Drug discovery
Prescribing the next generation of technologies to boost the biomedical sciences
Pulsed laser deposition system in Wako
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Drug discovery

Innovation in drug discovery technology

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Technology has been a major driver of progress in drug discovery. However, further improvements are needed to bring about new advances in the medical and pharmaceutical sciences. Tools and technologies derived from synthetic biology, genomics, functional imaging and other disciplines promise a more dynamic and integrated approach to biomedical research and will make a new era of drug discovery possible.

In the early days, natural products derived from plants and microorganisms served as the predominant source of bioactive compounds and drug candidates. Advances in chemistry made it possible to synthesize large families of compounds with a high degree of structural diversity that could be screened to identify molecules with potential biological activity. With the dawn of the biotechnology industry and human genome sequencing, chemistry has given way, in part, to biology. By using molecular biology, genomics and computation, researchers can now design drugs with desired properties and create medicines by manipulating basic biological building blocks, such as proteins and nucleic acids.

Still, productivity in the drug industry has languished in recent years as the classic approaches have begun to reach the limits of their effectiveness. New and more powerful technologies are critical to tackling the persisting fundamental questions in the life sciences.

RIKEN is committed to the realization of innovation for science and improved human health, leading its efforts with an integrated, non-traditional approach. The recently established RIKEN Center for Life Science Technologies brings together systems and structural biologists, omics scientists, molecular imaging specialists and engineers to collaborate in the pursuit of technological foundations for all areas of medical and pharmaceutical science.

A new therapeutic paradigm
To date, most drugs have been designed to inhibit or activate particular molecules with a very narrow specificity. These conventionally designed drugs usually follow the ‘one drug—one
target–one disease’ approach, which rarely captures the intricacies of true biology. Complex diseases have multiple ‘causes’ and drug discovery for such diseases requires a multi-target line of attack.

A new therapeutic paradigm is needed to effectively combat complex diseases—one that is based on ‘live science’. Live science is a methodology that develops and harnesses technology to go beyond static snapshots to clarify the dynamic processes that must be understood in order to achieve higher sensitivity and specificity in drug discovery (Fig. 1).

Among other advancements, live science methodologies could help to realize medical ‘regulatory molecules’. These molecules are drugs that rather than inhibiting or activating a single target would modulate an entire pathway implicated in a disease to yield the desired therapeutic benefits. Constructed with insights from structural and synthetic biology, regulatory molecules would be made using the same building blocks of conventional drugs. Ultimately, they would be cheap to manufacture, easy to deliver and have wide-reaching effects across several targets of interest.

The development of new technologies will be necessary to make efficient and economical regulatory molecules a reality. For instance, current projects at RIKEN and elsewhere are combining engineering principles with molecular biology to build networks of molecules that can be tweaked and perturbed to better understand whole systems.

**Integration and innovation**

Genomics also holds great promise for drug discovery in the near future. However, the insights gleaned from genome sequencing projects must also be connected to gene function. At RIKEN, teams are developing methods to decode the genomes of single cells to reveal biological heterogeneity at greater resolution in cancer cells, reprogrammed stem cells, neurons and other types of tissue.

RNA molecules in the cell that do not code for proteins—known as non-coding RNA—are another area to be explored. Also referred to as genomic ‘dark matter’ and only partially understood, non-coding RNA performs a regulatory role in many biological events and could explain the origin of complex disease traits such as obesity and heart disease. Efforts by large consortia, such as FANTOM (Functional Annotation of the Mammalian Genome) spearheaded by RIKEN, will continue to be instrumental in illuminating the non-coding RNA world and the roles it may play in complex diseases.

Improved drug discovery will also require advanced imaging modalities to better observe emergent symptoms and drug metabolism in real time at unprecedented resolution. Such modalities can be achieved both through novel innovations and by upgrading and combining existing approaches, such as positron emission tomography (PET), magnetic resonance imaging (MRI), optical imaging and electron microscopy. Work at RIKEN has created new labeling methods to visualize drug candidate molecules and track their efficacy, pharmacokinetics and pharmacodynamics in animal models and human study subjects including, for example, ways to follow drug absorption in the intestine and trace its subsequent distribution in tissue and ultimate excretion through the liver and related organs.

**The future of drug discovery**

Older models of research and development must be changed and refined, not only to enhance the process of drug discovery but also to reduce costs. Bringing a new drug to market from scratch typically takes 10 to 15 years and costs more than 100 billion yen. A fusion of techniques and the technology to support these new methods will drive the drug discovery field forward, thus reducing direct costs and increasing the overall efficiency of therapies.

Integrating disparate disciplines and emergent technologies will be challenging but can be done. For decades, the biomedical sciences have been held back by technical limitations, but now the time is ripe to develop live science and move beyond existing partial and static approaches toward whole-system and dynamic ones. Live science will enable researchers to answer some of the most interesting questions in the biomedical sciences today.

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Every naturally occurring heavy element on Earth has its origins in the stars of the Universe. Through a process known as nucleosynthesis, nuclear collisions inside massive celestial bodies fuse together lightweight atoms such as hydrogen and helium to produce heavier and heavier elements, such as carbon and silicon. The natural abundance of elements and their isotopic variants—atoms with an unbalanced number of protons and neutrons—is determined by the stability of the nucleus, which governs whether an astrophysical isotope will be long-lived or susceptible to rapid radioactive decay.

One way that physicists gauge the stability of atomic nuclei is with the concept of ‘magic numbers’. Similar to the way that electrons travel in tightly defined ‘shells’ around a central nucleus, protons and neutrons also occupy distinct orbits inside the nucleus. When the number of protons or neutrons completely fills a particular shell, corresponding to 2, 8, 20, 28, 50, 82 or 126 particles (the magic numbers), the isotope gains exceptional stability. Such nuclei take on a spherical shape and are difficult to excite due to a significant energy gap that must be overcome before the nucleus can accept new protons and neutrons.

Now, a research team led by David Steppenbeck from the University of Tokyo and Hiroyoshi Sakurai and Satoshi Takeuchi from the RIKEN Nishina Center for Accelerator-Based Science has uncovered evidence of a new ‘magic number’ of nuclear components in an exotic calcium isotope with 34 neutrons (\(^{54}\text{Ca}\)). The finding is significant because the isotope displays surprising stability despite its seemingly volatile nuclear structure.

