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on Parkinson's disease

RECREATING THE BRAIN

**TRASH TO
TREASURE**

Supplying
industry through
agricultural waste

**GOOD
VIBRATIONS**

A revolution
in light at the
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Editorial

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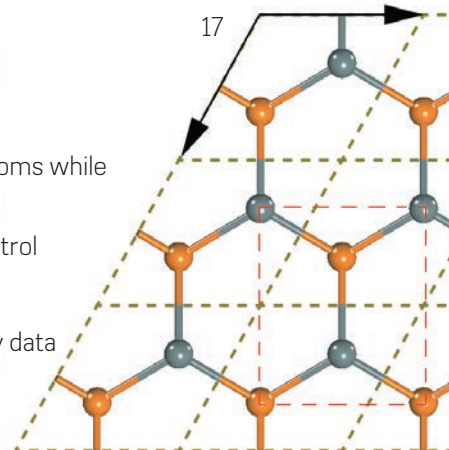


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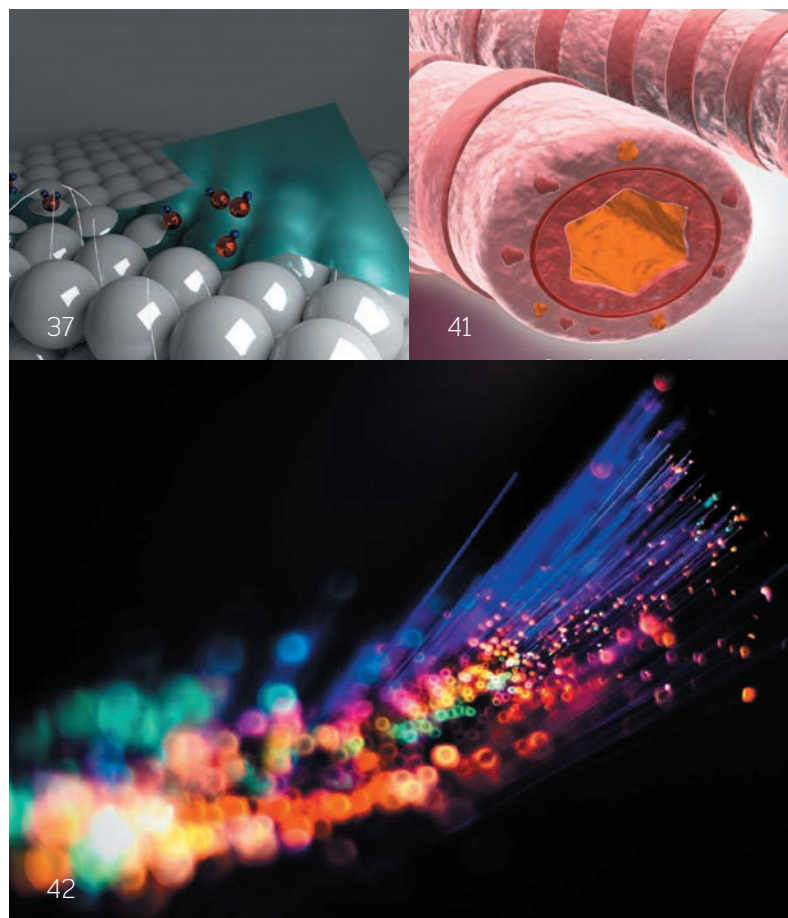
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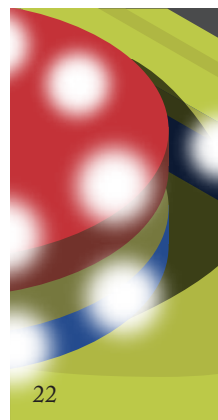
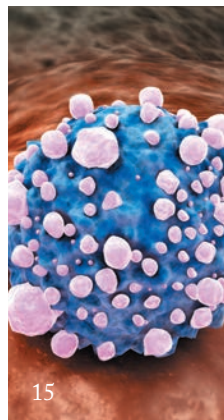
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[NOTES FROM THE EDITORS]

*Editorial board member, Boris Luk'yanchuk, introduces the latest issue of A*STAR Research*

Welcome to the second edition of *A*STAR Research* for 2017. Global uncertainties related to economic crises, political instabilities and terrorism have influenced and shifted research priorities in many countries in the last few months. While some countries have proposed radical changes — US President Donald Trump's double-digit cuts for the Environmental Protection Agency (EPA) and the National Institutes of Health (NIH) spring to mind — Singapore and A*STAR's approach has been to continue shifting our focus towards application-centric research and value creation.

Although I am not an expert in biomedical research, I was particularly interested in this issue's stories about lung cancer diagnostics (page 38), new DNA analysis methods (page 15) and the identification of Jekyll and Hyde-like genes in breast cancer cells (page 28). With Singapore's aging society, the feature on page 32 discussing how A*STAR scientists are pioneering the creation of models for age-related brain diseases, such as Parkinson's and Alzheimer's, is especially timely. Throughout the rest of the issue

there are many other stories on different types of cancers, viruses and vaccines (pages. 9, 18, 35). Many of the teams highlighted in this issue focus on developing practical methods that can eventually be of use in clinical laboratories.

Within the cluster of SERC stories, I would like to draw your attention to our feature on page 12 on fermenters for converting biomass, such as palm-oil waste, into high-value chemicals. The fabrication of a laser on a chip, discussed on page 22, is a promising step towards the development of room temperature optoelectronic devices. Models for heat transfer in stanene and smart city transportation systems are also presented on pages 17 and 4. On the infocommunications front, our researchers have also developed technology to improve video streaming in crowded mobile environments, as presented on page 45.

A*STAR's environment fosters the development of novel concepts and enables their rapid transition to innovative technologies. This is particularly clear in the third feature in this issue, on page 42, which discusses the work from the Data Storage Institute to realize the potential of small semiconductors in the emerging field of nanophotonics, and pave the way for better holographic displays and other new optical devices.

This is just a taste of the range of research covered in this issue, I hope you dive in and enjoy the rest of the magazine.



COVER IMAGE

Mini-brains offer wisdom of ages [p.32]

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[RESEARCH HIGHLIGHTS]



A*STAR researchers created a program that predicts public transport usage in Singapore based on land use and the location of amenities, an essential capability for smart city planning.

Urban planning

SMART CITY TRANSPORT SYSTEMS

A MACHINE-LEARNING PROGRAM USES LAND USE AND AMENITIES DATA TO PREDICT PUBLIC TRANSPORT USE IN SINGAPORE

From schools and shops to hospitals and hotels, a modern city is made of many different parts. Urban planners must take account of where these services are located when designing efficient transit networks. A*STAR researchers have developed a machine-learning program to accurately recreate and predict public transport use, or 'ridership', based on the distribution of land use and amenities in Singapore¹.

Traditional cities consist of an inner central business district (CBD), where most people work, surrounded by outer residential and

industrial zones. Unfortunately for commuters, the high volume of people traveling to and from the CBD can cause gridlock at peak hours. To alleviate some of this frustration, the Singaporean government is working on creating regional centers by the year 2030. The planners hope to encourage businesses to open at specified regional centers around the city-state, easing peak-time pressure and encouraging public transport use.

"We're aiming to understand the recipe for a smart city," explains Christopher Monterola

"SINGAPORE NEEDS AN EFFICIENT TRANSPORT SYSTEM TO SUPPORT PEOPLE'S ACTIVITIES GIVEN THE EXISTING AND PLANNED INFRASTRUCTURE."

at the A*STAR Institute of High Performance Computing, who led the project in collaboration with scientists across Singapore. "Singapore needs an efficient transport system to support people's activities given the existing and planned infrastructure. To guide planners,

we needed a model that could predict ridership under the regional centers plan.”

The team collected data from the city’s smartcard system on people tapping in and out of individual bus and subway stations over a period of a week — more than 20 million journeys in total.

The smartcard data was combined with city-wide information on how land was being used — for business, industry, residence, water or greenery — and high-resolution maps that identified individual amenities within a set radius of each station. Monterola’s team

trialled three different machine-learning models — computer programs that train themselves through repeated simulations — to find one that first accurately reproduced, and then predicted, transport ridership across the city.

“We found that a decision-tree model performed best, with good accuracy, computational efficiency and an easy-to-follow user display,” says Monterola. “Results indicated that an increase in amenities of up to 55 per cent across the city would increase ridership. Beyond this point, ridership begins to decline;

this is logical because if amenities are available locally, people walk instead.”

The high-resolution amenity data proved a much stronger predictor of ridership than general land-use details; a useful result for informing future urban planning and monitoring Singapore’s regional centers as they develop. The model could be applied to any city with access to similar high-resolution data, notes Monterola. ■

1. Hu, N., Legara, E. F., Lee, K. K., Hung, G. G. & Monterola, C. Impacts of land use and amenities on public transport use, urban planning and design. *Land Use Policy* 57, 356–367 (2016).

Nanomaterials

TURNING THE SCREW

A SIMPLE ETCHING TECHNIQUE OFFERS A MEANS FOR CREATING LEFT-HANDED AND RIGHT-HANDED NANOSTRUCTURES

What could be the world’s smallest screws have been fabricated by researchers from A*STAR.

The thread on a screw is among the ‘chiral’ structures whose mirror image is different from the original. When reduced to the nanometer scale, these structures could have an important role in nanosensor technology. However, making a screw out of a straight wire is no small task, even in the macroscopic world. Making it on the nanoscale has previously used bottom-up methods that grow or assemble the structure in a gas or solution. But such approaches can be complicated, slow and expensive.

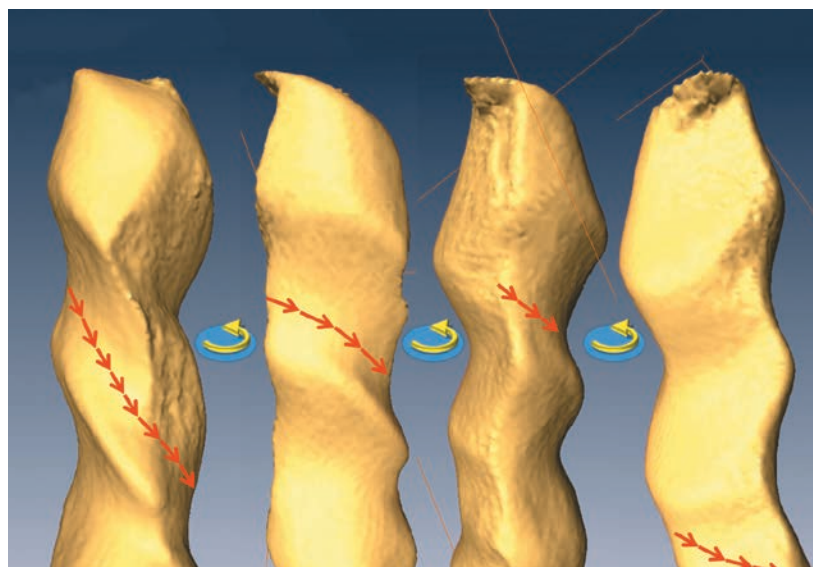
Jun Wei from A*STAR’s Singapore Institute of Manufacturing Technology

and co-workers from the A*STAR Institute of Materials Research and Engineering, Nanyang Technological University and Nanjing Tech University in China, developed a simpler method that uses etching techniques to convert a straight nanowire into a screw.

The team created 10-micrometer-long silver nanowires, 80 nanometers in diameter and with five sides. The structures were attached to a silicon substrate and then placed into a solution of silver nitride in ethylene glycol at 80 degrees Celsius for 20 minutes. The sample was then rinsed clean, and the process was repeated five times.

When the resultant wires were imaged using a scanning transmission electron microscope the team observed smooth ridges and grooves reminiscent of screw threads. Interestingly, such a structure was not evident when a single-step etch was used.

Etching usually works along specific crystallographic directions, leading to symmetric structures, so the team wanted to know how equivalent crystal facets could be etched in an anisotropic way. They propose that this unusual etching mode might begin with the creation of pits at the boundaries between the five crystallographic regions that make up the pentagonal nanowire. These pits



A simple etch method can create nanoscale screws for useful for sensors.

merge at an angle, driven by the propensity to minimize the surface energy, and thus create ridges and grooves that spiral around the nanowire.

"This selective etching is driven by a faster etching rate at some defect locations on the silver nanowire," says Wei. "Thus, we can

convert a regular structure into non-symmetrical one."

Such chiral nanostructures have a much larger surface area than a straight nanowire of similar size. This makes them potentially useful for sensing applications. "We next hope to use the nanoscrews in the fabrication

of sensors and transparent conductors," says Wei.

1. Tan, R. L. S., Chong, W. H., Feng, Y., Song, X., Tham, C. L., Wei, J., Lin, M. & Chen, H. Nanoscrews: Asymmetrical etching of silver nanowires. *Journal of the American Chemical Society* **138**, 10770-10773 (2016).

Anxiety

AGORAPHOBIC FLIES HELP STUDIES ON HUMAN ANGST

WHAT ANXIOUS DROSOPHILA CAN TELL US ABOUT THE FUTURE OF ANTI-ANXIETY TREATMENTS

About 1 in 14 people in the world suffer from an anxiety disorder. Research into the condition is a huge field, but despite the prevalence of the disorders and intense research, there are still no optimal drug treatments available. Now, a group at the A*STAR

Institute of Molecular and Cell Biology show that flies can be used to analyze basic anxiety mechanisms¹.

The image of a rat in a maze is a cliché thanks to the widespread use of these animals in neuropsychiatric research. But using rats

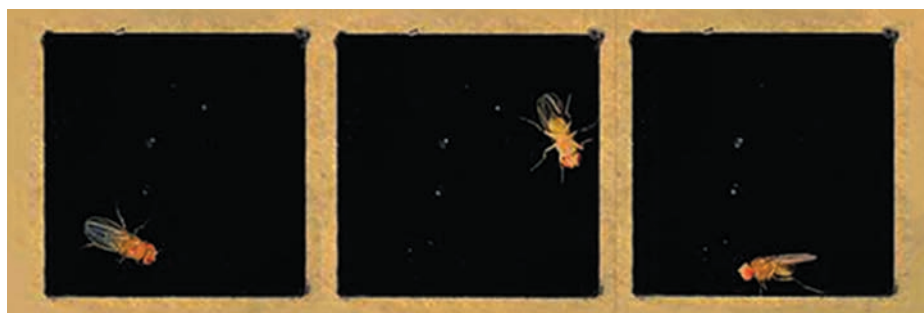
and mice to analyze anxiety mechanisms and discover drugs to treat anxiety has not lately been successful. "The proof is in the pudding: rodent research hasn't produced an effective new anxiolytic in at least 30 years," says Adam Claridge-Chang, head of A*STAR's Laboratory of Translational Neurogenetics.

Vinegar flies, *Drosophila melanogaster*, are a model system that has dominated the animal genetics field for more than a century. "I wondered if *Drosophila* could be also used to analyze anxiety," says Farhan Mohammad, the project's lead experimental scientist. Using automated video tracking to analyze the flies' behavior, the group found that the animals naturally stuck close to the walls of a container, exhibiting 'exposure avoidance' behavior.

When given diazepam, an anxiety-reducing tranquilizer, the flies were more likely to venture from the walls. Deleting a serotonin transporter increased the wall-hugging behavior, suggesting increased anxiety. Subjecting the flies to environmental stress such as heat, physical restraint and social isolation provoked similar effects. These findings match those from mouse models, verifying that flies have an anxiety-like state that is regulated by similar pathways to humans. The scientists hope their research will complement and guide work in rodents.

"THERE ARE NO HUMAN GENES KNOWN TO BE ASSOCIATED WITH ANXIETY DISORDERS."

Using vinegar flies will improve experimental reliability by allowing researchers to conduct tests more quickly and cheaply and with much larger sample sizes. Also, the genetic tools available to modify *Drosophila* are far more advanced than for their mouse counterparts. The team was also able to show



Flies walk on all surfaces but tend to stay near the edges of a chamber, a behavior that is thought to represent anxiety.

that a number of vinegar fly genes pertain to fly anxiety, including several genes related to serotonin, a neuromodulator.

“There are no human genes known to be associated with anxiety disorders,” Claridge-Chang notes, “we don’t know very

much about the genetic or molecular underpinnings of either anxiety or depression. However, clearly neuromodulators like serotonin have enormous effects on emotional state, so it is a priority to understand how these systems work.”

The team is now using their fly anxiety

assay to elucidate basic neurogenetic mechanisms of anxiety.

1. Mohammad, F., Aryal, S., Ho, J., Stewart, J. C., Norman, N. A. *et al.* Ancient anxiety pathways influence drosophila defense behaviors. *Current Biology* 26, 981–986 (2016).

Immunology

SEARCHING FOR THE SECRET OF YOUTH

NOT ALL TYPES OF T CELLS FOLLOW THE SAME TRAJECTORY AS WE AGE. UNDERSTANDING WHY COULD HELP IMPROVE IMMUNITY IN THE ELDERLY

Understanding why immune cells age at different rates could help improve immunity in the elderly.

As people age, their immune system gradually deteriorates and their ability to respond optimally to infections declines, a process called ‘immunosenescence’. A*STAR research shows that not all types of T cells, a type of immune cell that matures in the thymus, follow the same trajectory with age.

There are two main subtypes of T cells. The majority are α/β T cells, which help mediate immunity to infections. While γ/δ T cells represent approximately 1 to 10 per cent of all circulating T cells, they differ from α/β cells in that they recognize fewer foreign elements, or antigens, entering the body, explains immunologist Anis Larbi of A*STAR’s Singapore Immunology Network. They are, however, vital for fighting infections and targeting cancer cells.

“We wanted to understand whether all T cells were equal toward the process of cell differentiation and senescence,” says Larbi.

Larbi and colleagues in Singapore and Germany compared the blood levels of α/β T cells with those of a subtype of γ/δ T cells, called V δ 2 cells, in thirty-six 18- to 23-year-olds and seventy-two 55- to 85-year-olds. V δ 2 T cells represent 60 to 80 per cent of all γ/δ T cells.

When T cells emerge from the thymus, they are ‘naïve’ as they have not yet encountered an antigen. Once introduced, however, they turn into ‘memory’ T cells that can proliferate to deal with the current emergency and then survey the circulation for similar future ones. The team found significant differences between the two cohorts in the percentages of naïve and memory α/β and V δ 2 T cells and in the amounts of proteins, called cytokines, secreted by the cells. The data suggests that V δ 2 cells sustain their functionality with age, unlike other types of T cells.

“This study raises the question of whether V δ 2 T cells are resistant to

senescence,” explains Larbi. “Some cells potentially could be used as models to better understand senescence and identify pathways to resist the phenomenon, and not only on immune cells”.

Further studies are needed to understand the pathways that reduce susceptibility to senescence in T cells. Studies should also include other T cell populations to better understand the clinical importance and biological significance of immunosenescence, the researchers say. Studies that aim to control the proportion and function of V δ 2 T cells may be of clinical value. For example, a drug used to treat Crohn’s disease was found to selectively eliminate V δ 2 T cells and so may help moderate inflammatory diseases.

1. Tan, C. T. Y., Wistuba-Hamprecht, K., Xu, W., Nyunt, M. S. Z., Vasudev, A. *et al.* V δ 2+ and α/β T cells show divergent trajectories during human aging. *Oncotarget* 7, 44906–44918 (2016).

The accuracy of GelApp 2.0, designed by A*STAR researchers, has been enhanced by a cutting-edge image-processing algorithm. GelApp 2.0 can provide accurate gel band detection results from images taken under the unique conditions present in individual labs.

Biomedical science

GAME ON FOR MORE EFFICIENT ANALYSIS

SCIENTISTS USE A GAMING ALGORITHM TO ENHANCE A DNA SEQUENCING ANDROID APP

The accuracy of a smartphone app called GelApp, designed by A*STAR scientists to help analyze biomedical samples, has been greatly enhanced by the addition of a cutting-edge image-processing algorithm.

GelApp was developed in 2015 by intern Jia-Zhi Sim under the supervision of Samuel Ken-En Gan and Hwee Kuan Lee at the A*STAR Bioinformatics Institute. The app analyzes and labels outputs from 'gel electrophoresis' — a common laboratory technique that separates and identifies molecules, such as DNA and proteins, by passing a sample through a gel under electric charge. Individual molecules move through at different speeds, so they separate out and create a pattern of bands across the surface. Gel band images are traditionally analyzed by eye and labeled by hand; the size of each band indicates which precise molecules are present, and highlights where genes are truncated or proteins are altered.

"Eyeballing band-size means that subtler details might be missed, not to mention the time demanded by the task," explains Gan.

“While our first GelApp went a long way to enable automated analysis, there are still improvements to be made.”

The A*STAR team and their collaborators in France wanted to improve GelApp’s functional accuracy when faced with variations in experimental set-ups, cameras, lighting, reflections and blurring.

“We looked to machine learning and advances in computer gaming to find a solution,” says Lee. “We settled on a Monte Carlo Tree Search algorithm, used to great effect in Google’s Alpha-Go, which we trained using expertly labeled gel band images. The

algorithm then continues to learn from individual GelApp users according to their own laboratory set-up and inputs.”

GelApp uses image filters linked in a chain. Each filter heightens the sharpness and accuracy of band detection by reducing noise and pinpointing the horizontal edges of each band. The algorithm automatically selects the best five filters from a filter bank to provide the best match to manually analyzed images, before GelApp is used on new images.

The team found GelApp 2.0 enhanced band detection accuracy by around 56 per cent

for proteins and around 36 per cent for DNA compared with the original app.

“With better automation, there is less room for error, greater standardization and accuracy; this in turn enhances experimental reproducibility,” says Gan. The team hope to expand the use of Monte Carlo algorithms in image processing, for example in analyzing tissue images for disease diagnostics. ■

1. Nguyen, P.-V., Ghezel, A., Hsueh, Y.-C., Boudier, T., Gan, S. K.-E., & Lee, H. K. Optimal processing for gel electrophoresis images: Applying Monte Carlo Tree Search in GelApp. *Electrophoresis* 37, 2208–2216 (2016).

