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Spinning a new phase

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Antimatter cools off

Chilled antihydrogen could reveal wrinkles in nature's symmetry

Physicists have proposed a novel way to cool antimatter, which should make it much easier to study in detail. Trapping useful amounts of the substance may be the key to working out why matter dominates antimatter in making up the fabric of the universe. However, no efficient method of cooling and storing antimatter has yet been developed, limiting these experimental investigations.

Most of the atoms in existence are hydrogen, made of a positive proton circled by a negative electron. Yet in 1932, physicists discovered that the electron had a mirror particle with exactly the same mass, but carrying a positive charge. They called it the positron. As more of these antiparticles were found, it became clear that every particle in nature has a symmetrical antiparticle—an evil twin. If the two collide, they annihilate each other in a burst of energy.

In the early 1990s, scientists created the first anti-atoms by combining positrons with negative antiprotons to make antihydrogen (Fig. 1 and Fig. 2). However, this recipe produces hot anti-atoms that are unsuitable for sensitive measurements.

Scientists want to trap antihydrogen in a way that allows them to cool it down, so that they can compare it with hydrogen. Although the standard theory predicts that matter and antimatter are symmetrical, more sensitive tests might show some slight asymmetry. This would not only help to refine our understanding of nature, it may also answer the puzzle about the imbalance of antimatter and matter in our universe. Hot antihydrogen is simply no use for these studies, explains Yasunori Yamazaki, a physicist at the RIKEN Discovery Research Institute in Wako. Instead, the anti-atoms must be cooled almost to absolute zero (-273 °C), the coldest temperature possible.

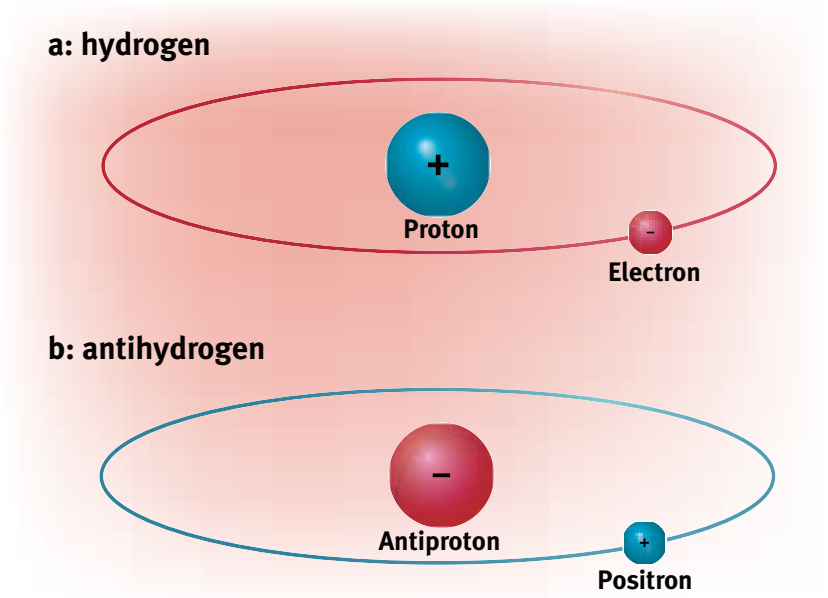


Figure 2: A graphical representation of the difference between hydrogen and antihydrogen.

Too precious for evaporative cooling

Hydrogen atoms can be chilled to these extremes relatively easily if they are contained within magnetic fields that act like a well. Changing the shape of this well by varying the magnetic fields allows the hottest atoms to spill over the sides of the trap, just like boiling water spurting out of the spout of a kettle. As the hot hydrogen atoms leave, they carry energy with them and leave cooler atoms behind. Subsequent collisions between the atoms remaining in the trap makes sure that there are always a few more 'hottest' atoms waiting to leak out over the lip of the trap.

But this process uses up trillions of hydrogen atoms, which are lost during the evaporative cooling process. That's no problem if you are trying to cool the most abundant element in the universe—but antihydrogen, on the other hand, is incredibly rare. "The total number of antihydrogen atoms ever synthesized may be around one million," estimates Yamazaki. Researchers simply cannot afford to lose most of their precious antihydrogen by evaporation. "It is practically possible to use evaporative cooling in the case of hydrogen atoms, but not in the case of antihydrogen atoms," says Yamazaki.

So he and his colleagues, including his doctoral student Yugo Nagata, from RIKEN, the University of Tokyo and the Harvard-Smithsonian Center for Astrophysics in Cambridge, US, have come up with an alternative cooling method.

Setting a new trap

When antihydrogen is synthesized it is hot and energetic, with the positron and antiproton relatively far apart—this separation means that the electrical attraction between them dominates any

ATHENA collaboration/CERN

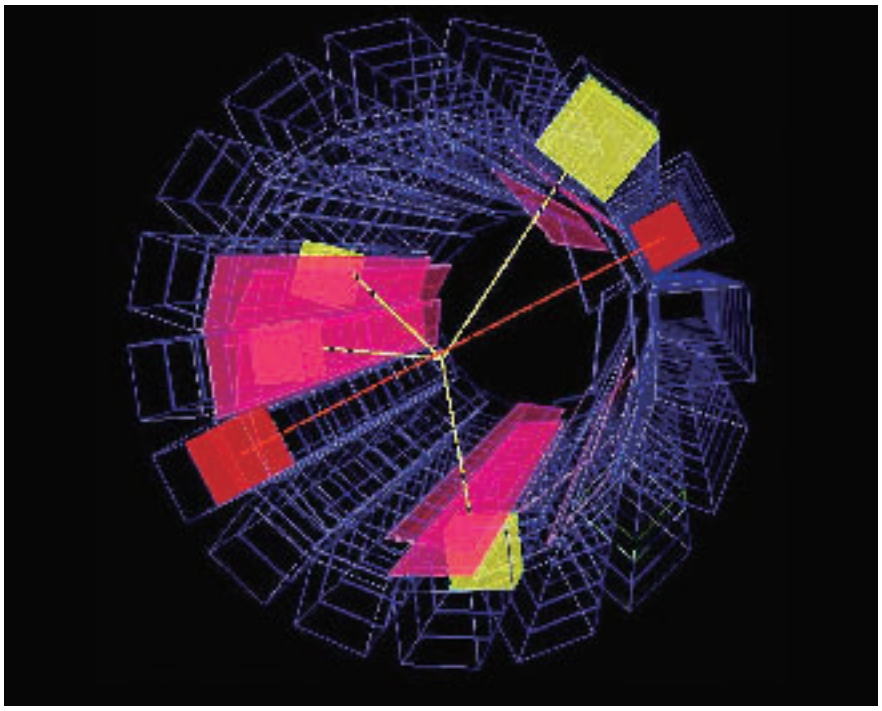


Figure 1: The first controlled production of cooled antihydrogen atoms was achieved by the ATHENA collaboration at CERN. The traces shown above schematically represent the annihilation pattern of the constituent antiparticles.

other subtle measurements that might point to an asymmetry. But over time, the hot anti-atoms will spontaneously lose energy by emitting radiation. During this process, the positrons fall closer towards the antiproton until they achieve their lowest energy level, or ground state. As reported in *Physical Review Letters*[1], Yamazaki and his colleagues have used quantum mechanical calculations to prove that this decay process could be harnessed to cool samples of antimatter, because it provides a way to efficiently eject energy from the trapped antimatter.

They propose using a 'cusp trap', similar to those used to contain hydrogen atoms but relying on a magnetic field formed by two solenoids with opposite currents. This will contain positrons, antiprotons and neutral antihydrogen atoms, all in the same spatial region. "In the cusp trap scheme, antiprotons and positrons can be accumulated very stably," says Yamazaki.

The scientists' calculations also show that the decay rate of the excited antihydrogen atoms—and hence the efficiency of cooling—gets higher in stronger magnetic fields, which are found at the edges of the trap. As well as efficient cooling, the trap could also store the antimatter effectively. "The lifetime of the antimatter is expected to be very long, something like 10 to 100 seconds or even longer, considering the results of previous experiments with sodium atoms," explains Yamazaki. That's important because "one must trap antihydrogen atoms for a while to get them into their ground state," he adds.

In other calculations, the team has also shown that by carefully controlling the temperature and magnetic fields within the system, it should also be possible to form beams of antihydrogen atoms. Overall, the scientists predict that cooling close to absolute zero should be possible using their technique.

"At the moment, I do not believe that this is difficult," says Yamazaki. "But still it should be experimentally proved before we say it's easy."

1. Pohl, T., Sadeghpour, H. R., Nagata, Y. & Yamazaki, Y. Cooling by spontaneous decay of highly excited antihydrogen atoms in magnetic traps. *Physical Review Letters* **97,** 213001 (2006).

About the researchers

Yasunori Yamazaki was born in Osaka, Japan, in 1949. He graduated from the Department of Physics, Osaka University, in 1973, received his master's degree in 1975, and in 1978 received his doctoral degree from the Department of Applied Physics at the same university. He was appointed as a research associate of the Tokyo Institute of Technology in 1978, an associate professor of the University of Tokyo in 1988, and a professor at the same university in 1993. He was also appointed as a chief scientist at RIKEN in 1997. His research subjects are the application of beam physics to various fields of natural science, such as biology, as well as the study of antimatter.