**Exotic evolution**

When the theory of magic numbers was developed over a half-century ago, researchers believed that nuclear shells were robust and constant. These notions began to change, however, with studies into short-lived exotic nuclei that have quite different properties from those of natural isotopes, which have balanced numbers of protons and neutrons. In exotic nuclei with significantly more neutrons than protons, the excess neutrons begin to fill normally empty orbital shells, producing extra forces not seen in stable isotopes. “These attractive and repulsive forces cause the orbitals to shuffle about,” explains Steppenbeck. “In some cases, the large energy gaps associated with the magic numbers of standard stable nuclei can disappear and new energy gaps—the onset of new magic numbers—can materialize.”

In order to better understand how changes in shell structure—or ‘shell evolution’—occur, researchers are increasingly turning to calcium isotopes. This element has a magic number of 20 protons, and evidence has emerged that an exotic calcium isotope with 32 neutrons (\(^{52}\text{Ca}\)) has a substantial energy gap and therefore forms a relatively inert nuclear core. Theoretical calculations have also suggested that \(^{48}\text{Ca}\), which has 20 protons and...
34 neutrons, should show magic-number stability, while other studies have cast doubt on this proposition.

“Prior to our experiment, we simply did not know whether the magic number of 34 neutrons would exist in calcium or not,” says Steppenbeck. “There are a great number of theories that predict different characteristics of the $^{54}$Ca excited states. Some predict a shell closure with 34 neutrons that is just as large as the traditional one seen with 28 neutrons, while others predict no shell closure at all. Clarifying which theories are correct was a major motivation behind our work.”

**Going for a knockout**

Resolving the uncertainty surrounding $^{54}$Ca isotopes is inherently challenging because the substance is extremely difficult to produce and study in the laboratory. Fortunately, the team had access to a facility with far from ordinary capabilities: the Radioactive Isotope Beam Factory (RIBF), jointly operated by RIKEN and the University of Tokyo. The superconducting ring cyclotron (Fig. 1) and powerful electromagnets at the RIBF allow researchers to generate beams of specific heavy isotopes traveling at around 60 percent of the speed of light. “No other facility in the world can currently match the high-intensity radioactive ion beams available at the RIBF,” notes Steppenbeck.

In their experiment, the team bombarded a beryllium plate with the isotopes scandium-55 ($^{55}$Sc) and titanium-56 ($^{56}$Ti), both of which have 34 neutrons but do not display magic-number stability. Occasionally, these collisions can knock out a single proton from $^{55}$Sc, or two protons from $^{56}$Ti, to form the elusive $^{54}$Ca isotope. By applying careful separation and purification techniques, the researchers produced a beam of $^{54}$Ca and proceeded to study its characteristics in detail.

To determine if 34 was indeed a magic number, Steppenbeck and his colleagues excited $^{54}$Ca to its higher-energy excited state and watched it decay back to its original state. During the decay process, the isotope emitted gamma rays that the team tracked with a high-efficiency detector array (Fig. 2). The gamma radiation signatures revealed a large energy gap between the ground state of $^{54}$Ca and its next shell level—clear evidence of a new magic number. Furthermore, the researchers note that $^{54}$Ca is ‘doubly magic’ because both of its proton and neutron shells are filled.

**A three-body solution**

This discovery promises to give physicists important insights into predictions of unstable nuclei. Investigating how calcium isotopes contribute to element-forming reactions, however, requires theoretical calculations that are currently at the frontier of computational capabilities. Steppenbeck notes that a key finding of the study is that complex ‘three-body’ forces between triplets of subatomic particles should be incorporated into the toolkits of theoreticians to enable experimental work to be reproduced with greater accuracy. “Understanding the properties of neutron-rich nuclei and the forces between protons and neutrons could lead to a better understanding of astrophysical processes.”


**Figure 2:** Distinct energy signatures uncovered by the Detector Array for Low Intensity Radiation 2 (DALI2) (pictured) at the RIBF show the presence of a new ‘magic number’ of neutrons for calcium isotopes.
Maintaining cerebellar circuits for motor skills

New insights into the control of neuronal circuitry could lead to treatments for an inherited motor disorder

The cerebellum is a region of the brain critical for balance, learning of motor skills and coordination of movements. In the outer layer of the cerebellum, individual Purkinje cells integrate inputs from the brain stem and hundreds of thousands of granule cells to produce the cerebellar ‘output’. Maintenance of the connections between Purkinje cells and associated parallel fibers is critical for proper cerebellar function, but very little is known about the underlying molecular mechanisms.

A team of researchers led by Katsuhiko Mikoshiba from the RIKEN Brain Science Institute in Wako has now identified a signaling molecule responsible for maintaining the integrity of these neuronal circuits in the mature cerebellum.

The type 1 inositol trisphosphate receptor (IP₃R1) is known to be expressed at high levels in Purkinje cells. Mutations in the IP₃R1 gene lead to uncoordinated movements, abnormal Purkinje cell structure and impaired signaling between Purkinje cells and parallel fibers in mice, and cause a human disease called spinocerebellar ataxia 15 (SCA15). Mikoshiba and his colleagues investigated the role of IP₃R1 in the mature cerebellum by genetically engineering mice specifically lacking the receptor in their Purkinje cells.

The researchers found that the mutant mice displayed impaired motor-skill learning and severely uncoordinated movements, or ataxia, as seen in patients with SCA15. Closer examination of the cerebellum under the microscope also revealed abnormalities in the mice’s Purkinje cells. While appearing to develop normally, in the adult animals these cells showed a dramatic increase in the density and length of their dendritic spines—the tiny finger-like protuberances that form connections with other cells (Fig. 1). All of the spines, however, formed fully functional connections with parallel fibers in the adult animals.

Previously, Mikoshiba’s group showed that IP₃R1 plays a critical role in a process called synaptic plasticity, by which connections between neurons are strengthened or weakened during learning. These new findings show that the receptor is also required for maintaining the proper spatial arrangement of connections in the adult cerebellum.

“Mice lacking IP₃R1 specifically in Purkinje cells display ataxia similar to SCA15 patients,” says Mikoshiba. He notes that since the abnormal maintenance of Purkinje-cell dendritic spines appears to be associated with severe ataxia in the mutant mice, defects in the maintenance of the cerebellar circuit might similarly be involved in SCA15 pathogenesis.

