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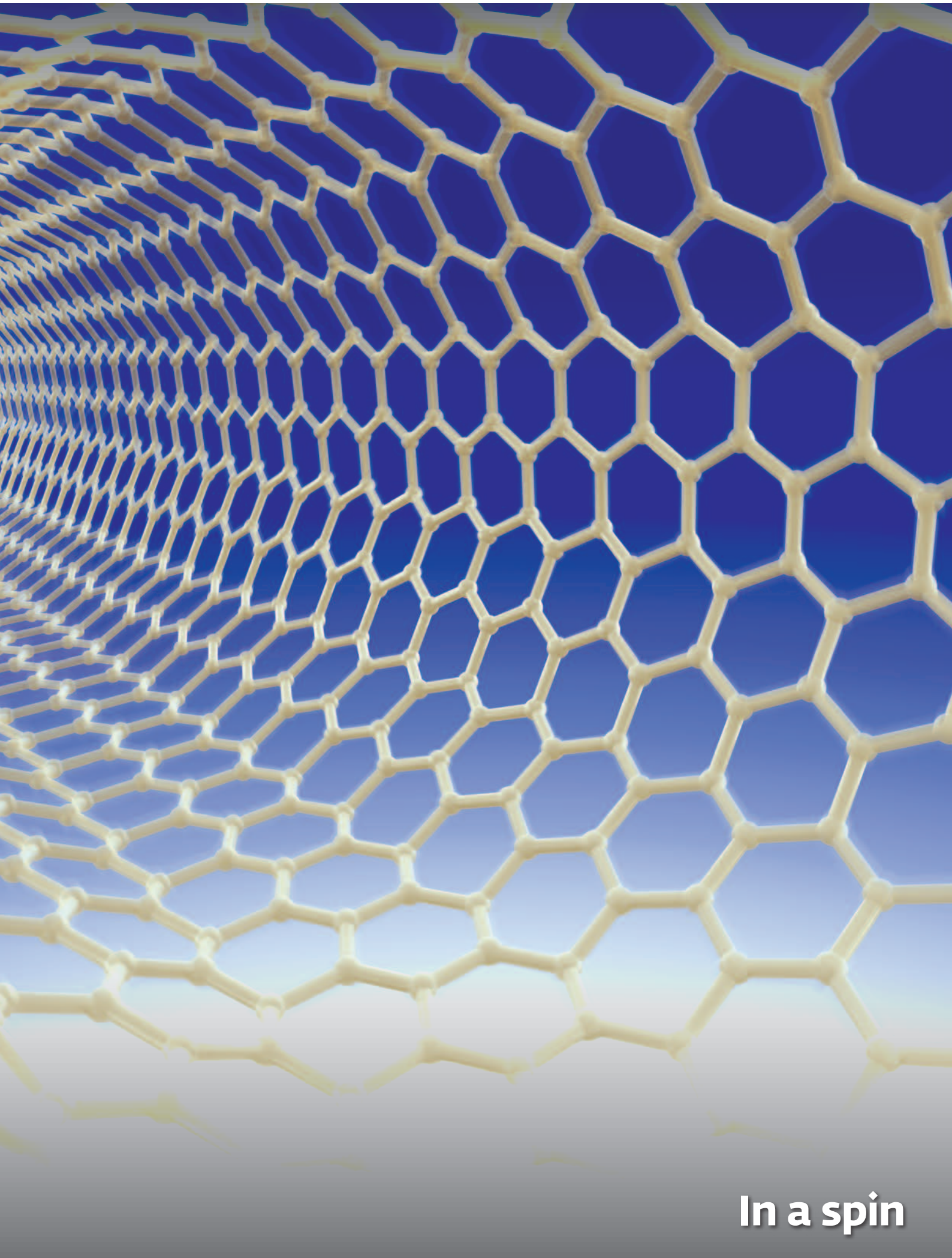
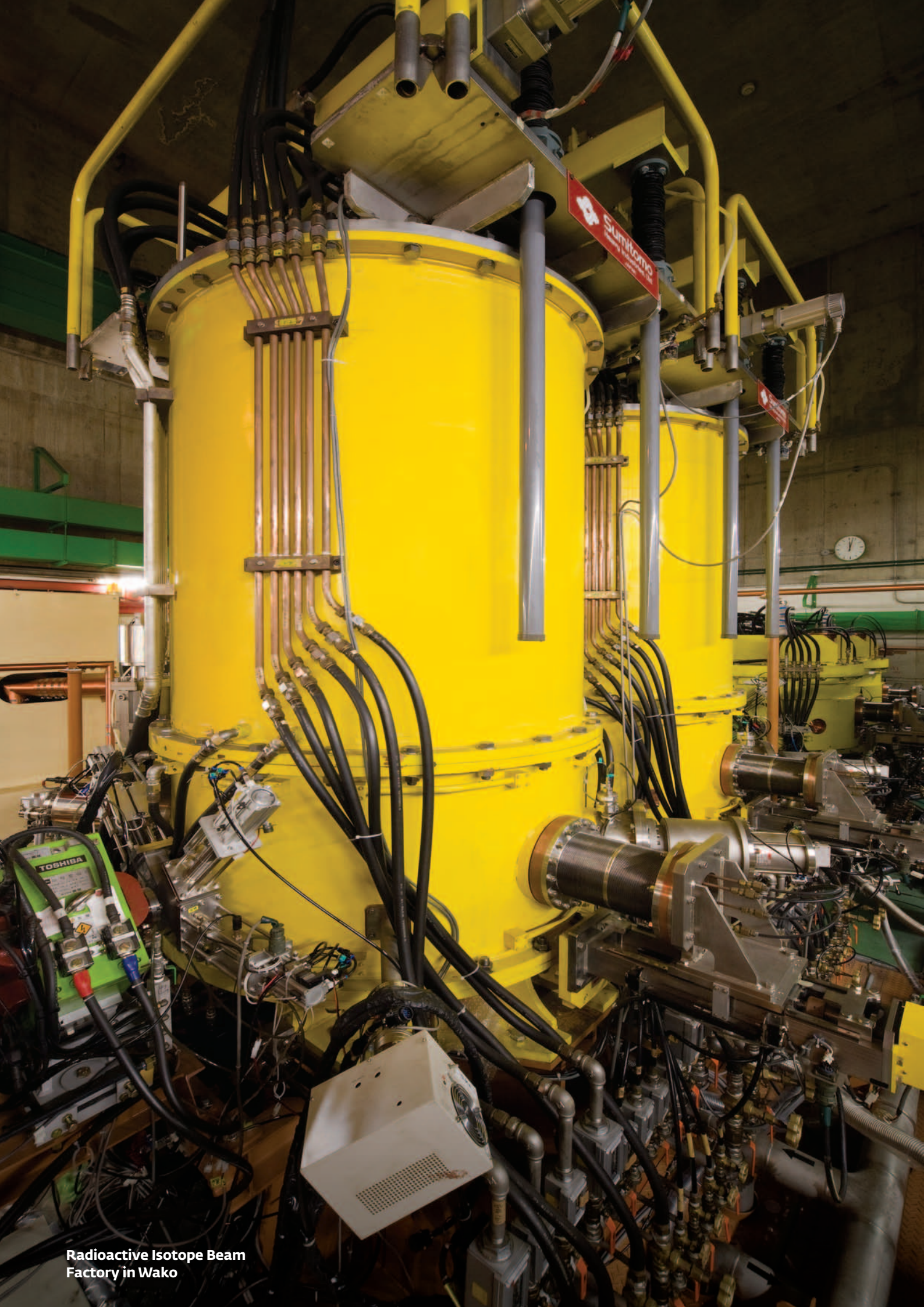


In a spin



Radioactive Isotope Beam Factory in Wako

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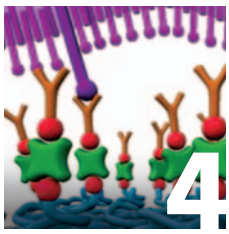

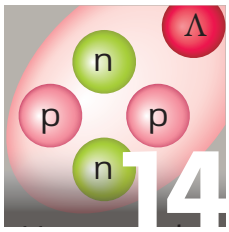

RIKEN RESEARCH

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Biology

Cancer cells get the cold shoulder

A temperature-responsive nanomaterial efficiently captures trace quantities of tumor cells from blood samples and releases them on demand for further study

Perhaps the most insidious property of cancerous tumors is their tendency to shed cells into the bloodstream, spreading the cancer by seeding new tumors in other parts of the body. Yet it is also this very property that could ultimately prove to be a cancer's undoing. Researchers around the world are developing efficient ways to capture circulating tumor cells (CTCs) from the bloodstream, and diagnostic tests based on these methods could be exploited by doctors to detect and treat tumors before they have the chance to take root and spread.

One group at the forefront of CTC-capture technology is the collaborative research teams of Hsiao-hua Yu at RIKEN's Yu Initiative Research Unit and Hsian-Rong Tseng at the University of California, Los Angeles (UCLA) in the US. These researchers have been involved in the refinement of a highly efficient CTC-capture material called 'NanoVelcro', and their latest work provides a means to not only capture CTCs, but also to release them for further study[1].

NanoVelcro, originally developed by Tseng and his colleagues at UCLA, exploits the ability of cells to adhere much more strongly to nanostructured surfaces than to flat ones. In the first iteration of the material, the team fabricated surfaces covered with vertically oriented silicon nanopillars, which were then coated with CTC-selective antibodies. Although CTCs exist within the blood at abundances as low as a few cells per milliliter, the material could capture these cells with up to 70% efficiency. When the same antibodies were attached to a flat surface, the capture efficiency reached only 14%.

