A*STAR RESEARCH

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A biomarker for sun-damaged skin

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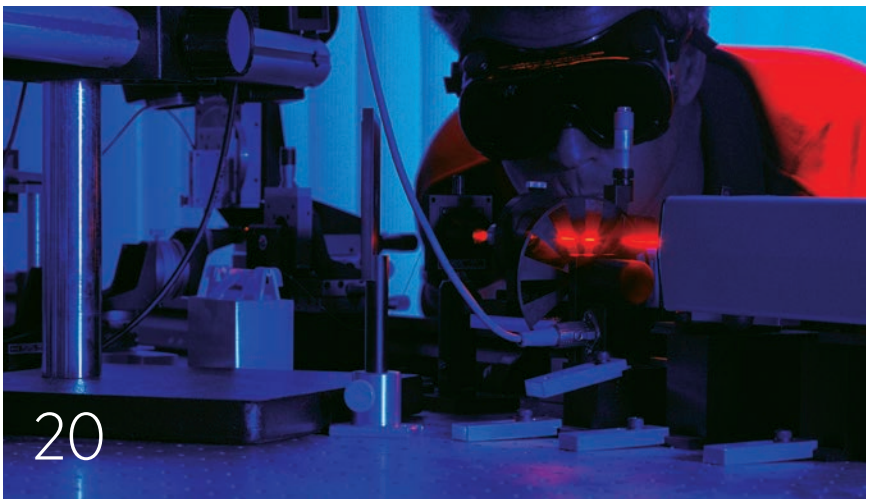

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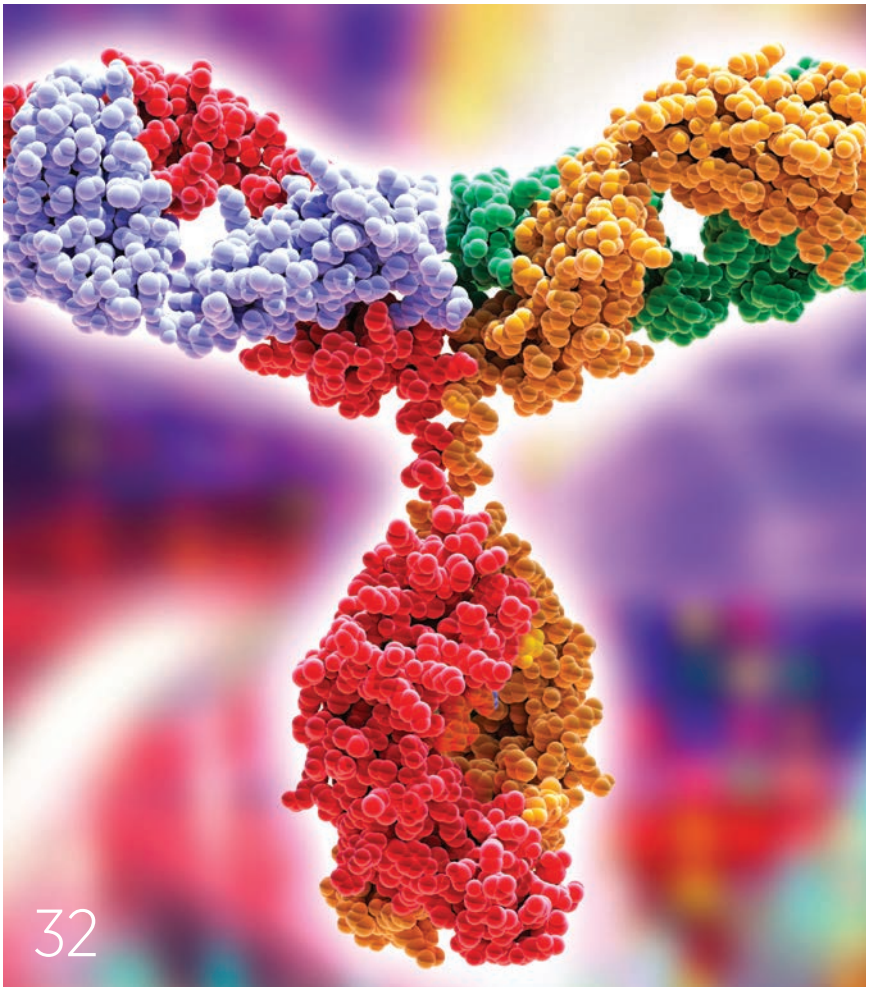


RESEARCH HIGHLIGHTS

NEXT ISSUE

NOTES FROM THE EDITORS

The Editorial Team introduces the latest issue of *A*STAR Research*

Antibiotic resistance has emerged as one of the biggest threats to global health, as bacteria acquire resistance faster than new antibiotics can be developed. Scientists have recently developed a polymer that can kill bacteria effectively without triggering the evolution of resistance, which could be the key to averting the antibiotic resistance crisis (page 8).

Our second feature looks at solving a very different problem that plagues us on a daily basis – that of battery capacity. Lithium-ion batteries essentially power our daily lives – from mobile phones to electric cars, and even entire homes in some cases – but they are fast approaching their physical limits. Two award-winning A*STAR researchers are exploring an alternative – lithium-sulfur batteries, a 50-year old technology that can potentially provide energy densities up to five times that of lithium-ion batteries (page 4).

We also have many more stories from diverse areas of research, such as:

STEM CELLS

- A subpopulation of chief cells that can become stem cells in response to injury has been identified, shedding light on how the stomach lining renews itself, and how gastric cancer begins (page 15).
- Researchers have found a way to generate large numbers of 'universal' natural killer cells from induced pluripotent stem cells, for applications such as immunotherapies (page 33).

CANCER TREATMENTS

- A single-base mutation has been discovered that can strongly predict whether an oral cancer patient will respond to specific treatments (page 50).
- Scientists have also devised a method to grow miniatures of patients' tumors in the lab, which could help doctors choose the most effective treatments for individual patients (page 49).

BIG DATA

- A fully automatic web-based system has been developed that empowers non-experts to analyze big data (page 28).
- Another team have devised a novel approach to predict cancer drivers with improved accuracy, and made their tool freely available online (page 22).

NANOSTRUCTURES

- Scientists have found a way to assemble quantum dots into nanoscale grids using focused electron beams, for applications ranging from biosensors to solar panels (page 41).
- A new printing technique based on silicon nanostructures has been invented, which could achieve resolutions of up to 100,000 dpi (page 31).

For our latest stories, please visit us at https://research.a-star.edu.sg/ or follow us on Twitter at: @astar_research. We hope you will enjoy reading the rest of this magazine.

Lithium-sulfur batteries could outperform lithium-ion batteries. [page 4]

COVER IMAGE
Antimicrobial resistance is a global issue [page 8]
© WLADIMIR BULGAR/ SCIENCE PHOTO LIBRARY; Texture: © 4khz/iStock/Getty

CHARGING FORWARD

WITH THE BATTERY REVOLUTION

Imagine energy densities five times that of lithium-ion batteries. An A*STAR team is leading the race to bring lithium-sulfur batteries to the masses

Theoretically, cars powered by lithium-sulfur batteries will be able to travel more than five times farther than those powered by lithium-ion batteries.

The desire to super-charge increasingly smart, portable electronic devices using more efficient, large-capacity batteries is palpable. "We hear very sensational reports. For example, that you will soon be able to charge your mobile phone batteries in just a few seconds," notes Guangyuan Wesley Zheng, a chemical engineer at the Institute of Materials Research and Engineering (IMRE) at A*STAR. "However, while there is a lot of noise and hype, it's very important research". Electric car batteries, he points out, are going to play a significant role in transportation. In fact, Macquarie Research dubbed 2017 'the year that electric cars came of age', with global

In 2017, battery whiz Zhi Wei Seh spoke to a mixed crowd about his research into batteries at the *MIT Technology Review*'s annual EmTech conference.

demand up 51 per cent on 2016. Zheng adds that "Boeing and Airbus are also talking about aircraft fully powered by electricity" and more battery-powered electrical appliances are becoming possible — "like vacuuming robots."

Lithium-ion batteries have been the dominant battery on the market since their commercial debut in the early 1990s, used in everything from mobile phones to cars. Tesla, for example, a Silicon Valley-based electric vehicle manufacturer, is now building a 'Gigafactory' to dramatically scale up the production of lithium-ion batteries.

However, lithium-ion batteries are reaching the limit of what they can achieve, says Zheng's colleague, Zhi Wei Seh, a materials scientist at IMRE. "So, instead of making incremental improvements on what lithium-ion batteries can theoretically do, we are exploring drastically different, revolutionary battery technologies."

FROM LITHIUM-ION TO LITHIUM-SULFUR BATTERIES

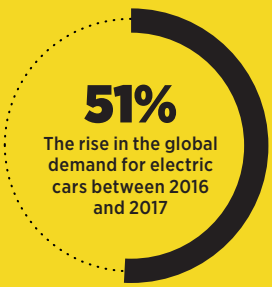
Many research groups are looking into the potential of lithium-sulfur batteries as an alternative to lithium-ion batteries, but Zheng and Seh are among the most ambitious, both recently named among the *MIT Technology Review*'s top 'Innovators under 35' in Asia Pacific.

Lithium-sulfur batteries are attractive because sulfur is cheap and abundant, and the batteries themselves theoretically have energy densities five times larger than lithium-ion batteries. "That means, if we put batteries of the same weight into a car, it could travel five times longer," Zheng explains. That possibility could also pave the way for applications that lithium-ion batteries cannot handle, such as high-energy stationary grid storage systems for renewable energy sources like solar and wind power.

Like all batteries, lithium-ion and lithium-sulfur batteries store energy in the form of chemical energy, which is converted to electrical energy during discharge. The basic configuration of a lithium-ion battery involves two electrodes made of different materials, both of which are able to store lithium.

When connected to a circuit during discharge, the negative electrode (anode) undergoes oxidation, while the positive electrode (cathode) undergoes reduction. The change in the chemical composition of the electrodes stimulates an electric current to move from the anode to the cathode through the circuitry of the device being powered, and lithium-ions to move from one electrode to another within the battery. When the ions run out the battery becomes flat. When a battery is plugged in to recharge, this process is reversed — the cathode is oxidized, while the anode is reduced — and both electrodes are returned to their original state.

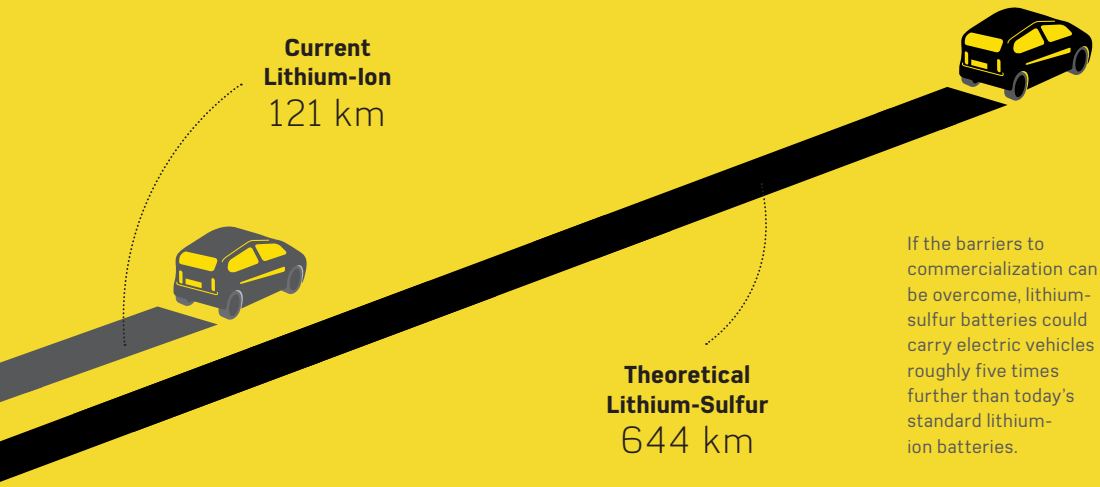
ELECTRIC CARS could benefit hugely from improved battery technology and demand for them is growing.



Energy density limits, which along with the lifetime of a battery determine the commercial proposition of a battery, hinge on the expense and ion-carrying limits of electrode materials. A lithium-ion battery's anode is generally made from graphite, and the cathode from a mix of expensive metal oxides such as cobalt, nickel and manganese. Researchers have been steadily improving battery performance by incrementally adjusting the composition of electrode materials, along with the battery cell design. Nonetheless, because of the basic limits of how lithium ions are stored in these materials, the energy density limit of lithium-ion batteries is still theoretically capped at roughly 420 watt-hours per kilogram (Wh/kg), just enough to power electric cars over distances expected of conventional cars.

But if we want to continue to charge forward lithium-sulfur batteries promise greater performance using a different storage mechanism, explains Seh. The basic function is similar to that of lithium-ion batteries: during discharging, the lithium metal anode is oxidized to form lithium ions that travel through the liquid electrolytes inside the battery to reach the sulfur cathode, where a reaction reduces sulfur to form lithium sulfide. As in lithium-ion batteries, the change in the composition of the electrodes stimulates a flow of electrons from one electrode to another through the circuitry of a device. Again, this process can be reversed during charging.

Unlike conventional lithium-ion batteries, in which ions are inserted into the electrode materials, lithium-sulfur batteries use a metal plating and stripping mechanism on the lithium anode and a conversion reaction, in which the electrode material is transformed from sulfur into lithium sulfide, on the sulfur cathode side[1]. Negating the need for ions to be inserted into electrode materials means that the theoretical energy density of a sulfur-lithium



If the barriers to commercialization can be overcome, lithium-sulfur batteries could carry electric vehicles roughly five times further than today's standard lithium-ion batteries.

battery is much, much higher, at 2,500 Wh/kg.

The possibility of lithium-sulfur batteries was discovered as early as the 1960s, but huge technical challenges still obstruct commercialization. On the cathode side, major problems include the low electronic conductivity of sulfur and lithium sulfide, as they are naturally insulating. Also, faster lifetime decay occurs during a battery's energy cycling because of the build-up of lithium deposits, and because intermediate materials known as lithium polysulfides, which are important to maintain the cathode's performance, dissolve quickly into the battery's electrolytes, leading to a continuous loss of important active materials.

Researchers have been trying to solve the first problem by coating sulfur particles in a conductive material. A breakthrough came in 2009 when a Canadian team developed a technology to encapsulate sulfur particles within a highly-ordered framework made of porous carbon, which is conductive[2]. However, as a sulfur particle's volume expands by as much as 80 per cent upon reacting with lithium, it often cracked the protective coating, and the problematic polysulfide dissolution continued. Stable performance could thus be achieved for only 20 charge-discharge cycles (by comparison, the lifetime of a conventional lithium-ion AAA battery has roughly 500–1,000 cycles). Nonetheless, since 2009, research into lithium-sulfur batteries has been accelerating.

Guangyuan Wesley Zheng is banking on a future in which drones can cover long distances powered by batteries. He's bidding to work on drone vaccine deliveries on Vanuatu for UNICEF.

INSPIRATION FROM AN EGG

Both Seh and Zheng made significant advancements in the science several years ago when they were PhD students at the lab of Professor Yi Cui at Stanford University. It was Seh who looked into the volume expansion problem. "I came up with an idea similar to an egg structure," Seh says. "Imagine the sulfur is an egg yolk and the white is empty space."

The team developed a technology to coat sulfur nanoparticles with titanium oxide, and then allow some dissolution of the sulfur to create a void inside the shell to accommodate the volume expansion of sulfur[3]. As a result, the capacity decay after 1,000 cycles was as small as 0.033 per cent per cycle, which represented the best performance of a lithium-sulfur battery at the time.

Inspired by this yolk-shell structure, the performance of lithium-sulfur batteries reported in literature improved greatly, Seh says, and now some groups have even demonstrated 10,000 cycles.

Zheng, on the other hand, led a project on one of the other issues that makes commercialization difficult, the lithium anode. The anode tends to form dendritic and mossy metal deposits within the battery, one of the major causes of the battery's short lifetime.

His team initially considered using a carbon thin film to coat the lithium anode, but the coating cracked after a few cycles. After several attempts, they resolved the issue using a hollow carbon nanosphere approach.

Unlike conventional approaches focused on modifying anode materials to prevent dendrite formation, their approach focused on accommodating shape changes of the anode. As the hollow carbon nanosphere coating is highly flexible and robust, it can withstand the large volumetric changes of the lithium anode during energy cycling, thus allowing the battery to continue functioning[4].

"The result provided a new perspective on how simple nanotechnology can help address long-standing problems in lithium batteries," Zheng says. His team has since made more iterations, including

using a self-healing polymer and other coating methods, and he says these results also show good cycling at higher current densities.

THE ROAD TO COMMERCIALIZATION

Since moving back to Singapore from Stanford in the USA and joining A*STAR in 2016, the two scientists have been exploring new angles of the lithium-sulfur battery question. Zheng is pushing hard for commercialization. He runs a San Francisco-based company called Volans-i in collaboration with a friend, Hannan Parvizian. They build drones, and operate a delivery service across long distances. Volans-i still use traditional lithium-ion batteries because lithium-sulfur battery packs are still in development, but they ultimately need a battery platform robust enough to "deploy drones around the world," Zheng says.

Their progress is impressive. Volans-i has received funding from prominent investors such as Y-Combinator, and hybrid battery and gasoline models have achieved a long-range cruise drone of up to 1,600 kilometers at a maximum speed of 322 kilometers per hour. UNICEF has also recently approached the company and invited it to bid in its drone project to distribute vaccines to people on the small Pacific island of Vanuatu.

Meanwhile, as many start-up companies and automakers are competing to develop prototypes of lithium-sulfur batteries, Zheng last year set up a company called Coulomb Innovation in Singapore with a friend to commercialize lithium-sulfur technology, with a focus on material production. Zheng is now preparing to scale up the production of sulfur nanoparticles as a first step to practical application. In addition, his team is making pouch-cell prototypes (the type of battery used in newer laptops, mobile phones and electric cars) of lithium-sulfur batteries in a laboratory at IMRE, while trying to produce a battery pack that can power some devices such as drones and small robots.

In 2018, Guangyuan Wesley Zheng was one of only 10 researchers to be named in the *MIT Technology Review*'s top 'Innovators Under 35' in the Asia Pacific. In 2017, Zhi Wei Seh also received the honor. Both have spoken about their work at the *Review*'s annual conference, EmTech Asia.

Seh's team is investigating batteries that use alternatives to lithium, such as magnesium and aluminum, which are not only plentiful and much cheaper, but have two to four times greater charge capacity. Using these materials could also help avoid the issue of dendritic deposits, which are caused by the reactivity of lithium metal and eat away at the effectiveness and lifetime of the batteries. However, magnesium and aluminum ions are larger and often reported in literature to be slow and sluggish, says Seh. His team is searching for the best way to manipulate these materials "to move ions very fast during charging and discharging."

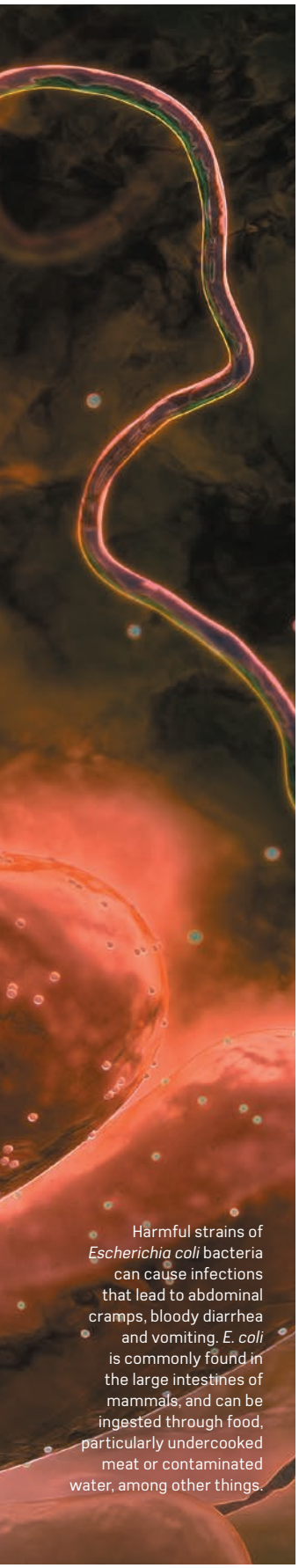
THIS COULD PAVE THE WAY FOR THINGS LITHIUM-ION BATTERIES CAN'T HANDLE, SUCH AS GRID STORAGE FOR SOLAR AND WIND ENERGY STORAGE.

Both scientists believe next-generation rechargeable sulfur-lithium batteries are very close to commercialization. "With our accumulated knowledge over the past few years, we are now seeking companies to work with, and we will have more prototype cells by the end of the year," Zheng says. Seh adds: "As for magnesium and aluminum batteries, we still don't fully understand these emerging battery technologies, including what is the most suitable electrode and electrolyte materials. But we hope to start making coin cells over the next 5–10 years and then build bigger cells that can really impact our lives." ■

For references, visit the online version of this article at: www.research.a-star.edu.sg/feature-and-innovation/7886

BEATING BACTERIA – LOOKING BEYOND ANTIBIOTICS

Genome specialists and bioengineers have come up with a polymer to fight superbugs, which could help solve the world's antibiotics crisis



Harmful strains of *Escherichia coli* bacteria can cause infections that lead to abdominal cramps, bloody diarrhea and vomiting. *E. coli* is commonly found in the large intestines of mammals, and can be ingested through food, particularly undercooked meat or contaminated water, among other things.

The standard treatment for a serious bacterial infection is a dose of antibiotics, which slow or halt the infection by hindering critical cellular processes within the bacteria. However, some bacteria have evolved devious mechanisms to protect themselves against antibiotics, for instance by producing enzymes that can destroy the antibiotic molecules, or by making themselves less permeable to the antibiotic. The most resilient become better at this every time they're exposed to the drugs. The class of multi-drug resistant bacteria that have become the most difficult, or, even impossible to treat, are known as superbugs. Last year, a UK government report suggested that, by 2050, drug-resistant infections could kill one person every three seconds.

In March 2018, a team working under Yi Yan Yang, a bioengineer from the Institute of Bioengineering and Nanotechnology (IBN), published a paper that could signal a break in the spiraling battle between antibiotics and superbugs. It looks at the properties of an infection-fighting synthetic polymer, the most eye-catching element of which is that the polymer doesn't appear to set off an adaptive response in bacteria at all.

In their study, Yang and her team showed that the polymer is biodegradable, non-toxic and can kill the *Acinetobacter baumannii* superbug within an hour. Genomic tests conducted by the Genome Institute of Singapore (GIS) then confirmed that the bacteria did not develop resistance to the polymer.

SERENDIPITY: HOW COMPUTER HARDWARE LED TO INFECTION TREATMENTS

Last year, the World Health Organization (WHO) released a list of 12 of the world's most dangerous superbugs — a move intended to underline them as the highest priorities for antibiotic research. Marie-Paule Kieny, the WHO's assistant director-general for health systems, was pointed in a WHO communiqué: "If we leave it to market forces alone, the new antibiotics we most urgently need are not going to be developed in time […] The pipeline is practically dry." However, a treatment that kills bacteria and doesn't stimulate resistance could represent a new chapter in the fight against superbugs.

