



## Enhancing translation for therapeutics

### HIGHLIGHT OF THE MONTH

## Trapped biomolecules yield critical information

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Philipp Gubler, Strangeness Nuclear Physics Laboratory

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## Biochemistry

# Trapped biomolecules yield critical information

A technique for encapsulating proteins within giant self-assembling cages could assist biochemists in the analysis of these important natural molecules

From respiration to reproduction, nature relies on proteins. These workhorse molecules control many of the chemical reactions essential for sustaining life. Understanding precisely how proteins perform these roles, or malfunction to cause disease, remains a challenging area of research. Now, a technique for capturing individual proteins developed by researchers in Japan promises to make their analysis easier<sup>1</sup>. It could also herald the harnessing of proteins for numerous industrial applications.

The sheer size of many proteins creates a challenge in handling them for study. Proteins are long-chain natural polymers, folded into bulky three-dimensional forms whose structures are often too complex to map using conventional chemical techniques. Encapsulating individual proteins within well-defined molecular cages could simplify their study. Here again, however, protein size presents a hurdle.

To trap such large structures, the researchers devised giant molecular cages designed to capture single proteins by self-assembling around them. The multi-disciplinary team performing the work included Makoto Fujita at the University of Tokyo, Takashi Kumasaka at the Japan Synchrotron Radiation Research Institute (JASRI) and Masaki Takata at the RIKEN SPring-8 Center. Their 12-sided cages consist of 2 components: palladium ions, which sit at the center of each face of the cage; and organic linkers that join neighboring palladium atoms to form the structure's sides. With each piece in place, the internal cavity of the self-assembled

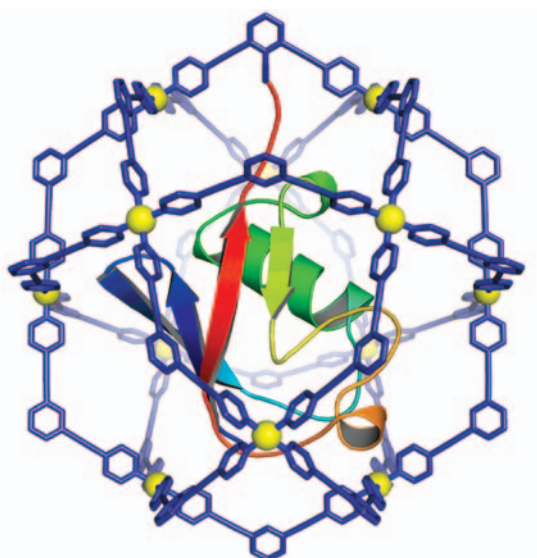


Figure 1: A schematic diagram of the ubiquitin protein (center, multicolored), trapped within a self-assembled cage made from palladium ions (yellow) and organic linkers (blue).

cages measures 6.3 nanometers across, which is large enough to accommodate many proteins.

As a test-case for their technique, Takata and colleagues examined ubiquitin, a heavily studied and well-understood regulatory protein common to the cells of plants, animals and many other organisms. Ubiquitin is also modestly sized, measuring approximately 4 nanometers in diameter, which fits easily within one of the cages.

### Keeping a tight grip

To encapsulate ubiquitin, the researchers first bonded it chemically to a single organic linker. When they added palladium ions and more linker units to this mixture, this first linker acted as an anchor point from which the rest of the cage self-assembled, piece by piece (Fig. 1). The team used several techniques

to confirm that a lone protein was trapped within each cage. For example, using analytical centrifugation, Takata and colleagues could weigh their cages. They found that the structures had a molecular weight of approximately 26,300—a figure that closely matches the theoretical molecular weight of 25,300 for a ubiquitin-filled cage.

Takata explains that they covalently bonded the protein to the first organic linker before forming the remainder of the cage to ensure reliable and robust encapsulation. Without the bond, Takata says, they would need to worry about preventing the protein from escaping the encapsulated state, or even failing to trap the protein in the cage. Although a relatively small protein such as ubiquitin might conceivably slip out of the cage, this would be less of a problem for larger proteins, Takata adds. “In the future, we

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envisage that the covalent bond could be cleaved after encapsulation, because huge proteins will not escape from the cage.”

For some applications, however, keeping a tight grip on the encapsulated protein should be advantageous. One promising use for the cages is to assist with x-ray crystallography. This powerful analytical technique uses a stream of x-rays to probe the structure of crystallized proteins. Detailed images of the protein’s structure can then be generated, sometimes down to the level of individual atoms. However, coaxing large, often globular structures such as proteins to form crystals can be very time-consuming, often requiring much trial and error. Many proteins of interest have never been successfully crystallized.

### A sweet solution

Takata and his colleagues showed that the encapsulation of proteins within cages that readily form crystals could offer a practical workaround. To obtain high-quality crystals, the researchers modified their cage by using organic linkers bearing sugar units on their internal face. These sugars gently wrapped around the trapped protein and held it relatively still without affecting its structure.

Although the giant cages were impossible to analyze using standard laboratory x-ray diffractometers, the team obtained crystal structures using the intense, stable x-ray beams generated by the RIKEN SPring-8 synchrotron. As

the encapsulated protein was not held rigidly within the cage, conventional analysis of the x-ray data generated only a hazy picture of the trapped ubiquitin. However, using the maximum-entropy method (MEM)—a powerful program for processing ambiguous structures—they succeeded in establishing the trapped protein’s position. They enhanced the resulting x-ray image using a computational model of the protein (Fig. 2).

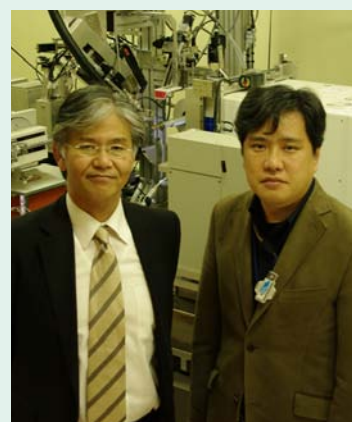
Although ubiquitin’s molecular composition is already well established, the researchers now plan to use their self-assembled cages to study proteins with unknown structures. “By artificial modification of the interior of the cage, we could encapsulate proteins that are difficult to crystallize by themselves,” says Takata. The researchers have already shown that by increasing the length of the organic linkers that form the cage, they can create cavities of up to 7.3 nanometers in diameter, thereby potentially trapping proteins larger than ubiquitin.

In further work, Takata and his colleagues plan to go beyond probing protein structure. They intend to investigate whether their cages can control the function of the protein trapped inside, says Takata. For example, many proteins are formidable catalysts that would be of great use in industrial settings such as pharmaceutical production. Encapsulation could be used to tailor the trapped protein’s reactivity to generate useful chemicals, while helping to protect the

protein from the harsh conditions used within industrial reactors. ■

1. Fujita, D., Suzuki, K., Sato, S., Yagi-Utsumi, M., Yamaguchi, Y., Mizuno, N., Kumasaka, T., Takata, M., Noda, M., Uchiyama, S. *et al.* Protein encapsulation within synthetic molecular hosts. *Nature Communications* **3**, 1093 (2012).

### ABOUT THE RESEARCHER



Masaki Takata (left) was born in Kure, Japan, in 1959. He graduated from the Faculty of Sciences, Hiroshima University, in 1982, and obtained his PhD in 1988 from the same university. After that, he was promoted to associate professor at Nagoya University, before moving into the role of professor at Shimane University in 1997, associate professor at Nagoya University in 1999, director of the Japan Synchrotron Radiation Research Institute (ASRI/SPring-8) in 2005, and chief scientist of the RIKEN SPring-8 Center in 2006. His research focuses on novel structural materials science using high brilliance synchrotron radiation x-rays at SPring-8.

Takashi Kumasaka (right) was born in Fukushima, Japan, in 1968. He graduated from the Faculty of Science of Tokyo Institute of Technology and obtained his PhD in 1996 from the same university. He was then employed as a research scientist by RIKEN and contributed to the construction of a SPring-8 beamline. After working as a lecturer at the Department of Life Science, Tokyo Institute of Technology for five years, he returned to SPring-8 as a group leader of JASRI in 2007. His research now focuses on the structural biology of cellular signal transduction and the methodology of crystallographic analysis using synchrotron radiation.

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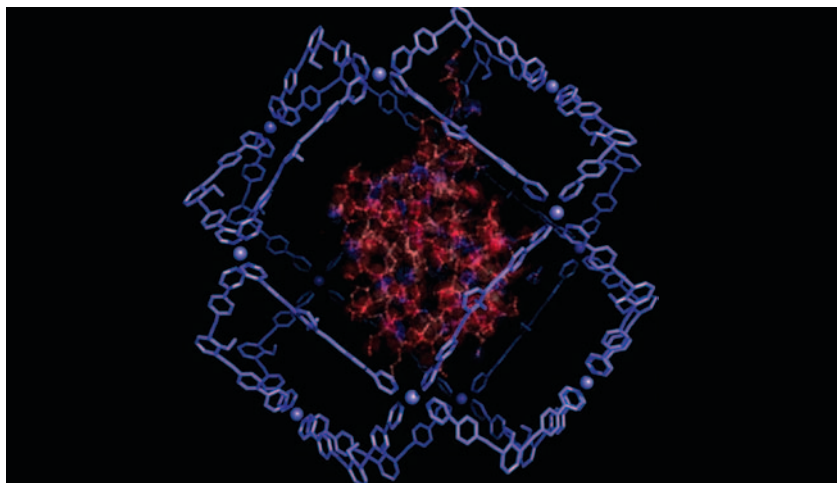


Figure 2: The crystal structure of ubiquitin (red), enhanced by computational modeling, inside the molecular cage (blue).



