# ASTAR ESSUE 10 | January - March 2018 ASTAR ESSUE 10 | January - March 2018

# UNLOCKING GRIDLOCK

Clearing the road for driverless cars

#### METFORMIN REPURPOSED

Diabetes stalwart could be adapted for cancer treatment

#### ON THE RECORD

Power-assisted crime fighting

#### ALLERGY EPIDEMIC GRIPS ASIA

Singapore's hay-fever levels not to be sniffed at



A\*STAR Research is a publication of the Agency for Science, Technology and Research (A\*STAR) — Singapore's lead government agency for fostering worldclass scientific research.

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#### **Editorial**

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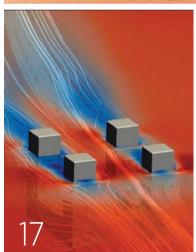
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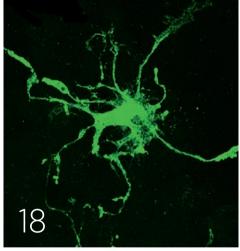
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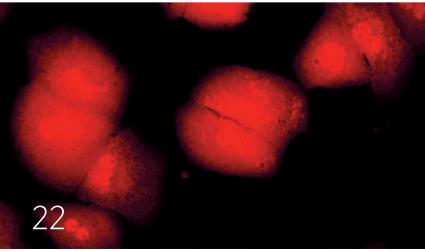
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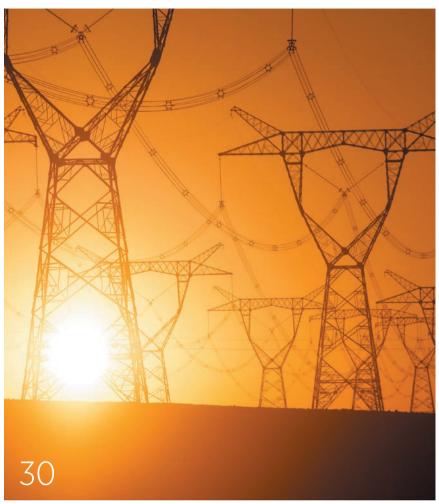
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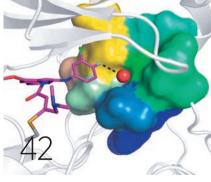
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## NOTES FROM THE EDITORS

Editorial board member, Arseniy Kuznetsov, introduces the latest issue of *A\*STAR Research* 

elcome to the second edition of *A\*STAR Research* for 2018, where we showcase some of the latest innovations and research from *A\*STAR*.

More than half of the world's population lives in urban areas — a proportion that is expected to grow to two-thirds by 2050. With overcrowding comes traffic jams and gridlocks, but smarter transport systems that are being developed and tested in Singapore could overcome these problems (read more in our cover story on page 8).

Several exciting inventions have also emerged from the field of nanophotonics and plasmonics. These include arrays of resonant dielectric papostructures

called metasurfaces, that could eventually replace bulky glass lenses, enabling higher imaging resolution and providing an excellent compact and lightweight solution for mobile device cameras and head-mounted displays (page 49). Another example is a new ultra-high resolution printing method based on resonant silicon nanostructures, which can now achieve a spread of colours beyond standard RGB (page 51). Additionally, a team of A\*STAR and NUS researchers have developed transducers that could transfer data directly from electrical signals to plasmonic circuits and back, avoiding speed reductions from electro-optical components (page 43).

Major advances have been made in medicine as well — including two

discoveries that have important implications for fetal and maternal health: the discovery of a hormone that could treat a dangerous complication of pregnancy (page 40), and the unravelling of mysteries surrounding the fetal immune system (page 35).

We also have a full-length feature that examines a health issue that affects many of us — allergies, and a common culprit that lurks in our homes (page 4). Another inflammation-related article reveals a surprising role for gut bacteria in chronic viral hepatitis, which may influence future treatments (page 20).

As usual, there are many more interesting stories in the rest of the magazine, and I hope you will enjoy reading them.





#### COVER IMAGE

Singapore is an ideal test bed for intelligent traffic innovation. [page 8] © RETOKA

olin Anderson/Photographer's Choice RE/G

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With dust mites likely causing allergic rhinitis symptoms in an enormous 40 per cent of Singapore's population, research in the city state is yielding some very interesting insights into the role of climate, genetics and childhood

People living in countries within the tropical belt (Singapore, Malaysia, Indonesia and others) with comparable climates showed similar evidence of exposure to two species of dust mite, Dermatophagoides pteronyssinus (right) and Blomia tropicalis.

irst, the bad news:
There's no simple cure
for allergies...
at least for now.
One of the most
widespread, allergic rhinitis
(or hay fever) affects as much
as five per cent of the world's
population. The hallmarks
are sniffling, itching and
sneezing, and it strikes
indiscriminately, regardless of
age or ethnic background.

No-one knows why some people develop this common allergy and not others, but scientists in Singapore have recently found some interesting clues in cell responses and childhood experiences.

Allergies are essentially glitches of the immune system. The human body responds to some usually harmless material by activating an immune response that releases a number of substances. The main culprit, histamine, sparks inflammation and is responsible for the most irritating symptoms, including itching and swelling.

In spring, people often suffer from what is known as seasonal or intermittent allergic rhinitis — triggered by pollen from trees and grasses, as well as fungal spores. Less seasonal is the allergic rhinitis triggered by dust mites, animal dander (such as pet fur), mold and cockroaches. The latter can be a huge burden

on busy cities in tropical climates — environments in which dust mites thrive.

**FEATURES** 

In fact, allergic rhinitis could be having a hefty effect on productivity worldwide. Roughly eight per cent of adults experience allergic rhinitis in the United States, while other national surveys have estimated prevalence rates of about six per cent in France and 29 per cent in the United Kingdom. More than just an irritation, common symptoms of allergic rhinitis are also loss of sleep, irritability and fatigue - all of which have an effect on performance at work and school.

#### **EIGHT-LEGGED CULPRIT**

A survey of 8,000 adults in Singapore published in *Allergy* in 2014 found an astonishing 40 per cent of those surveyed reported symptoms of allergic rhinitis.

In fact, by testing the blood of nearly 600 people, the team found a staggering 80 per cent of Singaporeans had antibodies, called Immunoglobulin E (IgE), that are specific to house dust mites.

Two species of dust mite that are often found in mattresses, bed linen, carpets and upholstered furniture were implicated: *Dermatophagoides* pteronyssinus and Blomia



An A\*STAR study has suggested that wheezing and eczema in infancy are correlated with having an increased risk of sensitization to airborne allergens, including allergic rhinitis (hay fever), later in childhood.

75% of Singaporeans have allergen antibodies

40% of Singaporeans show allergy symptoms

tropicalis. The unpleasant truth is that people are not allergic to the mites themselves, but their fecal matter.

"To verify our findings, we looked at a much larger sample size of more than 7,000 volunteers," says Anand Andiappan, a senior research scientist at A\*STAR's Singapore Immunology Network (SIgN), who worked on the study

with other colleagues at SIgN and collaborators at the National University of Singapore (NUS).

"Astonishingly, we found the figure was similarly high: 75 per cent of this larger group had antibodies against house dust mites." That means that three-quarters of Singaporeans potentially have a dust mite allergy. As part of the 2014 study, the researchers looked closely at the ethnic Chinese portion of respondents and found that climate, rather than genetics, appears to have the most impact on sensitization. Andiappan notes: "People who had just arrived in Singapore from mainland China seemed to have a much lower prevalence of the house dust mite allergy and allergic rhinitis symptoms compared to those who had stayed more than eight years." In some cases, he says, the difference was evident after just three years of living in Singapore.

By contrast, there was no significant difference in reactivity to dust mites between Chinese people from Malaysia and those born in Singapore. This suggested that climate is a significant factor in determining exposure levels and allergic response: People from countries in the tropical belt with similar climates — Singapore, Malaysia, Indonesia and others — show similar reactivity to dust mites, suggesting they had been exposed before. People from temperate climates, such as mainland China and Hong Kong, generally become more sensitive to house dust mites the longer they stay in Singapore.

Dust mites
thrive in the hot and
humid conditions
that are the norm
in tropical zones. In
temperate climates,
although they can
be a problem
in the

summer, they usually die off during the drier winter months.

To Olaf Rötzschke, a principal investigator at SIgN who is now leading the most comprehensive adult allergy cohort study conducted in Singapore, these findings were nothing short of "spectacular". He began to look more closely at the problem.

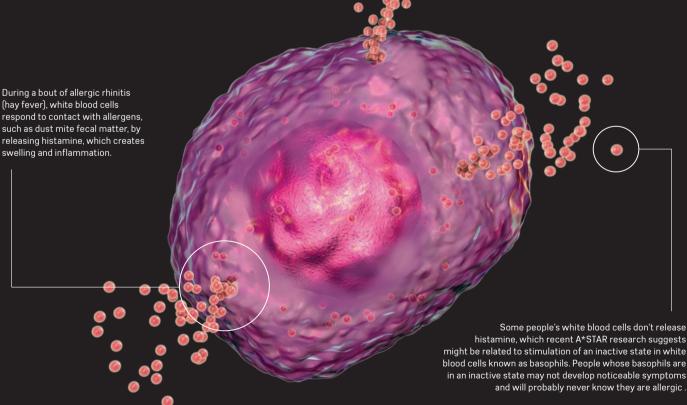
Usually, Rötzschke says, allergy studies are complicated by the sheer number of factors

that

could be involved, such as pollen, fungi and animal dander. "The advantage here in Singapore is that we have a perfect environment for analysis because the problem-causing allergen [dust mites] is so specific."

"We started out with a big exploratory study — we weren't even focusing on allergies at the beginning," says Rötzschke. "We effectively cast a net, and this fishing approach enabled us to build a huge database, so we could really dive into exploring immune responses and genomics."

Rötzschke points out that urbanization and lifestyle changes — namely, spending more time in indoors — may be the most significant factors behind an overall increase in the prevalence



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allergies in recent decades.

This tendency to stay in dust mite friendly environments is not limited to Singapore. "What we are seeing here will become more relevant to other countries," Rötzschke says. "So, I consider this a unique chance for us to become a world-leading center for allergy research."

#### MANY WON'T KNOW THEY'RE ALLERGIC

One intriguing conclusion drawn from Andiappan's 2014 study was that not everyone who tests positive for the house dust mite allergy (roughly 80 per cent) goes on to have symptoms (roughly 40 per cent).

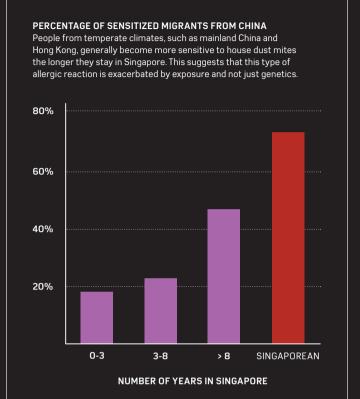
In 2017, Rötzschke and his team made a breakthrough on protection from allergic rhinitis based on a phenomenon called basophil anergy.

Basophils are cells in the blood that detect allergens and trigger allergy symptoms. In some cases, these basophils become inactive. This so-called 'anergy state' correlates with people who don't develop allergic rhinitis, which could be one explanation for why some people whose Immunoglobin E antibodies levels would suggest that they are allergic, don't develop symptoms.

Rötzschke says that it's possible that this process of shutting down basophils could provide a mechanism for targeted treatment of allergic rhinitis that involves "switching off" nasal allergies.

#### **EARLY RISK FACTORS**

No one knows yet if treatments targeting basophils will become a reality, but some people can



already train their immune system to respond better through exposure to small doses of an allergen over long periods. This method, called allergenspecific immunotherapy, is widely considered to be the best treatment option for allergies.

To give sufferers the best chance of benefitting from such treatment, more studies are focusing on immune response in early childhood to find ways to predict the likelihood of developing respiratory allergies later in life.

For example, a study in Sweden has shown that onset of eczema in the first year of life was linked to increased risk of developing asthma and allergic rhinitis later on. In the first large-scale study of its kind in Singapore, Evelyn Loo, co-workers at the Singapore Institute for Clinical Sciences and collaborators at NUS, among others, found that wheezing and eczema before 18 months is associated with an increased risk of senzitisation to allergens, including house dust mites, at three years of age.

They conclude that the common long-term effect of impaired airway function caused by early-onset wheezing or eczema might make children more vulnerable when exposed to allergens in later life.

The earlier people pick up on dust mite allergy symptoms, the better, agrees Andiappan. Most adults unfortunately seem to be self-diagnosing, he says, and ignoring symptoms or taking over-the-counter antihistamines, which only offer short-term relief. "As the house dust mite trigger doesn't immediately lead to severe reactions, people tend to be less cautious about it."

It's important Andiappan advises, to get yourself properly diagnosed via a skin prick or a blood test. "If you're constantly exposed to the allergen over a long period of time, it could affect disease treatment adversely, so our recommendation is early diagnosis."

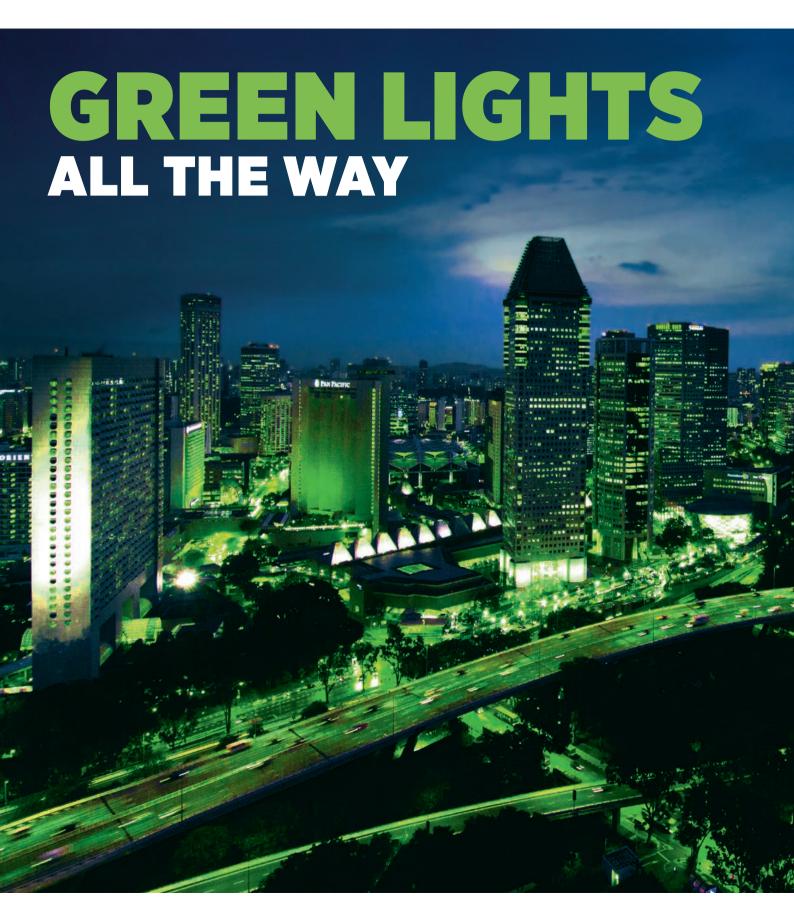
Following up with the group of people who 'carry' the allergy without adverse effects will be another crucial step for clinical research. This is where the study of epigenetics — changes in gene expression that do not involve changes to your DNA — will become increasingly important.

A curious side note is the fact that Rötzschke himself says he does not have allergic rhinitis, even though he has been based in Singapore for the last decade after spending many years in temperate climes both in Europe and the in US.

"I fall exactly into that area where I'm supposed to have a response to house dust mites, but I don't. Why am I not allergic?" he asks. "It may be that my genetics renders my basophils inactive." This broad question is among the many things he and his team will investigate next.

For references, visit the online version of this article at: https://www.research.a-star.edu.sg/ feature-and-innovation/7832

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Taxis utilized to their full potential, intersections with no traffic lights and devices that eliminate driver error — anything is possible in Singapore, one of the world's busiest test beds for intelligent traffic

e could increase traffic efficiency by up to 45 per cent," Yang Bo says, discussing a futuristic intersection without traffic lights he modeled at A\*STAR's Institute of High Performance Computing (IHPC) last year. Creating smoother traffic flows could also save millions of liters of fuel that would otherwise be consumed globally by vehicles idling at traffic lights each day. Indeed, a lot of fuel and commuter time could be saved by implementing the many ideas being modeled at the IHPC, including several to improve the 'hive brains' guiding the movements of taxis and ridesharing vehicles.

The IHPC is fortunate, as Singapore is a major test bed for intelligent transport systems. Its roads pulse with information and subtle adjustments: smart intersections vary their cycles according to the flow of traffic and intelligent ramp meters note the density of cars, while a congestion tax, politically contentious in other cities, is collected by electronic gantries daily. This is all managed by the city's Land Transport Authority (LTA) — who has 164 kilometers of expressways and road tunnel systems wired for data collection and video surveillance. The information is fed to operators who smooth flows and send assistance to motorists in trouble. Because of this coordinated effort, Singapore — one of the densest cities in the world — sits at a comfortable number 55 in TomTom's congestion world ranking, well behind less populous cities such as Sydney, San Francisco and Auckland.