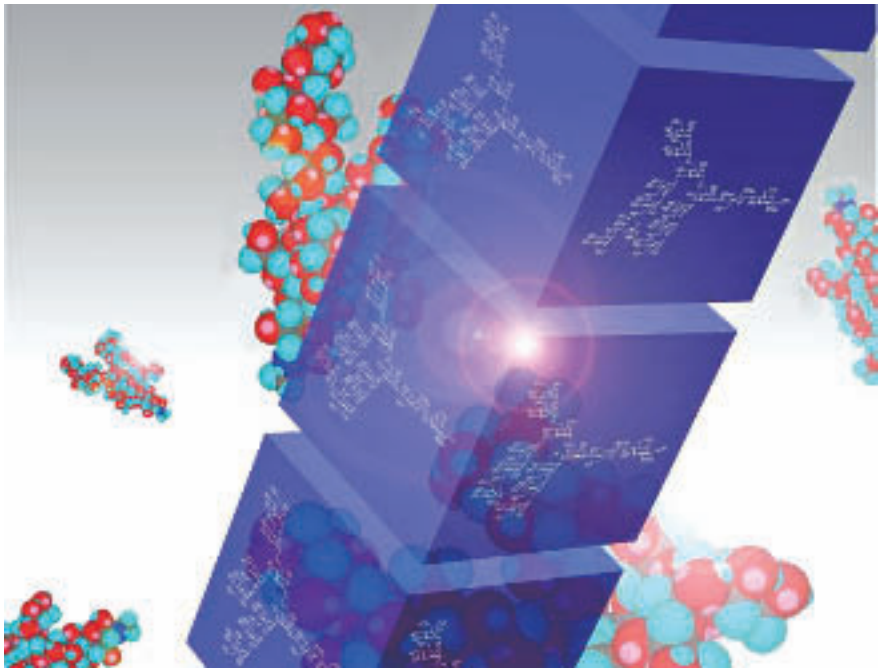
Yugo Nagata was born in Matsuyama, Japan, in 1980. He graduated from the Department of Natural and Environmental Science, Tokyo Gakugei University, in 2002, and is now studying for his doctoral degree at the Department of Basic Science, University of Tokyo. He has been the Junior Research Associate at the Atomic Physics Laboratory, RIKEN since 2006. His research interests are dynamic collision processes involving antimatter and strong fields.

One sugar or two?

A better understanding of enzyme function using synthetic carbohydrate substrates

Proteins are large organic molecules made up of amino acids linked together in a chain known as a polypeptide. These biological macromolecules are essential for life and are the basic components of our cellular machinery. Some of them are the catalysts that make the body's many different biochemical reactions work and others are the scaffolds that provide structural support in the cytoskeleton.

Polypeptide chains must be folded into the correct three-dimensional structure to produce a properly functioning protein. Moreover, some misfolded proteins are known to be responsible for certain neurodegenerative diseases such as bovine spongiform encephalopathy (mad cow disease) and Alzheimer's disease. In cells, the correct folding of polypeptides is sometimes aided by other proteins, known as chaperones.



American Society for Biochemistry and Molecular Biology (2006)

Figure 1: An artistic representation of synthetic glycan probes.

The sweet spot

In many cases, proteins are modified by the addition of carbohydrates—sequences of sugars such as glucose and mannose—to the amino acid backbone to form glycoproteins. These 'glycan' chains are important in many biological processes, including protein quality control, whereby protein folding, transport and degradation (of misfolded proteins) are mediated.

Yukishige Ito from RIKEN's Discovery Research Institute in Wako points out that, "although protein quality control is an important biological process, its molecular basis is not completely understood". Part of the problem is that it is difficult to gain a precise understanding of the exact roles that these carbohydrate groups play because glycoproteins obtained from natural sources, such as animal cells, are often mixtures—they differ in the number and arrangement of carbohydrate groups attached to them.

In order to get a more quantitative picture of how glycan chains are involved in these cellular processes, Ito and colleagues have chemically synthesized a series of pure glycan chains and used them to study glucosidase II—an enzyme involved in glycoprotein quality control (Fig. 1). "We have established a general method to make so-called 'high-mannose' glycans that can be used as synthetic probes to analyze enzyme function," comments Ito.

Breaking it down

In our cells, newly made polypeptides are often modified with a glycan chain comprising 14 separate sugars, linked together in a branched structure with three arms (Fig. 2). At the end of one of these arms are three glucose sugars, and the outer two are removed sequentially by two different glycan-processing enzymes.

Glucosidase I removes the outermost glucose unit to produce a glycan known as G2M9 (named for the two glucoses and nine mannoses present in the structure), and then glucosidase II removes the next one to give a glycan containing just a single glucose unit, G1M9. This monoglucosylated structure specifically binds to calnexin and calreticulin, chaperone proteins that ensure only correctly folded proteins are transported out of the endoplasmic reticulum. The last remaining glucose residue in G1M9 can also be removed by glucosidase II, resulting in the nonglucosylated glycan M9.

In their study, published in The *Journal of Biological Chemistry*[1], Kiichiro Totani from Ito's laboratory and colleagues linked the synthetic glycan chains to methotrexate, a drug that is used to treat cancer by binding to an enzyme called dihydrofolate reductase (DHFR) and inhibiting its activity. This approach has many advantages, the first of which is that the methotrexate provides a spectroscopic label that can be monitored

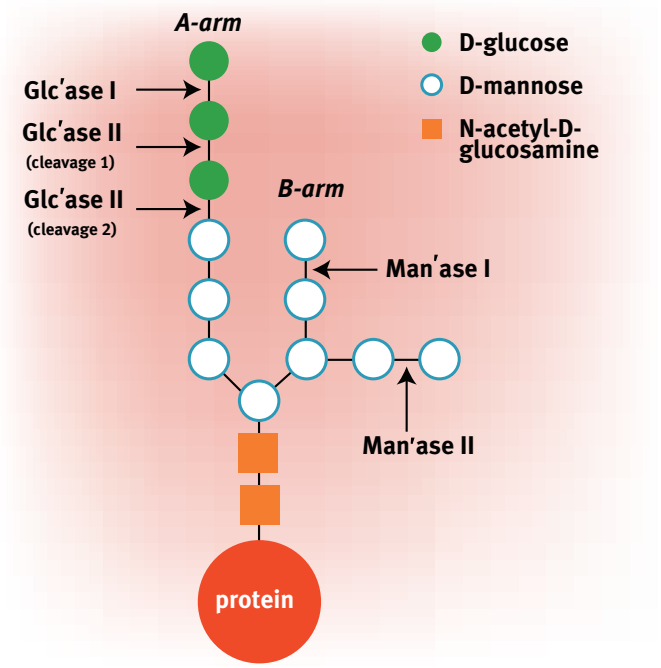


Figure 2: Glycoprotein processing in the endoplasmic reticulum.

in order to follow the progress of enzymatic reactions occurring on this substrate.

Even more importantly, however, it allowed the research team to study the glycan chains in two different environments. Methotrexate is known to mimic unfolded proteins in some fashion and so the glycan-methotrexate conjugates are a good model for natural glycoproteins prior to folding. On the other hand, simply by mixing the glycan-methotrexate conjugates with DHFR (to which methotrexate strongly binds), glycan-attached proteins are formed, which serve as a model system for mature folded glycoproteins.

One step at a time

The researchers show that glucosidase II trims the two glucose sugars from G2M9 in a sequential manner to form G1M9 and then M9, rather than transforming G2M9 directly into M9 by snipping off both glucose sugars in a single step. Moreover, glucosidase II is shown to remove the first glucose residue much more quickly than the second. In the presence of calreticulin, the rate at which glucosidase II removes the first glucose is unaffected, but the second is trimmed much more slowly. This result confirms that the monoglucosylated glycan G1M9 is trapped by calreticulin and, therefore, reacts very slowly with glucosidase II.

One of the biggest challenges of this work, however, is that conditions used for the biochemical analysis are quite different to those found inside cells. Determining the exact concentrations of all of the intracellular proteins involved in this system is far from easy, and Ito notes that, "it would be extremely difficult to reproduce physiological environments in test tubes".

Nevertheless, the reactivity of glucosidase II towards these artificial glycans provides valuable insight into how this enzyme works and, in particular, the rates at which it reacts with different substrates. There is no doubt that by carefully studying how glucosidase II interacted with structurally homogeneous glycans, a more detailed picture of how this enzyme behaves in the cell is emerging.

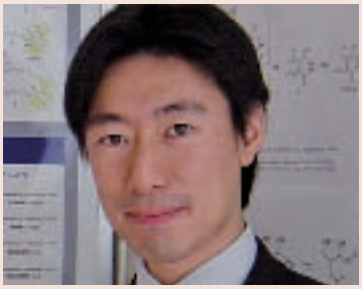
Moreover, by comparing the reactivity of closely related glycan chains, the researchers were able to confirm which ones are involved in particular pathways in the cell. This builds on earlier work from Ito's laboratory using other synthetic substrates to analyze the functions of other key proteins involved in protein quality control. "Continuation of our work will result in the thorough analysis of all proteins involved in this process," says Ito. ■

1. Totani, K., Ihara, Y., Matsuo, I. & Ito, Y. Substrate specificity analysis of endoplasmic reticulum glucosidase II using synthetic high mannose-type glycans. *The Journal of Biological Chemistry* **281,** 31502–31508 (2006).