# Metal goes organic

The observation of truly metallic behavior in an organic material heralds a new generation of electronic devices

When we think about metals, objects like copper wires and sheets of iron spring to mind. However, organic materials—those based, as all living matter, on carbon and oxygen atoms—can also exhibit metallic behavior. Some organic compounds have been established as good electric conductors, but these systems can be full-fledged metals as Reizo Kato of the RIKEN Advanced Science Institute, Wako, and co-workers in Japan and China have shown. They found unambiguous signatures in an organic compound which establish that the material behaves at low temperatures precisely like most metals<sup>1</sup>.

The interest in ‘organic metals’ is fuelled by the prospect of technological applications reaching from stretchable electronics to bio-integrated devices. However, almost 40 years after the first discovery of organic metals, a number of fundamental aspects remain to be explored in these materials. In particular, until now it has never been shown that organic metals behave according to

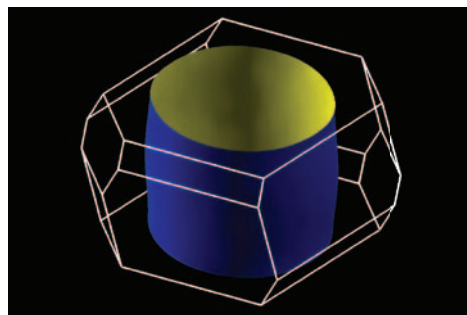
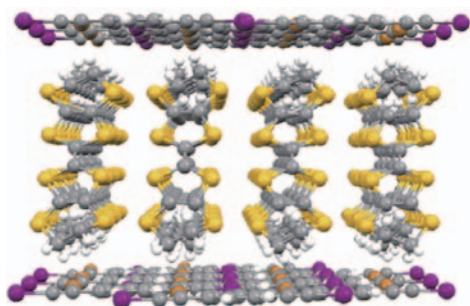
the so-called Fermi-liquid theory—the model that describes the behavior of most metals at low temperatures.

Kato and his colleagues have filled this gap. The team observed the signatures of a Fermi liquid in a compound known as (BEDT-TTF)<sub>3</sub>Br(pBIB) (Fig. 1, left). “In general, organic conductors are fragile and vulnerable to light irradiation,” says Kato. “But over a period of more than ten years our team has made methodological advances—in particular in the area of photoelectron spectroscopy—that allowed us to reduce the disruptive factors.” The team’s know-how enabled them to successfully conduct a series of experiments in which they showed that at low temperatures the electrons in (BEDT-TTF)<sub>3</sub>Br(pBIB) indeed behave in the same characteristic manner as they do in a conventional metal (Fig. 1, right).

These findings call for revisiting a number of earlier experiments that indicated that the electrons in organic materials behave differently from a Fermi liquid. But most importantly,

the work of Kato and his colleagues provides a sound foundation for understanding organic metals, which in turn should pave the way toward practical applications. “This project will provide important information for understanding electronic processes and designing organic materials,” says Kato. Among the organic metals, the (BEDT-TTF)<sub>3</sub>Br(pBIB) system and related compounds are particularly interesting as they are characterized by an architecture in which two-dimensional conducting layers are separated by insulating supramolecular networks. Such network structures may serve as the building blocks for functional molecular materials, including computing and memory elements for electronic devices. ■

1. Kiss, T., Chainani, A., Yamamoto, H.M., Miyazaki, T., Akimoto, T., Shimojima, T., Ishizaka, K., Watanabe, S., Chen, C.-T., Fukaya, A. *et al.* Quasiparticles and Fermi liquid behaviour in an organic metal. *Nature Communications* **3**, 1089 (2012).



**Figure 1:** The crystal structure of the organic metal (BEDT-TTF)<sub>3</sub>Br(pBIB) in real space (left) and a rendering of the ‘wave-number space’ (right), representing the space where conducting electrons exist.

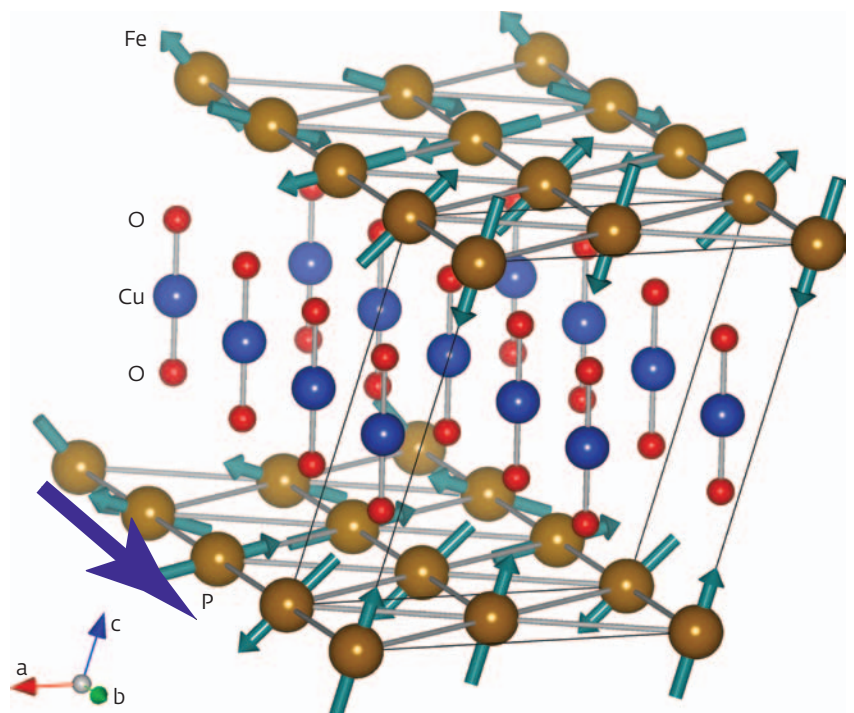
# A good coupling

A new way in which magnetism and electric polarization are coupled has been discovered in  $\text{CuFeO}_2$

Magnetism and electricity are two of the fundamental forces of nature. Combining them in a single multiferroic material in which one controls the other is not only of basic interest, but also relevant for practical applications. “Multiferroic materials can be used as magnetic sensors that change the sign of their electric polarization with a small magnetic field,” says Yoshikazu Tanaka from the RIKEN SPring-8 Center in Harima. After studying the properties of the multiferroic  $\text{CuFeO}_2$ , Tanaka and his colleagues have been able to verify a new mechanism by which magnetism and electricity can be coupled in a single material<sup>1</sup>.

There are different ways in which magnetism and electric polarization, so-called ferroelectricity, are coupled in multiferroics. An understanding of these effects is important, because not only are multiferroics quite rare, but a better understanding of their properties might also help to develop materials where these effects are suitably strong for applications.

The researchers studied the magnetic properties of  $\text{CuFeO}_2$  using a beam of x-rays from the synchrotron facility at the SPring-8 Center. The x-rays specifically probe the electronic states of the iron ions in the crystal that are related to its magnetic properties, and the experiments reveal that these electronic states extend throughout the material in a periodic manner. This arrangement is directly responsible for the multiferroic properties, as it breaks the crystal symmetry and leads to a shift of the electrically charged atoms in the crystal.



**Figure 1: Structure of  $\text{CuFeO}_2$ .** The magnetism of the iron atoms (brown, with green arrows showing magnetism) influences the chemical bonds with the other elements (blue: copper, red: oxygen) to create a ferroelectric polarization (purple arrow P).

At room temperature, each of the iron atoms is surrounded by a symmetric arrangement of oxygen atoms and the magnetic moments of the iron atoms are in disorder. However, at low temperatures the magnetic moments assume the shape of a screw (Fig. 1). Each magnetic moment slightly alters the energy of the chemical bonds in the crystal, depending on the relative orientation between the chemical bond direction and the magnetic moment. The resulting force then distorts the crystal structure and leads to an electric polarization.

Although such a coupling model between magnetism and ferroelectricity has been proposed theoretically, this work represents the first experimental

evidence for this particular mechanism. Moreover, although the practical applications for  $\text{CuFeO}_2$  itself are limited owing to the low temperatures at which this coupling occurs, the discovery could also guide the demonstration of similar materials which do have practical applications, explains Tanaka. “In future, this may lead to the discovery of other materials based on the same mechanism that work at room temperature.” ■

1. Tanaka, Y., Terada, N., Nakajima, T., Taguchi, M., Kojima, T., Takata, Y., Mitsuda, S., Oura, M., Senba, Y., Ohashi, H. *et al.* Incommensurate orbital modulation behind ferroelectricity in  $\text{CuFeO}_2$ . *Physical Review Letters* **109**, 127205 (2012).