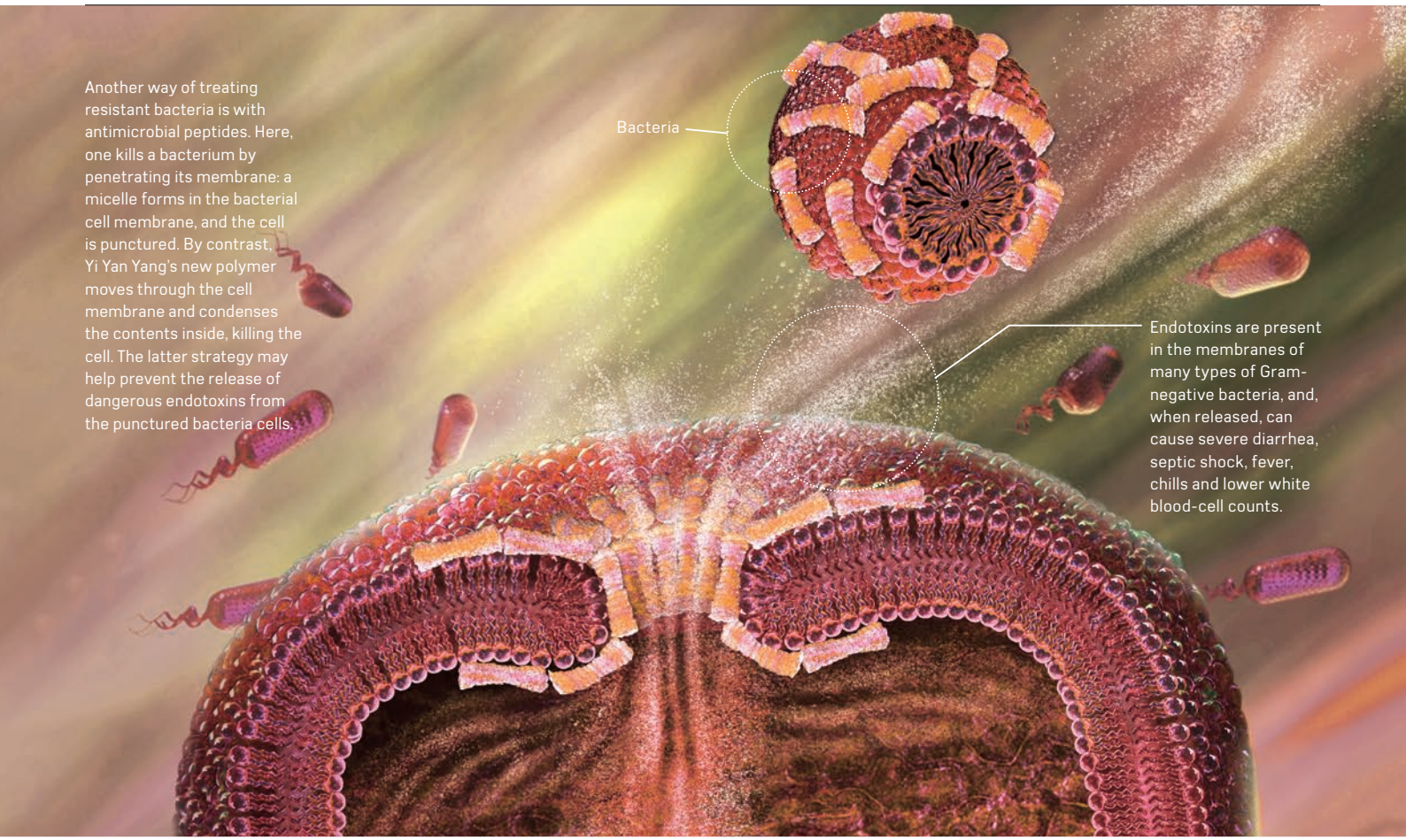
Yang was aware of the problem when her team started their polymer work, but superbugs also just pique her curiosity. Bacteria, she says, are not unlike our own mammalian cells in their ability to develop and learn. "They're very smart." If bacteria were in a classroom studying survival, superbugs would be top students, she says.

Yang, who is also interested in using nanotechnology for drug delivery, seeks and attracts interesting collaborators. The development of the most recent polymer began with multinational tech company IBM. While at a conference in Australia in 2007, Yang heard IBM's James Hedrick talk about a unique, positively charged synthetic polymer his team had accidentally made while working on creating silicon wafers for computer semiconductor technology. The polymer was of particular interest to Yang: its positive charge means it has the potential to become attached to negatively charged bacteria surfaces when introduced to the bloodstream, while leaving human cells alone. Today, Hedrick, the lead scientist for IBM Research's advanced organic materials group, is one of Yang's main collaborators.

Since then, Yang and a handful of other researchers have looked at various polymers, but each new iteration encountered problems. Biodegradability is one — without a route for excretion, non-biodegradable polymers could build up in a patient's body, potentially causing problems. Yang's new polymer is broken down via a chemical reaction with water, the products of which are excreted within days, meaning it's a much more promising candidate for drug development.

In 2016, a University of Melbourne study published in *Nature Microbiology* attracted attention when it found a similar lack of drug resistance in their polymer. Newspapers put a positive spin on the fact that it "ripped the bacteria apart". But Yang says that, actually, this is not really a good thing. "With polymers that break down the bacteria cell membrane, you worry that a lot of endotoxins will be released from the bacteria cells, and those endotoxins may cause sepsis." In other words, they could be toxic. This was exactly what stopped Yang from proceeding with drug trials for a polymer she published about in 2011 in *Nature Chemistry*. In large quantities, endotoxins – toxins present in the membranes of many types of Gram-negative bacteria – can cause severe diarrhea, septic shock, and even death. Smaller amounts may cause fever, chills, and can lower the number of white blood cells in the body, leading to a compromised ability to fight infection.

Yang's new polymer, by contrast, binds to bacteria



cell surfaces, moving through the cells' membranes into the cells. Once inside, the polymer condenses the proteins and DNA inside, killing the cells. The dead bacterial cells are then digested by macrophages, and preclinical tests showed that the products of the polymer are excreted within days.

Getting all these elements right took years of communication between Yang and chemists at IBM, who worked on the polymer design. They eventually settled on a guanidinium-functionalized polycarbonate, which was then synthesized in Yang's laboratory.

In the first stage of testing the researchers compared the polymer with imipenem, an important injected antibiotic used in hospitals to treat pneumonia, sepsis, joint infection and several other bacterial infections. They did this by repeatedly applying sub-lethal concentrations of the two agents on *A. baumannii* bacteria and looking for changes in the concentration at which the agents were effective. "Any increase in the effective concentration of antimicrobial agents against the resilient bacteria means that the bacteria have developed resistance against the antimicrobial agents," explains Yang.

With imipenem at sub-lethal concentrations, the group began to see an increase in the effective concentration levels of imipenem within eight to ten applications. By contrast, the same effective concentration of their polymer stayed roughly the same even after 30 applications at sub-lethal concentrations.

The group then proved that the polymer is not toxic to mice and is effective against five globally important superbugs: *E. coli*, *methicillin-resistant Staphylococcus aureus* (MRSA), *P. aeruginosa*, *K. pneumoniae* and *A. baumannii*.

Yang next looked to the genomes of the treated bacteria for more detail on the mechanisms behind their findings. It was the team of Paola Florez de Sessions at A*STAR's Genome Institute of Singapore who were tasked with analyzing the antibiotic and polymer-treated bacteria's genes using RNA sequencing.

In the imipenem-treated bacteria, the team observed an upregulation of genes associated with imipenem resistance, as well as those linked to resistance against other classes of antibiotics — a clear indication that the imipenem-treated bacteria had

NOTE ON THE TERM 'SUPERBUGS'

We'd like to note that, although commonly used, many microbiologists are trying not to proliferate the use and misuse of the term 'superbug'. The Mayo Clinic defines superbugs as "a term used to describe strains of bacteria that are resistant to the majority of antibiotics commonly used today". In this story, the term refers to bacteria that are resistant to many drugs.

developed cross-resistance, which eventually leads to multi-drug resistance. By contrast, none of these genes were upregulated in the polymer-treated bacteria. These results, together with the other studies, strongly reinforce the polymer's promise as an effective, broad-spectrum and long-lasting antibacterial agent.

THE BRAVE NEW WORLD OF MACROMOLECULAR THERAPEUTICS

GIS and gene sequencing will also be key to the next stage of research on the polymer. Hedrick has described the March paper as the beginning of a whole new class of treatments, which he calls 'macromolecular therapeutics'. While Hedrick may be correct in describing their findings as seminal, the field's newness means that to move toward drug development, Yang and GIS will have to continue to rigorously test the polymer, which they are doing in collaboration with the U.S. National Institutes of Health.

However, the hope is that — just as genome technology accelerates new discoveries — it can help to speed up the vetting of drugs as well. Florez de Sessions says GIS is keen to harness IBM's machine-learning technology to look in the treated bacteria sequence data for any other important patterns that might affect a person's health.

"The premise of this particular study is that the polymer is relatively inert compared to the standard-of-care antibiotics, imipenem or penicillin … or other antibiotics," she says. "So, you don't see a resistance, you don't see some of the things that you expect from antibiotics … but what about the things that we don't know?" What GIS can do, says Florez de Sessions, is help to show IBM what genome specialists already know about bacterial resistance regulation. Those are already well-annotated responses, she says. GIS and IBM can then run the treated bacteria's genomic data through a machine-learning program and highlight any other patterns that might be relevant to a person's health outcome.

IBM'S HEDRICK DESCRIBES IT AS THE BEGINNING OF A NEW CLASS OF TREATMENTS.

As for Yang, she thinks synthetic polymers have the potential to provide a better and cheaper response than one of the main alternatives to antibiotics — peptides (there are others alternatives, including immune modulation, antibody therapy and phage therapy). Peptides are "not actually that stable in vivo" and can easily be degraded by enzymes, she notes. "So they're high cost and unstable. That's why there are very few anti-microbial peptides in the clinic, and those peptides are only used in topical applications." Until now, polymers had been a relatively untapped direction for drug research. "Compared to peptide people there are still very few polymer people — although they're now increasing," Yang explains.

Yang and her team are looking for partners to start working on the comprehensive preclinical studies they will need before they can start to look at clinical trials. ■

For references, visit the online version of this article at: www.research.a-star.edu.sg/feature-and-innovation/7849

GENOMICS FOR SUPERBUG OUTBREAKS

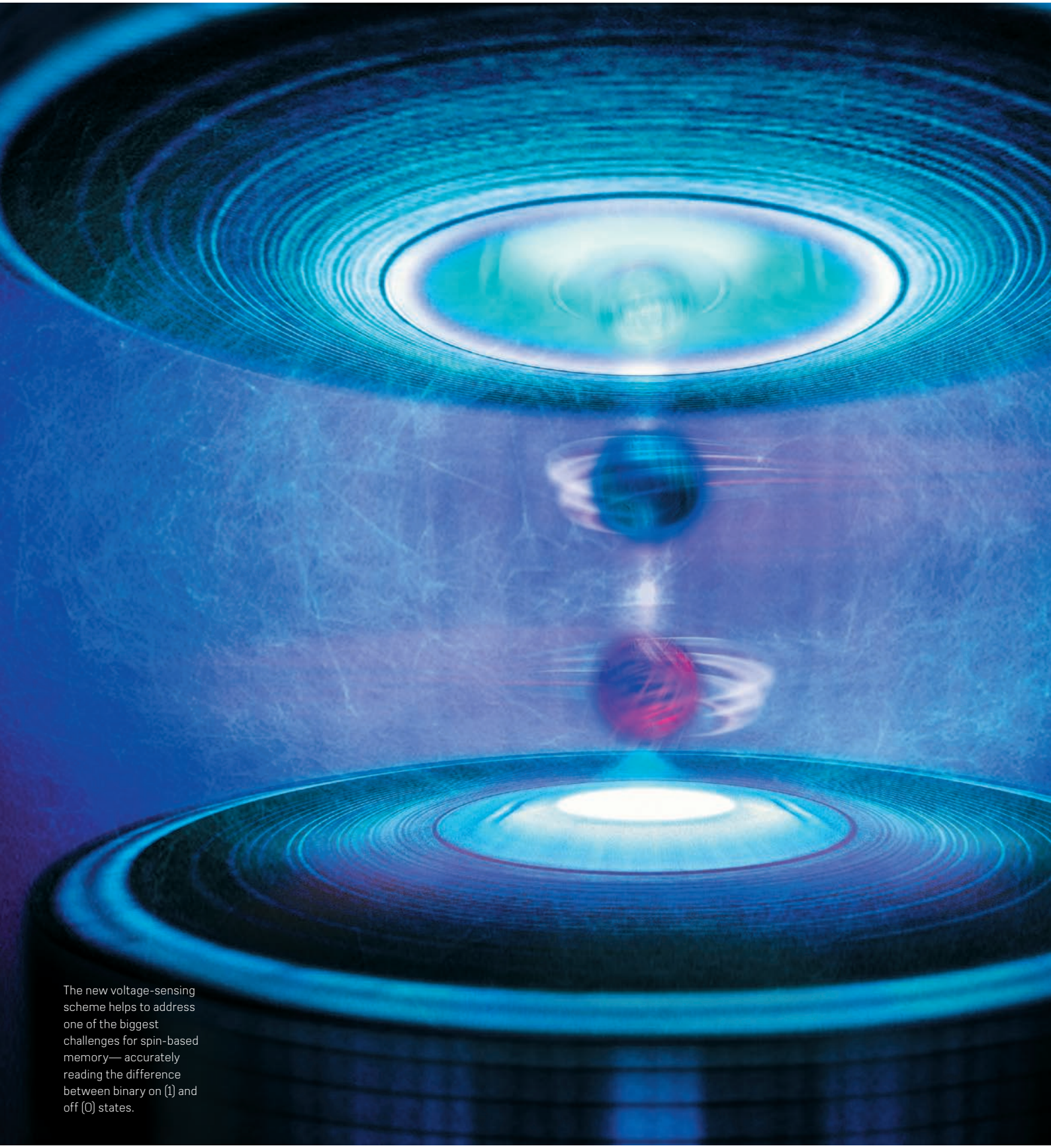
Genome technology's dramatic drop in price over the last few years has a lot to offer for the study of infection, says Florez de Sessions. She adds that some of GIS's current work supports economic arguments for using genomic analysis to isolate and halt superbug outbreaks. A recent GIS study showed that whole-genome sequencing was very effective at revealing the development of a *Streptococcus* outbreak in a male ward in a 200+ patient psychiatric hospital.

"Even though the upfront sequencing costs are more, if you have an outbreak in a hospital and say you have to keep screening and swab all patients once a month for six months and they're approximately $12 swabs, now, suddenly, by sheer numbers and sheer amount, it ends up being cheaper to do whole-genome sequencing."

While Florez de Sessions leads the GIS Efficient Rapid Microbial Sequencing (GERMS) platform, GERMS consists of three labs: Swaine Chen's lab focuses on infectious diseases; Niranjan Nagarajan's lab on computational and systems biology of microbes; and Florez de Sessions' lab on microbial sequencing.

Chen's lab was called upon in 2015 when Singapore had an outbreak of Group B *Streptococcus*. Throughout that year, a severe and invasive strain of this bacteria caused serious infections including meningitis, septic arthritis and spinal infection in over 300 people who were often young and otherwise healthy. However, many of these patients had recently consumed raw freshwater fish. After fishmongers and food handlers were screened and shown not to be carriers of the superbug, Chen's lab led the GERMS genome sequencing effort to identify the bacteria causing the infection.

By analyzing the genome sequences of bacteria isolated from sick patients and fish dishes, Chen and his team provided genomic evidence that traced the infections to two types of fishes, Song (Asian bighead carp) and Toman (snakehead fish), which are used in *yu sheng*, a raw fish dish often served with congee. This corroborated traditional epidemiological studies suggesting that the infections were emanating from those consuming contaminated raw fish. To prevent future outbreaks, Singapore's National Environment Agency has now banned food outlets from serving raw freshwater fish dishes. ■



The new voltage-sensing scheme helps to address one of the biggest challenges for spin-based memory— accurately reading the difference between binary on (1) and off (0) states.

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SPIN MEMORY

DYNAMIC SOLUTION FOR ACCURATE READING

A sensing scheme that responds dynamically to voltage fluctuations could improve accuracy when reading data from spin-based memory storage

A voltage sensing scheme developed by researchers from Singapore could improve the accuracy of reading data from spin-based memory systems with only minimal modifications. The scheme responds dynamically to voltage changes in the system, so that it can better discern whether it is reading a binary on (1) or off (0) state.

The cutting-edge data storage technology, called spin-transfer torque magnetic random-access memory (STT-MRAM), encodes data using the intrinsic angular momentum of electrons — their spin, instead of their charge. Quang-Kien Trinh, Sergio Ruocco from the A*STAR Data Storage Institute and Massimo Alioto from the National University of Singapore are at the forefront of global efforts to prove that STT-MRAM can provide a fast, high-density, low-power alternative to existing charge-based memories.

"STT-MRAM is the leading candidate for future non-volatile, universal memory technology," says Trinh. "It could serve in consumer devices, corporate data centers, and even high-end critical applications such as unmanned vehicles, aircraft, and military."

In STT-MRAM systems, data bits are stored as either 1s or 0s by flipping the orientation of magnetized 'bitcells'. To read a bitcell, the system compares its own reference voltage to the 'bitline' voltage across the bitcell — the 1 or 0 state is then identified based on the difference between the two voltages, called the read margin.

However, "the memory read operation is recognized as one of the major roadblocks of this emerging technology," according to Trinh. The reference voltage frequently unintentionally flips the bitcell, or reads the wrong memory state if the read margin is small.

Trinh, Ruocco and Alioto realized that they could avoid read errors if they were to sense the bitline voltage and adjust the reference voltage in response, so that the read margin always remains high.

"Our new dynamic reference scheme generates two reference values, one for reading logic 0 and another for reading logic 1," explains Trinh. "In logic 0 state, a small readout signal is compared to a large reference value, while in logic 1 state, a large readout signal is compared to a small reference value."

"STT-MRAM is the leading candidate for future non-volatile, universal memory technology."

The team's simulations suggest that their dynamic reference scheme could be incorporated into existing STT-MRAM systems with minimal modifications, and would reduce read errors by two orders of magnitude.

"We look forward to exploiting the synergy between our dynamic reference scheme and existing circuits," says Trinh. "We are also working on solutions to reduce the energy consumption and design complexity."

1. Trinh, Q.-K., Ruocco, S. & Alioto, M. Dynamic reference voltage sensing scheme for read margin improvement in STT-MRAMs. *IEEE Transactions on Circuits and Systems* **65**, 1269–1278 (2017).

OBESITY

HIGH-FAT DIET LINKED TO SKIN CONDITIONS

A high-fat diet may worsen inflammatory skin conditions such as psoriasis

A high-fat diet could aggravate skin diseases such as psoriasis by increasing the level of inflammatory cytokines in the skin, an A*STAR-led study finds.

Previous research has suggested that obesity is a trigger for psoriasis, a disease characterized by scaly, itches patches on the skin, thought to be associated with the immune system. So researchers from A*STAR, and colleagues in Japan, set out to explore the cause underlying the link between obesity and psoriasis.

Their study compared obese mice which had been fed a high-fat diet and lean mice on a normal diet. Both groups of mice were treated with a drug called imiquimod that can induce a psoriasis-like skin condition.

After five days of imiquimod treatment, they noticed that the obese mice that had been fed the high-fat diet exhibited significantly greater psoriasis-like symptoms than the mice on the normal diet.

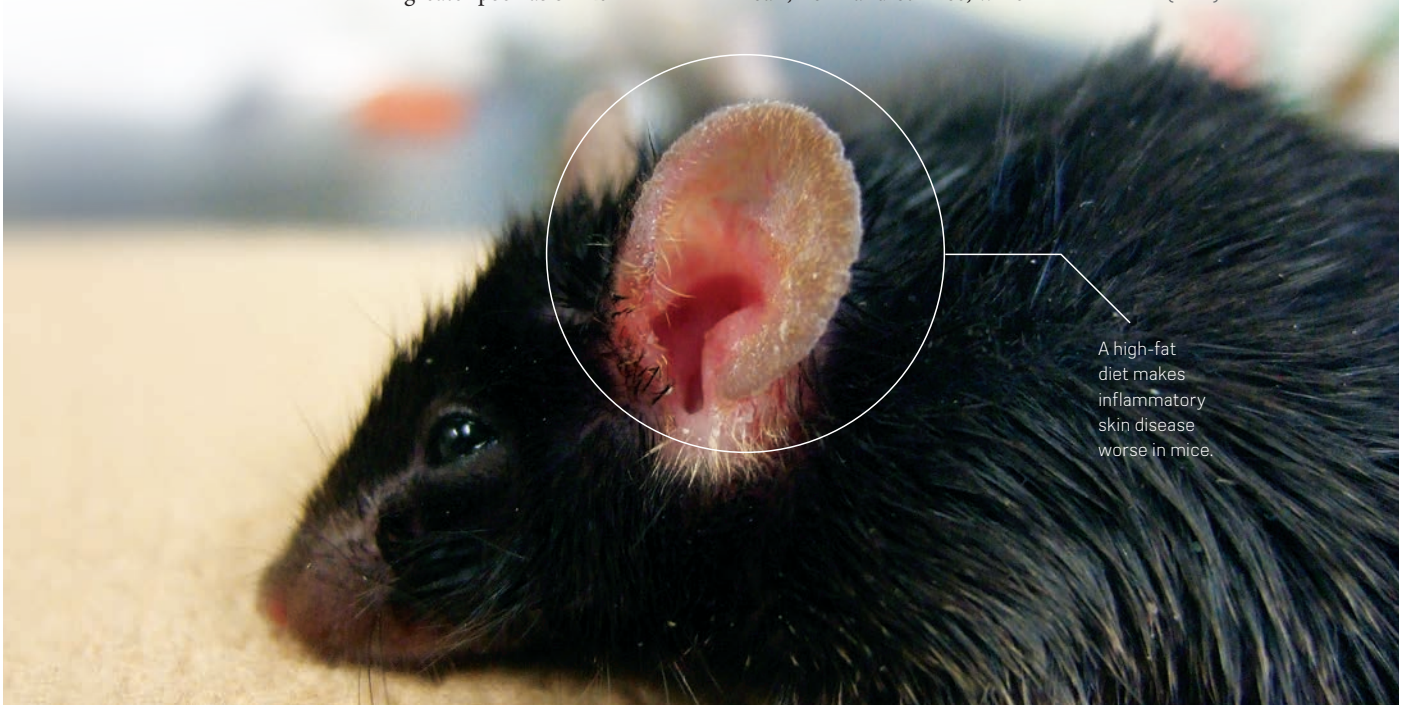
To understand this, the team took a closer look at what was happening to the immune cells in the skin of the obese mice. They discovered that the cells lining the blood vessels in the skin were putting out chemical signals that caused an increase in a type of immune cell called a T cell. This T cell produced another chemical messenger called IL-17, which is associated with increased inflammation.

But the researchers still weren't sure whether it was the obesity itself, or the high-fat diet that was responsible for the immune changes in the mice's skin. To answer this, they repeated the treatment on mice that were genetically engineered to be obese even without the input of a high-fat diet. They found that these mice responded to the imiquimod treatment in a manner similar to that of lean, normal diet mice, which suggested that it was in fact the high-fat diet, and not the resulting obesity, that was contributing to higher levels of skin inflammation.

While most people know that a high-fat diet is associated with heart disease and diabetes, not many would think about its potential to cause skin disease, says Satoshi Nakamizo, from the Singapore Immunology Network and Institute of Medical Biology at A*STAR.

"A high-fat diet acts on a large blood vessel such as the heart, causing arteriosclerosis, but it also acts on small blood vessels of the skin and causes skin inflammation," Nakamizo says. "Diet and weight management are important not only for systemic diseases but also for preventing skin diseases."

1. Nakamizo, S. Honda, T. Adachi, A. Nagatake, T. Kunisawa, J. *et al.* High fat diet exacerbates murine psoriatic dermatitis by increasing the number of IL-17-producing γδ T cells. *Scientific Reports* **7**, 14076 (2017).

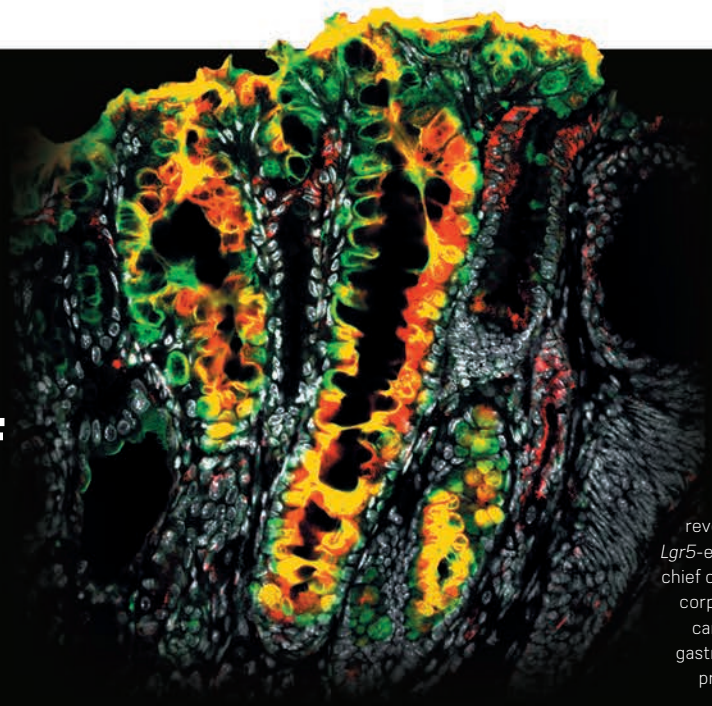


A high-fat diet makes inflammatory skin disease worse in mice.

