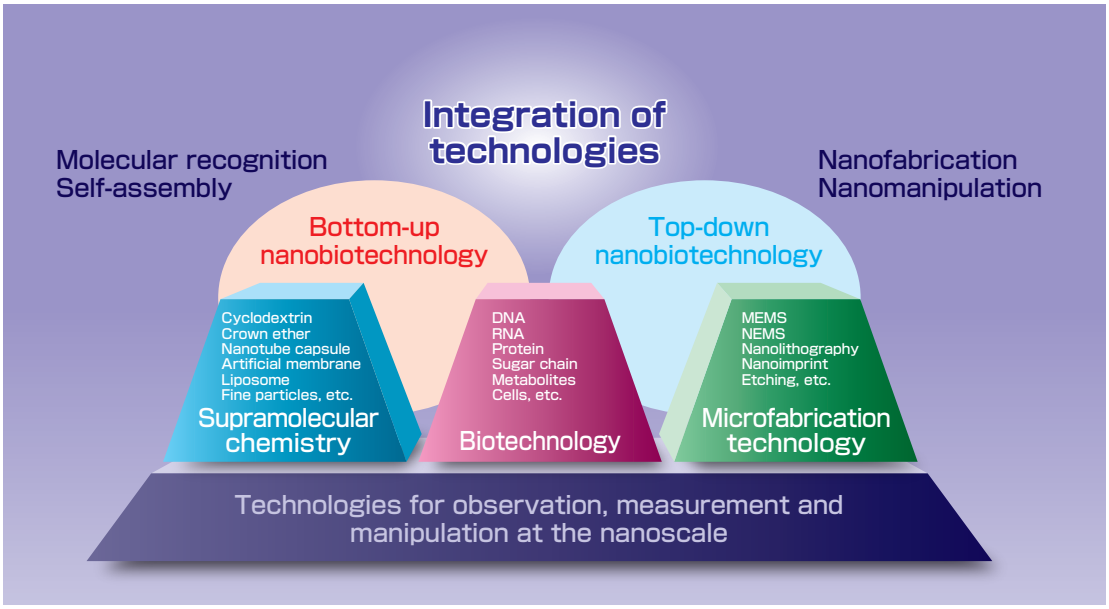


Figure: Creation of nanobiotechnology by interdisciplinary integration

discover unknown phenomena or universal laws (Type 1 Basic Research), research to combine multiple pieces of scientific knowledge and technology (Type 2 Basic Research), and research to build up products (Product Realization Research). These are also conducted in a unified and concurrent manner (Full Research). Particularly, nanobiotechnology may be said to be representative of research centered on Type 2 Basic Research because the research evolves by integrating different disciplines. Research Institute for Cell Engineering, Institute for Biological Resources and Functions, and other research units are currently conducting research and development of cell and enzyme chips, disease marker sensors, stress measurement chips, predictive diagnosis chips, and other products, and are fostering personnel to lead the nanobiotechnology field in the future. Collaboration Promotion Department intends to promote cooperation with the industrial, governmental, academic, and other sectors through technological integration beyond interdisciplinary boundaries.

## Fostering of Human Resources in the Nanobiotechnology Field

Research Institute for Cell Engineering
**Noboru Yumoto**

In the nanobiotechnology and other interdisciplinary fields, there is a serious shortage of personnel who can promote research and development. Thus, AIST established AIST Upbringing of Talent in Nanobiotechnology Course in FY 2003 (to FY 2007) by Promotion Budget for Science and Technology from Ministry of Education, Culture, Sports, Science and Technology (MEXT). This personnel fostering course consists of twelve full-time researchers belonging to AIST's five research units in the fields of life science, material and nanotechnology, and information technology and offers the curriculum as shown in the figure below. In addition to lectures and technical training, the trainees practice research under the common major theme of creating a nanobiomachine using motor protein to acquire hands-on skills and knowledge. Personnel training is given to professionals such as postdocs, graduate students, and engineers at companies, and a total of fifty-one persons were trained in the three years from 2003 to 2005. For personnel training, efforts have been made to keep track of corporate needs by questionnaire or other means, and the majority of those who completed in 2005 are already taking an active part at the companies.

For more information, please access http://unit.aist.go.jp/rice/link/nanobio/ (in Japanese).

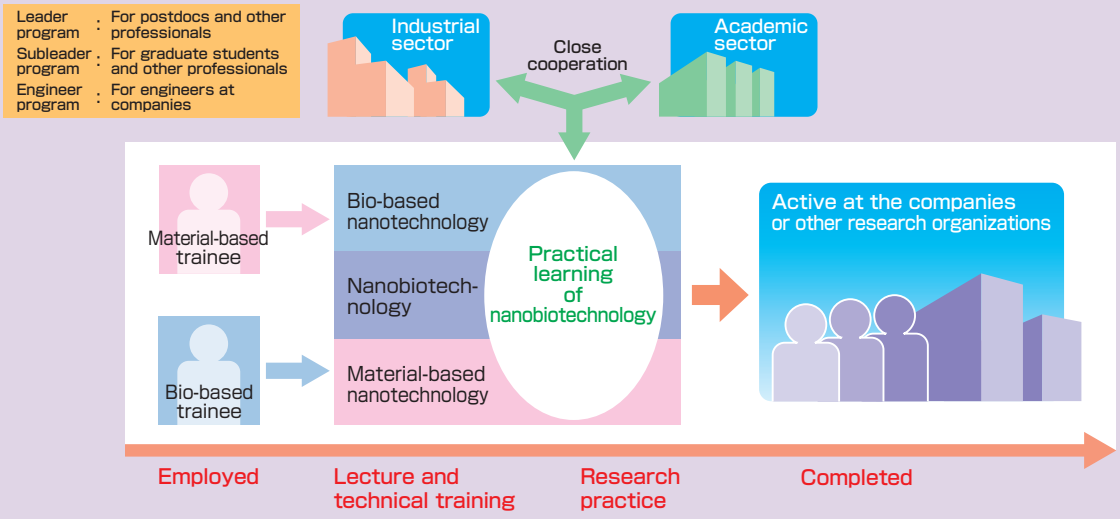


Figure: Curriculum of AIST Upbringing of Talent in Nanobiotechnology Course

# Cell Chip Applicable to Compound Profiling

Research Institute for Cell Engineering
**Masato Miyake**

## Importance of compound analysis

Today, we can find a wide variety of different types of chemical compounds such as pharmaceuticals, cosmetics, and food additives being used in all aspects of our everyday life. However, not all compounds have been thoroughly examined for their physiological action. If the biological effects of thalidomide, an antiepileptic drug notorious for its harmful effect, had been fully analyzed, the drug would not have caused severe malformations in infants born of mothers who had taken the drug during early pregnancy. It also might have been possible to predict at the first stage the usefulness of thalidomide in treating Hansen's disease and myeloma, which was found later.