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# How yeast responds to change

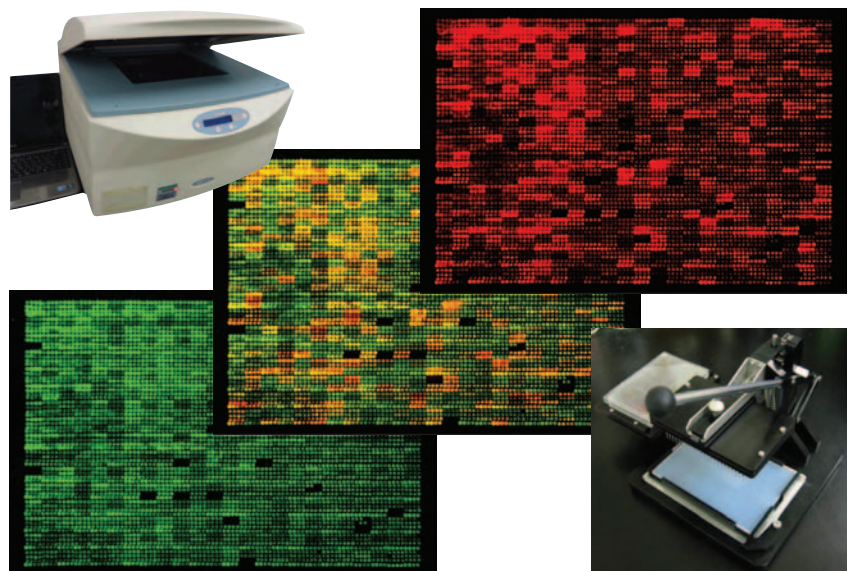
Development of a new protocol provides the key to measuring the complete set of yeast protein levels

A procedure that allows accurate measurement of the levels of over 99% of the proteins generated by different strains of fission yeast could open the way for new laboratory applications of the species. Researchers should now be able to determine the direct impact of environmental change, life-cycle stage, and gene mutations, deletions and activity on this model organism.

The protocol<sup>1</sup>, developed by Minoru Yoshida and Akihisa Matsuyama of the RIKEN Advanced Science Institute in Wako, together with colleagues from the University of Namur in Belgium, has successfully been manually implemented to study the effect of gene deletions. The researchers are confident, however, that it could easily be automated.

The advent of high-throughput genome sequencing has meant that complete genome sequences are readily available for many micro-organisms, allowing molecular biologists to deduce their protein sets—known as proteomes. This opens up the possibility of studying the proteome itself, and in particular how protein levels respond to changes in genes, age or the environment.

Fission yeast (*Schizosaccharomyces pombe*) has become a useful model for human genetic systems because it shares many similar genes and is easy to handle in the laboratory. The approximately 5000-gene fission yeast genome was first published in 2002, but several significant hurdles have prevented researchers determining accurate levels of all the proteins it generates. One obstacle was the yeast cell wall, which makes it



**Figure 1:** To uncover the fission yeast proteome, an infrared image scanner (top left) is used to simultaneously analyze two fluorescent signals—red and green (center panels). More than 6,000 membrane-mounted samples can be tested at once by the arrayer (bottom right).

difficult to extract proteins from the cell for analysis. In earlier work, Yoshida and co-workers successfully developed a means of bursting these cell walls to prepare mixtures of the proteins inside.

The new procedure allows comparison of the proteomes of different strains of yeast—typically a normal ‘wild-type’ strain with a genetic mutant or a strain raised in a specific environment. Specifically, it relies on tagging of open reading frames (ORFs)—the sections of chromosomes where proteins are encoded—with a small tag which is recognized by a fluorescently labeled antibody. The researchers introduced the tags to the strains of interest by means

of a ‘mass mating’ from which only yeast progeny containing tagged ORFs were selected. The level of each protein could then be determined using a fluorescence scanner (Fig. 1).

“We are now using the protocol to study the molecular mechanisms of ageing,” says Matsuyama. “Proteins whose levels reproducibly alter after the yeast cells stop division can provide us with markers for age.” ■

1. Bauer, F., Matsuyama, A., Yoshida, M. & Hermand, D. Determining proteome-wide expression levels using reverse protein arrays in fission yeast. *Nature Protocols* **7**, 1830–1835 (2012).

# Workings of the body clock

The neurotransmitter serotonin has been shown to link sleep-wake cycles with the body's natural 24-hour cycle

Almost all animals have a hard-wired 'body-clock' that controls biological function in cycles of approximately 24 hours. This is known as the circadian rhythm and, in mammals, it is controlled by signaling in a region of the brain called the suprachiasmatic nucleus (SCN). The SCN regulates a number of functions, including hormonal secretion, metabolism, brain activity and sleep.

Areas of the brain close to the SCN—the basal forebrain and pre-optic area (BF/POA)—control short sleep-wake cycles. These short cycles, which are unevenly distributed across 24 hours, are regulated by the circadian rhythm. In order to maintain the overall circadian sleep pattern, these sleep-wake cycles must therefore be linked to the rhythm generated by the SCN. A team led by researchers at the RIKEN Brain Science Institute, Wako, has demonstrated that the neurotransmitter serotonin is the key to this link<sup>1</sup>.

The team measured neural firing in the SCN and BF/POA of rats to monitor

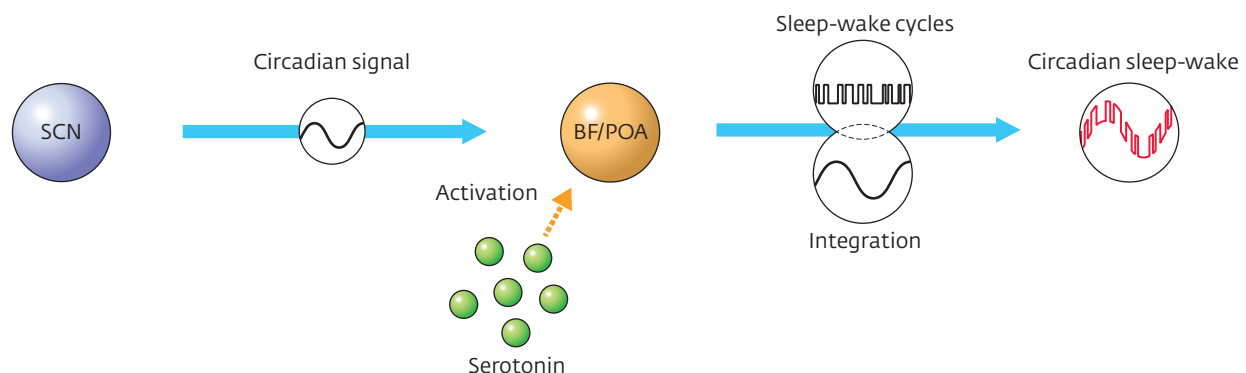
rhythmic activity and looked at how this changed when serotonin levels were reduced. The neurotransmitter was depleted in two separate ways: either an enzyme called TSOI was injected to degrade the precursor of serotonin and prevent its production, or an inhibitor of serotonin production called PCPA was added. Both methods had the same effect.

"After serotonin depletion, sleep-wake cycles became fragmented," said lead author Hiroyuki Miyamoto, "Sleep-wake phases were distributed throughout the day—that is, the circadian rhythm of sleep-wake cycles was lost." Underlying this was a disruption of rhythmic neural activity in the BF/POA, caused specifically by the reduction of serotonin levels. The same effect was not seen in the SCN, however, meaning that the circadian rhythm was unaffected while sleep-wake cycles were disturbed. Blocking serotonergic transmission locally in the BF/POA was also sufficient to disrupt sleep-wake cycles.

The researchers concluded that serotonin acts to link the two cycles (Fig. 1). "Since the BF/POA is a brain region that directly controls sleep-wake states, we think that coupling of the SCN and BF/POA activity rhythms by serotonin is critical for circadian sleep-wake rhythm," says Miyamoto.

The findings may also help in understanding similar brain rhythms in humans and how they may contribute to disorders. "Similar mechanisms may also work in human brains," says Miyamoto. "Dysfunction of the serotonin system has been implicated in depression and patients frequently complain of insomnia. Thus, our study may provide insights into the relationships between serotonin, sleep, circadian rhythms and depression." ■

1. Miyamoto, H., Nakamaru-Ogiso, E., Hamada, K. & Hensch, T.K. Serotonergic integration of circadian clock and ultradian sleep-wake cycles. *Journal of Neuroscience* **32**, 14794–14803 (2012).



**Figure 1: Serotonin links rhythmic activity in the basal forebrain and pre-optic area (BF/POA) to the circadian rhythm signalled by the suprachiasmatic nucleus (SCN), allowing sleep-wake cycles to be regulated over 24 hours.**



# Predicting a high risk of prostate cancer

A new genetic test can predict a man's risk of developing prostate cancer

Researchers in Japan have created a genetic test that will help doctors diagnose prostate cancer<sup>1</sup>. When given together with testing for prostate specific antigen (PSA), a widely used diagnostic biomarker for prostate cancer, the new assay could spare men from undergoing needless prostate biopsies.

“With a super-aging society coming to Japan and other Asian countries, there will be more prostate cancer patients and candidates, and more medical costs related to prostate cancer,” says Hidewaki Nakagawa of the RIKEN Center for Genomic Medicine in Yokohama, who led the study. “So we have to establish ‘personalized medicine’ approaches to screen, diagnose, and prevent prostate diseases more precisely and more efficiently.”

The genetic test includes 16 DNA markers that Nakagawa's team previously linked to prostate cancer in a 2010 study of Japanese men with the disease<sup>2</sup>. Using genetic data from around 4,000 Japanese men with prostate cancer and 7,000 healthy controls, Nakagawa and his colleagues showed that their risk assessment model was highly reproducible across cohorts and that its predictive performance was not influenced by PSA level.