CANCER

THE STOMACH'S 'CHIEF' SOURCE OF STEM CELLS

Stem cell reserves found in the stomach have implications for the genesis of gastric cancer



The study revealed that *Lgr5*-expressing chief cells in the corpus glands can become gastric cancer precursors.

A team of researchers at A*STAR has found that certain cells in the stomach, called chief cells, become stem cells in response to injury, providing a source of new cells[1]. As well as being significant for understanding how the stomach lining renews itself, the finding sheds light on how gastric cancer can begin.

The stomach wall is a hostile place for cells, as they are constantly subjected to mechanical stress and high acidity and must be continually replaced. The most likely source of such reinforcements is adult stem cells, but identifying these cells has been challenging.

"The identity of the gastric stem cells has been contentious," says Nick Barker of the A*STAR Institute of Medical Biology.

Now, Barker and his co-workers have located where these stem cells reside — at the bottom of small pits in the stomach known as corpus glands. They discovered in a mouse model that certain cells in this location express the gene *Lgr5*, a marker of stem cells in various organs, including the intestine and skin. The researchers also found that these cells are not normally stem cells; rather, they become stem cells in response to damage to the stomach wall.

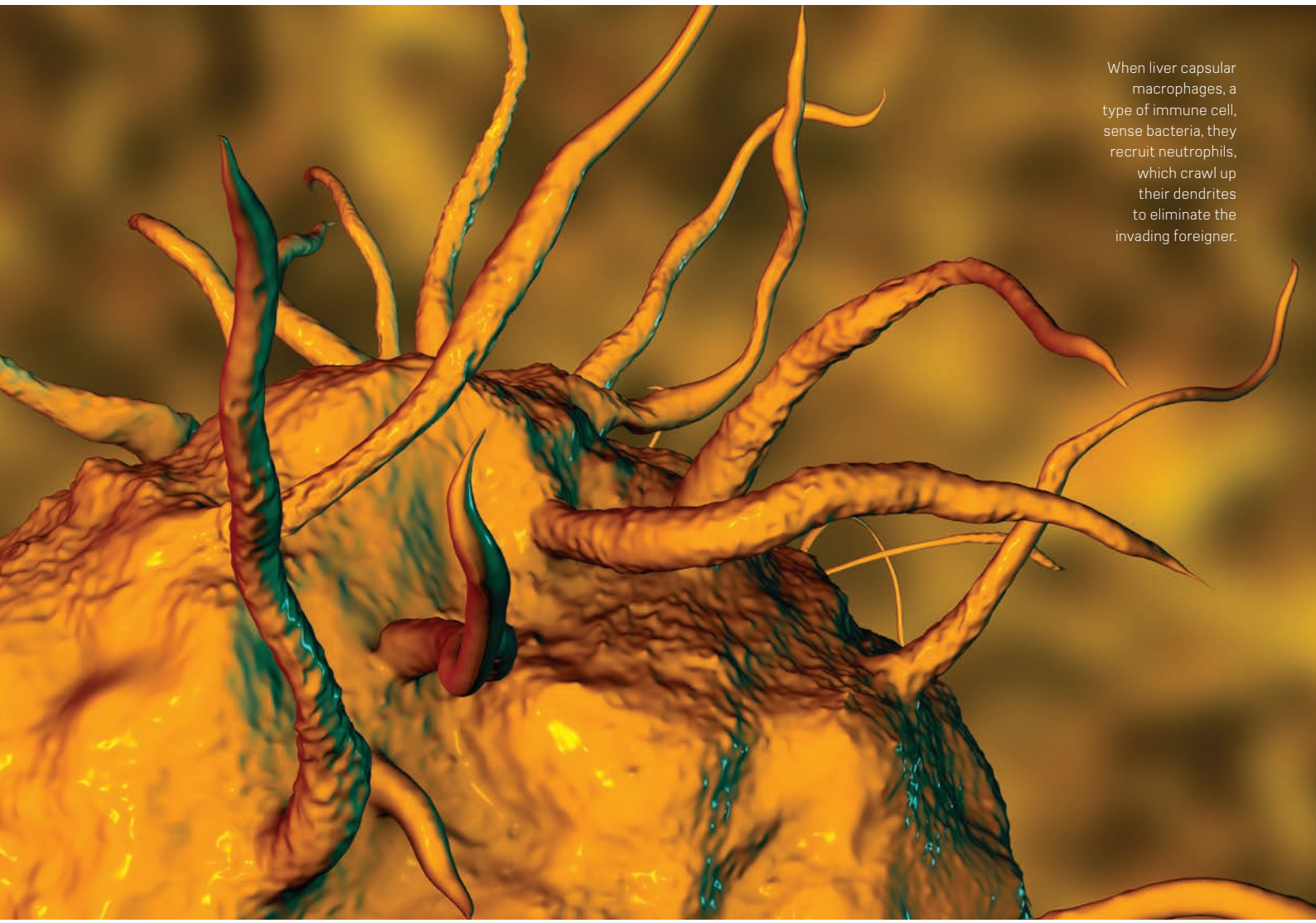
"The theory that chief cells can be activated to serve as the precursor to gastric cancer under damage conditions is controversial."

In addition, the team has implicated these cells in the genesis of gastric cancer — the fourth leading cause of cancer globally, according to the World Health Organization. They discovered that when these cells undergo a mutation, they become a major source of tumors at the base of the corpus glands (see image above).

"The theory that chief cells can be activated to serve as the precursor to gastric cancer under damage conditions is controversial," says Barker. "Indeed, a recent study concluded that other cells are the true precursors of cancer, not chief cells. In this study, we conclusively identified Lgr5^{+} cells as a source of spasmolytic polypeptide-expressing metaplasia (SPEM), helping to clarify the ongoing debate."

In the future, the team intends to explore exactly what causes the cells to become stem cells. "We are interested in better understanding the mechanism responsible for converting the normally quiescent, differentiated chief cells into proliferative, multipotent stem cells," says Barker. He notes that the same mechanism could be at play when the cells become cancerous. "We hope that these findings will contribute to translatable clinical outcomes," he adds.

1. Leushacke, M., Tan, S. H., Wong, A., Swathi, Y., Hajamohideen, A. *et al.* *Lgr5*-expressing chief cells drive epithelial regeneration and cancer in the oxyntic stomach. *Nature Cell Biology* **19**, 774–786 (2017).



When liver capsular macrophages, a type of immune cell, sense bacteria, they recruit neutrophils, which crawl up their dendrites to eliminate the invading foreigner.

IMMUNOLOGY

NEWLY IDENTIFIED CELLS IN LIVER PROTECTION ROLE

A subset of immune cells in the liver shields it from bacteria in the gut

A distinct population of immune cells found under the fibrous capsule that surrounds the liver has been identified by an international team. The cells, called liver capsular macrophages, protect the liver from bacteria in the peritoneal cavity that lies between the abdominal wall and the membrane covering abdominal organs.

Researchers had observed immune cells in the liver's capsule almost 30 years ago, but had not agreed on what type they were. Scientists from A*STAR's Singapore Immunology Network, in collaboration with colleagues in Australia, used a fluorescent imaging technique, called two-photon microscopy to investigate this further.

They found that their shape was different from Kupffer cells, an immune cell residing in the liver's tissue that protects it from bacteria circulating in the blood. The combination of molecules on their surface distinguished them from other types of immune cells, including Kupffer cells. When the team analyzed the cells' gene expression, they found it similar to — and so classified them as — macrophages, a type of white blood cell that engulfs

and destroys cellular debris, microbes, and cancer cells. The team also found that these 'liver capsular macrophages' were replenished by blood monocytes, unlike Kupffer cells that self-renew.

These newly identified cells have branches, or dendrites, that extend toward bacteria and other foreign substances. They are also involved in recruiting another type of immune cell, neutrophils, to the liver. Neutrophils crawl along the liver capsular macrophages' dendrites to reach the liver capsule and provide additional immune protection against infection.

"This study represents how two-photon microscopy can contribute to the discovery of new immune cell subsets," says A*STAR immunologist Lai Guan Ng, one of the study's co-authors. "Our study discovered that liver capsular macrophages serve as sensors for peritoneal bacteria and recruit neutrophils to fight infection. This study shows that the liver has two separate and non-overlapping niches occupied by distinct resident macrophage populations: Kupffer cells in the liver for mediating protection against systemic bacterial infection, and liver capsular macrophages for protection against intraperitoneal infection."

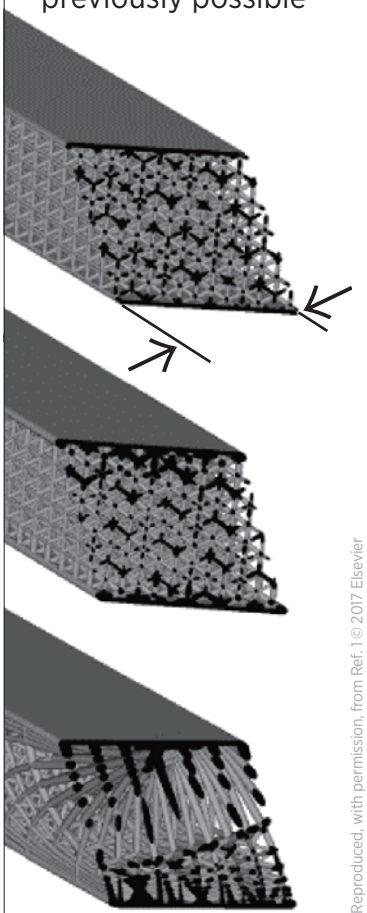
Ng says he is interested in further investigating how liver capsular macrophages respond to liver diseases, and how they are related to susceptibility to infection.

1. Sierro, F., Evrard, M., Rizzetto, S., Melino, M., Mitchell, A. J. *et al.* A liver capsular network of monocyte-derived macrophages restricts hepatic dissemination of intraperitoneal bacteria by neutrophil recruitment. *Immunity* **47,** 374–388 (2017).

MATERIALS

NATURE INSPIRES LIGHT, ROBUST LATTICE STRUCTURES

New technique for creating lightweight materials that are stiffer and stronger than previously possible



Lattice designs: (top) uniform benchmark, (middle) diameter graded and (bottom) spatially graded.

Advances in materials engineering have led to the development of lightweight structures that are both strong and stiff, which are transforming aerospace, automotive and medical industries. Conventional manufacturing techniques like casting and machining, however, limit the designs that can be fabricated as they are prone to inaccuracies and struggle to achieve the best results.

Now, scientists at A*STAR have invented a method that uses additive manufacturing techniques to create lightweight lattice structures with greatly improved stiffness and strength[1], paving the way for new materials for use in a wide range of applications including impact absorbent materials and sandwich structures.

The design and optimization of lightweight cellular and lattice structures is an emerging field made possible by advances in metal and polymer additive manufacturing, such as the ability to accurately print highly complex geometries.

By mimicking structures occurring in nature, Stephen Daynes and colleagues from A*STAR's Singapore Institute of Manufacturing Technology have developed a method to create these new robust materials in collaboration with researchers from the National University of Singapore.

"Lattice structures exceed the structural performance of conventional solid materials for use in lightweight sandwich cores, medical implants and a new class of lattice-type metamaterials with specific mechanical and thermal properties," explains Daynes. "Using a new biomimetic method, we were able to create cellular and lattice structures similar to those seen in bamboo and human bones."

The researchers determined the principal lines of stress, called isostatic lines, in the lattice using a method that combines topology and size optimization. This approach allows the size, shape and orientation of each cell in the structure to be tailored, significantly reducing the stress between neighboring lattice cells.

The researchers compared the performance of their graded lattice structure with a uniform lattice core and found that their optimized design had increased stiffness by 172 per cent and strength by 100 per cent.

"Our technique can create lightweight, functionally graded lattices that greatly improve the stiffness and strength of additively manufactured sandwich structures without increasing their mass," says Daynes. "These structures are particularly well suited to additive manufacturing processes since they are largely unconstrained by manufacturing complexity."

"We plan to apply the methodology to three dimensional stress fields, where employing spatially graded lattices can lead to novel and more weight efficient materials," says Daynes.

1. Daynes, S., Feih, S., Lu, W. F., & Wei, J. Optimisation of functionally graded lattice structures using isostatic lines. *Materials & Design* **127,** 215-23 (2017).

CELLS

GLARING LESSONS ON SKIN AGING

A protein can be used to measure the aging effect of sun exposure on skin cells

Levels of a protein called lamin B1 could be used to measure how sun exposure accelerates skin aging.

Lamins are components of the nuclear lamina, a fibrous layer on the nucleoplasmic side of the inner nuclear membrane, which is thought to provide a framework for the nuclear envelope and may also interact with chromatin. Researchers from A*STAR and their colleagues were studying the cells of patients with progeria, a genetic disorder that causes them to age and die prematurely, when they realized that these patients' senescent cells — those that have stopped growing and dividing — showed very low levels of lamin B1.

"Lamin B1 is expressed in every cell type of the human body, so if this protein disappears when cells age, it could be a useful new marker to identify aging cells," says Oliver Dreesen, from the A*STAR Institute of Medical Biology.

Researchers decided to use this knowledge to look at the effects of ultraviolet light on skin cell aging. To begin with, they exposed human skin cells in a petri dish to the same amount of ultraviolet B light that might be experienced by someone spending around 18 minutes in the Singaporean sun in the middle of the day.

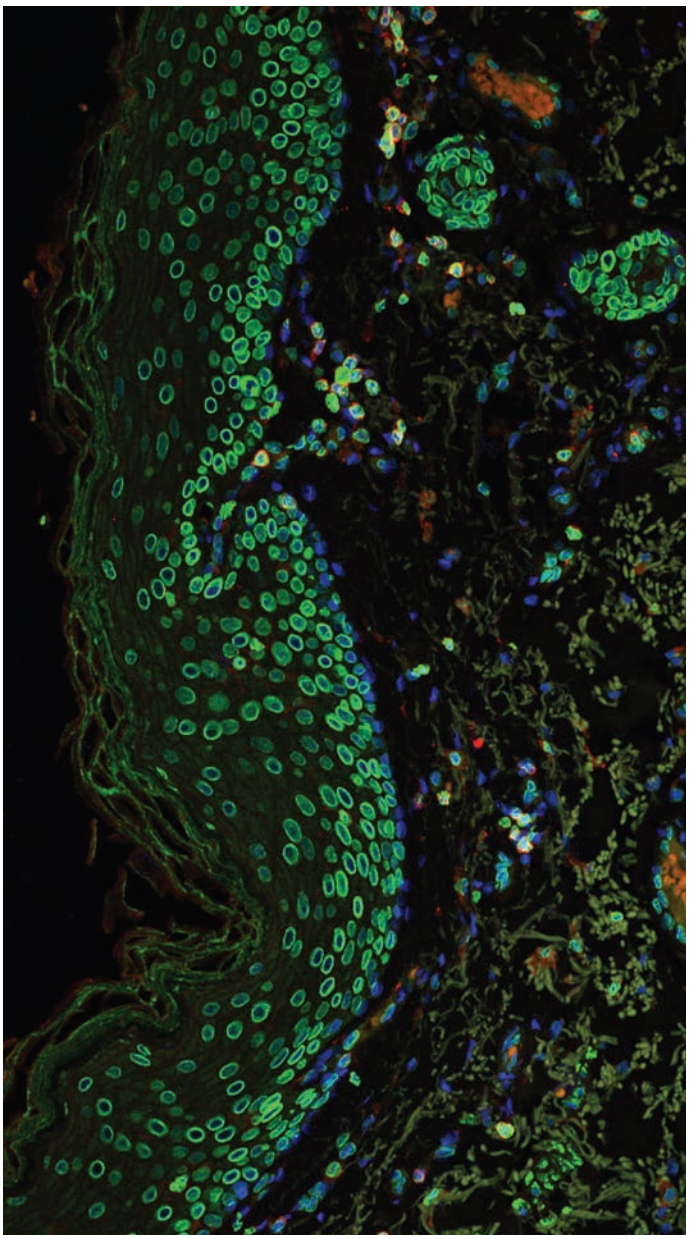
They found that in the UVB-exposed cells, the levels of lamin B1 dropped, while in cells that were protected with SPF50 sunscreen, the levels remained the same, suggesting that the UVB exposure was leading to cell senescence.

"Lamin B1 could be a useful new marker to identify aging cells."

The researchers also wanted to know how UVB exposure affected cells in different skin layers, so, in collaboration with Carlos Clavel, also from the Institute of Medical Biology, they looked at lamin B1 levels in mice whose skin was treated with UVB light daily for ten days.

"In our experiment, UVB light only affected the epidermis, the top layer of the skin, and it didn't seem to affect the dermis, which is the lower part," Dreesen says.

The researchers also noticed that once the mice's skin recovered from the UVB exposure and the skin regenerated, the levels of lamin B1 returned to normal.



Lamin B1 stained green in human skin cells.

As well as providing a way to measure sun damage of skin cells, lamin B1 levels could also help in the study of age-related skin problems such as actinic keratosis, which is caused by sun exposure. It could also help explore the impact of other environmental conditions, such as pollution, on skin aging.

"The long-term goal is to set up a toolkit that allows us to systematically look at how senescent cells contribute to pathological and cosmetic changes that occur in aging skin," Dreesen says.

1. Wang, A. S. Ong, P. F. Chojnowski, A. Clavel, C. Dreesen, O. Loss of lamin B1 is a biomarker to quantify cellular senescence in photoaged skin. *Scientific Reports* **7**, 15678 (2017).

MICROBIOME

GUT BACTERIA AFFECT THE BRAIN, EVEN IN THE WOMB

The effect of gut microbes on the mouse brain depends on both sex and stage of development

Immune cells in a mouse's brain react differently to changes in gut-dwelling bacteria depending on whether the mouse is male or female and whether it is a fetus or an adult, A*STAR researchers have found[1]. This discovery has potential implications for brain development and disorders.

Recently, scientists have found increasing evidence that the microbes in our digestive system, collectively known as the microbiome, can affect the brain via the metabolic products they produce.

Now, Florent Ginhoux from A*STAR's Singapore Immunology Network and an international team of researchers have found that this influence goes all the way back to the womb. By performing microscopy and genomic analyses, they have shown that, in mice, the absence of a microbiome in a mother affects the microglia, one of the major immune cell populations of the central nervous system, of her developing fetuses.

"This was really dramatic," says Ginhoux. "We never thought that a fetus, which is inside a mother, would be affected by her lack of a microbiome. We had previously believed that the brain of a fetus is a closed system, not subject to perturbations, and that it was only when the fetus goes out of the womb, that the environment can influence it."

This effect on microglia was much more pronounced in male fetuses than female ones. Conversely, in adult mice, females were more sensitive than males to the absence of the microbiome or its depletion through the use of antibiotics.

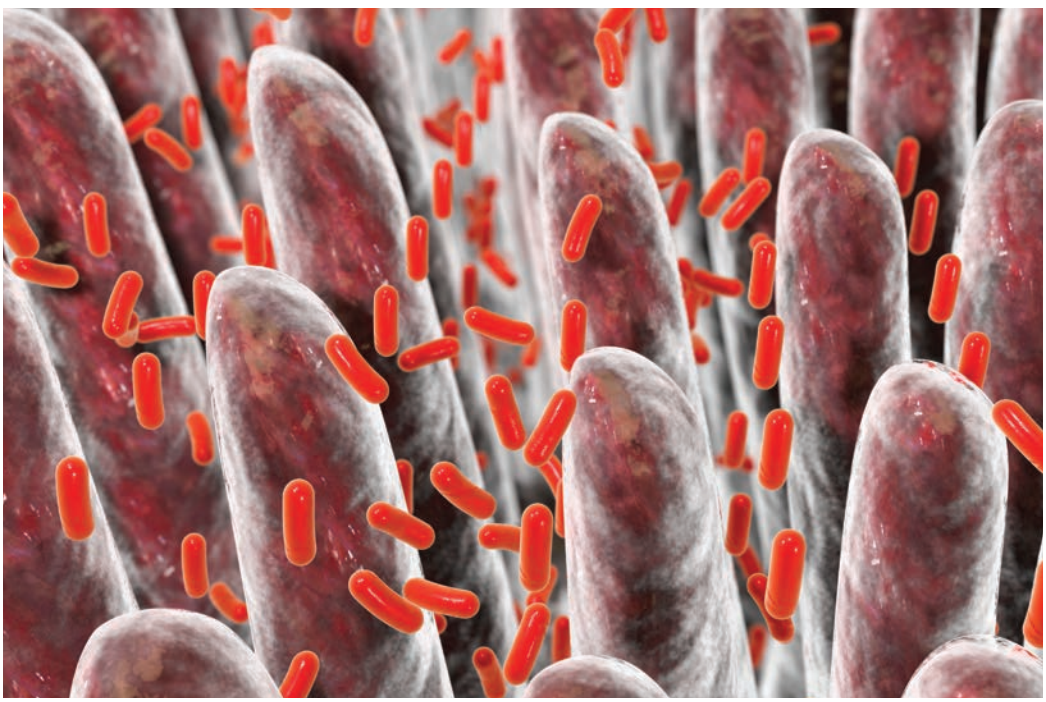
"This was really dramatic, we never thought that a fetus, which is inside a mother, would be affected by her lack of a microbiome."

The scientists are as yet unsure what is causing this difference. "At this stage, there is no really clear mechanism that explains why males are so sensitive to this change," says Ginhoux. "We think there may be a crucial window of development during which cells are very sensitive to changes in the environment, which is here exemplified by the absence of the microbiome."

But these differences are highly suggestive since men are known to be more susceptible than women to disorders such as autism, and the early onset of schizophrenia, both of which are thought to originate from dysregulated development in fetuses or early in life. On the other hand, women are more likely to suffer from depression and autoimmune diseases such as multiple sclerosis, which can develop in teenagers or adults.

The team intends to look at subtle changes to the microbiome that more accurately reflect those likely to occur in real life. They also plan to investigate how different treatments, such as antibiotics or changes to diet, can modulate microglia identity and function during pregnancy and adulthood. ■

1. Thion, M. S., Low, D., Silvin, A., Chen, J., Grisel, P. *et al.* Microbiome influences prenatal and adult microglia in a sex-specific manner. *Cell* **172**, 500–516 (2018).



A*STAR researchers have shown that the bacteria in the gut can affect the microglia in a mouse's brain.

A promising new type of laser spectroscopy developed at A*STAR exploits the strange phenomenon of quantum entanglement to make measurements of extremely fast atomic processes, even with low-cost lasers.

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LASER SPECTROSCOPY

MEASUREMENTS THROUGH ENTANGLEMENT

The extremely short relaxation times of atoms can be measured by using quantum-entangled photons

A new approach to laser spectroscopy based on quantum physics makes it cheaper and easier to observe ultrafast processes inside materials, say A*STAR researchers.

In general, spectroscopy involves shining a laser on a sample and observing the effect of the laser light to work out what is going on inside the sample. However, some processes in materials happen so quickly that very expensive lasers and sophisticated detector systems are needed to observe them. Powerful lasers can also damage samples.

Now, Dmitry Kalashnikov and colleagues at the A*STAR Data Storage Institute, Moscow State University, and the Russian Academy of Sciences, have demonstrated a method that exploits the strange phenomenon of quantum entanglement to measure extremely short-lived processes inside materials without expensive equipment.

"By measuring the time over which a sample responds to electromagnetic fields, we can learn about the connections between components in the substance," says Kalashnikov. "In particular, the so-called dephasing time describes how long different atoms or molecules of the substance respond as a coherent ensemble. For many substances this time lies within the femtosecond time scale (10^{-15} seconds) and is not easy to measure."

"In the future we will use this technology to study chemical and biological samples, with even shorter dephasing times."

Instead of investing in costly femtosecond lasers, Kalashnikov's team used a continuous-wave laser configured for Hong-Ou-Mandel (HOM) interference. This interference effect describes what happens when quantum-entangled photons, which are effectively indistinguishable and dependent on one another, hit a semi-transparent mirror called a beamsplitter.

