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Bioinformatics Institute (BII)
Bioprocessing Technology Institute (BTI)
Experimental Power Grid Centre (EPGC)
Genome Institute of Singapore (GIS)
Institute for Infocomm Research (I^2R)
Institute of Bioengineering and Nanotechnology (IBN)
Institute of Chemical and Engineering Sciences (ICES)
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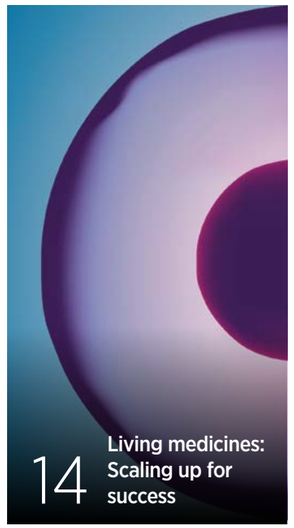

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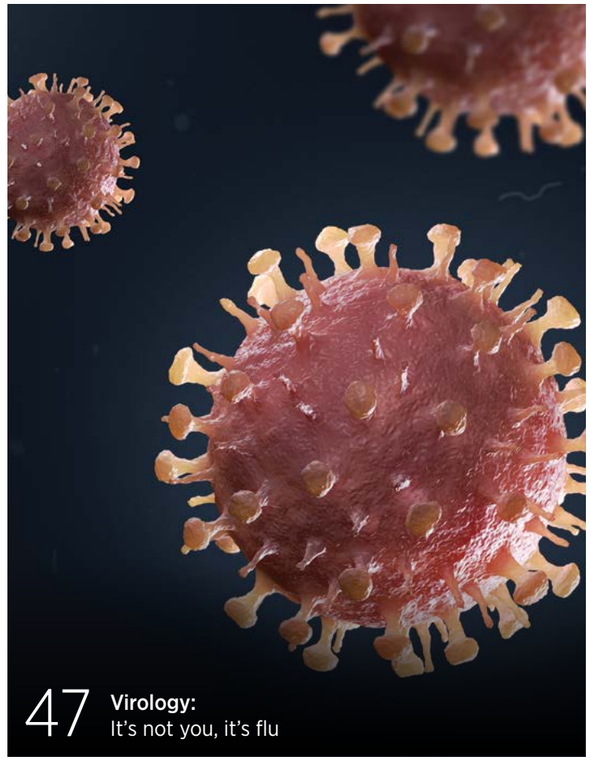
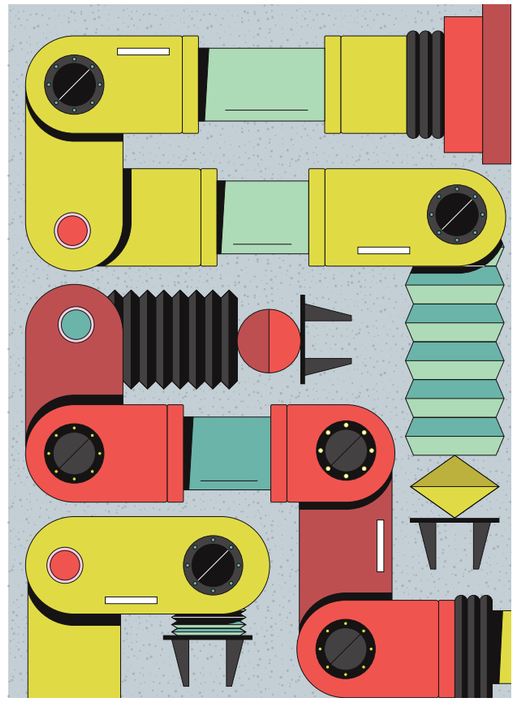


NOTES FROM THE EDITORS



It's often difficult to pinpoint exactly when an industrial revolution begins. What starts out as separate events and inventions gradually converge to drive massive progress in society, unlocking new possibilities for the future.

Now, with the expanding influence of the internet and rapid improvements in technologies such as robotics, machine learning and the Internet of Things, one can conclude that we are indeed experiencing a revolution of a different kind—the Fourth Industrial Revolution, as Professor Klaus Schwab, Founder and Executive Chairman of the World Economic Forum, calls it.

Promising to radically change the way the world operates, the Fourth Industrial Revolution is fueled by ideas and innovation. We are proud to say that there's no shortage of both at A*STAR.

In a modern take on crystal ball-gazing, our researchers at the Institute for Infocomm Research (I²R) are leveraging Internet of Things sensors to predict when machines break down, so that maintenance can be carried out pre-emptively. Meanwhile, at the Advanced Remanufacturing and Technology Centre (ARTC), scientists are developing robots that can work alongside humans in factories of the future. Read about these developments in our cover story, 'The building blocks of Industry 4.0 (p. 04).'

In addition to advancing traditional manufacturing, A*STAR researchers are also developing methods for bio-manufacturing, which involves the production of materials, molecules and medicines using biological systems. For example, stem cells have the potential to regenerate worn out tissues or replace dysfunctional body parts, but they need to be manufactured in the billions for the treatment of a single patient.

Producing stem cells in bulk presents unique challenges—ones that our scientists at the Bioprocessing Technology Institute (BTI) and the Singapore Institute of Manufacturing Technology (SIMTech) are tackling together with industry collaborators. We shine a spotlight on their efforts in the feature story, 'Scaling up for success (p. 14).' Readers interested to learn more about what stem cells could achieve in the clinic should also check out 'Weaponizing stem cells in the war on diabetes (p. 18).'

Other topics covered in this issue of *A*STAR Research* include cancer biology, immunology, materials science, mechanical engineering and more, so we hope you will discover something new for yourself as you browse through these pages. For our latest stories, do visit us at our recently revamped website: **research.a-star.edu.sg**, or follow us on Twitter at: **@astar_research** and LinkedIn at: **A*STAR Research**.

On the cover
Technologies such as robotics, artificial intelligence and the Internet of Things are ushering in a new industrial age. (p. 04)

THE BUILDING BLOCKS OF INDUSTRY 4.0

Researchers across A*STAR are developing the hardware and software needed to take factories to the next level in Industry 4.0.

Consider the smartphone in your pocket and all the things it enables you to do. From banking to entertainment, the mobile revolution has opened up entirely new economic sectors and now contributes trillions to the global economy. While each phone is an engineering marvel in its own right, the real potential of smartphones comes not only from hardware like integrated circuits and lithium-ion batteries, but also software like apps.

In the same way, the Fourth Industrial Revolution is not about any single piece of technology, but about how a wide range of different building blocks come together successfully to create new and improved ways of doing things. These Industry 4.0-enabling technologies run the gamut from data analytics to Internet of Things (IoT) devices and robotics, domains that are being tackled by different research groups across A*STAR.

Photo credit: Natalya Hora / Shutterstock

HARDWARE THAT IS SELF-AWARE

In the realm of robotics, for example, collaborative robots or cobots look poised to make a big impact on how things are manufactured. Promising to alleviate manpower shortages and take over work that is dangerous, dirty and dull, cobots are a relatively small but rapidly growing segment of the industrial robot market, says Alberto De San Bernabé, a Development Scientist at the Advanced Remanufacturing and Technology Centre (ARTC).

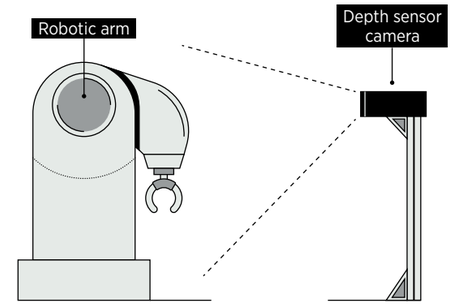
The problem when man and machine work together, however, is that safety becomes an issue. Currently, cobots are equipped with internal sensors to detect collisions, as well as external sensors to monitor nearby human operators. "These systems effectively turn a collision that could knock you out into a gentle push. However, cobots usually carry sharp tools, so most of the time it is much better to completely prevent the collision," De San Bernabé explained.

External safety sensors such as 2D light detection and ranging (LiDAR) are expensive and typically configured to slow down or stop the cobot when an object crosses predetermined static limits. Furthermore, they do not distinguish between trained operators and bystanders, unnecessarily reducing their speed and efficiency when people unexpectedly walk by, De San Bernabé added.

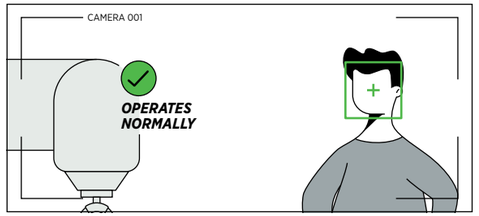
To improve the perception abilities of cobots, De San Bernabé and his team used the depth sensor camera of the Microsoft Kinect gaming accessory to reconstruct the 3D environment around the robot[1]. "Using the RGB-D camera, we were able to determine not only if there are humans in the frame but also the pose of their body, head and limbs. We used that information to determine the speed and direction of the person relative to the robot, feeding that data into an algorithm that dynamically calculates the safety limits."

Adopting dynamic limits is as safe as using static ones but more efficient, said De San Bernabé. For example, if both cobot and human are moving at low speeds, the minimum safety distance between them can be reduced, allowing the human to stand close to the robot. "If the limit was static, on the other hand, then the robot would stop once the human crosses the limit, even if it is in a situation considered safe by standards such as the ISO," he explained.

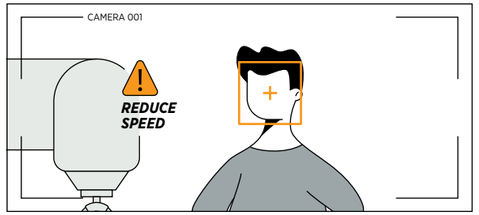
For collaborative robots to function safely alongside humans, a depth sensor camera may be used to monitor the moving robotic arm and its surroundings.



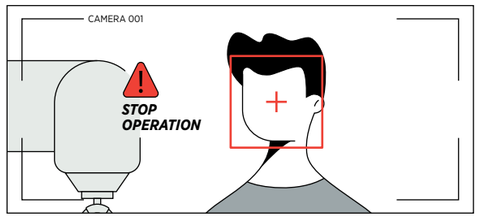
Data from the depth sensor camera is fed to a machine learning algorithm that judges the distance between a human and the robotic arm.



If a person is outside a dynamically defined safety zone, the robotic arm will continue with its task.



If a person is approaching the proximity limits of a dynamically defined safety zone, the robotic arm will reduce its speed.



If a person moves inside a pre-defined danger zone, the robotic arm will cease to operate.

MAKING THE CONNECTION

While De San Bernabé and his colleagues work on making individual robots smarter, other researchers at A*STAR are developing ways for smart devices to reach the next level of intelligence by communicating with one another. Connecting industrial Internet of Things (IIoT) devices is particularly challenging, said Min Li Huang and Boon Shyang Lim of the Institute for Infocomm Research (I²R), as the demands are high but the hardware is limited.

"The background noise and interference in industrial environments will affect the quality of the signal and packet reception, causing longer latency or higher packet loss rates. These are not acceptable for IIoT applications which are usually real-time and demand very low data loss rates, very high reliability and very low latency," Huang said. These issues are compounded by the fact that IIoT sensors are typically low-power devices or 'edge' devices that have limited processing and memory capabilities.

Working with Japanese heavy industry manufacturer IHI Corporation, Huang and the team under the leadership of Sumei Sun have developed an IIoT system that can capture sensor data and use it to anticipate machine maintenance needs. Called the 5G-ready Plug & Play IIoT Analytics Module, the technology comprises a wireless communications algorithm and a predictive maintenance algorithm based on machine learning.

Unlike other communications networks which require extensive human intervention to debug and optimize, the network management module developed by Huang is powered by an algorithm that allows it to self-configure and heal, allowing the researchers to achieve a reliability performance of 99 percent. Data collected from devices at the edge are then transmitted over this high reliability network to what are called edge gateways, which are devices with better processors and more memory to support edge analytics, before being sent to the cloud or a local server for storage or further processing.

The predictive maintenance algorithm, on the other hand, uses the sensor data collected to monitor machine condition, detect anomalies and predict the remaining useful life of the machine. A lightweight implementation of the model can be run directly on the edge devices, while results produced at the edge gateway can be used for real-time insights and decision making, Huang says.

"But what really sets us apart is that we are system- and platform-agnostic; our intellectual property can be plugged into any of the existing systems or platform tools available," she adds. For example, the network management module supports many different protocols—

IIoT supports many use cases and applications, each requesting for different communications performance and computing algorithms. Depending on the data types, the edge computing algorithms will need to adapt too.

— Sumei Sun, Head, Communications and Networks Cluster, I²R, A*STAR

including 5G—giving users the flexibility to choose the wireless protocol that best suits their needs.

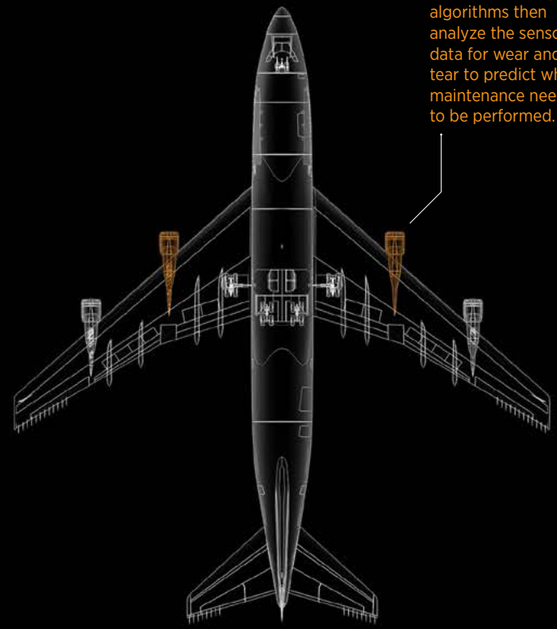
"IIoT supports many use cases and applications, each requesting for different communications performance and computing algorithms," said Sun. "Some use cases request for high data rates, such as high resolution images and videos, while others request for very low latency but also lower demands on bandwidth. Depending on the type of data, edge computing algorithms will also need to adapt. The module therefore supports multiple wireless protocols, including 5G. More edge computing functionalities will be enabled as well."

The team is deploying their system at Singapore Institute of Manufacturing Technology (SIMTech)'s Model Factory and ARTC's Next-Generation Hyper-Personalization Line, and is in discussion with a few partners for deployment, Sun shared.

MACHINE LEARNING TAKES TO THE SKIES

Nowhere do the twin concerns of safety and predictive maintenance come to the fore more than in the airline industry, where costly delays and cancellations can have a profound impact on a company's bottom line. With an eye towards using technology to address delays, Singapore Airlines (SIA) tapped on the expertise of researchers at I²R to set up the SIA-I²R Joint Lab.

"The partnership forms part of SIA's Digital Innovation Blueprint to boost our digital capabilities and accelerate the adoption of digital technologies in the aviation and travel industry, helping to transform the aviation industry for the future," said Hwa Peng Lau, Senior Vice President of Engineering at SIA.



Sensors constantly monitor the performance of aircraft components. Machine learning algorithms then analyze the sensor data for wear and tear to predict when maintenance needs to be performed.

Since deployment in 2018, the model has helped to identify three valve failures about one to two days before the failure occurred.

— Hwa Peng Lau, Vice President of Engineering at SIA

A single flight on an A380 or B777 airplane can generate vast amounts of data, with sensors measuring anything between 1,400 to 3,000 different parameters up to eight times per second. Making sense of so much data is simply not possible for humans, and until recently, even machines. But machine learning has changed the game, allowing researchers to probe the health of different aircraft components and estimate the likelihood of a failure, based on historical flight recorder data.

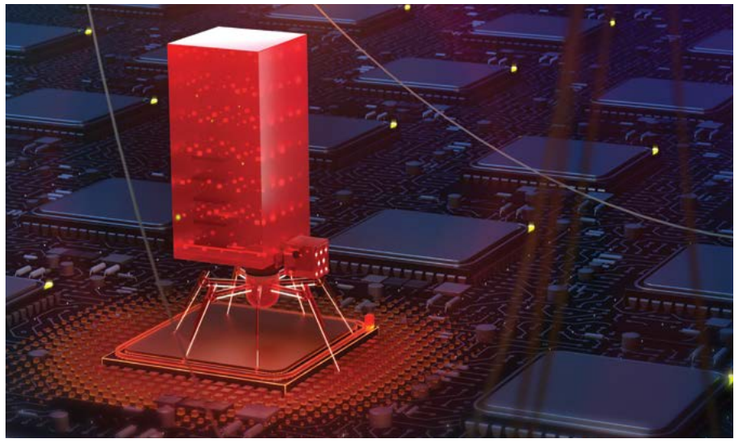
In particular, the algorithm developed by the joint lab measures the engineering health of a critical component known as the bleed pressure regulating valve, which controls the flow of hot, pressurized air from the engine to the cabin via the length of the wing. Pressure sensors upstream and downstream of this particular valve indicate whether it is sufficiently opened or closed. When combined with other algorithms, this algorithm has helped SIA mitigate more than 500 minutes of flight delay time, according to Lau.

The pressure regulating valve model has been deployed in an online decision support tool used by operational staff. Since 2018, the model has helped to identify three valve failures about one to two days before the failure occurred, Lau shares. "And as with all machine learning algorithms, it will become more accurate over time as we feed more data into it," Lau says, sharing that the team is continually adding more training events to improve the accuracy and lead time of their model.

"At the same time, as airline operations are dynamic, the team remains poised to embark on new predictive maintenance use cases related to emerging issues, possibly using appropriate new methods including unsupervised learning," he says.

"Through this applied partnership with I²R, our goal is to develop smart solutions that will help lower maintenance cost, reduce aircraft delays and aid us in enhancing our service standards." ★

1. De San Bernabé, A. and Araiza-Illan, D. Dynamic Regions to Enhance Safety in Human-Robot Interactions. 2018 *IEEE 23rd International Conference on Emerging Technologies and Factory Automation.*



CYBERSECURITY

Detecting malware on the fly

A*STAR researchers have devised a machine learning technique to help antivirus developers stay ahead in the cat-and-mouse game of Android malware detection.

More than two billion active devices run on Google's Android platform. Unfortunately, the popularity of the operating system has made it the target of malicious software, or malware—around 3.2 million new Android malware samples were identified by the end of the third quarter of 2018, according to German security software company G Data.

Cybersecurity experts have developed defenses against some of these bad actors, including machine learning and artificial intelligence tools that can recognize suspicious applications. However, most existing methods require expert analysis to predetermine specific malware features—an approach only possible for known cybersecurity threats.

"As malware keeps on evolving, any predetermined features will soon become outdated, but manually defining new features takes time and is not easy. Also, updating the batch learning-based classifiers requires retraining the malware detection model with new malware samples and all previous training samples, which is slow and resource intensive," said Li Zhang, a former Research Scientist at A*STAR's Institute for Infocomm Research (I²R), who is now with ST Engineering.

To overcome these limitations, Li and colleagues combined two techniques—n-gram analysis and online classifiers—to create a machine learning model for more efficient discovery of Android malware[1]. The method uses part of an application's code to generate n-grams, the equivalent of a fingerprint containing detailed information about the application.

A classifier algorithm then automatically assigns a score to the component parts of the fingerprint (sub-fingerprints) according to how closely each sub-fingerprint resembles malware. "A dedicated classifier is used to handle a specific category of information in the Android application. Such a design helps further improve the classification accuracy and reduce the model training time," Zhang explained. Importantly, their model is able to adapt itself based on new training samples without forgetting knowledge obtained from prior datasets—what is known as incremental learning.

Applying their approach to a benchmark dataset of more than 10,000 application samples, the researchers achieved a malware detection accuracy of 99.2 percent. Tested on a real-world dataset containing more than 70,000 samples, the model performed with 86.2 percent accuracy. Furthermore, when classifying malware, the technique obtained an accuracy of 98.8 percent on the top 23 malware families of the Debrin dataset, a well-annotated library of Android malware.

"Our framework can help security analysts or antivirus developers better cope with fast-evolving malware. Besides, the underlying model is linear and lightweight, which can even be deployed on phones to achieve real-time protection of Android users," said Zhang.

His team is now expanding the framework by also considering the runtime behaviors of Android applications, which will further improve malware classification accuracy. ★

ABOVE
Malware, like biological viruses, are constantly evolving, and cybersecurity countermeasures on mobile devices need to keep up.

Photo credit: archy13 / Shutterstock

1. Zhang, L., Thing, V. L. L., Cheng, Y. A scalable and extensible framework for android malware detection and family attribution. *Computers & Security* **(80)**, 120-133 (2019).

ARTIFICIAL INTELLIGENCE

Speeding up machine reading

A new model called DECAPROP is making machines more 'literate' by helping them to understand the contextual meaning of words.

Nearest Michelin-starred restaurant to your hotel? Check. The meaning of life? Check. When it comes to the all-encompassing task of answering any question we could have, search engines such as Google and Baidu never fail to deliver. At the heart of this all-knowingness is the ability to read and understand both the question and the encyclopedic volume of information on the internet.

While reading comprehension comes naturally to humans, designing machines that achieve similar capabilities remains a challenging task.