To investigate whether the test might be useful to risk-stratify patients in the clinic, the researchers considered men in the so-called ‘PSA gray-zone’. These men have somewhat elevated levels of PSA, yet only 20–25% of them actually have cancer. The rest typically suffer from less dangerous maladies, such as an enlarged prostate gland, and do not require aggressive treatment. Thus, doctors have long sought additional biomarkers that can identify



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**Figure 1: Adding genotyping to PSA testing could improve prostate cancer predictions.**

individuals at the highest risk of prostate cancer (Fig. 1).

Nakagawa and his colleagues showed that their test could serve this purpose. Among men with gray-zone PSA scores who, the model predicted, had at least a two-fold increased chance of developing cancer, 42% had positive diagnoses. In contrast, only 11% of men who the model predicted were at low-risk had cancer, despite elevated PSA levels.

Given the complications and cost associated with the prostate needle biopsies needed to confirm the presence of cancer following a gray-zone PSA test outcome, Nakagawa suggests that only men at high genetic risk should immediately take that next step, whereas men who score low on the genetic test might choose to continue monitoring their PSA levels before resorting to a needle biopsy.

“We are now negotiating with a company and hospitals about the feasibility of the test's clinical use,” Nakagawa says. “But first,” he notes, “we have to get more data about its precision and cost-effectiveness in a prospective large-scale study.” ■

1. Akamatsu, S., Takahashi, A., Takata, R., Kubo, M., Inoue, T., Morizono, T., Tsunoda, T., Kamatani, N., Haiman, C.A., Wan, P. *et al.* Reproducibility, performance, and clinical utility of a genetic risk prediction model for prostate cancer in Japanese. *PLoS One* **7**, e46454 (2012).
2. Takata, R., Akamatsu, S., Kubo, M., Takahashi, A., Hosono, N., Kawaguchi, T., Tsunoda, T., Inazawa, J., Kamatani, N., Ogawa, O. *et al.* Genome-wide association study identifies five new susceptibility loci for prostate cancer in the Japanese population. *Nature Genetics* **42**, 751–754 (2010).



# Genes mapped for itchy skin condition

A large-scale Japanese genomics project finds eight loci linked to atopic dermatitis

Around one in ten Japanese school children suffer from a debilitating form of eczema known as atopic dermatitis (AD). Despite clear signs that the condition is heritable, the genetic origins of the disease have remained elusive. Now, in a study of about 3,300 Japanese individuals with AD and some 15,000 unaffected controls, researchers have discovered eight loci with ties to the chronic inflammatory skin disorder<sup>1</sup>, a finding that could lead to new treatment options, particularly for Japanese people.

“Further investigation of the susceptible loci of atopic dermatitis could lead to the development of new therapeutic treatments,” says Mayumi Tamari of the RIKEN Center for Genomic Medicine in Yokohama, who led the study.

People with AD typically suffer from persistent, itchy and flaky rashes covering many parts of the body (Fig. 1). When two parents have AD, their offspring have a 70% risk of also developing the disease, with a mode of inheritance that appears to be complex, involving several genes.

To find those genes, Tamari and her colleagues decoded more than 600,000 single DNA letters spread across the entire human genome of their Japanese cohort. Other research teams working with individuals from China and Europe had previously reported seven susceptibility loci for AD. These cropped up in the Japanese group’s analysis, too. However, Tamari’s team also discovered eight newly identified risk loci in the Japanese population. These included genes implicated in innate-acquired immunity, inflammation and skin-related protection, as well



**Figure 1: Skin affected by atopic dermatitis.**

as parts of the genome associated with asthma and other allergies.

For example, one of these associated loci contained *IL1RL1*, an interleukin cytokine receptor gene. *IL1RL1* is expressed by T-helper cells and mast cells in the skin, and this region has also been identified recently as a susceptibility locus for bronchial asthma. This finding makes sense, notes Tamari, as more than 50% of children with severe atopic dermatitis also suffer from asthma and approximately 75% also have allergic rhinitis.

Topical steroids and the immunosuppressive agent tacrolimus are currently the mainstays of AD treatment. However, these therapeutic agents don’t work for everybody, and researchers have been on the hunt for new treatment options.

Interestingly, the researchers found a susceptibility locus on chromosome 20 that includes *CYP24A1*, a gene which is involved in vitamin D metabolism. Considering that vitamin D ointment is commonly used to relieve some of the dry skin symptoms associated with psoriasis, Tamari says that clinical studies should be conducted to see whether vitamin D ointment is effective for atopic dermatitis. ■

1. Hirota, T., Takahashi, A., Kubo, M., Tsunoda, T., Tomita, K., Sakashita, M., Yamada, T., Fujieda, S., Tanaka, S., Doi, S. *et al.* Genome-wide association study identifies eight new susceptibility loci for atopic dermatitis in the Japanese population. *Nature Genetics* **44**, 1222–1226 (2012).

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# Enabling rapid recall of familiar foes

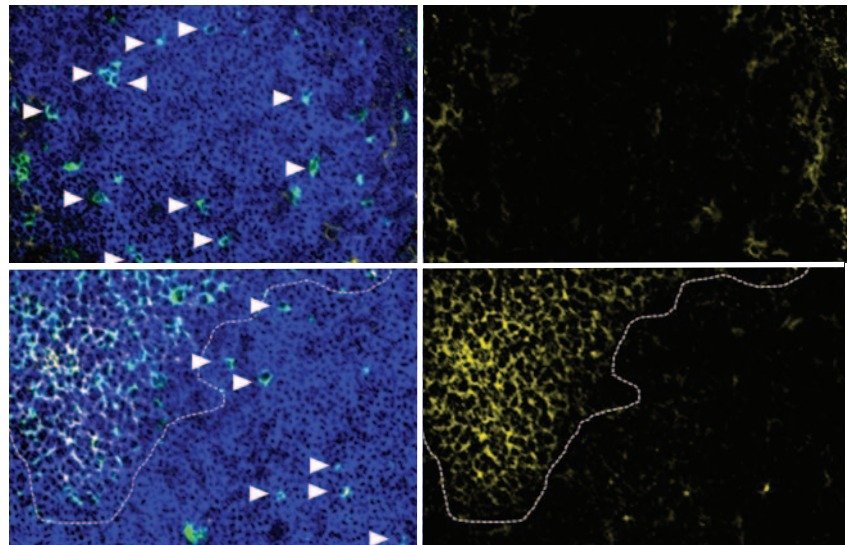
A ‘rapid response’ pathway for immune cell development may improve the body’s ability to recognize and fight back against recurring infectious threats

Efficient immune protection requires the ability to rapidly recognize intruders that the body has encountered in the past. This is achieved via ‘memory’ B cells, which develop following immune system activation by a virus, bacterium or other threat.

“Scientists have known about immunological memory for centuries,” explains Toshitada Takemori of the RIKEN Research Center for Allergy and Immunology in Yokohama, “but certain critical aspects of this process remain incompletely defined.” As a case in point, Takemori’s team and Klaus Rajewsky at Germany’s Max-Delbrück-Center for Molecular Medicine recently uncovered striking proof of a novel memory B cell production pathway with a potentially distinct role in immune defense<sup>1</sup>.

The initial appearance of an immunity-triggering antigen fuels interaction between B and T cells, which in turn yields activated B cells. These can either differentiate into cells that produce antibodies against the target antigen or migrate to structures called ‘germinal centers’ (GCs) where their antibody-encoding genes undergo extensive mutation. This somatic hypermutation (SHM) process generates antibodies with optimized target affinity and specificity, and the resulting cells mature into antibody-secreting plasma cells or memory B cells.

However, Takemori has observed evidence that some memory B cells never undergo SHM, apparently developing via a GC-independent pathway<sup>2</sup>. This has proven difficult to verify experimentally: mice lacking the *Bcl6* gene fail to develop GCs but also suffer other defects, making them a poor research model. To overcome



**Figure 1: Fluorescent labeling reveals that both conditionally *Bcl6*-deficient (top) and wild-type (bottom) mice are able to produce functional memory cells (left column, arrowheads). However, these genetically modified animals lack germinal center (GC) B cells (right column, yellow), revealing a GC-independent pathway for memory cell development.**

this, the researchers engineered rodents where *Bcl6* inactivation is limited to a subset of relevant cells.

These animals lacked GCs, but nevertheless generated memory B cells after an immune challenge in numbers roughly equivalent to normal mice (Fig. 1). Closer examination confirmed that the memory B cells produced by conditionally *Bcl6*-deficient animals did not undergo SHM. The researchers also isolated non-mutated memory B cells from wild-type animals, although these were eventually outnumbered by mutated memory B cells, indicating that these non-mutated cells represent a distinct subset of memory B cells that develop in advance of the GC maturation process. “Our analysis indicates that immunological memory is established as soon as possible after the onset of immune response,” says Takemori.

As non-GC memory B cells produce relatively low-specificity antibodies, the

researchers hypothesize that these cells may complement optimized, post-SHM memory B cells by broadly responding to related but distinct threats: for example, influenza viruses in general rather than one specific strain. “We are now determining whether the GC-independent memory pathway assists the GC-dependent pathway to protect hosts against viral infections,” says Takemori. ■

1. Kaji, T., Ishige, A., Hikida, M., Taka, J., Hijikata, A., Kubo, M., Nagashima, T., Takahashi, Y., Kurosaki, T., Okada, M. *et al.* Distinct cellular pathways select germline-encoded and somatically mutated antibodies into immunological memory. *Journal of Experimental Medicine* **209**, 2079–2097 (2012).
2. Takahashi, Y., Ohta, H. & Takemori, T. Fas is required for clonal selection in germinal centers and the subsequent establishment of the memory B cell repertoire. *Immunity* **14**, 181–192 (2001).