“We are now studying the precise mechanism of how IP₃R1 regulates Purkinje-cell spine maintenance. This may elucidate SCA15 pathogenesis and lead to the development of new therapies,” adds Mikoshiba.

Figure 1: Mouse Purkinje cells lacking IP₃R1 display a higher density and longer length of dendritic spines (right) compared with normal mice (left).

Magnets made from coils of superconducting wire can maintain current flows for years without losing energy. Such superconducting magnet systems provide significant advantages for technologies such as medical scanners, spectrometers, particle accelerators and high-speed trains. However, most superconducting materials only achieve their ideal conducting properties at temperatures far below zero.

A research team led by Hideaki Maeda from the RIKEN Center for Life Science Technologies has now developed an ultrathin insulation layer that enhances the efficiency of magnetic coils based on the promising high-temperature superconducting compound REBCO. Wire coils made out of REBCO, a rare-earth barium copper oxide, are capable of withstanding the intense stresses that occur when the magnet charges, allowing them to produce stronger magnetic fields than traditional superconducting wires. However, REBCO does not yet perform efficiently because it requires a thick electrical insulation layer, which lowers the maximum current density that can be produced by the conducting coil.

“The REBCO wires themselves are very thin—between 100 and 150 micrometers—but conventional layers of electrical insulation are about the same thickness. This reduces the effective amount of superconducting wire in every coil,” explains Maeda. “To achieve a higher current density in smaller coils, the insulation layer needs to be much thinner.”

Maeda and his team used a technique called electrodeposition to coat the REBCO wires with a uniformly thin layer of polyimide insulation. The process involves immersing wire in a bath of polyimide colloidal solution and establishing an electric field between the wire and an electrode in the bath. The electric field causes the polyimide particles to migrate to the wire and coat the superconductor’s surface.

Baking the wire in a furnace for a few minutes to harden the polyimide coating produced a conductor that was coated uniformly with an ultrathin layer of insulation ten times thinner than any previous insulation layer (Fig. 1). “With this new REBCO superconducting wire, we can achieve a current density that is double that of a conventionally insulated REBCO coil. We can also make the coils five times smaller than before,” explains Maeda. “As a result, the conductor can generate higher magnetic fields with smaller devices, opening up new potential applications.”

The researchers believe that their new REBCO coils could make it possible to produce a nuclear magnetic resonance spectrometer capable of generating magnetic fields stronger than 23.5 teslas—the magnetic field limit for conventional superconducting wires.

Mood and emotion are extremely complex aspects of behavior that are known to involve the neurotransmitters serotonin and norepinephrine. Both neurotransmitters are broken down by an enzyme called monoamine oxidase A (MAO-A), and drugs that interfere with this system, such as the anti-depressant fluoxetine (Prozac), have long been used to treat mood disorders. Jun Aruga and colleagues from the RIKEN Brain Science Institute in Wako have now shown that a ligase enzyme called Rines regulates MAO-A activity and could prove to be a promising therapeutic target for the treatment of such disorders.

The metabolism of MAO-A, like that of many other proteins in the cell, is regulated by what is known as the ubiquitin–proteasome pathway, which tags old, misfolded or otherwise unwanted molecules and dispatches them to a cellular trash can. Aruga and his colleagues hypothesized that this pathway would be critical for regulating MAO-A levels in the brain.

To investigate the role of the pathway, the researchers used genetic engineering to create mice lacking the gene encoding the Rines E3 ubiquitin ligase—an enzyme that determines which proteins will be tagged for destruction.

Deletion of the Rines gene had a dramatic effect on the emotional behavior of the mice. For example, mice lacking the gene were far more reluctant to explore a new environment and spent less time in open spaces compared to healthy mice, indicating that the mutant mice were more anxious and had an abnormal stress response.

The researchers then examined the brains of these mice and found that the altered emotional behaviors were associated with significantly reduced levels of serotonin and norepinephrine in the locus ceruleus, prefrontal cortex and amygdala—regions of the brain that regulate emotion and stress responses. This was accompanied by enhanced activity of MAO-A in the locus ceruleus, the main source of norepinephrine in the brain. The researchers also found that some of the abnormal emotional behaviors were abolished by MAO inhibitors.

“The next step is to clarify the change in emotional response abnormalities using the animals’ personal history,” says Aruga. “Studies in humans indicate that the prevalence of aggressive and antisocial behavior in adults with the low-level MAO-A variant is affected by their history of stress during childhood, so personal history and gene–environment interaction studies with the mutant mice would contribute to a more comprehensive understanding of the pathophysiology of aggression and antisocial behavior.”

Quantum dots make efficient decisions
A theoretical system triumphs over the best model of human decision-making in a slot machine contest

Even the simplest forms of life face an endless barrage of decisions—where to search for sustenance, for example, or how to avoid predators. Various mathematical models can mimic these decision-making processes, coming to the same conclusions that a living organism might reach. One of these models, known as the softmax rule, offers the closest approximation of a human trying to maximize their winnings from a bank of slot machines.

Masahiko Hara from the RIKEN Global Research Cluster, in collaboration with Song-Ju Kim from Japan’s National Institute for Materials Science and other researchers, has now developed a theoretical model based on quantum dots that outperforms the softmax rule at slot machine selection.

Quantum dots are tiny fragments of matter just nanometers in size. The model developed by the research team simulates the selection between two slot machines by using five of these dots arranged in a line—a small dot in the middle and a pair of medium and large dots on each side representing each of the two slot machines. Each machine has a different probability of hitting the jackpot.

The system chooses which slot machine to play by beaming a ‘control light’ at the large quantum dot on either the left or the right. The slot machine is then ‘played’ by shining a second light at the small dot in the center. This triggers a quantum excitation that is shared with the medium quantum dot in the chosen machine. The medium dot emits this energy as light, signalling which machine to play next.

After each play, the control light moves slightly toward the winning machine. By adjusting the intensity of the light in this way, the system soon settles on an optimal balance between the two machines. If one machine were to be four times more likely to ‘pay out’ than the other, the system could win more than 98 per cent of its games within 200 tries. If the machines’ payout probabilities change, the quantum-dot system also adapts and does so more quickly than the softmax rule. The system therefore represents a nanoscale device that can make decisions efficiently and adaptively by exploiting the intrinsic optical properties of quantum dots.