Cell cycle

KEEPING CELL DIVISION IN CHECK

A PROTEIN THAT ENSURES CORRECT CHROMOSOME SEGREGATION DURING CELL DIVISION CAN LEAD TO CANCER IF MUTATED

Researchers have discovered a protein linked to cancer helps ensure chromosomes are apportioned evenly after each round of

cell division. This protein, called *Mastl* in humans, is essential for creating two identical copies of the cell. Its discovery could lead

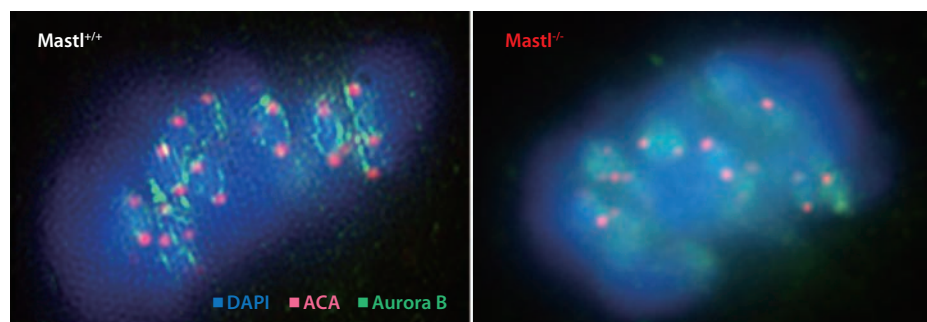
to new therapeutic strategies for targeting tumors of the breast, colon and other organs.

“The fidelity of chromosome segregation is essential to prevent cancer,” says study author Philipp Kaldis, a senior principal investigator at the A*STAR Institute of Molecular and Cell Biology. “It’s possible that *Mastl* inhibitors could be used in a therapeutic setting.”

Kaldis’ lab specializes in a group of enzymes that add chemical tags to proteins to signal when the cell is ready to pass into the next stage of its replication cycle. One of the proteins tagged in this way is *Mastl*, which is also known as Greatwall kinase in some non-human organisms. The textbook understanding of *Mastl*/Greatwall has been that the protein is needed for the cell to start the division, or mitosis, process. Researchers had observed this in fruit flies and in frog eggs. Yet, Kaldis and his colleagues observed a different process in mice, which are more relevant as models of human disease.

The researchers engineered mice so they could switch off the gene that encodes *Mastl*. When they did so, cells still progressed into mitosis — unlike in flies and frogs — but the chromosomes didn’t pair and separate correctly. This abnormality caused the DNA to unravel.

Kaldis’ group showed that this error occurs because *Mastl* is needed for a critical verification step in the cell cycle: the spindle assembly checkpoint. In this step, various proteins work together to ensure that all the chromosomes are correctly attached to the cell’s cytoskeleton so that, when segregation starts, each new cell gets



Mouse cells without *Mastl* show a mislocalization of Aurora B at the kinetochore, a protein structure, that usually marks the site of chromosome pairing during mitosis.

just one copy of each chromosome. Mastl thus acts like a patrolman, keeping watch over this checkpoint so that it's not skipped. To do this, it adds chemical tags that modulate the function of essential proteins involved in the process.

In cell culture, Kaldis and his team

observed that the loss of Mastl could be overcome with drug treatment, suggesting that this might be a way to treat cancers triggered by mutated Mastl. However, "Mastl has multiple functions in mitosis," notes Kaldis, "and we need to learn more about

these" before going ahead and blocking the protein in cancer patients. ■

1. Diril, M. K., Bisteau, X., Kitagawa, M., Caldez, M. J., Wee, S., Gunaratne, J., Lee, S. H. & Kaldis, P. Loss of the Greatwall kinase weakens the spindle assembly checkpoint. *PLoS Genetics* **12**, e1006310 (2016).

Computer memory

REDUCING READ ERRORS MORE THAN A BIT

A NEW CIRCUIT SCHEME WOULD GREATLY INCREASE THE ACCURACY OF HIGH-DENSITY SPIN-BASED DATA STORAGE

While we aspire to store increasing amounts of digital data on ever smaller devices, conventional memory technologies based on electron charge are reaching a physical limit on how much they can store in a given space. Alternative storage methods are urgently needed.

Kien Trinh, Sergio Ruocco and Massimo Alioto at the A*STAR Data Storage Institute and National University of Singapore are investigating a promising storage technique called spin-transfer torque magnetic random-access memory (STT-MRAM). In their pursuit, the

researchers have developed a voltage-boosting scheme for a STT-MRAM system, which greatly reduces errors incurred when reading data¹.

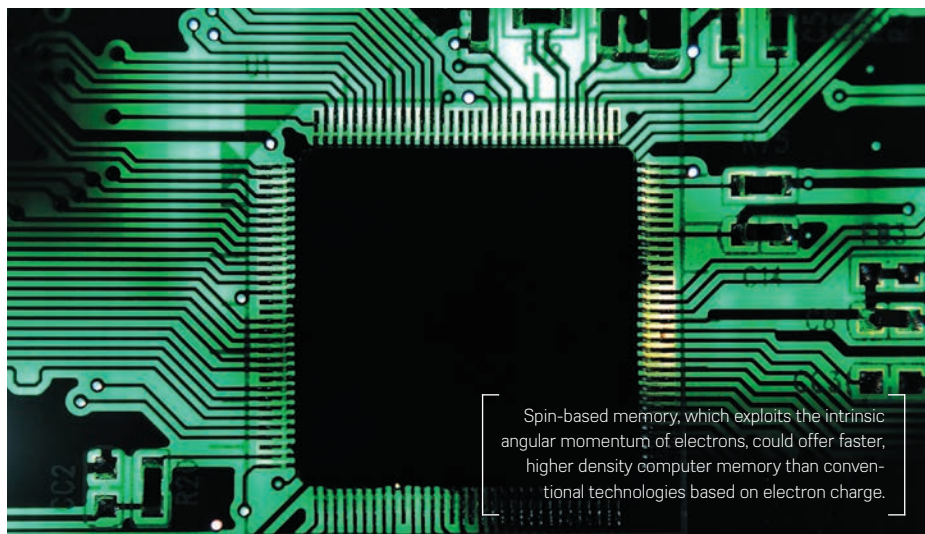
STT-MRAM works by exploiting electrons' intrinsic angular momentum, or spin, rather than their charge. Electron spin can take only two values — up or down — and a standard electrical current contains approximately equal numbers of each. STT-MRAM uses a spin-polarized current, which has more of one spin type than the other, to exert a torque on magnetized 'bitcells'. This flips

the bitcell orientation to high or low states and thereby writes binary data. To read data, the surrounding circuitry must then detect small changes in the resistance of the bitcell, a difficult task to achieve without errors.

"In the physical process of reading STT-MRAM, there is an established trade-off between read disturbance (the chance of unintentionally flipping the bitcell when you read it) and read decision (reading the wrong value currently stored in the bitcell)," says Trinh. "To lower the read disturbance, the read current has to be small. However, a larger read current helps us distinguish between the high and low resistance states of the bitcell." In other words, if you reduce one error, you increase the other.

Trinh and co-workers trialed a new read scheme in which the voltage in the system was boosted by switched capacitors. They performed extensive statistical simulations to find optimum electronic design settings that minimize the impact of natural variations.

"We achieved a rate of just one error per billion bitcell reads, compared to the conventional sensing scheme which has one per ten million," says Alioto. "What's more, our system is one of the first that can achieve so few errors while remaining suitable for low-power and low-voltage applications."



Spin-based memory, which exploits the intrinsic angular momentum of electrons, could offer faster, higher density computer memory than conventional technologies based on electron charge.

The team are hopeful they can prove their design concepts on real devices in the near future, and contribute to the commercialization of this emerging

technology. “We think STT-MRAM could be available on the consumer market within three to five years,” says Ruocco.

1. Trinh, Q.K., Ruocco, S. & Alioto, M. Novel boosted-voltage sensing scheme for variation-resistant STT-MRAM read. *IEEE Transactions on Circuits and Systems* **63**, 1652–1660 (2016).

Neuroscience

NURTURING NEURONS

A STRATEGY FOR EFFICIENTLY CONVERTING STEM CELLS TO NEURONS OFFERS A POTENT NEUROLOGICAL RESEARCH TOOL

Neurological disorders are especially challenging to study in the laboratory, in part because of limited access to fully functional human neurons. Now, a powerful technique for reliably producing a subset of neurons involved with common neurological disorders has been developed by a team of Singaporean researchers led by Hyunsoo Je of the DUKE-NUS Medical School¹.

Neurons can be classified based on the neurotransmitters that they release — for example, those that secrete glutamate are considered ‘excitatory’, while those releasing gamma-aminobutyric acid (GABA) are ‘inhibitory’.

The latter group of neurons plays a prominent role in conditions such as epilepsy, but these cells are generally poorly understood, says team member Alfred Sun, a postdoctoral fellow working with Bing Lim and Huck Hui Ng at the A*STAR Genome Institute of Singapore. “This is mainly because there is currently no efficient and fast protocol to make them in culture,” he says. Indeed, existing methods for converting stem cells into GABA-releasing neurons can take more than six months to complete.

The process by which stem cells naturally develop into mature, specialized cells is largely

driven by proteins known as transcription factors, which directly control gene activity. So the research team systematically treated cultured stem cells with different combinations of transcription factors that are known to participate in the formation of GABAergic neurons.

This allowed them to home in on three critical transcription factors, which formed the foundation for a potent neuron-generating cocktail. With their final recipe, the researchers were able to transform more than 90 per cent of treated stem cells into GABAergic neurons in just 35 days.

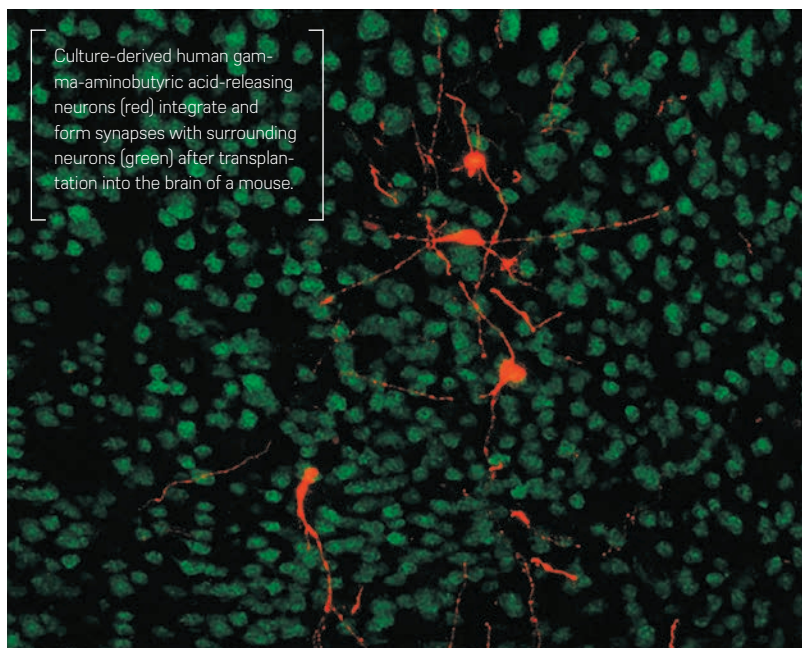
“THE RESULTANT CELLS SEEM TO BE REALLY FUNCTIONALLY MATURE.”

Sun was taken aback by the success of their protocol. “The resultant cells seem to be really functionally mature,” he says. He and his colleagues were able to discern a diverse array of subtypes of GABAergic neurons in their

stem-cell-derived cultures, which exhibited molecular characteristics and electrophysiological behavior that were essentially identical to that of their naturally occurring counterparts. Indeed, these neurons were even able to form functional inhibitory synaptic connections with other neurons both in culture and after transplantation into mice (see image).

This method cannot yet generate the full spectrum of GABAergic neurons, and Sun says his team is still learning how to produce one major subclass known as parvalbumin neurons. Nevertheless, the researchers have already begun to explore the clear clinical potential of their neuron-producing protocol. “We are interested in understanding epilepsy, autism and schizophrenia,” says Sun, “and we are already applying this method to model epilepsy.”

1. Sun, A. X., Yuan, Q., Tan, S., Xiao, Y., Wang, D. *et al.* Direct induction and functional maturation of forebrain GABAergic neurons from human pluripotent stem cells. *Cell Reports* **16**, 1942–1953 (2016).



Culture-derived human gamma-aminobutyric acid-releasing neurons (red) integrate and form synapses with surrounding neurons (green) after transplantation into the brain of a mouse.

From trash to treasure: INDUSTRIAL CHEMICALS FROM AGRICULTURAL WASTE

A*STAR's Biomass-To-Chemical and Bio-Renewable Chemicals from Biomass programs are leading the way on the use of waste biomass for the sustainable production of industrial chemicals

Each year more than half a billion tonnes of oil — more than an eighth of the total global oil consumption — are used to produce chemicals and plastics. The demand for oil leaves the petrochemical industry, with a market value slated to exceed US\$758 billion by 2022, critically exposed to oil price fluctuations and the uncertainty of dependence on a finite fossil resource. Researchers and chemical engineers around the world have been trying to find ways to use alternative raw materials such as agricultural waste, or 'biomass', to replace petroleum in the production of common industrial

chemicals as a step toward a more sustainable chemical industry.

In 2012, A*STAR brought together scientists working on research related to biomass feedstocks to form the Biomass-To-Chemical (B2C) programme. Led by the A*STAR Institute of Chemical and Engineering Sciences (ICES), in close collaboration with the Institute of Bioengineering and Nanotechnology (IBN), the B2C programme has worked toward developing a complete value chain, from raw biomass to commodity and specialty chemicals, as a commercially viable demonstration of biomass-based sustainable chemical production.

A local resource

Almost half of the world's petrochemical production occurs in Asia, which happens to have a rich source of waste biomass — the waste 'fruit' left over from palm oil production. Research at the ICES has focused on utilizing this cheap and local 'empty fruit bunch' (EFB) resource for sustainable chemical production.

"The biggest challenge we face is in cost-effectively converting EFB into the pure 'feedstocks' or basic chemicals needed for industrial chemical production," explains Wu Jinchuan, head of industrial biotechnology at the ICES. "For this we need to develop cheaper ways to obtain fermentable sugars from the biomass, and

Waste biomass from palm oil production, known as empty fruit bunch, represents an enormous untapped resource for industrial chemical production.



more powerful microbes for fermentation of the sugars to obtain useful feedstocks such as lactic acid in industrial yields.”

Lactic acid is a food preservative and curing and flavoring agent, but it is also used as a crude feedstock for the production of many other industrial chemicals. As a vital first link in the biomass-to-chemical value chain, a process has been developed by Wu’s team for efficiently converting EFB to high-yield lactic acid by enzyme-driven hydrolysis followed by fermentation using thermophilic bacteria that occur naturally in Singapore.

“With this process we have shown that we can produce high-yield lactic acid

from EFB,” says Wu. “Our next step is to lower the processing cost further by refining the pretreatment and fermentation technologies.”

From crude feedstock to industrial chemicals

Many industrial chemicals still rely on petroleum-based feedstocks. Finding a way to substitute a renewable feedstock like biomass-derived lactic acid in the production of a wide range of chemicals will be critical to driving a sustainable revolution in global chemical production.

The ICES Heterogeneous Catalysis division, under the leadership of Armando

Borgna, is working on the conversion of lactic acid to common industrial chemicals like acrylic acid, which is normally produced from petroleum-based propylene.

“Acrylic acid is an important commodity chemical used for superabsorbent polymers, plastics, and synthetic rubber, as well as in the manufacture of coatings, paint formulations and leather finishing,” says Choi Won Jae, head of the Bioprocess Engineering Centre and the B2C programme director. “There are intensive efforts going on worldwide to develop a biological-based method to produce it from a renewable feedstock. Our approach using lactic acid allows us to make use of

EFB, which is a non-food resource that is cheap and readily available as a waste derived from the world's largest palm oil mills here in southeast Asia."

The critical part of the lactic acid to acrylic acid conversion process is finding an efficient way to strip the water from lactic acid. Choi and his team have been studying the use of multi-element inorganic materials as a catalyst for this reaction, but the key challenge has been the low yield of acrylic acid from this process due to unwanted side reactions. Recent breakthroughs, however, have shown significant promise.

"Through structural engineering and advanced surface modification of the catalyst material, we have been able to increase the yield of acrylic acid to more than 80 per cent, which is by far the best performance ever reported for this reaction," says Choi.

To support the B2C programme, the ICES has brought all of these technologies together to establish an integrated biorefinery process specifically for the

production of acrylic acid from EFB. The process encompasses the entire value chain with research teams working on each operational unit, from biomass pretreatment, enzymatic hydrolysis and fermentation, to separation, purification and catalytic conversion of lactic acid to acrylic acid.

"At the ICES, we have world-class facilities, excellent scientists from all over the world and strong financial support from the Singaporean government," says Wu. "We have all the facilities needed for our research — Parr reactors for pretreatment of biomass to extract fermentable sugars, fermenters to convert sugars to various chemicals by microbial fermentation, and a range of supporting facilities such as automated liquid handling systems, robotic colony pickers, and a plasma generator for isolating microbes and genetically modifying them to improve their performance for chemical production."

Exploring chemical diversity

At the Institute of Bioengineering and Nanotechnology, scientists involved in the



Bench-scale fermenters for biomass conversion experiments at ICES.

biomass-to-chemical research coordinate with their colleagues at ICES through the Bio-Renewable Chemicals from Biomass programme led by Yugen Zhang.

"Here, we are working on the conversion of biomass resources to a wider range of industrial chemicals," says Zhang. "Chemicals such as adipic acid, maleic anhydride, acrylic acid, butadiene and furandicarboxylic acid, are very important for the polymer industry. Our scientists are doing cutting-edge research that capitalizes on our expertise in many different fields, including catalysis, organic chemistry and materials."

Under the IBN programme, researchers have developed highly efficient processes for a number of industrially important reactions, including the conversion of mucic acid to adipic acid — a fundamental step in the production of nylon — and of sugars to furandicarboxylic acid, which is an important emerging bioprocess with many potential applications in polymer production and medicine.

"In our research, we focus on increasing the selectivity of the reactions, which increases yield and lowers the cost of the overall process, which is the major challenge for the use of biomass in chemical production," says Zhang.

Despite the obstacles, the potential of industrial biomass-to-chemical production make it very much worth the effort and investment. "This research field is very exciting," says Wu. "Converting renewable resources to value-added chemicals is sustainable in a way that fossil fuels will never be. Even when the price of petroleum is low, biomass conversion is still commercially promising, particularly for specialty chemicals."

“Converting renewable resources to value-added chemicals is sustainable in a way that fossil fuels will never be.”



A 50 liter fermenter for biomass conversion at the ICES laboratory.

[RESEARCH HIGHLIGHTS]

Genetic changes in cancer cells, such as these pancreatic cancer cells, will be much easier to track thanks to a new DNA analysis method developed by A*STAR researchers.

Cancer genetics

REVEALING THE BLUEPRINTS

A SENSITIVE DNA PROFILING TECHNIQUE COULD IMPROVE SCIENTISTS' APPROACH TO FIGHTING CANCER DRUG RESISTANCE

A method for analyzing DNA in cells at a deeper level is set to give a clearer picture of the biology of tumors and could revolutionize the way they are treated.

Cancer claims millions of lives every year and researchers are racing to find effective treatments. However, no two cancers are the same, and drugs that are effective for one patient may not work for another.

A reason is that tumors contain millions of cells which all have slightly different characteristics. Understanding the subtle differences between cancer cells could shed light on how they evolve and reveal which treatment is most likely to be effective.

In 2013, Lih Feng Cheow and his colleagues from the A*STAR Institute of Molecular and Cell Biology developed a method for identifying

some of these differences, by measuring a cell signature called DNA methylation in single cells.

After writing new scripts for a system that is already used commercially to sequence DNA, Cheow and his team have automated their method to run on microchips that can treat and analyse 96 cells at the same time¹. They also expanded the method to measure even more cellular detail — DNA mutation and gene expression — concurrently.

The team tested their method to better understand a type of lung cancer called adenocarcinoma.

Using their technique, they could distinguish previously undiscovered signatures. They found that the subset of cells in the population that carried a key cancer mutation exhibited distinct patterns of DNA methylation.

The benefit of this method is that it is user-friendly, says Cheow. “We reengineered a commercial system so that anyone can just get this off the shelf.” After demonstrating the method works, Cheow says, “we’re now working with clinicians to look at the profiles of different cancer cells and to look at drug resistance.”

Cheow believes his method may also find use in clinical laboratories, where it could be used to screen embryos and increase the success rates of *in vitro* fertilization procedures.

“We want anyone to have easy access to this method and to do great science with it,” he says.

1. Cheow, L. F., Courtis, E. T., Tan, Y. *et al.* Single-cell multimodal profiling reveals cellular epigenetic heterogeneity. *Nature Methods* **13**, 833–836 (2016).

Fluorescence

INTO THE LIGHT

MODELING THE FLUORESCENCE ENHANCING CAPABILITIES OF MATERIALS PAVES THE WAY FOR MORE SENSITIVE BIOLOGICAL AND CHEMICAL TRACKING TECHNOLOGIES

The capacity of various noble metals and dielectrics to enhance fluorescence has been compared by A*STAR researchers, with a view to realizing more-sensitive technologies to creating new applications in biology and medicine¹.

Fluorescence occurs when an electron, after excitation from a fluorophore molecule, drops from the excited state back to its ground state and emits a photon of light. Utilizing this phenomena, fluorescent labeling, a highly sensitive and non-destructive technique, allows for binding to a specific region or functional group on a target molecule, such as a protein or enzyme.

Fluorescent labeling is commonly used for tracking biological or chemical compounds in mineralogy, forensics and medicine. Its application in DNA sequencing, molecular and cell biology, and the food safety industry is also attracting considerable interest, but relies upon light emitted by a single fluorophore, which is generally weak, thwarting its sensitivity.