Current reading comprehension technology hinges upon artificial neural networks—computer programs mimicking the human brain—which comprise several 'layers.' More layers enable the network to provide complex and informative results, such as the image of a person's face by detecting a series of clustering pixels, but it also comes at a price—slower information flow and the need for greater computational power.

To overcome the constraints associated with machine reading comprehension, researchers from the Institute of Infocomm Research (I²R) and Nanyang Technological University, Singapore, have designed a new architecture for training neural networks to read. Named Densely Connected Attention Propagation for Reading Comprehension, or DECAPROP for short, their model yields faster and improved learning, producing more accurate and efficient machine reading[1].

The study's lead authors, Yi Tay and his supervisor, Anh Tuan Luu, highlight a key feature of DECAPROP: the bidirectional attention connector (BAC), which enables the network to build contextual relationships between words in a given text. For instance, the word 'cold' could refer to an illness, the temperature or someone's behavior. Arriving at the correct interpretation would require a machine to recognize the context by processing the entire text.

"DECAPROP also increases the number of interaction interfaces, by matching layers in an asynchronous, cross-hierarchical fashion that leads to an improvement in performance," added Luu.

The researchers then put the model to the test using five datasets—NewsQA, Quasar-T, SearchQA, NarrativeQA and SQuAD—comprising hundreds of thousands of question-answer pairs. These datasets allow scientists to assess a neural network's ability to extract accurate answers to questions relating to long and complex text, such as news articles and even books or movie scripts.

"DECAPROP achieved exceptional performance on four datasets, achieving a significant gain of 2.6–14.2 percent absolute improvement in F1 score over the existing state-of-the-art," said Luu, the F1 score being a measure of a neural network's precision and recall ability.

The results, which have been presented in a paper at the 32nd Conference on Neural Information Processing Systems, pave the way for enhanced reading comprehension skills in machines, which could be applied in diverse fields such as healthcare, customer service and language translation.

"The modularity of the BAC makes it relevant to other models and domains, thus enabling a wider usage of this model in reading comprehension applications," explained Luu. "DECAPROP can, therefore, be used for any application that requires machine comprehension or question answering." ★

Photo credit: AlesiaKan / Shutterstock

1. Tay, Y., Luu, A.T., Hui, S.C., Su, J. (2018). Densely Connected Attention Propagation for Reading Comprehension. NIPS'18 *Proceedings of the 32nd International Conference on Neural Information Processing Systems*, 4911-4922 (2018).

LEFT
The DECAPROP model for reading comprehension developed by A*STAR scientists could help machines become more 'literate.'

INTERNET OF THINGS

On guard against wireless jamming

An A*STAR team has developed a 'guard node' approach to securing Internet of Things networks.

As the field of wireless communication advances, the sheer number and variety of smart devices and physical objects connected to the internet will only increase. In this Internet of Things (IoT) universe, we might observe medical sensors monitoring vital signs in patients in real time, and self-driving cars updating their location every millisecond to avoid collisions.

If society is to rely on these IoT systems for critical functions, protecting them from cyberattacks will be vital. A team of researchers at A*STAR's Institute for Infocomm Research (I^2R) has thus developed a strategy involving a 'guarding node' to keep IoT networks secure[1].

In a typical IoT setup, several remote devices transmit information wirelessly to a base station, which must separate the signal it receives into individual components, then decipher the original information sent by each device. "The base station has to do this 'blind,' without any prior knowledge about the true input signals it is meant to receive," explained Peng Zhang, a Research Scientist at I^2R who led the study.

"The guard node approach enables us to simultaneously detect the jamming attack, reject the jamming signals and recover the legitimate signals."

As Internet of Things networks become increasingly commonplace, it is crucial to devise strategies to protect them against crippling jamming attacks.

Photo credit: Rawpixel.com / Shutterstock

However, this leaves the network open to being jammed by an attacker transmitting a false signal using the same channel as one of the remote devices, which would contaminate the information received by the base station. This is where the guarding node comes into play.

Put simply, the guarding node can be preconfigured at the base station to inject a known guarding signal into other incoming signals from remote devices—think of this as a barcode being overlaid onto the received signal. When the base station recovers the individual incoming signals from the received signal, it should be able to derive the barcode correctly in a jamming-free environment.

Incoming signals that have been subjected to jamming will have the barcode tampered with in a specific manner. Importantly, by analyzing the pattern of tampering, the base station can derive the original signals using an algorithm. This guard node approach enables greater security over low latency connections, the researchers said.

The traditional response to a jamming attack also introduces communication delays as it requires two separate steps: jamming detection, followed by jamming countermeasures such as switching the communication frequency between the base station and the remote device.

"In contrast, the guard node approach enables us to simultaneously detect the jamming attack, reject the jamming signals and recover the legitimate signals, with just one snapshot of the received signal, preserving a network's communication speed and security," Zhang noted.

Moving forward, Zhang's team will work on simulating modulated signals that are typical of an industrial IoT environment and demonstrate performance enhancements linked to their guard node approach. ★

1. Zhang, P. and Sun, S. One Node to Guard All: Jamming-Resistant and Low-Latency Communication for IoT. *2018 IEEE Global Communications Conference (GLOBECOM), Abu Dhabi, United Arab Emirates*, 1-6 (2018)

SURFACE ANALYSIS

A smooth solution to a rough problem

Laser-based sensor measures the surface roughness of materials in real time.

To get an idea of how rough a surface is, scientists may run a physical stylus across it to record variations in surface height—what is known as a contact method of measurement. An alternative would be to use optical measurement instruments, a non-contact method that does not damage the surface and allows for higher resolution data.

However, as most optical profilers are large and desk-bound, assessments are typically performed on finished products, when correction of defects may be difficult or wasteful. To make real-time and on-site evaluation of surface roughness possible, scientists from A*STAR's Advanced Remanufacturing and Technology Centre (ARTC); Nanyang Technological University, Singapore; and the University of Strathclyde in Glasgow, UK; developed an optical sensor that can profile the surface of materials at high resolution, and in real-time[1].

Their device consists of a laser confocal sensor that concentrates light at a focal point on the material being assessed. Affixed to an industrial robotic arm, the laser-based sensor sweeps across the material surface, while a motion control system minimizes vibration caused by the positioning system or scanning mechanism, allowing for highly precise analysis of material surface properties.

"We found that single-point sensing is very suitable for roughness measurement, but because single-point confocal sensors typically have a small measurement range, we devised a way to increase the horizontal measurement range of the technique using data stitching," said Fang Cheng, Group Manager at ARTC, a lead author on the study.

With their system, the researchers were able to measure surfaces with a roughness average (Ra) of 0.2–7 micrometers, performing comparably to the Talysurf PGI 800 stylus profilometer, a widely used, high-accuracy standard instrument for surface roughness measurements. Essentially, Ra is the average value of a set of individual measurements of a surface's microscopic valleys and peaks. A patent application has been filed for the technology.

Moving forward, the team intends to further improve the system by assessing more roughness parameters aside from Ra. "We also need to include surface tracking technology to increase the vertical measurement range, which is critical for free-form surfaces, and provide solutions to isolate vibrations or remove vibration noise," Cheng added. The group is also looking to further enhance the present optical sensor's resolution of measurement and lower its cost of production. ★

1. Fu, S., Cheng, F., Tjahjowidodo, T., Zhou, Y., Butler, D. A Non-Contact Measuring System for In-Situ Surface Characterization Based on Laser Confocal Microscopy. *Sensors* **18**, 2657 (2018).

BELOW
Non-contact methods for evaluating surface roughness are advantageous because they avoid damaging the surface and provide higher resolution measurements.

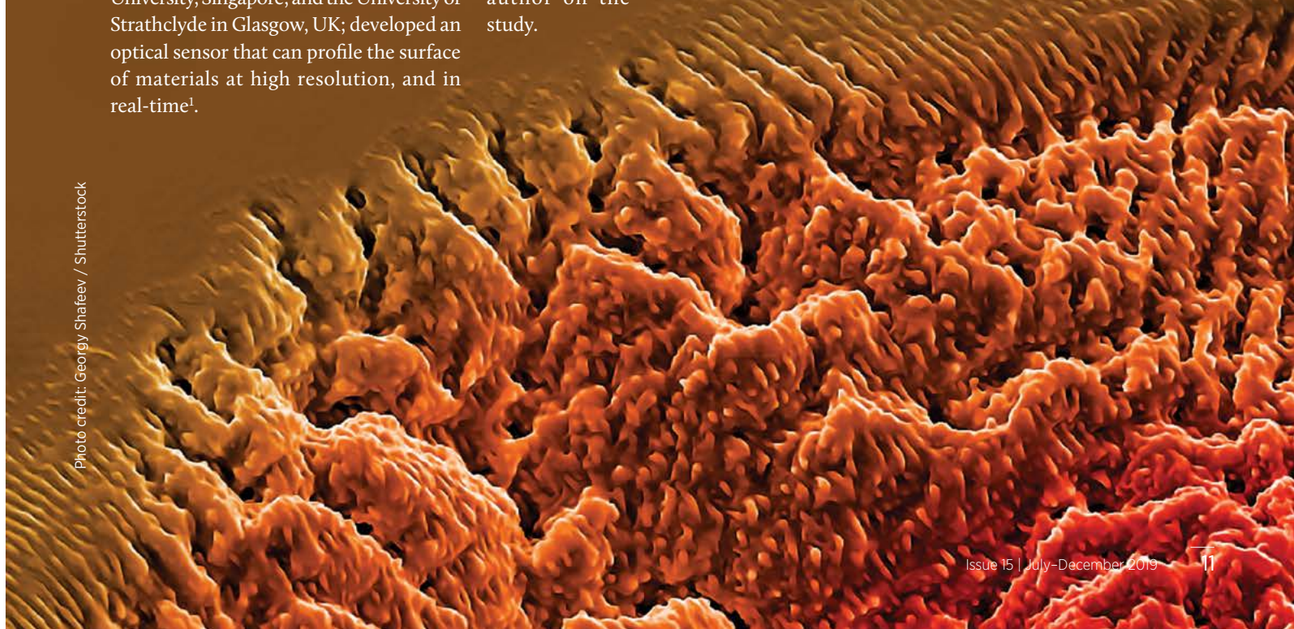
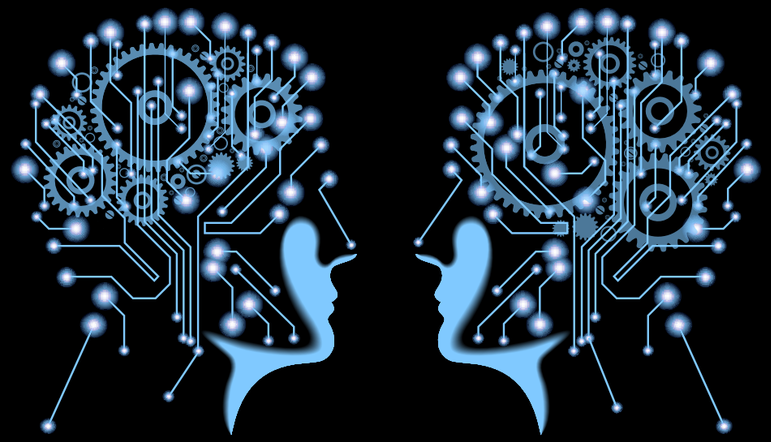


Photo credit: Georgy Shafeev / Shutterstock



MACHINE LEARNING

A shortcut for anomaly detection

A*STAR scientists have designed sophisticated machine learning techniques that are more efficient at identifying anomalies in datasets.

The rules of 'spot the difference' puzzles are simple: given two pictures—one original and one that has been subtly altered—a player must identify all the differences between them. Detecting all the 'anomalies' in the altered picture comes intuitively to humans, and with recent advances in machine learning, even computers can perform this basic task with relative ease.

Beyond fun and games, anomaly detection can be applied to more complex problems. For instance, a company that tracks the log-in activity on its e-payment platform would know the typical behavior of its users, but it also wants to flag any suspicious actions that may be taking place. Because of the large and multi-dimensional nature of datasets on user activity, more sophisticated machine learning techniques, such as generative adversarial networks (GANs), are required to accurately spot anomalies.

Essentially, GANs consist of two competing networks—a generator and a discriminator. The generator creates new data that mimics as closely as possible real-world data from random latent codes, while the discriminator seeks to distinguish between real-world data and those produced by the generator. The core idea behind GAN-based anomaly detection methods is that normal data (that the GAN is trained on) can be accurately reconstructed, while anomalous data cannot, much like how it is far easier for a human to sketch out a previously seen object than something completely new.

Reconstructing a particular data sample, however, requires a time-consuming optimization process to find its associated random latent code. "The only GAN-based anomaly detection method available at the time was extremely slow and impractical for use on large datasets," said Chuan Sheng Foo of the Institute for Infocomm Research (I^2R). "We wanted to develop a method that leveraged the power of GANs while still being fast."

The researchers thus used a class of GANs that simultaneously learns an encoder network to, in effect, predict the associated random latent code for a data sample. By sidestepping the optimization routine to find the code, it allows for speedier anomaly detection[1].

"Once the GAN is trained, it can be used to detect anomalies by calculating a threshold value based on a novel anomaly score that quantifies the distance between the original samples and their reconstructions; higher scores reflect more anomalous examples," Foo explained.

Applying this Adversarially Learned Anomaly Detection (ALAD) method to anomalies in image data as well as tabular data, the team demonstrated that their approach worked as well as, if not better than, other competing methods in terms of accuracy. ALAD was also much faster than the previous GAN-based techniques.

"We are exploring how ALAD and related techniques can be applied to time-series data such as sensor data from machines, for example. This could be useful for the predictive maintenance of machines," Foo said. ★

ABOVE
Generative adversarial networks behave like two opponents in a game. In this case, the game involves identifying anomalies in data sets.

1. Zenati, H., Romain, M., Foo C. S., Lecouat, B. & Chandrasekhar, V. Adversarially Learned Anomaly Detection. *2018 IEEE International Conference on Data Mining (ICDM)*.

Photo credit: VLADGRIN / Shutterstock

NANOTECHNOLOGY

Towards thinner and faster transistors

Nanoribbon field effect transistors could usher in the next generation of computing.

At the heart of every computer and smartphone, billions of microscopic silicon transistors etched into a tiny chip perform digital calculations at mind-boggling speeds. A transistor turns on or off the current flowing through it, depending on the input voltage it receives. Smaller transistors require only small voltages and can switch between states quickly, leading to increased performance.

The continued shrinking of silicon transistors has made computers faster, cheaper and more efficient over time, with Moore's Law predicting that twice as many transistors can be fitted into an integrated circuit every two years. "However, in the past decade, silicon transistors have become so small that their performance has degraded due to quantum effects," said Dharmraj Subhash Kotekar-Patil, a researcher at A*STAR's Institute of Materials Research and Engineering (IMRE).

Seeking to overcome these limitations, Kotekar-Patil and colleagues are exploring new materials to create the next generation of smaller, faster transistors. They focused their efforts on molybdenum disulphide (MoS_2), a transition metal dichalcogenide that is known to exhibit interesting electrical properties such as high charge mobility, high on/off ratio and low contact resistance[1].

"Commercializing these smaller, faster transistors would result in significant increases in the performance of computer processors."

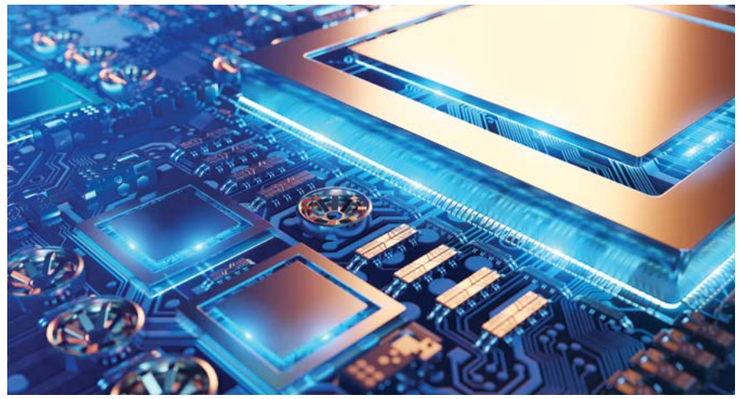


Photo credit: sdecoret / Shutterstock

In this study, the researchers optimized the stepwise process needed to manufacture nanoribbons of MoS_2 at high resolution—down to 50 nanometers—to produce field effect transistors (FETs), devices that direct current flow using an electric field.

"Previous work focused on MoS_2 nanoribbon FETs that are about 6–11 nanometers thick. We have now demonstrated the first nanoribbon FET in single layer MoS_2 that is only 0.7 nanometers thick, with FET properties outperforming previous reports," Kotekar-Patil said.

For instance, in terms of mobility, which is the measure of how fast charge carriers move in a material system, the team's nanoribbon FET displayed almost double the mobility of existing devices. The researchers also reported transistor switching speeds that are almost three times faster than earlier systems. Nonetheless, more research is required to grow and etch single layer FETs across an entire semiconductor wafer before the process can be carried out at an industrially relevant scale.

"Commercializing these smaller, faster transistors would result in significant increases in the performance of computer processors," said Kotekar-Patil. "In addition, MoS_2 nanoribbon transistors could be used to trap single electrons and use their spin properties to encode information for quantum computing, which is an ongoing and active area of research at IMRE." ★

LEFT
Smaller transistors can turn on or off more quickly, thereby allowing faster computing. Researchers are thus pushing the limits of transistor size into the nanometer range.

1. Kotekar-Patil, D., Deng, J., Wong, S. L., Lau, C. S., and Goh, K. E. J. Single Layer MoS_2 Nanoribbon Field Effect Transistor. *Applied Physics Letters* **114**, 013508 (2019).

SCALING UP FOR SUCCESS

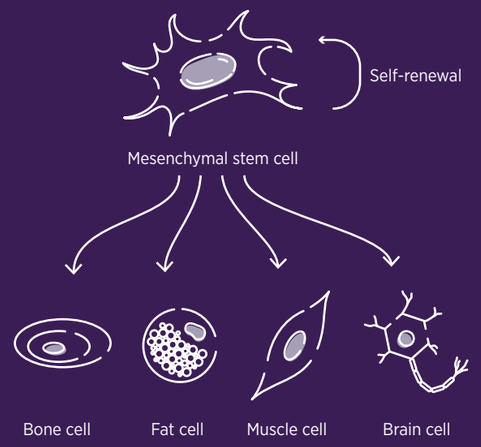
If cell-based therapies are to make it to the clinic, methods to grow stem cells in vast quantities will need to be developed. Here's how A*STAR scientists are tackling the challenge of manufacturing stem cells at scale.

Of the many characters Australian actor Hugh Jackman has played, Marvel Comics' Wolverine is arguably his most iconic role.

Underlying Wolverine's longevity in the often violent and cataclysmic comic-book universe is his powerful regenerative ability, conferred by something vaguely referred to as a 'healing factor.' Although greatly exaggerated for dramatic effect in the comics, 'healing factors' do exist in the human body and can take the form of stem cells.

In adults, stem cells can be found in a variety of organs, including bone marrow, liver, skin and skeletal muscle. Unlike mature cells that are specialized and that do not divide, adult stem cells retain their ability to self-renew and are pluripotent, which means that their fates and functions are not yet cast in stone. For example, mesenchymal stem cells (MSCs)—a type of stem cell typically found in umbilical cord blood and bone marrow—can give rise to bone, brain, fat and muscle cells.

When thought of as replacement parts for machines, stem cells represent a novel class of treatments that could help repair organs damaged by injury or disease. However, hurdles remain in bringing these living medicines to the clinic.



"As the cell therapy market expands and more cell therapies get approved, there is a need to be able to manufacture cells at a large scale in order to deliver these therapies to patients on time," May Win Naing, Head of the Bio-Manufacturing Programme at A*STAR's Singapore Institute of Manufacturing Technology (SIMTech), told *A*STAR Research*.

Some cell therapies that have received regulatory approval include TEMCELL® for the treatment of acute Graft versus Host disease in Japan, and Alofisel® for the treatment of Crohn's disease in Europe. Meanwhile, hundreds of other clinical trials involving stem cells are currently underway.

"Manufacturing cells for these applications must be carried out with safety, consistency, scalability, reproducibility and comparability in mind," Win Naing added.

A NUMBERS GAME

Scientists estimate that 1–10 million MSCs per kilogram of patient are required for each infusion of a cell-based therapy. The massive number of cells needed for multiple infusions (further multiplied by many patients) means that traditional cell culture methods fall short, primarily because of limitations in the surface area available for cell growth, as well as difficulties in maintaining a homogenous physical and chemical environment for the cells.

Seeking to overcome these challenges, researchers led by Steve Oh, Director of the Stem Cell Bioprocessing group at A*STAR's Bioprocessing Technology Institute (BTI), developed biodegradable microcarriers that can be used to expand MSC populations *in vitro*[1]. The research also involved collaborators at the Institute of Materials Research and Engineering (IMRE) and the Singapore Bioimaging Consortium (SBIC).

