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HIGHLIGHTS

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NAIST was founded in 1991 as a Japanese national university consisting solely of graduate schools in three integrated areas: information science, biological sciences, and materials science. At present, about 1,000 students —19% from overseas— are supervised by roughly 200 NAIST faculty.

With its cutting-edge facilities and a 5 to 1 student-to-faculty ratio, NAIST's world-leading education and research are a direct result of its rich, global environment and supportive infrastructure. Moreover, the outstanding achievements of NAIST's faculty and students are shared worldwide through patents, licenses, spin-off companies, and active exchange with overseas partners.

NAIST has quickly established itself as a world-class education and research center where young science and technology researchers become tomorrow's global leaders.

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NAIST Research Highlights

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Aims & Scope

NAIST Research Highlights showcases the most promising and important research achievements of each laboratory at NAIST and presents their current research and core technologies to the public. The publication distills highly technical research papers into short, easy-to-understand articles that appeal to a global audience of both specialists and non-specialists. *NAIST Research Highlights* aims to inform readers of the latest developments in NAIST's pioneering research, and to stimulate new and existing international collaborations. Digital versions of each issue can be accessed online at: http://ipw.naist.jp/iri/naura/en/highlight.html

NAURA

NAURA (NAIST URA team) publishes *NAIST Research Highlights* under the auspices of the "Program for Promoting the Enhancement of Research Universities", which is funded by Japan's Ministry of Education, Culture, Sports, Science and Technology (MEXT). Through this program, NAIST further supports its cutting-edge research while expanding into new interdisciplinary fields in science and technology toward becoming a globally recognized education and research center.

Innovative Research and Education Programs

NAIST constantly strives to renew its research and education programs toward producing science and technology researchers prepared to meet the demands facing tomorrow's global scientific community. These programs are regularly awarded external funding for their wide-ranging benefits.

Program for Promoting the Enhancement of Research Universities (2013-2023)

Japanese Ministry of Education, Culture, Sports, Science and Technology (MEXT) launched the Program for Promoting the Enhancement of Research Universities in October 2013, which is a new type of research funding in Japan that aims to enhance research capabilities by utilizing University Research Administrators (URA) who implement intensive reforms to strengthen the research environment at their respective universities. NAIST is one of 22 universities/ research institutions selected to receive support through this program.

NAIST continues to conduct frontier-opening research while expanding into new interdisciplinary fields in science and technology. With the establishment of a university-wide strategic research infrastructure, NAIST endeavors to leverage its abundant resources to attain the new research materials and facilities necessary for next-generation research, to disseminate its achievements and human resources around the globe, and to further expand its global research and education network in order to contribute to the overall advancement of science and technology. Projects being supported through this program include (1) the Creating New Research Streams Program which creates new research domains promising a high global profile, (2) the Sustainable Development of Research Capabilities Program which enhances NAIST's world-class research capabilities, and (3) the Joint International Research Program which raises the global visibility and standing of NAIST's research capabilities.

Formulating strategies and plans based on objective analysis data

Supporting the strategic acquisition of external competitive research funds

Enhancing the international collaborative research network

Reforming the research system to enhance NAIST's research capabilities

Top Global University Project (2014-2024)

In October 2014, NAIST was one of 37 universities selected to another prestigious MEXT initiative, the Top Global University Project. For a period of ten years, MEXT will support outstanding universities in their efforts to reform institutional governance and collaborate with top universities worldwide in order to strengthen international competitiveness.

Through the Top Global University Project, NAIST has committed to enhancing its international graduate courses by (1) including a joint degree scheme, (2) developing a new model for graduate education based on top-notch research, (3) reforming institutional governance and strategic agility, (4) creating a campus environment that supports trans-disciplinary education and cultural diversity, and (5) reorganizing its three graduate schools into a single entity toward establishing new, flexible research groups.

NAIST Graduate Schools

NAIST graduate education and research programs aim to be at the world-leading edge of research in science and technology, specifically in the core fields of information, biological and materials sciences, and actively engages in cross-disciplinary studies to explore and seek solutions in the most challenging areas.

Information Science

The core focus of the Graduate School of Information Science is on communication between society, people and computers, as well as the computing infrastructure for the Big-data era that will support sustainable growth and societal development well into the future. Our world-class faculty, staff, and curriculum contribute to the cultivation of researchers and engineers who will be leaders in tomorrow's universally connected society.

Computer Science	● Computing Architecture ● Dependable System ● Ubiquitous Computing Systems ● Mobile Computing	● Software Engineering ● Software Design and Analysis ● Internet Engineering ● Internet Architecture and Systems
Media Informatics	● Computational Linguistics ● Augmented Human Communication ● Network Systems ● Vision and Media Computing	● Interactive Media Design ● Optical Media Interface ● Ambient Intelligence
Applied Informatics	● Robotics ● Intelligent System Control ● Large-Scale Systems Management ● Mathematical Informatics	● Imaging-based Computational Biomedicine ● Computational Systems Biology

Biological Sciences

The final goal of the research in the Graduate School of Biological Sciences is to uncover various structures and functions of microorganisms, plants and animals at the molecular and cellular levels, and to clarify principles of the basic phenomena of life and biological diversity. Based on highly advanced basic research, we provide research and development that benefits human well-being, through which we train researchers to play active roles in the global community.

Plant Biology	● Intercellular Communications ● Plant Cell Function ● Plant Developmental Signaling ● Plant Metabolic Regulation ● Plant Growth Regulation	● Plant Morphological Dynamics ● Plant Stem Cell Regulation and Floral Patterning ● Plant Immunity ● Plant Developmental Biology
Biomedical Science	● Molecular Signal Transduction ● Functional Neuroscience ● Gene Function in Animals ● Functional Genomics and Medicine ● Molecular and Cell Genetics	● Tumor Cell Biology ● Molecular Immunobiology ● Molecular Medicine and Cell Biology ● Developmental Biomedical Science
Systems Biology	● Microbial Molecular Genetics ● Systems Microbiology ● Cell Signaling ● Applied Stress Microbiology	● Structural Biology ● Membrane Molecular Biology ● Gene Regulation Research ● Systems Neurobiology and Medicine

Materials Science

The main research area in the Graduate School of Material Science is "Photonic Nanoscience" which seeks to understand the mechanisms of materials on the electron, atomic, and molecular levels from the perspective of "seeing with light", "creating with light", and "transmitting with light". Researchers aim to create new materials, structures, and functions. We systematically educate students to become excellent leaders in research and development fields in the global society.

Physics	● Quantum Materials Science ● Surface and Materials Science ● Nanostructure Magnetism	● Laser Nano-Manipulation Science ● Theoretical Condensed Matter Physics
Information	● Photonic Device Science ● Information Device Science	● Microelectronic Device Science ● Sensing Device
Chemistry	● Advanced Polymer Science ● Synthetic Organic Chemistry	● Photonic Molecular Science ● Photofunctional Organic Chemistry
Biology	● Biomimetic Materials Science ● Complex Molecular Systems	● Supramolecular Science ● Biocompatible Materials Science
	● Organic Electronics ● Green Nanosystem	● Nanomaterials and Polymer Chemistry

As of July 1, 2015

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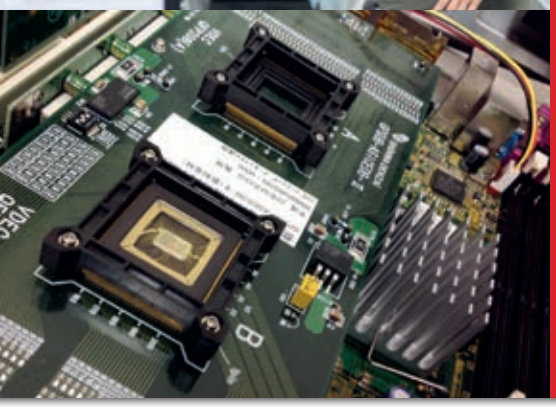

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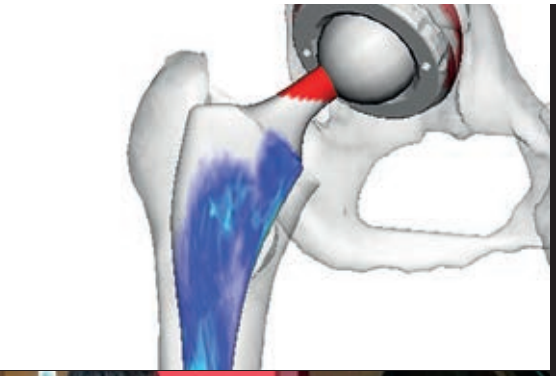
Information Science



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Research Highlights

Nara Institute of Science and Technology | Computing Architecture Laboratory www.naist.jp/en

Computer engineering

More tolerance, less waste

Microchip fault tolerance that consumes one-third less power may now be possible with new chip design

Modern microprocessors contain billions of transistors that have small capacitances (ability to store electrical charge) and low supply voltages. These properties make them prone to failure since they are vulnerable to background noise, cosmic radiation and environmental vibrations. Engineers can improve the reliability of a microprocessor by incorporating extra copies (or redundancies) of critical components in the system, so that when one component fails, another takes over.

However, conventional redundancy architectures, such as the triple modular redundancy (TMR), have all their components — including redundancies — hard-wired into the circuit. Consequently, the lifespan of devices is limited by their least reliable unit.

Now, Jun Yao, Yasuhiko Nakashima and colleagues at NAIST have proposed a low-cost redundancy architecture called Explicit Redundancy Linear Array (EReLA), which offers greater flexibility and better automation than conventional architectures[1]. Processors based on this novel design offer the same functionality, consume less power and have ten times longer lifetimes than conventional processors.

TMR has been the preferred architecture because it comes with configurable isolation (between input, output and power) and hot swap technologies (which allow systems to be switched without shutting down); the former enables defective components, or failures, to be detected and isolated, while the latter ensures smooth running of the system when a fault occurs.

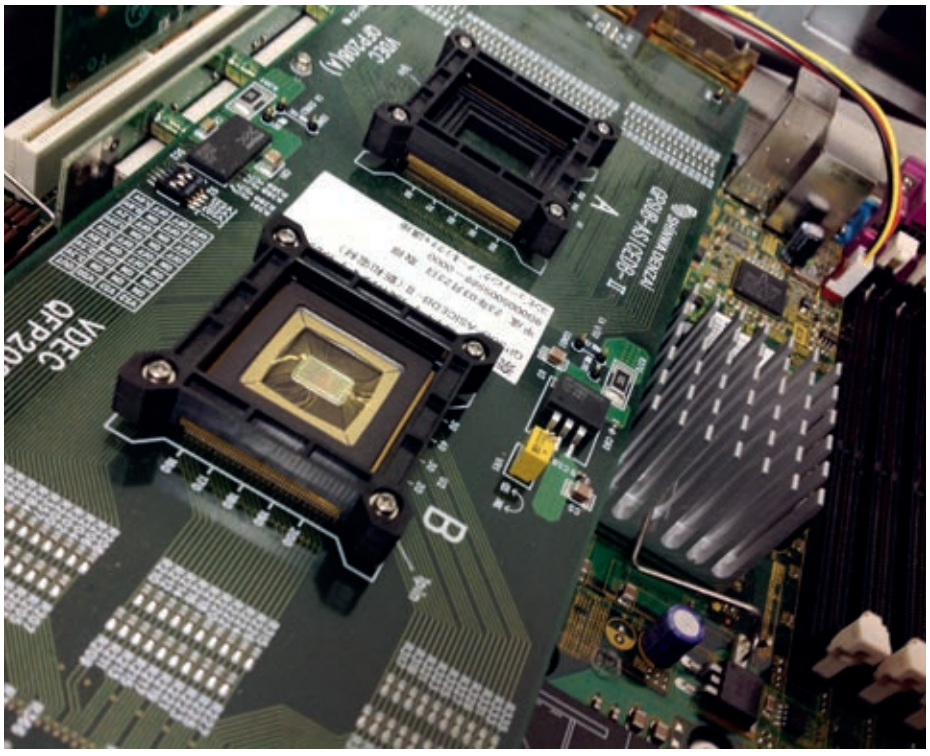
> **"The EReLA architecture provides a cost-effective way to maintain the growth in computer performance for the future."**

The researchers have incorporated two novel features in the design of EReLA: a fully automated method for locating failures in the system and a self-tuning mechanism that simplifies the hardware needed for diagnosis. To locate permanent failures in TMR, triple redundancies and voter logic are used: three systems perform a function and the result is processed by a majority-voting system to produce a single output — costing both time and money. The newly proposed EReLA does not require either.

"The EReLA can detect erroneous units by itself," says Nakashima. "It comes with an 'approximate computing mode' that can tolerate errors in non-important computations."

The researchers have built a prototype chip based on EReLA (see figure) and showed that its fault tolerance — a measure of how well the system performs in a failure — is similar to those based on TMR. In addition, the power consumption is reduced by a third due to the simpler hardware. These findings are significant for miniaturizing devices and developing low-cost, low-power, highly reliable computers.

"The mean time between failures of high-end computers is becoming shorter and shorter," says Nakashima. "The EReLA architecture provides a cost-effective way to maintain the growth in computer performance for the future."



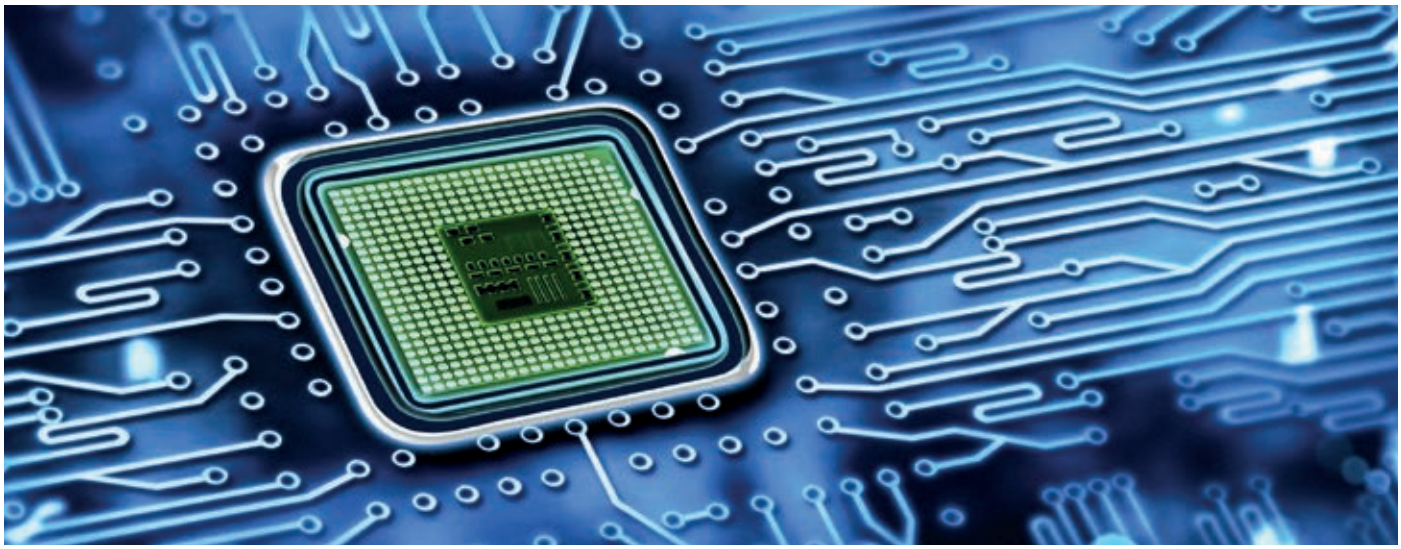
The new EReLA architecture offers a cost-effective, lower-energy-consumption method to boost computer performance.

Reference

1. Yao, J., Nakashima, Y., Saito, M., Hazama, Y. & Yamanaka, R. A flexible, self-tuning, fault-tolerant functional unit array processor. *IEEE Micro* **34,** 54–63 (2014).

More information about the group's research can be found at the Computing Architecture Laboratory webpage:
http://arch.naist.jp/index-e.html

Nara Institute of Science and Technology | **Dependable System Laboratory** www.naist.jp/en



Testing customizable computer circuits with unused memory blocks can improve fault detection and lower the strain on system resources.

Microchip design

Better built-in fault detection

A more effective way of testing integrated circuits can help manufacturers of digital devices improve reliability

A new 'built-in self-test' (BIST) computer architecture that offers better fault detection for custom-built circuits while using fewer system resources has been developed by researchers at Japan's NAIST[1]. The architecture enhances the safety and reliability of highly complex, made-to-order devices, such as medical electronics or optical transmission networks.

Field-programmable gate arrays (FPGAs) are a modern type of computer chip based around a series of programmable components known as 'logic blocks'. Device designers configure and wire the FPGA logic blocks to meet their needs, instead of ordering traditional application-specific integrated circuits (ASICs) from chip manufacturers. This flexibility, which can cut development costs and shorten times-to-delivery, has currently given FPGAs a US$4 billion slice of the lucrative semiconductor market.

Monitoring FPGAs for defects, such as transistor ageing, however, is problematic. ASIC-type chips have built-in tests, known as scan-based BISTs, which send pseudo-random data patterns through a series of 'flip-flop' circuits that can switch between one of two bi-stable states. By comparing the observed data signature with the expected results, this method can identify performance degradations or manufacturing defects. Unfortunately, FPGAs do not have built-in 'scan chains' that shift in and shift out the testing data from the primary microchip design. Instead, redundancies have to be introduced that use the chips' logic elements — a situation that can tax up to 50 per cent of a system's resources.

Tomokazu Yoneda, Michiko Inoue and colleagues at NAIST's Dependable System Laboratory discovered that self-testing with on-chip memories could lessen the load on FPGA logic elements. They configured unused memory blocks to run 'shift register' circuits that use clock signals to shift in or shift out data. Then, they carefully designed a way to insert the shift registers as 'test points' into computer architectures to maximise FPGA reliability.

When the team tested their concept with experimental circuits, they found a remarkable difference in delay test quality — a 5 to 6 per cent boost in fault detection compared to previous methods. "Test point insertion is a key factor in improved fault coverage," explains Yoneda. "Our method can find more resources and insert more test points, since it utilizes unused memory blocks."

Because memory blocks are inherent components of FPGA users, the new self-test design can be easily integrated into current chip design flows and produce significant savings. "Current dual- and triple-mode redundancy solutions for products that require high field reliability are not cost effective," says Yoneda. "This work is promising since it can provide high reliability without any redundancy."

Reference

1. Ito, K., Yoneda, T., Yamato, Y., Hatayama, K. & Inoue, M. Memory block based scan-BIST architecture for application-dependent FPGA testing. *FPGA '14 Proceedings of the 2014 ACM/SIGDA International Symposium on Field-programmable Gate Arrays* 85–88 (2014).

More information about the group's research can be found at the Dependable System Laboratory webpage:
http://dslab.naist.jp/index-e.html

Nara Institute of Science and Technology | **Ubiquitous Computing Systems Laboratory** www.naist.jp/en

Ubiquitous computing

Tailoring workout intensity

Smartphone technology can account for physical differences between individuals during walking to optimize exercise intensity

Even as fitness programs such as CrossFit and Zumba surge in popularity, walking remains one of the most reliable and effective forms of exercise for burning calories and building muscle mass. Walking is free, requires no specialized equipment, and can be done in small time increments. But achieving optimal heart rate is necessary for getting the most out of a walking workout and heart rate targets vary dramatically depending on a person's physical fitness, age, health, and other factors.

Now, researchers at the Nara Institute of Science and Technology have shown that existing smartphone technology has the capacity to account for these individual differences, called 'physical load', to allow walkers to achieve optimal heart rate. Keiichi Yasumoto and colleagues at the NAIST Graduate School of Information Science have proposed a method for estimating physical load and minute-by-minute changes during walking routes, taking into account the individual's physical condition and exertion level.

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NAIST research helps walkers achieve optimal heart rates using smartphone technology.

Walking that is too strenuous can result in heart problems or joint injuries, particularly in the elderly and in people with certain conditions; meanwhile, insufficient exertion yields little benefit. Yet, achieving the optimum exercise intensity is not clear-cut or unambiguous without specialised equipment, such as heart rate monitors. But cost and inconvenience of such equipment can keep some people from taking advantage of walking as an exercise regimen.

To get a more reliable measure of heart rate, the researchers constructed heart rate prediction models that rely on walking data collected by smartphones and heart rate data obtained by a monitor.

Existing smartphone applications are able to measure heart rate, but they rely on capturing finger or face images, a system that does not work well during walking. To get a more reliable measure of heart rate, the researchers constructed heart rate prediction models that rely on walking data collected by smartphones and heart rate data obtained by a monitor.

To account for fluctuations in heart rate due to changing exercise intensity during a walk, the NAIST team developed a novel technique to estimate oxygen uptake under varying walking speeds and gradients. Their technique also takes into account the physical condition of individuals. The team tested their method in 18 subjects and five walking routes, and found that they were able to predict heart rate accurately within a mean error of seven heartbeats per minute.

Reference

1. Sumida, M., Mizumoto, T., Yasumoto, K. Estimating heart rate variation during walking with smartphone. UbiComp 2013, 8-12 September 2013, Zurich, Switzerland.

More information about the group's research can be found at the Ubiquitous Computing Systems Laboratory webpage:
http://ubi-lab.naist.jp/

Nara Institute of Science and Technology | **Mobile Computing Laboratory** www.naist.jp/en

Transit systems

Swirl protocol cuts congestion

A system that directs traffic to circular routes in city centres reduces journey times

Poorly timed traffic lights are a cause of congestion in cities — wasting drivers' time, increasing air pollution and fuel consumption. A protocol, known as GreenWave, exists for controlling lights to cut congestion, but its effectiveness in city centres is poor. Now, researchers at the Nara Institute of Science and Technology (NAIST) have found a way to use GreenWave that, in simulations, significantly reduces journey times[1].

> **"The aim of GreenSwirl is to pump the traffic out of the city centre into the circular GreenWaves that surround the city."**

Most traffic lights work independently of those immediately before and after them, which means cars get stopped at red signals repeatedly. The GreenWave system makes lights on a major road turn green in a timed sequence, allowing a continued flow of cars across several lights without having to stop. But trials of the protocol in several cities worldwide have been disappointing — traffic crossings become congested, and queues of vehicles build up at intersections, waiting to turn.

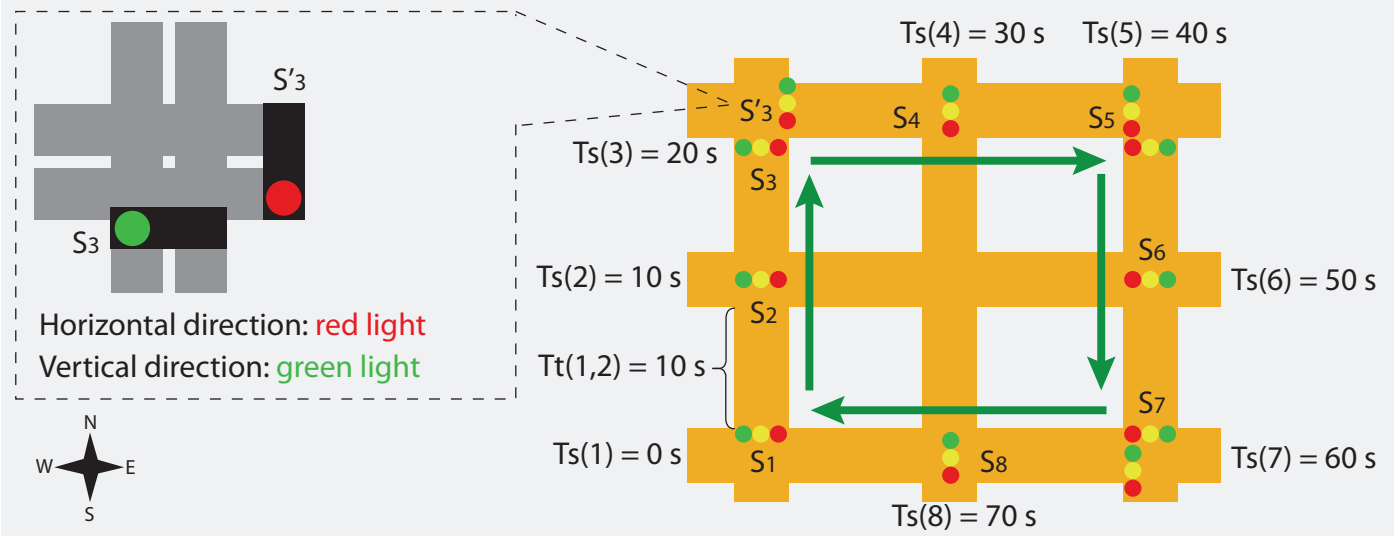
"GreenWave is usually implemented in the main streets going through the city centre. But the amount of traffic soon exceeds the capacity of the street, and any benefit is lost," says Naoki Shibata of NAIST. He and his colleagues had the idea of applying GreenWave, not to straight routes, but to circles surrounding the city centre. They call this approach 'GreenSwirl'.

"The aim of GreenSwirl is to pump the traffic out of the city centre into the circular GreenWaves that surround the city," says Shibata. Introducing GreenSwirl in isolation will not work, however, he cautions. "People will be reluctant to go through the swirls because they will see them as a detour." The answer is to jointly implement a navigation system, 'GreenDrive', which recommends the fastest route through the city to drivers with GPS systems in their vehicles. The team ran simulations, taking Manhattan in New York as an example. Using GreenSwirl alone reduced average travel time by 10–20 per cent; moreover, adding the GreenDrive navigation system reduced travel time by as much as 60 per cent in some scenarios.

Shibata thinks that, although drivers may initially be reluctant to divert to the swirls, they will soon learn that they will get to their destination more quickly. "Our objective is to smooth out the traffic over the city. The capacity of the whole network is maximized when the amount of traffic on each road segment is slightly less than its capacity. The best way for a driver to go will then be the route the navigation device suggests."

Reference

1. Xu, J., Sun, W., Shibata, N. & Ito, M. GreenSwirl: combining traffic signal control and route guidance for reducing traffic congestion. *Proceedings of the IEEE Vehicular Networking Conference (IEEE VNC 2014)*, 179–186, 2014.



Poorly timed traffic lights are a cause of congestion in city centres, but a system that directs traffic to circular routes can drastically reduce journey times.

More information about the group's research can be found at the Mobile Computing Laboratory webpage:
http://ito-lab.naist.jp/mediawiki/index.php/Main_Page/en

Nara Institute of Science and Technology | **Software Engineering Laboratory** www.naist.jp/en

NAIST researchers have found a new way to increase the cost-effectiveness of software testing by incorporating fault prediction.

Software engineering

Acceptance testing accelerated

Simulation approach helps optimize the cost-effectiveness of software testing

Quality assurance is crucial in software development: as programs become more complicated and increasingly integrated, it is essential to identify modules where faults are likely to occur. One way of doing this is 'acceptance testing', where developers check the software performs in accordance with the needs of the end user.

> **"Assessing the cost-effectiveness of this approach was, of course, a priority for them — it was then that I got the idea to incorporate a simulation approach."**

Akito Monden and colleagues at NAIST have collaborated with industry to develop a simulation approach to assess the cost-effectiveness of fault prediction in acceptance testing. The result is both academically stimulating and provides tangible benefits to software developers[1]. The idea for this work arose from an initial discussion between Monden and researchers from the Nippon Telegraph and Telephone West (NTT West) corporation about potential areas of collaboration. "I introduced them to several of our ongoing research topics, when they expressed interest in using the fault prediction technique in acceptance testing," Monden recalls. "Assessing the cost-effectiveness of this approach was, of course, a priority for them — it was then that I got the idea to incorporate a simulation approach."

In software engineering research, it was unprecedented to apply a simulation approach to optimize the cost-effectiveness of software testing using fault prediction results. Monden notes that the overwhelming majority of fault prediction research is focused on improving the fault prediction *accuracy*. While this is academically challenging, it is not a priority for industry practitioners. In contrast, the NAIST research team's work "exactly targets industry as it focuses on the reduction of 'cost' — or effort — of acceptance testing, which is the main interest of practitioners."

The simulation assessed resource allocation strategies for acceptance testing based on fault prediction results. Using data from NTT West as a test case, they found that the best strategy was to let the 'test effort' (or cost) be proportional to the number of expected faults in a module, multiplied by the common logarithm of the module size. By using this strategy, they found that the test effort could be reduced by an impressive 25 per cent.

This work is a success story for academic-industry collaboration, proving that addressing industrial priorities and probing areas of academic interest are not mutually exclusive pursuits. Monden explains that the collaboration with NTT West "acted as a bridge to transfer research findings, such as fault predictions, to address industry priorities, such as reduction of testing efforts." The team's next step is to test the technique on larger software systems.

Reference

1. Monden, A., Hayashi, T., Shinoda, S., Shirai, K., Yoshida, J., & Barker, M. Assessing the cost effectiveness of fault prediction in acceptance testing. *IEEE Transactions on Software Engineering*, **39**, 1345-1357 (2013).

More information about the group's research can be found at the Software Engineering webpage:
http://se-naist.jp/

Nara Institute of Science and Technology | **Software Design and Analysis Laboratory** www.naist.jp/en

Two new tools, Historage and Kataribe, are set to improve the productivity of many software companies.

Software design

Method histories made easy

Two new tools make it easier for developers to browse method histories and extract important information from source codes

The code repository is one of the most valuable tools in software development because it contains everything there is to know about the source code — including, but not limited to, bug reports, developer messages and file histories. Despite its usefulness, however, retrieving method histories (also known as fine-grained histories) from the data-rich code repository has never been easy.

To overcome this problem, Hajimu Iida and co-workers at NAIST have developed 'Historage', a code repository specifically designed to store fine-grained histories; and 'Kataribe', a hosting service for Historage repositories[1]. Together, these technologies make it easier for developers to browse method histories on the web and extract important information from source codes.

Iida and co-workers built Historage from Git, an open source distributed revision control system designed to handle everything from small to very large projects with speed and efficiency. Equipped with a superb branching system, Git can implement an almost endless number of workflows with relative ease.

The researchers developed a tool for converting a Git into a Historage repository. Historage stores method histories in much the same way Git stores file histories. For this reason, conventional mining software designed to extract file histories from Git repositories are equally applicable to extracting method histories from Historage repositories.

However, Historage is not so useful if developers cannot copy the code repository and have easy access to method histories. To tackle these issues, the researchers built Kataribe, a hosting service for Historage repositories that enables developers to browse method histories on the web and clone Historage repositories.

"We wanted Kataribe to be a developer-friendly web service," says Iida. "At the end, we decided to extend the existing Git hosting service (GitLab) to implement features of Kataribe, and that implementation had been a great challenge."

Iida and co-workers at NAIST's Graduate School of Information Science praised the institute for providing a cloud platform that facilitated the development of Kataribe. They could easily create a testing server using the computational resource without having to go through formal procedures. Several researchers have already used Kataribe to successfully reconstruct method histories.

The team plans to further the capabilities of Kataribe by expanding its dataset, web services and functionalities. In fact, they have already implemented a visualization feature for displaying the semantics of changes in the commit snapshot of a Historage repository. The innovative technologies are set to promote the reuse of source code and improve the productivity of many software companies.

Reference

1. Fujiwara, K, Hata, H., Makihara, E., Fujihara, Y., Nakayama, N. *et al.* Kataribe: A hosting service of Historage repositories. *Proceedings of the 11th Working Conference on Mining Software Repositories* 380–383 (2014).

More information about the group's research can be found at the Software Design and Analysis Laboratory webpage:
http://isw3.naist.jp/Contents/Research/cs-06-en.html

Nara Institute of Science and Technology | **Internet Engineering Laboratory** www.naist.jp/en

Cloud computing offers many benefits, but it can comprise security.

Cloud computing

Security vulnerabilities detected

A security analysis highlights the vulnerabilities of popular cloud management software, OpenStack

An analysis of the most widely used open-source cloud management software, OpenStack, by NAIST researchers confirm concerns about its security. In particular, it exposes OpenStack's susceptibility to security problems propagating between different parts (or 'trees') of its system[1].

Cloud computing offers individuals and companies many benefits, including convenient access to data and the ability for team members in different locations to simultaneously work on the same projects. But accompanying its rapid rise in popularity are concerns regarding security. In particular, software that provides free tools for constructing and managing cloud computing platforms for both public and private clouds — known as open-source cloud management software — has come under scrutiny.

The most popular open-source cloud management software platform is OpenStack. In addition to having a reputation for being easy to use, it is backed by some of the biggest names in information technology, including Dell, NEC, Hewlett-Packard and IBM. Furthermore, it is supported by an enthusiastic global community of volunteers. However, its security vulnerabilities have scored highly on the National Vulnerability Database, a publicly accessible database for computer-related vulnerabilities managed by the U.S. government.

To evaluate these concerns, Takeshi Okuda and colleagues at NAIST's Internet Engineering Laboratory performed a security analysis of OpenStack. They did these by performing a fault tree analysis, to evaluate the vulnerabilities of the cloud management software. A fault tree is a helpful tool for quantitatively analysing a system, as it generates a graphical representation of an undesirable event in a system based on Boolean logic.

Using this approach, the researchers were able to generate high-level vulnerability trees, which can be used by organizations wanting to quantify their own OpenStack cloud infrastructure. They found that the high degree of interconnectedness of OpenStack's architecture means that security problems in one tree can propagate to other trees.

However, the team was not able to evaluate OpenStack's security as completely as they had hoped, because the currently used naming system for vulnerabilities fails to account for subcomponents of main components.

The results were not a complete surprise to the researchers as they had "expected to find some sort of vulnerability propagation between the different components," explains Fall Doudou, a doctoral student from Senegal. "But the fact that the security quantification could not be more accurate came as a surprise."

The researchers are now planning to come up with a new naming system that takes into account all the details of the components of OpenStack while conforming with the current standards in the community.

Reference

1. Fall, D., Okuda, T., Kadobayashi, Y. & Yamaguchi, S. Towards a vulnerability tree security evaluation of OpenStack's logical architecture. *Lecture Notes in Computer Science* **8564,** 127–142 (2014).

More information about the group's research can be found at the Internet Engineering Laboratory webpage:
http://isw3.naist.jp/Contents/Research/cs-07-en.html

Nara Institute of Science and Technology | **Internet Architecture and Systems Laboratory** www.naist.jp/en

Networking

Efficient data sorting for wired cars

A more efficient system for data delivery should improve the performance of mobile apps for 'wired' vehicles

We are rapidly moving towards a world in which cars are continuously wired into mobile services that help drivers avoid traffic, accidents and construction to arrive — and park — at their destination. To facilitate these developments, researchers at NAIST have devised a system that could make it easier for such vehicular communication systems to obtain their data without overly burdening wireless networks[1].

In order to function, apps based on vehicular communication systems draw on traffic information and data transmitted from connected cars, pedestrians and other sources. Given that only a tiny fraction of this data will be of value to any user at a given time, an efficient system needs to act as a matchmaker — selectively delivering geographically relevant information from across the entire network. Atsuo Inomata, Kazutoshi Fujikawa and colleagues at NAIST have proposed a solution that could streamline this process and make it practical to bring vehicular communication systems and resources together on a nationwide scale.

Their approach is based on what is known as a publish–subscribe model. In this system, data-generating 'publishers' indiscriminately release resources into the network with classifying information that allows 'subscribers' (in this case, apps running on vehicular communication systems) to recognize whether those resources are relevant. For example, one vehicular communication system might report a traffic jam bringing cars to a standstill, but this will only be of interest to other drivers using a navigation app that might direct them onto the same highway.

Regulating the amount of information circulating in the network is a critical factor, however. "Larger scope is preferable for resource discovery, [but] may cause scalability issues," the authors write, while "smaller scope may fail to discover potential publishers."

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An efficient vehicular communication system that links mobile applications with useful navigational information can connect vehicles across a country as dense as Japan.

The team avoid swamping the system by limiting data publication to instances when a given vehicle changes its speed or direction, rather than continuously broadcasting. Communications between publishers and subscribers will depend on the interaction between mobile routers and devices with regional servers through what is known as the GeoNetworking protocol, a framework that makes it possible to assign a target address for data transmission based on geographic and topographic information.