Sticky surface

Precisely why nanostructured surfaces outperform flat ones for capturing cells remains an open question. "At this moment, we have no direct or scientific evidence on why a nanostructured surface allows improved efficiency on cell capturing," Yu says. The researchers are fairly confident that the phenomenon has more to do with the biophysiological response of cells to nanostructured surfaces than the effects of surface area or the number of antibodies present. Moreover, the effect is general and broadly observed. "It has been demonstrated on silicon nanopillars, titanium nanowires, and conducting polymer nanodots," says Yu.

The apparent universality of the phenomenon proved advantageous in the team's latest research. Although their antibody-coated silicon nanowires were effective for CTC capture, attempts to release captured cells through subsequent enzyme treatment were less successful: only 10% of cells remained viable upon release, and even fewer survived for long-term study.

"Much research effort has been focused on capturing CTCs with high efficiency. However, it is equally important to understand them biochemically," says Yu, noting that if the cells could be effectively detached and cultured, many potential studies could be carried out. For example, the physiological nature of these cells could be

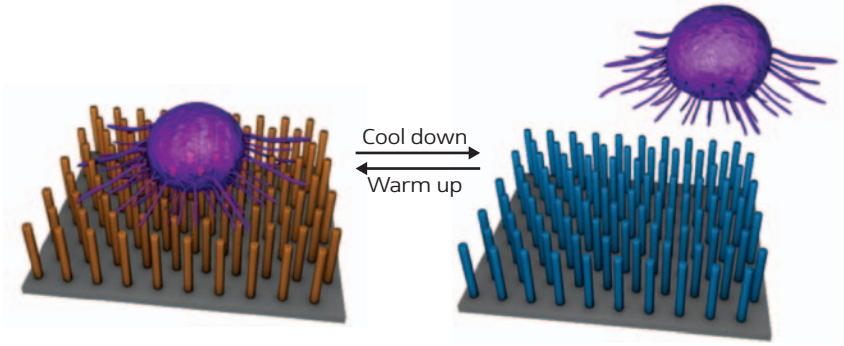

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Figure 1: At 37 °C (left), circulating tumor cells stick strongly to the nanostructure's surface, but are released unharmed when the material is cooled to 4 °C (right).

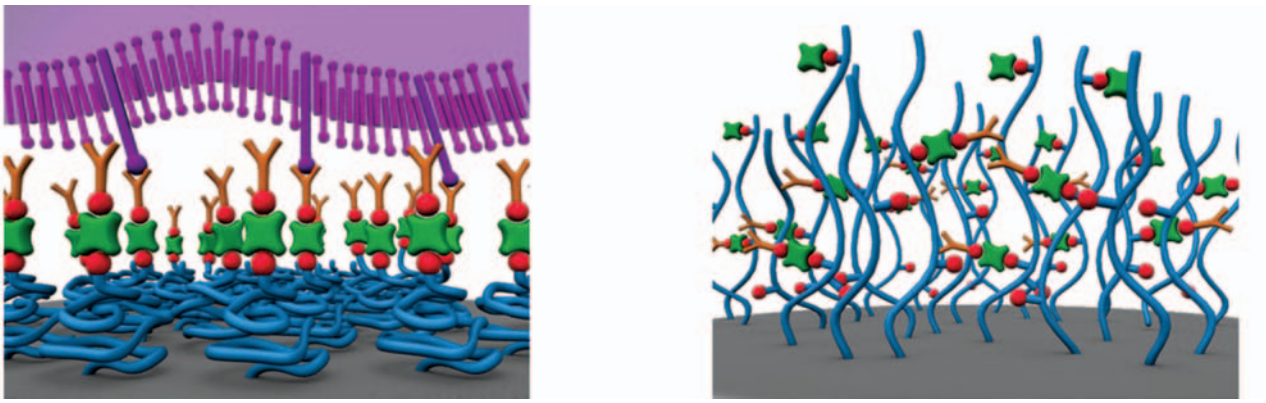

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Figure 2: A temperature-responsive polymer (blue) controls the nanomaterial's capture and release behavior. At 37 °C (left), the polymer coils up, presenting antibodies on its surface (brown) to capture passing tumor cells (purple). When cooled to 4 °C (right), the polymer unfurls, the antibodies are internalized and the tumor cell detaches from the surface.

probed, and their molecular and genetic readouts analyzed for new insights into tumor biology. In addition, further studies are needed simply to validate CTCs as robust and reliable indicators of cancer. "Overall, we need to know more about CTCs in order to establish them as a biomedical marker or diagnostic tool," he says.

In late 2011, Tseng spent a month at RIKEN as a visiting scientist. During that time, the team devised a solution to the cell-release problem, designing a new material that would release the cells in response to temperature change (Fig. 1). Before attaching the antibodies to their nanostructured surface, they first grafted strands of a temperature-responsive polymer known as PIPAAm onto the silicon nanowires. Using a molecule called biotin as a linker, they then attached CTC-selective antibodies onto each polymer strand.

"The thermoresponsive property of PIPAAm is one of the best-known cases of stimuli-responsive materials," says Yu. At temperatures of around 37 °C, PIPAAm is hydrophobic and the polymer strands form a densely coiled layer from which the attached antibodies project out into solution to capture passing CTCs. When cooled to around 4 °C, however, the polymer becomes hydrophilic and uncoils out into the surrounding solution. As a result, the antibodies holding the CTCs become internalized within the expanded polymer layer, and so the adhered cells become detached (Fig. 2).

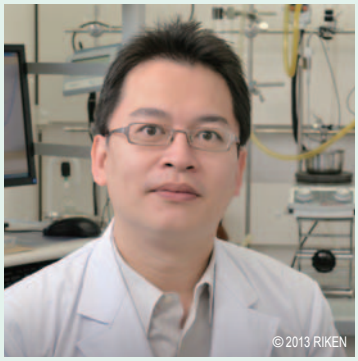
Ready release

To measure the performance of their stimuli-responsive surface, the researchers tested it using 1-milliliter samples of artificial blood, each spiked with 1,000 CTCs. They compared the performance of surfaces with different densities of biotin attached to the PIPAAm, and found that a 10% coating proved most effective both for CTC capture and subsequent release. This material was found to be just as effective as the first-generation NanoVelcro for capturing cells, if not more so, trapping over 90% of the CTCs in the blood sample at 37 °C. After transferring the material to the refrigerator for 30 minutes, over 90% of the cells were released. In addition, almost 90% of the released cells remained viable and thus suitable for further study. The material should therefore allow CTC capture to be validated as a non-invasive test for identifying cancers early in the critical window before a tumor begins to spread, the researchers say.

To further their research, Yu and his RIKEN colleagues plan to develop and test more smart surfaces for capturing CTCs and other cells of interest that only exist naturally at very low concentrations. "We are particularly interested in developing temperature-, electrical-, pH- and light-responsive nanomaterials to demonstrate their capability for rare-cell capture and release," says Yu. In addition, the team plans to carry out more fundamental research to investigate why cells and nanostructured surfaces interact so strongly.

1. Hou, S., Zhao, H., Zhao, L., Shen, Q., Wei, K. S., Suh, D. Y., Nakao, A., Garcia, M. A., Song, M., Lee, T., *et al*. Capture and stimulated release of circulating tumor cells on polymer-grafted silicon nanostructures. *Advanced Materials* **25**, 1547–1551 (2013).

ABOUT THE RESEARCHER



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Hsiao-hua (Bruce) Yu was born in Taiwan in 1974. He graduated from National Taiwan University in 1996 and obtained his PhD from the Massachusetts Institute of Technology in the US, where he also conducted postdoctoral research. In 2004, he was appointed as a team leader and senior research scientist at the Institute of Bioengineering and Nanotechnology, Singapore, where he investigated the use of organic conductive materials in biosensors. He joined the RIKEN Advanced Science Institute as an initiative research scientist in 2008. His research now focuses on efforts to develop organic conductive biomaterials through a combination of synthetic organic chemistry, electronic materials and biological investigations into the molecular and nanoassembled building blocks of organic materials.

In or out of phase?

Large-scale computer simulations show that an intriguing state of matter previously predicted in graphene-like materials might not exist after all

Virtually every material undergoes atomic-level ordering when cooled to temperatures approaching absolute zero. Liquid water, for example, is frozen into atomically ordered crystalline ice. However, condensed matter physicists have theorized that it may be possible to achieve a state called a quantum spin liquid, in which quantum-mechanical effects or the structure of the atomic lattice hinder the development of atomic order while retaining strong electronic interactions. Seiji Yunoki and colleagues from the RIKEN Center for Emergent Matter Science and the RIKEN Advanced Institute for Computational Science have now shown through detailed calculations that achieving the quantum spin liquid state may be more difficult than previously thought[1].

Recent theoretical studies have indicated that a quantum spin liquid phase could exist in two-dimensional materials where the constituent atoms are arranged in a hexagonal network. A prominent example of such a material is graphene, a single layer of carbon atoms with a honeycomb lattice structure.

Yunoki and his team modeled the honeycomb lattice structure by performing a series of quantum Monte Carlo simulations using Japan's new 'K computer'—one of the world's top three supercomputers. Monte Carlo-based simulations allow immense and complex physical systems to be calculated at a fraction of the usual computational time by sampling important parts of the system and using statistics to generate predictions. Yet even with such an efficient simulation code, it was only the massive computational power of the K computer that made these latest calculations possible.