#### ELIMINATING TRAFFIC LIGHTS FROM INTERSECTIONS

Yang proposed his lightless intersection in 2017, along with a colleague, Christopher Monterola. Cars would continuously move through these junctions, and while human drivers still steer, acceleration, deceleration and interactions with other cars are controlled by a beacon installed on the dash and at the intersection. He says efficiency gains are found in reducing redundant waiting for light changes and human factors such as "phantom traffic", in which drivers slow unnecessarily at the fringes of a congestion, with rippled effects slowing cars upstream.

It's a response to a hopeful, but uncertain future as autonomous vehicles begin to penetrate the market, Yang says. "The question is: How do we make a new system that has the least-required modification to vehicles until we fully implement driverless cars?" While tech behemoths such as Didi and Google race to develop systems to guide driverless cars through intersections, Yang says

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the aim of his model is to make something practical today and for the anticipated "long transition period" to fully driverless technology. "If you have a high technological barrier, say you have a system that needs an autonomous vehicle, it's going to take a long time to implement, because cars that aren't driverless will still be on the roads," he points out.

In 2016, the world's first driverless car policy emerged from the United States and outlined five levels of autonomous vehicle ranging from regular cars with driver assistance (Level 1) to fully automated machines with no steering wheel (Level 5). At

its simplest, Yang's intersection only require that vehicles to be at Level 1 — the intelligent car beacon could take over some aspects only in the intersection 'zone' and would protect passengers using an algorithm that was designed to create a mathematical repulsion between vehicles.

Because the zone would be

highly localized, this system is also easy to implement and requires minimal energy to run. Yang adds that his modeling of the intersection sees overall efficiently gains even if today's cars (he calls them 'legacy cars') make up 70 per cent of the traffic. He explains that for today's vehicles, these

shanghaiface/Gett



intersections mean using a system similar to when a driver stops and waits for the right moment to cross when he or she sees a stop sign. The caveat is "it will be a little slower for older cars, but that will encourage people to upgrade."

Without real-world implementation and data to account for vehicle-pedestrian interaction, Yang's intersection is still theoretical. But in Singapore, ideas like this can become reality; the city's advanced thinking on the road means it has bucked dominant world trends and actually improved its congestion levels in recent years.

The red carpet is also being laid out for companies diving into the autonomous vehicles market by the Singapore Autonomous Vehicle Initiative (SAVI), a joint partnership between the LTA and A\*STAR. They see Singapore as the logical home for the world's driverless statutory and technical firsts. At the moment, there are only a handful of official city-based driverless trials in the world; this year Singapore will host both the world's first driverless taxi trials for the MIT spin-off nuTonomy and commercial driverless car tests for electronics and software company, Delphi Technology.

#### **OPTIMIZING TAXI ROAMING**

This forward-thinking approach to its roads is typical. For decades now, Singapore's government has been extraordinarily clever with its road data. For example, each of the city's more than 20,000 taxis are required to transmit its GPS location and working status every 30 seconds to the LTA, so that it can monitor a key mode of transport in a city where fewer than one in 10 own a private car. Some of this data is also being harnessed by the IHPC to come up with ways to improve taxi occupancy.

In 2016, a team led by Qin Zheng, a senior scientist also at the IHPC, started by looking at a week's worth of taxi data. To examine the 3.6 million-odd data points, they worked with the Fujitsu-SMU Urban

Computing and Engineering Corporate Lab in a collaboration between A\*STAR, technology giant Fujitsu Limited (Fujitsu) and the Singapore Management University (SMU). Zheng's team used the taxi data to train a learning neural network they devised — called Fusion Architecture for Learning and Cognition with Alternative MemorY (FALCON.Amy) to predict pick-up hotspots for taxi drivers.

#### TIMES CHANGE FOR TAXIS

Singapore's average daily taxi ridership fell to 785,000 last year, an 18-per cent drop from 2016 according to data from Land Transit Authority.

AVERAGE DAILY TAXI RIDERSHIP



However, the results of a survey published by the Public Transport Council in February show satisfaction with taxi services has improved despite lower ridership. The number of commuters satisfied with taxi services rose 4 per cent between 2016 and 2017, and the mean satisfaction score increased by 0.4 points.

#### MORE COMMUTERS SATISFIED



2017

At peak demand, these street pickups become much more efficient than bookings systems, which include disruptors such as Uber and Asian counterparts, Grab and Didi. IHPC and other studies have shown that in high-demand conditions,

vehicles will often pass a number of potential passengers to get to a booked passenger.

The IHPC collaboration soon spawned an app using FALCON. Amy, called the Driver Guidance System (DGS). Within the year, the app was already directing taxis to roads where they're likely to find a fare, and being used in the National Taxi Association (NTA) SkillsFuture Training Programme.

Zheng says Singapore's taxi association is big supporter of the project. "They are very anxious to improve technology for their drivers," he says. While reducing empty taxi roaming alleviates congestion by decreasing unnecessary road use, it could also boost profitability for taxis struggling in an era when disruptive technologies are cutting into their bottom line.

The DGS was primed to predict fare locations using roughly two years' worth of taxi data. Numbers from a free trial for taxi drivers showed significant improvements for cab drivers during off-peak hours; those using the app decreased their average empty roaming time between fares in the city between midnight and 6am from 17 to 12 minutes.

Early feedback, collected through discussions with drivers who participated in the trial, was also predominantly positive, particularly for new taxi drivers who don't already know where to find fares. However, some taxi drivers reported a reluctance to miss out on booking fees.

This doesn't bother Zheng. While, it seems that companies, such as Uber and Grab are here to stay for the mid-term, he says the long-term thinking is that they will be used for another five to ten years, after which the city will move to driverless taxis.

"Imagine there are no drivers; then all we want is to optimize the social benefit," he says. In that future scenario, systems, including the one Zheng's team trained for this project, will be less constrained by individual drivers' needs and could form a part of very efficient future traffic systems run largely by advanced algorithms and artificial intelligence.

However, in countries such as Fujitsu's country of origin, Japan, the data they need to train a system like Zheng's will have to be drawn from a wider variety of stakeholders than in Singapore, including a number of different taxi or transport companies. But, while Singapore has one of the world's most centralized traffic data sources, the base information needed to run Zheng's program exists in any city with GPSlinked taxi-like services. "If we can get hold of Uber or Grab data we can apply it," he says.

#### **COULD THERE BE 60 PER CENT FEWER TAXIS?**

Yang adds that increasing vehicle sharing has the potential to further winnow traffic. One 2015 study showed that in New York, assuming people were willing to share taxis whenever possible, taxi demand could be reduced to just 15 per cent of the current fleet.

In Singapore, says Yang, if a ride-sharing algorithm he and his colleagues have developed were to be adopted by 50 per cent of taxis - in a system in which more than one person is an individual paying passenger - the 15,000 taxis on the road at any one time could be reduced to 6,000.

But he's quick to explain that this number needs to be taken with a grain of salt: "We're



Various innovations may lighten traffic by reducing the numbers of taxis needed on the road. However, the financial returns for taxi drivers could also increase if they are able to carry multiple, separately paying passengers at once and are able to be occupied for a greater percentage of the working day.

still in the process of looking at lots of information, particularly traffic conditions and commuter boarding and alighting behaviours." That's their next step, he says, "adding more and more information to make the simulation more accurate." Yang also points out that "the tricky thing about Singapore it is that it's a relatively rich society, so people don't really want to give up their privacy to save a few dollars."

But Yang's work shows that ride-sharing taxis may find a foothold during peak hours and bad weather, when passengers are faced with surges in

demand. His model suggests that during busy periods, gains in shorter wait times could far outweigh the time cost of accommodating another taxi passenger, not to mention the real cost savings.

Taking all of those taxis off the road will also reduce traffic and travel times, says Yang. But those benefits will only become evident if advanced modeling like the IHPC's "can convince policy-makers to cultivate a culture of ride sharing", he says. "Our results show that a good algorithm for taxi ride-sharing can really

"Our results show that a good algorithm for taxi ridesharing can really help."

help," he explains, "because increased ride-sharing is not only for the common good, but can also be immediately helpful to individuals as well."

A culture of hyper-organized solutions has always been Singapore's strategy for dealing with limited space. As the world approaches a watershed for driving technology, a dense and still fast-growing population means that Singapore is incentivised to remain the world's most cutting-edge place for intelligent traffic.

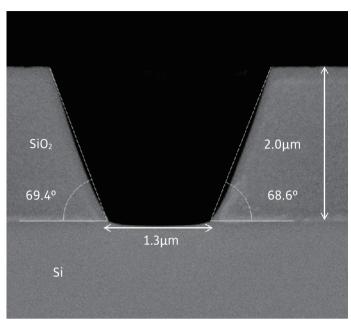
The government also continues to invest heavily in advanced technology: in 2016, for example, the LTA announced a new S\$556 million (US\$423 million) project to install units into all Singaporean cars so a satellite tracking system can determine congestion fees and automatically deduct charges for curb-side parking. Driverless cars are perhaps still a decade away, but Singapore and its researchers are positioning themselves as front runners in the road race towards the smartest cities of the future.

For references, visit the online version of this article at: https://www.research.a-star.edu.sg/ feature-and-innovation/7831



## A BETTER ANGLE ON MICROSCOPIC DEVICES

A flexible, low-cost technique could lead to the mass production of microelectromechanical systems for use in a range of applications



Tapered contact opening fabricated by new two-step plasma etching process.

Making increasingly small microelectromechanical systems (MEMS) has proved very challenging, limiting their anticipated potential. Now, researchers at A\*STAR have developed a versatile and cost-effective technique for making devices with much greater precision and reliability for use in biotechnology and medical applications.<sup>1</sup>

MEMS are used in applications ranging from airbag systems and display screens to inkjet cartridges. They are tiny devices that combine mechanical and electrical components. Current manufacturing technologies, however, are expensive and lack the precision for making devices with micron and sub-micron scale features.

This led Vladimir Bliznetsov and colleagues from the A\*STAR Institute of Microelectronics to develop a versatile and low-cost method for fabricating MEMS at dimensions not previously possible, edging closer to the mass production of smaller and more reliable devices for a range of new applications.

Combining these processes into a two-step method achieves greater control over the etching process and produces micron-sized vias with smooth, tapered walls.

"MEMS are following the general trend of miniaturization in electronics, with devices that are reducing in size from the tens of microns to one micron or less," says Bliznetsov. "But the techniques used to manufacture top metal contacts to devices with such microscopic dimensions are costly and unreliable."

Current methods for creating channels, called vias, with tapered sidewalls in MEMS

at scales of five microns or less, are not dependable. The etching method, with sidewall polymerization, is not suitable as the width at the bottom of the vias shrink considerably. Another method, involving the transfer of a photoresist profile into an etched layer, limits the maximal depth of vias due to excessive loss of the photoresist mask resulting in unacceptably rough sidewalls.

To overcome these restrictions, the researchers developed a two-step plasma etching process. This combines firstly photoresist tapering, which modifies the photoresist from a vertical to a tapered profile, and then an oxide etching with sidewall polymerization with better selectivity to photoresist. This produces vias with minimal dimensions down to 1.5 microns, and smooth sidewalls with angles of around 70 degrees.

"We combined two effects which are usually harmful

during the etching process — accelerated corner sputtering and sidewall polymerization," explains Bliznetsov.

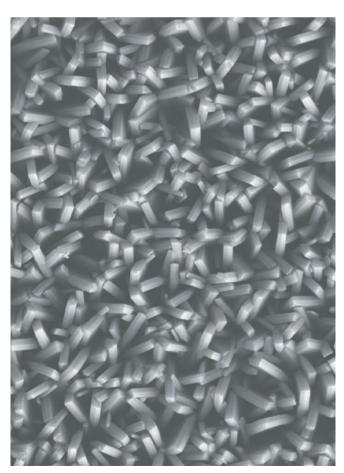
Combining these processes into a two-step method achieves greater control over the etching process and produces micronsized vias with smooth, tapered walls. And by minimizing the sidewall angle of the vias, it is possible to fabricate devices that can be better protected and have more reliable metal contacts, greatly improving their performance.

"Precise control of sidewall angle has use in many applications, and we are now planning to fabricate functional magnetic memory cells, which require pillars of magnetic material with sidewalls having a specific angle," says Bliznetsov.

Bliznetsov, V., Li, B., Lee, J. W., & Lin, H. MEMS industry-worth etching to fabricate tapered structures in SiO<sub>2</sub>: Journal of Microelectromechanical Systems 26, 1400–1407 (2017).

#### **TAKING A STAB AT MICROBES**

Arrays of tiny, rigid, and sharp pillars mimic natural antimicrobial surfaces by binding and breaking bacterial cells open



A\*STAR researchers have developed positively charged nano-dagger arrays (pictured) that effectively kill microbes, such as E. coli and Staphylococcus aureus.

A powerful solution to the global spread of antimicrobial resistance could soon become available, thanks to A\*STAR researchers, who have come up with a physical and environmentally friendly alternative to biochemically active antibacterial agents.

Typically transmitted by contact with contaminated surfaces, bacterial infections pose serious health threats in medical settings. Small molecular antibacterial agents, which are commonly used in antiseptics, disinfectants, and preservatives, and other consumer care products, can prevent cross-infection by annihilating bacteria on frequently touched surfaces. However, their overuse contributes to antimicrobial resistance. These toxic and persistent substances can also harm the environment by disrupting the ecological balance of soils and endangering aquatic life.

In response to this, Yugen Zhang and Yuan Yuan, from the Institute of Bioengineering and Nanotechnology have developed nanostructured surfaces that destroy bacteria through physical, rather than biochemical interactions. These surfaces mimic the antimicrobial patterns formed by ultra-small pillars on cicada wings. "In addition to being clean and safe, this technology does not require externally applied chemicals," says Zhang.

The researchers added a zinc-based solution to various surfaces, including rubber, glass, wood, and metal foil, then immersed the surfaces in an aqueous solution containing amine-rich 2-methylimidazole to form a so-called zeolitic imidazolate framework coating. The coating consisted of an array of tiny, positively charged dagger-like crystals that grew perpendicularly to the substrates.

"We used inexpensive materials and a simple method to create this nano-dagger structure on different types of surfaces," says Zhang noting that his team had to try numerous formulas before finding the right growth conditions.

Regardless of the coated substrate, the nano-dagger arrays effectively killed the antibiotic-resistant bacteria Escherichia coli and Staphylococcus aureus as well as the fungus Candida albicans, demonstrating their broad applicability. They also retained their antibacterial activity when exposed four consecutive times to E. coli over two months, proving their durability.

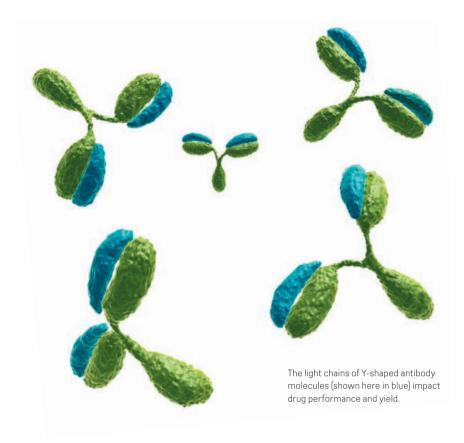
According to Zhang, the positive charges positioned on the nano-daggers first attract bacterial cells that bear negatively charged membranes, making them stick to the coated surface. Next, the sharp nano-dagger tips rip the cell membranes open through electrostatic and gravitational forces.

"We are really excited about the excellent bacterial killing property of this technology and believe that it will have wideranging applications in real life," says Zhang. His team is currently working on developing nano-dagger surface prototypes and other antimicrobial nanopatterned surfaces using different materials.

1. Yuan, Y. & Zhang, Y. Enhanced biomimic bactericidal surfaces by coating with positively-charged  ${\sf ZIF}$ nano-dagger arrays. Nanomedicine: Nanotechnology, Biology, and Medicine 13, 2199-2207 (2017).

#### SHEDDING 'LIGHT' **ON ANTIBODY DESIGN**

The light chain subunits of therapeutic antibodies impact drug performance and yield



Antibody-based drugs have long been a mainstay of treatment for many conditions, especially cancer and autoimmune diseases, but there may still be room for improving these complex biological therapies. An A\*STAR study has found that changes to a part of antibodies that's often overlooked by protein engineers and drug companies can have dramatic effects on target binding and manufacturing yield.

An antibody is made up of four polypeptide chains: two 'heavy' chains form the Y shape of the molecule, and two 'light' chains sit alongside them. To date, most of the modification techniques in therapeutic antibody design have focused on the heavy chains. The light chains were generally considered less important, even though it has been demonstrated that they're also involved in binding target molecules and that the 'framework' regions of light chains provide structural support to allow target contact.