In addition to demonstrating the theoretical feasibility of their system, the researchers note that their models demonstrate that this approach could be affordably expanded to efficiently relay data between large numbers of vehicles — "even in the highly-populated scenario in which all vehicles in Japan support the proposed system." As a next step, the researchers will be moving on to real-world implementation and testing.

Reference

1. Noguchi, S., Matsuura, S., Inomata, A., Fujikawa, K. & Sunahara, H. Wide-area publish/subscribe mobile resource discovery based on IPv6 GeoNetworking. *IEICE Transactions on Communications* **7**, 1706–1715 (2013).

More information about the group's research can be found at the Internet Architecture and Systems Laboratory webpage:
http://isw3.naist.jp/Contents/Research/cs-08-en.html

Nara Institute of Science and Technology | **Computational Linguistics Laboratory** www.naist.jp/en

Machine translation

Better bilingual dictionaries

By combining two computational methods, NAIST researchers have enhanced the accuracy of machine-readable bilingual dictionaries

Machine-readable bilingual dictionaries aid computer translation, but existing frameworks are plagued with inaccuracies. To remedy this situation, researchers at Japan's NAIST have devised a method for generating more accurate bilingual dictionaries for machine translation; this method combines two existing computational techniques — topic modelling and word alignment[1].

"Bilingual dictionaries that focus on specific domains, for example medicine or tourism, are useful for many applications," explains Kevin Duh, who worked with NAIST colleagues Yuji Matsumoto and Xiaodong Liu on the project. "Existing topic models automatically group words into specific domains, so we included this as an integral component of our method. We are the first to use topic models for this purpose."

A topic model is a mathematical framework that predicts the proportion of words related to different topics in a particular text. It then generates groups of words that are likely to correspond to individual topic areas. The team applied topic models to English and Japanese 'real-world' documents (or 'corpora') written on the same subject, taken from the online encyclopaedia Wikipedia. This gave them lists of English and Japanese words that correspond to individual topics. Although these lists have the advantage of being easier to deal with than entire original texts, topic modelling did not help the researchers correctly identify which English words translated into which Japanese words in each list.

"Let's say each topic list contains about 500 English words and 500 Japanese words," says Duh. "There are then 500 × 500 translation possibilities in each list. Our insight came when we realized that this problem is similar to the word alignment problem in the field of statistical machine translation."

Word alignment is a computational technique used for linking words that are close translations of one another within texts. Matsumoto's team carried out word alignment after running topic models, enhancing the chances of accurate bilingual translation. In fact, they discovered that bilingual dictionary extraction became more accurate when they incorporated more subject-specific documents from multiple languages. Additional language data meant that each topic model became more and more specific, allowing word alignment to 'zone in' on more precise translations relevant to the original texts.

"Including multiple word phrases, such as compound nouns, is an important next step because they are so common in languages," explains Duh. "This is a surprisingly difficult task, because it dramatically increases both the computation time and the number of translation candidates. However, it will enhance the usefulness of our method."

Reference

1. Liu, X., Duh, K. & Matsumoto, Y. Multilingual topic models for bilingual dictionary extraction. *ACM Transactions on Asian Language Information Processing* **9**, 39 2014.

Multilingual Wikipedia entries on the same subject were used to create more accurate bilingual dictionaries by combining existing topic model and word alignment techniques.

More information about the group's research can be found at Yuji Matsumoto's webpage:
http://www.naist.jp/en/about_naist/offices/administration_bureau/yuji_matsumoto/index.html

Nara Institute of Science and Technology | Augmented Human Communication Laboratory www.naist.jp/en

Software

Segmentation makes translation twice as fast

Segmentation algorithms show promise in boosting both the speed and accuracy of simultaneous speech translation

An automated learning system developed by researchers in Japan that identifies the most accurate way to break down a sentence for simultaneous interpretation has cut waiting times in half compared to conventional methods[1]. The system can be used to improve the performance of speech translation software, including mobile apps.

> **"We basically let the computers come up with their own ideas."**

Traditional systems translate speech one sentence at a time, which means listeners have to wait until the end of a sentence before receiving the translation. To speed this up, researchers have proposed dividing sentences into smaller units, but finding appropriate dividing points has been tricky. Most suggestions are based on human intuition, such as detecting a pause in rhythm and intonation, or predicting the location of commas. However, these markers can be confusing for automated systems. A quickening of speech or a dramatic pause for emphasis, for example, could reduce translation speed and accuracy.

Now, a team of researchers led by Graham Neubig, Yusuke Oda and Satoshi Nakamura at NAIST has developed a method that does not rely on heuristics. "In a way, the method was born out of frustration, since it was proving harder and harder to improve on existing methods," says Neubig. "We basically let the computers come up with their own ideas."

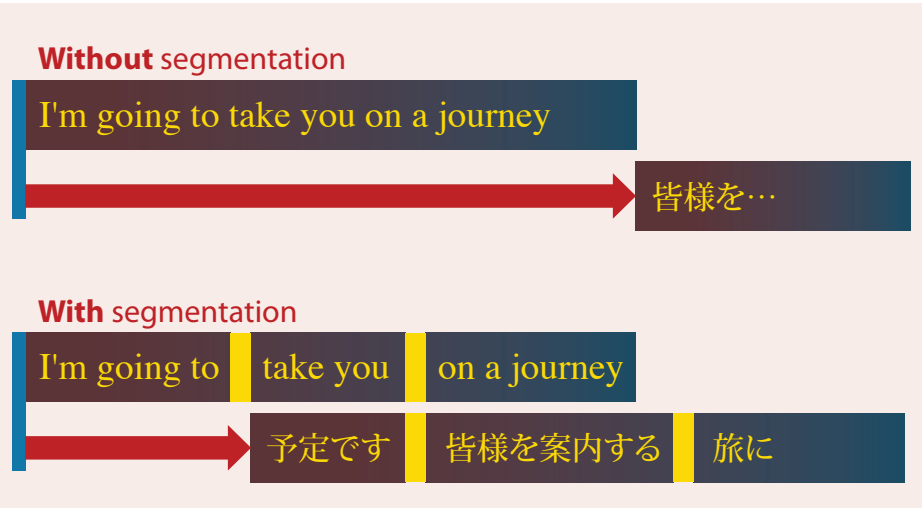
The researchers combined a greedy algorithm, a grouping strategy and dynamic programming to develop a system for computing the accuracy of different segmentation approaches and selecting the optimal translation breakdown. They then experimentally assessed their computational system using English-to-German and English-to-Japanese speech translation data.

The resulting translation speeds were two to three times faster than those of sentence-based methods, while maintaining the same accuracy. In some cases, the accuracy was slightly improved — a surprising result since segmentation removes the context and flexibility afforded by sentence-scale translation. "Current machine translation systems are occasionally confused by longer sentences," explains Neubig.

> **"Human simultaneous interpreters are remarkably clever about guessing what the speaker is going to say in the future, and using these estimates to help improve the simultaneity of their interpretation."**

His team is currently working on improving the machine-learning system by incorporating prediction techniques often employed by their real-life counterparts. "Human simultaneous interpreters are remarkably clever about guessing what the speaker is going to say in the future, and using these estimates to help improve the simultaneity of their interpretation."

The research is well timed given the proliferation of real-time translation applications, such as the preview version of Skype Translator, and the introduction of automated systems in universities. Neubig hopes to apply the new technology to various situations. "Being able to translate faster, while maintaining a high level of accuracy, is essential to achieving smoother conversations or more complete understanding of lecture contents," he says.



Segmenting sentences into smaller units can increase the speed of simultaneous translation technology.

Reference

1. Oda, Y., Neubig, G., Sakti, S., Toda, T. & Nakamura, S. Optimizing segmentation strategies for simultaneous speech translation. *Proceedings of the 52nd Annual Meeting of the Association for Computational Linguistics (Short Papers)* **2,** 551–556 (2014).

More information about the group's research can be found at the Augmented Human Communication Laboratory webpage:
http://ahclab.naist.jp/index_en.html

Nara Institute of Science and Technology | **Network Systems Laboratory** www.naist.jp/en

Wireless data

Dual strategy to boost underground Wi-Fi

Combining two different signal systems promises to enable clearer Wi-Fi reception in underground commuter trains

Underground train passengers may soon be able to access Wi-Fi wherever they are, thanks to the discovery by NAIST researchers that leaky cables can be stacked up in tunnels to transmit and receive digital signals.

Several major cities, including Tokyo and London, offer Wi-Fi to commuters at underground stations by installing antennas at various locations in public areas. However, it is much challenging to obtain a signal inside a tunnel. This is because solid objects like trains block the high-frequency radio waves that Wi-Fi uses to transmit information when the waves are funnelled down a tunnel.

Underground train passengers may soon be able to access Wi-Fi wherever they are, thanks to the discovery by NAIST researchers that leaky cables can be stacked up in tunnels to transmit and receive digital signals.

One way to overcome this problem is to run a 'leaky coaxial cable' down a tunnel. Such a cable resembles a hose pipe in that it has many small holes drilled in it. But instead of leaking jets of water, the cable releases — and can receive — digital information through the tiny holes. Such cables have already been installed in some underground systems, but because they transmit limited amounts of data, the Wi-Fi signal tends not to be very strong. Consequently, the amount of data that can be downloaded and uploaded is limited and transfer speeds are slow.

Now, Minoru Okada and his colleagues at NAIST in Japan have proposed overcoming this problem by combining leaky coaxial cables with another system known as multiple-input multiple-output (MIMO), which is used in many home Wi-Fi systems[1]. In a classic radio system, one antenna transmits a signal and another antenna (for example, one in a laptop computer or a smartphone) receives it. In MIMO, multiple antennas are used at each end and different data streams are sent simultaneously; this strategy enhances both the speed and the amount of data that is transmitted. Processors at either end then detect tiny differences in the signals, which are caused by distortions as the signals bounce off different objects, and use these differences to disentangle the different data streams.

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Passengers on underground trains may soon be able to access fast Wi-Fi thanks to a strategy proposed by NAIST researchers.

Okada and co-workers have mashed these two systems together by combining two leaky cables to create an underground MIMO system. So long as the holes in the cables release the Wi-Fi signals in different directions, the differences in the data streams should be large enough to distinguish the different data streams.

The additional capacity that MIMO adds to the system means that if multiple people wanted to download an attachment from their emails at the same time, they could do so more quickly.

Reference

1. Hou, Y., Tsukamoto, S., Ariyoshi, M., Kobayashi, K. & Okada, M. 4-by-4 MIMO channel using two leaky coaxial cables (LCXs) for wireless applications over linear-cell. *2014 IEEE 3rd Global Conference on Consumer Electronics (GCCE 2014)* 125–126 (2014).

More information about the group's research can be found at the Network Systems Laboratory webpage:
http://isw3.naist.jp/Contents/Research/mi-03-en.html

Nara Institute of Science and Technology | **Vision and Media Computing Laboratory** www.naist.jp/en

Diminished reality in action: Left panel is the original image, right panel is the resulting image with signpost removed via the new diminished reality process.

Image processing

Seamlessly removing objects from images

New state-of-the-art image processing methods may allow objects to be removed from still photography — and even video

Image processing is no longer just about removing cellulite from celebrities or lens flare from lakes. Beefed-up computing power, combined with increasingly sophisticated image processing techniques, has made it possible to remove unwanted objects from video in real-time. "Real-time object removal allows us to simulate various scenarios, such as furniture arrangement and city planning, using real video images," notes Norihiko Kawai.

> **"Diminished reality methods have not been deeply investigated – there are still many issues to be addressed."**

Kawai, along with colleagues from the Graduate School of Information Science at NAIST, have used two approaches for the removal of objects from the foreground of an image: 'in-painting' for static images and, more recently, 'diminished reality' for video images.

Diminished reality refers to the real-time removal of objects from videos. It is the opposite of augmented reality: the addition of computer-generated objects to videos in real-time. "Whilst augmented reality techniques have been widely developed, diminished reality methods have not been deeply investigated – there are still many issues to be addressed," explains Kawai.

In response, Kawai and his colleagues decided to build on the success of image in-painting — an established technique where an object is removed from the image foreground, with the empty space being filled-in using an actual or realistic background texture — and apply it to diminished reality.

His team developed a diminished reality process based on image in-painting, but a sophisticated one that uses multiple two-dimensional planes to approximate a three-dimensional background. First the scene to be modified is analysed and a key frame, with the object to be removed, identified and an appropriate mask is fitted. After analysis, two processes are run concurrently: image in-painting for the one key frame, and real-time overlay of that in-painted texture for subsequent frames.

The results have been impressive, allowing for an almost seamless removal of objects (see figure). Without the panel on the left of the image in the figure above, it is unlikely the viewer would know there had ever been a signpost at the beginning of the footpath. Surprisingly, the computational load of this powerful method is comparable to related techniques.

Further complications lie ahead for this technique, such as removing target objects that are in motion, and dealing with increasingly complex background textures. However, Kawai looks forward to embracing the challenge of addressing these issues: "Adding virtual objects and removing real objects from video enriches our interactions with media for both entertainment and practical use," he says.

Reference

1. Kawai, N., Sato, T. & Yokoya, N. From image inpainting to diminished reality. In *Virtual, Augmented and Mixed Reality. Designing and Developing Virtual and Augmented Environments*. Lecture Notes in Computer Science **8525**, 363–374 (2014).

More information about the group's research can be found at Norihiko Kawai's webpage:
http://yokoya.naist.jp/~norihi-k/research-e.html

Nara Institute of Science and Technology | **Interactive Media Design Laboratory** www.naist.jp/en

Computer vision

Augmented reality zoom now possible

A novel algorithm improves the registration between virtual and real images in dynamic augmented reality

Augmented reality adds computer-generated images, video and sound to our own perception of the world around us. For example, the technology could aid navigation by superimposing street directions onto the view of a user through a head-mounted display. Researchers at Japan's NAIST have now developed a method that enables zooming in augmented-reality systems, making them useful for an even broader range of applications[1].

A common way to implement augmented reality is to overlay virtual information on a camera image and then relay this information to the user through a display device such as glasses or goggles. This 'video see-through' technology requires the computer-generated image to be accurately aligned with the objects in the user's field of view.

> **"Taketomi and co-workers ... have now demonstrated an algorithm that can simultaneously estimate both intrinsic and extrinsic camera parameters during camera zooming."**

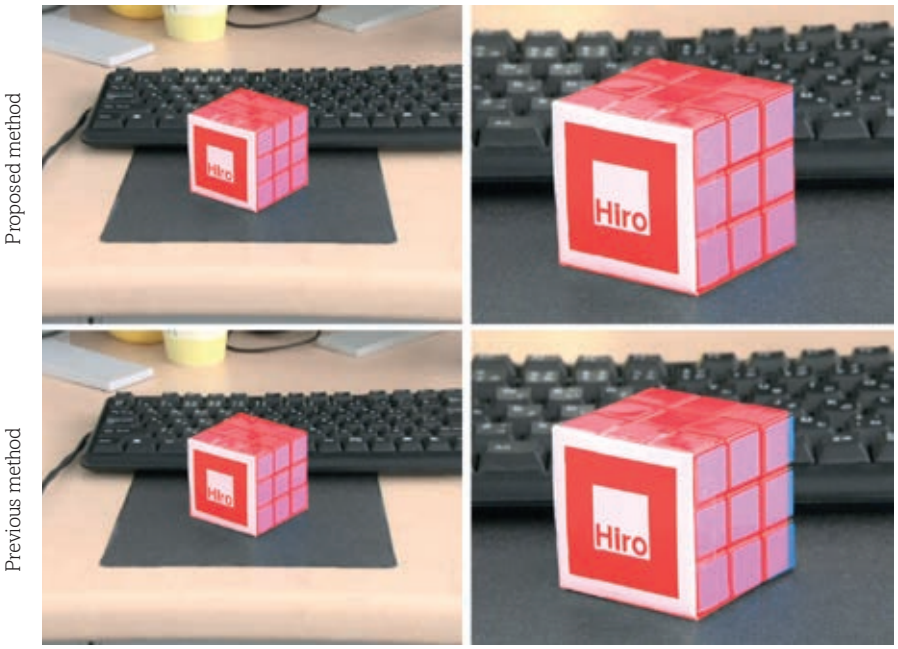
One way to perform this geometric registration between real and virtual worlds is to position the artificial image relative to specific markers by using an algorithm that can estimate various operational parameters of the camera. Such parameters can include extrinsic ones, such as position and orientation, and intrinsic ones, such as focal length and lens distortion.

"Many methods for estimating camera pose have been proposed," says NAIST researcher Takafumi Taketomi. "However, these methods assume fixed intrinsic camera parameters, which prevents zooming because the focal length changes depending on zoom values."

Taketomi and co-workers from the NAIST Interactive Media Design Lab together with colleagues from Capcom and the Tokyo Institute of Technology have now demonstrated an algorithm that can simultaneously estimate both intrinsic and extrinsic camera parameters during camera zooming.

The team's scheme starts with a pre-calibration procedure. This involves assessing the intrinsic camera properties at each level of magnification. The calculation then accurately estimates the intrinsic and extrinsic parameters online. This approach uses the conventional marker-based method, but improves it by adding a consideration for reprojection errors and the continuity of zoom values.

The team demonstrated their technique by superimposing a virtual cube on a real Rubik's cube. Whereas a displacement could be seen between the two when zooming in using a traditional camera-parameter estimation technique, far improved alignment was possible using the new scheme (see figure).



Augmented reality superimposes a virtual cube on a real Rubik's cube. The alignment between the two using the novel scheme (top right) is much improved relative to previous methods (bottom right).

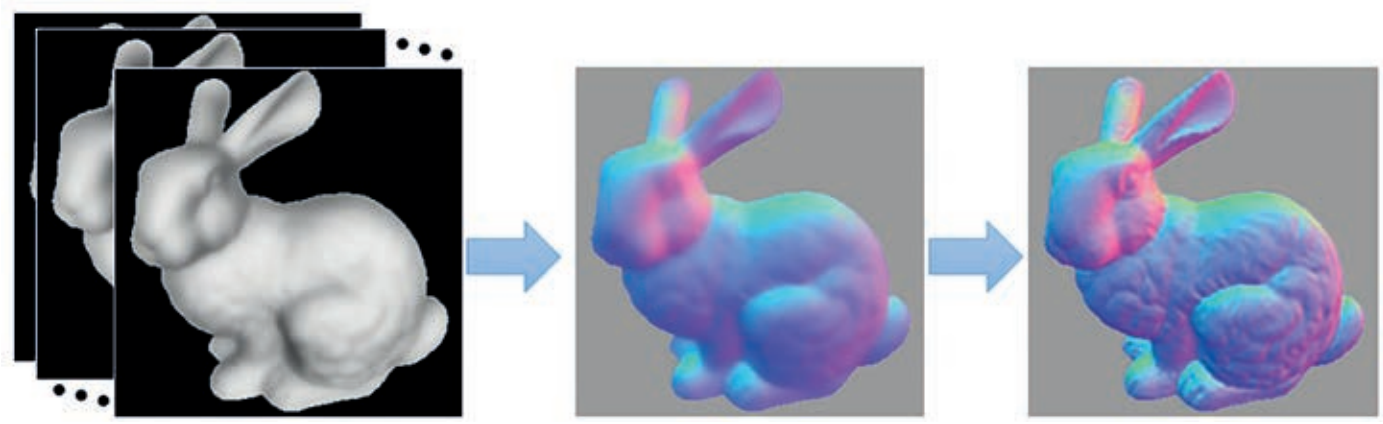
Camera zooming is an important technique for video production, and so this method could allow augmented reality to be used in entertainment-industry applications. "Our method is designed for specific cameras at the moment," explains Taketomi. "In the next step, we want to extend this method for more general camera settings, so that it can be applied to mobile devices with a camera and display."

Reference

1. Taketomi, T., Okada, K., Yamamoto, G., Miyazaki, J. & Kato, H. Camera pose estimation under dynamic intrinsic parameter change for augmented reality. *Computers & Graphics* **44**, 11–19 (2014).

More information about the group's research can be found at the Interactive Media Design Laboratory webpage: http://imd.naist.jp/

Nara Institute of Science and Technology | **Optical Media Interface Laboratory** www.naist.jp/en



A new technology makes it possible to turn multiple pictures of translucent objects (left panel) into sharp and realistic 3D images.

Visual computing

Resolving translucent 3D objects

Decoding light information with an innovative imaging technique can help robots better navigate their three-dimensional surroundings

Researchers from NAIST have developed a method to measure the three-dimensional (3D) shapes of everyday objects with translucent properties, such as food, clothing and dishes. The findings from Yasuhiro Mukaigawa and colleagues may make it simpler for robots to sense important environmental clues — the difference between a slippery bar of soap and a fragile vase, for example — that are critical for operating in real-life conditions.

Humans can determine if an object has a smooth or bumpy surface just by looking at it. But computers have a harder time making this judgment because of their reliance on flat, two-dimensional (2D) images. One way to overcome this problem is to illuminate an object and then capture images as the light source moves over it. This 'photometric stereo' technique deduces the surface normal vectors that project perpendicularly from each surface point by using the different shading angles seen in the images. Algorithms then turn the surface 'normals' into 3D reconstructions.

Photometric stereo has applications ranging from quality assurance to facial recognition. However, translucent materials are tricky to resolve with this technology: the random reflections of light inside such objects make it hard to relate shading patterns to surface geometry, and images appear blurry. To compensate, researchers have tried to develop models to remove sub-surface light scattering components from true surface shadings.

Implementing scattering models, however, requires a way to simultaneously inspect the material's optical properties along with its 3D shape. Mukaigawa and his co-workers solved this problem by recording how a thin ray of light reflects off a diffuse, uniformly scattering surface before projecting the beam over the translucent target. Separating the incident light radiance from the photometric stereo measurements using computer algorithms revealed the target's 'convolution kernel' — a mathematical image processing unit that holds information about surface geometry and sub-surface scattering.

Using a straightforward setup — a standard digital SLR camera, light projector and polarizers — the researchers examined intricate figurines carved from substances such as soap and marble (see figure). Their approach generated true-to-life 3D images that are remarkably sharper than conventional photometric stereo pictures of translucent objects. Mukaigawa notes that the simplicity and speed of this method could lead to improved types of computer vision technologies in the future.

"Many objects in our environment are translucent, but even expensive commercial sensors cannot measure their 3D shapes," he says. "If vision systems can know these shapes with our simple equipment, robots have a better chance of working in our living environment."

Reference

1. Inoshita, C., Mukaigawa, Y., Matsushita, Y. & Yagi, Y. Surface normal deconvolution: photometric stereo for optically thick translucent objects. In *Lecture Notes in Computer Science* Volume 8690, 2014, pp 346-359.

More information about the group's research can be found at the Mukaigawa Lab webpage:
http://omilab.naist.jp/

Nara Institute of Science and Technology | **Ambient Intelligence Laboratory** www.naist.jp/en

Robotics

Appearance plays role in social perception

Robots doing menial jobs can be perceived as more capable than disguised humans

Advanced social skills may not be necessary for robots undertaking dull, stressful and dirty tasks. Researchers at Japan's Nara Institute of Science and Technology (NAIST) have found that for menial tasks, people significantly preferred machines — even compared to a human in disguise performing the same job. This may be because robots are thought to lack feelings of anger, pain and self-respect.

"Even if a robot's appearance is to some degree human-like, people do not perceive it as essentially human," say researchers at Japan's ATR Intelligent Robotics and Communication Laboratories, including its former director Norihiro Hagita, now at the Ambient Intelligence Laboratory at NAIST[1]. These findings could inform the design of robots acceptable to urban societies.

From clearing bombs in a battlefield to fabricating parts with monotonous precision, robots are used for dangerous, dirty and dull jobs — for this, they need few social skills. In more urban settings, robots are being developed to engage with and assist senior citizens, students and office workers. Studies show that while people prefer robots for repetitive or hazardous tasks, for tasks involving intelligence and communication they prefer humans. The research team set out to assess the acceptance of robots for tasks that involve a mixture of drudgery and social interaction. They prepared nine separate videos of a robot, a human, and a human dressed up as a rabbit; each was performing a dull, stressful or dirty task at a popular shopping mall. Tasks included endlessly guiding visitors at an information counter, responding to an angry and threatening customer, and scooping keys out of a garbage bin after a shopper accidently dropped them there. The researchers invited 30 university students to watch the videos and fill out a questionnaire on the appropriateness of each task for each subject; and to assess how 'human' they felt each character looked and how they deserved to be treated. Participants judged the robot to be the best for all three tasks. They also assigned the robot the lowest ranking for human-like attributes, with the human, as expected, scoring highest. However, the researchers were surprised to find that participants did not rate the person dressed as a rabbit on the same scale as the human, despite there being a real human inside the costume – suggesting the importance of appearance in assigning human-like cognitive ability.

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Robots have traditionally been used for dangerous jobs in remote environments such as bomb disposal, but could increasingly find their way into social settings to fulfil dull, stressful and dirty tasks.

The study offers useful insights into acceptable roles for robots in daily urban life and supports the ongoing development of guidance robots for social environments, such as tour guides in museums and exhibition spaces.

Reference

1. Hayashi, K., Shiomi, M., Kanda, T. & Hagita, N. Are Robots Appropriate for Troublesome and Communicative Tasks in a City Environment? IEEE *Transactions on Autonomous Mental Development* **4**, 150–160 (2012).

More information about the group's research can be found at the Ambient Intelligence Laboratory webpage:
http://isw3.naist.jp/Contents/Research/mi-07-en.html

Nara Institute of Science and Technology | **Robotics Laboratory** www.naist.jp/en

Robotics

Gestures and 'interruptivity' boost interaction

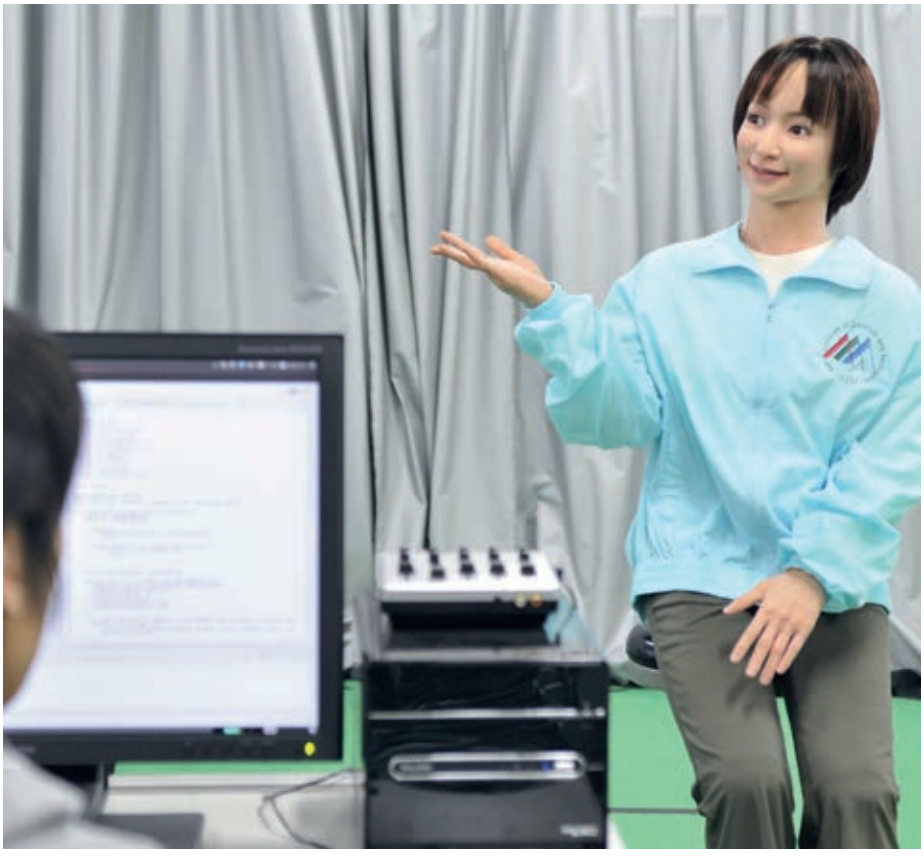
Life-like gestures and self-correction allow better communication with an android

Helpful androids — robots in human form — cannot yet be found in shopping malls, but researchers from NAIST are busy working to make this possible. They have programmed an android to make realistic gestures and recognize the speaker in a group of people facing it.

Robots that look like humans need to behave in a very life-like way. Otherwise, a human interacting with them will experience 'the uncanny valley'; a feeling of revulsion experienced when an object is nearly, but not quite, like a human being — such as seeing a corpse or zombie.

"The goal of this research is to take androids beyond the 'uncanny valley'," says Jun Takamatsu, a robotics scientist at NAIST[1]. Using motion capture film techniques, his group recorded the normal gestures of ten volunteers and these movements became the basis for the android's gestures. "The gesture that the android should use when answering each type of question was defined and stored in a database," explains Takamatsu.

The Actroid-SIT android they have programmed looks like a shop mannequin. 'She' turns to face any person who holds a microphone. As the person speaks, the android looks at them and makes small appropriate movements, such as nods and bows. Actroid-SIT can glance to the side, turn her head and look directly at someone. She makes meaningful gestures as she answers their questions — pointing to the bathroom, for example.



The Actroid-SIT android made by Kokoro Company Ltd and programmed by the NAIST team.

> **"This research has solved the issue of generating natural gestures in real time. We believe this is a very important first step."**

The team measured the android's effectiveness on 1,662 people attending an exhibition, who encountered the android standing behind a roped-off barrier. When Actroid-SIT could make gestures, more than 60% of the subjects who approached her then spoke to her; a significantly greater proportion than when her gestures were disabled.

The android also sports 'motion interruptivity': when she fails to recognise a speaker's remark or detects an unusual response from a person, she stops the gesture she is making and selects a different gesture and spoken response.

People at the exhibition talked to the android for significantly longer than when motion interruptivity was active.

"We are interested in service robots, which need to react to human demands appropriately," says Takamatsu. "This research has solved the issue of generating natural gestures in real time. We believe this is a very important first step."There is a long way to go, but I can imagine androids like this providing information to people in shopping malls, train stations and airports."

Reference

1. Y., Kondo, K., Takemura, J., Takamatsu , T.,Ogasawara. A gesture-centric android system for multi-party human-robot interaction. *Journal of Human-Robot Interaction* **2**, 133–151 (2013).

More information about the group's research can be found at the Robotics Laboratory webpage:
http://robotics.naist.jp/en/

Nara Institute of Science and Technology | **Intelligent System Control Laboratory** www.naist.jp/en

A new artificial intelligence system can considerably boost efficiency in an optical grid.

Applied informatics

Raising optical grid efficiency

Dynamic decision-making tools can improve the flow of information over ultrafast optical grid networks

Computers that share processing power and data storage through a grid of high-speed fibre-optic cables can maximize their productivity with a new artificial intelligence system developed at Japan's NAIST[1].

Takuji Tachibana and colleagues at Kenji Sugimoto's Intelligent System Control Laboratory have worked out a technique for dynamic, on-demand allocation of computer and optical-grid resources that can benefit applications ranging from massive particle accelerator experiments to consumer video editing.

Before individual computers can access the optical grid, they must make contact with a machine known as a job manager. This computer system establishes 'lightpaths' (direct connections between nodes that can transfer thousands of terabytes per second) for the requested processing task. Because the lightpaths and computing power available are finite, the job manager must carefully manage the network connections to ensure that computations run at smooth and stable rates for all users.

Although much attention has been paid to optimizing lightpath connections, Tachibana and his co-workers realized that improving connections between computers and the optical grid could considerably boost efficiency. For example, one problem with the job manager is that it needs to store a constant number of tasks in its memory buffer at all times to complete job executions without delays. Most existing job managers, however, lack the computing resources to satisfy this requirement.

To solve this, the researchers used proportional-integral-differential (PID) control theory to preserve the memory buffer by regulating the tasks sent to the job manager. Then, they implemented two kinds of model-predictive-control (MPC) methods to intelligently handle the needs of both lightpath generation–release processes, as well as computing client demands. "PID control has been used in industry for decades, and is widely recognized as practical," says Sugimoto. "MPC is rather new, but the quality of this method is attracting much attention."

The team's simulations showed that their approach can make the number of tasks stored in the buffer closer to ideal quantities than other, contemporary controllers. Furthermore, it had the ability to dynamically adapt to lower-specification computers appearing in the network — features that can speed implementation of existing hardware into future optical grid systems.

Sugimoto has confidence that this approach could improve communication between information technology researchers, as well as within the optical grid. "Studies of communication and control used to be closely related, but they have progressed in different ways," Sugimoto notes. "This work brings them together through the problem of optical grid."

Reference

1. Matsui, G., Tachibana, T., Kogiso, K. & Sugimoto, K. Dynamic resource management in optical grid. *IEEE Transactions on Control Systems Technology* **22**, 1607–1614 (2014).

More information about the group's research can be found at the Intelligent System Control Laboratory webpage:
http://genesis.naist.jp/?lang=en

Nara Institute of Science and Technology | **Large-Scale Systems Management Laboratory** www.naist.jp/en

Multihop wireless networks allow for more versatile and dynamic deployments of wireless devices, such as sensors, but can be undermined by an overuse of network-coding algorithms.

Wireless networks

Delays undermine multihop Wi-Fi

Modelling shows that delays caused by a network-coding algorithm are a drain on the performance of multihop wireless networks

Wireless networking is nearly ubiquitous in our daily lives, but the technology is more than a mere convenience. For scientific applications — such as industrial and environmental sensor networks — wireless connectivity has made it possible to connect and network devices in previously unimaginable locations while retaining the ability to view data remotely in real-time.

To maintain wireless connectivity, the wireless device needs to be in 'talking' distance to a base station — typically, in near line-of-sight and within a few tens of metres. Where these requirements are difficult to meet, such as around the summit of a volcano or within the labyrinthine heart of an industrial complex, the devices themselves can be used to capture and retransmit data from nearby devices so that the data eventually finds its way to a wireless endpoint — and from there to a wide area network like the Internet. This type of 'multihop' wireless network has become popular, but as the networks become larger and carry more data, there is a need for greater throughput and improved network performance.

Shoji Kasahara from Japan's Nara Institute of Science and Technology, with colleagues from Kyoto University, has now shown that the performance increases obtained from a promising throughput-enhancing scheme called 'network-coding' are actually limited by the additional processing time incurred in the encoding process[1]. Network-coding is a data transmission algorithm that takes two or more received packets of data and encodes them into a single packet for broadcast to neighbouring multihop nodes. This offers a theoretical boost to throughput, but as the algorithm is currently implemented as a network process rather than in hardware, the encoding step requires computation, which can add milliseconds to each packet transmission.

Kasahara and colleagues found through mathematical analysis of a generalized three-node multihop network that throughput is only increased above that of a network without network coding if the computational delay is less than 1,500 microseconds. Moreover, the performance benefits achieved at shorter computational delays are significantly undermined by this 'coding overhead'. "The main achievement of this work is that the result was obtained in a general setting, which makes the finding applicable to any transmission situation," explains Kasahara.

Since most multihop networks are implemented on low-power remotely deployable sensor devices, network coding may not be as attractive as might first appear. In order to realize the true potential of this promising wireless transmission scheme, hardware-based network coding – with negligible coding overhead – is essential.

Reference

1. Yazane, T., Masuyama, H., Kasahara, S. & Takahashi, Y. Effect of network-coding overhead on end-to-end throughput for multihop wireless networks. *Performance Evaluation* **70,** 14–27 (2013).

More information about the group's research can be found at the Large-Scale Systems Management Laboratory webpage: http://isw3.naist.jp/Contents/Research/ai-03-en.html

Nara Institute of Science and Technology | **Mathematical Informatics Laboratory** www.naist.jp/en

Neuroimaging

Improved modelling for three-dimensional scanning

A new computational method can build accurate three-dimensional images of brain activity from near-infrared spectroscopy data

For some medical applications, near-infrared spectroscopy (NIRS) imaging can provide cheaper, more portable alternatives to magnetic resonance imaging (MRI). In particular, NIRS is useful for tracking changes in the blood supply to different parts of the brain. But improving the mathematical models used to interpret NIRS data and construct three-dimensional (3D) images of brain activity has been a key challenge, and one which NAIST researchers have advanced.