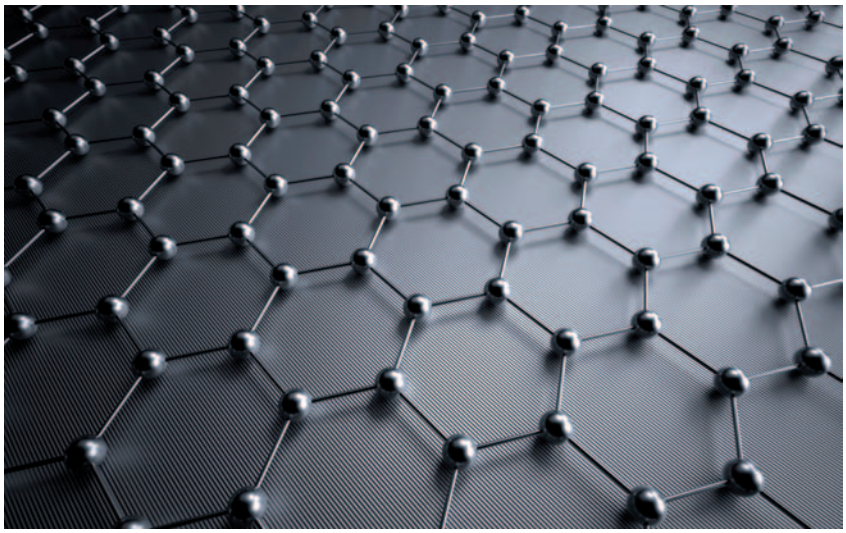

Figure 1: The quantum spin liquid state, predicted to exist in two-dimensional hexagonal lattice systems, may not occur in such structures after all.

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"The accuracy of the numerical results is largely determined by the system size," explains Yunoki. "The effects we are looking for are small and so we needed large-scale simulations." Yunoki's team was able to model a honeycomb lattice that included as many as 2,592 atomic sites—four times more than in previous studies.

The simulations showed that the spin state in the honeycomb lattice moves directly from a semi-metal phase to an antiferromagnetic-insulator phase, where adjacent spins point in opposite directions. Importantly, whereas the quantum spin liquid state was suggested in previous studies on smaller systems, the researchers found no evidence of an intermediate spin liquid phase in their simulations.

"We believe that a spin liquid phase is an important quantum state of matter, which could have a huge impact on condensed matter physics if we can find a true spin liquid in a realistic quantum system," says Yunoki. "Our next step is to find a spin liquid, and we strongly believe that the power of the K computer will help significantly."

1. Sorella, S., Otsuka, Y. & Yunoki, S. Absence of a spin liquid phase in the Hubbard model on the honeycomb lattice. *Scientific Reports* **2**, 992 (2012).

X-rays in focus

Atomically precise mirrors open up new opportunities for x-ray free-electron laser applications

X-ray free-electron lasers (XFELs) are powerful analytical light sources that can be used for applications ranging from fundamental materials science to the study of biological processes. A collaborative team including researchers from the RIKEN SPring-8 Center (RSC), Osaka University and the Japan Synchrotron Radiation Research Institute have now developed an XFEL mirror system that is able to focus the x-ray beams to a very small spot, providing ultrahigh light intensities that will enable a range of novel experiments[1].

X-rays easily pass through most materials, which makes controlling x-ray beams using conventional optics such as glass lenses very difficult, and alternative lens designs have tended to reduce the efficiency of x-ray experiments. "Reduced efficiency and higher absorption of x-rays in the lens damages the optics because absorbed x-rays excite and ionize the atoms in the optical materials," says Makina Yabashi from the RSC.

A promising method for controlling x-rays is based on reflection, where x-rays are reflected off polished surfaces in a similar manner to the way a satellite dish focuses television signals. The team's design consists of a pair of elliptical mirrors that reflect x-rays at low angles, providing both horizontal and vertical control of the focused light (Fig. 1).

However, the quality of the reflected beam is strongly dependent on the quality of the reflective surfaces. As x-ray light has a very short wavelength, extraordinarily smooth surfaces are required for the reflection system to be effective. The researchers achieved remarkable atomically smooth surfaces through a combination of techniques including elastic emission machining—a chemical etching technique that provides atomic precision. Fabricated at the Yamauchi Laboratory at Osaka University, the mirrors used in the reflection system had a surface roughness of just 0.13 nanometers on average over the entire mirror length of 40 cm. The mirror itself was fabricated from carbon-coated quartz—carbon has only weak absorption of light at x-ray wavelengths and so does not get damaged by the intense x-ray beams hitting the mirror surface.

The mirrors were tested at the Japanese XFEL facility, known as the SPring-8 Angstrom Compact Free Electron Laser (SACLA). The researchers achieved a focus size of just 1 micrometer, effectively increasing the XFEL intensity by a factor of 40,000. The new mirror system is expected to open up a number of new applications for the XFEL facility. "Applications include the analysis of small particles or nanoparticles, and the study of fundamental physical processes at extremely intense light fields," says Yabashi.

1. Yumoto, H., Mimura, H., Koyama, T., Matsuyama, S., Tono, K., Togashi, T., Inubushi, Y., Sato, T., Tanaka, T., Kimura, T. *et al.* Focusing of X-ray free-electron laser pulses with reflective optics. *Nature Photonics* **7**, 43–47 (2013).

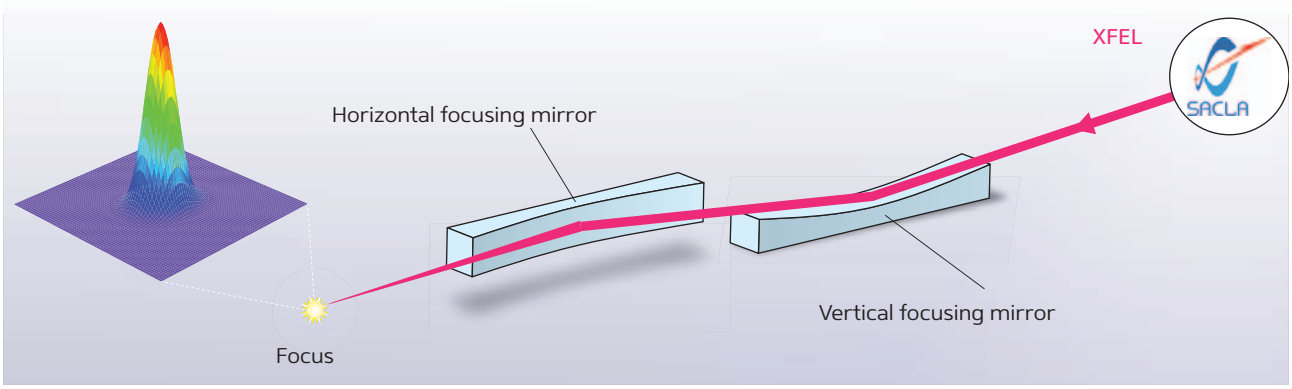

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Figure 1: The elliptical mirror system for the SACLA XFEL facility. Two high-precision mirrors focus x-ray radiation from the XFEL into spots of only 1 micrometer in diameter.

Gold atoms caught in the act

Femtosecond 'snapshots' reveal a dramatic bond tightening in photo-excited gold complexes

Metal complexes are becoming increasingly important as the photochemical building blocks of functional molecular systems such as sensors and photoelectrochemical cells. Of particular interest are metal complexes that involve gold atoms in the +1 valence state, due to their ability to self-assemble into larger units. The assembly process, known as aurophilic interaction, is enhanced by photoexcitation—an effect recently exploited by chemists to link individual gold(I)–dicyanide complexes into phosphorescent oligomer chains through careful control of complex concentrations and light exposure. However, the fundamental structural details of this reaction have yet to be understood.

Tahei Tahara and colleagues from the RIKEN Molecular Spectroscopy Laboratory, in collaboration with scientists from Toyama University, have now used ultra-fast laser spectroscopy to observe the movements of gold atoms in real time during the photochemical reaction of gold(I)–dicyanide[1].

To make their observations, Tahara and his colleagues used a laser spectroscopy technique that is capable of resolving changes in molecular emission and absorption at the femtosecond scale—a method that the researchers had previously used to observe the flattening of a propeller-like copper complex. This time, the team studied how 'trimers' of three linked gold(I)–dicyanide molecules are affected by light. Theory predicts a rapid decrease in distances among gold atoms when electrons are pushed into a photoexcited state. However, no experimental evidence exists for this sort of bond tightening.

In response to laser excitation, the gold(I)–dicyanide trimer produced a new absorption signal that progressively grew more intense and shifted to higher energies over a period of 2 picoseconds, which the team ascribed to an excited-state trimer undergoing a geometric change. Density functional theory (DFT) calculations revealed this timeframe to be consistent with a bent-to-linear transformation of the trimer and tight gold–gold bond formation.

By scrutinizing the first picosecond of spectral data given off by the excited-state trimer, the researchers saw that the peak absorption signal oscillated sharply in femtosecond increments. Tahara notes that this kind of modulation is clear evidence of a rapid shortening of inter-atomic gold distances (Fig. 1)—an interpretation backed by DFT computations correlating the oscillation frequencies to gold–gold stretching vibrations. The team's results also show that the bent-to-linear transformation must occur before the gold(I) complexes can link into longer oligomers. The researchers anticipate that the fundamental insights gained using ultra-fast laser spectroscopy will prove critical to chemists' understanding of the light-induced self-assembly of metal complexes.

1. Iwamura, M., Nozaki, K., Takeuchi, S. & Tahara, T. Real-time observation of tight Au–Au bond formation and relevant coherent motion upon photoexcitation of $[Au(CN)_2^-]$ oligomers. *Journal of the American Chemical Society* **135**, 538–541 (2013).