#### "The light chain and its framework regions can impact key factors of a good therapeutic antibody."

To evaluate the importance of the light chain in antibody performance, a team led by Samuel Ken-En Gan from the A\*STAR Bioinformatics Institute and the p53 Laboratory deleted two amino acids in the framework region of the light chain of trastuzumab, an antibody drug for treating breast cancer, sold under the brand name Herceptin. Either deletion on its own, the researchers found, led to a reduction in antibody secretion

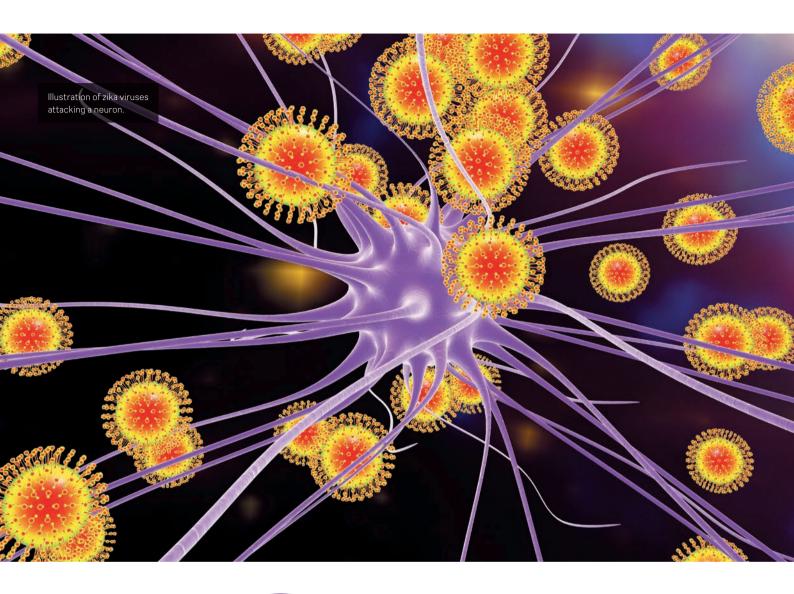
from a recombinant cell culture system, although it did not severely affect the ability of trastuzumab to bind its target, a protein called human epidermal growth factor receptor 2 (Her2). Delete both amino acids, however, and both antibody production and Her2 binding decreased significantly.

Structural modeling and experiments conducted by members of Gan's Antibody Product and Development lab, including co-first authors Chinh Tran-To Su and Wei-Li Ling, revealed that the deletions impaired the interaction between trastuzumab and a protein used for antibody purification. That explains the yield reduction, while decreased affinity for Her2 explains why the deletions also obstruct antibody performance.

"This study shows that the light chain and its framework regions can impact key factors of a good therapeutic antibody, even at sites that are not known to be directly involved in binding or purification," he says.

Although these initial findings demonstrate only the negative consequences of altering an antibody's light chain, Gan and his team, in work not yet published, have also found ways to enhance both production and target binding through other light chain modifications. "This is only the first in a series of papers from my lab to show that we need to consider the antibody as a whole and not just as a sum of different parts," Gan says.

1. Su, C. T., Ling, W. L., Lua, W. H., Poh, J. J. & Gan, S. K. The role of Antibody Vk Framework 3 region towards Antigen binding: Effects on recombinant production and Protein L binding Scientific Reports 7, 3766 (2017).





#### A NEW WEAPON **AGAINST THE ZIKA VIRUS**

An existing laboratory technique can detect Zika virus infection and may help make diagnostic tests more accessible

A new test for the Zika virus could help limit future outbreaks, especially in areas without access to sophisticated diagnostic methods.

An international team led by Lisa F. P. Ng of the A\*STAR Singapore Immunology Network found that an existing flow cytometry technique can accurately detect the presence of a key Zika virus antigen.

Most people infected with the mosquito-borne virus have no symptoms or very mild effects. However there is strong evidence that it can cause severe birth defects. While the number of new cases has dropped

significantly since the major outbreak in the Americas in 2015-16, outbreaks are thought to be cyclical.

The current gold standard diagnostic test identifies the presence of Zika virus RNA in blood or urine. To be sure of detecting infection, however, blood samples must be taken within seven days of the onset of symptoms or within 14 days for urine. There are also tests for antibodies against the virus in blood, but these tests can fail to distinguish Zika from closelyrelated flaviviruses like dengue.

A team of researchers from Singapore, Estonia and Russia

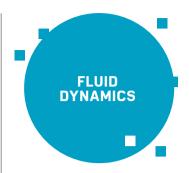
made a synthetic version of NS3, a protein that helps the Zika virus replicate. They injected these proteins into rabbits to generate NS3 antigen-specific antibodies, which they then added to blood samples taken from 47 Zika patients at Tan Tock Hospital in Singapore.

The researchers used a flow cytometry technique called fluorescence-activated cell sorting (FACS) to measure the proportion of white blood cells containing Zika virus antigen in the patient samples. After tests on samples from healthy controls, the group set the threshold for Zika virus infection at 0.5 per cent.

Detection rates of less than 10 per cent, 10–40 per cent and more than 40 per cent were characterized respectively as low, medium and high.

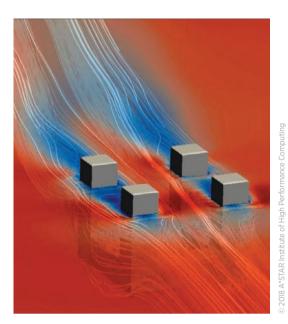
All the samples from patients in the high detection group were taken between two and five days after the onset of symptoms, suggesting this may be the optimum window for the test. Further tests also showed the technique can differentiate between Zika virus and similar viruses.

"The current detection methods all have limitations," says Ng. "Using FACS to detect Zika virus NS3 antigen in the blood could offer a relatively efficient and fast complementary method. It can be done using fresh blood samples and requires minimal processing, and so could be especially useful in settings where there is no access to the equipment and trained technicians required by current molecular tests."



#### COMPUTING THE EBB AND FLOW

Designing deep-sea oil rigs could be improved by simplified wave simulations



The interaction between waves and the wide legs below a floating structure, as indicated above, is complicated.

Evaluating the impact of waves on deep-sea oil rigs has been made easier by an A\*STAR-developed computational technique that should increase the operational lifetime of floating platforms and make them cheaper to manufacture.

Offshore oil platforms and wind turbines conventionally rest securely on the seabed. But if they are to operate in deeper waters, floating structures are required. Given the enormous investment needed to build and position these platforms, it is essential that they can withstand waves, storms and even tsunami. Potential designs are typically tested by building a model and trialing it in a wave tank — but this is expensive, and results obtained under such controlled conditions are not always transferable out at sea.

Computer simulations provide a cheaper approach. The rapid development of high performance computers has

made it possible to solve the complicated three-dimensional nonlinear equations that describe fluid flow around and through a complex arrangement of objects. But the calculations are time consuming, and engineers must often make a compromise between accuracy and efficiency. To address this trade-off, Xin Lu and co-workers from the A\*STAR Institute of High Performance Computing developed a method that reduced computing time by building on a technique known as domain decomposition.

Their method divides the area to be simulated into subdomains in which different types of calculations are performed: a near field subdomain in which the viscous flow model is applied, and a farfield domain in which a model that calculates the system's potential energy is applied. The computational effort per subdomain is much smaller than the full domain, but cou-

pling the subdomains is difficult. Lu and the team achieved this coupling by overlapping the regions in what they call buffer zones. "This overlapped zone technique eliminates the need for sub-iteration in each time step, thus speeding up the modeling," explains Lu.

The team use their overlapping domain decomposition technique to model the propagation of a solitary wave, to measure the impact of the wave on a partially submerged body and to recreate a wave as it breaks on a beach. "This new method is able to deliver comparable results in just half or even a third of the original computing time," says Lu. "And when optimized, the computing time is expected to be further reduced by 80 per cent."

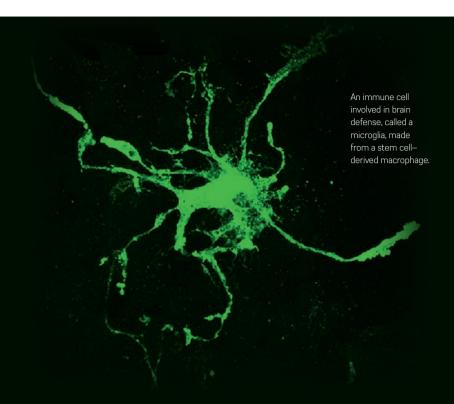
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Lum, F.-M., Lin, C., Susova, O. Y., Teo, T.-H., Ng, L. F. P. et al. Sensitive detection of Zika virus antigen in patients' whole blood as an alternative diagnostic approach. *Journal of Infectious Diseases* 216, 182–190 (2017).

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).

#### USING A MOUSE TO MAKE AN 'IMAC'

A new method for making specialized immune cells from stem cells offers a tool for modeling inflammatory diseases in the lab



A specific type of white blood cell, considered the sentinel of the immune system, can now be made in almost limitless volumes in the laboratory.

A two-step protocol, developed by A\*STAR researchers, for turning reprogrammed stem cells into various types of tissuespecific white blood cells, known as macrophages, could help scientists design new medicines for inflammatory diseases of the lungs, brain and other organs.

The first step in the process involves converting induced pluripotent stem cells into a kind of primordial macrophage. Florent Ginhoux and his colleagues at the A\*STAR Singapore Immunology Network devised a recipe for doing this with either mouse or human cells, creating what they called iPSC-derived primitive macrophages — or simply iMacs.

These iMacs were genetically and functionally similar to early macrophages found in the developing embryo. And by making iMacs from the iPSCs of someone with Familial Mediterranean Fever, a rare inherited autoimmune disease, they showed that these cells provide a tractable system for studying the ways in which a genetic disease can cause immune signaling to go awry.

Ginhoux's team next coaxed the iMacs to form the kinds of mature macrophages found in different tissues of the adult body. In one experiment, the researchers mixed the iMacs in a lab dish with neurons that had also been made from the same batch of iPSCs. After about a week of co-culturing, the iMacs started to develop tentacle-like projections called dendrites, suggesting they had morphed

into a type of immune cells, known as microglia, involved in brain defense.

These cells provide a tractable systen for studying the ways in which a genetic disease can cause immune signalling to go awry.

The researchers could also form these lab-made microglia — or iMicro cells, as they called them — by implanting iMacs directly into the brains of newborn mice. Putting iMacs into the lungs of mice also resulted in lung-specific macrophages.

With the protocols all worked out, these kinds of tissue-resident immune cells now provide a valuable tool for studying diseases characterized by macrophage dysregulation in different organ systems. For example, the iMicros could help scientists shed light on the immune contributions to neurodevelopmental disorders such as autism and neurodegenerative disorders such as Alzheimer's disease.

"[These cells] will provide insights into the mechanism of primitive macrophage maturation into microglia," says Ginhoux. "Furthermore, the co-culture model has the potential to serve as an *in vitro* drug screening system for the development of novel therapies against brain diseases."

 Takata, K., Kozaki, T., Lee, C. Z. W., Thion, M. S., Otsuka, M. et al. Inducedpluripotent-stem-cell-derived primitive macrophages provide a platform for modeling tissue-resident macrophage differentiation and function. Immunity 47, 183–198.e6 (2017). 018 A\*STAR Singapore Immunology Netw

#### **OF MICE AND MEN**

A rare mutation causes severe growth retardation and spine malformation in humans and mice



Mutations in a gene associated with cellular proliferation cause a newly described disease characterized by severe growth retardation, spine malformation, dysmorphic facial features and intellectual disabilities, according to an international study led by A\*STAR researchers.

The team found that nine individuals from five unrelated families in Turkey, Algeria, Tunisia and Saudi Arabia with identical clinical features all carry mutations that inactivate the cyclin-dependent kinase 10 (CDK10) gene. Mutations are rare in CDK genes, which encode proteins that regulate cell growth and division and are carefully kept in check to prevent tumor formation.

To investigate the effects of losing the function of CDK10, the researchers generated mice lacking the Cdk10 gene. These mice displayed severe

growth retardation and some skeletal defects that resemble those observed in patients with CDK10 mutations, or what has been termed Al Kaissi Syndrome. "The mouse model provides compelling data that CDK10 is the gene causing disease, and confirms the human genetic data" said Philipp Kaldis, at the A\*STAR Institute of Molecular and Cell Biology and lead author of the study.

Despite Cdk10 being an essential gene for mouse development, fibroblast cells from Cdk10-deficient mice and from patients divided normally when isolated and grown in the lab. However, analyses of various organs revealed multiple defects in the mice lacking Cdk10. In the affected organs (which included kidneys, lungs, brain, intestine and muscle) more than 600 genes were expressed differently compared with healthy control

mice. A significant number of these genes are involved in lipid metabolism which suggests that the impaired conversion of fats into energy may underlie the growth retardation observed in mice and humans bearing CDK10 mutations. This raises the potential for mitigating some of the features associated with Al Kaissi Syndrome through dietary interventions.

"Without the mouse model we would not have uncovered a new role for CDK10 in cilia growth and metabolism."

The expression of genes involved in the formation of tiny hair-like, sensory cellular protrusions called cilia was affected in organs of Cdk10deficient mice - their fibroblasts developed longer cilia than the healthy controls. The functional consequences of Cdk10's effect on cilia, however, remain to be investigated.

This study is a great example of how mouse models contribute to our understanding of human developmental disorders. "Without the mouse model that recapitulates some of the features of Al Kaissi Syndrome we would not have uncovered a new role for CDK10 in cilia growth and metabolism" explains Kaldis.

Identifying the genetic causes of rare conditions not only helps physicians with diagnoses, but offers researchers a starting point for developing new treatments.

1. Windpassinger, C., Piard, J., Bonnard, C., Alfadhel, M., Lim, S. et al. CDK10 mutations in humans and mice cause severe growth retardation, spine malformations and developmental delays. The American Journal of Human Genetics 101, 391-403 (2017).

## GUT BACTERIA IMPLICATED IN LIVER INFLAMMATION

The effect of gut bacteria on specific immune cells is behind persistent liver inflammation in chronic viral hepatitis

Persistent liver inflammation in sufferers of chronic viral hepatitis is likely caused by interactions between pro-inflammatory immune cells in the liver and products from gut bacteria, according to new work involving A\*STAR researchers. The findings identify new therapeutic targets.

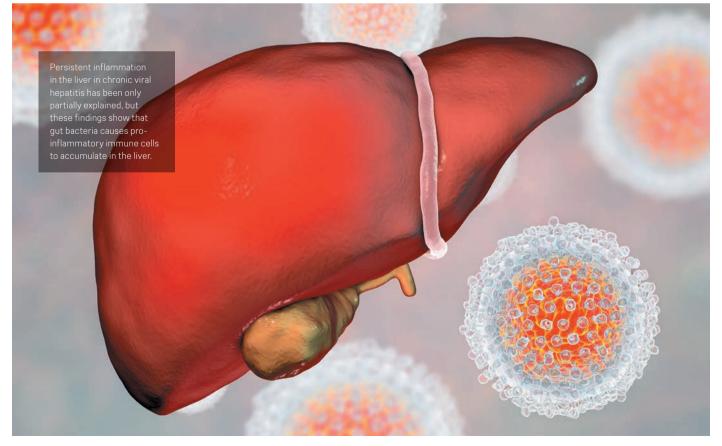
Chronic viral hepatitis is characterized by persistent inflammation of the liver, but the mechanism that maintains this inflammation has been poorly understood. The degree of inflammation does not correspond to the extent of viral activity in the liver, or to the activity of immune cells that specifically target the virus, as might be expected.

The new work, a collaboration between the laboratories of Antonio Bertoletti at the DukeNUS Medical and Singapore Institute of Clinical Sciences, the laboratory of Qingfeng Chen at the A\*STAR Institute of Molecular and Cell Biology and Charles-Antoine Dutertre in the laboratory of Florent Ginhoux from the A\*STAR Singapore Immunology Network, aimed to determine the mechanisms underpinning the inflammation. The researchers focused on a

different group of immune cells called macrophages, which are increasingly recognized as significant in liver disease.

They analyzed the molecular and functional profiles of macrophages in the livers of patients with chronic viral hepatitis and those of healthy people. In the livers of patients, there was an excess of macrophages with a specific profile that promotes inflammation. "Unlike macrophages found in a healthy liver, these macrophages have the capacity to continuously produce pro-inflammatory mediators when they encounter bacterial products," explains Dutertre.

The team studied this phenomenon further in mice engineered to have human liver cells and human immune cells. The mice were infected



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with hepatitis B virus, and consequently accumulated the same pro-inflammatory macrophage population in the liver as that seen in patients. Treatment of the mice with antibiotics to reduce the gut bacteria reversed the accumulation of these macrophages. "This demonstrated that bacterial products that leak from the gut into the liver are responsible for the accumulation of the proinflammatory macrophages," says Dutertre.

The results identify a novel mechanism that maintains inflammation in chronic viral hepatitis. Dutertre and colleagues say that their findings suggest new therapeutic approaches, in addition to targeting the virus, that involve modifying the intestinal bacteria or depleting the proinflammatory macrophages in the liver.

The researchers are now investigating the mechanism behind the effects of bacterial products on macrophages in the liver. So far they have identified certain soluble mediators that play a pivotal role. "Our hypothesis is that by inhibiting these soluble mediators, we could limit the accumulation of the proinflammatory macrophages and resolve liver inflammation in patients with chronic viral hepatitis," concludes Dutertre.

1. Tan-Garcia, A., Wai, L. E., Zheng, D., Ceccarello, E., Jo, J. et al. Intrahepatic CD206+ macrophages contribute to inflammation in advanced viralrelated liver disease. Journal of Hepatology 67, 490-500 (2017).