When a specific part of the brain is activated, the flow and volume of oxygenated blood in that location peaks dramatically in response. Haemoglobin, the protein in red blood cells that transports oxygen, takes different forms when it is oxygenated and deoxygenated, and these forms absorb near-infrared light to different extents. Hence, NIRS systems that emit and then detect near-infrared light can indicate which parts of the brain are active.

The complex process of combining NIRS data to build 3D images is called diffuse optical tomography (DOT). Recently, Kazushi Ikeda and his colleagues at the NAIST Graduate School of Information Science, together with scientists across Japan, succeeded in improving NIRS-DOT images by enhancing the computational efficiency of the DOT approach[1].

Generally, DOT methods use information from previous states of the brain to calculate new states; in the language of Bayesian statistics, these are called the prior and posterior states, respectively. Conventional DOT models utilize the 'minimum norm estimation' to calculate the posterior state — but this cannot fully account for localized brain activities.

The major advance made by Ikeda and co-workers was to introduce a new type of input state for DOT modelling, called the 'automatic relevance determination' (ARD) prior. They also employed a variational Bayes method to calculate the posterior state more efficiently.

A similar approach had previously been employed for processing data from another brain imaging technology, magnetoencephalography (MEG). However, MEG depends on the brain's magnetic permeability, which is almost constant everywhere, whereas NIRS depends on the optical transparency, which varies with position. This meant the problem faced by the NAIST team was considerably more difficult to solve.

By performing a wide series of numerical experiments using synthetic brain data, Ikeda and his co-workers showed that their new DOT method could create improved 3D images that were robust against variations in optical transparency across the brain. Since their study was published, they have been working to confirm the method using real brain data recorded by more expensive techniques such as MRI.

Near-infrared spectroscopy data can be used to generate three-dimensional images that indicate which parts of the brain are active at different times.

Reference

1. Miyamoto, A., Watanabe, K., Ikeda, K. & Sato, M. Variational inference with ARD prior for NIRS diffuse optical tomography. *IEEE Transactions on Neural Networks and Learning Systems* (Available online at http://ieeexplore.ieee.org/xpl/articleDetails.jsp?arnumber=6839041).

More information about the group's research can be found at the Mathematical Informatics Laboratory webpage:
http://isw3.naist.jp/Contents/Research/ai-04-en.html

Nara Institute of Science and Technology | Laboratory of Imaging-based Computational Biomedicine www.naist.jp/en

Surgery

Digital tool boosts preoperative skill base

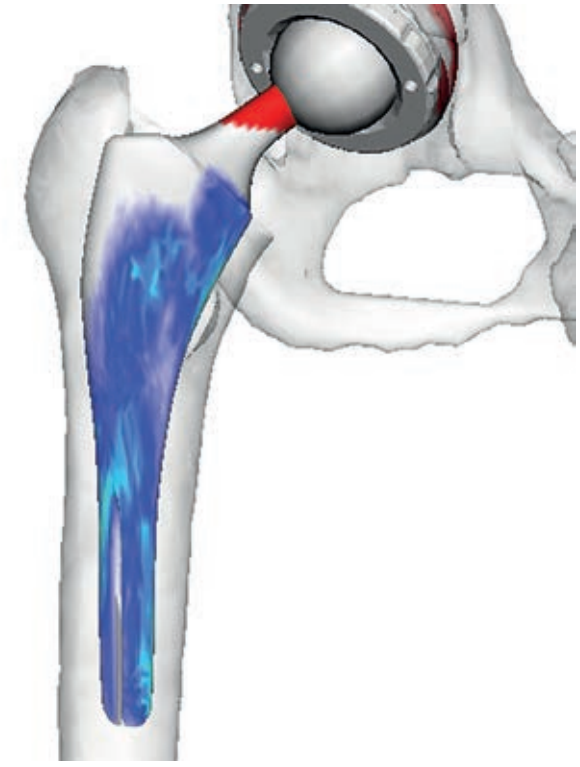
Pooling the experience of many surgeons, an artificial intelligence tool can help novice surgeons prepare better preoperative plans

Researchers in Japan are developing automated systems that capture the skills of experienced surgeons to help novices plan for total hip replacements and other surgical techniques. "Most patients want to have surgery performed by an experienced surgeon rather than a beginner," says Yoshinobu Sato of NAIST, who is part of a team developing artificial intelligence systems to give all surgeons the benefits of experience gained over many previous procedures.

Optimal planning for total hip replacement surgery requires careful consideration of each patient's anatomy and requirements. The precise size of the implant and its position relative to the patient's bones must all be considered to prepare the preoperative plan and select the best implant.

Working with colleagues at Osaka University and Kobe University, Sato — now at the NAIST Graduate School of Information Science — compiled computer-based 'atlases' that gathered and analyzed past surgical experience. The data came from selected preoperative plans that had already been prepared and used by experienced surgeons. The digital atlases analyzed the surgeons' plans for the exact pattern of contact between the implant and the femoral canal inside each patient's femur bone, which received the stem of the implant. The information in the digital atlases was then fitted to each new patient's data to generate a preoperative plan based on the experience of many skilled surgeons, rather than just one.

The researchers tested their system by selecting 40 individual cases, and comparing the automatically generated plans with those prepared by experienced surgeons using existing interactive systems. They found that their automated system effectively reproduced the plans of highly experienced surgeons[1]. This confirms that the system could assist less-experienced surgeons in matching the skills of the best.



An automatically constructed surgical preoperative plan can help less-trained practitioners prepare for surgery.

Sato says that accumulating surgical data from all over the world in this way could develop what he calls a 'super-experienced surgeon' using statistical machine learning. The methods have now moved out of the theoretical and experimental stages. Although the data have not yet been published, Sato reports "promising initial results" from preliminary clinical evaluations conducted by orthopaedic surgeons at Osaka University Hospital.

"We would now like to evaluate our system at many hospitals via the Internet," he adds, reflecting the obvious potential of digital automated systems to travel effortlessly to many locations on demand, unlike an individual skilled surgeon. And with some actual surgical procedures also becoming automated and robotically performed, there seems scope for fully automating every critical step, from planning to surgery, in suitable cases.

Reference

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More information about the group's research can be found at the Laboratory of Imaging-based Computational Biomedicine webpage: http://isw3.naist.jp/Contents/Research/ai-05-en.html

Nara Institute of Science and Technology | **Computational Systems Biology Laboratory** www.naist.jp/en

Making sense of large amounts of data is helping NAIST researchers understand metabolites.

Metabolomics

Matching structure to function

A systems biology approach pairs hundreds of structurally distinct metabolite groups to their corresponding functions

Organisms — from bacteria and plants to humans — generate a diverse array of chemicals called metabolites, which play many important roles, such as inducing growth, serving as antimicrobial agents and fighting the growth of tumours.

"The result could aid in the identification of the functions of newly discovered metabolites and the design of new drugs."

Now a team of researchers, including Shigehiko Kanaya from NAIST, have used databases they have developed that classify the chemical structure and biological function of thousands of metabolites across many species, to group metabolites with similar structures into those with similar functions[1]. The result could aid in the identification of the functions of newly discovered metabolites and the design of new drugs.

Metabolomics — the study of metabolites — is a very complex field. Some metabolites are unique to a particular species, while some are seen across multiple species. Some metabolites have a unique function; some have multiple functions. The extent to which clear themes can be identified in how metabolites function will allow researchers to fashion a better understanding of newly discovered metabolites. Systems biology approaches that aim to make sense of large amounts of data are key drivers in understanding metabolomics.

Kanaya and colleagues therefore took a systems approach to the question of how metabolite structure could provide insights into metabolite function. They began by studying the structure of over two thousand metabolites across many species and grouping together those metabolites that had similar chemical structures.

The team identified 671 structurally distinct groups among the metabolites studied. They then categorised each metabolite according to 140 different biological functions from a metabolite activity database, and looked for relationships between a given chemical structure group and a given biological function. This analysis revealed the existence of 983 structure–function pairings.

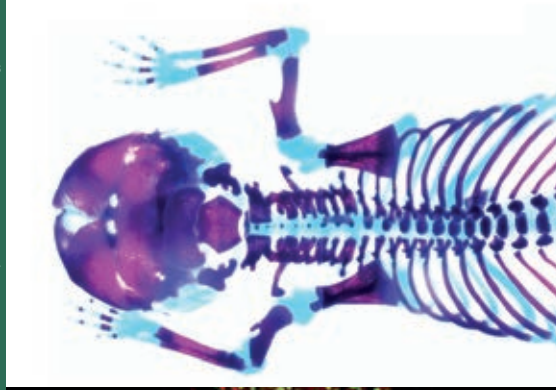
Although more than 70% of the structural groups could be clearly linked to one or more biological activities, the remaining structural groups could not be definitively classified in this manner, in part because the metabolites that made up those structural groups had multiple divergent functions.

These analyses will provide a guide for researchers in the field of metabolomics to be able to link a newly identified metabolite for which a structure is known with a biological function, and could aid in the development of new medicines.

Reference

1. Ohtana, Y., Abdullah, A.A., Altaf-Ul-Amin, M., Huang, M., Ono, N. *et al.* Clustering of 3D-structure similarity based network of secondary metabolites reveals their relationships with biological activities. *Molecular Informatics* **33,** 790-801 (2014).

More information about the group's research can be found at the Computational Systems Biology Laboratory webpage:
http://isw3.naist.jp/Contents/Research/ai-06-en.html



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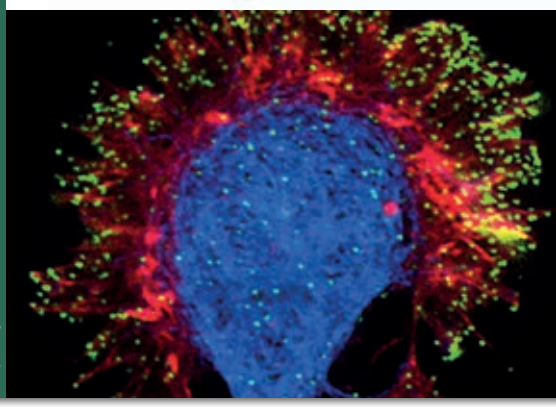
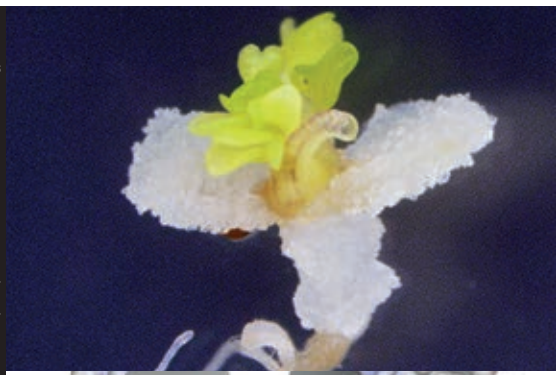


Naoyuki Inagaki

Biological Sciences

Research Highlights



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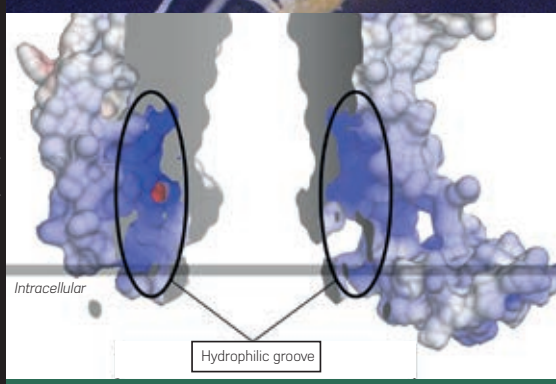

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Nara Institute of Science and Technology | **Intercellular Communications Laboratory** www.naist.jp/en

Plant genetics

Self-recognition in pollination

Studies reveal a new way for plants to distinguish self from non-self during pollination

Genetic diversity is crucial for ensuring the fitness of a species. One way plants promote diversity is by preventing inbreeding — also known as self-fertilization. Previous research demonstrated that *Petunia* uses a non-self-recognition system in which a pollen protein called S-locus F-box (SLF) interacts with a protein in the style S-RNase. S-RNase is toxic, and it prevents pollen-tube growth unless it is degraded by an SLF protein. Thus, pollen SLF functions by recognizing non-self S-RNases and degrading them, thereby allowing growth to continue.

However, researchers have been puzzled as to why the genetic diversity of the SLF gene is much lower than that of S-RNases in *Petunia*. According to Seiji Takayama of NAIST in Japan, "it remained a mystery how SLF recognizes a large repertoire of non-self female determinants." To shed light on this enigma, Takayama and colleagues sought to determine the molecular mechanism of self-recognition in *Petunia*.

> **"It was tough work to show that each SLF recognizes and detoxifies a subset of non-self S-RNases."**

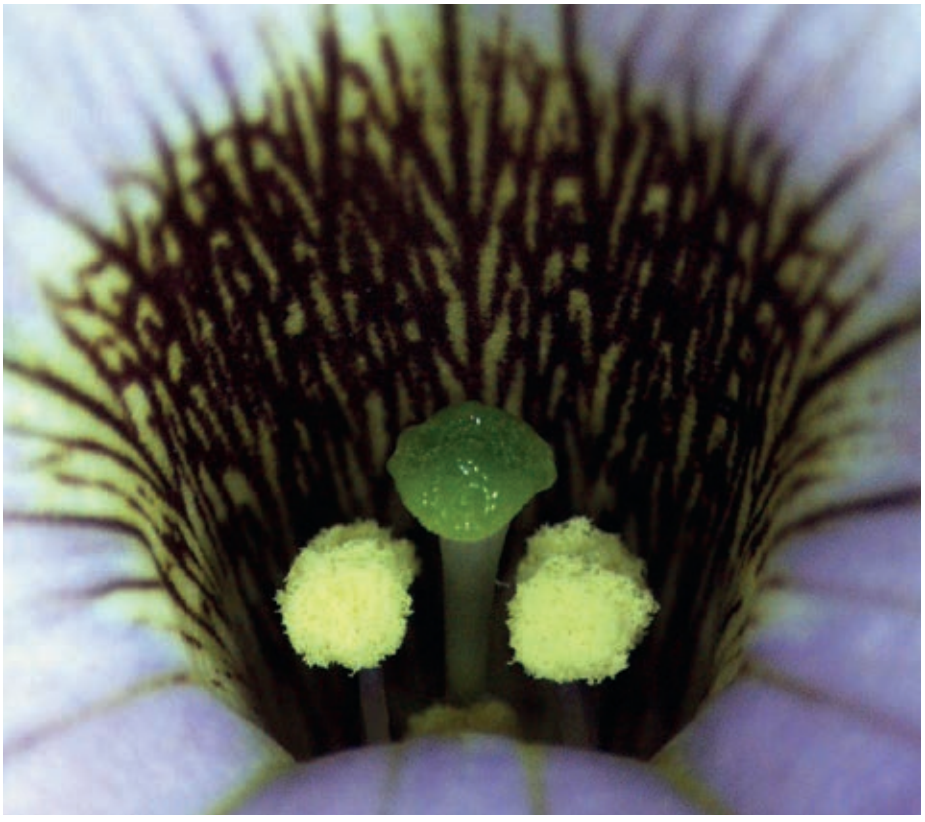
The scientists solved this mystery when they discovered that, unlike pollen of other plant species, *Petunia* pollen expresses not one, but multiple SLF-like proteins. At the start of their work, the team discovered several SLF-like genes that are expressed in pollen. But this was just the beginning. "It was tough work to show that each SLF recognizes and detoxifies a subset of non-self S-RNases," Takayama explains. "To search for the actual targets [S-RNases] of each SLF, we had to make several hundred transgenic plants."

Ultimately, they showed that these SLF proteins work together to recognize and degrade diverse S-RNases to promote non-self recognition. According to Takayama, the data provided convincing evidence that *Petunia* uses a 'collaborative non-self recognition model', which had never been described for other types of plants.

Since making these pioneering discoveries, the team has continued to uncover the molecular mechanism of collaborative non-self recognition. Takayama notes that during the research, "we identified six SLF genes." They then "thoroughly searched for SLFs using next-generation sequencing and found that 16–20 SLFs collaboratively function to detoxify non-self S-RNases."

They also "found evidence of gene conversion events," Takayama says. He considers these events to be "essential to the constitution of this 'non-self recognition' system and also to contribute to self-compatible mutation." The results of this work were recently published in *Nature Plants*.

All of this work was facilitated by NAIST which, according to Takayama, provided "a quiet and calm atmosphere suitable for scientific research, properly maintained research facilities, and leading-edge shared equipment."



Close-up image of a petunia flower.

Reference

1. Kubo, K. *et al.* (2010) Collaborative non-self recognition system in S-RNase-based self-incompatibility. *Science* **330,** 796-799 (2010).

More information about the group's research can be found at the Intercellular Communications Laboratory webpage:
http://bsw3.naist.jp/eng/courses/courses102.html

Nara Institute of Science and Technology | **Plant Cell Function Laboratory** www.naist.jp/en

Cell biology

Enzyme shatters cell skeleton

The process by which stress-activated enzymes destabilize a cell's molecular scaffold could one day be directed against cancer

Plant and animal cells are supported by a framework of tubular proteins, called microtubules, that are crucial in many cellular processes. In plants, environmental stress can cause this framework to break down, but the molecular mechanisms underlying this disintegration have remained elusive. NAIST researchers have now discovered that a protein known as PHS1 plays a key role in dismantling the microtubule scaffold[1].

Takashi Hashimoto and colleagues at NAIST's Plant Cell Function Laboratory studied the function of the protein by using mutants of *Arabidopsis* — small flowering plants related to cabbage — that were deficient in PHS1. They found that PHS1 contains two parts: a kinase and a phosphatase (see figure).

The kinase causes the transfer of a phosphate group to microtubule proteins, which destabilizes the entire microtubule skeleton.

The kinase is usually inactive, but Hashimoto's team showed that it is permanently active in mutants with defective phosphatase domains. This suggests that the phosphatase acts as a switch controlling the kinase. Furthermore, when the researchers applied stress such as a high salt concentration, they found that the phosphatase 'switched on' the kinase. This, they report, "provides an efficient strategy for reversibly and rapidly regulating microtubule stability in response to biotic and abiotic signals."

Although proteins with multiple functions are fairly common, 'autoregulated' proteins such as PHS1, with two opposing domains are rare, according to Hashimoto. "I do not recall any kinase having a juxtaposed phosphatase domain," he notes.

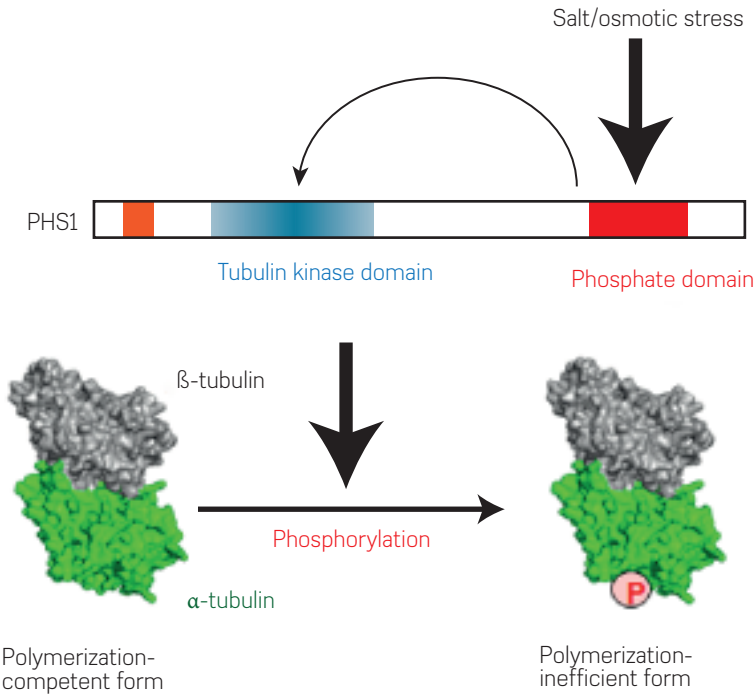
> **"Our current preferred idea is that PHS1 acts to halt plant growth temporarily under water stress, allowing the plant to adapt to changing water resources. This is one of the most important questions we want to address."**

The functional significance of PHS1 in nature remains unclear and is something the team is actively investigating. "Our current preferred idea is that PHS1 acts to halt plant growth temporarily under water stress, allowing the plant to adapt to changing water resources. This is one of the most important questions we want to address," says Hashimoto.

PHS1 is thought to be unique to, but universal in, the plant kingdom. "All sequenced plant genomes have PHS1 genes," says Hashimoto, who has recently shown that mosses use the same system.

The protein also provides a useful tool for studying microtubule-based cellular processes and, because microtubules are central to all cell division, it could potentially be used to stop cancer cells proliferating. Tests on cultured monkey cells have shown that PHS1 can also destabilize mammalian microtubule arrays.

Hashimoto reasons that, since PHS1 also acts on animal microtubules, "it could be targeted to cancer cells to dysfunction their microtubules, thereby stopping proliferation." In the shorter term, his research provides useful experimental tools for studying cellular processes.



PHS1 comprises two domains: a kinase and its suppressor, a phosphatase. Environmental stress causes phosphatase to release the kinase, which then transfers a phosphate group to a tubulin molecule. This phosphorylation of tubulin results in the breakdown of cellular microtubule arrays.

Reference

1. Fujita, S., Pytela, J., Hotta, T., Kato, T., Hamada, T. *et al.* An atypical tubulin kinase mediates stress-induced microtubule depolymerization in *Arabidopsis*. *Current Biology* **23**, 1969–1978 (2013).

More information about the group's research can be found at the Plant Cell Function Laboratory webpage:
http://bsw3.naist.jp/eng/courses/courses103.html

Nara Institute of Science and Technology | **Laboratory of Plant Developmental Signaling** www.naist.jp/en

Plant science

Key propagation protein identified

A key protein required for embryonic development and growth in plants has been uncovered

The development of an embryonic plant from a fertilized seed follows a programmed pattern of cell divisions. This allows the differentiation of cells into specific cell types, which enables healthy growth of tissues and the correct development of the plant.

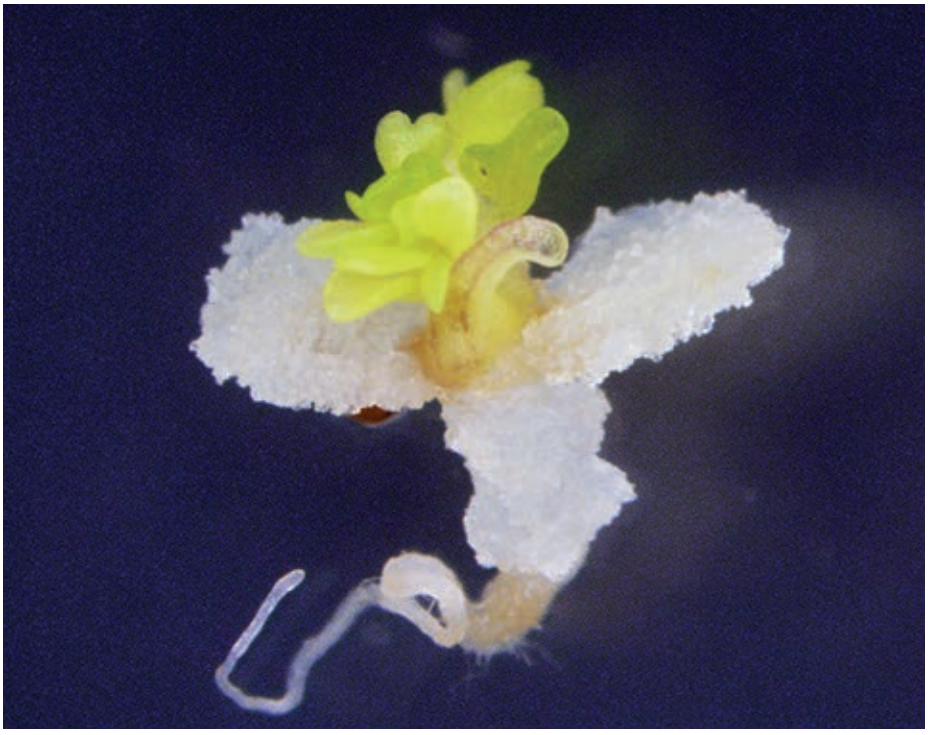
A small number of transcription factors contribute to this process, but little is known about the mechanisms and genes that control the complex organisation of cells over time in seed plants (a process known as pattern formation). NAIST researchers have uncovered the critical role of protein RKD4, and the genes that respond to it, in the early development of *Arabidopsis* plants[1].

> **"When we stopped the overexpression, we thought it might result in new shoots or root formation; instead we found embryos appearing a week later."**

"We originally began genetic screening to isolate a patterning regulator for post-embryonic roots," explains Keiji Nakajima, from the NAIST Graduate School of Biological Sciences, who worked on the study with his colleagues. "Instead, we uncovered a mechanism responsible for embryogenesis rather than root patterning. The protein RKD4 seems to be even more important than simple patterning regulators, because it can actually reprogram cells in the earliest stage of plant growth."

It took the NAIST team some time to understand what they had stumbled across, because at first they were focusing on the plant roots. It was only when Nakajima analyzed microarray data from RKD4 overexpressing plants, that they uncovered the role of RKD4.

The researchers found that RKD4 preferentially accumulates in developing seeds. Mutant strains without RKD4 showed severe germination defects — many plants simply did not grow, and those that did had truncated roots with disrupted cell structure or no roots at all.



The protein RKD4 triggers embryogenesis in *Arabidopsis* plants: knocking out RKD4 means the plant does not develop properly, whereas overexpressing RKD4 can lead to the reprogramming of cells and the creation of new embryos (above).

However, the overexpression of RKD4 had an unexpected effect, as Nakajima explains: "We created transgenic seedlings with high levels of RKD4, which resulted in the overproliferation of young leaves and root tissues, as well as triggering the expression of early-embryo-specific genes. When we stopped the overexpression, we thought it might result in new shoots or root formation; instead we found embryos appearing a week later."

The overexpression of RKD4 essentially reprogrammed differentiated cells and gave them the potential to produce embryonic, or undifferentiated, cells. The team concluded that RKD4 is an important regulator, which primes cells to activate embryogenesis, and promotes the expression of genes required for kick-starting the correct growth and patterning of plant embryos (see figure).

The discovery may prove useful for applications such as controlling the propagation of endangered plant species. Nakajima's team is currently extending the research to investigate evolutionary processes in plants.

Reference

1. Waki, T., Hiki, T., Watanabe, R., Hashimoto, T. & Nakajima, K. The *Arabidopsis* RWP-RK protein RKD4 triggers gene expression and pattern formation in early embryogenesis. *Current Biology* **21,** 1277–1281 (2011).

More information about the group's research can be found at the Laboratory of Plant Developmental Signaling webpage:
http://bsw3.naist.jp/nakajima/English/

Nara Institute of Science and Technology | **Laboratory of Plant Metabolic Regulation** www.naist.jp/en

Plant genetics

Wood-forming genes active in mosses

Key genes for wood development are expressed in mosses, hinting at an ancient evolutionary story

Despite the distance between mosses carpeting a forest floor and the trees above, researchers have discovered that both groups express key wood-development genes. Although mosses lack wood, the genes originally played a central role in the migration of plants to the land.

When plants moved onto land, the absence of water presented them with the challenge of holding themselves upright and keeping aerial tissues hydrated. The evolution of special water-conducting cells, known as xylem, solved both problems, providing a continuous network of stiff tubes that support the body and deliver water. Vascular plants used this evolutionary innovation to carry themselves ever higher in the competition for light, from little flowers to towering trees in which xylem tissue forms the bulk of the biomass in the form of wood. Unlike vascular plants, mosses transport water through hydroids instead of xylem, and have remained small.

However, a group of key xylem development genes are active in the moss *Physcomitrella patens,* according to research carried out by a team at the Nara Institute of Science and Technology (NAIST) and other Japanese institutes[1]. The VND/NST/SMB (VNS) gene family regulates the differentiation of woody cells in vascular plants. While the *P. patens* genome is known to include several VNS-like genes, their function has remained unclear. The researchers studied the genes' expression patterns and used knock-out mutants to figure out their role in the development of *P. patens*.

"I didn't expect such clear results at first," says Misato Ohtani of NAIST's Graduate School of Biological Sciences. Several of the genes are expressed in tissues consisting of hydroids and other cells responsible for water transport and structural support. Mutant *P. patens,* with multiple VNS genes knocked out, have defective hydroids together with decreased water uptake.

The team is now looking downstream of the VNS genes to understand how different water-conducting tissues evolved in mosses and vascular plants. "I first guessed that *P. patens* would lack the second master regulators of woody cells, MYBs, and the biosynthetic genes for lignin," says Ohtani. To her surprise, homologs of both groups are expressed downstream of the VNS genes in *P. patens*, suggesting that a primitive form of the VNS-MYB pathway evolved before the divergence of mosses and vascular plants and became specialized in both lineages.

"Amazing!" says Ohtani. "We're starting to use several plant species, including a kind of algae, to explore this issue." Understanding how the VNS genes regulate the differentiation of water-conducting cells in different species could provide versatile tools for manipulating plant biomass.

Wild-type *P. patens* growing in soil.

Reference

1. Xu, B., Ohtani, M., Yamaguchi, M., Toyooka, K., Wakazaki, M. *et al.* Contribution of NAC transcription factors to plant adaptation to land. *Science* **343,** 1505-1508 (2014).

More information about the group's research can be found at the Laboratory Of Metabolic Regulation webpage:
http://bsw3.naist.jp/demura/?cate=137

Nara Institute of Science and Technology | **Plant Growth Regulation Laboratory** www.naist.jp/en

Plant biology

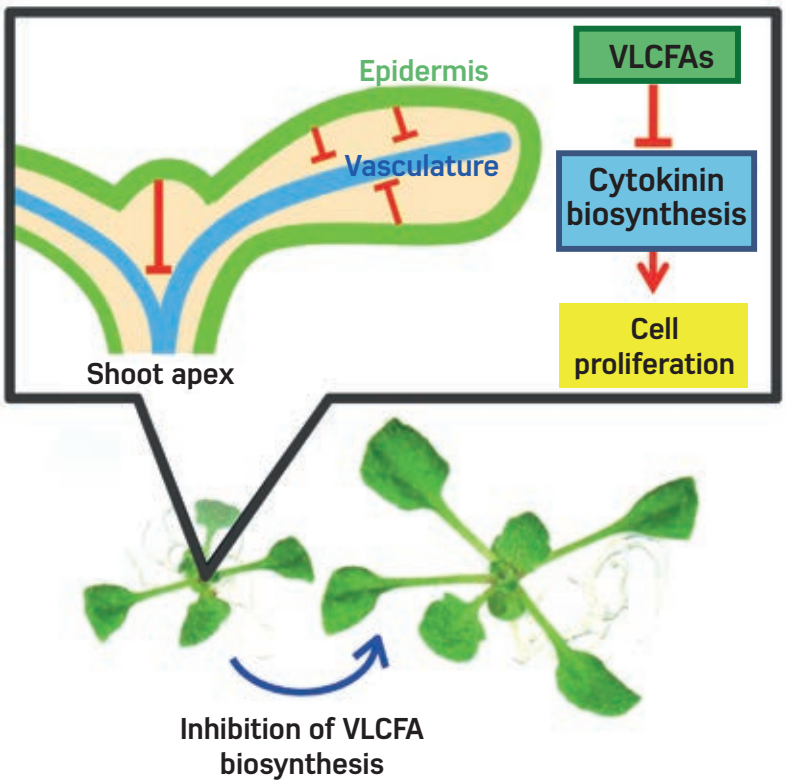
Surface signals control growth

The signalling processes governing plant organ growth have been found to originate within the surface layers of the plant

The outermost cell layer in plants, known as the epidermis, plays an important role in interactions between the plant and the environment. The epidermis also communicates with cells in inner layers to maintain optimal organ size by accelerating or restricting cell proliferation. Recent investigations by researchers at NAIST indicate that the epidermis is capable of sending signals to deeper plant tissues in order to control cell proliferation and shoot organ growth.

The epidermis is covered by a hydrophobic barrier called the cuticle, which protects plants from environmental hazards such as drought, and also helps guard against pathogen attacks. The cuticle is made of the polymer 'cutin', and a substance called 'cuticular wax', which is made up of very-long-chain fatty acids (VLCFAs).

Masaaki Umeda and co-workers at the NAIST Graduate School of Biological Sciences, together with scientists across Japan, have shown that VLCFA synthesis in the epidermis, and subsequent VLCFA-derived signalling to inner cell layers, is important for shoot organ growth regulation in the small flowering plant known as thale cress (*Arabidopsis thaliana*)[1].



Signals derived from very-long-chain fatty acids (VLCFAs) play an important role in maintaining organ size during continuous plant development. VLCFAs synthesized in the epidermis of the *Aribidopsis thaliana* plant can suppress the cytokinin accumulation around the vasculature and proliferation of cells in internal plant tissues.

"Knocking out the genes that are required for VLCFA synthesis in the plants resulted in severely impaired cuticular wax layer formation," explains Yoko Okushima, a member of Umeda's team. "This had such an impact on plant development that it was difficult to ascertain the exact outcomes related to low VLCFA content."

To examine the effect of disrupting VLCFA synthesis in more detail, Umeda's team gradually reduced the levels of VLCFA in *Arabidopsis* plants using different doses of a synthetic VLCFA inhibitor called cafenstrole, an active ingredient in commercial herbicides. The results showed that reducing VLCFA content increased the expression of a protein called IPT3, which promoted biosynthesis of a growth hormone called cytokinin in the inner plant layers. This led to excess cell proliferation.

"VLCFA synthesis in the epidermis suppresses this excess cell proliferation in internal tissues," explains Okushima. "Signals derived from VLCFAs regulate cytokinin biosynthesis inside the plant and determine shoot organ growth. Interestingly, the mild inhibition of VLCFA synthesis using a low concentration of cafenstrole actually produced larger aerial organs without inhibiting cuticular wax layer formation. VLCFA synthesis may therefore be a possible target for technologies aimed at increasing plant biomass."

Umeda and his team are continuing their investigations into this interesting regulatory signalling pathway. Their current studies aim to uncover how VLCFAs regulate cytokinin biosynthesis and cell proliferation via intercellular signalling.

Reference

1. Nobusawa, T., Okushima, Y., Nagata, N., Kojima, M., Sakakibara, H. & Umeda, M. Synthesis of very-long-chain fatty acids in the epidermis controls plant organ growth by restricting cell proliferation. *PLOS Biology* **11,** e1001531 (2013).

More information about the group's research can be found at the Plant Growth Regulation webpage:
http://bsw3.naist.jp/eng/courses/courses105.html

Nara Institute of Science and Technology | **Plant Morphological Dynamics Laboratory** www.naist.jp/en

An *Arabidopsis* flower.

Plant development

Understanding how flowers and leaves form

Insights into the molecular mechanisms governing how buds develop leaves and flowers could help improve agronomy and horticulture

Genes and molecular mechanisms that allow the plant hormone auxin to control the formation of leaves and flowers have been identified by NAIST researchers. Understanding these complex systems involved in plant development could contribute to advances in plant breeding and genetic engineering of benefit to agriculture and horticulture.

> **"Our findings enable us to manipulate plant development, including organ growth orientation and plant architecture."**

As we watch plants grow and buds form and then develop into leaves and flowers, we can't fail to be impressed by the neatly ordered and beautiful structures created by the hidden chemistry within. Masahiko Furutani and colleagues at the NAIST Graduate School of Biological Sciences investigated the ebb and flow of chemicals that create the impressive botanical architectures we can all admire. They have worked with *Arabidopsis*, small flowering plants widely used as model organisms to investigate plant genetics and genetic engineering (see figure). "The molecular mechanisms that control these dynamic changes were unknown," says Furutani.

The researchers did know that the formation of *Arabidopsis* leaves and flowers is triggered by localised accumulations of auxin, a small and relatively simple plant growth factor or plant hormone. The development of organs such as the parts of a flower are initiated in a spiral manner in the outer layers of the region of undifferentiated tissue at the tip of a growing shoot, known as the meristem. Furutani and his colleagues found that a family of genes known as the MAB4 genes promote organ development by establishing a downwards flow of auxin[1]. This involved the creation of an 'auxin sink' mechanism to promote the localised removal of auxin needed to generate the necessary gradients in auxin concentration. The crucial role of MAB4 genes in establishing the auxin sink is one of the key insights from this research.