# Prioritizing protein production

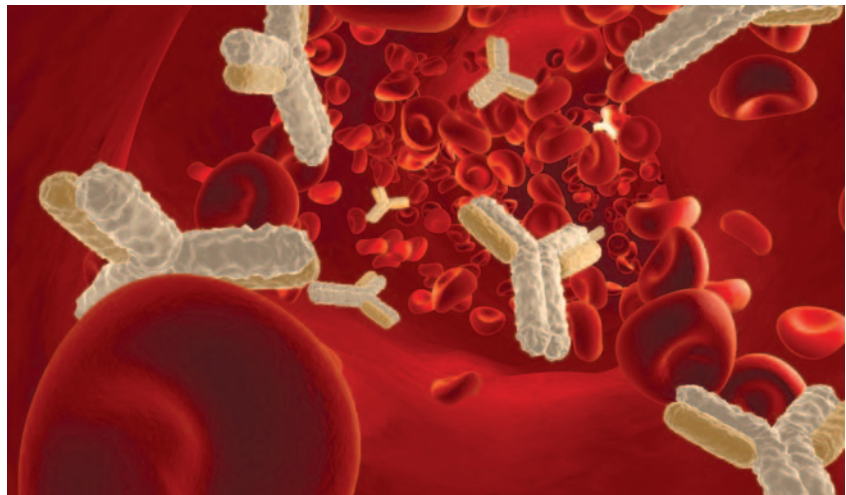
A surprising natural mechanism for selectively stimulating protein production may offer scientists a powerful tool for clinical and biotechnology applications

Every protein arises from translation of an RNA molecule, which was itself transcribed from a gene sequence somewhere in the genome. However, not every gene yields a protein, and over the past decade genome researchers have encountered a stunning variety of non-protein-coding genes that outnumber their protein-coding counterparts by a substantial margin.

New research from a team of Italian researchers, in partnership with Piero Carninci of the RIKEN Omics Science Center in Yokohama, has now uncovered a surprising functional role for certain of these non-coding transcripts that could offer important benefits for clinical research and therapeutic applications<sup>1</sup>.

As part of FANTOM, a multinational genomics consortium based at RIKEN, Carninci's group found that 'antisense' genes, encoding RNAs that arise from transcribing protein-coding genes in the 'wrong' direction, are widespread throughout the genome<sup>2</sup>. These antisense transcripts bind protein-coding 'sense' transcripts via the same chemistry that drives interaction between complementary DNA strands. "More than 70% of the genes show some sort of antisense transcription," says Carninci, "and searching for function was the next step."

Carninci worked with Stefano Gustincich of the International School of Advanced Studies in Trieste, Italy, to investigate an antisense transcript of *Uchl1*, a gene linked with Parkinson's and Alzheimer's diseases. Antisense transcripts typically inhibit translation of their sense counterparts, but the researchers were surprised to find that antisense *Uchl1* actually stimulated



**Figure 1: By generating custom antisense genes, researchers may be able to selectively stimulate production of medically useful proteins such as therapeutic antibodies.**

production of the UCHL1 protein in neurons. "This was completely unexpected based on studies so far," says Carninci.

They subsequently identified a sequence element within antisense *Uchl1* called SINEB2, which appears to be directly responsible for boosting protein synthesis. Additional experiments indicated that this antisense transcript is normally produced as part of a stress response pathway, forcing cells to prioritize increased production of essential proteins when biochemical resources are limited. Given the established involvement of UCHL1 in preventing onset of neurodegenerative disease, such a mechanism could provide critical nervous system protection in the event of a physiological crisis.

Having identified the core functional components of *Uchl1*, Carninci is excited by the possibility of exploiting this mechanism for medically relevant protein targets (Fig. 1). "We see immediate applications to human health

because we can substitute the antisense part to enhance translation of any protein," he says. He and Gustincich recently launched a company called TRANSINE Technologies, based at the RIKEN Omics Science Center, through which the researchers hope to better understand and fine-tune this potentially powerful clinical tool. "We see the chance to create a good biotechnology product," says Carninci. ■

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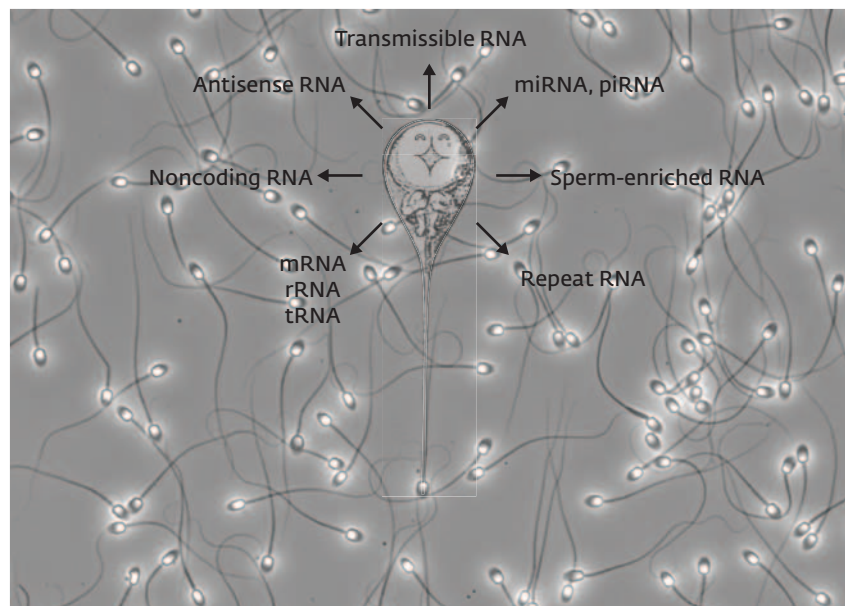
# Uncovering the role of sperm RNA

Sperm contain small RNAs that may play a part in early post-fertilization development

Sperm give new embryos more than just a copy of their genes: they also contribute RNAs, molecules similar to DNA that play many essential roles in switching genes on or off, and in regulating how much protein is produced from each gene. However, very little is known about sperm RNAs or their functions. Now, a RIKEN team led by Mitsuoki Kawano of the Omics Science Center, Yokohama, Japan, has identified two novel RNAs that may influence the early development of the embryo<sup>1</sup>.

Kawano, now at Niigata University of Pharmacy and Applied Life Sciences, and colleagues surveyed the RNA complement of mouse sperm using deep sequencing, which identifies both the sequence and frequency of each RNA. The analysis generated over 350,000 RNA sequences, including fragments of ‘coding’ RNAs that are made into proteins (messenger RNAs) and ‘non-coding’ RNAs that perform functions, such as translating genes into proteins (ribosomal RNAs) or silencing genes (Piwi-interacting RNAs (piRNAs) and microRNAs (miRNAs)) (Fig. 1).

The team isolated and examined two of the most abundant of the small noncoding RNAs, dubbed spR-12 and spR-13, which are composed of fewer than 22 bases, or building blocks, and whose precise functions have yet to be determined. To help understand how they might function, Kawano’s group determined how long these small RNAs persist. Both are present in sperm, the newly fertilized egg, and the very early stages of the embryo. Their relative longevity and presence in the nucleus



**Figure 1: Many types of RNAs are known to be present in sperm and studies are now progressing to reveal their functions.**

may indicate that they play a role in setting gene expression patterns in the new embryo.

Examination of their structure revealed that spR-12 and spR-13 are part of a unique set of RNAs. “They are different from the well-known piRNA population from testis and miRNA,” says Kawano. Further analysis has revealed a distinct signature for these mature-sperm-enriched small RNAs, which represent a novel class of abundant small RNAs that can be grouped into distinct families. The researchers speculate that these RNAs are produced in a unique manner: unlike other RNAs formed during spermatogenesis, they are trimmed down from their longer precursor piRNA relatives.

Knowledge of which RNAs are usually present in healthy sperm could be used to confirm success of vasectomies, diagnose male infertility, and help to pinpoint environmental factors contributing to the rise in male infertility. “Future work will reveal whether these sperm-enriched small RNAs contribute to development of a fertilized egg,” says Kawano. The researchers are also interested in investigating how factors such as diet, smoking, and stress affect the small RNA profiles in human sperm. ■

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# Finding cancer's escape route

By determining the structural effects of tumor-causing mutations, scientists obtain valuable information for drug discovery

For many patients with non-small-cell lung carcinoma (NSCLC), tumorigenesis is fueled by mutations that hyperactivate the epidermal growth factor receptor (EGFR) signaling protein. These individuals may benefit from treatment with drugs such as gefitinib, a chemical inhibitor of EGFR, although additional mutations in EGFR can render the cancer drug-resistant.

Accordingly, scientists are struggling to overcome NSCLC recurrence. “The mutations related to drug sensitivity and those that cause drug resistance cannot be understood without the structures of these EGFR variants,” explains Shigeyuki Yokoyama, director of the RIKEN Systems and Structural Biology Center in Yokohama. By teaming up with Tadashi Yamamoto’s group at the University of Tokyo, Yokoyama and colleagues have now made major headway in clarifying the roots of resistance and how they might be exploited with future drugs<sup>1</sup>.