This is pushing the search for technologies that amplify the fluorescence, spurring Bai Ping and colleagues from the Electronics and Photonics Department at the A*STAR Singapore Institute of High Performance Computing to compare the fluorescence enhancing capabilities of dielectric

nanoparticles and silver and gold plasmonic metal nanoparticles.

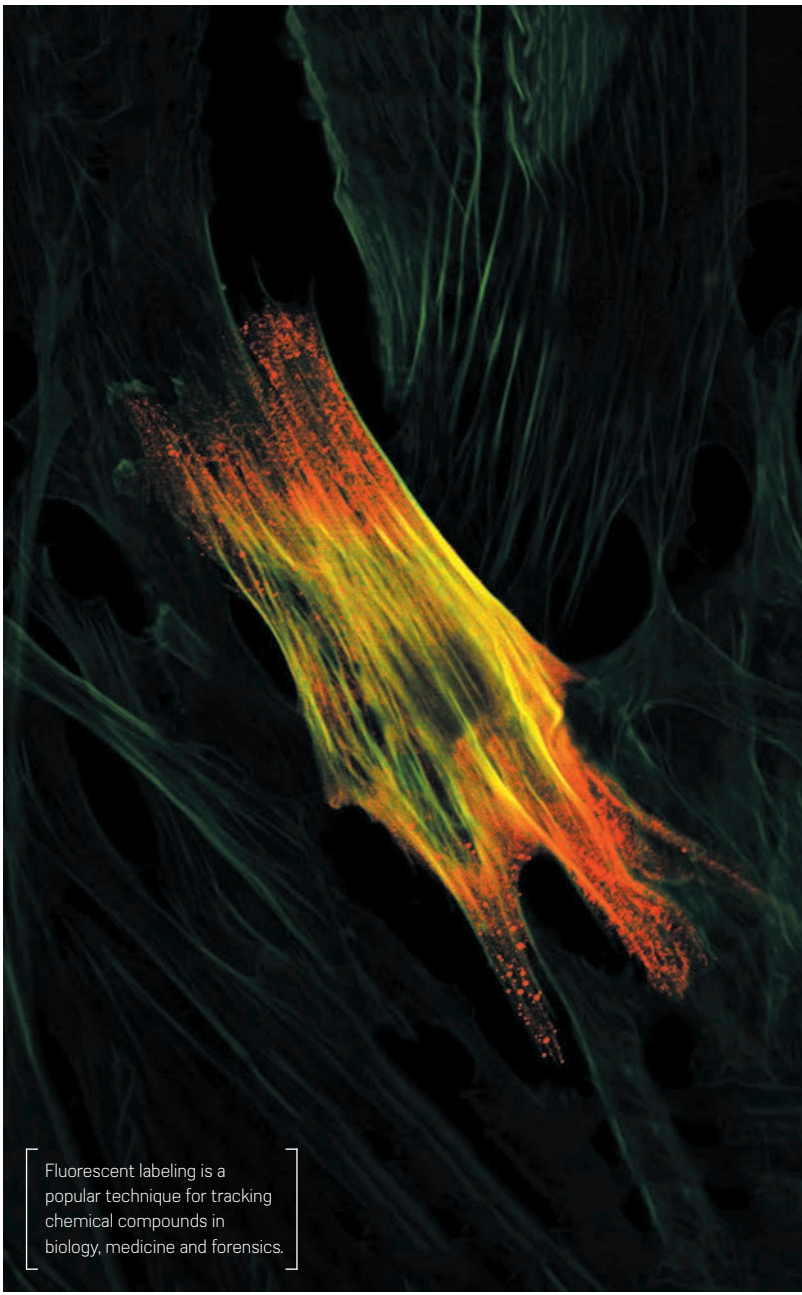
"Previously, metals have been used because they are able to confine the light into a small area, producing a stronger signal," explains Bai. "But when the metal is placed close to the fluorophore, some of the light is reabsorbed by the metal — called quenching — reducing its fluorescence-enhancing capabilities."

As dielectric materials do not undergo quenching, particularly in the visible light

range, they have also been used; but they have poorer confinement capabilities than metals.

"A hybrid that combines the advantages of both materials is needed," Bai says. "Our work compares the performance of both materials by taking their structures and operating environments into account, providing for an objective comparison."

Because of the tiny distances between the materials and the fluorophores, an experimental comparison is very challenging. The researchers used a simulation



Fluorescent labeling is a popular technique for tracking chemical compounds in biology, medicine and forensics.

based on a simple spherical nanoparticle model, and observed the fluorescence enhancement in an air and water environment. This allowed them to observe the different physical confinement characteristics for each material.

“Our results show that in air the dielectric is better, but in water the metals perform better,” says Bai. “This provided us with knowledge to explore new materials and structures that could combine the advantages of

both materials, with the potential for more-sensitive technologies.”

1. Sun, S., Wu, L., Bai, P. & Png, C. E. Fluorescence enhancement in visible light: Dielectric or noble metal? *Physical Chemistry Chemical Physics* **18**, 19324–19335 (2016).

Materials

STANENE'S THERMAL SURPRISE

ELECTRONS PLAY A KEY ROLE IN HEAT TRANSPORT THROUGH 2D TIN SHEETS

Heat travels through atom-thin sheets of tin in a very unusual way, A*STAR researchers have found¹. The discovery could help develop applications for the material, including thermoelectric refrigeration or power generation.

Graphene, a layer of carbon just one atom thick, was first isolated in 2004. Since

then, researchers have created a plethora of other ‘2D’ analogs of graphene using different atoms. Stanene, with its tin atoms arranged in a slightly corrugated hexagonal pattern (see image), arrived in 2015. Hangbo Zhou and colleagues at the A*STAR Institute of High Performance Computing have

now studied how this cousin of graphene conducts heat.

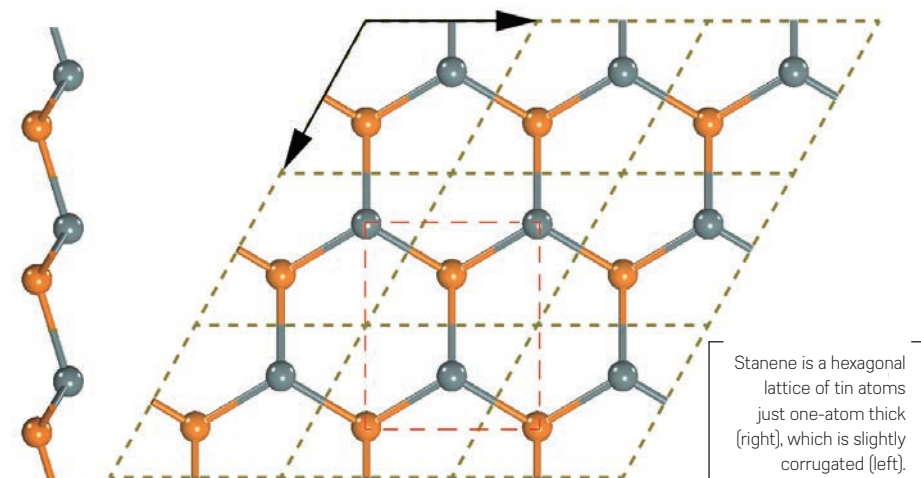
In solid materials, heat is generally carried by electrons or through vibrations between atoms. As these vibrations travel through the material, they behave rather like a particle, known as a phonon. At room temperature, graphene mostly conducts heat with phonons, whereas metals largely rely on electrons. But in stanene, the balance between these two mechanisms was unknown.

The A*STAR team calculated the phonon and electron thermal conduction in stanene at various temperatures and found that stanene has a much lower phonon thermal conduction than graphene. Indeed, at room temperature, electron thermal conduction in stanene is roughly the same as its phonon conduction.

"THE VIOLATION OF THE LAW MAY PROVIDE AN ALTERNATIVE ROUTE TO ACHIEVING HIGH-EFFICIENCY THERMOELECTRIC MATERIALS."

They also found that stanene deviates from the Wiedemann–Franz law, which states that electron thermal conduction depends on the temperature and the electrical conductivity of the material. In stanene, however, the contribution of electron thermal conduction to overall heat transfer also depends on the material’s ‘chemical potential’—a measure of how much energy is required to add one more electron to the material. Crucially, the researchers found that chemical potential also affects electron thermal transport in graphene and some other 2D materials.

The surprising findings could make stanene useful in thermoelectric devices, in which a temperature gradient creates a voltage between two parts of a material, or vice versa.



“The Wiedemann–Franz law is one of the major factors that limits the thermoelectric efficiency of conductors,” says Zhou. “The violation of the law may provide an alternative route to achieving high-efficiency thermoelectric materials.”

The calculations suggest that stanene’s thermal transport properties could be tuned by altering its chemical potential, he adds, for example by adding traces of other atoms.

The team now hopes to calculate how efficiently stanene can generate thermo-

electric power, and the size of the voltage generated by a temperature difference in the material.

1. Zhou, H., Cai, Y., Zhang, G. & Zhang, Y-W. Quantum thermal transport in stanene. *Physical Review B* **94**, 045423 (2016).

Chikungunya

CHILDREN SHRUG OFF DISEASE SYMPTOMS WHILE STILL INFECTIOUS

MILD SYMPTOMS COULD MEAN THAT CHILDREN ARE BRINGING THE MOSQUITO-BORNE DISEASE TO THE PLAYGROUND

Children recover from chikungunya viral infection more quickly than adults, which could make them hidden carriers of the disease, finds a team of immunologists and pediatricians in Singapore and Malaysia¹.

Unlike adults, who typically stay indoors while the virus is active, children feel less wretched and continue to play outdoors, exposing themselves to mosquitoes. “Some kids

still carry the virus in their blood even when they don’t feel sick anymore, which is scary,” says Lisa F. P. Ng, who led the study at the A*STAR Singapore Immunology Network (SIgN). “It is important to clinically manage children from serving as reservoirs of infection.”

Chikungunya is a painful viral disease transmitted via mosquitoes that also carry dengue and the Zika virus. Identified in Tanzania in 1953,

the virus has since spread worldwide. There are no specific antiviral treatments for chikungunya and vaccines are stalled in preclinical trials.

Infected adults typically suffer from fever, rashes and debilitating joint pain that can persist for months to years. But clinicians at Sarawak General Hospital in Malaysia, led by Ooi Mong How, noticed that children did not suffer as much. They approached Ng and her team to explore the immunological basis for the chills and aches.

The team studied blood samples from 86 chikungunya-infected children aged 1 week to 11 years, who visited the hospital between 2009 and 2010. They compared these samples with those of 64 infected adults in Singapore. The children expressed higher levels of small proteins known as cytokines, which communicate information between immune cells. “They have a very active immune response at an early stage, and then it clears off fairly quickly,” explains Ng. The spiked cytokine activity could explain the children’s improved clinical outcomes.

The children’s results revealed a more complex picture. More than half still had the virus



Children infected with the chikungunya virus could infect mosquitoes that spread the disease.

circulating in their blood when discharged from hospital. Compared to those who had hastily rid their bodies of the virus, these children had lower cytokine levels and complained more of joint pain. The results further supported the conclusion that clinical severity is closely associated with immunological

response and virus elimination. “Children with early viral clearance have milder disease,” says Ng. But more concerning for Ng was the seemingly healthy, yet still infectious, group. “You think they are safe, but they are not.”

Ng is keen to compare these results with other infected infant populations. “The virus

is here to stay, so the best thing we can do is to try to understand it.”

1. Simarmata, D., Ng, D. C. E., Kam, Y.-W., Lee, B., Sum, M. S. H. *et al.* Early clearance of Chikungunya virus in children is associated with a strong innate immune response. *Scientific Reports* **6**, 26097 (2016).

Materials

CRACKING THE CODE FOR FISSURE CONTROL

SCIENTISTS FIND A WAY TO CONTROL THE WAY CRACKS FORM AND SPREAD TO MAKE A COATING FOR ELECTROCHROMIC MATERIALS

A micrograph showing the highly ordered cracked surface of a metal oxide thin film.

Cracks in a material typically compromise its strength and integrity, so research focus has traditionally been on preventing their occurrence and spread. An A*STAR team has now taken a different approach, prompting and directing the propagation of cracks on thin films to make highly ordered patterned coatings for electrochromic materials¹.

The transmission of light by electrochromic materials alters in response to brief bursts of electrical charge. They have optical uses ranging from the windows in Boeing 787-9 Dreamliners which change color at the touch of a button, to privacy glass around hotel bathrooms which switch between clear and opaque, to auto-dimming rear view car mirrors.

To feasibly expand the potential uses for these materials, scientists must reduce the amount of electrical power needed to modulate their optical property changes, explains team leader Sing Yang Chiam from

the A*STAR Institute of Materials Research and Engineering. To achieve this, “devices will require a greater surface area of contact for enhanced interaction”, he says. “If you use nanoparticles for a large surface area, scattering makes for poor optical properties. Using a film with controlled cracks allows us to increase the surface area for better electrical efficiency, without sacrifice of the optical properties.”

Chiam’s team’s first step was to grow a thin NiO/Ni(OH)₂ film on top of a regular array of pillars fixed to a rigid substrate. Such a structure introduced strain at predetermined and regular points on the film. For example, spots with no support from any pillars were mechanically weak. The team found that briefly air-drying the newly formed films was sufficient to trigger the crack formation at these locations. Further dehydration in a furnace caused the material to shrink and cause significant crack propagation. Electron microscopy images showed that the

cracking pattern on the surface was so ordered that it looked “artificially squarish” (see image), Chiam says. An unprecedented level of fragmentation control at the submicron and nanometer scale had been achieved.

Finally, the team checked the electrochromic performance of the films using cyclic voltammetry measurements to measure their switching and optical properties. “The resultant structures yielded excellent electrochromic performance with high-coloration efficiency and stable cycling stability,” Chiam confirms.

“While the demonstrated enhancement is in electrochromics, I think the significance of the work is in the discovery of a method to order and control fragmentation at such a scale,” he adds.

1. Guo, L., Ren, Y., Kong, L. Y., Chim, W. K. & Chiam, S. Y. Ordered fragmentation of oxide thin films at submicron scale. *Nature Communications* **7**, 13148 (2016).

Meta-analysis

ANXIETY OVER ANXIETY RESEARCH

**RESEARCHERS FIND EVIDENCE
OF SHELVED NEGATIVE
RESULTS IN PRECLINICAL
STUDIES OF ANXIETY**

A systematic review of rodent studies of anxiety drug targets has found a possible reason for thwarted drug development in the field: researchers might not reveal all the data they collect¹.

“In a perfect world of open data, researchers would publish every single datum,” says Adam Claridge-Chang, who led the investigation at the A*STAR Institute of Molecular and Cell Biology (IMCB). “But there is a stigma attached to negative results, so these data are often censored by the researchers themselves, even though they are useful.”

Claridge-Chang’s in-depth probe into pre-clinical data could lead to better treatments for the cluster of mental health disorders that affect more than 7 per cent of the global population.

Treatments for anxiety have been fraught with problems. In the early twentieth century, pharmaceutical companies began selling barbiturates, which put patients at risk of lethal overdose. These were followed by diazepam (first sold as Valium), which can be habit-forming and can cause severe withdrawals.

Anxiety researchers often don't publish negative results to avoid the stigma attached to them.

A new class of drugs was released in the 1990s called selective serotonin reuptake inhibitors (SSRIs). These drugs, including Prozac and Zoloft, increase serotonin levels in the brain by blocking the proteins that pump them into neurons. But scientists have grave doubts about their effectiveness.

Claridge-Chang's group at A*STAR studies anxiety in the vinegar fly, a powerful genetic model. When they turned to the mouse and rat literature for guidance, they found many contradictory results. This lack of consensus was especially striking, as preclinical studies of rodents typically form the basis for psychiatric drugs entering clinical trials.

To make sense of the background, team members Farhan Mohammad and Joses Ho analyzed more than 300 mouse and rat studies published between 1985 and 2015 for ten types of anxiety drug targets, including the targets of SSRIs. Eight of the interventions were found to have strong effects on anxiety in the animals.

However, when the researchers plotted the published data on a graph, they found an unexpectedly skewed pattern. "Where dots should have been, they weren't," explains Ho. Medical statisticians show that such skewed distributions usually indicate that researchers are shelving statistically

insignificant results, a phenomenon called publication bias.

This was not the only inconsistency: mutant mice lacking the SSRI target protein had higher anxiety levels, even though SSRIs are prescribed as anti-anxiety medications. Yet the literature did not reflect this. "This is a direct contradiction, but about half of the authors didn't even mention it in their papers," says Claridge-Chang.

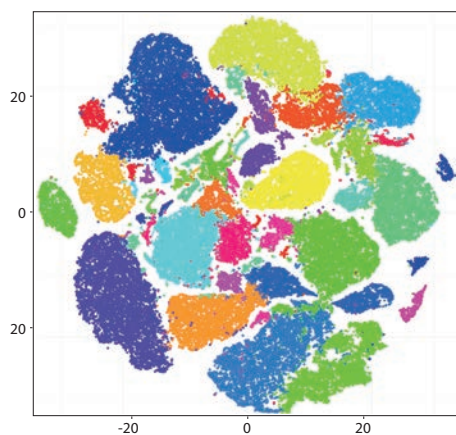
1. Mohammad, F., Ho, J., Woo, J. H., Lim, C. L., Poon, D. J. *et al.* Concordance and incongruence in preclinical anxiety models: Systematic review and meta-analyses. *Neuroscience & Biobehavioral Reviews* **68**, 504–529 (2016).

Bioinformatics

NEW TOOL TO CLEAN FLOW CYTOMETRY DATA

A MORE INTUITIVE AND EFFICIENT SOLUTION TO GET RID OF ANOMALIES IN IMMUNOLOGICAL STUDIES

A*STAR researchers have developed a new bioinformatics tool called flowAI, which provides a more objective, efficient and intuitive solution to the quality control of data



The complexity of the immune cells in human whole blood analyzed by flow cytometry.

acquired via a common biological technique called flow cytometry¹.

Flow cytometry is the first-choice technology in immunology and other biological fields to characterize physical and functional properties of cells. Beyond separating cells according to their size and granularity, flow cytometry can distinguish specific cells by the proteins present on their membrane, which are recognized by antibodies labeled with different fluorescence colors.

Although this technique allows up to 20 parameters to be analyzed simultaneously, its inefficient data analysis is often performed manually, which is time consuming and relies on high expertise and subjective interpretation.

"Being actively involved in several activities of the International Society for the Advancement of Cytometry (ISAC), we have realized that one growing demand is to improve the automatic analysis of flow cytometry data," explains

Anis Larbi, principal investigator at the Singapore Immunology Network. "We believe that high-quality data lead to more-accurate results and better downstream computational analyses."

FlowAI is a software package, which uses the statistical language known as R, and is available on the open-source project Bioconductor. It allows users to discard poor-quality data either automatically via an algorithm or manually using a graphical user interface. FlowAI eliminates anomalies caused by debris, air intrusion in the fluidic system, technical issues, voltage instability and so on, which create abrupt changes in the speed of the fluid, instability of signal acquisition over time and data outliers. The analysis generates a report that indicates the percentage of cells that did not pass the quality checks and graphs showing where the anomalies were detected.

A*STAR scientists tested flowAI with 4,469 flow cytometry files from 11 different datasets and also compared the flowAI automatic

method with other software packages used for flow cytometry data analysis and quality control, namely flowJo and flowClean. Among these, flowAI was the fastest, the most stringent toward anomalies and the most intuitive to use.

“Scientists who want to look deeper into the cellular complexity often need to distinguish

extremely rare cells, which may be lost with an unreliable quality control. We expect that flowAI will help scientists to remove background noise and achieve a more accurate detection of these rare cells and an easier characterization of the source of ambiguous results. We recommend flow cytometry users

to try flowAI and let us know what they think,” suggests Larbi.

1. Monaco, G., Chen, H., Poidinger, M., Chen, J., de Magalhães, J. P. & Larbi, A. flowAI: Automatic and interactive anomaly discerning tools for flow cytometry data. *Bioinformatics* 32, 2473–2480 (2016).

Optoelectronics

MIX AND MATCH LASERS

COMBINING SILICON WITH AN OPTICALLY ACTIVE MATERIAL ENABLES TINY LASERS COMPATIBLE WITH INDUSTRIAL FABRICATION TECHNIQUES

Combining silicon with a light-producing semiconductor may help develop micrometer-scale lasers, say Doris Keh-Ting Ng and her colleagues from the A*STAR Data Storage Institute¹.

Silicon has revolutionized the manufacture of electrical devices. This abundant semiconductor is easily processed into tiny

components, such as transistors, using methods that are scalable to industrial levels, thus enabling the production of hundreds of thousands of elements on a single chip. Electronic engineers would like to further expand the functionality of these integrated circuits by enabling them to create, manipulate and detect light.

These optoelectronic devices could speed up processing of digital information, and lead to micrometer-scale lasers, for use in barcode scanners for example. The problem, however, is that silicon is not an efficient light generator.

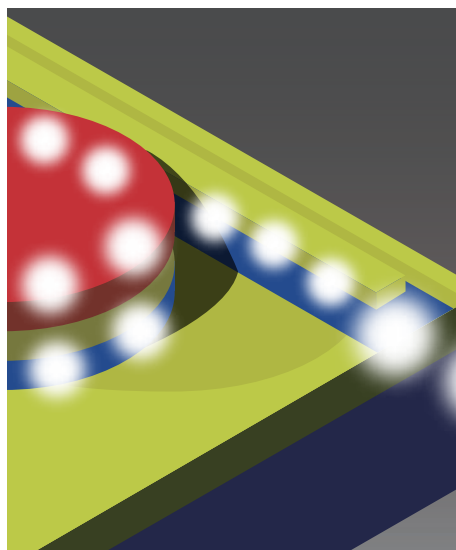
Ng’s team designed and produced a laser compatible with silicon fabrication techniques by combining silicon and another semiconductor material that can produce light: indium gallium arsenide phosphide (InGaAsP). “Our results demonstrate a promising approach for efficient and compact active optoelectronic devices on silicon using a very thin III–V semiconductor layer,” says Ng.