The actual flow of auxin from cell to cell is mediated by carrier proteins embedded in the cell membranes, known as PIN proteins. The Furutani's team investigated the role of these proteins in the auxin gradients, and the mutual interactions among auxin, the MAB4 genes and the PIN proteins. Their findings allowed them to propose a new model to describe the details of auxin gradient formation and auxin transport during organ formation in the plants.

Although focused on *Arabidopsis*, the research is likely to have wider relevance for understanding plant development in general. "Our findings enable us to manipulate plant development, including organ growth orientation and plant architecture," says Furutani, explaining the ultimate motivation for unravelling these complex molecular interactions.

Reference

1. Furutani, M., Nakano, Y. & Tasaka, M. MAB4-induced auxin sink generates local auxin gradients in *Arabidopsis* organ formation. *Proceedings of the National Academy of Sciences of the United States of America* **111**, 1198–1203 (2013).

More information about the group's research can be found at the Plant Morphological Dynamics webpage:
http://bsw3.naist.jp/eng/courses/courses106.html

Nara Institute of Science and Technology | **Plant Immunity Laboratory** www.naist.jp/en

Plant disease

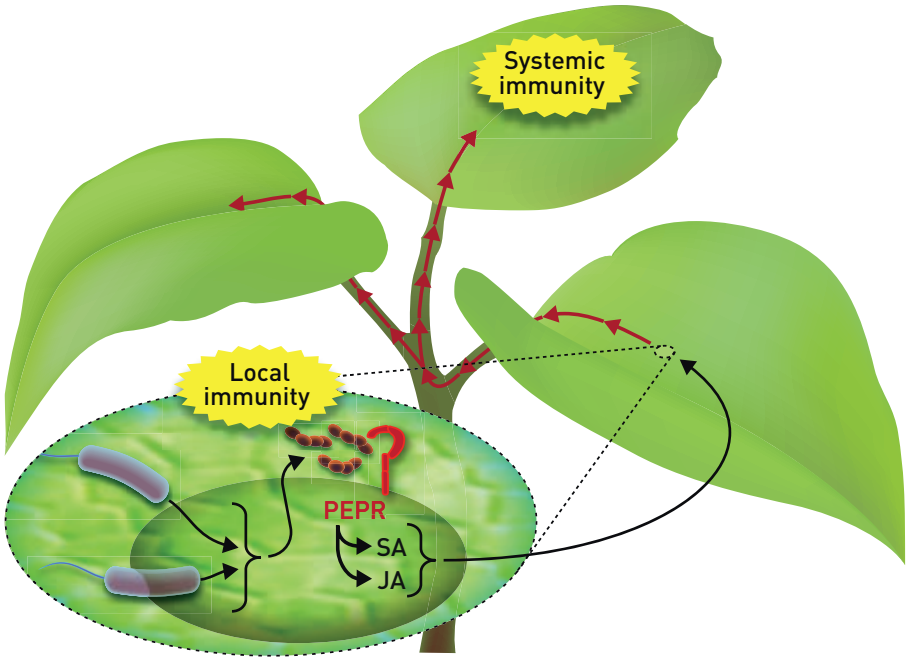
Understanding plant immunity, both near and far

A signaling pathway, linking local and systemic immunity in plants, could be used to create disease-resistant crops

Plants employ a two-fold defense against disease: local immunity immediately surrounding a pathogen, and systemic immunity further away. However, the molecular pathways linking pathogen recognition with immunity are poorly understood. NAIST researchers are investigating a cellular component central to both local and systemic immunity, providing a potential target for developing disease-resistant crops[1].

Plant immunity develops through detection of both pathogen molecules and endogenous danger signals. The latter include small protein fragments called PROPEPs, which bind to receptors (PEPRs) on the cell surface. The NAIST team, led by Yusuke Saijo, formerly of the Max Planck Institute for Plant Breeding Research, Cologne, Germany, is investigating how PEPRs coordinate local and systemic immunity.

> **"The components of the *Arabidopsis* PEPR pathway are evolutionarily conserved in higher land plants including important crops."**



The NAIST team's proposed model for the role of PEPRs in plant immunity. Pathogen attack causes the release of Pep peptides which are received by PEPRs. This activates hormonal salicylic (SA) and jasmonic acid (JA) pathways, resulting in both local and systemic immunity to the pathogen.

Using mutant *Arabidopsis* – small flowering plants related to cabbage and mustard – Saijo's team determined that PEPRs contribute to local immunity, through perception of PROPEPs. They then tested systemic immunity by inoculating PEPR-defective mutants with a pathogen and looking for defense-response markers in uninfected parts of the plant. The mutants had reduced numbers of markers, indicating that PEPR has a critical role in systemic immunity, which is in turn vital to plant survival. As Saijo explains, "In contrast to animals, plants don't have specialized mobile immune cells." Systemic immunity enables pathogens to be resisted, "not only in the infected cell, but also in its neighbours and distant organs. Our work indicates that local action of the PEPR pathway strengthens an as-yet-unidentified systemic signal from the pathogen-challenged site, enhancing systemic immunity in distant, unchallenged tissues."

The team also catalogued the genes switched on by PEPRs. They found that PEPRs co-activate two branches of the plant immune system, mediated by the hormones salicylic and jasmonic acid. However the detailed mechanisms underlying systemic immunity remain to be elucidated, and the lab — which prides itself on its intellectual flexibility and international outlook — continues to investigate the molecular-genetic and biochemical pathways involved.

These results suggest that PEPRs provide a critical control center in plant defense, connecting internal and external triggers, co-activating salicylic and jasmonic acid pathways that would typically antagonize each other, and linking immediate local defense with long-distance systemic immunity (see figure). According to Saijo, "The components of the *Arabidopsis* PEPR pathway are evolutionarily conserved in higher land plants including important crops."

He continues: "The systemic immune system can enhance plant disease resistance against a broad spectrum of pathogens in a sustainable and cost-efficient manner. Thus, our work shows the potential of endogenous signals, such as PROPEPs, and PEPRs, as tools to increase disease resistance in crops."

Reference

1. Ross, A., Yamada, K., Hiruma, K., Yamashita-Yamada, M., Lu, X. *et al*. The Arabidopsis PEPR pathway couples local and systemic plant immunity. *The EMBO Journal* **33,** 62–75 (2014).

More information about the group's research can be found at the Plant Immunity Laboratory webpage:
http://bsw3.naist.jp/eng/courses/courses111.html

Nara Institute of Science and Technology | **Plant Development Biology Laboratory** www.naist.jp/en

Plant genetics

Understanding female reproductive organ formation

A deceptively simple appearance belies the complex genetic interactions that occur during the formation of Arabidopsis thaliana*'s female reproductive organs*

Flowers are not simply nature's decorations, they serve a more practical purpose — as plant reproductive organs. A plant's male reproductive organs, the stamens, are collectively known as the androecium; whereas the stigma, style and ovary are collectively known as the gynoecium, the female counterpart. Mitsuhiro Aida, from the Graduate School of Biological Sciences and colleagues investigated the genetic interactions involved in the formation of the closed structure of the gynoecium.

"Much of our daily food comes from fruits or seeds, all of which are products of the gynoecium," notes Aida. What makes the gynoecium special is its closed structure; this shields developing seeds from changing environmental conditions, allowing them to flourish. Flowering plants with this closed gynoecium structure have proved to be the most successful plant group because "a delicate fertilization process is able to take place in a more regulated manner under more stable conditions than is possible for plants without a gynoecium," explains Aida.

The *Arabidopsis thaliana* flower, a popular model for plant studies.

> **"Elucidating the functions of *CUC1/CUC2* genes and their regulators in gynoecium development will give us clues on how flowering plants evolved to elaborate reproductive organ systems."**

He and colleagues were interested in understanding how the gynoecium formed as a closed structure via the fusion of developmental units similar in form to leaves, known as carpels. Previously, they had been working on the *CUP-SHAPED COTYLEDON* genes, *CUC1* and *CUC2* — investigating their function as developmental regulators. While *CUC1* and *CUC2* influence the development of seeds, over-expression of the genes can be 'too much of a good thing' and stop adjacent organs from fusing, which would impede the formation of closed gynoecia.

As closed gynoecium are common in nature, Aida recalls, "We hypothesized that there should be a factor that maintains appropriate expression of *CUC1* and *CUC2* to ensure proper gynoecium development." They identified the *SPATULA* gene, *SPT* as the most likely candidate since loss of *SPT* function had been observed to result in plants with split gynoecia. Interestingly, the *SPATULA* gene was named after the strange shape of the mutant gynoecia.

This was followed by an extensive study of *CUC1*, *CUC2* and *SPT* expression and corresponding gynoecium structures amongst wild type and mutant variants of *Arabidopsis thaliana*[1], a Eurasian small flowering plant also known as the thale cress.

The most significant finding was the domain-specific effect of *SPT* on *CUC* expression — suppressing it in the apical domain (thus ensuring closure in the top section of the gynoecium), while allowing it in the basal domain — facilitating seed development in the bottom section of the gynoecium.

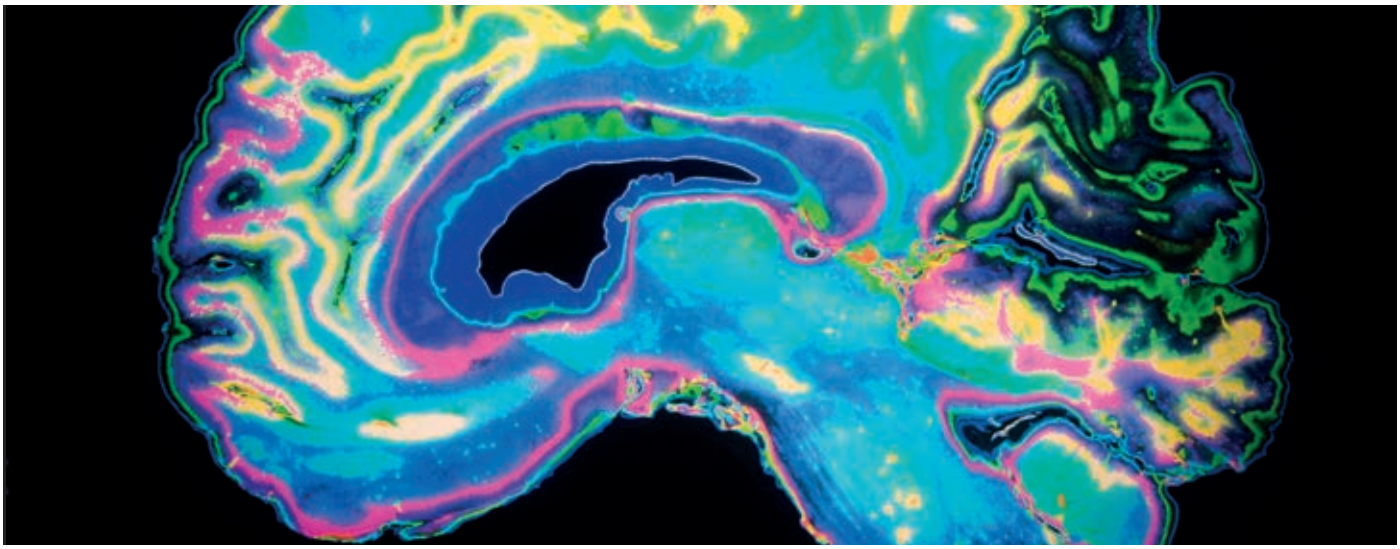
It is possible that these interactions may be responsible for the closed gynoecia in other flowering plants. "Elucidating the functions of *CUC1/CUC2* genes and their regulators in gynoecium development will give us clues on how flowering plants evolved to elaborate reproductive organ systems," notes Aida.

Reference

1. Nahar, M. A-U., Ishida, T., Smyth, D. R., Tasaka, M. & Aida, M. Interactions of *CUP-SHAPED COTYLEDON* and *SPATULA* genes control carpel margin development in *Arabidopsis thaliana*. *Plant Cell Physiology* **53,** 1134–1143 (2012).

More information about the group's research can be found at the Plant Development Biology webpage:
http://bsw3.naist.jp/eng/courses/courses108.html

Nara Institute of Science and Technology | **Molecular Signal Transduction Laboratory** www.naist.jp/en



A healthy brain, like this one, has many folds and grooves. A discovery by NAIST researchers could help treat lissencephaly, during which these features fail to develop.

Embryology

Protein does double duty in cell migration

Doublecortin protein has two different roles in controlling how the brain develops

As the fetal brain develops, young brain cells known as neuronal progenitor cells need to migrate from where they are formed to their final home in the cerebral cortex. When that fails to happen, the brain's folds and wrinkles don't develop properly; a condition known as 'smooth brain', or lissencephaly, which leads to developmental delays, seizures and respiratory problems.

> **"Dual and coordinated regulation of two cytoskeleton systems by one molecule was a very exciting finding."**

A number of different proteins and signalling molecules are involved in controlling that migration, and a team of researchers at Japan's NAIST has mapped one pathway that controls when and how the cells get moving.

Within that pathway, they found a surprise: a protein called Doublecortin (DCX) has two different functions when it comes time for the cell's internal scaffolding — the cytoskeleton — to begin moving the cells along on their journey.

"Dual and coordinated regulation of two cytoskeleton systems by one molecule was a very exciting finding," says Hiroshi Itoh, a molecular biologist at NAIST who led the work.

Cellular migration is triggered when a signalling molecule called pituitary adenylyl cyclase-activating peptide (PACAP) binds to a receptor that spans the membrane of the neuronal progenitor cell. That receptor, part of an important class of proteins called G-protein coupled receptors, induces the activation of protein kinase A (PKA) in the cell. PKA then changes the behaviour of DCX, by adding a phosphate group to one particular area of the protein.

Up until this point, DCX has been holding the cell rigid by bundling one part of the cytoskeleton, the microtubules, together. But once it is phosphorylated by PKA, DCX releases the microtubules and switches its attention to another part of that scaffolding, a protein called actin. It induces the actin to form long filaments that push against the cell membrane from the inside and, freed from the stabilising effect of the microtubules, the cell begins to move towards its final home in the developing brain.

The discovery could help with the treatment of lissencephaly by allowing doctors to treat a fetus with drugs that promote proper migration of neuronal progenitor cells if genetic testing reveals a mutation in the phosphorylation site on DCX, says Itoh.

Itoh's team is now investigating how another protein kinase, called CDK5, might be involved. CDK5 is known to regulate neuronal cell migration and, like PKA, also phosphorylates DCX, but at a different point on the protein. They are analysing the relationship between these two different versions of phosphorylated DCX in controlling cell migration.

Reference

1. Toriyama, M., Mizuno, N., Fukami, T., Iguchi, T., Toriyama, M. *et al. The Journal of Biological Chemistry* **287**, 12691-12702 (2012).

More information about the group's research can be found at the Molecular Signal Transduction webpage:
http://bsw3.naist.jp/eng/courses/courses202.html

Nara Institute of Science and Technology | **Functional Neuroscience Laboratory** www.naist.jp/en

Neuroscience

Signals that strengthen the brain

Insights into an important signalling system for neural plasticity may enhance understanding of bipolar disorder and schizophrenia

The brain is capable of modifying itself over time by creating and strengthening synapses — the structures that carry electrical signals between neurons. This process, known as neural plasticity, enhances cognitive functions including learning and memory. Researchers at NAIST are at the forefront of investigations into the mechanisms surrounding cognitive functioning, and a major study in 2012 uncovered an important signalling system in neural plasticity involving an enzyme called neuropsin and a protein called NRG-1[1]. The dysfunction of the neuropsin-NRG-1 signalling system may be involved in psychiatric illnesses such as bipolar disorder and schizophrenia.

> **"Neuropsin is thought to influence cognitive brain function, and has been implicated in working memory and bipolar disorders."**

"About 20-30 years ago, neural and synaptic structures were thought to be rigid and immovable, even in neural plasticity processes like memory acquisition," explains Sadao Shiosaka, of the NAIST Laboratory of Functional Neuroscience and one of the authors of the study. "We hypothesized that neural plasticity actually induces dynamic neural change, particularly in an area called the synaptic cleft, found between pre- and post-synaptic neurons."

The main problem with studying signalling systems in the synaptic cleft is that the specific enzyme-protein interactions happen very quickly and cannot easily be isolated and examined. Shiosaka and his team therefore pioneered a new method to uncover the protein substrates processed by neuropsin in the brains of mice.

"Neuropsin is thought to influence cognitive brain function, and has been implicated in working memory and bipolar disorders," explains Shiosaka. "We modified neuropsin so that it had a mutation which allowed binding to target proteins, but did not then release them. This allowed us to identify several of neuropsin's targets, including NRG-1."

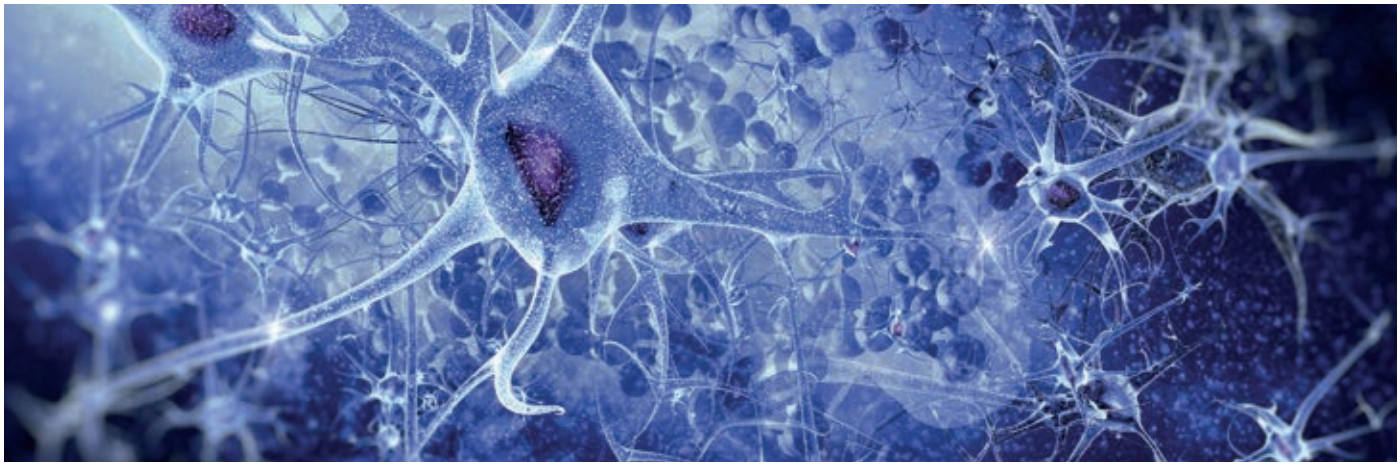
The team analysed samples taken from the hippocampus in the mice. They found that neuropsin processes NRG-1, which in turn activates the so-called ErbB4 receptor to stimulate a type of neuron called GABAergic neurons.

Signalling from ErbB4 allows for the strengthening of synapses between neurons, enhancing the brain's ability to learn from repeated activities, a process known as early long-term potentiation. When Shiosaka and his team created neuropsin-deficient mice, they found that the mice displayed both behavioural and neuronal hyperexcitability, as well as impairments in early long-term potentiation.

These results show that neuropsin-NRG-1 processing is a vital part of neural plasticity, and that functioning neuropsin pathways allow for the strengthening of synapses and normal cognitive function. Shiosaka and his team believe that their work could help further understanding of bipolar disorder and schizophrenia, and may one day provide a remedy for plasticity-related conditions such as hallucination and delusion.

Reference

1. Tamura, H., Kawata, M., Hamaguchi, S., Ishikawa, Y. & Shiosaka, S. Processing of neuregulin-1 by neuropsin regulates GABAergic neuron to control neural plasticity of the mouse hippocampus. *The Journal of Neuroscience* **32,** 12657–12672 (2012).



A major signalling pathway involved in strengthening synaptic connections between neurons in the brain has been identified. Its disruption may be connected with psychiatric disorders such as schizophrenia.

More information about the group's research can be found at the Functional Neuroscience webpage:
http://bsw3.naist.jp/eng/courses/courses205.html

Nara Institute of Science and Technology | **Gene Function in Animals Laboratory** www.naist.jp/en

Genetics

Stress response gene underlies loss of vision

A key gene protects retina cells from oxidative stress, but also causes them to age prematurely

Age-related macular degeneration is the leading cause of severe, irreversible vision loss in people over 55. Its basis remains poorly understood, and no effective treatment exists. Recent research at NAIST has shown how a stress response gene plays a role, linking the genetic and environmental factors behind this debilitating disease.

In 2006, two research groups discovered a link between the *HtrA1* gene and age-related macular degeneration. Since then, increasing evidence has demonstrated that high *HtrA1* levels in the retinal pigment layer play a central role in the disease – but the gene's function in this tissue has remained a mystery. The retina is exposed to high levels of physiological stress in the form of oxidative damage, prompting Masashi Kawaichi's team at NAIST to investigate whether *HtrA1* activity modifies the retina's stress-response.

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NAIST researchers are studying the link between the *HtrA1* gene and stress response of the retina.

> **"We believe that the same senescence process is a major part of the physiological function of HtrA1 in ossification and its pathological function in arthritis, both of osteoarthritis and rheumatoid arthritis."**

The team used cultures of embryonic mouse cells and human retinal cells to test how *HtrA1* responds to oxidants such as hydrogen peroxide. Their experiments showed that cells under oxidative stress activate *HtrA1* expression; this protects them from dying due to the stress, but also causes them to senesce earlier[1]. The researchers believe that increased senescence resulting from persistent exposure to oxidative stress eventually causes retinal damage in the form of macular degeneration.

HtrA1 is also expressed in a variety of other tissues, such as cartilage, ligaments and tendons, and has been linked with diseases from pre-eclampsia to arthritis. Oxidative stress is known to play a role in causing arthritis, raising the possibility that similar mechanisms may underlie the two conditions. "We believe that the same senescence process is a major part of the physiological function of *HtrA1* in ossification and its pathological function in arthritis, both of osteoarthritis and rheumatoid arthritis," says Kawaichi.

Unfortunately, the diversity of *HtrA1*'s roles also presents a challenge to efforts to transform these findings into improved treatments. "Some researchers believe that *HtrA1* is a tumour suppressor gene. A decrease in *HtrA1* expression levels is frequently associated with malignant transformation or metastasis of ovary cancers, gastric cancers, melanoma, and so forth," explains Kawaichi.

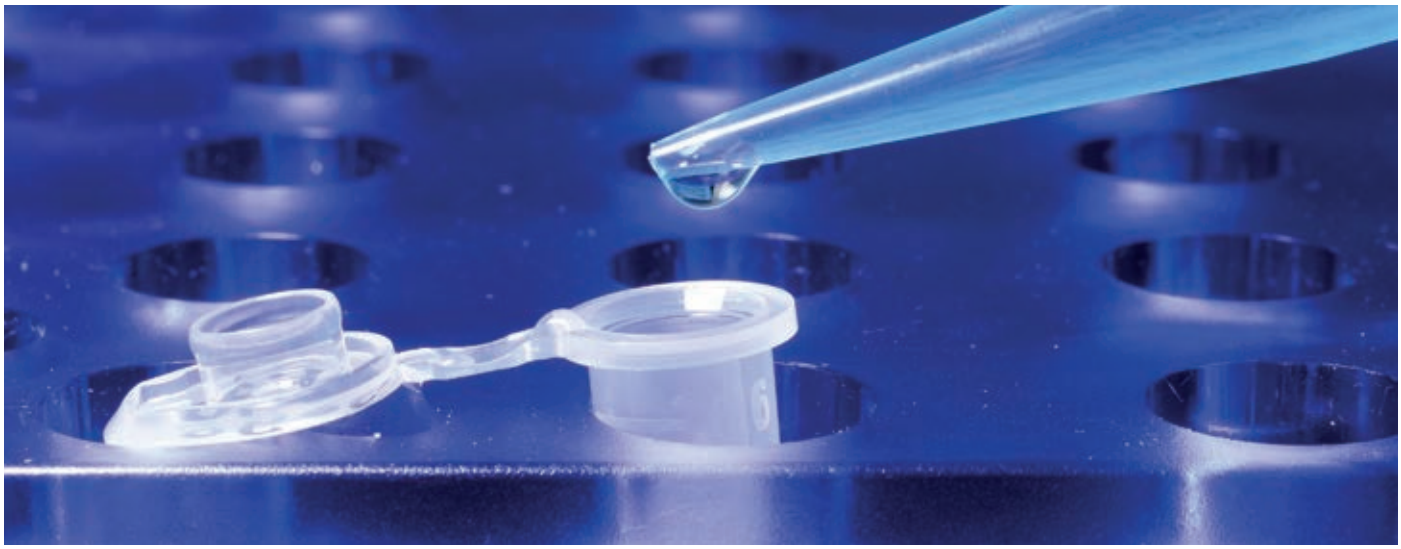
"General administration of potent inhibitors of *HtrA1* could have side effects, particularly in the long term. If we could have an effective inhibitor which could be used for ocular instillation or topical injection into joints, we hope those would provide promising remedies to these prevalent diseases," Kawaichi adds.

Reference

1. Supanji, Shimomachi, M., Hasan, M. Z., Kawaichi, M. & Oka, C. *HtrA1* is induced by oxidative stress and enhances cell senescence through p38 MAPK pathway. *Experimental Eye Research* **112,** 79-92 (2013).

More information about the group's research can be found at the Gene Function in Animals Laboratory webpage:
http://bsw3.naist.jp/eng/courses/courses206.html

Nara Institute of Science and Technology | **Functional Genomics and Medicine Laboratory** www.naist.jp/en



NAIST researchers are exploring the quality control function of the Upf1 enzyme in the protein production process.

Cell biology

Key quality control element revealed

An RNA-scanning mechanism may help cells prevent production of potentially toxic truncated proteins

A mutation affecting a single nucleotide can be sufficient to interrupt the synthesis of a protein, resulting in a truncated product that may potentially disrupt the normal function of the cell. Researchers led by Yasumasa Ishida of NAIST have uncovered a 'quality control' mechanism that help detect and eliminate these prematurely-shortened RNA molecules[1].

Within every messenger RNA (mRNA) resides the recipe for building a protein, composed of a series of three-nucleotide 'codons' that each encode a particular amino acid. These instructions are concluded by one of three different 'stop codons', which terminate protein production. Genetic alterations can convert an amino acid-encoding codon into a stop codon, interrupting the translation of the affected mRNA prematurely. These molecules are selectively recognized and eliminated via a mechanism known as 'nonsense-mediated decay'.

Ishida's team wanted to explore how Upf1, a 'molecular motor' enzyme with the capacity to travel along RNA and DNA strands, contributes to this process. Before an mRNA can be translated into protein, it undergoes a splicing process that removes various non-protein-coding segments; these splice sites are marked by assemblies of proteins known as the exon-junction complex (EJC), which get removed as the mRNA undergoes translation. In principle, a prematurely terminated mRNA with a nonsense mutation will retain at least some of these EJCs intact. Ishida and colleagues experimented with various synthetic mRNAs to examine how interactions between Upf1 and EJC contribute to nonsense-mediated decay.

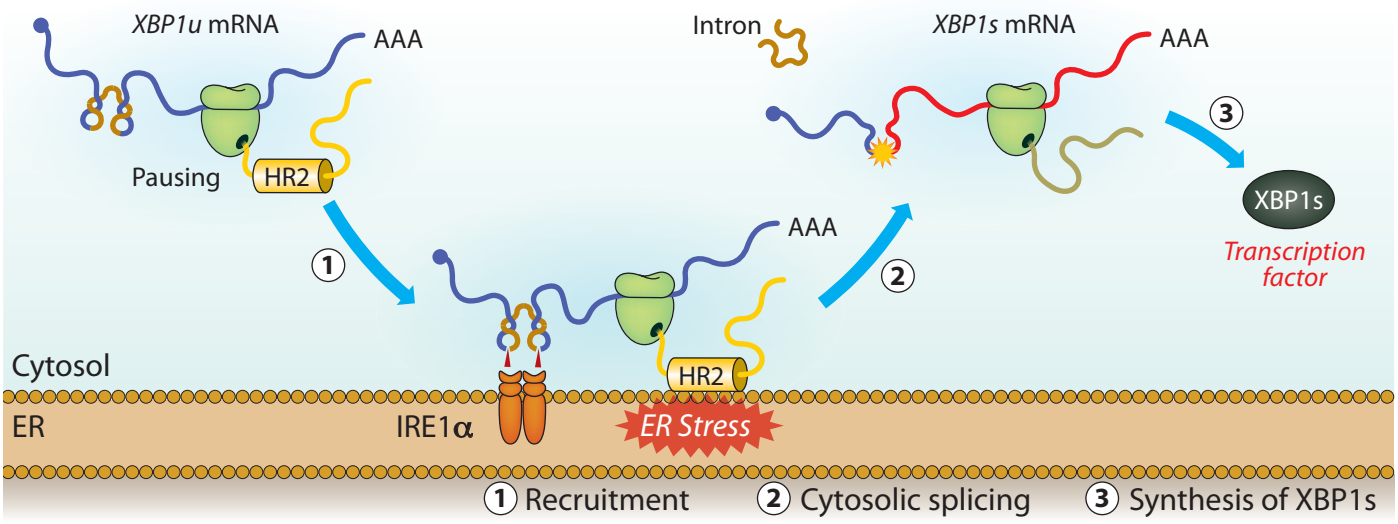
Upf1 is associated with the protein translation machinery, and Ishida's team found evidence that this protein uses its motor activity to essentially 'reel in' the mRNA strand after protein translation becomes stalled. If this halt is due to a true stop codon, the translation machinery disassembles, and the mRNA is released intact. In the event of a nonsense mutation, Upf1 scans until it encounters an EJC; this interaction sets the nonsense-mediated decay process in motion, eliminating the damaged mRNA strand.

These data suggest that Upf1 fulfils a critical quality control function to restrict the production of defective — and possibly toxic or tumorigenic — proteins. If future studies validate this model, it could also fill in critical gaps in our understanding of the protein production process. "People still do not know how a normal cycle of protein translation really ends," explains Ishida. "Our 'reeling-in' model could beautifully explain both ribosome recycling, which is the last step of a normal cycle of protein translation, and nonsense-mediated decay in a unified manner."

Reference

1. Shigeoka, T., Kato, S., Kawaichi, M. & Ishida, Y. Evidence that the Upf1-related molecular motor scans the 3'-UTR to ensure mRNA integrity. *Nucleic Acids Research* **40,** 6887–6897 (2012).

More information about the group's research can be found at the Functional Genomics and Medicine Laboratory webpage:
http://bsw3.naist.jp/eng/courses/courses211.html



The NAIST team's proposed model for the action of XBP1u in translational pausing, mRNA splicing, and production of active XBP1s.

Molecular biology

Ribosome pause has critical role

A novel system in animal cells halts protein-synthesis to generate molecules that protect against disease

A little-known process in cell genetics — translational pausing — may be more important than previously thought. Scientists at NAIST have uncovered a new system that protects against the accumulation of misfolded proteins in a cellular compartment called the endoplasmic reticulum (ER). It may be involved in diabetes and neurodegenerative disorders.

Proteins, the building blocks of life, are assembled in a multi-stage process. First, the DNA code is 'transcribed' into a complementary molecule, messenger RNA (mRNA). This is then 'translated' into proteins at a structure called a ribosome. Normally, proteins are not functional until released from the ribosome. However, a team led by Kenji Kohno uncovered one — XBP1u — which plays a crucial role before it is even fully translated[1]. As XBP1u emerges from the ribosome, it drags the entire complex — ribosome, mRNA and protein — to the ER's external membrane. Here, a protein called IRE1α splices a section out of the *XBP1* mRNA (see figure). The modified mRNA translates into a new protein, XBP1s, which induces genes that prevent damaging misfolded proteins from accumulating in the ER.

> **"We want to know exactly how this translational pausing occurs and how translation is then restarted."**

Kohno's team identified a section of XBP1u, HR2, which causes the complex to attach to the ER membrane. Because HR2 lies close to the end of XBP1u, there is a risk that translation of XBP1u may be completed, and the protein released, before HR2 has had time to do its job. The scientists showed — by separating out the cell's proteins according to size and charge (a method called electrophoresis) — how this is prevented: another section of XBP1u causes translation to pause before XBP1u is complete.

This leaves the protein attached to the complex both during movement to the ER membrane and mRNA splicing. In sum, says Kohno, "Translational pausing of XBP1u enhances the distribution of its mRNA to the surface of the ER, which increases the efficiency of cytoplasmic splicing of XBP1u, producing a new mRNA encoding XBP1s."

Using mutants with reduced or extended translational pausing, the team showed the importance of the pausing process to both membrane targeting and efficient splicing of the mRNA. They also found that the pausing sequence continues to function when attached to other proteins, so could be used to study protein folding in general.

The easy availability of novel equipment and strong communication between laboratories has made this ground-breaking research possible. "Now," says Kohno, "we want to know exactly how this translational pausing occurs and how translation is then restarted."

Reference

1. Yanagitani, K., Kimata, Y., Kadokura, H. & Kohno, K. Translational pausing ensures membrane targeting and cytoplasmic splicing of XBP1u mRNA. *Science* **331**, 586-589 (2011).

More information about the group's research can be found at the Molecular and Cell Genetics webpage:
http://bsw3.naist.jp/eng/courses/courses207.html

Nara Institute of Science and Technology | **Tumor Cell Biology Laboratory** www.naist.jp/en

Cell biology

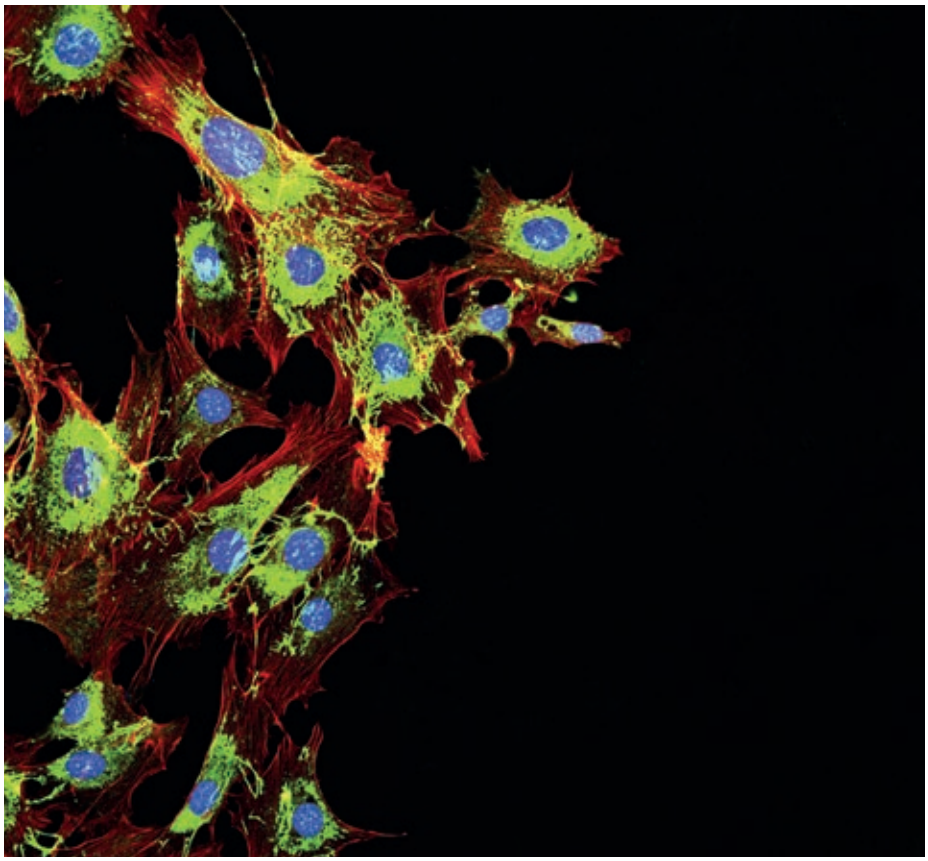
Regulator of tumour suppression found

Novel protein promotes degradation of cell cycle inhibitor p27 by facilitating its translocation from the nucleus to the cytoplasm

Cancer is a group of diseases characterized by out-of-control cell division. The cyclin-dependent-kinase (Cdk) inhibitory protein p27 helps prevent the development of cancer by slowing or stopping cell division, particularly that of tumour cells. Although mutations in p27 are rarely found in human tumours, reduced expression of the protein has been associated with poor survival of patients with breast and colorectal cancers.