#### A CATALYST FOR CHANGE IN CHEMICAL ETCHING

A simple, versatile and low-cost technique for etching nanoholes in silicon could underpin new filtration and nanophotonic devices

Metal-assisted chemical etching, or 'MacEtch', is used to fabricate a range of nanostructures, but movement of the catalyst during vertical etching processes hamper its wider use. Now, a team led by A\*STAR have developed a technique that improves catalyst stability, paving the way for wider application.1

MacEtch is a wet etching method for fabricating nanostructures from patterned metal film. The simplicity, versatility, and cost effectiveness of MacEtch in silicon and other semiconductors have led to its use in the manufacture of a wide range of products, from electronic and optoelectronic devices to biological and chemical sensors, as well as energy harvesting technologies. These applications, however, use relatively large mesh catalyst structures.

When catalysts with smaller dimensions are used, forces acting on the catalyst cause it to move during the etching process, which limits their use in the fabrication of structures with high aspect ratios, such as nanoholes.

"Previously, it has been very difficult to achieve directional isolate catalyst etching, and [this] has been a major roadblock in its development," explains Sing Yang Chiam from A\*STAR's Institute of Materials Research and Engineering. "Small feature sizes are especially important for fabricating filtration devices, but at these dimensions, etching becomes very challenging."

Now, a technique for controlling the catalyst during the etching process, allowing for the fabrication of nanoholes in silicon with unprecedented aspect ratios, has been developed by Chiam and colleagues in collaboration with the National University of Singapore and the University of Illinois at Urbana-Champaign in the United States.

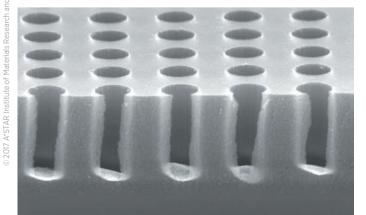
The researchers investigated isolate catalyst etching of regular gold disks of identical array spacing and catalyst thickness, formed using laser interference lithography. This allowed the team to study precise and isolated effects of the etching parameters, such as the etchant and doping concentrations, to understand the interface forces on the catalyst.

They found that higher ratios of hydrofluoric acid to hydrogen peroxide, or higher p-type silicon doping levels, reduce catalyst motion, and attributed this to a lowering of the interface Van der Waals forces caused by the creation of porous silicon.

The researchers demonstrated their technique by fabricating large-area, regularly ordered, nanoholes arrays in silicon with an aspect ratio of around 12. This new method enables the fabrication of new biological and water filters, and nanophotonic devices.

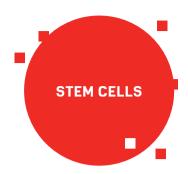
"We plan to use our findings to make a simple filtration device, and then see how much further we can take deep trenching," says Chiam.

An example of directional etching for nanohole arrays of less than 500nm in diameter.



1. Kong, L., Zhao, Y., Dasgupta, B., Ren, Y., Kedar, H. et al. Minimizing isolate catalyst motion in metal-assisted chemical etching for deep trenching of silicon nanohole array. ACS Applied Materials & Interfaces 9, 20981-20990 (2017).

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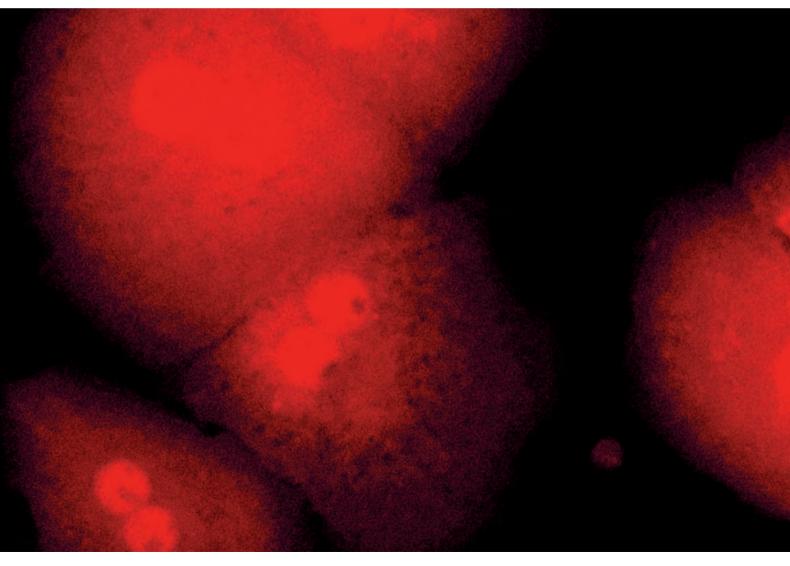
#### KEEPING STEM CELLS NAÏVE

Two proteins maintain embryonic stem cell pluripotency through different means

Two 'finger-like' proteins employ different mechanisms to help safeguard the ability of embryonic stem cells to differentiate into a variety of cell types, according to an A\*STAR-led study. This finding could help researchers develop new ways to regenerate lost or damaged tissue.

PRDMs are a family of 17 proteins with finger-like structures that contain zinc, which are involved in regulating gene expression and modifying the structure of chromatin, the material that forms chromosomes. Ernesto Guccione, of A\*STAR's Institute of Molecular and Cell Biology, and colleagues throughout Singapore investigated the roles of two PRDMs, PRDM14 and PRDM15, in mouse embryonic stem cell (ESC) development.

They found that both proteins were critical for ESC self-renewal — the process that maintains their 'naïve state', allowing ESCs to perpetuate the stem cell pool and mature into any cell type — but that they did this through different mechanisms.



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PRDM14 helps keep stem cells naïve by turning off a group of genes, called *DNMTs*, which code for a family of enzymes that add methyl groups to DNA. PRDM15, however, was not involved in DNA methylation.

The team found that PRDM15 conserved the naïve state of ESCs by turning on genes involved in regulating two signaling pathways that communicate how the cell should function.

"I found it interesting, from an evolutionary standpoint, that different members of the same family, which bind to completely "Our findings could be incorporated into the routine screening strategies for families showing developmental defects."

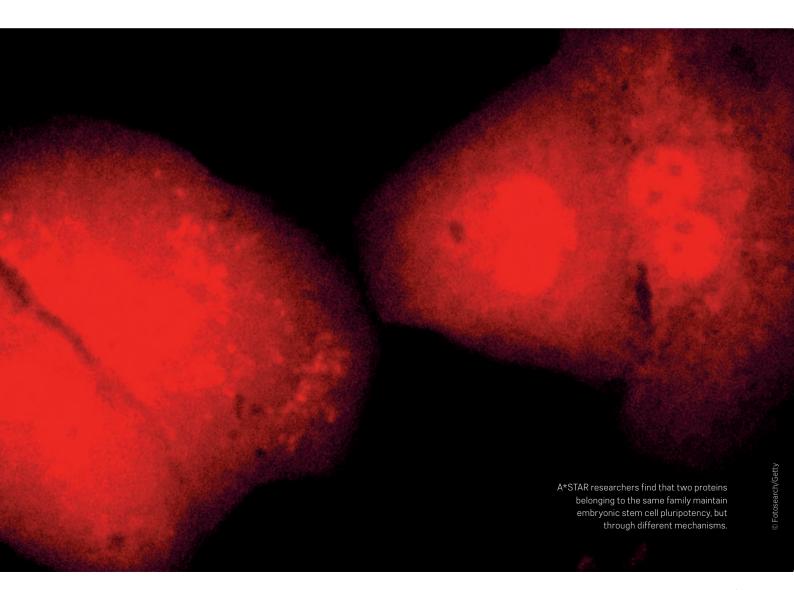
distinct targets, have evolved to regulate similar pathways, in a non-redundant fashion, to prevent embryonic stem cell differentiation," says Guccione.

Previous studies had determined 'downstream'

details of what happens within embryonic stem cells when three different signaling pathways are switched on and off, but much remained unknown about the 'upstream' factors, including those discussed in this study, that affect these pathways. Understanding these signaling pathways is important for determining optimal culturing conditions for mouse ESCs, understanding the cues that regulate normal embryonic development in living organisms, and finding ways to reprogram the body's cells to regenerate other tissue types.

Guccione and his colleagues are now collaborating with geneticists to investigate whether mutations in PRDM15 lead to developmental defects in humans. "We think our findings could be incorporated into the routine screening strategies for families showing developmental defects," he says.

 Mzoughi, S., Zhang, J., Hequet, D., Teo, S. X., Fang, H. et al. PRDM15 safeguards naïve pluripotency by transcriptionally regulating WNT and MAPK-ERK signaling. Nature Genetics 49, 1354–1363 (2017).



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#### **BRIDGING** THE SECURITY **DIVIDE FOR CHAT APPLICATIONS**

Successful decrypting demonstrations show that chat-app designers should improve the ways they protect users' personal data

A\*STAR researchers have successfully recovered decryption keys for two popular chat-apps — WeChat and WhatsApp. With these decryption keys, they could potentially collect users' personal data and private information.

WeChat is particularly popular in Asia with more than 700 million users and uses an open-source library called SQLCipher to encrypt data. By contrast, WhatsApp, owned by Facebook Inc., with more than one billion active users worldwide uses an encrypted backup database file usually stored on a device's SD card.

Vrizlynn Thing, who led the project at the A\*STAR Institute for Infocomm Research explained that many messaging apps use 'end-toend' encryption — only the sender and receiver can read messages and they are encrypted for third parties. "Now, we've confirmed that a technique called information flow analysis can reveal decryption keys for current and future versions of chat-apps, assuming the app design and use of external encryption libraries stay the same," added her colleague, Zhongmin Dai.

This information flow analysis technique is used in mobile forensics to filter pertinent details from the vast volumes of data flowing within devices. Using this method, Thing's team was able to pinpoint the decryption keys for both apps, even though the chat-apps used different encryption techniques. The researchers then used this information to simulate the key generation processes, which allowed them to access data from the devices.

Through this project, the team was able to assess the robustness of the chat-apps and suggest a variety of preventative methods.

"Chat-app servers should verify more than one piece of information from an incoming decryption key request before releasing the key," said Thing, "they should make an association between a device phone number and the user account, for example." She points out, however; that their experiments were carried out on exploitable devices with escalated privilege. Even so, she urges users to keep their devices and applications updated to protect them from security risks.

<sup>1.</sup> Dai, Z., Sufatrio, Chua, T-W., Balakrishnan, D. K., & Thing, V. L. L. Chat-app decryption key extraction through information flow analysis. Cryptology and Information Security Series 15, 3-18 (2017).

#### WHAT A **HUNGRY FLY CAN TELL US ABOUT OBESITY**

Understanding how a fly modulates its feeding behavior could help fight obesity



Turning various neural circuits on and off in the brain of the vinegar fly could help A\*STAR researchers develop new treatments for obesity, diabetes, and heart disease1.

In changing external environments, animals, including humans, maintain a steady physiological energy state by altering both their internal metabolism and feeding behavior. If energy supplies are low, for example, the brain produces hunger signals to drive food-seeking behavior to replenish the animal's reserves.

In a recent study, Adam Claridge-Chang, and colleagues from A\*STAR's Institute of Molecular and Cell Biology and the Singapore Institute of Manufacturing Technology looked at how inhibiting neuromodulator cells that release dopamine and serotonin would affect feeding and metabolic functions1.

"When we started this project, I expected to find a lot of studies in this important area, but was surprised to find just three papers with five experiments. Our study more than doubles the available information."

"We can shut off specific modulator circuits and examine the effect this has on feeding."

"We wanted to know, if we turn off these different circuits, what will happen to the feeding behavior," Claridge-Chang says. "If we see a dramatic impact on feeding, could that be a way of treating the compulsive eating related to obesity?"

To find out, the research team silenced five different neuromodulator

systems in genetically manipulated vinegar flies, Drosophila melanogaster.

"We used transgenes that combine the fly's own gene switch with a protein that silences electrical activity in neurons. This way, we can shut off specific modulator circuits and examine the effect this has on feeding."

The insects were then assessed by monitoring 11 parameters: activity; climbing ability; individual feeding; group feeding; food discovery; both fed and starved respiration; fed and starved lipid content; and fed/starved body weight.

Claridge-Chang says the results from these experiments indicate that individual neuromodulatory system effects on feeding behavior, motor activity and metabolism are not related to each other. As an example, in one experiment,

oxygen intake - which should have mirrored food intake actually decreased while food intake increased.

The group's original hypothesis was that disrupting neuromodulators would have coordinated effects on feeding and other aspects of physiology, but this was refuted — an outcome that Claridge-Chang says could lead to important new insights. "The dissociation of phenotypes implies that different modulatory circuits have disconnected or even oppositional effects on different physiological functions. We will test this hypothesis in future experiments."

1. Eriksson, A., Raczkowska, M., Navawongse, R. Choudhury, D., Stewart, J. C. et al. Neuromodulatory circuit effects on Drosophila feeding behaviour and metabolism, Scientific Reports 7, 8839 (2017).

A microfluidic chip injection mold.

#### **A SMOOTHER FINISH ALLOWS FLUIDS TO FLOW**

A magnetic method for polishing metals enables mold templates with microscale features

Injection molding enables large-scale production of polymer and plastic materials with micrometer-sized features. Now, A\*STAR scientists have developed a method for creating mold templates with high precision and few defects.

A fluid behaves very differently when it is confined to micrometer-scale channels. This phenomenon already has several applications such as enabling the analysis of small samples of blood.

These microfluidic systems are small and portable, easy to use without expert knowledge, and disposable because they are cheap to produce. But this disposability means that microfluidic chips need to be quickly mass produced.

Now, Jiang Guo and his colleagues from the A\*STAR Singapore Institute of Manufacturing Technology have developed a method for fabricating molds that can

quickly create microfluidic channels in polymer substrates. "The technology addresses a critical problem in mold insert fabrication for microfluidic chip production, and will enhance local industry," says Guo.

#### The polish reduced the roughness of the surface by a factor of four, leaving a mirrorlike finish.

Injection molding involves shaping a material while in a molten state using a metal template. It is cheap, fast, and useful for creating microfluidic chips. However, engineering a mold with precise micrometerscale features and smooth surfaces is challenging as burrs and tool marks create defects. A post-polishing process can fix some of these imperfections, but it is difficult for polishing tools

to access the recessed corners of microstructured surfaces and remove unwanted material uniformly.

Guo and his colleagues started by milling their template for a microfluidic channel 100 micrometers in depth and width on a special aluminum alloy. The channel was 100 millimeters in length and included two fluid inlets, one fluid outlet and a serpentine channel as reaction chamber. They then polished the template using a method known as magnetic field-assisted finishing. Two magnetic rollers rotating in opposite directions on either side of the mold create a magnetic field. This field controlled a magnetic abrasive made of carbonyl iron powder and alumina particles bound together by oil, which removed any unwanted material and smoothed the surface.

their template before and after

this magnetic polish. They observed that the process preserved the height of the microstructure, although the edges were more rounded after polishing. The polish reduced the roughness of the surface by a factor of four, leaving a mirrorlike finish. "The next step will be to use the polished mold template for actual injection molding," says Guo.

The researchers compared

<sup>1.</sup> Guo, J., Liu, K., Wang, Z. & Tnay, G. L. Magnetic field-assisted finishing of a mold insert with curved microstructures for injection molding of microfluidic chips. *Tribology International* 114, 306-314 (2017).



#### **HEPATITIS B EXPLOITS THE** LIVER'S WEAKNESSES

In revealing the liver's 'weak spot' for hepatitis B virus replication, A\*STAR scientists are paving the way for new drug development efforts

In a surprising discovery, researchers from the Singapore Immunology Network (SIgN), A\*STAR, have found that the liver is the main site of hepatitis B (HBV) replication — not only because it contains material that helps the virus proliferate, but also because most other tissues of the body contain proteins that actively repress HBV replication.

"We've been working on this project for a number of years

because HBV is very prevalent in Asia," says Ee Chee Ren, leader of the SIgN team that made the discovery.

"HBV enters the liver cells through specific receptors and then deposits its genetic material into the nucleus," explains SIgN's Hui Ling Ko, the paper's first author. The viral DNA then hijacks the host cell's machinery to make new copies of HBV. While

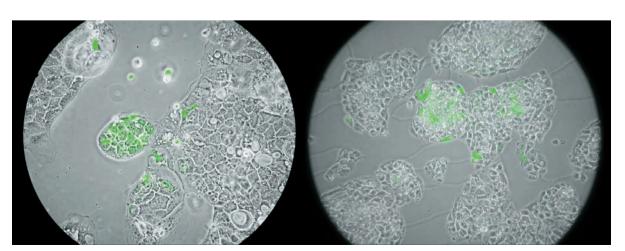
it's known that liver cells contain factors that facilitate HBV replication, Ko and her team found that the cells also lack functional levels of the proteins Slug and SOX7. These proteins are found in most other human tissues, where they bind to the HBV genome and block the binding of the cells' proteins that initiate and promote viral replication. "Slug and SOX7 are involved in embryonic development and that's when they're most active," says Ko. After development, it is thought that the proteins are no longer required in the liver, and so get 'turned off.'

"This provides us with a dramatically improved platform to create a drug screening assay for HBV."

The team is now using tools including CRISPR-Cas9, the pioneering gene editing technology, to produce cells without any functioning Slug or SOX7 proteins. This nullifies the cells' defenses against HBV infection so that they can be used to test the efficacy of new drugs. "The reason for the very successful development of antiviral drugs for hepatitis C was the availability of robust, cell-based screening assays. Hepatitis B has always been lacking in this area," says Ren. "When we introduce HBV into these Slug-deficient cell lines, they show a large increase in the viral load. This provides us with a dramatically improved platform to create a drug screening assay for HBV."