A crucial consideration in any laser structure is optical feedback: the ability to trap light within the structure to drive further light generation. In conventional lasers, this is done by placing a mirror on either side of the light-generating region. Instead, Ng and the team used a cylindrical device geometry. This trapped some of the generated light at the walls

of the device and forced it to propagate round inside the cylinder. This is called a whispering-gallery mode because the same effect traps sound waves in a circular room such as a cathedral dome.

The team started with a silicon substrate, onto which they deposited a thin layer of silicon oxide. The optically active InGaAsP film, just 210 nanometers thick, was fabricated separately and bonded on top of the silicon oxide. The team then etched through some of the material to create cylinders either 2 or 3 micrometers in diameter. The 3-micrometer devices emitted laser light with a wavelength of 1,519 nanometers, very close to that used in commercial optical communications systems.

A unique feature of this device is that the whispering-gallery mode extends over both the silicon and the InGaAsP regions. The InGaAsP provides light amplification while the silicon passively guides the light. “Next, we hope to apply these ideas to devices operating at room temperature,” says Ng. “Operation at higher temperature will require fine-tuning the laser design and fabrication.”



A microlaser comprised of a cylinder of indium gallium arsenide phosphide (red) on silicon (blue) could enable integrated optical circuits.

1. Lee, C.-W., Ng, D. K.-T., Tan, A. L. & Wang, Q. Hetero-core III–V/Si microlaser. *Optics Letters* 41, 3149–3152 (2016).

Nutrition

MEASURING DISEASE RISK

UNDERSTANDING ETHNIC DIFFERENCES IN ABDOMINAL FAT DISTRIBUTION AT BIRTH COULD LEAD TO BETTER GUIDELINES FOR MATERNAL LIFESTYLE AND NUTRITION



The superficial subcutaneous tissue (green), the deep subcutaneous tissue (orange), and the internal adipose tissue (magenta) of Singaporean infants of three different ethnic backgrounds were measured and compared.

Abdominal fat in adults has long been associated with increased risk of metabolic and cardiovascular disease. Now, a Singaporean team has studied more than 300 infants and found the amount of abdominal fat they carry varies depending on ethnic background¹. This may be the first step in establishing a link between abdominal fat in newborns and disease later in life.

Investigators from A*STAR, the KK Women's and Children's Hospital and the National University Health System conducted magnetic resonance imaging abdominal scans on 333 Singaporean newborns of Chinese, Malay and Indian ethnicities who were part of the Growing Up in Singapore Towards Healthy Outcomes (GUSTO) birth cohort. They found significant differences in the amounts of abdominal fat between ethnicities. The findings support the notion that maternal lifestyle during prenatal development may alter glucose metabolism and body composition, contributing to the higher risks for cardiac and metabolic diseases (like diabetes) seen in Indian and Malay adults in Singapore.

"Our findings of ethnic differences in abdominal adiposity at birth are important as they not only reflect the influence of genetic factors unique to ethnic groups, but also the importance of ethnic-specific maternal

lifestyle and nutrition during pregnancy," says Yung Seng Lee from the A*STAR Singapore Institute for Clinical Sciences.

The study investigated differences in three different fat compartments in the abdomen: abdominal superficial subcutaneous tissue, which is located just under the skin and surrounds the abdominal cavity; abdominal deep subcutaneous tissue, which is clearly separated from the superficial fat by a sheath of fibrous tissue and is located on either side of the posterior abdominal wall; and internal adipose tissue — the minute amount of fat that surrounds the abdominal organs.

The researchers compared the volumes of each fat compartment and found that Indian and Malay newborns had greater volumes of deep subcutaneous fatty tissue than the Chinese babies.

"Several reports have found that deep subcutaneous adipose tissue is strongly associated with insulin resistance and cardiovascular outcomes in type II diabetic adults. The possible relevance of this in the babies is intriguing, but we need to continue to track the fat depot distribution over time to substantiate its significance," says Mya Thway Tint of the Yong Loo Lin School of Medicine.

If further research supports these findings, they will highlight the importance of

"THIS MAY HELP TO STRATEGIZE ETHNIC-SPECIFIC EARLY INTERVENTIONS SUCH AS LIFESTYLE MODIFICATIONS AND NUTRITIONAL GUIDELINES FOR WOMEN [WHO ARE PLANNING TO BECOME PREGNANT]."

maternal health and well-being both before conception and during pregnancy, explains Tint. Further studies are needed to investigate correlations between maternal lifestyle and abdominal fat distribution in newborns. "This may help to strategize ethnic-specific early interventions such as lifestyle modifications and nutritional guidelines for women [who are planning to become pregnant]," she says.

The team will track changes in abdominal fat volume in the newborns when they reach four and six years of age to help further understand the impact of ethnic variation in adiposity, as well as other maternal and developmental factors, on subsequent risks of cardiometabolic diseases.

1. Tint, M. T., Fortier, M. V., Godfrey, K. M., Shuter, B., Kapur, J. *et al.* Abdominal adipose tissue compartments vary with ethnicity in Asian neonates: Growing Up in Singapore Towards Healthy Outcomes birth cohort study. *The American Journal of Clinical Nutrition* 103, 1311–1317 (2016).



[AWARD-WINNING IMAGE]

Thankiah Sudhaharan and Jaron Liu (alumni) from the A*STAR Institute of Medical Biology won the Thermo Fisher Scientific Cell-ebrate Science Imaging Contest with this super-resolution microscopy image of hair-like protrusions in NIE-115 mouse neuroblastoma cells.

Neuroblastoma is a type of cancer that develops from immature nerve cells. In humans, it is the most common cancer in children under 1 year of age.

Dr. Thankiah Sudhaharan and Dr. Jaron Liu (alumni), A*STAR Institute of Medical Biology



Patterns of repetition in DNA could be associated with the risk of developing kidney inflammation.

Genomics

COPY THAT? THE GENETICS OF KIDNEY FAILURE

GENETIC REPETITIONS DETERMINE THE RISK OF DEVELOPING A COMMON KIDNEY DISEASE

Counting the number of times a string of letters appears in the genome could bring us closer to predicting kidney failure, suggests an international team of researchers¹. They found that fewer copies of a gene which produces an important defense protein increases a person's risk of developing a common form of kidney inflammation.

The findings could help explain why Chinese people are more susceptible to the condition known as immunoglobulin A nephropathy (IgAN). "Chinese populations have the highest prevalence of IgAN in the world," says Jianjun

Liu, who led the study at the A*STAR Genome Institute of Singapore. "IgAN is a leading cause of kidney disease in this population."

Several genome-wide association studies have been conducted to identify single-nucleotide variations associated with the risk of developing IgAN. One of these large-scale fishing exercises recently identified a specific region on chromosome 8 linked to IgAN. "The contribution of this locus to the IgAN risk equals the sum of all the other genetic risk factors that have been discovered so far," says Liu. He and his team wanted to explore

this region further by quantifying patterns of repetition, known as copy number of variations, in a specific gene called *DEFA1A3*. The number of times a gene repeats can influence disease development and progression.

Using a sophisticated genomic mining technique, the researchers compared the number of *DEFA1A3* genes present in DNA of more than 1,000 individuals with IgAN, and a similar number of healthy individuals. They found that the IgAN patients had significantly fewer repetitions of the *DEFA1A3* gene, which was associated with an increased

risk of developing the disease. Variations in this gene number could also predict whether IgAN patients would progress to end-stage kidney disease. The *DEFA1A3* gene encodes an essential anti-microbial compound called α -defensin, which is released by immune cells during inflammatory responses.

Liu and his colleagues replicated the analysis in a Caucasian population and found a similar

trend: fewer copies of the α -defensin gene in individuals with IgAN. Interestingly, however, the reduced occurrence of a four-nucleotide deletion observed in the Chinese IgAN group, which correlated with IgAN risk, was not seen in the Caucasian group. “We believe this locus contributes to the difference in prevalence between Caucasian and Chinese populations,” says Liu. “However, more studies of this locus, particularly

in the Caucasian population, will be needed to confirm and quantify this contribution.”

The α -defensin protein expressed in this region of the genome could offer potential targets for IgAN therapy, he adds.

1. Ai, Z., Li, M., Liu, W., Foo, J., Mansouri, O. *et al.* Low α -defensin gene copy number increases the risk for IgA nephropathy and renal dysfunction. *Science Translational Medicine* 8, 345ra88 (2016).

Molecular biology

VIRAL GATECRASHERS HAVE TRICK TO BOOST NUMBERS

A BLOCKING MECHANISM IS USED BY A MYSTERIOUS CLASS OF RETROVIRUSES TO FORCE THEIR HOST TO ALLOW THEM TO REPLICATE

Viruses hijack a body's cellular machinery for their own reproduction. Scientists have shown how one class of virus uses a trick to override natural signals that would otherwise stop them from replicating.

Researchers from the A*STAR Institute of Molecular and Cell Biology have identified the mechanism through which one such virus — Moloney murine leukemia virus (MMLV) — is able to effectively ignore the RNA messages

known as stop-codons, which tell the body to stop translating genetic code into proteins.

The project's lead researcher, Haiwei Song, explained that MMLV is one of a family of retroviruses, a type of virus that can insert itself into host DNA to replicate.

“MMLV belongs to the gammaretroviruses, one genus of retroviruses recently implicated in human diseases,” he says. But the exact role of gammaretroviruses in causing

disease remains mysterious.

In 2006, the gammaretrovirus XMRV was identified in samples from men with prostate cancer.

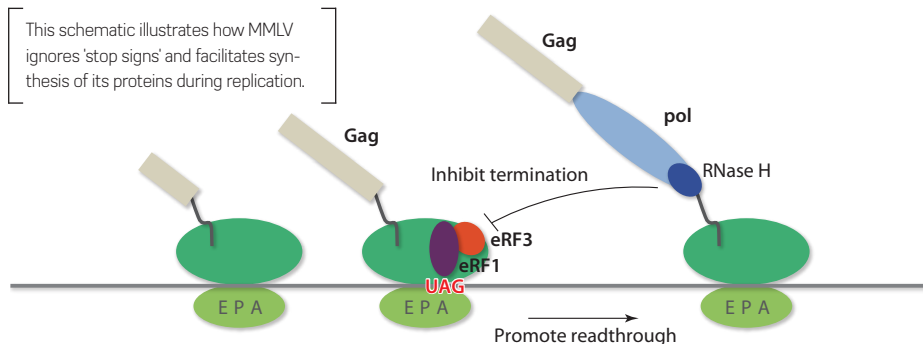
Some studies have found murine leukemia virus (MLV), as well as XMRV, occurs at much higher rates in people with chronic fatigue syndrome than in healthy controls; however, others have not found this difference.

“We don't know the potential role of XMRV and MLV in causing diseases such as prostate cancer and chronic fatigue syndrome, and the frequency of gammaretrovirus infection among healthy people,” Song says. But a link common to all retroviruses is their ability to ignore RNA stop-codons.

“Without this ability, the retroviruses will simply not survive,” Song says. “So it is a very important target for antiviral intervention.”

Identifying the mechanism gammaretroviruses use to ignore stop-codons could lead to new treatments, Song says.

This schematic illustrates how MMLV ignores 'stop signs' and facilitates synthesis of its proteins during replication.



MMLV contains the enzyme reverse transcriptase that allows its RNA to be converted to DNA in the host. Song's research shows that this enzyme binds to a protein (peptidyl release factor 1) in its host that would otherwise be responsible for reading the stop-codons. This thwarts its ability to bind and prevents it from working.

The research also provides insight into the workings of the world's most notorious retrovirus, human immunodeficiency virus (HIV).

It was previously believed that a reverse transcriptase interaction would be common across all retroviruses. However, Song and his team found that the HIV reverse transcriptase did not bond to peptidyl release factor 1.

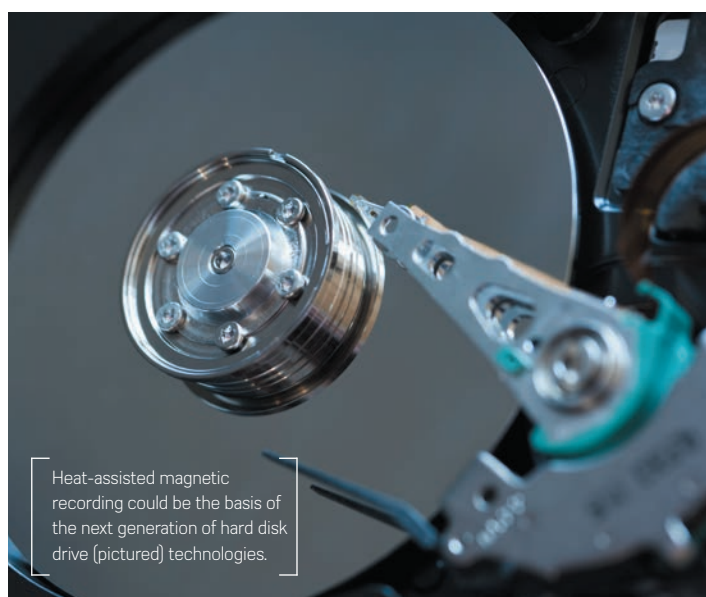
"This means that any anti-gammaretrovirus interventions would not work on HIV," Song says.

1. Tang, X., Zhu, Y., Baker, S. L., Bowler, M. W., Chen, B. J. *et al.* Structural basis of suppression of host translation termination by Moloney Murine Leukemia Virus. *Nature Communications* 7, 12070 (2016).

Data storage

LASER HEATING HITS THE SPOT

LASER-BASED MEASUREMENTS REVEAL JUST HOW SMALL EACH MAGNETIC 'BIT' COULD BE USING NEXT-GENERATION HARD DISK TECHNOLOGY KNOWN AS HEAT-ASSISTED MAGNETIC RECORDING



Heat-assisted magnetic recording could be the basis of the next generation of hard disk drive (pictured) technologies.

A method for accurately measuring the thermomagnetic properties of heat-assisted magnetic recording (HAMR) media reveals what the minimum bit size and ultimate data density might be for the next-generation storage technology¹.

Existing hard disk technology is approaching fundamental physical limits on the amount of data that can be stored on magnetic disks. One of the most promising technologies capable of breaching these limits is HAMR, which heats small areas to allow for smaller magnetic bits and higher data densities. The minimum possible bit size has been the subject of considerable debate. Yang Hongzhi and Yunjie Chen from the A*STAR Data Storage Institute (DSI) have now developed a method using two lasers to put this debate to rest.

"The basic idea of HAMR is to use a tiny laser spot to heat the magnetic material on the

disk to its critical 'Curie' temperature, which makes it more easily writable," explains Chen.

Writability sets the upper limit on data density as it determines how small an area can be magnetically 'switched' using the weak magnetic field of conventional data writing heads. By heating the magnetic disk to a certain temperature, a material with an intrinsically finer-grained magnetic fabric can be used, resulting in smaller bits. One of the unknowns surrounding the technology is how far each bit would need to be separated in order to maintain reliable switching without affecting neighboring bits.

"The switching field distribution at the heating temperature is directly related to how narrow a magnetic transition can be recorded, which will decide the data density that could be achieved," says Chen. "Using a lab-built multifunctional HAMR writing and measurement system here at the DSI, we developed a method that allows us to accurately measure the

thermomagnetic properties of HAMR media at the Curie temperature."

The team's approach uses two laser beams, one to spot-heat the media to exactly the right temperature, and the other to measure the magnetic signal based on an unusual interaction between magnetism and light known as the magneto-optic Kerr effect.

Using this approach, the researchers were able to run a range of tests on experimental HAMR media, providing unprecedented insight into its thermomagnetic response. "We expect this test method to be helpful for characterization and development of HAMR media as the major candidate for the next generation of hard disk drive technologies."

1. Yang, H. Z., Chen, Y. J., Leong, S. H., An, C. W., Ye, K. D. & Hu, J. F. Measurement of magnetic property of FePt granular media at near Curie temperature. *Journal of Magnetism and Magnetic Materials* 423, 27–33 (2017).

Metastatic breast cancer (pictured). Oxygen deprivation is a key factor in switching the function of major cancer genes from tumor-promoting to tumor-suppressing in triple-negative breast cancer.

Cancer

JEKYLL AND HYDE GENES IDENTIFIED

THE PARADOXICAL ROLES OF WELL-KNOWN CANCER GENES ARE MEDIATED BY OXYGEN LEVELS IN BREAST CANCER

Oxygen deprivation, or hypoxia, has been identified by A*STAR researchers as a key factor in switching the function of major cancer genes from tumor promoting to tumor suppressing in a breast cancer subtype, suggesting the need for differential therapies in cancer treatments.

A number of key genes are associated with promoting or suppressing tumor formation and/or migration and invasion in several human cancers. Polycomb repressive complex 2 (*PRC2*) and enhancer of zeste 2 (*EZH2*) are two genes that appear to be significant in both promoting and suppressing tumor formation. A team led by Qiang Yu from the Genome Institute of Singapore at A*STAR were surprised to find that hypoxia was the key factor for promoting *EZH2*-mediated tumor invasion and, therefore, for poor clinical outcomes in triple-negative breast cancer (TNBC).

These findings may have significant consequences for developing future therapies. "Different cancers are driven by different mechanisms, and some signaling components, such as the *EZH2/PRC2* complex, can be both tumor suppressing and tumor promoting,"

says Yu, “and therefore context dependency is always an important factor when it comes to targeted therapies.”

Yu and his team discovered this paradox by examining the chemical pathways of these genes in breast cancer cells. They showed that hypoxia leads to impaired *PRC2* expression but promotes *EZH2* partnering with another tumor-promoting gene, *FoxM1*. Together *EZH2* and *FoxM1* increase expression of the cancer migration promoting gene, matrix metalloproteinase (*MMP*), and enhance tumor migration.

“The double face of *EZH2* as both a tumor

suppressor and an oncogene, and hypoxia to facilitate a switch of the dual functions were surprising,” explains Yu. “This helped to explain how hypoxia can promote growth and invasion.”

Examining these factors in breast cancer was important because *PRC2* and *EZH2* appeared to be expressed at differential levels in breast cancer subtypes, suggesting that they may not always function together. In particular, they have opposite expression levels — *PRC2* is low and *EZH2* is high — in the TNBC subtype, which is highly aggressive and kills more patients than any other breast cancer. Therefore, the switching

of partners by *EZH2* from *PRC2* to *FoxM1* may be responsible for mortality.

This mechanism may also explain the apparently paradoxical nature of *EZH2* in other cancer types. “This may be also seen in other instances, as non-canonical *EZH2* activity has also been documented in other cancers,” notes Yu.

1. Mahara, S., Lee, P. L., Feng, M., Tergaonkar V., Chng, W. J., Yu, Q. HIF1- α activation underlies a functional switch in the paradoxical role of *Ezh2/PRC2* in breast cancer. *Proceedings of the National Academy of Sciences of the USA* **113**, E3735 (2016).

Materials

NANOSTRUCTURED COATINGS TAKE A BITE OUT OF POLLUTANTS

LOW-COST IRON HYDROXIDE COATINGS WITH UNIQUE FIN-LIKE SHAPES CAN CLEAN HEAVILY CONTAMINATED WATER WITH A SIMPLE DIPPING PROCEDURE

An A*STAR team has found a way to turn iron hydroxides into an environmentally friendly coating that repeatedly absorbs large amounts of pollutants, such as dyes, from drinking water at room temperature¹.

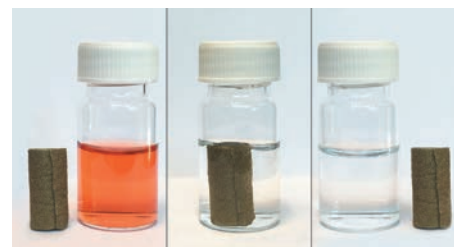
Conventional activated charcoal treatments have trouble removing heavy metals and bulky organic compounds from water. Instead, iron hydroxides are being increasingly used because they can form stable chemical bonds to these unwanted pollutants. Researchers have recently found that turning iron particles into miniscule nanomaterials boosts their active surface areas and enhances chemical absorption processes.

Separating iron hydroxide nanomaterials from water, however, remains difficult. Commercial filtration systems and experimental magnetic treatments introduce significant

complexity and cost into treatment plants. Failure to remove these substances may lead to acute or chronic health issues if they are ingested.

To improve handling of the nanosized iron hydroxides, Sing Yang Chiam from A*STAR's Institute of Materials Research and Engineering and co-workers decided to attach them to a solid, sponge-like support known as nickel foam. This type of material could safely trap and remove contaminants by immersion into dirty water, and then be regenerated with a simple chemical treatment. But immobilizing the nanoparticles also diminishes their valuable high surface areas — a paradox the team had to solve.

“We were not totally convinced that a coating approach could perform as well as traditional powders and particles,” says Chiam. “So we were really pleased when some



Iron-based coatings with elongated nanoscale ‘fins’ can easily absorb large amounts of dye molecules from contaminated water.

nice test results came through.”

The A*STAR team found their answer by synthesizing iron hydroxide coatings with a hierarchy of structural features, from nano- to micrometer scales. To do so, they turned to electrodeposition, a green synthesis method that

deposits aqueous metal ions on to nickel foam at mild voltages. After optimizing the uniformity and adhesion of their multiscale coatings, they tested their material in water contaminated by a 'Congo red' dye pollutant. Within half an hour, the water became almost colorless, with over 90 per cent of the dye attached to the special coating.

Close-up views of the coating's nanostructure using scanning electron microscopy revealed that elongated, fin-like protrusions were key to recovering active surface area for high-performance pollutant removal. "Even though these coatings have some of the highest capacities ever reported, they are only operating at a fraction of

their theoretical capacity," says Chiam. "We are really excited about tapping their potential." ■

1. Liu, J., Wong, L. M., Gurudayal, Wong, L. H., Chiam, S. Y. *et al.* Immobilization of dye pollutants on iron hydroxide coated substrates: Kinetics, efficiency, and the adsorption mechanism. *Journal of Materials Chemistry A* 4, 13280–13288 (2016).