The model’s optical energy transfer system has already been used in quantum-dot systems, says Hara, and the team is now trying to build a working version of their decision maker. Hara notes that such systems might offer an extremely efficient way to make decisions in trial-and-error tasks.

Dravet syndrome is a rare and severe form of epilepsy caused primarily by inherited loss-of-function mutations in a gene called SCN1A. This gene encodes a sodium ion channel known as Nav1.1 and is required for the proper function of brain cells. However, exactly which neurons go awry in the brains of Dravet syndrome sufferers remains poorly understood.

Working with mouse models of the disease, a team of researchers led by Kazuhiro Yamakawa from the Laboratory for Neurogenetics at the RIKEN Brain Science Institute has now demonstrated that the loss of functional Nav1.1 in inhibitory neurons leads to seizures and other symptoms of Dravet syndrome, while similar Nav1.1 impairment in excitatory neurons can have a beneficial effect.

Yamakawa and his colleagues engineered a series of transgenic mouse lines with the aim of selectively disrupting the SCN1A gene in one of three neuronal subtypes at a time—forebrain excitatory neurons, global inhibitory neurons or a subpopulation of inhibitory neurons called parvalbumin cells. For each transgenic mouse line, the researchers determined the severity of epileptic symptoms and the levels of Nav1.1 expression in various brain regions involved in Dravet syndrome.

The effects of Nav1.1 deletion in inhibitory neurons were profound. The researchers found that mice lacking the Nav1.1 channel in just the global inhibitory neurons had more severe epileptic seizures than mice in which the channel was missing in all three neuronal cell types. Mice lacking the Nav1.1 channel in just the global inhibitory neurons also died prematurely. By examining the degree of Nav1.1 loss needed to elicit disease symptoms, Yamakawa’s team showed that reducing Nav1.1 levels by even a small amount in parvalbumin cells proved sufficient to bring about spontaneous seizures and loss of motor function.

Unexpectedly, many of these symptoms were ameliorated by additionally eliminating the Nav1.1 channel in excitatory neurons. In this way, the researchers revealed that mice lacking Nav1.1 in both inhibitory and excitatory neurons had a much lower death rate than mice lacking the sodium channel in inhibitory neurons alone. “We have shown for the first time the protective effect of deleting Nav1.1 in excitatory neurons on the pathology of Dravet syndrome,” says Yamakawa.

The findings could have important implications for the development of future treatments for this currently incurable disease. “Our results highlight the importance of targeting specific neuronal populations when considering potential therapeutic approaches,” notes Yamakawa.

Quantum mechanics provides such a different description of the world compared to classical physics that even Albert Einstein had problems comprehending the implications of the theory. However, sometimes the predictions attributed to quantum-mechanical effects alone actually conform to the framework and predictions of classical physics. Franco Nori, Konstantin Bliokh and colleagues from the RIKEN Center for Emergent Matter Science have now derived a classical theory explanation for a light beam experiment previously explained only through complex quantum-mechanical arguments.

One of the fundamental principles of quantum mechanics is that certain properties of a quantum-mechanical object, such as a photon or electron, cannot be measured simultaneously with precision. The position of these particles, for example, cannot be determined at the same time as its momentum: measuring one property causes a certain ‘fuzziness’ in the determination of the other.

A few years ago, an experiment in which both the path of photons and their interference patterns were measured simultaneously drew considerable attention. “This was because the experiment seemingly overcame the fundamental restrictions of quantum mechanics. Simultaneous measurements of the path information and interference picture are impossible in standard quantum theory, like the simultaneous determination of the coordinates and momentum of a particle.”

The results of the two-slit interference experiment—as it was known—were brought into line with quantum mechanics by arranging the measurements such that the results were averaged over several experiments conducted using a number of photons. This means that the precise position of a single photon was not actually measured. Instead, its properties were retrospectively deduced by making many measurements on identical particles.

Explaining these experiments required complicated quantum theory arguments. Nori and his colleagues have now presented an alternative viewpoint. “We give a classical-optics interpretation of this experiment and other related problems,” says Bliokh.

Key to the researchers’ classical interpretation is a description of the experiment based on the momentum density of light (Fig. 1). Because many photons are averaged, the results can be regarded in the context of the way light waves would be treated in classical theory.

This approach, according to the researchers, can also explain how a number of other effects seen in the complex propagation of classical light similarly provide measurements of photon trajectories. Even though quantum physics can sometimes be very unintuitive, it is often surprising how many of these effects can also be explained by classical theory.


Figure 1: Reconstruction of photon trajectories (left) from the measured transverse momentum of light (right) in a vortex beam.

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Engineered light bending on a larger scale

A ‘nanoimprinting’ technique makes it possible to fabricate visible-light-bending metamaterials at unprecedented scales.

Artificial materials containing arrays of metal nanostructures can interact with light in useful and interesting ways. One of the most interesting possible uses of such ‘metamaterials’ is to bend light around objects, rendering them invisible. However, metamaterials usually only interact with light over a very narrow range of wavelengths—typically long wavelengths far beyond those visible to the human eye.

Takuo Tanaka from the RIKEN Metamaterials Laboratory, in collaboration with Shoichi Kubo and colleagues at Tohoku University, has now demonstrated a scalable fabrication method that greatly eases the production of metamaterials that can interact with light at visible wavelengths.

Tanaka and his team created a silica-based metamaterial containing an array of split-ring resonators—thin gold rings with two small breaks at the top and bottom (Fig. 1). A similar design previously proved successful when used at longer wavelengths in the microwave region. In principle, modifying the structure to function at visible wavelengths only requires the resonators to be made smaller to match the shorter wavelengths of visible light. However, the features required for such visible-light metamaterials are below 100 nanometers in size. Metamaterials are commonly fabricated by electron-beam lithography, which involves using a beam of electrons to draw out each resonator one at a time. This process is painstakingly slow, particularly for the production of the millions of small features needed to create a visible-light metamaterial of a practical size.

Instead, the researchers fabricated their structures through a process known as nanoimprint lithography. This technique involves transferring a master mold of the desired design onto a thin polymer film and then using standard metallization techniques to recreate the pattern in gold. In this way, the team was able to create split rings approximately 212 nanometers across and 54 nanometers high.