The SIgN team made another unexpected discovery during their studies: human colon, lung and stomach cells also lack Slug and SOX7. The scientists are now collaborating with clinicians at Singapore General Hospital to launch a thorough investigation into whether these tissues could act as a hidden reservoir of HBV, potentially complicating disease treatment.

1. Ko, H. L., Lam, T. H., Ng, H., Toh, J., Wang, L. W. et al. Identification of Slug and SOX7 as transcriptional repressors binding to the hepatitis B virus core promoter. Journal of Hepatology 68, 42-52 (2017).



In the absence of protective proteins Slug and SOX7, hepatitis B infection thrives in liver cells, indicated by green staining in this image.

#### **CLINGING ON TO MEMORY**

Immune cells hold their memory of how to respond to allergens in a surprising way













Previously unexposed mice were first injected in their right ears with DP or DN IgG1 B cells and then they were intravenously injected with an antigen. Those injected with DP, on the left, showed a local anaphylactic reaction in their ear (blue). Those injected with DN, on the right, did not. This demonstrates that only the DP cells are producing high affinity IgE. Understanding how the immune system remembers allergycausing antigens could help prevent severe reactions.

When a predisposed person is initially exposed to an allergy-causing antigen, specific antibodies, called immunoglobulin E (IgE), are produced without leading to an allergic reaction. If exposed to the allergen a second time, the person may become ill. "But noone has found the memory IgE cells believed to be responsible for the illness," says bioinformatics researcher and molecular viral epidemiologist Michael Poidinger from A\*STAR's Singapore Immunology Network (SIgN).

Jin-Shu He and colleagues at SIgN collaborated with researchers in Singapore and the US to decipher the function of IgE memory. Instead of finding IgE memory cells, they discovered that the memory cells of immunoglobulin G1 (IgG1), another antibody, held the memory of IgE responses.

The team injected mice with one of two antigens, eliciting a primary allergic immune response. They then isolated a type of immune cell from the mice's serum, called memory B cell, specific to IgG1. They found three subsets of this memory B cell: double positive (DP), single positive (SP) and double negative (DN) depending on the types of receptors on their surface. These cells were then injected into mice that hadn't been previously exposed to the antigens; one of the two antigens was administered, and a secondary allergic immune response occurred.

The researchers found that DP IgG1 memory B cells from the donor mice — those that held the memory for either of the two antigens - produced, as expected, a type of white blood cells called plasma cells in the recipient mice, which secreted IgG1 antigen-specific antibodies. Surprisingly, however, they also generated plasma cells that secreted IgE with a high affinity for their specific antigen.

SP and DN IgG1 memory B cells also produced IgE-secreting plasma cells. But the secreted IgE had only a low affinity for their antigens.

"Knowing how **IgE** memory is generated, we can ask questions about drugs and preventative treatments."

It remains unknown what causes the same double positive IgG1 memory B cells to generate IgG1 or IgE plasma cells. But the study reveals that IgG1 memory B cell subsets are important in the memory of IgE responses.

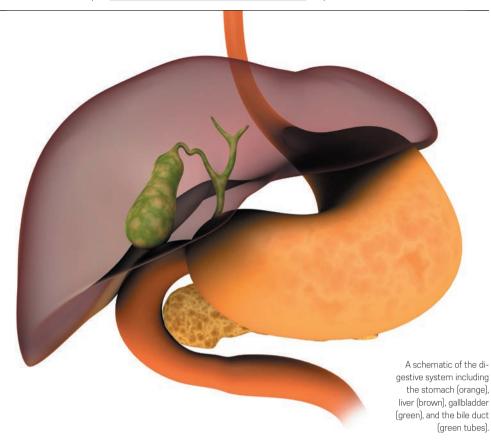
In a secondary allergic reaction, allergens bind to IgE attached to the surface of another type of white blood cells, called mast cells, which then release chemicals to cause anaphylaxis. The team hypothesize that low affinity IgE compete with high affinity IgE for space on the surface of mast cells, and may help limit severe allergic reactions.

"This is an early phase study for this aspect of IgE and allergy," says Poidinger. "Knowing how IgE memory is generated, we can ask questions about drugs and preventative treatments."

1. He, J. Subramaniam, S., Narang, V., Srinivasan, K., Saudners, S. P. et al. IgG1 memory B cells keep the memory of IgE responses. Nature Communications 8, 641 (2017).

#### CLASSIFY AND CONQUER

Molecular dissection of bile duct cancer reveals subtypes with different origins and varying potential treatments



Researchers have discovered that cholangiocarcinoma (CCA), a form of liver cancer also known as bile duct cancer, consists of several molecular subtypes with distinct potential therapies and prognoses.

An international collaboration led by Patrick Tan of A\*STAR's Biomedical Research Council, analyzed tumors collected from almost 500 bile duct cancer patients in 10 countries, in a study that is part of the International Cancer Genome Consortium. By examining a large, diverse group, the researchers were able to uncover patterns missed in earlier investigations, such as one that included only North American patients, all of whom were free of the liver-fluke infection that is a common catalyst for bile duct cancer in Southeast Asian patients.

Using modern highthroughput DNA sequencing, the team investigated the genomic sequences of the tumors and their epigenomic and gene expression profiles. They divided the cancers into four groups with different patterns of mutations and other genetic defects, two of which were associated with liver-fluke infection. "The current way of classifying CCAs is by their anatomical subtype. Our data shows that if you look at molecular data, you get more clinically relevant subtypes that have different potential therapies and prognoses," says Bin Tean Teh of the National Cancer

"About a third of patients can be treated using existing therapies, if experimental drugs were included it would be even more."

Centre Singapore, one of the study's senior authors.

One of the clusters was characterized by changes in genes associated with immune response, suggesting that it might be a candidate for immunotherapy treatment. Likewise, the characteristics of some of the tumors indicated that they might respond to therapeutic interventions currently in use or under development. "We've shown that about a third of the patients can be treated using existing therapies, if experimental drugs were included it would be even more", says lead author, Apinya Jusakul. Currently, surgery is the only treatment for bile duct cancer.

The molecular clusters can also guide researchers in understanding the development of CCA. Two of the clusters had an excess of methylation, a DNA modification. Differences in the methylation patterns offer clues about the processes acting in each cluster. "Based on molecular data, we can speculate about the different mechanisms leading to different DNA changes in the development of CCA. You really can't see that from just the anatomical information," says co-lead author Chern Han Yong.

These findings demonstrate that distinct molecular subtypes can result from different cancer triggers. Learning more about these subtypes and how they link with various carcinogenic processes may reveal the mechanisms behind these cancers and guide the development of therapeutic interventions.

 Jusakul, A., Cutcutache, I., Yong, C. H., Lim, Y. Q., Huang, M. N. et al. Wholegenome and epigenomic landscapes of etiologically distinct subtypes of cholangiocarcinoma. Cancer Discovery 7, 1116–1135 (2017). CONTENTS | FEATURES | RESEARCH HIGHLIGHTS | NEXT ISSUE



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#### FREQUENCY FINGERPRINTS INCREASE CRIME-FIGHTING POWER

A system that recognizes electrical power grid fluctuations hidden in audio recordings provides a powerful forensic tool for police investigators

Audio and video recordings are important sources of evidence in criminal investigations, especially as more electronic devices are in use now than ever before. However, for recordings to be admissible, investigators often need to determine the time they were made, which can be difficult. Now, a team led by Vrizlynn Thing at the A\*STAR Institute for Infocomm Research (I2R), in collaboration with the Singapore Police Force, has developed a new system that reliably estimates the time of recordings by identifying small fluctuations in the frequency of the electrical power grid.

The 'electrical network frequency' (ENF) of power grids is centered around 50 or 60 Hertz, and is picked up in audio recordings as a background hum. The ENF shifts up and down randomly,

which provides each recording with a unique fingerprint that can be compared to the long-term records captured continuously and maintained at forensic labs.

"The science behind electrical network frequency pattern matching has been proven to be reliable, like fingerprints and DNA."

"The random fluctuations are consistent across different places within the same power grid," explains team member Lilei Zheng. "As a consequence, recordings captured in different places at the same time will have ENF fingerprints showing the same fluctuations."

By visually inspecting the ENF, human investigators can reliably match recorded fluctuations to a time in the long-term records, but this is a laborious task best done by a computer. In response, the I<sup>2</sup>R team developed a similarity criterion called bitwise similarity (bSim) that mimics the way humans judge the similarity of two signals.

The team tested bSim by using it to identify the timing of 187 audio recordings made around Singapore using various mobile phones.

They found that bSim greatly outperformed previous similarity metrics, which were thrown into doubt by small deviations even when the general shapes of the signals were clearly similar. "bSim enables us to focus our attention on the overlapped parts instead of being drawn away by the deviated parts," says Zheng.

"The science behind ENF pattern matching has been proven to be reliable, like fingerprints and DNA," says Thing. "It has been used in courts in various jurisdictions and the cases cut across many different crimes. We hope to extend our work from audio recordings to videos, which not only contain audio but may also enable us to 'see' the ENF through variations in lighting."

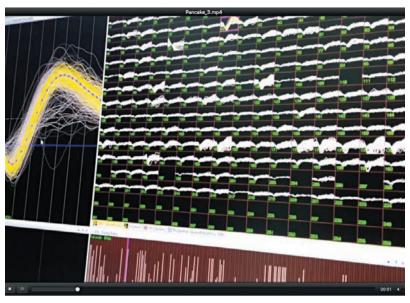
"This innovative solution towards audio authenticity verification developed by I<sup>2</sup>R has already proven itself in actual use, and we are excited about the potential it holds," says a statement from the Singapore Police Force.

 Zheng, L., Zhang, Y., Lee, C. E. & Thing, V. L. L. Time-of-recording estimation for audio recordings. *Digital Investigation* 22, S115-S126 (2017).

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## DIVERTING DISTRACTION

To avoid getting sidetracked by distractions, neurons in the brain's short-term memory center reorganize their activity patterns



Electrical signals in the brain's short-term memory center (as shown above), located in the prefrontal cortex, morph when faced with distracting stimuli.

The ability to remember something like a grocery list or telephone number even under bombardment from loud noises, incoming text messages or other distractions requires an intricate shape-shifting act in the brain.

A Singaporean team has found that the neuronal response patterns responsible for retaining short-term memories reorganize into a different configuration in the face of distracting stimuli — a process dubbed 'codemorphing'.

"This code-morphing capability may be an important factor underlying cognitive flexibility," says Camilo Libedinsky, a neuroscientist at the A\*STAR Institute of Molecular and Cell Biology who co-led the research.

The team's findings may help computer scientists develop more brain-like neural network algorithms for artificial intelligence, or help biomedical researchers better understand diseases such as Parkinson's, schizophrenia and dementia, all of which involve memory deficits.

The brain's center for short-term memory storage sits behind the forehead in the lateral prefrontal cortex. The neurons there were once thought to be unaffected by disruptions, but Libedinsky's team has shown otherwise.

There is something special about short-term memory retention in the prefrontal cortex.

Working with Shih-Cheng Yen, an electrical engineer at the National University of Singapore, Libedinsky and colleagues trained two longtailed macaques to perform a simple visual task. Using drops of juice as a reward, the monkeys were shown a grid on a screen and trained to remember the location of a red square that flashed briefly, without getting thrown off by the subsequent flash of a green square.

Each monkey had a suite of electrodes implanted in the brain, both in the prefrontal cortex and in the frontal eye field, a nearby region involved in controlling visual attention and eye movements. This allowed the researchers to track neuronal activity as the monkeys performed the memory test.

The team found that neurons in the prefrontal cortex became activated following the initial stimulus, maintaining the memory of the location, but this activity — the 'code' used by the brain to remember the red square's location — morphed after the monkeys saw the green square, creating a different code held by the same group of neurons, all without losing any information or impacting the memory in any way.

This was not the case in the frontal eye field, however. There was no evidence of code-morphing ability in this visual part of the brain. This told the researchers that there was something special about short-term memory retention in the prefrontal cortex. They traced this cognitive capacity to a unique set of neurons with the ability to multitask.

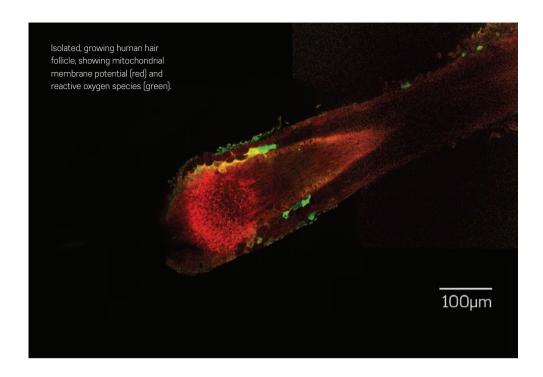
"These results will be of interest to researchers in a number of fields," says Aishwarya Parthasarathy, a postdoctoral research fellow at A-STAR and the study's first author, noting that all areas of memory-related research — cognitive neuroscience, artificial intelligence and neuropsychiatry — could benefit from the discovery.

Parthasarathy, A., Herikstad, R., Bong, J. H., Medina, F. S., Libedinsky, C. & Yen, S. C. Mixed selectivity morphs population codes in prefrontal cortex. *Nature Neuroscience* 20, 1770–1779 (2017).



#### **GETTING TO** THE ROOT OF **HAIR LOSS**

The age-old search for a cure for hair loss may be close to an end



Understanding the energy it takes to grow a strand of hair could hold the key to ensuring women, in particular, end their lives with a wonderful bouffant.

Recent research by A\*STAR scientist Thomas Dawson and his team, in collaboration with researchers from the Medical University of South Carolina Pharmacy School Department of Drug Discovery, suggests that the slowing of the metabolism as we age could be a driver of 'chronogenetic alopecia' or agerelated hair loss, a condition that predominantly affects women.

Dawson, from A\*STAR's Institute of Medical Biology, says that the majority of hair research to date has focused on balding men - even medical books from Cleopatra's reign contained formulas to cure hair loss.

His latest study uses cuttingedge laser microscopy on plucked human and bovine hair to better understand hair growth at a molecular and metabolic level.

In particular Dawson's team is examining the role that mitochondrial metabolism and its by-product, reactive oxygen, play in the bioenergetics of the hair follicle.

"People who go on crash diets, students undertaking exams, all will lose hair — only a small change in metabolism makes a noticeable difference."

"As we age mitochondrial energy production slows, so we end up with a reduced ability to make good hair," he says. "Any time there is a screw up in your metabolism you lose hair. People who go on crash diets, students undertaking exams, all will lose

hair — only a small change in metabolism makes a noticeable difference."

Hair growth is an energyintensive process, Dawson says, with an average human growing almost two meters of hair over their body per hour.

"It takes about 670 kilojoules of energy to grow one gram of hair, which is the equivalent of six minutes of intense exercise using both arms and legs," Dawson points out.

He believes the cells that create the hair multiply and synthesize biomass so quickly that they burn enormous amounts of energy much like "driving a car with both feet down hard on the gas".

"The motor is running absolutely flat out and as a result there is excess reactive oxygen generation in the hair follicle that actually damages the structure. The follicle then loses its ability to continue to operate at full form over time."

The team also uncovered a previously unknown region in the hair shaft which they called the 'ring of fire' because it is a major source of reactive oxygen species.

Dawson says this study shows that hair follicles and growth are more complex than previously thought, and that quelling the formation of reactive oxygen species and maintaining mitochondrial metabolism could be key to improving hair quality as we age.

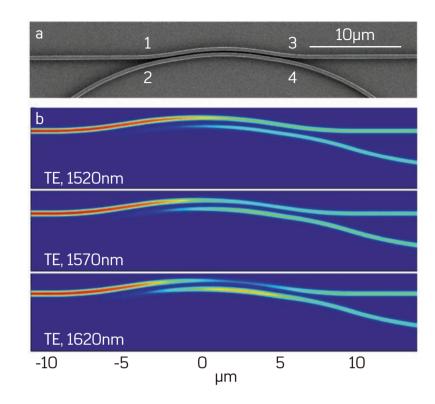
"If we use materials such as leave-on creams or lotions that alter metabolism you can change the way hair grows and make follicles survive longer and produce better hair."

1. Lemasters, J, Ramshesh, V. K., Lovelace, G. L., Lim, J., Wright, G. D., Harland, D. & Dawson, T. L. Jr. Compartmentation of mitochondrial and oxidative metabolism in growing hair follicles: A ring of fire. Journal of Investigative Dermatology 137, 1434-1444 (2017).

#### **A SIMPLER PATH**

A fresh design approach greatly simplifies a key component of photonic circuits by optimizing the coupling of straight and curved light paths

An electron micrograph of the fabricated coupler (a), and the optical energy in the two waveguides (b).



A core element of circuits handling optical signals can now be made smaller and simpler, and with functionality over a wider range of wavelengths, because of a mathematical model developed by A\*STAR researchers¹. The new design methodology is expected to have widespread use in photonic integrated circuits and contribute to more compact photonic devices.

Optical signals, such as those transmitted over optical fiber, require photonic elements and circuits to be useful. One of the most important photonic functions is 'coupling', which allows the signal to be split into two different paths or used to drive other dependent circuits. Coupling, however, is generally only reliable over a narrow band of wavelengths, demanding a complicated design and fabrication process and bulkier circuits.

