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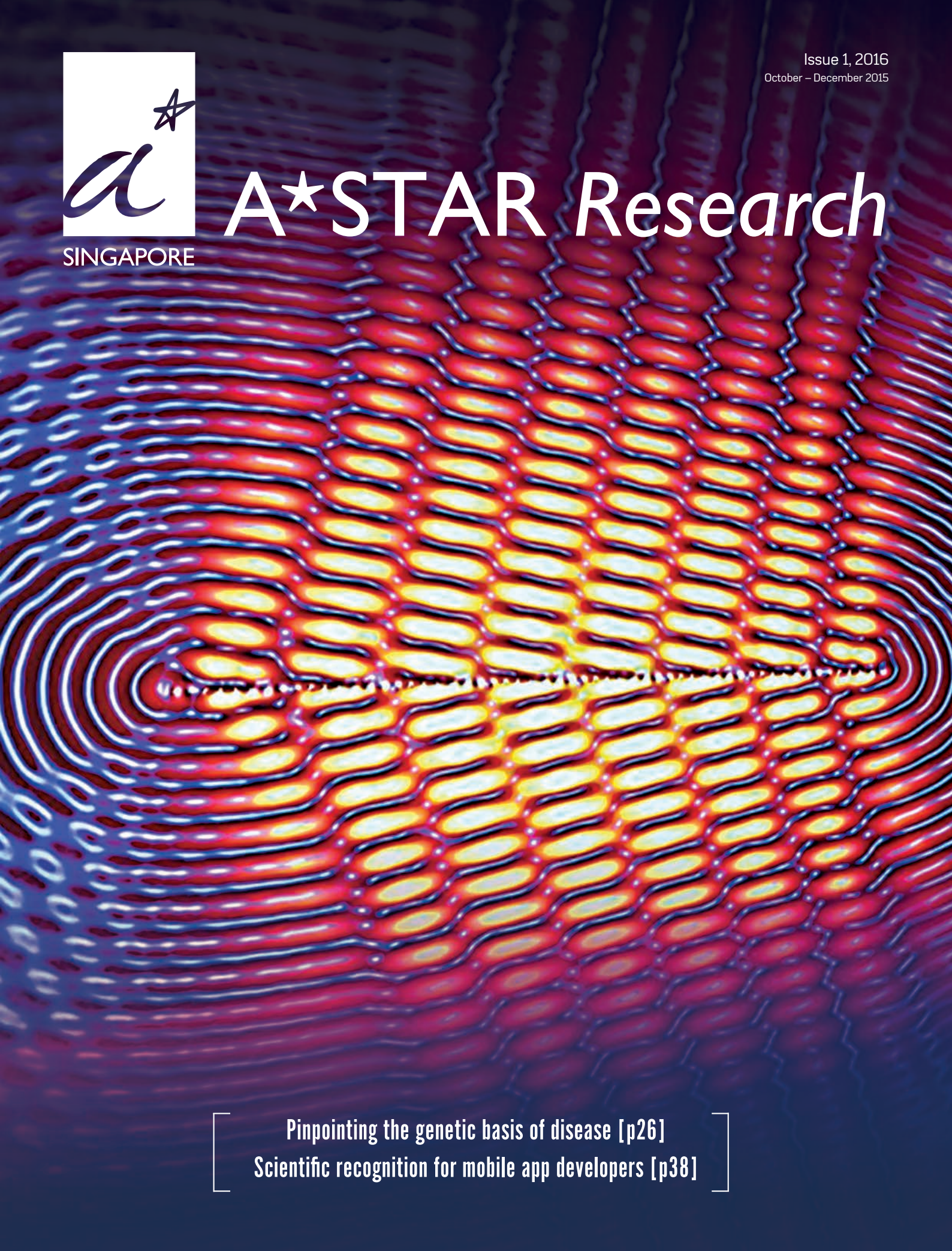

Pinpointing the genetic basis of disease [p26]

Scientific recognition for mobile app developers [p38]

A*STAR Research

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Agency for Science, Technology and Research
1 Fusionopolis Way, #20-10 Connexis North Tower Singapore 138632, Singapore

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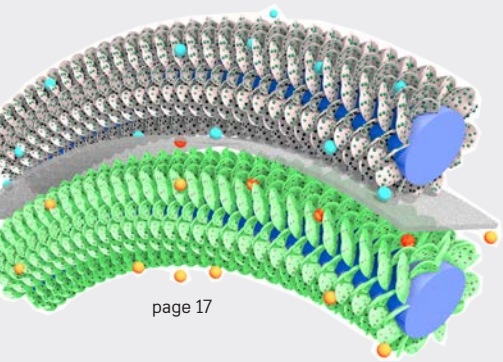

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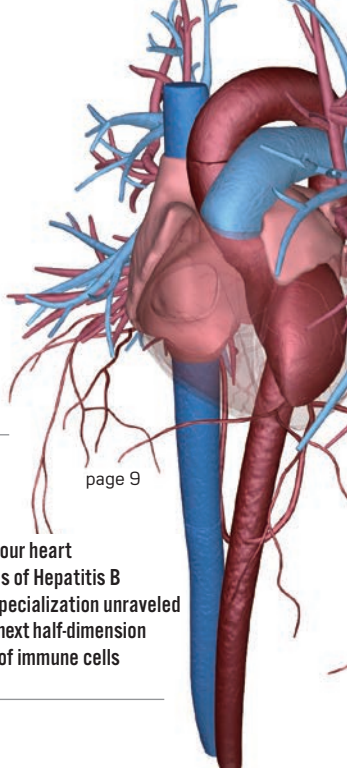

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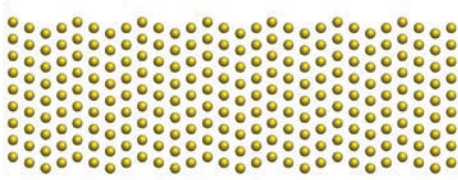

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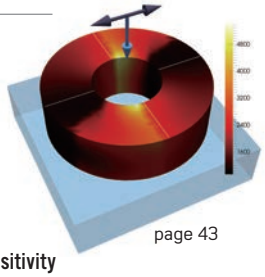

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A*STAR Research
SINGAPORE

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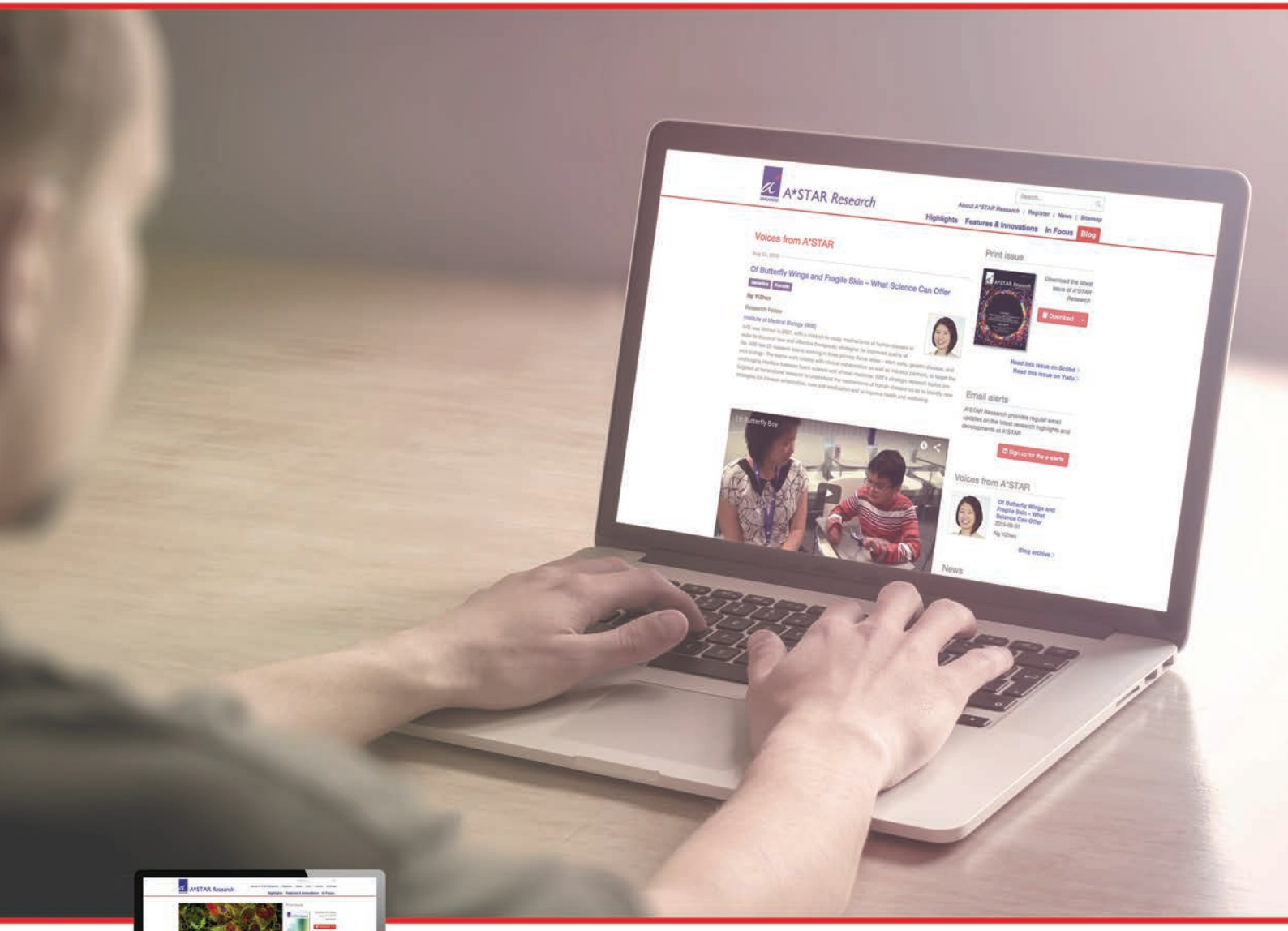

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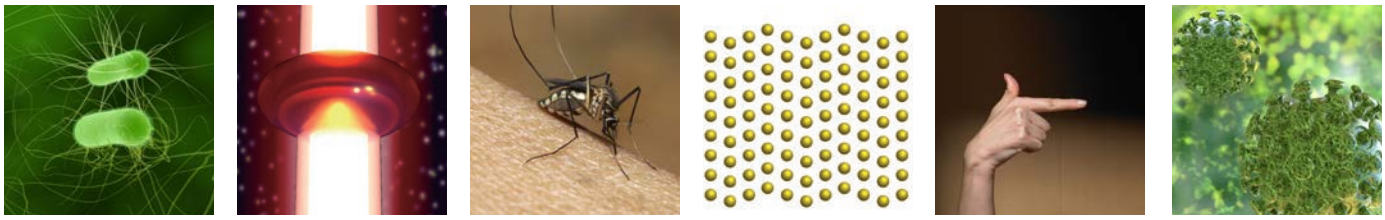


[NEW YEAR, NEW LOOK]

Sir David Lane, Editor-in-Chief, introduces the latest issue of *A*STAR Research*

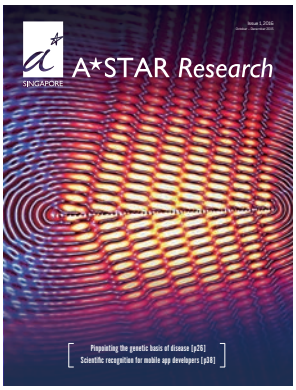
Welcome to the first edition of the new format of *A*STAR Research*.

After seven years of publishing the latest research from A*STAR, we — and you, our trusted readers — felt it was time to undergo a redesign. To allow us to communicate our cutting-edge science in a more timely fashion, the magazine is now going to be a quarterly rather than a biannual publication. This new, lighter edition will be easier to carry with you as you travel the world.

From protein crystals that grow inside cells to breakthroughs in pain management, A*STAR is making wonderful progress that we are excited to discuss with all of you in our great new format. Looking ahead to 2016, we already have some fantastic new stories ready to share. Science is progressing with tremendous speed as many of the promises of earlier decades of research are now paying off in a big way. It is marvelous to see that once-deadly diseases are now being managed by new antibody drugs with quite amazing success while improvements in image analysis are allowing us to see deeper into the structure of life and the nature of disease than ever before. The Internet of things is beginning to enter all of our lives and cars without drivers are on their way, sooner rather than later.

The Singapore government is dedicated to funding a vigorous research and technology base to create a prosperous, safe and effective economy and A*STAR is at the forefront of this task.

It is going to be a great ride and we are glad that you can join us on it.



COVER IMAGE

A*STAR researchers capture waves of surface plasmons on a gold film lined with rows of nanoscale slits. [p48]

Stem cells grow in higher density on microcarriers.

Stem cells:

GROWING STEM CELLS AT LARGE SCALE

TO PRODUCE MESENCHYMAL STEM CELLS IN LARGE NUMBERS WILL REQUIRE CAREFUL TUNING OF THEIR GROWTH MEDIA

Mesenchymal stem cells (MSC) have a vital therapeutic role, yet can still only be manufactured in relatively small batches. A*STAR researchers have made advances in finding a better growth media[1].

MSCs that can develop into bone, cartilage, muscle or fat cells show promise in treating human disease and are in late stage clinical trials for more than a dozen indications, explains Steve Oh from A*STAR. "In total there are around 400 trials globally" says Oh, but "if any of these trials lead to blockbuster therapies there is not enough serum available to sustain manufacturing of the cells."

Currently MSCs are grown in a single layer on a culture dish in media often containing animal serum. To scale up production cells will have to be grown in larger volumes and in media that does not contain animal serum as this can vary from batch to batch.

A team led by Oh and Kah Yong Tan, also at A*STAR's Bioprocessing Technology Institute, wanted to know how effective current serum-free media formulations are at sustaining MSC growth. They compared proliferation of seven MSCs lines in six different

media compositions, either in a dish or on small beads, called microcarriers, which can support large numbers of cells.

Their findings were disappointing, but not surprising. No single media supported growth of all MSC lines and a medium that performed well in monolayer cultures did not necessarily lead to growth on the microcarrier.

"The MSCs come from a variety of sources; the bone marrow, the umbilical cord, adipose tissue," explains Oh, "it is likely that growth and attachment requirements will be different and need to be optimized for each cell line."

The work was done in static microcarrier cultures with no agitation. Scaling up production really requires the cells to be stirred. "To switch cells from static to shaker culture is critical," explains Oh "and then we need to look at functional aspects as well as stem cell markers."

Oh is currently working with a company to develop a generic medium that will support more MSC lines. While a single medium to grow all MSC lines may not be realistic Oh hopes that two or three formulations will support the growth of most lines.

Oh and Tan's work lays the groundwork for finding the optimal media. The next step is to identify a definitive method of how to grow MSCs on a scale needed for therapy.

1. Tan, K. Y., Teo, K. L., Lim, J. F., Chen, A. K. L., Reuveny, S. & Oh, S. K. W. Serum-free media formulations are cell line-specific and require optimization for microcarrier culture. *Cytotherapy* **17,** 1152–1165 (2015).

Immunology:

ENZYME HELPS DETECT FOREIGN DNA

THE IMMUNE SYSTEM RELIES ON AN ENZYME CALLED BRUTON'S TYROSINE KINASE TO SENSE NUCLEIC ACIDS FROM PATHOGENS

The human immune system responds to DNA from pathogens by triggering the production of a defense molecule known as interferon. A research team led by A*STAR scientists has now pinpointed an enzyme integral to this process, called Bruton's tyrosine kinase (BTK), which provides a potential new target for drug development[1].

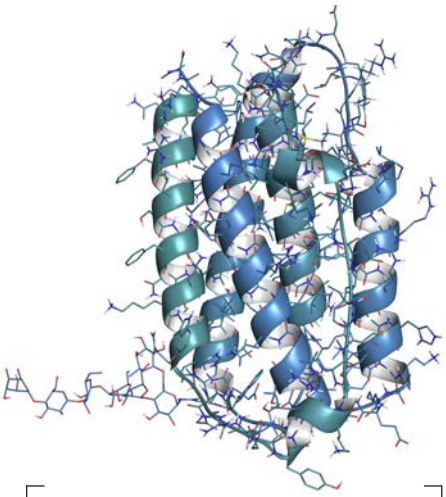
The presence of bacterial and viral nucleic acids inside the cell is generally undesirable, so a protein called DDX41 responds to microbial invaders by binding this foreign DNA. DDX41 then recruits another protein called STING to help launch an immune defense. STING, an acronym for stimulator of interferon genes, does what its name suggests and turns on the genes that code for interferon, a type of cytokine.

However, a team led by Kong Peng Lam, executive director of the A*STAR Bioprocessing Technology Institute, discovered this pathway from DNA sensing to interferon production also relies on BTK.

"Our findings indicate that agents targeting BTK activation could yield new and broad-based antimicrobial agents," says Lam.

Lam's lab group at A*STAR, together with scientists from the National University of Singapore, studied mouse cells that lacked the gene necessary for making BTK. They treated these cells with bacterial DNA or infected them directly with pathogens such as Listeria and malaria. In each instance, interferon production was impaired, indicating the essential role for BTK in fighting off infections.

The researchers then looked at human cells to identify the mechanism by which BTK works with DDX41 and STING to promote antimicrobial immunity. They showed that when pathogens invade cells, BTK physically attaches to DDX41 and adds a phosphorylation tag to a residue of the protein. This phosphorylation primes DDX41 to bind the foreign DNA and also stabilizes DDX41's interaction with STING, which in turn activates the genes necessary to generate interferon.



Production of interferon, an essential defense molecule, is linked to Bruton's tyrosine kinase.

Pharmaceutical companies are already working on drugs that modulate BTK activity. One BTK inhibitor, called ibrutinib, has been approved for the treatment of leukemia and lymphoma. Others, which are for people suffering from autoimmune diseases such as rheumatoid arthritis and lupus, are

undergoing clinical trials.

According to Koon-Guan Lee, an immunologist at A*STAR and first author of the study, the discovery that BTK also plays a role in warding off pathogens "further suggests that BTK inhibitors could potentially be used to dampen the cytokine storms seen in sepsis or in severe infections that could otherwise kill the host."

1. Lee, K.-G., Kim, S. S.-Y., Kui, L., Voon, D. C.-C., Mauduit, M. *et al.* Bruton's tyrosine kinase phosphorylates DDX41 and activates its binding of dsDNA and STING to initiate type 1 interferon response. *Cell Reports* **10,** 1055–1065 (2015).

Molecular biology:

REMOVING GENES WITHOUT A TRACE

DNA REPAIR MECHANISM MANIPULATED TO DELETE GENES WITHOUT LEAVING A SCAR

Genes may now be deleted without creating a scar in certain strains of *Escherichia coli* and other microorganisms, thanks to A*STAR researchers[1]. The technique makes it easier to string together several genetic engineering steps without interference caused by a deletion scar.

Scientists currently delete genes by manipulating a process known as homologous recombination. Nucleotide sequences change places with the target gene during homologous recombination and are left behind as a genetic scar, undermining the effectiveness of subsequent deletions. As scars accumulate, the recombination process is more likely to recognize them than the target gene, disrupting the deletion attempt.

A*STAR researchers have successfully deleted genes from Escherichia coli without leaving a scar.

The scar-free deletion trick developed by Hua Zhao and colleagues at the A*STAR Institute of Chemical and Engineering Sciences utilizes a natural DNA repair mechanism. Gene duplication events or errors during replication occasionally lead to the formation of a mirrored DNA sequence known as an inverted repeat. Since the repeated segments in an inverted repeat are complementary, they bind to each other and form a loop structure. While short loops have a biological role, longer loops can damage the genome and are therefore cut out by repair machinery.

"The key insight was the extreme instability of inverted repeats in the *E. coli* genome, which we and others observed. That prompted us to explore its application in gene deletion," says Zhao.

To delete a gene, Zhao's team prepares a DNA fragment, which includes an inverted repeat of part of the target gene. They then insert the fragment into the genome adjacent to the gene. The inverted repeats form a loop, and the repair machinery swoops in to snip them out. Since the repair process does not always happen, the team also engineers a selection marker into the fragment, enabling them to detect colonies in which it has been cut out.

Zhao's team successfully repeated their method on three different *E. coli* genes. They also tested inverted repeats of different lengths to determine which worked best. While shorter repeats were less likely to be excised, longer repeats did not integrate into the genome as often.

Engineering *E. coli* to produce biochemicals often involves the deletion of multiple genes. According to Zhao, approaches presently only allow four genes to be deleted in sequence. "After that, further deletions create trouble because of recombination between the deletion scars. Our new method doesn't introduce scars, so recombination won't be a problem for multiple deletions."

1. Tear, C. Y., Lim, C. & Zhao, H. Excision of unstable artificial gene-specific inverted repeats mediates scar-free gene deletions in *Escherichia coli*. *Applied Biochemistry and Biotechnology* **175,** 1858–1867 (2015).

Dengue fever:

VIRUS VERSION CONTROL

A PROTEIN VACCINE DESIGNED TO TARGET ALL FOUR VARIATIONS OF THE DENGUE VIRUS PROVES EFFECTIVE IN MOUSE TRIALS

Dengue fever, spread by mosquitoes, has proven difficult to immunize against, but a new vaccine being tested at A*STAR shows great promise.

A breakthrough in the search for safe immunization against dengue fever has emerged after trials at A*STAR showed a new vaccine without live viruses induces an effective immune response[1].

Dengue fever is a debilitating tropical disease spread by mosquitoes, against which a global research effort has failed to develop a universally approved vaccine. Crucially, the dengue virus comes in four variations, known as serotypes, and for a vaccine to be effective it must target all four serotypes at once. Cuban scientists developed a new potential vaccine and sought input from Katja Fink and colleagues at the A*STAR Singapore Immunology Network (SIgN).

"Several dengue vaccine candidates have been developed, but they all have shortcomings," says Fink. "Vaccines that contain live viruses can produce a strong immune response, but cause unwanted side effects. From a safety point of view it is preferable to have protein-only approaches."