Previous studies have identified several mechanisms for the regulation of p27: for example, phosphorylation by the cyclinE/Cdk2 complex; degradation by the ubiquitin-proteasome pathway; and sequestration by the transcription factor c-Myc. In 1999, Junya Kato and co-workers at Japan's Nara Institute of Science and Technology (NAIST) identified another important mechanism for p27 regulation – they found a protein called p38 that specifically binds p27 and promotes its degradation in mammalian cells[1].

Kato and co-workers at NAIST's Graduate School of Biological Sciences screened a mouse T-cell lymphoma library to identify genes encoding proteins that are able to interact with p27. Among them was a mouse gene that shared 91.5 per cent of its sequence identity with the human gene *Jab1*. This gene – the mouse homologue of *Jab1* – encodes a protein with a relative molecular weight of 38 kilodalton.



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The nucleus (blue) and cytoplasm (green) of fibroblasts.

The researchers dubbed the protein p38, and found that it binds to p27 but no other proteins (eg., cyclinB1, cyclinE and Cdk2). Moreover, they showed that overexpression of p38 in mouse fibroblasts resulted in the translocation of p27 from the nucleus to the cytoplasm — the place where p27 is broken down by degradation machinery.

Taken together, the findings showed that p38 is a negative regulator of the cell cycle inhibitor p27. The reduction or absence of p27 in the nucleus can lead to a host of detrimental effects, including, but not limited to, the promotion of cell cycle progression and the disruption of controlled cell division. Although not well understood, these processes are likely contributing factors to the poor survival of cancer patients observed in clinical studies.

"Our findings affected many fields and led to a host of new discoveries, for example, the combination of the upstream of proteolytic machinery with cell cycle regulation and intracellular translocation with proteolytic regulation," says Kato. "We have recently uncovered a novel mechanism by which *Jab1* contributes to cancer. The discovery will form the basis of our future development in drugs for treating cancer."

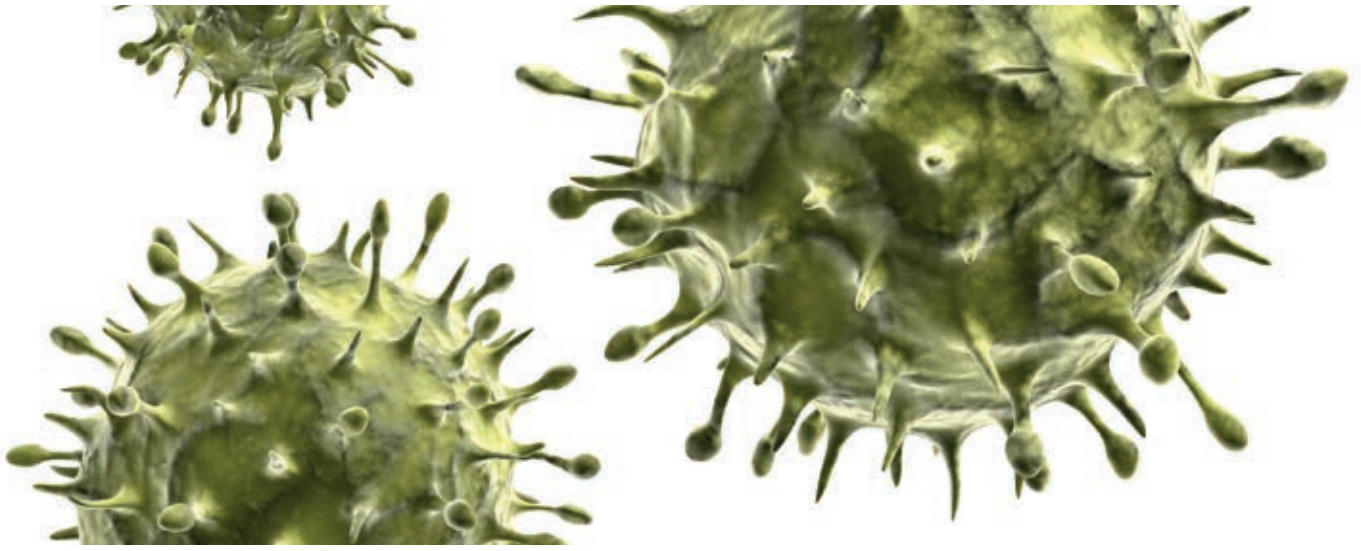
Kato and co-workers are still figuring out the precise role of p38 in the regulation of p27. Nevertheless, the protein may serve as a potential therapeutic target for the treatment of breast and colorectal cancers.

Reference

1. Tomoda, K., Kubota, Y. & Kato, J. Degradation of the cyclin-dependent-kinase inhibitor $p27^{Kip1}$ is instigated by Jab1. *Nature* **398,** 160–165 (1999).

More information about the group's research can be found at the Tumor Cell Biology Laboratory webpage:
http://bsw3.naist.jp/kato/?cate=205

Nara Institute of Science and Technology | **Molecular Immunobiology Laboratory** www.naist.jp/en



Influenza virus.

Antiviral defence

Understanding the role of a messenger

A key component of the innate immune response may improve vaccines and anti-tumour assistance

The first line of defence against pathogen infection, known as 'innate immunity', could be boosted using synthetic copies of a small signalling molecule, called phosphatidylinositol-5-phosphate (PtdIns5P), which has been investigated by NAIST researchers.

The molecule could be paired with adjuvants, substances added to vaccines to provide additional assistance in fighting off infection. "This research suggests the possibility for developing a new vaccine adjuvant," says Taro Kawai, who led the NAIST team.

> **"Innate immunity is also important in anti-tumour immunity and so in future we hope we can also develop anti-tumour adjuvants."**

When a virus invades the body, various parts are recognized by receptor molecules. These trigger inside the cells complex multi-step signalling pathways that activate elements of the innate immune system. It is a general defence system not directed at any specific microorganisms; unlike 'acquired immunity', which produces targeted antibodies against unique invaders.

One of the most prominent aspects of innate immunity triggered by the signalling pathways is the production of interferon proteins. These are so-named because they can interfere with the multiplication of viruses and stimulate other cells into fighting the infection.

Kawai and colleagues at the NAIST Graduate School of Biological Sciences and other research centres in Japan investigated a part of the innate immune response that stimulates the production of type 1 interferon. The pathway leading to this interferon was known to involve two crucial proteins: a transcription factor that regulates gene activity, known as interferon regulatory factor-3 (IRF3); and an enzyme called TBK1 that adds phosphate groups to IRF3 during the interferon-producing pathway.

The researchers say that the detailed mechanisms for regulating what they call "the TBK1-IRF3 axis" remain unclear, but they have been able to identify that ptdIns5P plays a crucial role. In particular, the work has revealed that PtdIns5P acts as a molecule that carries a signal from receptors into other parts of the cell, a process known as "second messenger" activity.

Kawai explains that using this insight to create a new vaccine adjuvant will require a suitable synthetic analogue of PtdIns5P, to make the adjuvant soluble in water and able to penetrate into cells. The team has already made and tested one such analogue and found that it can boost immune responses in mice.

Turning to a wider possibility, Kawai adds, "Innate immunity is also important in anti-tumour immunity and so in future we hope we can also develop anti-tumour adjuvants."

Reference

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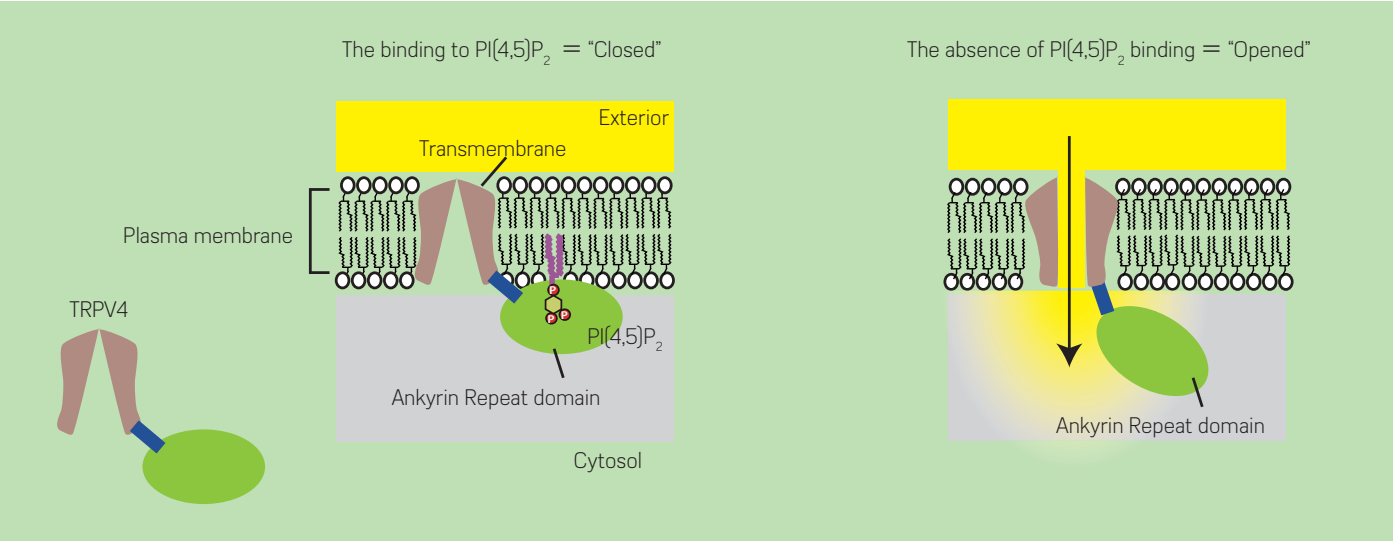
More information about the group's research can be found at the Molecular Immunobiology Laboratory webpage:
http://bsw3.naist.jp/eng/courses/courses209.html



The protein structure of PIP2 in complex with TRPV4 ARD.

Cell biology

Ion channel mutations may cause disease

Mutations in the lipid-binding domain of one ion channel subfamily promotes function and cell death linked to a variety of inherited diseases

Transient receptor potential channels (TRPs) are a large group of ion channels found mostly in the plasma membranes of animal cells. One is TRPV4, a non-selective positive ion channel that allows the passage of several different cation species, such as sodium and calcium (Na+ and Ca2+). It has multiple regulatory sites and is activated by physical stimuli including heat, chemicals and shear stress.

Recent genetic studies on some families have linked mutations in TRPV4's ankyrin repeat domain (TRPV4 ARD) to inherited diseases, such as spinal muscular atrophy (SMA) and Charcot-Marie-Tooth disease. However, the molecular mechanisms underlying the pathogenesis of these diseases are unclear.

Shiro Suestsugu at NAIST and colleagues have now examined the binding affinity of TRPV4 ARD to various membrane lipids using functional assays[1]. They found that TRPV4 ARD binds most strongly to phosphatidylinositol-4,5-bisphosphate ($PI(4,5)P_2$), a membrane lipid known to regulate various intracellular signalling cascades and TRPV4 channel activity. Structural analyses revealed that TRPV4 ARD consists of six ankyrin repeats, which it uses to recognise $PI(4,5)P_2$.

"We decided to study TRPV4 because its mutations have implications for several hereditary diseases," says Suetsugu.

The researchers confirmed that disease-associated mutations cause protein unfolding and, they found, decrease the ability of TRPV4 ARD to bind $PI(4,5)P_2$. The lack of interactions between TRPV4 ARD and $PI(4,5)P_2$, in turn, increases TRPV4 channel activity, raises Ca^{2+} levels and promotes cell death due to increased cytotoxicity. The latter is probably an important factor contributing to the pathogenesis of SMA and Charcot-Marie-Tooth disease.

Suetsugu pointed out that the crystallization of TRPV4 ARD with $PI(4,5)P_2$ head group has been one of the greatest challenges in their study because the domain is relatively unstable.

In a sense, ARD is very much like the 'off-switch' for non-selective cation channels. It suppresses channel activity, prevents cations from permeating through the cell membrane and maintains the balance of Ca^{2+} level within the cell. Understandably, a broken off-switch could lead to all sorts of problems associated with malfunctioned cation channels.

Previous studies have identified ankyrin repeat domains (ARDs) in more than 400 human proteins with diverse binding specificities and affinities to proteins. The finding by Suetsugu and his team represents the first demonstration of the lipid-binding ability of ARD. Moreover, it may have important implications for the development of gene therapy for treating inherited diseases.

"We are very much interested in the functions of other ARDs," says Suetsugu. "Examining these widely expressed but poorly characterised ARDs will be the next step for our study."

Reference

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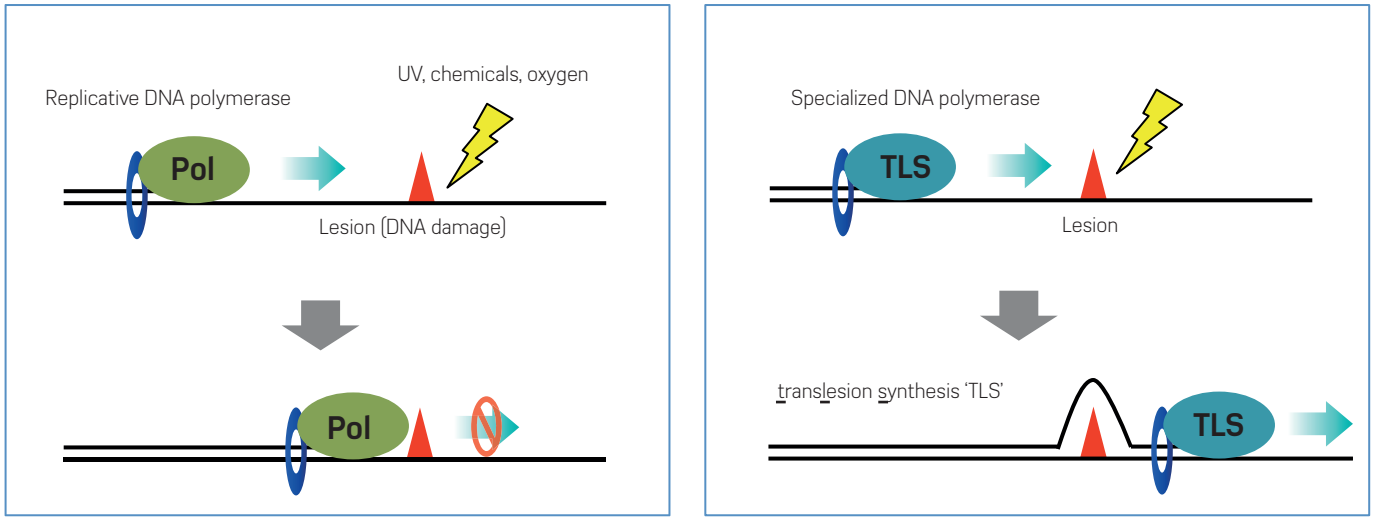
More information about the group's research can be found at the Molecular Medicine and Cell Biology Laboratory webpage:
http://bsw3.naist.jp/suetsugu/?cate=278

Nara Institute of Science and Technology | **Microbial Molecular Genetics Laboratory** www.naist.jp/en



After a replicative DNA polymerase stalls at a lesion site (left), a specialized polymerase for translesion synthesis (TLS) helps overcome the DNA damage (right).

Genetics

Repair enzyme also reboots genome copying

DNA polymerase IV enzyme involved in damage tolerance also aids in DNA synthesis

A special bacterial enzyme involved in repairing the genome also helps restart DNA replication after the process has stalled[1]. The finding, from researchers at NAIST in Japan, could shed light on the source of genetic mutations — a major cause of cancer and other age-related diseases.

When the cell is exposed to a dangerous chemical, sometimes a piece of DNA will form a bond with the chemical agent. This creates what is known as a DNA lesion. These lesions will block the genetic copying machinery, but fortunately the cell has a class of enzymes to deal with these kinds of obstruction.

Humans and other eukaryotes use one set of enzymes, while bacteria and other prokaryotes use another. Through a process known as translesional synthesis (TLS), these specialized enzymes help overcome DNA lesions so that the standard gene copying enzyme can continue its normal function.

In the rod-shaped bacterium *Escherichia coli*, an enzyme called DNA polymerase IV was not thought to be involved in TLS for major lesions (see figure). However, a team led by Hisaji Maki at NAIST, along with collaborators in France and the United States, has now discovered multiple new functions of this enzyme.

> **"Polymerase IV can transiently and efficiently work in the replication fork, instead of the normal type of DNA polymerase."**

The researchers exposed a circular piece of *E. coli* DNA to a compound found in coal tar that forms bonds with DNA. They then recreated the entire DNA copying process, and — in a first — watched what polymerase IV did at the replication fork, the junction where the double-stranded DNA splits apart into two single strands during replication.

"Unexpectedly, we observed that polymerase IV entered into the replication machinery very effectively," says Asako Furukohri, a biochemist at NAIST and one of the co-first authors of the study.

Polymerase IV not only aided with gene synthesis over the damaged DNA — it helped with the entire process of resuming gene copying. "Polymerase IV can transiently and efficiently work in the replication fork, instead of the normal type of DNA polymerase," Furukohri says. "Our finding suggests the possibility that polymerase IV may play some role in genomic DNA replication." Polymerase IV is known to make more copying mistakes than standard replication enzymes, and the mutations it introduces have been linked to drug resistance in bacteria. The human equivalent of polymerase IV has also been implicated in different forms of cancer. Knowing the role of polymerase IV in genomic replication is thus "an important issue," says Furukohri, because it could reveal the genomic triggers of mutation-driven diseases.

Reference

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More infornmation about the group's research can be found at the Microbial Molecular Genetics Laboratory webpage:
http://bsw3.naist.jp/eng/courses/courses301.html

Nara Institute of Science and Technology | **Systems Microbiology Laboratory** www.naist.jp/en

Systems Microbiology

Gene function revealed in *E. coli* library

A new library of thousands of bacterial strains could help scientists understand gene function in cells

The systematic deletion of each gene in one strain of a bacterium widely used in recombinant DNA studies has allowed researchers at Japan's NAIST to create a library that could be crucial in discovering how cells work.

Although many components of the molecular machinery inside cells are now well understood, less is known about how these individual components work together as a system. The genome is the blueprint for this system. However, despite the entire genetic sequences of many organisms having been determined, it is still unclear what each gene does — even in simple organisms like bacteria. Ascertaining gene functions is crucial for gaining a complete understanding of how cells work.

To assist scientists study the functions of individual genes, a team of researchers led by Hirotada Mori at the NAIST Graduate School of Biological Sciences has produced a library of thousands of strains of the K-12 variant of the bacterium *Escherichia coli*; each strain in this library has a single gene deleted[1]. Mori's team used an efficient, state-of-the-art technique to realize this, enabling them to succeed where previous attempts had failed.

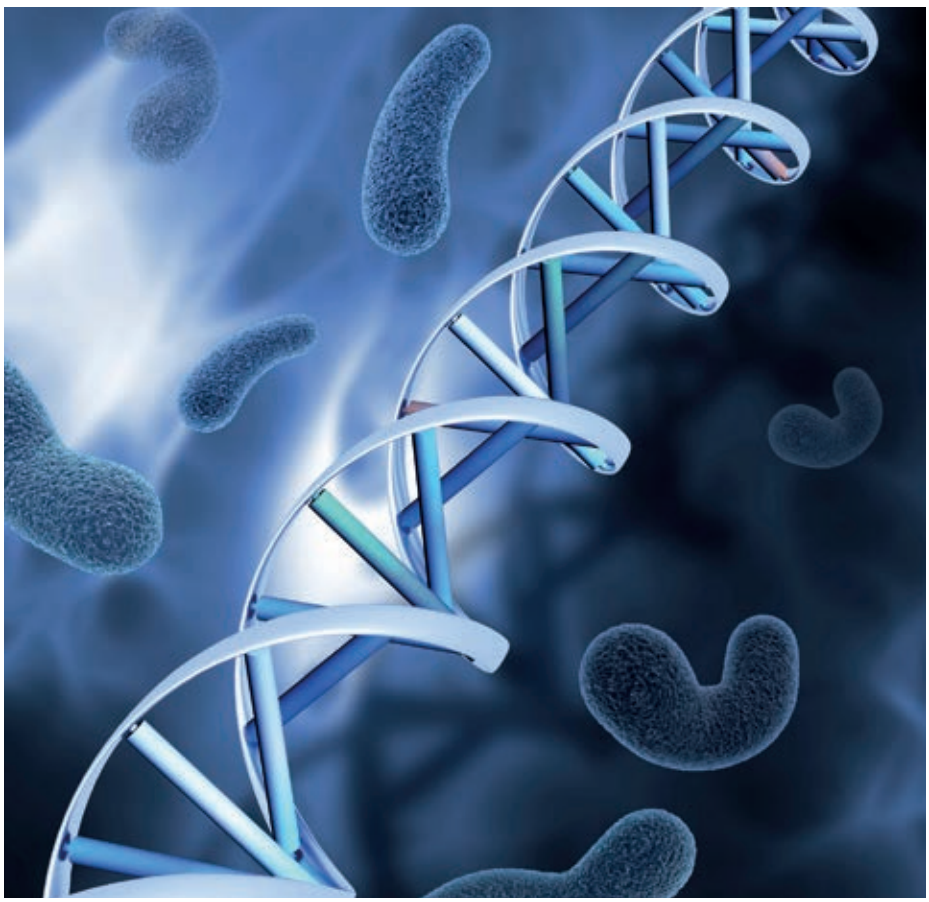
The team substituted one gene at a time with another gene that gave cells resistance to the antibiotic Kanamycin, enabling selective growth of the modified K-12 cells. The modified cells were then collected. The antibiotic-resistance gene can be cut out if it is desired to avoid polar effects — leaving the DNA as though the gene had never existed.

The researchers repeated the process 4,288 times — once for each K-12 gene — and successfully produced 3,985 unique K-12 strains. The failure to produce bacteria in which the approximately 330 remaining genes were deleted indicates that these genes are essential for the cell's survival.

The team collated the bacterial strains into a library, which they called the Keio collection, and made it available to all researchers. "Making these resources open to the public is so important for science," explains Mori. "Research can be performed in parallel across the world, and accumulation of the results will accelerate research to understand cells."

The research not only produced a complete library, but it allowed the team to perfect their technique and build on it. "Three years were required to complete the first library," says Mori, "but experience allowed us to construct a second, improved deletion library in six months. We have also developed a method to generate double-knockout strains of bacteria by combining two deletion strains."

Mori says that these strains with two genes deleted will enable researchers to study the interactions of genes, with the potential to provide even greater insight into how cells work.



Systematic deletion of genes in K-12 bacteria created thousands of different strains that make up the Keio collection.

Reference

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More information about the group's research can be found at the Systems Microbiology Laboratory webpage:
http://bsw3.naist.jp/eng/courses/courses302.html

Nara Institute of Science and Technology | **Laboratory of Cell Signaling** www.naist.jp/en

Cell signaling

Yeast enzyme regulator recognized

A highly conserved cellular pathway is triggered by the presence — and absence — of glucose in yeast

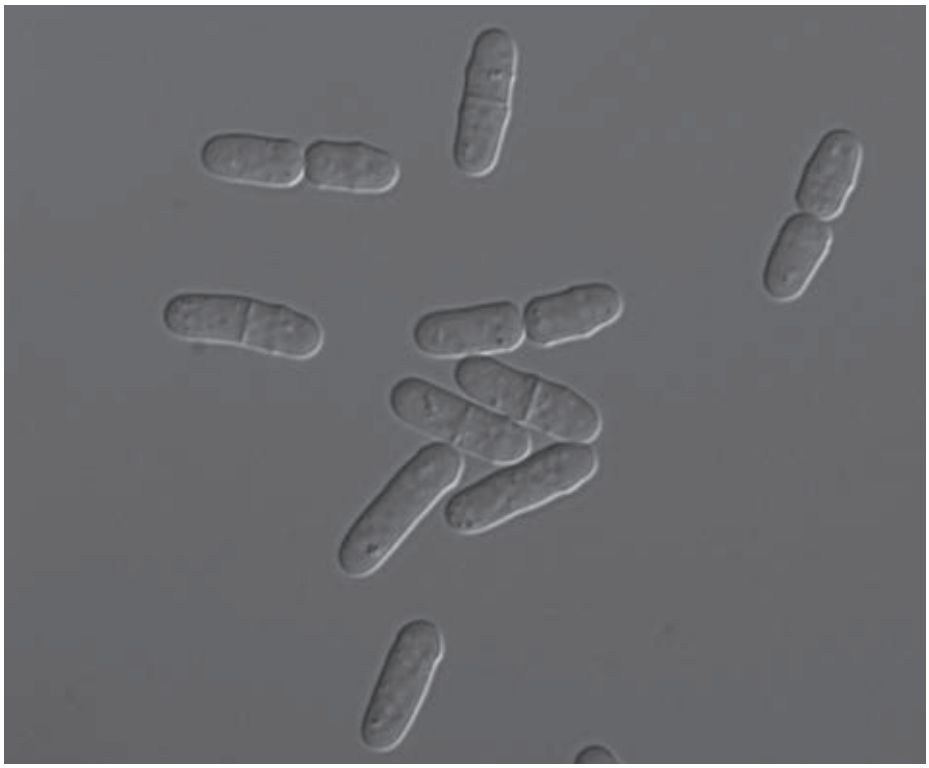
Glucose both positively and negatively controls the activity of an enzyme in a model yeast essential for cell growth, division and stress resistance, find NAIST researchers[1]. This finding could help uncover the regulatory mechanism driving the enzyme's human homologue, which is active in certain cancers.

The enzyme target of rapamycin (TOR) has been conserved during the evolution of various species, indicating its critical importance for the viability of life forms. TOR forms two protein complexes, each of which is controlled by, and controls, a distinct set of upstream and downstream proteins. Recent studies have shown that the essential nutrients nitrogen and carbon activate the TOR complex 1 (TORC1) protein pathway — but the critical trigger for TORC2 had remained elusive.

Previously, the NAIST researchers had demonstrated that a protein belonging to the small GTPase family of enzymes Rhy1 interacts with TORC2 to promote the phosphorylation of another enzyme — Gad8[2]. But they were keen to uncover the whole story. "Nobody knew what stimuli or signals regulate fission yeast Ryh1 and TORC2," explains NAIST's Hisashi Tatebe, who led the study.

After testing the TORC2–Gad8 pathway's responsiveness to nitrogen, which proved tenuous, the researchers considered glucose. They transferred cells of the fission yeast *Schizosaccharomyces pombe* (see figure) from standard growth media containing 3 per cent glucose to media with only 0.02 per cent glucose. Within 5 minutes, the glucose-starved yeast cells stopped dividing and showed no sign of Gad8 phosphorylation.

To identify the role of Ryh1 in the glucose-induced signaling process, the team tried the same experiment with yeast models lacking the Ryh1 protein. Given sufficient glucose in its diet, the mutant yeast showed reduced phosphorylation, confirming the importance of Ryh1 in activating the TORC2–Gad8 pathway.



An important cellular pathway for cell growth, division and resistance to stress in the fission yeast *Schizosaccharomyces pombe* is triggered by both the presence and absence of glucose.

However, after about 30 minutes of surviving under glucose stress, both normal and Ryh1-lacking yeast strains made a surprising recovery, phosphorylating Gad8 at rates similar to those under glucose abundance. This finding suggests an alternative, Ryh1-independent, mechanism of TORC2–Gad8 activation, triggered by the virtual absence of glucose.

"It was totally unexpected that the fission yeast TORC2 pathway is regulated by the paired positive and negative regulatory mechanisms," says Tatebe.

The researchers will conduct genetic screens in yeast to investigate the mysterious resurgence and are eager to find a small GTPase in humans that plays a similar regulatory role to Ryh1 in yeast. "TORC2 activity is indispensable in various cancer cells, but not in normal cells," says Tatebe. "Our findings may offer many clues about TORC2 in humans."

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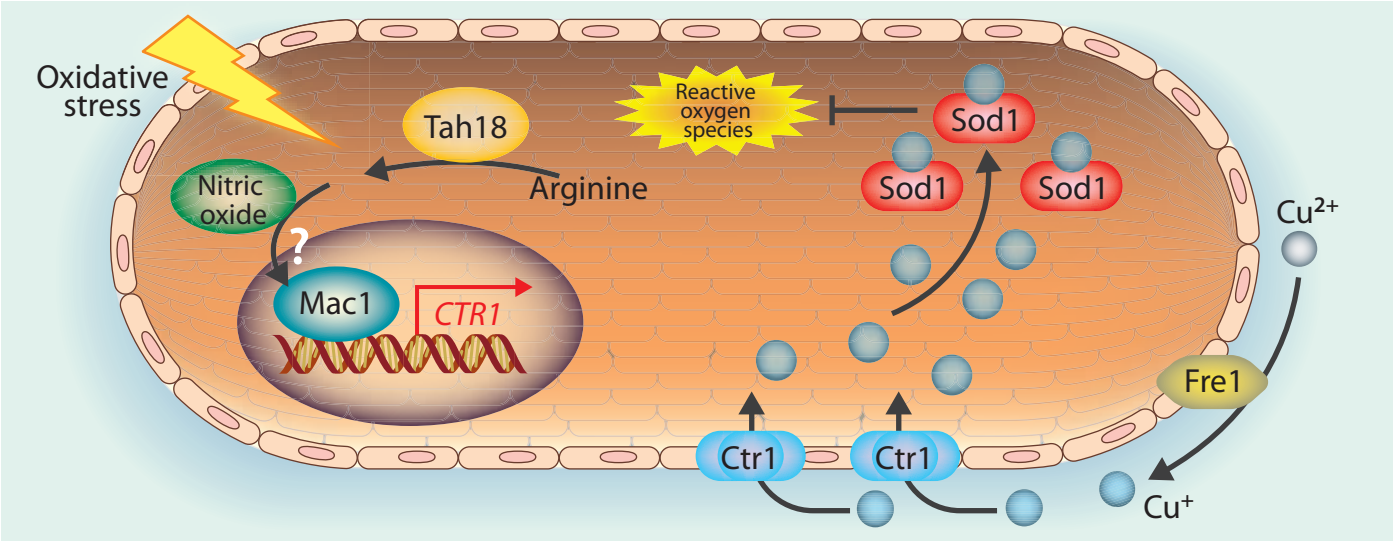
More information about the group's research can be found at the Laboratory of Cell Signaling webpage:
http://bsw3.naist.jp/shiozaki/?cate=207

Nara Institute of Science and Technology | **Laboratory of Applied Stress Microbiology** www.naist.jp/en



Nitric oxide (NO) produced by yeast cells in response to oxidative stress triggers an influx of copper (Cu) through the activity of the regulatory protein Mac1.

Microbiology

Yeasts with better stress tolerance

Copper helps yeast deal with temperature extremes, pointing to gains in fermentation and biofuels

Yeast responds to the stress of high temperatures by activating a genetic pathway involved in copper uptake and metabolism, researchers at Japan's NAIST have found[1]. The discovery could help bioengineers develop heartier yeast varieties for use in the food and biotechnology industries.

"Environmental stresses induce growth inhibition or cell death, which limits the fermentation ability of yeast," says Hiroshi Takagi, a molecular microbiologist at NAIST who led the study. "Our findings could contribute to the construction of new yeast strains with higher stress tolerance, leading to the effective production of breads, alcoholic beverages and bioethanol."

In 2013, Takagi and his colleagues discovered a novel mechanism by which the budding yeast *Saccharomyces cerevisiae* — widely used in wine-making, baking and brewing — deals with the potential damage wrought by outside pressures such as elevated temperatures. They found that the microorganism produces nitric oxide (NO), a molecule that confers protection against the damaging reactive oxygen species created in the wake of extreme heat[2]. Takagi's team showed that a protein called Tah18 was involved in NO production, but the mechanism underlying how NO aids in stress tolerance remained unclear.

The researchers therefore analyzed the gene expression profile of yeast cells exposed to NO in the laboratory. They have now discovered that genes involved in the transport of heavy metal ions, such as copper, are activated by the NO treatment. These genes are under the control of a regulatory protein called Mac1. Since Mac1 is important for tolerance to other types of stress, Takagi's team suspected that it might play a role in NO-mediated stress responses as well.

Indeed, the researchers found that NO produced under stressful conditions of high temperatures activated Mac1 in the yeast. This set in motion a molecular cascade that increased the amount of copper inside the cell; and the influx of copper in turn triggered an enzyme called Sod1, a type of 'superoxide dismutase' that destroys toxic free radicals and thus helps to boost cell viability (see figure).

Putting all the pieces together, Takagi says: "Our main conclusion of this work is that NO enhances the activity of Sod1, which is one of the most important antioxidative enzymes." This knowledge could pave the way for synthetic biologists to craft stress-resistant yeast strains for the fermented breads, beverages and biofuel sectors[3]. These designer yeast strains could be a huge economic boost for the fermentation industries.

References

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More information about the group's research can be found at the Laboratory of Applied Stress Microbiology webpage:
http://bsw3.naist.jp/eng/courses/courses305.html

Nara Institute of Science and Technology | **Structural Biology Laboratory** www.naist.jp/en

Plant growth

Receptor role in protection unmasked

How a plant hormone receptor helps a vital process necessary for maintaining healthy plant growth is finally understood

Hormones play a crucial role in regulating the growth and development of plants. One group, called gibberellins (GAs), is involved in seed germination and promoting new cells in stems, leaves and flowers. Only in the past decade have researchers begun to uncover the precise mechanisms involved in GA signalling in plants, because very little has been known about the structure and behaviour of GA receptors.

In 2008, researchers at NAIST, working with scientists in the United States, established the first structural model of a gibberellin receptor called GID1A, found in thale cress plants (*Arabidopsis thaliana*), and with that revealed a key process involved in protecting healthy plant development[1].

There are 136 known GAs, but only a few of them function as hormones; those that do are known as bioactive GAs. Without the presence of bioactive GAs, plants are stunted and dwarfed; but if too much is present, the cells in the plant stems are elongated – producing plants that are tall and often infertile.

Toshio Hakoshima and NAIST co-workers conducted a series of crystallographic and biochemical studies of GID1A and observed how it is activated by binding to a bioactive GA. Their aim was to verify how GA and GID1A both bind to, and trigger, the degradation of a negative transcriptional regulator of GAs called GID1-DELLA. DELLA proteins can disrupt the regulation of GA, causing significant damage to plant growth.

"Hakoshima and his team deepened understanding of how cellular functions can be regulated by signalling from hormones and their receptor molecules."

They found that GID1A has a compact form with a so-called 'N-terminal extension', as well as a pocket for GA incorporated into its structure (see figure). The N-terminal senses GA and, once GA binds to GID1A, folds back on itself to cover the GA pocket. In this way, the N-terminal acts as a shield to protect GA.

Following the initial binding, GA activates GID1A, triggering conformational change – an alteration in the shape of the macromolecule – in the folded N-terminal extension, creating a binding surface for the GID1-DELLA protein. Once GID1-DELLA is bound to GID1A, the receptor sends out an explicit signal, targeting GID1-DELLA for degradation by other proteins.

Through this work, Hakoshima and his team deepened understanding of how cellular functions can be regulated by signalling from hormones and their receptor molecules. Their findings could help influence crop-selection and the manipulation of plant growth. For example, it may be possible to develop derivatives of GAs to use as growth regulators in some crop plants.