A broadband coupler that provides reliable performance over a wide range of useful wavelengths is something of a holy grail for photonics engineers.

To overcome this obstacle to photonic circuit miniaturization, Jun Rong Ong and colleagues from the A\*STAR Institute of High Performance Computing, in collaboration with researchers from the Singapore University of Technology and Design, went back to the drawing board to find a simpler approach to designing these critical coupling elements for broadband operation.

"Couplers are the basic building blocks of photonic circuits," explains Ong. "However, it has been very difficult to try to make them compact, with low power loss and broadband operability, using standard processes."

A coupler works by running a second optical path, known as a waveguide, alongside the signal path. The optical signal induces a secondary signal in the adjacent waveguide, passing with it some of the signal's energy. However, the ratio of power splitting changes depending on the wavelength of the signal, so a broadband coupler that provides reliable performance over a wide range of useful wavelengths is something of a holy grail for photonics engineers.

"We distilled the key ingredient that makes a directional coupler a broadband splitter – a combination of straight and bent waveguides – and thoroughly explored and exploited it," says Ong.

Ong's team came up with a design methodology based on a mathematical 'transfer matrix method' to determine the coupler section lengths, radii, and waveguide cross-sections that would give the desired operation with low wavelength sensitivity. Using this approach, the team designed and fabricated a 50/50 power splitter with a wide bandwidth and length of just 20 micrometers.

"We were able to improve the broadband performance of the photonic power splitter through a simple modification of existing designs, and have achieved a balanced 50/50 output which is difficult by other designs," Ong says.

 Chen, G., Ong, J.R., Ang, T.Y.L., Lim, S.T., Png, C.E., & Tan, D.T.H. Broadband silicon-on-insulator directional couplers using a combination of straight and curved waveguide sections. Scientific Reports 7, 7246 (2017).

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### **FETAL IMMUNE** SYSTEM DEVELOPED, BUT TOLERANT

More mysteries of the developing immune system have been revealed in a study of fetal dendritic cells

The fetal immune system is fully developed and functional from as early as 16 weeks gestation, but has a mechanism to keep it suppressed until after birth, according to an A\*STARled study. Their findings could shed light on the immunological mechanisms underlying fetal-maternal health problems such as preeclampsia.

"People thought that the fetal immune system was defective because it was not mature, but we've discovered that it's highly dynamic, highly functional, and in fact represents another specialization," says Florent Ginhoux, from the Singapore Immunology Network at A\*STAR, who co-led the study with Jerry Kok Yen Chan from the KK Women's

and Children's Hospital, Singapore.

Ginhoux and co-authors analyzed fetal tissues donated under strict ethical guidelines — for dendritic cells, which are immune cells that sample the environment for potential threats and pass that information to the T cells and B cells to ward off those threats.

"People thought that the fetal immune system was defective because it was not mature, but we've discovered that it's highly dynamic, functional and represents another specialization."

The researchers discovered that not only do dendritic cells exist as early as 12 weeks gestation, but, when stimulated by molecules that interact with the toll-like receptors found on the surface of the cells, mimicking the interaction with a bacterial or viral attack, they behave almost exactly the same as adult cells.

"We found that the cells in the tissue were able to migrate to the lymph nodes very early, which means they were able to talk to T cells, so everything looks similar to what we find in adults," Ginhoux says.

But this posed further questions: if a fetal immune system responded to threats in exactly the same way as an adult immune system, the resulting inflammation could theoretically cause harm to the developing fetus.

The research team

compared genes from fetal and adult dendritic cells, and found one very significant difference; fetal dendritic cells over-express an enzyme called arginase-2, which is known to block the T cell secretion of a key inflammatory signaling

molecule

called TNF-

alpha during inflammation.

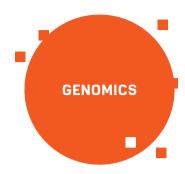
They tested this by co-culturing adult and fetal dendritic cells with T cells, and showed that only the adult dendritic cells triggered production of large amounts of TNFalpha from the T cells, while fetal dendritic cells inhibited it through release of arginase-2.

The results could help our understanding of pregnancy conditions such as preeclampsia and gestational diabetes, which Ginhoux suggests could be the result of a disruption in this careful balance of immune activation and tolerance in the fetus.

1. McGovern, N., Shin, A., Low, G., Low, D., Duan, K. et al. Human fetal dendritic cells promote prenatal T-cell immune suppression through arginase-2. Nature 546, 662-666 (2017).



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#### MAPPING RNA EDITING

A large-scale study explores how RNA editing varies between different tissue types and primates

In a large-scale study of more than 8,500 human samples and hundreds of primate and mouse samples, A\*STAR researchers have investigated how RNA editing allows for the variable expression of genes in different human organs, and those of other mammals¹. The mammalian atlas will help scientists determine what genes

and cell types are important in various diseases.

Almost every cell in your body contains nearly identical copies of your DNA, which is why DNA profiling is so useful in forensic analysis. But how that DNA is expressed varies greatly in different kinds of cells, resulting in the diversity of body tissues.

A cell produces RNA by copying stretches of its DNA, and then RNA is subjected to various kinds of processing which gives rise to some of the variability observed between different types of cells. It also goes some of the way toward explaining the complexity of humans despite the fact that we do not have vastly more protein-coding genes than other mammals.

"RNA editing diversifies the transcriptome," explains Meng How Tan of the A\*STAR Genome Institute of Singapore. "If every variation of a transcript was genetically encoded, our genome would be enormous."

By drawing on the resources of the Genotype-Tissue
Expression (GTEx) Consortium,
Tan and his colleagues have conducted a survey of RNA editing in more than 8,500 human samples (corresponding to 53 tissue types in 552 individuals). They also analyzed hundreds of samples from the mouse and four other primates: chimpanzee, macaque, baboon and marmoset. The team focused on the RNA editing process

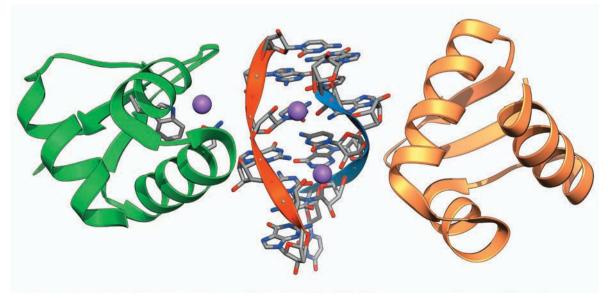
that converts the nucleotide adenosine to inosine, the most common kind of RNA editing in animals.

The results revealed several surprises. For a start, the greatest amount of editing of RNA that codes for proteins was found to occur in the arteries, and not in the brain, as previously thought. Also, the comparison between species revealed that RNA editing profiles are more similar between different organs in a single species than they are between the same organ in different species.

The team is now exploring how diseases occur when RNA editing goes wrong. "There are over a million editing sites in humans, but for the most part, no-one knows what their functions are," says Tan. "We are now actively investigating how RNA editing is dysregulated in various diseases, and we have ongoing collaborations with clinicians in Singapore."

 Tan, M. H., Li, Q. Shanmugam, R., Piskol, R., Kohler, J. et al. Dynamic landscape and regulation of RNA editing in mammals. Nature 550, 294–254 (2017).

RNA being edited by an enzyme. The enzyme ADAR1 (green and gold) converts adenosine into inosine in the genetic sequence.



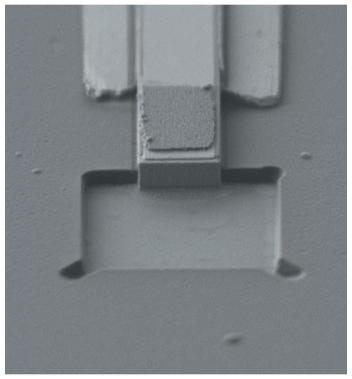
#### **ESCAPING SILICON**

An alternative fabrication scheme for microscale semiconductor lasers could expand the applications of on-chip lasers beyond conventional silicon

A systematic study of a simple and general structure for on-chip semiconductor lasers by A\*STAR researchers sets the scene for much broader application of integrated semiconductor lasers beyond conventional siliconbased systems<sup>1</sup>.

The ability to use, manipulate and sense light is applicable to many technologies, from data interconnection and fiber optics to optical sensors and optical storage systems. Tiny lasers are routinely integrated into microchips for these 'optoelectronics' applications using a well-understood siliconbased laser structure, but alternative and potentially simpler structures in non-silicon systems have yet to be explored in detail.

One such non-silicon-based application is a new type of data storage system called heatassisted magnetic recording (HAMR), which researchers at the A\*STAR Data Storage Institute have been working on as a next-generation data storage technology. HAMR uses integrated lasers for fast and precise micro-spot heating of a magnetic medium, but requires the laser to be formed on aluminum-titanium-carbide (AlTiC) rather than silicon. This presented Chee-Wei Lee and his colleagues with a significant problem, since the silicon substrate plays in integral role in



An etched facet semiconductor laser with an air gap reflector.

producing the laser light.

"We needed to develop a generic integration scheme that would allow us to fabricate laser devices on different substrates, not just silicon," says Lee. "For this, a facet reflector structure is very useful, but low facet reflectivity is a problem, and using different reflectors usually means a more complicated fabrication process and greater chance of device failure."

The lasers used in such applications turn electrical

current into a light emission. They do this by taking light produced by a stack of ultrathin layers of a light-emitting semiconductor (in this case aluminum-gallium-indium-arsenide), and multiplying light at the target wavelength using a resonant cavity formed between two reflectors.

By designing a faceted laser structure considering process integration, Lee and his team developed a fabrication scheme that can accommodate different "We needed to develop a generic integration scheme that would allow us to fabricate laser devices on different substrates, not just silicon."

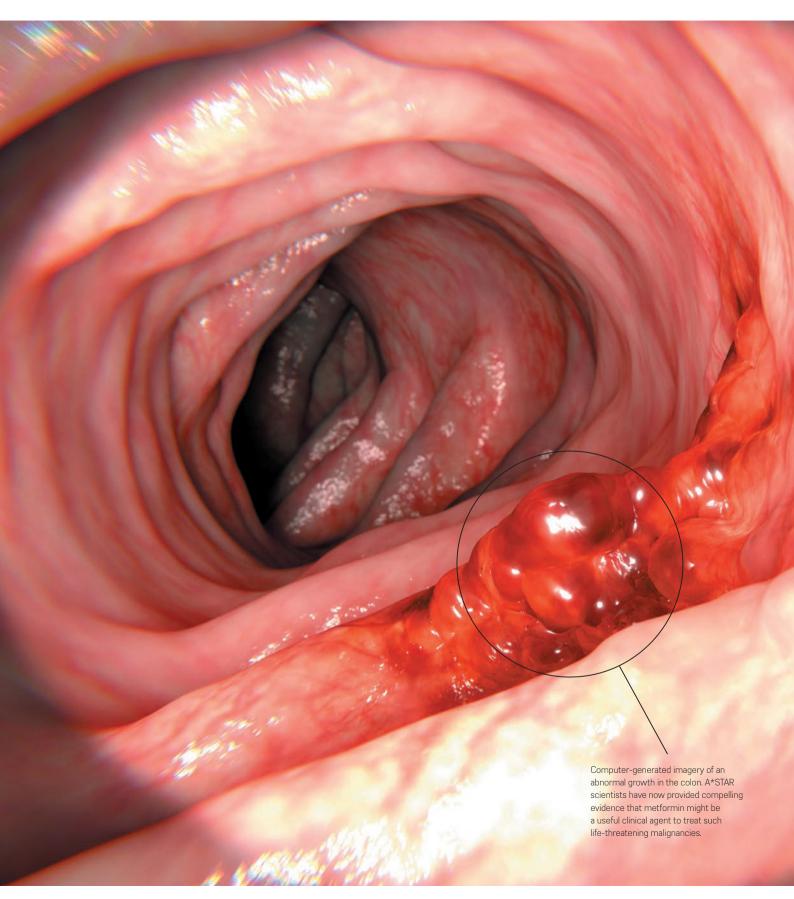
types of reflectors without additional processing steps. The team then used this fabrication scheme to test facet reflectors made by depositing a thin gold film, by chemical modification of the surface, or by etching an air gap (see image).

Studies of the different laser structures fabricated in the A\*STAR laboratory and supporting simulations revealed that a thin gold layer, less than 100 nm thick, afforded the best performance in terms of facet reflectivity, minimum lasing current, emission efficiency and output power.

"We expect our results to serve as benchmark for research and development on etched facet lasers with different reflectors," says Lee.

Lee, C.-W., Ng, D. K.-T., Ren, M. Fu, Y.-H., Kay, A. Y. S. et al. Comparison of III-V/Si on-chip lasers with etched facet reflectors. Applied Optics 56, 5086–5091 (2017).

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#### **MORE EVIDENCE FOR METFORMIN**

A popular diabetes drug could one day be used to treat malignant tumors

A\*STAR researchers have provided strong evidence, using patient tumor grafts, that metformin, a common diabetes drug, might help fight colorectal cancer in humans.

Metformin's potential as a tumor-suppressing agent, to prevent the growth of breast, colon, lung and prostate cancers, has been demonstrated in pre-clinical studies. However, the experimental models used in these studies do not accurately recreate the natural manifestations of the disease, and require toxic levels of metformin to demonstrate beneficial effects.

Min-Han Tan and a group of researchers from A\*STAR's Institute of Bioengineering and Nanotechnology (IBN), the Biological Resource Centre, and the Genome Institute of Singapore, with collaborators from hospitals across Singapore, have now tested metformin's cancer-fighting abilities on a model of colorectal cancer that is more representative of how the disease appears in humans.

The team took samples of cancer tissue from two patients and implanted them in mice, then assessed how the tumors responded to metformin as well as 5-fluorouracil, the current front-line treatment for colorectal cancer.

They found that metformin inhibited tumor growth by at least 50 per cent after 24 days and, when combined with 5-fluorouracil, inhibited the tumor grafts from one patient by 85 per cent. The experiments used concentrations of metformin equivalent to that used to treat diabetes in humans.

In previous studies, scientists typically injected cancer cells into host animals rather than transplanting tissue directly. In these models, high glucose, insulin, and growth factor levels are needed to establish cell cultures for injection. Tan's group suspects that this artificial environment meant previous studies needed higher levels of metformin to stunt tumor growth as, in this study, they were able to

demonstrate response using therapeutic doses of the drug: "We tested a wide range of concentrations down to the physiological," says Tan. "It was important to show that there was a response at that level, as many studies have not documented that."

"Our study shows that metformin has a possible activity against colorectal cancer."

The IBN-led collaboration discovered that metformin enacted its therapeutic benefits by activating a cellular pathway implicated in the inhibition of cancer, and by reducing cancer cell oxygen consumption. Using next-generation genetic sequencing, the team also provided evidence that direct patient tissue grafts in mice retain the genetic, molecular, and tissue features of the original tumor - making them ideal platforms to study colorectal cancer and its treatment.

It's believed that this is the first investigation into metformin and colorectal cancer using patient tumor grafts. Tan says that future studies could shed light on metformin's relevance as a therapeutic agent for cancer: "Our study shows that metformin has a possible activity against colorectal cancer, using gold-standard materials, and provides a mechanism to explain this. Further clinical trials are now needed."

1. Suhaimi, N-A. M., Phyo, W. M., Yap, H. Y., Choy, S. H. Y., Wei, X. et al. Metformin inhibits cellular proliferation and bioenergetics in colorectal cancer patient-derived xenografts. Molecular Cancer Therapeutics 16, 2035-2044 (2017).

Gaertner/Science Photo Library/Getty

# HORMONE DISCOVERY OPENS PATH FOR NEW PRE-ECLAMPSIA TREATMENT

Early diagnosis and treatment of pre-eclampsia could soon be possible due to the discovery of the pregnancy hormone ELABELA

A hormone secreted by the placenta during pregnancy may play a key role in the development of pre-eclampsia; a major worldwide cause of maternal and fetal death.

A\*STAR researchers first discovered the hormone, called ELABELA, or ELA, in 2013 and showed, in zebrafish, that it was essential for normal embryonic development of the heart and cardiovascular system. Further work revealed that, in mammals, ELA was produced by the placenta, and when administered to rats and mice, caused their blood pressure to drop.

"So if you consider the two — a hormone specifically expressed in the placenta, that has blood pressure-lowering properties — one would expect that its absence would trigger gestational hypertension, a key symptom of pre-eclampsia," says Bruno Reversade, research director at the A\*STAR Institute of Medical Biology.

In this new international study led by A\*STAR, researchers crossed male and female mice that each carried only one copy of the ELA-coding gene to produce 'knockout' mice. Around half of these knockout mice showed severe embryonic cardiovascular malformations and did not survive to birth. Despite their



"We have uncovered a completely new signaling pathway, a hormone and its receptor, in the pathogenesis of pre-eclampsia."

inability to produce ELA, the other half did survive.

But when the female knockout mice grew to adulthood and became pregnant, they showed high blood pressure and protein in their urine, classic signs of preeclampsia, while their fetuses also had the lower birth weight typical of babies born to mothers with the condition.

However, Reversade says the most exciting discovery of the research was that treating the pregnant knockout female mice with ELA hormone reversed their pre-eclampsia symptoms and increased the birth weight of their offspring.

"We have uncovered a completely new signaling pathway, a hormone and its receptor, in the pathogenesis of pre-eclampsia. Because it's a hormone, it could be developed into a drug as is insulin for diabetes," Reversade says.