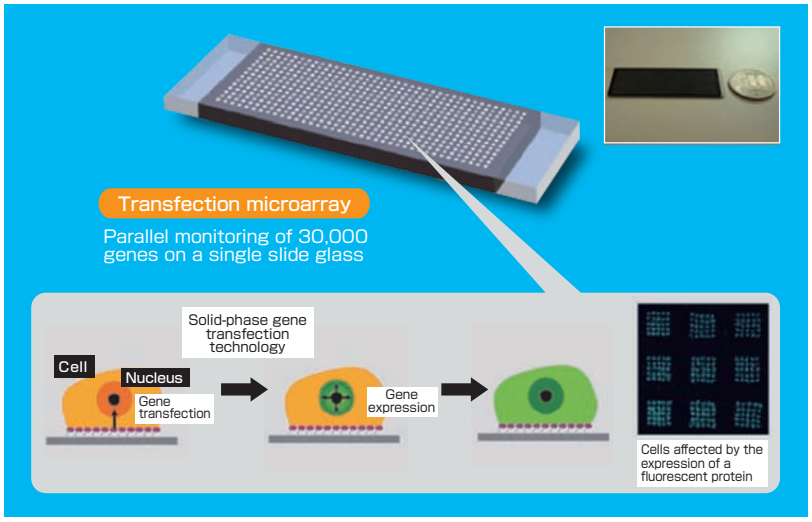


Figure 1: Appearance of transfection microarray and principle of gene transfection

## Development of a profiling tool

The Cell Informatics Research Group developed some devices using cells of human origin designed for fast and detailed compound profiling (feature analysis) as a tool to support the search for new uses of compounds. One of these devices is a transfection microarray (Figure 1). In searching for a combination of RNA-interfering agents (for example, siRNA) that have similar action to, work together with, or interfere with a certain compound, this device can be used to search for such a combination fast among RNA-interfering agents targeting the entire human gene and thereby identify the target of that compound. Thus, it becomes possible to predict a compound that has similar or collaborative effects. In addition, an enzyme microarray (Figure 2) allows researchers to accelerate the search for a compound that specifically inhibits the combination of certain enzymes. The group is also conducting research and development of device and analysis technology to improve the accuracy of prediction of the biological action of compounds or their combination.

Compound profiling using the transfection microarray is commercialized by an AIST technology transfer venture, CytoPathfinder, Inc. (http://www.cytopathfinder.com/eng/), which serves mainly domestic and foreign pharmaceutical and chemical manufacturers.

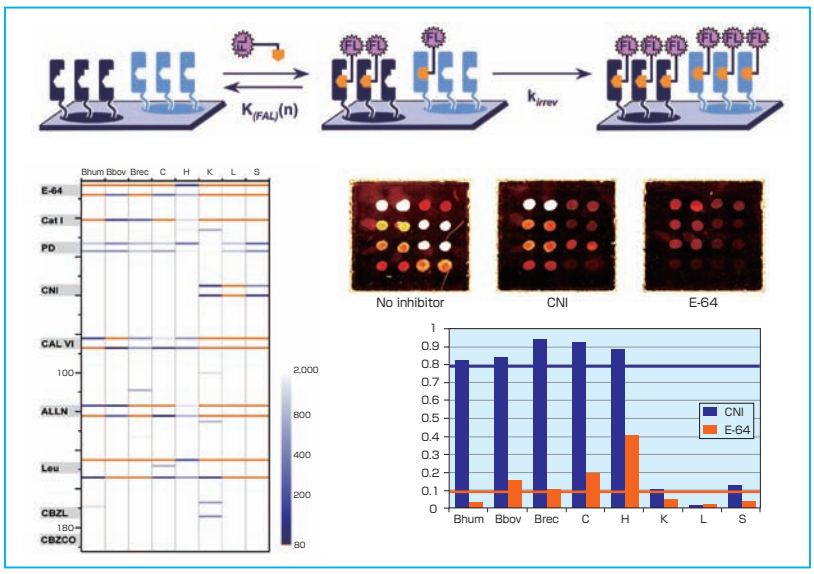


Figure 2: Parallel analysis of inhibitory effects of various compounds on the cathepsin family, using the enzyme microarray (Source: Nature Biotechnology, 23(5), 622–627 (2005))

# Stress Measurement Chip: Aiming at Mental Disease Prevention

Human Stress Signal Research Center
**Shin-ichi Wakida**

## Dilemma over stress marker measurement

Levels of catecholamines or other substances in the blood are known to serve as stress markers. For healthy people who are unaccustomed to blood drawing, however, blood drawing itself is a strong stress stimulus and causes stress responses such as a blood pressure rise. So, stress cannot be evaluated correctly, which is an intrinsic contradiction in stress measurement.

## Aiming at a multicomponent measurement Lab-on-a-Chip

As shown in Figure 1, we are conducting basic research in an organized way, such as (1) development and demonstration research of Lab-on-a-Chip devices to measure stress-related substances in saliva, which can be taken as a noninvasive specimen, (2) research and development of Lab-on-a-Chip technology to measure stress-related substances in the blood in a minimally invasive manner, and (3) advanced fluid control technology to realize these techniques.

## Development of a saliva stress measurement Lab-on-a-Chip device

We have chosen secretory immunoglobulin A (s-IgA), cortisol, and other substances in saliva, which are responsible for the biological defense function, as stress markers, and are developing a prototype of an electrophoretic Lab-on-a-Chip device and conducting basic research on a centrifugal Lab-on-a-Disk device.

## Development of a saliva NO assay Lab-on-a-Chip device

We have chosen nitrogen monoxide (NO), which is responsible for the biological defense function, as an oxidative stress marker, and developed Lab-on-a-Chip technology for rapid assay of salivary NO metabolites. Existing NO assay kits use the Griess method and take more than 2 hours to measure the total content of nitrate and nitrite ions, which are metabolized rapidly.

Noting the fact that NO metabolites have absorption bands in the ultraviolet region, we have conducted further development following the strategy of separating on the basis of slight differences in physical properties by electrophoresis. As a result, we have achieved good quantitativity and reproducibility.

## Demonstration research using saliva specimens

Saliva specimens were taken from subjects undergoing exercise stress approved by the Human Engineering Ethics Committee. By merely diluting the specimens 10 times, we realized separation and analysis of nitrate and nitrite ions for as short a time as 15 seconds, as shown in Figure 2. Furthermore, saliva specimens extracted from the high and low exercise intensity groups were used to analyze the relationships between the NO metabolite content and various exercise parameters. As a result, we obtained a preliminary result suggesting that the salivary NO metabolites would be an exercise stress indicator.

## Clinical research on saliva NO measurement started

We are now conducting joint research with a circulatory system clinical laboratory and are working together to acquire fundamental clinical data not only of saliva measurement but also of blood measurement and breath measurement.

