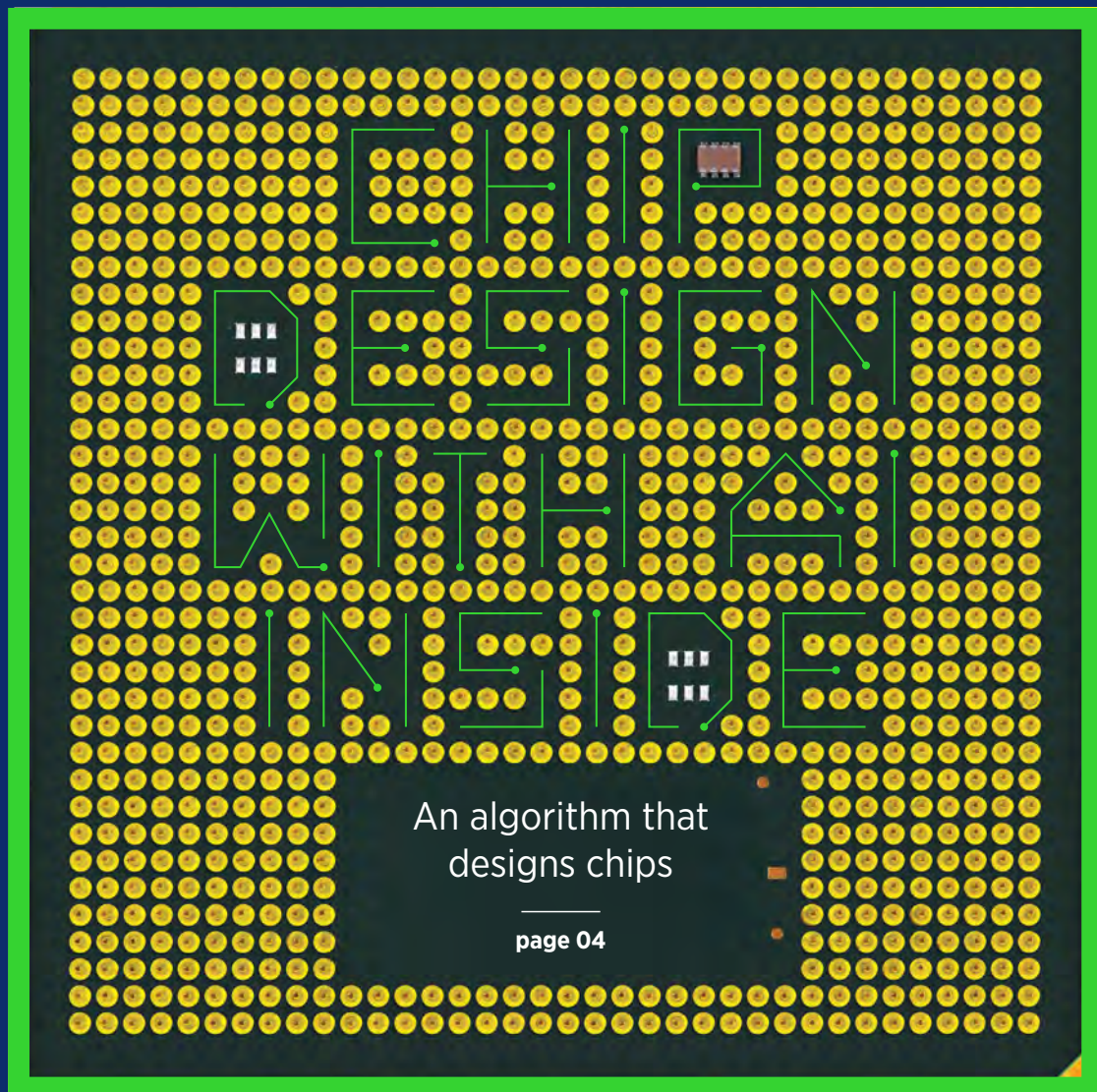


A★STAR RESEARCH

Issue 17 | April - June 2020



DEFYING CONVENTION IN CANCER DRUG DISCOVERY

Targeting cancer cells from within

page 22

RETHINKING BREAST CANCER RISK

A scoring system for early detection

page 42

A*STAR RESEARCH

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*A*STAR Research* is published quarterly, presenting research highlights and feature articles. All articles are first published online on the *A*STAR Research* website and available free to all readers. Register online to receive our monthly e-newsletter by email.