Reference

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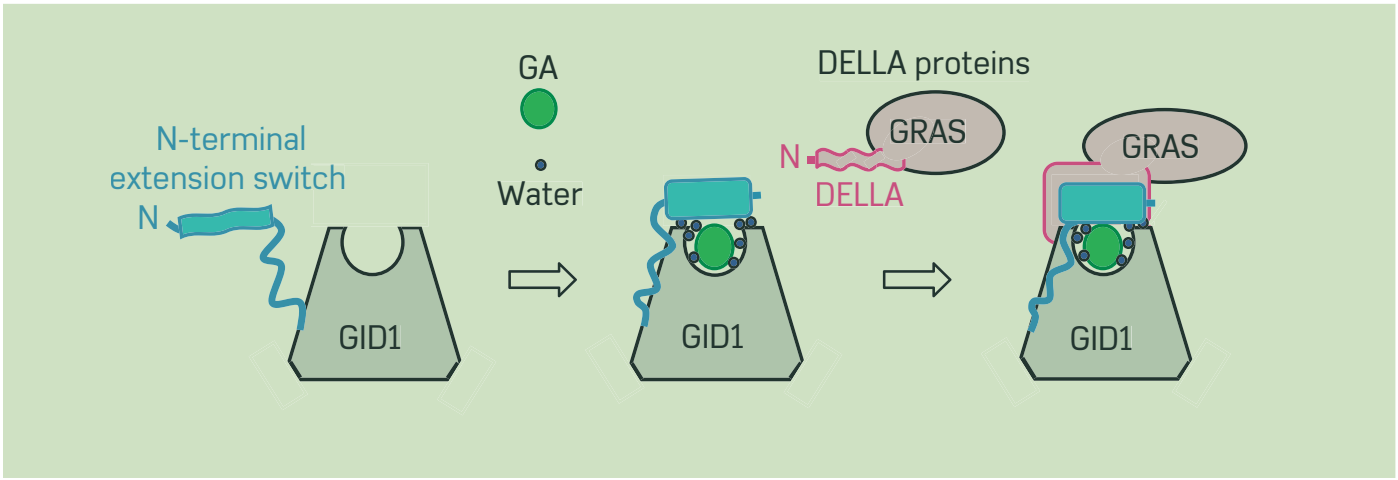


The structural mechanism by which receptor GID1A binds with gibberellin hormones (GAs) and subsequently binds to and targets GID1-DELLA proteins for degradation. The process helps protect correct plant growth and development.

More information about the group's research can be found at the Structural Biology Laboratory webpage:
http://bsw3.naist.jp/eng/courses/courses306.html

Nara Institute of Science and Technology | **Membrane Molecular Biology Laboratory** www.naist.jp/en

Cell function

Protein groove aids membrane assembly

The crystal structure of a membrane protein in soil bacteria is described for the first time

Cell membranes are semi-permeable barriers, consisting of a bilayer of lipids with embedded proteins that perform vital functions. These proteins are so important that they have become the target of 60 per cent of the medicinal drugs used today.

For newly synthesized membrane proteins to properly function, they must be accurately inserted, folded and assembled into the membrane.

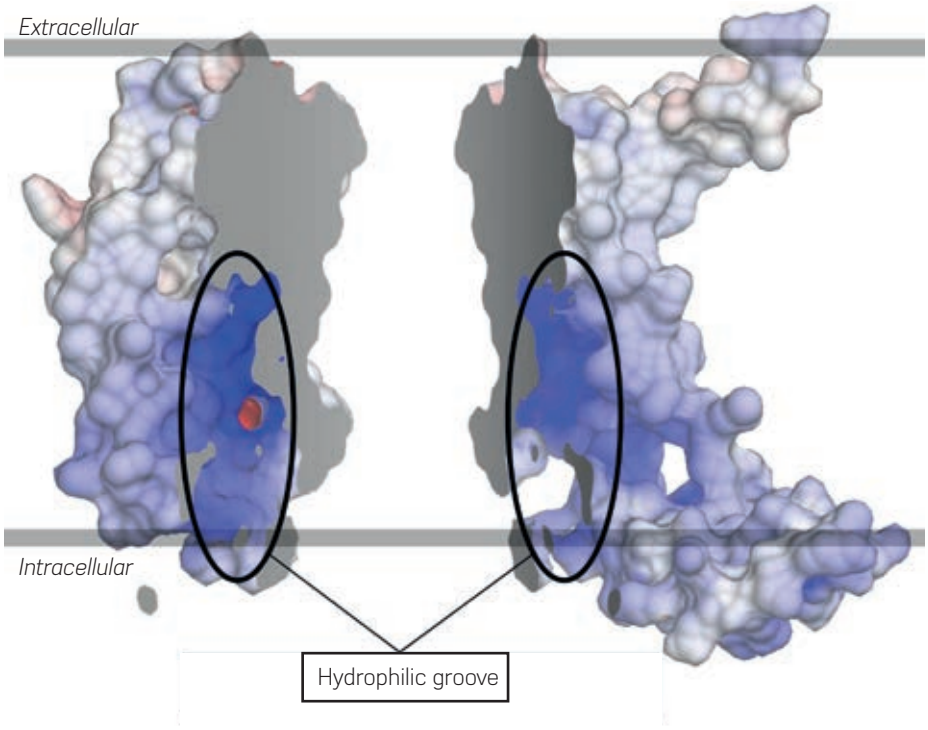
To help understand how this process occurs, Tomoya Tsukazaki and colleagues at NAIST described, for the first time, the crystal structure of YidC, a protein in the cytoplasmic membrane of *Bacillus halodurans*, a gram-positive bacterium found in soil[1]. "We determined the first high-resolution structure of the YidC–Oxa1–Alb3 family of membrane proteins, and we propose an insertion mechanism for membrane proteins," says Tsukazaki.

"Our findings pave the way for further investigations into the molecular mechanism of YidC."

YidC is involved in the folding and insertion of many membrane proteins, including one involved in the release of energy, and is thus essential for cell viability.

Tsukazaki's team collected diffraction data on YidC crystals at Japan's SPring-8 synchrotron facility, and refined the structure to a 2.4 ångström resolution. They then created computer simulations mimicking the physical movement of YidC in a lipid bilayer. To better understand YidC's role, they compared the effects of mutating the *yidC* gene to the effects of mutating another gene involved in the membrane insertion process.

Previous studies suggested YidC formed a channel that facilitated membrane insertion of protein substrates, says Tsukazaki. His team's investigations revealed, instead, that YidC contains a novel fold in which five independently functioning transmembrane helices form a groove that is open toward the lipid bilayer and cytoplasm, but closed on the extracellular side. The groove has an affinity for water (is hydrophilic) and positively charged (see figure).



The membrane protein YidC's hydrophilic, positively charged groove facilitates membrane insertion of proteins.

A substrate protein interacts with the positively charged cytoplasmic region of YidC, and is then captured in YidC's hydrophilic groove; the positively charged groove attracting the negatively charged residue on the substrate. An arginine residue that exists in the groove participates in recognizing the substrate protein, which is then released by the groove into the membrane, partially facilitated by the membrane potential attracting the negatively charged residues on the substrate.

"Our findings pave the way for further investigations into the molecular mechanism of YidC," says Tsukazaki.

"YidC also facilitates the proper folding of membrane proteins inserted by a protein-conducting channel — the SecYEG translocon — and is essential for cell viability. We are trying to determine the structure of the complex of YidC and Sec proteins in order to fully understand the conserved mechanism of membrane protein biogenesis."

Reference

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More information about the group's research can be found at the Membrane Molecular Biology Laboratory webpage:
http://bsw3.naist.jp/eng/courses/courses309.html

Nara Institute of Science and Technology | **Gene Regulation Research Laboratory** www.naist.jp/en

The axial skeleton of a newborn mouse.

Embryology

Signal loop sets vertebrae numbers

A signalling feedback loop regulates the molecular clock that controls the number of vertebrae in backbones

Researchers at NAIST are unravelling the regulatory mechanism determining the number of vertebrae formed in the embryo, a critical process in the development of back-boned animals.

Embryonic development in animals includes a carefully choreographed interaction between a moving chemical gradient and a ticking molecular clock to measure out segments (called somites) along the body's length. The segmentation clock involves oscillations in gene activity that regulate a principal cell-to-cell communication mechanism, known as the Notch signalling pathway, as well as Notch intracellular domain (NICD). By tuning these oscillations, each species develops a specific number of somites, determining how many vertebrae it has.

> **"I feel that the Nara Institute has an atmosphere in which we can wrestle with fundamental questions."**

To better understand this process, a team of researchers at NAIST and other institutes investigated the role of a negative Notch regulator, Nrarp. Since Notch controls the expression of Nrarp, the two form a feedback loop regulating NICD levels. The researchers studied mice lacking Nrarp to discover how this feedback loop regulates somite formation.

"Nrarp knock-out mice are almost normal, but they have small anomalies in their axial skeletons," says Yasumasa Bessho, from NAIST's Graduate School of Biological Sciences. In addition to these defects, the mutant mice have fewer vertebrae than normal. Measuring the segmentation clock showed that it ran slightly slower in the mutant mice, a decrease that was linked to higher Notch levels.

When the team used a drug to reduce Notch levels, the segmentation clock ran faster and more somites were formed. Based on their findings, the researchers speculate that the Notch-Nrarp feedback loop provides robustness to the segmentation clock.

During somite formation, "most of the cells are synchronized with the gene oscillations, but some of them are out of phase because of perturbations from the environment, such as chemical agents or temperature changes, or intrinsic perturbations, such as cell division," explains Bessho. Since the out-of-phase cells don't disrupt development, something must resynchronize them with their neighbours. Bessho and his colleagues suspect that Nrarp plays an important role in this resynchronization mechanism.

The team is now testing this hypothesis by checking whether Nrarp knock-out mice are more sensitive to a drug that causes embryonic abnormalities. So far, the results suggest that Nrarp is necessary for robust somite development, but research is still ongoing.

"Nowadays, this kind of basic research may be neglected," says Bessho. "However, I feel that the Nara Institute has an atmosphere in which we can wrestle with fundamental questions."

Reference

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More information about the group's research can be found at the Gene Regulation Research Laboratory webpage:
http://bsw3.naist.jp/eng/courses/courses308.html

Nara Institute of Science and Technology | **Laboratory of Systems Neurobiology and Medicine** www.naist.jp/en

Neurobiology

How nerve cell axons become mobilised

A signalling pathway stimulated in the tips of growing axons triggers the generation of traction forces between an axon and its substrate

Axons are the long, slender projections of the neurons that make up the nervous system and conduct electrical impulses. As they grow towards their targets in the developing nervous system they need to generate traction forces, which allow them to grab onto their substrate and extend themselves to their final location. How do they do it?

A research team led by Naoyuki Inagaki at Japan's Nara Institute of Science and Technology has found that chemo-attractant molecules activate a signalling pathway in growing axons, allowing the generation of mechanical forces that permit axons to propel themselves toward their destination.

Netrin is an extracellular chemo-attractant molecule that induces the growth of developing axons and the researchers thought this could be because netrin induces traction force generation within the tip of the axon. To test this, Inagaki and colleagues grew neurons on elastic gel embedded with fluorescent beads in the presence of netrin and observed the displacement of the beads. This suggested that the axons were grabbing on to the substrate in order to propel themselves forward.

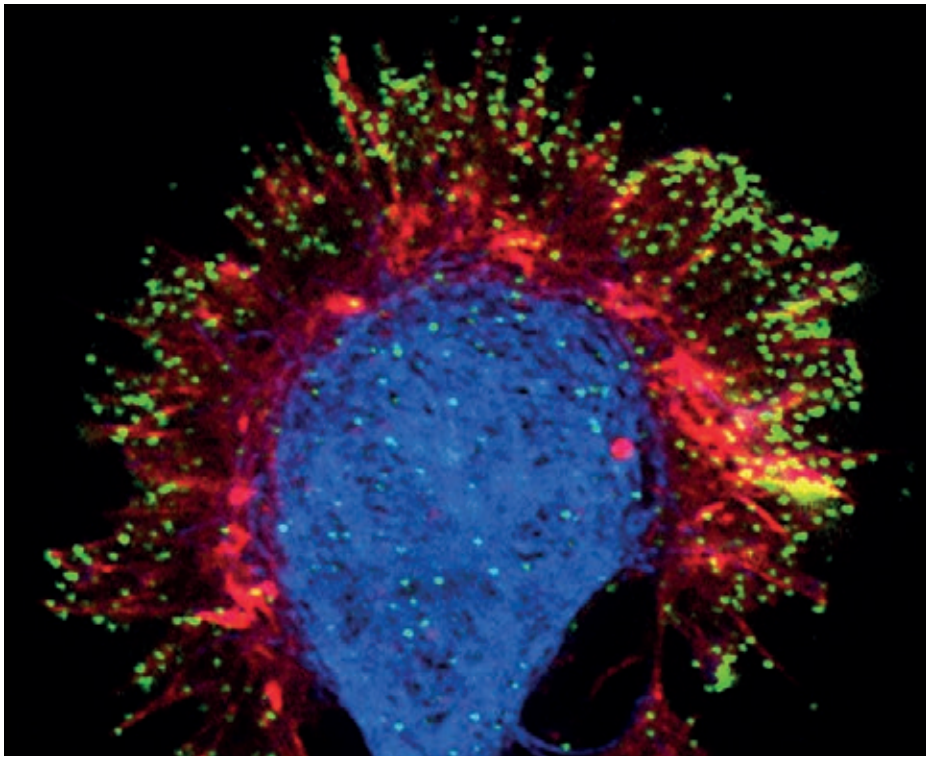
Netrin activates a wide range of intracellular signalling molecules, some of which — such as actin — may be responsible for strengthening the link between structural molecules within the axon and the substrate on which the axon is growing. Pak1 is an enzyme within the axon tip that adds phosphate groups to other proteins to regulate their activity and shootin1 is a linker molecule that couples actin to the substrate.

> **"Our findings provide a key molecular mechanism for understanding the mechanical forces that are involved in human brain development."**

The researchers found that Pak1 can add phosphate groups to shootin1 and that this addition could be increased by adding netrin to the neuronal cultures. When shootin1 contains these phosphate groups that have been added by Pak1, it leads to the strengthening of the link between actin and the substrate.

When Inagaki's team lowered the expression of shootin1 within the neurons, the growing axons seemed to slip on their substrate and netrin was not as effective at inducing axonal outgrowth. If they expressed a modified Pak1 that blocked its ability to add phosphate groups to shootin1, axon growth was also inhibited. However, when they expressed a modified shootin1 that already contained these phosphate groups, traction force generation and axon outgrowth could be restored.

Taken together, the data indicate that the generation of the mechanical forces required for axons to be able to grow requires the induction of a signalling pathway that involves the activation of Pak1 and shootin1. "Our findings provide a key molecular mechanism for understanding the mechanical forces that are involved in human brain development," says Inagaki.



Naoyuki Inagaki

A fluorescence image of an axonal growth cone (green, shootin1 phosphorylated by Pak1; red, actin filaments; blue, microtubules), showing the sites where the signal-to-force transduction for axon outgrowth occurs.

Reference

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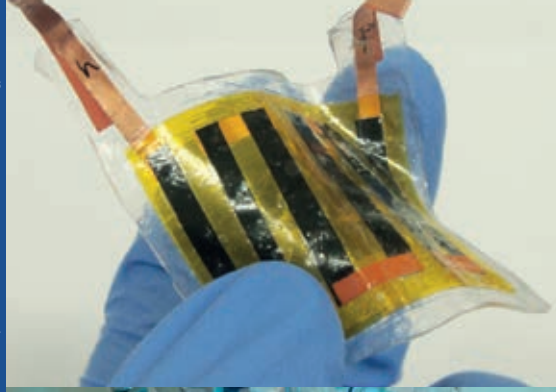
More information about the group's research can be found at the Inagaki Lab webpage:
http://nippon.naist.jp/inagaki_g/english/



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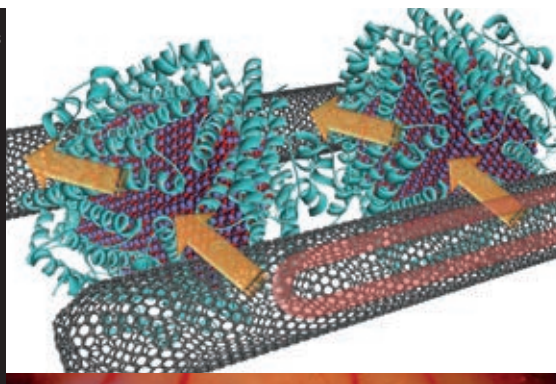
Materials Science

Research Highlights



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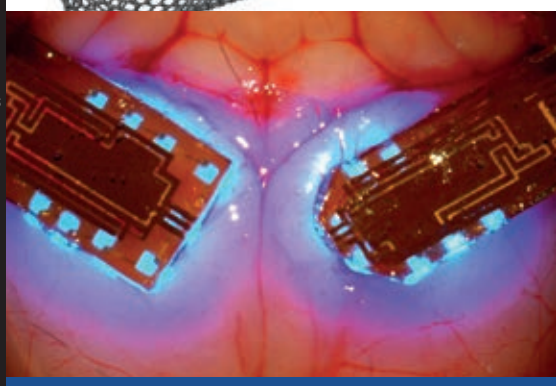

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Nara Institute of Science and Technology | **Quantum Materials Science Laboratory** www.naist.jp/en

Optical devices

Making simpler, cheaper organic crystals

Molecular materials could simplify the fabrication and reduce the cost of lasers

Cheap and efficient sources of light could play an important role in future communication systems. NAIST researchers have now synthesized molecular crystals that could be used in bright, but easily manufactured, laser devices[1].

Most optoelectronic light sources, such as light-emitting diodes and lasers, are made from inorganic materials such as gallium arsenide, which are expensive. Organic-molecule-based materials offer a cheaper alternative because they can be produced by solution and printing processes rather than complicated methods like the deposition of crystalline overlayers on substrates, known as epitaxy. As an added bonus, organic-based devices can be fabricated on curved or even flexible substrates.

But much development is still needed before these sources match the performance and versatility of their inorganic counterparts. An electrically powered laser made using a molecular active region is particularly challenging, for example.

Hisao Yanagi from NAIST's Quantum Material Science Laboratory, working with colleagues in Japan and Italy, have now synthesized a novel organic-molecule-based material, and demonstrated that it can produce laser light, paving the way to an electrically driven molecular source of intense optical radiation.

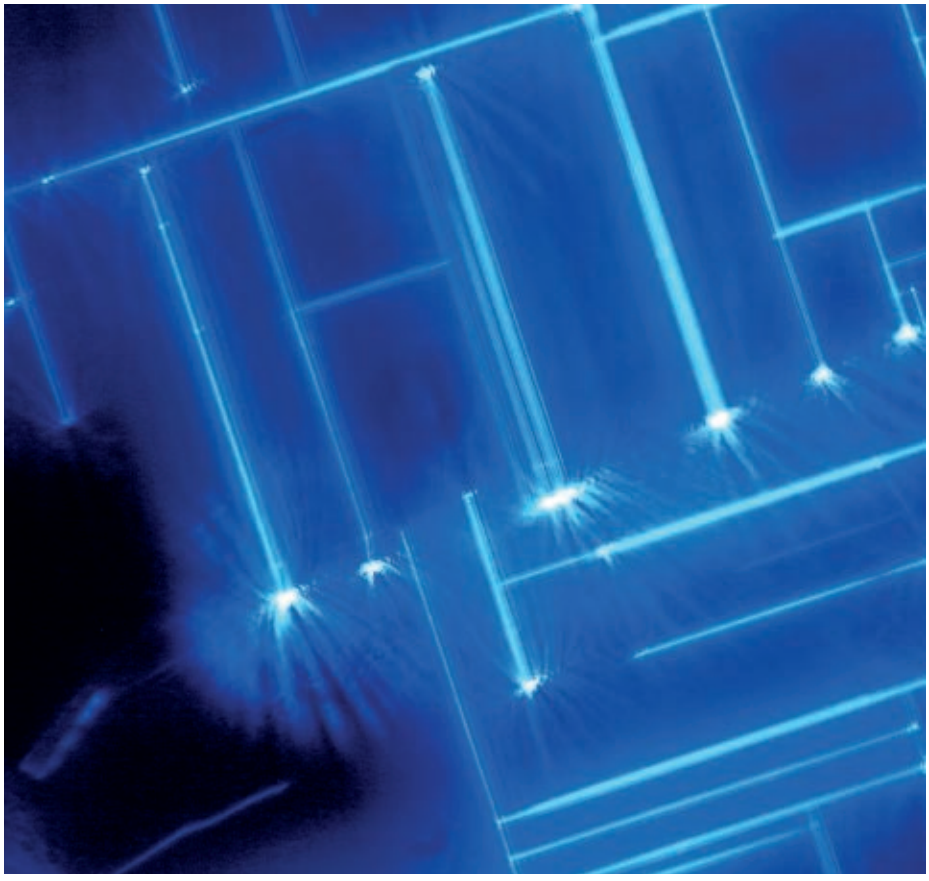
"One of the advantages of molecular materials is that their emission colour is tunable by molecular modification," explains Yanagi. "So we prepared blue-light-emitting molecular crystals and investigated their laser properties."

The researchers chose to use a molecule in a class of organic semiconductors known as thiophene/phenylene co-oligomers (TPCOs). Scientists are focusing a great deal of attention on TPCOs because they are robust and have excellent semiconducting properties.

Yanagi's team observed spectrally pure and bright emission with a wavelength of 500 nanometres when they illuminated their sample with ultraviolet radiation. This so-called optically pumped lasing is the first step in the development of electrically driven devices.

The most important part of many semiconductor light emitters is the p–n junction: an interface between a positively and negatively charged regions where the light is produced. These charge regions arise in inorganic materials because of atomic impurities. In molecular materials, however, p and n regions are created by adding 'electron accepting' or 'electron withdrawing' molecular groups to the active molecule.

Most previous TPCOs have been p-type. Yanagi and co-workers managed to create an *n*-type organic semiconductor crystal by substituting in cyano groups, creating a molecular crystal called BP1T-CN. This work is the first demonstration of optically pumped lasing in an n-type TPCO. "We next hope to fabricate light-emitting diodes with this material," says Yanagi.



NAIST researchers hope to soon fabricate light-emitting diodes based on BP1T-CN, an n-type TPCO organic semiconductor crystal, which emits bright blue light when illuminated with ultraviolet radiation.

Reference

1. Mizuno, H., Maeda, T., Yanagi, H., Katsuki, H., Aresti, M. *et al.* Optically pumped lasing from single crystals of a cyano-substituted thiophene/phenylene co-oligomer. *Advanced Optical Materials* **2**, 529–534 (2014).

More information about the group's research can be found at the Quantum Materials Science Laboratory webpage:
http://mswebs.naist.jp/LABs/optics/eng/index-e.html

Nara Institute of Science and Technology | **Surface and Materials Science Laboratory** www.naist.jp/en

Semiconductors

120-fold boost in photoluminescence

Analysing and refining semiconductor surfaces dramatically increases the efficiency of luminescent devices

Careful preparation of semiconductor surfaces can greatly enhance the efficiency of semiconductor materials widely used in light-emitting diode (LED) displays and many other applications, according to research conducted at NAIST in Japan. "Luminescence efficiencies can be increased by more than 100 times," says Ken Hattori, one of the researchers in the study.

Hattori and colleagues at NAIST, working with collaborators at Osaka University, investigated the semiconductor gallium nitride. "Blue light from gallium nitride is now used everywhere in displays and other applications," says Hattori. However, typically only a very thin surface region — no deeper than a few hundred nanometres — emits light. This is because material near the surface reabsorbs light created deeper in the semiconductor.

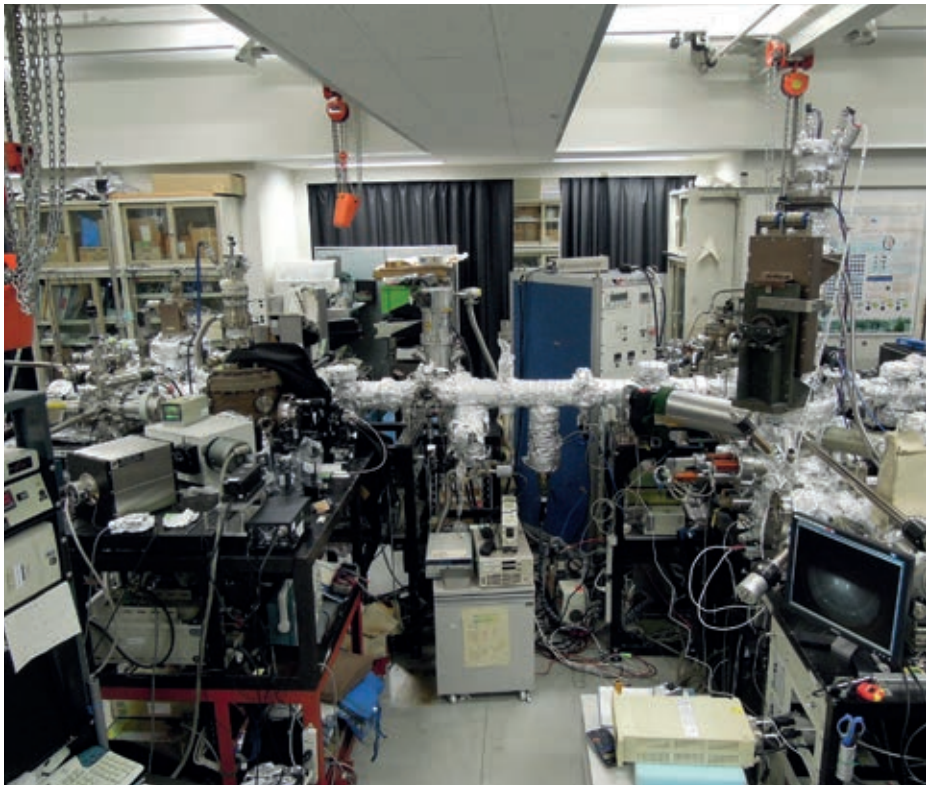
> **"Improving the quality of the layers near the gallium nitride surface will be key to making much higher performance luminescence devices."**

Mechanical polishing is currently the final stage in the commercial production of gallium nitride crystals, but this process can damage layers in the crucial region near the surface. The relationship between the quality and chemistry of the surface layers, and the material's luminescence efficiency, had not been well studied, a deficiency this research addressed.

The team used sophisticated surface-science techniques to examine the effects of removing physically damaged layers and oxide-containing layers under ultrahigh vacuum conditions. These techniques included specialized chemical etching and physical treatments with ultrapure water and gas. The researchers also investigated the role of hydrogen contamination, since hydrogen can penetrate crystals from solutions used in their production.

The team found that removing physically damaged layers and oxides near the surface and reducing the concentration of hydrogen in the crystals resulted in an approximately 120-fold improvement in photoluminescent efficiency[1]. While semiconductor manufacturers have already attempted to remove damaged and oxide-containing layers, they used a less sophisticated process that is clearly much less efficient than that developed by the NAIST team and their colleagues.

"Improving the quality of the layers near the gallium nitride surface will be key to making much higher performance luminescence devices," explains Hattori. Given the plethora of LEDs and similar systems in all areas of modern technology, this research could lead to considerable industrial and economic benefits. The researchers believe that the findings could be applied to other semiconductor materials. Their next step is to conduct similar studies on other semiconductors to see if the striking enhancements achieved with gallium nitride can be replicated more generally. NAIST has one of the biggest complex surface-analysis systems in the world (see figure). "This gives us significant advantages," says Hattori.



The NAIST complex surface-analysis system is one of the biggest and most sophisticated in the world.

Reference

1. Hattori, A. N., Hattori, K., Moriwaki, Y., Yamamoto, A., Sadakuni, S. *et al.* Enhancement of photoluminescence efficiency from GaN(0001) by surface treatments. *Japanese Journal of Applied Physics* **53,** 021001 (2014).

More information about the group's research can be found at the Surface and Materials Sciences Laboratory webpage:
http://mswebs.naist.jp/LABs/daimon/index-e.html

Nara Institute of Science and Technology | **Advanced Polymer Science Laboratory** www.naist.jp/en

Polymer chemistry

Handedness theory usurped

Right circularly polarized light does not necessarily give left-handed molecules

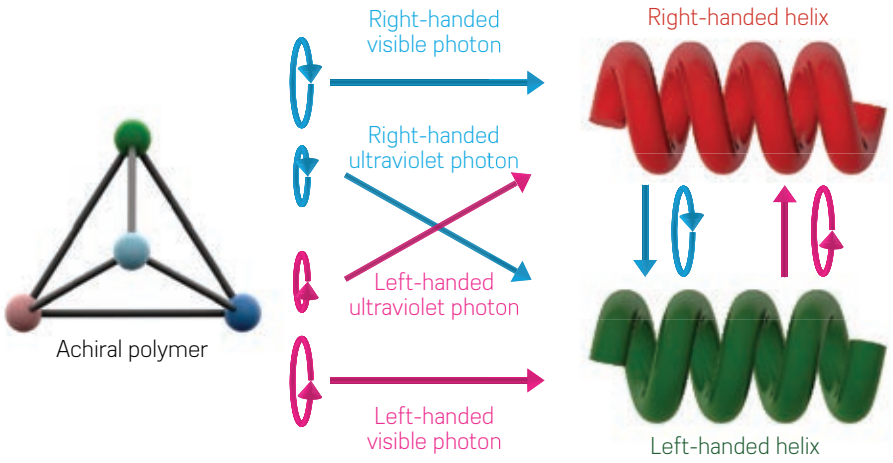
It had long been accepted that right circularly polarized light is needed to produce right-handed polymer molecules. But to the surprise of researchers at NAIST, right circularly polarized light can also give rise to left-handed polymer molecules, and vice versa: polymer handedness depends on other factors, including the wavelength of the light and the refractive index of the solvent.

Chemists often want to produce either a left- or right-handed version of a chemical. The two forms, which are known as enantiomers, are identical except that they are mirror images of each other — like left-handed and right-handed gloves. However, they react very differently with other enantiomers, which are frequently found in biological systems.

"The researchers anticipate that their findings will lead to the production of various photoluminescent substances and polymeric materials that can be used for re-writable optical memory, efficient erasure, and long-term data storage."

An attractive way to produce enantiomers is to use circularly polarized light, as this method can induce handedness without using expensive chemicals. Up until now, conventional wisdom had dictated that left-handed circularly polarized light preferentially produces left-handed molecules, and vice versa. Furthermore, the handedness of a molecule was thought to be independent of the wavelength of the light used. Now, Michiya Fujiki and co-workers at NAIST have overturned both these assumptions[1].

Using a green-emitting chain-like polymer known as PF8T2, the researchers found that the handedness of PF8T2 depends on both the wavelength and the handedness of the circularly polarized light used. Specifically, they found that when a right-handed circularly polarized light in the visible region is used, right-handed PF8T2 was produced; whereas when right-handed circularly polarized light in the ultraviolet region was used, left-handed PF8T2 was formed (see figure).



NAIST researchers find that left circularly polarized light can give rise to right-handed molecules and vice versa.

"In the future, we will try to induce and control the helix sense of polymers and the chirality hand of small molecules with a 100 per cent yield driven by circularly polarized light, spinning electron beams, anti-neutrinos and magnetic and electric fields."

They also found that, by precisely controlling the refractive index of an inexpensive organic solvent, they could increase the yield of PF8T2 up to 10 per cent — the highest yield ever reported for an enantiomer using this light-based synthesis method.

The researchers anticipate that their findings will lead to the production of various photoluminescent substances and polymeric materials that can be used for re-writable optical memory, efficient erasure, and long-term data storage. They also expect that the findings will facilitate efficient switching using a circularly polarized light source once a detector for circularly polarized light has been developed.

"In the future, we will try to induce and control the helix sense of polymers and the chirality hand of small molecules with a 100 per cent yield driven by circularly polarized light, spinning electron beams, anti-neutrinos and magnetic and electric fields," Fujiki states. "This has probably been the ultimate goal of chirality-related scientists in chemistry and physics over the last two centuries."

Reference

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More information about the group's research can be found at Michiya Fujiki's laboratory webpage:
http://mswebs.naist.jp/LABs/fujiki/english/index-e.html

Nara Institute of Science and Technology | **Photonic Device Science Laboratory** www.naist.jp/en

Photonics

Implant sensor images brain activity in vivo

Image sensor implant makes it possible to record the neural activity of a living mouse as it moves

A fluorescence imaging system that can be implanted in the brains of living and freely moving mice allows the neural activities of mice to be monitored during different behaviours. The system, which was developed by researchers at NAIST in Japan, has the potential to provide fresh insights into how neural networks function in the brain.

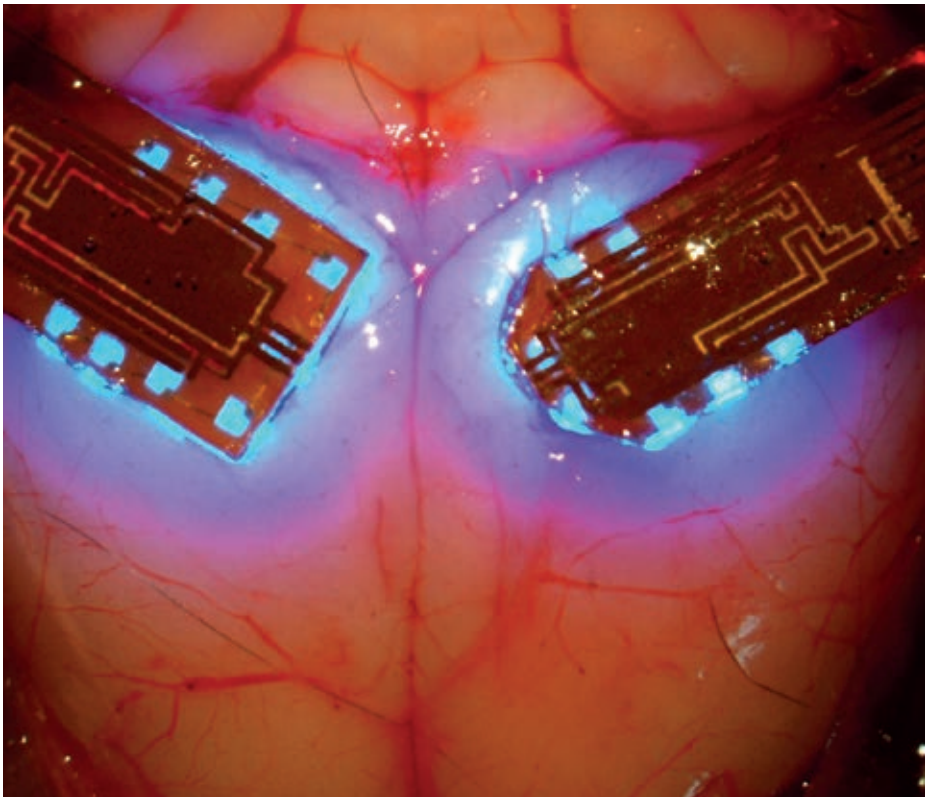
The implantable fluorescence imaging system, fabricated by Jun Ohta of NAIST's Graduate School of Materials Science and his colleagues, consists of a set of blue/green light-emitting diodes (LEDs) and a tiny image sensor — a smaller and simpler version of those commonly found in digital cameras. The image chip contains 120 × 268 pixels and is just 1 millimetre wide and 3.5 millimetres long.

Light from the LEDs excites fluorescence in brain cells that have been doped with a voltage-sensitive dye. Consequently, the intensity of the excited fluorescence varies with changes in the membrane potential, which is related to neural activity. The emitted fluorescence is then captured by the image sensor and transferred via a cable to a computer and additional electronics where it is analysed. This is performed at a rate of 60 frames per second, enabling movies of neural activity to be recorded.

> **"In contrast, the NAIST imaging system can function even when the subject is moving."**

While techniques such as conventional fluorescence microscopy and functional magnetic resonance imaging are capable of recording activity in different parts of the brain, they require the subject to remain perfectly still. In contrast, the NAIST imaging system can function even when the subject is moving.

"The system we have developed makes it possible to take real-time images [of the brain activity] of small experimental animals such as mice and rats in a freely moving condition," explains Ohta, "which is important for studying learning and memory functions."



Two image sensor chips implanted in the two visual cortices of the brain of a mouse.

By installing two sensors in the left and right hemispheres of the brain, the team has mainly used the system to study activity in the visual cortex of mice. However, Ohta says the system could be used to investigate other functional areas of the brain.