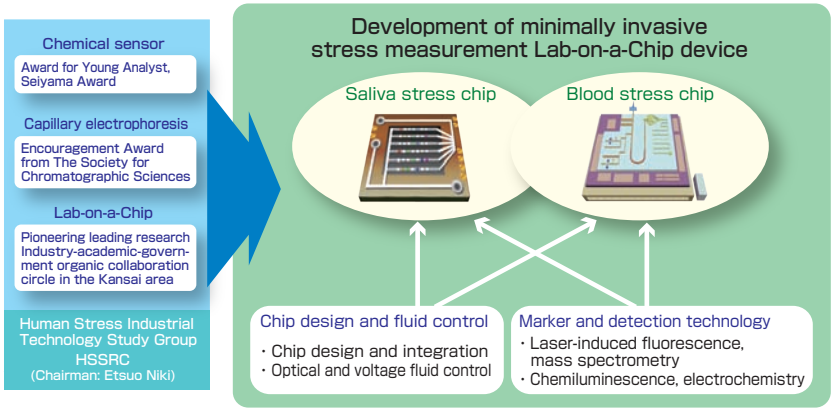


Figure 1: Schematic of R&D of a stress measurement Lab-on-a-Chip device and element technology and research structure for it

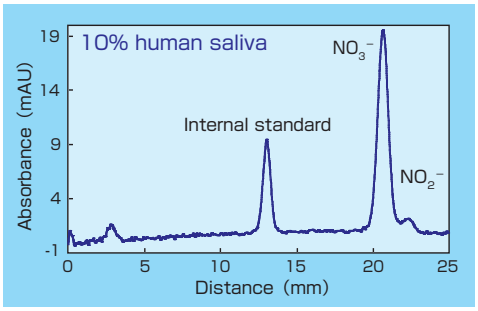


Figure 2: Example of 15-second separation and analysis of NO metabolites in 10% diluted human saliva

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# New Fluorescence Detector Achieving Size Reduction of Biochip Devices

Health Technology Research Center
**Mitsuru Ishikawa**

## Why light detection devices cannot be made more compact

Blood glucose and urine glucose meters have recently become commercially available for testing for diabetes at home. A urine glucose meter is about the same size as an electronic thermometer. Such measurements are made electrochemically and electrochemical devices are very compact. Why is it that light detection devices are not used for such purposes? A general feature of light detection is the ability to measure multiple wavelengths simultaneously with high sensitivity. We hear that developers of one company recognize this feature of light detection but ultimately choose electrochemical devices instead because it allows them to be made more compact. Using a light detection device involves an optical system made up of lenses and mirrors besides a light source. In the optical system, such optical elements must usually be placed taking a certain distance between them to keep a good S/N ratio. This ends up with a somewhat large optical system.

## New fluorescence detection device that allows for compact implementation of light detection

Electrophoresis is a basic technique for analyzing biomolecules. If this technique could be combined with fluorescence measurement in microchip form, excellent performance would be realized in terms of reliability, speed, and detection sensitivity of analysis. A compact electrophoresis biochip that can be easily held between the fingertips (Figure 1) has now been successfully implemented in practical form by our research team. However, it seems that the measuring devices other than this chip cannot be made palm-sized because they include a microscope, laser, and a CCD camera. Therefore, it is very difficult to develop an electrophoresis-based diagnostic device that is as compact as the urine glucose meter.

The possibility to overcome this difficulty was provided by a new integrated fluorescence detection device (Figure 2) developed at the Nanoelectronics Research Institute (Toshihiro Kamei). A combination of this compact detector with microlenses and a compact light source for optical communication can dramatically reduce the optical system size. Joint development is now underway to develop an electrophoresis device for home diagnosis in a combination of these components with a biochip. This development is based on the interdisciplinary integration of biological measurement with device fabrication.

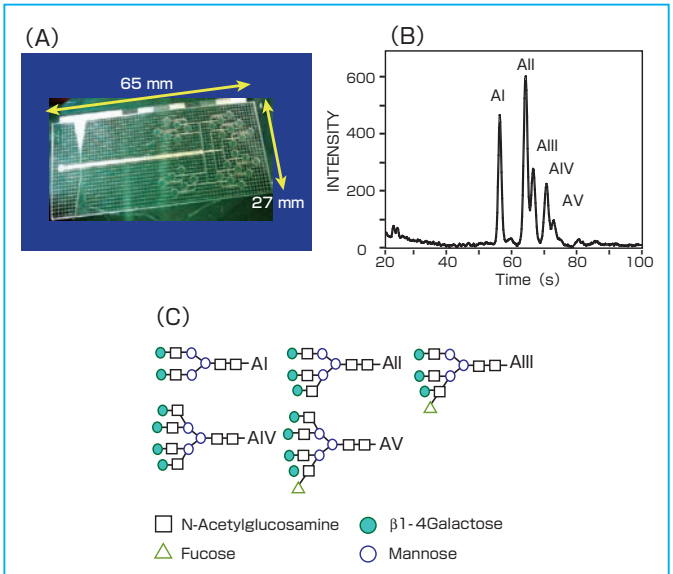



Figure 1 (A) Appearance of electrophoresis biochip, (B) Five different sugar chains analyzed using this chip, and (C) Structure of sugar chains used for analysis. ((B) and (C) are reproduced from Anal. Chem., 2006, 78, 1452-1458 with permission.)

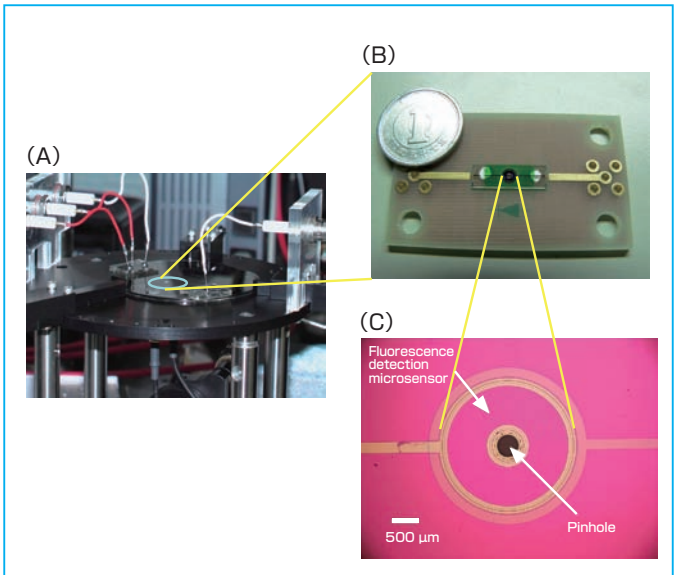



Figure 2 (A) Integrated fluorescence detection device in combination with an electrophoresis biochip, (B) Magnified view of the integrated fluorescence detection device, and (C) Magnified view of the photodiode of the device. ((C) is reproduced from Appl. Phys. Lett., 2006, 89, 114101 with permission.)

# Fully-automatic Two-dimensional Electrophoresis for Proteomic Analysis

Research Center of Advanced Bionics
**Kenji Yokoyama**

## Proteomic analysis tool

Proteome research analyzing proteins comprehensively is very important in understanding life phenomena that cannot be clarified by gene analysis alone. Proteomic analysis tools that have been used so far include two-dimensional electrophoresis. After separating proteins by two-dimensional electrophoresis, they are identified using a state-of-the-art mass spectrometer.

However, because the procedure of two-dimensional electrophoresis is not automated, reproducible results cannot be obtained unless the analysis is performed by a fully trained technician. The analysis takes 20 hours or more even if a small gel is used and almost three days for a large gel. Thus, the efficiency of analysis is very poor.