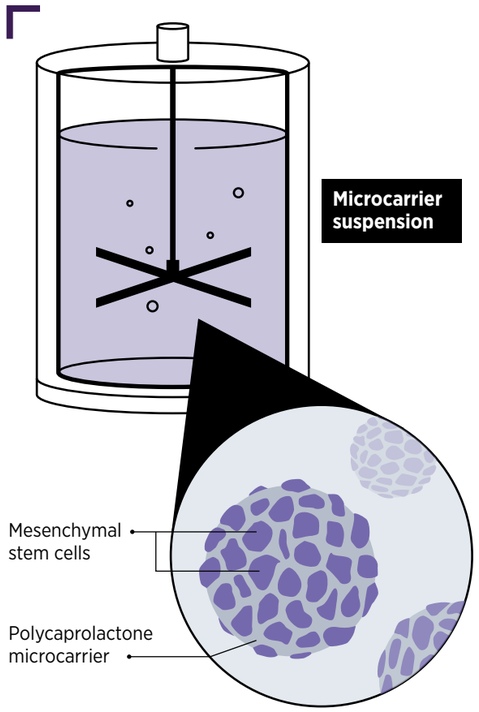
Fundamentally, microcarriers are tiny, porous particles with a large surface area to volume ratio for MSC attachment and growth. "Our microcarriers are made of polycaprolactone—a cheap, consistent material source widely used as a biodegradable construct for scaffolds and reconstruction of bone," said Oh, adding that his team coated the microcarriers in a layer of three proteins that promote MSC adhesion and spreading.

With a density of just 1.06 g/cm^3, these coated microcarriers were easily suspended under constant stirring in well-defined culture media—liquid containing a precise combination of nutrients and other chemicals that encourage MSC growth while preventing MSCs from losing their pluripotency. Using their system,

the researchers achieved cell densities of nearly half a million cells per milliliter of cell culture—about a ten-fold increase over conventional culture techniques. More than 90 percent of those cells were viable and expressed markers typical of MSCs.

Further, when applied to a defect inflicted on the calvarial bone, or skullcap, of rats, MSCs delivered with the coated microcarriers enhanced bone healing as compared to MSCs or microcarriers alone. This result suggests that the coated microcarriers serve as a support matrix, acting synergistically with the MSCs to regenerate bone. Another crucial observation was the absence of inflammatory cells at the site of MSC-on-microcarrier delivery, indicating that there was no rejection of the graft.

Having demonstrated the feasibility of high-density MSC culture using coated microcarriers and proved their therapeutic efficacy in rats, the researchers are now looking to scale up their approach. "We will be working with IMRE to mass produce the biodegradable microcarriers to kilogram levels for ten-liter scale bioprocessing," Oh said. The ultimate goal, he added, is to create a bioreactor capable of producing billions of MSCs of consistent quality for use in the clinic.



SEPARATING THE GOOD FROM THE BAD

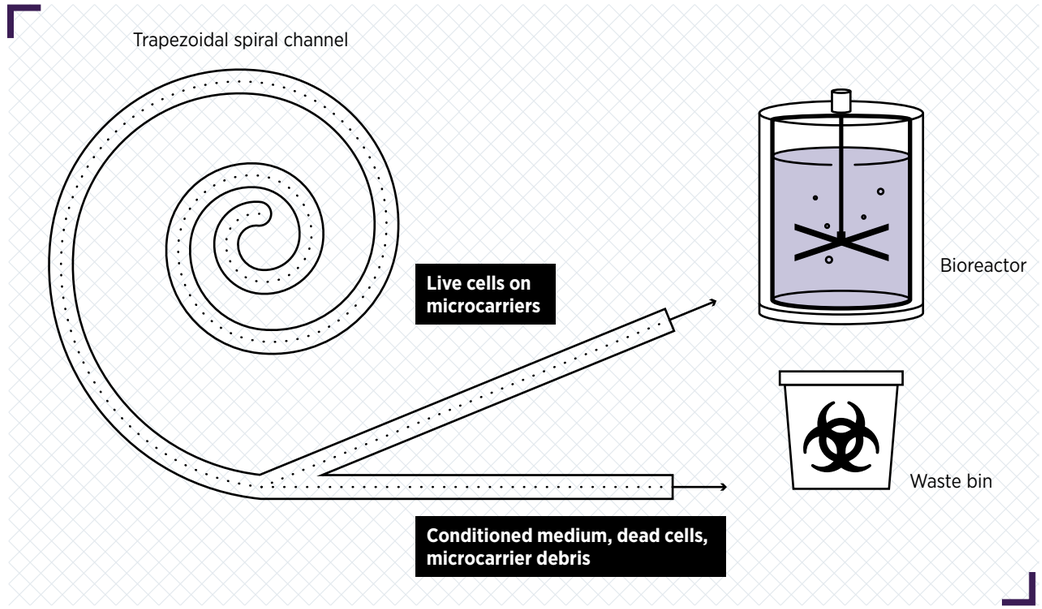
But microcarrier properties are not the only considerations when building a good bioreactor, said Oh. "Mixing becomes an issue if the microcarrier concentration is increased, and this puts an upper limit on cell densities in culture," he explained. "Furthermore, cell expansion in bioreactors is inevitably accompanied by some unwanted byproducts such as particulate contamination, including dead cells and microcarrier debris."

To develop methods for sifting out unwanted contaminants in bioreactors without compromising the quantity and quality of the cell-bearing microcarriers, Oh collaborated with Win Naing's team at SIMTech. Instead of using a membrane as a filter, the scientists designed a spiral microfluidic system that relies on flow forces within liquids as a sorting mechanism[2]. The research also involved Chun Yang, Professor at Nanyang Technological University, Singapore, as well as an industry partner, Whirlcell Technologies.

Existing microfluidic systems for processing biological samples typically deal with particles about ten micrometers in size. However, microcarriers—especially those with cells attached, or those that clump—are at least ten times larger. The researchers thus had to increase the dimensions of their device and optimize its flow-control properties, depending on numerical simulations to facilitate their design. The outcome was a trapezoidal spiral channel with an outer wall that was taller than the inner wall.

Applying their scaled-up microfluidic system to a prototype bioreactor for MSCs, Win Naing's team was able to replace 'old' or conditioned cell culture medium with fresh medium via a single-loop trapezoidal spiral channel. They reported 1–2 percent losses of MSC-bearing microcarriers during the medium replacement process, but showed eight-fold cell expansion over the culture period, a result comparable to standard spinner flask cultures. Notably, the size of microcarrier clumps in their prototype bioreactor was smaller, which makes for easier separation and harvesting of MSCs later on.

"Our scaled-up inertial microfluidic device relies only on hydrodynamic forces—shear force and centrifugal force—in curved channels to separate culture medium from microcarriers. Simultaneously, the isolated microcarrier-cell complexes are recycled to the bioreactor continuously, without any interruption to the operation of the bioreactor," said Win Naing. The technique developed in this study could be applicable to the mass-culturing of immune cells for immunotherapy, or even lab-grown meat, she added.



NICHES BUT NOT SILOS

Recognizing that their research projects are links in a broader manufacturing value chain for cell-based therapy, both Oh and Win Naing see great benefit in collaborating and combining the strengths of their respective labs.

"The Bio-Manufacturing Programme at SIMTech works closely with collaborators such as BTI and the National Cancer Center Singapore. We pursue feedback and conduct extensive validation studies to benchmark innovative manufacturing platforms and technologies for scaling up and scaling out cell therapies," Win Naing commented. "Our capabilities at SIMTech include design and simulation software, prototyping equipment, automation and bonding technologies."

All this is complemented by BTI's focus on the bioprocessing aspects of cell therapies. The key thrusts at BTI revolve around the development of serum-free media for microcarrier cultures and more optimal feeding strategies to achieve higher yields and better harvesting conditions. David Fiorentini, Vice President for Scientific Affairs at Biological Industries, a multinational biotechnology company headquartered in Israel, notes that these objectives align well with industry needs.

"We entered into a collaboration with A*STAR's BTI three years ago to develop culture medium, auxiliary solutions and processes for three-dimensional culture of MSCs in a microcarrier suspension culture system. The development work, which is still ongoing at BTI, will support the requirements for a high number of quality-assured cells to be used in clinical applications," he said.

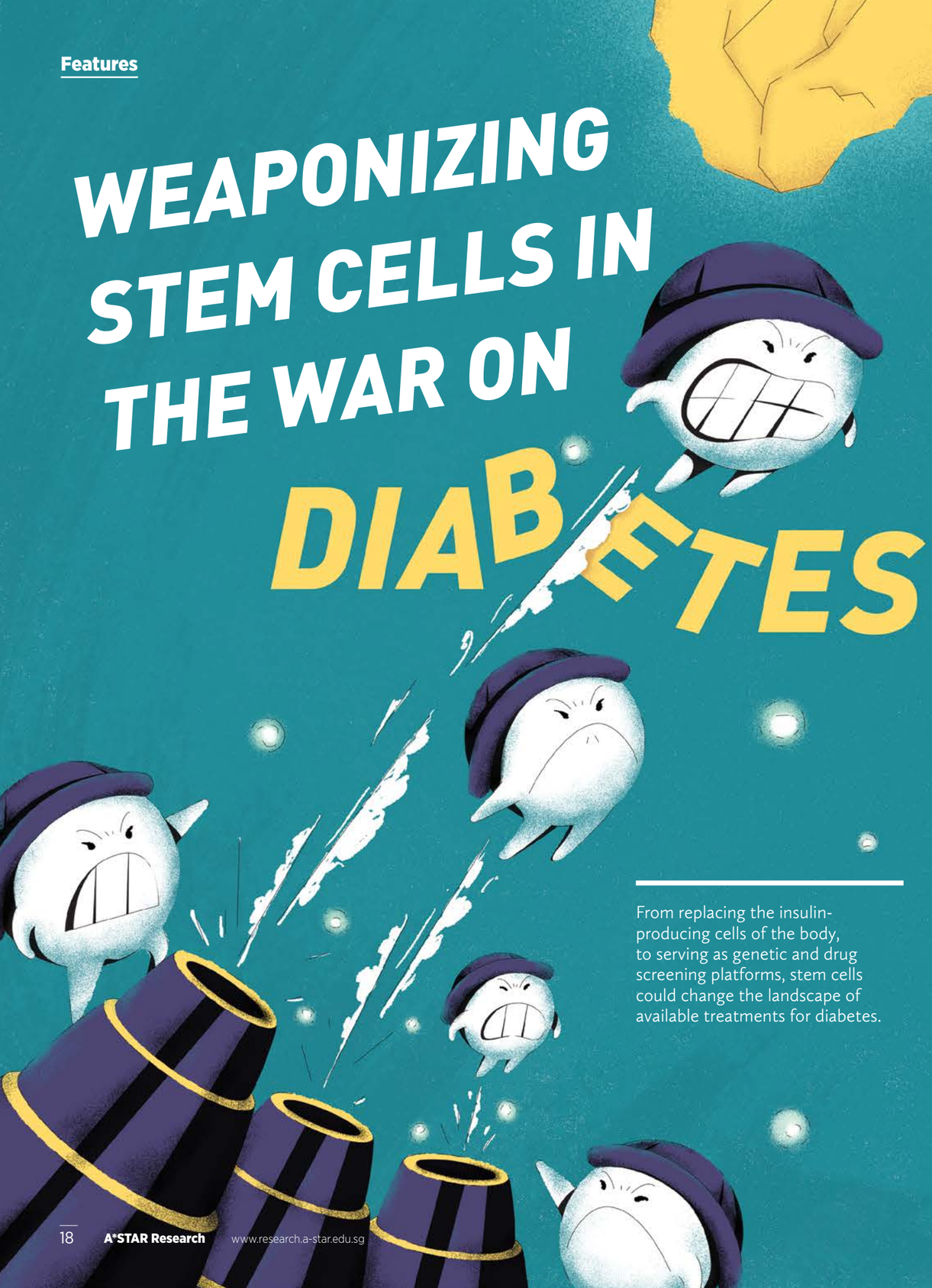
Meanwhile, the Bio-Manufacturing Programme at SIMTech has also inked collaborations with local manufacturers Cal-Comp Precision and MClean Technologies to expand its technical capabilities at the intersection of engineering and biology, said Win Naing.

So just as Wolverine didn't succeed on his own but was aided by the diverse strengths of his fellow X-Men, a multidisciplinary approach bridging academia and industry is the preferred strategy for unlocking the potential of cell therapies.

"Close collaboration between clinicians, regulators, engineers and scientists—that's what is needed to ensure cell therapies are successful," Oh concluded. ★

1. Lam, A. T., Sim, E. J., Shekaran, A., Li, J., Teo, K. L. *et al*. Sub-confluent culture of human mesenchymal stromal cells on biodegradable polycaprolactone microcarriers enhances bone healing of rat calvarial defect. *Cytotherapy* **21(6)**, 631-642 (2019)
2. Moloudi, R., Oh, S., Yang, C., Teo K. L., Lam, A. T. *et al*. Scaled-Up Inertial Microfluidics: Retention System for Microcarrier-Based Suspension Cultures. *Biotechnology Journal* **14(5)**, e1800674 (2019)

WEAPONIZING STEM CELLS IN THE WAR ON DIABETES



From replacing the insulin-producing cells of the body, to serving as genetic and drug screening platforms, stem cells could change the landscape of available treatments for diabetes.

There's no sugar coating it—diabetes is shaping up to become one of the greatest health challenges in modern times. According to the World Health Organization (WHO), one in eleven individuals suffers from diabetes, and the condition was the direct cause of 1.6 million deaths in 2016, based on the latest publicly available data. WHO has since designated diabetes as one of four priority noncommunicable diseases (alongside cancer, respiratory and cardiovascular diseases) to be addressed by global health authorities.

Closer to home, more than 400,000 Singaporeans live with the diagnosis of diabetes, says the Ministry of Health. This constitutes ten percent of the local disease burden. Meanwhile, a separate study by the National University of Singapore projected that by 2050, Singapore would be home to one million diabetics if current trends continue. Wary of the insidious consequences of unchecked chronic illness, Singapore's Health Minister Gan Kim Yong declared a "war on diabetes," calling for a concerted national effort to prevent disease onset and better manage disease symptoms.

STEM THE SUGAR TIDE

Despite presenting as a simple disease, diabetes in fact comes in numerous forms. Type I diabetes arises due to an autoimmune reaction that destroys insulin-producing cells called beta cells, located in the pancreas. On the other hand, type II diabetes (T2D) occurs because cells no longer respond to insulin. Lesser known is monogenic diabetes—a rare form of diabetes that is caused by mutations in a single gene.

"Over time, however, pancreatic beta cell failure and beta cell death is a common denominator for all types of diabetes," said Adrian Teo, a Principal Investigator at A*STAR's Institute of Molecular and Cell Biology (IMCB), adding that while obesity is a major contributing factor to diabetes in the West, the main contributing factor in Asia is generally pancreatic beta cell failure. "Although current diabetes medication can help to control blood glucose levels for extended periods of time, they do not cure or even improve pancreatic beta cell health."

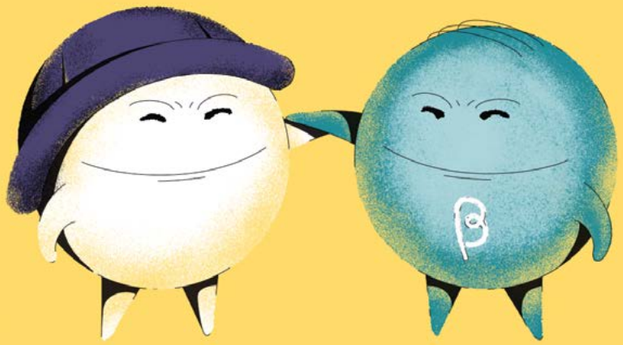
This is why Teo's team sees potential in harnessing the power of stem cells to beat back the scourge of diabetes. Unlike most cells in the body, stem cells have the ability to self-renew and can be differentiated into a variety of cell types, including pancreatic beta cells. Hence, stem cells could potentially be used to replace the defunct pancreatic beta cells of diabetic patients, restoring their insulin production and glucose-regulating capabilities.

Rather than rely on embryos as a source of stem cells, Teo envisions obtaining blood cells and fibroblasts—a type of cell in the skin—from diabetic patients, then reprogramming them into human induced pluripotent stem cells (hiPSCs). Subsequently, gene editing may be carried out to correct diabetes-associated mutations or gene variants in these hiPSCs before they are differentiated into pancreatic beta cells and transplanted back into the patient.

"This method potentially allows for the creation of a near-unlimited supply of pancreatic beta cells for cell replacement therapy," said Blaise Su Jun Low, a final year PhD student in Teo's lab. "Because patients will be transplanted with their own cells, graft rejection is less likely to occur."

A DIFFERENT MODE OF DISCOVERY

Beyond cell replacement therapy, hiPSCs can also help shed light on the underlying molecular mechanisms of diabetes. For example, Teo's research group uses hiPSCs from patients diagnosed with maturity-onset diabetes of the young (MODY)—a subtype of monogenic diabetes—to understand how certain gene networks control pancreas and liver development. Both organs are crucial for normal glucose metabolism.



The broad experimental setup is as follows: first, the researchers induce hiPSCs from MODY patients to differentiate into foregut endoderm and human pancreatic precursors—the parts of human embryos that eventually give rise to the pancreas and liver, and then to pancreatic beta-like cells. The team then compares the gene expression pattern of MODY hiPSC-derived foregut endoderm, pancreatic precursors and beta-like cells to that of normal individuals.

With this approach, Teo's team discovered that mutations in a gene called *HNF4A* result in reduced overall expression of genes specifying pancreas and liver development in MODY1 patients[1]. Importantly, Teo noted that this discovery would not have been possible using mouse models, since mice with one mutant copy of *HNF4A* do not develop diabetes, unlike the situation in humans.

"Currently, there are more than 14 forms of MODY, each caused by mutations in a different gene (e.g. *HNF4A, HNF1A, PAX4* and *INS*)," Teo explained. "Interestingly, gene variants found in many of these MODY genes are associated with T2D, the most common form of diabetes that affects approximately 90 percent of the diabetic population." The findings from MODY patients may therefore be relevant to the pathophysiology of T2D as well, Teo added.

Furthermore, by using hiPSCs as a platform for genetic screens, researchers may be able to better stratify patients into different treatment groups. At the same time, novel drug targets may be identified based on such screening methods. This brings the ideal of precision medicine for diabetes closer to reality—no longer will a one-size-fits-all solution be applied to all patients, but medicines will be prescribed based on underlying genetic defects that are unique to each diabetic person.

SWEETENING THE DEAL

The use of hiPSCs in genetic screening and drug discovery for diabetes is already underway in many labs around the world. On the other hand, therapy involving hiPSC replacement of dysfunctional pancreatic beta cells still has some way to go before it can be approved for use in the clinics.

Low cautioned that protocols for differentiating hiPSCs into pancreatic beta cells are not yet 100 percent efficient, and some residual pluripotent cells may still lurk among differentiated pancreatic beta cells. If these pluripotent cells are also transplanted along with the pancreatic beta cells, they could result in a teratoma—a tumor that could lead to life-threatening complications, she said.

The precise function of hiPSC-derived pancreatic beta cells has yet to be fully verified, Teo continued. "They need to function just like *bona fide* human pancreatic beta cells or islets," he warned. "Otherwise the glucose levels of the individual will not be properly regulated, posing health risks."

Yet another concern lies with the safety of gene-editing technologies such as the popular CRISPR/Cas9 system when correcting diabetes-associated gene mutations. Unless undesirable or unexpected off-target consequences of CRISPR-mediated genome editing can be ruled out, the use of gene-edited hiPSCs for cell replacement therapy will likely remain limited, said Teo.

In spite of these challenges, Teo's group remains optimistic and undeterred. "For hiPSC-based cell therapy without any genome editing, there are currently a few clinical trials ongoing to evaluate the ability of these hiPSCs to mature into pancreatic beta cells and regulate blood glucose levels," he said. "Our lab and many others in the world are now working on optimizing the pancreatic differentiation protocol to eliminate residual hiPSCs and improve the functionality of hiPSC-derived pancreatic beta cells such that they behave similarly to *bona fide* insulin-secreting cells in the islets of the human pancreas."

In fighting the good fight against diabetes, Teo underscored the importance of close collaboration between the lab and clinic. "We believe that partnerships with clinicians and pancreas transplant surgeons are a win-win model that can help us translate our scientific efforts toward eventual therapeutic value for our patients and society," he emphasized. ★

Fluorescent image of a pancreatic islet containing beta cells responsible for producing insulin.

1. Ng, N. H. J., Jasmen, J. B., Lim, C. S., Lau, H. H., Krishnan, V. G. *et al*. HNF4A Haploinsufficiency in MODY1 Abrogates Liver and Pancreas Differentiation from Patient-Derived Induced Pluripotent Stem Cells. *iScience* **16**,192-205 (2019).

IMMUNOLOGY

Delivering a renewable source of Kupffer cells

A*STAR researchers have devised a method to generate a renewable source of mature human Kupffer cells for liver toxicity bioassays.

Residing within the liver are specialized immune cells called Kupffer cells that protect the host by engulfing and destroying pathogens. Despite beneficial functions such as detoxifying chemicals and drugs, Kupffer cells can also cause liver inflammation in response to viruses, alcohol and drugs.

To accurately recapitulate these biological processes in liver toxicity bioassays, researchers need a renewable and sustainable source of mature human Kupffer cells. Capitalizing on the differentiation potential of induced pluripotent stem cells (iPSCs), scientists led by Hanry Yu at A*STAR's Institute of Bioengineering and Nanotechnology (IBN) devised a method to generate mature human Kupffer cells from iPSCs, replacing the current reliance on primary Kupffer cells (pKCs) obtained from adult human livers[1].