The researchers have several ideas for future work. One idea is to create a wireless system; this would eliminate the need to have a cable connected to the animal, which considerably limits its movement. Another plan is to develop a microimaging device that can be implanted in the limbic system — a deep brain region associated with emotion and addiction and hence important for studying mental diseases.

Reference

1. Novel implantable imaging system for enabling simultaneous multiplanar and multipoint analysis for fluorescence potentiometry in the visual cortex. Kobayashi, T., Motoyama, M., Masuda, H., Ohta, Y., Haruta, M. *et al. Biosensors and Bioelectronics* **38,** 321–330 (2012).

More information about the group's research can be found at the Photonic Device Science Laboratory webpage:
http://mswebs.naist.jp/LABs/pdslab/index-e.html

Nara Institute of Science and Technology | **Information Device Science Laboratory** www.naist.jp/en

Data storage

Improving memory using hot spots

Localized hot spots are the key to the operation of low power consumption electronic memories

An increasing reliance on electronics for processing information has driven a need for devices that can store all this data. NAIST researchers have now used thermal imaging to better understand the storage mechanism in one possible type of memory device[1], and provide insight that could help reduce the power consumption of such components in the future.

Non-volatile memory — devices that retain their data when power is switched off — should squeeze as much information into as small a space as possible, be cheap to produce, and not consume too much energy. Resistive random access memory (ReRAM) is one technology that fulfills these requirements. Resistive devices operate by harnessing the ability of some materials to switch between high and low electrical resistance states. This means that ReRAM offers rapid reading and writing of data, and can be easily integrated with existing silicon-based electronics technologies.

> **"Our future work will attempt to establish a relationship between heat and electrical characteristics of the device."**

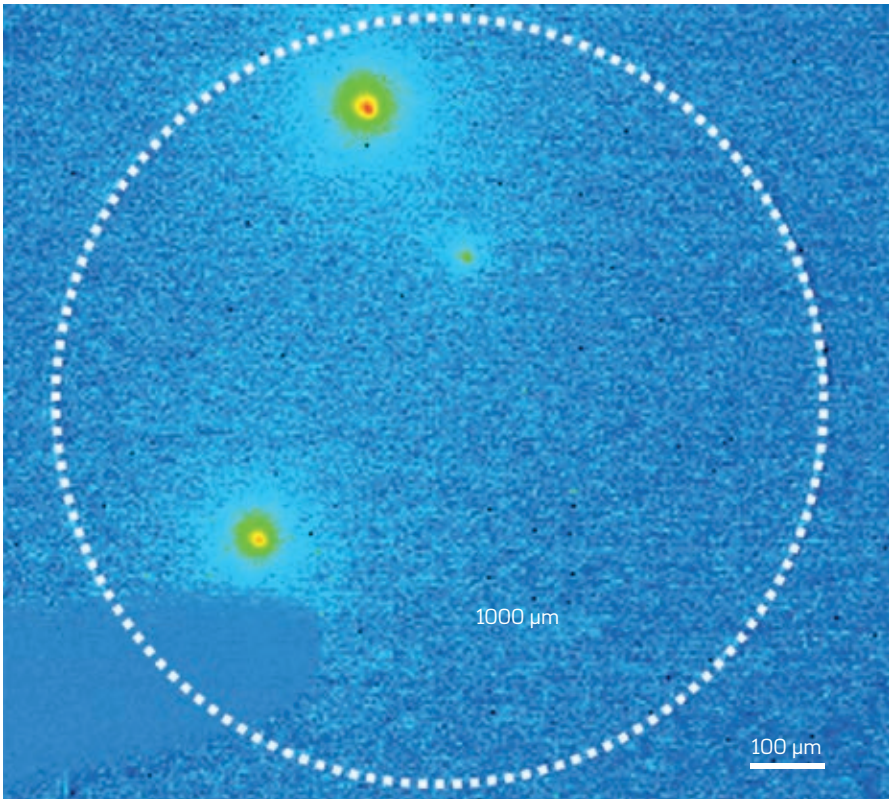
"But the operation mechanism of resistive switching memories is not fully understood," says NAIST researcher Yukiharu Uraoka, from NAIST's Information Device Science Laboratory. "So we chose to investigate the heat generated in the device to see if it affected the performance of the memory."

Uraoka and his co-workers studied the changes in ReRAM during switching by monitoring the surface temperature. They created their ReRAM by sandwiching a 30 nanometre-thick layer of amorphous indium gallium zinc oxide (a-IGZO) between two platinum electrical contacts. They used an infrared detector to create a spatially resolved thermal map of the device as they applied a voltage of up to 1.5 volts across the electrodes.

The team noticed that the voltage created hot spots on the surface of the device (see figure). These localized regions, with a maximum temperature of 186 °C, were a sign of electrical pathways, or current filaments, that pass through the a-IGZO. These filaments significantly alter the resistance of the thin film, thus enabling the switching that is central to the operation of a ReREM.

Current filaments have been observed in resistive materials in the past using techniques such as scanning electron microscopy or electron energy loss spectroscopy. But these methods weren't able to observe the filaments as the voltage was applied — which means they didn't provide much information on how the filaments are formed.

"Our method can detect the location of conductive filament, a main contributor to the resistive switching," says Uraoka. "Our future work will attempt to establish a relationship between heat and electrical characteristics of the device."



Hot spots imaged on the surface of a resistive random access memory 1,000 micrometres in diameter are a signature of current filaments that significantly alter the electrical resistance of the device.

Reference

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More information about the group's research can be found at Yukiharu Uraoka's webpage:
http://mswebs.naist.jp/LABs/uraoka/PUBLIC/staffs/uraoka/uraoka.html

Nara Institute of Science and Technology | **Synthetic Organic Chemistry Laboratory** www.naist.jp/en

The photoreactor used to produce cyclobutanes.

Photochemistry

Microflow set-up boosts output

Photochemical reactions in a flow rather than a batch reactor lead to easy scaling up

Most chemical reactions require energy to start them. In almost all cases, that energy is heat, but for certain reactions, light energy must be used instead. Known as photochemical reactions, these include one of the most effective ways of producing cyclobutane rings — cyclic structures containing four carbon atoms, which are frequently found in naturally occurring compounds with useful biological properties, such as insect pheromones.

Now, Yasuhiro Nishiyama and his team at NAIST's Graduate School of Materials Science in Japan, along with Michael Oelgemöller at Australia's James Cook University, have shown that photochemical reactions like these can be done very effectively in a flow chemistry set-up[1].

Most industrial chemical processes are run in a so-called 'batch set-up', which is not unlike the procedure for baking a cake: reactants are combined in known proportions and mixed for a set time. Each reaction batch produces a fixed amount of product. But this way is not very amenable to photochemical reactions: "Light cannot penetrate sufficiently into large batch reactors or high-concentration solutions," explains Nishiyama. "Thus, almost all photoreactions show poor conversion in batch reactors."

> **"If we need to scale up, we can also run multiple reactors in parallel, a process called numbering up."**

The team investigated reactions that produce cyclobutanes from two alkenes (a class of hydrocarbons containing only carbon and hydrogen) in a microflow set-up. In this set-up, the reaction tubes have an internal diameter of just 1 millimetre and are wrapped around the light source, allowing the whole apparatus to be cooled easily (see figure).

Of particular interest is that one of the reagents — ethylene — is a gas. In a batch process, this would add a further difficulty, since the reaction can only occur at the surface between the gas and liquid reagents. In flow, however, pumping a gaseous and a solution reagent into a small tube produces small gas bubbles in the solution as it flows through the tube. This is known as slug flow, and it ensures that there is a very thin film of reactant solution at the edge of the tube, which is easily penetrated by light. The small bubbles create a large surface area at which the reaction can occur.

Photoreactions performed in the flow set-up are slightly more efficient and selective than those performed in a small batch reaction. Importantly, making a large amount of product is much easier: simply running the reactor continuously produces more product. "If we need to scale up, we can also run multiple reactors in parallel, a process called numbering up," says Nishiyama.

Reference

1. Terao, K., Nishiyama, Y., Tanimoto, H., Morimoto, T., Oelgemöller, M. & Kakiuchi, K. Diastereoselective [2+2] photocycloaddition of a chiral cyclohexenone with ethylene in a continuous flow microcapillary reactor. *Journal of Flow Chemistry* **2**, 73–76 (2012).

More information about the group's research can be found at the Synthetic Organic Chemistry Laboratory webpage:
http://mswebs.naist.jp/english/courses/1414/

Nara Institute of Science and Technology | **Biomimetic Materials Science Laboratory** www.naist.jp/en

Silicon chemistry

Hybrid skin for stable agents

Spherical vesicles formed from lipid bilayer membranes with an outer silica network show excellent morphological stability

Cerasomes – spherical vesicles with a lipid bilayer membrane and ceramic surface and an internal aqueous compartment – have exciting potential for use in biomedical and electronic devices. These organic–inorganic hybrid structures are made from the spontaneous self-assembly of amphiphilic molecules (attracted to both water and fat) to form a bilayer structure, followed by condensation reactions on the structure's exterior to form a silica-based network.

NAIST researchers Jun-ichi Kikuchi, Kazuma Yasuhara and Keishiro Tahara are investigating new ways to make and modify these hybrid materials to create gene delivery agents or magnetic structures that can be manipulated by external magnetic fields[1].

They have prepared and characterized cerasomes formed from lipids with different molecular components — including those with cationic groups (positive ions) — and shown how their structure and stability, across a pH range and in the presence of surfactants, are influenced by the cerasomes' composition and the reaction conditions used to make them.

The molecular components of a cerasome-forming lipid comprise a hydrophobic tail, a connector unit (for example, an amide or urea function), and an ethyoxysilyl group, which must be removed by hydrolysis before assembly of the lipid can occur. The cerasomes are between several tens of nanometers and microns in diameter, and can exist as single-walled or multiwalled vesicles.

Cerasomes, a close relative of phospholipid-containing vesicles (namely liposomes), differ as a result of the siloxane framework that covalently links several of the amphiphilic lipids together. "The cerasomes behave as biomembrane models, but they have a much enhanced morphological stability compared with conventional liposomes," says Kikuchi. The siloxane framework that connects the surface together is the key stabilizing factor and, in addition, imparts the chemical and biophysical characteristics of silica particles to the cerasomes.

The surface of these hybrid structures can be easily adapted to a range of chemical species including organic molecules, titanium dioxide, hydroxyapatite, and metals, which opens up many possible functions. For example, the titanium-dioxide-coated cerasomes are photocatalytically active, and the hydroxyapatite coating enhances the biocompatibility of the vesicles.

The metal-coated cerasomes, or metallosomes, are formed by electroless plating of palladium or gold onto the outer silica surface. "Metallosomes have practical advantages as a new type of organic–inorganic–metallic material in various applications, including energy conversion and information processing," says Kikuchi. "For example, the magnetic cerasome can behave as a molecular vehicle manipulated by an external magnetic field."

In addition, cationic cerasomes have been developed as gene delivery agents with high transfecting capabilities and low toxicity in various cell lines[2]. The clinical translation of these cerasomes is a future goal in this area.

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2. Tahara, K., Moriuchi, T., Tsukui, M., Hirota, A., Maeno, T. *et al.* Ceramic coating of liposomal gene carrier for minimizing toxicity to primary hippocampal neurons. *Chemistry Letters* **42,** 1265-1267 (2013).

NAIST chemists have prepared and characterized cerasomes formed from lipids with different molecular components.

More information about the group's research can be found at the Biomimetic Materials Science Laboratory webpage:
http://mswebs.naist.jp/english/courses/1417/

Nara Institute of Science and Technology | **Laboratory of Bioenergetics and Biophysics** www.naist.jp/en

Biophysics

Enzyme's special bonds finally revealed

High-resolution crystallography uncovers the first view of bonds important for enzyme structure and function

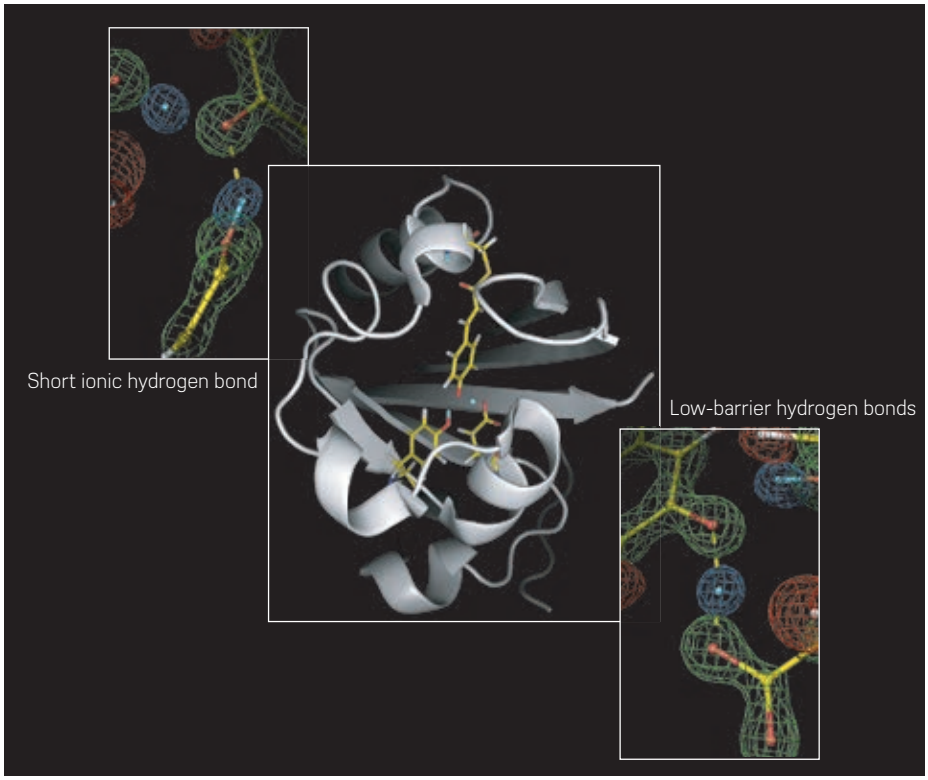
In 1994, a pair of papers published in *Science* suggested that a special class of intramolecular bonds known as low-barrier hydrogen bonds (LBHB) play an important role in enzymatic reactions. But it took until 2009 for a high-resolution crystallography technique developed by Japan's NAIST to allow scientists to finally observe these elusive bonds.

LBHBs are exceptionally short hydrogen bonds, resulting in the hydrogen ion, a proton, being more evenly shared between the partners. This confers additional flexibility to the molecule, enabling it to change shape more easily during reactions. However, the short length of LBHBs also makes them difficult to detect directly, leading to some debate about their existence. Observation of an LBHB calls for very high-resolution crystallography to determine the position of the proton and the donor and acceptor atoms.

> **"The resolution we attained was the highest in the world. Nobody had ever conducted such high-resolution neutron crystallography."**

Mikio Kataoka's team at NAIST was able to overcome these challenges in collaboration with researchers at other Japanese institutes. They developed a method to grow large crystals of the light-sensitive photoactive yellow protein (PYP) in heavy water, enabling them to determine its structure at an unprecedented resolution with neutron diffraction[1]. "The resolution we attained was the highest in the world. Nobody had ever conducted such high-resolution neutron crystallography," says Kataoka, adding that the team had to develop a new analytical method to handle their data.

Work on the research spanned five years, including two years to develop the crystallization conditions. "A talented and ambitious student selected the project as his doctoral research," observes Kataoka. "NAIST allows us to tackle such time-consuming projects."



Visualization of a portion of the molecular structure of photoactive yellow protein, with insets showing two different kinds of hydrogen bond.

The team was able to detect the position of 87% of the hydrogen ions in PYP, and determine that one of them — buried within the protein's interior — forms an LBHB. In addition to easing the protein's conformation change in response to light, the LBHB serves to stabilize a negative charge, which would otherwise be energetically unfavourable in the interior environment of the protein.

Kataoka is now using time-resolved crystallography to follow changes in the LBHB during PYP's photoreaction. "Before practical applications, we must clarify the properties of LBHB and the principle of LBHB formation. Basic research will be still required," he says.

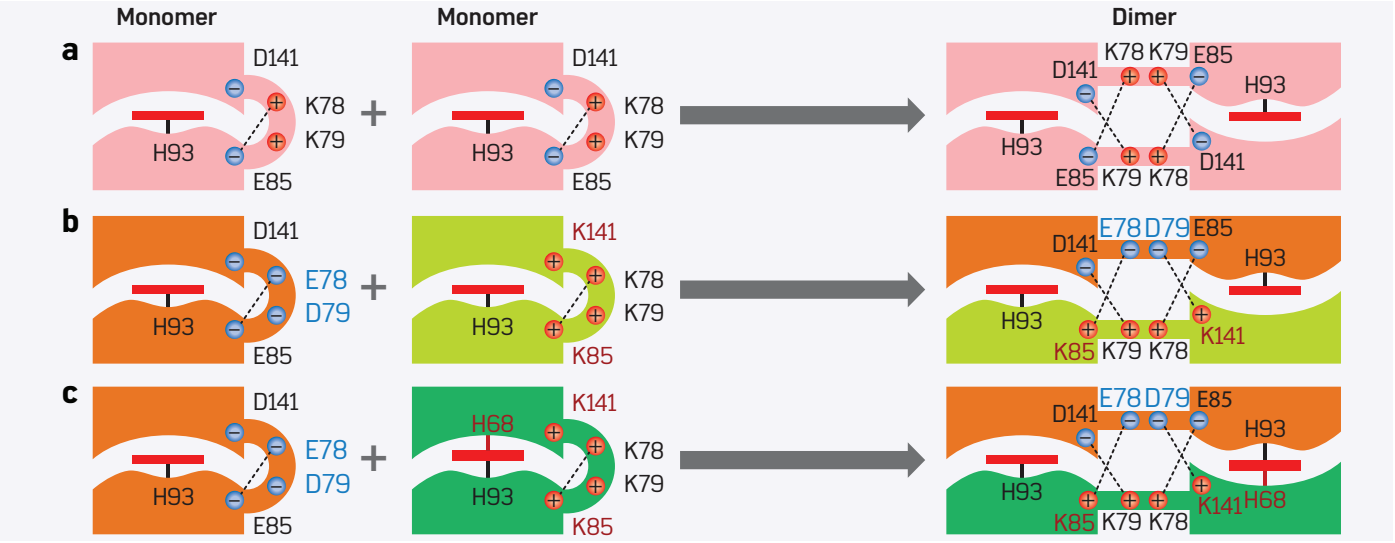
A better understanding of LBHBs may significantly improve our ability to engineer molecules. "The formation of LBHBs is a key element for efficient proton transfer," explains Kataoka. "If we can control the formation of LBHBs, it will allow us to design artificial enzymes with high efficiency and artificial proton pumps, as well as [more] efficient medicine."

Reference

1. Yamaguchi, S., Kamikubo, H., Kurihara, K., Kuroki, R., Niimura, N. *et al.* Low-barrier hydrogen bond in photoactive yellow protein. *Proceedings of the National Academy of Sciences USA* **106,** 440–444 (2009).

More information about the group's research can be found at Mikio Kataoka's webpage:
http://www.naist.jp/en/about_naist/offices/administration_bureau/mikio_kataoka/index.html

Nara Institute of Science and Technology | **Laboratory for Supramolecular Science** www.naist.jp/en



Myoglobin dimer formation using (a) the natural protein monomer, (b) two mutant monomers obtained by switching the positions of the bridging residues (E78/K85 and D79/K141) and (c) two mutant monomers with switched bridging residues and different heme active sites (H93 and H68/H93).

Artificial proteins

Growing enzyme diversity using mutations

A targeted protein mutation strategy opens the door to multifunctional artificial enzymes

Site-specific modifications and pairing of natural proteins are expected to generate artificial enzymes capable of catalysing multiple reactions simultaneously, reveals new research by NAIST[1].

The work of enzymes relies on protein folding. Existing protein design methods have produced several artificial enzymes that promote oxidation, reduction, and hydrolysis reactions. These methods have rested on metal ions or other additional compounds called co-factors to control protein folding and achieve these functions. Other self-assembly approaches have led to similar results by forming protein pairs, or dimers. However, the variety of synthetic enzymes remains limited.

To expand this artificial enzyme library, Shun Hirota, from the Graduate School of Materials Science, and co-workers have developed a new strategy that produces various dimers by exchanging specific residues in natural proteins. As a proof-of-concept, they applied this domain swapping approach to iron-containing proteins responsible for oxygen transport and storage in the body, known as hemeproteins.

Having recently discovered that domain swapping governed the polymerization and oligomerization of hemeproteins, such as cytochrome *c* and myoglobin, Hirota's team decided to exploit this phenomenon to create new proteins containing a few monomer units, or oligomers. "Domain swapping with mutation allows us to construct artificial proteins possessing active sites with different structures," he adds.

> **"Domain swapping with mutation allows us to construct artificial proteins possessing active sites with different structures."**

By examining the X-ray crystallographic structure of the natural myoglobin dimer in detail, Hirota's team identified four bridges between each protein subunit. These bridges resulted from electrostatic interactions between positively and negatively charged residues, holding the dimer together. Consequently, to control the dimerization, the researchers engineered two different myoglobin mutants by switching the positions of the charged residues in each mutant (see figure). Specifically, they replaced two positively charged residues by two negatively charged ones in one mutant, and two negatively charged residues by two positively charged residues in the second mutant. These two mutants gave a stable dimer upon pairing.

Moreover, to generate a dimer with two distinct active sites, Hirota and co-workers altered one of the heme sites in the second myoglobin mutant before coupling it with the first mutant. A spectrometric analysis of the resulting dimer demonstrated that the two sites exhibited different reactivities upon exposure to a mild reducing agent. These results suggested that the sites operated independently — evidence for the usefulness of domain swapping in the design of multi-heme proteins.

"We are currently designing other artificial hemeproteins comprising different active sites by domain swapping," says Hirota. By expanding their library, the researchers are also planning to produce multifunctional enzymes.

Reference

1. Lin, Y.-W., Nagao, S., Zhang, M., Shomura, Y. , Higuchi, Y. & Hirota, S. Rational design of heterodimeric protein using domain swapping for myoglobin. *Angewandte Chemie International Edition* **54,** 511–515 (2015).

More information about the group's research can be found at the Laboratory for Supramolecular Science webpage:
http://mswebs.naist.jp/LABs/hirota/member1_e.html

Nara Institute of Science and Technology | **Biocompatible Materials Science Laboratory** www.naist.jp/en

Medical implants

Anti-fouling polymers demonstrate star quality

Star-shaped polymer coatings show promise in fending off bacteria and blood clots in medical implants

Two polymers, designed by researchers at the Nara Institute for Science and Technology (NAIST), may herald a new class of 'anti-fouling' coatings for medical implants such as heart valves and catheters[1].

The problem with implants, made from silicone or polyethylene terephthalate (PET), is that cells tend to stick to them. When blood platelets (which promote blood clotting) attach to an artificial heart valve, for instance, there is risk of thrombosis, while adherent bacteria can cause secondary infections.

> **"We designed the hetero-star as a first step, and expected they would show moderate inhibition of platelet and bacterial adhesion. But they exhibited much higher inhibition than we expected."**

The researchers, from NAIST's Graduate School of Materials Science, suspected that a coating of star-shaped polymers — in which polymer chains radiate from a central polymer ball — would make it difficult for bacteria and platelets to attach. "We wanted to build a brush-like surface," says corresponding author Tsuyoshi Ando, "but without the cumbersome steps of surface chemistry." Rather than attaching the star-polymer 'brush bristles' using covalent chemical bonds — difficult to do, as PET is quite inert — they designed star-polymers with hydrophobic chains that were naturally attracted to the PET surface (see figure). "We chose polymethyl methacrylate (PMMA) as our model hydrophobic compound for attachment to the PET," says Ando. The team synthesized three star-polymer varieties: some made purely of PMMA, some made from poly 2-hydroxyethyl methacrylate (PHEMA), and 'hetero' star-polymers containing both PMMA and PHEMA. They then coated small strips of PET with the polymers.

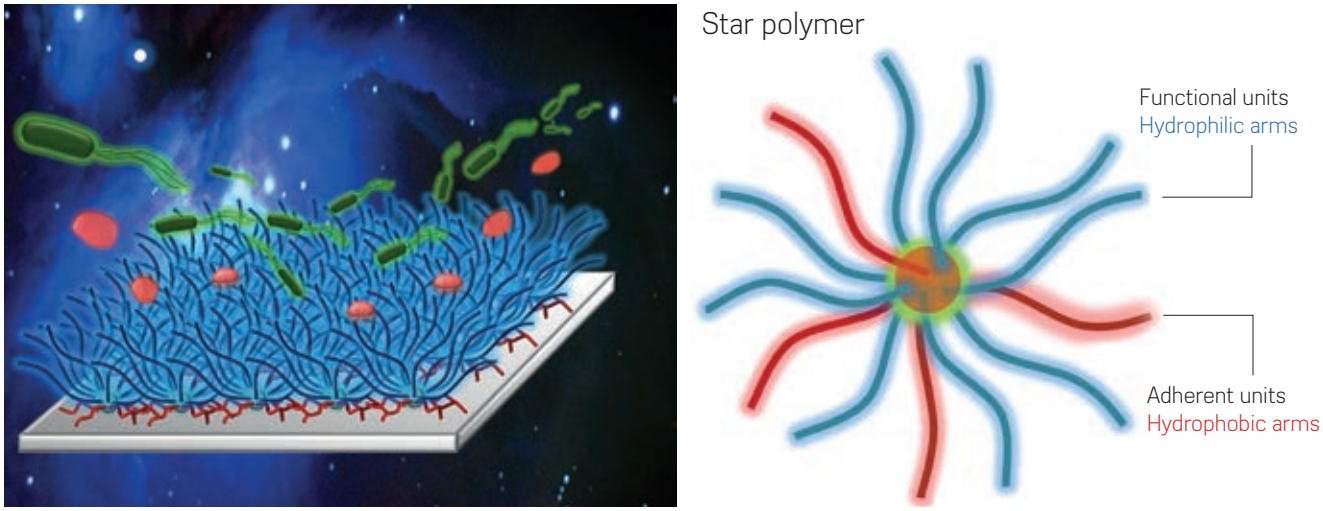
Ando's team had expected the hydrophobic PMMA stars to attach well to the PET surface, and indeed it proved the most durable coating trialled. However, the PMMA stars were not very good at preventing platelet attachment.

The hetero stars provided the best of both worlds: the PMMA chains allowed them to cling to the PET surface, while their PHEMA chains discouraged clotting and bacteria. "We had some surprises," says Ando. "We designed the hetero-star as a first step, and expected they would show moderate inhibition of platelet and bacterial adhesion. But they exhibited much higher inhibition than we expected." The PHEMA stars inhibited platelet adhesion effectively too, and to the team's surprise, they attached well to the PET surface. "They were still attached even after seven days of being washed with a surfactant." says Ando. "We think these two 'stars' are promising materials for non-platelet adherence."

Ando thinks improved versions of these star-polymers could prevent bacterial 'fouling' not only in devices in contact with body fluids, but "also on surfaces like the toilet bowl or sink."

Reference

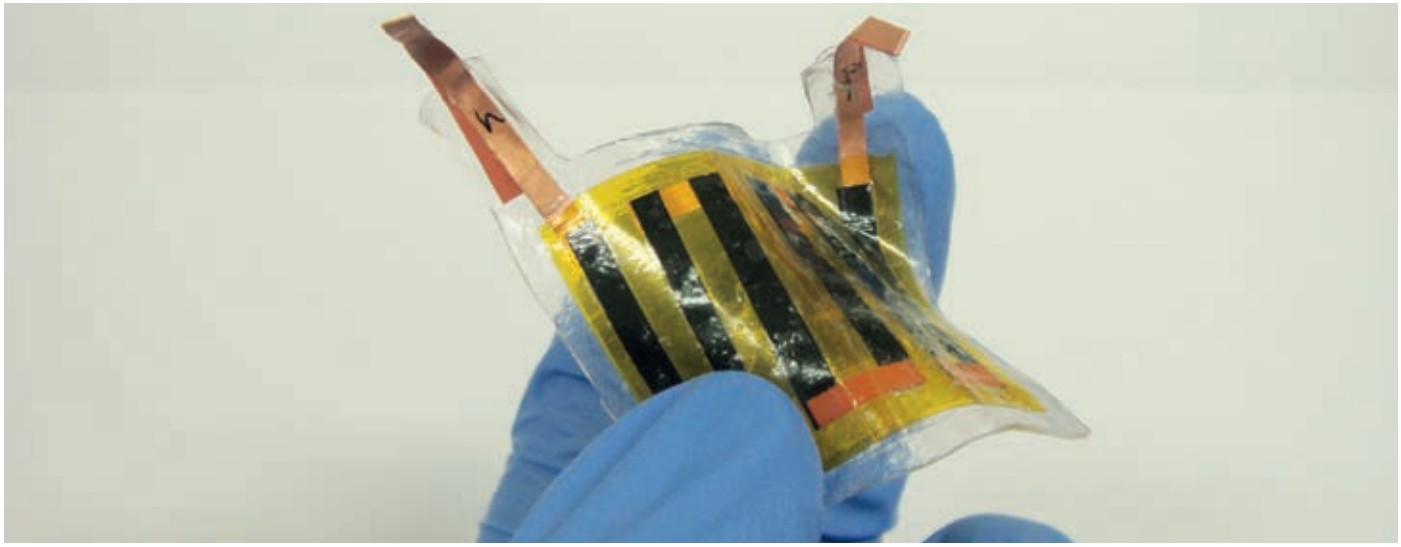
1. Totani, M., Ando, T., Terada, K., Terashima, T., Kim, I. Y. *et al.* Utilization of star-shaped polymer architecture in the creation of high-density polymer brush coatings for the prevention of platelet and bacteria adhesion. *Biomaterials Science* **2**, 1172–1185 (2014).
2. Totani, M., Ando, T., Terada, K., Terashima, T., Kim, I. Y. *et al.* Inside front cover. *Biomaterials Science* **2**, 1138–1138 (2014).



Schematic of (left) the 'brush-like' star-polymer surface and (right) a star-shaped heteropolymer.

More information about the group's research can be found at the Biocompatible Materials Science Laboratory webpage:
http://mswebs.naist.jp/LABs/tanihara/index-e.html

Nara Institute of Science and Technology | **Photonic Molecular Science Laboratory** www.naist.jp/en



A bendable thermoelectric device made from modified carbon nanotubes can generate electricity from a temperature gradient.

Semiconductors

Generating power from waste heat

Additives improve the thermoelectric efficiency of carbon nanotubes

Bendable devices that convert waste heat into useful electricity have become much more efficient, thanks to films made of tiny carbon nanotubes developed at NAIST in Japan[1]. These thermoelectric films generate a voltage when one side is hotter than the other and could be used in a variety of applications, including battery-free sensors and energy-saving technologies. "More than 5 per cent of the fuel consumption of gasoline-powered vehicles could be saved by thermoelectric heat recovery systems in the near future," says Tsuyoshi Kawai of the Laboratory for Photonic Molecular Science at NAIST, who led the research.

Most thermoelectric devices are solid, inflexible materials made from highly toxic elements. So Kawai and his colleagues created lightweight and flexible thermoelectric films made of hollow straws of carbon atoms mere billionths of a metre wide, called single-walled carbon nanotubes (SWNTs). These films (see figure) can closely hug the curved surfaces of an engine or pipe, maximizing the power they generate.

Unlike a metal wire, SWNTs do not carry current as a flow of electrons. Instead, the nanotubes rely on the movement of positively charged 'holes' left behind by missing electrons — they are known as p-type semiconductors. The efficiency of thermoelectric devices made solely from p-type materials is very low — unless teamed with n-type semiconductors, which have a surfeit of electrons. But researchers have previously struggled to create stable and flexible n-type thermoelectric materials.

Kawai's team studied 33 different carbon-based molecules to assess whether they could transform SWNTs into n-type semiconductors. They found 18 molecules that could. The best was triphenylphosphine (tpp), whose phosphorus atoms inject electrons into the SWNTs. The researchers filtered the tpp-SWNTs from a liquid to create air-stable n-type films, and paired them with p-type SWNTs that had been enhanced with another additive, tetracyanoquinodimethane (TCNQ). The device was sealed inside a polymer and wrapped around a pipe that was hotter at one end than the other.

The team found that a temperature difference of 20 degrees Celsius generated 110 nanowatts of power at a voltage of 6 millivolts. "We have demonstrated the first example of flexible thermoelectric sheets with this bipolar structure," says Kawai.

The team's survey of different additives also revealed which molecules are best suited to fine-tuning the behaviour of the SWNTs, which is helping to design even more efficient and stable n-type materials. "We are now cooperating with some industrial companies for future commercial applications," says Kawai.

Reference

1. Nonoguchi, Y., Ohashi, K., Kanazawa, R., Ashiba, K., Hata, K. *et al.* Systematic conversion of single walled carbon nanotubes into n-type thermoelectric materials by molecular dopants. *Scientific Reports* **3,** 3344 (2013).

More information about the group's research can be found at the Photonic Molecular Science Laboratory webpage:
http://mswebs.naist.jp/LABs/kawai/english/index.html

Nara Institute of Science and Technology | **Ultrafast Photonics Laboratory** www.naist.jp/en

Optical memory

Laser switchable across waveguides

Optical buffer memories may be improved with a vertical-cavity surface-emitting laser that can switch light into different waveguides

A design of a semiconductor laser that emits light into different sets of waveguides depending on the polarization of the lasing beam has been theoretically investigated by NAIST researchers[1].

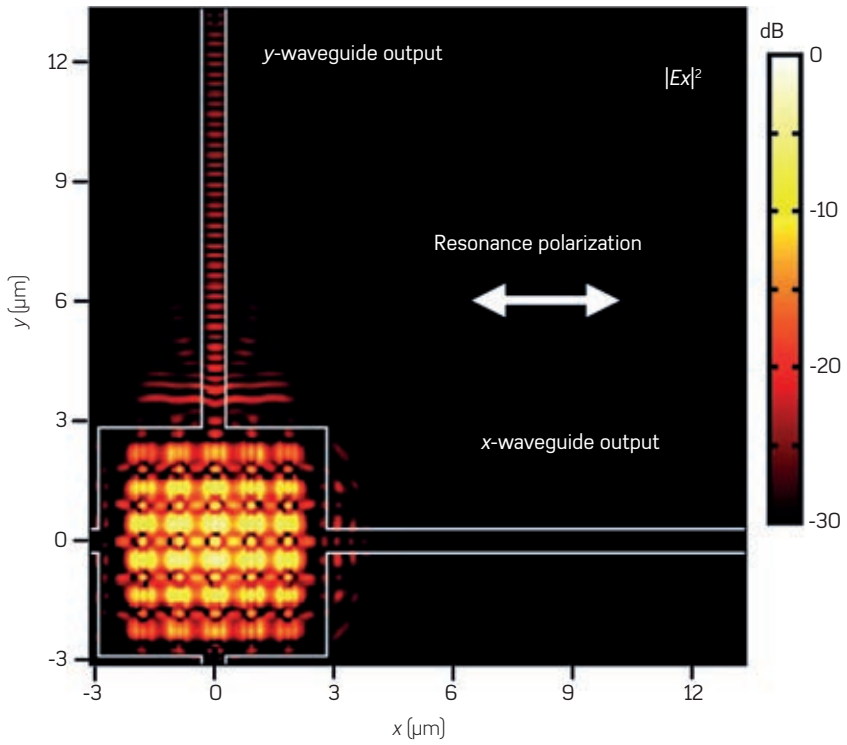
Hitoshi Kawaguchi's team at NAIST used three-dimensional finite-difference time-domain modelling to study the behaviour of a vertical-cavity surface-emitting laser (VCSEL) coupled to two sets of silicon waveguides arranged in orthogonal directions to each other (see figure). The coupling takes place via a square-shaped, polarization-independent, high-index-contrast subwavelength grating (HCG) that is also made in silicon and forms the bottom mirror of the VCSEL.

> **"For applications requiring a larger waveguide output power, the reflectivity of the DBR will be increased by increasing the number of the DBR layers."**

When employed with a polarization bistable VCSEL, such an arrangement may prove useful for creating compact and efficient all-optical buffer memories on a silicon chip that can temporarily switch and store optical data streams without the need for free-space optics.