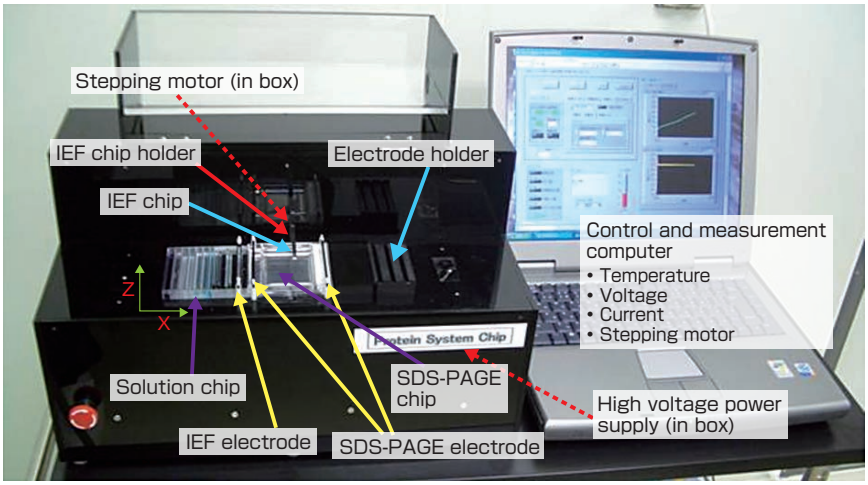



Figure 1: Fully-automatic two-dimensional electrophoresis system

## Development of a new tool

We are developing a system that can perform two-dimensional electrophoresis in a fully-automatic fashion. It takes only one hour or so for the analysis and detection using the system. Figure 1 shows our prototype of such a fully-automatic two-dimensional electrophoresis system. In this mechanism, the isoelectric focusing (IEF) chips are placed on the holder for electrophoresis of the first dimension.

The chip holder holds a dry IEF chip (a support plate to which an IEF gel strip is fixed) and moves it into the protein sample bath. Then, the chip is moved into the swelling bath and then the IEF bath. There IEF takes place by applying a predetermined voltage. We employed an intermediate staining method in which the protein is stained between IEF and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). In this staining method, the chip is moved into the washing bath on completion of IEF and then transferred to the staining bath where the protein is stained with Cy5 or other fluorescent dye.

After washout of excessive dye, SDS equilibration treatment is carried out. The IEF chip is transferred to the SDS-PAGE gel start point of the second dimension, where the gels are brought into close contact with each other to start SDS-PAGE. This system has a CCD camera for detection, which allows real-time visualization of the separation during SDS-PAGE.

## For practical implementation

We used this system to perform two-dimensional electrophoresis. It took 10 minutes for sample immersion and gel swelling, 20 to 30 minutes for IEF, 10 to 20 minutes for staining, washing, and SDS equilibration, and 20 to 30 minutes for SDS-PAGE and detection. That is, operations that previously took more than 20 hours could be performed in 60 to 90 minutes in our system. In the conventional system the gel is manually moved from the first dimension electrophoresis to the second dimension, while this system allows the fully-automatic two-dimensional electrophoresis.

We conducted an investigation using mouse liver lysates as a protein sample and succeeded in obtaining superior reproducibility and equal separation performance to a commercially available manual device (for small gels). This system is much better than conventional methods because results of two-dimensional electrophoresis can be obtained quickly and in a fully-automatic way.

# Heart Disease Marker Sensor Using Self-assembled Monolayers

Institute for Biological Resources and Functions
**Osamu Niwa**

## Disease diagnosis by heart hormone

A heart hormone called B-type natriuretic peptide (BNP), which is biosynthesized and secreted in cardiac muscle cells, is expected to have a significant beneficial effect on the diagnosis, prediction, and prognosis of heart disease.

However, because its concentration in blood is extremely low with about 10 pg/mL (3 pM) in healthy people, conventional immunochromatography is so low in sensitivity that radioimmunoassay or a large measuring apparatus such as a fluorescence detection system must be employed. A highly-sensitive sensing method is demanded that measures a sample with extremely low concentrations using a small, easy-to-use device in order to measure disease markers quickly on-site for diseases requiring urgent treatment such as heart disease.

## Development of new markers

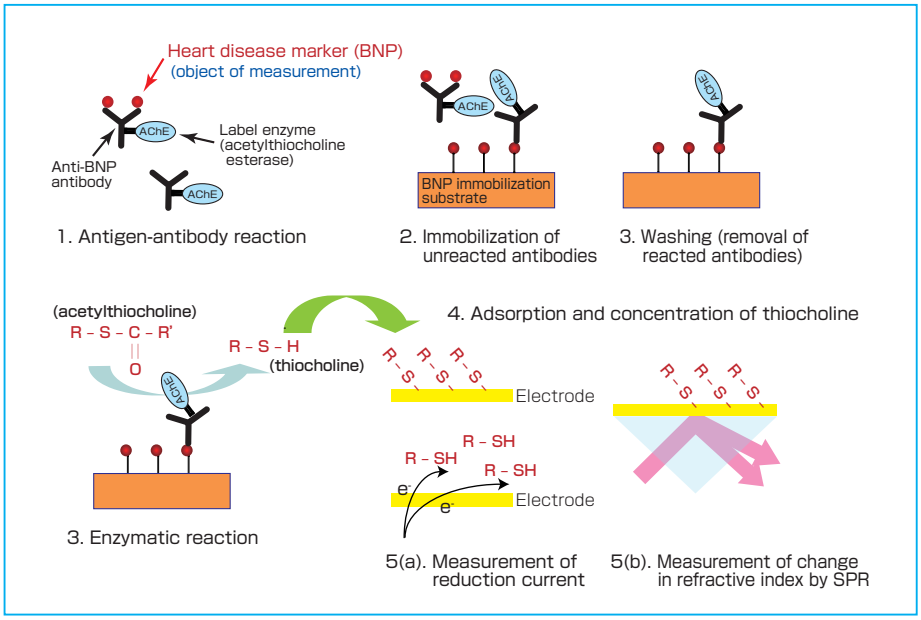
Peptide disease markers such as BNP usually use antigen-antibody reaction for measurement. The key is to amplify the reaction of extremely low amounts of molecules as much as possible in the measurement process. In the field of surface science, thiol compounds having an SH group at the end of an organic molecule are known to form self-assembled monolayers of nanometer order on the surface of metals such as gold and silver. An anti-BNP antibody was therefore used, which was labeled with acetylthiocholine esterase (AChE), an enzyme that generates a thiol compound by enzymatic reaction. Unreacted labeled antibodies are captured on the substrate following antigen-antibody reaction with disease markers. When acetylthiocholine is introduced after the removal of reacted antibodies by washing, thiol compounds are generated as a result of decomposition by the enzyme from the captured unreacted antibodies. When introduced onto a noble metal film, thiocholine forms a monolayer, resulting in high concentration of the enzymatic reaction product. BNP can be measured with high sensitivity if electrical current is measured when the product is subjected to electrochemical reduction or if the change in surface refractive index is measured by the surface plasmon resonance (SPR) method (Figure).

## Highly-functional system test using heart disease markers

To improve the detection limit through the measurement of extremely low quantities, the efficient capture of labeled antibodies and the concentration of the enzymatic reaction product (thiol compounds), a system has been developed which puts together a chip with siloxane polymer microchannels and a portable SPR instrument. This sensing chip enables immunoassay of heart disease markers to realize an extremely high sensitivity of 5 pg/mL within 30 minutes.

**Relevant information**

AIST TODAY (Japanese), Vol. 7, No. 2, 20–21 (2007)



# Safer Gene Therapy with a Novel Gene Transfer Vector

Biotherapeutic Research Laboratory
**Mahito Nakanishi**

## Obstacle to practical gene therapy

Causes of various diseases have been identified at the molecular level through advances in bioscience. However, there are diseases of known cause that are incurable with current treatment strategies, such as hereditary metabolic diseases, which cause the inability to maintain normal physical function due to inherent abnormal genes. Methods for treating such diseases are still very limited. Gene therapy is a state-of-the-art medical technique that involves the introduction of foreign therapeutic genes into cells in the human body to prevent, control or cure diseases. The therapy is expected to become practical as a radical treatment for such intractable diseases.