Tanaka and his colleagues demonstrated that their metamaterial magnetically interacts with red light. More important, however, is the scalability of their fabrication technique. Whereas techniques such as electron-beam lithography are limited to producing arrays of just several hundred square micrometers in area, Tanaka and his co-workers managed to create an array of 360 million split-ring resonators across a 5-millimeter square using their nanoimprint technique. “This is, to the best of our knowledge, the world’s largest two-dimensional split-ring resonator array metamaterial for visible light,” explains Tanaka. “Our next step will be to create much larger metamaterials, to make them three dimensional, and to reduce the operation wavelength.”

Information about odorant molecules in the environment helps animals to find food, select mates and avoid predators. Yoshihiro Yoshihara and colleagues from the RIKEN Brain Science Institute have now identified a protein called Goofy within sensory neurons in the noses of mice that helps to sharpen their sense of smell.

Goofy is expressed in the olfactory epithelium (Fig. 1), the inner surface of the nose where odors are initially detected by receptors on olfactory sensory neurons. Yoshihara and his colleagues homed in on this particular protein because it contains a motif called a signal peptide, which is also found on many other transmembrane and secreted proteins known to have important functions in regulating the sense of smell.

In mice genetically engineered to lack Goofy, the researchers observed that the neurons in the olfactory system maintained their normal connections with each other, suggesting that Goofy does not play a role in controlling the formation of the olfactory neural circuitry. However, they found that adenylyl cyclase III—a key enzyme involved in olfactory signaling in olfactory sensory neurons—was mislocalized in Goofy-deficient mice. This indicates that Goofy is required for correct trafficking of this protein. “It is likely that the proper localization of these molecules is crucial for the normal functioning of sensory systems,” says Yoshihara.

Olfactory neurons contain long membranous extensions called cilia that stretch into mucus in the nose to detect odorant chemicals. Goofy-deficient mice had shorter olfactory cilia compared to normal mice, suggesting that Goofy regulates the development of cilia in olfactory neurons. Additionally, the researchers found that olfactory epithelium cells from Goofy-deficient mice produced weaker electrical responses than normal cells when exposed to various odorants. Goofy-deficient mice also exhibited less fear behavior compared to normal mice when exposed to an odor from foxes—one of their natural predators. According to the researchers, these findings show that Goofy is required for mice to maintain their keen sensitivity to odorant information in their environment.

“A fully functioning olfactory system is important for our health and increases our quality of life,” Yoshihara says. “Because the human genome also contains the Goofy gene, which is most likely expressed in our nose, the present findings may have important implications for human disorders of olfactory perception, including anosmia—the loss of the sense of smell,” he explains.


Figure 1: The Goofy protein (red) is expressed in many olfactory sensory neurons (green).
Organic semiconductors made from small aromatic molecules can be dissolved and screen-printed onto many substrates, including plastics, opening the path for 'flexible' electronic devices such as low-cost polymer solar cells. Kazuo Takimiya and colleagues from the Emergent Molecular Function Research Group at the RIKEN Center for Emergent Matter Science, in collaboration with researchers from Hiroshima University, have now developed a synthetic procedure that makes it easier to tailor the chemical structure of an important organic semiconductor.

Takimiya and his team were studying molecules known as diacene-fused thienothiophenes when they discovered their new synthetic procedure. Diacene-fused thienothiophenes are composed of interlocking benzene and sulfur-containing aromatic rings and are more durable, and have higher charge carrier mobilities, than most other organic semiconductors. Although current schemes to make these compounds are relatively straightforward, they are also difficult to modify. Thus, chemists have a hard time producing derivatives based on this ring system with more desirable properties.

The researchers devised a creative synthesis that, instead of relying on bulky aromatic precursors, generates diacene-fused thienothiophenes from small molecules through two consecutive ring-forming reactions. First, they generated an active reagent called phenylsulfenyl chloride that joins to a benzene–acetylene molecule and transfroms it into a three-ring system. Then, they used selective carbon–hydrogen bond activation to set off a rare intramolecular coupling that produces a molecule with four fused rings known as benzothieno-benzothiophene (BTBT). Takimiya explains that this approach produces excellent yields and makes it possible to scrutinize numerous BTBT derivatives by making simple changes to the starting reagents.

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Trials revealed that this technique was particularly useful for extending the ring structure of BTBT-type molecules (Fig. 1). For example, by substituting double- and triple-fused benzene molecules into the synthetic procedure, the team linearly constructed the BTBT substructure to form five, six and seven aromatic rings. Intriguingly, these new derivatives have an asymmetric structure that may dramatically improve their solubility—an important processing feature for printed electronics and one that is difficult to achieve using existing synthetic techniques.

Lengthening the BTBT framework to an eight-ringed symmetric structure also yielded a potent new organic semiconductor with excellent thermal stability and a charge carrier mobility five times higher than that of BTBT. “This mobility is among the highest recorded for thin film organic field-effect transistors, meaning that this molecule could be a candidate for real flexible electronics applications in the future,” says Takimiya.

Figure 1: A synthetic procedure that selectively elongates the aromatic ring structures of organic semiconductors may prove useful for the development of flexible electronics.

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Figure 1: A synthetic procedure that selectively elongates the aromatic ring structures of organic semiconductors may prove useful for the development of flexible electronics.

Intestinal bacteria show ‘community spirit’

The collaborative effects of multiple bacterial strains in the gut may help prevent the onset of certain inflammatory diseases.

At first, it may sound alarming to learn that a population of bacteria in your gut is conspiring to suppress your immune system—however, this is actually good news. By identifying the strains responsible, a research team led by Kenya Honda of the RIKEN Center for Integrative Medical Sciences may have uncovered a promising avenue of treatment for certain inflammatory disorders.

Immune cells known as regulatory T (T<sub>reg</sub>) cells are in part responsible for preventing the immune system from overreacting to foreign molecules or attacking healthy tissue. It is well established that immune function is affected by the diverse microbial community within the digestive tract, and Honda’s team previously discovered that bacteria belonging to the genus Clostridium act on this particular immune pathway in mice to exert a strong anti-inflammatory effect.