"When HOM interference was discovered in the 1980s it became the true manifestation of quantum mechanics," says Kalashnikov. "When two entangled photons hit a beamsplitter from different sides they always exit together from one or another beamsplitter outputs; they never exit separately."

After assembling and aligning their HOM setup, the team could measure the level of entanglement in a beam by placing two photodetectors at the beamsplitter outputs. If both detectors clicked at the same time, then there was no entanglement, but if only one detector clicked, the photons were entangled.

"When a sample is placed into the path of one of the photons, it causes the photon to become less identical to its twin," explains Kalashnikov. "Therefore, the distribution of clicks changes, and we can estimate the dephasing time." Through this method, the researchers measured dephasing times as short as 100 femtoseconds, in neodymium-doped crystals and silicon nanodisks.

"In the future we will use this technology to study chemical and biological samples, with even shorter dephasing times," says Kalashnikov. "Our work could also be important for developing quantum computer memory."

1. Kalashnikov, D., Melik-Gaykazyan, E. V., Kalachev, A. A., Yu, Y. F., Kuznetsov, A. I. & Krivitsky, L. A. Quantum interference in the presence of a resonant medium. *Scientific Reports* **7**, 11444 (2017).

ConsensusDriver is a new computational method that integrates the results from multiple analyses to improve prediction of cancer drivers.

MUTATIONS

PINPOINTING CANCER DRIVERS

A consensus-based computational approach improves prediction of gene mutations linked to cancer

Combining different analyses has enabled A*STAR researchers to better identify gene mutations, which could lead to improved personalized treatments.

A major issue in cancer genomics has been distinguishing between harmless mutations and 'cancer driver' mutations that give rise to or exacerbate cancer. Many computational methods have been developed to tackle this challenge, spurring Niranjan Nagarajan of A*STAR's Genome Institute of Singapore to compare the performance of different approaches. "We've been excited about the concept that you can integrate diverse molecular profiling data for tumors and pinpoint the key alterations among thousands," says Nagarajan.

Using data from 3,400 tumors spanning 15 cancer types, the team assessed 18 prediction methods which represented five different ways of approaching the problem. The performance of the five approaches varied, with differing results based on the implicit tradeoffs in each. The methods were generally robust to messy data and weren't misled by fake mutations introduced by the researchers. However, they also failed to predict any drivers in 10 per cent of the patients.

"We've been excited about the concept that you can pinpoint the key alteration among thousands."

Many of the methods predicted different drivers, which led the team to develop a consensus approach that integrated results from the different methods, known as ConsensusDriver, which

consistently outperformed the individual methods. In addition, ConsensusDriver can determine whether unfamiliar mutations are cancer drivers.

"Most groups approach the question from a basic cancer biology perspective of finding key cancer genes. Our interest has always been to go beyond this and see how far we can move towards personalized medicine," says Nagarajan. "If methods like ConsensusDriver were used for precision oncology, it would double the number of patients for whom we would be able to recommend treatments," though he adds that precision oncology remains a distant goal requiring extensive clinical validation.

The team has made their research tools freely available online, providing the community with a convenient toolbox to run the 18 predictions methods as well as ConsensusDriver. With improved predictions from consensus-based methods, researchers may be able to find new drug targets by identifying genes which are frequently drivers across different cancer types.

Meanwhile, Nagarajan's team is developing sophisticated machine-learning techniques to predict drug response in individual patients, as well as validating the predictions using cell cultures and exploring the value of single-cell approaches in predicting cancer drivers and treatments.

1. Bertrand, D., Drissler, S., Chia, B. K., Koh, J. Y., Li, C. *et al.* ConsensusDriver improves upon individual algorithms for predicting driver alterations in different cancer types and individual patients. *Cancer Research* **78**, 290-301 (2018).

CHEMISTRY

BUILDING A BETTER ION CHANNEL

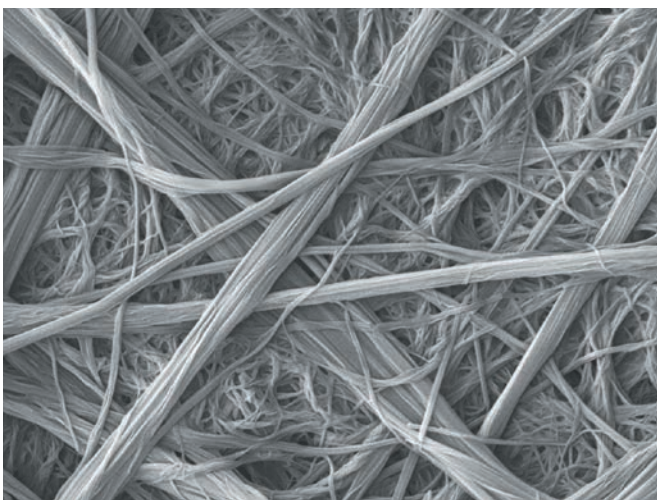
A synthetic channel with a strong preference for potassium ions offers rapid transport through an artificial membrane

Artificial ion channels developed by A*STAR researchers could pave the way for new kinds of antibacterial agents and biomedical sensors[1].

Ion channels are biochemical superhighways that enable ions of metals such as potassium and sodium to zoom in and out of cells. Crucially, the channels are typically very selective, allowing only one type of ion through and barring others. For example, the naturally-occurring KcsA potassium ion channel can transport 100 million ions per second, and only lets one sodium ion through for every 10,000 potassium ions.

"But protein-based ion channels are costly and difficult to manipulate," says Huaqiang Zeng at the A*STAR Institute of Bioengineering and Nanotechnology. "Synthetic versions are therefore being developed to mimic and eventually surpass the functions exhibited by naturally-occurring protein channels." However, it has been difficult to develop artificial channels that have a strong selectivity for potassium over sodium ions.

Zeng and colleagues have now developed ion channels that offer rapid potassium ion transport with a selectivity that is among the highest reported for any artificial ion channel. The channel is formed from a series of identical molecules that stack on one another. Each molecule contains three components. At one end is a crown ether, a large ring of carbon and oxygen atoms; in the middle is an amino acid, which contains chemical groups that allow the molecules to stack in a specific pattern; and at the other end is a long, carbon-based 'tail'. These molecules can self-assemble so that the crown ether rings line up to form a tube, which acts as an ion channel.



This scanning electron microscope image shows that the artificial ion channels self-assemble into fibers.

The researchers created a library of molecules using various amino acids, different lengths of alkyl chains, and crown ethers that contained five or six oxygen atoms. Then they formed membranes from the stacked channels, and tested their ion transport properties.

The most selective channel they studied contained a crown ether with five oxygen atoms, a phenylalanine amino acid, and an alkyl chain containing eight carbon atoms. This could transport 30 million ions per second, and was about ten times more selective for potassium ion than sodium ions. This offers a much better performance than previous artificial potassium ion channels based on crown ethers or other molecular scaffolds.

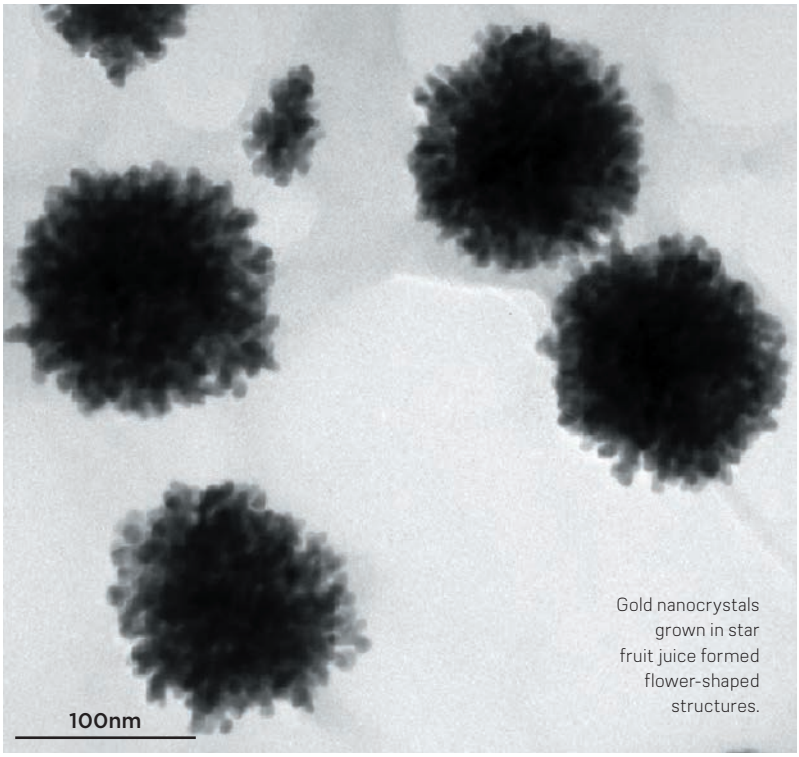
The molecules' three components can be readily altered to fine-tune the channels' properties, so Zeng is optimistic that his team can improve their performance further. They hope to test their optimized systems in medical applications, such as antibacterial agents or hair growth promoters.

1. Ren, C., Shen, J. & Zeng, H. Combinatorial evolution of fast-conducting highly selective K+-channels via modularly tunable directional assembly of crown ethers. *Journal of the American Chemical Society* **139**, 12338–12341 (2017).

CANCER TREATMENT

PHOTOTHERMAL THERAPY BLOOMS

Flower-shaped gold nanocrystals are too hot to handle for tumor cells



Gold nanocrystals grown in star fruit juice formed flower-shaped structures.

Gold nanoflowers grown in starfruit juice are promising agents for photothermal cancer therapy. When injected into a tumor and irradiated with near-infrared laser light, the nanoflowers heat up and kill the cancer cells around them.

Although photothermal therapy is an established idea, the agents developed so far have had drawbacks, explains Enyi Ye from the A*STAR Institute of Materials Research and Engineering, who co-led the current work. The challenge is to achieve high efficacy, stability and biocompatibility in the same nanocrystal, which all relate to the way the nanocrystals are grown.

A nanomaterial's shape dictates its light capturing ability. During the synthesis of nanocrystals, when gold ions are mixed with a reducing agent to precipitate crystal formation, chemicals called capping agents can be added to direct the crystals' growth.

"Capping agents will attach to different facets of the nanocrystal nuclei," says Ye. "They regulate the crystal's growth and stabilize the final nanocrystals." However, conventional capping agents are not biocompatible, so they need to be eliminated before the nanocrystals are used for therapy. Removal, however, can be difficult and may damage the nanocrystal's shape, impairing its light absorption.

Ye and his colleagues tried an alternative 'green' approach, using a natural material they hoped would contain the biological equivalent of capping agents. This would negate the need to remove the compounds before nanocrystal injection into the patient.

"I selected starfruit juice because it is available all the time in tropical areas, and because starfruit is rich in vitamin C, which serves as the reducing agent, and polyphenolic antioxidants," says Ye. As the team had predicted, the vitamin C initiated and directed nanocrystal growth, and the polyphenolic compounds acted as capping agents, coating the flower-shaped nanocrystals with a stabilizing, biocompatible surface.

"We will continue to develop green methods to prepare multifunctional nanocrystals."

The gold nanoflowers showed strong near-infrared light absorption, and were non-toxic to cells. When 808 nanometer near-infrared laser light was shone on cells mixed with nanoflowers, 30 seconds of illumination killed every cell within the laser spot. In tests with mice, photothermally-treated animals' tumors gradually disappeared — whereas in animals given either laser irradiation only, or nanoflower injection alone, the tumor continued its rapid growth.

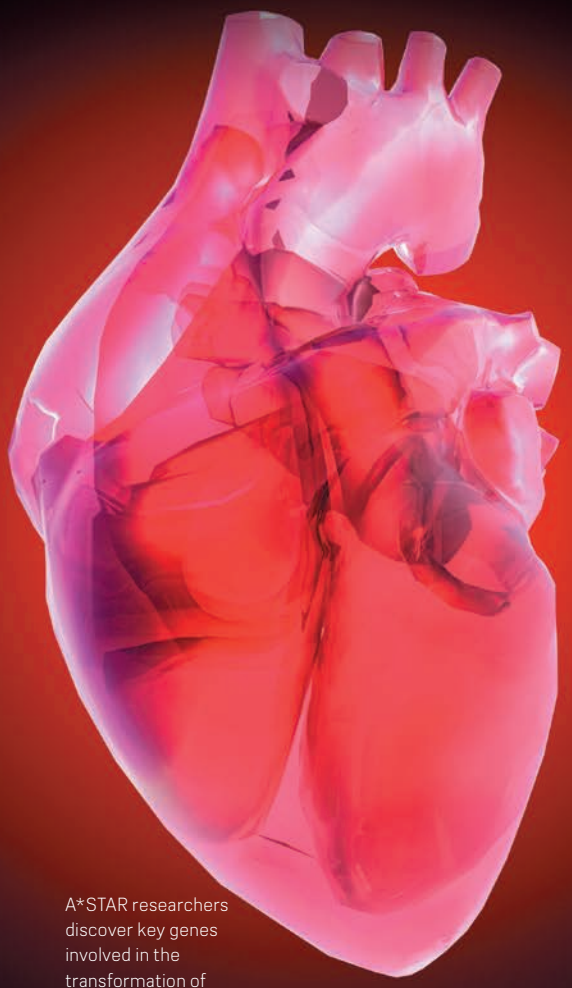
The team now plans to take the research in two directions, says Ye. "We will continue to develop green methods to prepare multifunctional nanocrystals. We would also like to further exploit the practical applications of the gold nanoflowers in areas such as wound healing and bacterial infection control," he says. ■

1. Yang, D.-P., Liu, X., Teng, C. P., Owh, C., Win, K. Y. *et al.* Unexpected formation of gold nanoflowers by a green synthesis method as agents for a safe and effective photothermal therapy. *Nanoscale* **9,** 15753–15759 (2017).

STEM CELLS

AIMING FOR THE HEART

Genetic switch activates transformation of stem cells into heart muscle cells



A*STAR researchers discover key genes involved in the transformation of embryonic stem cells into heart muscle cells.

The discovery of a genetic 'switch' that triggers stem cells to turn into heart cells is a major step in finding treatment for damaged hearts.

Researchers from A*STAR and their colleagues in India have been investigating the molecular and genetic processes by which human embryonic stem cells differentiate into the body's many types of cells — in particular, cardiomyocytes, or heart muscle cells.

"The effort is underway globally to find ways to differentiate these stem cells into beating functional heart muscle cells so that they can be used for cell-based therapies to treat structural abnormalities," says Prabha Sampath, from the A*STAR Institute of Medical Biology.

In this study, researchers used a method called transcriptome profiling, which examines the activity of a wide range of genes in a cell, to determine which are more active when human embryonic stem cells differentiate into heart cells.

Two particular genes, *NR2F2* and *EZH2*, show increased expression during the differentiation process. The proteins that these genes code for suppress the activity of another gene called *OCT-4*, which is responsible for keeping stem cells in their undifferentiated state.

"*NR2F2* recruits *EZH2* to gene *OCT-4*, and potentially suppresses its expression, propelling the cells towards differentiation," Sampath says. "With the down-regulation of this gene, the cells start differentiating into cardiomyocytes."

The NR2F2 protein has not previously been linked to cardiac differentiation, but mutations in the *NR2F2* gene have been known to cause the development of a type of congenital heart defect. "While it's unlikely to be the only mechanism involved in the differentiation of stem cells into cardiac cells, it's an important step in the process", says Mohsin Bin Bashir, also from the Institute of Medical Biology. "If we can understand how a stem cell becomes a cardiomyocyte, we have more chance of creating these cells in a controlled fashion."

That process could also be used in reprogramming adult stem cells, where normal cells are taken from a patient, reprogrammed back into their stem cell state, then differentiated into whatever cell type is needed for treatment.

"You can convert them into cardiomyocytes and put them back into the patient, and one of the advantages with that [process is that] there would be no immune rejection because these cells come from the patient themselves," Bashir says.

1. Pursani, V., Pethe, P., Bashir, M., Sampath, P., Tanavde, V., *et al.* Genetic and epigenetic profiling reveals EZH2-mediated down regulation of OCT-4 involves NR2F2 during cardiac differentiation of human embryonic stem cells. *Scientific Reports* **7**, 13051 (2017).

CELL BIOLOGY

PUTTING MUSCLE NUCLEI IN THEIR PLACE

Microtubules anchored to proteins in the nuclear membrane position muscle cell nuclei

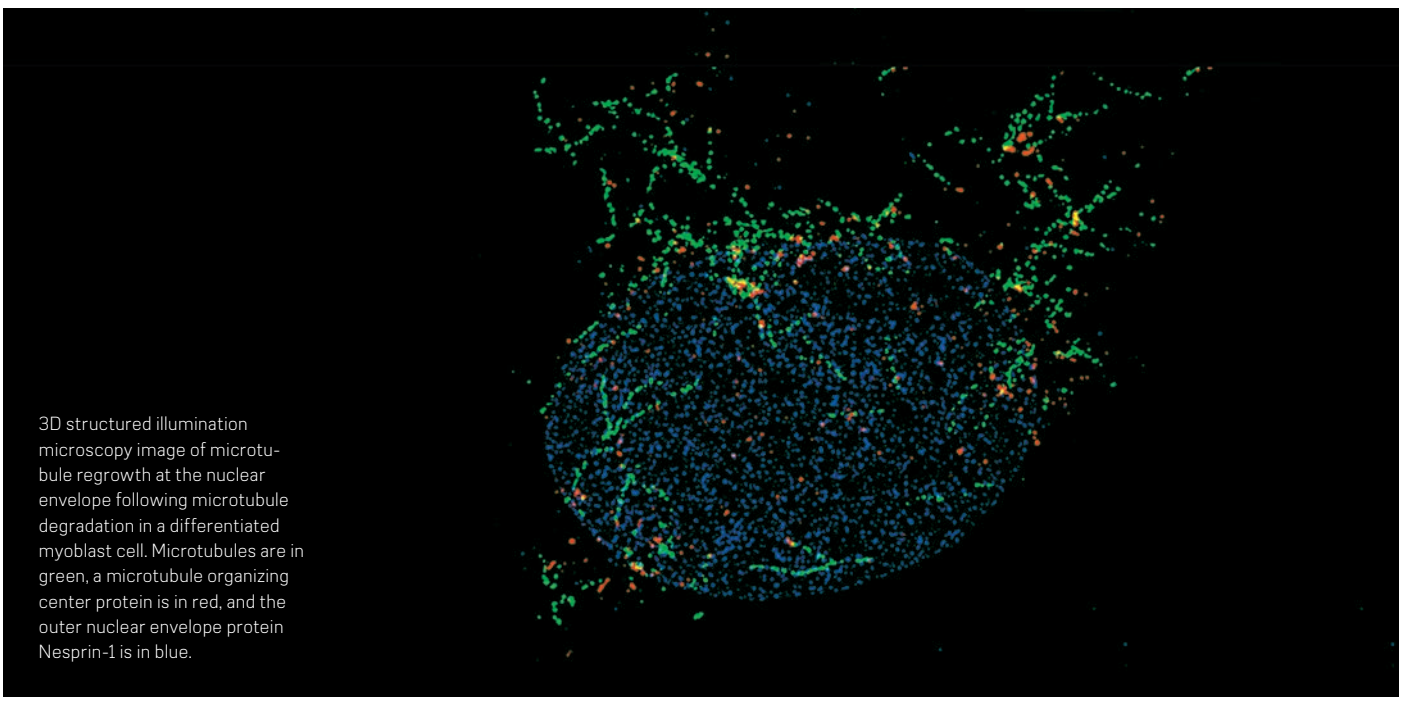
Scientists at A*STAR have revealed how microtubules, part of a cell's 'skeleton', position the multiple nuclei in muscle cells. Malfunctions in this crucial developmental process are linked to muscular dystrophies, a group of debilitating diseases that cause progressive weakness and loss of muscle mass.

Brian Burke of A*STAR's Institute of Medical Biology was drawn to the topic more by accident than by design. His research focuses on the genes that encode proteins that are active in the 'nuclear envelope', a membrane around the cell nucleus. In muscle cells, the nuclear envelope contains proteins usually associated with the centrosome, a cytoplasmic structure which organizes the microtubules, but for years this remained a niche subject. "It was just an odd phenomenon that no-one working in the centrosome field understood the significance of, and no-one outside the field was aware of," he says.

Yin Loon Lee, who studied the centrosome during his PhD before joining Burke's lab, brought the topic to his attention. Burke's recent research had revealed that protein pairs in the nuclear envelope, known as LINC complexes, play a role in positioning the nucleus in many cells. "It seemed like a good bet that these [complexes] might be involved in anchoring centrosomal proteins at the muscle cell nucleus," explains Burke.

"It seemed like a good bet that these [complexes] might be involved in anchoring centrosomal proteins at the muscle cell nucleus."

To test this idea, Lee and Burke fished out proteins that interact with the LINC protein Nesprin-1 in early- and late-stage muscle cells. Comparing



3D structured illumination microscopy image of microtubule regrowth at the nuclear envelope following microtubule degradation in a differentiated myoblast cell. Microtubules are in green, a microtubule organizing center protein is in red, and the outer nuclear envelope protein Nesprin-1 is in blue.

the two lists revealed 446 proteins that interact with Nesprin-1 specifically in late-stage muscle cells, including four centrosomal proteins. Meanwhile, researchers in France had performed an RNAi screen to identify genes involved in microtubule nucleation at the nuclear envelope. "The only candidate they came up with was Nesprin-1," says Burke. The teams decided to collaborate to solve the mystery.

Lee engineered cells lacking either Nesprin-1 or its LINC partners. In these cells, three of the four centrosomal proteins remained in the cytoplasm rather than accumulating at the nuclear envelope. The same mislocalization was apparent in cells from patients with a congenital muscular dystrophy caused by a Nesprin-1 mutation. By depleting each of the three centrosomal proteins individually, the team discovered which protein was required for microtubule formation at the nuclear envelope.

In muscle cells with a missing or defective copy of Nesprin-1, the nuclei clumped together rather than spreading out. Understanding how Nesprin-1 and centrosomal proteins position the nuclei of muscle cells may help clarify the development of muscular dystrophies.

1. Gimpel, P., Lee, Y. L., Sobota, R. M., Calvi, A., Koullourou, V., *et al.* Nesprin-1a-dependent microtubule nucleation form the nuclear envelope via Akap450 is necessary for nuclear positioning in muscle cells. *Current Biology* **27**, 2999–3009 (2017).

QUANTUM MATTER

A SURFACE THAT MAKES LIGHT WORK

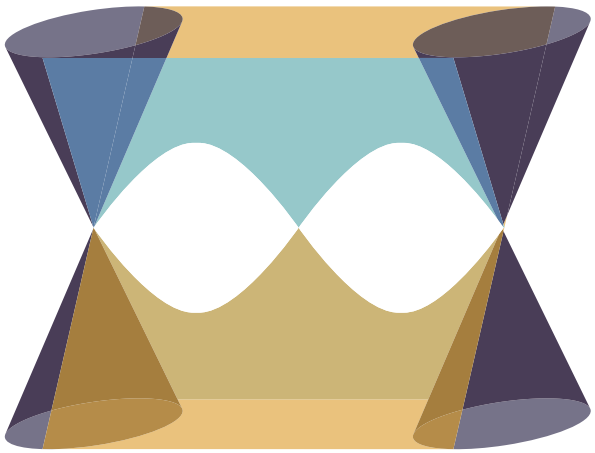
The quantum states on the surface of conducting materials can strongly interact with light

An exotic state of matter that is dazzling scientists with its electrical properties, can also exhibit unusual optical properties, as shown in a theoretical study by researchers at A*STAR.

Atomically thin materials, such as graphene, derive some of their properties from the fact that electrons are confined to traveling in just two-dimensions. Similar phenomena are also seen in some three-dimensional materials, in which electrons confined to the surface behave very differently from those within the bulk — for example, topological insulators, whose surface electrons conduct electricity even though their bulk electrons do not. Recently, another exciting class of materials has been identified: the topological semimetal.

The difference in insulator and conductor electrical properties is down to the bandgap: a gap between the ranges, or bands, of energy that an electron traveling through the material can assume. In an insulator, the lower band is full of electrons and the bandgap is too large to enable a current to flow. In a semimetal, the lower band is also full but the lower and upper bands touch at some points, enabling the flow of a small current.