> "SEVERAL DENGUE VACCINE CANDIDATES HAVE BEEN DEVELOPED, BUT THEY ALL HAVE SHORTCOMINGS."

One promising candidate is the dengue virus E protein, which includes epitopes — protein regions that are recognized and targeted by the immune system — found on all four dengue serotypes. A specific part of the E protein, called DIII, is targeted by particularly potent neutralizing antibodies. However, DIII tends to attract only one type of lymphocyte, called B cells. B cells are good for neutralizing viruses on repeated infection, but a strong response also requires T cells to kill infected cells, to support the B cells and establish immune memory.

Researchers at the Center for Genetic Engineering and Biotechnology in Havana combined DIII with the capsid protein (C) found on all four serotypes, which has effective T-cell epitopes, to produce an aggregate vaccine named DIIIC and asked Fink's group to analyze exactly what sort of immune response it would induce.

Extended trials on immunized mice revealed that the DIIIC vaccine induced a so-called Th1-type response, which helps to promote cytotoxic T cells and regulate the production of antibodies from B cells. Moreover, the antibodies induced by DIIIC were still at useful levels 120 days after immunization.

"In natural dengue infection an imbalance towards a Th2 response appears to be associated with more severe disease and a stronger Th1 response is preferred, so it is encouraging to see an efficient, long-lasting Th1 response to DIIIC," says Fink. Her team hopes to continue collaboration with their Cuban counterparts to understand and develop this promising vaccine for clinical trials using A*STAR's state-of-the-art facilities.

1. Zuest, R., Valdes, I., Skibinski, D., Lin, Y., Toh, Y. X. *et al.* Tetravalent dengue DIIIC protein together with alum and ODN elicits a Th1 response and neutralizing antibodies in mice. *Vaccine* **33**, 1474–1482 (2015).

Consuming carbohydrates like Basmati rice with a relatively low glycaemic index could help to prevent the onset of Type 2 diabetes among Asian populations.

Nutrition:

INSULIN PRODUCTION ON OVERDRIVE

SINGAPOREAN CHINESE, MALAYS AND INDIANS HAVE DIFFERENT INSULIN RESPONSES TO A BOWL OF RICE

Following a carbohydrate-rich meal, Singaporean-Indians have more insulin released into their bloodstreams than their Chinese and Malay compatriots to maintain the same blood sugar levels, according to a clinical study by researchers at the A*STAR Singapore Institute for Clinical Sciences (SICS)[1]. The results suggest that consuming fewer carbohydrates and choosing those with a lower impact on blood glucose levels could benefit populations — including Singaporean-Indians — that have a high prevalence of type 2 diabetes.

"The global literature on blood glucose levels is largely based on Caucasian subjects consuming Western foods," says the director of the SICS Clinical Nutrition Research Centre, Christiani Jeyakumar Henry, who led the study along with Verena Tan. "Our study and our center is focusing on the metabolic response of what we call the Asian phenotype and Asian foods."

The researchers selected 75 healthy Singaporean males — 25 from each ethnic group — and gave them a serving of either Jasmine rice, Basmati rice or a control course of glucose. The subjects' blood glucose and insulin levels were measured before each meal, and then at fifteen- and thirty-minute intervals after eating.

Overall, the Indians were found to have

significantly higher blood insulin levels than the Chinese and Malay participants for up to two hours after every meal. The researchers hypothesize that Indians, who are more resistant to the effects of insulin, secrete excess amounts of the hormone to maintain normal glucose levels. This insulin surge in turn makes them more resistant, "like a boxer receiving one-too-many punches," says Henry. "It's a vicious cycle."

The team also found that the subjects' blood glucose and insulin levels varied depending on the consumed food's glycaemic index, better known as its GI, and its insulinaemic index. Jasmine rice has a higher glycaemic and insulinaemic index than Basmati rice, and therefore resulted in higher levels of glucose and insulin, regardless of ethnicity.

Considering the high proportion of carbohydrates in the Asian diet, choosing rice varieties and other foods with lower glycaemic and insulinaemic indices could help prevent the onset of type 2 diabetes, which is a large and growing public health crisis in the region.

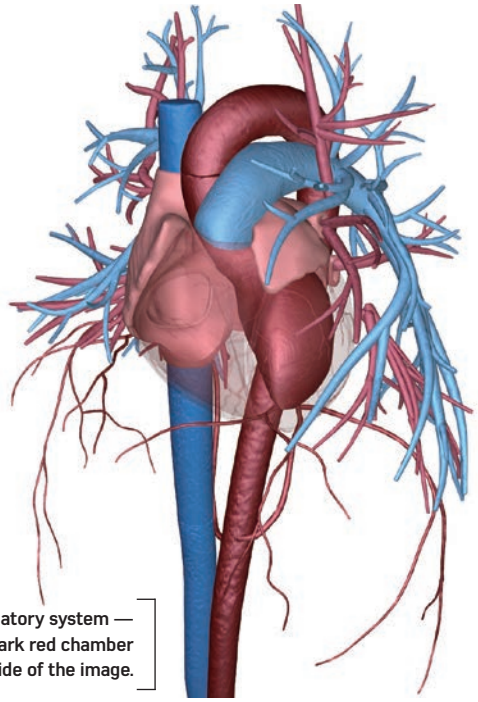
Henry and his team at the Clinical Nutrition Research Centre have recently published the glycaemic index values of 15 popular foods consumed in South-east Asia, including the fried Chinese breadstick, *youtiao*, the coconut-infused rice dish, *nasi lemak*, and iced green tea[2]. "We are conducting fundamental research to help Asians make more informed decisions about the foods that they eat," says Henry.

1. Tan, V. M. H., Wu, T., Henry, C. J. & Lee, Y. S. Glycaemic and insulin responses, glycaemic index and insulinaemic index values of rice between three Asian ethnic groups. *British Journal of Nutrition* **113**, 1228–1236 (2015).
2. Sun, L., Lee, D. E. M., Tan, W. J. K., Ranawana, D. V., Quek, Y. C. R., Goh, H. J. & Henry, C. J. Glycaemic index and glycaemic load of selected popular foods consumed in Southeast Asia. *British Journal of Nutrition* **113**, 843–848 (2015).

Cardiac modeling:

LOOK INSIDE YOUR HEART

MAGNETIC RESONANCE IMAGING PROVIDES INSIGHT INTO MYOCARDIAL MECHANICS AFTER A HEART ATTACK



The heart circulatory system — the left ventricle is the dark red chamber shown on the right side of the image.

Quantifying the damage caused to specific parts of the heart by cardiac arrest is key to providing effective treatment and accurate prognoses for millions of people worldwide. Now, A*STAR researchers have developed a computational method that uses magnetic resonance imaging data to assess the extent of damage to the left ventricle, the heart's powerhouse[1].

Heart attacks — medically known as myocardial infarctions — damage the heart tissue, leading to scarring, impaired function and sometimes death. "Heart attacks are very complicated," explains Teo Soo-Kng of the A*STAR Institute of High Performance Computing, "but in general, when the left ventricle is affected it usually leads to worse outcomes for the patient as that is the largest and most powerful pump in the heart."

After an infarction, clinicians need an accurate measure of the damage sustained to the left ventricle to decide on the best possible treatment. Clinical assessments based on changes in the amount of ejected blood during a heartbeat, called the 'ejection fraction', do not provide enough region-specific information about the damaged tissue. On the other hand, cardiac magnetic resonance imaging combined with a contrast agent is able to measure tissue scarring and provide region-specific information. However, such an approach only provides a semi-quantitative measure of the damage incurred.

Teo and his A*STAR colleagues decided to combine the best of both worlds to develop a robust, quantitative and region-specific measure that could be clinically useful. Using magnetic resonance imaging scans from clinical partners the team constructed a three-dimensional model of the left ventricle.

They then used this to compute the 'regional' ejection fraction and the change in left ventricle surface area during a heartbeat — or 'regional area strain'. These measures enable clinicians to quickly pinpoint which segments of the left ventricle have been compromised by a heart attack.

"Our method is not just useful for diagnostics, but also potentially for monitoring," Teo remarks. "It could ultimately be used for clinicians to assess how well the patient responds to treatment — for example, whether the degeneration of the heart function stabilizes or continues." This is reflected by the team's ongoing trials with clinical partners, focused on relapse and heart failure.

Teo and the team are also implementing a server infrastructure for the technique that would increase its usefulness for time-sensitive clinical applications. "We are trying to reduce the time frame. At the moment it can range from days to a week — but we're aiming to get it down to around half an hour or even less," he says.

1. Teo, S.-K., Vos, F. J. A., Tan, R.-U., Zhong, L. & Su, Y. Regional ejection fraction and regional area strain for left ventricular function assessment in male patients after first-time myocardial infarction. *Journal of the Royal Society Interface* **12**, 20150006 (2015).

Immunology:

THE TWO FACES OF HEPATITIS B

VIRUS TRAINS NEWBORNS' IMMUNE CELLS, ENHANCING THEIR ABILITY TO RESPOND TO PATHOGENS

Exposure to the hepatitis B virus (HBV) *in utero* can help 'mature' a human neonatal immune system and may improve the capacity of human neonatal immune cells to respond to secondary infections, an international research team has shown[1].

Exposure to HBV in utero can 'train' neonatal immune cells to respond more effectively to a secondary infection.

Chronic infection with HBV affects approximately 300 million people worldwide and can lead to liver disease, cancer, and death. Transmitted via blood or bodily fluid, the virus is most commonly spread from mother to child, especially in Asia. Mother-to-child transmission often leads to chronic infection and, based on evidence from mouse models, is thought to be associated with an 'immunotolerant' state in newborns.

This study suggests that HBV may behave differently in humans than it does in mice. "HBV does not infect mouse liver cells, therefore the significance of mouse models in relation to HBV pathogenesis is questionable," notes Michelle Hong from the A*STAR Singapore Institute for Clinical Sciences. The 'immunotolerance' hypothesis is also undermined by the high success rate of neonatal HBV vaccinations; backed up by the recent finding that chronic HBV-infected young adult patients, clinically labeled as 'immunotolerant', have a normal immune response.

> "CONTRARY TO CURRENT BELIEF, INFANTS EXPOSED TO HBV ARE NOT IMMUNE-TOLERANT BUT THEY HAVE MORE MATURE IMMUNE SYSTEMS."

Hong, along with Antonio Bertoletti, and colleagues from Singapore, Italy and the UK examined the immune cells in the umbilical cord blood of newborns to see whether HBV exposure *in utero* alters immune response. "Contrary to current belief, infants exposed to HBV are not immune-tolerant but they have more mature immune systems," she says, "we were able to detect more activated innate and

adaptive immune cells, and a stronger immune response upon unrelated bacterial challenge in a process called trained immunity."

"In trained immunity, the first stimulus/infection acts like a 'trigger' that primes the innate immune response," explains Hong. "While this declines rapidly after the infection, it often remains above basal levels. When exposed to a second stimulus — either the same pathogen or a different one — you have a much stronger innate immune response."

"We suggest that HBV exposure *in utero* confers a selective advantage to these babies to counteract a second infection during their early years."

Hong notes this dual role of HBV was a surprising discovery, "HBV is pathogenic in later adulthood but during the early years it seems to be protective in a sense, since it confers some benefits to the host."

Hong and colleagues are now studying the immune response in chronic HBV-infected pediatric patients. "The ages between two to 12 years is a 'black box': it's not really clear what happens during this early stage of infection," she says.

1. Hong, M., Sandalova, E., Low, D., Gehring, A. J., Fieni, S. *et al.* Trained immunity in newborn infants of HBV-infected mothers. *Nature Communications* **6,** 6588 (2015).

Stem cells:

CONTROLS OF SPECIALIZATION UNRAVELED

RESEARCHERS SHOW HOW HUMAN EMBRYONIC STEM CELLS MAINTAIN THE POTENTIAL TO DIFFERENTIATE INTO ALMOST ANY CELL TYPE, BUT ENTER A DEATH PATHWAY IF DAMAGED

Human embryonic stem cells with fluorescence reporters, which aided the discovery of novel regulators of stem cell potency. Red and green fluorescence demarcate different phases of the cell cycle.

Two phases of the cell cycle of human embryonic stem cells have been shown, for the first time, to actively employ pathways that maintain pluripotency — the potential to develop into almost any type of cell in the body[1].

Embryonic stem cells are derived from a group of cells present in an embryo before its implantation in the womb. For differentiation of these cells there must be a breakdown of the processes that maintain pluripotency. Several studies have identified pathways that regulate the cessation of mouse embryonic stem cells' pluripotency, however the process for human embryonic stem cells (HESCs) has remained a mystery.

Researchers at A*STAR's Genome Institute of Singapore used RNA to 'knock down' specific genes in HESCs, a method called high-throughput RNA interference screening. By using this method under a variety of conditions, they could identify pathways that regulate the exit of HESCs from pluripotency into specialized differentiation.

"We found a lot of different pathways, but I think the most exciting one was the involvement of the cell cycle," says cell biologist, Kevin Gonzales.

The previous dogma, explains Gonzales, was that the G1 phase — the first of four phases of cell division — was the only part of the cell cycle actively regulating pluripotency. The G1 phase is known to receive signals and express factors that encourage the differentiation of HESCs into specialized cells.

However, the A*STAR team found that the subsequent S and G2 phases of the cell cycle also have particular pathways that maintain pluripotency. In fact, the researchers believe that the absence of these pathways in the G1 phase make this stage more responsive to cues that induce cell differentiation.

The team also found that HESCs tend to maintain pluripotency, rather than proceed to differentiation, when their DNA is damaged. HESCs can better repair DNA damage when they are pluripotent. If repair fails, the cells enter a death pathway: an easier process when they are pluripotent than when differentiating. This status means differentiating cells will not give rise to damaged cells, says Gonzales, essential in the context of an embryo because

it prevents the production of a large number of cells in the body with DNA damage.

This study was basic research, rather than having a particular application, says Gonzales. But these new understandings are significant as they can help to fine-tune laboratory protocols on how to control the differentiation of HESCs, he says.

1. Gonzales, K. A. U., Liang, H., Lim, Y.-S, Chan, Y.-S., Yeo, J.-C. *et al.* Deterministic restriction of pluripotent state dissolution by cell-cycle pathways. *Cell* **162**, 564–579 (2015).

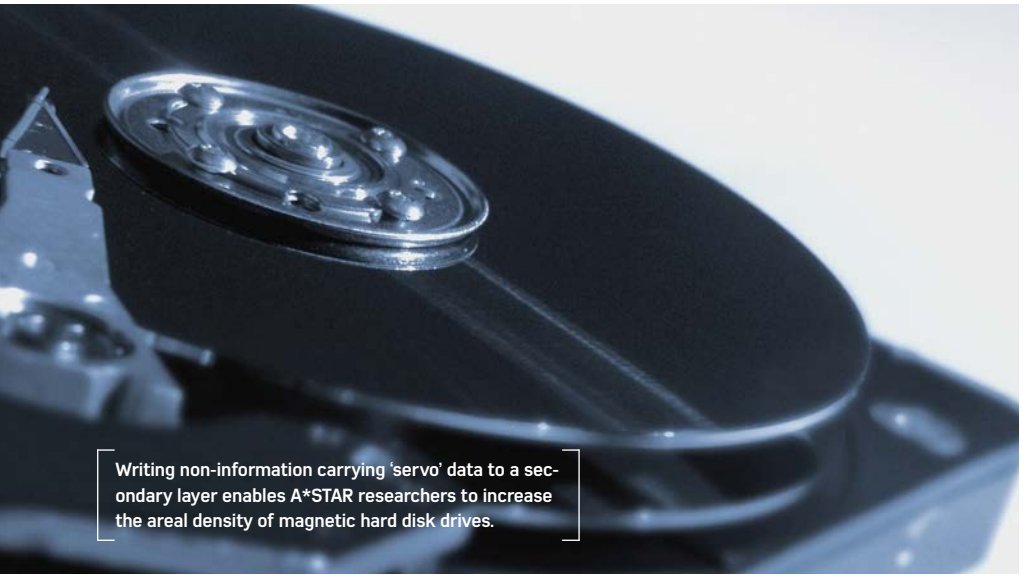
Data storage:

EXPLORING THE NEXT HALF-DIMENSION

A 2.5-DIMENSIONAL MAGNETIC RECORDING SCHEME COULD HELP BREAK THE DATA DENSITY BARRIER

It could soon be possible to squeeze more information onto magnetic hard disk drives using new technology developed by A*STAR researchers that thwarts physical limits on data density by moving head tracking bits to a deeper, second magnetic recording layer [1].

Hard disk drives consist of a stack of thin spinning magnetic 'platters' that are read and written to using a magnetic read/write head that moves at high speed just a few nanometers above the platter surface. With all other mechanical components now optimized after many years of development, the density of magnetic bits on the platter has become the crucial determinant of performance and capacity. Researchers and engineers have devised increasingly sophisticated and physically esoteric ways of raising this bit density, but a range of mechanical and physical barriers has presented them with a hard limit of about 1 terabyte per square inch.



Writing non-information carrying 'servo' data to a secondary layer enables A*STAR researchers to increase the areal density of magnetic hard disk drives.

Yunjie Chen and colleagues from the A*STAR Data Storage Institute have now found a way to cram in more usable data within these physical limits by moving 'servo' data — data bits required for mechanical stability of the read/write head but which do not carry file data — to a deeper secondary magnetic layer on the disk.

"Servo data provides a position error signal to maintain accurate read/write head tracking. Moving this data to another physical layer could free up more disk area for information storage and also enhance servo tracking, which could lead to higher track density," explains Chen.

The '2.5-dimensional' magnetic recording scheme demonstrated by Chen and his team uses a magnetic head with laser heating to allow non-information-carrying servo data to be written to and read from a second deep magnetic layer while maintaining the read/write performance required for normal data operations in the top layer.

"The main technical challenge we needed to overcome was writing to the bottom servo layer, which has been difficult due to the larger spacing between the servo layer and the write head," says Chen. "Laser heating lowers the magnetic coercivity, which allows the deeper bits to be switched using a relatively weak external magnetic field."

Using a heat assisted-magnetic recording system and multilayer disks which the team fabricated, the researchers demonstrated that various servo bit densities and patterns could be achieved. With the potential to raise effective data density by up to 25 per cent, the dedicated deep servo layer approach is a promising advance for hard disk technology.

1. Chen, Y. J., Yang, H. Z., Leong, S. H., Santoso, B., Shi, J. Z. *et al.* Heat assisted recording on bottom layer of dual recording layer perpendicular magnetic recording media for two and a half dimensional (2.5D) magnetic data storage. *Journal of Applied Physics* **117**, 17C106 (2015)..

Immunology:

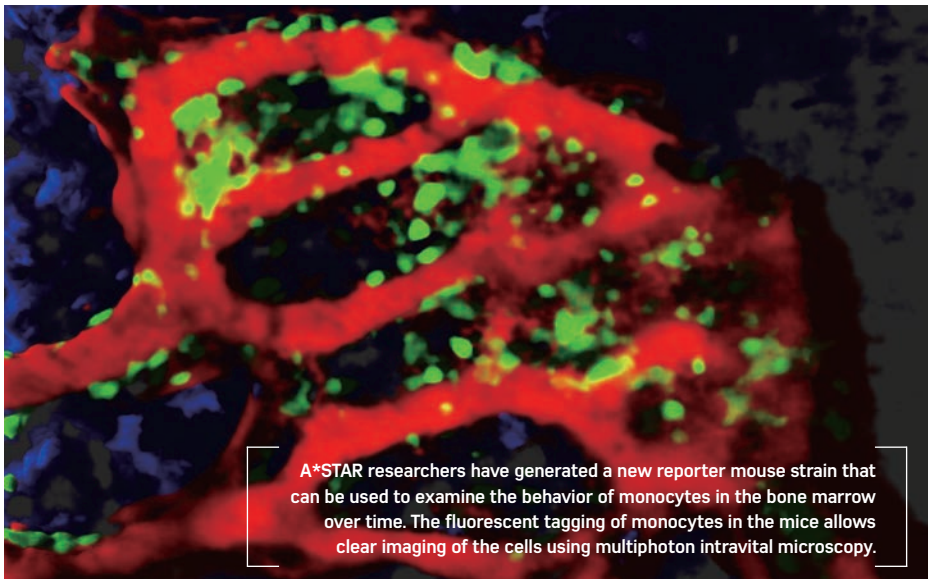
A CLEAR VIEW OF IMMUNE CELLS

A MOUSE STRAIN GIVES THE MOST DETAILED VISUALIZATION YET OF IMMUNE CELLS IN THE BONE MARROW

White blood cells called monocytes, that play a vital role in stable immune responses, can now be visualized over time inside the bone marrow using a new reporter mouse developed by A*STAR researchers[1].

Monocytes mediate inflammation and trigger the body's defense mechanism in response to injury and disease. From the bone marrow, monocytes are sent out into the bloodstream, where they differentiate into tissue macrophages — large cells that 'consume' pathogens. This process is accelerated during disease or infection, and its disruption is implicated in inflammatory diseases such as cancer.

Despite their critical role, little is known about how monocytes are recruited and mobilized in the body. The ability to view monocytes leaving the bone marrow over time would be invaluable (see image). To achieve this goal, A*STAR researcher Lai Guan Ng at the Singapore Immunology Network, and co-workers from institutions across Singapore, adopted multiphoton intravital microscopy, a deep-tissue imaging technique that picks up signals from the fluorescent tagging of specific cell types.



A*STAR researchers have generated a new reporter mouse strain that can be used to examine the behavior of monocytes in the bone marrow over time. The fluorescent tagging of monocytes in the mice allows clear imaging of the cells using multiphoton intravital microscopy.

> "IN CONTRAST, MULTIPHOTON INTRAVITAL MICROSCOPY CAN MONITOR INDIVIDUAL CELLS OVER TIME, REVEALING CRITICAL DETAILS AND PROVIDING A COMPREHENSIVE PICTURE OF THE ENTIRE BIOLOGICAL PROCESS."