As surgical operation requires scalpels and surgical suture, gene therapy requires a nanoscale drug delivery system (DDS) to deliver a drug, i.e. gene. However, unlike the conventional DDS used to deliver low-molecular-weight drugs, a DDS (vector) for gene therapy must have the capability to carry a drug (gene) into cells. There are two major types of vectors that have been used in clinical studies for gene therapy. One is viral vectors engineered to carry therapeutic genes by recombinant DNA technology and the other is non-viral vectors carrying a combination of DNA molecules, produced in *E. coli*, and chemical substances. The delay in developing a vector with high gene transfer efficiency and safety has acted as a barrier against the practical application of gene therapy.

## Nanotechnology helps passage through barriers

To express a gene introduced into a cell, advanced technology is necessary that allows efficient passage of the gene through cellular barriers, such as cell and nuclear membranes, without injuring them. We have successfully laid the foundation for the development of highly functional non-viral vectors by developing a number of tools. One tool is a fusogenic liposome that can fuse directly with the cell membrane and transfer its content. The tools also include a nanoparticle that passes through cell membrane by the action of a peptide called PTD (protein transduction domain) (Eguchi, et al., 2001), and a nanoparticle that passes through the nuclear membrane actively by the action of a short peptide (nuclear localization signal) with which the nuclear protein is transported into the nucleus (Akuta, *et al*., 2002; Eguchi, *et al*., 2005).

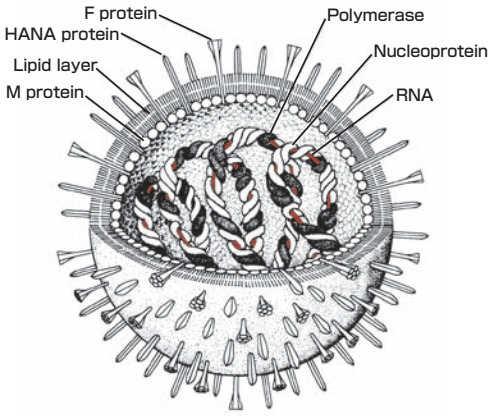



Figure 1: The structure of Sendai virus, which was used as material for the development of the RNA replicon by the Biotherapeutic Research Laboratory. It is a nanoparticle of about 240 nm in diameter having a lipid bilayer outside and genomic RNA inside.
(Reproduced with permission from Nikkei Science Inc.)

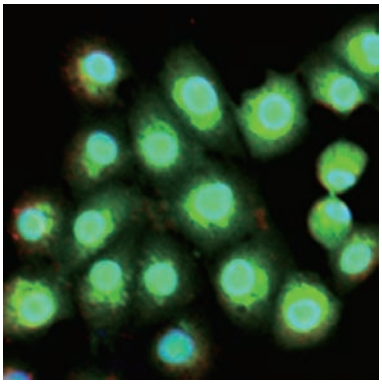


Figure 2: Simian cells emitting stable, green fluorescence for an extended period due to the expression of the enhanced green fluorescent protein (EGFP) gene carried on an independent RNA replicon (Photographed by Ken Nishimura)

## Safer gene therapy using RNA vector

When long-term persistent expression of an introduced gene is required, as in hereditary metabolic diseases, a platform must be developed to carry and maintain genetic information stably in cells in addition to the development of a carrier for genes. In conventional DNA-based gene expression technology, foreign genes are stabilized by inserting them into chromosomes. However, random insertion of genes into chromosomes is inefficient and can cause safety problems including carcinogenesis. We thus tried to create a new platform for carrying genes and have successfully developed an independent RNA replicon that allows long-term stable gene expression even if the genes have not been inserted into chromosomes by using RNA instead of DNA as the genetic body.

AIST aims to contribute to the practical application of gene therapy through the development of high-performance vectors for gene therapy based on such results.

# Smart Capsule with Highly-functional Nano Spaces

Nanotechnology Research Institute
**Masahiro Fujiwara**

## Microcapsule

Hollow and porous fine particles in nanometer or micrometer size are like minute capsules (microcapsules) because they can contain and release various compounds as appropriate. In AIST, we conduct research into various nanobiotechnologies by incorporating various drugs or biomolecules into these minute capsule spaces composed of inorganic materials. For example, an inorganic spherical hollow particle has been successfully synthesized on a one-step method by making skillful use of water and oil interfaces.[1]

## Application to a drug delivery system

Figure 1-A, which presents an electron microscope image of silica microcapsules, indicates a large empty space inside each microcapsule. Protein and DNA molecules can be enclosed directly in the empty space if they are present together when synthesizing the microcapsule. Figures 1-B and -C present an optical microscope image (Figure 1-B) and a fluorescence microscope image (Figure 1-C) at almost the same point of a silica microcapsule encapsulating bovine serum albumin (BSA) containing fluorescent dye, respectively. These images indicate that there is fluorescent BSA in the microcapsule.[2] As the BSA contained is larger in size than the pore in the capsule shell, it will not be released outside unless the capsule is broken. Therefore, application to technologies, such as responsive drug delivery system or immobilized enzyme technologies, is expected.

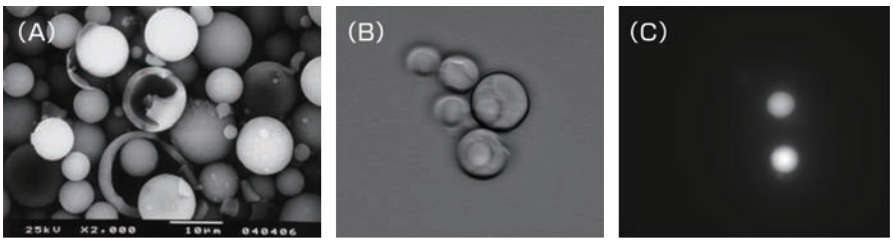



Figure 1: An electron microscope image (A) of a representative silica microcapsule. An optical microscope image (B) and fluorescence microscope image (C) at the same point of the silica microcapsule encapsulating BSA containing fluorescent dye. Glittering circular areas are observed in capsules.

## New capsule technology

If a molecular gate that will open and close reversibly in response to external stimulation is installed at the outlet of a nanopore of silica that is uniform in size, storage in the pore and outward release of the enclosed compounds can be controlled by opening and closing the gate. For example, controlled releases are achieved by photoirradiation in a gate of a coumarin molecule that dimerizes reversibly by light[3] as well as by oxidation-reduction reaction in the disulfide moiety where the linkage is cleaved reversibly by oxidation and reduction[4] (Figure 2).

Thus, materials with a minute empty space are expected to be applied to various nanobiotechnologies as smart capsule materials that can control the transportation of molecules and biopolymers freely at the nano level.