About the researchers

Yukishige Ito was born in Kobe, Japan, in 1954. He graduated from the Faculty of Pharmaceutical Sciences, the University of Tokyo, in 1977, and obtained his PhD in 1982 from the same university. After two years postdoctoral training at the Department of Chemistry, Massachusetts Institute of Technology in Cambridge, USA, he returned to Japan as a research scientist at RIKEN, where he started his career in carbohydrate chemistry. He was promoted to senior scientist in 1996 and to chief scientist in 1998. Since then, he has been director of his own research group. His research focuses on the synthesis and functional analysis of glycoprotein-related compounds and the development of methodologies for efficient and selective synthesis of oligosaccharides.

Kiichiro Totani was born in Tokyo, Japan, in 1974, and graduated from the Department of Applied Chemistry, Keio University in 1997. After he obtained his PhD from the same university in 2002, he became a special postdoctoral researcher at the Synthetic Cellular Chemistry Laboratory at RIKEN. Since 2005, he has been working at the same RIKEN laboratory as a researcher employed by the Japan Science and Technology Agency. His research interests are synthesis and molecular recognition of sugar derivatives, including oligosaccharides and glycoproteins.



Flipping spins create unusual quantum phase

Mathematical model prompts investigations of solid helium

Ordinary matter can be classified into three different phases—solid, liquid or gas—depending on how its atoms and molecules are arranged. But scientists are now discovering a whole range of different 'quantum' phases in the subatomic world.

Tsutomu Momoi, a theoretical physicist at RIKEN's Discovery Research Institute in Wako, and colleagues in France and the UK have devised mathematical models that could show experimental physicists where to look for new types of quantum phases[1,2].

Unlike conventional phases of matter, quantum phases differ not in the positions of their constituent particles, but in the distribution of the quantum properties (such as spin) that each particle possesses.

When a charged particle spins, it generates a magnetic field that can point 'up' or 'down', just like the north and south poles of a bar magnet (Fig. 1). Neighboring particles prefer to alternate their magnetic poles to minimize the repulsion between them. But when three magnetic particles are fixed in a triangle, it is impossible for each one to have the opposite polarity to both its neighbor: the system is said to be frustrated.

In some materials, spins constantly fluctuate as they try to accommodate their neighbors, even at the coldest possible temperature. Momoi and colleagues have created a mathematical model of one such 'spin liquid' where four particles flip their spins, one after another in a never-ending cycle, in order to ease their frustration.

Unusually, these spins can be arranged at more subtle angles than simply 'up' or 'down'. "New degrees of freedom come out instead," says Momoi.

Figure 1: Charged particle spins of a bar magnet generate a magnetic field.

The team found that the new spin arrangements are carried by a trio of magnons, a type of quantum particle that describes the spread of magnetic disorder through a system. "Usually magnons scatter each other, but we found that these three magnons form a bound state," explains Momoi.

This produces a magnetic field within the quantum phase which has eight poles, a hidden degree of freedom that has never been investigated before, says Momoi.

The model suggests that experimental physicists could actually find these effects in solid films of helium-3 atoms. "Finding new phases gives us the chance of creating a new frontier of physics," he adds. "Understanding how to control them will be useful in developing technology of magnetic materials in future." ■

1. Momoi, T., Sindzingre, P. & Shannon, N. Octupolar order in the multiple spin exchange model on a triangular lattice. *Physical Review Letters* **97**, 257204 (2006).

2. Shannon, N., Momoi, T. & Sindzingre, P. Nematic order in square lattice frustrated ferromagnets. *Physical Review Letters* **96**, 027213 (2006).

Selection with silicon

Organic chemists develop a new one-pot reaction to make compounds containing carbon-silicon bonds

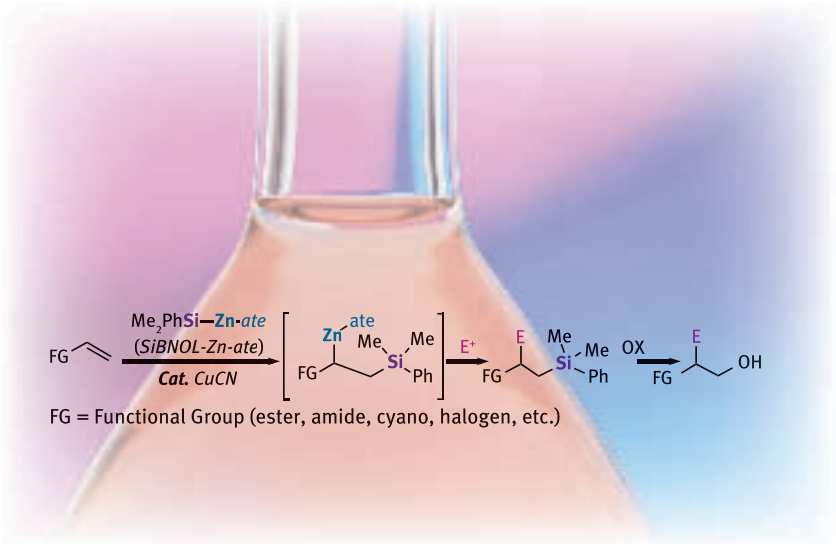


Figure 1: Regio- and chemoselective silylation of functionalized terminal alkenes.

A new, simple synthetic method to selectively prepare compounds containing carbon-silicon bonds has been developed. These compounds—known as organosilyl compounds—exhibit unique structural, electrical, optical and chemical properties. It is these properties that make these compounds so attractive to researchers and they are used in various ways in material sciences, biotechnology, and organic synthesis.

The challenge for organic chemists is the selective synthesis of specific organosilyl compounds which also contain other reactive groups. To date, only a few practical methods have been developed. Now, Masanobu Uchiyama from the RIKEN Discovery Research Institute, Wako, and colleague Shinji Nakamura of The University of Tokyo, have developed a selective method they believe will be of use to many organic chemists[1].

This new method treats alkenes, simple carbon-carbon double bonds, with complex catalysts containing silicon-zinc bonds to give the organosilyl compound. In the reaction, the silicon atom adds preferentially to the very end of the double bond and where there are two carbon-carbon double bonds in a molecule only the terminal one reacts leaving the other intact. This means that a range of starting compounds can be used. The method can also be modified to give compounds that have other functional groups adjacent to the silicon atom (Fig. 1).

Practically speaking the reaction is also attractive as this is the first example of a 'one-pot' method to generate such compounds. The reaction takes place smoothly using an equivalent amount of reagent to starting compound. Uchiyama hopes this reaction could be applied to starting materials that, for example, once reacted, would form specifically just one of two possible mirror-image products, known as enantiomers.

"One of our aims is to provide novel approaches to functionalizing organic compounds by means of development of the chemistry of newly designed complexes. Success here will provide powerful tools for designing and creating new functionalized molecules," says Uchiyama.

Uchiyama and Nakamura began research in this area in 2004—designing complexes and catalysts containing silicon-zinc bonds to synthesize functionalized carbon-carbon double and triple bonds[2,3]. Tools for the selective introduction of various functional groups to organic molecules are still quite limited in their applicability. A fact that motivates the work undertaken in Uchiyama's laboratory where they focus on the development of breakthrough synthetic processes to create new materials based on synthetic organic chemistry, physical chemistry and computational chemistry. The next step is to understand in detail how the reaction works and determine its scope. ■

1. Nakamura, S. & Uchiyama, M. Regio- and chemoselective silylmetalation of functionalized terminal alkenes. *Journal of the American Chemical Society* **129**, 28–29 (2007).
2. Nakamura, S., Uchiyama, M. & Ohwada, T. Chemoselective silylzincation of functionalized terminal alkynes using dianion-type zincate (SiBNOL-Zn-ate): regiocontrolled synthesis of vinylsilanes. *Journal of the American Chemical Society* **126**, 11146–11147 (2004).
3. Nakamura, S., Uchiyama, M. & Ohwada, T. Cp_2TiCl_2-catalyzed regio- and chemoselective one-step synthesis of gamma-substituted allylsilanes from terminal alkenes using dianion-type aincate (SiSiNOL-Zn-ate). *Journal of the American Chemical Society* **127**, 13116–13117 (2005).

Calculated approach to catalysis

Computer model helps uncover the mysteries behind the selective introduction of metal atoms to small organic molecules

A theoretical study by chemists in Japan and the US has revealed the origin of differences in selectivity of a chemical reaction between two types of bimetallic reagents used to introduce metal atoms to aromatic rings. For decades, the choice of catalyst for a successful organometallic reaction has depended on which reactive group components were on the starting material. Moreover, a complete understanding of some of the difficulties encountered and of the underlying reaction mechanisms was lacking in many cases.

Now, Masanobu Uchiyama from the RIKEN Discovery Research Institute, Wako, and colleagues, which includes collaboration with Keiji Morokuma from Emory University, US, and Kyoto University, use a computational model that provides a rationale for the differences in the mechanisms between the two bimetallic reagents studied[1]. The first reaction involved an intermediate in which the two metal atoms were the same, as for traditional alkyllithiums or Grignard reagents; and the second involved an intermediate containing two different metal atoms, in this case a lithium-zincate complex—known as an 'ate complex'.