"Current approaches track monocyte recruitment using cell counting methods, and only provide static snapshots of what is happening," explains Ng. "In contrast, multiphoton intravital microscopy can monitor individual cells over time, revealing critical details and providing a comprehensive picture of the entire biological process."

Ng and his team tested three genetically-modified mice to determine the best reporter mouse for tracking monocytes. One of the mice, labeled *Cx3cr1*$^{gfp/+}$, produced reasonable images of cell activity in the bone marrow. Dendritic cells — immunity messenger cells — were also tagged by the same fluorescent marker, leading to an unclear confused signal and difficulty in singling out individual monocytes.

"We realized we needed to create a new reporter mouse that labels fewer dendritic cells," says Ng. "We crossed the *Cx3cr1*$^{gfp/+}$ reporter mouse with another strain that produces less dendritic cells. Using this crossbred mouse, we could visualize the monocytes in greater detail than ever before."

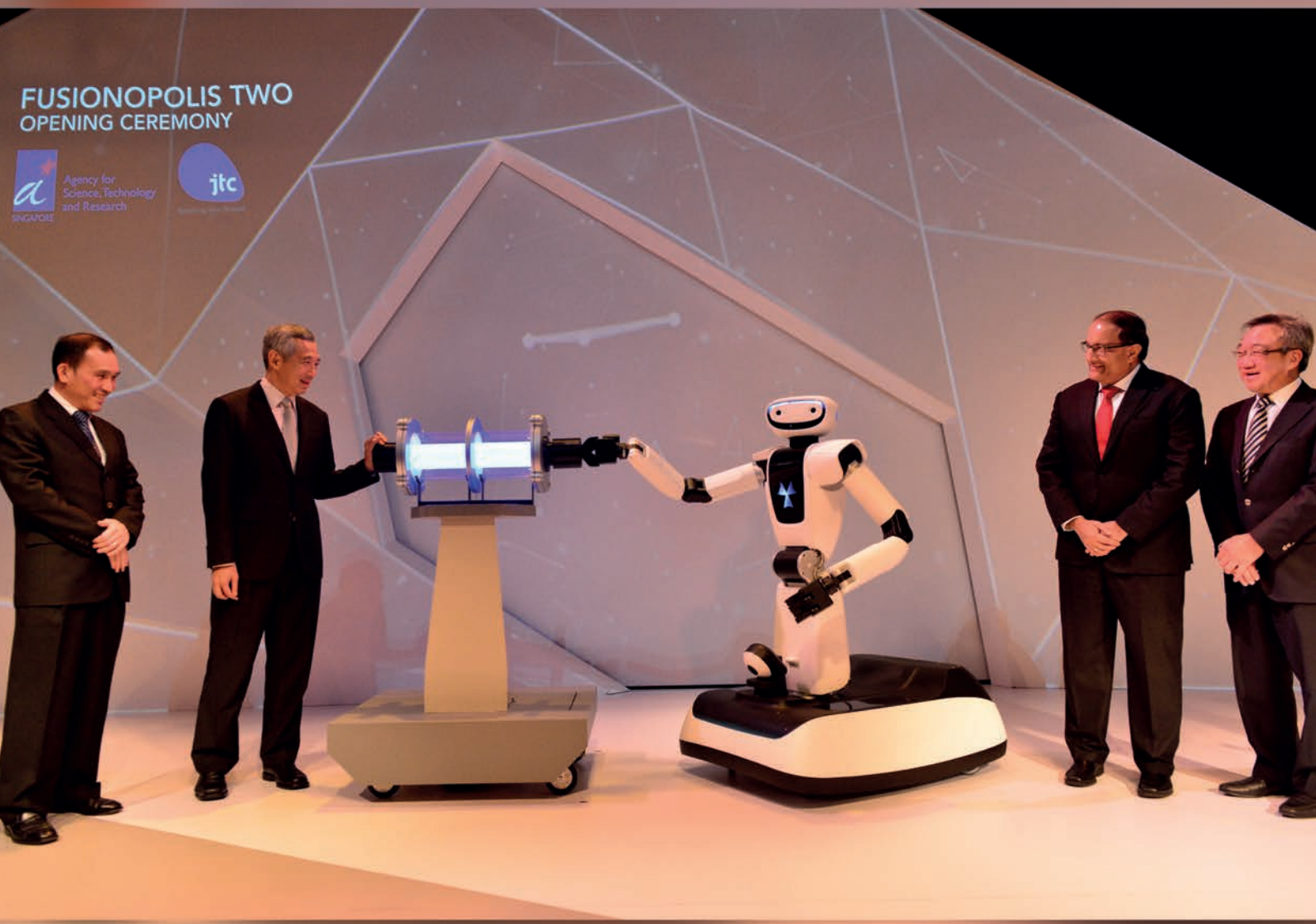
The team found that most monocytes mobilized in response to a trigger signal, such as molecules on the surface of bacteria. Some monocytes were unable to leave the bone marrow however, indicating a retention mechanism is at play. This mechanism may provide a pharmacological target that could mobilize cells from the bone marrow.

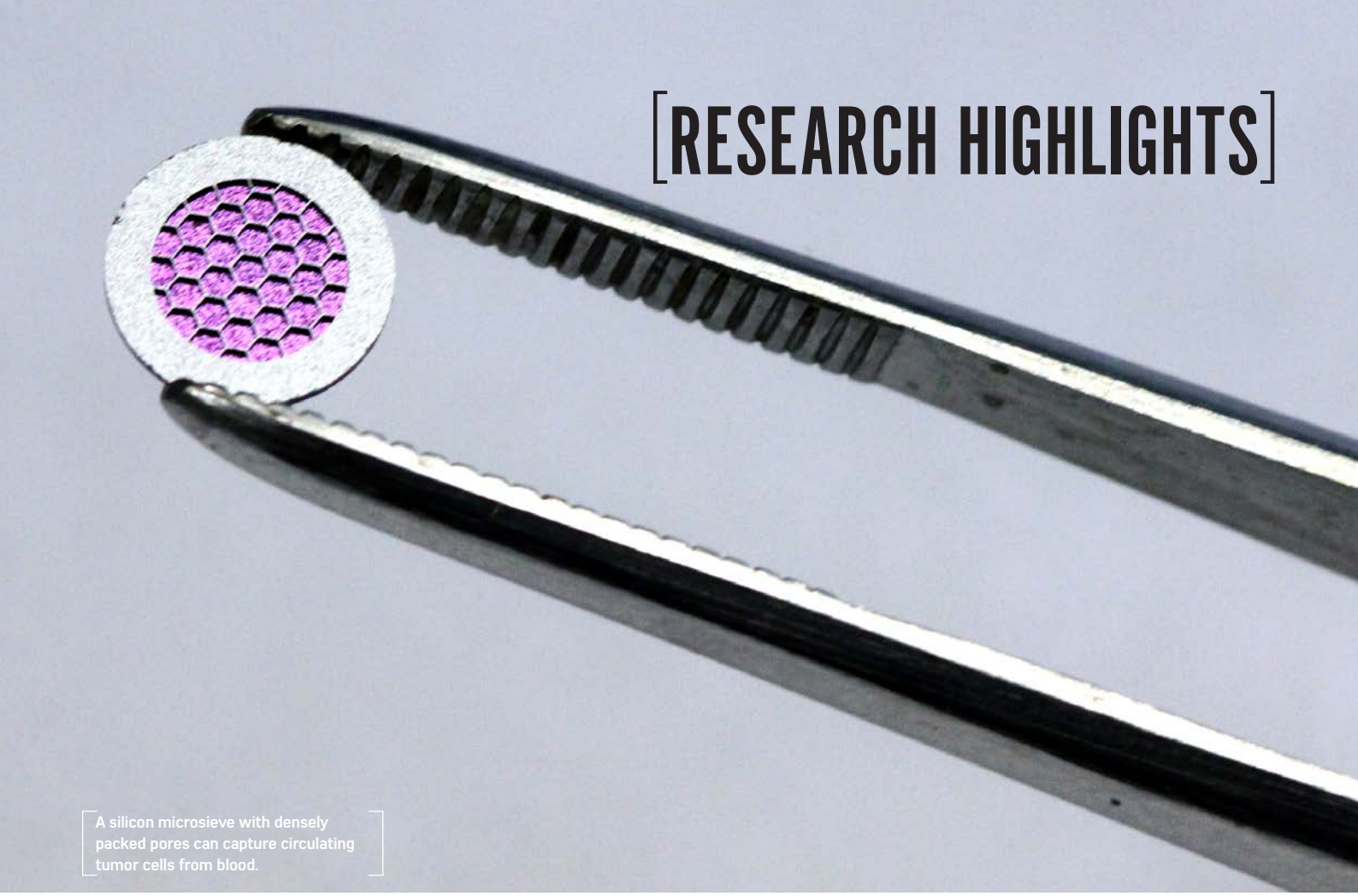
"Our approach could help us understand monocyte behavior in multiple diseases and metabolic conditions," says Ng. "It may also prove a powerful tool to address the efficacy of drugs targeting monocyte mobilization, and may be applied to other cell types involved in immunity in the future."

1. Evrard, M., Chong, S. Z., Devi, S., Chew, W. K., Lee, B. *et al.* Visualization of bone marrow monocyte mobilization using *Cx3cr1*$^{gfp/+}$*Flt3L*$^{-/-}$ reporter mouse by multiphoton intravital microscopy. *Journal of Leukocyte Biology* **97**, 611–619 (2015).

[PICTURE STORY]

Prime Minister Lee Hsien Loong assisted by Olivia the Robot, officially opens Fusionopolis Two, the newest addition to Singapore's R&D hub, one north. From left to right: Mr Lim Chuan Poh, Chairman of A*STAR; Mr Lee Hsien Loong, Prime Minister of Singapore; Olivia the Robot; Mr S. Iswaran, Minister for Trade and Industry (Industry) and Dr Loo Choon Yong, Chairman of JTC Corporation.





A silicon microsieve with densely packed pores can capture circulating tumor cells from blood.

Cancer:

FILTERING FOR FAST ANALYSIS

TRAPPING CELLS THAT TUMORS RELEASE INTO THE BLOODSTREAM ALLOWS FAST AND SENSITIVE GENETIC ANALYSIS

A simple and non-invasive method to detect cancer-related mutations without direct biopsy sampling of tumors has been developed by A*STAR researchers.

The technique uses a tailor-made filter to collect cancer cells circulating in the bloodstream after being shed from the tumor mass. These circulating cells can serve as a liquid biopsy. The study was led by Min-Han Tan and Jackie Y. Ying from the Institute of Bioengineering and Nanotechnology (IBN) of A*STAR, with collaborators from the Genome Institute of Singapore of A*STAR and other research centers in Singapore.

Genetic analysis of cancer cells is crucial for characterizing a cancer type and selecting the most suitable treatment options for a patient. Genetic results also play a role in monitoring the effectiveness of treatment and establishing a likely prognosis, explains Tan.

Many tumors shed cells into the bloodstream and several methods have been developed to try to capture these cells to assist in diagnosis, analysis and treatment. All previous attempts, however, have significant deficiencies, particularly in efficiently separating the cancer cells from other cells circulating in the blood.

"We used special microfabrication technology, which results in very uniform and precisely controlled pore size and depth for isolating the circulating cancer cells," explains Ying.

The silicon micro-sieves (see image) developed using this technique comprise a two-layered structure, with an upper thin porous membrane for cell filtration and a lower layer of thick honeycomb rings to support the membrane.

To prove the validity of their novel approach, the research team used blood samples taken from patients with bowel cancer, the most common type of cancer in Singapore. But, Tan says, their method is also likely to be applicable to a wide range of tumors, such as those in breasts, lungs and kidneys.

Having isolated the cancer cells, detailed genetic analysis was able to identify two specific types of mutation associated with bowel cancer[1]. The analysis process begins directly on the sieve, and is completed within the sampling system, which significantly simplifies and speeds up the procedure. The new method also proved more sensitive than earlier alternatives, confirming that it could be of considerable clinical significance for improved diagnosis and treatment.

The team is now working towards developing the technology for clinical use.

IBN's non-invasive genetic test was licensed to AITbiotech Pte Ltd, a Singapore-based biotechnology company, in January 2015 for development into a ready-to-use test kit. AITbiotech provides molecular diagnostics and genomic services to hospitals and research laboratories.

1. Suhaimi, N.-A. M., Foong, Y. M., Lee, D. Y. S., Phyo, W. M., Cima, I. *et al*. Non-invasive sensitive detection of *KRAS* and *BRAF* mutation in circulating tumor cells of colorectal cancer patients. *Molecular Oncology* **9**, 850–860 (2015).

Immunology:

TELLING MOSQUITO-BORNE VIRUSES APART

A NOVEL DIAGNOSTIC TEST FOR CHIKUNGUNYA VIRUS ALSO HELPS DISTINGUISH THE ILLNESS FROM OTHER MOSQUITO-BORNE DISEASES

The Chikungunya virus (CHIKV) causes similar symptoms to other mosquito-borne viruses, such as dengue. A*STAR researchers have developed a detection assay to identify the presence of CHIKV-specific antibodies for more accurate diagnoses.

A detection assay that identifies the presence of viral-specific antibodies in patients with Chikungunya virus (CHIKV) has been developed by A*STAR researchers[1]. The technique can also help differentiate between CHIKV and other mosquito-borne viruses.

The alphavirus CHIKV and the dengue flavivirus (DENV) cause similar symptoms in the early stages of infection, including fever and joint pain. This similarity makes it difficult for doctors to discern which disease a patient has and administer the appropriate treatment. Simultaneous outbreaks of both diseases in parts of Asia, Africa and the Americas has intensified the need for an effective method for differentiating them.

"We previously demonstrated that the early, naturally-acquired antibody response in patients is directed against a specific part of a CHIKV protein - the E2EP3 epitope," explains Lisa F. P. Ng from the A*STAR Singapore Immunology Network (SIgN). "We realized E2EP3 could play a role in future diagnostic and preventive applications, and this inspired us to conduct the present study."

The team wanted to develop an easy-to-use, accurate detection assay to improve on current techniques for diagnosing CHIKV. They theorized that the presence of anti-E2EP3 antibodies in patients would be a useful indication of CHIKV, particularly if the antibodies were specific to the illness.

"We decided to test the E2EP3 antibody response in patient samples collated from different CHIKV outbreaks, and samples from other mosquito-borne viruses such as DENV,"

explains Ng. "We developed an E2EP3-based assay to determine the prevalence and activity of the antibodies in the samples."

The researchers analyzed 260 blood samples collected from 38 patients with CHIKV during the Singapore outbreak of 2008–2009. From this, 72 per cent of CHIKV patients tested positive for anti-E2EP3 antibodies in the early stages of the illness, rising to 100 per cent as the disease progressed. These antibodies showed strong propensity to neutralize CHIKV, and the new assay was far more accurate than the existing CHIKV diagnosis method.

The team from SIgN and the Environmental Health Institute (National Environment Agency) compared these results with samples from patients infected with different CHIKV strains, other non-CHIKV alphaviruses, and flaviviruses such as DENV. The other alphavirus samples reacted to E2EP3, but only seven per cent of the flavivirus samples reacted. Crucially, none of the flavivirus samples were able to neutralize CHIKV, regardless of the presence of anti-E2EP3 antibodies.

"With this assay we can rapidly diagnose early-stage CHIKV and distinguish it from DENV," says Ng. "Doctors will be able to administer the correct treatment quickly, reducing the costs of unnecessary medication and hospitalization."

1. Kam, Y.-W., Pok, K.-Y, Eng, K. E., Tan, L.-K., Kaur, S. *et al*. Sero-prevalence and cross-reactivity of Chikungunya virus specific anti-E2EP3 antibodies in arbovirus-infected patients. *PLoS Neglected Tropical Diseases* **9**, e3445 (2015).

Supercapacitors:

GRAPHENE'S STABILIZING INFLUENCE

GRAPHENE AND METAL NITRIDES IMPROVE THE PERFORMANCE AND STABILITY OF ENERGY STORAGE DEVICES

Supercapacitors can be charged and discharged tens of thousands of times, but their relatively low energy density compared to conventional batteries limits their application for energy storage. Now, A*STAR researchers have developed an 'asymmetric' supercapacitor based on metal nitrides and graphene that could be a viable energy storage solution[1].

A supercapacitor's viability is largely determined by the materials of which its anodes and cathodes are comprised. These electrodes must have a high surface area per unit weight, high electrical conductivity and capacitance and be physically robust so they do not degrade during operation in liquid or hostile environments.

Unlike traditional supercapacitors, which use the same material for both electrodes, the anode and cathode in an asymmetric supercapacitor are made up of different materials. Scientists initially used metal oxides as asymmetric supercapacitor electrodes, but, as metal oxides do not have particularly high electrical conductivities and become unstable over long operating cycles, it was clear that a better alternative was needed.

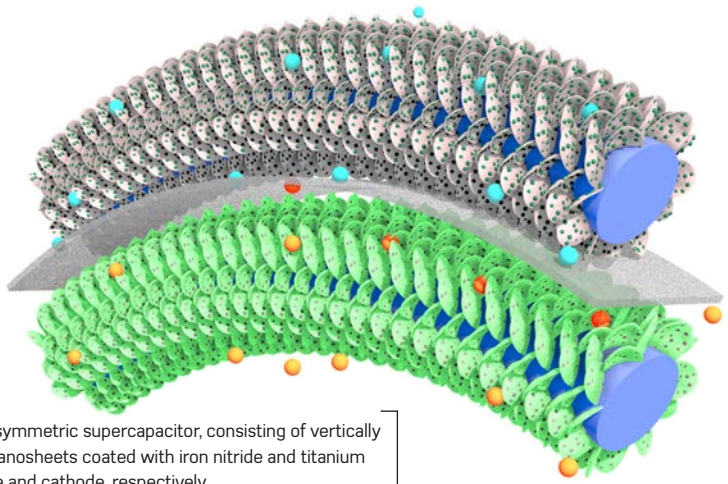


Illustration of the asymmetric supercapacitor, consisting of vertically aligned graphene nanosheets coated with iron nitride and titanium nitride as the anode and cathode, respectively.

Metal nitrides such as titanium nitride, which offer both high conductivity and capacitance, are a promising alternative, but they tend to oxidize in watery environments that limits their lifetime as an electrode. A solution to this is to combine them with more stable materials.

Hui Huang from A*STAR's Singapore Institute of Manufacturing Technology and his colleagues from Nanyang Technological University and Jinan University, China, have fabricated asymmetric supercapacitors which

incorporate metal nitride electrodes with stacked sheets of graphene.

To get the maximum benefit from the graphene surface, the team used a precise method for creating thin-films, a process known as atomic layer deposition, to grow two different materials on vertically aligned graphene nanosheets: titanium nitride for their supercapacitor's cathode and iron nitride for the anode. The cathode and anode were then heated to 800 and 600 degrees Celsius respectively, and allowed to slowly cool. The two electrodes were then separated in the asymmetric supercapacitor by a solid-state electrolyte, which prevented the oxidization of the metal nitrides.

The researchers tested their supercapacitor devices and showed they could cycle 20,000 times and exhibited both high capacitance and high power density. "These improvements are due to the ultra-high surface area of the vertically aligned graphene substrate and the atomic layer deposition method that enables full use of it," says Huang. "In future research, we want to enlarge the working-voltage of the device to increase energy density further still," says Huang.

1. Zhu, C., Yang, P., Chao, D., Wang, X., Zhang, X. *et al.* All metal nitrides solid-state asymmetric supercapacitors. *Advanced Materials* **27**, 4566–4571 (2015).

Immunology:

A MILDER STRAIN OF CHIKUNGUNYA VIRUS?

THE CHIKUNGUNYA VIRUS THAT HAS HIT THE AMERICAS IS LESS SEVERE THAN THE STRAIN THAT WREAKED HAVOC IN LA REUNION

The Chikungunya virus, transmitted via mosquito bite, causes severe joint pain.

The strain of Chikungunya virus (CHIKV) that has traveled across the Americas in the last two years is potentially milder and caused less severe symptoms than the strain responsible for the major Indian Ocean outbreaks of 2004–06, according to new research from A*STAR scientists and their international collaborators[1].

CHIKV is transmitted to humans by mosquitoes and brings high fever, headache and serious joint pain. There is currently no vaccine or treatment. The disease was in the spotlight in 2004 following outbreaks in Africa, India and Southeast Asia. Almost 270,000 people were infected in 2005–06 on La Reunion — about a third of the Indian Ocean island's population.

In late 2013 CHIKV arrived in the Americas, initially to the island of St Martin. Within a year more than a million people were infected across the Caribbean, South and Central America.

Three separate strains of the virus are known, originating from West Africa, East-Central-South African (ECSA) and Asia. French scientists led by Xavier de Lamballerie, of the Aix-Marseille University, identified the Caribbean CHIKV as being of the Asian strain rather than the ECSA genotype behind the Indian Ocean outbreaks.

In collaboration with the French team, Lisa Ng of the A*STAR Singapore Immunology Network and co-workers infected batches of connective tissue cells called fibroblasts taken from mice with two different CHIKV isolates to investigate the responses more closely.

Laboratory tests that measured the level of virus in the samples over a 24-hour period confirmed that the Asian-Caribbean strain replicates more slowly than the ECSA version.

Ng and colleagues also found that mice infected with Asian-Caribbean CHIKV had lower concentrations of virus in their blood and significantly less joint swelling.

"The results were very consistent," says Ng. "We had no idea which would cause more severe swelling until we saw those first results."

"This suggests the symptoms of patients with Asian-Caribbean CHIKV might be somewhat milder to those with the ECSA strain, and that therefore their clinical management might also be different."

The researchers also found important differences in immune system reactions to infection, with ECSA CHIKV triggering greater responses from type 1 T helper (Th1) cells,

which promote inflammation, and from natural killer cells, which target and kill infected cells.

"This suggests that if doctors could control the number of Th1 and NK cells, they could perhaps control the swelling and damage," adds Ng.

1. Teo, T.-H., Her, Z., Tan, J. J. L., Lum, F.-M., Lee, W. W. L. *et al.* Caribbean and La Réunion Chikungunya virus isolates differ in their capacity to induce proinflammatory Th1 and NK cell responses and acute joint pathology. *Journal of Virology* **89**, 7955–7969 (2015).