"pKCs are expensive, exhibit donor variability, can have a low yield after tedious isolation procedures, and cannot be expanded in culture. They are also restricted by their availability, which depends on the amount of human tissue available," said Farah Tasnim, a Senior Research Scientist at IBN and the first author of the study.

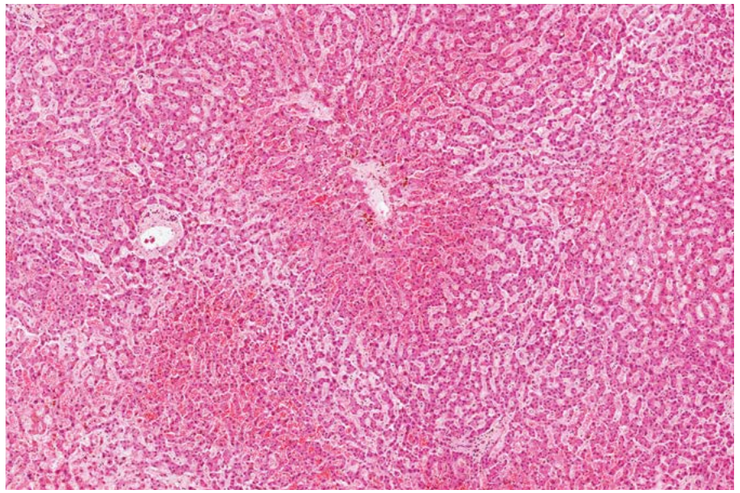
To produce induced Kupffer cells (iKCs), the researchers generated immature immune cells known as macrophage precursors from iPSCs. They then differentiated the macrophage precursors into iKCs using primary human hepatocyte conditioned media. The ability to mature iPSCs into Kupffer cells with an adult-like state in this study is an important breakthrough, since iPSC-derived cells typically retain an immature phenotype.

Using microarray analysis and immunostaining, Yu's team observed that the expression of genes and proteins governing inflammatory and immune function in iKCs were comparable to that of pKCs. The iKCs also functioned similarly to pKCs—they were capable of engulfing fluorescent beads and could produce inflammatory mediators in response to lipopolysaccharide, a substance found on the surface of bacteria.

"Liver toxicity studies can now incorporate inflammation or immune-mediated aspects without being challenged by cell source issues. In addition, more physiologically relevant models for liver conditions, such as fibrosis, viral hepatitis, cholestasis, steatohepatitis, alcoholic/non-alcoholic liver disease and drug-induced liver injury, can be created," Yu said.

Tasnim added that with their method, both iKCs and pKCs can be obtained from the same donor for use in liver toxicity bioassays, expanding the utility of such tests.

The team plans to use iKCs to create a disease model for non-alcoholic steatohepatitis (NASH), a major cause of liver disease globally. ★



Kupffer cells are a specialized subset of immune cells that reside in the liver. Because of their scarcity, they are difficult to obtain for liver toxicity assays in the lab.

Photo credit: Convit / Shutterstock

1. Tasnim, F., Xing, J., Huang, X., Mo, S., Wei, X., *et al.* Generation of mature Kupffer cells from human induced pluripotent stem cells. *Biomaterials* **192**, 377-391 (2019).

STEM CELLS

Growing a 'spine' on a chip

Microfluidic device allows for precise control of growth factor gradients, mimicking natural spinal cord development *in vitro*.

During embryonic development, molecular signals direct the cells of a fetus to divide, move and differentiate, eventually giving rise to all the organs necessary to sustain life outside the mother's womb. In vertebrates, the spinal column is one of the most important structures to be defined along a directional axis of head to tail, and within the spinal column resides a variety of motor neurons connecting the brain to the muscles of the body.

Boon Seng Soh and colleagues at the Institute of Molecular and Cell Biology (IMCB) have now devised a microfluidic device that mimics the molecular cues directing the development plan of motor neurons in a spinal column[1]. The device, which they have named the microHIVE platform, consists of a linear chamber in which stem cells can be seeded and flanked on both sides by an interlocking honeycomb lattice of hexagonal microstructures (microhexagons).

Specific growth factors can then be introduced into the chamber via the honeycomb lattice in a spatially-controlled manner. As the growth factors flow through the honeycomb lattice, they branch out and mix at junctions between the hexagonal microstructures, giving rise to growth factor gradients.

"We know that growth factor gradients are important for tissue patterning in living organisms, so we collaborated with bioengineers to generate these gradients, allowing us to coax the stem cells to self-organize and achieve spatial diversification *in vitro*," Soh explained.

"Our 3D spinal cord model derived from human pluripotent stem cells can be used to study and model spinal cord injury."

By varying device parameters such as the number of fluid inlets for growth factor delivery, the size of each microhexagon and the packing density of the microhexagons, the researchers were able to generate precise gradient profiles of retinoic acid and GDF11—key growth factors involved in patterning the spinal column from head to tail.

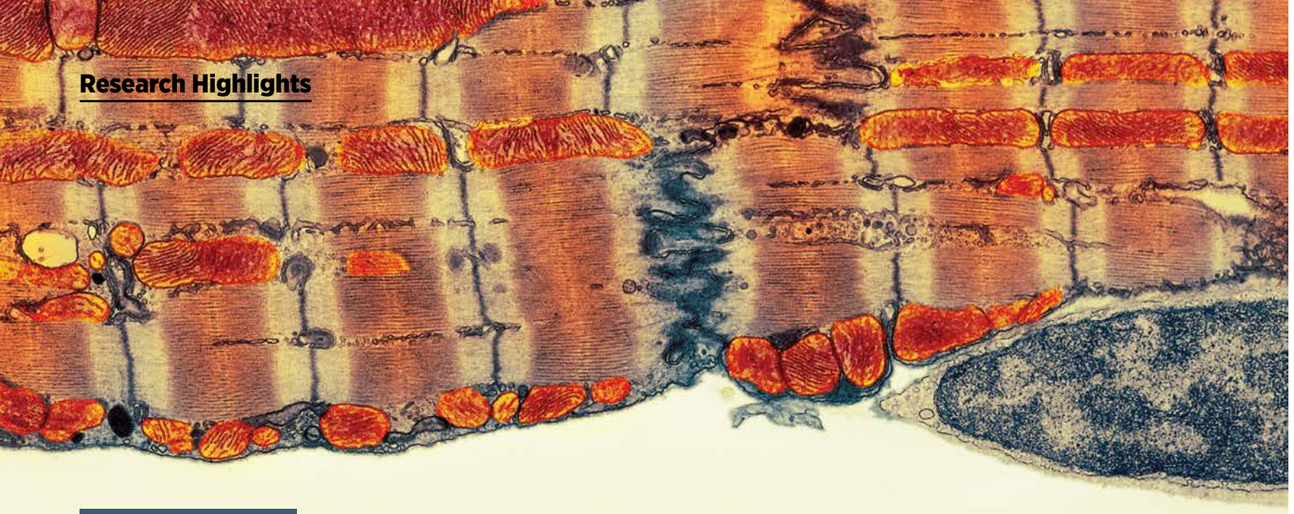
Notably, stem cells exposed to the retinoic acid and GDF11 gradient profile as determined by the microHIVE platform differentiated into motor neuron subtypes in the same arrangement as would have been observed in a developing fetus.

"Our 3D spinal cord model derived from human pluripotent stem cells can be used to study and model spinal cord injury," said Soh. "This system could potentially be used to test or optimize cell-based therapy for spinal cord injury, or serve as a drug screening platform for diseases such as spinal muscular atrophy, which is characterized by muscle wastage due to the death of spinal motor neurons." ★

RIGHT
With the right growth factor gradients, stem cells can be coaxed to form a model of the spinal cord *in vitro*.

1. Lim, G. S., Hor, J. H., Ho, N. R. Y., Wong, C. Y., Ng, S. Y., *et al.* Microhexagon gradient array directs spatial diversification of spinal motor neurons. *Theranostics* **9(2)**, 311-323 (2019).

Photo credit: Nerthuz / Shutterstock



CELL BIOLOGY

Getting mature human heart muscle cells to multiply again

By triggering the Wnt signaling pathway, A*STAR scientists were able to reactivate proliferation in mature human heart muscle cells.

An estimated 17.9 million people die from cardiovascular disease yearly, making it the leading cause of death worldwide. During cardiovascular injury, heart muscle cells—or cardiomyocytes—die and are not replaced, thereby impairing overall cardiac function.

"Only 1–2 percent of mature cardiomyocytes were found to be proliferative in a person's lifetime," said Boon Seng Soh, a Group Leader at the at the Institute of Molecular and Cell Biology (IMCB).

Seeking to preserve or restore cardiac function post-injury, scientists have been exploring ways to improve the efficiency of cardiomyocyte cell division, otherwise known as cell proliferation. Soh's team has now discovered a pathway that reactivates proliferation in mature cardiomyocytes[1].

The researchers obtained cardiomyocytes from adult mice, as well as derived terminally-differentiated cardiomyocytes from human embryonic stem cells. Typically, as cells become more differentiated, they lose their capacity to proliferate. However, when the researchers activated the Wnt signaling pathway—a molecular pathway regulating embryonic heart development—in terminally-differentiated cardiomyocytes, the cardiomyocytes became 'dedifferentiated' and capable of cell division once more.

Importantly, Soh's team demonstrated two ways to trigger Wnt signaling—using an antibody, or a small molecule called CHIR99021.

"The antibody binds to N-cadherin, a molecule found on the surface of cardiomyocytes, resulting in the release of proteins known as β-catenin from the cell membrane, which in turn activates Wnt signaling," Soh explained. "Meanwhile, CHIR99021 prevents β-catenin degradation, which achieves the same effect as activating Wnt signaling."

Unlike the antibody against N-cadherin, which could cause cardiomyocytes to lose their cell-cell contacts, CHIR99021 does not directly interfere with cell-cell adhesion, which makes it more feasible as a therapeutic for heart repair and regeneration. Nonetheless, Soh's team noted that CHIR99021 alone could not restore proliferation for injured cardiomyocytes.

"Some of the injured cardiomyocytes may have commenced the process of programmed cell death and thus will not respond to CHIR99021 or up-regulation of Wnt signaling. Therefore, we suggest the use of CHIR99021, in combination with another drug such as simvastatin, to inhibit apoptosis and promote Wnt signaling to achieve tissue regeneration," said Soh.

The team plans to use nanoparticles to deliver the drugs in a targeted and controlled manner to cardiomyocytes. ★

ABOVE
The ability to get mature heart muscle cells to multiply again has implications for the treatment of cardiovascular injury.

1. Fan, Y., Ho, B. X., Pang, J. K. S., Pek, N. M. Q., Hor, J. H., *et al.* Wnt/β-catenin-mediated signaling reactivates proliferation of matured cardiomyocytes. *Stem Cell Research & Therapy* **9**, 338 (2019).

Photo credit: Thomas Deerinck, Ncmir / Science Photo Library

DIAGNOSTICS

A brighter approach to molecular genetics

A*STAR scientists have created an expanded genetic alphabet containing fluorescent artificial DNA bases for use in diagnostics.

Whether it is to diagnose an infectious disease or identify microbial contaminants in our water supply, quantitative polymerase chain reaction (qPCR) is the go-to technique of many research labs. In essence, qPCR allows for the amplification and quantification of DNA molecules based on the incorporation of a fluorescent probe.

However, to detect the fluorescence of existing probes, highly sensitive—and therefore expensive—equipment is required to obtain a readout of gene expression. To overcome this barrier to widespread use, a research team led by Ichiro Hirao at A*STAR's Institute of Bioengineering and Nanotechnology (IBN) developed an expanded genetic alphabet system of artificial DNA bases tagged with molecules that fluoresce intensely when in close proximity[1].

Hirao first came up with the expanded genetic alphabet at RIKEN, Japan, creating two artificial DNA bases—'Ds' and 'Px'—that pair up, the same way base pairing occurs in the natural DNA bases, between G and C, and A and T. "By increasing the number of base components using unnatural bases, we can produce new DNA molecules with increased functionality," he said.

Expanding on this idea, Hirao and colleagues began modifying their novel genetic bases to incorporate fluorescent molecules. The researchers synthesized a short DNA strand, called a primer, containing 'Ds,' and another unnatural base, which they called 's'. Meanwhile, 'Px' was attached to a fluorescent probe, Cy3.

Put simply, during the DNA amplification step in qPCR, 'Ds' and 'Px' pair up, bringing Cy3 close to 's,' resulting in the emission of bright fluorescence visible to the naked eye. Using their technique, the group was not only able to detect the target DNA, but also measure the copy number of DNA molecules based on the intensity of the fluorescence, thereby enabling quantitative, visual PCR.

Finally, the researchers demonstrated that their system could successfully distinguish between bacterial antibiotic resistance genes that differed only by a single nucleotide. They noted that quantitative, visual PCR would be particularly useful for field applications, especially in situations where rapid detection, ease of use and specificity are paramount.

"We would like to use this method to develop diagnostic kits for infectious diseases, such as dengue and Zika. For instance, the dengue virus has four serotypes, and the combination of real-time PCR with our method can be used to identify the dengue serotype of patients at health clinics within a short period of time," said Hirao. The group is now working on an improved PCR method that more precisely amplifies target DNA sequences. ★

"The dengue virus has four serotypes, and the combination of real-time PCR with our method can be used to identify the dengue serotype of patients at health clinics within a short period of time."

1. Yamashige, R., Kimoto, M., Okumura, R. and Hirao, I. Visual detection of amplified DNA by polymerase chain reaction using a genetic alphabet expansion system, *Journal of the American Chemical Society* **140**, 14038–14041 (2019).

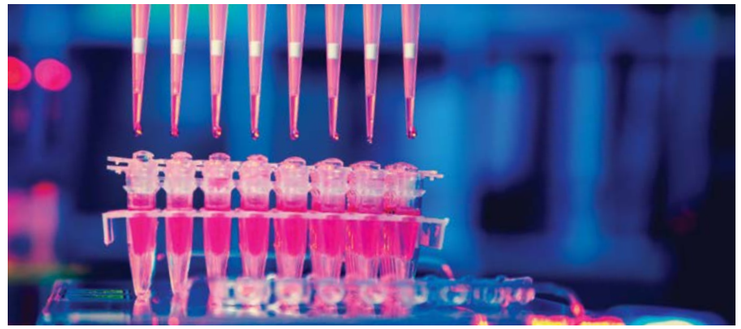


Photo credit: science photo / Shutterstock

Fluorescent molecular probes bright enough to the naked eye could greatly enhance medical diagnostics.

DIAGNOSTICS

One test to determine them all

A*STAR researchers have devised a method that can be used to detect and distinguish closely related flaviviruses with 100 percent accuracy.

When disease outbreaks occur, identifying the microbe that caused the infection is critical for the correct treatment to be administered. Health authorities also rely on accurate and rapid diagnostics to coordinate containment strategies.

However, some infectious organisms, such as those belonging to the flavivirus family, are difficult to distinguish from one another using conventional antibody methods, and remain undetectable by RNA-based diagnostics even at a late stage of infection. Examples of viruses in this family include four strains of the dengue virus, Zika virus, yellow fever virus and Kunjin virus.

"Each flavivirus diagnosis also requires a specific test, and combining multiple tests within a single diagnostic reaction often compromises detection sensitivity," said A*STAR's Jayantha Gunaratne.

Seeking to improve flavivirus identification, Gunaratne and his team at the Institute of Molecular and Cell Biology (IMCB), in collaboration with the National Environment Agency's Environmental Health Institute and the National Centre for Infectious Diseases, developed a proteomic mass spectrometry (MS)-based assay that can simultaneously detect all seven of the above-mentioned flaviviruses, with high sensitivity and specificity[1].

Gunaratne's team first focused on the virus non-structural protein 1 (NS1), a protein not found in humans but expressed by flaviviruses. Using their technique, they determined that each flavivirus, including the four strains of the dengue virus, has a unique NS1 peptide sequence that could be used in a way similar to fingerprints for diagnosis.

The researchers proceeded to validate their approach in serum samples from infected patients, demonstrating that they could distinguish the four strains of dengue virus with 100 percent accuracy. The assay could also be used to pinpoint exactly which flavivirus species or strains were culpable for co-infected samples.

Importantly, the assay remained sensitive and specific when applied to samples from patients with secondary flavivirus infections, during which the virus' NS1 peptide may be bound to immune proteins. The researchers noted that a step in their protocol likely resulted in the dissociation of immune complexes from the NS1 peptide, thereby preventing the immune proteins from interfering with the assay.

"Proteomic MS offers single amino acid resolution when analyzing peptide sequences, so I was confident that the technology could be exploited in the diagnosis and typing of flaviviruses," Gunaratne said. "Our findings could pave the way for a more efficient response towards outbreaks, especially for new emerging flaviviruses such as the Zika virus."

Gunaratne's team plans to develop an end-user friendly package to bring this assay into the clinic, as well as explore the possibility of expanding this application to other diseases. ★

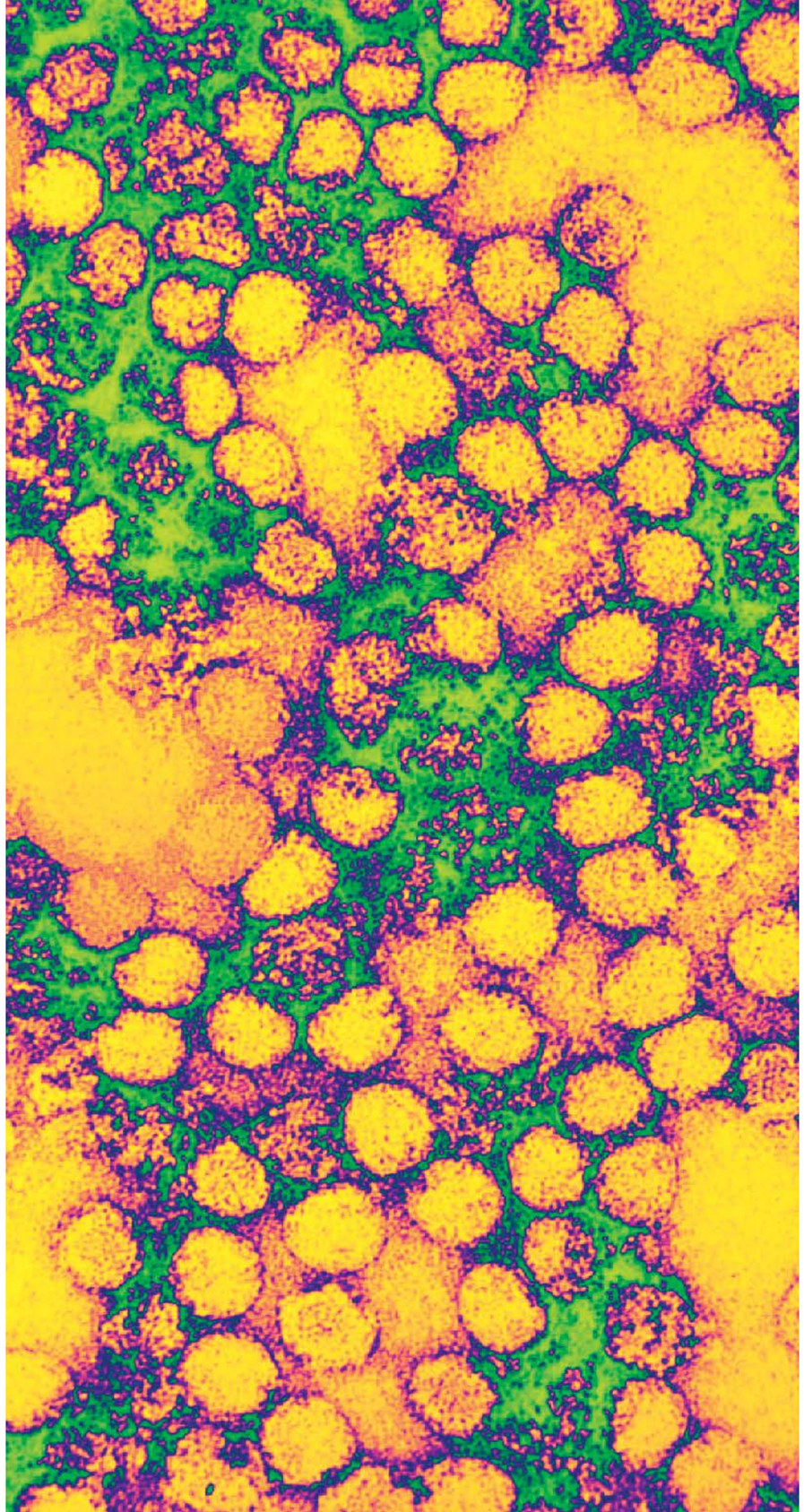


Photo credit: Cdc / Science Photo Library

RIGHT
A colored transmission emission microscopy image of yellow fever viruses, which are related to other pathogens such as the Zika virus and the four serotypes of dengue virus. Accurately distinguishing these infectious microbes is important for disease monitoring and diagnosis.