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EDITORIAL

Agency for Science, Technology and Research

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ISSN 2010-0531

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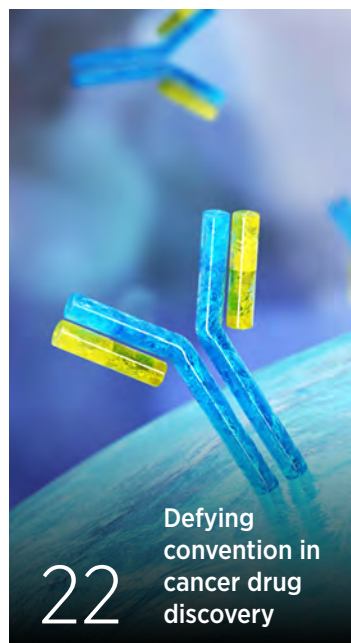
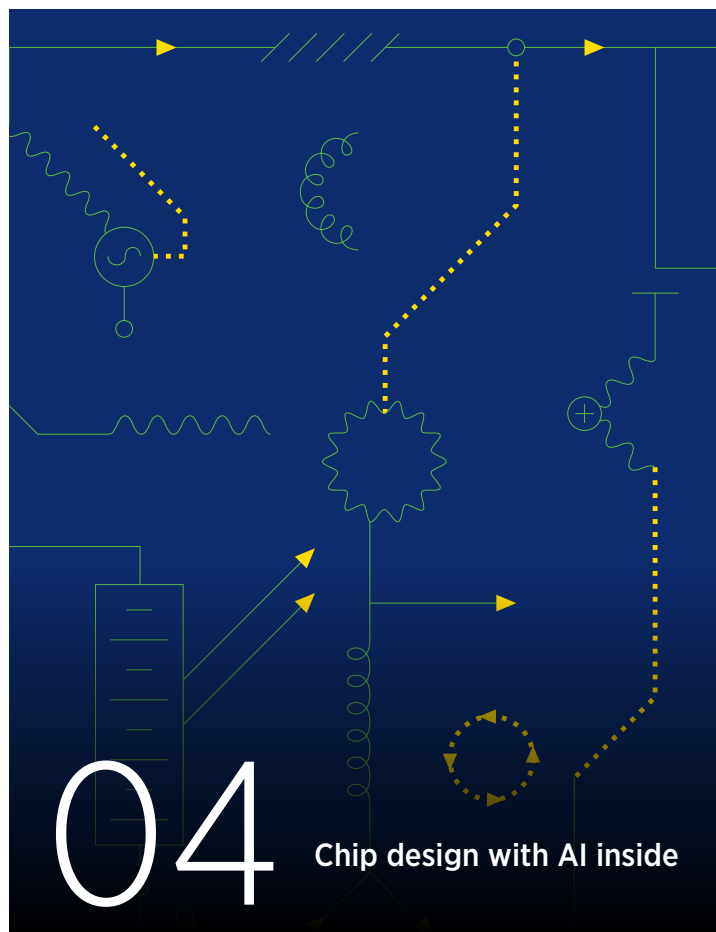
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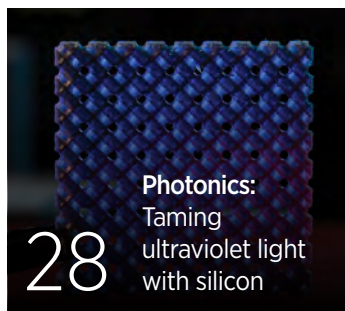
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NOTES FROM THE EDITORS

Whether you are reading this on your phone or computer, this message was brought to you by integrated circuits, or in layman's terms, chips. Chips have faithfully grown smaller and more powerful over the years, putting at our fingertips computing power that could only be dreamed of by the engineers that put Apollo 11 into space. Although today's chips are much more sophisticated than before, designing them is still a remarkably labor-intensive process, involving considerable trial and error.

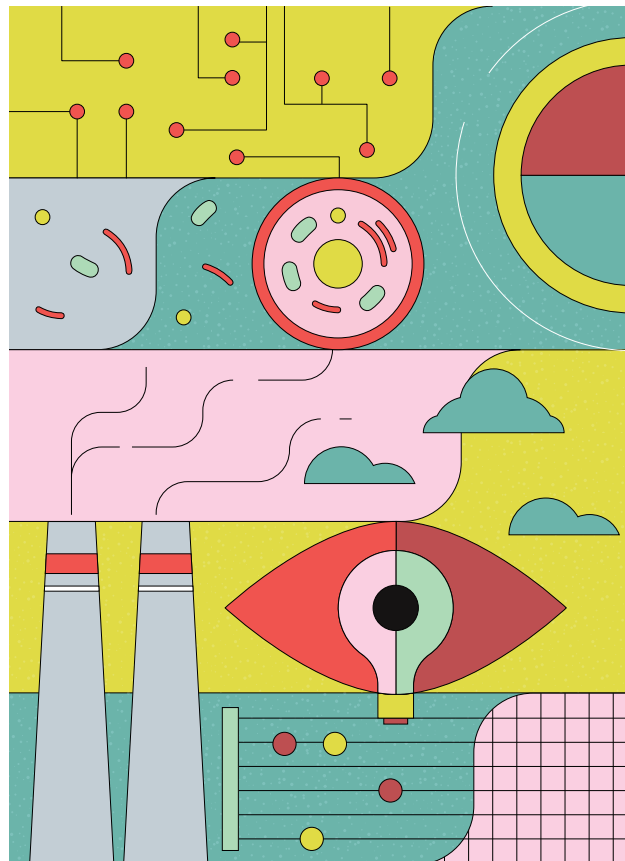
A new machine-learning algorithm by researchers from A*STAR's Institute of Microelectronics (IME) and Institute for Infocomm Research (I²R) is now changing that. Their approach, based on semi-supervised learning, enabled them to shorten the design process from one week to just one day, resulting in a chip that performed twice as well as the best human-optimized design. This breakthrough, featured in our cover story, 'Chip design with AI inside (p. 04),' looks set to transform the way foundries approach chip design.