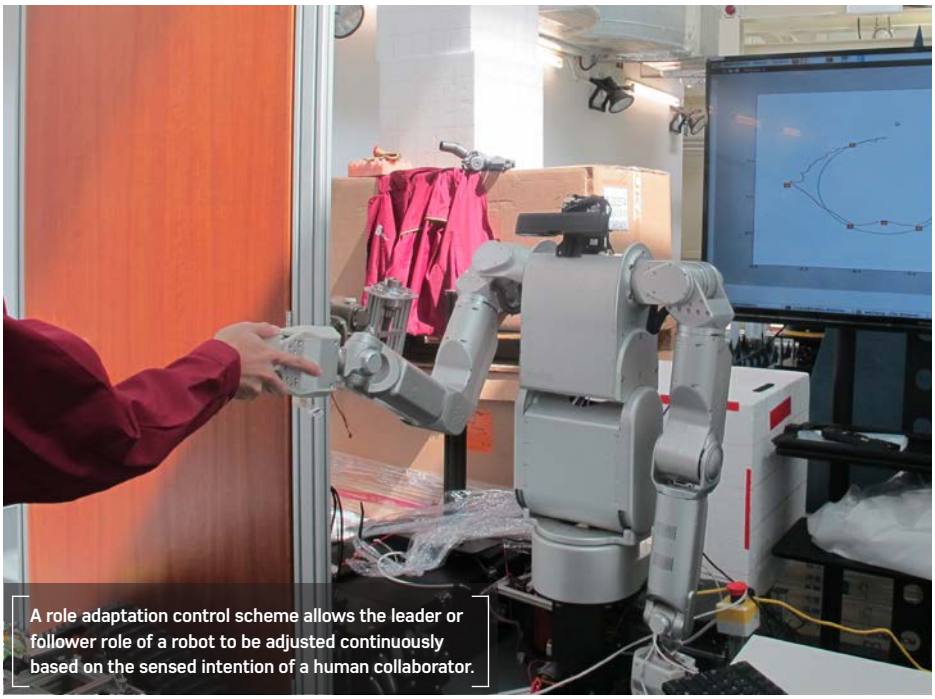
Robotics:

WHO'S THE MASTER?

CONTINUOUS ADAPTATION MAKES FOR MORE NATURAL INTERACTIONS BETWEEN HUMANS AND ROBOTS IN SHARED TASKS

A robot's role in a shared task could be continuously adjusted during the activity, thanks to a new adaptive robot control system developed by A*STAR researchers that can sense whether a human operator wants to lead or follow[1]. The innovation takes human–robot interactions to a new level of sophistication and opens a range of applications for robots that were previously too difficult to achieve.

Humans are assisted by robots in everything from automotive manufacturing through to delicate surgical procedures and even search and rescue operations, but there remains much room for improvement and expansion of their roles. "Robots are still not as intelligent as we expect and this is particularly clear when it comes to human–robot interactions," says Li Yanan from the A*STAR Institute for Infocomm Research (I^2R).



A role adaptation control scheme allows the leader or follower role of a robot to be adjusted continuously based on the sensed intention of a human collaborator.

Though extremely useful for performing repetitive or high-precision tasks and for bearing heavy loads, in many applications robots can only be engaged when guided by a human operator to the start location or object. Once engaged, the robot often takes autonomous control of the task. However, as the role of a robot for any task is usually preset and difficult to change during operations, robots are difficult to manage in tasks where its function might switch frequently, such as in high-mix, low-volume applications like welding, polishing and painting.

To extend the utility of robots to improve such nuanced human–robot collaborations, Li and colleagues from the I^2R set out to develop a control code that sensed the intent of the human operator, based on the force they exerted on the robot, and adjusted the role of the robot automatically.

"In the code, to estimate the human operator's motion intention so that the robot control strategy could be changed, we used game theory, which provides useful tools for analyzing the interactive behavior of players involved in a game," says Li. "The main technical hurdle, however, was the next step — namely the design and development of an intuitive human–robot interface for experimental validation."

The mathematically derived control algorithm determines the intention of the human operator based on the human–robot interaction force, and changes the proportion of control sharing between the human and the robot accordingly. In the team's experiments (see image), the role adaptation control scheme gave better performance results than fixed roles schemes in a range of tasks. "Next, we plan to evaluate this control scheme in a real-world application," says Li.

1. Li, Y., Tee, K. P., Chan, W. L., Yan, R., Chua, Y. & Limbu, D. K. Continuous role adaptation for human-robot shared control. *IEEE Transactions on Robotics* **31**, 672–681 (2015).

A*STAR researchers uncover evidence that the relationship between *HIF3A* methylation levels and weight begins before birth. Their findings may hold significant implications regarding the development of weight-related disorders such as obesity later in life.

Obesity:

ADDING WEIGHT TO THE BIRTH STORY

HIGH LEVELS OF DNA METHYLATION AT A PARTICULAR GENETIC LOCATION ARE LINKED TO GREATER BIRTH WEIGHT AND HIGHER ADIPOSITY

The theory that weight gain is partly predetermined by our DNA, and not just a result of adult habits, has gained traction among obesity researchers. Now, scientists at A*STAR have discovered that epigenetic variations at a genetic region linked to obesity are directly linked to birth weight and an infant's 'adiposity' or baby fat[1].

Our DNA sequence is specified at conception, while epigenetic factors controlling DNA are modified by environment *in utero* and after birth. Variations in DNA methylation levels — the mechanism used by cells to regulate gene expression — have been linked to the development of certain diseases. A recent study of Caucasian adults found a link between methylation levels of the hypoxia inducible factor 3 gene (*HIF3A*) — a gene that responds to decreases in cellular oxygen levels — and body mass index.

"We wanted to build on this study to see if variations in *HIF3A* methylation levels in babies are linked to birth weight," says Joanna Holbrook, who led an international team of scientists with her colleague Neerja Karnani at the A*STAR Singapore Institute for Clinical Sciences (SICS). "If it did, it would suggest that the path to obesity starts early and is not just a consequence of adult behaviors."

The team used DNA samples collected from the umbilical cords of 991 participants in the ongoing Growing Up in Singapore Towards healthy Outcomes (GUSTO) project — a large-scale study of Asian families in Singapore conducted by KK Women's and Children's Hospital, the National University Health System, and the SICS, which examines influences on childhood health and development from pregnancy onwards that started in 2008.

As in the adult study, Holbrook, Karnani and co-workers focused on *HIF3A*, also examining genetic variants linked with the gene. They found that higher *HIF3A* methylation was associated with greater birth weight and infant adiposity. The correlation between weight and *HIF3A* was significant across all genotypes, but the association was strongest in the CC-genotype. This indicates that some people's weight may be linked more strongly to higher *HIF3A* methylation levels than others.

"*HIF3A* levels and weight are already connected at birth," says Holbrook. "Something happens between conception and birth to dictate this, but so far we have not pinpointed the exact cause."

The breakthrough suggests that *HIF3A* could be a useful biomarker for metabolic development. Holbrook adds that a more beneficial breakthrough would be to find a biomarker that shows whether or not an intervention to alter developmental trajectory has been successful.

"Obesity is a complex disorder, and this finding is just the tip of the iceberg," says Karnani. "It highlights the need for an exhaustive investigation of the epigenome both at birth and in the early years."

1. Pan, H., Lin, X., Wu, Y., Chen, L., Teh, A. L. *et al. HIF3A* association with adiposity: The story begins before birth. *Epigenomics* **7**, 937–950 (2015).

Mathematics anxiety:

COUNTING IN *ADAGIO*

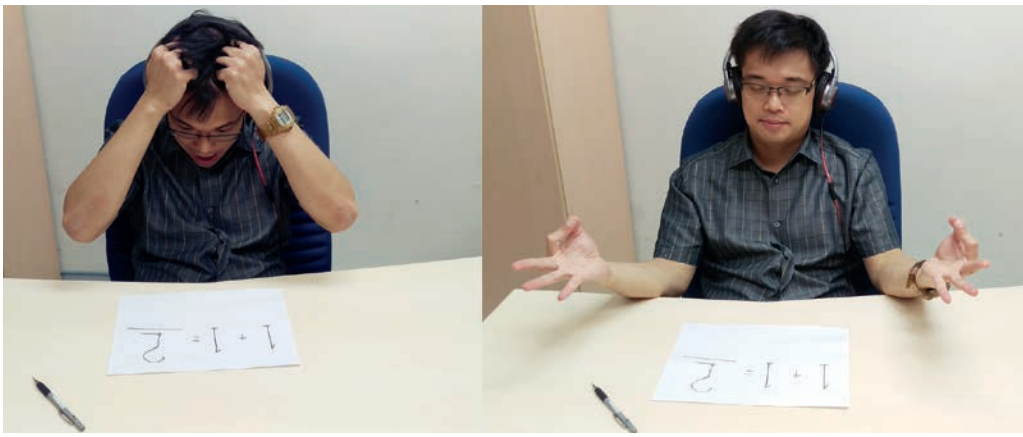
LISTENING TO SLOW AND STEADY MUSIC CAN HELP ALLEVIATE A FEAR OF TESTING IN NUMBERS

Sedative music can reduce the heart rate and blood pressure of people with anxiety about mathematics, suggests Samuel Gan from the A*STAR Bioinformatics Institute[1].

Relaxing music is commonly used to calm the jittery nerves of patients going into surgery. The music is thought to synchronize the listener's heartbeat to a slow tempo of 60 to 80 beats per minute. But while most studies report that subjects report reduced anxiety after listening to calming music, only a few note the associated physiological changes.

"You might need to first become aware of the music and perceive yourself to be relaxed before that awareness is translated into a physiological response of the body," explains Gan, who is also affiliated with Australia's James Cook University in Singapore. Gan admits to having suffered from the phobia in his youth. "I avoided maths all the way — that is why I became a scientist."

Gan and his psychology student at James Cook University wanted to establish a conclusive link between music and its effect on the body. They recruited 105 undergraduate psychology students, aged 19 to 31, to take a timed maths exam. All students reported on their anxiety levels before, during and after the test, and regular measurements were taken of their heart rate and blood pressure.



It can take up to 30 minutes for music to have a relaxing effect on the mind and body of people with mathematics anxiety.

One group was lulled by Beethoven's

Moonlight Sonata: another group listened to Saint-Saëns's sprightly *Allegro Moderato, Symphony No. 3*. A control group was not provided with music.

"Many students seemed to be quite stressed out by the maths questions," says Gan. "Some of them even breathed a sigh of relief at the end." Statistical analysis of the students' self-assessments showed that sedative music reduced their anxiety but stimulating music sustained it. Their heart rates, however, were not as easily swayed by the music, and did not show any change.

"We were a little disappointed with our preliminary analysis," says Gan. "So we dug deeper into our data using different statistical tests and found that there was a physiological effect, characterized by a decrease in systolic blood pressure."

A review of earlier studies suggested an optimal musical relaxation time of 30 minutes. To explain his inconclusive results, Gan proposed a 'Perception to Physiology' or 'P2P' model, which states that time, familiarity with and fondness for the sedative music helps to decrease blood pressure.

Gan plans to conduct further studies to verify the model. "The implications are not limited to mathematics anxiety."

1. Gan, S. K., Lim, K. M.-J. & Haw, Y.-X. The relaxation effects of stimulative and sedative music on mathematics anxiety: A perception to physiology model. *Psychology of Music advance* online publication, 19 June 2015 (doi: 10.1177/0305735615590430).

Rehabilitation:

ELECTRIC ASSISTANCE FOR STROKE RECOVERY

ROBOTIC THERAPY WORKS BETTER IF STROKE PATIENTS' BRAINS ARE STIMULATED BY ELECTRICITY

Research that could help stroke victims with severe disabilities to regain control over their limbs has been produced by a team at A*STAR. They have shown that stimulating the brain with electric current can help stroke victims use brain–computer interfaces (BCIs) to interact with therapeutic robotic systems[1].

While repetitive exercises can help some stroke survivors regain control over their motor functions, not all patients are physically capable of executing the required movements. An alternative is 'motor imagery', in which patients can try to repair impaired neurological pathways by imagining movements without physically executing them. Studies have shown motor imagery to be an effective neurological rehabilitation technique.

"Patients can imagine the movement of their limbs, but their thoughts cannot be seen by doctors that want to monitor their progress," explains Kai Keng Ang from the A*STAR Institute for Infocomm Research. "A BCI lets us detect a patient's thoughts using electroencephalogram (EEG) recordings, and then a feedback system moves the limb using robots. This shows patients if they have imagined the movement correctly."

Set up for a brain computer interface rehabilitation system incorporating transcranial direct current stimulation.

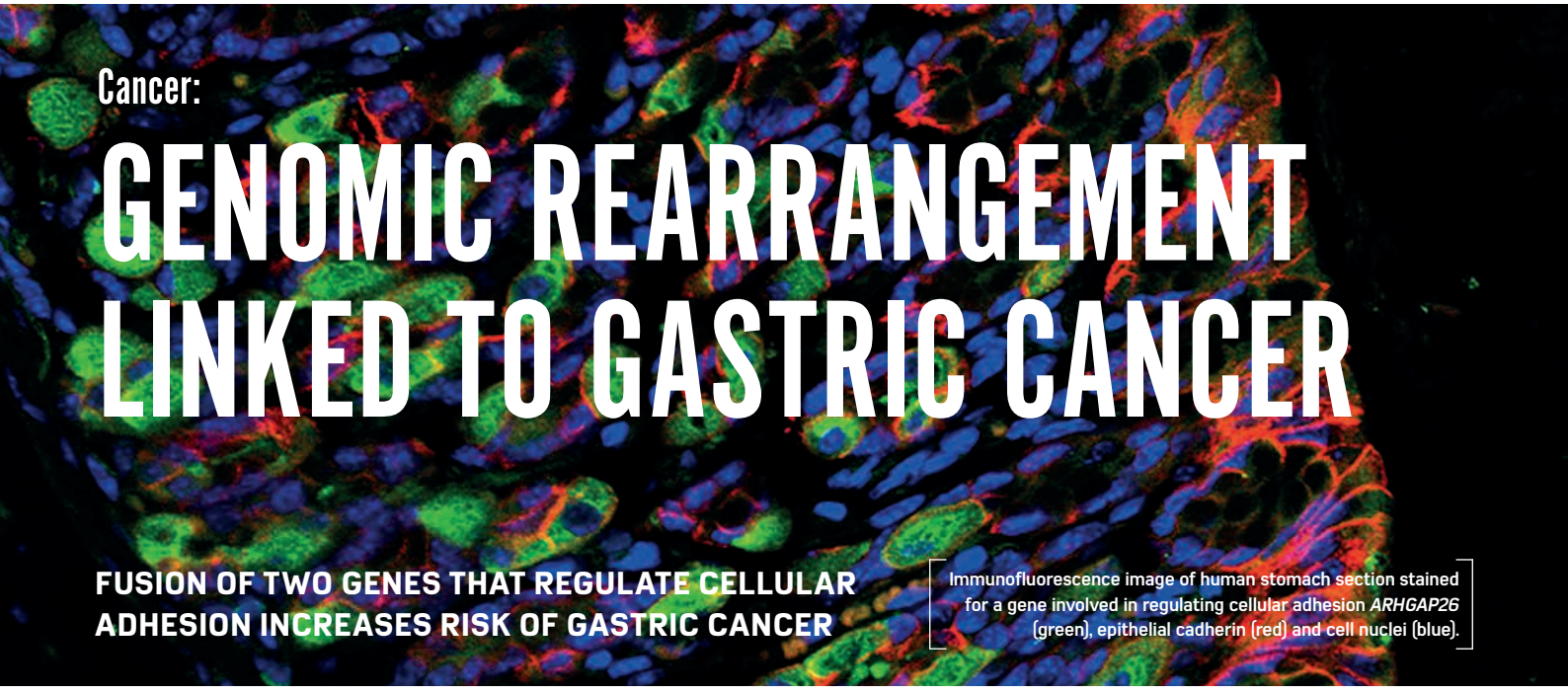
The combination of motor imagery and BCI feedback can help the brain rebuild damaged neural pathways, but is not successful with every patient. Ang and co-workers, in collaboration with researchers across Singapore and in Australia, wanted to investigate whether patients could get better at using a BCI if their brain was first subjected to transcranial direct current stimulation (tDCS) — the application of an external electric current to the skull.

The researchers selected 19 stroke patients with impaired arm movements. They randomly assigned 10 patients to receive tDCS for 20 minutes and 9 patients to receive 'sham-tDCS' — in which current was only applied for the first 30 seconds to give the sensation of stimulation. The patients then underwent several BCI trials in which they were asked to imagine either moving their arm, or remaining idle.

The trials showed that the group receiving tDCS were significantly more accurate than the sham-tDCS group in generating the required motor imagery signals. The tDCS group also showed a higher indication of event-related desynchronisation, a well-established neurophysiological observation that when we move one of our hands or imagine the movement, the amplitude of certain EEG frequencies in the opposite hemisphere of the brain decreases.

The researchers hope that tDCS could soon be combined with existing rehabilitation techniques, and help more patients access the benefits of BCI therapy. "We are working on algorithms to improve the detection of motor imagery from EEGs, and conducting research on using tDCS with BCI for lower limb rehabilitation, such as walking," says Ang.

1. Ang, K. K., Guan, C., Phua, K. S., Wang, C., Zhao, L. *et al.* Facilitating effects of transcranial direct current stimulation on motor imagery brain-computer interface with robotic feedback for stroke rehabilitation. *Archives of Physical Medicine and Rehabilitation* **96**, S79–S87 (2015).



Cancer:

GENOMIC REARRANGEMENT LINKED TO GASTRIC CANCER

FUSION OF TWO GENES THAT REGULATE CELLULAR ADHESION INCREASES RISK OF GASTRIC CANCER

Immunofluorescence image of human stomach section stained for a gene involved in regulating cellular adhesion *ARHGAP26* (green), epithelial cadherin (red) and cell nuclei (blue).

Researchers at A*STAR have identified a genomic rearrangement linked to the development and spread of gastric cancer[1].

The DNA in our cells is subject to constant wear and tear from copying mistakes during replication to breaks which sever a chromosome. Extensive repair mechanisms helps cells cope with this damage, but the process is not perfect — when fragments of two broken chromosomes join to form a hybrid chromosome, the genes adjacent to the break sites may fuse. These 'fusion genes' are associated with a higher cancer risk.

"I'm amazed what a mutational load every cell in our body has to face every day, and the repair systems and redundancy that let us tolerate so much damage," says Axel Hillmer of the A*STAR Genome Institute of Singapore, who led the study. "These mechanisms, however, can also result in mutations which cause cancer."

To identify fusion genes related to gastric cancer, Hillmer's team compared the genomes of cells from tumors to genomes of normal cells from the same patients. Five of these 'fusions' were more common than expected in the gastric cancer samples, suggesting they increase the likelihood of tumor formation.

One of the fusions in the gastric cancer sample combined two genes involved in regulating cellular adhesion, *CLDN18* and *ARHGAP26*. The team used a variety of approaches to investigate the changes wrought by the fusion gene and discovered that it reduces cellular adhesion and aggregation. This weakens the connection between cells in the gastric epithelium, allowing stomach acids to leak out, which leads to chronic gastric inflammation and increases the risk of gastric

cancer. Once the cells reach a cancerous state, the fusion gene also enhances their invasiveness and their propensity to metastasize. The fusion therefore plays an important role in both the early and late stages of cancer progression.

"This isn't a classic oncogene, but it has other features that make it important for cancer. That makes it special — it's uncommon to find a cancer gene that isn't an oncogene or a tumor suppressor," says Walter Hunziker, co-senior author of the study.

Unlike oncogenes, which make a cell cancerous, the fusion gene contributes to conditions that are likely to lead to cancer. Though the impact of oncogenes makes them easier to detect and characterize than more subtle players such as these fusion genes — which Hillmer suspects may be more common than realized — both hold promise for cancer diagnosis.

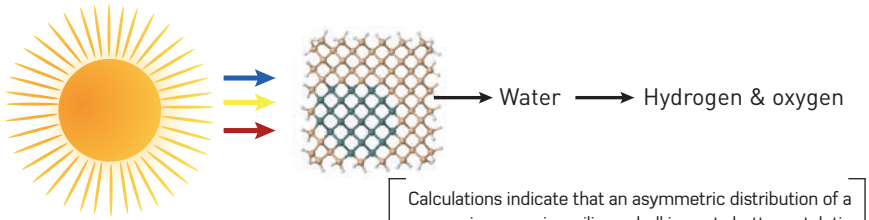
As the *CLDN18–ARHGAP26* fusion was not detected in normal gastric tissue, the researchers are hopeful about its potential as an early biomarker for gastric cancer, following further validation.

1. Yao, F., Kausalya, J. P., Sia, Y. Y., Teo, A. S. M., Lee, W. H. *et al.* Recurrent fusion genes in gastric cancer: *CLDN18–ARHGAP26* induces loss of epithelial integrity. *Cell Reports* **12**, 272–285 (2015).

Photocatalysis:

PROSPECTING FOR GREEN CATALYSTS

CALCULATIONS INDICATE THE BEST PLACES TO LOOK FOR NANOWIRE CATALYSTS TO ACCELERATE GREEN REACTIONS



Calculations indicate that an asymmetric distribution of a germanium core in a silicon shell imparts better catalytic properties to nanowires than symmetric distributions.

A*STAR scientists have created a 'prospector's guide' to help researchers locate the best germanium–silicon nanowires for catalyzing important clean-energy reactions[1].

Using sunlight to split water into its constituent elements, or to convert carbon dioxide into carbon monoxide or hydrocarbons, are among the most viable methods for reducing greenhouse gas emissions. To realize their potential, both clean-energy reactions require catalysts.

Silicon is advantageous because its properties are well studied and it is an abundant material, but the 'band gap' between its conduction and valence bands is too narrow to effectively catalyze these reactions. This deficiency can be overcome in two ways: by 'nanosizing' silicon or by experimenting with different alloys of silicon.