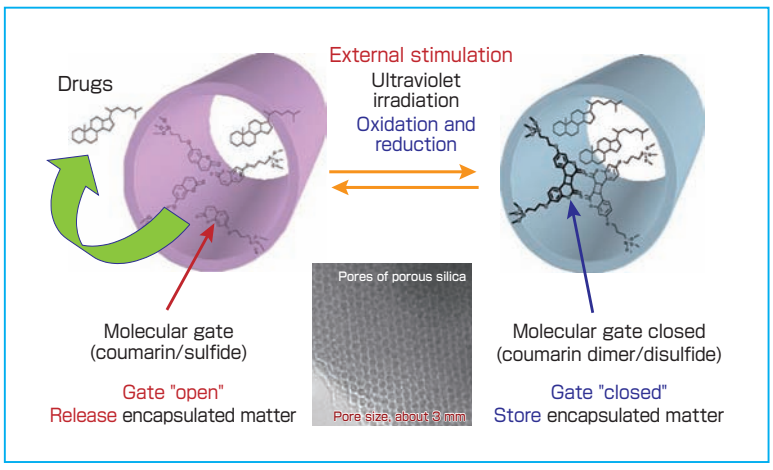



Figure 2: External stimulation-responsive drug delivery using porous silica

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# Sensing and Manipulation of Cells

Research Center of Advanced Bionics
**Toshiyuki Kanamori**

## From population to individual

Bioinformatics has established its usefulness in understanding the genome and the focus of its research is now shifting to the proteome. After that, the focus will shift further to the cellome.

Another important point is that the viewpoint of research is shifting from population mean to individual analysis as represented by single nucleotide polymorphism detection and single molecule measurement. This is an inevitable trend if biodiversity and biocomplexity are considered.

In response to this situation, we are developing techniques to sense and manipulate individual cells.

## On-demand two-dimensional cell manipulation techniques

Light is advantageous in manipulating a cell of several ten micrometers in size in a closed space because it can be radiated instantly and locally from a distance. We already developed a cell culture surface on which cell adhesion could be changed by light irradiation and reported that this technique can be applied to cell selection and precision pattern culture (Figure 1).


Figure 1: Pattern culture (top) and precision co-culture (bottom) of cells by on-demand two-dimensional cell manipulation techniques

Furthermore, we developed an instrument that radiates light in an arbitrary pattern onto individual cells being observed through an optical microscope, and plan to set up a venture shortly to sell this instrument together with photoresponsive cell culture dishes and other products.

## A microchip handling cells

Considering that the cell size is several ten micrometers, microprocesses are advantageous in handling individual cells precisely. We aim at establishing techniques to integrate various elemental technologies, including the on-demand two-dimensional cell manipulation techniques described above, in a microchip and handle cells in it.

We have already succeeded in forming a particular cell spot (colony) in a channel in the microchip (Figure 2, left). Microvalves are needed to introduce cells into compartments in the microchip or inject a drug solution or other chemicals into compartments. Here again, we give attention to light and are developing an optically-controlled microvalve that can be operated by light from outside (Figure 2, right).

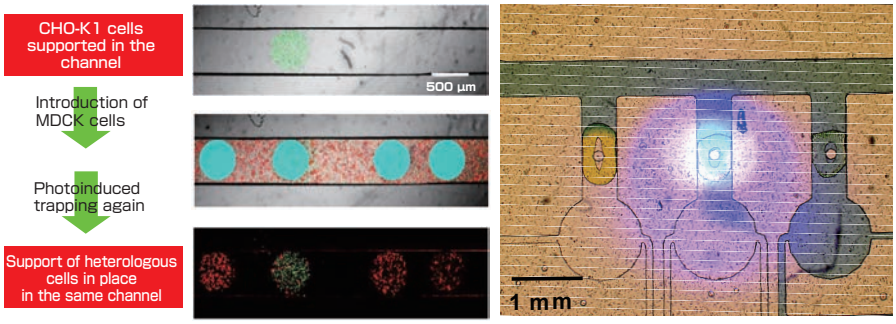


Figure 2: Cell spots (left) and optically-controlled microvalves (right) in a microchannel (1 mm wide)

In the near future, it will become possible to inject a group of cells into individual compartments or culture cells in the form of a spot in the channel to give stimuli to these cell groups or cells with a particular drug solution or to perform cell assays for drugs in them.

Relevant information
http://www.aist.go.jp/aist_e/aist_today/2006_19/hot_line/hot_line_15_2.html
AIST TODAY (Japanese), Vol. 5, No. 10, 20–21 (2005)

# Cell Surgery: Cell Manipulation with a Nanoscale Needle

Research Institute for Cell Engineering
**Chikashi Nakamura**

## Nanoscale needle for cell manipulation

The human somatic cell is about 20 to 30 μm in diameter, so a very thin needle (nano-needle) about 200 nm in diameter is required for an insertion operation without killing the cell. The cell does not die even after an insertion operation for more than one hour, so it is expected that the cell can be analyzed or used after the insertion action. We named this cell manipulation technique using nano-needles "cell surgery" and are currently developing it.

Using an atomic force microscope (AFM) instrument, the nano-needle is manipulated and the very weak force acting between the needle and cell is observed. In this way, we can observe the inserted state of the needle. As shown in Figure 1, a typical pyramidal AFM probe is sharpened using a focused ion beam into a needle of 200 nm in diameter. As shown by the confocal fluorescence images, the typical AFM probe is forced into the cell involving the cell membrane at the probe tip while the nano-needle is inserted in the cell smoothly and reaches the inside of the cell nucleus without deforming the cell. With the typical probe, the repulsion simply increases when it is pushed against the cell. When the nano-needle was pushed against it, the phenomenon in which the repulsion was quickly relaxed was observed. This relaxation means that the needle got through the cell membrane successfully. The nano-needle insertion requires no particular cell modification and ensures that the success or failure of insertion action can be detected. Therefore, the cell can be used in a natural state after manipulations.

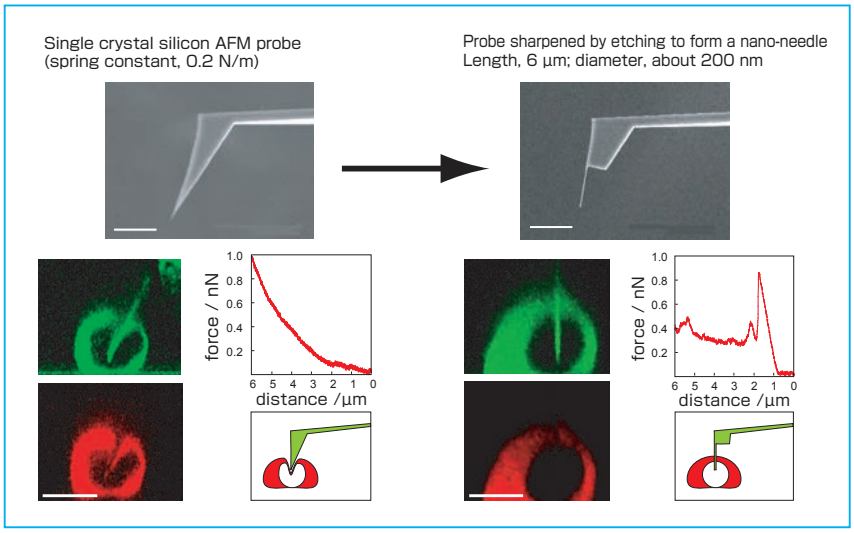


Figure 1: Insertion of a nano-needle into a cell and mechanical response (scale bar, 10 μm)

## Efficient gene transfer technique using a nano-needle

The human bone marrow-derived mesenchymal stem cell is a flat cell of several micrometers in thickness in adhesion culture and has the problem of a very low gene transfer efficiency. We performed a transfer operation after modifying a nano-needle of 200 nm in diameter with polylysine and making it adsorb plasmid phrGFP. With the conventional microinjection technique using a glass capillary, the transfer efficiency was 10% or less. On the other hand, we succeeded in gene transfer with a high efficiency of 70% or more using the nano-needle. Gene transfer using the nano-needle ensures needle insertion not only into a cell but also into the cell nucleus. This is thought to have achieved the high gene transfer efficiency.