“We showed that they were responsible for triggering production of T<sub>reg</sub> cells in the colon of mice,” says Honda, “and that oral administration of these strains protected mice against colitis and systemic allergic responses.”

Honda and his colleagues have now verified the existence of an equivalent bacterial population in humans. They obtained a stool sample from a healthy volunteer and subjected it to the same purification regimen that yielded the Clostridia subpopulation identified in mice. When these bacteria were transplanted into the colons of ‘germ-free’ mice, in which the normal population of gut bacteria is entirely absent, they exerted a potent immunomodulatory effect. Through systematic analysis of this microbial cohort, the researchers zoomed in on a specific subset of 17 distinct Clostridia strains.

These strains collectively secrete a host of signaling molecules that promote T<sub>reg</sub> cell activation. “None of the organisms alone were nearly as potent as when they were in consortium,” says Honda. “This suggests that cooperation between the strains is essential to their therapeutic effects.” The collective benefit also appears to pertain to humans; analysis of the gut ‘microbiome’ in healthy patients versus individuals with ulcerative colitis revealed that all 17 strains were present at significantly lower levels in the latter group.

Accordingly, oral administration of this 17-microbe ‘cocktail’ greatly mitigated intestinal inflammation in mouse models of allergic diarrhea and ulcerative colitis (Fig. 1), suggesting the potential for a more ‘natural’ treatment of such conditions in humans. “A substantial number of patients don’t benefit from existing drugs, which also have considerable adverse effects,” says Honda. “We want to clinically test our hypothesis that reconstituting these bacteria to normal levels in patients may help to restore immune tolerance and resolve chronic inflammatory processes.”

Figure 1: Inflammatory damage to the intestinal epithelium in a mouse model of colitis (left) is greatly reduced after oral administration of a mixture of 17 Clostridia strains (right).
The elements are born of stars

“The latest research estimates that the Universe was created about 13.8 billion years ago. At that time, it contained only small amounts of the elements lithium, hydrogen and helium,” says Tamagawa.

Today, there are more than 90 naturally occurring stable elements, all of which were created by the activity of stars. “The stars themselves were formed from hydrogen or helium several hundred million years after the birth of the Universe. Other elements were created within stars by nuclear fusion. In addition, some heavy stars end their lives in a huge ‘supernova explosion’, which also creates many different elements. In the Universe, stars are in a never-ending cycle of life and death,” Tamagawa continues. “Our goal is to reproduce the creation of elements and the drama of this endless cycle.”

The High Energy Astrophysics Laboratory, based at the RIKEN Nishina Center for Accelerator-Based Science, observes and studies celestial bodies that emit x-rays, which include neutron stars, black holes and supernova remnants, as well as galaxy clusters and active galactic nuclei. Such x-rays are emitted by celestial bodies with extremely high temperatures in the tens of thousands to tens of millions of degrees.

Vela Junior: A supernova remnant

When a supernova explosion occurs, stellar materials and new elements that are created at the time of the explosion are violently expelled into outer space. Known as supernova remnants, these materials frequently collide with gas in interstellar space, forming shock waves that continue to expand outwards for tens of thousands of years. Often, a high-density celestial body, such as a neutron star or a black hole, is left behind at the center.

Vela Junior is an example of such a supernova remnant. “We successfully measured how fast the Vela Junior supernova remnant expanded in a year, and our results overturned conventional theory about the remnant’s age,” says Satoru Katsuda, a postdoctoral researcher in the High Energy Astrophysics Laboratory.

On 18 June 1962, Scorpius X-1—a celestial body emitting intense x-rays—was discovered, marking the birth of x-ray astronomy. Fifty years on, researchers use x-ray astronomy satellites to observe the x-rays emitted by exploding supernovae, neutron stars and black holes in order to understand the mechanisms of high-energy astronomical phenomena. As part of these efforts, Toru Tamagawa’s laboratory is working at the leading edge of x-ray astronomy, helping to develop ASTRO-H, Japan’s latest x-ray satellite, and the GEMS x-ray polarimeter.
Vela Junior was discovered in 1998 by ROSAT, a German x-ray satellite. Concurrently, the Compton Gamma Ray Observatory (CGRO), a NASA gamma-ray astronomy satellite, detected gamma rays being released from the decay of titanium-44 ($^{44}$Ti) in Vela Junior.

The researchers found the results of the separate analyses to be surprising. “Gamma rays emitted during the decay of $^{44}$Ti allow us to estimate when a supernova explosion occurred or the age of the supernova remnant,” explains Tamagawa. “The CGRO observation showed that Vela Junior was comparatively young—about 700 years old—and about 0.2 kiloparsecs, or 0.65 light years, away from the Earth. However, other observations showed that the neutron star at the center of Vela Junior was several thousand years old and about 1 kiloparsec, or 3.26 light years, away from the Earth, leading to conflicting results.”

**Determining the age and distance of Vela Junior**

To calculate the rate at which the Vela Junior supernova remnant is expanding, Katsuda used data provided by XMM-Newton (X-ray Multi-Mirror Mission-Newton), a European Space Agency (ESA) x-ray observatory. Almost every year since 2001, ESA used XMM-Newton to observe the upper-right portion—the north-west section of the celestial sphere—of the x-ray image of Vela Junior to calibrate its detectors. Katsuda was able to compare the images observed in 2001, 2003, 2005 and 2007 and extract the differences (Fig. 1). “A black streak (indicated by the arrow) could be observed, which suggests that this region was expanding between 2001 and 2007,” says Katsuda.

The analysis showed that the upper-right edge of Vela Junior expanded outwards by about 0.84 arcseconds in one year. On seeing the value, Katsuda thought that the expansion rate was too slow. “A supernova remnant first expands at a great speed, then the rate of expansion gradually slows down. If Vela Junior was 700 years old, the expansion rate should have been at least five times faster than the measured result,” he notes. “I compared the result with the expansion rates of supernova remnants with known ages and estimated that Vela Junior was about 1,000–3,000 years old and 750 parsecs from the Earth. This age is the same as the estimated age of the neutron star at its center.” Hence, Katsuda believed that the gamma rays associated with the decay of $^{44}$Ti were unlikely to be originating from Vela Junior.