This lack of a full bandgap means that topological semimetals should theoretically exhibit very different properties from those of the more conventional topological insulators.



The upper and lower electron bands in a semimetal touch at places known as Dirac points.

To prove this, Li-kun Shi and Justin Song from the A*STAR Institute of High Performance Computing used an 'effective Hamiltonian' approximation to show that the two-dimensional surface states in semimetals, known as Fermi arcs, possess a light–matter interaction much stronger than that found in other gapless two-dimensional systems, such as graphene.

"Typically, the bulk dominates material absorption," explains Song. "But we show that Dirac semimetals are unusual in that they possess a very optically active surface due to these peculiar Fermi arc states."

Shi and Song analyzed a proto-typical semimetal with a symmetric band structure where the electronic bands touch at two places, known as Dirac points, and predicted the strength with which incident radiation induces electron transitions from the lower band to the upper one. They found that surface absorption depends heavily on the polarization of light, being 100 to 1,000 times stronger when light is polarized perpendicular — rather than parallel — to the crystal's rotational axis. This strong anisotropy offers a way of optically investigating and probing the topological surfaces states of Dirac semimetals.

"Our goal is to identify more unconventional optics that arise due to Fermi arcs," says Song. "Topological semimetals could host unusual opto-electronic behavior that goes beyond conventional materials."

1. Shi, L.-K. & Song, J. C. W. Large optical conductivity of Dirac semimetal Fermi arc surface states. *Physical Review B* **96**, 081410 (2017).

You don't have to be an expert to gain insights from big data, thanks to a new web-based, machine-learning tool.

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MACHINE LEARNING

DATA ANALYSIS TOOL EMPOWERS THE PEOPLE

Despairing of data sets? Here's an automatic, cloud-based system designed for data rookies

Data production doubles each year, but data scientists, who wrangle insights from reams of data, are in short supply. To bridge this gap, a team at A*STAR has developed a fully automatic, web-based system that puts the power of big data analysis in the hands of laypeople[1].

Uncovering patterns and relationships hidden in vast data sets requires a machine learning pipeline or 'workflow' — a string of algorithms and processes called operators. But not every workflow is appropriate for every situation. So how does the non-expert know which to use? To help, Theint Theint Aye, from the A*STAR Institute of High Performance Computing and her colleagues have produced an analytics system (called the Layman Analytics System, or 'LAS') for the novice.

Say you have a data set to analyse. The first part of the LAS — the workflow recommender — compares your data set's metadata to that of existing data sets in a repository. It then selects the best-performing workflows based on those similar repository data sets and passes them to the second part: the workflow optimizer.

"Non-experts usually take days to generate a good workflow, in LAS, the average time was just over 3 hours."

Here, 'genetic programming' refines the workflow. Operators are randomly replaced, analogous to random genetic mutations in DNA. Mutated workflows are then crossed with each other, which involves swapping pairs of operators between them.

The process then repeats — 'fittest' workflows are selected, mutated and crossed — for a predefined number of generations (based on empirical experience). The result: an automatically generated tailor-made workflow.

The system is web-based using cloud infrastructure, so there is no need to install special software or use dedicated computing power.

To evaluate whether the LAS generated appropriate workflows, Aye's team tested it on 114 data sets from the University of California's Irvine Machine Learning Repository and benchmarked against OpenML, an open-source, online machine learning platform.

For 87 data sets (about 76 per cent of the total), LAS-produced workflow accuracy was above the 50th percentile of OpenML's performance. This figure could improve over time too, Aye says. Users can plug their data sets and workflows back into the repository, providing a richer stock from which the workflow recommender can later draw.

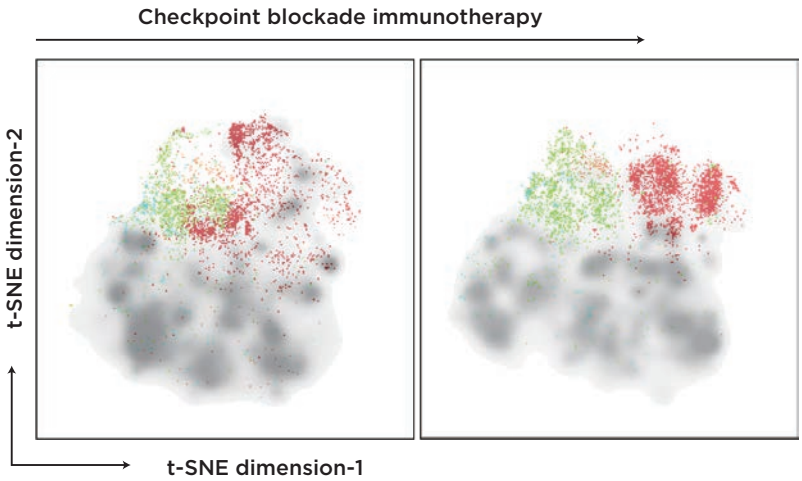
Non-experts usually take days to generate a good workflow, however, in LAS, the average time to produce a workflow in 15 generations was just over 3 hours. In the future, implementing a faster search technique, or heuristic, could further cut processing time. "Obviously, we would want to run it as efficiently as possible and also have good accuracy values," Aye says, adding that a graphics processing unit might also boost the LAS's speed.

1. Aye, T. T., Lee, G. K. K., Su, Y., Zhang, T., Lee, C. *et al.* Layman analytics system: a cloud-enabled system for data analytics workflow recommendation. *IEEE Transactions on Automation Science and Engineering* **14**, 160–170 (2017).

CANCER TREATMENTS

UNDERSTANDING INDIVIDUAL IMMUNE RESPONSES

A technique that identifies and characterizes different immune cell groups in individual patients could revolutionize cancer treatments



A plot of all the different possible phenotypes of T cells without (left) vs. with (right) immune checkpoint blockade immunotherapy. The colored dots represent T cells that are tumor neoantigen specific and colored by which tissue they were derived from: (red) tumor, (green) dLN (draining lymphnode - the lymph-node closest to the tumor - peripheral immune tissue), (blue) ndLN (non-draining lymphnode - peripheral immune tissue) and (orange) spleen (peripheral immune tissue).

A pioneering technique developed by A*STAR researchers can identify and profile specific groups of immune cells that target cancer cells in individual patients. This approach could open the door to personalized cancer treatments.

Cancer cells produce many antigens that trigger immune responses from the body. One group of immune cells called $CD8^+$ cytotoxic T cells recognize and bind to antigens attached to the surface of cancerous cells. Often, mutations unique to individual patients create mutant antigens, or 'neoantigens', which lead to a tumor-specific immune response. Scientists hope to harness these responses to create personalized cancer treatments, and possibly neoantigen-based cancer vaccines.

Now, Evan Newell and co-workers at A*STAR's Singapore Immunology Network, along with scientists at Washington University in St Louis, USA, have developed an approach that can efficiently identify and characterize neoantigen-specific $CD8^+$ T cells in mice. Their technique may help determine whether a tumor will respond to specific immunotherapies.

"Our technique could also identify relevant antigens to develop personalized cancer vaccines."

"In theory, we might predict how someone will respond to certain immunotherapies by looking at the characteristics of the immune cells that are circulating in the patient's blood," explains Newell. "However, this is challenging, because there are huge numbers of circulating cells and only a tiny proportion of them are neoantigen-specific $CD8^+$ T cells."

Newell's team hypothesized that patients who were receptive to effective immunotherapy would have tumor-specific T cells with specific characteristics. The team's technique combines mass cytometry with heavy metal staining to simultaneously screen and profile the multiplicity of such cells in blood and tumor samples.

"Other approaches require that you stimulate the T cells with their specific antigens to identify them," says Newell. "Instead, we used isolated proteins from the antigens called peptide-MHCs (major histocompatibility complex), which the T cell receptors bind to. In addition to formulating the proteins carefully to promote stable interactions, we labelled them with heavy metals so that they were detectable in high-throughput mass cytometry."

In experiments on mice with sarcoma tumors that responded well to particular immunotherapies, the team analyzed T cells targeting 81 possible tumor antigens. They identified T cells specifically associated with two neoantigens in spleens, tumors and lymph nodes. Depending on the type of immunotherapy used, the neoantigen-specific T cells displayed different phenotypes during treatment, whereas other T cell populations were mostly unaffected.

"It's possible we could identify tumor-specific T cells that could either be expanded and re-infused into patients to boost their immune response, or used as biomarkers to predict responses to any given immunotherapy," says Newell. "Our technique could also identify relevant tumor antigens to develop personalized cancer vaccines." ■

1. Fehlings, M., Simoni, Y., Penny, H. L., Becht, E., Loh, C. Y. *et al.* Checkpoint blockade therapy reshapes the high-dimensional phenotypic heterogeneity of murine intratumoural neoantigen-specific $CD8^+$ T cells. *Nature Communications* **8**, 562 (2017).

PRINTING

TINY DROPLETS FOR BIG COLOR IMPACT

High-resolution full color images can be formed using silicon-nanostructure pixels

Color prints produced on contemporary printers have a resolution of a few thousand dots per inch (dpi), but an alternative strategy that harnesses the power of nanotechnology can improve this resolution by an order of magnitude.

Depositing ink droplets on a surface to create color pictures is a centuries-old technology. A*STAR researchers are now testing a new method, which uses an array of nanostructures that reflect light of the desired color. As these structures, or pixels, are much smaller than ink drops, a resolution of up to 100,000 dpi could, in principle, be achieved.

Nanostructures influence light through so-called optical resonances. In the case of metals, these optical resonances are due to the excitation of plasmons — light strongly couples to spatially-confined electrons on the surface, and is either absorbed or reflected depending on its wavelength. The peak reflectivity wavelength, and thus the apparent color of the pixel, is tunable by changing the dimensions of the nanostructures.

Plasmonic materials are often noble metals, such as gold and silver, or aluminum. But these materials are constrained by price, spectrum coverage, or the low purity of the color they reflect.

Ramón Paniagua-Domínguez from the A*STAR Data Storage Institute and co-workers investigate semiconductor nanostructures made of silicon. They measure the optical properties of an array of discs with diameters ranging from 50 to 250 nanometers under illumination conditions suited for a practical implementation.

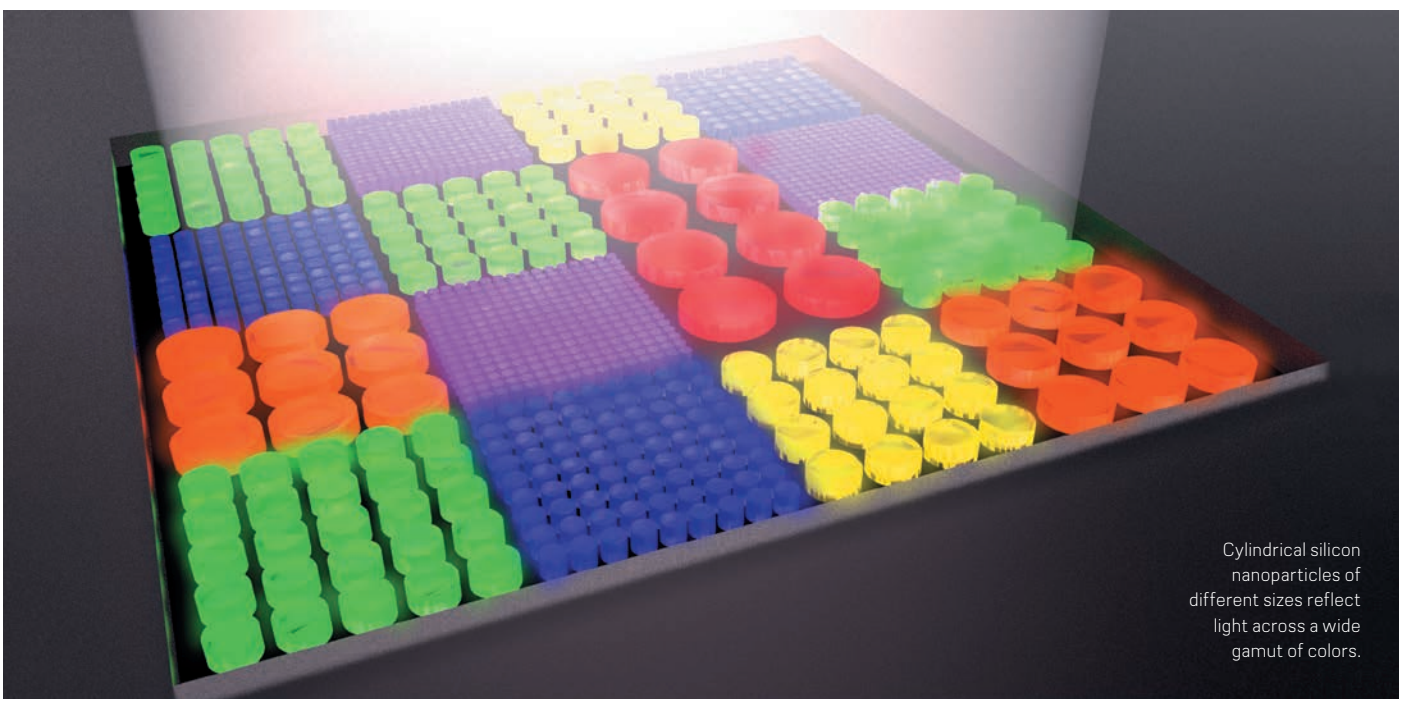
"We compared the quality of colors generated by silicon particles with those from silver and aluminum plasmonic particles," says Paniagua-Domínguez. "We showed that the colors obtained are of far better quality in terms of hue, gamut and intensity."

The improvement is because the colors from silicon do not stem from plasmonic resonances as they do in the noble metals, but rather from geometrical resonances that originate from bound electrons. Consequently, silicon is less affected by absorption losses than silver or aluminum and so can produce a sharper reflectance spectrum, meaning a better color purity.

The technology for fabricating silicon nanostructures is well developed due to its broad adoption in the manufacture of electronics. Thanks to this they were able to reproduce masterpieces such as Edvard Munch's *The Scream* in an area smaller than one square-millimeter.

"Our focus is on expanding the color gamut to go beyond the standard widely adopted in the display industry," says Paniagua-Domínguez. "We will also explore mechanisms to actively control the resonances, and therefore the color, of the particles, to bring this technology closer to application in ultra-high-definition displays."

1. Flauraud, V., Reyes, M., Paniagua-Domínguez, R., Kuznetsov, A. I. & Brugger, J. Silicon nanostructures for bright field full color prints. *ACS Photonics* **4**, 1913–1919 (2017).



Cylindrical silicon nanoparticles of different sizes reflect light across a wide gamut of colors.

THERAPEUTICS

ENGINEERING A SAFER ANTIBODY DRUG

By taking advantage of the body's natural diversity, antibody drugs can be made less toxic by having a more localized effect

An innovative approach to protein engineering could help make antibody-based drug therapies less toxic and more effective.

Working with two antibody drugs taken by thousands of women with breast cancer each year, A*STAR researchers have shown the possibility of transforming these agents — which normally circulate in the bloodstream, but can also harm healthy heart tissue — into alternative forms that may have more localized tissue distribution. This tweak should result in fewer side-effects, while potentially reducing the dosage needed, which could help lower the cost of treatment.

"It's proof-of-concept that such antibody modification can be undertaken," says Samuel Gan, head of the Antibody and Product Development group at the Bioinformatics Institute and the p53 Laboratory at A*STAR.

"There is a whole range of possibilities to using more localized antibodies as prophylactics."

Antibodies are also known as immunoglobulins, and they come in five main 'isotypes': IgA, IgD, IgE, IgG and IgM. Most therapeutic antibody drugs are of the IgG isotype that circulate in the bloodstream, but Gan's team showed they could remodel IgG antibodies into some of the other isotypes that may have greater tissue precision.

Their finding lays the groundwork for future antibody therapies that can be safer for patients without compromising yield or potency.

The researchers worked with trastuzumab and pertuzumab, two antibody drugs marketed under the brand names Herceptin® and Perjeta®, that selectively bind to different parts of human epidermal growth factor receptor 2 (HER2), a protein that is implicated in approximately 20 per cent of all breast cancer cases. They transformed these IgG antibodies into all five human isotypes and the four subtypes, and examined what impact that change had on drug production and binding to the HER2 target.

IgD versions of the antibody drugs proved inferior, both in terms of yield and HER2-targeting, but IgE and IgA versions both generally retained the effectiveness of the original therapies — which raises the possibility of developing safer versions of trastuzumab and pertuzumab based on these alternate isotypes. Most interestingly, the findings also show that constant regions in antibodies can influence in the antibody's ability to bind, a finding that is often not noticed and often neglected in antibody research.

As Gan points out, both IgE and IgA antibodies are likely to be more active in the ductal tissues of the breast, where HER2-expressing tumor cells

Changing the isotype of Immunoglobulin can make it easier to target specific parts of the body.

are also found, and they could be combined with low doses of blood-targeting IgG antibodies to guard against circulating metastatic cells while lowering the risk of other side-effects such as cardiac toxicity.

Additionally, Gan suggests that immunotherapies could be developed with IgA and IgE antibodies to better guard against mucosal infections such as influenza or AIDS. "There is a whole range of possibilities to using more localized antibodies as prophylactics," he says.

1. Lua, W. H., Ling, W. L., Yeo, J. Y., Poh, J. J., Lane, D .P. & Gan, S. K. The effects of antibody engineering CH and CL in trastuzumab and pertuzumab recombinant models: Impact on antibody production and antigen-binding. *Scientific Reports* **8**, 718 (2018).

CANCER

A SUSTAINABLE SOURCE OF NATURAL KILLERS

Generating large numbers of universal immune cells could transform cancer immunotherapy

A scalable method of generating universal 'off-the-shelf' natural killer (NK) cells for cancer immunotherapies has been devised by A*STAR researchers. Their technique could ensure that future NK cell-based cancer treatments can be used for most patients.

NK cells are a group of small white blood cells from the innate immune system that help kill virus-infected cells and malignant cancer cells. Scientists can harvest NK cells and use them to directly target cancer cells. However, existing techniques generate limited numbers of NK cells from selected donors that are suitable for specific patients only.

"Current donor-dependent NK cell harvesting methods carry the risk of graft-versus-host disease, because traces of other cells and molecules from donors that are mixed in with the NK cells can react to patients' normal cells. This can limit the use of therapies considerably," says Shu Wang at the A*STAR Institute of Bioengineering and Nanotechnology, who led the study. "We wanted to devise a new method of generating large numbers of pure, universally-suitable NK cells that could widen the use of such immunotherapies."

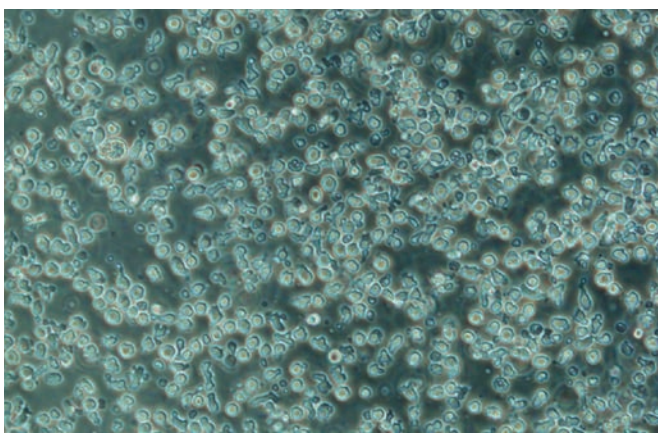
"We may now have an invaluable cell source for a wider group of patients."

The team needed a readily available, sustainable and non-controversial source of stem cells from which they could derive massive numbers of NK cells. So they used peripheral blood cells to generate induced human pluripotent stem cells, iPSCs, which can differentiate into every type of cell in the body, including NK cells. This is the first time peripheral blood cell-derived iPSCs have been used to generate NK cells.

Wang's team designed a new protocol to derive NK cells from iPSCs, with a focus on robustness and scalability. They co-cultured iPSCs with bone marrow connective tissue cells — these activate the signaling processes needed for cell differentiation and commitment to a specific cell type, which resulted in large yields of NK cells.

"An unexpected bonus of our protocol was that most derived NK cells were free of a particular group of inhibitory receptor proteins that can limit universal application in patients," says Jieming Zeng, the first author of the study. "This means that we may now have an invaluable cell source for a wider group of patients."

Scaling up the manufacture of NK cells will require considerable further investment, particularly with its reliance on the connective tissue cell lines that may prove expensive to provide in the form of cell banks. The team will continue to streamline their technique, and believe the breakthrough will inform the development of universal 'off-the-shelf' cancer treatments.



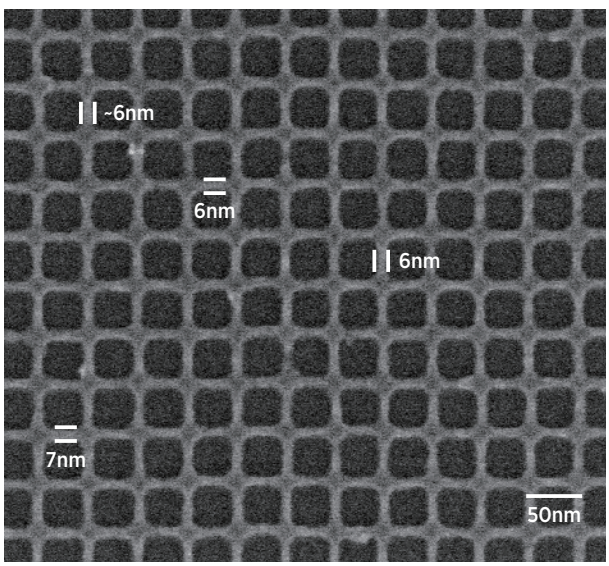
A novel method to generate natural killer cells from peripheral blood cell-derived stem cells (above) may transform immunotherapies for different cancers.

1. Zeng, J., Tang, S. Y., Toh, L. L. & Wang, S. Generation of 'off-the-shelf' natural killer cells from peripheral blood cell-derived induced pluripotent stem cells. *Stem Cell Reports* **9**, 1796–1812 (2017).

LITHOGRAPHY

A BRIGHT IDEA FOR ON-DEMAND NANOPATTERNS

Focused electron beams can simultaneously synthesize optically active nanocrystals and pattern them into intricate surface arrays



Light-emitting ZnS nanostructures can be patterned into nearly any shape, including grids as above, using a multipurpose photoresist.

An A*STAR team has assembled zinc sulfide (ZnS) quantum dots into nanoscale grids and arrays of lens-like disks by performing electron-beam lithography on a multipurpose thin film[1]. The photoluminescent properties of these patterns could make them useful components in applications such as biosensors and solar cells.

While individual ZnS nanoparticles have intriguing optical properties, due to the effects of quantum coupling, their light-emitting capabilities become more potent when positioned into ordered assemblies. Instead of conventional bottom-up approaches that use wet chemicals to generate nanoparticle ensembles on silicon chips, many researchers now approach this problem from the top down, using nanoscale lithography to strip away unwanted material and write quantum dots directly on to surfaces.

"The nice thing about photoluminescent ZnS nanocrystals is that they can be arranged into practically any shape."

Carving shapes into semiconductor surfaces smaller than 10 nanometers is a particular expertise of M. S. M. Saifullah from the A*STAR Institute of Materials Research and Engineering, and colleagues. They direct high-powered electron beams on to special thin films called 'resists'. Areas of the resist exposed to the focused beams undergo chemical changes that enable tiny features to stay in place while the surrounding film is washed away by solvents.

In most electron-beam lithography techniques, the patterned resist is transferred to another substrate and a chemical etching step generates the final nanoscale shapes. Saifullah and the team, however, had a different strategy. "We developed a resist that can decompose and form a metal sulfide right under the electron beam," he notes. "This was a challenge because most resists don't have such functionalities."

The team found a compound called zinc butylxanthate that could meet their needs. In this molecule, zinc and sulfur atoms are connected to long-chain organic groups that potentially can be detached using the energy from an electron beam. Experiments with the new resist proved the efficiency of this conversion process: by gradually increasing the electron beam exposure, the starting film was transformed into ZnS nanocrystals with a conversion rate of almost 100 per cent

The A*STAR-led team exploited the properties of the zinc butylxanthate resist to produce lines of ZnS nanocrystals with diameters as thin as 6 nanometers. Then, after characterizing the structures with electron microscopy, they made another serendipitous discovery — the nanopatterns emitted bright photoluminescent light when exposed to ultraviolet radiation. Defect states on the nanocrystal surfaces were pinpointed as the cause of the new optical behavior.