If further development of this technique makes it possible to recover the function of cells or make desired cells without changing the genetic characteristics of the cells, this will lead to regenerative medicine by safe autologous cell transplantation.

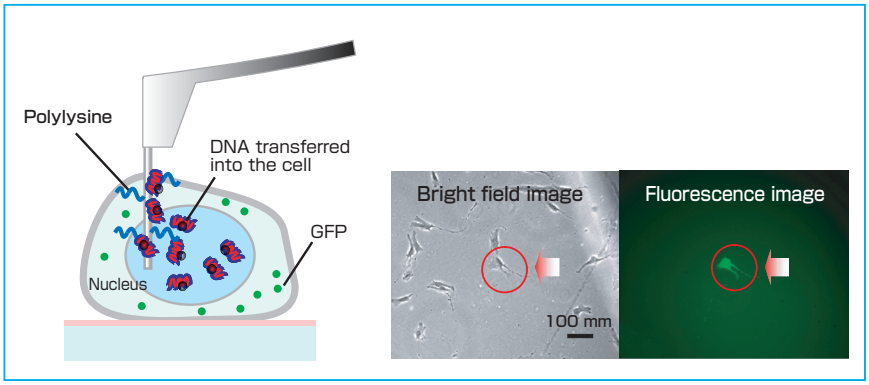


Figure 2: DNA transfer into a mesenchymal stem cell with a nano-needle

# Aiming for the Development of Diagnostic Techniques for Cancer Metastasis

Institute for Biological Resources and Functions
**Tomoko Okada**

## Cancer metastasis to bone marrow

It is not too much to say that the reason why people continue to die of cancer in spite of remarkable progress in the diagnostic and therapeutic techniques for cancer is that its metastasis cannot be prevented. Metastasis to bone (bone marrow), among others, occurs frequently in breast cancer, prostate cancer, and multiple myeloma. It causes severe pain or bone fracture in patients, resulting in a marked reduction in their quality of life. However, the mechanism of metastasis has not been fully elucidated.

## Aiming at the clarification of the metastatic mechanism

We established a bone marrow metastasis model for murine myeloma cancer cells to study the mechanism of bone marrow metastasis. It was expected that cancer cells metastasized to bones, kind of special tissue, with the help of osteoclasts, cells destroying bones under physiological conditions. So, focusing on the interactions among cancer cells, bone marrow-derived endothelial cells, and osteoclasts, relationships among these cells in bone marrow metastasis were examined.

It was revealed that an osteoclast differentiation factor (ODF), which is essential for the differentiation and induction of osteoclasts from precursor cells, was found to be expressed on the surface of bone marrow-derived endothelial cells. It also became clear that when cancer, endothelial, and precursor cells were co-cultured, osteoclast induction was enhanced only in the presence of both cancer and endothelial cells. Co-culture of endothelial and cancer cells resulted in the increase in the expression of both mRNA and protein of ODF on the surface of endothelial cells. In other words, metastatic cancer cells were found to stimulate endothelial cells to express more ODF on their surface, leading to indirect promotion of osteoclast differentiation and induction[1].

To examine whether the same thing occurs *in vivo*, the cancer cells were injected to mice through the tail vein, and their femurs were analyzed. As a result, there was an increase in the number of osteoclasts in the murine femurs receiving the cancer cells, indicating enhanced induction of osteoclasts *in vivo* by these cancer cells[1], [2].

We are currently working at creating a highly bone marrow metastatic model of breast cancer, which has been increasing among Japanese women[3].

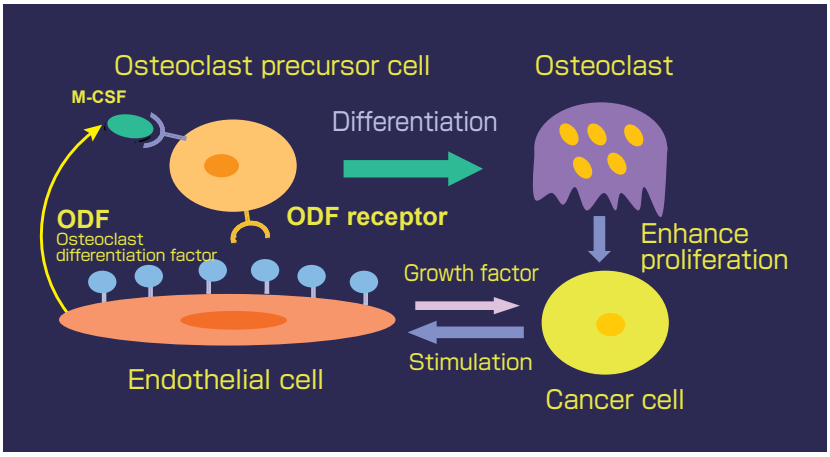


Figure: Hypothesis of the mechanism of osteoclast induction

## Use of nanobiotechnology for the diagnosis of metastasis

We are considering applying nanobiotechnology to develop a technology to diagnose such metastasis to other organs. In recent years various nanocarriers have been developed and are expected to be applied to cancer diagnosis and treatment. We are aiming to develop a nanocarrier that can be used to identify the site of metastasis as well as to localize (target) a drug to primary cancer site, in collaboration and cooperation with researchers in the field of material science.

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[2] Tomoko Okada *et al*.: Osteoclast induction by bone marrow metastatic myeloma cells was mediated by M-CSF production from endothelial cells. Proc. 96th annual meeting of American Association for Cancer Research, 46, 1105 (2005)

[3] Tomoko Okada *et al*.: Establishment of highly metastatic murine breast cancer cell line to bone marrow, UICC World Cancer Congress 2006, 19, 476 (2006)

# Motor Protein Used as a Nanoactuator

Research Institute for Cell Engineering
**Taro Uyeda**

## Nanomotor in biological organisms

Organisms have a group of enzymes called motor proteins. For example, protein filaments called microtubules run through nerve axons, and the motor protein called kinesin transports membrane vesicles filled with neurotransmitters along the microtubules. Each molecule of the motor protein works as a motor, which is thus quite small. In addition, motor proteins have various features not found in artificial motors, such as the potential to form large structures by self-assembly, which are general properties of proteins. Applied research has been conducted all over the world to use those motor proteins as nanoactuators.