In another example of out-of-the-box thinking, the Institute of Molecular

and Cell Biology's Qi Zeng shares how Intra-ImmuSG did what everyone else said could not be done: use an antibody to go after targets inside cancer cells rather than on their surface. In 'Defying convention in cancer drug discovery (p. 22),' she describes how the team discovered PRL3-zumab and started Intra-ImmuSG, which is now conducting Phase II trials.

Last but not least, we turn the spotlight on Jingmei Li, a Senior Research Scientist at the Genome Institute of Singapore (GIS), in 'Rethinking breast cancer risk (p. 42).' Find out more about Li, who won the 2017 Young Scientist Award at the President's Science and Technology Awards (PSTA), for her work on uncovering the genetics behind breast cancer, particularly in Asian populations.

Visit our website: research.a-star.edu.sg, or follow us on Twitter at [@astar_research](https://twitter.com/astar_research) and LinkedIn at A*STAR Research for the latest updates!



On the cover

A machine-learning algorithm from A*STAR could make the design of integrated circuits less labor-intensive (p. 04).

CHIP DESIGN WITH AI INSIDE



A machine-learning algorithm that takes the trial and error out of chip design could help us extend Moore's Law for a few more years.

In less than a decade, artificial intelligence (AI) has gone from an obsession of a few ivory-tower academics to runaway commercial success, potentially adding around US\$13 trillion to the global economy by 2030 according to a McKinsey projection. One reason that AI is taking off now rather than when it was first conceptualized in the late 1950s is the availability of affordable computational power, in turn, made possible by steady advances in chip design.

But for all the technological advances ever smaller and more powerful integrated circuits (IC) have ushered in, designing the chips themselves remains a time-consuming and labor-intensive task. Although electronic design automation (EDA) software automating the placement of transistors on a chip has been available since the 1980s, the input of experienced human engineers is still required in what is largely a trial-and-error process, together with EDA tools to find the optimized sweet spot.

"More specifically, a large number of simulations and verifications are manually performed during the conventional design process. If the specification in any design cycle is not met, the designers have to redesign and verify the performance through simulation again," explained Salahuddin Raju, a Scientist at the A*STAR Institute of Microelectronics (IME).

"Many EDA companies have joined the AI bandwagon, offering specific AI capabilities across different design tools. However, their approach is not flexible enough to include the design styles of various chip design houses and does not provide learning together in a cohesive manner with the designers. Moreover, AI-assisted EDA tools are sold at a premium, forcing customers to be locked into contracts with specific EDA vendors and increasing the cost of the chip design," explained Rahul Dutta, a Principal Research Engineer at IME.

But what if AI could be used to design chips instead, irrespective of the underlying EDA tools and design? In a virtuous circle, a team of A*STAR researchers from IME and the Institute for Infocomm Research (I²R) has now developed a machine-learning framework which works in tandem with the EDA tools to capture the experience of seasoned chip designers, using it to reduce the cost of designing new chips while simultaneously exploring new design spaces.

SMILE will definitely change the way circuit designers look at design.

— Dr. Kevin Chai
Senior Scientist and Head of IC Design, Institute of Microelectronics, A*STAR

THE INCREDIBLE SHRINKING CHIP

For the last fifty years, chips have become simultaneously smaller and more powerful in keeping with Moore's law. Moving from the 180nm process to 90nm in the mid-2000s, for example, effectively allowed chip makers to squeeze double the number of transistors on the same chip. Smaller chips mean shorter distances traveled within the chip, resulting in greater speed while shrinking transistor sizes mean less energy consumption. Both these factors combined to make chips cheaper as they got smaller.

But this size-cost relationship has begun to break down. These days, making chips even smaller has become so expensive and complicated that it may no longer make financial sense to keep developing smaller processes. Manufacturing costs aside, it is the design of new chips that takes up a sizeable portion of the total cost, with paying for EDA software estimated to contribute nearly half of the total development cost. Semiconductor consulting firm IBS predicts that shifting from 16nm to 10nm processes increased the

cost of chip design by approximately US\$174.4 million, while moving even further to 7nm processes would cost nearly US\$300 million.

"Furthermore, with the increased circuit complexity in advanced technology nodes, circuit design criteria has become more stringent and designers have to go through more iterations to achieve multiple design goals," Raju said. "As a result, productivity suffers, firms incur more cost and it takes more time to bring the product to market."

Despite the costs involved, IC foundries can ill-afford to compromise on their hardware design. Unlike software that can be shipped in a less-than-perfect state and subsequently patched, defective chips cannot be fixed once produced, potentially costing companies eye-watering sums. A hardware bug in Intel's flagship Pentium chips discovered in 1994 reduced the company's profits by 37 percent, going down in history as one of the costliest mistakes in hardware design.