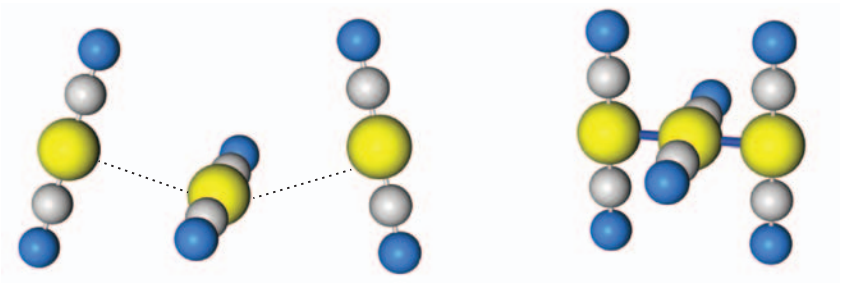

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Figure 1: A gold(I)–dicyanide trimer chain (left) changes from a bent geometry to a linear structure with short gold–gold bonds (right) on photoexcitation.

A stable model for motor neuron disease

Increased stability of a misfolded protein is linked to the age of onset of a common form of motor neuron disease

Neurodegenerative diseases are often characterized by the aggregation of misfolded proteins, which accumulate to form insoluble clumps within or around nerve cells. In the adult motor neuron disease amyotrophic lateral sclerosis (ALS), for example, such aggregations are formed by misfolding of the TDP-43 protein (Fig. 1). The mutation responsible for the inherited form of ALS is known to originate in the gene encoding the TDP-43 protein, but the relationship between the biochemical properties of TDP-43 and the progression of ALS was unclear.

New research by Koji Yamanaka and colleagues from the Laboratory for Motor Neuron Disease at the RIKEN Brain Science Institute has now revealed that increased stability of mutant TDP-43 is associated with earlier onset of ALS[1].

Yamanaka and his colleagues isolated the human *TDP-43* gene and used genetic engineering to introduce seven different mutations that have previously been identified in patients with inherited ALS. They then introduced the mutated genes into neurons growing in culture dishes in order to induce the cells to synthesize the mutated proteins.

They found that the mutated versions of TDP-43 were far more stable than the normal form, with half-lives up to four times that of the normal protein. This increased stability also made the mutant TDP-43 molecules more toxic to the cells.

The researchers then screened the clinical information of 81 patients with the inherited form of ALS to determine whether the stability of the mutated protein is related to the age of disease onset. The results showed that patients carrying TDP-43 mutations with a longer half-life developed the disease at an earlier age.

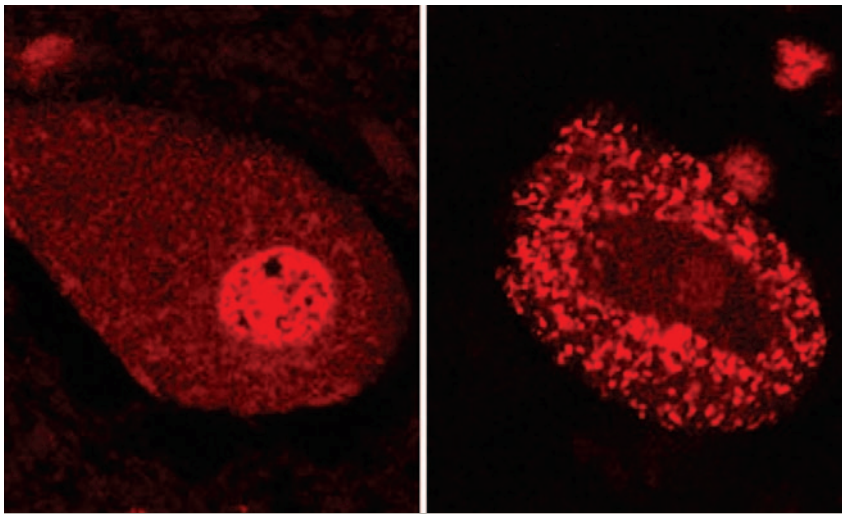

Figure 1: TDP-43 (red) in motor neurons in a patient with ALS (right) compared with a normal motor neuron cell (left).

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Misfolded proteins are normally recognized and targeted for destruction by a cell structure called the proteasome before they can cause cellular damage. Yamanaka's group found that stabilized TDP-43 protein molecules inhibit proteasome activity, thus adding to the growing body of evidence that this clearing mechanism fails in neurodegenerative diseases. They also found that stabilized TDP-43 protein loses the ability to control its own mRNA transcripts, thereby further accelerating its accumulation.

The cell culture experiments demonstrate a new model that can be used to control the stability of TDP-43, and which could provide further insights into the importance of protein stability for the mechanisms of disease development and progression.

"Elucidating the mechanisms and consequence of stabilization will provide a mechanistic view of how motor neuron degeneration is initiated in ALS," says Yamanaka. "We are now looking at the mechanisms of toxicity, and the therapeutic means to prevent neuron death."

1. Watanabe, S., Kaneko, K. & Yamanaka, K. Accelerated disease onset with stabilized familial Amyotrophic Lateral Sclerosis (ALS)-linked mutant TDP-43 proteins. *The Journal of Biological Chemistry* **288**, 3641–3654 (2013).

RNA defects in motor neuron disease

A structure that edits messenger RNA transcripts is defective in two different forms of motor neuron disease

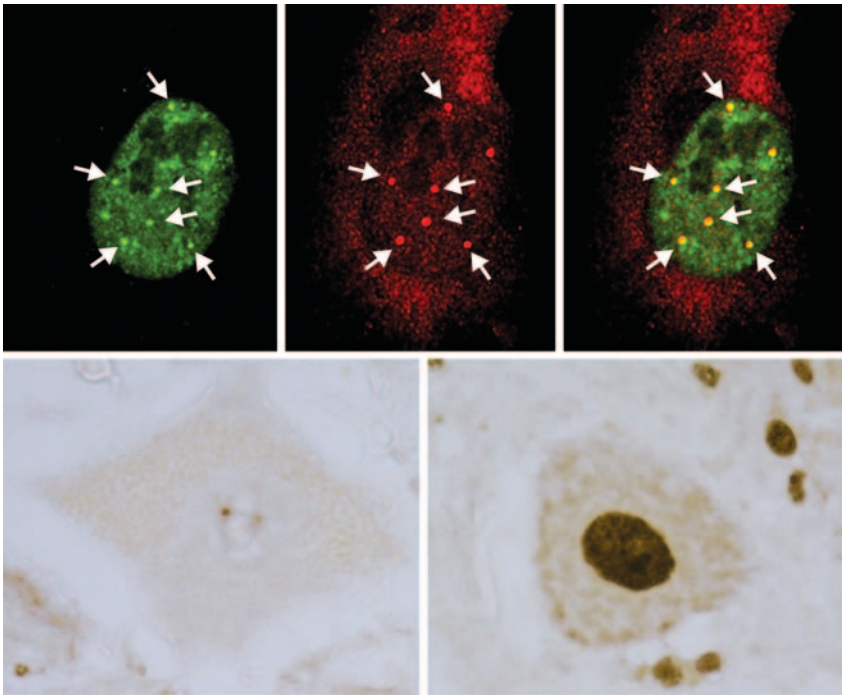

Figure 1: (Upper) TDP-43 (green) and SMN (red) proteins localize in nuclear gems in cultured cells (arrows). (Lower) Spliceosome subunits accumulate abnormally in the nuclei of motor neurons from an ALS patient (right), but not in normal motor neurons (left).

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Amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA) are degenerative motor neuron diseases in which the key mutated genes are involved in RNA metabolism. This similarity suggests that a common dysregulation of some aspect of RNA metabolism in motor neurons may underlie both disorders, although the exact cellular effects of the neurodegenerative mutations are unknown. Koji Yamanaka, Hitomi Tsuiji and colleagues from the RIKEN Brain Science Institute and other institutions in Japan have now obtained evidence that a cellular structure that edits messenger RNA (mRNA) transcripts is defective in both of these motor neuron diseases[1].

ALS is associated with mutations in the SOD1, TDP-43 and FUS/TLS protein-encoding genes, and spinal muscular atrophy (SMA) with mutations in a gene called *SMN1*. After determining that the TDP-43, FUS/TLS and SMN proteins are all localized to structures known as 'gems' inside the nucleus (Fig. 1), the researchers investigated whether these proteins might perform a similar function, which may indicate that the associated diseases share common RNA processing defects.

Yamanaka and his colleagues performed a series of biochemical experiments using lab-grown neurons and cancer cells, as well as neurons isolated from genetically engineered mice lacking the *FUS* gene. They found that eliminating TDP-43 expression in cultured cells prevented the formation of nuclear gems, and that gems were absent from neurons isolated from the mutant mice. Gem formation requires an interaction between TDP-43, SMN and FUS proteins, and this interaction is mediated by one end of the TDP-43 protein.

The researchers also found that all three proteins are involved in maintenance of the spliceosome, a large multi-component structure found in the nucleus. The spliceosome consists of multiple protein and RNA subunits, and controls splicing—the process by which non-coding sequences are removed from mRNA transcripts before they are translated into the strings of amino acids that make up proteins.

In ALS patients, the team found that ALS motor neurons had spliceosome defects—gems were missing from the nucleus and the RNA subunits of the spliceosome accumulated abnormally. Motor neurons from SMA patients, on the other hand, had significantly reduced levels of spliceosome RNA subunits in the nucleus.

The findings suggest that loss of spliceosome integrity plays an important role in neurodegeneration in both diseases. "Defective spliceosomes cause abnormal protein expression patterns, which can lead to motor neuron death," says Yamanaka. "This could be a new therapeutic target for neurodegenerative diseases, and we are now initiating efforts to develop a new class of drugs."

1. Tsuiji, H., Iguchi, Y., Furuya, A., Kataoka, A., Hatsuta, H., Atsuta, N., Tanaka, F., Hashizume, Y., Akatsu, H., Murayama, S. *et al.* Spliceosome integrity is defective in the motor neuron diseases ALS and SMA. *EMBO Molecular Medicine* **5**, 221–234 (2013).