COMMENTARY BY PROTEOMICS EXPERT RUEDI AEBERSOLD,

Professor of Molecular Systems Biology at ETH Zurich, Switzerland

Mass spectrometry (MS) is one of the most versatile, sensitive and quantitatively accurate analytical techniques, and has been widely used for decades, primarily for the analysis of small molecules. More recently, it also has become the method of choice for the analysis of proteins and proteomes. Because it is the sequence and not the composition of amino acids that determines the identity of a peptide or protein, even highly accurate mass measurements of peptides are insufficient to determine their identity.

Confident peptide identification, therefore, requires tandem mass spectrometric techniques which determine not just the peptide mass, but also the amino acid sequence of peptides. Several tandem mass spectrometric methods have been developed, each with different performance profiles.

Among these, targeted MS is characterized by quantitative accuracy, high degree of reproducibility, relative insensitivity to the composition of the sample matrix, low limits of detection and a wide dynamic range. Targeted MS was named method of the year in 2012 by *Nature Methods* and its favorable properties make it well suited for clinical applications, particularly for diagnostic tests which need to be highly precise, sensitive and reproducible.

A recently published, pioneering manuscript from the Gunaratne lab highlights the successful exploitation of targeted mass spectrometry for the diagnosis of flavivirus infections, a global healthcare threat. Flavivirus infections have no definitive treatment, making precise diagnosis pivotal for efficient disease management strategies.

The current gold standards, primarily based on immunoassays and viral RNA, have several limitations including cross-reactivity and limited extendibility to multiple flaviviruses without compromising sensitivity. The targeted-MS assay developed by the authors circumvents these shortcomings and enables an efficient response to newly emerging flavivirus outbreaks, with accurate diagnosis and virus typing possible shortly after the detection of a new virus.

Capitalizing on the power of MS to distinguish highly similar peptides, the authors selected viral non-structural protein 1, which is secreted and stably present in patients' blood for extended periods, to conclusively identify and distinguish different flavivirus infections down to the serotype level. The authors show that this single-shot, multiplexed assay can even diagnose secondary- and co-infections from a small amount of blood, a result that has been challenging to achieve with existing flavivirus diagnostics.

The present study is a powerful and well-constructed example of the application of advanced MS technology to an important clinical question. It promises a clear path for translation and broad utility. Further, the approach is readily extendible to new viruses or virus subtypes without the need for expensive and slow antibody development. The study therefore makes a unique contribution to the flavivirus field and points the way towards the conclusive detection of other types of virus.

In light of these advantages, various MS labs around the world are developing automated and simplified workflows to tailor this technology for diagnostics. It can therefore be expected that this outstanding work will make a contribution to public health.

1. Wee, S., Alli-Shaik, A., Kek, R., Swa H. L. F., Tien, W. P., Lim, V. W. *et al*. Multiplex targeted mass spectrometry assay for one-shot flavivirus diagnosis. *PNAS* **116(14)**, 6754-6759 (2019).

IMMUNOLOGY

More than a gut feeling about mosquito-borne viruses

Deciphering the immune response in mosquitoes to the O'nyong nyong virus may lead to new broad-spectrum antiviral targets.

Notorious for their ability to transmit diseases such as malaria and dengue hemorrhagic fever, mosquitoes are a threat to global health. Curiously, the *Anopheles* mosquito is a potent vector for the malaria parasite but is known to be less effective than the *Aedes* and *Culex* mosquitoes at transmitting viruses. In fact, the *Anopheles* mosquito is known to transmit just one virus—the O'nyong nyong virus (ONNV), which belongs to a family of alphaviruses that include the Chikungunya virus.

Seeking to understand how ONNV manages to circumvent the *Anopheles* mosquito's immune system where other viruses have failed, scientists led by Guillaume Carissimo at A*STAR's Singapore Immunology Network (SIgN) studied the expression of immune-related genes in ONNV-infected mosquitoes[1]. The research was done in collaboration with the Pasteur Institute in France.

The researchers first determined that the ideal moment to collect mRNA from the mosquitos was three days after the insects had been fed a bloodmeal containing ONNV. At this time point, the 'battle' between ONNV and the mosquito's immune system is taking place in the mosquito's midgut, which allows the team to assess which genes are expressed differently during this critical phase of first contact.

"Our earlier findings showed that the immune pathways in mosquitoes are compartmentalized, which means that it is important to study the immune response in the midgut before systemic infection takes place," said Carissimo.

The team found that a "surprisingly narrow" range of genes was differentially regulated upon ONNV infection of the mosquito midgut—they observed changes in the expression of only 30 mRNA transcripts and just one microRNA transcript between uninfected and ONNV-infected mosquitoes. Digging deeper, the researchers identified that two key regulators of the mosquito's innate immune response—the transcription factors STAT and Rel2—were responsible for altering the expression of vast and distinct clusters of mRNA and microRNA genes.

These findings could pave the way for novel methods of mosquito control, Carissimo noted. "There are very intriguing and interesting possibilities that could be harnessed and modified to recognize and neutralize pathogens in modified vectors," he said.

Carissimo's team plans to continue studying mosquito-virus and human-virus interactions, with a special emphasis on the Chikungunya virus and ONNV. "Implementing systems and technologies to study the conserved and specialized virus-hosts interactions will yield attractive targets for broad-spectrum antiviral targets, as well as fundamental knowledge on how viruses can modulate immune responses in humans," he said. ★

ABOVE
The immune system of the mosquito's midgut is the first line of defence against viruses such as the O'nyong nyong virus and the Chikungunya virus.

1. Carissimo, G., Pain, A., Belda, E. and Vernick, K. D. Highly focused transcriptional response of *Anopheles coluzzii* to O'nyong nyong arbovirus during the primary midgut infection. *BMC Genomics* **19**, 526 (2018).

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X-RAYS

Future bright for mini synchrotrons

Shooting electrons into hybrid light-matter particles on a surface could be the key to a new wave of compact X-ray machines.

Colliding a stream of electrons with laser light near an array of tiny silver structures could be the recipe for a new X-ray source in medical imaging and security scanning. This is according to findings from a study led by Liang Jie Wong at A*STAR's Singapore Institute of Manufacturing Technology (SIMTech), in collaboration with MIT in the US, Technion University in Israel and the University of Mons in Belgium[1].

"Based on our theoretical predictions, our lab-scale experiment will be able to generate an X-ray brightness comparable with that used for medical imaging," Wong said. "With some tweaks, we are optimistic we can reach synchrotron brightness. We're very excited about that."

Synchrotrons are X-ray sources whose radiation is bright enough to allow detailed study of tiny structures such as proteins or complex crystals. However, they are large installations—typically tens of meters in scale—that require entire buildings to house them.

Wong and his team envisage a table-top apparatus for their X-ray generators, which rely on the interaction between a laser at wavelengths between infrared and ultraviolet, and electron energies around five mega-electron volts, a regime achievable by current state-of-the-art electron guns.

"If we do manage to scale up, the impact will be quite revolutionary. Instead of just having a few synchrotrons available, you can put a high-brightness X-ray source in every lab and hospital."

The arena for the interaction between the laser and the electrons is an array of microscopic silver structures on a glass slide. The laser is directed at the surface at an angle, creating surface waves called plasmon polaritons. The electrons are then shot parallel to the surface into the surface waves, which interact with the free electrons, causing their trajectories to undulate and X-rays to be generated.

The up-conversion to X-ray energies is a result of the properties of plasmon polaritons, hybrid particles formed by coupling electrons and photons. These hybrid particles are strongly confined on the surface, which concentrates the intensity of the laser. As the spatial dimension is greatly reduced, the polariton's momentum is greatly increased at a given energy, resulting in the conversion from few-electron volt (eV) plasmon polaritons into kilo-eV X-rays, using mega-eV electron energies.

"It's an electrodynamical process that no one had predicted," Wong said.

The team explored a range of configurations for the metamaterial, with groups of structures ranging in size and spacing, from 5–26 nanometers in diameter and spaced regularly around 90 nanometers apart.

The results showed it was possible to control the spatial and temporal characteristics of the X-rays by changing parameters such as the geometry of the metasurface, or the shape of the electron wave-packets. The ability to control the beam features is a huge benefit because X-rays are challenging to focus and steer: they tend to pass through most materials without interacting.

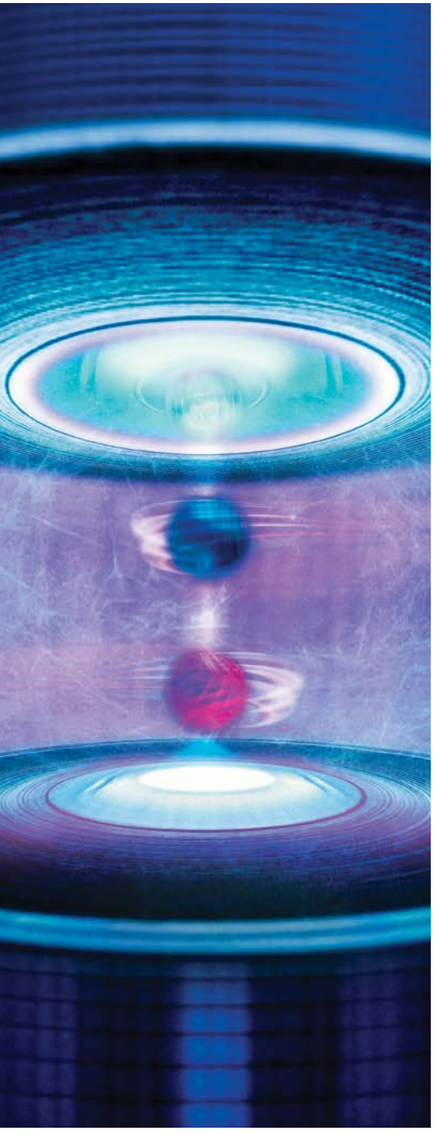
Wong and his co-workers now plan to conduct proof-of-principle experiments with the new X-ray source.

"If we do manage to scale up, the impact will be quite revolutionary. Instead of just having a few synchrotrons available, you can put a high-brightness X-ray source in every lab and hospital," he said. ★

ABOVE
Bulky and expensive equipment known as synchrotrons are typically required to produce X-rays.

1. Rosolen, G., Wong, L. J., Rivera, N., Maes, B., Soljačić, M. and Kaminer, I. Metasurface-based multi-harmonic free electron light source, *Light: Science & Applications* **7**, 64 (2018).

Photo credit: FlashMovie / Shutterstock



ABOVE
Transferring spin currents from one material to another has proven challenging due to the scrambling of currents that occurs during the transfer process.

1. Liang, C., Xinbo, W., Weifang, Y. Jianwei, Y., Ming, Y. *et al.* Far out-of-equilibrium spin populations trigger giant spin injection into atomically thin MoS_2. *Nature Physics* **15**, 347–351 (2019).

SPINTRONICS

A shot in the arm for spintronics

A spin injection technique that is more than 10,000 times more efficient than existing methods could allow for the development of ultrafast spintronic computers.

A*STAR scientists have developed simple and efficient method for transferring the currents produced by spinning electrons from metals to semiconductors, laying the groundwork for faster and more energy-efficient spintronic computers[1].

Although advances in electronics have led to increasingly powerful computers, the electrical resistance of silicon limits the rate—known as the clock speed—at which computers can process information to just a few gigahertz or several operations per nanosecond.

Using the spin of electrons instead of their charge to convey information could overcome this limitation. Additionally, these so-called spin currents consume less energy than electric currents, making spintronic computers more energy efficient than conventional electronic computers.

Transferring spin currents from one material to another, however, has proved very challenging as the currents can become scrambled during transportation, destroying the information they carry.

Now, Justin Song from A*STAR's Institute of High Performance Computing (IHPC) and colleagues from the Institute of Materials Research and Engineering (IMRE), in collaboration with an international team of researchers, have developed a simple technique that significantly improves the efficiency at which spin currents are injected from a magnetized metal into a semiconductor.

"While methods exist for spin injection from metals into semiconductors, a key issue is that they typically operate close to equilibrium," Song explained. "As a result, when a simple ferromagnet/semiconductor interface is used, the spin injection efficiency is low."

By heating a cobalt-based ferromagnet with pulses of light from a laser, the researchers were able to create spin-polarized electrons with a range of different energy levels in the cobalt, a state referred to as 'out-of-equilibrium,' and in which their spins mostly point in the same direction.

This out-of-equilibrium state generates spin-current pulses that last for less than one trillionth of a second—enough time for them to diffuse from the cobalt into a semiconductor made from single-atom layers of molybdenum disulfide, which then converts the spin current into an electric current.

"Possibly the most striking aspect is that all this was demonstrated using a simple metal-semiconductor interface, without the complicated and costly structural engineering used in other spintronics," said Song.

The work establishes a new and simple technique for spin injection that is more than 10,000 times more efficient than what is available now. It also represents a significant step towards the development of ultrafast spintronic computers that use spin currents as carriers of information, with clock speeds more than a thousand times faster than those in existing conventional computers.

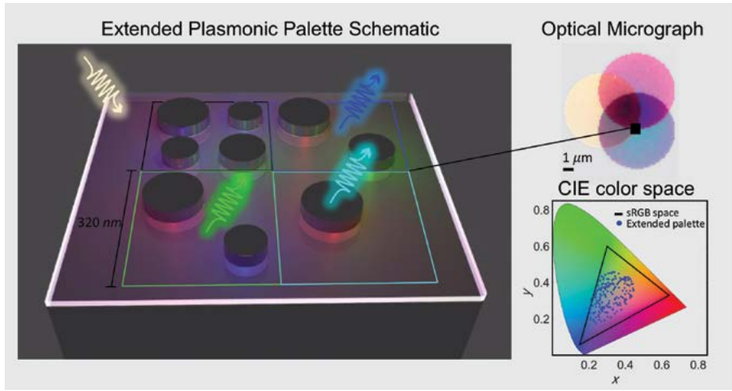
"We are now investigating how pushing systems into an out-of-equilibrium state can lead to effects not possible at equilibrium," said Song. ★

Photo credit: Richard Kail / Science Photo Library

NANOTECHNOLOGY

Creating colors without dyes

Nanofabricated metallic structure arrays produce a kaleidoscope of bright colors.



For most of human history, the only available colors were those conferred by dyes extracted from nature. Some dyes were so scarce and expensive that they came to be associated with wealth and status, like the purple of royalty. With the mid-1800s came the first synthetic dyes, which made colors more accessible to everyone, but also introduced significant environmental hazards, as well as health risks. For instance, workers in the aniline dye industry were found to be at greater risk of bladder cancer.

Finding a way to produce color without resorting to dyes might seem like a non-starter to many people, but not to a group of researchers led by Joel Yang at A*STAR's Institute of Materials Research and Engineering (IMRE).

"With the chemical dyes used in everyday consumer products, colors are created by absorption and scattering of the light caused by various chemical compounds and elements. Depending on the wavelength of the light that is absorbed or scattered, various colors emerge," Yang explained.

In contrast, plasmonic color generation involves the use of metallic nanostructures—something like studs on the top of Lego blocks. When light hits such a surface, it sets in motion the collective oscillation of free electrons, also known as plasmon resonances, on the surface of the metallic nanostructures, causing light to be absorbed and scattered differently, thereby producing different colors. However, the span of colors is limited due to losses in the metals.

Seeking to expand the color palette of plasmonic color printing technology, Yang's team sandwiched a 30-nm-thick aluminum oxide film between a 100-nm-thick aluminum layer and 40-nm-thick aluminum disks[1].

They found that by varying the diameter of the disks, the distance between discs and the arrangement (square and hexagonal patterns) of disks, they could increase the color spectrum of their plasmonic color printing system by relying on an effect where each disk absorbs light within an area significantly larger than its size.

"We achieved about 50 percent coverage of the standard red, green, blue (RGB) color space, which is considerably more than previous works," said Soroosh Daqiqeh Rezaei, a graduate student in Yang's lab and the first author of the study.

The researchers further showed that the hexagonal arrangement of disks achieved higher color saturation as compared to the square arrangement. They hope that their findings could bring plasmonic color generation closer to commercial use.

"Plasmonic color prints possess ultra-high resolution—you can pack more than 100,000 pixels into one pixel of the iPad Retina display," said Rezaei. "Therefore, plasmonic color prints can be used to store information and provide anti-counterfeiting features, in addition to providing fade-resistant colors. We are also working on incorporating plasmonic pixels in display technologies and optical sensors."

Importantly, the researchers noted that scaling up their technology would not be hazardous to the environment. "In this work, we have only used aluminum and aluminum oxide, which are cost-effective, earth-abundant and stable," said Yang. "Colors from precious metals or chemical dyes instead might be more vibrant but could be less sustainable or environmentally friendly." ★

ABOVE
Light hitting these metallic disk-like nanostructures gets absorbed and scattered differently, resulting in a spectrum of colors being produced.

1. Rezaei, S.D., Hong Ng, R.J., Dong, Z., Ho, J., Koay, E.H.H. *et al.* Wide-Gamut Plasmonic Color Palettes with Constant Subwavelength Resolution, *ACS* Nano **13**, 3580-3588 (2019).

Photo credit: © 2019 American Chemical Society

AUGMENTED REALITY

Making light behave in useful ways

By integrating nanoantennas with liquid crystals, A*STAR researchers have created a metasurface that allows fine dynamic control over the properties of light.

WHY THIS MATTERS

- Useful as liquid crystals may be as selective light modulators, their resolution and efficiency fall short of the requirements for next-generation optical devices.

From holograms to augmented reality devices and optical sensors, a common enabling principle behind these innovations is the ability to precisely manipulate light. Over the years, researchers have created materials that allow them to control how light behaves. A familiar example would be liquid crystals, used in television and mobile phone displays.

However, the resolution of liquid crystal-based spatial light modulators leaves much to be desired—each pixel ranges from three to tens of micrometers. Next-generation light detection and ranging (LIDAR) and display technologies require higher resolution and wider steering angles than currently afforded by liquid crystals.

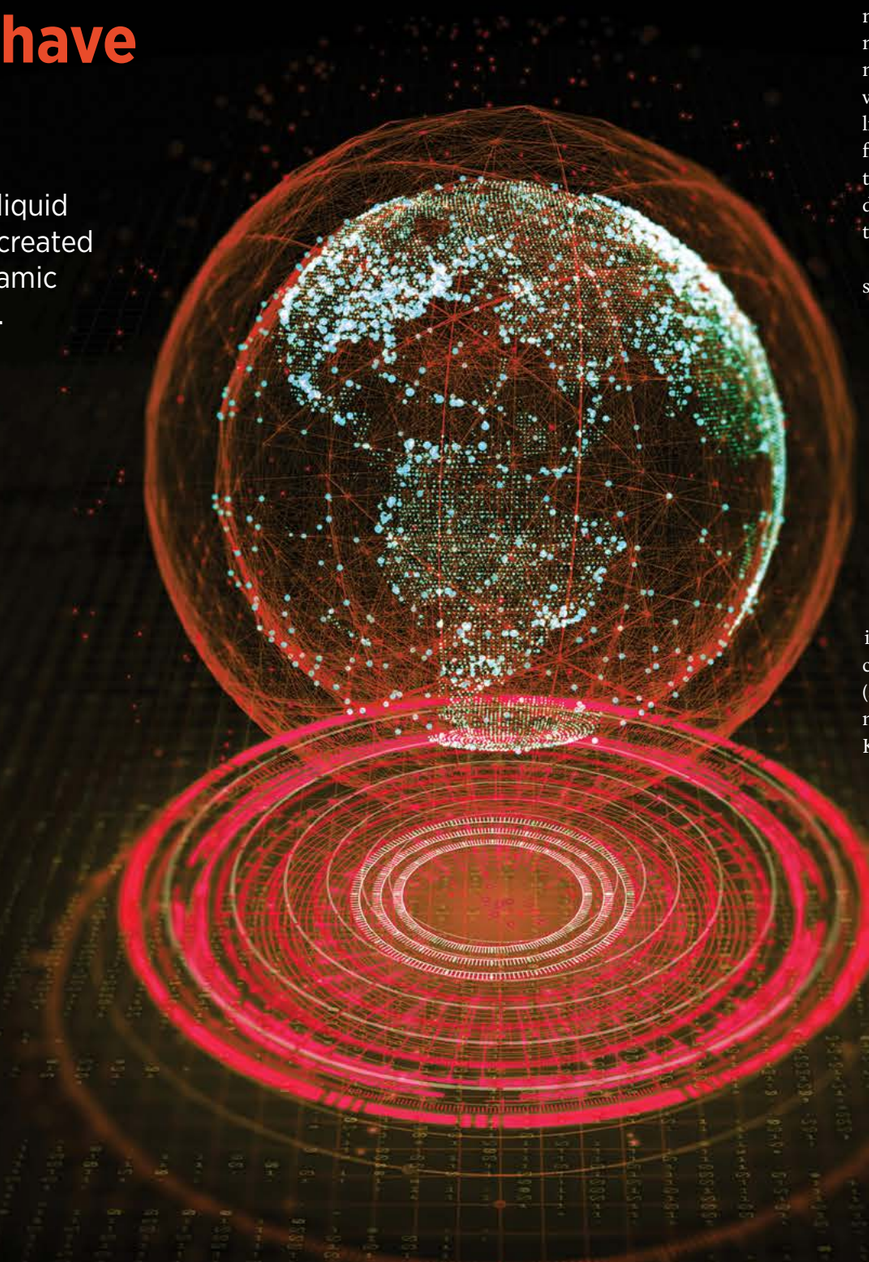


Photo credit: spainter_vfx / Shutterstock

Researchers led by Arseniy Kuznetsov at A*STAR's Institute of Materials Research and Engineering (IMRE) have now overcome these restrictions using metasurfaces—materials comprising nanostructures that are smaller than the wavelengths of light[1]. "When an incident light beam is passing through or reflected from a metasurface, its phase and amplitude transform in a desired fashion to achieve different functionalities, as determined by the nanostructures," Kuznetsov explained.