LESS DATA, MORE LEARNING

To reduce the cost and time taken to design new chips, the team led by Kevin Chai, Senior Scientist and Head of IC Design at IME, turned to AI, specifically, a subset of machine learning known as semi-supervised learning. In supervised learning, the algorithm is trained using a set of inputs paired with the desired outputs, requiring a large amount of pre-labeled data. In the case of chip design, input features are the design variables of the circuit, such as transistor length, width, bias and temperature, while the outputs are design goals such as power consumption, bandwidth, other performance criteria and chip area.

"When a design specification or desired output is set, the learning model proposes the input parameters for the design. The design is then verified by computation- and time-intensive EDA simulations," Chai said. "To reduce the number of simulations required, we used a semi-supervised learning model that can be trained with a small amount of labeled data and a large pool of unlabeled data."

The resulting AI algorithm and EDA automation, created under the Smart IC Design with Learning Enablement (SMILE) program, reduced the amount of labeled data required by 90 percent compared to supervised

learning. “As previously mentioned, EDA software has been around for a long time, and great strides have recently been made in the field of machine learning; it was integrating both advances that was the key challenge,” Chai said.

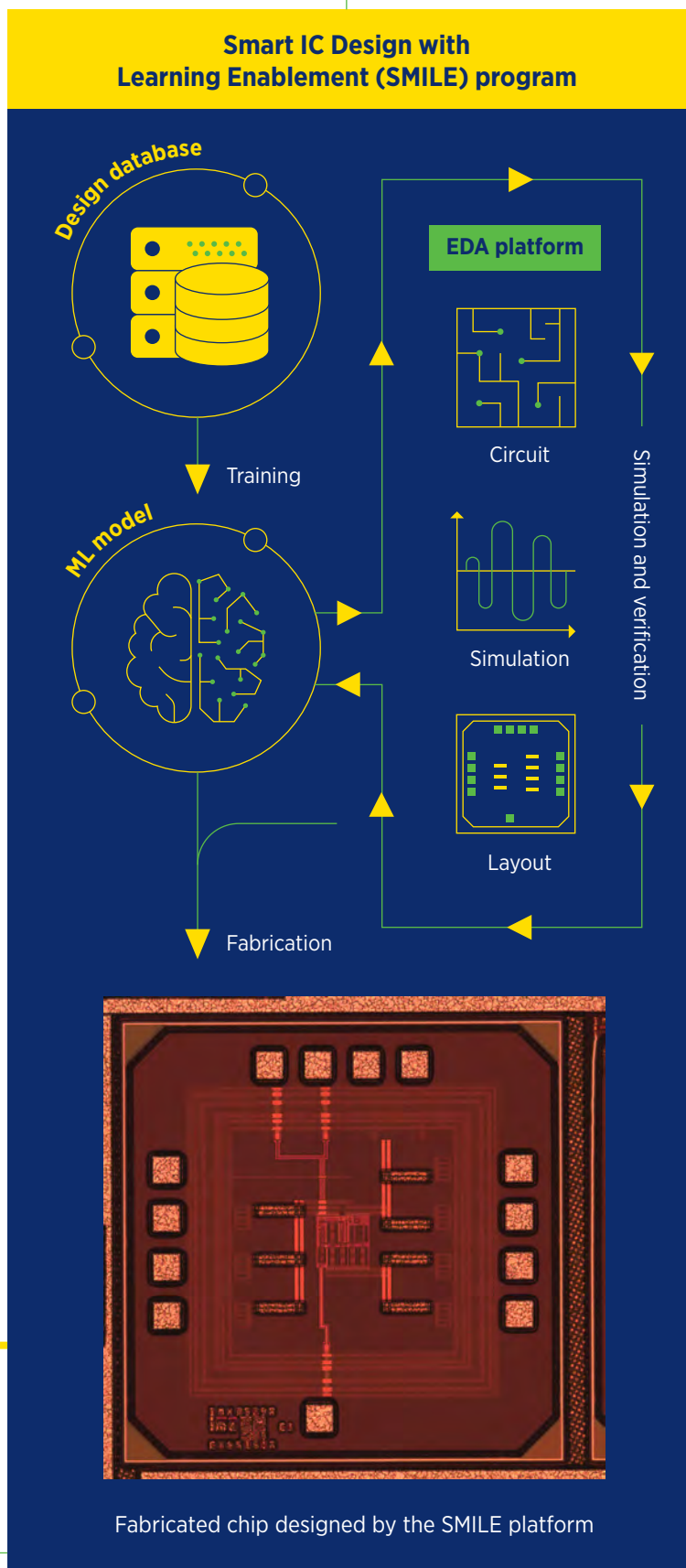
“In the whole design iteration process, there is no human designer in the loop. The circuit designer just has to select the circuit topology and design specifications at the initial stage and the rest of the design is performed by a tight integration of the EDA tool with the AI framework,” Rahul added.

DATA-DRIVEN CHIP DESIGN

The resulting AI was able to complete a complex design in just one day, whereas a human designer would ordinarily have required one week. Furthermore, when the AI-designed chip was fabricated by a foundry and tested at A*STAR laboratories, its performance was found to be twice that of the best human optimized design. This performance was achieved through balancing chip design trade-offs in speed, area and power.