Unearthing onions' sulfurous secrets

Clever use of high-resolution mass spectrometry allows the rapid cataloging of sulfur-containing compounds in plant extracts

Figure 1: Rapid cataloging of sulfur-containing compounds in extracts from plants such as onions has been demonstrated using a FT-ICR mass spectrometer (pictured).

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Plants are a rich resource of bioactive compounds, many of which have inspired therapeutic drugs. Yet countless plant compounds, potentially with medical uses, still remain to be identified. Kazuki Saito, Ryo Nakabayashi and colleagues from the RIKEN Center for Sustainable Resource Science have now developed a technique for rapidly cataloging subsets of compounds in plant extracts based on mass spectrometry data as a first step toward a fully automated system for cataloging novel plant compounds[1].

Mass spectrometry is an analytical technique that enables identification of the elements or molecules in a sample, making it a powerful tool for determining unknown compounds. Those compounds that contain certain 'heteroatoms' such as oxygen, nitrogen and sulfur produce a spectral 'fingerprint' that can be resolved in high-resolution mass spectra. Saito and his team hypothesized that this characteristic could be exploited to quickly catalog compounds in plant extracts.

To test their approach, the researchers profiled sulfur-containing compounds in onion extracts. Sulfur-containing compounds produce a pair of adjacent peaks in the mass spectrum—one 20 times stronger than the other—associated with the ratio of the two most common naturally occurring isotopes of the sulfur atom.

Using an ultrahigh-resolution, highly mass-accurate instrument called a Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer (Fig. 1), Saito and his colleagues were able to identify 67 sulfur-containing ions in the onion extract. Then, using isotope chemistry, they were able to establish the number of carbon atoms in each structure.

The researchers analyzed extracts from two sets of onions: one grown under a normal atmosphere, and another grown under an atmosphere in which the carbon in CO_2 was replaced with the heavier carbon isotope, carbon-13. By measuring how much heavier a plant compound was when formed from carbon-13, the researchers could calculate its carbon count and so determine its complete atomic make-up. In a final step, the team established the full chemical structure of some of the sulfur-containing compounds by comparing their data with that of known compounds.

"We are now planning to develop an automated structural assignment system," says Nakabayashi. The researchers hope to automate this time-consuming process by incorporating an additional analysis technique called nuclear magnetic resonance (NMR), developing a database of high-quality mass spectrometry and NMR reference data, and establishing a computational algorithm for checking each compound against this database to establish its chemical structure. The researchers also plan to extend their existing technique to compounds containing other heteroatoms, such as oxygen, nitrogen, bromine and chlorine.

1. Nakabayashi, R., Sawada, Y., Yamada, Y., Suzuki, M., Hirai, M. Y., Sakurai, T. & Saito, K. Combination of liquid chromatography–Fourier transform ion cyclotron resonance-mass spectrometry with ^{13}C-labeling for chemical assignment of sulfur-containing metabolites in onion bulbs. *Analytical Chemistry* **85**, 1310–1315 (2013).

Giving antitumor immunity a helping hand

A two-pronged approach to immune activation could lead to vaccines that effectively shut down tumor expansion

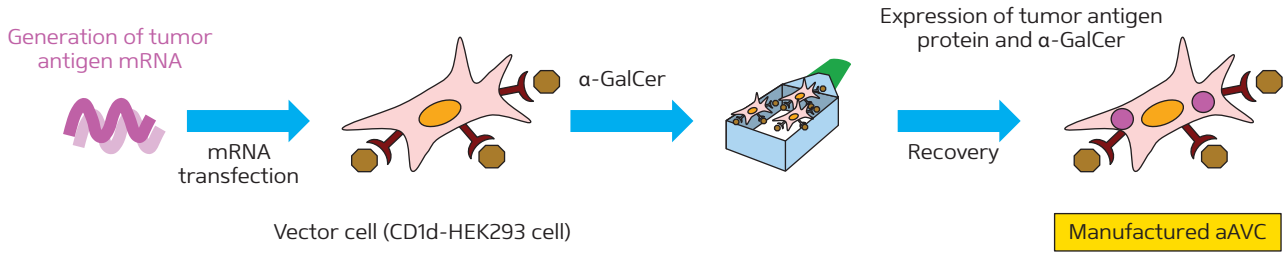

Figure 1: Artificial adjuvant vector cells (aAVCs) were produced by introducing mRNA-encoding tumor-specific antigens to human kidney cells dosed with the synthetic compound α-galactosylceramide (α-GalCer), which activates NKT cells.

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Tumor cells often express proteins that set them apart from their healthy neighbors. These very same proteins can also help the immune system to recognize and destroy the cancer. Several research groups and companies have already demonstrated proof-of-concept for antitumor therapeutic vaccines based on this principle, typically employing 'retrained' dendritic cells (DCs) harvested from a patient's own immune system. To date, however, such vaccines have demonstrated only limited effectiveness in beating back tumor progression. Shin-ichiro Fujii, Kanako Shimizu and colleagues from the RIKEN Integrated Medical Science Center have now revealed research that could supercharge the potency of future cancer vaccines[1].

DC-based vaccines directly stimulate 'adaptive' immunity, which is directed against specific molecular targets. "DCs play a pivotal role in determining the character and magnitude of an immune response," explains Fujii, "and the loading of patient DCs with tumor-specific antigens is one of the most promising current immunotherapeutic strategies."

Using a mouse model, Fujii's team demonstrated that the natural antitumor DC response can be considerably ramped up by stimulating invariant natural killer T (iNKT) cells from the 'innate' immune system, which triggers a more generalized response against disease.

Key to their iNKT cell-based strategy is the use of artificial adjuvant vector cells (aAVCs), derived from human kidney cells (Fig. 1). The aAVCs are dosed with mRNA encoding a tumor-specific antigen to yield a protein that trains DCs to recognize their 'enemy'. The aAVCs are also treated with α-galactosylceramide, a synthetic glycolipid that is a potent activator of iNKT cells. The iNKT cells go on to accelerate maturation of host DCs, which in turn promote an adaptive immune response against the selected tumor antigen.

Initial experiments demonstrated that aAVCs producing the protein ovalbumin activated DCs in mice and effectively stalled growth of grafted tumors expressing this protein. Importantly, this treatment proved more effective than a conventional vaccine approach using DCs injected with ovalbumin mRNA. Subsequent experiments confirmed that aAVCs could also elicit immunity against the melanoma antigen MART-1. "Even the injection of one cell could evoke both the innate and adaptive immune response," says Fujii.

Further tests using dog models revealed a clear immune response with no notable adverse effects, even in animals receiving multiple doses of aAVCs. These promising preclinical results could potentially pave the way for human clinical trials in the near future. "We have some candidate tumor antigens," says Fujii, "and now we need to examine the possibility of using this technique for these antigens in terms of efficacy and safety."

1. Shimizu, K., Mizuno, T., Shinga, J., Asakura, M., Kakimi, K., Ishii, Y., Masuda, K., Maeda, T., Sugahara, H., Sato, Y. *et al.* Vaccination with antigen-transfected, NKT cell ligand-loaded, human cells elicits robust *in situ* immune responses by dendritic cells. *Cancer Research* **173**, 62–73 (2013).

Asymmetry makes the protein pump go round

First high-resolution structures provide clues to the mechanism of an important molecular rotary motor

The protein vacuolar ATPase (V-ATPase) is an attractive drug target due to its crucial role in several disease conditions including osteoporosis and the spread of cancer. It is found within the membranes of many organelles where it pumps protons from one side of the membrane to the other. A team of researchers led by Takeshi Murata and colleagues from the RIKEN Center for Life Science Technologies has now produced the first high-resolution structures of a bacterial version of this important protein, revealing previously unknown details of its pumping mechanism[1].

V-ATPase in the bacterium *Enterococcus hirae* is closely related to its human equivalent, consisting of nine different protein-chain subunits. One part of the protein, V_0, is hydrophobic and sits in the membrane, while the other part, V_1, is hydrophilic and juts out into the cell body. As a result of previous work in which a team led by Murata produced, purified and assembled the protein subunits comprising V_1 in the laboratory, V_1 was revealed to consist of a hexagonal barrel-like complex built from three catalytic 'A' subunits alternating with three non-catalytic 'B' subunits (Fig. 1). Another complex, known as DF, fits into the center of the barrel and rotates.

In their most recent work, Murata's team solved x-ray crystallography structures for the A_3B_3 complex, as well as for the A_3B_3 complex attached to the DF complex and in association with an analog of ATP—a biological energy carrier. Much to their surprise, they found that although the three A chains all had the same sequence, as did the three B chains, the A_3B_3 complex fitted together in an asymmetric fashion.

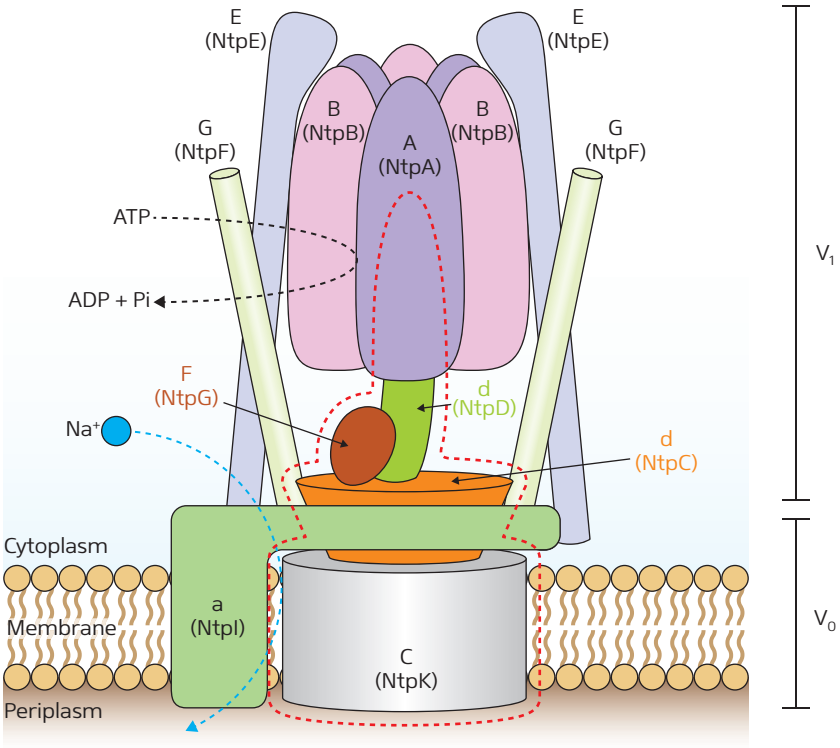

Figure 1: Schematic model of V-ATPase in *Enterococcus hirae*, showing the A_3B_3 barrel complex (top center) and the DF complex (immediately below).