The researchers performed structural and biochemical analysis of an EGFR variant containing the gefitinib sensitivity-inducing G719S mutation, either alone or with the additional resistance mutation T790M (Fig. 1). Remarkably, they determined that although G719S binds strongly to gefitinib, G719S/T790M binds the drug even more tightly. “This appears to be contradictory to the drug resistance phenotype,” says Yokoyama.

Further investigation offered potential explanations for this paradox. First, the T790M mutation appears to stabilize a network of amino acids that maintain EGFR in a continuously active state. Additionally, EGFR must bind molecules of

adenosine triphosphate (ATP) to perform its signaling activities, and the researchers determined that G719S/T790M has a markedly increased capacity for ATP binding relative to G719S. This enhancement of ATP binding caused by the T790M mutation, could therefore render EGFR resistant to gefitinib in spite of its strong affinity for the drug.

Yokoyama and Yamamoto also identified the mechanistic basis for the strong drug response observed for both G719S and another common gefitinib-sensitive EGFR variant, L858R. In both cases, they identified specific rearrangements that essentially widened the protein’s ATP-binding site, creating sufficient space for gefitinib to bind and interfere with signaling.

Collectively, these structural findings could prove invaluable for uncovering new vulnerabilities in drug-resistant cancers. Yokoyama and colleagues recently used computer simulations to identify vulnerabilities in the G719S/T790M double-mutant<sup>2</sup>. These new data should enable

even more accurate drug design against EGFR as well as other cancer-linked signaling proteins in the future. “We are planning to increase inhibitor specificity based on structure determination of the complexes between drug-resistant EGFR mutants and various compounds,” says Yokoyama. “This structure-based drug discovery should yield more powerful and useful anti-cancer drugs.” ■

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**Figure 1: Structure of the gefitinib-resistant EGFR double mutant bound to a chemical analogue of ATP (represented by the stick diagram), where green spheres represent the G719S mutation and yellow spheres represent the T790M mutation. These mutations keep the receptor active by stabilizing a network of amino acids known as the 'hydrophobic spine' domain (blue spheres).**

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## ZHAOMIN HOU

Chief Scientist  
Organometallic Chemistry Laboratory  
RIKEN Advanced Science Institute

# Creating new catalysts and producing new materials

**“New Catalysts, New Reactions, New Materials”**—this is the slogan for the research activities at the Organometallic Chemistry Laboratory in the RIKEN Advanced Science Institute. **“In my laboratory, we are working to develop new and original catalysts, to carry out new reactions that have not been conventionally feasible using these new catalysts, and to produce new materials through these new catalysts and reactions. Our ultimate goal is to develop catalysts that will allow chemical bonds to be broken and formed at any desired site,”** says Zhaomin Hou, the laboratory’s chief scientist. Utilizing rare earth metals, for which few catalyst-related applications have previously been found, Hou has been successful in developing a number of new catalysts. Currently, he is also working on developing hydrogen-absorbing materials as an extension of his catalyst research.

### New catalysts to change society

“Mankind has acquired the ability to produce a wide variety of substances through chemistry. However, the desired substance generally cannot be obtained without many steps of chemical reactions, which can consume vast amount of energy, produce unwanted byproducts and place great burdens on the environment. We are working to develop catalysts that can selectively produce the desired substances with minimal intermediary steps,” says Hou.

A catalyst is a substance that promotes a chemical reaction while remaining unchanged before and after the reaction. “If a new catalyst is developed, it will be possible to carry out an otherwise

unfeasible chemical reaction to produce a new substance. The development of powerful catalysts can have major impacts that will change society.” In the last decade alone, nine scientists—including the president of RIKEN, Ryoji Noyori—have received the Nobel Prize in Chemistry for their achievements in developing new catalysts, speaking to the recognized importance of this field.

There are a great many types of catalysts, and the Organometallic Chemistry Laboratory mainly develops organometallic complexes. An organometallic complex has an active metal at its center, which acts on reactive molecules to promote chemical reactions, and an organic substance (ligand) around the metal,

which controls the mode of the reaction. “When the central metal is replaced with another, the complex exhibits different behavior. To date, a wide variety of catalysts have been created by changing the metal. However, for the creation of a more sustainable society, it is necessary to continue to develop new catalysts.”

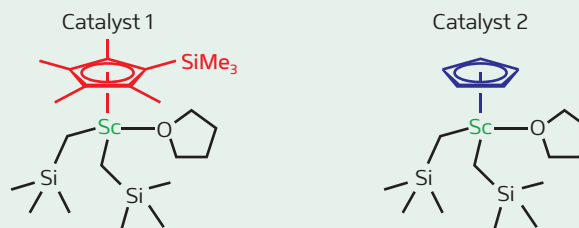
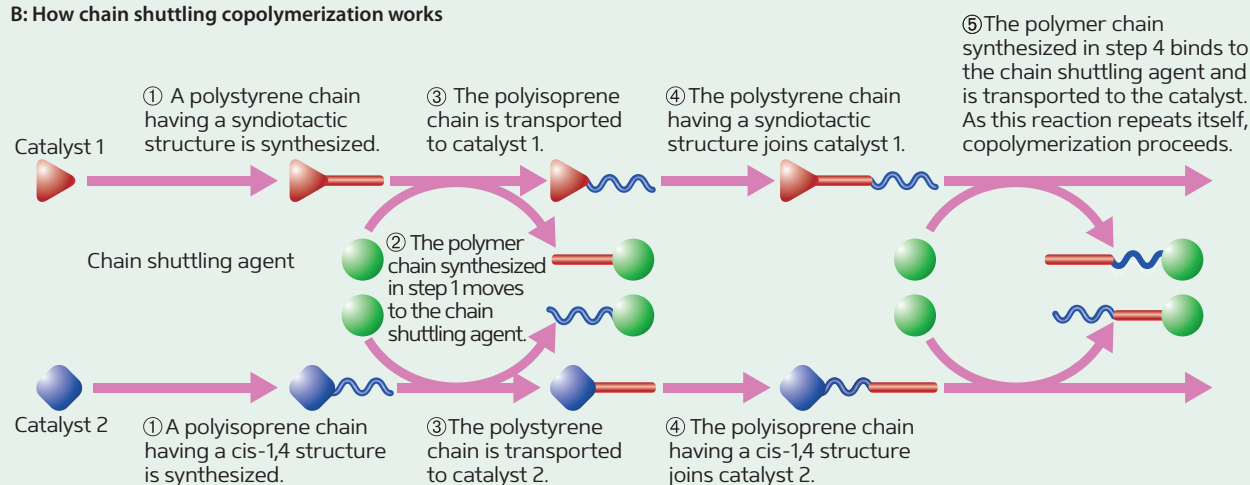
### Exploring rare earth metals to produce catalysts

One of the major aims of Hou’s laboratory is to develop catalysts using metal elements called rare earth metals. “While indispensable to advanced modern technologies, these elements are unfamiliar to many chemists and the general public,” says Hou.

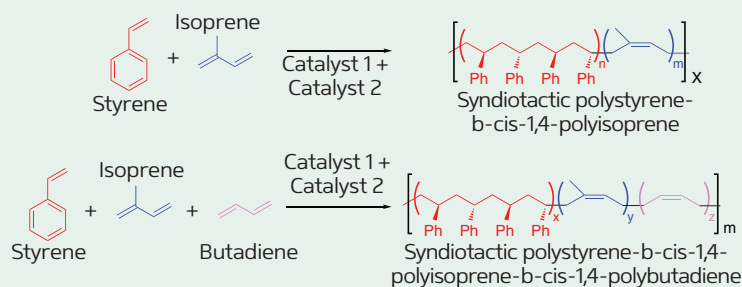


**A: Rare earth polymerization catalysts**

Catalyst 1 is capable of synthesizing polystyrenes with a syndiotactic structure, which are hard and highly resistant to heat and chemicals. Catalyst 2 is capable of synthesizing polyisoprenes and polybutadienes having a cis-1,4 structure; polymers having a cis-1,4 structure are soft and highly elastic.

**B: How chain shuttling copolymerization works****C: Structures of polymers synthesized by chain shuttling**

Adding a chain shuttling agent to catalysts 1 and 2 allows styrene and isoprene to be copolymerized in a stereoselective way (upper process). Copolymerization of three components, i.e., styrene, isoprene and butadiene, is also possible (lower process). The chain shuttling agent used here is triisobutylaluminum ( $i\text{Bu}_3\text{Al}$ ), prepared by binding an alkyl group to the aluminum atom.



**Figure 1: Chain shuttling copolymerization**

Theoretically, a highly functional polymer possessing the nature of two or more molecular species can be synthesized by polymerization. However, it is usually difficult to carry out such a copolymerization reaction selectively. In chain shuttling, by using more than one catalyst capable of selectively polymerizing individual molecular species and a chain shuttling agent, it has become possible to polymerize several molecular species in a stereoselective manner.

In organic synthesis, organometallic compounds containing a main-group metal, such as lithium or magnesium, were the first to be used as reaction reagents. In later years, organometallic complexes containing a d-block transition metal—elements belonging to groups 4 to 12 in the periodic table, such as titanium or palladium—emerged as catalysts and have been widely used to date. Breaking tradition, Hou has been working with rare earth metals since he was a graduate school student in the 1980s. “Rare earth metals are distinct from the main-group metals and transition metals. I conceptualized that by making the best use of their characteristics, it would be possible to create new catalysts that can be used to carry out reactions that would be impossible with

conventional catalysts, thus leading to the production of new substances.”