## Manipulating motor proteins

To allow kinesin to move *in vitro* after taking it out of the cells, a system with a configuration reversed from the *in vivo* configuration has been used conventionally. In that system, kinesin is adsorbed onto a glass surface on which fluorescence-labeled microtubules move. However, the system does not allow the microtubules to do useful work to the outside because the microtubules move in random direction on the glass surface. We therefore created tracks on the glass surface by lithography as shown in Figure 1, and thereby succeeded in limiting the microtubule movement to one dimension. Moreover, moving almost all microtubules in one direction was realized by adding arrowhead-shaped "rectifier" patterns along the linear tracks. Microtubules moving in one direction along a track are expected to be used as a minute belt conveyor. To that end, various peripheral technologies must be developed, including a technology to control motor activity locally, a technology for external control of the traveling direction of microtubules at the junction of the track, a technology to bind a load to be carried to a moving microtubule and release the load at a destination, and a technology to sustain the movement for an extended period.

At AIST, specialists from various fields have established the Upbringing of Talent in Nanobiotechnology Course, led by Noboru Yumoto, Director, Research Institute for Cell Engineering, with the support of the Special Coordination Funds for Promoting Science and Technology to further technological development while training interdisciplinary personnel.

For example, Nomura and Tatsu (Biomolecular Engineering Research Group, Research Institute for Cell Engineering), who have skills in the technology for a "caged peptide," which is activated by ultraviolet irradiation, identified a peptide that inhibited the motor activity of kinesin reversibly, and have developed a system that stops microtubular movement reversibly by ultraviolet irradiation that "uncages" the peptide (Figure 2).

In addition, Konishi and Kubo (Molecular Neurophysiology Group, Neuroscience Research Institute), experts in protein engineering, have succeeded in the development of a chimeric kinesin molecule which is switched on by calcium ions.

Kato and Shibakami (Lipid Engineering Research Group, Institute for Biological Resources and Functions), who are skilled in organic synthesis, bound cyclodextrin chemically to microtubules. Moreover, they have succeeded in binding and dissociating azobenzene to and from cyclodextrin-conjugated, moving microtubules by reversible photo-modulation of the affinity between cyclodextrin and azobenzene. Taira and Kodaka (Molecular Recognition Research Group, Institute for Biological Resources and Functions) bound oligonucleotides to moving microtubules, and demonstrated that oligonucleotides with complementary sequences can be transported. Because even a single base-pair mismatch prevented this transport, this system may be helpful in the analysis of single nucleotide polymorphisms (SNPs), which will enable tailoring treatment regimens to individual patients. These and other novel technologies should be combined to realize micro-devices and systems in the future.

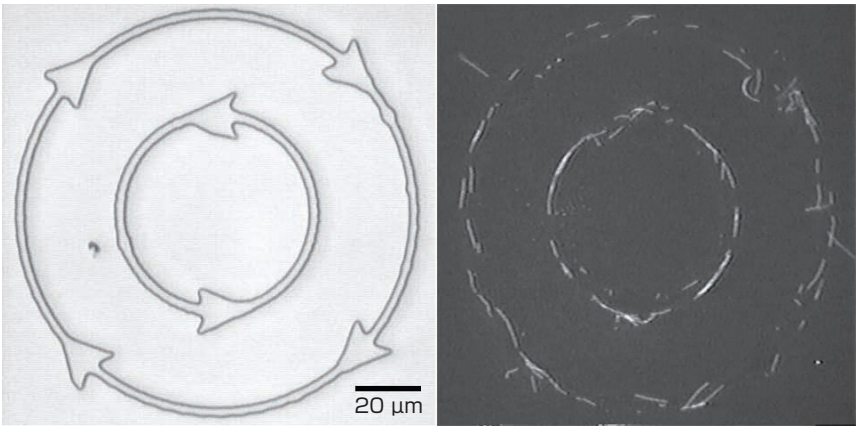


Figure 1: One-dimensional unidirectional movement system of microtubules
The resist applied on a glass surface was processed by lithography to form narrow groove-shaped tracks (transmission microscope image on the left). Selective adsorption of kinesin molecules onto the bottom surface of the track resulted in the successful confinement of microtubular movement within the track (the fluorescence microscope image on the right represents fluorescent microtubules). Unidirectional movement was achieved by adding arrowhead-shaped "rectifier" patterns. (Modified and reproduced from *Biophys. J.* 81:1555-1561 (2001))
Actual circling movement can be seen at http://staff.aist.go.jp/t-uyeda/motility/biomotors/.

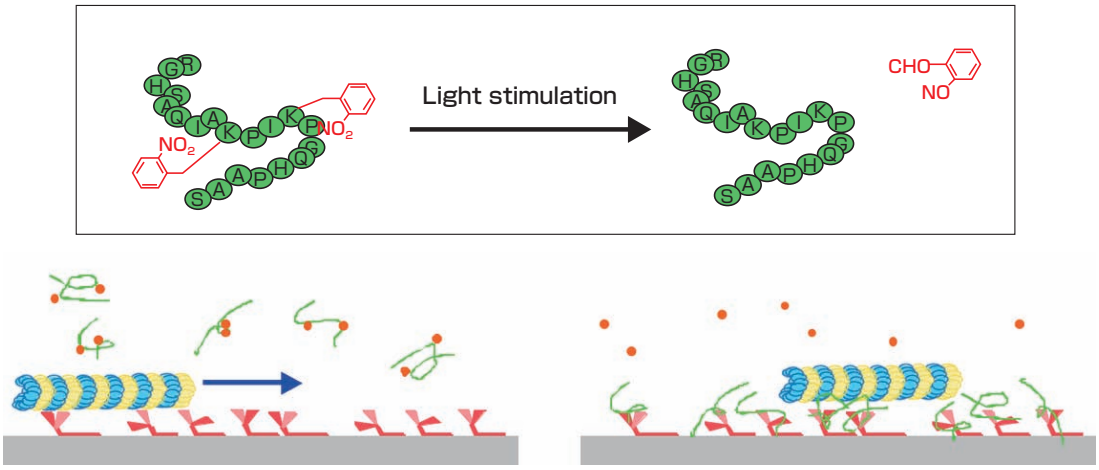


Figure 2: Microtubules can move normally in the presence of caged peptides (lower left). But the movement of microtubules is inhibited (lower right) when the caged peptide turns into a peptide with inhibitory activity (upper) due to light stimulation.
(By courtesy of Dr. Yoshiro Tatsu)

## Use pre-assembled motile structures

Rather than using purified motor proteins as nano-components of micro devices, a more biological approach is to modify motile biological structures and use them as pre-assembled motile units in an artificial environment.

For example, Hiratsuka and Uyeda (Gene Function Research Center), with the cooperation of Miyata (Osaka City University) and Tada (Advanced Semiconductor Research Center), are working at the development to use the gliding bacteria *Mycoplasma mobile*, which moves at high speed (3 μm/s) continuously on a substrate, as a microactuator.

They developed a technology for unidirectional circling movement of *Mycoplasma* cells in a minute (20 μm in diameter) circular track, and bound a microrotor created using the MEMS technology to circling cells, resulting in the creation of a rotary micromotor driven by the bacteria (Figure 3). As we relied on cattle and horses for a long time before man-made vehicles such as cars are available, micro-cattle and horses may play an important role for some time in the field of nanobiotechnology.

(Positions cited are as of the time of publication).


Figure 3: An electron micrograph (scale bar, 5 μm) of a rotary micromotor driven by *Mycoplasma mobile*. (Reproduced from Proc. Natl. Acad. Sci. USA 103: 13618-13623 (2006))
Actual rotational movement can be seen at http://www.pnas.org/cgi/content/full/0604122103/DC1#M1.

# Nanobiotechnology




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