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The A_3B_3 complex should contain three pockets between the A and B subunits, into which ATP can bind. When the ATP analog was added, however, the researchers found it bound in only two of the pockets because of the asymmetry. Adding the DF complex increased the asymmetry, causing the three pockets to develop different morphologies—particularly with respect to positioning of a projection involving the amino acid arginine, which assists the breaking of an ATP phosphate bond to release the energy that powers the rotary ion pump. They also found that the shape of the complex appears to change during rotation.

"This structural study will be useful for designing future drugs targeting V-ATPase," says Murata. "We now want to perform further experiments to understand the rotational mechanism more precisely."

1. Arai, S., Saijo, S., Suzuki, K., Mizutani, K., Kakinuma, Y., Ishizuka-Katsura, Y., Ohsawa, N., Terada, T., Shirouzu, M., Yokoyama, S. *et al.* Rotation mechanism of *Enterococcus hirae* V_1-ATPase based on asymmetric crystal structures. *Nature* **493**, 703–707 (2013).

Elucidating the structure of hypernuclei

EMIKO HIYAMA

Associate Chief Scientist
Strangeness Nuclear Physics Laboratory
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On Earth, stable atomic nuclei normally consist of protons and neutrons and the interactions between them are well understood. In space, however, 'hypernuclei'—atomic nuclei containing less familiar subatomic particles known as hyperons—are thought to exist. Recently, particle accelerators have enabled artificial production of hypernuclei and Emiko Hiyama, associate chief scientist at the Strangeness Nuclear Physics Laboratory, RIKEN Nishina Center for Accelerator-Based Science, is working hard to clarify their structure.

Three-body and four-body problems are difficult

"I would like to apply the 'Infinitesimally-shifted Gaussian lobe' computation method to many different areas of physics to open up new fields, thereby predicting, discovering, and analyzing new phenomena," says Hiyama, discussing her laboratory's research plan (Fig. 1). "The computation method can lead to advances, and then be applied to a new problem in another field to elucidate new physical phenomena. This has long been my strategy, and it appears to be becoming reality at RIKEN."

Hiyama describes the Infinitesimally-shifted Gaussian lobe method in further detail. "This method rigorously analyzes *n*-body problems such as the quantum mechanical three-body or four-body problem," she says, where quantum mechanics refers to the physics of components of the microscopic world, including atomic nuclei. An *n*-body problem deals with the interactions between *n* number of particles. "Decisions are easy to make when only two people are involved, but when there are three people, the problem becomes more complex because of their various viewpoints, and the problem becomes even more complicated when there are four people involved. Similarly, *n*-body problems become much more difficult as the number *n* increases, even though the two-body problem is comparatively easy," explains Hiyama.

In the two-body problem there are two possible cases: one with two tightly combined particles and another with two loosely combined particles. In the three-body problem, however, there are five possible cases: the first with three tightly combined particles; the second with three loosely combined particles; and the remaining three cases with two tightly combined particles plus a single loosely combined particle. In the four-body problem, the number of cases increases to nine including the first with four tightly combined particles, the second with four loosely combined particles, the third with two groups of two tightly combined particles, and the fourth with three tightly combined particles plus a single loosely combined particle. To solve these problems, all possibilities have to be considered using the Schrödinger

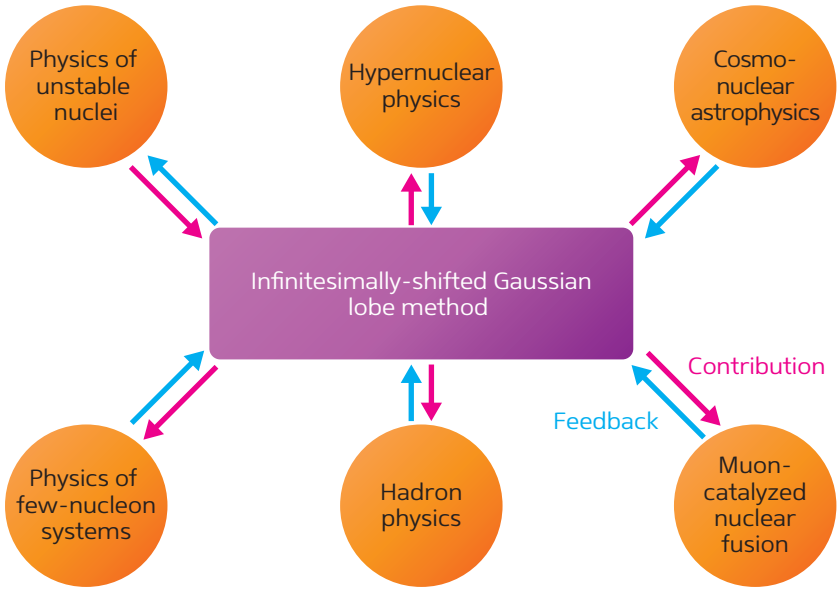

Figure 1: Research strategy of the Strangeness Nuclear Physics Laboratory

Focusing on the Infinitesimally-shifted Gaussian lobe method, the strategy aims to apply the method to many different fields of physics such as hypernuclear physics and cosmo-nuclear astrophysics, thus contributing to the understanding of these fields of physics. It also aims to use feedback for further development of the computation method. Modified from: Gaussian expansion method for few-body systems and its applications to atomic and nuclear physics. *Progress of Theoretical and Experimental Physics*, 01A204 (2012). Licensed under CC by 3.0 at dx.doi.org/10.1093/ptep/pts015. © 2012 E. Hiyama

equation, which describes the behavior of quantum mechanical particles. Specifically, a complex 'sixth-order partial differential equation' is required for the three-body problem, with a 'ninth-order partial differential equation' for the four-body problem.

"It is very difficult to solve *n*-body problems when *n* is three or larger, and this is one of the leading challenges in nuclear physics. Kyushu University, where I studied, has already developed a computation method for solving the three-body problem," says Hiyama.

In the early 1990s, while Hiyama was at Kyushu University, only three research institutes had succeeded in accurately solving the three-body problem: one in the US, one in Russia, and Kyushu University in Japan, which developed a computation method in 1988. These institutes came up with the same answer to the problem but differed in computing speed; while the American and Russian computational methods required 10 hours to calculate the answer, Kyushu University's Gaussian expansion-based method took just 3 minutes.

"As a student at Kyushu University, I attended a lecture on nuclear physics by Masayasu Kamimura, who developed the Gaussian expansion method. I was attracted to the idea that anyone who had mastered this computation method could soon perform world-leading research as the method is easy for anyone to use."

Developing the Infinitesimally-shifted Gaussian lobe method

Hiyama joined the nuclear theory laboratory when she was a fourth-year undergraduate student and then continued her studies in graduate school under Kamimura. "In my first year as a graduate student, I really focused on the three-body problem. However, I found it difficult to use the computation method because the calculation of spherical surface harmonics was very difficult."

Instead, Hiyama decided to seek a simpler computation method that did not involve spherical surface harmonics, which she soon found. "The Gaussian lobe method was developed in the 1960s, and it was introduced in a textbook as the easiest for undergraduate students to use. As I read the textbook, I thought I had found the answer until I realized that the method is good for graduate students as a problem-solving exercise but cannot be applied to the three-body problem. I was greatly disappointed. However, I persuaded myself that I could succeed in finding a way to solve the problem."

After many discussions with Kamimura, in 1996, Hiyama finally succeeded in developing the Infinitesimally-shifted Gaussian lobe method, based on the existing Gaussian lobe method. "The new method is not only easier to use than the Gaussian lobe method, but also allows us to solve the complex three-body and four-body problems which we could not solve with the conventional Gaussian lobe method."

Participating in an international computation test

In 2001, an international computation test for the four-body problem was held. At that time, only seven groups in the world, including Hiyama's group, had succeeded in solving the four-body problem. The test was planned in order to evaluate the reliability of their approaches because each group had their own unique computational method. The groups were required to calculate the energy and wave functions of the atomic nucleus of helium-4, which consists of two protons and two neutrons, and they simultaneously emailed their answers to the researcher in charge. A paper describing the results was then submitted to the *Physical Review* journal, meaning that each participating group only learned of the other groups' answers upon publication.

"This test was very stressful because various answers might have emerged, making it difficult to find the right answer," she confesses. "The worst-case scenario was that the other groups had the same answer [as each other], and it was far from ours. I read the paper with a pounding heart, but I found that the answers of the seven groups were almost the same, which proved that our computation method and approach was reliable."

Hiyama then went on to improve the computation method in order to solve the five-body problem. Currently, only five groups in the world, including Hiyama's, are able to solve this problem. The larger the number that *n* represents in the *n*-body problem, the fewer the number of groups that can solve the problem: three can tackle the six-body problem and only a single group can do the ten-body problem—currently the largest.