In the 1980s, only a few researchers were involved in research into rare earth metals, let alone in their use as catalysts. “Rare earth metal catalysts soon break down upon contact with air or moisture, so they are quite difficult to handle. Although I had trying times, everything that I attempted to do in the area of rare earth catalysts always led me to new discoveries because no one else had investigated it, which was very fun for me.”

**Highly functional synthetic rubber**

One of the exciting new catalysts born in the Organometallic Chemistry Laboratory is a polymerization catalyst.

The term ‘polymerization’ refers to binding molecules together to produce a compound with a larger molecular weight. The starting molecular compound is called a monomer, and the resulting high-molecular compound is called a polymer. In 2005, Hou succeeded in developing a new polymerization catalyst with the rare earth metal yttrium, and used it to create a synthetic rubber that surpasses the functionality natural rubber.

The primary component of natural rubber is a polyisoprene (a polymerization product of isoprene) which has a cis-1,4 structure. Molecules with cis-1,4 structures account for about 98% of polyisoprenes chemically synthesized using conventional catalysts. “Because isoprene has two double

bonds in its molecular structure, polymers with a wide variety of structures can be produced depending on which of the two double bonds reacts and how it reacts. To create a highly functional synthetic rubber, it is necessary to control the reaction to unify the polymer structure.” The ratio of cis-1,4 structures differs by only 2% between natural rubber and artificial rubber made with traditional catalysts, but this small difference has a major effect on the frictional and tensile strengths, elasticity, and other properties of the rubber; to date, synthetic rubber has been unable to surpass natural rubber in terms of functionality.

The polyisoprene prepared using the yttrium catalyst developed by Hou has a completely cis-1,4 structure. “The molecular weight and polyisoprene length of natural rubber is variable even though it has the same structure. In contrast, our polyisoprene has an almost constant molecular weight. Furthermore, natural rubber contains impurities that can cause allergies, whereas our rubber is free from impurities and boasts features exceeding those of natural rubber.”

Why did it become possible to synthesize uniformly structured polyisoprenes? “One reason was that our catalyst is a ‘single-site’ catalyst. Because the portion involved in the reaction is monofunctional, the reaction always occurs with the same binding position and number,” explains Hou. “The other reason was that we changed the ligand structure.” Specifically, he employed a phosphinamide group, which is not

used in conventional polymerization catalysts. More remarkably, when he changed the ligand to amidinate, Hou succeeded for the first time in the world in synthesizing a polyisoprene with an isotactic-3,4 structure. “I was very excited by the dramatic change that was induced by merely altering the ligand.”

### New and better polymers

Polymerization of two kinds of monomers, called ‘copolymerization’, makes possible the production of high performance polymers possessing the properties of the two monomers. However, the use of conventional catalysts is unlikely to achieve the desired copolymerization.

In 2004, Hou developed a scandium catalyst carrying a cyclopentadienyl ligand, which he then used to create a high performance polymer by copolymerizing styrene and ethylene in a stereoselective manner. Polystyrene, comprised of styrene units bound to each other in a “syndiotactic” regular steric structure, is highly resistant to heat and chemicals and possesses such desirable characteristics as excellent dimensional stability and low dielectric constants. However, it is brittle and too hard to permit easy processing. By using the scandium catalyst, Hou was able to introduce ethylene into a polystyrene with a syndiotactic structure, and thereby produce a tough, soft and highly workable polymer material with new functions.

This achievement has paved the way for the synthesis of novel functional

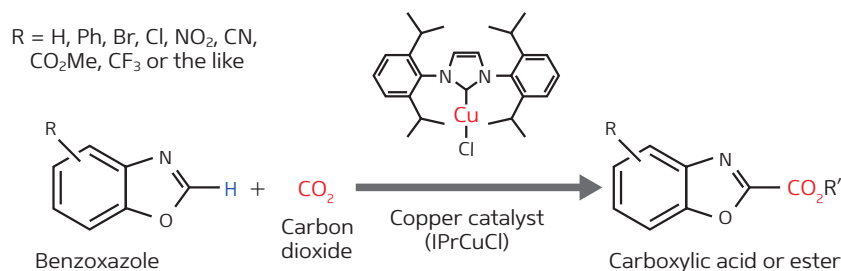
high-molecular materials. “However, synthesis with only one catalyst is subject to limitations,” says Hou. He attempted to copolymerize styrene and isoprene to produce a high-performance polymer possessing both hard and soft natures, but the catalyst exhibited almost no selectivity for isoprene, although it was excellently stereoselective for styrene polymerization. “Hence, I decided to try a combination of two kinds of catalysts.”

Based on the rare earth metal scandium, Hou developed two catalysts: one that polymerizes styrene into a polystyrene having a syndiotactic structure (catalyst 1) and another that polymerizes isoprene into a polyisoprene having a cis-1,4 structure (catalyst 2) (Fig.1, part A). “In fact, the combination of these two catalysts is insufficient. The key is to add a chain shuttling agent.”

The styrene polymerized by catalyst 1 binds to the chain shuttling agent and is transported to catalyst 2 (Fig. 1, part B); at catalyst 2, isoprene is polymerized, binds to the chain shuttling agent, and is transported to catalyst 1; at catalyst 1, styrene is again polymerized in a recurring cycle. Through this reaction, Hou was able to synthesize a polymer consisting of a polystyrene with a syndiotactic structure and a polyisoprene with a cis-1,4 structure, a major achievement that was announced in October 2011 (Fig. 1, part C, upper process).

Chain shuttling is a technique that initially came into use around 2006 with a catalyst containing a group 4 d-block transition metal, but this was the first time in the world that chain shuttling had been performed with a rare earth metal catalyst. “The simpler the chemical reaction, the better; thus, first you search for a single catalyst. However, just like the fact that the ability of a single person alone is subject to limitations, teamwork is sometimes necessary for catalysts.”

Hou also successfully applied this method to synthesize a 3-component polymer consisting of a polystyrene having a syndiotactic structure, a



**Figure 2: Conversion of carbon dioxide to useful organic substances using a copper (Cu) catalyst**

When reacting the heterocyclic aromatic compound benzoxazole and carbon dioxide (CO<sub>2</sub>) using a copper catalyst (IPrCuCl), the carbon-hydrogen (C-H) bond between the oxygen atom (O) and the nitrogen atom (N) in the benzoxazole becomes activated and replaced by CO<sub>2</sub>, resulting in conversion of the starting materials to a carboxylic acid or its ester.

polyisoprene with a cis-1,4 structure and a polybutadiene having a cis-1,4 structure (Fig. 1, part C, lower process).

“All the catalysts I use in my studies contain a rare earth metal, and I have great hope for these particular studies, which I just recently started. I believe that it is possible to create new high-molecular materials that are difficult to synthesize using conventional catalysts by combining a rare earth metal catalyst and a group 4 transition metal catalyst.”

### A heterogeneous polymetallic hydride for hydrogen absorption and release

Research into rare earth metal catalysts sometimes leads in directions that surprise even Hou. One such example is new research into hydrogen-absorbing materials.

Hydrogen is drawing increasing attention as a clean and sustainable source of energy. However, because it is gaseous and hence voluminous under normal temperature/pressure conditions, a material that efficiently and safely absorbs large amounts of hydrogen must be developed before hydrogen can be widely adopted as an energy source.

While Hou was conducting an experimental study to develop a new catalyst in which rare earth catalyst ligands were replaced by hydrogen, he produced a tetranuclear rare earth hydride compound (a hydride is a negatively charged hydrogen atom) containing four rare earth metals and eight hydrides. “The tetranuclear rare earth hydride compound exhibited an extremely high reactivity that would never occur with a mononuclear catalyst, which contains only one metal: the four metals cooperate to break strong bonds which cannot be broken by a monomolecular catalyst. For example, we also succeeded in breaking the carbon-oxygen triple bond in carbon monoxide to produce ethylene, a task that has been difficult to achieve using any conventional catalyst.” While this compound is expected to find major advances as a catalyst, Hou noticed

further potential for it as a hydrogen-absorbing material.

Known hydrogen-absorbing materials include lanthanum-nickel alloys; by allowing hydrogen to be absorbed on a metal in the form of hydride, the volume can be reduced to less than one-thousandth. However, it remains unclear how such alloys absorb and release hydrogen. Having already determined the positions of individual atoms in the tetranuclear rare earth hydride compound by x-ray structural analysis, Hou thought that the hydrogen absorption/release efficiency might be improved through an understanding of the mechanism for hydrogen absorption and release from the structure.

The rare earth metal and the hydride are tightly bound, and it is therefore difficult to absorb and release hydrogen. Hence, Hou combined a d-block transition metal compound, which was already known to facilitate the absorption and release of hydrogen, to synthesize “a heterogeneous polymetallic hydride cluster”. When this heterogeneous polymetallic hydride cluster is thermally degassed under reduced pressure, one hydrogen molecule is released. X-ray structural analysis revealed that the hydride at the center and the yttrium-yttrium binding hydride bind together, resulting in the release of a hydrogen molecule. He also found that when hydrogen was blown through the cluster at normal temperature under normal pressure, the heterogeneous polymetallic hydride cluster that had released hydrogen was restored to its original structure. This new understanding of the mechanism for hydrogen absorption and release is expected to help develop a highly efficient method of hydrogen storage.