In this study, his team fabricated nanoscale antennas (nanoantennas) made of the semiconductor titanium dioxide and integrated them with liquid crystals to form a hybrid metasurface. They then applied a voltage to the liquid crystals, which in turn alters the resonance range of the nanoantennas to allow fine-tuning of the properties of the resultant light beam.

"Importantly, only a very thin layer of the liquid crystal—less than one micron—around the nanoantenna is required to perform this tuning. This is very different from conventional liquid crystal-only spatial light modulators (SLMs) where the liquid crystal layer is much thicker, typically five microns," said Kuznetsov.

By miniaturizing the system components into the nanometer range, the researchers also demonstrated, for the first time, light modulation by a metasurface at the level of individual pixels. The efficiency of light modulation in their 28-pixel device exceeded 30 percent, superior to prior demonstrations which performed in the efficiency range of just 1–2 percent.

"We were also able to achieve a beam-steering angle of 22 degrees using our pixels of approximately one-micron width, compared to only 0.8 degrees allowed by currently existing transmissive SLMs," Kuznetsov added. This improvement in the steering angle can already be applied to technologies such as LIDAR in autonomous vehicles, the researchers said.

Going forward, Kuznetsov's team plans to use the high beam-steering angle of their metasurface in 2D pixels for producing holographic images. ★

IMPACT

Nanoantennas made of titanium dioxide surrounded by a thin layer of liquid crystals allow for much smaller pixel sizes and wider beam-steering angles. These properties are important for sensors in autonomous vehicles and holographic displays, among other applications.

BELOW
Light passing through or reflected off a surface covered in nanostructures can take on interesting properties.

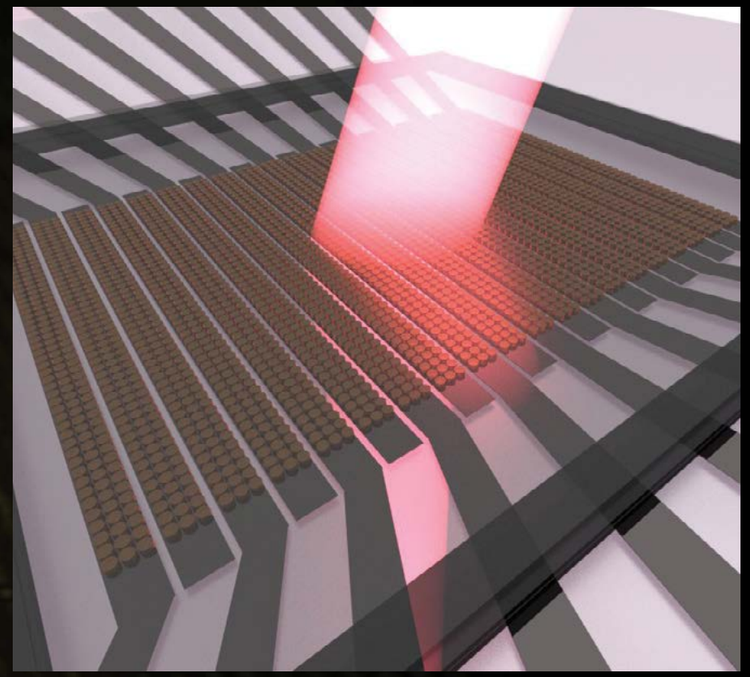
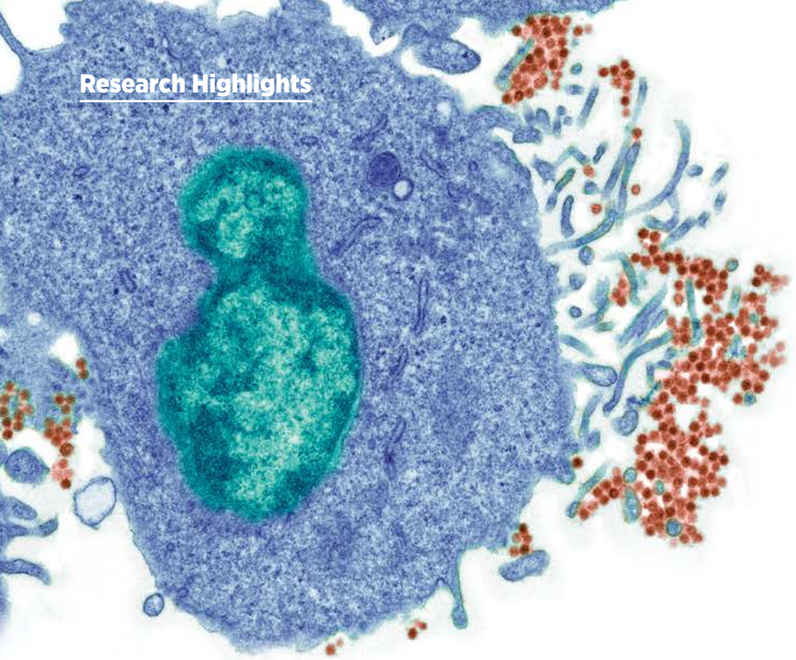


Photo credit: © 2018 Institute of Materials Research and Engineering

1. Li, S. Q., Xu, X., Veetil, R. M., Valuckas, V., Paniagua-Domínguez, R. *et al*. Phase-only transmissive spatial light modulator based on tunable dielectric metasurface. *Science* **364(6445)**,1087-1090 (2019).



CANCER BIOLOGY

Sniffing out high-risk EBV strains

A*STAR scientists have identified two Epstein-Barr virus strains associated with a high risk of causing nasopharyngeal carcinoma.

Since it was discovered over 50 years ago, the Epstein-Barr virus (EBV) has been linked to cancers such as nasopharyngeal carcinoma (NPC), a cancer of the nose that is also known as 'Cantonese cancer.' Although EBV infects an estimated 95 percent of the population, only a small percentage of people with the infection develop NPC. The reason the virus causes cancer in some individuals but not others is poorly understood.

Now, an international team of scientists led by Jianjun Liu from A*STAR's Genome Institute of Singapore (GIS) has discovered for the first time two strains of EBV that are associated with an increased risk of NPC[1].

To determine the unique genetic code of different EBV strains, the team first performed large-scale, whole-genome sequencing on EBV isolates from patients diagnosed with EBV-associated cancers—namely NPC, gastric carcinoma and lymphomas—and compared the genetic sequences to healthy controls. The samples were collected from NPC-endemic areas in southern China, such as Guangdong and Guangxi provinces, as well as from other non-endemic regions in China.

After comparing the genetic sequences between the EBV isolates from patients and those obtained from healthy people living in similar regions, the team found two EBV-coding variants that are associated with high risk for developing NPC. The team also performed principal component and phylogenetic analyses to investigate the origin of these risk variants.

"Such analyses can help us understand how these strains or risk variants arise in human populations," explained Liu.

The two viral strains associated with an increased NPC risk harbor genetic variation in the BALF2 gene, which codes for a DNA binding protein integral for viral DNA replication and viral propagation. These two variant strains are absent or extremely rare in non-Asian people from Africa and western countries such as Australia, the United States and the United Kingdom.

"Our results from the phylogenetic analysis showed that the variants originated in Asia or China, and underwent a clonal expansion," said Liu, further noting that "the most important aspect of our discovery is that it explains the NPC endemics in Southern China."

EBV is a human virus that spreads via bodily fluids like semen, blood and saliva, making it easily transmittable. The discovery of these risk variants thus allows for the development of screening kits to identify high-risk individuals for clinical evaluation and early diagnosis.

"Our findings have also revealed a new target for the development of vaccines and therapies against the virus," Liu added.

Expanding on the present findings, the research team is now working with two cancer centers in Singapore to determine if these virulent strains exert similar effects in Southeast Asia. ★

ABOVE
A colored transmission electron microscopy image of the Epstein-Barr virus.

Photo credit: Steve Gschmeissner / Science Photo Library

1. Xu, M., Yao, Y., Chen, H., Zhang S., Cao, S. M. *et al.* Genome sequencing analysis identifies Epstein–Barr virus subtypes associated with high risk of nasopharyngeal carcinoma. *Nature Genetics* **51(7)**, 1131-1136 (2019).

CANCER BIOLOGY

Starving liver cancer cells into submission

Researchers reveal that the buildup of branched-chain amino acids fuels the growth and aggressiveness of liver cancer cells.

Proteins are an essential part of a healthy diet, but liver cancer cells have found a way to make use of certain amino acids from proteins to fuel their growth at the expense of the body. These findings were published by scientists from A*STAR's Singapore Bioimaging Consortium (SBIC), National Cancer Centre Singapore, Duke Molecular Physiology Institute and the University of Rhode Island[1].

Branched-chain amino acids (BCAAs) like leucine, isoleucine and valine are essential amino acids that cannot be produced by the body and must be obtained from food. However, researchers have now found that cancer cells reprogram their metabolic networks to accumulate BCAAs, which can increase tumor cell growth by activating the protein mTORC1.

"Now that we know chronic BCAA supplementation increases the risk and severity of liver cancer, we should evaluate patients' BCAA catabolic efficiency before administering BCAAs for long-term treatment."

Photo credit: Sebastian Kaulitzki / Shutterstock

Liver cancer cells gain a survival advantage by suppressing the expression of enzymes that break down branched-chain amino acids.

The team first compared the transcriptomes of tumor and non-tumor liver tissues in both humans and mice, identifying the loss of BCAA metabolism as a key difference between healthy and diseased tissue. The high levels of BCAAs in tumor cells were attributed to the suppression of catabolic enzymes that under normal circumstances break down BCAAs.

To confirm the role of BCAAs in tumor formation, the researchers reduced the amount of BCAAs in the diet of mice with liver cancer and found a significant reduction in liver tumor size. Correspondingly, the extent of suppression of these catabolic enzymes in human tumor samples correlated with their aggressiveness, degree of metastasis, as well as the patient's clinical outcome.

It is currently common practice to supplement the diet of liver cirrhotic patients with BCAAs to reduce the risk of developing hepatic encephalopathy, which is brain damage caused by a decline in liver function. However, these new findings may alter the way liver disease patients are treated.

"Now that we know chronic BCAA supplementation increases the risk and severity of liver cancer, especially in high-risk patient groups (i.e., those with liver diseases), we should evaluate patients' BCAA catabolic efficiency before administering BCAAs for long-term treatment," said Weiping Han at SBIC, team leader of the study. "We are also planning dietary interventions in patients with advanced liver diseases."

With the identification of BCAA metabolism as a new target for liver cancer prevention and therapy, the team now intends to explore the applicability of these findings in gastrointestinal malignancies such as colorectal and gastric cancers. ★

1. Ericksen, R. E., Lim, S. L., McDonnell, E., Shuen, W. H., Vadiveloo, M., *et al.* Loss of BCAA Catabolism during Carcinogenesis Enhances mTORC1 Activity and Promotes Tumor Development and Progression. *Cell Metabolism* **29**, 1151-1165 (2019).

CANCER BIOLOGY

How cancer cells tip the Bim balance in their favor

Cancer cells evade Taxol-induced cell death by downregulating the expression of a protein known as BimEL.

Each time a cell divides, a tightly orchestrated dance of DNA and proteins takes place to ensure that each daughter cell contains the right amount of genetic material. A structure known as the mitotic spindle is assembled to capture the condensed chromosomes and partition them equally into the daughter cells.

Anti-microtubule drugs such as paclitaxel (trade name Taxol) and vincristine kill rapidly dividing cancer cells by altering the dynamics of the mitotic spindle, thereby stopping chromosomes from being properly partitioned into daughter cells. This eventually results in programmed cell death, or apoptosis, of the cancer cells.

In this study, A*STAR researchers led by Uttam Surana at the Institute of Molecular and Cell Biology (IMCB) identified a mechanism by which cancer cells evade apoptosis and become resistant to treatment with anti-microtubule drugs[1]. They first demonstrated that resistance to anti-microtubule drug treatment was not due to mitotic slippage—the process by which cells prematurely exit mitosis to avoid cell death—as generally believed. Rather, the cancer cells downregulate the expression of a protein called BimEL.

"Bim is an activator of apoptosis, and BimEL refers to the 'extra-long' isoform of the protein," said Surana. Therefore, by downregulating BimEL expression, the signal to initiate apoptosis is weakened in cancer cells, allowing them to survive treatment with anti-microtubule drugs.

The researchers next sought to identify how cancer cells downregulate BimEL expression. They showed that cancer cells target BimEL for destruction via the activity of cullin-RING ubiquitin ligases—proteins that add a molecular 'throw away' tag to cellular components. Alternatively, or simultaneously, cancer cells stall BimEL production by blocking the transcription of BimEL mRNA.

"Hence, Bim expression can be used as a biomarker to guide therapy—if a tumor does not show Bim expression, anti-microtubule drug treatment will not be of any benefit. Also, therapeutic agents that augment the expression of Bim would be desirable 'companion drugs' to induce cell death in cancer cells that are resistant to anti-microtubule drugs such as Taxol," Surana explained.

In the future, Surana's team is interested in detailing the fates adopted by cancer cells that have escaped anti-microtubule drug-induced cell death. "Understanding these mechanisms may lead to further strategies to induce cell death, during the subsequent division cycle, in cells that have initially escaped Taxol-induced mitotic death," Surana said. ★

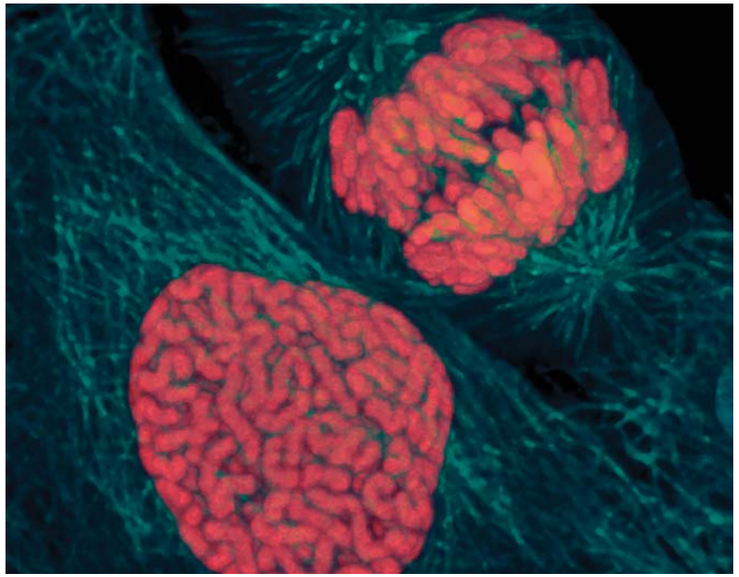


Photo credit: David Becker / Science Photo Library

LEFT
During cell division, the segregation of genetic material is tightly controlled, failing which, programmed cell death occurs. Some cancer cells evade this lethal fate by lowering their expression of the Bim protein.

1. Ruan, W., Venkatachalam, G., Sobota, R. M., Chen, L., Wang L. C., *et al*. Resistance to anti-microtubule drug-induced cell death is determined by regulation of BimEL expression. *Oncogene* **38**, 4352–4365 (2019)

CANCER BIOLOGY

Chipping away at drug resistance

A platform for rapidly profiling mutations and gene expression offers valuable insights into cancer biology.

A tumor can contain remarkable diversity, where even neighboring cells may differ greatly in terms of the mutations they contain. A device that can overcome this heterogeneity by enabling efficient analysis of both DNA and RNA in individual cancer cells has been developed by researchers led by Say Li Kong and Axel Hillmer of the A*STAR Genome Institute of Singapore (GIS).

"The simultaneous sequencing of a single cell's genome and transcriptome is a powerful means to dissect genetic variation and its impact on gene expression," Kong explained. Such data can enable more accurate evaluation of how tumor cells evolve over the course of disease progression and acquire resistance in response to treatment. However, most strategies for parallel analysis of DNA and RNA are overly complex and only enable screening of small numbers of cells at a time.

Kong and Hillmer developed a platform they call CORTAD-seq, short for 'concurrent evaluation of RNA and targeted DNA sequencing.' Much of the work is performed on a chip which contains multiple tiny fluid-filled chambers. Within these chambers, individual tumor cells are broken up and their RNA and genomic DNA are isolated and prepared for sequencing[1].

As a demonstration, the researchers used CORTAD-seq to study an experimental model of acquired drug resistance. They treated a cultured lung cancer cell line with gefitinib, a commonly used drug, and performed a comparative analysis of pre-exposure cells and cells collected after different durations of exposure. CORTAD-seq enabled them to analyze thousands of genes and detect genomic mutations in more than 500 individual cells. This gave intriguing insights into how cells acquire gefitinib resistance.

Many of the resistant cells studied had acquired a mutation known as T790M, which is commonly seen in patients with gefitinib-resistant tumors. However, Kong notes that getting this mutation alone did not seem to have a major impact on gene expression. "Our study did not find tremendous transcriptomic differences between cells with or without the T790M mutation," she said, adding that this indicates that other mutations or epigenetic changes may be necessary in addition to T790M to produce complete drug resistance.

Kong is now looking to evaluate the performance of CORTAD-seq in a variety of other tumor cell types, but also hopes that other groups will take the opportunity to test this method for themselves. "With its simplicity, moderate reagent costs, easy accessibility and excellent data quality, CORTAD-seq could potentially benefit a broad community of researchers," she said. ★

"With its simplicity, moderate reagent costs, easy accessibility and excellent data quality, CORTAD-seq could potentially benefit a broad community of researchers."

Photo credit: Dr Gopal Murti / Science Photo Library

BACKGROUND
Concurrent sequencing of both the genome and transcriptome of cancer cells could be useful in understanding how drug resistance arises in tumors.

1. Kong, S.L., Li, H., Tai, J.A., Courtois, E.T., Poh, H.M. *et al*. Concurrent single-cell RNA and targeted DNA sequencing on an automated platform for comeasurement of genomic and transcriptomic signatures. *Clinical Chemistry* **65**, 272–281 (2019).

EPIGENETICS

Crippling cancer cell growth

Disruption of the methionine cycle in tumor-initiating cells disarms their tumorigenic capabilities, researchers say.

WHY THIS MATTERS

- Cancer relapse often occurs because of drug-resistant tumor-initiating cells that remain after treatment is stopped.
- Exposing the vulnerabilities of tumor-initiating cells could lead to better treatment outcomes in cancer patients.

Successful eradication of cancer requires targeted therapy against tumor-initiating cells (TICs). Although these cells are a minority in tumors, TICs are often resistant to chemotherapy, causing cancer to return with a vengeance after treatment.

Now, researchers have found a novel point of weakness in these TICs, opening up new pathways for treatment. In a multi-institutional collaborative effort, scientists under the direction of Wai Leong Tam at A*STAR's Genomic Institute of Singapore (GIS) discovered that TICs require the amino acid methionine to grow. Conversely, inhibiting the metabolism of methionine in these cells severely crippled their tumor-initiating capabilities[1].

After performing metabolomic and metabolite tracing analyses, the researchers identified differences in the epigenetic landscape of TICs and the cancer cells they produce. Likened to an on-off switch, epigenetics controls the expression of genes and subsequent production of functional proteins. The amino acid methionine plays a crucial role in epigenetics as its conversion to S-adenosyl-L-homocysteine (SAH) by the enzyme MAT2A regulates gene expression via a process known as histone methylation.

"As the methionine cycle directly influences cellular methylation rates, we naturally thought that this would be a key starting point in characterizing tumorigenic programs in TICs," explained Tam.

Starving TICs of methionine resulted in a significant reduction of tumor growth after transplantation into mice. Similarly, treating tumor-transplanted mice with the MAT2A inhibitor FIDAS-5 stopped the growth of these tumors. Analysis of human lung adenocarcinoma revealed increased expression of MAT2A in the tumors compared to normal lung tissue, demonstrating the clinical relevance of the team's earlier findings.

"The efficacy of the inhibitor on patient-derived xenografts *in vivo* was a key proof-of-concept that inhibiting methionine-cycle activity is a viable therapeutic approach to targeting TICs in human tumors," said Zhenxun Wang, a senior postdoctoral fellow who co-led the study.

"Treatment resistance and cancer relapse, which may be mediated by the presence of TICs, contribute towards the incurability of many cancers," Wang added. "By targeting the root of tumors, that is, the TICs, we envisage a more durable tumor response and the possibility of overcoming the problems of resistance and relapse."

Forging ahead, the team is now embarking on a drug discovery effort to develop novel drugs that inhibit methionine-cycle activity effectively in human tumors. As different subpopulations of carcinoma cells within a tumor have unique metabolic dependencies, the team is also working on identifying other metabolic genes that can be future targets for cancer therapy. ★

IMPACT

Enzymes involved in methionine metabolism represent novel drug targets for potentially destroying tumor-initiating cells.

LEFT
Tumor-initiating cells are addicted to the amino acid methionine, a vulnerability that can be targeted to address cancer relapse. This cartoon was made for GIS by Pedro Veliça, author of the Pedromics cartoon series.