"We found that the output waveguide can be switched by changing the lasing polarization of the VCSEL with an extinction ratio of 11.9," says Kawaguchi. The light coupled more strongly to the waveguide when it was perpendicularly orientated to the polarization of the resonant mode, rather than parallel. The strongly coupled mode was found to be transverse electric, or perpendicular to the propagation direction of the beam.

The modelling assumes a VCSEL operating at 1.55 micrometres in wavelength, consisting of an active gain region composed of a series of InGaAsP (indium gallium arsenide phosphide) quantum wells sandwiched between the HCG below, and a Distributed Bragg Reflector (DBR) above. The DBR is made from an alternating stack of 32 pairs of thin layers of InP (indium phosphide) and InGaAsP.



Simulation showing light exiting the VCSEL via the y waveguide only. By changing the lasing polarization, the output light switches to the x waveguide.

The HCG has a greater than 99 per cent reflectivity for wavelengths around 1.55 micrometres, while the DBR has a reflectivity of around 90 per cent. The HCG is bonded to the rest of the VCSEL structure using benzocyclobutene. According to the researchers, the output power coupled to the waveguide is similar to the emission from a conventional VCSEL, which is less than 1 per cent of optical power inside the laser cavity.

"This output will be sufficient for all-optical memory applications because the memory works with a very low input power of 80 nanowatts," explains Kawaguchi. "For applications requiring a larger waveguide output power, the reflectivity of the DBR will be increased by increasing the number of the DBR layers."

Their simulations also suggest that the output power of the waveguide is roughly proportional to the waveguide width.

Reference

1. Tsunemi, Y., Ikeda, K. & Kawaguchi, H. Lasing-polarization-dependent output from orthogonal waveguides in high-index-contrast subwavelength grating vertical-cavity surface-emitting laser. *Applied Physics Express* **6**, 092106 (2013).

More information about the group's research can be found at the Ultrafast Photonics Laboratory webpage:
http://mswebs.naist.jp/LABs/kawaguchi/index-e.html

Nara Institute of Science and Technology | **Laboratory for Photofunctional Organic Chemistry** www.naist.jp/en

Photovoltaics

Semiconductor sandwich solution for solar cells

Improved chemical process offers simpler route to promising organic photovoltaics

An improved technique for making flexible organic solar cells with higher efficiencies, using a sandwiched configuration that is more cost-effective to manufacture, could help to boost their application, according to research from Japan's NAIST.

Organic photovoltaic (OPV) devices, which rely on carbon-based molecules to harvest light, are lightweight and perform well in relatively dim conditions, but currently have lower efficiencies than conventional solar cells. "Owing to these characteristics, OPVs are likely to find applications first as indoor or portable devices, rather than in large-scale power plants," says NAIST's Mitsuharu Suzuki.

He and his colleagues have developed a way to make OPVs that contain three layers of semiconducting molecules. One of the outer layers is an n-type semiconductor, which has a surfeit of electrons; the other is p-type, which has a deficit of electrons. The filling inside this semiconductor sandwich is an 'inter layer' made from a mixture of p- and n-type materials. When light hits these cells, it frees electrical charge from the inter layer so that electrons — and the holes they leave behind — can flow to the outer layers, thereby generating a current.

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A technique for fabricating flexible solar cells with three layers of semiconductor molecules offers more efficient organic photovoltaics.

> **"Our proof-of-concept system will be further elaborated in the very near future, so that much higher efficiencies can be achieved."**

Building these cells can be troublesome and expensive, not least because the crucial organic molecules often dissolve into neighbouring layers during production. Suzuki's team solved this problem by forming some of the layers from precursor molecules containing chemical groups called ketones.

When the researchers exposed these precursors to a bright light, it triggered a reaction that stripped away the ketones and generated an insoluble semiconductor layer. After testing a range of different semiconductor combinations, the team made a cell with an efficiency of 2.89 per cent — among the highest recorded for this category of device[1].

Using this approach meant that the researchers could build their cells using solutions of the semiconductor molecules, a process that is more cost-effective than alternative vacuum evaporation. The strategy avoids extensive heat treatments, so that the semiconductors can be coated directly onto thin plastic films. And tailoring the layers in this way also allowed the researchers to use materials that have ideal properties for each different layer of the cell.

Suzuki notes that access to high-quality instruments and well-trained operational staff at NAIST's facilities in Ikoma, Japan, was also crucial to the success of their research.

His team of researchers are now tweaking their molecules to absorb a greater quantity of light, and to carry current more effectively. "Our proof-of-concept system will be further elaborated in the very near future, so that much higher efficiencies can be achieved," says Suzuki.

Reference

1. Yamaguchi, Y., Suzuki, M., Motoyama, T., Sugii, S., Katagiri, C. *et al*. Photoprecursor approach as an effective means for preparing multilayer organic semiconducting thin films by solution processes. *Scientific Reports* **4**, 7151 (2014).

More information about the group's research can be found at the Laboratory for Photofunctional Organic Chemistry webpage: http://mswebs.naist.jp/LABs/env_photo_greenmat/en/Yamada_Group/HOME.html

Nara Institute of Science and Technology | **Nanostructure Magnetism Laboratory** www.naist.jp/en

Layers of magnetic and non-magnetic materials are used in the read heads of computer hard drives.

Materials science

Bringing magnetism to gold

The magnetic alignment of iron carries further into an adjacent non-magnetic gold layer than anticipated

Many physical phenomena can arise at the interface between two different materials, and they are often important for many applications. In computer hard drives, the electrical resistance of a non-magnetic material that is surrounded by magnetic layers is very sensitive to external magnetic fields, which is used to read the magnetic information on the hard drive.

Studying related magnetic structures, Nobuyoshi Hosoito and colleagues from the NAIST Graduate School of Materials Science, the Japan Synchrotron Radiation Institute in Sayo and the Nara National College of Technology have discovered that the magnetic polarization can extend further into the non-magnetic layer than previously anticipated[1]. "We have shown that gold atoms within a few nanometres from the interface with an iron layer behave more like a ferromagnet than a non-magnet," says Hosoito.

The magnetic information carried by elementary particles, such as the electron, is known as their 'spin'. Whereas conventional electronic devices, such as transistors, make use of the electrical charge of an electron, researchers are working on devices that use the spin instead, because this offers benefits such as a potentially lower energy consumption. In particular, the combination of magnetic and non-magnetic materials enables new avenues for controlling magnetic properties.

An important aspect of these devices is to understand how far the magnetism of a magnet extends into an adjacent non-magnet — in this case, that of iron into a layer of gold. This is possible using resonant X-ray magnetic scattering, which probes the relative orientation of the electron spins in the atomic states of gold with high precision.

The expectation was that, away from the interface with the iron alignment, the spins within the gold layer drops off rapidly towards the random orientations of a non-magnet. However, the measurements show that the spin alignment extends further into the gold than expected. This is due to the enhancements from the interface between the iron and the gold, explains Hosoito. "Electrons approaching this interface are reflected differently according to the orientation of their spins. This creates a polarization."

The creation of an enhanced magnetic polarization inside non-magnets could lead to new fundamental physical phenomena, where the aligned spins inside the non-magnet can interact with other effects that typically do not exist in magnetic materials, such as superconductivity, to achieve new functionalities. More practically, in technical devices such as computer hard drives, the manipulation of magnetic fields by complex magnetic/non-magnetic structures could deliver its own advantages for the manipulation of magnetism in enhanced storage devices.

Reference

1. Hosoito, N., Ohkochi, T., Kodama, K. & Suzuki, M. Charge and induced magnetic structures of Au layers in Fe/Au bilayer and Fe/Au/Fe trilayer films by resonant X-ray magnetic reflectivity at the Au L_3 absorption edge. *Journal of the Physical Society of Japan* **83,** 024704 (2014).

More information about the group's research can be found at the Nanostructure Magnetism Laboratory webpage:
http://mswebs.naist.jp/LABs/hosoito/English/English.html

Nara Institute of Science and Technology | **Theoretical Condensed Matter Physics Laboratory** www.naist.jp/en

NAIST researchers theoretically examined how terahertz spectroscopy would help analyse electrons and electron holes, as well as electronic plasma, and how they behave around the exciton Mott transition – the point at which electron-hole pairings disappear.

Terahertz spectroscopy

Probing exciton transitions

Electron interactions in semiconductors have been theoretically calculated using terahertz absorption spectra

Terahertz radiation — between the mid-infrared and microwaves regions — is an important transition point on the electromagnetic spectrum, and can be used to visualize the boundary between quantum and classical physics. It is a band with many potential applications, from biotechnology to the development of low-energy optoelectronic devices.

Researchers at NAIST conducted a theoretical study examining electronic plasma (an electrically-charged gas made up of free-flowing carriers) and paired electron-hole structures in optically excited semiconductors, seeking to understand how terahertz spectroscopy might help visualize phenomena[1].

"The issue of electron interactions is very difficult and has been studied for five decades," states Takeshi Inagaki, who worked on the study with colleague Yen Thi Hai Le at the NAIST Graduate School of Materials Science. "We needed a new way to tackle this problem."

When light shines on an optically excited semiconductor, electrons in the valence band are excited to the conduction band, leaving holes in the valence band. This process is analogous to the movement of electrons through different energy bands in a hydrogen atom. As the negatively-charged electrons move, Coulomb's law means they remain bound to their positively-charged electron holes — creating electron-hole pairs called excitons.

Inagaki and Le used numerical analysis to examine three-dimensional electron systems in cuprate semiconductors under terahertz (THz) radiation. They were particularly interested in electron interactions around the exciton Mott transition; the point at which electron-hole pairs disappear because the density of the electrons and holes, and the density of the ionized electronic plasma, have reached certain critical levels.

"We developed a computer program to calculate THz absorption spectra for different exciton densities," explains Inagaki. "NAIST has a high-spec computing system which made this job fairly straightforward."

A previous experimental study had shown that the spectral component left by electron movements from the 1s to 2p energy band remained the same regardless of exciton density. The researchers were able to explain this phenomenon using their new theory.

They also found that the exciton Mott transition was clearly visible using THz radiation, observing a sudden increase in the densities of both the electronic plasma and the electron-hole pairs just before the spectra from exciton structures abruptly disappeared. At a certain level of ionization density, both excitons and electronic plasma co-exist, the semiconductor becomes metallic and electric current flows.

"Understanding the nature of the system near the exciton Mott transition is very useful," states Inagaki. "It will help us incorporate electron interaction effects into new, low-energy devices."

Reference

1. Le, Y. T. H. & Inagaki, T. J. Density dependence of the terahertz absorption spectra in optically excited semiconductors. *Physica Status Solidi B* advance online publication, 25 November 2014 (doi: 10.1002/pssb.201451191).

More information about the group's research can be found at the Theoretical Condensed Matter Physics Laboratory webpage: http://mswebs.naist.jp/english/courses/1438/

Nara Institute of Science and Technology | **Laser Nano-Manipulation Science Laboratory** www.naist.jp/en

Biophysics

Detecting forces at cellular scales

A platform for measuring nanoscale vibrations could help map the mechanics of cellular growth and development

Throughout the life of an organism, its cells are continually moving, stretching and reorganizing; accurate detection of the forces underlying these processes could give scientists deeper insights into many biological processes. A highly sensitive measurement system developed by researchers at NAIST in Japan now makes it possible to precisely detect such biomechanical movements at the nanometre scale[1].

> **"We are developing a technology to estimate the distribution of the mechanical properties throughout the sample, which is impossible to do by conventional methods."**

Yoichiroh Hosokawa is an applied physicist with a deep interest in exploring the tiny forces that affect cell growth and behaviour. "If we could successfully estimate the distribution of mechanical forces in a plant or animal embryo," he says, "we could combine this with microscopic imaging data to achieve new insights into biomechanics and mechanobiology."

In prior research, his group developed a strategy for generating tiny forces by using the extremely short but powerful pulses of light produced by a femtosecond laser. By using a microscope to focus these laser pulses into water, Hosokawa's team was able to generate nanoscale explosions.

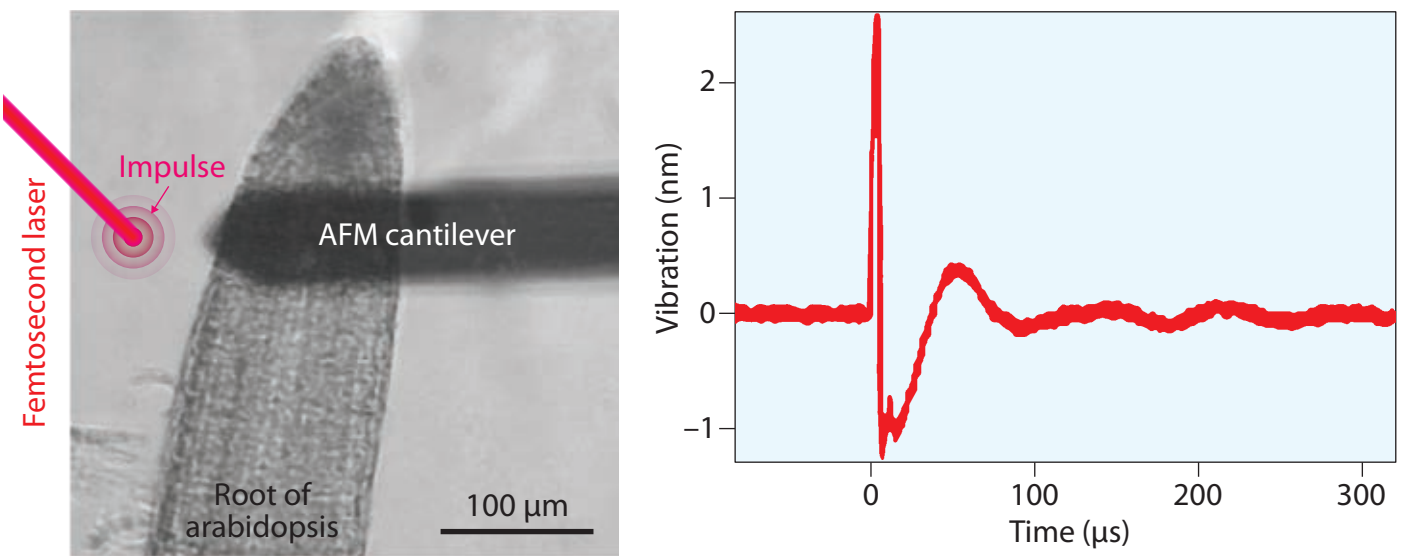
In subsequent work, Hosokawa and his colleagues applied such femtosecond laser explosions to produce external forces, which they used to analyse the stiffness of a stem and root of a plant (see figure). To do this, they immobilized a segment of a thale cress plant in water and focused a femtosecond laser pulse on a point in the water some distance away from the plant. The resulting explosion generated a stress wave that spread outward from this focal point and struck the plant stem. The movement and deformation in the stem induced by the impact of the stress wave was detected by an atomic force microscope, whose curved silicon probe (or 'cantilever') was placed in contact with the surface of the stem. By analysing how the probe bent, the researchers were able to derive information about the stiffness of the plant.

This set-up allowed the researchers to detect minuscule movements — equivalent to introducing a bend of less than one-thousandth of a degree to the stem. Importantly, since the probe that detects the force is separated from the source of the physical stress, it becomes possible to do much more sophisticated three-dimensional analyses of how forces affect a biological specimen.

Such measurements could, for example, help chart the internal forces at work as cells divide and reorganize in a developing embryo. "We are developing a technology to estimate the distribution of the mechanical properties throughout the sample, which is impossible to do by conventional methods," says Hosokawa.

Reference

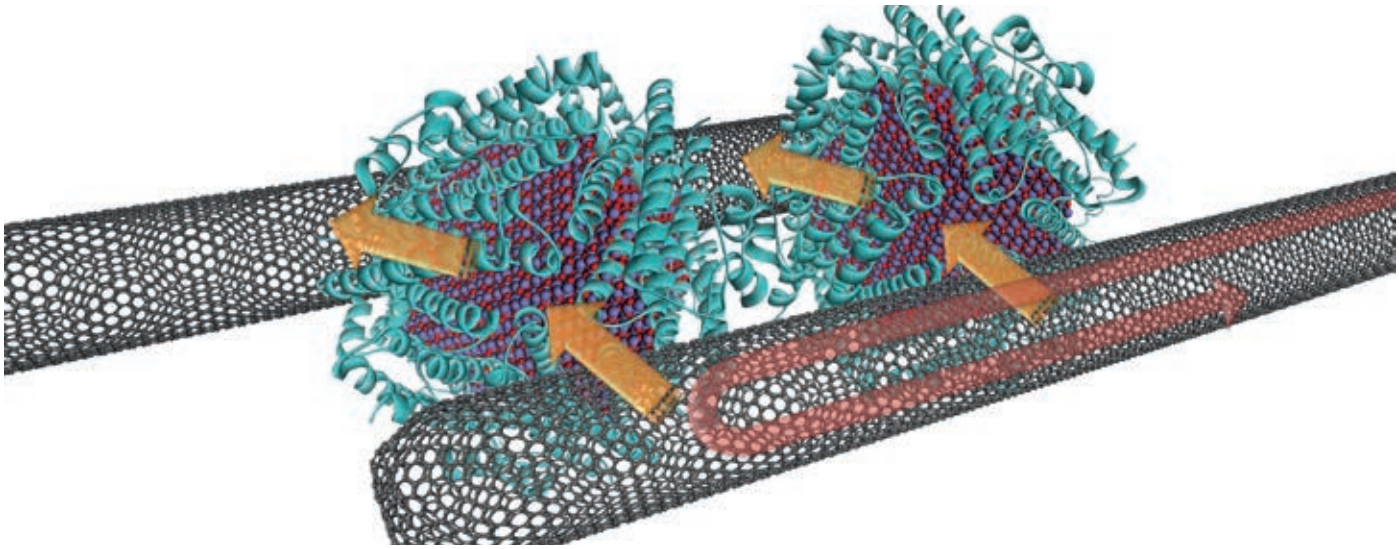
1. Takenaka, M., Iino, T., Nagatani, A. & Hosokawa, Y. Nanoscale bending movement of biological micro-object induced by femtosecond laser impulse and its detection by atomic force microscopy. *Appl. Phys. Express* **7**, 087002 (2014).



An atomic force microscope (AFM) probe is positioned atop a plant root (left) immersed in water. An extremely short laser pulse targeted some distance away creates a shockwave by heating and vaporizing water. The resulting vibrations in the plant root can be measured from the movement of the AFM probe (right).

More information about the group's research can be found at the Laser Nano-Manipulation Science Laboratory website:
http://mswebs.naist.jp/english/courses/1447/

Nara Institute of Science and Technology | **Organic Electronics Laboratory** www.naist.jp/en



Carbon nanotubes (CNTs) linked by protein molecules (blue) with a semiconducting core (purple). Orange arrows show electric current flow from hot to cold CNTs. Red arrow indicates phonon scattering.

Semiconductors

Making waste heat into energy

Combining carbon nanotubes and protein molecules produces electricity from low-temperature heat sources

Thermoelectric devices convert heat into electrical energy without the need for moving parts, meaning they are long-lasting, reliable and environmentally-friendly. It is now even possible to harvest energy from low-temperature waste heat, thanks to advances in nanoscale components such as carbon nanotubes.

Carbon nanotubes (CNTs) are strong, light and excellent conductors of electricity, making them promising candidates as thermoelectric components. Their thermal conductivity when in pure form, however, is too high. For this reason, researchers at NAIST are working to improve the thermoelectric properties of CNTs for use in tiny power generators and even wearable electronics.

Effective thermoelectric devices require materials with high electrical conductivity, low thermal conductivity, and a high 'Seebeck coefficient' — the proportion of voltage generated by temperature differences across the material. The higher the coefficient, the better the device performs.

Masakazu Nakamura and co-workers at the NAIST Graduate School of Materials Science developed a composite CNT material by inserting biomolecules with semiconducting cores at the junctions between CNTs[1]. The CNTs retain high electrical conductivity, but the biomolecules help suppress thermal conductivity and enhance the Seebeck coefficient.

"Conventional modifications to improve thermoelectric properties in CNTs, such as impurity doping, usually improve one or two of these parameters," explains Nakamura. "Our proposed method controls the three parameters independently, quite different from any previous approach to thermoelectric materials design."

The team's main aim was to create a nanostructure which blocks heat transfer but enhances electron mobility. To do this, they used the unique functions of a cage-shaped protein molecule designed at another NAIST laboratory.

"Luckily, the ideal protein molecule for my work had already been developed by a colleague, so I neatly side-stepped one of the biggest challenges in this study," explains Nakamura. "Peptides sticking out of the molecule's surface help the structure to self-assemble with the CNTs. The soft shell of the molecule prevents the heat transfer from one CNT to another, while the semiconducting core helps the electric current flow through the CNT junctions effectively."

Heat scattered at the biomolecular junctions lowers the thermal conductivity of the CNTs, and the steep temperature gradient at the CNT/molecule interface increases the Seebeck coefficient. This combined effect enhances the thermoelectric conversion efficiency of the CNTs.

"We need various methods for energy harvesting in the future," says Nakamura. "I want to create thermoelectric stickers, thermoelectric clothes — even walls should be able to produce energy from waste heat."

Reference

1. Ito, M., Okamoto, N., Abe, R., Kojima, H., Matsubara, R. *et al.* Enhancement of thermoelectric properties of carbon nanotube composites by inserting biomolecules at nanotube junctions. *Applied Physics Express* **7**, 075102 (2014).

More information about the group's research can be found at the Organic Electronics Laboratory webpage:
http://mswebs.naist.jp/LABs/greendevice/index_e.html

Nara Institute of Science and Technology | **Green Nanosystem Laboratory** www.naist.jp/en

Materials science

Characterizing atomic orbitals

Pulses of corkscrew-like X-rays prove useful for probing the properties of atomic orbitals

The properties of a solid material depend on the behaviour of its electrons: some electrons are localized near the nucleus, whereas others travel throughout the crystal lattice. Researchers at Japan's NAIST have now shown that an unusual property of electromagnetic radiation can provide previously unavailable information on these valence band electrons, enabling more detailed material characterization[1].

An atom is made up a nucleus surrounded by electrons. The outermost electrons, or valence electrons, largely determine how an atom interacts with molecules and other atoms. So it is important to fully understand the properties of these electrons.

"Copper is nonmagnetic, whereas nickel, which has one less electron than copper, is ferromagnetic," says NAIST researcher Fumihiko Matsui. "If we can selectively excite a valence electron, then we can explore the behaviour of key atomic orbitals responsible for these electronic properties."

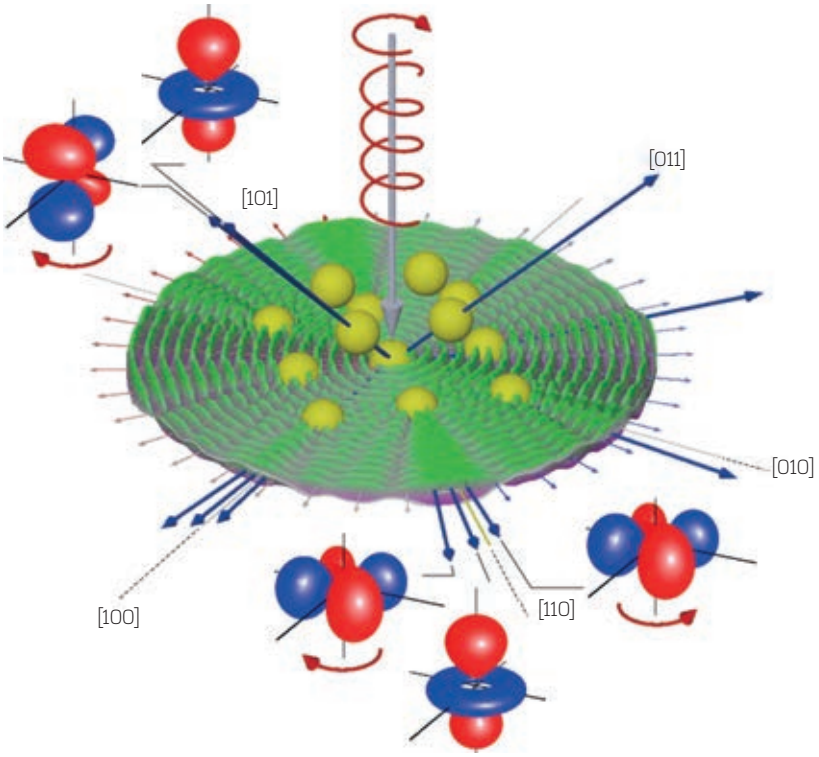
To achieve this, Matsui and his colleagues from NAIST's Green Nanosystem Laboratory and the Japan Synchrotron Radiation Research Institute have applied their original method called atomic stereography. In this technique, X-ray pulses provide the energy needed for an electron near the core of an atom to escape from its nucleus. The X-rays used by the team have a property known as angular momentum, which gives them a corkscrew-like shape. This angular momentum is transferred to the escaping photoelectron. By measuring the kinetic energy and emission angle of these photoelectrons, the researchers can build a 'stereograph' of the atomic arrangement around the excited atom.

> **"Our aim is to take out valence electrons from a crystal surface and study the property of the newly created excited state."**

Further electrons, known as Auger electrons, can also escape from the atom as the electrons shuffle around to fill the gap left at the core. Measuring the energy of these Auger electrons can provide further information about the material's electronic structure. Matsui and his co-workers have now shown that, contrary to expectation, Auger electrons can also have angular momentum.

Matsui's team fired a corkscrew-like beam of X-rays at a copper sample and measured the angular distribution of the emitted Auger electrons. From these maps, they were able to identify the non-negligible circular properties of the Auger electron. This observation suggests that the hole left behind by the photoexcited core electron is slightly angular-momentum polarized, which is then passed on to the Auger electron.

"Our aim is to take out valence electrons from a crystal surface and study the property of the newly created excited state," says Matsui. "By combining Auger electron measurement and atomic stereography, we have developed a new technology that enables such physics."



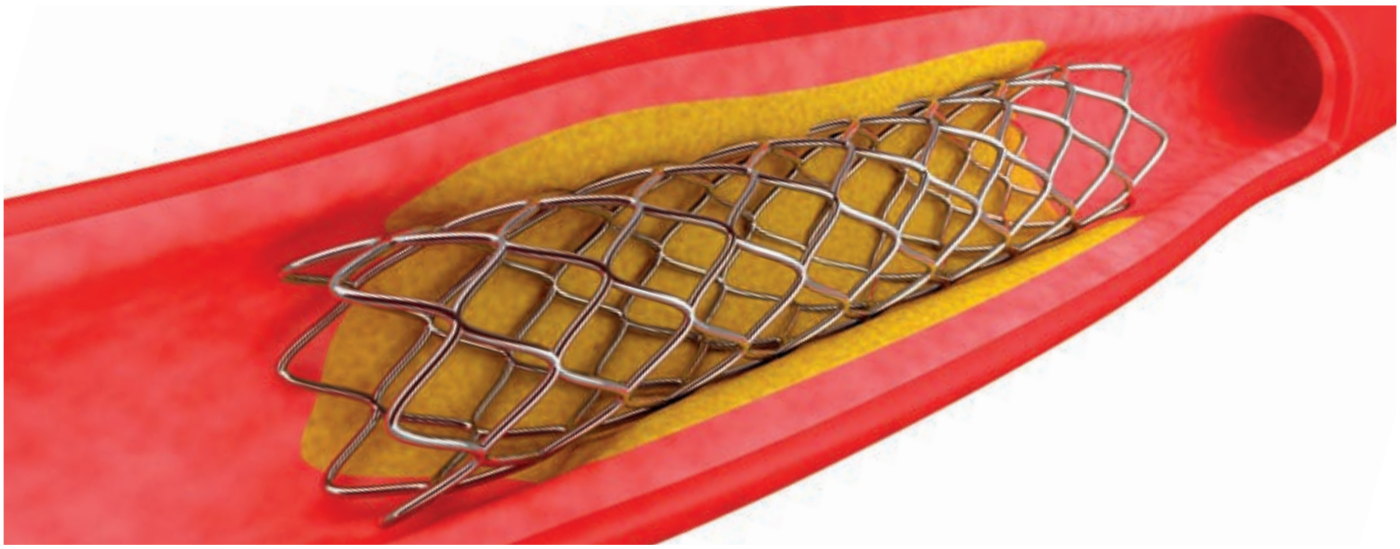
Corkscrew-like X-rays (red) enable electrons to escape from the surface of copper at various angles.

Reference

1. Matsui, F., Fujita, M., Ohta, T., Maejima, N., Matsui, H. *et al.* Selective detection of angular-momentum-polarized Auger electrons by atomic stereography. *Physical Review Letters* **114,** 015501 (2015).

More information about the group's research can be found at the Green Nanosystem Laboratory webpage:
http://mswebs.naist.jp/LABs/matui/index-e.html

Nara Institute of Science and Technology | **Nanomaterials and Polymer Chemistry Laboratory** www.naist.jp/en



Block copolymer films are promising candidates for stent coatings for controlled drug release.

Polymer chemistry

Switchable surface wettability

Switchable spin-coated surfaces hold promise for controlled drug release in medical applications

Controlled drug release is essential for a range of medical treatments, from contraceptive implants to chemotherapy and vaccinations, and a popular option is the use of drug-eluting stents (see figure). The key to this controlled release is being able to manipulate the surface characteristics of the stent coatings — namely, their wettability and degradability.

Hiroharu Ajiro, formerly of Osaka University, now leading the Nanomaterials and Polymer Chemistry Laboratory at Japan's NAIST, investigated different block copolymer films to see which afforded 'switchable' wettability (transitioning between hydrophilic and hydrophobic behaviour) and slow degradability.

Block copolymers are large polymer chains consisting of 'blocks' of simpler polymers. Ajiro and colleagues decided to focus on spin-coated films consisting of block copolymers of L-lactide (LA) and trimethylene carbonate (TMC) derivatives with methoxyethyl groups. They then systematically investigated the surface structural control of their films using contact angle measurements, X-ray photoelectron spectroscopy and degradation behaviour analysis.

There were some challenges that the researchers had to overcome to fabricate these coatings, including designing the monomers and synthesizing the polymers to use as 'blocks'. Ajiro emphasizes that it was particularly important to attain a balance, "both between the hydrophilic and hydrophobic properties, and between the soft and hard domains of the polymers."

Ultimately the researchers prevailed, producing controllable coatings based on poly(TMC) and poly(LA) — with contact angle measurements showing dynamic changes between hydrophobicity and hydrophilicity[1]. The films were tested in two different solvents: water and hexane, exhibiting hydrophilic and hydrophobic behaviour, respectively. This switchable wettability is believed to be due to the interactions in the copolymer between the TMC polymer main chain and the methoxyethyl functional groups — the reorientation of the copolymer making different moieties aggregate on the surface, thereby influencing its wettability.

Understanding the degradation of the films is of particular importance for biomedical applications: the time the film takes to break down must be appropriate for its intended use. Ajiro's team compared the degradation speed of amorphous and crystalline poly(LA) films to their coating, and found that the degradation was markedly slower for the crystallized block copolymer film — making it a much more appropriate coating for long-term drug release.

Ajiro, who is also a researcher with the Precursory Research for Embryonic Science and Technology (PRESTO) programme of the Japan Science and Technology Agency, notes that these films hold great promise for a range of applications, not just in the medical field but for anything that requires "a switchable coating surface without degradation conditions."

Reference

1. Ajiro, H., Takahashi, Y., Akashi, M. & Fujiwara, T. Surface control of hydrophilicity and degradability with block copolymers composed of lactide and cyclic carbonate bearing methoxyethoxyl groups. *Polymer* **55,** 3591–3598 (2014).

More information about the group's research can be found at the Nanomaterials and Polymer Chemistry Laboratory webpage: http://mswebs.naist.jp/english/courses/1844/

NAIST as an International Research University

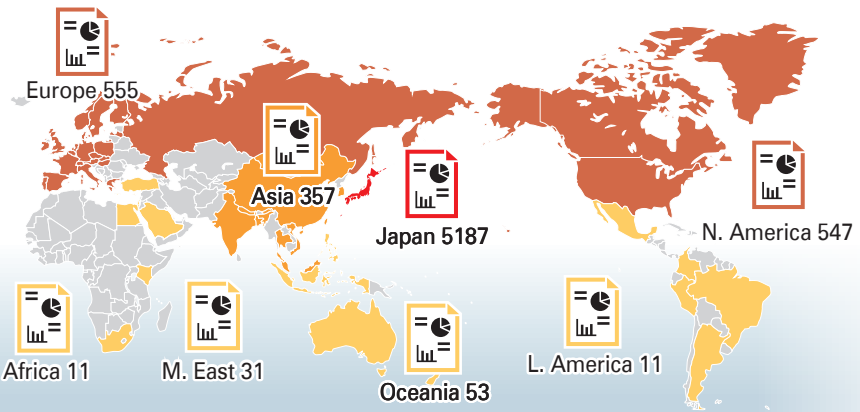
NAIST research collaborations around the globe

Joint research publications with collaborators in **56** countries/regions

Number of joint papers

1-99 | 100-499 | 500-999 | 1000-

data source: Thomson Reuters InCites Benchmarking (1993-2015)



International students in NAIST

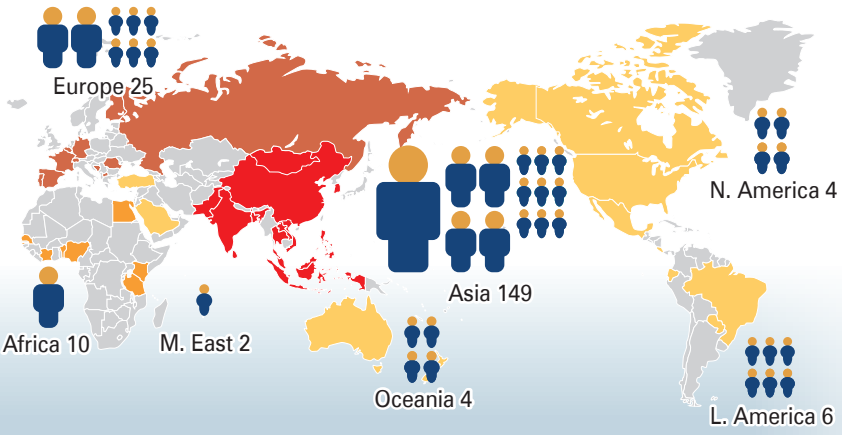
International Students comprise about **18.6%**

Total: **200** students from **43** countries/regions

Number of international students

1-9 | 10-19 | 20-29 | 30-

As of July 1, 2015



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4 research fields

- Plant & Animal Sciences
- Molecular Biology & Genetics
- Biology & Biochemistry
- Chemistry

data source: Thomson Reuters InCites Essential Science Indicators (2005-2015)

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RESEARCH HIGHLIGHTS 2015

NAIST is located in Ikoma City, in Japan's historic Nara Prefecture. Home of the first official capital of Japan, Nara Prefecture has an incredibly rich history as a center for international trade and relations. In addition to its prolific ancient heritage, Nara Prefecture is also conveniently located in close proximity to Kyoto and Osaka, and just 90 minutes from Kansai International Airport.

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Cover story

Researchers at NAIST unraveled the functions of genes responsible for wood development expressed in mosses (*P. patens*). These results will provide versatile tools for manipulating plant biomass. [p30]

The illustration on the cover is a traditional Japanese lighting tool called a "*tourou*" (lantern), a lampshade protecting its flame from the wind. Introduced to Japan together with Buddhism, the *tourou* entered a massive production period around the Nara era (A.D.710-794). This traditional Nara craft illuminated the night with a jumping flame and led to the discovery of a new world. Researchers at NAIST value all local customs and practices nurtured in the ancient capital of Nara and continue to uncover new topics while working tirelessly to identify solutions to any and all challenges that may arise.





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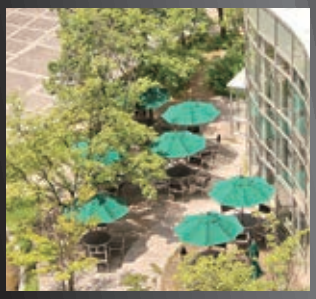
無限の可能性、ここが最先端





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