These differences have implications for chemists wishing to develop better methods for improved reactivity and selectivity. The teams' work also gives an insight into why reactions sometimes result in a product containing two metal atoms as opposed to one.

Their research emphasizes the importance of applying established theoretical methods to rational catalyst design (Fig. 1). Uchiyama understands that traditional approaches to design new reactions based on past experiences, or trial and error, are often expensive and time-consuming. He believes a dual approach combining the benefits of theoretical and experimental chemistry should be encouraged: an approach that will result in breakthrough synthetic processes, use fewer chemicals and be more cost effective.

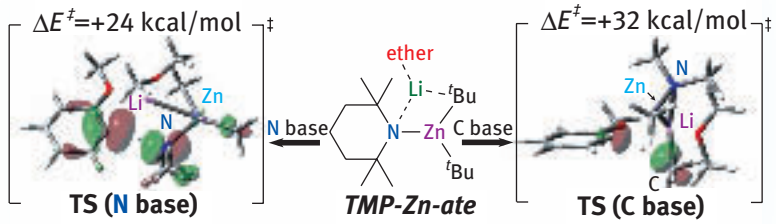


Figure 1: Visualization of reaction pathways and mechanism of TMP-Zincate has been studied using a computational model.

This work is the result of a collaboration that started in 2001 between Uchiyama and several colleagues from The University of Tokyo and RIKEN. The collaboration has already resulted in five other publications. Uchiyama acknowledges that his colleagues play a central role in this research. "We have been working hard the past several years to clarify the unknown reaction pathways and mechanisms of small molecules and macromolecules," he says.

The next steps in Uchiyama's work, the 'flexible and command' construction of aromatic compounds with specific functional group arrangements, will be of interest to medicinal and materials chemists. "We intend to develop new transformations on aromatic rings that have unique reactivity and selectivity using these results in combination with our experiences as organometallic chemists," says Uchiyama. ■

1. Uchiyama, M., Matsumoto, Y., Usui, S., Hashimoto, Y. & Morokuma, K. Origin of chemoselectivity of TMP zincate bases and differences between TMP zincate and alkyl lithium reagents: A DFT study on model systems. *Angewandte Chemie International Edition* **46**, 926–929 (2007).

Using FOX to hunt for gene function

Researchers develop a new technique for understanding genomes

Molecular biologists from RIKEN and two Japanese universities have developed a new system for rapidly determining the function of individual genes in plants.

The method is based around creating transgenic *Arabidopsis thaliana* plants by adding an extra copy of a single gene external to the chromosomes. It has been dubbed the FOX—Full-length cDNA Over-eXpressing gene—hunting system by the research team. The protein products of the extra genes modify the plants by adding to the metabolic activity of their cells. Already, the researchers have used their technique to gain information about two genes that cause changes in leaf color.

The FOX hunting system provides an alternative to the two conventional techniques of investigating gene function—knocking out or activating individual genes within the chromosomes *in situ*.

In a recent paper in *The Plant Journal*[1], the researchers describe how they used RIKEN's collection of full-length DNA copies of about 10,000 individual *Arabidopsis* genes to develop their technique. They normalized this library—that is, ensured there were equal numbers of each gene copy—and introduced them back into *Arabidopsis* plants together with genetic material to ensure they were expressed. The transgenic plants were grown for two generations and monitored for mutants in various categories, such as shape, leaf color and fertility. The introduced DNA was then able to be recovered from the mutants and analyzed to determine which gene caused which changes.

During the course of their development work, the researchers created more than 15,000 fertile plant lines (Fig. 1). They also carried out a series of tests to screen the progress and efficiency of their new technique.

Figure 1: Examples of the range of mutants produced by the FOX system after two generations.

The group undertook a detailed study of two light-green leaf-color mutants to see how much information on the function of individual genes their new technique could provide. They were able to extract, sequence and classify the genes responsible for the changes, as well as match them to variations in plant structure, growth and form. In one case the gene selected affected the development of chloroplasts and subsequent photosynthetic activity; in the other, while the precise role of the gene remained a mystery, it was clearly important as preventing it from functioning tended to be lethal.

"The ultimate goal of our work is to improve plants by introducing genes from other organisms," says team leader, Minami Matsui from RIKEN's Plant Science Center in Yokohama. "Using such genes, you can add new functions to plants that have never been achieved by conventional breeding." ■

1. Ichikawa, T., Nakazawa, M., Kawashima, M., Iizumi, H., Kuroda, H., Kondou, Y., Tsuhara, Y., Suzuki, K., Ishikawa, A., Seki, M., Fujita, M., Motohashi, R., Nagata, N., Takagi, T., Shinozaki, K. & Matsui, M. The FOX hunting system: an alternative gain-of-function gene hunting technique. *The Plant Journal* **48**, 974–985 (2006).

Steering mechanism for nerve circuit formation revealed

A recent study extends our understanding of how nerve cells reach out towards attractive stimuli

New work sheds light on the processes responsible for guiding the tips of nerve cells in the right direction. Nerve cells receive messages called impulses through one end, the dendritic tree, and dispatch impulses out the other end, the axon. To ensure that messages reach the desired destination, nerve cells send out axons that elongate toward their appropriate targets. Growth cones, the tips of these axons, 'sense' the location of the targets. In turn, targets make their presence known by sending appropriate positional cues.

Previous work indicates that these cues trigger fluxes of calcium within growth cones. A team led by Hiroyuki Kamiguchi, a scientist at RIKEN's Brain Science Institute in Wako, identified events linking calcium flux with directional movement of growth cones[1].

The researchers hypothesized that the direction of axon elongation might shift with preferential addition of new membrane material to one side of the growth cone. Testing this hypothesis required tracking the movement of vesicles, which are sources of new membrane material found within nerve cells, after calcium levels increase in specific regions within the growth cone.

Indeed, nerve cell vesicles labelled with a fluorescent molecule preferentially migrated towards calcium released on one side of the growth cone. This directed-trafficking of vesicles required microtubules, which transport cargo within many cell types.

Vesicles moving in the direction of calcium release contained VAMP2, a member of the SNARE family of proteins, which act like 'zippers' to enhance fusion between two closely opposed membranes. The researchers observed such fusion of the migrated vesicles with surface membranes of the growth cone (Fig. 1). Application of tetanus neurotoxin, which prevents VAMP2-directed fusion, prevented the growth cone turning in the direction of calcium release.

Importantly, VAMP2 inhibition also blocked the growth cone turning towards myelin-associated glycoprotein and nerve growth factor, which are calcium-dependent 'natural' stimuli encountered by growth cones within the body.

Identification of the steps linking calcium flux to vesicle movement remains for future study. Regardless, these data provide another link in the chain of events responsible for dispatching nerve cell messages in the correct direction.

"This work provides a simple answer to the long-lasting question of how a growth cone changes its direction of locomotion. Further investigation of steering mechanisms will enable us to manipulate the direction of axon elongation within the body. This technology could contribute to the development of therapeutic strategies by which regenerating axons can be guided toward appropriate targets to achieve functional recovery after nervous system injury," says Kamiguchi. ■

1. Tojima, T., Akiyama, H., Itofusa, R., Li, Y., Katayama, H., Miyawaki, A. & Kamiguchi, H. Attractive axon guidance involves asymmetric membrane transport and exocytosis in the growth cone. *Nature Neuroscience* **10**, 58–66 (2007).

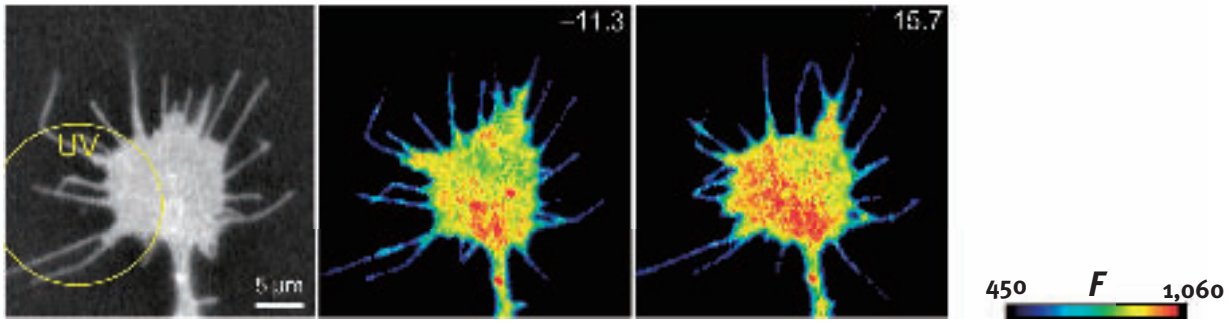


Figure 1: Vesicle fusion is enhanced preferentially in areas of calcium flux. In the left panel, the yellow circle denotes the area of calcium elevation. In the two right panels, red indicates the fusion between VAMP2-containing vesicles and surface membranes of the growth cone. Numbers represent seconds after the onset of calcium release.