1. Wang, Z., Yip, L. Y., Lee, J. H. J., Wu, Z., Chew, H. Y. *et al*. Methionine is a metabolic dependency of tumor-initiating cells. *Nature Medicine* **25**, 825-837 (2019)

Photo credit: NIBSC / Science Photo Library

GENETICS

Clearing the air on cilia development

A*STAR researchers have uncovered the sequence of genetic events behind the development of motile cilia in airways.

Like janitors who keep corridors and walkways clean, multiciliated cells (MCC)—cells with hair-like structures known as cilia—keep the airways of land-dwelling animals free of particulate matter and disease-causing bacteria. MCCs perform a sweeping motion to prevent unwanted material from entering the lungs, and individuals with defective MCCs are at increased risk of respiratory diseases.

In the present study, scientists led by Sudipto Roy at A*STAR's Institute of Molecular and Cell Biology (IMCB) wanted to understand how MCCs develop. "Two regulatory proteins— GMNC and MCIDAS —have been shown to be required for MCC development in previous studies," said Roy. "However, what remains unclear is how the proteins actually function in MCC development: do they function together at the same developmental point in the MCC differentiation pathway, or at different steps?"

To answer these questions, the researchers generated MCIDAS mutant mice and found that MCIDAS-defective MCCs produced only a single cilium, unlike normal mice whose MCC bear hundreds of cilia. In addition, they discovered that MCIDAS-defective MCCs could not form multiple basal bodies—the structures found at the base of cilia[1].

Because basal body formation is known to be controlled by a signaling pathway called the deuterosome-dependent (DD) pathway, Roy's team proceeded to investigate the interaction between MCIDAS and the DD pathway. MCIDAS overexpression in cells strongly activated the expression of genes in the DD pathway to trigger basal body formation. Another regulatory protein involved in cilia formation, GMNC, similarly activated the DD pathway, albeit to a weaker extent.

The researchers further showed that although GMNC was able to induce MCIDAS expression, the reverse was not true. This finding suggests that GMNC acts at an earlier developmental step to MCIDAS.

"Our results show that there is a genetic hierarchy in that GMNC functions upstream of MCIDAS. Therefore, they are deployed in a stepwise manner for regulating the gene expression program during MCC differentiation," said Roy. His team is currently studying MCIDAS mutant cells to assess which pathways are dysregulated in the absence of MCIDAS function.

"Both GMNC and MCIDAS have the ability to program MCC development, which makes them good candidates for devising therapeutic strategies that restore damaged ciliary epithelia in severe lung disease," Roy concluded. ★

ABOVE
The hair-like structures of cells, known as cilia, perform a sweeping function. In the lungs, this sweeping motion helps expel foreign materials and microbes from the body.

1. Lu, H., Anujan, P., Zhou, F., Zhang, Y., Chong, Y.L. *et al*. Mcidas mutant mice reveal a two-step process for the specification and differentiation of multiciliated cells in mammals. *Development* **146**, dev172643 (2019).

DEVELOPMENTAL BIOLOGY

Writing the roadmap for pancreas development

A protein named GATA6 is key to the development of a normal pancreas in humans.

When setting out on a vacation, we usually plan in advance the route we will be taking and the time that we will arrive at our destination. Similarly, in biological systems, cells must arrive at defined locations and at specific times for normal organ development to occur. However, the roadmap for organ development remains incomplete, and while model organisms such as mice are useful for exploring the genes involved in organ development, some interspecies differences may not be accounted for.

"It's becoming increasingly obvious that human and mouse embryology are not the same," said Ray Dunn, a Principal Investigator at the Institute of Medical Biology (IMB). For example, in humans, the loss of one functional copy of the GATA6 gene results in an extremely rare condition called pancreatic agenesis, where the person is born without a functional pancreas. In mice, however, pancreatic agenesis only occurs when both copies of the GATA6 gene—and both copies of another highly related gene, GATA4—are non-functional.

Seeking to better model and understand pancreatic development in humans, Dunn's team at IMB studied human induced pluripotent stem cells from GATA6-heterozygous individuals (provided by Professor Andrew Hattersley's lab at the University of Exeter, UK) and a human embryonic stem cell line in which they introduced a range of different GATA6 mutations[1].

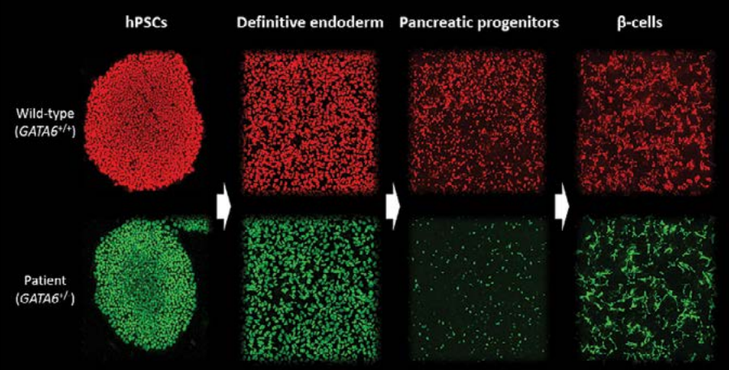


Photo credit: © 2018 A*STAR Institute of Medical Biology

The researchers evaluated the ability of the various stem cells to generate definitive endoderm (DE), from which the pancreas and other organs in the gastrointestinal tract emerge, using markers unique to different stages of DE differentiation. They found that GATA6 mutant stem cells showed significantly lower expression of the DE markers CXCR4 and SOX17, and this corresponded with fewer DE cells forming. "This means that GATA6 is required very early on in the formation of the human pancreas," explained Dunn.

"It's sort of like a relay race, figuring out who comes first, and who the baton is passed on to."

The team used techniques such as RNA sequencing and chromatin immunoprecipitation sequencing to show that the GATA6 protein cooperates with other proteins—EOMES and Smad2/3—to activate the expression of genes promoting DE formation in the various stem cell lines.

"There are still many different holes in that story," Dunn said. "We're trying to decode what are the direct transcriptional targets of GATA6 and its partners so we can sequentially understand and mirror *in vitro* the probabilities of what happens during human gestation. It's sort of like a relay race, figuring out who comes first, and who the baton is passed on to." ★

LEFT
Immunostaining showing how the loss of one functional copy of the GATA6 gene in humans impairs the development of pancreatic progenitor cells.

1. Chia, C. Y., Madrigal, P., Denil, S. L. I. J., Martinez, I., Garcia-Bernardo, J. *et al*. GATA6 Cooperates with EOMES/SMAD2/3 to Deploy the Gene Regulatory Network Governing Human Definitive Endoderm and Pancreas Formation. *Stem Cell Reports* **12**, 57-70 (2019).

IMMUNOLOGY

On the trail of two types of white blood cells

Nerve bundles and blood vessels each have their own specific subtypes of macrophages.

People who grow up and reside in different countries typically end up developing different cultures and habits. The same could be said of immune cells in the body, which reside in different tissue 'niches.'

Led by Florent Ginhoux of A*STAR's Singapore Immunology Network (SIgN), a team of scientists has discovered two distinct groups of interstitial macrophages (a type of white blood cell) that coexist and occupy specific compartments of tissues in the body.

"Macrophages are immune cells implicated in maintaining the normal functioning of tissues, from neuronal activity in the brain to the clearance of dying cells in all tissues," Ginhoux explained, adding that macrophages differ in their properties and behavior depending on their location within tissue niches. His team thus sought to understand what those differences are and how those differences arise.

Using a technique called single-cell mRNA sequencing, the researchers observed that macrophages surrounding nerve bundles and those near blood vessels had different 'signatures,' based on the genes they expressed and the proteins they displayed[1]. Macrophages associated with blood vessels were characterized as Lyve1^{hi}MHCII^{lo}, while those in close proximity to nerve bundles were characterized as Lyve1^{lo}MHCII^{hi}.

The researchers further demonstrated that Lyve1^{hi}MHCII^{lo} macrophages were critical for maintaining blood vessel integrity, suppressing inflammation and reducing the extent to which collagen fibers are deposited in tissue. In mice whose Lyve1^{hi}MHCII^{lo} macrophages had been removed, the team recorded increased blood vessel leakiness and worsened inflammation which delayed healing in the heart and lungs, ultimately resulting in greater scarring of the tissue.

On the other hand, Lyve1^{lo}MHCII^{hi} macrophages were more prone to interacting with another subset of immune cells known as CD4$^+$ T cells. Upon activation by the Lyve1^{lo}MHCII^{hi} macrophages, the CD4$^+$ T cells increase in number and turn into regulatory T cells (T_{regs}), which are known to play a role in preventing autoimmune reactions.

Importantly, Ginhoux's team found that Lyve1^{hi}MHCII^{lo} and Lyve1^{lo}MHCII^{hi} macrophages have a common origin—both are derived from immature cells called blood monocytes. "This in turn implies that the tissue microenvironment—either the blood vessels or nerve fibers—is the driver of monocyte-specific differentiation into either Lyve1^{hi}MHCII^{lo} or Lyve1^{lo}MHCII^{hi} macrophages," said Ginhoux.

Since nerve bundles and blood vessels are common 'sub-tissue niches' in many parts of the body, the two distinct macrophage populations likely play important roles in health and disease. Ginhoux and colleagues thus intend to explore more deeply how the two types of macrophages contribute to the development of cancer, for instance.

"We are also trying to better understand the role of nerve bundle-associated Lyve1^{lo}MHCII^{hi} macrophages. This subset of macrophages would be well placed to contribute to focal chemokine/cytokine gradients, which could promote tissue tolerance by modulating the activity of T_{regs}," Ginhoux said. ★

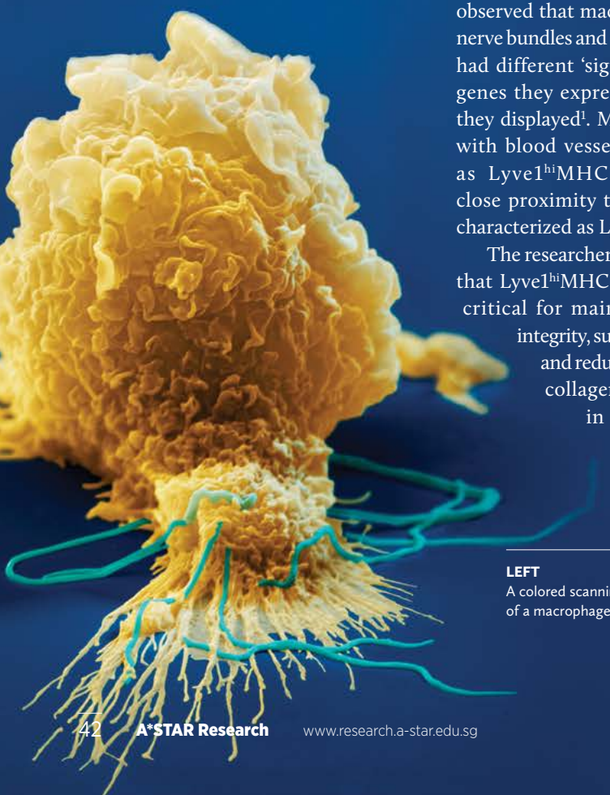


Photo credit: Eye Of Science / Science Photo Library

LEFT
A colored scanning electron microscopy image of a macrophage attacking bacteria.

1. Chakarov, S., Lim H. Y., Tan, L., Lim, S. Y., See, P., *et al*. Two distinct interstitial macrophage populations coexist across tissues in specific subtissular niches. *Science* **363**, eaau0964 (2019).

IMMUNOLOGY

Battling bacteria that get under the skin

A subset of skin-resident immune cells secrete the protein VEGFα to help mount a robust response to bacterial invasion.

As the primary barrier between the human body and the environment, our skin is exposed to millions of microbes daily, some of which can cause infection. Like gatekeepers to a fortress, the immune system must mount an appropriate response to bacterial invaders.

A*STAR researchers led by Florent Ginhoux at the Singapore Immunology Network (SIgN) have now discovered how a minor subset of immune cells, called conventional dendritic cells (cDCs), play a key role in fighting off skin infections such as *Propionibacterium acnes* (*P. acnes*), which are responsible for outbreaks of acne.

"cDCs are the sentinels of the immune system in all tissues, including the skin. They are the first line of defense against pathogens and hence play crucial roles at all stages of the immune response," said Ginhoux.

Although skin-resident cDCs are known to be activated in response to danger signals generated upon skin infection, the downstream actions of their activation remained unclear.

In this study, Ginhoux's team profiled the immune cell populations that accumulate over time in mice whose ears had been injected with *P. acnes*[1]. They found that a type of cDC, called cDC1, helps recruit neutrophils—another group of immune cells—from the blood to the skin, thereby fortifying the immune response against infection. Probing deeper, the researchers used single-cell mRNA sequencing to reveal that cDC1 could be further categorized into two subgroups, one of which had elevated expression of a protein called VEGFα.

VEGFα is a known attractant for neutrophils, and the specific deletion of the VEGFα gene in cDC1 resulted in impaired neutrophil recruitment in response to *P. acnes* infection in mice. These findings indicate that VEGFα-secreting cDC1 is necessary for mounting an effective immune response to bacterial infection of the skin.

"Since cDC1s can activate neutrophils to control the intensity of the ensuing immune response, we believe that they represent a new and unique cellular target to modulate skin immunity. We could activate them in conditions that require a stronger immune response or inhibit them in conditions that require dampening of immunity," Ginhoux explained. For example, targeting VEGFα could offer a new therapeutic window for skin autoimmune diseases such as psoriasis or atopic dermatitis, he said.

Going forward, Ginhoux's team intends to explore if cDC1 has similar functions in other kinds of human skin infections, or where neutrophils' recruitment to tissue plays a crucial role in a protective or harmful immune response. ★

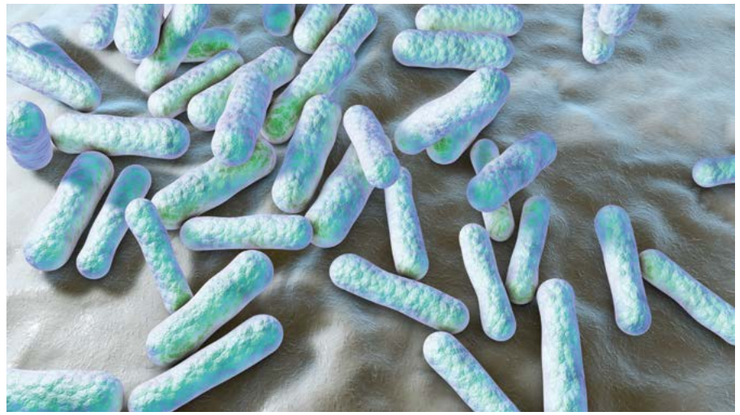


Photo credit: Kateryna Kon / Shutterstock

LEFT
Skin microbes such as *Propionibacterium acnes* are responsible for outbreaks of acne.

1. Baptiste, J., Amit, A. P., Lau, M. C., Goh, C. C. Rasha, M., *et al*. A Subset of Type I Conventional Dendritic Cells Controls Cutaneous Bacterial Infections through VEGFα-Mediated Recruitment of Neutrophils. *Immunity* **50**, 1069-1083 (2019).

MECHANICAL ENGINEERING

Sound barriers inspired by origami

A*STAR scientists have designed foldable sound barriers by borrowing concepts from origami, the art of paper folding.

WHY THIS MATTERS

- Urban living often entails exposure to high levels of noise from traffic, construction and so on.
- Most noise barriers developed to date are heavy and bulky, and therefore cumbersome to deploy.

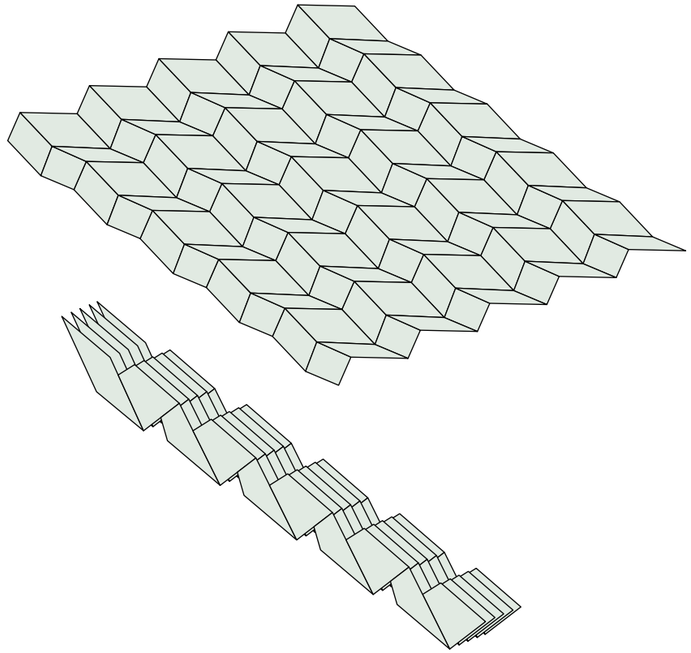
While public awareness about the dangers of air pollution is high, problems arising from noise pollution have captured less attention despite being no less dangerous. Sound barriers, commonly used to limit noise transmission, are often seen enveloping construction sites and roads. Despite being effective, they are heavy, rigid and require significant manpower to assemble.

Seeking to overcome these restrictions, Xiang Yu at A*STAR's Institute of High Performance Computing (IHPC), together with colleagues from the National University of Singapore and Hong Kong Polytechnic University, are borrowing concepts from origami, the art of paper folding.

"The strategy here is to use rigid plates and flexible hinges to form what is known as a Miura-ori sheet, which is easily reconfigurable," explained Yu, the lead author of the study. Miura-ori is the name of a particular fold used in origami. With a reversible, single-degree-of-freedom foldability, Miura-ori structures can be folded for easy transportation, then quickly deployed into a 3D structure[1].

The researchers first used a technique called finite element modeling to evaluate how various properties of an origami sound barrier, such as the folding angles and origami pattern, would affect the acoustic shadow zone, or the area protected from noise. Next, to assess whether their mathematical predictions would hold up in reality, they created miniature 3D-printed prototypes of foldable sound barriers, varying the folding configurations and recording the actual acoustic performance of each prototype.

Yu's team also explored incorporating a micro-perforated membrane—a 0.5 mm-thick carbon fiber sheet with 0.42 mm-diameter holes drilled into it—in their origami sound barrier designs. The membrane is attached to the front surface of the origami sound barrier and can enhance the barrier's sound absorption properties.



ABOVE
Two different configurations of the proposed foldable sound barrier, designed based on origami concepts.

Photo credit: Mykolastock / Shutterstock

"Our work offers fundamental insights into how folding would affect the acoustic performance of sound barriers, and opens up new opportunities for designing innovative origami-inspired acoustic devices," said Yu.

"Although this study is developed based on a specific type of origami cell—Miura-ori—the vast origami library can enable other designs with particular geometric features that are acoustically beneficial," he said. ★

IMPACT

Compact yet easily deployable sound barriers could help reduce noise pollution in urban areas, making for better quality of life in cities.

1. Yu, X., Fang, H., Cui, F., Cheng., L., Lu, Z. Origami-inspired foldable sound barrier designs. *Journal of Sound and Vibration* **442**, 514-526 (2019).

ENVIRONMENT

Simulations shed light on air filter deployment

Using computational tools, A*STAR researchers are learning how best to deploy air treatment systems across large urban areas.

High concentrations of particulate matter (PM) in the air, especially those with diameters less than 2.5 micrometers, have been linked to health problems and reduced economic productivity. Although technologies such as PM filters are currently available to purify the air, the question of how best to deploy these filters on a massive scale remains unanswered.

"One could assess the performance of these filters by carrying out wind or water tunnel experiments, or by directly installing large filter systems in cities and performing air pollution measurements. The former needs experimental facilities and the latter is very expensive," said Venkata B.L. Boppana, a Scientist with A*STAR's Institute of High Performance Computing (IHPC), who is collaborating with Corning Singapore Holdings to simulate the deployment of filters across large urban areas.

Seeking to clear the air on the judicious use of PM filters in urban areas, the researchers first selected a 3D map of a typical town in the North-East of Singapore for their simulations. The area of interest is intersected by two roads, and vehicular traffic on those roads generate PM.

The team then developed a computational fluid dynamics (CFD) model that accounts for a wide range of parameters such as road conditions, the height of surrounding buildings and the volumetric airflow rate of large filter units (called Corning Air Treatment Systems).

Using this model, the team was able to simulate the distribution and concentration of PM in the area. "With the current model and configuration, we found that the clean air zone extending from the center of an air treatment unit is up to a few tens of meters, and that the addition of flanking noise barriers—ten-meter walls on either side of roads—can almost double the maximum reduction in PM levels," Boppana explained.

Given the complex interplay among the various parameters used in the simulation, and the assumptions used in the computational model, Boppana cautioned against generalizing the findings of this study across other geographical settings. Nonetheless, having demonstrated that CFD tools can help to optimize the deployment and efficacy of air treatment units in urban settings, the researchers intend to include even more parameters in their model to increase the accuracy of simulations.