With these impressive results, the SMILE platform has already attracted interest from players in the semiconductor industry, such as fabless IC design companies, Chai said. However, he notes that the technology is still in the development phase, and will require further validation and generalization to make it compatible with a wide range of circuit topologies before it can be deployed commercially.

Nonetheless, AI is undoubtedly the future of chip design, Chai continued. “SMILE will definitely change the way circuit designers look at design,” he said. “Gone are the days where much is dependent on experience and heuristics. Designers of new chips will be greatly aided by ‘data-oriented’ design strategies, thus greatly reducing the number of simulation iterations, the time taken to reach design targets and the costs of design optimization.” ★



BEHAVIOR

Sounding the alarm when things are fishy

A*STAR researchers uncover how bacteria and the immune system generate alarm behaviour in fish.

When someone on the street delivers a high-pitched scream, it changes the behavior of those nearby—some may flee, while others may approach to render help. This mechanism of ‘warning the herd’ works for creatures that can vocalize, but what about those that cannot, like fish?

It turns out that fish have their own means of signaling danger, which goes by the term Schreckstoff. The word, coined by Nobel laureate Karl von Frisch, originates from the German words ‘schreck’ (appropriately meaning ‘fright’ or ‘terror’) and ‘stoff’ (meaning ‘substance’). How Schreckstoff evolved in fish is a mystery, one that A*STAR researchers, working in collaboration with scientists from Nanyang Technological University, Singapore, and the University of Oregon, US, are attempting to unravel.

“When injured, fish release substances from their skin that cause fear in other

members of their shoal,” explained Suresh Jesuthasan, a Principal Investigator at the Institute of Molecular and Cell Biology (IMCB), A*STAR. “These substances are detected by the fish’s olfactory system (responsible for the sense of smell), and lead to a dramatic change in swimming behavior, which can include high-speed escape, freezing or hiding.”

In this study, Jesuthasan’s team demonstrated that bacteria are a component of Schreckstoff in zebrafish. When the group introduced bacteria into tanks containing zebrafish, they noted alarm behavior in the fish.

But bacteria are just one part of the equation—the group’s findings also indicate that Schreckstoff is a by-product of the fish immune response. “Fish are in constant contact with bacteria. To prevent these bacteria from causing an infection, fish have an immune system in the skin,” Jesuthasan said.

A type of cell, known as a club cell, is responsible for consuming these bacteria and alerting the immune system in the skin. If a fish suffers an injury to the skin, the club cells present at the site of injury burst, releasing the accumulated bacteria into their immediate environment.

“In this case, bacteria can produce something that triggers fear. The findings are also relevant to the field of mucosal immunity—that is, immunity in the gut or airway epithelium—as they point to the existence of a surveillance mechanism that has not been reported in other vertebrates yet,” Jesuthasan said.

He added that the findings not only reinforce the growing view that bacteria influence behavior, but also suggest an evolutionary advantage for Schreckstoff: any fish that is able to link the release of bacteria with danger would be more likely to survive.

Jesuthasan now wants to explore the implications of the mechanism behind Schreckstoff. “It would be interesting to see how the immune mechanisms uncovered in this study are relevant to the gut and airway, and in other species,” he concluded. ★

BOTTOM

A type of immune cell found in the skin of zebrafish protects it against bacterial infection. When the immune cells burst due to injury, they release the trapped bacteria, causing nearby fish to exhibit alarm behavior.

1. Chia, J. S. M., Wall, E. S., Wee, C. L., Rowland, T. A. J., Cheng, R. K., *et al.* Bacteria evoke alarm behavior in zebrafish. *Nature Communications* 10, 3831 (2019).



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BIOCHEMISTRY

Finding the missing link for a rare heritable disease

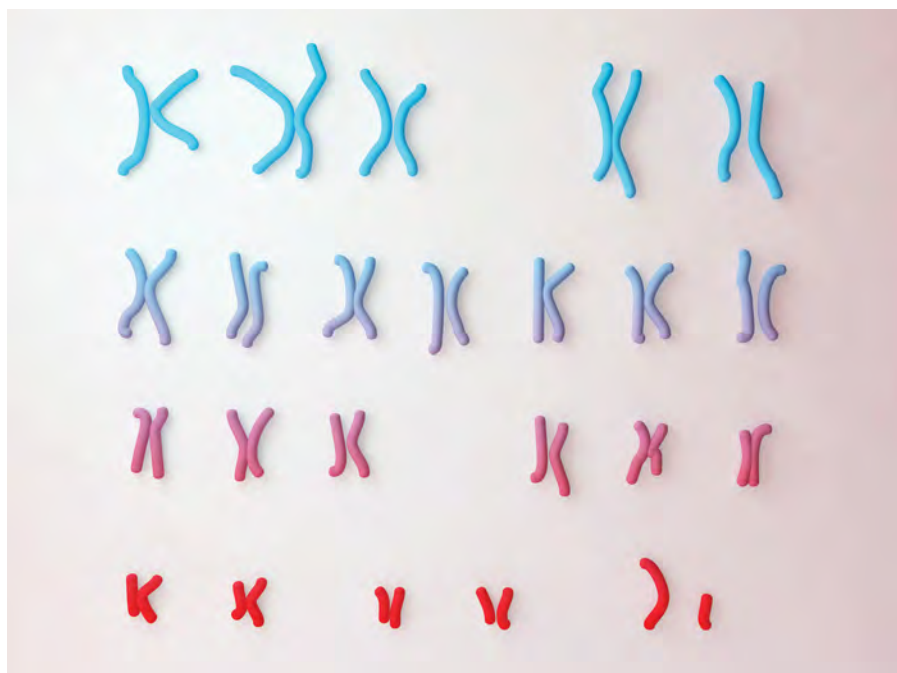
Mutations accelerating the degradation of the protein gelsolin are responsible for amyloid plaque buildup in a rare type of amyloidosis.