### Producing high-value-added substances from carbon dioxide

Hou is also conducting research towards synthesizing high-value-added substances from carbon dioxide (CO<sub>2</sub>). He has succeeded in converting CO<sub>2</sub> into useful organic substances—i.e., carboxylic acids and their esters—using a

catalyst based on copper, an inexpensive metal. Specifically, he broke the carbon-hydrogen (C-H) bond in the heterocyclic aromatic compound benzoxazole using his copper catalyst and introduced CO<sub>2</sub> into it (Fig. 2). While studies into the production of useful substances from CO<sub>2</sub> are being conducted worldwide, one problem concerns how to directly activate the stable and sparingly reactive C-H bond and allow it to react with CO<sub>2</sub>. Hou selected the heterocyclic aromatic compound as the recipient of CO<sub>2</sub> because it is easy to activate due to the presence of the C-H bond between the oxygen atom and the nitrogen atom. “We are proceeding to develop a catalyst that is capable of activating all C-H bonds and converting CO<sub>2</sub> into a high-value-added substance,” says Hou.

Another goal of his work is to develop a technology for effective use of nitrogen, which accounts for about 80% of the atmosphere. He aims to synthesize ammonia—an important component of fertilizer and numerous other products—from nitrogen with reduced energy consumption and no environmental load.

Hou hopes that his findings will help to shape a greener future. “Through my research, I want to contribute to a more sustainable society.” ■

### ABOUT THE RESEARCHER

Zhaomin Hou was born in Shandong Province, China, in 1961. He graduated from the China University of Petroleum in Shandong, China, in 1982, and obtained his PhD in chemistry in 1989 from Kyushu University, Fukuoka, Japan. After postdoctoral training at RIKEN (1989-1991) and the University of Windsor in Windsor, Canada (1991-1993), he joined RIKEN as a research scientist. He was promoted to senior scientist in 1997 and to chief scientist in 2002. Since then, he has been directing the Organometallic Chemistry Laboratory. His research focuses on the development of new catalysts for more efficient selective chemical transformations and the synthesis of novel functional materials.





## PHILIPP GUBLER

Foreign Postdoctoral Researcher  
Strangeness Nuclear Physics Laboratory  
RIKEN Nishina Center for Accelerator-  
Based Science

# Exploring research opportunities at RIKEN

### How and when did you join RIKEN?

I am originally from Switzerland. Upon completing my PhD studies at the Tokyo Institute of Technology in early 2012, I joined RIKEN via the Foreign Postdoctoral Researcher (FPR) program.

### What is your field of research?

I am a theoretical physicist working in the area of hadron physics. Specifically, I am interested in the “strong interaction” which governs the properties of nuclei at the center of atoms. As part of the Strangeness Nuclear Physics Laboratory led by Associate Chief Scientist Emiko Hiyama, my research explores the outcome of matter when it is heated to extremely high temperatures. Such hot matter is experimentally realized in heavy-ion collisions, during which we expect a new form of matter—the “quark-gluon plasma”—to emerge.

Although this subject is technically very challenging, I look forward to making real progress during my time here at RIKEN.

### Why RIKEN?

The research environment provided here is very attractive, and there are several top-notch labs—both theoretical and experimental—at RIKEN led by distinguished scientists in the fields

related to my research. Furthermore, the FPR position gives me a lot of freedom to pursue my scientific goals and generous grant support, which allows me to purchase necessary equipment and to attend international conferences.

### What is the best thing about working at RIKEN?

People at RIKEN are very receptive towards new ideas and opinions, which gives me a sense of freedom. I really appreciate the regular exchange of ideas not only between me and my colleagues, but also with researchers from different labs with overlapping interests. This provides me with many opportunities to learn about techniques and tools used in other areas of research, and thus ideas about new approaches that could be developed for my own work.

### What has been a memorable experience for you during your time at RIKEN?

At an event called the “Discovery Evening” I was given the opportunity both to present my own work and to explain the latest developments in nuclear and particle physics to researchers from a variety of disciplines.

As I normally present my research only to people in my field, this was a unique

experience which helped me to put the work done at our lab into a broader perspective and, at the same time, to find out a little bit more about the projects that other researchers at RIKEN are working on. All in all, it was a unique and illuminating experience.

### Would you recommend the FPR program?

Absolutely. Coming to RIKEN is a great opportunity to pursue one’s scientific goals and to enrich one’s life experience by living in Japan. Life for non-Japanese researchers is facilitated by the many people at RIKEN who speak English, and there are people working full-time to help with daily life issues, such as making travel arrangements, or finding accommodation and helping with official documents and paperwork, as well as providing assistance with academic matters, such as grant applications. RIKEN is a supportive and very international institution, and a rewarding place to pursue one’s research.

### CONTACT INFORMATION

For details about working at RIKEN, please contact the RIKEN Global Relations Office:

Tel: +81-(0)48-462-1225

E-mail: [gro-pr@riken.jp](mailto:gro-pr@riken.jp)

## RIKEN scientists awarded Baelz Prize

On 13 November 2012 a team from the RIKEN Center for Genomic Medicine (CGM) won second place at the awards ceremony of the 49th Baelz Prize 2012. The theme of the 2012 prize was autoimmune diseases, and Kazuhiko Yamamoto, laboratory head of the Laboratory for Autoimmune Diseases at the CGM in Yokohama, was awarded second place in the Baelz Prize along with team members Senior Scientist Yuta Kochi, Senior Scientist Akari Suzuki, and Senior Visiting Scientist Ryo Yamada, and Visiting Scientist Yukinori Okada of the Laboratory for Statistical Analysis. The team received the award for their paper entitled, "From genetics to functional insights into autoimmune diseases."

The Baelz Prize was established in 1964 by the German pharmaceutical company Boehringer Ingelheim in commemoration of the German doctor Erwin von Baelz, who was a leading figure in the promotion of German–Japanese ties and played a significant role in the development of medicine and medical education in Japan during the late 1800s. The Baelz Prize also aims to celebrate the long history of Germany–Japan relations, to promote collaboration between the two countries in the area of medicine, to deepen the friendship between the two countries, and to support medical research in Japan.



Kazuhiko Yamamoto (second from left), laboratory head of the Laboratory for Autoimmune Diseases at the CGM, receives second place in the Baelz Prize on behalf of his team.

"Genetic information is important for understanding the mechanisms of autoimmune diseases," explains Yamamoto. "In order to identify disease-associated genes, a genome wide association study (GWAS) is a rewarding strategy. We have been involved

in such genetic analyses of autoimmune diseases for more than 10 years with the collaboration of many researchers and staff within our research center at RIKEN and from other institutions. Our award is a consequence of these collaborative efforts." ■

### FANTOM5 Meeting and the OSC Award

The third international meeting of the FANTOM5 consortium was held at the RIKEN Yokohama Institute from 29 October to 2 November 2012. The Functional Annotation of the Mammalian Genomes (FANTOM5) consortium is an international research association organized by the RIKEN Omics Science Center (OSC). About 154 researchers from 14 countries and 45 research institutes participated in the consortium's third meeting, which focused on the theme of the time courses of

cellular changes, and participants engaged in lively discussions on the main theme and other topics.

At the event, the 2012 OSC Award (FANTOM Prize) was presented to FANTOM collaborator David A. Hume, professor at the Roslin Institute and Royal (Dick) School of Veterinary Studies, the University of Edinburgh. The OSC Award (The Award for Distinguished Contribution) is given to researchers and staff as an expression of gratitude and respect to recognize a great contribution to the establishment and development of the OSC. Hume earned this award because of his excellent

and ongoing contribution to FANTOM since its earliest beginnings, when the consortium was first established in 2000. The award ceremony was held during the consortium meeting on 1 November. Hume also gave a memorial lecture that traced the history of FANTOM and reflected on the vital contributions made by collaborating researchers over the past 12 years, as well as looking ahead to future goals for the further development of the FANTOM consortium. ■



Participants gathered from around the world to attend the third international meeting of the FANTOM consortium.

### RIKEN to host a booth at AAAS Annual Meeting

RIKEN will be exhibiting at the 2013 American Association for the Advancement of Science (AAAS) Annual Meeting in Boston, US, from 14 to 18 February. The booth is one of several in this year's 'Japan Pavilion', an exhibition showcasing the latest research at top Japanese universities and research institutions. In addition, on 15 February RIKEN, the Japanese Ministry of Education, Culture, Sport, Science and Technology (MEXT) and the University of Tsukuba will hold a joint workshop to provide detailed information about the three major Japanese research organizations and the career opportunities they offer to foreign scientists. ■



[www.riken.jp](http://www.riken.jp)

RIKEN, Japan's flagship research institute, conducts basic and applied experimental research in a wide range of science and technology fields including physics, chemistry, medical science, biology and engineering. Initially established as a private research foundation in Tokyo in 1917, RIKEN became an independent administrative institution in 2003.

RIKEN RESEARCH is a website ([www.rikenresearch.riken.jp](http://www.rikenresearch.riken.jp)) and print publication intended to highlight the best research being published by RIKEN ([www.riken.jp](http://www.riken.jp)). It is written for a broad scientific audience and policy makers interested in science and aims to raise global awareness of RIKEN and its research.

For further information on the research presented in this publication or to arrange an interview with a researcher, please contact

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