How surface cells band together

RIKEN researchers discover a key role for the Tuba protein

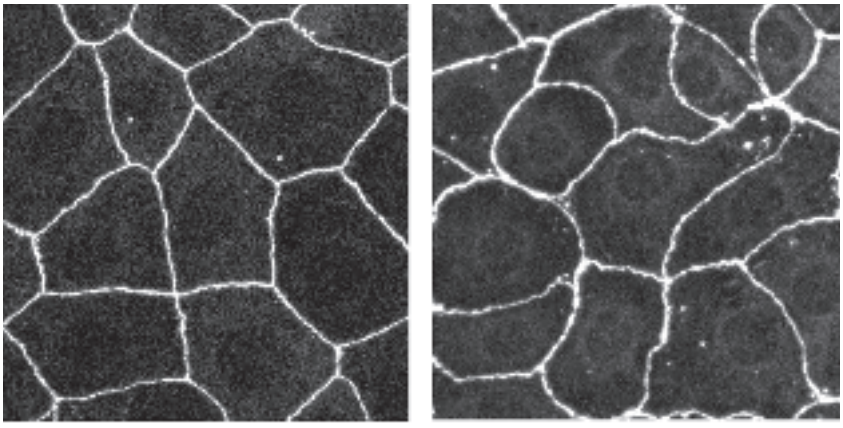
Researchers from RIKEN and Kyoto University have unraveled details of how an enzyme known as Tuba regulates the joining together of tissue surface or epithelial cells.

Epithelial cells characteristically form a honeycomb-like structure (Fig. 1) such that membrane contact areas between neighboring cells are held to a minimum. This suggests that the cells pack together under some form of tension. The honeycomb appearance is thought to be important in the functioning of the surfaces—minimizing light scattering in the lenses of the eye, for instance. Formation and remodeling of such surfaces is a significant facet of development.

The cells themselves tend to be columnar in shape. In the region closest to the surface—the apical region—the membranes of neighboring cells are pushed together and adhere to one another in a complex of two kinds of junction. Nearest the surface a tight junction forms, where the membranes are so closely joined they form an impermeable barrier to fluid, preventing the passage of molecules and ions between cells.

Immediately beneath the tight junction is a looser junction, where cell membranes are anchored to each other by complexes of three families of proteins—cadherins, catenins and flexible actin filaments which extend into the bodies of the adjoining cells. Signalling proteins, such as Tuba, can help to organize such complexes by bringing together the interacting proteins.

The research team at RIKEN's Center for Developmental Biology in Kobe reports in *The Journal of Cell Biology*[1] that staining studies show Tuba to be concentrated in the tight junction area. When Tuba synthesis is disrupted by RNA interference, the team found, the honeycomb packing of human intestinal epithelial cells becomes flabby, and takes on a curved, distorted appearance (Fig. 1).



Rockefeller University Press

Figure 1: The typical 'honeycomb' appearance in an epithelial cell line from the human intestine (left). The distorted appearance when Tuba synthesis is disrupted by RNA interference (right).

Tuba is a guanine nucleotide exchange factor (GEF) known to activate Cdc42, a compound which regulates the assembly of actin filaments and cadherins. The researchers discovered that Tuba inactivation leads to disruption of the network of actin and cadherin fibers involved in holding neighboring membranes together under tension. They suggest that Tuba stimulates Cdc42 and other compounds to enhance assembly and delivery of actin and cadherins to the junction area.

Several studies have implicated other GEFs in the cell-cell adhesion process. "How the different GEFs share the cell assembly regulation roles is an intriguing topic and one we are looking to tackle in the future," says the paper's lead author, Tetsuhisa Otani. ■

1. Otani, T., Ichii, T., Aono, S. & Takeichi, M. Cdc42 GEF Tuba regulates the junctional configuration of simple epithelial cells. *The Journal of Cell Biology* **175**, 135–246 (2006).

Two for the price of one

Two different hormones can impinge on the same signaling pathway to effect different cellular responses

Molecular biologists at the RIKEN Genomic Sciences Center in Yokohama have taken a whole-cell systematic approach, combined with mathematical modeling, to show how different hormones can bind similar cell surface receptors and result in complementary responses by the cell[1].

Cellular responses to external stimuli can be quite different. An example of this plasticity is the response of the breast cancer cell line, MCF-7, to the hormones known as epidermal growth factor (EGF) and heregulin (HRG). These hormones both play important roles in controlling cell function.

The research team, led by Mariko Hatakeyama, shows that in the MCF-7 cell line EGF evokes cell proliferation while HRG promotes differentiation (Fig. 1). This difference occurs even though both hormones bind the same type of cell-surface receptors and stimulate the similar intracellular signaling pathway. However, the team also shows that EGF produces a transient activation of its receptor and its downstream signaling pathway, while that of HRG lasts much longer.

In spite of these differences in duration of activation and signaling, both hormones invoke the expression of similar sets of genes, and at similar rates, at least at early time points. However, the longer duration of signaling by HRG eventually leads to a feedback loop, such that HRG stimulates cell differentiation rather than proliferation.

The difference between EGF- and HRG-induced responses is due to a small level of change in gene activity over a large number of genes, rather than a very large change in just a few key genes. Thus, the sum of all these changes brings about a dramatic difference in later transcription and cell response. "In our view, magnitude and duration of gene expression together with signaling pathways coordinate to determine cell fate during early transcriptional relay," says Hatakeyama. Exactly "how the difference in gene expression magnitude occurs and what may break this control" is the next research project of the team, she notes.

Hatakeyama also points out that other cell types might display this same mechanism and that it may be a general trend in biology. The analogy she likes to use is the human–chimp genome story; that is, the fact that humans and chimps are so similar in their genomes and yet so different phenotypically is probably explained by small changes over hundreds of genes—rather than a large change over a few genes. More experiments using this system-wide approach to look at other hormone responses in other cell lines should show if she is right. ■

1. Nagashima, T., Shimodaira, H., Ide, K., Nakakuki, T., Tani, Y., Takahashi, K., Yumoto, N. & Hatakeyama, M. Quantitative transcriptional control of ErbB receptor signaling undergoes graded to biphasic response for cell differentiation. *The Journal of Biological Chemistry* **282**, 4045–4056 (2007).

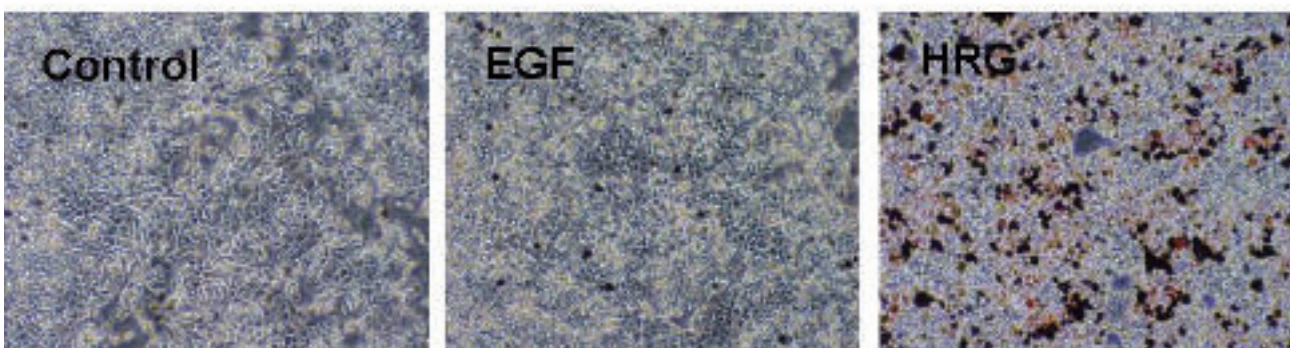


Figure 1: Treatment of breast cancer cells with heregulin (HRG) leads to cellular differentiation, as visualized by Oil Red staining of lipid droplets, whereas epidermal growth factor (ERG) does not.

Death to allergy-inducing cells

How attenuated tuberculosis infection stems allergy

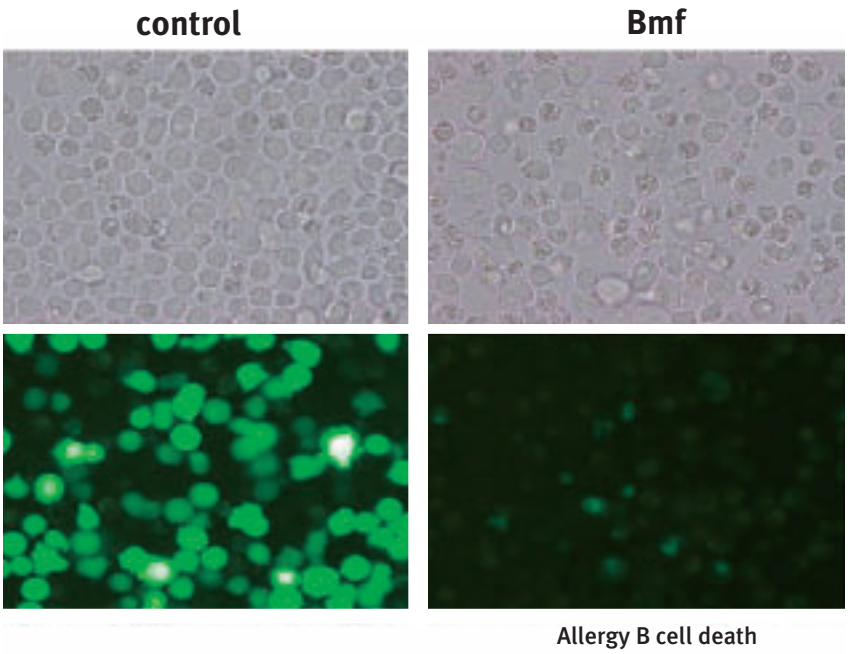


Figure 1: A microscopic image showing that allergy-inducing B cells (green cells, left) undergo cell death when the death promoting factor Bmf is active (right).