"The nice thing about photoluminescent ZnS nanocrystals is they can be arranged into practically any shape," says Saifullah. "In the future, we would like to combine these nanostructures with plasmonics." ■

1. Lu, X., Denver, D., Chandar, J., Chen, Y. & Lou, J. An overlapping domain decomposition based near-far field coupling method for wave structure interaction simulations. *Coastal Engineering* **126**, 37–50 (2017).

IMMUNOLOGY

MONOCYTES IN THE MIX

Understanding how monocyte cells generate specialized immune system cells may help target immunity against cancer

An international research team has helped unravel important details of how blood cells called monocytes develop into the crucial 'helper' cells of the immune system[1]. "The work should ultimately allow better and more personalized treatment," says team member Florent Ginhoux of the A*STAR Singapore Immunology Network.

The immune system is a complex network of interacting cells circulating throughout the body, protecting us from invading microorganisms and from diseases originating from within. Scientists and clinicians are learning how to select and modify immune system cells to target them more effectively at specific diseases, especially cancer.

"Monocytes have many faces," says Ginhoux, explaining how these white blood cells perform various roles in immunity, and develop or differentiate into a range of more specialized cells. Monocytes circulating in the blood can ingest and destroy foreign invaders and promote inflammation of diseased tissues. One of their most crucial roles, however, is to develop further into specialized 'antigen-presenting cells'. These interact with parts of foreign organisms or diseased cells and display molecular tags called antigens on their surface, which can interact with other cells of the immune system. This antigen-presenting activity initiates an immune response against the source of the antigens, whether invading micro-organisms or diseased cells such as cancer cells.

By understanding how monocytes differentiate into these vital antigen-presenting cells, the team, led by Joachim Schultze and Andreas Schlitzer of the University of Bonn, hope to learn how to stimulate their activities more effectively to fight cancer and other diseases.

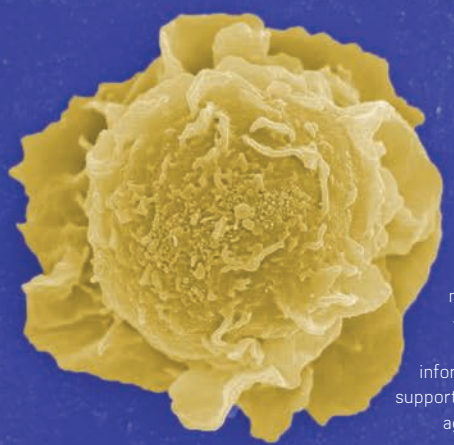
"We now understand the molecular make-up and clinical potential of the cells much better."

Their work, performed with cultured human cells, revealed details of the molecular signals that regulate the differentiation of monocytes into a complex mixture of cell types. The team also identified time-dependent phenomena that control the developmental fate of monocytes. The wide variety of cell types produced from the monocyte population was a particularly significant surprise.

"Through this work we now understand the molecular make-up and clinical potential of the cells much better," says Ginhoux. He explains that a logical next step will be to examine similar cells that are being used clinically, to see if they also display the mixed cell-type characteristics found in the lab studies. This may reveal ways to improve the clinical use of the cells by identifying and producing the most effective specific cells for the treatment of each disease and hopefully individual patients.

"Drilling down to the single cell level will bring better treatments," Ginhoux emphasizes.

1. Sander, J., Schmidt, S. V., Cirovic, B., McGovern, N., Papantonopoulou, O. *et al.* Cellular differentiation of human monocytes is regulated by time-dependent Interleukin-4 signaling and the transcriptional regulator NCOR2. *Immunity* **47**, 1051-1066 (2017).



A human monocyte cell — these cells may hold key information about supporting immunity against cancer.

Relocation of N-acetylgalactosaminyltransferases from the Golgi apparatus to the endoplasmic reticulum makes cancer cells more invasive. (above) Illustration of cancer cells breaking through tissue.

© Callista Images/Getty

CANCER

RELOCATED ENZYMES MAKE CANCER MORE MOBILE

Relocation of enzymes involved in protein modification makes it easier for cancer to spread

Enzymes that are involved in protein modification are relocated in cancer cells, altering protein processing and helping cancer to spread, according to new work by A*STAR researchers. The mechanism, which was studied in liver cancer, reveals novel potential targets for cancer treatments.

During their production, many cell surface proteins undergo glycosylation — a process which involves attachment of sugar molecules, called glycans, to specific sites on proteins. This normally takes place in the Golgi apparatus, after the protein has been transferred from the endoplasmic reticulum (ER) where it was made. "Glycosylation is a poorly understood process," explains Frederic Bard from the A*STAR Institute of Molecular and Cell Biology, who led the new study, "yet it is involved in virtually every aspect of human biology and it has long been known that glycosylation is altered in cancer."

In liver cancers, some of the enzymes involved in glycosylation have been shifted from the Golgi to the ER. Altered glycosylation in cancer is associated with more invasive — and therefore more deadly, tumors — but the underlying mechanisms are unclear. Bard and colleagues investigated these mechanisms in liver cancer, focusing on the initial step of glycosylation in which the glycan N-acetylgalactosamine (GalNAc) is attached to proteins by enzymes called N-acetylgalactosaminyltransferases (GALNTs). The researchers had previously proposed that relocation of GALNTs from the Golgi apparatus to the ER affects glycosylation in cancer, and was investigating whether there was further evidence to support this case.

The team first demonstrated that GALNTs are active at the ER in liver tumor cells from mice and humans, rather than being active at the Golgi apparatus as they are in healthy cells. Furthermore, genetic targeting of GALNT1 to the ER in mice with liver cancer reduced survival from 23 weeks to just 10 weeks.

Further experiments in the mice and cultured cells revealed that relocation of GALNT1 to the ER caused increased glycosylation of the protein matrix metalloproteinase 14 (MMP14). This in turn led to breakdown of the extracellular matrix that keeps cells in place, making it easier for cancer cells to break away from the primary tumor and invade other tissues. "Glycosylation activates the mechanisms by which cancers destroy normal tissue, making space for their own growth," explains Bard.

"Glycosylation is a poorly understood process, yet it is involved in virtually every aspect of human biology."

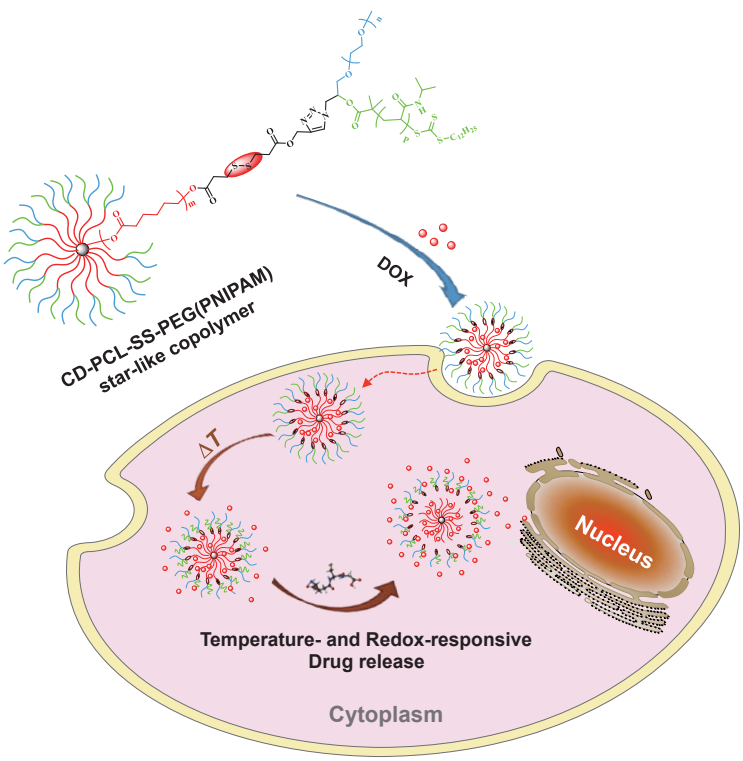
The researchers say their findings suggest that GALNTs, and the mechanisms that control their relocation to the ER, are potential therapeutic targets for cancer treatments. Identification of similar mechanisms could reveal further potential targets. "While we focused on MMP14, we have preliminary evidence that many cell surface proteins are regulated by hyperglycosylation," says Bard, "We are currently exploring how these other proteins contribute to tumor development.

1. Nguyen, A. T., Chia, J., Ros, M., Hui, K. M., Saltel, F. *et al.* Organelle specific O-glycosylation drives MMP14 activation, tumor growth, and metastasis. *Cancer Cell* **32,** 639–653.e6 (2017).

DRUG DELIVERY

REACH FOR THE STARS

Star-shaped nanoparticles that release their drug payload only after entering cells could improve current treatments



The change in temperature and chemical environment inside the cell triggers the nanoparticle to release its drug payload.

A*STAR researchers have developed nanoscale drug delivery particles that can sense their surroundings, and release their payload only after entering a cell, a discovery that could make many existing medicines more effective.

The new nanoparticles, developed by Zibiao Li from the A*STAR Institute of Materials Research and Engineering and his collaborators, are a significant upgrade from previous generations of polymer-based drug delivery nanoparticles. Early examples typically consisted of simple polymer chains with a polar, hydrophilic head and a non-polar, hydrophobic tail. In water, these chains naturally aggregate into spheres, with their hydrophobic tails all pointing inward to form a non-polar core. The core formed a good site for drug molecules to nestle. In the bloodstream, however, these aggregates tend to be torn apart.

Li and his colleagues used the latest polymer synthesis techniques to create single-molecule nanoparticles. Rather than a self-assembled aggregate of separate polymers, the team synthesized a more robust structure in which the polymer chains were strongly covalently bonded to a central core. The synthesis began with beta-cyclodextrin, a circular sugar molecule with 21 hydroxyl groups on its surface. The hydroxyl groups formed the chemical anchors from which the team constructed the nanoparticle's multiple long, Y-shaped, multifunctional polymer arms.

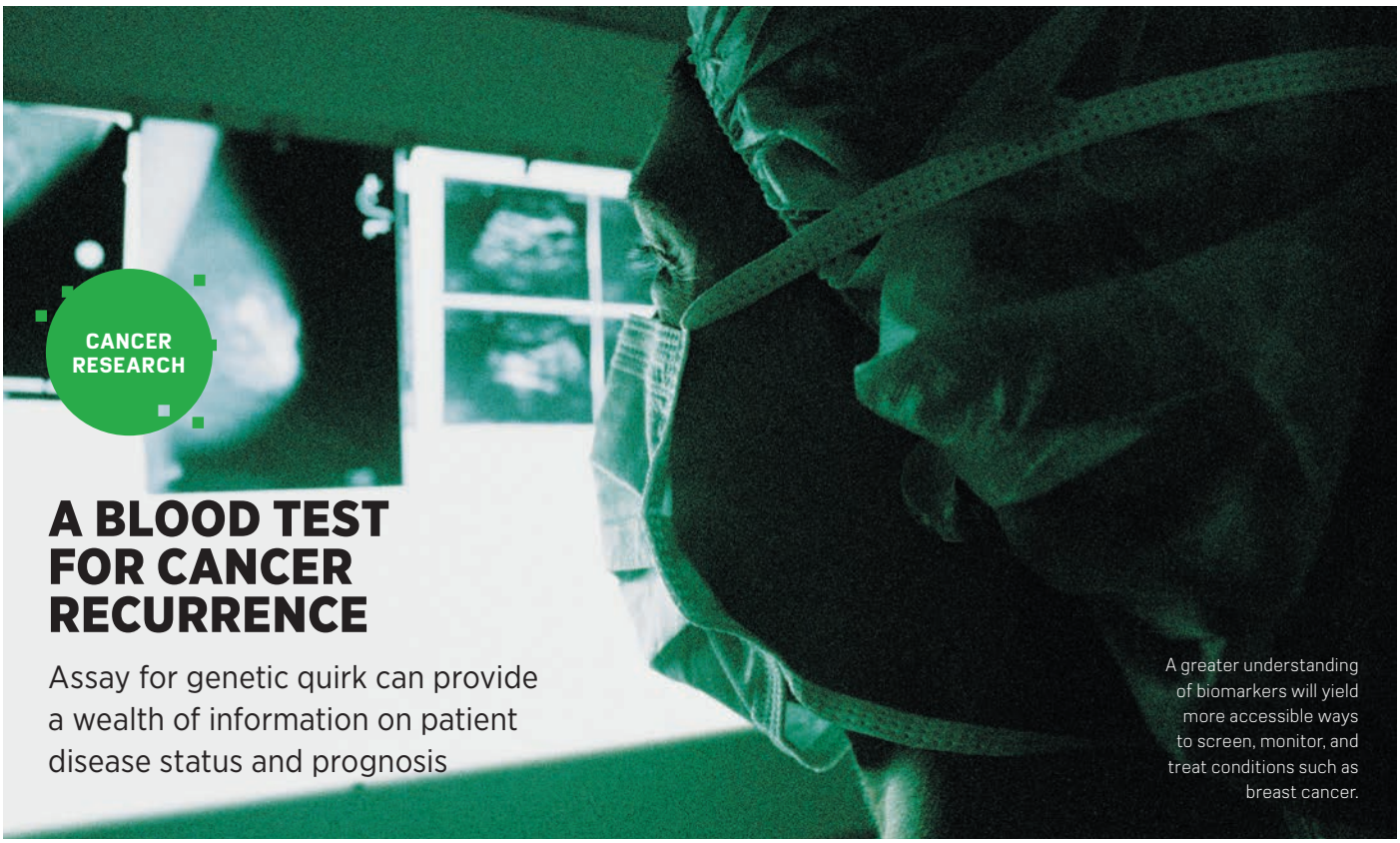
"The greatest challenge in making the [nanoparticle] was to integrate different synthetic methodologies into one macromolecular design," says Li. At one of the ends of each Y-shaped branch, the team attached a temperature-sensitive polymer called PNIPAM. At room temperature the PNIPAM polymer extends outward, but collapses once body temperature, 37 degrees Celsius, is reached, allowing the nanoparticle's drug molecule cargo to escape.

Mid-way along each polymer arm, the team installed a sulfur-sulfur bond. When the particles enter a cell, they meet high levels of glutathione, a molecule that cleaves sulfur-sulfur bonds. Thus the nanoparticle's outer polymer coat is snipped off, and the drug is released even faster.

When the team tested this effect with an anti-cancer drug called doxorubicin, the dual-action effect was clear. "The change from room temperature to body temperature, and the effect of glutathione, showed synergistic and fast release of the drug," Li says. At lower temperatures, or in the absence of glutathione, drug release was significantly slower, he says.

"The next plan is to integrate new functionality, for precision release of the drug for targeted cancer therapy," says Li. One end of each Y-shaped branch could be functionalized with a molecule that sticks selectively to cancer cells, delivering the drug precisely where it is needed.

1. Fan, X., Wang, X., Cao, M., Wang, C., Hu, Z. *et al.* "Y"-shape armed amphiphilic star-like copolymers: design, synthesis and dual-responsive unimolecular micelle formation for controlled drug delivery. *Polymer Chemistry* **8**, 5611 (2017).



CANCER RESEARCH

A BLOOD TEST FOR CANCER RECURRENCE

Assay for genetic quirk can provide a wealth of information on patient disease status and prognosis

A greater understanding of biomarkers will yield more accessible ways to screen, monitor, and treat conditions such as breast cancer.

Despite great advances in medicine, cancer remains a death sentence for many. "Patients are still dying from breast cancer relapse," says Jian Yuan Goh, of A*STAR's Genome Institute of Singapore. The researcher and his colleagues, a team from Singapore, China, the United States, and Denmark, have recently identified a genetic aberration in a particularly aggressive subtype of breast cancer cells that, when quantified, can provide insights into a patient's cancer status, chances of relapse, and treatment progress. This discovery paves the way for an alternative to invasive and expensive biopsy testing.

One model of cancer's proliferation involves the presence of chemotherapy-resistant tumor-initiating cells (TICs). "Even after chemotherapy and surgery, these cells can survive, regrow, and cause recurrence," says Goh. The team, led by A*STAR's Qiang Yu, found that TICs possess an abnormally high number of copies of a specific section of DNA. This 'copy number amplification' results in an overproduction of the proteins coded for by that section of genetic material.

"What we're trying to do is provide a cost-effective way for clinicians to track breast cancer recurrence."

Goh and his colleagues have produced a 'liquid biopsy' assay that detects this genetic quirk within circulating fragments of tumor DNA found in blood. A presence of TIC-linked DNA allows clinicians to inform patients of the increased risk of breast cancer recurrence, as well as monitor the progress of treatment and the emergence of tumor resistance to therapy, based on the changing levels of the biomarker. The researchers also found the amplification to be correlated to cancer metastasis.

TIC presence might signal a bleak prognosis for patients if it didn't also offer a unique avenue of treatment. With an abnormally high number of a specific set of genes, TICs overproduce the S100A family of proteins. As these proteins provide TICs with their chemo-resistant properties, the researchers are now investigating pharmacological interventions that might block this pathway and provide a lifeline in otherwise incurable cases of breast cancer. "We're in discussions to start a clinical trial with the drug pacritinib, using our biomarker to guide treatment," explains Goh. "Pacritinib is not yet approved for use in breast cancer, so we first need to see whether tumors respond to this treatment."

"What we're trying to do is provide a cost-effective way for clinicians to track breast cancer recurrence. The alternative — genetic sequencing — is comprehensive, but very expensive. You can't imagine a patient coming in every month to be sequenced for 3,000 dollars, but, if we develop this assay, testing will be a lot more affordable," says Goh.

1. Goh, J. Y., Feng, M., Wang, W., Oguz, G., Yatim, S. M. J. M. *et al.* Chromosome 1q21.3 amplification is a trackable biomarker and actionable target for breast cancer recurrence. *Nature Medicine* **23**, 1319–1330 (2017).

ULTRAFAST LASERS

NEW APPROACH TO GENERATING ULTRASHORT PULSES

Extremely short mid-infrared laser pulses will help scientists explore ultrafast processes in atoms

A laser synthesizer developed by an A*STAR researcher and his overseas collaborators produces infrared pulses shorter than the wave period, which could enable scientists to probe how electrons move inside atoms and solids. Importantly, these pulses are readily absorbed by many atoms and molecules because their wavelengths lie in the mid-infrared range.

Processes within atoms, such as electrons moving from one energy level to another, happen extremely quickly, on time scales of femtoseconds (10^{-15} second) or even attoseconds (10^{-18} second). Consequently, researchers need very short, high-power laser pulses to observe these events. One way to generate them is to shine highly intense, ultrashort infrared pulses on to nonlinear crystals.

Houkun Liang at A*STAR's Singapore Institute of Manufacturing Technology and his colleagues in the United States and Germany realized they needed to rethink conventional approaches for generating ultrashort mid-infrared pulses.

"To get a pulse that is shorter than the wave period, you need a very broad spectral bandwidth," explains Liang. "Previously, researchers had combined pulses with different spectral coverages to obtain such huge bandwidths, but this is extremely difficult. The relative phase and amplitude of the individual pulses have to be highly precisely controlled, which makes

A new laser pulse synthesizer developed at SIMTech produces extremely short mid-infrared pulses that enable observations of ultrafast processes within solids.

the laser system very complex because it requires a lot of noise-control apparatus."

To simplify things, Liang and his colleagues used an optical parametric amplifier — a device that produces two pulses, which cover different spectral ranges and whose amplitudes and phases can be fixed relative to one another. The team constructed their amplifier to have a very short time delay between the two pulses, meaning they naturally combine into a wide-bandwidth pulse with no need for noise control. The resulting pulse could be made even shorter than the period of the wave because constructive interference occurs at its center, while destructive interference 'trims' the pulse at its edges.

Perhaps the most useful characteristic of these mid-infrared pulses is that when they are directed on to certain solids, they can stimulate the emission of higher energy photons in the deep-ultraviolet or extreme-ultraviolet regions. These higher energy photons can then be used to study the fastest processes inside atoms, which occur on attosecond scales. Liang's team demonstrated this with their system by using the mid-infrared pulses to generate high-energy photons in thin silicon films.

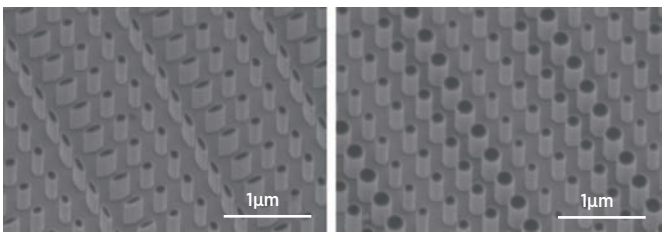
"We are interested in using the pulse synthesizer to generate isolated attosecond electron pulses from different materials," says Liang. These would allow observations of even faster processes within materials.

1. Liang, H., Krogen, P., Wang, Z., Park, H., Kroh, T. *et al.* High-energy mid-infrared sub-cycle pulse synthesis from a parametric amplifier. *Nature Communications* **8**, 141 (2017).

PHOTONICS

LIGHT-BENDING NANO-PATTERNS FOR LEDS

Direct nanoscale patterning of LED surfaces brings new possibilities for the control of light



Nanopillar arrays fabricated directly on a GaN surface.

Nanoscale patterns designed to bend, deflect and split light can now be fabricated directly on light-emitting diode (LED) surfaces using an innovative etching method developed by A*STAR researchers1. The new fabrication scheme creates new possibilities for the facile control of light output.

Recent advancements in LED lighting have transformed daily life and cutting-edge technology — from efficient room lighting, to TV and mobile device backlights, and the tiny optical circuits that drive global fiber-optic networks.

The light-emitting component of LEDs is a surprisingly simple structure, typically a thin layer of a dielectric material such as gallium nitride (GaN) on a crystalline sapphire substrate. This structure means the light emitted by LEDs is inefficiently dispersed in all directions, including back into the substrate on which the light-emitting layer is fixed. So, while researchers have made tremendous advances in light-emitting efficiency, there remains room for improvement.

Egor Khaidarov and colleagues from A*STAR's Data Storage Institute and Nanyang Technological University have now found a way to pattern GaN with nanoscale features that can control the behavior of light.

"We have demonstrated that metasurfaces — surfaces patterned with features typically smaller than the wavelength of emitted light — can be fabricated directly on a standard GaN-on-sapphire platform," says Khaidarov. "Most importantly, we have shown that with good design, it is possible to create the metasurfaces without the need for an additional layer, while still maintaining a high level of emission efficiency."

Metasurface modifications of LEDs have been attempted in the past. These included patterning an additional layer with a very different refractive index than the underlying GaN-on-sapphire substrate to keep the light in the metasurface layer and enhance the light-matter interactions. The problem with patterning GaN directly — a major benefit for fabrication — is a weakness of interactions because of the lack of refractive index contrast.

"To overcome this, we worked with very deep structures with a large aspect ratio, effectively arrays of nanopillars, to reduce the influence of the substrate on the metasurface's optical modes," explains Khaidarov (see image).

The resulting design, however, posed a major challenge for fabrication, requiring the team to develop a precise nanofabrication procedure involving electron beam lithography and fast, high-temperature reactive ion etching.

"With our design concept we have, in principle, full control of the output properties of light, which allows us to fabricate more complex optical components such as lenses, vortex beam generators, polarimeters and holograms," says Khaidarov.

1. Emani, N. K., Khaidarov, E., Paniagua-Domínguez, R., Fu, Y. H., Valuckas, V. *et al.* High-efficiency and low-loss gallium nitride dielectric metasurfaces for nanophotonics at visible wavelengths. *Applied Physics Letters* **111**, 221101 (2017).