Rare diseases are often understudied, making treatments for them even rarer. One such disease is familial amyloidosis, Finnish type (FAF), so named because it was initially observed only in the Finnish population.

FAF is characterized by the abnormal build-up of a protein called amyloid in the organs, leading to blindness, paralysis and heart problems, among other symptoms. Although scientists know that FAF is associated with mutations in the gene that produces a protein called gelsolin, exactly how these mutations result in amyloid build-up has remained elusive.

Seeking the missing link, researchers at A*STAR's Institute of Molecular and Cell Biology (IMCB) and the Bioinformatics Institute (BII) used X-ray crystallography to obtain a molecular snapshot of mutant and normal gelsolin. Unexpectedly, they observed that mutant and normal gelsolin were not only structurally similar to each other, but also functionally intact in terms of their biological activity.

Probing deeper, the group traced the root of the problem to the stability of domain-domain interactions within mutant gelsolin. Gelsolin comprises six different domains, with domain 2 containing a cleavage site for the enzyme furin. The cleavage site in domain 2 is normally blocked by domain 3.



The molecular and genetic origins of rare diseases such as familial amyloidosis, Finnish type, are often poorly understood.

The researchers discovered that the interaction between domains 2 and 3 was particularly weak in mutant gelsolin, which resulted in the furin cleavage site being more readily exposed. The cleavage of gelsolin by furin then sets off a chain of events leading to the build-up of amyloid.

"Furin makes the first cut in gelsolin, which leads to the progressive degradation of the gelsolin. Some of the resulting peptide fragments eventually self-assemble into amyloids which cause the symptoms of FAF," said Robert Robinson, a Research Director at IMCB who led the study.

These findings highlight a potential strategy to control the molecular mechanisms underlying FAF onset and

progression, and pave the way for better diagnostic tools to detect the disease and monitor its severity.

"Our findings tell us that we need to stabilize domain 2, which we believe can be achieved by a nanobody binding to domain 2, as we have shown in a previous study. The results also indicate that we should be looking for other mutations in gelsolin when assessing if an individual is likely to suffer FAF symptoms," Robinson explained. ★

1. Zoragati, H., Larsson, M., Ren, W., Sim, A. Y. L., Gettemans, J. *et al.* The role of gelsolin domain 3 in familial amyloidosis (Finnish type). *Proceedings of the National Academy of Sciences of the United States of America* **116**, 13958-13963 (2019).

BRAIN-COMPUTER INTERFACES

Mind over matter for stroke rehabilitation

Mental practice of upper limb movement and visual feedback could help improve physical rehabilitation among stroke patients.

WHY THIS MATTERS

- Some 50% of stroke patients remain disabled after being discharged from hospital.
- Brain-machine interfaces that register brain signals could help in the recovery of stroke patients' upper limb function.

Being able to move around freely is essential to having a sense of autonomy, but a stroke could snatch this ability from us in an instant. Therefore, for patients who have suffered a stroke, recovering motor function is a crucial component of therapy.

While the standard treatment for motor rehabilitation is conventional physiotherapy, statistics point to a clear need for more effective methods—nearly half of all stroke survivors remain disabled and consequently suffer from a poorer quality of life.