Worldwide—but particularly in industrialized countries—incidence of allergy is on the rise. Although the reasons for this are not clear, some scientists have speculated that excessive use of antibiotics and heightened cleanliness prevent beneficial infections early in life that would normally drive immune system development in a way that lessens the possibility of allergy in adulthood.

A collaboration of scientists led by Masaru Taniguchi of the RIKEN Research Center for Allergy and Immunology, Yokohama, now demonstrates[1] that vaccination with *Mycobacterium bovis* bacillus Calmette Guerin (BCG), the current vaccine against human tuberculosis, stimulates the immune system to dampen allergic responses.

Working with mice as a well-characterized model of induced allergy, the team characterized the types of immune cells that respond to BCG vaccination. "BCG, ethically approved in Japan, was used to mimic bacterial infection in allergy patients or healthy volunteers," says Taniguchi.

The team found that NKT cells—a special subset of immune cells found especially in the liver—were responsible for the anti-allergy effect. Using genetically modified mice that lack all NKT cells, they demonstrated that NKT cells are required to suppress allergy after BCG vaccination.

They also found that interleukin 21 (IL-21) expressed by the NKT cells was important, and that another cytokine, IL-12 made by dendritic cells that respond directly to BCG, was required to stimulate the NKT cells to produce IL-21.

Exactly how BCG stimulates dendritic cells is not clear. "Because BCG is a live vaccine, many candidate BCG proteins can stimulate dendritic cells," says Taniguchi. "We are currently collaborating with biochemists to purify BCG-derived products and to identify essential molecules."

The key to control of allergy after BCG vaccination is the IL-21 produced by NKT cells, which affects allergy-producing B cells in a way that causes them to die. The team found that IL-21 induces activation of the death-promoting factor Bmf in B cells, which blocks the death-preventing factor Bcl-2 from keeping the cells alive (Fig. 1).

The importance of the study by Taniguchi and colleagues lies in the characterization of mechanism by which BCG decreases allergy, which may have therapeutic implications. It also shows that infection (or vaccination) can reduce allergy.

"It is easy to imagine that the IL-21 pathway in B cells provides a target for developing anti-allergy drugs," notes Taniguchi. "If we can successfully identify a low molecular weight compound or a small molecule that stimulates the pathway, we can develop a drug for a treatment of allergy." ■

1. Harada, M., Magara-Koyanagi, K., Watarai, H., Nagata, Y., Ishii, Y., Kojo, S., Horiguchi, S., Okamoto, Y., Nakayama, T., Suzuki, N., Wen-Chen Yeh, Akira, S., Kitamura, H., Ohara, O., Seino, K. & Taniguchi, M. IL-21–induced Bε cell apoptosis mediated by natural killer T cells suppresses IgE responses. *The Journal of Experimental Medicine* **203**, 2929–2937 (2006).

Watching hardworking electrons

Masaki Takata

Chief Scientist
Structural Materials Science Laboratory
RIKEN Harima Institute
RIKEN SPring-8 Center

New materials, such as fullerene and carbon nanotubes, are attracting considerable attention because of their potential to achieve major breakthroughs in science and technology and industries in all areas, including information and communications, energy, the environment, medicine, machinery, and architecture. The function of a material depends on the state of the electrons contained in the material. However, it has been difficult to clearly observe the electrons in these new materials. Masaki Takata, Chief Scientist at the Structural Materials Science Laboratory in the SPring-8 Center of the RIKEN Harima Institute, is collaborating with other scientists on an extensive examination of the electrons in materials. The scientists are using synchrotron X-rays from SPring-8 with a unique analytical approach based on information theory. They expect this to lead to a major revolution in the development of new materials.

Visualizing structures from trace amounts of powder

"I was a boy astronomer. I used to photograph Mercury and other celestial bodies using my father's camera I smuggled out of the house," says Takata, looking back on the past. "I like observing things. Even now I enjoy photography as a hobby."

Takata specializes in visualization of the atoms and electrons that form materials. After spending some time using electron microscopy for his research at graduate school, he shifted to using Xrays as his research tool of choice. Around 1990, under Makoto Sakata of Nagoya University, he began analyzing the structure and electron distribution of atoms that constitute functional materials using trace amounts of their powder. "X-ray crystallography often involves the use of large single crystals as samples to obtain better measurement data," says Takata. "But most new materials under development are only available in the form of trace amounts of powder." He adds that determining the structures of materials with powder samples would allow new materials to be developed based on the findings. "We have been engaged in extensive exploration, not only of atomic arrangements, but also of electron distributions in substances using powder samples," he continues. "The properties of a substance depend on the electron configuration. What I want to know most in the context of developing new materials is the electron distribution."

To this end, Takata and others use the maximum entropy method (MEM), a technique that has emerged from information theory. "Since MEM is somewhat difficult to understand, only two scientists—Professor Sakata and I, myself—were using it for X-ray crystallography in Japan around 1990," says Takata. In fact, as he points out, no researchers worldwide were utilizing the MEM technique during this period, with the exception of a small amount of activity in France.

So what is MEM? "Using X-rays, we cannot obtain direct observations of magnified views as we can with optical microscopy, which employs visible light," Takata explains. This is because the sort

of lenses used for optical microscopy will not converge scattered light at X-ray wavelengths to produce images. "We use MEM in place of the lens." When an X-ray beam is directed onto a sample, the X-rays are scattered by the electrons in the subject material. Measured data from the scattered X-rays are converted to images using a computer. It should be noted, however, that there is more than one possible candidate for the image derived from the measured data. Which image to select?—this problem is solved by MEM. "Remember science experiments at school. Any experiment is unavoidably accompanied by measurement errors; a plot of the distribution of measured data shows dispersion," says Takata. "For example, assume we draw a line here [through a set of data points]. There are then a variety of positions where we could draw the line. MEM offers the simplest line that involves no subjective judgment. Additionally, MEM enables us to extend the line even to a region where measured data are unavailable." However, Takata adds that if the precision of the measured data is poor, only obscure images can be selected, even using MEM. "Hence, we have made efforts to improve the precision of measured data, including the early adoption of imaging-plate technology."

Elucidating the structure of metallofullerene

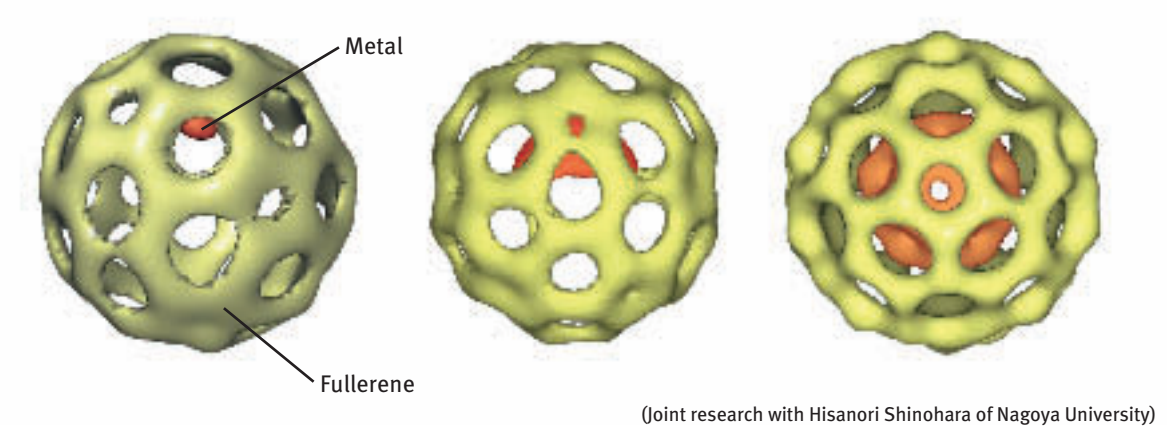
In 1995, for the first time in the world, Takata succeeded in visualizing how metal atoms are incorporated into fullerene (Fig. 1). Fullerene, which was discovered in 1985, is a molecule comprising carbon atoms gathered in a cage form like a soccer ball. "The first expectation was that artificially placing metal atoms in the fullerene cage might result in various new properties, including superconductivity," says Takata. In fact competition was stimulated worldwide to analyze the structure of a synthetic compound of fullerene and metal atoms. However, it took a great deal of time to determine whether the metal atoms adhere to the outside of the cage or are incorporated in the cage. The fine molecular structure of the compound was difficult to analyze because its powder could only be produced in very small amounts.