Katsuda was prompted into making his discovery by a report about a supernova remnant whose synchrotron radiation changed in intensity year after year. Synchrotron radiation is the light emitted when charged particles, such as electrons or protons, are accelerated within a magnetic field. In space, many particles fly around at relativistically fast speeds, and although researchers are not definitively sure of the source of their acceleration, supernova remnants are considered to be the major candidate.

“Because of this report, I thought that I should investigate whether or not Vela Junior exhibited a similar variation in intensity,” says Katsuda. However, he was disappointed to find no variations in the intensity of the x-rays from Vela Junior. Feeling frustrated, Katsuda looked at the difference image once again and found the black streak. “I was so surprised and pleased when it revealed information on the expansion of a supernova remnant hidden in the difference image,” he recalls.

In addition, Katsuda successfully measured the expansion rate of numerous other comparatively bright supernova remnants, including the SN 1006 supernova, which emerged in the year 1006 and is described in Teika Fujiwara’s Meigetsuki, and Tycho’s Supernova (SN 1572)—discovered by Danish astronomer Tycho Brahe in 1572.

“Measurement of the expansion rate has proved to be a powerful tool that allows us to derive the age and distance of a supernova remnant as well as the density of the surrounding region. The density information is particularly important for the analysis of the mechanism of a supernova explosion because it reflects the state of the star immediately before the supernova explosion,” he explains.
Proving the existence of black holes

A black hole is a celestial body that is denser than a neutron star, and its massive gravitational field prevents even light from escaping it. Shinya Yamada, also a postdoctoral researcher in the High Energy Astrophysics Laboratory, is working to prove their existence.

“Black holes are thought to be present at the center of galaxies and to form binary systems with other stars like the Sun. These ideas are, however, based on observations of the motion of stars or of x-rays emitted from high-temperature gases when they are absorbed into a region; they only demonstrate that a huge mass is packed into a very small region,” something that does not prove the existence of black holes, says Yamada. “To ultimately prove the existence of black holes, we need to take a picture of one directly. However, we have no way of doing this at the moment.”

Black holes are very small and when viewed from the Earth they appear to be only several microarcseconds in size—equivalent to viewing a human hair on the surface of the Moon. In order to be observed with NASA’s Chandra X-ray Observatory, which is equipped with the most sensitive equipment currently available, researchers would need to increase the resolution by up to six orders of magnitude, something that they are unlikely to achieve within the next 100 years. Thus, Yamada instead decided to take advantage of the intensity variation of x-rays. “The smaller something is, the shorter its period of variation. I decided to visualize the region around a black hole by accurately measuring the intensity variation and temperature of x-rays emitted from the gases absorbed into a black hole.”

Observing the gases that fall into celestial bodies

Yamada decided to observe Cygnus X-1, an intense x-ray emitting celestial body that was discovered in 1971 (Fig. 2). “Based on the fact that the intensity of the x-rays emitted from Cygnus X-1 varies over a short period of time, Minoru Oda—a former president of RIKEN who also discovered Cygnus X-1—believed that it must be a black hole. Since then, more than 40 years have passed with no decisive evidence having been obtained, which leaves Cygnus X-1 as a candidate black hole,” explains Yamada.

To make his observations, Yamada used Suzaku, a Japanese x-ray satellite that followed four predecessors: Hakuchô, Tenma, Ginga and ASCA. Suzaku is equipped with a hard x-ray detector that allows the x-rays that are emitted from the gases absorbed into black holes to be observed with high sensitivity.

Suzaku revealed that the intensity of the Cygnus X-1 x-rays fluctuated wildly over a short time and also contained several peaks known as ‘shots’. “A shot is the x-rays emitted at the moment when a mass of gas falls into a black hole. But the variation occurred over such a short period of time in Cygnus X-1 that we were unable to analyze a single shot in detail. However, we have developed a unique technique in which multiple shots are superposed, thus successfully measuring the temperature variation of gases that fall into its center,” says Yamada. “As a result, we found that the gas that falls into the center is suddenly heated to 1 billion degrees or higher in the last one hundredth of a second. This phenomenon provides real proof that Cygnus X-1 is indeed a black hole,” (Fig. 3).

Yamada is certain that Cygnus X-1 can only be a black hole and not another high-density celestial body, such as a neutron star. “A neutron star has a surface, whereas a black hole that absorbs everything has no surface. The temperature of the gas that falls into a neutron star decreases because of radiation from the surface. The fact that the gases were suddenly heated up to 1 billion degrees means that Cygnus X-1 has no surface and therefore must be a black hole.”

Yamada was heavily involved in the development of the hard x-ray detector mounted onto Suzaku and was also in charge of verifying the detector’s performance both before and after its launch. “Soon after launch, we were unable to obtain accurate and reliable data due to many changing conditions,” he says. “It took me five years to calibrate Suzaku so that we could achieve the observation of Cygnus X-1.”

ASTRO-H: A next-generation x-ray satellite

As well as using currently operating x-ray satellites, Tamagawa’s High Energy Astrophysics Laboratory is preparing
for upcoming research projects with ASTRO-H, an x-ray satellite to be launched in 2015.

Coordinated by the Institute of Space and Astronautical Science (ISAS) and the Japan Aerospace Exploration Agency (JAXA), the development of ASTRO-H has involved many x-ray astronomy researchers in Japan. “We are developing a microcalorimeter designed to accurately measure the energy of x-rays in collaboration with ISAS, Tokyo Metropolitan University and Kanazawa University. We expect that our microcalorimeter will enable us to improve on the observations of supernova remnants in more detail,” predicts Katsuda.

Each of the elements emits x-rays of different energy levels called ‘bright lines’. By using a device with a higher energy resolution capability, we will be able to precisely determine the locations in space where particular elements were created and how they were scattered into outer space,” says Katsuda. “As the microcalorimeter uses the Doppler effect and is capable of detecting even a slight energy variation in bright lines, we will also be able to observe the very slow motion of an object and understand whether the object is approaching or moving away. Ultimately, this will enable us to understand the expansion of supernova remnants in more detail,” predicts Katsuda.

**Figure 3: Superposed shot analysis of x-rays from Cygnus X-1**
The moment when gas is absorbed into a black hole corresponds to the time 0 seconds. As the gas approaches the black hole, it gains gravitational energy; x-rays are brightest at time 0. Immediately after the phenomenon begins, the number of x-ray photons with an energy level of 100 kiloelectronvolts (keV) increases drastically. The energy of 100 keV corresponds to 1 billion degrees.