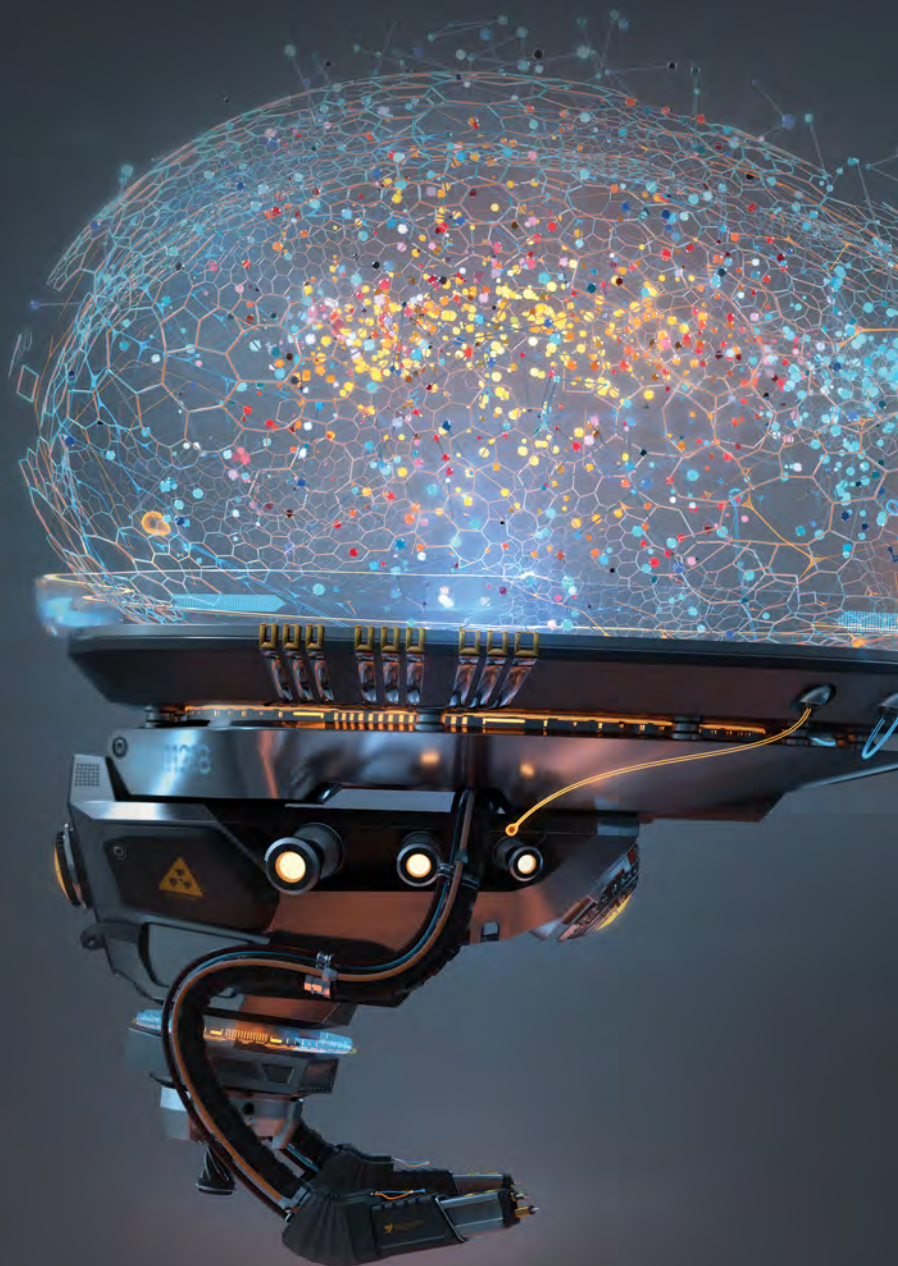


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As an alternative approach to conventional physiotherapy for stroke patients, scientists at A*STAR's Institute for Infocomm Research (I²R) have proposed a combination of motor imagery and a brain-computer interface (BCI). Their research was carried out in collaboration with researchers at Nanyang Technological University, Singapore, and clinicians at Tan Tock Seng Hospital and the National University Hospital.

The non-invasive approach—named the Neurostyle Brain Exercise Therapy Towards Enhanced Recovery, or nBETTER for short—involves the use of electroencephalograms (EEGs) to monitor brain electrical activity. The EEG readouts allow the team to observe the patient's mental practice of movement, or motor imagery. Brain signals from motor imagery are subsequently relayed by the BCI to move a virtual limb displayed on a computer screen, providing visual feedback to the patient.

"Stroke patients have difficulty performing physical movement of the impaired upper limb, but they can imagine moving the limb," explained Kai Keng Ang, a Senior Scientist at I²R and an author on the study. Because this approach does not require actual movement from the patient, it bypasses stroke patients' physical limitations at the start of rehabilitation.

Using the Fugl-Meyer Assessment as the standard for scoring motor function, the team conducted a small trial to compare the clinical efficacy of nBETTER with that of standard arm therapy (SAT).

They found that patients who underwent nBETTER over a span of 24 weeks had an average improvement score of 5.8, compared to 3.6 for those who underwent SAT.

"Our findings suggest a role for BCI in detecting imagination of movement and providing visual feedback, perhaps by using virtual reality or augmented reality," Ang noted. "This is how BCI can be used to complement existing rehabilitation practices."

However, the researchers also observed mental fatigue in patients undergoing nBETTER, which could reduce rehabilitation efficacy. They suggest that the fatigue is caused by the monotony of the mental practice and the sustained attention required. "Going forward, we intend to use soft robotics to provide stroke patients with touch and motion feedback during MI-BCI therapy, in addition to visual feedback," Ang said. ★

IMPACT

Stroke patients who used the A*STAR scientists' brain-machine interface system over a span of 24 weeks scored better on a motor function assessment than those receiving standard arm therapy.

BELOW

A*STAR scientists have developed a brain-computer interface to help stroke patients regain upper limb function.