Why, then, did Takata succeed in the structural analysis of metallofullerene for the first time in the world? "Just before the successful analysis, I conceptualized an analytical method that combined MEM and the Rietveld method," recounts Takata. He explains that in those days, only the structure of fullerene on its own was known. The Rietveld method derives the true structure by comparing such available information and theoretical models with actual measured data. First, the structure of fullerene alone is compared with the structure estimated by MEM from the measured data. Because the measured data include the contributions of metal atoms, the two structures would be expected to differ. "Then I attempted to locate the discrepancy, and found a distribution of electrons in the fullerene that was inconsistent with the measured data," he adds. "When applying metal atoms to that portion, the structure of metallofullerene was precisely derived."

A new material that exhibits property change on gas adsorption

Determining whether the results of a structural analysis are correct requires a level of judgement that is augmented by intuition and sharpened by experience. "The derivation of the true structure of a substance is very beautiful. If the structure obtained is incorrect, it has an unnatural appearance which makes us uneasy," says Takata. He is now working on structural analysis using X-rays at the highest possible intensity produced by SPring-8 in combination with his unique analytical approach, which incorporates the MEM-Rietveld method.

"We were all astonished and excited at the incredibly beautiful structure," says Takata, looking back on his first success in structural analysis of polyporous chelate polymer carrying adsorbed oxygen. The polyporous chelate polymer is a new material that was prepared from metal ions and organic molecules by Susumu Kitagawa and others at Kyoto University. This material has regularly arranged small pores of nanometer size (one billionth of a meter), which permits a high degree freedom in the design of the size and shape. As such, the polyporous chelate polymer had been attracting attention as a material capable of adsorbing gases efficiently just by reducing its temperature.



(Joint research with Hisanori Shinohara of Nagoya University)

Figure 1 : **Examples of metallofullerene containing internalized metal atom, visualized by X-ray structural analysis.**

"However, it was not known how the gas molecules were adsorbed," Takata recounts. It was hypothesized that the gas molecules randomly adhered to the inner walls of the pores. Actual structural analysis, however, revealed that the oxygen molecules were floating in series in each pore." (See upper panel on front cover; oxygen molecules shown in red.) The analytical method was able to indicate the number of electrons belonging to each molecule and atom. "The number of electrons belonging to the oxygen molecule was found to be 16 [the electron number of atomic oxygen]," Takata reveals. "Hence, the electrons did not transfer from the oxygen molecule, but were floating, supported by a very weak force known as intermolecular interaction."

The major point to note from this analysis is the finding that magnetism develops with the adsorption of oxygen molecules. Individual oxygen molecules have magnetism. However, because the oxygen atoms in a gas, and hence their magnetic dipoles, are oriented randomly, gaseous oxygen as a whole does not exhibit magnetism. Structural analysis suggests that when oxygen molecules are adsorbed, they are arranged linearly so that magnetism might be developed as a result of uniform orientations of the oxygen dipoles. "Actual measurements detected weak but significant magnetism," Takata notes. These results strongly stimulated the imagination of scientists who were investigating the physical properties of the new materials. The polyporous chelate polymer was found to present a new material phenomenon in that it exhibits property change on gas adsorption. Furthermore, it was found that altering the kind of gas to be adsorbed and the size and shape of the pores produced a broad range of new properties and functions. "For example," says Takata, "the polyporous chelate polymer exhibits ferroelectricity on adsorbing gaseous carbon monoxide."

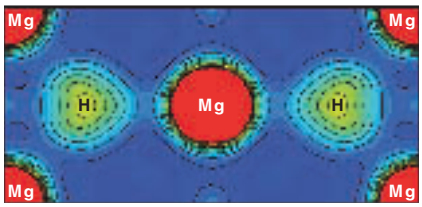
The results of this structural analysis has led to polyporous chelate polymers becoming a major research theme in nanotechnology, including fullerene and carbon nanotubes. Moreover, polyporous chelate polymers have immediate potential for industrial applications. "This is because these polymer can easily be produced chemically, under ordinary conditions, at one atmospheric pressure, and at ambient temperature, in contrast to expensive fullerene and carbon nanotubes, which are still difficult to prepare in large amounts at low cost," explains Takata.

Visualizing hydrogen using X-rays

Hydrogen is expected to provide a source of clean energy that does not emit carbon dioxide and other greenhouse gases. However, a major problem arises concerning the storage of hydrogen, which is gaseous at normal temperatures. For example, vehicles powered by fuel cells will not become feasible until a way is found to load the hydrogen fuel into the car in a compact way. Although hydrogen-absorbing substances that are capable of storing hydrogen in compact volumes at high density are under development, it has been difficult to visualize hydrogen in any material using X-rays. This is caused by hydrogen atoms scattering X-rays weakly, because each atom has only one electron. Takata, collaborating with scientists from Toyota Central R&D Labs, succeeded in visualizing hydrogen absorbed on magnesium (Fig. 2). It was found that hydrogen is bound to magnesium through not only ionic bonds, but also covalent bonds, which are weaker than ionic bonds. At present, new hydrogen-absorbing alloys are being developed by improving the relationship between the metal–hydrogen bonding strength and hydrogen-absorption efficiency.

Visualizing the behavior of electrons during reactions

"Since its construction in 1997, SPring-8 has been updated to provide enhanced performance, including synchrotron radiation," says Takata. "However, we remain unable to utilize the radiation to



(Joint research with Toyota Central R&D Labs, Inc.)

Figure 2: **Hydrogen (H) absorbed in magnesium (Mg)**

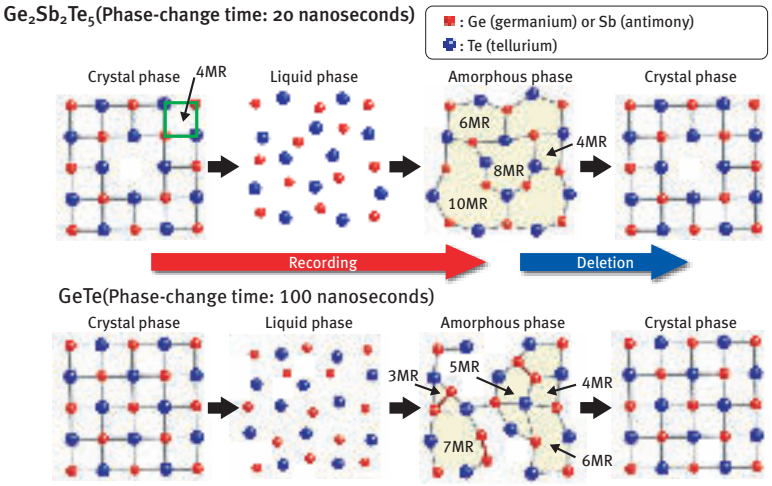


Figure 3: **Phase-change models. MR stands for membered ring.**

the fullest. Taking my work, for example—an even broader range of phenomena could be visualized by making use of light in the X-ray region."

Takata and others are striving to visualize reaction processes behind material functions using X-ray pulses from SPring-8 to imitate stroboscopic light from ordinary cameras. One of the materials under investigation is DVD-RAM (DVD random-access memory). When the surface of this type of rewritable optical disc is exposed to laser light, the crystalline phase, in which atoms are regularly arranged, dissolves to form a liquid phase. The liquid phase immediately cools down to form an amorphous phase, in which atoms are arranged irregularly. The crystalline phase can be restored by illuminating the amorphous phase with laser light. Light reflectance differs between the crystalline and amorphous phases. Information is recorded on DVD-RAM by assigning the integer '0' or '1' to differences in the light reflectance. "In certain materials, phase change occurs in the extremely short time of 20 nanoseconds (two hundred millionths of a second)," notes Takata. However, he adds that the reason why the phase change is so fast remained unknown because no one had ever been able to watch the reaction pathway.

In October 2006, Takata and others demonstrated the structural difference between materials showing fast phase change and those showing slow phase change in a joint research program with Noboru Yamada and others at Matsushita Electric Industrial Co., Ltd. (Fig. 3). In materials with a fast phase change, the crystalline and amorphous phases are structurally alike. In materials with a slow phase change, the two phases are not alike. It seems that the fast transition may result from a consistency in the basic structure during the crystalline and amorphous phases.

"In our ongoing study, however, we have only succeeded in watching the beginning and end of the phase change. I want to visualize the entire reaction process of the phase change to help develop a new type of DVD-RAM that offers even faster recording speeds," says Takata. "As I mentioned before, the properties of materials can vary when electrons are exchanged in reaction processes. Making use of the advanced X-rays from SPring-8 would enable us to visualize these exchanges in times of the order of picoseconds (one trillionth of a second).

Takata and others are also working to visualize electron spins by using the polarity of the X-rays from SPring-8. Electron spins represent a motion like the rotation of a celestial body, occurring in two directions: either upward or downward. The spin direction of the electrons in a material determines the properties of the material as a whole, such as its magnetism and the nature of the electric currents produced. "Now we are acquiring a firm footing for the development of a methodology for visualizing a three-dimensional distribution of electron spins using SPring-8," says Takata. "To visualize how the distribution of electrons and the directions of spin change in reaction processes that take place in an extremely short time, hence to visualize all the behavior patterns of electrons that govern physical properties —this is my ultimate goal."

On the way to this ultimate goal, Takata expects to see the development of a series of new materials that will improve people's lifestyles and benefit society. ■

About the researcher

Masaki Takata was born in Japan in 1959. He obtained his PhD in physics from Hiroshima University in 1988. He now works as chief scientist at the RIKEN Harima Institute and concurrently serves as director at the Division of Research and Utilization at JASRI (Japan Synchrotron Radiation Research Institute), which runs SPring-8. His scientific research focus is on materials science, using synchrotron radiation X-rays to probe the structures of inorganic compounds, organometallic compounds, proteins, and other materials.