Imaging

SPIKY NANOSTRUC-TURES CAPTURE LIFE'S FINE DETAILS

ASSEMBLING NANORODS INTO COMPLEXES SHAPED LIKE SEA URCHINS MAY ENABLE REAL-TIME IMAGING OF CELL COMPONENTS, INCLUDING DNA

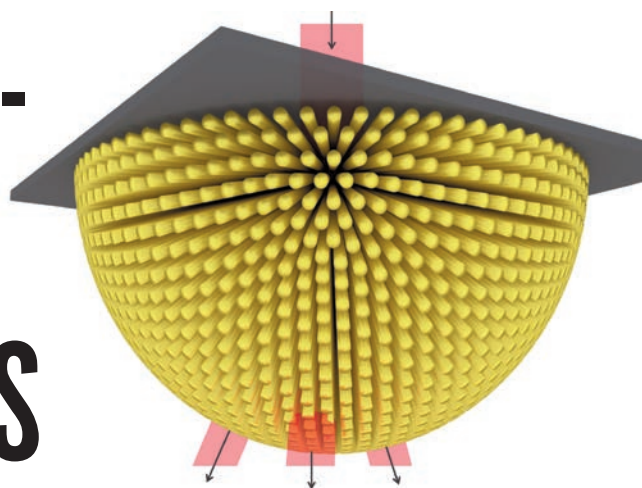
Optical microscopes that use lenses to bounce photons off objects have trouble distinguishing nanometer-scale objects smaller than the imaging beam's wavelength, such as proteins and DNA. An innovative 'hyperlens' designed at A*STAR can overcome optical diffraction limits by capturing high-resolution information held by short-lived or evanescent waves lurking near a target's surface¹.

Hyperlens devices — composed of thin stacks of alternate metal and plastic layers — have raised prospects for capturing living biological processes in action with high-speed optics. Key to their operation are oscillating electrons, known as surface plasmons, that resonate with and enhance evanescent waves that appear when photons strike a solid object. The narrow wavelengths of evanescent beams give nanoscale resolution to images when

the hyperlens propagates the images to a standard microscope.

Mass production of current hyperlenses has stalled however because of their intricate fabrication — up to 18 different layer depositions may be required, each with stringent requirements to avoid signal degradation. "For perfect imaging, these layers need precisely controlled thickness and purity," says Linda Wu from the A*STAR Singapore Institute of Manufacturing Technology. "Otherwise, it's hard to magnify the object sufficiently for a conventional microscope to pick up."

Wu and her co-workers proposed a different type of hyperlens that eliminates the need for multiple interfaces in the light propagation direction — a major source of energy loss and image distortion. The team's concept embeds a hemispherical array of nanorods into a central



A hemispherical hypersphere designed at A*STAR can capture nanometer-scale details in optical images thanks to its sea-urchin-shaped geometry.

insulating core, giving the hyperlens a shape similar to a thorny sea urchin. This geometry enables more efficient harvesting of evanescent waves, as well as improved image projection.

"For the sea-urchin geometry, the nanosized metallic structures align in the same direction as the light propagation direction, and they are much smaller than the wavelength of applied infrared light," explains Wu. "Therefore, the light doesn't 'see' any obstacles, and propagates effectively and naturally, without loss."

The researchers' simulations revealed the spiky hyperlens could separate the complex wave information into its component frequencies and then transmit this data to the microscope as an intense, easy-to-spot band. This approach was also efficient — it proved capable of resolving intricate objects, 50 to 100 nanometers wide, without the need for image post-processing.

Wu notes that fabricating sea-urchin hyperlenses should be much simpler than multilayered structures. “The nanosized metallic structures could be formed using pores and

templates into flexible lenses, with no real size limitations,” she says. “This hyperlens could be an important tool for real-time biomolecular imaging.”

1. Bisht, A., He, W., Wang, X., Wu, L. Y. L., Chen, X. *et al.* Hyperlensing at NIR frequencies using a hemispherical metallic nanowire lens in a sea-urchin geometry. *Nanoscale* 8, 10669–10676 (2016).

Genomics

WHY SEAHORSES ARE SUCH MODERN MALES

GENETIC STUDY REVEALS HOW SEAHORSES LOST THEIR TEETH AND PELVIC FINS, AND ACQUIRED MALE PREGNANCY

The first complete sequence of a seahorse genome finds the genetic roots of the tropical creature’s unusual shape and characteristics¹.

The findings could also explain many features common to the entire animal kingdom, including hind-limb and mineralized-teeth development in humans. “Fish and humans have a similar set of genes,” says Byrappa Venkatesh at the A*STAR Institute of Molecular and Cell Biology, who led the study. “Investigating the seahorse genome can help us to understand human biology and human disease better.”

While classified as fish, seahorses more closely resemble the ‘horse caterpillar’ alluded to in their Latin name, *Hippocampus*. Instead of scales, seahorses are covered in rugged, bony armor. They also lack the tail fin and pelvic fins needed for propulsion and steering, preferring to trot upright, anchoring on to grasses and corals with their coiled tails.

Their tiny, toothless mouths suck food up elongated snouts; and seahorse fathers nurse their young in a brood pouch.

Venkatesh and his colleagues wanted to find the parts of the seahorse genome that give rise to these peculiar features.

They collected samples from a species endemic to the Asia–Pacific region — the tiger tail seahorse — and conducted whole-genome shotgun sequencing, which involves sequencing fragments of DNA and reassembling the strands in a computer. They then compared the seahorse



genome with those of other fish, including the zebrafish, stickleback and Nile tilapia.

The researchers found that much of the seahorse’s strange anatomy could be explained by the loss of specific genes. Seahorses lack *tbx4*, an important gene for pelvic fin formation and the development of hind legs in land animals. They are also missing crucial genes that encode the enamel proteins in teeth, and have a sparse repertoire of receptors required for smell. “This suggests that seahorses don’t use the sense of smell as extensively as other fishes for avoiding predators or finding food and mates,” says Venkatesh.

Seahorses have also acquired several genetic abilities. In a more detailed analysis of the male brood pouch, the researchers counted significantly higher expression levels of a

cluster of novel pregnancy-associated genes called *patriscacins*.

Most surprising for Venkatesh was the genome’s speed of evolution from a common fish ancestor. “Seahorses are very sluggish animals, but if you look inside their DNA, it has been changing very rapidly compared to other fish.”

The team hope to extend their genomic analysis to study the fish’s population size and change over time. Most seahorse species on the IUCN Red List of Threatened Species are categorized as vulnerable, with decreasing or unknown population trends. “Genome sequencing will help in coming up with measures to conserve their stock.”

1. Lin, Q., Fan, S., Zhang, Y., Xu, M., Zhang, H. *et al.* The seahorse genome and the evolution of its specialized morphology. *Nature* 540, 395–399 (2016).

Mini-brains offer wisdom of ages

Researchers at A*STAR are growing better models of aging brain diseases in the search to stop the decline

Dopamine-secreting neurons in mini models of the midbrain produce the dark pigment neuromelanin

Huck Hui Ng and his team were observing a plate of mini brains when they noticed something that made them think of the renowned French anatomist Felix Vicq d'Azyr.

In 1786, D'Azyr published a treatise of the human brain, in which he described for the first time the presence of “dark spots” buried deep in the brain stem, the brain’s control center.

Huck Hui’s team was seeing the same cloudy speckles materialize in their nursery of tiny midbrains¹. Since D'Azyr’s time, neurologists had discovered that the dark pigments, called neuromelanin, were produced by dopamine-secreting neurons, and that damage to these neurons causes Parkinson’s disease.

“We were very excited,” says Huck Hui at the A*STAR Genome Institute of Singapore. “It was the first time that neuromelanin had been detected *in vitro*.” The mottled bulbs, derived from stem cells, signaled to Huck Hui and his collaborators, Hyunsoo Shawn Je at Duke–NUS Medical School and Eng King Tan at Singapore’s National Neuroscience Institute, that they were on course to determine the biological basis for Parkinson’s. “It marked the start of a very exciting journey in trying to understand Parkinson’s disease.” The discovery also fueled increasing confidence in the use of stem cells to study complex diseases in the human brain.

Neurodegenerative diseases such as Alzheimer’s and Parkinson’s are characterized by a progressive deterioration of the nervous system. These diseases can cause declines in mental capacity, a condition known as dementia, as well as reduced control over movement, resulting in the characteristic Parkinson’s tremors. Up to 10 million people globally have Parkinson’s disease and more than 45 million suffer from Alzheimer’s or some other form of dementia. With 8 million cases of dementia diagnosed every year, those numbers are expected to triple by 2050. And the cost of caring for these patients is no small burden — estimated at US\$815 billion in 2015 alone. “We hope our work will have an impact on the lives of patients afflicted with these diseases,” says Huck Hui.

Black stuff

Living models of human tissue made from stem cells try to copy what our bodies naturally do. For years, researchers rushed to create model cell lines for every occasion — neurons, heart muscle cells, red blood cells. These human cell

cultures, they believed, could better mimic the progression of disease in the human body than any other organism, especially the popular laboratory mouse.

But cells in the body do not exist in segregated communities; they mix together to form complex organs. A new approach to stem cell derived models emerged around 2010, which tried instead to grow whole organs on three-dimensional (3D) scaffolds. Scientists produced rudimentary intestines, eyes and pituitary glands, but the brain remained difficult to recreate. That is, until 2013, when a group of researchers in Europe contoured its outer folds. The publication inspired Huck Hui and his collaborators in Singapore to attempt a similar model for the midbrain — a critical region in the pathogenesis of Parkinson’s disease.

They started with a batch of human embryonic stem cells, feeding and coaxing them into shape over several months. “We had to guide the differentiation in a very precise way, telling the embryonic cells what to do at every stage. It is a pretty lengthy and painful protocol,” says Huck Hui.

The researchers made their first real breakthrough in 2014: the cells formed tissue-like structures, two millimeters wide, resembling the midbrain. “We were excited, but the excitement was followed by a period of skepticism,” says Huck Hui. It was not enough just to have a blob of cells sitting in the laboratory, they needed to determine whether it was brain-like in function.

They found that the neurons were definitely living, firing electric pulses back and forth when stimulated. And the neurons were not just any samples — they produced dopamine. The real surprise came when the two-month-old miniature organs, or organoids, formed dark deposits of neuromelanin. “This proved to us that our organ system was very different from what others had done in the past,” says Huck Hui.

The team is now trying to create mutant versions of the organoids that replicate what happens in the motor systems of patients with Parkinson’s, which is characterized by a dramatic loss of dopamine-secreting neurons.

In a clump

Similar work on stem cell models of the diseased brain is already underway in other A*STAR labs. Shi-Yan Ng at the A*STAR Institute of Molecular Cell Biology (IMCB)

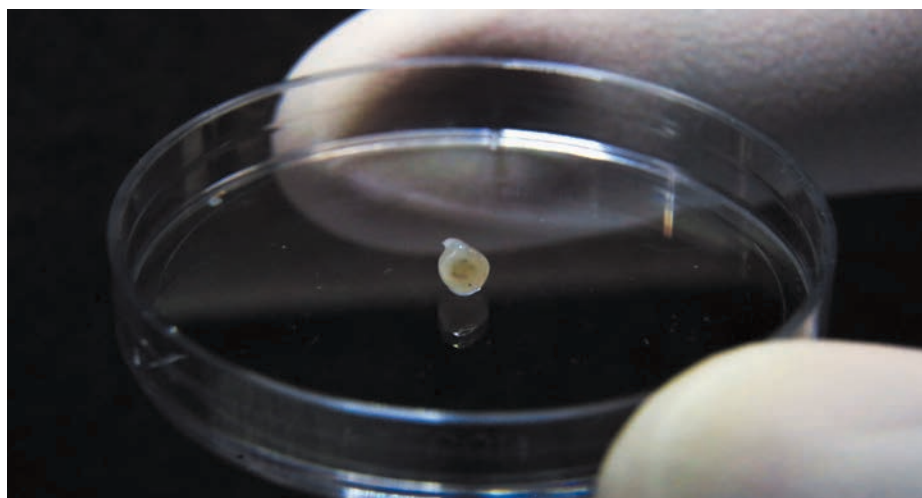
is using stem cells derived from patients with neurodegenerative diseases to develop 3D models of the brain. “Mini brains offer better models of aging diseases because they can mimic what is happening in a 60 year old’s brain, unlike the fetal neurons represented by 2D cultures,” says Shi-Yan, who is looking for a very specific change which kills neural cells in the diseased brain.

Sick neurons do not die without a sign. Their demise is precipitated by a gradual toxic accumulation of proteins: clusters of alpha-synuclein in Parkinson’s, clumps of amyloid-beta in Alzheimer’s, and a combination of many different proteins in amyotrophic lateral sclerosis (ALS).

Researchers believe that these lethal clusters can spread like an infectious plague between neurons, and even to supportive glial cells. Shi-Yan’s models could help to explain if and how these toxins seep through brain tissue. The results could have implications for surgery because sterilizing instruments does not eliminate the proteins. Depending on how the proteins are found to aggregate, surgeons may be guided to destroy their instruments after operating on patients with Parkinson’s disease.

These are early days for Shi-Yan, but so far, she has been able to create spherical brain organoids of Parkinson’s disease, in which the neurons die at a faster rate than in healthy organoids. More intriguing, however, is that the diseased cells accumulate alpha-synuclein proteins in dense clusters — something that has never been seen before in a cell model.

The methods developed by Shi-Yan could also be used to predict whether a healthy young adult test subject will develop Parkinson’s disease in 50 years. Researchers could take samples of their cells, age them by a few decades, and watch for any pathogenic effects. “We’d be able to see neurons dying within a month, which is really quick,” she says.



One of the tiny midbrains produced by Huck Hui Ng’s team.

In the blood vessels

Researchers could detect neurodegeneration even earlier if they looked beyond the nervous system. Christine Cheung at the IMCB has taken a hint from the famous words of 17th century English physician, Thomas Sydenham: “A man is as old as his arteries.”

“Blood vessels are like silent killers,” says Christine. This is especially apparent in a form of cognitive decline caused by blocking the brain of its blood supply, such as during a stroke. “In vascular dementia, blood vessel damage occurs before the neurons begin to die,” says Christine. “With the current focus on preventative medicine, there is cause for looking at early events.” Christine is growing stem cell models of blood vessels to better understand how one system affects the other.

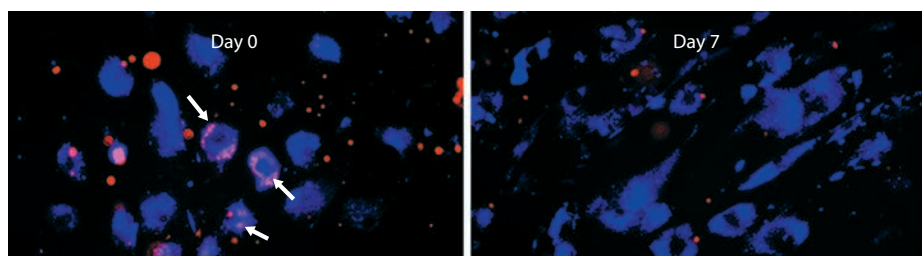
Not all blood vessels are the same, however. Unlike blood vessels to the other organs, those to the brain have to meet a higher demand for nourishment to feed our thoughts, memories and coordinated movements. The vessels are structurally distinct,

and are believed to play a role in draining the brain of the toxic amyloid-beta proteins found in Alzheimer’s patients. But these functions are not visible using standard models of blood vessels.

In 2014, Christine grew the first brain-specific line of blood vessels². She derived them from a specific type of embryonic tissue known as the neural crest, made with stem cells. The blood vessel cells clearly showed that the brain’s vasculature is essential for clearing out excess amyloid-beta proteins. Vessels exposed to low-oxygen conditions, typical in a stroke, could not pump the proteins out as efficiently. “Without organ-specific blood vessel cells to model disease processes, it is hard to have an accurate picture of what could have gone wrong.”

Christine sees huge potential for introducing her vascular cells to the 3D organoids developed by Huck Hui and Shi-Yan for tissue engineering applications.

“We are all taking small steps toward the long-term goal of coming up with more effective drugs for neurodegenerative diseases,” says Shi-Yan. “In a few years, someone will piece all these disparate studies together like a jigsaw puzzle and finally solve it.”



Blood vessel cells are capable of clearing amyloid-beta proteins (red), which are transported to organelles (blue) for enzymatic degradation.

1. Jo, J., Xiao, Y., Sun, A. X., Cukuroglu, E., Tran, H.-D. *et al.* Midbrain-like organoids from human pluripotent stem cells contain functional dopaminergic and neuromelanin-producing neurons. *Cell Stem Cell* **19**, 248–257 (2016).
2. Cheung, C., Goh, Y. T., Zhang, J., Wu, C. & Guccione, E. Modeling cerebrovascular pathophysiology in amyloid- β metabolism using neural-crest-derived smooth muscle cells. *Cell Reports* **9**, 391–401 (2014).

[RESEARCH HIGHLIGHTS]

A more precise method for identifying vaccine targets against the malaria parasite could save millions of children from infection.

Malaria

TAKING THE WITCHCRAFT OUT OF VACCINE DEVELOPMENT

STUDYING THE BODY'S IMMUNE RESPONSE TO MALARIA INFECTION COULD HELP SCIENTISTS FIND LIFE-SAVING VACCINES

Three malaria proteins that trigger an immune response in infected individuals have been identified by A*STAR researchers¹. These proteins could underpin a new vaccine against the world's deadliest parasitic disease.

Half a million people, mostly young children, are killed by malaria annually. Despite almost a century of research and development, no commercial vaccine exists for malaria.

Part of the problem is the complexity of the parasite, says Laurent Rénia, who led the study at the A*STAR Singapore Immunology

Network. Compared with viruses, which have a maximum of 50 genes, the malaria parasite has 5,000 genes and 14 chromosomes. It also changes shape, reinventing itself as it moves from humans or monkeys to mosquitoes and back to the mammalian host. "Everything that works for viruses, doesn't work for malaria," says Rénia. "We need to think differently."

To start with, researchers need to be less haphazard in selecting potential vaccine targets. "Vaccine studies to date have been conducted like witchcraft, with no clear criteria for

deciding why one protein candidate is better than another," says Rénia. "We are trying to put a bit of rationality into the process."

In 2009, Rénia and a team of researchers in the Netherlands discovered that individuals exposed to a few bites from infected mosquitoes, while taking the antimalarial drug chloroquine, developed long-lasting immunity. Rénia wanted to determine the specific parasitic proteins that trigger this immune response. These antigens, he reasoned, could offer a legitimate target for potential vaccines.

He collaborated with an international team to engineer mammalian cells that express a range of malarial antigens on their surfaces. The team exposed the cells to blood samples taken from two groups of a total of 14 individuals: those who had been treated for long-lasting immunity and those who had not. The immunized individuals produced antibodies that recognized three malaria antigens, which were generally absent in the non-immunized group.

The researchers then tested these antigens' potential as vaccine targets. They introduced one of the antigens to human liver cells growing in a dish and then exposed the cells to rabbit antibodies that recognize and block the protein's activity. The antibodies protected the liver cells against parasitic invasion.

During an infection, the malaria parasite first incubates and amplifies in the liver, before flooding the bloodstream and attacking red

blood cells. Blocking the infection at this early stage could save lives.

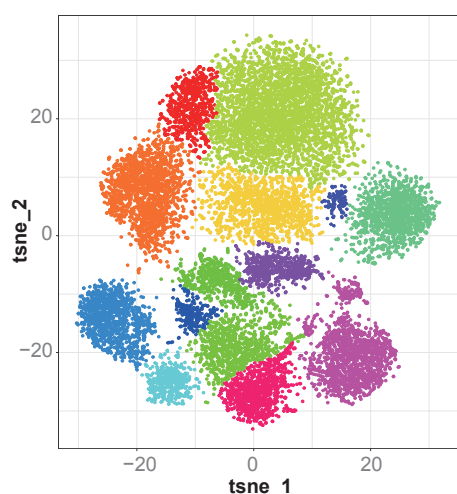
Rénia now wants to replicate the experiment on a larger group to see if the same three proteins resurface as provokers of an immune response. ■

1. Peng, K., Goh, Y. S., Siau, A., Franetich, J.-F., Chia, W. N. *et al.* Breadth of humoral response and antigenic targets of sporozoite-inhibitory antibodies associated with sterile protection induced by controlled human malaria infection. *Cellular Microbiology* **18**, 1739–1750 (2016).

Bioinformatics

A NEW KIT FOR CYTOMETRY ANALYSIS

CYTOFKIT HELPS RESEARCHERS MAKE SENSE OF MASS CYTOMETRY DATASETS TO UNCOVER CELL SUBSETS



cluster

- CD4 CM
- CD8 EM
- Less diff
- CD4 Eff
- CD8 Naive
- gamma delta
- CD4 EM
- gamma delta Vd2-
- MAIT
- CD4 Naive
- gamma delta Vd2+
- NK
- CD8 Eff
- late CD4 Eff
- NKT

A new software package offers easier analysis and interpretation of experiments that use mass cytometry, a sophisticated method for determining the properties of cells. The tool — called cytofit — enables scientists to identify different subpopulations of cells within a sample of immune cells, cancer cells or other tissue types.

Flow cytometry remains the go-to method for biological investigations that require single-cell resolution. But because the technology relies on fluorescent tags to detect different markers within the cell, only a limited number of labels can be applied before the light signals start to bleed into one another.

Mass cytometry helps solve this problem. By using metal labeling, the technique allows scientists to measure many more characteristics simultaneously within individual cells. But sorting through all the data it produces can be challenging, and most researchers agree that better analytic tools are needed.

Jinmiao Chen and her colleagues at the A*STAR Singapore Immunology Network made cytofit in response to this need.

The package combines state-of-the-art bioinformatics methods and in-house novel algorithms to help anyone make sense of mass cytometry data. “It provides a very user-friendly graphical interface and interactive visualization of analysis results,” says Chen. “Anybody, including bench scientists and non-bioinformaticians, can use it without any training.”

The software involves four main steps: first, cytofit performs data preprocessing according to the users' specifications; second, the software automatically identified different matching subsets of cells; third, it allows visualization of the data with color-labeled cell types; and lastly, it infers the relatedness between cell groups.