UV PROTECTION

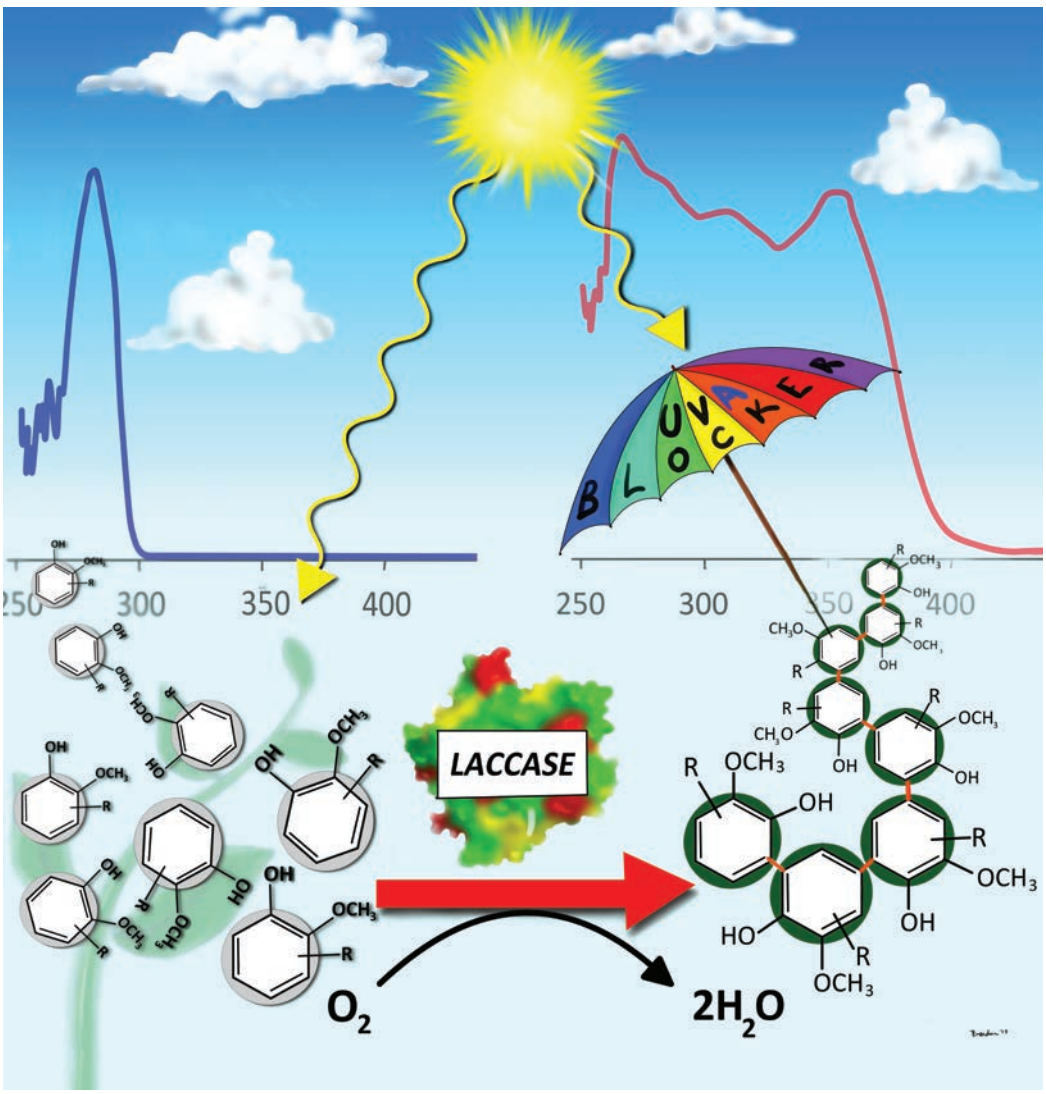
LIGNIN'S STRENGTHS IN SMALLER PACKAGES

Adapted polymer offers plant-based protection from the Sun's harmful rays

Lignin, the natural polymer that gives tree trunks their strength, could be the source of a new range of skincare products, new research suggests.

The lignin polymer contributes to plants' physical rigidity, and also absorbs UV rays, helping to protect the plant's more delicate cellulose structures from sun damage. Researchers at A*STAR have found a way to make short-chain lignin-like structures that retain the parent molecule's UV blocking ability, but can be more easily incorporated into sunscreens and other skin products[1].

Although lignin itself is a readily-available renewable resource, the natural polymer's use in performance materials and personal care products is limited by two key factors, explains Balamurugan Ramalingam from the A*STAR Institute of Chemical and Engineering Sciences, who led the current work. High molecular weight natural lignin polymers are inherently insoluble in common solvents, and disperse poorly into mixtures of other materials, making it difficult to work with.



A lignin-like short chain oligomer (right) absorbs UV light far more effectively than its component monomers (left).

"We were particularly pleased by the broader UV blocking ability and higher molar absorptivity exhibited by the oligomers."

The lignin polymer can be broken down into its component small molecule monomers to improve workability, but this removes natural lignin's UV-absorbing characteristics. Ramalingam and his coworkers suspected that the sweet spot for making UV blockers might be to form lignin-like 'oligomers', short-chain structures rather than the long-chain polymers of natural lignin.

The team showed they could form lignin-like oligomers either by chemically

breaking natural lignin into smaller pieces, or by linking lignin monomers into short chains. Tests confirmed the lignin-like oligomers offered the best of both worlds, combining good solubility and dispersion with useful sunscreen properties. "We were particularly pleased by the broader UV blocking ability and higher molar absorptivity exhibited by the oligomers in comparison with respective monomers," Ramalingam says. The oligomers' UV blocking ability also appeared to be robust and long-lived in accelerated UV weathering tests.

The lignin-like oligomers have several potential advantages over existing small molecule UV blockers that are used to stabilize plastics and in sunscreens, Ramalingam says. "In contrast to synthetic small molecule UV stabilizers, the present lignin-like oligomers are bio-derived and presumably non-toxic," he says. As oligomers are physically larger structures than small molecule UV blockers, they are also less likely to leach out of plastics into the environment, or penetrate the skin when used in sunscreens.

"We are currently evaluating the possibility of incorporating lignin-like oligomers into commercial sunscreens, towards developing nature-inspired personal care products," Ramalingam says.

1. Lim, J., Sana, B., Krishnan, R., Seayad, J., Ghadessy, F. J. *et al.* Laccase-catalyzed synthesis of low-molecular-weight lignin-like oligomers and their application as UV-blocking materials. *Chemistry — An Asian Journal* **13**, 284 (2018).

LASERS

PULSES OF LIGHT WITH A TILT AND A TWIST

An exact mathematical solution reveals how to tilt a laser pulse and move intensity peaks at faster than the speed of light, or even backwards

A breakthrough in the mathematical solution for manipulating pulses of light clarifies the physics of 'tilted' laser pulses, and shows how they can be used to produce exotic effects1. The solution, developed by researchers from A*STAR and the Massachusetts Institute of Technology (MIT), could advance applications such as terahertz lasers, X-ray generation and ultrafast electron imaging.

Pulsed lasers are commonly used in industrial and research applications to deliver precision doses of high-intensity laser energy, which for example can prevent the target from being damaged while still heating or exciting it to a certain energy state. Some of the latest techniques use ultrashort pulses which last just a few femtoseconds — one quadrillionth of a second — to excite atoms and molecules and observe how they respond.

One of the methods used to enhance the interaction between a laser pulse and the target atom or molecule is to tilt the wavefront of the pulse so that the energy delivery becomes slightly spread over time and in space, like the angle on the blade of a snow-plow. This effect can also be used to match pulses from different sources, which at such ultrashort pulse-lengths can be extremely challenging. Until now, the mathematics used to calculate the parameters and behavior of tilted laser pulses have only been approximations, with limited insight into the physics involved.

"It is interesting to study very short tilted-pulse-front pulses not only because of the new physics they contain, but also because such pulses can allow us to achieve target intensities with much lower energies, which could translate to cost savings," says Liang Jie Wong from A*STAR's Singapore Institute of Manufacturing Technology (SIMTech). "However, it is notoriously challenging to obtain exact analytical solutions for realistic pulses."

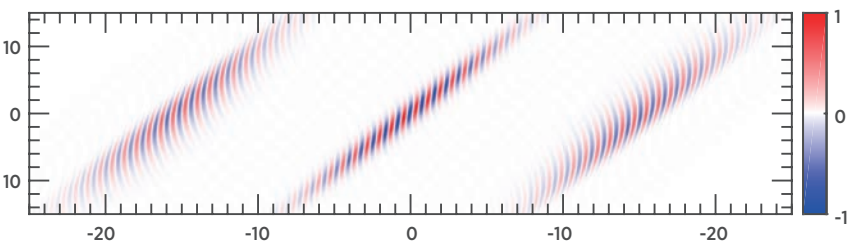
Wong and collaborator, Ido Kaminer, from MIT and the Technion, Israel, originally set out to develop exact mathematical solutions for a new type of laser pulse called a needle pulse, but ended up with something much bigger.

"This result was mostly serendipity," says Wong. "We simply played around with possible mathematical expressions that could solve the equations exactly."

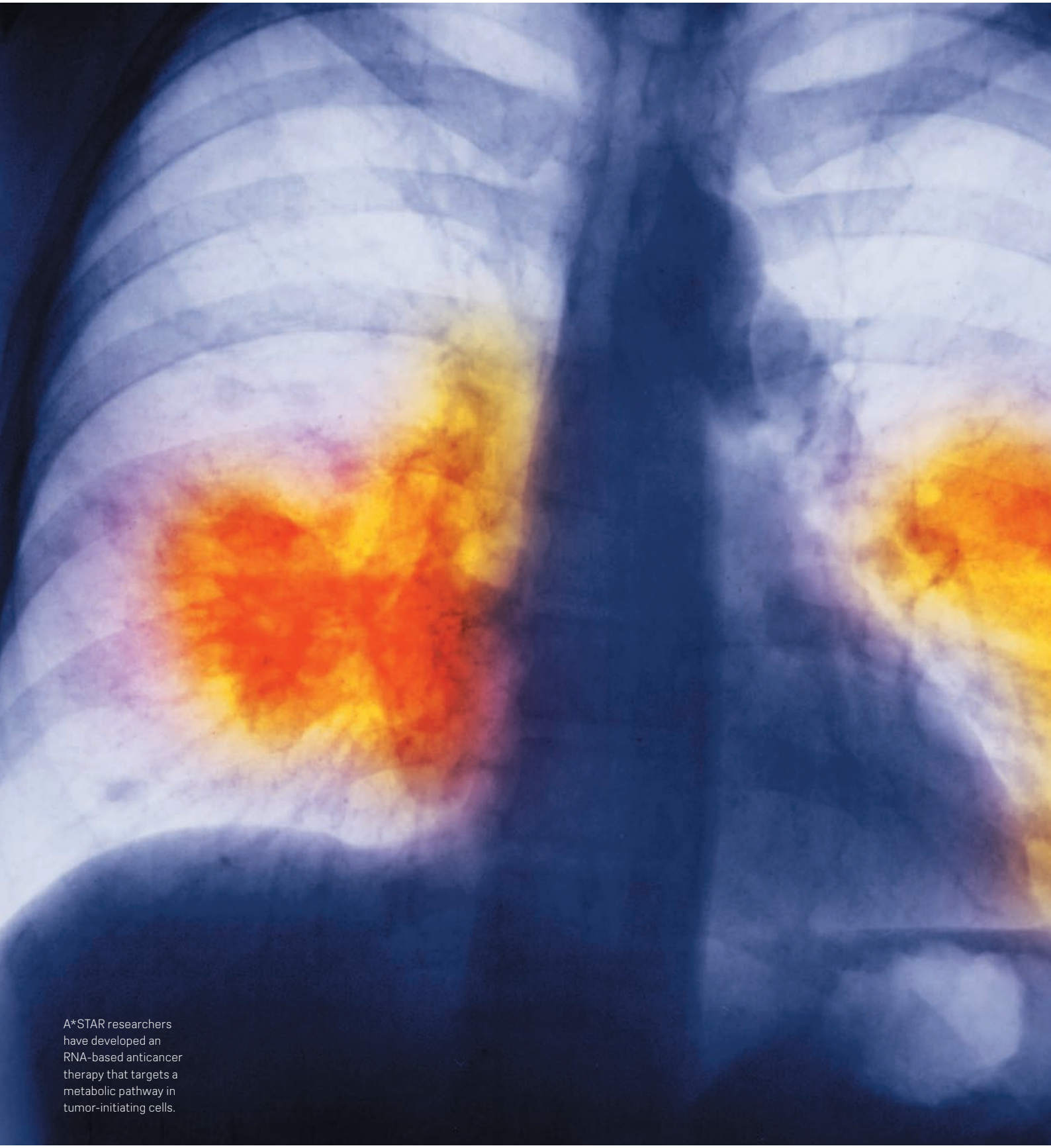
The new mathematical solution not only describes tilted pulses with exact physical precision (see image), allowing for rigorous study for optimization of existing applications, but also reveals that a beam of tilted-pulse-front pulses can be tailored for some exotic physics.

"We can create wave packets with intensity peaks that can propagate at faster or slower than the speed of light, and even backwards," says Wong.

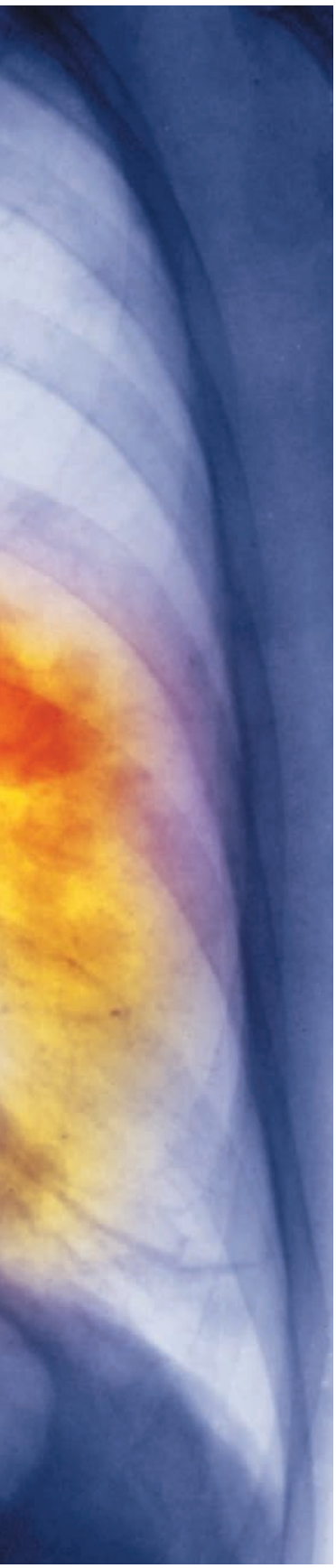
1. Wong, L. J. & Kaminer, I. Ultrashort tilted-pulse-front pulses and nonparaxial tilted-phase-front beams. *ACS Photonics* **4**, 2257–2264 (2017).



A progression of femtosecond tilted-pulse-front pulses.



A*STAR researchers have developed an RNA-based anticancer therapy that targets a metabolic pathway in tumor-initiating cells.



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CANCER THERAPEUTICS

TARGETING THE ROOT OF THE PROBLEM

Tumor growth in a mouse model of lung cancer is reduced using an RNA-based therapeutic that inhibits a metabolic pathway in tumor-initiating cells

RNA-based therapeutics that prevent a key metabolic enzyme from being expressed in tumor-initiating cells (TIC) hold promise for the treatment of lung cancer, an A*STAR team has shown.

As implied by their name, TIC — also known as cancer stem cells — are a subset of tumor cells with the capacity to self-renew and start new tumors. Abnormal metabolism is a key feature of TIC as the high proliferation rate and tumorigenic potential of these cells require changes to the metabolic pathways that are normally used by healthy cells to produce energy and biosynthetic precursors.

The discovery of elevated expression of normal or mutant forms of metabolic enzymes in a variety of cancers has created great interest in cancer metabolism, explains Uttam Surana from the Institute of Molecular and Cell Biology, and Dave Wee from the Institute of High Performance Computing. Targeting TIC metabolism is emerging as a promising strategy to thwart the progression of various cancers.

Previous studies have shown that TIC of non-small cell lung carcinoma (NSCLC) tumors contain high levels of glycine decarboxylase (GLDC), an enzyme that breaks down the amino-acid glycine. Overexpression of GLDC stimulates the generation of tumors and the proliferation of TIC, and high GLDC levels are associated with poor survival rates in NSCLC patients.

"They offer important advantages over small molecule drugs, which have a higher risk of resistance and toxicity."

No therapeutic agents have been developed against GLDC until now. Surana and Wee identified short synthetic RNA sequences (steric hindrance antisense oligonucleotides, or shAONs) that supressed the production of GLDC protein in human lung cancer cells, hindering their proliferation and preventing tumor formation.

Working with colleagues at A*STAR's Genome Institute of Singapore, the team showed that when the GLDC-targeting shAON sequences were injected into mice bearing NSCLC enriched with TIC, tumor growth was reduced by 60 per cent compared with mice injected with scrambled control sequences.

The shAONs disrupt a key step in the process through which the gene that encodes GLDC is translated into protein. "We have designed three shAONs that efficiently interfere with the process that normally removes non-protein coding sequences from the GLDC pre-mRNA transcribed from the gene's DNA template," Surana explains. "The resultant aberrant transcripts are primed for degradation and GLDC protein production is dramatically reduced".

Future work will determine whether these shAONs can be delivered through the nose to target tumors grafted in the lungs of mice, and test their efficacy against other types of cancer in which an upregulation of GLDC contributes to TIC metabolism, such as breast and thyroid cancers. Because of shAONs' high target specificity, they offer important advantages over small molecule drugs, which have a higher risk of resistance and toxicity.

1. Lin, J., Lee, J. H. J., Paramasivam, K., Pathak, E., Wang, Z., *et al.* Induced-decay of glycine decarboxylase transcripts as an anticancer therapeutic strategy for non-small-cell lung carcinoma. *Molecular Therapy Nucleic Acids* **9**, 263-273 (2017).

REHABILITATION

AN ADAPTIVE APPROACH TO STROKE RECOVERY

A new method for reading brain waves improves the usefulness of motor imagery exercises

A*STAR scientists have found a new way to give stroke patients instructive feedback during rehabilitation exercises aimed at restoring mobility.

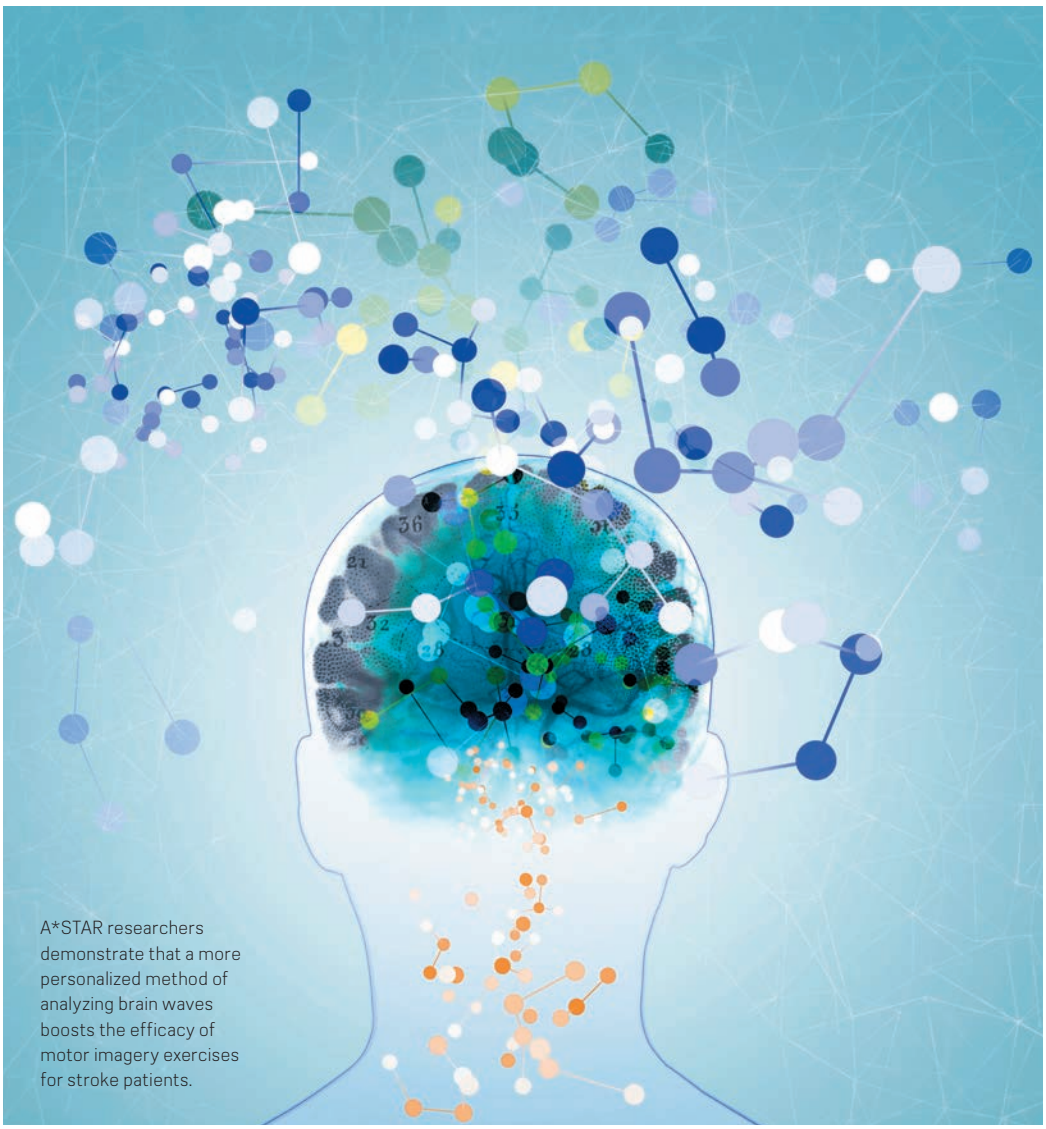
For stroke patients, the long road to recovery starts with rehabilitation exercises, including visualizations in which people imagine enacting motions they are physically unable to perform. This 'rewires' their brains, and leads to real-life bodily improvements. The exercises only work if you're performing them correctly, and since visualization takes place entirely in the mind's eye, it has been difficult for clinicians and patients to know if the correct brain connections are being made.

"This feedback will help stroke patients in rehabilitation restore brain functions and improve motor recovery."

To get a glimpse inside the brain, patients typically wear caps fitted with electrodes that track neural activity via electroencephalography (EEG) readings. Now scientists from the A*STAR Institute for Infocomm Research have validated a more accurate way of turning those EEG signals into clinically meaningful feedback for stroke sufferers.

"This feedback will help stroke patients in rehabilitation restore brain functions and improve motor recovery," says study author, Kai Keng Ang, an A*STAR senior scientist who heads the Neural and Biomedical Technology Department.

Ang and his colleagues — including Cuntai Guan, formerly with A*STAR and now a professor at Nanyang Technological University in Singapore — previously ran a clinical trial in which stroke patients relearned hand grasping and knob manipulation through combined mental and physical training. Traditionally, patients have had to teach themselves over the course of several sessions to produce predefined EEG rhythms in their brains while performing a motor imagery exercise, but



A*STAR researchers demonstrate that a more personalized method of analyzing brain waves boosts the efficacy of motor imagery exercises for stroke patients.

Ang's team used a newer machine-learning strategy that automatically calibrates the EEG patterns associated with a particular mental exercise to an individual's unique brain waves while engaged in that task.

This strategy helps correct for brain differences between individuals, and avoids the hassle of multiple preparative sessions. However, it doesn't account for the fact that individuals themselves can differ in their brain activity associated with a motor imagery exercise from one rehab appointment to the next.

It is this gap that is filled by the newest adaptive strategy. It works much the same as the machine-learning model, but it doesn't stop at just one calibration session. It keeps updating its model of how the patient's EEG patterns match motor imagery exercises as new information comes in after each and every session, thereby correcting for day-to-day changes in neural connectivity.

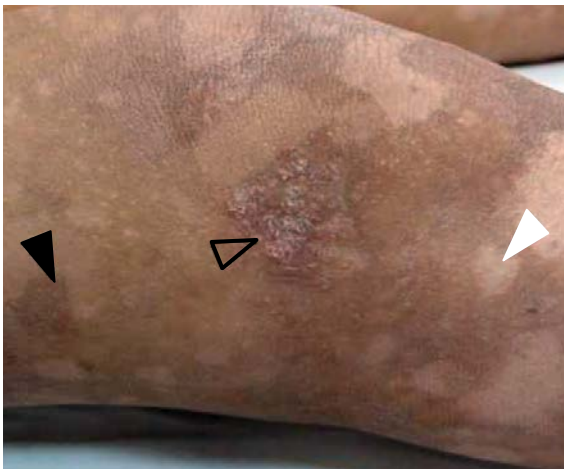
Ang and Guan have retrospectively applied the adaptive model to the clinical data from their earlier trial. As they report in their new paper, this would have significantly improved motor imagery detection in the patients. The researchers have also tested the adaptive strategy on 11 additional stroke patients in another clinical trial last year. "We are now analyzing the results and will be reporting the findings soon," Ang says.