'Asian Research Forum on Emerging and Reemerging Infections—2007' held in Nagasaki

Emerging and reemerging infections are now causing great concern because of the threat they pose to public health. Rapid social and environmental changes, such as forest destruction, which increases the opportunity for human contact with wild animals, the globalization of goods, and the increased distances people travel, ever more frequently, are assumed to be causing these infections to emerge.

On January 15 and 16, the RIKEN Center of Research Network for Infectious Diseases and the Ministry of Education, Culture, Sports, Science and Technology held an international conference called 'the Asian Research Forum on Emerging and Reemerging Infections—2007' in Nagasaki. Researchers from four of the research centers in Thailand, Vietnam, China, and Japan, and special guests from abroad and from internal institutions reported their recent research outputs. 'Mosquito-borne Infections' was one of the focuses in the forum.

The following reports attracted particular attention: Roger Nasci from the Centers for Disease Control and Prevention, USA, showed how West Nile virus (WNV) has been on an inexorable march west and south through the USA, and has brought serious social damage since 1999. According to Nasci, this has been due to the rapid growth of the virus, the very high rate of infection from mosquitoes to birds, the absence of immunity against WNV in North American birds, and the ability of WNV to survive the winter inside mosquito eggs.

Thomas Wellems from the National Institute of Allergy and Infectious Diseases reported the comprehensive genomics of the malaria parasite, including identification of the point mutation of the pumping membrane protein that is responsible for resistance to an anti-malarial drug, chloroquine, and the genes determining the parasite's virulence. He further addressed human genetic polymorphism, which affects the severity of the disease.

Vu Sinh Nam from the Vietnam Administration of Preventive Medicine reported the usefulness of predacious copepods of a genus, Mesocyclops, to

prey on mosquito larvae that are dengue vectors. They found this greatly reduced the number of mosquitos for the five years from 1998. Their strategy originated with community-based research in Eastern Asia. It is sustainable in Vietnam and can also be applied in other regions.

Almost 200 participants not only listened eagerly to the presentations, but also showed avid interest in the 45 posters with many enthusiastic discussions taking place. ■

RIKEN has won Nano-fabrication Technology Award at nano tech 2007

For 3 days, from February 21 to 23, RIKEN exhibited at the 'nano tech 2007 International Nanotechnology Exhibition & Conference' in Tokyo, where a total of almost 50,000 people presented their latest results. These research achievements included 'Nanodevices with Carbon Nanotube Building Blocks' and 'Nano-scale controlled plastic electronics' in the fields of nanotechnology and nanoscience. RIKEN won the Nano-fabrication Technology Award owing, in part, to successful nanoscale semiconductor fabrication achieved by a research team at the Center of Intellectual Property Strategies, together with Tokyo Oka Kogyo Co. Ltd. By developing a new coating process and coating materials, the team realized a film several nanometers thick with a remarkably improved etching resistance, making it ideally suited for use in resist patterns. Moreover, RIKEN successfully transferred this technology to the industrial sector through joint research based on the RIKEN 'Integrated Collaborative Research Program with Industry. ■

Protein 3000 project over—aim achieved

On February 28, the Protein 3000 project, facing its conclusion at the end of March, held a symposium to release its research results and talk about the future of protein research. Almost 1,000 people attended the symposium and there were lots of discussions.

In 2002, the Ministry of Education, Culture, Sports, Science and Technology (MEXT) started this national project to obtain deep insight into the biological network by solving over 3,000 protein structures and determining functions of biological and medical importance. The research topics included investigating three-dimensional structures and molecular functions, elucidating the molecular mechanisms of biologically and medically important phenomena, developing technologies suitable for large-scale structural biology studies, and discovering chemical compounds to control proteins, thus paving the way toward rational drug design. While almost 90 laboratories in universities and research institutions joined this project, the RIKEN Structural Genomics/Proteomics Initiative (RSGI) (http://protein.gsc.riken.jp; http://www.rsgi.riken.go.jp) conducted systematic and comprehensive research to elucidate protein structures and functions at the RIKEN Genomic Sciences Center and the SPring-8 Center.

The research of the RSGI targets a wide range of diverse proteins from many species and determined over 2,500 structures. The primary targets are medically valuable proteins from human, mouse, and *Arabidopsis thaliana*, especially those involved in signal transduction or nucleic-acid binding. Proteins from bacteria and archaea were also studied to investigate proteins playing roles, for example, in replication, recombination, transcription, and translation of the fundamental genetic system. Together with another 500 protein structures studied by other research centers, the project has succeeded in achieving its initial aim to clarify over 3,000 structures.

Above all, a research group of Shigeyuki Yokoyama, a deputy director of RSGI, made remarkable progress in establishing a standardized method for high-yield cell-free protein synthesis, the key for high-throughput protein production. For nuclear magnetic resonance (NMR) analysis, protein samples are labeled with ^{15}N and subjected to heteronuclear single quantum correlation screening. Constructs selected by the screening step are produced by larger-scale protein synthesis with ^{13}C or ^{15}N labels. Selenomethionine incorporated proteins for multi-wavelength anomalous diffraction phasing are then prepared for X-ray crystallography. Robotic techniques have been introduced and large-protein preparation facilities have also been established.

During the latter half of the project, RSGI placed emphasis on expressing difficult proteins, such as protein complexes and integral membrane proteins. Many protein-expression systems were combined, including the cell-free protein-synthesis method and cell-based expression using yeast, insect, and human cells.

According to Yokoyama, from now on, they will try to make an even stronger collaboration between medical, pharmaceutical, biological and industrial sectors, so that they can contribute to the general understanding of lifescience and the development of new drugs by chemical design, based on protein structures, clarified in this project and future research. ■

Millennium Projects Blast Off—Genomics at RIKEN, Part II

Since 2000, RIKEN has established several life science research centers that play a leading role in the world's post-genomic research endeavors

Information gained from genomic analysis is crucial to developing long-awaited advances in medical treatments and the biotechnology industry. As competition in the post-genomic research era intensified, RIKEN ushered top-level researchers and energetic young scientists from around the country to its life science research centers established at the beginning of this millennium.

In 1997, when the UK surprised the world by producing the first cloned animal, Dolly the sheep, Japan's Council for Science and Technology Policy compiled a report outlining the long-term direction of the nation's life science research.

In 1999, a government panel recommended that Japan develop research centers in three important fields: genomic analysis to study polymorphism and diseases, developmental biology and plant biology. In the same year, five Japanese ministers made an unusual joint announcement on a basic guideline to nurture the fledgling biotechnology industry—a strategy that influenced other key areas of life science projects at RIKEN (see *RIKEN RESEARCH* **2 (3),** p18). This occurred when genomic analysis research, notably the Human Genome Project, was progressing faster than originally predicted. The government chose RIKEN as the lead organization to implement these government plans.

In 2000, RIKEN first decided to set up two new life science research centers in 2000 at its Yokohama Institute—the home of the Genome Sciences Center since 1998. One was the Plant Science Center, which aims to investigate genomic functions in, and the metabolic systems of, model plants such as *Arabidopsis* and rice, in order to improve plant production in both volume and quality.

The other center, the SNP (single nucleotide polymorphism) Research Center (SRC), was established to realize tailor-made medicines based on genetic information. In 2002 while developing high-throughput technologies to effectively analyze SNPs (genetic variations), the center successfully identified a gene related to heart infarction, suggesting that genome-wide scanning of SNPs targeting disease cases is effective.

In 2001, the Research Center for Allergy and Immunology (RCAI) was added at Yokohama as part of the government's plan to revive Japan's stagnant economy and stimulate sustainable growth. The center is designed to unveil mechanisms of the complex immune system, which are little known despite a long history of immunology research in Japan. Specifically, the RCAI aims to develop effective vaccines and immune therapies and the capability to control adverse reactions in organ transplant patients.

Figure 1: The Center for Developmental Biology, one of RIKEN's new life science centers this millennium, is at the core of Kobe's plans to make the port city Asia's medical hub.

Meanwhile, in the late 1990s RIKEN was planning to build the Center for Developmental Biology (CDB). Since its opening in 2000, the CDB's researchers have been studying development and regeneration of animal cells as well as regenerative medicine for humans. The center has also been positioned as the core of Kobe city's project to make it the center of the advanced medical industry (Fig. 1).

High-quality biological resources are essential to support life science research. In 2001, RIKEN established the BioResource Center (BRC) in Tsukuba to offer a variety of biological resources ranging from cells, genes, DNA and experimanetal animals to experimental plants and microbes. It allows RIKEN and other researchers to use these resources.

These five new centers bring the total number of RIKEN's life science research centers to seven, including the Brain Science Institute in Wako. Before these centers opened, RIKEN scrambled to appoint top-level researchers as directors and chief scientists from universities and research institutes nationwide, but its persistence and passion to create a world-class research system moved many renowned scientists to relinquish their tenure positions and join RIKEN. RIKEN has thus established a robust framework for implementing Japan's national post-genome research strategy, and is currently forging ahead aggressively to become the world's center of excellence in this field. ■

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