Now, Teck Leong Tan and Man-Fai Ng at the A*STAR Institute of High Performance Computing have used computer simulations to explore the effect of varying the diameter of germanium–silicon nanowires and also the ratio of silicon to germanium on the catalytic properties of the alloy.

Explaining the aim of the study, Tan says, "we thought that by varying both the nanowire diameter and the alloy composition, we could generate a larger design space for engineering a material with the optimal band gap and band structures to photocatalyze clean-energy reactions such as solar water splitting and carbon dioxide reduction."

> "THE COMBINATION OF THE THREE TECHNIQUES PROVIDES A POWERFUL METHODOLOGY FOR HIGH-THROUGHPUT SCREENING OF ALLOY NANOSTRUCTURES FOR DESIRABLE PROPERTIES. IT CAN BE ADAPTED TO OTHER APPLICATIONS TO ACCELERATE THE DISCOVERY OF NOVEL MATERIALS."

The pair combined three established computational methods to perform their calculations: density functional theory, cluster expansion and Monte Carlo simulations. While this combination of techniques has been used before, the researchers discovered a simple correlation that enabled them to predict band gaps accurately using simpler computational methods. This considerably reduced the computational cost, enabling more alloy nanostructures to be screened than usual.

The results indicate that germanium–silicon nanowires with diameters of 3 nanometers or smaller are suitable photocatalysts for both water splitting and carbon dioxide reduction. Their calculations also predict that nanowires with asymmetric core–shell structures (see image) will be more effective than those with conventional symmetric ones. Finally, nanowires with diameters between 2 and 3 nanometers should have band gaps that are well matched to the spectrum of sunlight, making them effective light harvesters.

According to Tan, this demonstrates that "the combination of the three techniques

provides a powerful methodology for high-throughput screening of alloy nanostructures for desirable properties. It can be adapted to other applications to accelerate the discovery of novel materials."

The two scientists are eager to collaborate with experimentalists to confirm the predictions generated by their calculations. They also intend to apply the technique to other promising semiconductor nanowire alloys.

1. Tan, T. L. & Ng, M.-F. Computational screening for effective $Ge_{1-x}Si_x$ nanowire photocatalyst. *Physical Chemistry Chemical Physics* **17**, 20391–20397 (2015).

Fluid mechanics:

A SLIPPERY SLOPE

INVESTIGATING HOW DROPLETS MOVE AROUND ON A SURFACE SHOWS US WHY IT IS IMPORTANT TO SET BOUNDARY CONDITIONS

Not everyone ponders sets of partial differential equations when watching droplets slide down a window on a rainy day, but, thanks to new research from A*STAR, those who are so inclined now have what they need to construct a robust physical and mathematical explanation of what they see[1].

A 'contact line' is formed when a fluid meets a solid wall. "Contact lines are ubiquitous in nature," explains Weiqing Ren of the A*STAR Institute of High Performance Computing. Everyday life is replete with them, from the condensation on the outside of a frosty glass on a hot day through to the milk split on the kitchen table. We engage daily with what physicists call the 'moving contact line problem' without even realizing.

The motion of liquid droplets on a solid surface can now be robustly described using a first-principles hydrodynamic model.

Explaining how a droplet spreads out on a surface seems like it should be simple, yet the moving contact line problem has been contentious. This debate is largely about how 'slip', or the movement of the contact line, should be treated in the mathematical models[2].

The classical hydrodynamic model assumes that fluids do not slip on a solid surface. This assumption is an example of a restriction placed on the solutions of differential equations at the edge of a given domain or area, known as a 'boundary condition'.

As the 'no-slip condition' leads to unrealistic, or 'unphysical' behavior at the contact line, theoreticians have proposed a number of modifications to overcome this issue. Ren notes, however, that "most of these models are phenomenological in nature and it is not clear whether they correctly describe the real physics near the moving contact line."

Ren and colleagues combined mathematical and computational methods — including continuum theory, molecular dynamics and multiscale techniques — with two sets of boundary conditions: one for the macroscopic regime where the droplet's motion can be safely described using the no-slip condition, and another for the microscopic regime around the contact line where slip plays an important role. These components were drawn together to form a sophisticated 'first-principle' hydrodynamic model that describes exactly how a liquid drop moves on a surface.

Aside from explaining common occurrences — such as a detergent's ability to remove an oil drop from a solid surface or the interaction of an ink drop with paper during ink-jet printing — the team's model also provides insights to support device design in developing fields such as microfluidics. "This model helps us to examine many important physical phenomena using analytic and computational tools," says Ren.

1. Ren, W., Trinh, P. H. & E, W. On the distinguished limits of the Navier slip model of the moving contact line problem. *Journal of Fluid Mechanics* **772**, 107–126 (2015).
2. Ren, W., Hu, D. & E, W. Continuum models for the contact line problem. *Physics of Fluids* **22**, 102103 (2010).

PINPOINTING THE GENETIC BASIS OF DISEASE

GENOME-WIDE ASSOCIATION STUDIES CAN INFORM US ABOUT A RANGE OF DISEASES, INCLUDING PSORIASIS

Genome-wide association studies involve sifting through large volumes of genetic data to find small disease-related differences in the hope of using that information to establish more effective treatments.

The Human Genetics group at the A*STAR Genome Institute of Singapore.

The human genome contains 3.2 billion nucleotides, chained together in long, linear sequences of DNA. Differences in single nucleotides known as single-nucleotide polymorphisms (SNPs) account for a lot of the genetic variation in a population, and can be associated with disease. Jianjun Liu, head of the Human Genetics group at the A*STAR Genome Institute of Singapore (GIS), is using genome-wide association studies to scan large volumes of genetic data for that single unit out of place in people suffering psoriasis, a condition characterized by scaly, flaky or itchy skin that affects approximately three per cent of the world's population.

"Genome-wide association studies can sharpen our understanding of disease mechanisms, and help us to develop new treatments or strategies for identifying people at high risk of developing a disease," says Liu.

Liu joined the GIS in 2002, becoming head of the Human Genetics group in 2007 and deputy director of GIS research programs in 2012. He has spearheaded the institute's involvement in huge international collaborations to identify the genetic basis of disease inheritance and susceptibility. "Human genetics analysis is a powerful tool for dissecting many different kinds of disease with significant family risk, and thus genetic susceptibility."

Liu searches for observable traits, known as phenotypes, which clearly demonstrate a disease's genetic basis, and then sets about finding doctors with expertise in that disease and clinical resources. "Once those two conditions are in place, I apply my tools for human genetic analysis to help doctors gain a first-hand understanding of the kind of variants and related genes that are involved in the disease environment," he explains. Liu has taken this approach to study a wide range of diseases, from cancer and neurological conditions, such as schizophrenia, through to autoimmune disorders.

> "GENOME-WIDE ASSOCIATION STUDIES CAN SHARPEN OUR UNDERSTANDING OF DISEASE MECHANISMS, AND HELP US TO DEVELOP NEW TREATMENTS OR STRATEGIES FOR IDENTIFYING PEOPLE AT HIGH RISK OF DEVELOPING A DISEASE."

Liu's recent interest in psoriasis was piqued when he noted that the condition has an estimated heredity of up to 80 per cent. "It was very natural for me as a geneticist to pick psoriasis," he explains.

Many genetic studies have looked at the disease, but Liu was the first to conduct a trans-ethnic genome-wide meta-analysis across different ethnic populations for psoriasis. Liu and his team compared genetic data from seven independent Chinese and Caucasian population samples, consisting of information on about 4 million SNPs in more than 5,000 patients and 8,000 healthy controls, to see if there were any significant psoriasis-related genetic differences between patients and healthy controls as well as the Chinese and Caucasian cohorts.

Surprisingly, the team discovered that of the 45 genetic loci associated with psoriasis susceptibility — four of which were discovered during the course of the study — ten were only found in the Caucasian population[1]. This goes some way towards explaining why Caucasians are approximately ten times more likely to experience psoriasis than the ethnic Chinese population.

Liu notes that this type of cohort-based study is extremely useful for understanding differences in genetic susceptibility to disease at the ethnic-population level and for identifying high risk groups.

Moving forward, Liu emphasizes the need for more studies focusing on non-European populations, including Asian populations, and for collaborative research involving molecular biologists, physicians and geneticists. He hopes to work closely with clinicians to design more effective, targeted treatments that account for ethnic differences.

1. Yin, X., Low, H. Q., Wang, L., Li, Y., Ellinghaus, E. *et al.* Genome-wide meta-analysis identifies multiple novel associations and ethnic heterogeneity of psoriasis susceptibility. *Nature Communications* **6**, 6916 (2015).

A 3D rendered illustration of a coronavirus: these are a family of viruses that represent a significant threat to public health.

Immunology:

HOSTING UNWELCOME GUESTS

A TRAWL THROUGH THE HUMAN GENOME FOR FACTORS IN THE REPLICATION OF BRONCHITIS VIRUS IN HUMAN LUNG CELLS REVEALS PATHWAYS THAT CAN BE TARGETED FOR THERAPEUTIC DEVELOPMENT

Viral replication and spread throughout a host organism employs many proteins, but the process is not very well understood. Scientists at A*STAR have led a collaborative study to learn which host factors play a key role in viral replication[1]. The aim was to identify host pathways and processes that operate at various stages of infection by a bronchitis virus that could be targeted to fight viruses.

Led by Frederic Bard at the A*STAR Institute of Molecular and Cell Biology, the researchers infected human lung cancer cells with a special bronchitis virus that causes the cell to glow when it replicates. The bronchitis virus belongs to a family, known as coronaviruses, which represent a significant threat to public health: they cause diseases such as severe acute respiratory syndrome (SARS), Middle East respiratory system (MERS), as well as the common cold.

The method by which a virus can infect cells and replicate requires multiple steps. The first step is for the virus to bind to receptors on the host cell membrane, which then folds to enclose the virus in a process called endocytosis. Next the virus is uncoated so that its genome can enter the host cell cytoplasm to allow for host factors to create viral proteins. Finally, the viral particles are generated and secreted from the host cell to allow for viral spread.

To determine which factors in the host cell are required for viral replication the researchers systematically knocked down the expression of more than 21,000 human genes in the cells. They identified 83 factors in eight different host cellular compartments that are recruited by the virus.

The pertinent pathways include ones that play a role in the movement of vesicles from

one part of the cell to another, that splice immature RNAs into more mature forms, and that degrade or refold proteins.

One protein identified by the researchers is called valosin-containing protein (VCP), which they were surprised to find plays a powerful role in viral replication. To determine which part of viral replication it regulates, they looked at which stage the viral assembly got stuck.

"BLOCKING VIRUSES IN ENDOSOMES COULD HELP THE CELLS TO DEGRADE THEM MORE EFFECTIVELY AND THE BODY TO GET RID OF THEM WITHOUT MUCH TOXICITY."

They found that knocking down VCP still permitted viral entry into the cell, but caused viruses to get stuck in vesicle structures called early endosomes, rendering the viral genome unable to move into the cytoplasm. "Blocking viruses in endosomes could help the cells to degrade them more effectively and the body to get rid of them without much toxicity," explains Bard.

1. Wong, H. H., Kumar, P., Tay, F. P. L., Moreau, D., Liu, D. X. *et al.* Genome-wide screen reveals valosin-containing protein requirement for coronavirus exit from endosomes. *Journal of Virology* **89**, 11116–11128 (2015).

Nanomaterials:

ALL THAT GLITTERS IS STILL GOLD

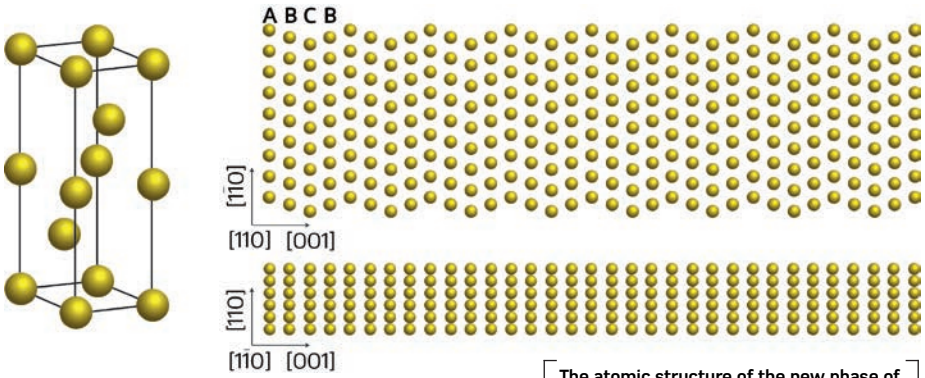
NANOSCALE RIBBONS OF A NEW PHASE OF GOLD HAVE BEEN PRODUCED WITH A DIFFERENT CRYSTALLINE STRUCTURE

A new and stable phase of gold with different physical and optical properties from those of conventional gold has been synthesized by A*STAR researchers[1], and promises to be useful for a wide range of applications, including plasmonics and catalysis.

Many materials exist in a variety of crystal structures, known as phases or polymorphs. These different phases have the same chemical composition but different physical structures, which give rise to different properties. For example, two well-known polymorphs of carbon, graphite and diamond, arranged differently, have radically different physical properties, despite being the same element.

Gold has been used for many purposes throughout history, including jewelry, electronics and catalysis. Until now it has always been produced in one phase — a face-centered cubic structure in which atoms are located at the corners and the center of each face of the constituent cubes.

Now, Lin Wu and colleagues at the Institute of the A*STAR Institute of High Performance Computing have modeled the optical and plasmonic properties of nanoscale ribbons of a new phase of gold — the 4H hexagonal phase (see image) — produced and characterized by collaborators at other institutes in Singapore, China and the USA. The team synthesized nanoribbons of the new phase by simply heating the gold (III) chloride hydrate ($HAuCl_4$) with a mixture of three organic solvents and then centrifuging and washing the product. This gave a high yield of about 60 per cent.



The atomic structure of the new phase of gold synthesized by A*STAR researchers.

The researchers also produced 4H hexagonal phases of the precious metals silver, platinum and palladium by growing them on top of the gold 4H hexagonal phase.

The cubic phase looks identical when viewed front on, from one side or from above. In contrast, the new 4H hexagonal phase lacks this cubic symmetry and hence varies more with direction — a property known as anisotropy. This lower symmetry gives it more directionally varying optical properties,

which may make it useful for plasmonic applications. "Our finding is not only is of fundamental interest, but it also provides a new avenue for unconventional applications of plasmonic devices," says Wu.

The team is keen to explore the potential of their new phase. "In the future, we hope to leverage the unconventional anisotropic properties of the new gold phase and design new devices with excellent performances not achievable with conventional face-centered-cubic gold," says Wu. The synthesis method also gives rise to the potential for new strategies for controlling the crystalline phase of nanomaterials made from the noble metals.

1. Fan, Z., Bosman, M., Huang, X., Huang, D., Yu, Y. *et al.* Stabilization of 4H hexagonal phase in gold nanoribbons. *Nature Communications* **6,** 7684 (2015).

Nutrition:

STEAMING AHEAD FOR A LOW-GI LOAF

ON THE GLYCEMIC INDEX, STEAMING PRODUCES HEALTHIER BREAD THAN BAKING

Asian-style steamed bread has a lower glycemic index (GI) than western-style baked bread, A*STAR researchers have found[1]. This preparation method could be more widely adopted as a useful weapon in the battle against diseases such as obesity, heart disease and diabetes, they suggest.

The four types of bread made and tested by the A*STAR team. Top left, western baked bread; top right, modified baked bread (made with steamed bread ingredients); bottom left, oriental steamed bread; bottom right, modified steamed bread (made with baked bread ingredients).

Carbohydrates are a vital source of energy in the human diet, typically contributing up to 70 per cent of total energy intake. But, not all carbohydrates are equally wholesome. The glycemic index of food is used to measure how rapidly carbohydrates are converted to blood glucose — their 'glycemic response'. High-GI foods cause a rapid spike in blood glucose, while low-GI foods, considered 'healthier,' increase blood glucose gradually and sustain it for longer.

A team led by Jeyakumar Henry, of the A*STAR Institute for Clinical Sciences, examined the GI of bread, a carbohydrate-rich staple usually made from wheat flour. The GI of bread can vary significantly based on several factors, including the way in which it is cooked.

Henry and team compared bread baked in an oven, the traditional method in western countries, with the Asian-style bread, which is usually steamed. To assess the impact of both the ingredients and manufacturing methods, they also compared bread made with western ingredients but prepared using Asian methods (including steaming), and bread made with Asian ingredients processed with western methods (including baking).

The researchers tested the four types of bread in the laboratory, and by measuring the glycemic response of volunteer consumers — if they could stop themselves from eating it first! "It was hard to keep my hands off the freshly-baked bread," said Henry.

> "GIVEN THE WORLDWIDE INTEREST IN INTERNATIONAL CUISINE, IT MAY NOT BE LONG BEFORE WESTERN CONSUMERS TAKE TO STEAMED BREAD, PARTICULARLY WHEN THEY KNOW IT HAS ADDITIONAL HEALTH BENEFITS."

Both lab analysis and consumer blood tests revealed that the highest GI was found in bread made with Asian ingredients under western methods, and the lowest GI was found in bread made with western ingredients under Asian methods. Overall, processing, including cooking method, had a greater impact on GI than ingredients. Thus, the A*STAR team concluded, steaming is a healthier way of making bread than baking.

Henry believes the implications may be far-reaching: "Given the worldwide interest in international cuisine, it may not be long before western consumers take to steamed bread, particularly when they know it has additional health benefits."

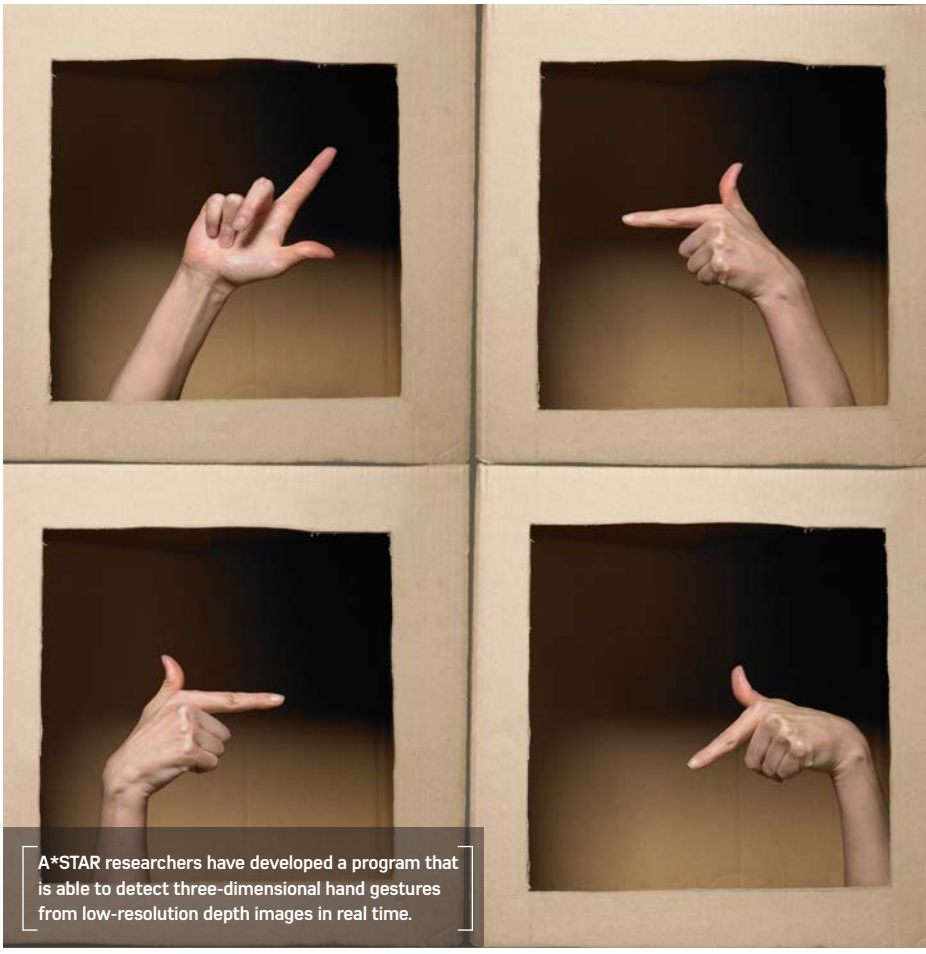
This research points to a whole new approach to reducing the GI of foods via processing and cooking methods, in addition to the established modification of the raw ingredients. Both the Health Promotion Board and commercial bread manufacturers in Singapore have already expressed an interest in the findings.

1. Lau, E., Soong, Y. Y., Zhou, W. & Henry, J. Can bread processing conditions alter glycaemic response? *Food Chemistry* **173**, 250–256 (2015).

Robotics:

READING THE SIGNS

TECHNOLOGY FOR FAST AND EFFICIENT DETECTION OF HAND POSES COULD LEAD TO ENHANCED HUMAN-COMPUTER INTERACTIONS



A*STAR researchers have developed a program that is able to detect three-dimensional hand gestures from low-resolution depth images in real time.

A mobile phone that responds to hand signals rather than the touch of a button may soon be possible thanks to technology developed by A*STAR researchers that efficiently detects three-dimensional human hand movements from two-dimensional images in real time[1]. Combining this technology with devices such as laptops or mobile phones can facilitate robot control and enable human-computer interactions.

Extracting correct three-dimensional hand poses from a single image, especially in the presence of complex background signals, is challenging for computers. As the computer has to determine the general position of the hand, as well as each finger, the extraction of hand movements requires the analysis of many parameters. Optimizing these parameters at the same time would be extremely computationally demanding.

To simplify these calculations, Li Cheng and colleagues from the A*STAR Bioinformatics Institute developed a model that breaks this process into two steps. First, the general position of the hand and the wrist is determined. As a second step, the palm and individual finger poses are established using the anatomy of the human hand as a guide for the computer to narrow its options. Separating the task into two steps reduces the overall complexity of the algorithm, and accelerates computations.