This discovery also opens up the possibility of using ELA, or more specifically the observation of lower ELA levels, to enable early diagnosis of pre-eclampsia, which affects 5–8 per cent of all pregnancies.

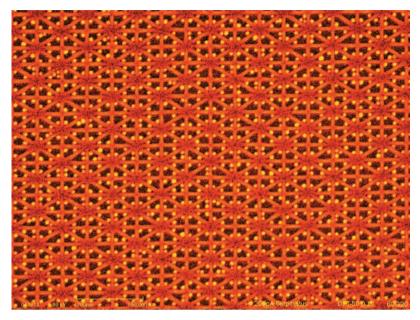
"One of the biggest unmet needs in the field is that we don't have early predictive diagnostic biomarkers of when a woman is susceptible to develop preeclampsia," Reversade says.

Researchers are now examining this hormone in humans, starting with a study comparing ELA levels in pregnant women with preeclampsia and those with normal blood pressure.

 Ho, L., van Dijk, M., Tan Jian Chye, S., Messerschmidt, D. M., Chng, S. C. et al. ELABELA deficiency promotes pre-eclampsia and cardiovascular malformations in mice. Science 357, 707–713 (2017).

## LET LIQUID FINGERS POINT THE WAY

Tiny gold spheres can be manipulated on surfaces with nanometer precision using the effects of solvent evaporation



A scanning electron micrograph of 8 nanometre-sized gold nanoparticles on a triangular template.

Unlocking the molecule-detecting capabilities of gold nanoparticles often requires positioning techniques that are beyond the limits of conventional lithography.

An A\*STAR team now demonstrates that a combination of topographical templates and localized traps left by evaporating liquids can fabricate arrays of nanoparticles with controllable separations below five nanometers¹.

Left alone, nanoparticles tend to agglomerate due to their high entropy. Because keeping gold nanospheres set distances apart is critical for applications, including optical bioimaging, researchers are developing ways to fabricate hundreds of thousands of these objects automatically. One promising route, known as directed self-assembly, deposits liquid suspensions of reagents on to substrates with predefined small-scale patterns. Liquid capillary action then draws

the nanoparticles inside the templates pushing them to their target locations.

Mohamed Asbahi from the Institute of Materials Research and Engineering at A\*STAR recalls trying to control selfassembly within square-shaped templates when he and his co-workers made an intriguing discovery. "We were increasing cavity sizes in the templates, and expected to see more nanoparticles inside trying to optimize their arrangement," he says. "But with toluene as the solvent, only four nanoparticles were trapped at each corner of a square — no matter how large the cavity."

To explain this behavior, the researchers developed a virtual model to simulate interactions between the deposited nanoparticles and solvent within confined cavities. These computations showed that after the liquid begins drying out, the shape of the retreating interface played a key role

in positioning. For example, elongated liquid 'fingers' within square templates forced nanoparticles to move to corners where the solvent volume is the largest.

"We were surprised by this effect before we understood the physics behind it."

"We were surprised by this effect before we understood the physics behind it," says Asbahi. "But after we predicted irregular cavities were more successful at directing nanoparticles than equilateral ones, we chose to validate our explanations with triangular templates."

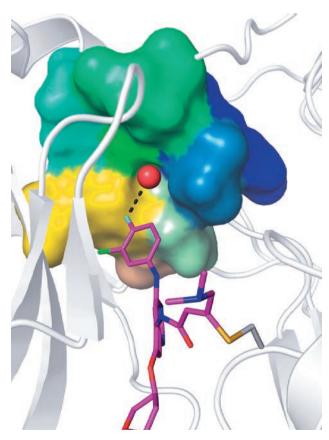
Working with stateof-the-art electron beam lithography, the researchers fabricated templates containing thousands of triangles just a few nanometers in scale. Comparing equilateral to right-angled triangles revealed the potential of asymmetrical patterns — up to three nanoparticles could be trapped and positioned at various nanoscale separations in right-angled templates.

Further experiments demonstrated that particular liquids can have different impacts on nanoscale patterns. While toluene tends to 'pin' to the template structure and trap nanoparticles at lower-than-normal densities, hexane solvents produce fully-packed surfaces. Asbahi notes that the control offered by this technique could be sufficient for integration into prefabricated circuits and plasmonic nanostructures.

 Asbahi, M., Dong, Z., Wang, F., Saifullah, M. S. M., Yang, J. K. W. & Chong, K. S. L. Second order directed positioning of nanoparticles induced by the main terminal meniscus shape in irregular template cavities. *Nanoscale* 9, 9886–9882 (2017).

# ALL IN A MOLECULE OF WATER FOR EFFECTIVE CANCER THERAPY

Molecular simulations reveal why certain cancer therapeutics may bind — or fail to bind — to mutant proteins



Molecular simulation of afatinib (magenta) bound to EGFR<sup>19ded</sup> (gray), with a single water molecule (red sphere) mediating interactions (dotted line) between the drug and the protein. Other mutation-prone amino acids within the drug-binding pocket are individually colored.

A single molecule of water is the reason certain mutations are more responsive to a 'targeted' cancer therapy, according to molecular-scale simulations carried out at the A\*STAR Bioinformatics Institute.

Targeted therapies that take down tumors by selectively interfering with the defective proteins that promote their survival and growth have revolutionized cancer therapy. For example, the drug afatanib is a targeted agent given to lung cancer patients with certain mutations in the gene encoding the epidermal growth factor receptor (EGFR). Recent clinical studies suggest that afatanib may confer survival benefit specifically in patients with the EGFR<sup>19del</sup> mutation rather than in patients with a different mutation, known as EGFR<sup>L858R</sup>, when compared with patients receiving only standard chemotherapy.

Daniel Shao-Weng Tan, a clinical oncologist from A\*STAR's Genome Institute of Singapore and the National Cancer Center of Singapore, who participated in these trials in Singapore, was confounded by this disparity, so he consulted scientist Chandra Verma at the Bioinformatics Institute to rationalize their findings. Verma and Srinivasaraghavan Kannan, a specialist in molecular modeling at the Bioinformatics Institute, teamed up with Tan to identify differences in the structure and dynamic behavior of these two mutant proteins that might explain the distinct response profiles.

It turns out that the critical difference was a mere molecule of water. Afatanib binds to a pocket on EGFR that is missing five amino acids in the *EGFR*<sup>19del</sup> mutant. Molecular dynamics simulations carried out at the Bioinformatics Institute and

the National Supercomputing Centre, Singapore, showed that this deletion creates a more physically constrained structure, which can snugly accommodate both afatanib and a single water molecule in a very stable arrangement. In contrast, the mutant EGFR<sup>L858R</sup>, also found to characterize lung cancer, contains just a single amino acid substitution, and is predicted to hold two water molecules in its pocket. However, these are in a weaker and relatively less stable arrangement, resulting in weakened interactions with afatanib that likely reduce the drug's affinity and hence effectiveness.

"Our hypothesis is based on understanding the physics of the system and could offer a compelling rationale for the clinical observations with afatanib," says Kannan. Intriguingly, he and Verma were also able to home in on other binding pocket mutations (single nucleotide polymorphisms, or 'SNPs') that occur alongside *EGFR*<sup>19del</sup> that might likewise influence the efficacy of afatanib.

The researchers are now examining whether these predictions hold in real-life experiments and see this as a promising general strategy for predicting patient response to targeted treatments. "We are trying to develop a robust pipeline to examine the structural effects of mutations on drug interactions," Kannan says. "This will be a very valuable approach to complement our engagement with the national precision medicine efforts."

Kanna, S., Pradhan, M. R., Tiwari, G., Tan, W.-C., Chowbay, B. et al. Hydration effects on the efficacy of the epidermal growth factor receptor kinase inhibitor afatinib. Scientific Reports 7, 1540 (2017).

# FASTER DATA TRANSFER THROUGH PLASMONS

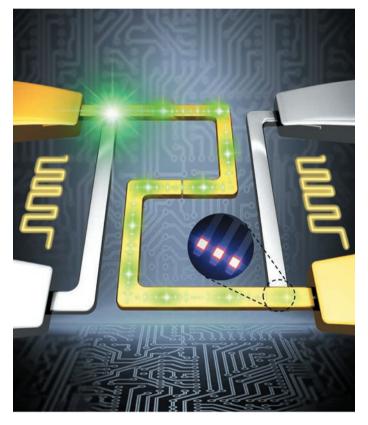
A transducer that converts electrical signals directly into surface plasmons could allow rapid data transfer and link photonic devices to electronics

Photonic and electronic devices could soon be successfully integrated thanks to new transducers developed by a team of Singaporean researchers that can generate, manipulate and read small packets of energy called surface plasmon polaritons (SPPs).

Photonic devices, which use light rather than electric charges to carry information, can operate thousands of times faster than conventional electronics, although they tend to be large and difficult to integrate with microchips.

The team of researchers led by Hong-Son Chu at the A\*STAR Institute of High Performance Computing and Christian Nijhuis at the National University of Singapore believe that SPPs, electromagnetic surface waves that exist at the interface of two materials, could be used to seamlessly link photonic devices and electronics.

"SPPs essentially contain light confined to dimensions smaller than its wavelength, and they function like photonic elements, carrying information at high speeds," says Chu.
"However, the SPPs offer the best of both worlds because they have the operational speed of optical elements as well as a small size suitable for nano-electronics applications. We have developed the first on-chip electronic-



This illustration shows two tunnel junctions coupled together by a plasmonic waveguide in the center. When a voltage is applied to the source junction, tunneling electrons excite surface plasmons, which propagate along the plasmonic waveguide and modulate the tunneling current at the detector junction. Inset: A light emission image of tunnel junctions, showing plasmons scattering from the end of the plasmonic waveguide.

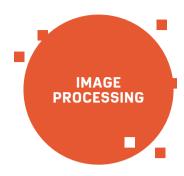
plasmonic transducers that operate at optical frequencies, and we achieved an electronto-SPP conversion efficiency of more than 10 per cent."

Most existing plasmonic devices require light sources such as LEDs to generate SPPs. This indirect method is quite slow. Chu and coworkers realized it would be much faster to produce SPPs by direct electrical means, so they designed transducers comprising aluminum and gold electrodes, separated by a two nanometer-thick layer of aluminum oxide that acts as an insulating 'quantum tunneling' barrier. Electrons that make the quantum leap across this gap will either generate or detect SPPs.

By joining two transducers with a plasmonic waveguide, so that one acted as a source and another as detector (see image), the researchers observed about 1 in 7 of the tunneling electrons coupling to a SPP. Although the reasons for this high tunneling rate are uncertain, Chu and coworkers suggest that SPPs at the junctions might induce an oscillating electric field, which changes the effective size of the tunneling gap and therefore the number of electrons that can cross the gap and interact with SPPs.

"By doing away with the need for light sources and detectors, devices based on this mechanism would be intrinsically fast," says Chu. "Our work has attracted interest from research communities and industries, with potential applications in three-dimensional integrated circuits and high bandwidth memory devices. For example, there is a need for small, high-speed interconnectors to improve processing speeds."

 Du, W., Wang, T., Chu, H.-S. & Nijhuis, C. A. Highly efficient on-chip direct electronic-plasmonic transducers. Nature Photonics 11, 623–627 (2017).



### EYES ON THE ROAD FOR RETINA MAPPING

An image processing algorithm designed to follow directional features shows promise for mapping the blood vessel networks in the eye

More accurate and efficient mapping of retinal blood vessels using a path-following image processing scheme, developed by an A\*STAR-led research team, could help improve retinal scanning and medical diagnosis<sup>1</sup>.

The blood vessels found on the retina at the back of the eye are an important diagnostic indicator for many clinical disorders including diabetes, high blood pressure, arterial hardening, and occlusion of retinal arteries. However, tracing retinal blood vessels is a time-consuming process requiring training and skill, which would be better performed by a reliable automated process that

can efficiently map the vessel network.

"We have spent years analyzing retinal blood vessels, where a challenge is always to single out each vessel from the rest or to separate artery from vein vessels," says Cheng Li from the A\*STAR Bioinformatics Institute. "We have developed an algorithm that can trace a network from a few marked or 'labeled' nodes, and it works especially well for large-scale networks of, say, millions of nodes even with very few known labels."

In their theoretical study, Li and his team explored the use of a well-established algorithm in image processing, called the Markov chain, to better follow the complex branching networks of blood vessels in the retina.

A Markov chain is a statistical representation of a sequence, in this case of connected nodes, where an element in the sequence is independent of everything that came before it. For a blood vessel, this means that its direction of branching from a given point could be entirely random and not dependent on the path of the vessel that came before it. Li's team took this further to adopt

an absorbing Markov chain, which 'locks in' the traced path up to the current node, and then applies a random walk algorithm to probe an image for the next blood vessel direction.

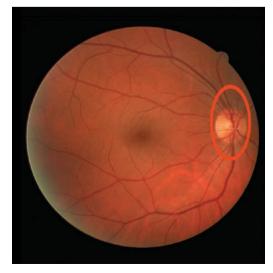
In this way, their image processing algorithm can start from a labeled node, such as a major branch, and trace the blood vessels to form a connected network in a way similar to how a physician would tackle the problem.

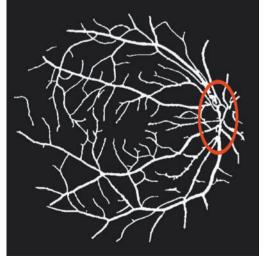
In application to real retinal images, the algorithm outperformed other state-of-theart approaches, and matched the accuracy of human tracing.

"We developed this algorithm out of our very practical biomedical imaging experience in blood vessel tracing over a number of years," says Li. "Our approach is simple, easy to implement, and has many important applications including image classification, and network and link analysis."

De, J. Zhang, X., Lin, F. & Cheng, Li.
Transduction on directed graphs
via absorbing random walks. IEEE
Transactions on Pattern Analysis and
Machine Intelligence, advance online
publication 11 Aug 2017.

A retinal image and the blood vessel network traced by the 'absorbing random walk' image processing algorithm.





### PICKING WINNERS FROM MILLIONS OF POSSIBILITIES

A clever simulation scheme helps identify the most promising compositions of two-dimensional materials



A high-throughput scan of possible compositions for a new class of materials known as MXenes gives researchers invaluable direction for picking the best candidate from the millions of possible material recipes<sup>1</sup>. The simulation study by researchers from the A\*STAR Institute of High Performance Computing is a significant advancement in the field of MXenes, which have exciting potential in next-generation energy storage applications.

Two-dimensional (2D) materials are a relatively new class of materials that display a wide range of unusual properties associated with their ability to constrain the movement of electrons and energy in a 2D plane. The MXene alloys are a very recently discovered class of 2D materials, which could conceivably consist of any of millions of possible

arrangements of transition metals (like molybdenum or titanium), carbon and nitrogen. These characteristics are reflected in the name 'MXene' — the 'M' represents metal atoms, the 'X' denotes carbon and nitrogen, while the 'ene' suffix signals the materials' 2D atomic structure.

"Our approach enables quick evaluation of the formation energies of millions of MXene alloy structures."

"Since MXenes are new, there's still much to be learned about their structure and properties," says Teck Leong Tan from A\*STAR. "As MXene alloys are formed by mixing different types of transition elements at different compositions, the alloying possibilities in MXenes

are huge. So we developed a high-throughput computational method to predict the probable structures and stable phases of different MXene alloys across all compositional ranges and temperatures."

Although there are many possible MXene alloy compositions, most will not be stable. The challenge faced by material scientists has been how to efficiently sweep through the huge number of alloy configurations to identify those with the lowest formation energy and hence highest stability. Conventional 'first principles' calculation approaches are too computationally intensive for such a scan to be feasible.

"Our approach uses what's called a cluster expansion method to 'learn' the effective interactions between atoms, thus enabling quick evaluation of the formation energies of millions of MXene

alloy structures," says Tan.

The scan, conducted in collaboration with Drexel University in the US, revealed that molybdenum-based MXenes mixed with vanadium, tantalum, niobium or titanium, appear to form the most stable ordered structures. Titanium however tends to form stable 'asymmetric' ordered structures that were previously not considered viable.

"Our scan allows us to predict the structures of MXene alloys that are yet to be fabricated and estimate the likelihood of their fabrication from a thermodynamics viewpoint. And for known MXene alloys, our predicted structures are consistent with experimental results."

Tan, T.L., Jin, H.M., Sullivan, M.B., Anasori, B., & Gogotsi, Y. Highthroughput survey of ordering configurations in MXene alloys across compositions and temperatures. ACS Nano 11, 4407–4418 (2017).

### HEALING DAMAGED HEARTS

Scientists believe they have discovered why heart muscle cells regenerate so slowly, opening potential for inducing damaged hearts to repair themselves Researchers at A\*STAR have identified the 'brakes' that keep heart muscle cells from dividing and healing damaged heart tissue<sup>1</sup>. This finding raises the possibility of developing treatments that target these brakes to stimulate heart repair.

More people die of cardiovascular disease than of any other cause. One reason the disease is so often fatal is that heart muscle cells, or cardiomyocytes, are very slow to divide and replenish themselves. Consequently, damage to the heart is often irreversible. Scientists have long searched for a way to induce cardiomyocytes to regenerate at higher rates.