"I want to improve our computation method so as to solve the six-body problem within a year or two, and eventually I want to solve the eleven-body problem which nobody has ever solved," says Hiyama excitedly. "I have pride in the computation method that I have developed and improved. Our method has one advantage not found in other methods: it can deal with both the strong interactions between closely spaced particles, and the weak interactions between widely spaced particles, enabling us to precisely solve the interactions between particles regardless of their structure."

Contracting hypernuclei

"I am now focusing on the application to hypernuclear physics," says Hiyama. "A normal atomic nucleus consists of two types of particle: protons and neutrons. The hypernucleus is an atomic nucleus consisting of protons and neutrons plus 'hyperons' (Fig. 2), which were named in the sense that they go 'beyond' the atomic nucleus."

To understand hyperons, one must explore their structure. Protons and neutrons consist of three quarks, which can be classified into six types—including 'up', 'down' and 'strange'. A proton consists of two up quarks and one down quark, whereas a neutron consists of one up quark and two down quarks. A hyperon also consists of three quarks but includes at least one strange quark. Hyperons are classified into lambda (Λ), sigma (Σ), xi (Ξ) and omega (Ω) types (Fig. 2). They are not found on Earth because their strange quark is unstable and has a lifetime of less than a nanosecond. Therefore on Earth, hyperons can only be created by colliding high-energy particles in an accelerator.

Collectively, protons and neutrons are known as 'nucleons'. Interactions between nucleons are well understood, thanks to the meson theory proposed by Hideki Yukawa and elastic scattering experiments that examine changes in the motion of nucleons by bombarding target nucleons with other nucleons. However, hyperon–nucleon and hyperon–hyperon interactions are poorly understood. "More than 4,000 elastic scattering experiments on the interactions between nucleons have been reported, but only 40 hyperon–nucleon experiments and no hyperon–hyperon experiments at all. This is why we need to precisely solve the interactions between the particles in the hypernucleus. Then we can propose what experiments are needed."

Hiyama used the Infinitesimally-shifted Gaussian lobe method to precisely analyze the interactions between hyperons and predicted that the atomic nucleus of lithium-6 contracts by 20% when a Λ hyperon is added to the nucleus. Subsequently, another researcher performed experiments to create and examine a lithium-6 hypernucleus that contained a Λ hyperon, showing that the atomic nucleus contracts by 19%. Additionally, it provided a groundbreaking example of how theory-based prediction can lead to progress toward the understanding of hypernuclei through the proposal of experiments, and subsequent experimental verification.

In 2009, KEK, the High Energy Accelerator Research Organization, succeeded in creating a beryllium-11 atomic nucleus consisting of four protons and five neutrons—plus two Λ hyperons. This was only the eighth case of the creation of a 'double hypernucleus' in the world. In 2010, Hiyama was the first to succeed in precisely solving the interactions between these double hypernuclei.

Given that there are 11 subatomic particles in a hypernucleus (left, Fig. 3) and the Infinitesimally-shifted Gaussian lobe method cannot be applied beyond the five-body problem, Hiyama had to develop a new approach. "I simplified the hypernucleus consisting of 11 subatomic particles into one with 2 helium nuclei consisting of 2 protons and 2 neutrons, 1 neutron, and 2 Λ hyperons (right, Fig. 3)," she explains. "Of course, solving the hypernucleus as a system consisting of 11 subatomic particles is much better. However, in terms of accuracy, we knew that there is no significant difference in the results between the 5-body system and the 11-body system. I think it is important in theoretical research to

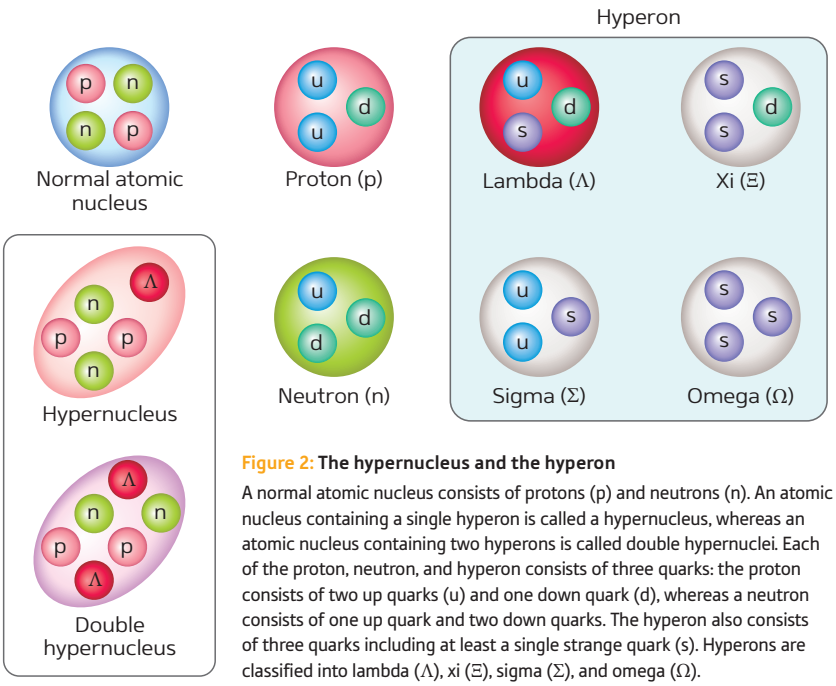

Figure 2: The hypernucleus and the hyperon

A normal atomic nucleus consists of protons (p) and neutrons (n). An atomic nucleus containing a single hyperon is called a hypernucleus, whereas an atomic nucleus containing two hyperons is called double hypernuclei. Each of the proton, neutron, and hyperon consists of three quarks: the proton consists of two up quarks (u) and one down quark (d), whereas a neutron consists of one up quark and two down quarks. The hyperon also consists of three quarks including at least a single strange quark (s). Hyperons are classified into lambda (Λ), xi (Ξ), sigma (Σ), and omega (Ω).

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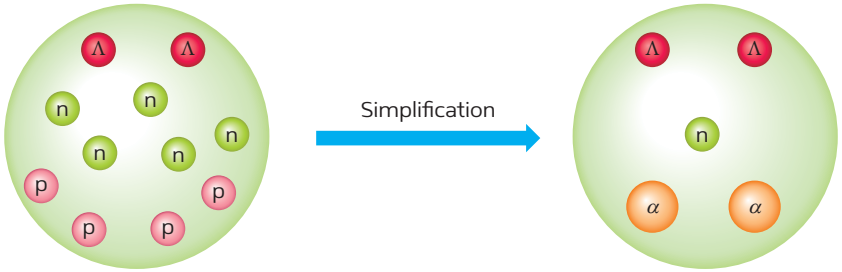

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Figure 3: Schematic representation of the nucleus of a beryllium atom containing two hyperons
The atomic nucleus consists of 11 subatomic particles: 4 protons (p), 5 neutrons (n), and 2 lambda (Λ) hyperons (left). The nucleus is a simplified 5-body system consisting of 2 helium atomic nuclei (α: 2 protons + 2 neutrons), 1 neutron, and 2 Λ hyperons (right).

identify to what degree we can simplify a complex system so that we can solve the system within a practical period of time."

The results showed that the beryllium atom's nucleus contracted by 8% when 2 hyperons were added. However, the experiment to confirm how much a double hypernucleus contracts has not yet been performed. In 2014, KEK will begin to create a large number of double hypernuclei in the Japan Proton Accelerator Research Complex (J-PARC), as part of an experiment to confirm the contraction by comparing theoretical and experimental results.

Hiyama is greatly looking forward to the J-PARC experiment: "No hypernuclei containing Ξ hyperons have yet been discovered. Searching for such hypernuclei is also an important research subject. I am a joint researcher in the J-PARC experiment and am planning to predict which Ξ hyperon-containing hypernuclei should be discovered and to guide the direction of the experiment."

Probing the interior of a neutron star with hypernuclei research

"Hypernuclear physics can lead to cosmo-nuclear astrophysics," says Hiyama, demonstrating that her research is also relevant to other areas of physics. At the end of its life, a fixed star with a mass of more than 10 times that of the Sun undergoes a supernova explosion, which removes most of its matter. Sometimes, a small but extremely heavy neutron star is left at the center. The neutron star measures just 10 kilometers in diameter yet its mass is 1.5 times that of the Sun. It is this extraordinarily large density that allows the hyperons it contains to exist in a stable state.

"What is the inner structure of the neutron star? That is the hottest topic in cosmo-nuclear astrophysics today," argues Hiyama. "Our calculations of the interactions between subatomic particles in a hypernucleus are expected to raise our understanding of the inner structure of the neutron star."

Combining theory with experiment

As a theoretician, Hiyama notes that there are perceived differences in the working practices of theoretical and experimental researchers. "Many people imagine that theoreticians stay in a laboratory, writing mathematical calculations all day," she suggests. "Actually, many theoreticians spend much of their time in discussions with other researchers and collaborators. This is because they need to confirm their lines of study through discussion, as they can easily deviate from the right direction if they work in isolation."

Fortuitously, collaborators go hand-in-hand with experts who specialize in experiments. "Theoreticians make predictions, whereas experimentalists confirm the predictions. It is wonderful when a prediction is confirmed by a single experiment," says Hiyama. "If not, there may be another physical phenomenon. It is also fascinating to probe the phenomenon. Thus, what we need is to repeatedly modify a formulated theory and confirm its predicted results by experiment. A theory cannot be developed without being confirmed by experiment, and conversely, experiments cannot be developed without formulating theories. Thus, both theory and experiment are essential to the development of physics."