Chen's team tested the tool's performance on mass spectrometry results collected from a sample of white blood cells. As they reported in *PLOS Computational Biology*, the software correctly identified known subpopulations of cells and further segregated these subsets to reveal additional cell types. In collaboration with A*STAR immunologist Evan Newell, the researchers also showed that cytofit revealed

Cytofit allows users to visualize the different subtypes of cells in their sample, as above.

many types of follicular helper T cells from blood and tonsils. Plus, says Chen, “we have tested the utility of cytofit on a large number of other datasets not mentioned in the paper.”

Cytofit is also gaining popularity with scientists around the world. “It now has

more than 4,000 users,” says Chen. Her lab continues to improve and upgrade the tool in response to user feedback.

The software — which works both on flow and mass spectrometry datasets alike — is freely available through

Bioconductor, an open-source software framework for biologists.

1. Chen, H., Lau, M. C., Wong, M. T., Newell, E. W., Poidinger, M. & Chen, J. A bioconductor package for an integrated mass cytometry data analysis pipeline. *PLOS Computational Biology* **12**, e1005112 (2016).

Electrochemistry

GOING CARBON FREE BOOSTS BATTERY LIFE

DROPPING THE CARBON FROM A KEY BATTERY COMPONENT COULD FINALLY ENABLE LONG-LIFE, LOW-COST GRID-CONNECTED BATTERIES FOR RENEWABLE ENERGY STORAGE

Zinc–air batteries are one of the most promising solutions for the large-scale storage of intermittently generated renewable electricity from solar, wind or tidal: they are non-flammable and inexpensive and have a very high energy density.

But the lifetime of current zinc–air devices is far too short to be commercially viable, because oxygen attacks and corrodes their carbon-based components. Researchers at A*STAR have now developed a carbon-free version of one of the battery’s key components, the oxygen electrocatalyst¹.

Conventional rechargeable batteries store all electrochemically active materials within the device. Metal–air batteries, however, use oxygen from the surrounding air as the active cathode material, significantly boosting the battery’s storage capacity. To incorporate oxygen into the battery’s electrochemical cycle, these batteries use an oxygen electrocatalyst, which requires good electrical conductivity for fast electron transfer. Various metals and other catalytic materials have been tried as the electrocatalyst, but virtually all have to be laced with carbon to raise their electrical conductivity. Over time, the carbon corrodes, eventually leading to device failure.

Yun Zong and Zhaolin Liu from the Institute of Materials Research and Engineering

(IMRE) at A*STAR and their colleagues have now developed a highly active oxygen electrocatalyst that contains no carbon.

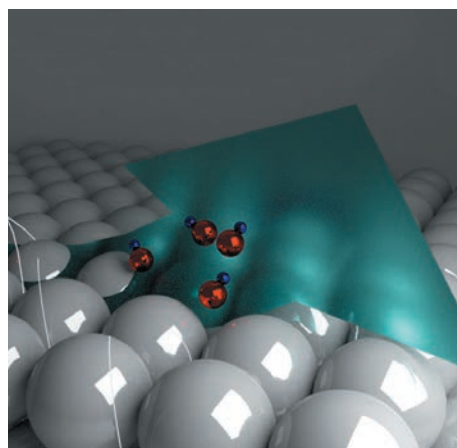
This material — nickel-doped lanthanum strontium manganite (LSMN) — is a member of the perovskite family, a recently discovered group of electrochemically active materials that are also causing a stir as potential solar panel materials. “The high intrinsic electrical conductivity of LSMN means that carbon is not needed as additive for conductivity enhancement,” Zong says.

By alternating the ratio of nickel to manganese in the material, Zong was able to tune the perovskite’s performance. The best-performing formulation, containing 10 per cent nickel, matched the electrocatalytic performance of palladium on carbon, the current benchmark electrocatalyst. Yet without the carbon, the stability of the material was greatly enhanced. The team tested LSMN over 300 electrochemical cycles and saw negligible performance degradation.

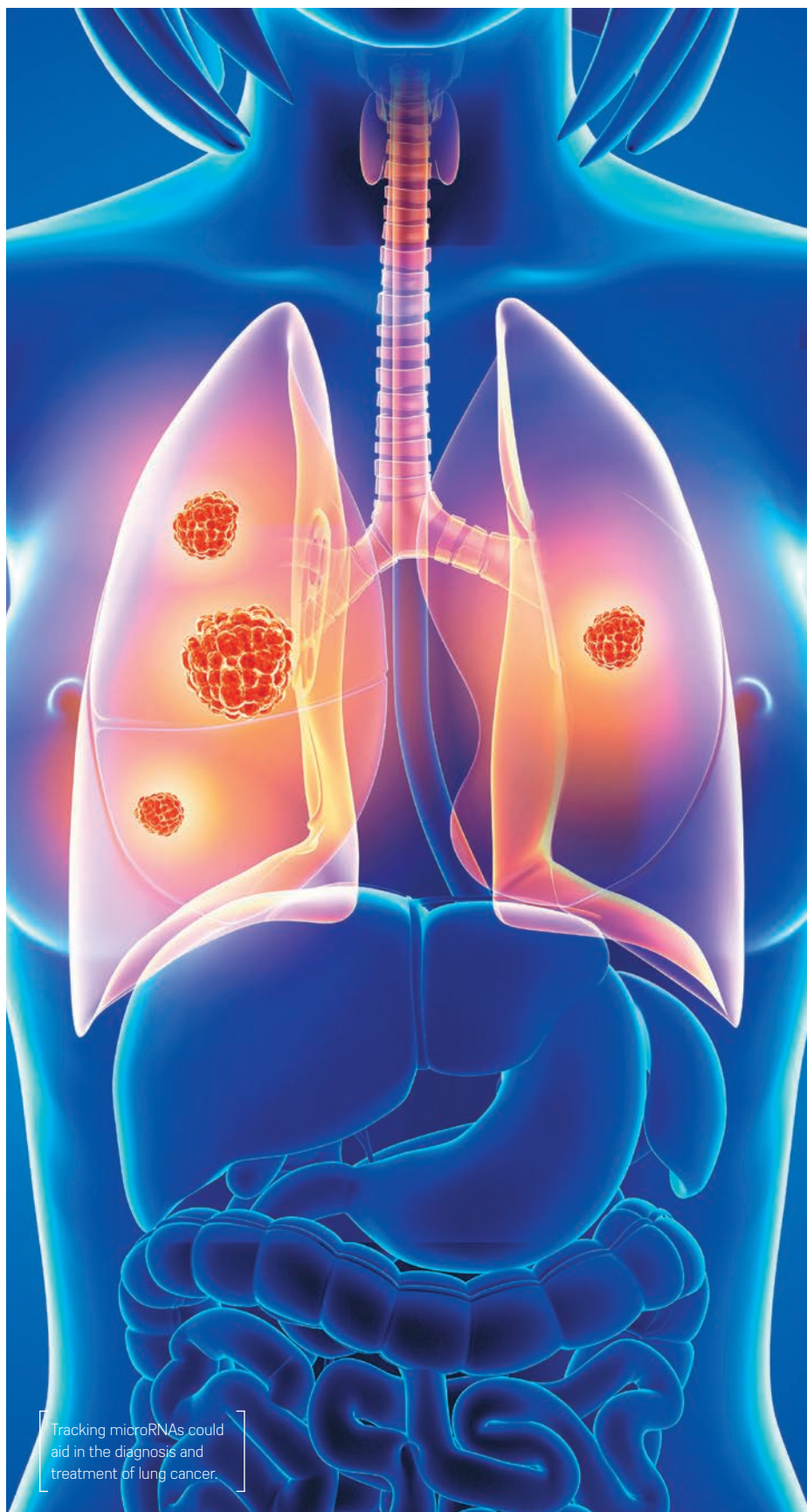
The next hurdle to overcome, Zong explains, is changing the process. In current metal–air battery designs, the catalyst is formed layer by layer on to a mat of carbon. “This defeats the purpose of using carbon-free catalysts, as underlying carbon may still suffer corrosion,” Zong says. One possibility is to replace the carbon mats with a nickel foam, on to which the carbon-free electrocatalyst could be grown *in situ*, he adds. “Our group is developing metal–air batteries where all components are essentially carbon free.”

The team is also working on carbon-free versions of other battery technologies, says Zong.

1. Ge, X., Du, Y., Li, B., Hor, T. S. A., Sindoro, M. *et al.* Intrinsically conductive perovskite oxides with enhanced stability and electrocatalytic activity for oxygen reduction reactions. *ACS Catalysis* **6**, 7865–7871 (2016).



Oxygen (red) and water (red and blue) molecules react on the carbon-free electrocatalyst surface (gray) as the metal–air battery is charged.



Tracking microRNAs could aid in the diagnosis and treatment of lung cancer.

Cancer

SMALL RNAS OFFER BIG HOPE FOR LUNG CANCER TREATMENT

A PAIR OF MICRORNAS IMPLICATED IN THE SPREAD OF LUNG CANCER COULD LEAD TO NEW DIAGNOSTICS AND THERAPIES

Lung cancer kills more people than any other form of cancer — partly because it is often diagnosed at such an advanced stage that few treatment options are possible. A team led by A*STAR has now discovered a pair of small, noncoding RNA molecules that could enable earlier detection and potentially new therapies¹.

These two microRNAs “can behave as an early warning signal that the disease has disseminated beyond the primary tumor to a distant

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site,” says Wai Leong Tam, a researcher at the A*STAR Genome Institute of Singapore who led the study. “This can prompt oncologists to perform more thorough examinations or implement new treatment regimens for patients.”

Lung tumors contain a variety of different cell types, including a rare subset known as tumor-initiating cells (TICs), or cancer stem cells considered to be key drivers of disease relapse and spread throughout the body. Tam and his A*STAR colleagues teamed up with physicians from two nearby cancer centers in Singapore to identify regulatory microRNAs that are essential for TIC function.

The researchers obtained biopsies from patients diagnosed with non-small cell lung cancer, isolated TICs from the tumors, and then compared the expression pattern of microRNAs

in these cells with those from non-TIC tissue taken from the same patient samples.

Tam’s team found a slew of microRNAs that were either expressed at much higher or lower levels in the TICs. However, they focused in depth on just two, miR-1246 and miR-1290. These microRNAs were the most up-regulated and had never been characterized before. Observations and experiments showed that both play a critical role in helping seed new tumors at distant sites outside the lungs.

The researchers tracked the levels of miR-1246 and miR-1290 in patients undergoing therapy. “As expected, the higher expression levels of those two microRNAs in malignant tissues consistently predicted poorer survival outcomes in a large cohort of lung cancer patients,” says Wencai Zhang, who worked on

the study as a research scientist at A*STAR, prior to moving to his current position as a research fellow at Harvard Medical School in the United States.

The microRNAs could serve as a useful predictive biomarker of expected patient outcomes. They might also provide promising drug targets. The researchers wiped out the microRNAs with a special kind of drug known as a locked nucleic acid in mouse models and saw reduced tumor growth. The same kinds of drugs are now being used in humans for other diseases, and could prove helpful in treating lung cancer. ■

1. Zhang, W. C., Chin, T. M., Yang, H., Nga, M. E., Lunny, D. P. *et al.* Tumour-initiating cell-specific miR-1246 and miR-1290 expression converge to promote non-small cell lung cancer progression. *Nature Communications* 7, 11702 (2016).

Materials

TESTING THE WATER

THEORETICAL MODEL REVEALS HOW DROPLETS GROW AROUND TINY PARTICLES ON A SURFACE

A mathematical model that predicts how water condenses around tiny particles could help to improve chemical industrial processes, including the production of drug tablets, fertilizers and catalysts¹.

Previous condensation models differ in their rate predictions, depending on

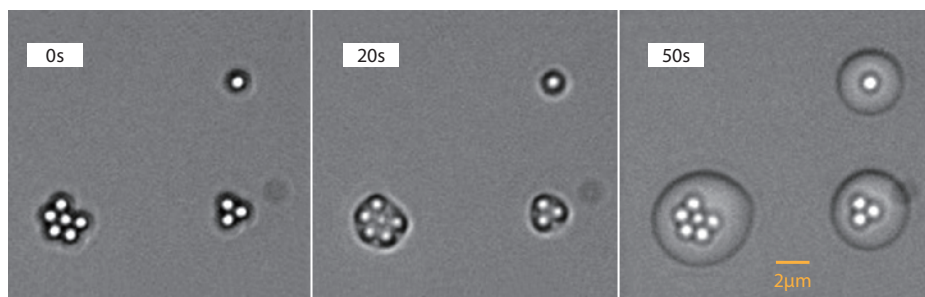
factors such as the shape and composition of the surface that the droplet grows on. Fong Yew Leong of the A*STAR Institute of High Performance Computing wanted to develop a more realistic theoretical model to help his collaborators understand their experimental condensation results. “This

is where modeling and computation gets really useful, in providing physical insights that can’t be obtained from experiments,” says Leong.

He and his colleagues modeled a water droplet growing in the crevice between a micrometer-sized particle and a flat surface. Their model considers factors such as particle size, the surface tension of the droplet, and how much the underlying surface attracts or repels water.

The model shows, for example, that a growing droplet covers a water-attracting (hydrophilic) surface more quickly than a water-repelling (hydrophobic) surface. The volume of a droplet initially increases more slowly on a hydrophobic surface, but then speeds up as the droplet becomes more convex. “The droplet doesn’t shrink during condensation; it instead wets the particle completely,” says Leong.

The team carried out experiments to test their model, filming how water condensed around micron-sized silicon dioxide particles on a glass slide (see image). They saw that water always condensed in the crevice between a particle and the slide, rather than forming standalone droplets on the surface, and found that the droplets’ growth was almost the same as that predicted by their model. The researchers also adapted the model



Water droplets grew around clusters of silica particles, rather than forming standalone droplets on the surface.

to predict the growth of droplets around clusters of particles.

These results demonstrate that it is not possible to accurately simulate condensation based on a single factor, the team says. Indeed, it appears that there is a

competition between the particle and the substrate that determines how fast each one is covered in water as the droplet condenses. "It points to significant implications for wetting at small scales," says Leong. The team now hopes to model condensation

and liquid interactions at even smaller length scales.

1. Quang, T. S. B., Leong, F. Y., An, H., Tan, B. H. & Ohl, C.-D. Growth and wetting of water droplet condensed between micron-sized particles and substrate. *Scientific Reports* **6**, 30989 (2016).

Catalysis

A BREATH OF FRESH AIR

THREE-LAYER NANOPARTICLE CATALYSTS IMPROVE ZINC-AIR BATTERIES

Nanoparticles containing three different layers of material can help to boost the performance of a zinc-air battery, A*STAR researchers have found¹.

Zinc-air batteries are cheap, have a high energy density, and last for a very long time. Their use of a water-based electrolyte makes them safer than other batteries, so they are often found in medical applications, such as hearing aids and heart-monitoring devices.

The battery's negative electrode contains zinc metal, which gives up electrons when it reacts with hydroxide ions in the electrolyte. Those electrons generate a current as they flow to the positive electrode, where they react with oxygen from the air to produce more hydroxide ions.

The sluggishness of the reaction with oxygen limits the battery's voltage output and its performance at high current. Finding a catalyst to speed up the reaction could yield higher power and energy densities, opening a wider range of potential applications.

Yun Zong and Zhaolin Liu of the A*STAR Institute of Materials Research and

Engineering and colleagues have developed a nanoparticle catalyst that could fit the bill. The particles are 20–50 nanometers across, with a cobalt core encased by an inner shell of cobalt oxide, which is surrounded by an outer shell of pyrolyzed polydopamine (PPD), a form of carbon 'dotted' with nitrogen atoms. These nanoparticles are coated on a porous carbon support that acts as an electrode. Their structure helps to prevent them from leaching cobalt or clumping together, and the protective outer shell also makes the nanoparticles more durable.

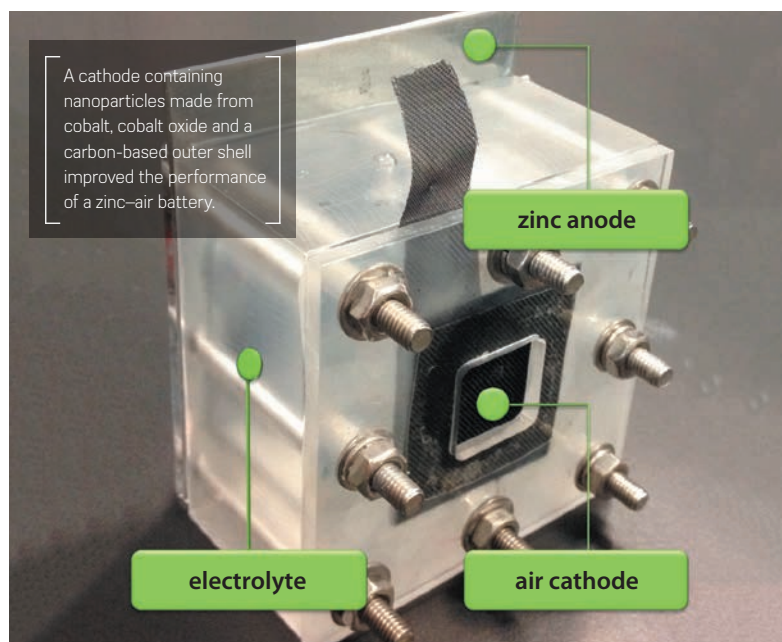
These three-layer nanoparticles efficiently transformed oxygen to hydroxide in a single step. The team suggests that nitrogen atoms in the PPD shell help to attract and make oxygen atoms more reactive on their way to catalytically active sites in the cobalt oxide and PPD. Meanwhile, the cobalt core and PPD shell help electrons to flow efficiently to the oxygen atoms. In contrast,

similar particles containing only cobalt and cobalt oxide, or PPD alone, transformed oxygen in a two-step process that produced hydroperoxide, an undesirable and corrosive intermediate.

The researchers tested their electrode in a zinc-air battery (see image), and found that it could produce a current of 5 milliamps per square centimeter of electrode at 1.36 volts for 5 days, outperforming an electrode that relied on a conventional platinum catalyst.

"The next stage of this research includes the simplification of the synthetic route to facilitate large-scale synthesis of the nanoparticles, and exploitation of other catalytic systems following the similar strategy," says Zong.

1. Wang, Z., Li, B., Ge, X., Goh, F. W. T., Zhang, X. *et al.* Co@Co₃O₄@PPD core@bimetallic nanoparticle-based composite as an efficient electrocatalyst for oxygen reduction reaction. *Small* **12**, 2580–2587 (2016).



Drug development

POTENTIAL ASTHMA TREATMENT WORTH ITS SALT

INVESTIGATIONS INTO NOVEL SOLID FORMS OF THE ANTI-INFLAMMATORY DRUG OXAPROZIN MAY LEAD TO A NEW COMBINED ASTHMA THERAPY

An exploratory study by A*STAR scientists into novel solid forms of the anti-inflammatory drug oxaprozin may lead to improvements for the asthma drug, salbutamol, and help reduce inflammation of the airways.

Many drugs, in their original 'parent' form, are not ideal for use in the human body. For example, poor solubility can limit a drugs' ability to disperse in the bloodstream, as is the case for oxaprozin, a widely used anti-inflammatory. Other drugs dissolve

"WE CREATED FIVE NOVEL CRYSTALLINE FORMS OF OXAPROZIN, INCLUDING THREE MOLECULAR SALTS MADE WITH DIFFERENT ORGANIC MOLECULES."

too quickly, lose their potency and require multiple doses, such as salbutamol — a drug used in asthma inhalers to open restricted airways. A solution can be to incorporate

two drugs into one solid form to create more effective medications.

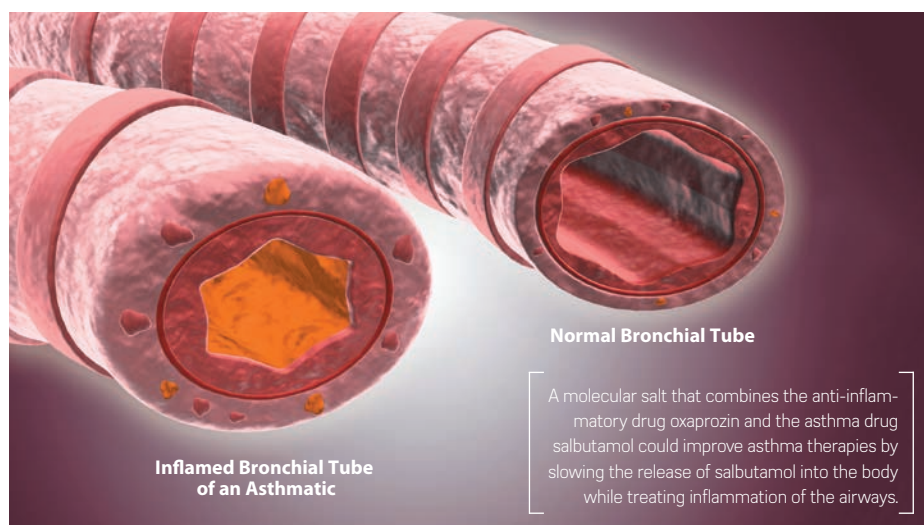
"With discoveries of new active pharmaceutical ingredients dwindling, combining two or more ingredients in a single dose is increasingly common for treating complex diseases such as HIV/AIDS and cancer," says Srinivasulu Aitipamula, from the team at the A*STAR Institute of Chemical and Engineering Sciences. "To find a more soluble version of oxaprozin that could be used in solid form, we created five novel crystalline forms of oxaprozin, including three molecular salts made with different organic molecules."

Molecular salts are ionic compounds formed by strong bonding between oppositely charged ions — atoms that have either lost or gained electrons resulting in a positive or negative charge. Aitipamula's team used X-ray crystal diffraction to determine the crystal structure of each solid and examined the resulting effects on oxaprozin's physical and chemical properties¹.

While the team did not succeed in altering oxaprozin solubility significantly, one molecular salt incorporating oxaprozin and salbutamol showed great promise for creating an extended-release, anti-inflammatory asthma therapy.