Now, Roger Foo and his co-workers at the A\*STAR Genome Institute of Singapore have identified the culprit responsible for the slow regeneration rates of cardiomyocytes — a long noncoding ribonucleic acid (RNA) the team labeled 'SingHeart'.

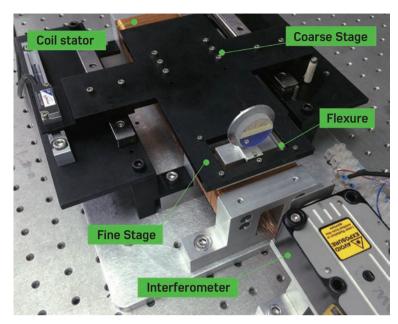
Noncoding RNA does not code for proteins and previously had no known useful role, leading some to call it 'junk RNA'. But there is now recognition that noncoding RNA plays an important role in modifying the expression of genes that code for proteins. In the case of SingHeart, Foo and co-workers found that the noncoding RNA regulates genes that control the ability of cardiomyocytes to multiply.

"This finding has the potential to change forever the way heart failure patients are treated," comments Foo. "Right now, drugs for patients with cardiovascular disease only stem the progress of the disease. Regenerative treatments that target SingHeart could reverse the course of the disease, which would be a revolutionary way to treat heart failure."

The researchers discovered SingHeart by analyzing gene expression in single cardiomyocytes derived from healthy and diseased hearts of both mice and humans. Their analysis revealed that, in diseased hearts, certain cardiomyocytes activate genetic programs related to cell division — the first time that different subpopulations of cardiomyocytes have been shown to have different gene expression in response to stress. Further analysis showed that SingHeart plays a role in blocking genes responsible for cell division in cardiomyocytes.

The team is now exploring whether this finding can be used to develop new treatments for cardiovascular disease. "We're very hopeful this will lead to future clinical treatments," says Foo. "If not through SingHeart, then through other molecules that my team are also hunting for."

1. See, K., Tan, W. L. W., Lim, E. H., Tiang, Z., Lee, L. T. et al. Single cardiomyocyte nuclear transcriptomes reveal a lincRNA-regulated de-differentiation and cell cycle stress-response in vivo. Nature Communications 8, 225 (2017).



An integrated nanopositioning device can achieve high precision with a simpler and less expensive design than typically used.

Probing intricate cells or high-tech microchip components requires 'nanopositioners' that can scan large areas with extremely small steps. Normally, these positioners have separate fine- and coarse-movement stages, but A\*STAR researchers have introduced a parallel-actuated system that integrates the two units for improved energy efficiency<sup>1</sup>.

When engineers design bearings for guiding moving parts, they typically work with tolerances of a few micrometers. Nanopositioning devices, however, need precision standards up to a thousand times greater. To achieve this kind of precision motion, piezoelectric crystals that slightly expand or contract with voltage stimulation are usually combined with an active feedback sensor. Then, the fine-motion crystal is mounted on to the moving part of a linear actuator capable of large displacements in a single stroke.

This type of configuration, known as serially-actuated dual-stage design, has inherent problems according to Daniel Tat Joo Teo from the Singapore Institute of Manufacturing Technology (SIMTech) at A\*STAR. "In a traditional serially-actuated design, power cables are linked to the fine-motion stage to energize the actuators. These cables cause substantial disturbance, and limit the performance of the entire motion system."

Teo and his collaborators from SIMTech and the National University of Singapore considered a different approach to constructing nanopositioners. Instead of conventional piezoelectric actuators, they chose a magnetic linear motor as a means to establish fine motion. Then, they coupled this motor with hinge-like flexure bearings attached to the coarse translator. The flexure system offers nanometer-scale movement without dry friction and critically,

can be controlled with the same electromagnetic machinery used to drive the coarse motor — eliminating the weight and expense of troublesome cables.

"The parallelactuated dualstage design gives reasonably good performance even with a low-cost coarse stage."

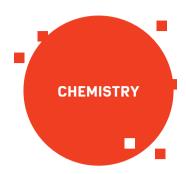
The challenge with this integrated design, notes Teo, lies in finding the right combination of mechanical characteristics such as stiffness and damping. While conventional positioners can analyze the properties of controllers, actuators, and mechanical stages independently, the team had to find a way to optimize these three elements concurrently. To do so, they established an analytical model capable of predicting how

applied force 'ripples' through the nanopositioner and impacts small-scale movements.

Fabrication of an experimental prototype revealed the potential of the researchers' integrated positioner and analytical model. As measured by a laser interferometer, the device could move in 20 nanometer steps without being sensitive to millimeter-scale coarse movements — findings that may usher in a new era of inexpensive nanopositioning devices.

"The parallel-actuated dualstage design gives reasonably good performance even with a low-cost coarse stage," says Teo. "We plan to commercialize the system to benefit our manufacturing industry."

 Zhu, H., Pang, C. K. & Teo, T. J. A flexure-based parallel actuation dual-stage system for large-stroke nanopositioning. *IEEE Transactions* on Industrial Electronics 64, 5553– 5563 (2017).



#### **AN ALL-IN-ONE CATALYST**

Palladium catalyst speeds up two separate reactions, making useful molecules in a single process

A palladium catalyst developed by A\*STAR researchers offers a reliable and efficient way to create a molecular structure that is commonly found in medicines and electronic materials1.

The structure is known as a stilbene fragment, which comprises two benzene rings, connected by two carbon atoms with a double bond between them. The benzene rings are able to carry a host of other chemical groups, so stilbenes make up a large and diverse family of molecules, which can be used in potential treatments for central nervous system disorders and as the active material in certain organic light-emitting diodes (OLEDs) used in advanced displays and lighting.

Until now, stilbenes have typically been made in a two-step process, with each step relying on different palladium catalysts and reaction conditions. The first step, known as a Suzuki coupling reaction, adds a two-carbon unit to a

benzene molecule. After the product of this reaction, styrene, is extracted and purified, a second step, called a Heck reaction, tacks another benzene ring on the other end of the carbon-carbon double bond. The product of the first step, or 'the intermediate', however, is often unstable, making it difficult to handle and lowering the overall yield of the process.

"The savings of time, solvent, reagents, waste and purification materials can be seen as a greener approach."

Howard Jong of the A\*STAR Institute of Chemical & Engineering Sciences and collaborators at the University of Ottawa, Canada, have now shown that a single palladium catalyst can carry out both of these steps in one reaction vessel, which avoids the need to isolate

the troublesome intermediate. This 'one-pot' Suzuki-Heck reaction offers a more efficient way to make stilbenes, potentially saving costs and reducing waste associated with purification of the intermediate product.

The catalyst partners palladium with a bulky phosphorus-containing molecule called Cy\*Phine. The team tested the catalyst's performance in the Suzuki coupling reaction using 48 different reaction conditions, varying the solvent and other reagents. After identifying the recipe that gave the best results, the researchers repeated this optimization process for the one-

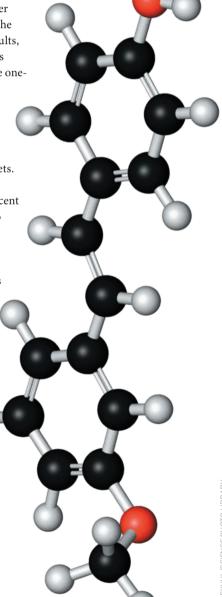
pot Suzuki-Heck reactions. This gave them a reliable method that was used to make a broad range of model compounds that resembled commercial targets. The reactions typically gave average yields of 64-91 per cent for each step, comparable to performing the reactions separately. "However, being able to perform one-pot reaction sequences provides a quicker and more economical strategy," says Jong. "The savings of time, solvent, reagents, waste, and

purification

Pterostilbene (pictured), a defensive chemical found in blueberries, is an example of a functional molecule that contains the stilbene motif.

materials can be seen as a greener approach." The team now hopes to improve the method and use it to make stilbene derivatives for organic electronics.

1. Das, U. K., Clément, R., Johannes, C. W., Robins, E. G., Jong, H. & Baker, R.T. One-pot Suzuki-Heck relay to prepare industrially valuable intermediates using the Pd-Cy\*Phine catalyst system. Catalysis Science & Technology 7, 4599 (2017)

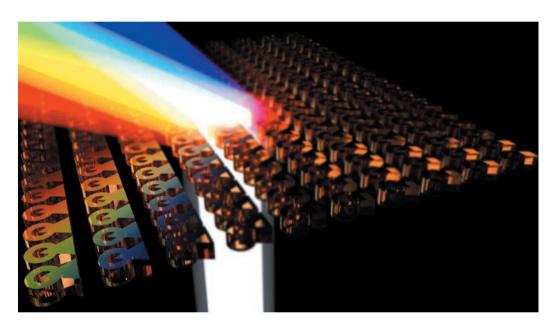


**OPTICS** 

A flatter alternative is found in metasurfaces, which consist of an array of structures, each smaller than the wavelength of light, engineered to modify the characteristics of incident light. As an optical beam hits this surface, it scatters off the subwavelength elements, forming an output beam with chosen properties. This can, for example, be used to bend the incoming beam into a new direction. However, the efficiency with which the light is redistributed into the correct direction decreases sharply for increasing angles, making

# FLAT-PACKED OPTICAL COMPONENTS

Nanostructured thin-films that can bend light by large angles could be a replacement for bulky glass optical components



A metasurface with fish-like elements redirects an incoming beam of light into a new direction.

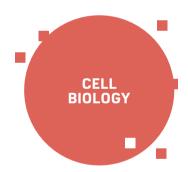
bending light at very large angles difficult.

Paniagua-Domínguez and the team have achieved efficient optical channeling at any desired angle using a metasurface comprising an array of asymmetric nanoantennas. Just like normal antennas, they alter the scattering directivity patterns by suppressing or enhancing emission at different angles. "Our new approach goes beyond the standard design, which is to use phase mapping," explains Paniagua-Domínguez. "We foresee that these metasurfaces may out-perform traditional bulk optics not only in terms of efficiency, but also functionality."

The researchers demonstrated this concept experimentally by etching their nanoantenna array design into a thin film of titanium dioxide on a glass substrate. One design they investigated had a structure that loosely resembled a fish, with a ring surrounding the tip of a triangle. The dimensions of the fish were all below 300 nanometers, much smaller that the wavelength of the incident light. With this novel approach they were able to bend more than 50 per cent of the energy of an incoming beam of green light at an angle of up to 73 degrees. Moreover, and contrary to previous approaches, this structure demonstrated broadband operation, effectively bending light across wavelengths in the green and blue parts of the spectrum.

"Based on this concept, we are now working towards a flat lens with an extremely large numerical aperture," says Paniagua-Domínguez. "That is, a lens that can focus light into a very small spot or resolve very small objects or features."

 Khaidarov, E., Hao, H., Paniagua-Domínguez, R., Yu, Y. F., Fu, Y. H., Valuckas, V., Yap, S. L. K., Toh, Y. T., Ng, J. S. K. & Kuznetsov, A. I. Asymmetric nanoantennas for ultrahigh angle broadband visible light bending. *Nano Letters* 17, 6267–6272 (2017).



### **PUTTING THE 'BONES' IN ORDER**

Tubular bridges organize the skeletons of cells in the early embryo

Scientists at A\*STAR have discovered how cells in the nascent embryo organize the 'bones' that make up the skeleton of the cell, known as microtubules. While this discovery has resolved one mystery, it also raises a range of new questions.

"This was a chance discovery. It's not something we or others could have hypothesized based on previous work," says Nicolas Plachta of A\*STAR's Institute of Molecular and Cell Biology, who led the study. A structure

known as the centrosome serves as a microtubule-organizing center (MTOC) in most animal cells, including older embryos. Plachta's team was examining the microtubules during the first few cell divisions in mouse embryos, which lack centrosomes, when they noticed that neighboring cells were connected by microtubule bridges. Microtubule bridges usually form between dividing cells during mitosis, but they are normally broken down afterwards. "But these

were retained, so that got us interested," says Plachta. "Is there something special about these bridges and these cells in the early embryo?"

To find out, Plachta's team disrupted the bridges with a laser pulse. This led to the breakdown of microtubules in the cells and a change in cell shape. Destroying the MTOC in other cells has similar consequences, suggesting to the team that the bridges may substitute for the MTOC in the early embryo.

Further observation revealed

that the bridges grew outwards from the center of the cell during division and then back into the cells afterward. A network of microtubules grew out of each bridge, and the bridge and network proved to be crucial for the transport of adhesive proteins to the cell membrane.

"This was a chance discovery. It's not something we or others could have hypothesized based on previous work."

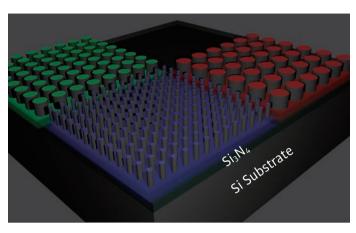
The team is also investigating why the bridges persist rather than breaking down as they do in other cells. "We think that the mechanisms that normally cut the bridges — the proteins and molecular machinery — are all there in the early embryo, but just aren't active. Something represses them in the early embryo," explains Plachta.

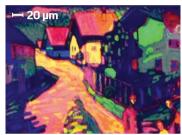
In addition to organizing microtubule growth and providing a scaffold for transport, these bridges may also mechanically connect embryonic cells to coordinate the dynamics of their growth and division during development. "We don't know much about that at the moment, but we're studying how it might work," says Plachta. "Each cell is connected to its sister through this shared skeleton. When a cell changes shape or gets squeezed, how does the microtubule skeleton change in that cell and in the sister cell connected to it?"

<sup>1.</sup> Zenker, J. White, M. D., Templin, R. M., Parton, R. G., Thorn-Seshold, O. et al. A microtubule-organizing center directing intracellular transport in the early mouse embryo. Science 357, 925-928 (2017).

### FLOATING NANOSTRUCTURES FOR BETTER COLOR

Extremely high-resolution color images are produced using silicon nanostructures that imitate particles floating in free space





(above) A schematic of the surface the team created featuring tiny silicon panostructures

(left) The team's reproduction of Kandinsky's Murnau Street with Women.

The detail in color images is limited by the size of pixel that can be printed. Recent advances in nanotechnology have opened the possibility of printing at incredible resolutions of around 100,000 dots per inch, by using metallic nanostructures that resonate in response to incident light. However, the colors that these systems produce are limited, and cannot fill the standard Red Green Blue (sRGB) palette used in most devices and the Internet.

Now, a team including Joel Yang, Arseniy Kuznetsov and Zhaogang Dong at the A\*STAR Institute of Materials Research and Engineering (IMRE), and Data Storage Institute (DSI) have produced a new printing system with nanostructures made from silicon, achieving a spread of colors even wider than sRGB!

"We have been working on ultra-high resolution color printing for the past five years, and now we have solved the problem of limited color saturation," says Yang. Unlike previous efforts with nanostructures made from precious metals such as silver and gold, the team used silicon, which is cheap, readily compatible with existing electronics, and has useful optical properties.

"We have been working on ultrahigh resolution color printing for the past five years, and now we have solved the problem of limited color saturation."

The researchers used electron-beam lithography and reactive-ion etching to produce tiny silicon disks, 130 nanometers high, on a 70-nanometer silicon nitride layer. The nitride acts as an 'anti-reflective' coating, effectively tricking the incident light into behaving as if it is not there and the nanodisks are floating in free space.

"An important requirement for ideal color reflection is to meet Kerker conditions, where incident light excites magnetic and electric dipoles in a silicon particle in such a way that the particle scatters light entirely in one direction," explains Yang. "In free space, the uniform surroundings of the particle allow Kerker conditions to be met. A substrate will generally break spatial symmetry, but if the substrate does not reflect, the situation is equivalent to light propagating through free space."

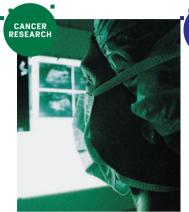
Yang, Dong and co-workers experimented with several different sizes and spacings of nanodisks, as well as nanorings, on their substrate, until they were able to achieve high-resolution images with over 120 per cent of the sRGB color palette — the best quality color from any nanostructure system of this resolution. They demonstrated the system by accurately reproducing a print of Wassily Kandinsky's famous painting *Murnau Street with Women*.

Yang and Dong are hopeful that their new design for nanostructure printing could eventually be accessible to everyone via a desktop device, and could also have applications in miniaturized displays, data storage and security prints for identifying counterfeits.

 Dong, Z., Ho, J., Yu, Y. F., Fu, Y. H., Paniagua-Dominguez, R. et al. Printing beyond sRGB color gamut by mimicking silicon nanostructures in free-space. Nano Letters 17, 7620–7628 (2017).

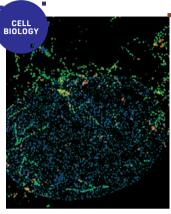
### **NEXT ISSUE**

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



#### A BLOOD TEST FOR **CANCER RECURRENCE**

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



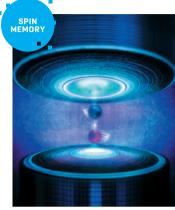
#### **PUTTING MUSCLE NUCLEI IN THEIR PLACE**

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



#### **REACH FOR THE STARS**

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



#### **DYNAMIC SOLUTION** FOR ACCURATE READING

A sensing scheme that responds dynamically to voltage fluctuations could improve data reading accuracy.



# **Push the frontier** with an A\*STAR scholarship

Agency for Science, Technology and Research

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.