Cheng notes that their method is efficient and outperforms competing techniques, "we can estimate three-dimensional hand poses efficiently with processing speeds of more than 67 video frames per second and with an accuracy of around 20 millimeters for finger joints." This speed is faster than usual video signals and therefore allows for real time analysis. The model extracts information for a broad range of poses, under different lighting conditions, and even if a person wears gloves.

Processing video signals in real time may open the door to better human-machine interactions, says Cheng. "For example, instead of the touchscreen technologies used in current mobile phones, future mobile phones might allow a user to access desired

apps by simply presenting unique hand poses in front of the phone, or by typing on a 'virtual' keyboard without having to access a physical keyboard."

The field of image processing and the extracting information from hand poses or other types of human motion has become very competitive, adds Cheng, noting that the two-step model developed by the researchers could well lead to more sophisticated ways of rendering three-dimensional human forms and gestures.

1. Xu, C., Nanjappa, A., Zhang, X. & Cheng, L. Estimate hand poses efficiently from single depth images. *International Journal of Computer Vision* **116**, 21–45 (2016).

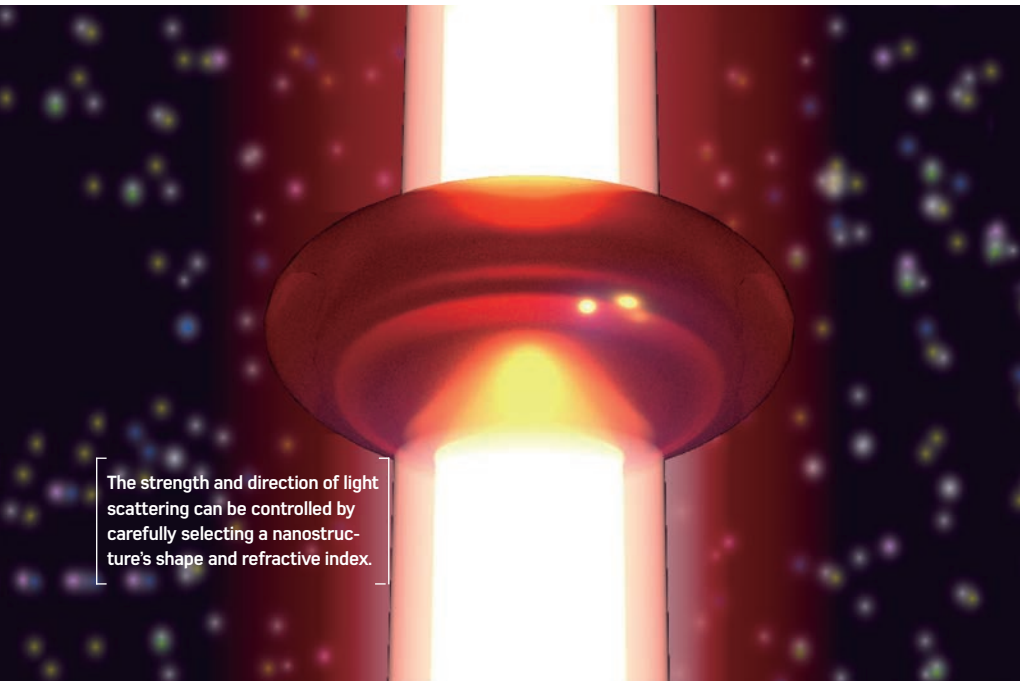
Optics:

STEERING THE FLOW OF LIGHT

TINY PARTICLES WITH VARIED SHAPES SCATTER LIGHT IN USEFUL AND UNUSUAL WAYS

A study into the way nanoparticles scatter light could lead to simpler and smaller optical nanoantennas with improved directivity and efficiency — crucial components for the next generation of advanced photonic devices[1].

Boris Luk'yanchuk and co-workers from the A*STAR Data Storage Institute together with a collaborator in St. Petersburg University, Russia, undertook a detailed numerical investigation of the light scattering characteristics of dielectric nanoparticles of different shapes with high refractive indices (larger than 2). They focused particularly on nanoscale spheres and spheroids.



The strength and direction of light scattering can be controlled by carefully selecting a nanostructure's shape and refractive index.

By carefully selecting the nanoparticle's shape and refractive index, the team discovered they could use the interference between the particle's electric and magnetic dipole resonances to control and optimize the strength and direction of its light scattering.

For example, they found that for a spheroid particle with a refractive index of 3.5, scattering in the forward direction can be maximized if the spheroid's major axis is just over twice the length of its minor axis.

Steering the flow of light by manipulating the nanoparticle's aspect ratio is potentially useful in many applications, for example to maximize the light captured by a solar cell or to make artificially-engineered metasurfaces that can cause light to flow in interesting and unusual ways.

"Dielectric particles with optimized shapes which behave as very efficient directional antennas can be used in sensing devices, transmission lines, metasurfaces with numerous uses and in many other devices such as negative refractive index lenses, optical cloaking devices or nanolasers," explained Luk'yanchuk, the lead researcher in the study.

> "THE FASCINATING PROPERTIES OF DIELECTRIC MATERIALS WITH HIGH REFRACTIVE INDICES ARE RELATED TO THEIR ABILITY TO HAVE BOTH ELECTRIC AND MAGNETIC DIPOLES."

"The fascinating properties of dielectric materials with high refractive indices are related to their ability to have both electric and magnetic dipoles. It makes it possible to produce different interference phenomena in their scattering, like Fano resonances for example. We are trying to use these effects to produce different types of nanoscale devices and metasurfaces."

According to Luk'yanchuk, the physics of this scattering is valid and scalable across the electromagnetic spectrum and thus their approach could be applied not only at optical and infrared frequencies, but at microwave frequencies as well, provided that suitable

transparent particles with a sufficiently high refractive index are used.

"At optical frequencies, for example, there are many suitable materials like titanium dioxide, silicon, germanium, gallium arsenide and other group IV and III-V semiconductors," commented Luk'yanchuk. "The possible limitation would be frequencies higher than the visible range, like the ultraviolet, where no high-index transparent dielectrics are readily available."

1. Luk'yanchuk, B. S., Voshchinnikov, N. V., Paniagua-Dominguez, R. & Kuznetsov, A. I. Optimum forward light scattering by spherical and spheroid dielectric nanoparticles with high refractive index, *ACS Photonics* **2**, 993–999 (2015).

Virology:

PUTTING A BLOCK ON INFLAMMATION

LIMITING IMMUNE RESPONSE CAN REDUCE JOINT INJURY CAUSED BY THE MOSQUITO-BORNE CHIKUNGUNYA VIRUS

Identifying methods to reduce inflammation caused by the Chikungunya virus can help to develop new therapies for this disease.

Increasing the proportion of an anti inflammatory immune cell subtype can reduce damage to joints in mice infected with Chikungunya virus, research by A*STAR reveals[1]. These findings are significant for the potential treatment of people infected by the virus, and have implications for related conditions.

The Chikungunya virus is transmitted to humans by mosquitoes, and causes crippling joint pain and fever. The number of people infected by this virus has grown rapidly worldwide over recent years, in both tropical and temperate regions.

When humans are infected by viral pathogens such as Chikungunya, the immune system activates to clear the infection. However, uncontrolled inflammation can result in tissue damage, leading to the painful symptoms experienced by patients. Identifying methods to reduce inflammation could help with the development of new therapies against these pathogens.

Previous studies by Lisa Ng and colleagues from the Singapore Immunology Network had shown that during infection by Chikungunya virus a type of immune cell, called a CD^{4+} T cell, migrates to the joint and causes injury. However, the researchers also noticed that removal of this subset reduces joint damage which indicates that controlling CD^{4+} T cells could be a promising approach to treat humans infected with Chikungunya virus.

T regulatory cells (Tregs) are a subset of immune cells that blunt responses of CD^{4+} T cells. The numbers of Tregs in mice can be increased by treatment with a particular cytokine complexed with an antibody to which it binds. Ng's team used this method to increase the number of Tregs in mice, and then infected them with Chikungunya virus.

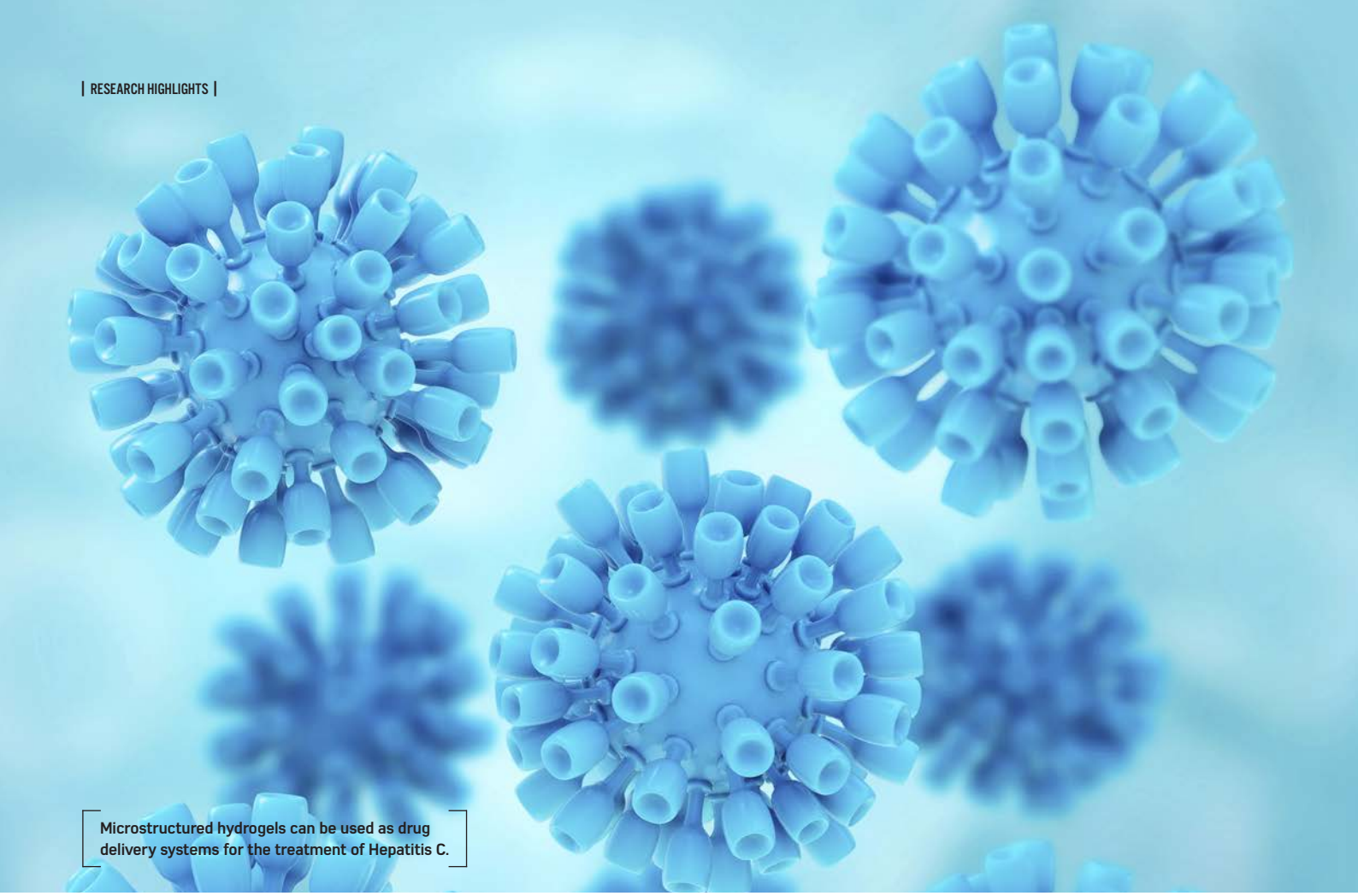
Joint injury caused by the virus was found to be less severe in mice which have more Tregs. Further investigation showed that increasing the number of Tregs helped prevent the appearance of CD^{4+} T cells at the joint. As CD^{4+} T cells are the ones that cause damage, joint injury is considerably reduced when these cells are absent.

When the researchers repeated this approach in mice without Tregs, this cytokine–antibody complex was unable to inhibit joint inflammation. This indicates that Tregs plays a key role in the protective effect of the cytokine-antibody complex.

Methods to increase Treg numbers in Chikungunya-infected patients could represent novel therapeutic approaches to fight joint inflammation and damage caused by these pathogens.

Chikungunya virus belongs to the arbovirus family of viruses. "Involvement of these same immune pathways that we identified could be relevant for other arboviruses that induce joint inflammation", explains Ng.

1. Lee, W. W. L., Teo, T.-H., Her, Z., Lum, F.-M., Kam, Y.-W. *et al.* Expanding regulatory T cells alleviates Chikungunya virus-induced pathology in mice. *Journal of Virology* **89**, 7893–7904 (2015).



Microstructured hydrogels can be used as drug delivery systems for the treatment of Hepatitis C.

Drug delivery:

IN CONTROL WITH HYDROGELS

MICROSTRUCTURED HYDROGELS CAN NEGATE THE PROBLEM OF PREMATURE RELEASE WHEN USED AS DRUG DELIVERY SYSTEMS

An improved design of hydrogel implants for sustained drug delivery has been developed by A*STAR researchers and successfully tested in a mouse model as a treatment for hepatitis C[1].

Hydrogels are cross-linked polymer networks that absorb water or biological fluids. Due to their compatibility with living tissues and ability to preserve embedded proteins in their natural state, they are a good vehicle for delivering protein drugs into the body. A significant problem, however, is the early and uncontrolled release of the embedded proteins.

"We have found a way to regulate the rate and duration of drug release," says Motoichi Kurisawa of the A*STAR Institute of Bioengineering and Nanotechnology (IBN), who worked with researchers from the A*STAR Institute of Molecular and Cell Biology.

The IBN team created hydrogels based on a complex carbohydrate called dextran. Their key breakthrough is the use of polymer polyethylene glycol to incorporate micrometer-sized domains within the gel. These domains act as reservoirs for a protein drug when the drug molecules are also linked to polyethylene glycol. The chemical interactions between the modified protein and the reservoir domains allow a slow and steady release of the protein, a considerable improvement on existing alternatives.

By tweaking the dimensions of the microstructures in the gel, the researchers could also control the rate at which the drug is released, which further increases the system's flexibility. Another advantage is that the nontoxic hydrogels degrade naturally after releasing their cargo.

Tests on the hydrogels bathed in surrounding fluid demonstrated sustained release of the protein interferon over three months, which is much longer than less sophisticated hydrogels can achieve.

The real test, of course, is performance in a living system. Kurisawa and his colleagues demonstrated their technology as a potential way to treat hepatitis C. This serious liver disease kills around 500,000 people each year worldwide, but damage can be limited by interferon. Existing treatment requires eight weekly injections in hospital, which is time-consuming, painful and causes side-effects including depression and fatigue.

The new interferon-loaded hydrogel was implanted in mice — that serve as a model of hepatitis C in humans — and the results were encouraging. "This single administration prevented liver disease as effectively as the current procedure of repeated injections," says Kurisawa. The next stage will be clinical trials in humans.

Kurisawa says the team is seeking a suitable healthcare partner to bring the technology to market. He also points out that the system could in principle be applied to deliver many other drugs.

1. Bae, K. H., Lee, F., Xu, K., Keng, C. T., Tan, S. Y. *et al.* Microstructured dextran hydrogels for burst-free sustained release of PEGylated protein drugs. *Biomaterials* **63,** 146–157 (2015).

Robotics:

NAVIGATING THE UNKNOWN

A COMPUTER ALGORITHM THAT COPIES THE NAVIGATION FUNCTIONALITY OF HUMANS AND ANIMALS HELPS ROBOTS NAVIGATE UNFAMILIAR SPACES

This robot uses neural schemes similar to humans to navigate an office environment.

A robot with a navigation system that mirrors the neural scheme used by humans and animals to find their way around has been developed by A*STAR researchers[1].

The human navigation function is operated by two types of brain cells — place cells and grid cells. Place cells become active in the brain when we recognize familiar places, while grid cells provide us with an absolute reference system, so we can determine exactly where we are on a map.

The way sailors used to navigate through tracking of relative movement, however, is essential for finding a way through unfamiliar areas, explains Miaolong Yuan from the A*STAR Institute for Infocomm Research team. "A sailor will use cues such as the stars or landmarks to determine where their ship is on a map, and then, as the ship moves, will update its location on the map by observing only speed and direction."

The human brain uses grid cells, which provide a virtual reference frame for spatial awareness to handle this type of relative navigation. Each time we move through and pass one of the virtual grid points that the brain has set up, the respective grid cell becomes active, and we know our relative movement in relation to those coordinates. By using both place and grid cells for navigation, humans and animals are able to accurately move through the environment.

Yuan and the team have implemented the same neural scheme for robots, using computer programs that simulate the activity of place and grid cells in the brain. Crucial to the computational algorithm is the strength of the feedback mechanism between the grid cells and place cells, and the calibration of the visual signals is integral to the map building process of the computer algorithm.

The algorithm was tested in a robot (see image) that explored a 35 meters × 35 meters indoor office environment. The robot was able to detect loops in the path through the office space and, by using visual cues to recognize areas visited repeatedly, built its own neurological map of the office. The computer navigation system assists the robot in situations where

it is lost in a new environment, says Yuan. "Cognitive maps can help the robot when it is lost, because they can provide global topological information of the navigating environment to help the robot localize itself."

1. Yuan, M., Tian, B., Shim, V. A., Tang, H. & Li, H. An entorhinal-hippocampal model for simultaneous cognitive map building. *Proceedings of the Twenty-Ninth AAAI Conference on Artificial Intelligence* 586–592 (2015).

Energy materials:

TELLURIUM ELECTRODES BOOST LITHIUM BATTERIES

TELLURIUM ELECTRODES HAVE HIGHER ENERGY DENSITIES AND MAY BE CHARGED AND DISCHARGED FASTER THAN CONVENTIONAL ELECTRODE MATERIALS

A*STAR researchers have demonstrated that electrodes made from tellurium can improve the energy storage and power output of rechargeable lithium-ion batteries[1].

Cathodes in conventional lithium-ion batteries typically contain iron, cobalt and manganese oxides and have a relatively limited energy density. In principle, cathodes in which lithium ions react with oxygen, sulfur or selenium could offer much higher capacities. In practice, however, these elements are not suitable electrode materials, as oxygen-based cathodes are inefficient, and sulfur and selenium electrodes are poor electrical conductors.

Cathodes made from tellurium nanowire mats can increase the energy storage capacity of lithium-ion batteries.

Zhaolin Liu and Yun Zong of the A*STAR Institute of Materials Research and Engineering decided to investigate tellurium — a metalloid element with similar chemistry to sulfur and selenium — as an electrode material. They discovered it was a much better conductor than sulfur and selenium and offered energy densities that were almost as large.

Liu conceded that tellurium is as rare as platinum and not cheap. "Such a battery is definitely not suitable for use in mobile phones or electric vehicles, but it may target high-end applications with special requirements, such as the power source for implantable cardiac pacemakers."

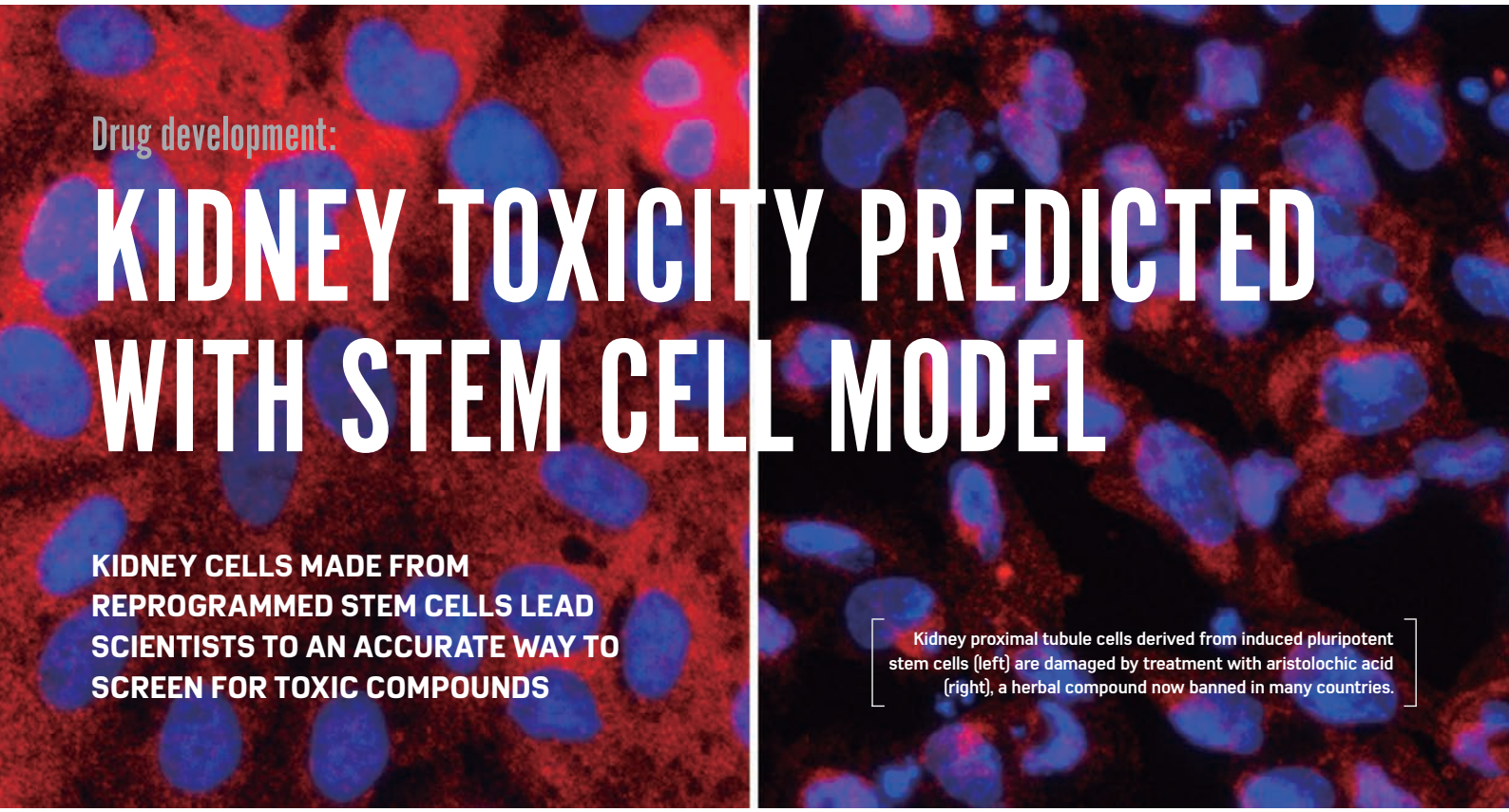
The researchers heated tellurium to 500 degrees Celsius until it melted into a porous carbon electrode, and then tested its performance as a cathode in four different liquid electrolytes. A common solvent called dimethyl sulfoxide gave the best results. They found that when lithium reacts with tellurium as the battery discharges, it forms a compound that is soluble in dimethyl sulfoxide. In contrast, lithium forms insoluble compounds when it reacts with sulfur and selenium, which causes the cathode to expand and damages its structure.