"By incorporating salbutamol and oxaprozin into one solid, we were able to slow the rate of salbutamol dissolution," says Aitipamula. "The solubility of a solid in water depends on the number of hydrogen bonds that it can form with water molecules. All the potential hydrogen bonding sites of salbutamol and oxaprozin were involved in creating the salt, meaning there were no sites left for water to interact with."

The strong crystal lattice in the oxaprozin-salbutamol salt means the molecules are held together firmly, facilitating a controlled release of salbutamol over time. Incorporating oxaprozin into an asthma therapy would also mean patients would no longer have to take supplementary anti-inflammatory drugs. "We will continue to expand our investigations into other active ingredients and create combined formulations for targeting different diseases," says Aitipamula.



Normal Bronchial Tube

Inflamed Bronchial Tube of an Asthmatic

A molecular salt that combines the anti-inflammatory drug oxaprozin and the asthma drug salbutamol could improve asthma therapies by slowing the release of salbutamol into the body while treating inflammation of the airways.

1. Aitipamula, S., Wong, A. B. H., Chow, P. S., & Tan, R. B. H. Novel solid forms of oxaprozin: Cocrystals and an extended release drug-drug salt of salbutamol. *RSC Advances* 6, 34110–34119 (2016).

A REVOLUTION IN LIGHT AT THE SMALL SCALE

Surprising optical effects in semiconductor nanoparticles promise to realize the latent potential of nanophotonics

Light behaves in rather tame and predictable ways when interacting with everyday objects — it travels in straight lines, rebounds when it hits shiny surfaces, and gets bent by lenses. But weird and wonderful things start to happen when light interacts with very small objects. Nanoparticles, for example, which are collections of atoms as small as a virus, can act as mini-antennas, and small disks of silicon can set off strange ‘modes’ of light that render the disks invisible.

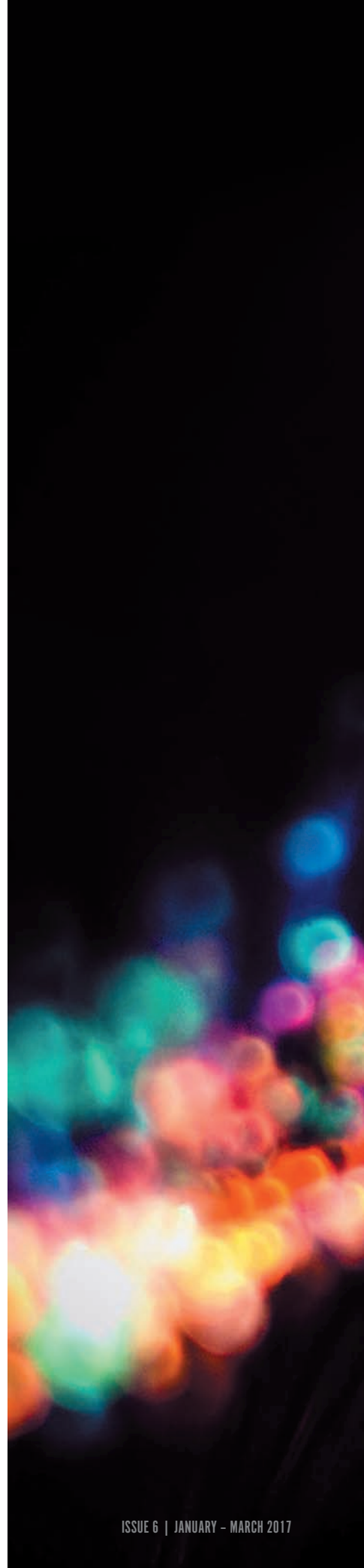
A new area of optics has emerged in recent years to study these strange phenomena. “Nanophotonics, a branch of optics dealing with light at nanoscale dimensions, has become a hot research topic over the last decade or so,” notes Arseniy Kuznetsov of the A*STAR Data Storage Institute. “It holds a lot of promise for various new applications, ranging from high-speed information transmission and holographic display technologies to bioimaging and genome sequencing.” Kuznetsov’s team is leading developments in a subfield of nanophotonics, which could ensure its widespread practical application.

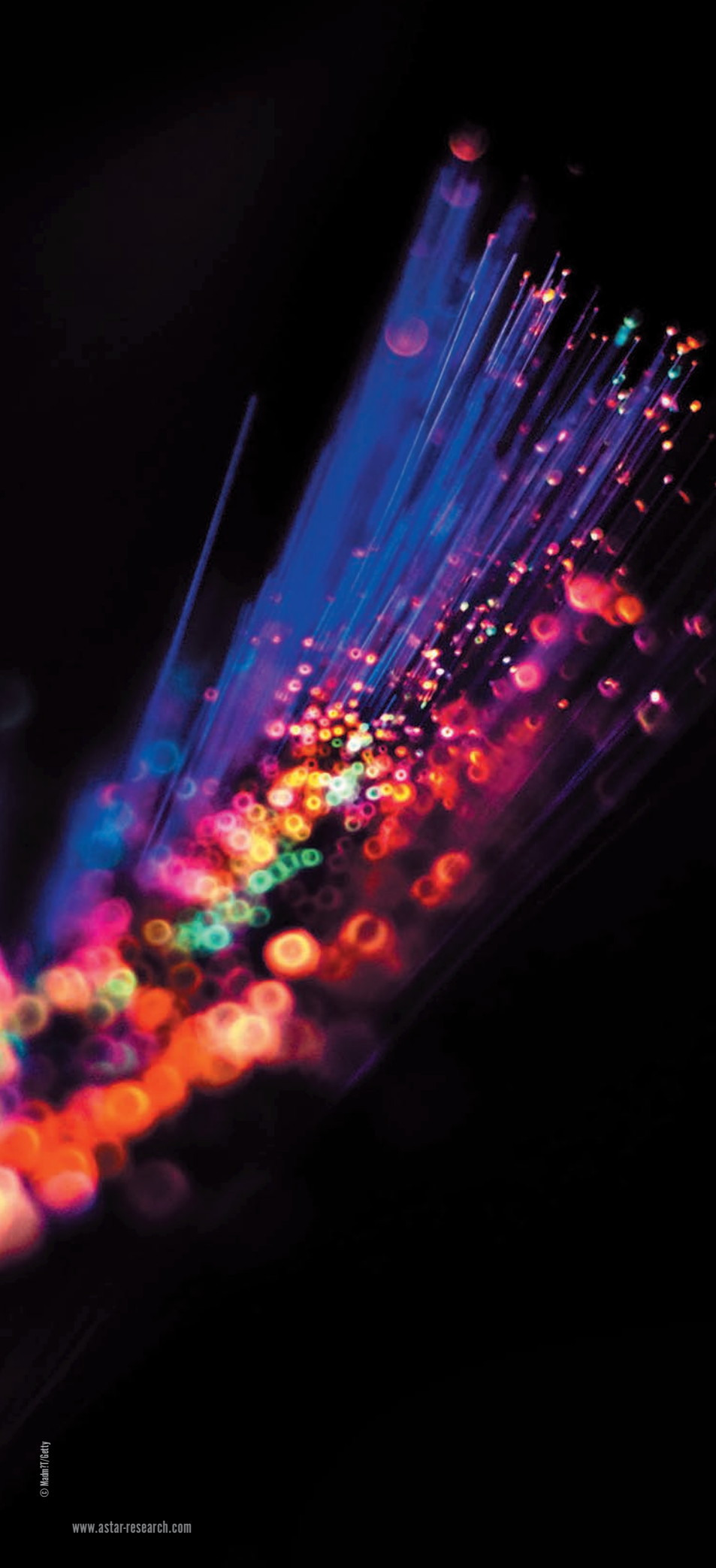
LIGHT ON TINY SCALES

Traditionally, nanophotonics has focused on tiny metal structures such as gold and silver

nanoparticles. Light’s oscillating electric field causes the free electrons in metals to oscillate collectively. At certain particle sizes, this can give rise to an effect known as surface plasmon resonance. Resonance is a general phenomenon in which a system exhibits a much larger response at certain frequencies — for example, an opera singer can cause a wine glass to shatter by singing at the pitch that it resonates at. Surface plasmon resonance refers to the specific resonance effect produced by surface plasmons, which are a collection of charged oscillations — the study of which is known as nanoplasmonics. While a very new research area, nanoplasmonic effects have been exploited for centuries — stained-glass windows in medieval cathedrals owe their color to surface plasmons excited in metal nanoparticles embedded in the glass.

Despite the high expectations for nanoplasmonics in areas such as information technology, security, energy, high-density data storage and the life sciences, it has resulted in relatively few practical applications. One reason for this disappointing outcome is that metal nanostructures lose a lot of light to absorption. “A deeper understanding of these resonances has brought a general understanding of major drawbacks related to unavoidable high losses in





resonant metallic nanostructures,” comments Kuznetsov. Furthermore, metals commonly used for plasmonics such as silver and gold are incompatible with standard methods for manufacturing semiconductor components, making them difficult to produce.

A QUIET REVOLUTION

But now a quiet revolution is underway in this area. The focus is shifting away from metals and toward electrically insulating and partially insulating materials known as dielectrics and semiconductors, which are ‘optically dense’ so that light travels considerably slower in them than in air. Examples of such materials include the semiconductors silicon, germanium and gallium arsenide, and titanium dioxide.

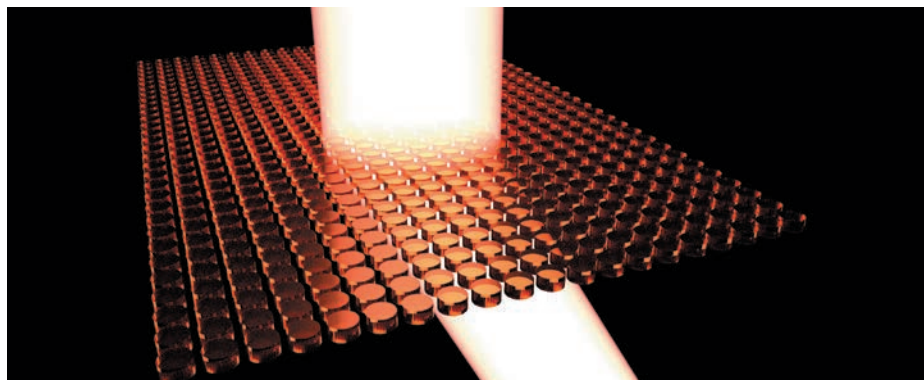
“The shift from metals to dielectrics is already happening,” says Kuznetsov. “Many leading teams in plasmonics have already started to work with resonant dielectric nanostructures.”

Though still in its infancy, the transition has revealed many benefits. “After the demonstrations of resonances in dielectric nanoparticles in 2012, the field took off,” says Kuznetsov. “Many advantages over conventional plasmonics have now been found.”

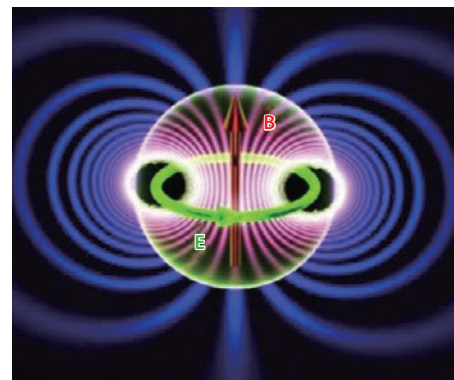
LEADING THE WAY

Kuznetsov and his team at A*STAR are at the vanguard of this revolution. They employ a three-pronged approach. “In many cases, we generate a theoretical concept, show it in simulations and then demonstrate it experimentally. However, sometimes the reverse process occurs — unexpected experimental observations lead to theory development to provide their physical understanding,” explains Kuznetsov.

The team members have realized some remarkable firsts in this young field. Physicist Boris Luk’yanchuk started the ball rolling in 2010 when he and colleagues in Germany published a seminal paper showing that, theoretically, silicon nanoparticles with sizes ranging from 100 to 200 nanometers might have both strong electric and magnetic resonances at visible-light frequencies — a low-loss alternative to plasmonic nanostructures¹. In a subsequent paper, Luk’yanchuk, together with researchers in Australia, proposed novel metal–dielectric hybrid structures where light could propagate due to interactions of magnetic moments, which is not possible in chains of metallic particles². Finally, in 2015, the A*STAR group showed that



An artistic view of a dielectric metasurface, a phased array of dielectric nanoantennas, controlling the properties (phase and amplitude) of light.



An artistic view of a magnetic dipole resonance in a high-refractive-index dielectric sphere.

similar types of optically induced interactions of magnetic moments exist in chains of silicon particles³. “Such magnetic interactions of silicon particles can far outperform waveguides based on plasmonics and conventional silicon photonics,” says Luk’yanchuk.

Luk’yanchuk, Kuznetsov and their team have experimentally demonstrated these resonances in silicon nanoparticles⁴. The team was also the first to experimentally show unique directional light scattering by silicon nanoparticles, which demonstrates their promising nanoantenna properties⁵. And the researchers were the first to experimentally show large enhancement of the electric and magnetic fields of light in close proximity to dielectric antennas made from two silicon nanoparticles placed very close to each other⁶.

According to *Google Scholar*, the papers describing these findings have been cited more than 1,000 times, reflecting the enormous impact that the team’s work has had in the field. Such is their reputation in this area that a recent review they wrote on the emerging field was published in the prestigious journal *Science*⁷.

In a 2015 study, the team, together with researchers from Australia and Germany, experimentally demonstrated a very unusual optical effect in nanoscale disks of silicon — patterns of radiation that do not emit or scatter light⁸. Such radiation modes could be used to produce tiny nanoscale lasers. The team has also showed how arrays of such silicon disks can precisely control the phase and amplitude of light, forcing it to bend, focus, or create high-resolution holographic images^{9,10}.

In 2016, the Institute of Physics Singapore awarded Luk’yanchuk the World Scientific Physics Research Award and Gold Medal for his outstanding contributions to physics research in the country. That same year, Kuznetsov was chosen as the recipient of the Institution of Engineering and Technology’s A F Harvey Engineering Research Prize for “his outstanding contributions in the field of lasers and optoelectronics and his pioneering research on a new branch of nanophotonics: optically resonant dielectric nanostructures and dielectric nanoantennas.”

A BRIGHT FUTURE

The team is excited about the potential of dielectric nanostructures. “We hope that resonant dielectric nanostructures will finally give rise to real-life applications from resonant nanophotonics,” says Kuznetsov. They anticipate that many areas of technology could be strongly affected by this development.


“Three-dimensional holographic displays for smartphones and high-resolution virtual and augmented reality devices might be developed based on dielectric nanoantennas. Substrates containing resonant dielectric nanoparticles could make bioimaging and genome sequencing more efficient and faster. And rapid computers based on light may appear with resonant dielectric nanoparticle components inside,” says Kuznetsov. “Some of these new and amazing applications may become reality in the next 5 to 8 years,” he predicts. While light may be predictable on large scales, the future is looking anything but tame for this emerging technology. ■

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3. Bakker, R. B., Yu, Y. F., Paniagua-Domínguez, R., Luk’yanchuk, B. & Kuznetsov, A. Silicon nanoparticles for waveguiding. *Frontiers in Optics 2015*, OSA Technical Digest [online] FM1B.2 (2015).
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[RESEARCH HIGHLIGHTS]



A*STAR researchers have developed a video Quality of Experience (QoE) scheme that can greatly improve streaming video in crowded mobile environments.

Wireless networks

THE IMPORTANCE OF BEATING BUFFERING

STREAMING VIDEO OVER MOBILE NETWORKS COULD BE GREATLY IMPROVED BY SMART PRIORITIZATION OF VISUALLY IMPORTANT DATA

By prioritizing the delivery of rich visual data, A*STAR researchers have demonstrated that the quality of streaming video can be vastly improved on even the most crowded wireless networks¹.

Video streaming is one of the most demanding tasks on mobile networks, not only because of the large amount of data that needs to be transmitted, but because even the faintest stutter or artifact in video playback can dramatically degrade the experience. Network engineers are continuously looking for new

ways to maximize video quality in increasingly congested wireless environments with many users vying for limited bandwidth.

There are already methods for guaranteeing a certain transmission rate to maintain the quality of streaming video and audio. Known as Quality of Service (QoS) protocols, these methods work well in many cases, but generally require a large allocation of bandwidth to each user, which might not be available on crowded mobile networks. Peng Hui Tan, Maodong Li and colleagues from the A*STAR Institute

for Infocomm Research instead studied how it might be possible to rate the importance of discrete video ‘packets’ to reduce the bandwidth needed to maintain a certain Quality of Experience, or QoE.

“QoE refers to the performance metric used to gauge the experience of the end user,” explains Tan. “We need to translate a given QoE into a set of parameters for QoS, which is then implemented in the network communication protocol. We found that by passing information across the different layers

of communications, from video playback application to network transmission, we could enhance the QoE through more efficient allocation of network resources.”

The researchers developed an efficient method to derive an ‘importance index’ for each video packet based on the video bit rate, which varies packet-to-packet depending on how much new information needs to be displayed — for example, slow scenes

with little movement require lower bit rates, while fast action scenes require very high bit rates.

By prioritizing video packets — each a fraction of an individual frame of video — based on bit rate and other network parameters, then inserting this priority in the QoS scheme in real time, the team was able to significantly enhance the perceived quality of streaming video among multiple users in a

laboratory environment with limited wireless bandwidth.

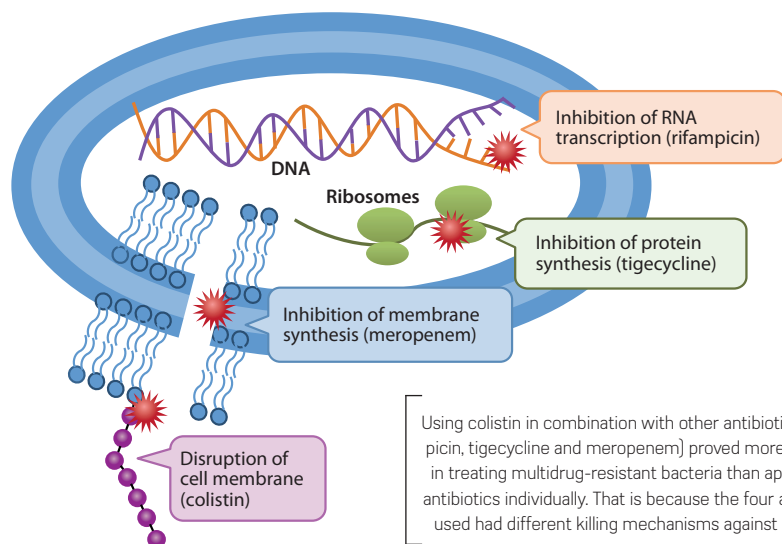
“For the end user, video quality will be improved with less distortion, while service providers can accommodate more users with the same network resources,” says Tan. ■

1. Li, M., Tan, P. H., Sun, S. & Chew, Y. H. QoE-aware scheduling for video streaming in 802.11n/ac-based high user density networks. *IEEE Vehicular Technology Conference (VTC Spring)*, 15–18 May 2016.

Antibiotics

OVERCOMING DRUG-RESISTANT LUNG INFECTIONS

TAILORED COMBINATIONS OF ANTIBIOTICS THAT KILL MICROBES IN DIFFERENT WAYS ARE A POWERFUL WEAPON AGAINST DRUG RESISTANCE



Using colistin in combination with other antibiotics (rifampicin, tigecycline and meropenem) proved more effective in treating multidrug-resistant bacteria than applying the antibiotics individually. That is because the four antibiotics used had different killing mechanisms against microbes.

Triple-pronged attacks on microbes that cause life-threatening lung infections are much more effective than individual antibiotics, A*STAR researchers have found¹. Using cocktails of antibiotics is promising for addressing the rising menace of multidrug-resistant microbes.

Multidrug-resistant bacteria are the plague of the twenty-first century and are predicted

to become the leading cause of death by 2050, surpassing even cancer and diabetes, says Desmond Heng Wen Chien of the A*STAR Institute of Chemical and Engineering Sciences. “It is imperative that we act now to stem the rise of antimicrobial resistance and to mitigate its impact with more robust, but safer therapies,” he urges.

In particular, superbugs that cause respiratory lung infections are posing an increasingly ominous threat. They have dire clinical outcomes, with mortality rates reaching as high as 80 per cent for some infections. Furthermore, they have begun to spread from hospitals to the community at large. The last line of defense against such superbugs is the highly toxic antibiotic known as colistin, but there are recent reports of infections that are resistant even to that.

Now, Heng and his co-workers have shown that using three tailored combinations of colistin with two other antibiotics can effectively combat lung infections caused by multidrug-resistant superbugs. They found that all three formulations were highly effective against the multidrug-resistance pathogens in the laboratory. In concocting these combinations, the team drew on initial laboratory screening data and existing clinical data to obtain more effective and robust formulations.

Since the four antibiotics used in these cocktails employ different mechanisms to kill bacteria (see image), their ternary combinations exhibit significant synergistic and additive effects. Their combined effectiveness is much greater than

their individual application. The triple-killing mechanism of these ternary combinations, and the potential to rotate the three combinations during therapy, makes it much harder for microbes to develop resistance to them. Using other antibiotics in combination with colistin has the added advantage that it reduces the amount of colistin needed and the toxicity to the patient.

The antibiotics are easy to self-administer. “Our formulations are designed to be delivered into the deep-lung region by a portable, easy-to-use dry-powder inhaler, which is faster, more direct and more convenient than other modes of treatment,” says Heng.

The team intends to conduct *in vivo* studies in animals and then humans in

collaboration with hospital clinicians. The researchers note that the same strategy could be applied to fight other drug-resistant bacteria.

1. Lee, S. H., Teo, J., Heng, D., Ng, W. K., Zhao, Y. & Tan, R. B. H. Tailored antibiotic combination powders for inhaled rotational antibiotic therapy. *Journal of Pharmaceutical Sciences* **105**, 1501–1512 (2016).