"Factors like vegetation, thermal stratification, turbulence from moving vehicles, air treatment system configurations and wind direction all play a role in the spread of pollutants. Quantifying the performance of these air treatment systems by incorporating all these variables is quite complex but worth pursuing in order to clean up polluted cities," Boppana said. ★

BACKGROUND
Computational fluid dynamics simulations can help city planners decide how best to deploy particulate matter filters to reduce air pollution.

1. Boppana, V. B. L., Wise, D. J., Ooi, C. C., Zhmayev, E. & Poh, H. J. CFD assessment on particulate matter filters performance in urban areas. *Sustainable Cities and Society* **46**, 101376 (2019).

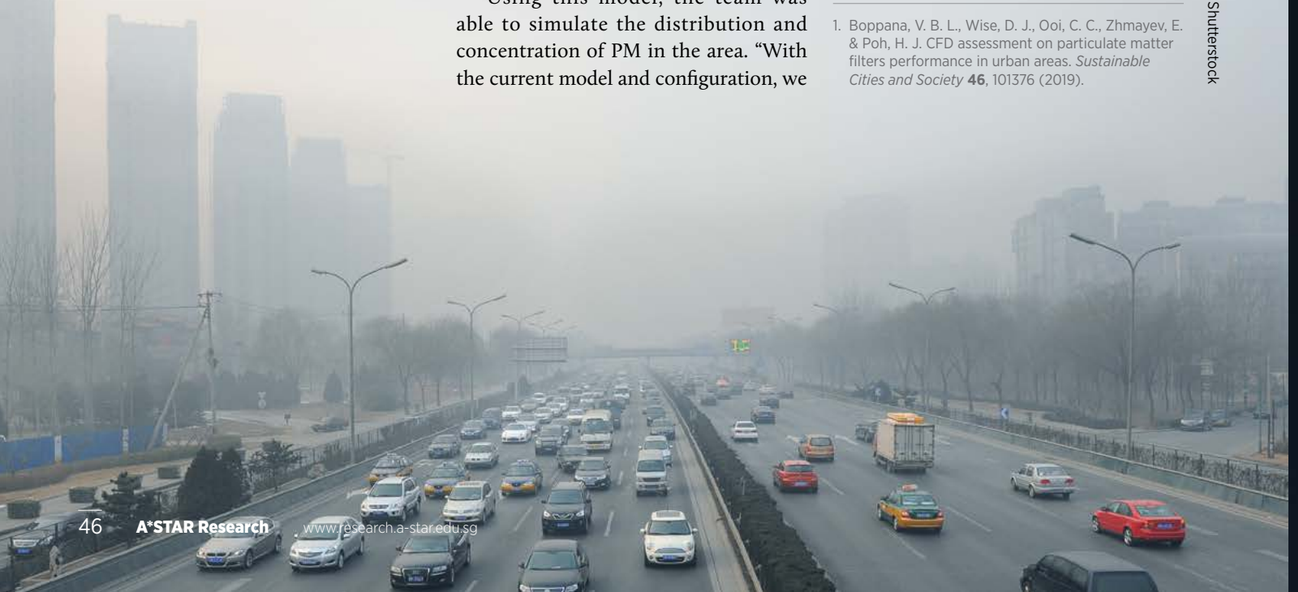


Photo credit: Hung Chung Chih / Shutterstock

VIROLOGY

It's not you, it's flu

The host immune system has a limited impact on the evolution of the influenza virus, A*STAR researchers find.

The aching muscles, nasal congestion and sore throat associated with being down with the flu can be prevented by vaccination, but you must get a new shot every year. This is because viruses evolve rapidly via a process known as antigenic drift, where a key surface protein called hemagglutinin is altered to evade detection by the host's immune system.

Scientists are still figuring out the precise mechanisms by which antigenic drift occurs, and previous studies have suggested that the host immune system is the primary determinant in driving influenza virus evolution. "If this is true, mutation patterns in young, immunologically naive children and older adults who have had influenza infections before should be prominently distinct," said Alvin Han, a graduate student in Sebastian Maurer-Stroh's lab at the Bioinformatics Institute (BII).

To test this hypothesis, the team, in collaboration with Colin Russell from the University of Amsterdam, analyzed 26,725 high quality publicly available hemagglutinin sequences across all four subtypes of seasonal influenza viruses circulating in humans from January 2009 to July 2016. They built a 'family tree' of influenza viruses and compared pairs of hemagglutinin sequences, taking into consideration the age of the hosts in which the viruses were identified. This allowed them to obtain the distribution of hemagglutinin mutations observed in young children and older adults[1].

"We found that viruses infecting children are just as likely to develop mutations as those infecting adults," Han explained. "Even adjusting for any potential confounding factors such as waning immunity due to old age, we still could not find any imprint of individual host selection pressure on influenza virus evolution. This study thus indicates that individual immune selection has a limited role in the antigenic evolution of influenza viruses."

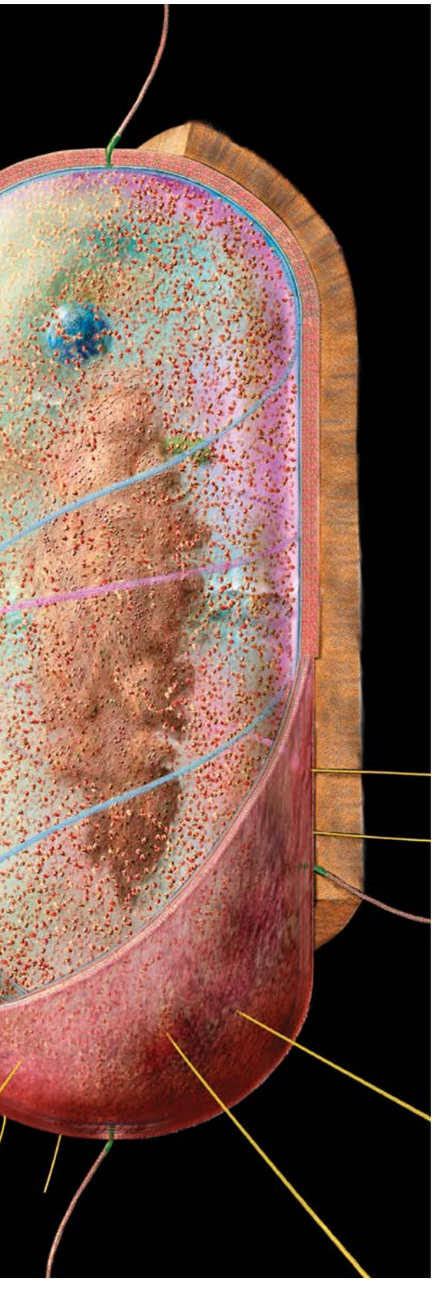
The researchers noted that influenza is usually an acute infection in humans. Hence, the duration of infection may not be long enough for the immune system to mount a substantial strain-specific response that drives the viruses towards generating immune-evasive mutants that become fixed in the virus population.

"If individual immune selection is not the primary driving force behind the antigenic drift of influenza, antigenic change should then be linked more closely to selection during transmission, with population immunity playing a key role. Therefore, public health measures and interventions targeting transmission are crucial to not only limit viral spread, but also virus evolution," said Han. ★

ABOVE
The influenza virus mutates rapidly to generate variants that can evade the human immune system.

1. Han, A. X., Maurer-Stroh, S. and Russel, C. A. Individual immune selection pressure has limited impact on seasonal influenza virus evolution. *Nature Ecology & Evolution* **3(2)**, 302-311 (2019).

Photo credit: dreamerb / Shutterstock



Peptidoglycans are polymer chains of sugars and amino acids that constitute the bacterial cell wall. They may shed off into the bloodstream and trigger more severe autoimmune reactions.

IMMUNOLOGY

How bacteria influence host immunity

When parts of a bacteria's cell wall enter into circulation, its host experiences more severe autoimmune reactions.

Invisible to the naked eye, bacteria cover every inch of our bodies and even live inside us, forming communities known as microbiomes. Many of these micro-organisms are essential to health. For instance, probiotics in the gut facilitate the release of nutrients from food and prevent harmful bacteria from taking up residence in the intestines.

"It is well accepted that the human microbiome influences many aspects of host physiology, including activities in sterile, extraintestinal organs such as the brain and joints. It is unclear, however, how microbes in the gut and on the skin exert a systemic effect," said Yue Wang, a Research Director at A*STAR's Institute of Molecular and Cell Biology (IMCB).

In collaboration with colleagues in China and the Netherlands, Wang's team sought to understand the influence that bacteria exert on the overall, or systemic, immune response of the body. They homed in on the molecule peptidoglycan (PGN), a major component of the bacterial cell wall[1].

To confirm that the microbiome is the main source of blood PGN, the researchers compared PGN levels between microbiota-colonized mice and antibiotic-treated or germ-free mice, which have minimal to no microbiota. They observed that microbiota-colonized mice had significantly higher PGN levels compared to antibiotic-treated and germ-free mice.

The team then embedded an osmotic pump under the skin of normal mice to release PGN, raising the blood PGN levels of treated mice to four to eight times that of control mice. Elevated blood PGN levels resulted in more severe autoimmune arthritis, as demonstrated by greater paw swelling as well as cartilage erosion in treated mice compared to control mice.

Seeking to counter the PGN-mediated autoimmune disease, the researchers injected mice with a monoclonal antibody called 2E7 that binds to and neutralizes circulating PGN. 2E7-treated mice experienced less severe autoimmune disease compared to control mice, hinting at the therapeutic potential of this approach.

Nonetheless, Wang noted that bacteria produce many forms of PGN subunits which get processed into a variety of products in the body. "Therefore, it is necessary to determine the forms of PGN subunits in circulation and their biological activities," he explained.

"We also want to modify 2E7 for use in humans. We plan on carrying out large-scale clinical studies to establish a link between the concentration and forms of circulating PGN with immune-mediated disease," he added. ★

Photo credit: Russell Kightley / Science Photo Library

1. Huang, Z., Wang, J., Xu, X., Wang, H., Qiao, Y., *et al*. Antibody neutralization of microbiota-derived circulating peptidoglycan dampens inflammation and ameliorates autoimmunity. *Nature Microbiology* **4**, 766-773 (2019).

IMMUNOLOGY

A RIPple effect during infection

Researchers have obtained a high-resolution structure of the RIP2 protein complex and detailed its interactions with other immune proteins.

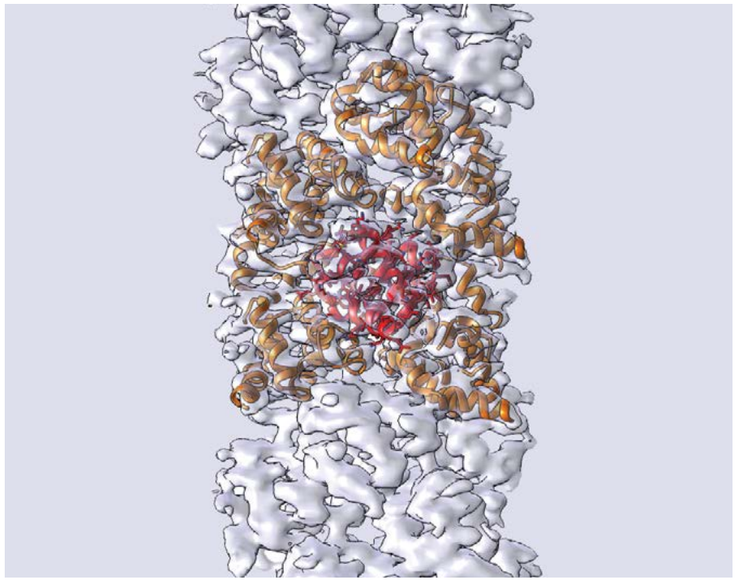


Photo credit: © 2018 Nanyang Technological University

Like fingerprints at a crime scene, infectious organisms such as bacteria leave traces of their presence in the body. Immune cells pick up on these traces and trigger a sequence of molecular events that eventually allows them to identify and destroy the invading microbe. One important protein involved in this process is the receptor interacting protein-2 (RIP2), which relays the 'danger' signal to other proteins inside the immune cell.

To understand how RIP2 orchestrates such intracellular events, scientists from Nanyang Technological University, Singapore (NTU) and A*STAR sought to identify the protein partners of RIP2. They found that for RIP2 to be activated, it must first bind to another protein called the nucleotide-binding oligomerization domain protein (NOD), which comes in two forms (NOD1/2) and is responsible for upstream detection of bacteria[1].

Thereafter, RIP2 physically attaches itself to other RIP2 molecules—a process termed oligomerization—to form a filamentous helical structure termed the RIPosome. The researchers revealed that a part of each RIP2 protein—what is known as the caspase-activation-and-recruitment-domain (CARD)—is essential for oligomerization to take place.

The team also used a technique called cryo-electron microscopy to visualize the structure of the RIPosome at a resolution of 4.1 Å. By mutating various parts of NOD1/2 and RIP2 proteins, the researchers were able to show that NOD1/2 specifically approaches and binds to RIP2 in a top-down orientation.

"The correct orientation of the interaction between NOD1/2 and RIP2 will give us a strong indication of the stability and natural biological state of these two proteins during an innate immune response," explained NTU's Bin Wu, the corresponding author on the study. Such information is crucial to understanding not only the normal immune response to bacterial infections, but also autoimmune disorders in which the immune system wreaks havoc by attacking the body's own cells.

"Mutations in NOD1/2 and RIP2 are associated with autoimmune diseases such as Crohn's disease and lupus. From a pharmacological perspective, peptide inhibitors that bind to the interacting sites of these two proteins can be synthesized, acting as competitive inhibitors for the treatment of autoimmune diseases," Wu explained.

Going forward, the researchers intend to map out the 3D structure of the NOD1/2–RIP2 complex. They are also interested to find out how NOD1/2-RIP2 signaling may interact with other immune pathways when mounting a defense against invading pathogens. ★

ABOVE
A 4.1 Å resolution rendering of the structure of the RIPosome, the protein complex responsible for relaying 'danger' signals inside immune cells during infection.

1. Gong, Q., Long, Z., Zhong, F. L., Teo, D. E. T., Jin, Y., *et al*. Structural basis of RIP2 activation and signaling. *Nature Communications* **9**, 4993 (2018).

BIOINFORMATICS

Predicting protein allergens accurately

The AllerCatPro analysis workflow helps researchers predict the likelihood of a protein producing an allergic response in people.

Be it a stuffy nose or a bad rash, symptoms caused by allergies can make one feel miserable. Despite numerous studies, understanding why some people develop allergies remains complex, and so is accurately predicting what possibly could be an allergen in food or personal care products.

Furthermore, as databases of known allergens grow, existing predictive methods become more prone to falsely identifying non-allergenic proteins as potential allergens, said Sebastian Maurer-Stroh at A*STAR's Bioinformatics Institute (BII). "As an example, some older methods would suggest that 90 percent of our own human proteins should be classified as allergens," he said.

Hence, his team, in collaboration with G. F. Gerberick and Nora Krutz from consumer goods company Procter & Gamble (P&G), sought to develop a method that can predict the allergenic potential of a protein with higher accuracy. The researchers first analyzed five major databases of known allergens to build a library of 4,180 proteins associated with allergy. Using this library, they were able to explore and refine the criteria for predicting protein allergenicity[1].

Traditionally, if a queried protein contains a six-amino-acid sequence that matches with a known allergen, it would have already been classified with allergenic potential. However, the field is well aware that this criterion may not be stringent enough, resulting in many false positives.

"Our new method allows us to determine the allergenicity potential of proteins with a 37-fold increase in specificity, and with 100 percent sensitivity."

The new method, called AllerCatPro, thus implements a new criterion from information theory: low complexity sequences commonly found in many proteins are first filtered out from the prediction workflow, thereby reducing random noise in downstream analyses. In addition, AllerCatPro compares the 3D structure of queried protein sequences against those in the aforementioned library to score for molecular shapes associated with allergenicity, therefore moving the previously linear window comparison into the more relevant 3D space.

"Our new method allows us to determine the allergenicity potential of proteins with a 37-fold increase in specificity, and with 100 percent sensitivity," said Maurer-Stroh. The workflow of AllerCatPro has been published and applied to identify proteins with low allergenic potential. It is also used by P&G to conduct what is known as weight-of-evidence Type I allergy risk assessments on botanicals or natural ingredients that may contain proteins.

"Our joint interest is to make the method available widely to facilitate acceptance by the scientific community and regulators, as well as to gather feedback which will help us to continuously improve on the current method," said Maurer-Stroh of the collaboration. AllerCatPro is also freely accessible on a web server (https://allercatpro.bii.a-star.edu.sg/).

Going forward, Maurer-Stroh and his team, together with A*STAR's Innovations in Food and Chemical Safety (IFCS) Programme, plan to apply AllerCatPro to the safety assessment of proteins found in novel foods, such as those replacing meat with alternative protein sources. By getting regulatory bodies such as the Singapore Food Authority and companies in the food and nutrition sector on board, Maurer-Stroh and his team hope that AllerCatPro will contribute towards Singapore's vision of ensuring national food security and safety. ★

ABOVE
It can be tricky deciphering which proteins in food and consumer products cause allergies. The AllerCatPro analysis platform is now available online to provide scientists with useful clues.

1. Maurer-Stroh, S., Krutz, N. L., Kern, P. S., Gunalan, V., Nguyen M. N. *et al.* AllerCatPro - Prediction of protein allergenicity potential from the protein sequence. *Bioinformatics* **(epub ahead of print)** (2019).

Photo credit: maramorosz / Shutterstock

DRUG DISCOVERY

Fly screen captures behavioral effects of venom

Injecting flies with snail venom could help us discover new biological drug candidates.

Counter-intuitive as it may sound, venom is a treasure trove of potential drugs. In recent years, a plethora of peptides—short chains of amino acids—have been extracted from venomous organisms, though their precise physiological functions are poorly understood.

For example, while a single family of sea snails (cone snails) boasts a million venom peptides, fewer than two percent have been characterized. Just one drug has been developed from a cone snail peptide—and is currently used to treat pain in HIV and cancer patients—but there could be many more just waiting in the wings.

In the present study, researchers led by Adam Claridge-Chang at the A*STAR Institute of Molecular and Cell Biology (IMCB) selected two peptides, Tv1 and Tsu1.1, from two types of venomous snails—*Terebra variegata* and *Terebra subulata,* respectively—to investigate their physiological effects[1]. Tv1 was the first terebrid peptide to be structurally characterized, yet its function remained unclear. "We were interested to see whether Tv1 affects pain perception in a whole animal model," said Mande Holford at Hunter College, US, a collaborator on the study.

The researchers injected the peptides into fruit flies and observed the flies' behavior. They noted that injections of Tv1 made flies less likely to avoid dangerously high temperatures, suggesting their pain threshold was increased. On the other hand, injections of Tsu1.1 made flies eat more meals, suggesting a role in appetite control.

Several thousand flies were used during this study, and the results suggest that flies could be used to study the physiological effects of venom peptides on a large scale. "Screening in this way would not be possible using mice," Claridge-Chang explained, adding that venom-inspired drugs are already commercially available.

"The biggest success story is exenatide, which is used to treat diabetes," he said. "We expect that peptides, including venom peptides, will become a major biological drug category."

The researchers envision that their technique will speed up the discovery of other potentially bioactive peptides that are hidden in the vaults of animal venom. They also shared that their screening method will complement optogenetic studies, in which light is used to manipulate brain cell activity. ★

1. Eriksson, A., Anand, P., Gorson, J., Grijuc, C., Hadelia, E. *et al.* Using Drosophila behavioral assays to characterize terebrid venom peptide bioactivity, *Scientific Reports* **8**, 15276 (2018).

Terebra subulata is a cone snail whose venom may potentially contain useful drugs.

Photo credit: zaferkizilkaya / Shutterstock

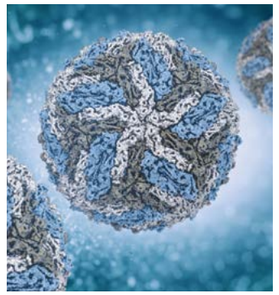
NEXT ISSUE

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

DEEP LEARNING

HELPING DRIVERLESS CARS SEE RED (AND GREEN AND AMBER)

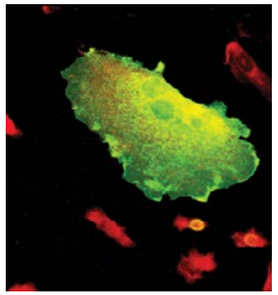
High dynamic range imaging combined with deep learning approaches could improve the ability of autonomous vehicles to recognize traffic lights.



VIROLOGY

THE LONG AND SHORT OF VIRUS GENOMES

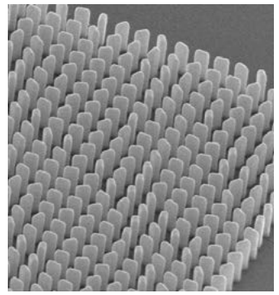
Short- and long-range interactions that give structure to virus genomes also influence virus replication and infectiousness.



CANCER BIOLOGY

TARGETING CANCER FROM THE INSIDE OUT

In times of stress, cancer cells move a protein normally found inside the cell to the cell surface, making it accessible to antibodies for immunotherapy.



PHOTONICS

UV-BASED ANTI-COUNTERFEITING IN THE LIMELIGHT

Surfaces bearing nanoscale patterns for manipulating UV light could be the next frontier in anti-counterfeiting technology and nanophotonic devices.

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RESEARCH

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