Although their lithium-tellurium battery initially showed a lower capacity than its sulfur and selenium analogs, it charged and discharged more quickly. It also maintained its capacity for much longer, exceeding its rivals' capacity after 50 charging cycles.

The team then developed a cathode made entirely from tellurium nanowires just seven nanometers wide, which they laid together to form a mat. This formed a flexible tellurium cathode with an energy density of 1,800 milliwatt hours per cubic centimeter, which allowed it to store 50 per cent more energy than a conventional lithium cobalt oxide electrode of the same size. It also retained more than 98 per cent of its capacity after 80 charging cycles.

"For our next step, we plan to partially substitute tellurium with low-cost sulfur to develop a hybrid system with higher capacity," says Liu.

1. Ding, N., Chen, S.-F., Geng, D.-S., Chien, S.-W., An, T. *et al.* Tellurium@ordered macroporous carbon composite and free-standing tellurium nanowire mat as cathode materials for rechargeable lithium–tellurium batteries. *Advanced Energy Materials* **5**, 1401999 (2015).



Drug development:

KIDNEY TOXICITY PREDICTED WITH STEM CELL MODEL

KIDNEY CELLS MADE FROM REPROGRAMMED STEM CELLS LEAD SCIENTISTS TO AN ACCURATE WAY TO SCREEN FOR TOXIC COMPOUNDS

Kidney proximal tubule cells derived from induced pluripotent stem cells (left) are damaged by treatment with aristolochic acid (right), a herbal compound now banned in many countries.

A platform that could help pharmaceutical, chemical and food companies screen for safe compounds for kidneys has been set up by A*STAR scientists, who have created the fastest and most efficient protocol for coaxing stem cells to become kidney proximal tubular cells (PTCs)[1].

Daniele Zink from the A*STAR Institute of Bioengineering and Nanotechnology (IBN) and colleagues have, for the past several years, sought to transform human stem cells into PTCs. These cells come from the functional unit of the kidney known as the nephron, and they are particularly vulnerable to toxicity because of their roles in compound transport, metabolism and clearance.

Damage to PTCs can lead to major health problems such as acute kidney injury or chronic kidney disease. Therefore a system that can predict kidney toxicity in the laboratory before experimental medicines, cosmetics or chemicals ever reach human patients is a valuable research tool.

"This new screening platform would reduce costs for industry and help to develop safer compounds," says Zink.

In collaboration with IBN executive director Jackie Y. Ying, Zink's team first successfully made PTCs with embryonic stem cells. In parallel, Zink's team developed the first predictive renal *in vitro* screening platform. Combining the technologies resulted in the first stem cell-based screening platform for kidney toxicity. But the differentiation process was inefficient. So, the researchers turned to induced pluripotent stem cells (iPSCs), formed by reprogramming adult cells to an embryonic-like state, and developed a one-step protocol for turning these stem cells into the desired kidney cells in just eight days. The proximal tubular cells were more than 90 per cent pure — good enough for toxicity testing.

Zink's lab combined the iPSC-derived PTCs with their screening technology and analytic methods developed by Lit-Hsin Loo's group at the A*STAR Bioinformatics Institute. Using this screening platform, the researchers looked at 30 compounds — some known to be safe, others toxic — and found that the platform predicted kidney toxicity with high accuracy. They also studied the cellular pathways of drug-induced injury and found that the iPSC-derived kidney cells correctly recapitulated known toxicity mechanisms.

"We have developed the first animal-free renal screening platforms and the only iPSC-based model for the accurate prediction of kidney toxicity in humans," says Zink.

Zink and her team are now using the same approach to create personalized drug screening platforms. Meanwhile, they are adapting the process to be more compatible for use at an industrial scale. "We will continue working with Ying's lab on developing automated and microfluidic platforms," Zink says.

> "WE HAVE DEVELOPED THE FIRST ANIMAL-FREE RENAL SCREENING PLATFORMS AND THE ONLY IPSC-BASED MODEL FOR THE ACCURATE PREDICTION OF KIDNEY TOXICITY IN HUMANS."

1. Kandasamy, K., Chuah, J. K. C., Su, R., Huang, P., Eng, K. G. *et al.* Prediction of drug-induced nephrotoxicity and injury mechanisms with human induced pluripotent stem cell-derived cells and machine learning methods. *Scientific Reports* **5**, 12337 (2015).

SCIENTIFIC RECOGNITION

FOR MOBILE APP DEVELOPERS

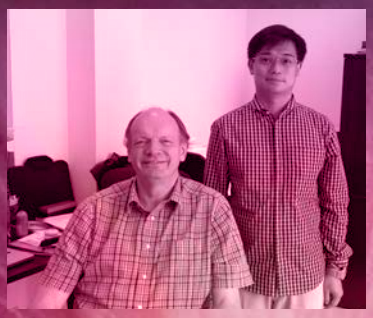
A*STAR AND SPRINGER LAUNCH AN OPEN-ACCESS JOURNAL TO ENCOURAGE THE DEVELOPMENT OF SMART TOOLS FOR SMART SCIENCE

With the enormous increase of processing power in mobile phones, the attempts by mobile gamers to extend the length of a line of black pixels to triumph in the game of Snake are now a quaint memory, "Some mobile phones are now almost as powerful as computers," says Samuel Gan from the A*STAR Bioinformatics Institute (BII). "With apps like Dropbox, and Microsoft office, the phone has become a portable office."

For scientists, smartphones hold potential for a pocket laboratory. And who better to develop these convenient and time-saving tools than scientists themselves? But these budding developers have been discouraged by a lack of scientific journals in which to publish the fruits of their labor. Familiar with the problem, Gan has led the way for a new open-access journal, launched by A*STAR and Springer in October 2015, to give scientist developers the academic recognition they deserve.

POCKET LABS

Gan first became interested in using the mobile platform for scientific analysis in late 2013. He was frustrated at being unable to analyze sequencing data while on a business trip far from his laboratory in Singapore. To overcome this, he and his staff, Nguyen Phi Vu created a new mobile phone app where scientists can view and analyze DNA sequencing files, called DNAApp. Although the app was downloaded by scientists from all over the world and caught the attention of more than 80 news agencies, it received a lukewarm reception from many in the academic community. Publishing the work was a difficult process. Some reviewers loved the idea but others did not regard it as a real area of research. "There was a polarization among scientists over the idea of publishing a mobile phone app in a scientific journal," Gan recalls. His team eventually succeeded in publishing their



The editors-in-chief of *Scientific Phone Apps and Mobile Devices*, Sir David Lane (seated) and Samuel Gan (standing).

A*STAR researchers have launched an open-access journal focused on scientific applications for mobile devices and peripherals.

results, but then faced similar difficulties in trying to get subsequent apps, such as GelApp, which measures the size of gel electrophoresis bands, published.

The user feedback on DNAApp and GelApp, however, was overwhelmingly positive. "There's nothing more exciting than for a scientist to know that what they're developing is being used," says Gan, noting the instant feedback and requests for additional features from app users as far away as Africa and the Middle East. Still, Gan and his team felt that something was missing — acknowledgment from the academic community, which is essential for a successful career in research. "These are scientific contributions, and they deserve scientific recognition," Gan explains.

Gan contacted a number of large publishing houses and after many meetings came to an agreement with Springer to create an open-access journal affiliated with the BII, with an editorial board headed by himself and Sir David Lane of the A*STAR p53 laboratory.

Thanks to Gan's determination and almost one year of hard work, *Scientific Phone Apps and Mobile Devices* was launched in October 2015. It covers research- and science-related apps and peripheral devices such as add-on sensors. "We are also looking at 'wearables', like Google Glass and the iWatch."

SMART SCIENCE

Gan hopes that by providing a way for app development to be recognized by the academic community, more researchers will be willing to invest their time in identifying gaps in their respective fields and be inspired to make apps to solve these problems.

Singapore is a logical choice for the journal's home. It has the highest level of smartphone use in the world, with nine out of ten Singaporeans having access to a smartphone. "If we move fast enough in pursuing this area, and contribute enough resources, Singapore could be the world leader in this field as it matures into an academic field in its own right," says Gan.

A*STAR and SERI researchers have developed a multidisciplinary fragment-based approach that provides antimicrobials against multidrug-resistant pathogens, such as *Staphylococcus aureus*.

Antimicrobials:

BREAKING THROUGH THE BACTERIAL MEMBRANE

ANTIMICROBIALS GENERATED USING A MULTIDISCIPLINARY FRAGMENT-BASED STRATEGY DESTROY DRUG-RESISTANT BACTERIAL MEMBRANES

A weapon in the battle against antibiotic resistance has been developed by A*STAR and SERI researchers, who have come up with a strategy for rational design of antimicrobials against multidrug-resistant pathogens, such as the gram-positive methicillin-resistant *Staphylococcus aureus* (MRSA)[1].

Antibiotic resistance is a worldwide growing problem. Traditional antibiotics target specific intracellular microbial proteins, which are easily mutated. These mutations then alter recognition sites, which prevent drug molecules from killing bacteria or controlling their growth.

Membrane-active antimicrobials are expected to thwart this resistance by selectively penetrating and disrupting bacterial membranes, which are more difficult to reconfigure than proteins. However, the mechanism for this disruption remains unclear. A lack of general design principles has limited the development of membrane-active antimicrobials as a viable tool.

Now, teams led by Chandra Verma from the A*STAR Bioinformatics Institute and collaborator Roger Beuerman from the Singapore Eye Research Institute (SERI) have developed a combined computational and experimental strategy for the rational design and synthesis of these next-generation antimicrobials.

The researchers focused on the bacterial inner membrane to generate anti MRSA drug prototypes. By dividing the membrane into fragments according to wettability, they identified one hydrophobic — or water-repelling — section, sandwiched between two negatively charged regions. Next, they constructed a model comprising a hydrophobic core bearing positively charged terminal groups, which interact with the fragments. Finally, they derived the prototypes from this model using the natural substance xanthone as the core.

The prototypes caused the bacterial membranes to leak, which demonstrated their antimicrobial activity. The higher leakage and permeation they displayed in the presence of bacterial membranes compared with their mammalian analogs was consistent with a low toxicity toward mammalian membranes.

The researchers discovered that the antimicrobial action mechanism followed an adsorption–translocation–disruption sequence. Lead author, Jianguo Li, explains that the drug molecules initially took on a U-shaped configuration, promoting both electrostatic interactions between their terminal groups and outer membrane layer, or 'leaflet', and hydrophobic core insertion. Their accumulation gradually neutralized the outer leaflet, inducing membrane deformation and electrostatic attraction from the inner leaflet. This caused one of the antimicrobial terminal groups to cross the hydrophobic membrane section to interact with the inner leaflet, changing drug configuration and altering both membrane interfaces.

"A certain number of membrane-active antimicrobials currently in clinical trials, such as XF-73, LTX-109, and brilacidin, match our model," says Li. "This is quite exciting because it is the first reported instance of membrane-based fragment assembly," he adds.

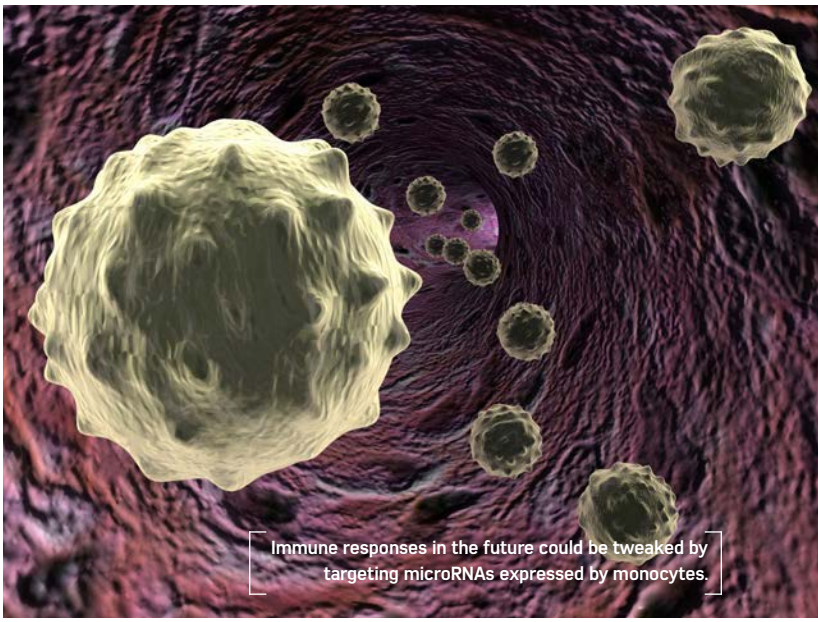
The team is currently working on the development of antimicrobials that simultaneously target gram-positive and gram-negative pathogens. "We hope that the same concept can be extended to cancer cell membranes," says Verma.

1. Li, J., Liu, S., Koh, J.-J., Zou, H., Lakshminarayanan, R. *et al.* A novel fragment based strategy for membrane active antimicrobials against MRSA. *Biochimica et Biophysica Acta (BBA) – Biomembranes* **1848,** 1023–1031 (2015).

Immunology:

MASTERS OF MONOCYTES

REVEALING THE DIFFERENCES BETWEEN SUBSETS OF IMMUNE CELLS IN THE BLOOD COULD YIELD NEW THERAPIES FOR INFECTIOUS AND AUTOIMMUNE DISEASE



Immune responses in the future could be tweaked by targeting microRNAs expressed by monocytes.

The tweaking of genetic material that instructs immune cell function may be able to slow or stop the progression of disease, research by A*STAR suggests[1].

White blood cells called monocytes comprise 5–10 per cent of immune cells in the blood, and play an important role in protecting the body against foreign invaders by consuming viruses and bacteria, and alerting other immune cells to potential threats. Until recently it was assumed that there was just one kind of monocyte, but research indicates that there are at least two, which can be differentiated by the presence or absence of a molecule called CD16 on their surface.

CD16 positive cells are particularly interesting, because they are prolific in people with infectious diseases like HIV, inflammatory conditions such as sepsis, and autoimmune disorders like multiple sclerosis. "Nobody really knows the function of these cells or the consequence of this cellular expansion during disease processes," says Siew Cheng Wong of the Singapore Immunology Network at A*STAR. "Are these cells helping to control disease or does the expansion of these cells contribute to the pathogenesis?"

To further investigate CD16 cells, Wong and her colleagues turned to pieces of genetic material called microRNAs, which

simultaneously control the expression of multiple genes. They identified 66 microRNAs which are expressed differently between CD16 positive and negative cells.

Further experiments revealed that many of the microRNAs regulate genes involved in the migration of cells to different sites around the body, and programmed cell death. Others, such as miR-345, seem to control the magnitude of the immune response by regulating production of proteins called transcription factors, which bind to genes and regulate how they are transcribed into messenger RNA. "Our data suggest these microRNAs could make a substantial contribution to regulating the functions of human blood monocytes," says Wong.

Now that these master regulators have been identified, the next stage is to investigate how up-regulating or blocking their activity might influence monocyte activity and the course of disease. Already, companies are developing drugs that might target microRNAs involved in other diseases, such as those affecting the heart. In future, individuals' immune responses could also be tweaked by targeting microRNAs expressed by monocytes. "MicroRNAs have multiple targets and so might be more useful as a point of therapy than just targeting a single gene or protein," says Wong. "We would be hitting a master regulator of the cell."

1. Dang, T.-M., Wong, W.-C., Ong, S.-M., Li, P., Lum, J. *et al.* MicroRNA expression profiling of human blood monocyte subsets highlights functional differences. *Immunology* **145,** 404–416 (2015).

Bladder cancer:

CLOSER TO ROUTINE URINE SCREENING

A HIGHLY RELIABLE URINE TEST HAS BEEN DEVELOPED FOR THE EARLY DETECTION OF A COMMON TYPE OF BLADDER CANCER

Urine test kits as simple to use as the pregnancy test could soon be available for a common type of bladder cancer.

A panel of five biomarkers that can detect the presence of transitional cell bladder carcinoma in urine with 100 per cent specificity has been developed by A*STAR researchers[1]. The noninvasive technique could be developed into simple home kits for routine screening of the disease, which accounts for nine out of ten patients with bladder cancer.

Prashant Kumar, who led the study with Jean Paul Thiery, both formerly at the A*STAR Institute of Molecular Cell Biology, said early diagnosis and vigilant monitoring for recurrence could save lives. "So we wanted to design something similar to the pregnancy test," Kumar says.

Current methods for diagnosing bladder cancer are painful, expensive and not very effective in that they cannot detect an early, yet fast-spreading transitional cell carcinoma called 'carcinoma *in situ*' that grows like moss along the bladder lining. Cystoscopy, for example, is performed by inserting a tube through the urethra to carve out tissue samples, and urine cytology relies on trained lab technicians. Commercially available biomarkers approved by the US Food and Drug Administration are more convenient but less reliable: they often result in false-positives (low sensitivity) or false-negatives (low specificity), particularly when blood appears in the urine.

Kumar and his colleagues used advanced mass-spectrometry-based quantitative proteomics to compare the proteins present in urine samples of healthy individuals with that of patients at various stages of the disease. They identified five proteins that were secreted

by the cancer patients at much higher levels. "We chose five biomarkers to account for the heterogeneity of bladder cancer," says Kumar.

The researchers extended their analysis to hundreds more patients, including those with chronic ailments such as bladder inflammation, benign prostatic hyperplasia, diabetes, hypertension, asthma and gastritis, and many different types of cancer, from lung to prostate, tongue, colon, pancreas, breast and renal cancer.

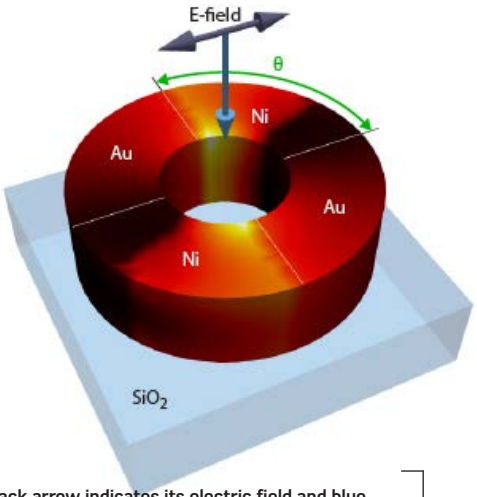
"Our results were really promising," says Kumar. The protein biomarkers showed high sensitivity and specificity for early and late stage transitional bladder carcinoma, even detecting cases of carcinoma *in situ*. They were not affected by blood proteins in urine, which can occur in cases of infection, inflammation, or injury of the urinary tract.

The researchers plan to conduct follow-up studies on a larger cohort of thousands of patients internationally. They are also in discussion with biomedical and pharmaceutical companies to develop monoclonal antibodies for detecting the biomarkers using a simple dipstick. "We want to create an affordable diagnostic kit that can be used for routine home screening by at-risk individuals."

1. Kumar, P., Nandi, S., Tan, T. Z., Ler, S. G., Chia, K. S. *et al.* Highly sensitive and specific novel biomarkers for the diagnosis of transitional bladder carcinoma. *Oncotarget* **6**, 13539–13549 (2015).

Plasmonics:

DOUBLING UP IMPROVES NANORING DESIGN



When a light pulse (black arrow indicates its electric field and blue arrow shows propagation direction) is irradiated on a nanoring made of nickel and gold on silica substrate (blue), the nanoring generates a magnetic pulse via the thermoelectric effect.

A NEW NANORING DESIGN SHOWS POTENTIAL FOR GENERATING SHORT MAGNETIC PULSES AND COULD BE USED TO EXPLORE MAGNETIC SWITCHING IN MATERIALS

A bimetallic nanoring that generates a short magnetic pulse when irradiated by a laser pulse has been theoretically studied by A*STAR researchers. It shows exciting potential for investigating magnetic switching and realizing rapid data storage[1].

Unlike their electrical and optical equivalents, ultrashort magnetic pulses have proved very difficult to generate. These pulses are needed to explore magnetic switching in materials — a process that underpins virtually all of today's data storage technology. However, most methods for generating magnetic pulses use large-scale particle accelerators or are limited to specific materials and do not produce tightly confined magnetic fields.

Now, Guillaume Vienne and colleagues at the A*STAR Data Storage Institute have theoretically proposed a nanoring that consists of four alternating gold and nickel sectors (see image). Their calculations predict that this nanoring will generate magnetic pulses shorter than a trillionth of a second when irradiated by an ultrashort laser pulse.

The ring is essentially a nanoscale version of the setup used by physicist Thomas Seebeck in 1821 when he discovered that a temperature difference produces an electric voltage in certain materials — now known as the thermoelectric effect. The nanoring operates in a similar way to Seebeck's setup in that heating it produces an uneven temperature distribution, which results in current flow and generates a magnetic field.