"I want to solve an *n*-body problem that nobody has ever solved," says Hiyama, when discussing her research goals. She points out that to achieve the target, it is not just necessary to develop computation methods—the performance of computers must also be improved. "Unfortunately, even RIKEN's K computer, an ultrahigh-speed supercomputer, is insufficient for what I need. I am looking forward to future developments because I want to discover the largest number of the *n*-body problem that can be analyzed."

Looking forward, Hiyama is now fascinated by a new topic: cold atoms. "Recently, I figured out the structure of a four-body system consisting of four helium-4 atoms at an extremely low temperature. The system consists of three closely related atoms and a single, widely spaced atom. I started the research in 2011, and have already published three papers. I am planning to apply our calculation method to five-body systems, but the calculations are still at the preparatory stage. Nonetheless, I am already looking forward to revealing our future findings."

ABOUT THE RESEARCHER

Emiko Hiyama was born in Fukuoka, Japan, in 1971. She studied nuclear physics at Kyushu University, completing a bachelor's degree followed by a master's degree in 1995 and the award of her PhD in 1998. A move to the RIKEN Muon Science Laboratory as a special postdoctoral researcher followed. In 2000 she became an associate researcher at KEK, the High Energy Accelerator Research Organization, and in 2004 joined the Department of Physics at Nara Women's University as an associate professor. In 2008 she returned to RIKEN as an associate chief scientist. Her research focuses on the structure of nuclei with and without strangeness quarks from the view point of the few-body problem.

Supporting RIKEN's contribution to society

Yoshinobu Aota

Manager
Sendai Administrative Office

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How did you join RIKEN and what kind of support did RIKEN provide you with?

I was working in a different industry but became interested in joining RIKEN when I learned about its excellent working environment. As it was my first experience of working in an administrative position, I was very nervous at the beginning. However, I started to feel more comfortable thanks to my comprehensive training and the office's relaxing atmosphere. I was encouraged to undertake courses and external training and what I learned during this time is still relevant to my work today. RIKEN has a diverse employee training program that includes an e-learning system and courses developed by external experts.

Please tell us about your work at RIKEN.

My first role involved handling payment-related tasks such as calculating salaries and income and resident taxes. After four years in Wako, I was relocated to Sendai to engage in accounting, safety control and facility management. In my office in Sendai, there were fewer workers to handle a similar volume of tasks than at the other offices and it was a challenge to multitask. However, the variety of roles taught me about the different sides of RIKEN and I soon realized that multiple tasks were related to each other. In 2010, I was promoted to manager and I now mainly oversee budget management and community relations.

What has been the most memorable event for you during your time at RIKEN?

The RIKEN Sendai Facility was greatly affected by the Great East Japan Earthquake in 2011. In the darkness after the power cut, I worked hard to understand the damage sustained and to secure the budget so that our researchers could continue their work. In response to the tsunami, nuclear disaster and surrounding rumors, many non-Japanese employees returned to their home countries and some of those who were supposed to start working at RIKEN chose not to come to Japan. However, some foreign researchers who passionately wished to work at RIKEN did still join us at Sendai. I am very pleased that they continue to perform research in Japan.

What is the best thing about working at RIKEN?

RIKEN is featured in the media almost every day. This is a reflection of our hard work to solve various issues and the fulfillment of our responsibility to contribute to society. I find it rewarding to feel that I am supporting research that benefits our society. The working environment at RIKEN is also ideal because researchers and support staff work closely together to support each other.

What would you say to other people considering joining RIKEN?

RIKEN is a great place for someone who is willing to take on challenges since a framework to nurture such talents has been laid out. Support staff can utilize their abilities to handle diverse tasks, while researchers will find themselves in a stimulating environment and able to create new ideas through internal and external collaborations. In addition, a high level of flexibility allows us to seize opportunities to pursue our own work interests. If we maintain a sincere attitude to our work, our efforts will be rewarded.

CONTACT INFORMATION

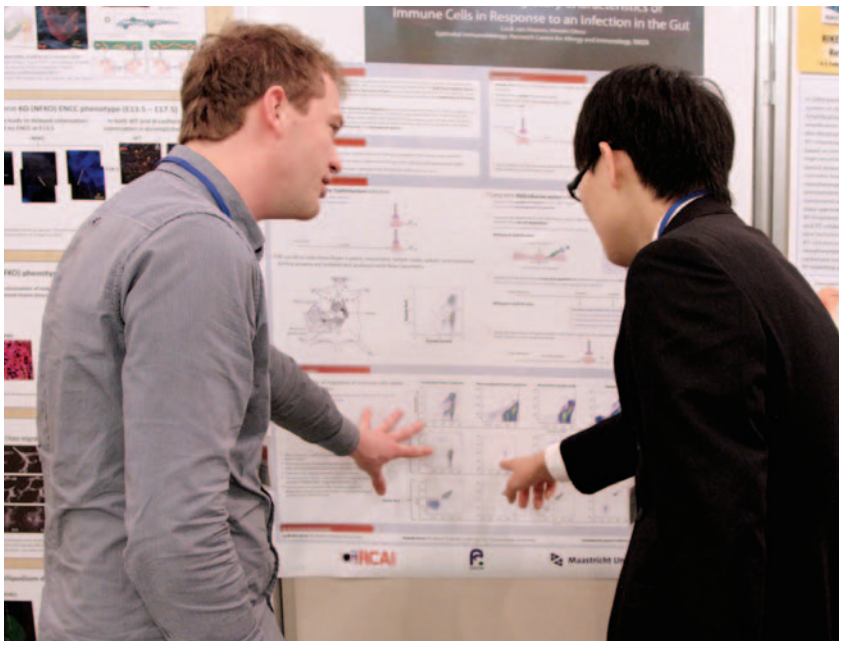
For details about working at RIKEN, please contact the RIKEN Global Relations and Research Coordination Office:
Tel: +81 48 462 1225
E-mail: pr@riken.jp

Biennial RIKEN Joint Retreat addresses the theme of behavior

The 5th Biennale RIKEN Joint Retreat was held on 4–5 February 2013 at the Yamaha Resort Tsumagoi in Shizuoka, situated roughly halfway between the Kanto and Kansai regions of Japan where RIKEN's largest campuses are located. More than 150 researchers working in a variety of biological fields across eleven RIKEN centers attended. The retreat was organized around the theme of "Behavior—from molecules, cells to organisms," and divided into sessions that focused on different parts of this puzzle.

A major goal of the retreat was to allow attendees to discover what others in related fields were working on within RIKEN. In the first session—centered on molecular behavior—three presenters discussed findings on the movement of molecules and how it contributes to cell behavior. During the second session, which looked at cellular behavior, the presenters examined issues that included epigenetic behavior and chromosome dynamics, and in the afternoon of the final day, two presenters discussed the behavior of organisms as a whole. A complementary session focused solely on the work of young researchers.

Interspersed with the scientific presentations were sessions intended to promote collaboration between labs at the different RIKEN centers—a second important goal of the retreat. To this effect, one of the first day's sessions reported on existing successful collaborations within RIKEN across the areas of epigenetics, superorganism metabolism and cellular interactions. In addition, a presentation was given on the Incentive Research Project, which provides grants for collaborative projects inside RIKEN. As a result of the discussion at the retreat, ideas were put forward for collaborations in the area of medical research, which will be a priority for RIKEN throughout the next five-year term. In between the sessions, participants were given the opportunity to view posters to gain a broad overview of each other's work. ■



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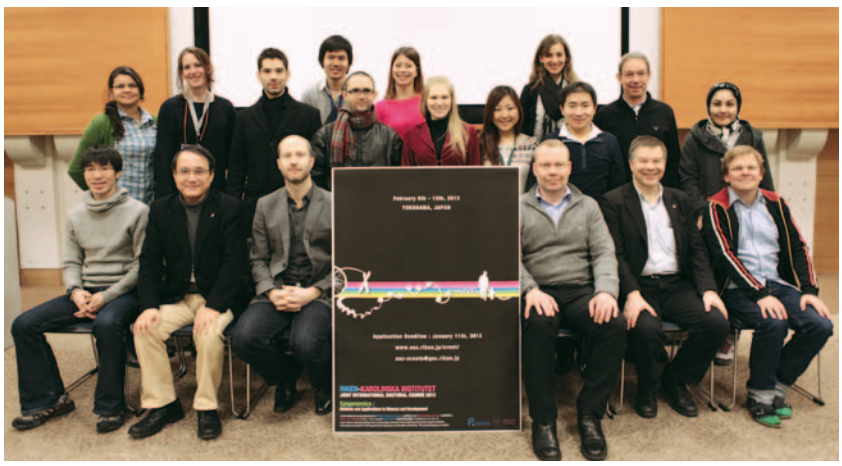
The retreat's poster sessions and presentations allowed attendees to learn about RIKEN research and seek future collaborations.

RIKEN hosts joint doctoral course on epigenomics

The RIKEN Omics Science Center* hosted a joint international doctoral course with the Karolinska Institutet of Sweden on the topic of "Epigenomics: Methods and applications to disease and development", in Yokohama on 6–13 February 2013. The course, held annually since 2010, provides doctoral students with an opportunity to learn about molecular biology from cutting-edge researchers and strengthen connections between Sweden and Japan.

Thirteen doctoral students of a variety of nationalities and currently studying biological disciplines at graduate schools in either country participated in the course, which consisted of lectures, discussions, group work and an examination.

Throughout the week, the students contributed to lively discussions on epigenetic themes including histone modification, DNA methylation, non-coding RNA function and analysis technologies with lecturers—all leaders in their field—from the Karolinska Institutet, the University of Tokyo and RIKEN. At the end of the course, each student was presented with a certificate of completion from the Karolinska Institutet. ■



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Doctoral students from Sweden and Japan took part in the eight-day course on epigenomics.

* Incorporated into the RIKEN Center for Life Science Technologies in April 2013

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