1. Ang, K. K. & Guan, C. EEG-based strategies to detect motor imagery for control and rehabilitation. *IEEE Transactions on Neural Systems and Rehabilitation Engineering* **25**, 392–401 (2017).

GENETICS

MUTATION LINKED TO RARE SKIN DISEASE

Impaired melanin production underlies an inherited pigmentation disorder



A patient with hypo- and hyper-pigmented macules (white and black arrowheads, respectively) and atopic eczema (open arrowhead).

A genetic mutation that affects the production of the skin pigment melanin is associated with a previously unknown form of Cole disease — a rare condition characterized by unusually light-colored and dark-colored skin areas as well as spots of thickened skin on the palms of the hands and the soles of the feet.

A team led by Bruno Reversade at the A*STAR Institute of Medical Biology analyzed the DNA of eight patients from three unrelated families in Sousse, Tunisia, presenting the clinical features of Cole disease. All patients developed the symptoms within the first three months of life and went on to develop eczema.

Until now Cole disease has been reported to follow a dominant pattern of inheritance, which means one copy of an altered gene in each cell is enough to cause pigmentation abnormalities. However, parents and siblings of these eight patients carrying one copy of an altered gene were unaffected, indicating that their form of the disease requires two copies of the altered gene and thus, follows a recessive pattern of inheritance.

The team found that the eight patients had a common mutation in the gene encoding ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1), a protein that regulates the production of extracellular inorganic pyrophosphate (PPi). PPi prevents the accumulation of calcium in the body and has a key role in bone development and the formation of abnormal calcium deposits in soft tissues, including skin.

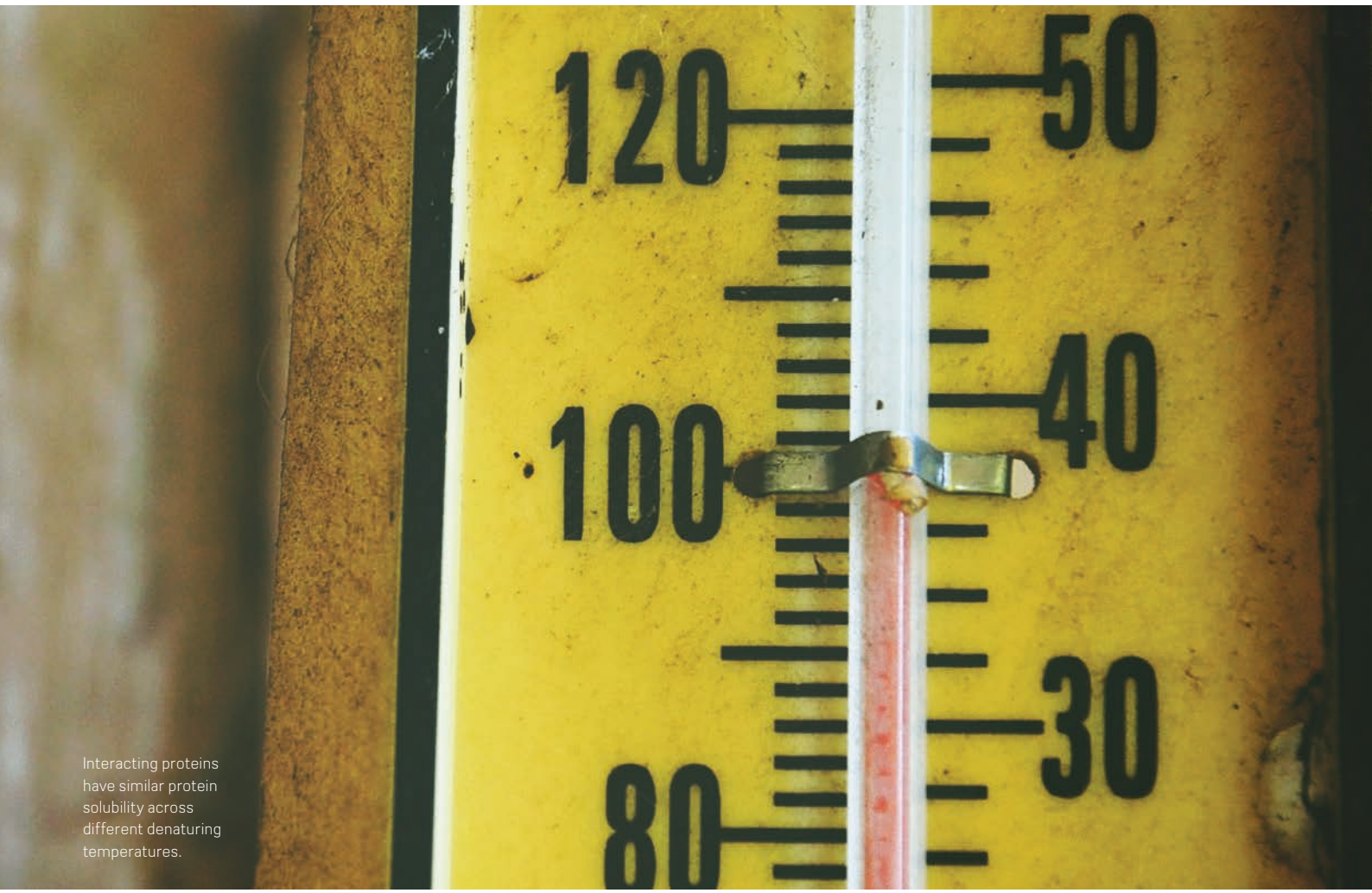
Interestingly, the identified mutation differs from others in the same gene that have been associated with the dominant form of Cole disease. Previous studies have shown that mutations affecting the SMB2 domain of ENPP1 protein disrupt the transport of melanin from the cells in which it is produced (melanocytes) to epidermal cells in response to insulin.

With the help of L'Oréal Research, Reversade and colleagues identified a mutation in a different region of the ENPP1 protein. As Reversade explains: "We found a mutation that affects the SMB1 domain of ENPP1 and interferes with the protein's ability to pair with itself".

Further examination of the skin from a patient and their unaffected sibling revealed that in the patient's skin there was an upregulation of melanin production in the darker skin regions and a downregulation in the lighter colored areas compared with normal skin, indicating that the SMB1 mutation impairs melanin production.

"The association of different ENPP1 mutations with Cole disease suggests that there is a spectrum of skin pigmentation anomalies that share some clinical features, but have different patterns of inheritance and severity" Reversade concludes.

1. Chourabi, M., Liew, M. S., Lim, S., H'mida-Ben Brahim, D. & Boussofara, L. *et al.* ENPP1 mutation causes recessive Cole disease by altering melanogenesis. *The Journal of Investigative Dermatology* **138**, 291–300 (2018).



Interacting proteins have similar protein solubility across different denaturing temperatures.

PROTEOMICS

TURNING UP THE HEAT ON PROTEIN INTERACTIONS

Deep data analytics drive the development of a technique that sheds light on drug action by monitoring the dynamics of thousands of protein complexes simultaneously within intact cells

Protein-protein interactions are at the heart of all cellular functions and biological processes. These interactions are carefully regulated in space and time to meet the cell's requirements and are often disrupted in disease states.

An international study led by Chris Soon Heng Tan at the A*STAR Institute of Molecular and Cell Biology describes a new technique that uses powerful data analytics to infer protein-protein interaction dynamics from the melting behavior of protein complexes inside cells[1].

Methods to capture snapshots of protein-protein interaction networks in cells have been described before, but as Tan explains, "until now there has been no way of monitoring the dynamics of these protein complexes in a high-throughput and untargeted manner".

Exposing proteins to increasing temperature causes them to precipitate out of solution. Thermal proximity coaggregation (TPCA) is based on the idea that proteins that are part of a stable protein complex will coprecipitate, by virtue of close proximity, and have a similar precipitation profile across different temperatures (or melting curve).

In isolation, different proteins are likely to have different melting curves, but

the team showed that in more than 350 well-characterized human protein complexes, the melting curves of interacting proteins are statistically similar. Thus, by quantifying similarity between melting curves, the TPCA method can be used to determine which proteins are likely to interact with each other and form stable complexes across different samples.

"We were quite surprised that the TPCA signatures were so strong and detectable," admits Tan. TPCA signatures were found to correlate with the amount of interaction between two proteins. Accordingly, they show that some complexes change their melting curves depending on the cell type or cell cycle stage, suggesting that TPCA could be used to identify changes in protein interactions under different conditions.

When explaining the advantages of TPCA, Tan says that when compared with current methods, TPCA does not rely on the availability of appropriate affinity reagents, such as antibodies, nor does it require genetic engineering. This allows it to be applied to tissues and clinical samples to identify protein complexes that are driving disease progression and that could serve as potential prognosis markers or therapeutic targets.

The team is already using the technique to study the molecular effects of drugs and synthetic chemicals, and plans to extend the technique to study the progression of selected human diseases.

1. Tan, C. S. H., Go, K. D., Bisteau, X., Dai, L., Yong, C. H. *et al.* Thermal proximity coaggregation for system-wide profiling of protein complex dynamics in cells. *Science* **359,** 1170–1177 (2018).

ONCOLOGY

CLONED TUMORS USED FOR A CANCER CRYSTAL BALL

Growing models of patients' tumors in dishes will allow doctors to optimize individual treatments

A powerful new approach to individualized cancer treatments has been demonstrated by A*STAR researchers. It involves growing micro-models of tumors outside a patient's body, testing their response to various treatments, then applying the most effective ones[1].

Traditionally, medical treatment has been based on a 'one size fits all' mentality, but clinicians are increasingly realizing that patients often respond differently to the same therapy. This has led them to pursue the concept of personalized medicine, which seeks to give the right drug to the right patient at the right dose and at the right time.

To date, personalized medicine has largely been driven by genomics. This means that researchers sequence the DNA of the tumors from many patients, identify key mutations and then develop therapies and drugs that target these genes. While this approach is very important for making new discoveries, it is expensive and takes many years before it benefits patients.

Ramanuj DasGupta at the A*STAR Genome Institute of Singapore and co-workers have now demonstrated a complementary approach that provides information with the potential that can benefit patients immediately.

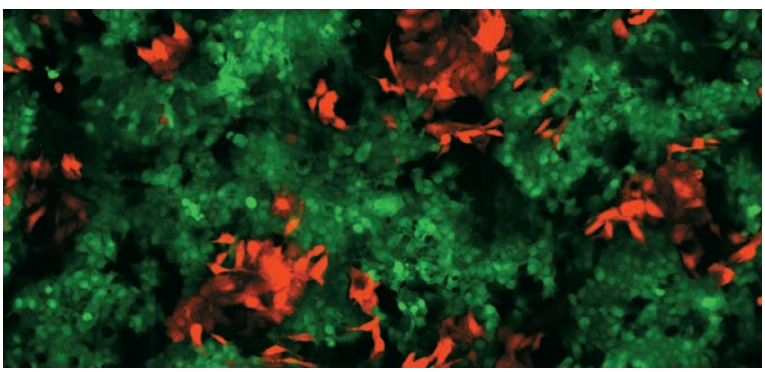
The researchers took tumor cells from 24 patients with head and neck cancers and grew models of the tumors in dishes. They then tested these micro-tumor models to see how susceptible they were to a range of treatments, including chemotherapy, radiotherapy and targeted anti-cancer drugs. Of the 24 patients, two went on to receive tailored treatments in clinical trials. Both patients responded very well to their respective treatments.

These results demonstrate that patient tumors can be used to generate critical information that can help clinicians prescribe the best treatment options. "It is akin to making a cancer 'crystal ball' that can help to predict whether a tumor will respond to standard chemotherapy; how the tumors might progress under treatment; whether the cancer will recur, and, if so, whether alternative treatments can be administered to the patient," explains DasGupta.

The researchers are excited about the potential of their approach. "Our work highlights the tremendous promise of precision medicine for treating cancer," says DasGupta. "Our dream is to make cancer into a chronically managed disease so that patients can enjoy a good quality of life while being treated with the most effective drugs. We strongly believe that our approach will go a long way toward realizing this dream. This is just the beginning."

The team wants to extend the study to include many more patients, as well as finding multiple indicators of cancers.

1. Chia, S., Low, J.-L. Zhang, X., Kwang, X.-L., Chong, F.-T. *et al.* Phenotype-driven precision oncology as a guide for clinical decisions one patient at a time. *Nature Communications* **8,** 435 (2017).

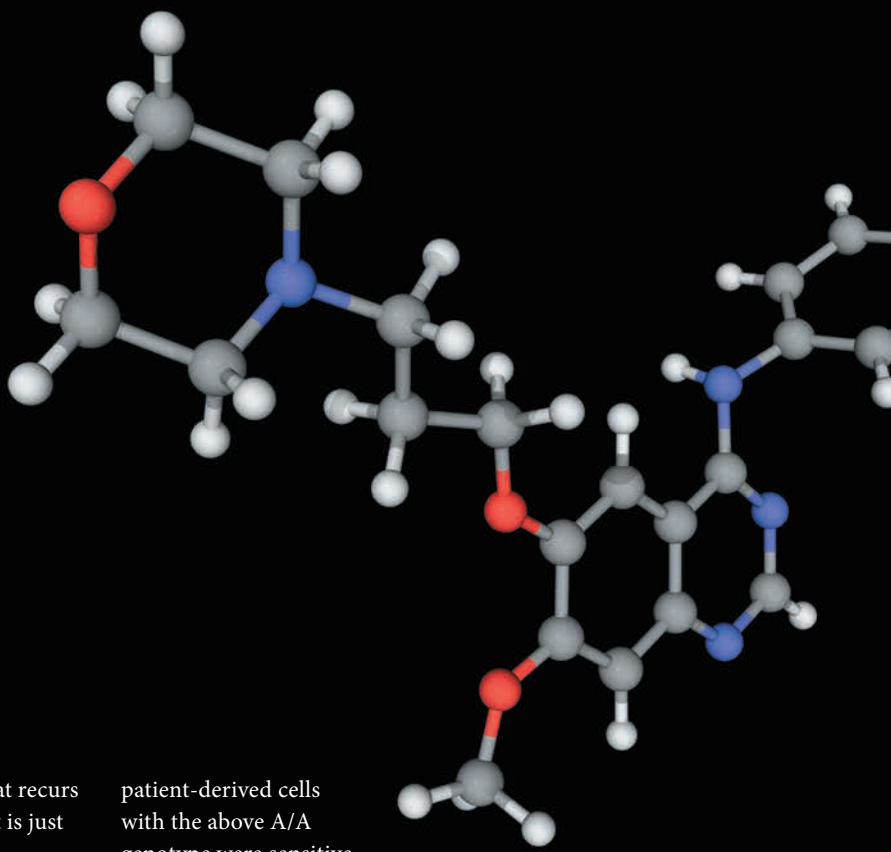


The expression of different proteins stained in red and green indicates the variation in the make-up of a patient-derived model of a tumor. Such models can be used to determine the most effective treatment for individual patients.

CANCER BIOLOGY

THE MOLECULAR PATH TO BETTER ORAL CANCER CARE

A better understanding of how genetics influences responses to mouth cancer drugs could lead to improved treatment



A single letter DNA mutation is a big determinant of whether patients with advanced oral cancer respond to treatments. Researchers from the National Cancer Centre Singapore (NCCS) and A*STAR who uncovered the mechanisms behind this effect hope their findings will help doctors target treatment more effectively.

Oral squamous cell carcinoma (OSCC) is characterized by the uncontrolled growth of thin, scale-like squamous cells in the outer layer of the mouth. Only around 50 per cent of patients who are treated through surgery or radiotherapy are cured, and the average duration of survival of those with advanced OSCC that recurs following treatment is just six to nine months.

Epidermal growth factor receptors (EGFRs) play important roles in driving the progression of some OSCCs. Drugs that target them, however, only work in a small number of patients.

A 2012 clinical trial led by Daniel Tan at NCCS and A*STAR's Genome Institute of Singapore had found that the EGFR-blocking drug gefitinib worked well in two patients with two copies of the EGFR coding gene with an adenine (A) nucleobase in place of the more common guanine (G) at a particular location.

More recently, tests by Gopal Iyer, also at NCCS, and Tan showed that OSCC patient-derived cells with the above A/A genotype were sensitive to gefitinib and erlotinib, another EGFR blocker. Those with the G/G or G/A variants exhibited resistance to the drugs.

> "We were pretty surprised it had such a dramatic effect."

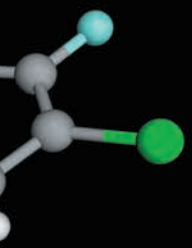
Editing the DNA of the G/G genotype cells to become G/A at the same location increased their sensitivity to the drugs 70-fold. "We were pretty surprised it had such a dramatic effect," says Iyer.

The genetic mutation occurs in a section of DNA that modulates the stability of a long non-coding RNA (lncRNA) known as EGFR-AS1. Gene expression tests showed that levels of this lncRNA were significantly higher in G/G genotype cells than in A/A cells.

When cells with the G/G genotype were exposed to small interfering RNAs that reduced their production of EGFR-AS1, their sensitivity to EGFR-blocking drugs increased significantly.

They also found that the tumors of seven patients with the A/A genotype shrank following treatment with EGFR-inhibiting drugs.

While the mechanism underlying this effect is not fully understood, the group



An illustration of the gefitinib drug molecule.

has demonstrated that cells of the G/A and A/A genotypes produced higher ratios of one of four variants of EGFR relative to another, and that EFGR-AS1 helps mediate this difference.

Iyer said that new RNA-interference therapies could be developed to target cancers dependent on EGFR signaling. The group is conducting a larger human trial to better understand the biomarkers that could provide for improved targeting of existing treatments.

1. Tan, D. S. W., Chong, F. T., Leong, H. S., Toh, S. Y., Lau, D. P. *et al.* Long noncoding RNA EGFR-AS1 mediates epidermal growth factor receptor addiction and modulates treatment response in squamous cell carcinoma. *Nature Medicine* **23,** 1167–1175 (2017).

CATALYSIS

POROUS SILICA PROTECTS NICKEL CATALYST

Nanoparticles avoid deactivation and convert biomass-derived gas into methane

By wrapping nickel nanoparticles in a protective shield of porous silica, A*STAR researchers have developed a highly active and robust catalyst that could help to produce methane from biomass[1].

Biomass is a potentially carbon neutral feedstock to make fuels or other useful chemicals. Through a process called gasification, biomass is converted to a mixture, known as syngas, comprising carbon monoxide, carbon dioxide and hydrogen. Syngas can be turned into a range of other chemicals, including methane, which may be used as a transportation fuel or town gas, or burned to generate electricity.

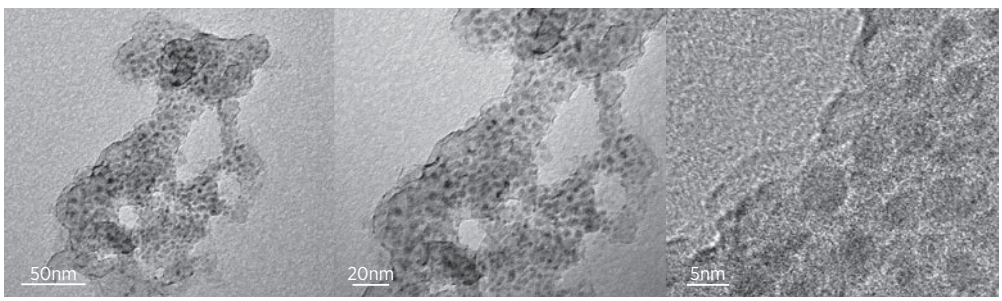
Various catalysts convert syngas to methane. Nickel is one of the most common, due to its high activity and moderate cost, and it is typically supported on another material such as alumina or silica. But the catalyst can become deactivated during this high temperature methanation reaction, either through a build-up of carbon called coking, or by a process called sintering in which catalyst particles clump together. Moreover, any traces of sulfur compounds in the syngas can very quickly switch off nickel's catalytic activity, so syngas must go through an expensive cleaning process to remove sulfur before methanation.

Luwei Chen of the A*STAR Institute of Chemical and Engineering Sciences and colleagues have now embedded nickel nanoparticles in porous silica, which allows gases to access the catalyst, but prevents the problems that cause deactivation.

They prepared the catalyst by mixing particles of nickel hydroxide with tetraethyl orthosilicate. After further processing, they activated the nickel by reacting it with hydrogen at 600 degrees Celsius, forming particles that contained about 40 per cent nickel by weight. The researchers tested their catalyst with syngas derived from a gasification process, and with a simulated syngas, both of which contained sulfur. Using techniques such as transmission electron microscopy, X-ray diffraction and thermal gravimetric analysis, they found that the catalyst experienced very little sintering or coking during the reaction, unlike a commercial catalyst that was tested using the same syngas samples. "Porous silica protects by isolating each particle, to prevent sintering," says Chen.

The nickel-silica catalyst also withstood sulfur impurities for three times as long as its commercial rival before deactivating. Improving the sulfur resistance of the catalyst in this way could lead to significant cost savings in the syngas cleaning process. The researchers are now collaborating with IHI, a Japanese engineering company, to scale up their synthesis of the catalyst, and the methanation process.

1. Kamata, H., Tian, Z. Q., Izumi, Y., Choong, C. K. S., Chang, J. *et al.* Dispersed and high loading Ni catalyst stabilized in porous SiO_2 matrix for substituted natural gas production. *Catalysis Today* **299,** 193–200 (2018).



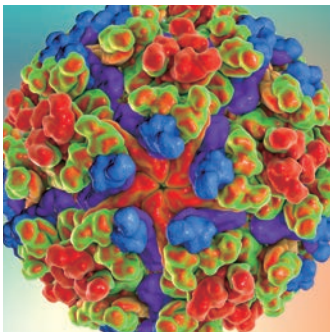
Transmission electron microscopy images of (left, center) nickel-silica catalyst and (right) a commercial catalyst.

NEXT ISSUE

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

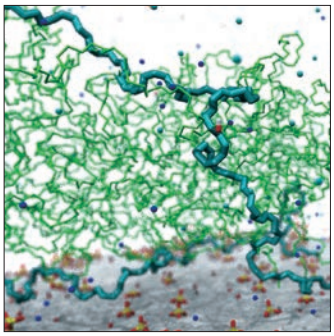
POWER SOURCES
SHEDDING LIGHT ON TOMORROW'S SOLAR ENERGY

Improved forecasting of sunlight could help increase solar energy generation



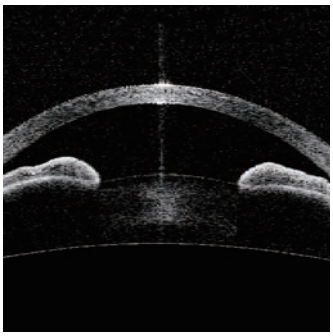
INFECTIOUS DISEASES
DOUBLING DOWN ON INFECTION

A new understanding of the way chikungunya virus protects mice against malaria could lead to improved patient care



CONCRETE
A HARD LOOK AT POLYMERS IN CEMENT MIX

Computer simulations have zoomed in on the role of superplasticizers in concrete performance



GLAUCOMA
A BIRD'S EYE VIEW FOR IMPROVED DIAGNOSIS

An algorithm paves the way for accurate, rapid diagnosis of closed-angle glaucoma

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X-ray sources used in medicine and other industries have remained virtually unchanged for over a century. Leveraging the unique properties of novel 2D materials, Dr. Wong Liang Jie and a team of collaborators have conceived a method to generate intense, continuously tunable X-rays on a microchip scale. The laser beam-like quality of the X-ray output also allows for more precise pinpointing of medical and dental X-rays, enabling lower dosages and leading to safer, more efficient and less costly X-ray sources in the future.

Dr. Wong Liang Jie

Scientist, Singapore Institute of Manufacturing Technology
PhD and Postdoctoral Fellow, Massachusetts Institute of Technology

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