The nanoring is heated by irradiation with a laser pulse. Its small size gives rise to resonant collective oscillations of conduction electrons, known as plasmon resonance. This results in efficient and uneven heating, producing hot and cool spots in the ring between which currents flow as a result of the thermoelectric effect. Finally, these currents generate a magnetic pulse.

Vienne sees the nanoring as being unique. "There simply isn't any source that produces a magnetic field that is so localized in both time and space. So it's a kind of a new object," he enthuses.

The four-sector nanoring has two significant advantages over a previous design that had only two sectors. It generates a higher

current and hence a higher magnetic field. In addition, the four-sector nanoring has a lower maximum temperature, which is fortunate since the ring will melt if its maximum temperature exceeds its melting point.

The A*STAR team is currently collaborating with scientists at Southampton University in the United Kingdom to fabricate and characterize such a nanoring.

The researchers are enthusiastic about its potential. "There is much debate about how fast magnetization can be switched," says Vienne. "Our nanoring should advance that debate."

1. Vienne, G., Chen, X., Teh, Y. S., Ng, Y. J., Chia, N. O. & Ooi, C. P. Novel layout of a bi-metallic nanoring for magnetic field pulse generation from light. *New Journal of Physics* **17**, 013409 (2015).

Chemistry:

A DASH OF NICKEL SETS DOMINOES IN MOTION

A SYNTHETIC PROCESS THAT COMBINES MULTIPLE COMPONENTS THROUGH QUICK, CONSECUTIVE REACTIONS IS PROMISING FOR DRUG MANUFACTURE

Using a simple nickel salt, A*STAR researchers have developed a mild, one-pot 'domino' reaction that can attach different hydrocarbon components to specific sites on a carbon–carbon double bond[1] — a chemical trick essential for the production of helical molecules and anti cancer medications such as tamoxifen.

When chemists build complex organic molecules for applications such as drug synthesis, they usually perform reactions step by step to ensure good yields and minimal by-products. But having two or more bond forming transformations occur consecutively in the same beaker could save enormous amounts of time and materials. To achieve this goal, laboratories are building precursors that, once activated, undergo a series of quick chain reactions to form the final product — a process that parallels a row of tumbling dominoes.

Jin Zhao, Andy Hor, Tamio Hayashi and co-workers from the A*STAR Institute of Materials Research and Engineering and the National University of Singapore aimed to find an improved domino reaction for producing carbon–carbon double bonds known as tetra-substituted alkenes. "These types of compounds have really interesting physical, structural, and electronic properties," explains Zhao. "You can find them in biologically active natural products, while others have use as molecular switches."

To achieve their domino reaction, the researchers wanted to attach two types of hydrocarbons to a third component bearing a carbon–carbon triple bond known as an alkyne. They postulated that if the first hydrocarbon contained a special organometallic group called a Grignard reagent, it could quickly add to the alkyne through a 'carbometalation' step. Then the second hydrocarbon, in the form of an organic halide, would couple to the reactive intermediate and give a tetra-substituted alkene.

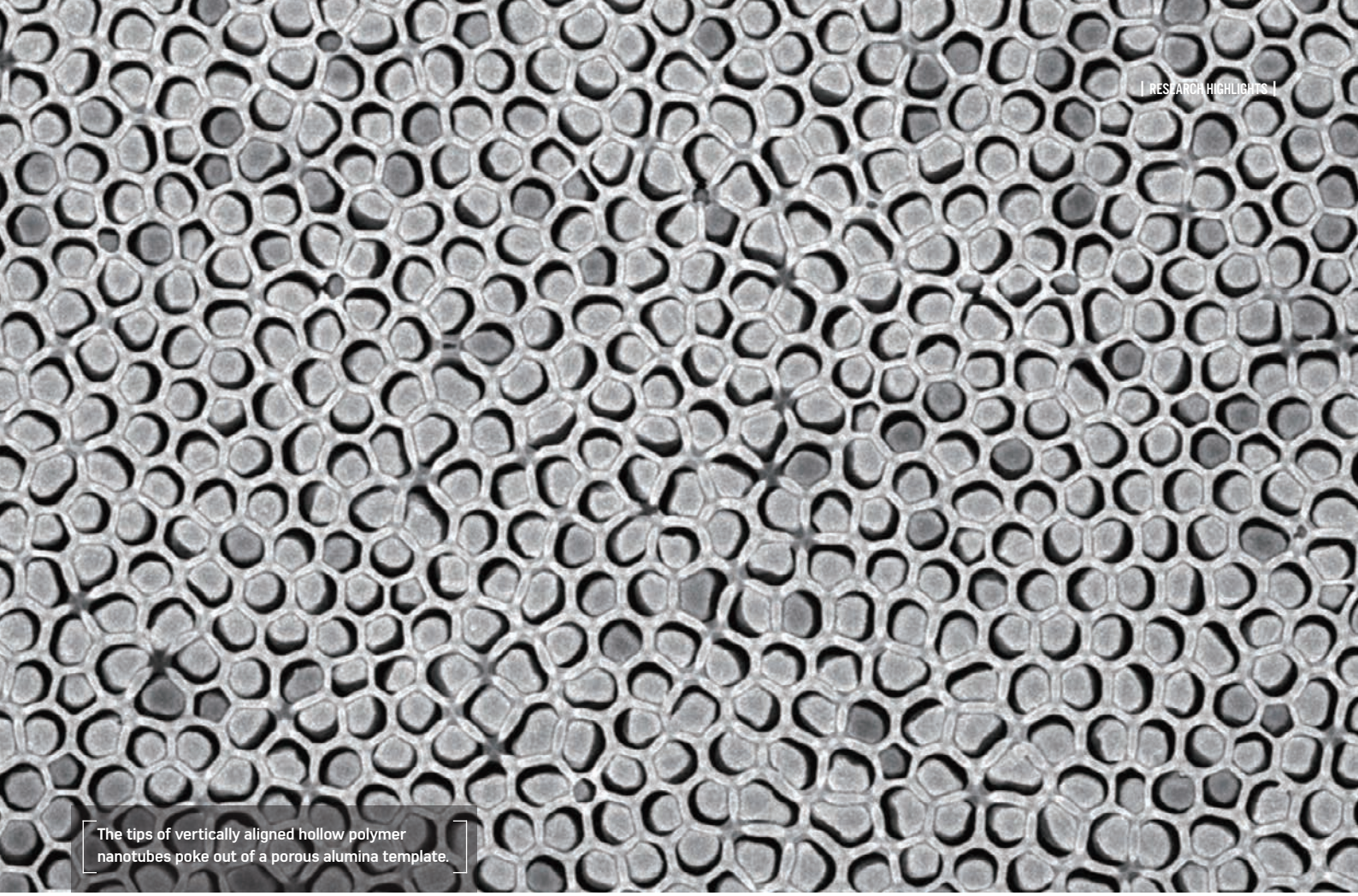
Controlling the selectivity of this multi step process was a significant challenge for the team. "The most serious problem in this reaction would be possible side reactions, where the Grignard reagent reacts with the organic halide before reaching the alkyne," explains Zhao.

To overcome this problem, Zhao and her co-workers drew on past experiences catalyzing Grignard chemistry with nickel chloride, an inexpensive salt that speeds up certain room temperature reactions. When the team mixed the three components with a pinch of nickel chloride catalyst, the results were impressive: high yields of tetra-substituted alkene targets were obtained by simply stirring for a few hours. Mechanistic studies revealed that the high selectivity of this domino process arose from an extremely fast carbometalation step.

A multi step domino reaction can improve synthesis of the breast cancer medication tamoxifen.

"This work provides a simple and selective method for synthesizing tetra-substituted alkenes, notably the drug tamoxifen" adds Zhao.

1. Xue, F., Zhao, J., Hor, T. S. A. & Hayashi, T. Nickel-catalyzed three-component domino reactions of aryl Grignard reagents, alkynes, and aryl halides producing tetrasubstituted alkenes. *Journal of the American Chemical Society* **137**, 3189–3192 (2015).



The tips of vertically aligned hollow polymer nanotubes poke out of a porous alumina template.

Materials:

STANDING UP FOR SENSITIVITY

ARRANGING POLYMER NANOTUBES IN A VERTICAL ARRAY ENHANCES PIEZOELECTRIC PROPERTIES FOR ACOUSTIC SENSORS

An array of hollow piezoelectric polymer nanotubes grown by A*STAR researchers could be used as an extremely sensitive acoustic sensor.

The tubes are made of a piezoelectric polymer called poly(vinylidene fluoride-co-trifluoroethylene), or P(VDF-TrFE) — to which applying a voltage causes a change of shape; conversely, the polymer generates a voltage when it is pressed or twisted. Piezoelectric polymers are considerably more flexible than other piezoelectric materials, and are highly responsive to pressure.

Forming piezoelectric materials into nanotubes can enhance their properties, but flexible polymer nanotubes tend to aggregate into bundles.

Kui Yao and colleagues at the A*STAR Institute of Materials Research and Engineering, and the National University of Singapore, have now developed a method to create vertical arrays of hollow P(VDF-TrFE) nanotubes, significantly increasing their piezoelectric capabilities[1]. "For the first time, we have demonstrated enhanced piezoelectric performance in a high quality P(VDF-TrFE) nanotube array," says Yao.

The team first made a template — a thin sheet of anodized alumina with vertical pores up to 4 micrometers deep and 350 nanometers wide — and added a coating of P(VDF-TrFE). Heating to 250 degrees Celsius melted the polymer into the pores, coating its walls. They repeated the cycle 15 times to create a polymer coating 60 nanometers thick.

They covered the polymer-loaded template with a thin gold electrode, then flipped the structure over and mounted it on a glass substrate. They used an acid to etch away part of the alumina, exposing the tips of the hollow polymer nanotubes inside (see image), and capped them with another gold electrode.

X-ray diffraction and infrared spectrometry revealed that the electrical polarization of the polymer was aligned with the nanotube's axis,

which increased the overall polarization in that direction by 1.5 times. "The dominant mechanism for enhanced piezoelectric performance is based on this unique molecular orientation and the nanotube's structure," says Yao.

The researchers found that an alternating voltage changed the nanotubes' strain almost twice as much as a standard P(VDF-TrFE) film. They also hypothesized that applying a small stress to the structure could produce a voltage many times larger than conventional piezoelectric materials, and more than three times that of a standard piezoelectric polymer film. "These are important indicators of a piezoelectric material's performance for electromechanical applications such as energy harvesters, sensors and transducers," says Yao. "We are now working towards the demonstration of acoustic sensors using the P(VDF-TrFE) nanotube array, with enhanced sensitivity compared to conventional piezoelectric films."

1. Liew, W. H., Mirshekarloo, M. S., Chen, S., Yao, K. & Tay, F. E. H. Nanoconfinement induced crystal orientation and large piezoelectric coefficient in vertically aligned P(VDF-TrFE) nanotube array. *Scientific Reports* **5**, 09790 (2015).

Genetics:

ALGORITHM UPDATE IMPROVES ESTIMATION OF ANCESTRY

REFINEMENT OF AN ALGORITHM FOR DETERMINING GENETIC ANCESTRY COULD HELP IDENTIFY GENETIC FACTORS IN DISEASE

LASER 2.0 can determine genetic ancestry even from small amounts of fragmented genetic information.

A statistical algorithm for determining genetic ancestry has been improved by A*STAR researchers, an advance that could increase the sensitivity and accuracy of studies that aim to link genetics with disease.

Developments in genetic sequencing technology have accelerated the collection of genetic information, providing new opportunities to identify genetic mechanisms of disease. However, study designs must account for the genetic ancestry of included individuals.

"Genetic association studies seek to identify genes that are linked with genetic diseases or traits," explains Chaolong Wang from the A*STAR Genome Institute of Singapore. "Such efforts can be complicated by the underlying genetic ancestry in study samples because different populations have distinct genetic backgrounds."

These genetic backgrounds, as well as environmental factors, can influence disease susceptibility and accurate ancestry information among study populations helps avoid links between genes and disease being missed or misidentified. "Knowledge of individual ancestry can help researchers better pinpoint genes that are truly associated with disease," Wang says.

Wang and colleagues previously developed an algorithm designed to determine the ancestry of an individual from a small percentage of their genetic sequence. The algorithm, called 'Locating Ancestry from Sequence Reads', or 'LASER' 1.0, could establish continental ancestry, such as distinguishing between European and Asian ancestry. However, it was not refined enough to pinpoint fine-scale ancestry, such as the country of origin within Europe, when little genetic information was available from each person. The team have now

developed LASER 2.0, which compares genetic information from individuals in an extensive ancestry reference dataset[1].

The team used LASER 2.0 to analyze genetic data that was previously studied with LASER 1.0. The new algorithm could estimate fine-scale European ancestry much more accurately than the original. The researchers also showed that when the available genetic data are insufficient, LASER 2.0 can use reference data to 'guess' some of the missing data, effectively increasing the amount of information for analysis.

LASER 2.0 could also accurately determine ancestry using genetic data collected from different sources or generated with different techniques. This ability is the most significant improvement over LASER 1.0 because it enables more data to be collated and analyzed, thereby increasing sensitivity to genetic associations with disease.

"LASER 2.0 can help reduce spurious associations being made by modeling the differences in ancestry within the study sample," explains Wang. "The facilitation of integrative analysis of genetic data from different sources should accelerate discovery in large-scale disease association studies. Our method could also provide insight into relationships between ancient and modern human DNA."

1. Wang, C., Zhan, X., Liang, L., Abecasis, G. R. & Lin, X. Improved ancestry estimation for both genotyping and sequencing data using projection procrustes analysis and genotype imputation. *The American Journal of Human Genetics* **4,** 926–937 (2015).

Medical imaging:

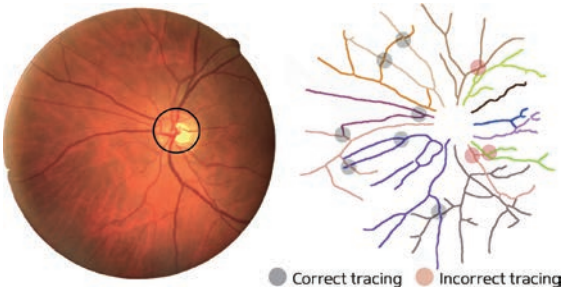
TRACING PATHS THROUGH A BOTTLENECK

COMPUTATIONAL ANALYSIS AUTOMATICALLY IDENTIFIES THE PATHS OF NEURONS AND BLOOD VESSELS IN MEDICAL IMAGES

Automatic tracing of filamentary structures in medical images could improve drug screening and clinical diagnosis, and might also be useful for image analysis in other fields. Software to perform this complex task has been developed by A*STAR researchers in collaboration with co-workers in Singapore and China[1]. The system has been used to examine images of neurons in brain tissue samples and blood vessels in the retina.

Clinical investigators often need to identify single neurons in images when assessing the effects of drugs being developed to treat neurological conditions such as Parkinson's and Alzheimer's diseases. Identifying the path of individual blood vessels in the back of the eye is used to diagnose damage caused by diabetes. Automating image analysis could make it faster and easier for clinicians to use, without requiring assistance from a technical expert. Some semi-automated systems already exist, but they frequently require human intervention to trace the complete paths of individual filaments in each image.

Automatic image analysis is a step closer, thanks to new software capable of automatically tracing the path of filamentary structures in medical images, such as blood vessels in the retina.



"A major bottleneck in automating the process has been the problem of filament crossovers," says Li Cheng of the A*STAR Bioinformatics Institute. He explains it can be difficult for any automated system to trace a single filament past the points where they crossover or touch branches from neighboring neurons or blood vessels.

Cheng's team, along with co-workers at other A*STAR Institutes, Nanyang Technological University in Singapore and Beijing Institute of Technology in China, tackled the problem with sophisticated image analysis techniques. In particular, a procedure called directed graph theory helped them trace the filamentary structures pixel by pixel. Their software has been tested on database images of neurons and retinas, which has confirmed that it could be developed into a high throughput automated system.

"Our tracing system significantly outperforms existing semi-automated procedures," Cheng says, adding, "Having developed this powerful system we are now keen to deploy it to real-world challenges." To pursue that aim the team want to highlight its possibilities to potential users worldwide. Patenting and commercialization will follow in due course if the initial promising indications are verified.

The software might also be applied to

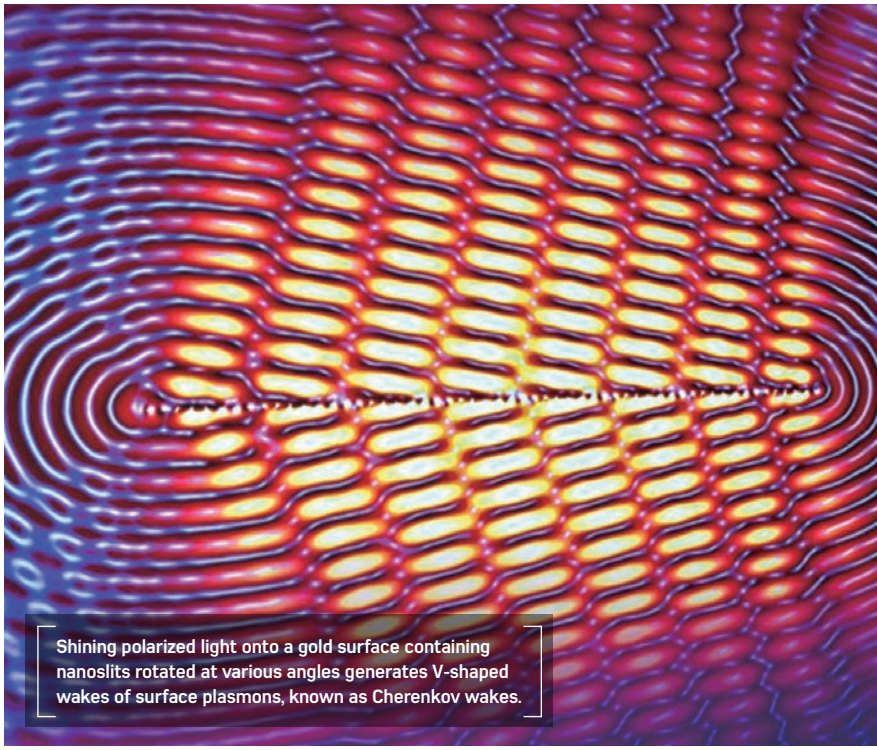
other medical and biological problems such as the movement of materials within cells. "We hope it will eventually lead to the faster identification of better drugs for saving patents' lives," says Cheng. He points out that it may also be able to trace features in the built and natural environment, such as road networks.

1. De, J., Cheng, L., Zhang, X., Lin, F, Li, H. *et al.* A graph-theoretical approach for tracing filamentary structures in neuronal and retinal images. *IEEE Transactions on Medical Imaging* **35**, 257–272 (2016).

Plasmonics:

LEFT IN THE WAKE

WAVES OF CHARGE SCUDDING ACROSS GOLD SURFACES ARE SHOWN TO CREATE WAKES THAT ARE READILY MANIPULABLE



Shining polarized light onto a gold surface containing nanoslits rotated at various angles generates V-shaped wakes of surface plasmons, known as Cherenkov wakes.

Two-dimensional, controllable light-like waves on a metallic surface, created by A*STAR researchers and co-workers at Harvard University, and analogous to the wake of a boat moving through water, have potential applications in nanoscale photonics.

A wake forms behind an object moving through a medium faster than the speed that a wave travels in that medium. One example is the sonic boom created by a supersonic jet. The optical version of this phenomenon, known as Cherenkov radiation, occurs when a charged particle moves faster than the speed of light in a medium. The eerie blue glow given off by nuclear reactors immersed in cooling water is caused by this effect.

Now, Patrice Genevet at the A*STAR Singapore Institute of Manufacturing Technology and his co-workers have generated the two-dimensional equivalent of Cherenkov radiation in a gold film containing a row of nanoscale slits oriented at various angles[1].

When polarized light is shone obliquely on the gold film (see image), it excites free electrons (the 'water') in the gold: this produces a wave of charge (the 'boat') that travels faster along the surface than light-like waves known as surface plasmons. Consequently, the wave of charge leaves V-shaped waves of surface plasmons in its trail (the 'wake').

These waves were difficult to capture as they are confined to the surface of the gold. The team addressed this by using a near-field scanning microscope to 'lift' the waves off the surface, allowing their intensity to be measured.

Using an ensemble of nanostructured apertures, the scientists were even able to steer these waves by varying the nanoslit angles and the incident angle of the light beam. "We had a feeling that the running wave of charge could be manipulated to control the angle of surface plasmon wakes," says Genevet. "On seeing the first experimental near-field images, we realized our intuition was correct. There is nothing more gratifying than taking the vision of a physical effect and making it a reality." This controllability will be important for realizing practical applications of the effect.

In particular, the effect could be used to create new types of surface-plasmon-based optical components, such as plasmonic holograms and directional plasmonic lenses, Genevet says. He is also excited about the potential to manipulate light on tiny scales. "We are fortunate to be doing this research when nanotechnologies are really taking off," Genevet says. "Photonics at nanoscale is having a remarkable impact on optics, and our findings will hopefully help to better understand the excitation mechanisms of surface electromagnetic waves."

1. Genevet, P., Wintz, D., Ambrosio, A., She, A., Blanchard, R. & Capasso, F. Controlled steering of Cherenkov surface plasmon wakes with a one-dimensional metamaterial. *Nature Nanotechnology* **10**, 804–809 (2015).
2. Chen, H., Duan, Z. & Chen, M. Metamaterials: Steering surface plasmon wakes. *Nature Nanotechnology* **10**, 736–737 (2015).

Agency for Science, Technology and Research
SINGAPORE

The Agency for Science, Technology and Research (A*STAR) is Singapore's lead government agency dedicated to fostering world-class scientific research and talent for a vibrant knowledge-based economy.

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- Data Storage Institute (DSI)
- Experimental Therapeutics Centre (ETC)
- Genome Institute of Singapore (GIS)
- Institute of Bioengineering and Nanotechnology (IBN)
- Institute of Chemical and Engineering Sciences (ICES)
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