Neuroscience

DROPPING LIKE FLIES

LIGHT-SENSITIVE MOLECULE SILENCES NEURAL CIRCUITS FOR BRAIN RESEARCH



A*STAR researchers have made genetically modified flies that drop mid-flight when struck by light¹. The optogenetic trick gives scientists an important new way to study the brain's workings.

The brain is abuzz with activity. Electrical signals carried by ions move along busy neuronal circuits to enable activities as simple as breathing and as complex as recalling memories. When these circuits do not behave correctly, they can result in brain disorders like major depression. This is why researchers need flexible tools to see what happens when certain neurons are activated or suppressed.

One such tool is optogenetics, which uses light to control genetically modified cells. While a popular approach for studying what happens when certain neurons are excited or activated, optogenetic techniques for investigating neuronal inhibition — the silencing of brain activity — are more limited.

To solve this puzzle, Adam Claridge-Chang from the A*STAR Institute of Molecular and

Cell Biology looked into light-sensitive proteins called anion channelrhodopsins (ACRs) previously isolated from an algae.

ACRs act like a gate: when illuminated by a specific wavelength of light they let more negatively charged ions into the neurons. In 2015, this physiological activity had been shown to inhibit brain-cell activity in a Petri dish. So the team decided to test these proteins in a living animal — the vinegar fly, *Drosophila*, an important model for biomedical research.

Choosing the right behavioral tests to validate the ACRs, however, was not trivial. “With an activator, you have a wide range of choices, because when you activate those cells the animal should do something,” says Claridge-Chang. “It’s a bit harder when you’re trying to remove a function — you have to prove that the animal stopped doing something.”

Movement was the obvious choice. When flies carrying the ACR genes were illuminated with specific wavelengths while crawling on a vertical wall, they almost immediately dropped

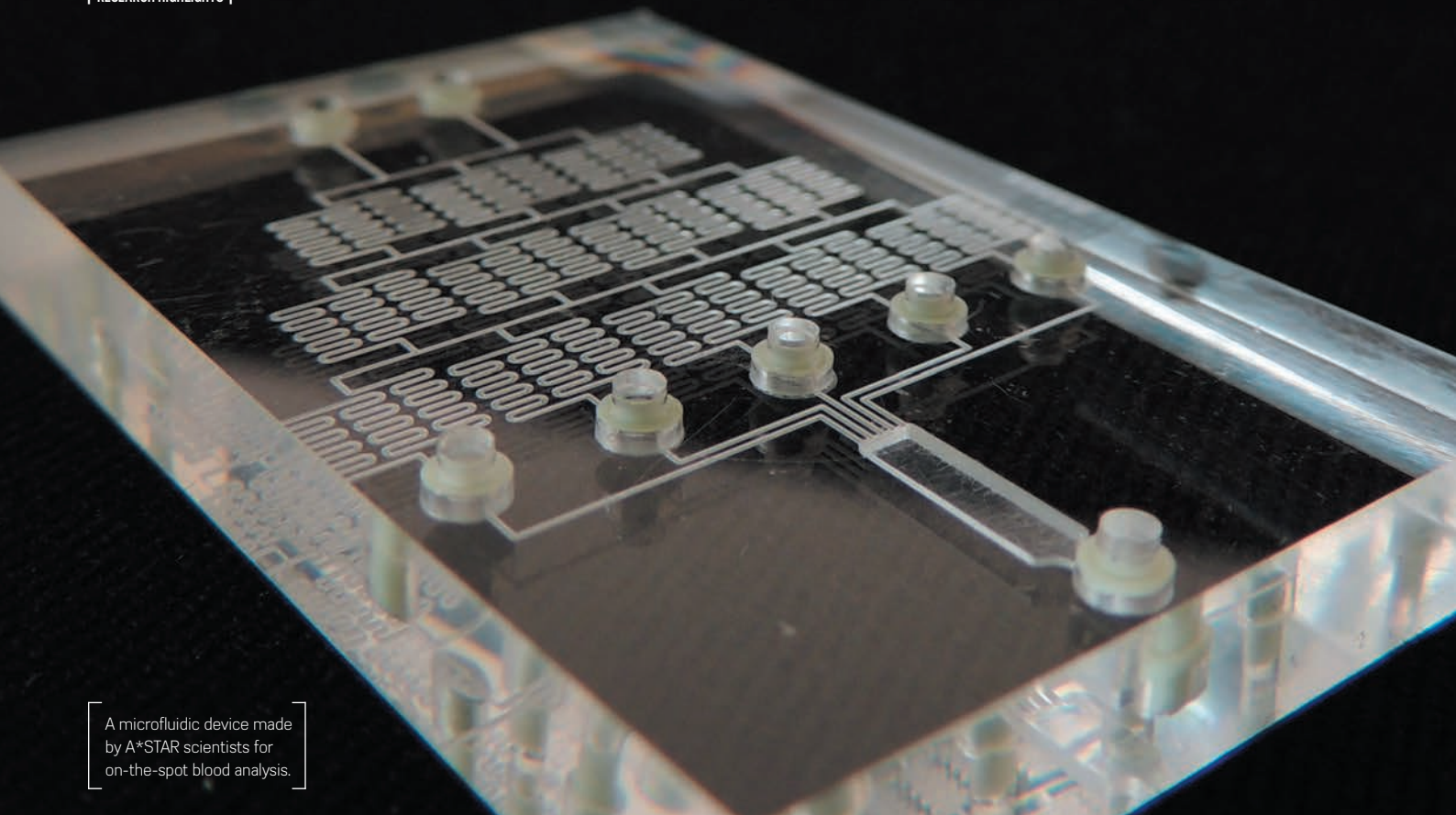
to the ground, lying motionless. “The paralysis happens extremely fast, within tens of milliseconds,” explains Claridge-Chang.

Another test focused on the flies’ sweet tooth. Flies typically cannot resist sugar, and so the team targeted taste receptors and suppressed their ability to detect sweetness. When illuminated, the flies did not lick sugar droplets placed in front of their mouths (see image).

The team released their findings prior to publication, and it exploded on social media within the *Drosophila* community. Since then, more than a dozen labs have started using the lab’s ACR flies to independently validate the team’s findings and analyze brain function.

Moving forward, Claridge-Chang’s team will use the flies to investigate how emotional behaviors are affected in disorders like anxiety and depression.

1. Mohammed, F., Stewart, J. C., Ott, S., Chlebnikov, K., Chua, J. Y. *et al.* Optogenetic inhibition of behavior with anion channelrhodopsins. *Nature Methods* **14**, 271–274 (2017).



A microfluidic device made by A*STAR scientists for on-the-spot blood analysis.

Microfluidics

A MODULAR VALVE SIMPLIFIES DIAGNOSTIC CHIP FABRICATION

RAPID AND CHEAP ON-THE-SPOT DIAGNOSES FOR DISEASES SUCH AS TUBERCULOSIS AND CANCER ARE A STEP CLOSER THANKS TO A NEW MODULAR VALVE FOR MICROFLUIDIC CHIPS

Swapping delicate microscopic flow valves for a universal modular valve system has enabled A*STAR researchers to dramatically decrease the cost and complexity of microfluidic diagnostic chips — business card-sized devices that can analyse blood on the spot for a range of disease biomarkers.

“Microfluidic chips are advancing point-of-care diagnosis for many diseases,” says Alicia Toh from A*STAR’s Singapore Institute of Manufacturing Technology (SIMTech). “Inside these chips, tiny microvalves precisely

direct microlitres of fluid through a series of microchannels for automated analysis. However, integrating microvalves into the microchannels is complex and highly susceptible to fabrication defects, which translates into a higher cost per device. In the medical diagnostic sector, the race is on to lower the cost per diagnosis by producing cheaper microfluidic diagnostic chips.”

Toh and her colleagues Zhiping Wang and Zhenfeng Wang addressed the problem by moving the microvalves off the main

microfluidic chip, and created a modular valve that is fitted to the surface of the chip after fabrication. The valves consist of a microfluidic channel that connects to surface ports on the chip, and an air chamber that allows the channel to be pinched by increasing the air pressure. The team demonstrated that their modular valves could precisely manipulate chemical concentrations through fluidic routing, which is critical in many advance diagnostic applications.

“By mass producing these microvalve modules separate from the microfluidic

chip and testing valve function prior to chip integration, we can achieve much lower defect rates, which boosts yields and results in a much lower cost per device,” says Toh. “This technology will reduce waste and help contribute to sustainable manufacturing practices for microfluidic chips.”

Getting the valve design right, however, was complicated. The team used state-of-the-art software to predict the microscopic

interactions between the flexible elastomeric silicone membrane and the fluid in the micro-channel. Using materials that are compatible with the latest microfluidics technologies was also a big constraint.

“The industry is rapidly moving toward more cost effective thermoplastic materials,” says Toh. “By using compatible materials, we can achieve reliable integration without additional surface modification or adhesives.”

Toh and her team are now exploring the production of microvalve modules using a variety of novel materials. “Greater adoption of microfluidic technology will mean that we could see our modular microvalves being used in a wide spectrum of applications,” she says. ■

1. Toh, A. G. G., Wang, Z. & Wang, Z. Modular membrane valves for universal integration within thermoplastic devices. *Microfluidics and Nanofluidics* **20**, 85 (2016).

Biofouling

STICKING TO THE STORY AT THE MOLECULAR LEVEL

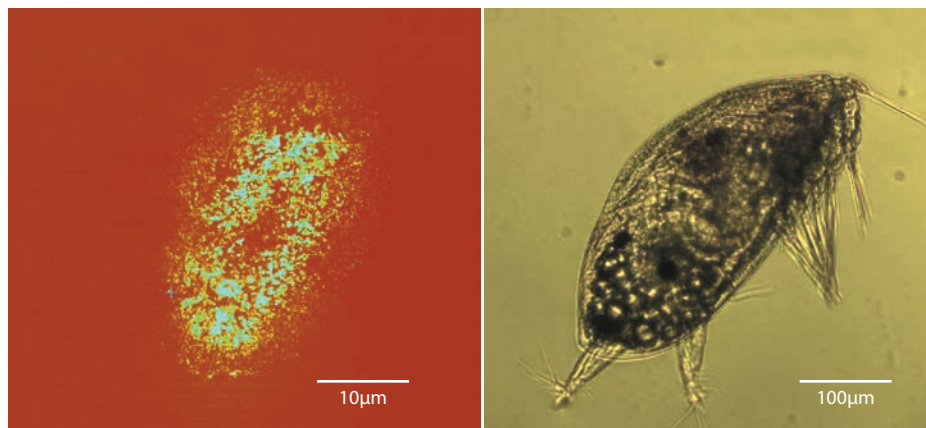
MOLECULAR INSIGHT INTO PROTEIN NET CHARGE MAY EXPLAIN AND HELP SOLVE THE HARMFUL BUILD-UP OF ORGANISMS IN THE MARINE ENVIRONMENT

A deeper understanding of protein adhesion to solid surfaces may shed new light on biological phenomena such as marine biofouling. In their quest for non-toxic, microorganism-repelling surfaces, A*STAR researchers evaluated the relationship between charge and pH for an adhesive protein that exists in minute quantities in the footprint of barnacle larvae and showed it influences their ability to attach to surfaces¹.

The measurements led to a specific pH value known as the isoelectric point (pI), at which the protein net charge equals zero. The pI provides pH ranges for protein solubility and also gives valuable information on a protein's affinity to charged surfaces, which is essential for protein separation, sensing, and nonspecific adsorption. Proteins lose or gain protons depending on the acidity of their surroundings, which alters their net charge. They typically present a positive net charge under highly acidic conditions and a negative charge in highly basic environments. This enables attractive interactions with oppositely charged substances. At pI, the zero net charge promotes protein aggregation.

Several approaches to determine pI values already exist but tend to be time consuming and require high water solubility and concentration. Under the Innovative Marine Antifouling Solutions program, a team led by Julius Vancso from the A*STAR Institute of Chemical Engineering Sciences and Dominik Jańczewski from the A*STAR Institute of Materials Research and Engineering has developed a strategy that delivers protein pI values using atomic force microscopy (AFM).

“AFM has become an enabling platform to visualize as well as manipulate and study matter at a molecular scale,” explains Vancso, noting



AFM image of cyprid barnacle larva footprint protein (left) and microscope image of the cyprid barnacle larva (right).

that his team has used AFM to investigate macromolecular behavior for about 25 years. The researchers anchored a few protein molecules to AFM probes approximating one micrometer across and assessed their adhesion force to charged surfaces at well-defined pH values while pulling the probes off these surfaces.

After validating this approach for various well-known proteins, the team tackled the pI

value of the footprint protein. According to Vancso, this protein stimulates the attachment of a larva, which triggers colonization and further build-up by other larvae. The protein exhibited a pI value of 9.6 to 9.7, consistent with its positive charge in seawater and its adhesiveness to the negatively charged immersed surfaces.

This proof-of-concept experiment minimized protein amount requirements. “We

hope that we contributed to the solving of this notoriously difficult and very essential issue,” says Vancso. They expect that protein scientists will adopt their technique. ■

1. Guo, S., Zhu, X., Jańczewski, D., Lee, S. S. C., He, T. *et al.* Measuring protein isoelectric points by AFM-based force spectroscopy using trace amounts of sample. *Nature Nanotechnology* **11**, 817–823 (2016).

Dengue

MOLECULAR 'MOVIE' REELS VIRAL ENVELOPE INTO SHAPE

COMPUTER SIMULATIONS REVEAL EVERY CURVE OF THE DENGUE CAPSULE

The near-spherical outer structure of the dengue virus has been recreated in remarkable detail by a team of bioinformaticians in Singapore¹. The virtual model could show researchers how the virus fuses with and infects human cells at the molecular level. “We want to understand the relationship between structure and dynamics along the pathway of fusion

and infection, with a view to developing new vaccines and therapies,” says Peter Bond, who, together with Chandra Verma, led the study at the A*STAR Bioinformatics Institute.

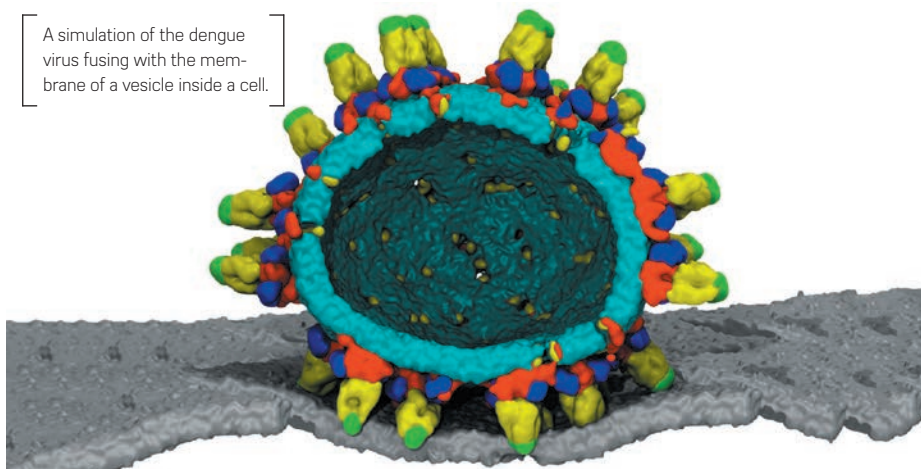
Dengue is a mosquito-borne virus that infects an estimated 400 million people a year, resulting in 21,000 deaths worldwide. It is a flavivirus — the same family as the Zika

virus, Japanese encephalitis and yellow fever. Flaviviruses share a common structure: a single-stranded RNA genome encased in a capsule made up of a fatty lipid sandwich stuffed with proteins called envelopes and membranes.

Once inside human cells, the smooth outer shell of the dengue virus forms spikes and fuses with the membrane of transport vesicles called endosomes, infecting the cell through the release of the viral genome. Researchers are particularly interested in how the external envelope proteins facilitate this process. “These proteins are the first thing to come into contact with our immune system,” says Bond. “If we are going to protect ourselves, we need to recognize and, ideally, neutralize them.”

The problem with standard experimental techniques such as cryoelectron microscopy for visualizing biological systems is that they can only detect ordered, uniform solids. Dengue’s lipid membranes, however, are in a free-flowing state, somewhere between solid and liquid. Bond and Verma’s teams overcame this hurdle using computational modeling, which applies

A simulation of the dengue virus fusing with the membrane of a vesicle inside a cell.



Newton's laws of motion to a static structure "to create a movie that zooms in on all the jiggling atoms."

They simulated the viral shell, and played with different components to see what happened with the overall structure. "We used a Frankenstein-style approach to chop off bits of protein from the virus and see how it affected the morphology — a difficult job in the wet lab."

Surprisingly, the membrane's curves seemed to be held in place by scaffolding proteins external to the membrane. And the positioning of envelope and membrane proteins was secured by specific interactions with negatively charged lipids in the fatty membrane, a finding that could be exploited for treatment development.

The results have important implications for the fusion process (see image), which Bond and

Verma plan to study in more detail through a larger Singapore-wide collaboration supported by the Ministry of Education. The teams are also expanding their scope to other flaviviruses, including Zika.

1. Marzinek, J. K., Holdbrook, D. A., Huber, R. G., Verma, C. & Bond, P. J. Pushing the envelope: Dengue viral membrane coaxed into shape by molecular simulations. *Structure* **24**, 1410–1420 (2016).

Cancer

BUSTING MYTHS ON TUMOR CELL CLUSTERS

A SURPRISING FINDING SHOWS THAT SOME CLUSTERS OF CELLS COMMONLY FOUND IN THE BLOODSTREAM OF COLORECTAL CANCER PATIENTS ARE ACTUALLY NON-CANCEROUS

Clusters of human cells originating from blood vessels captured from the bloodstream by using a custom-designed microdevice.

In a major debunking of scientific orthodoxy, A*STAR researchers have discovered a new type of tumor-derived cells that are non-cancerous floating in the bloodstream¹. This finding promises to open novel, non-invasive ways to detect and monitor the spread of cancer in the body.

For decades, clusters of cells circulating in the bloodstream of cancer patients have been regarded as invasive cancerous cells shed by the tumor. They have been implicated in spreading cancer to other parts of the body, resulting in secondary tumors that are often harder to kill than the initial tumor.

Now, Min-Han Tan of the A*STAR Institute of Bioengineering and Nanotechnology (IBN) and his co-workers have exonerated some clusters of circulating tumor cells, finding that they are not actually cancerous.

The discovery goes against half a century of

received wisdom that all varieties of circulating tumor cells are malignant. "This was an absolutely surprising result," comments Tan. "When we first set out on this study, we expected to find that these clusters were cancerous."

The researchers used a custom-designed microdevice, which was developed by Jackie Y. Ying's team at IBN, to trap cell clusters in blood samples of 80 patients with colorectal cancer (see image). Analysis of the cells' DNA and RNA revealed that the cells originated from the innermost lining of blood vessels that line the tumor, rather than from the tumor itself. Tan and the team also found that these clusters detached intact from blood vessels near a tumor and were not formed by individual cells coming together in the bloodstream.

By monitoring these clusters, doctors should be able to glean vital information about cancer in other parts of the body during the course of

treatment. "What I think excites everyone is the chance to measure the vascular health of a tumor non-invasively, which has never hitherto been possible," explains Tan. "One can imagine administering drugs and evaluating the impact of such agents on the tumor vasculature."

It may even be possible to use the clusters to diagnose certain cancers. "Unexpectedly, we found that these clusters commonly occur even in the early stage of colorectal cancer, which opens up an opportunity to investigate using these cell clusters for cancer screening," adds Tan.

In the future, the team intends to study these circulating cell clusters in other types of cancers. They also plan to develop improved ways to capture and characterize these clusters.

1. Cima, I., Kong, S. L., Sengupta, D., Tan, I. B., Phyo, W. M. *et al.* Tumor-derived circulating endothelial cell clusters in colorectal cancer. *Science Translational Medicine* **8**, 345ra89 (2016).

[VOICES FROM A*STAR]

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*Voices from A*STAR* is a monthly blog published on the *A*STAR Research* website. It features a personal account of the challenges and rewards of a life in science by A*STAR researchers from a range of disciplines. Staff interested in contributing to the *Voices from A*STAR* blog are encouraged to contact the Managing Editor.



Sandhya Sriram

Programme Management Officer; SBIC

“Having worked with antioxidants and oxidative stress (harmful free radicals) for about a decade now, I am convinced that antioxidants are the way to go to prevent or relieve the symptoms of certain diseases — they also help to keep you energetic by detoxifying your body.”



Kaval Kaur

Research Fellow; SigN

“Vaccine formulations can no longer be restricted to contain solely dead or weakened whole viruses or bacteria. Instead, scientists must make informed decisions by identifying the components of disease-causing agents that are most relevant for our immune system to recognize and develop long-lasting memory.”

[NEXT ISSUE]

Here's a sneak peek of the material covered in the next issue of *A*STAR Research*



Immunology:

A UNIVERSAL LANGUAGE FOR IMMUNOLOGICAL SENTINELS

A framework for characterizing dendritic cells should bring greater consistency and reliability to immunological research

Survival tactics:

‘SCARY STUFF’ STUDY FINDS DISTRESS CHEMICALS IN NEW FISH ORDER

A fish alarm system could be more universal than originally thought

Precision engineering:

OPTIMIZING DRILL DESIGN WHEN THE CHIPS ARE DOWN

Modeling the motion of chips produced in gun drilling shows a simple angle change could lead to better gun drill design



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.

A*STAR actively nurtures public-sector research and development in biomedical sciences, physical sciences and engineering, and spurs growth in Singapore's key economic clusters by providing human, intellectual and industrial capital to our partners in industry and the healthcare sector.

A*STAR currently oversees the following research institutes, consortia and centers and supports extramural research with universities, hospital research centers, and other local and international partners.

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Bioinformatics Institute (BII)
Bioprocessing Technology Institute (BTI)
Clinical Imaging Research Centre (CIRC)
Data Storage Institute (DSI)
Experimental Power Grid Centre (EPGC)
Experimental Therapeutics Centre (ETC)
Genome Institute of Singapore (GIS)
Institute of Bioengineering and Nanotechnology (IBN)
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Institute of High Performance Computing (IHPC)
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