The phenomenon lasts for a hundredth of a second until the gas falls into the black hole and its temperature increases sharply.

**The world’s first high-sensitivity x-ray polarimeter**
The Gravity and Extreme Magnetism Small Explorer (GEMS) is a NASA-run project and the world’s first satellite for measuring the polarization of x-rays. Our gas electron multiplier foil was adopted by NASA and they decided to install it in the core part of the x-ray polarimeter of GEMS,” explains Tamagawa.

X-ray polarimetry should enable researchers to visualize the wavefront of a shock wave from a supernova remnant. This phenomenon is closely related to particle acceleration, the direction of the magnetic field, the shape of the disc around a black hole, the rotation of the black hole and the warping of time–space around the black hole. Although due to be launched in 2014, the GEMS satellite project was recently suspended because of increases in development costs. “As our device is almost ready, I will once again propose it to NASA so that it can be used when GEMS is finally launched,” says Tamagawa. “The success of GEMS will open up a new era in x-ray polarization astronomy.”

**ABOUT THE RESEARCHER**
Toru Tamagawa was born in Hyogo, Japan, in 1970. He graduated from Tohoku University in 1993 and obtained his PhD from the University of Tokyo in 2000. Immediately changing his research direction from nuclear physics to astrophysics, Tamagawa joined the Cosmic Radiation Laboratory at RIKEN as a postdoctoral researcher. In 2010, he was appointed associate chief scientist and director of the High Energy Astrophysics Laboratory. His research interests include the nuclear matter of neutron stars and nucleosynthesis in supernova explosions. His laboratory is developing unique devices for satellites that are opening up a new area of astrophysics research known as high-resolution x-ray spectroscopy and polarimetry.
Decoding the parent–infant bond

KUMI KURODA
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How did you join RIKEN?
I obtained my PhD at Osaka University’s Graduate School of Medicine, and in 2003 I visited RIKEN for the first time to attend the RIKEN Brain Science Institute (BSI) annual Summer Program for young neuroscientists. The following year I joined the Laboratory for Molecular Dynamics of Mental Disorders, headed by Dr. Tadafumi Kato, as a special postdoctoral researcher.

What attracted you to RIKEN?
When I joined RIKEN, I felt that the BSI was the best place for neuroscience research in Japan and one of the top institutes worldwide—something that I still believe today.

The BSI rivals other research institutes across many criteria: facilities, budget, size, quality and maintenance of its laboratory animal services, and opportunities to meet world-class researchers. The institute also hosts academic forums, social events and retreats, which help to create a comfortable environment for researchers. This, of course, would not be possible without the institute’s extremely efficient support system and friendly administrative staff. The BSI is like an academic’s paradise. Put simply, I fell in love with it.

Please tell us more about your research at RIKEN.
In my current research, I focus on understanding the neural mechanisms responsible for affiliation—or bonding—between mammalian parents and their infants, to serve families in difficulty.

Parents are equipped with an innate motivation to nurture their infants and guarantee their survival and well-being. Correspondingly, infants are born with attachment instincts, such as suckling, crying and following their caregivers. These drives are hardwired into the brain and refined through postnatal learning. My unit hopes to determine the neural mechanisms involved through a variety of techniques, including behavioral assays, brain-area mapping, neural circuit analyses and the identification of relevant biochemical molecules and intracellular signaling pathways.

What has been the highlight of your time at RIKEN so far?
Since joining RIKEN, I have had the opportunity to interact with eminent brain science academics affiliated with the BSI, including Founder and Special Advisor Masao Ito, Director Susumu Tonegawa, Special Advisor Takao Hensch and my former mentor Dr. Kato. My research has been shaped by their leadership, as well as regular engagement with other principal investigators and researchers through formal and informal events. I am honored to be a member of an institute with such a prestigious history.

What would you say to other people considering joining RIKEN?
RIKEN offers a lot of support for men and women with children. For example, it runs an on-campus childcare facility called ‘RIKEN Kids’, where I enrolled my son for almost one-and-a-half years. Personally, I find that the key to an agreeable work–life balance is to plan your career goals and then trace back the steps required to achieve them, a strategy known as backcasting. Using this technique could help young researchers to balance their research ambitions with other responsibilities.

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K computer scores big in the HPC Challenge Award Competition

The K computer—RIKEN’s supercomputer—received top marks in the 2013 HPC Challenge Award Competition, an annual contest where supercomputers from around the world are judged on their performance and productivity. The results were announced at SC13, an international conference for high performance computing, networking, storage and analysis, held in the United States in November.

The competition is divided into two classes; Class 1 assesses performance according to four of the most challenging benchmarks, which were selected from seven criteria designed to test the capabilities of high-performance computing systems. The K computer ranked first in the Class 1 awards for the third consecutive year, achieving top scores in three of the four categories.

Class 2 measures the overall productivity, or ‘elegance’, of programming language implementation. The metric is of interest to researchers worldwide since the pace of research can be accelerated by the development of high-speed applications that run in large-scale computation environments.

RIKEN and Chinese institutions host joint materials science symposium

On 11–12 November 2013, RIKEN organized a two-day joint Symposium on Materials Science in collaboration with four Chinese research institutions: the Institute of Chemistry, Chinese Academy of Sciences; the Institute of Physics, Chinese Academy of Sciences; Peking University; and Tsinghua University.

Materials science—a key area of collaboration for RIKEN—is expected to offer solutions to some of the most critical challenges facing society today, including energy and the environment. Over 100 researchers from RIKEN and the guest institutions attended the symposium, held at RIKEN’s campus in Wako, to share and discuss research in the field pertinent to the overarching theme of "Contribution to a sustainable society."

Parallel programming language XcalableMP, the implementation of which was developed by RIKEN and the University of Tsukuba, helped the K computer to secure a win in the Class 2 awards—a first for Japan.

The K computer was developed by RIKEN and Fujitsu as part of the High-Performance Computing Infrastructure initiative led by Japan’s Ministry of Education, Culture, Sports, Science and Technology (MEXT). The supercomputer’s computational power is being harnessed to advance research in a wide range of fields, including the life sciences and materials science, as well as for disaster prevention, manufacturing and unraveling the mysteries of the Universe.