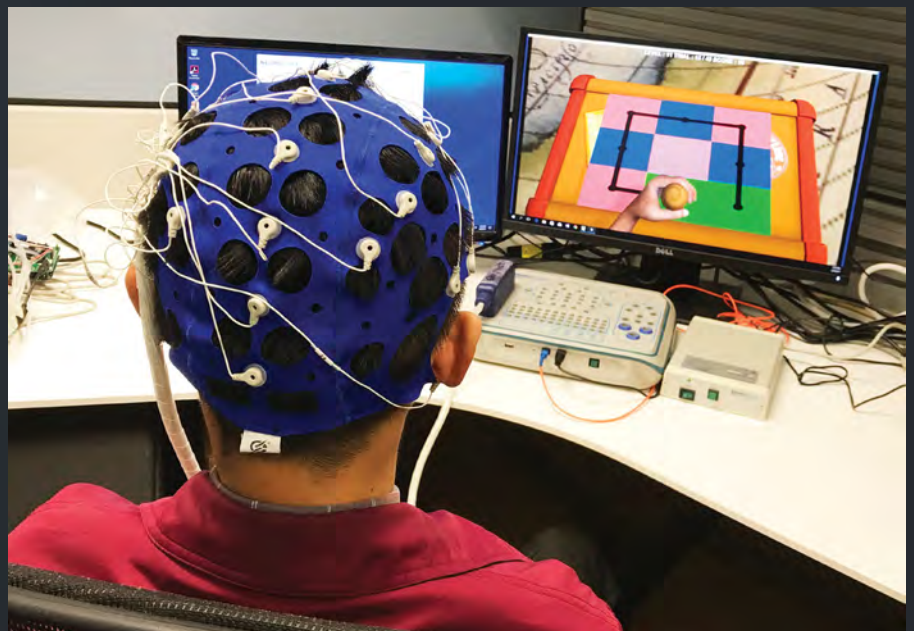


Photo credit: © 2019 A*STAR Institute for Infocomm Research

1. Foong, R., Ang, K. K., Quek, C., Guan, C., Phua, K. S. *et al.* Assessment of the Efficacy of EEG-based MI-BCI with Visual Feedback and EEG Correlates of Mental Fatigue for Upper-Limb Stroke Rehabilitation. *IEEE Translational Biomedical Engineering* (2019).

BIOINFORMATICS

How the flu virus hops from one host to another

A*STAR researchers have identified specific sites in the surface protein of influenza viruses that allow the pathogen to adapt to different host organisms.

From birds to cats, pigs to humans, many animals fall ill with their own variant of the flu virus, also known as the influenza virus. But every once in a while, an influenza variant manages to cross the species divide. For example, the virus strain responsible for the 1997 outbreak of avian influenza in Hong Kong was detected in humans—the strain had essentially adapted to a new host organism.

To understand how host adaptation occurs, Sebastian Maurer-Stroh and colleagues at the Bioinformatics Institute (BII), A*STAR, with collaborators in Singapore, the Netherlands and the US, are diving deep into the protein sequences of influenza viruses. Of particular interest to them is the hemagglutinin protein of influenza viruses, which plays a crucial role in infection. What they seek to pinpoint are passage bias sites—positions in the hemagglutinin protein that help the influenza virus better adapt to a new cellular environment.

Analyzing more than 80,000 influenza hemagglutinin sequences where information on passage in cells of different species was available, the researchers identified 54 common passage bias sites in the hemagglutinin of influenza variants.

“These 54 sites lie in three structural regions of the hemagglutinin protein: the receptor-binding site which enables the

“Our data and FluSurver tool will be valuable to researchers who track emerging influenza viruses that have the potential to cause local epidemics or global pandemics.”

virus to enter the host cell, the region that affects the pH-dependent structural changes of the protein, and the N-terminal signal peptide of the protein,” Maurer-Stroh said. Of the three, the impact of N-terminal signal peptide mutations is the least well understood in the context of host adaptation, he added.

The research group also demonstrated that some passage bias sites in the hemagglutinin proteins show signs of epistasis, which means that these adaptation mutations do not appear independently. Hence, the sum of all mutations is likely to determine productive adaptation. These findings indicate that it is difficult to generalize transmissibility behavior of a virus based on previous experiments that do not consider the effects of multi-variant interactions.

“Our data and FluSurver tool will be valuable to researchers who track emerging influenza viruses that have the potential to cause local epidemics or global pandemics,” said Maurer-Stroh.

In addition, because influenza vaccines are produced by growing human viruses in egg cells, those viruses typically undergo mutations that adapt them to better growth in chickens rather than in humans. Maurer-Stroh is working on another project with colleagues at the Genome Institute of Singapore (GIS), A*STAR, to understand how this can sometimes affect the efficacy of the vaccine.

“We will further examine the role of the understudied signal peptide region of hemagglutinin in host adaptation. We also plan to identify cell passage adaptive sites for other viral proteins and continue our work on the interplay between egg adaptation and vaccine efficacy,” he said. ★

ABOVE

Mutations to the hemagglutinin protein give rise to influenza virus variants that can adapt to new host organisms.

1. Lee, R. T. C., Chang, H. H., Russell, C. A., Lipsitch, M. and Maurer-Stroh, S. Influenza A Hemagglutinin Passage Bias Sites and Host Specificity Mutations. *Cells* **8(9)**, 958 (2019).

MALARIA

A new dimension to studying malaria

A three-dimensional liver organoid model for studying dormant malaria parasites may pave the way for better antimalarial drugs.

Once thought to be caused by ‘mala aria’—Italian for ‘bad air’—from swampy areas, malaria is now attributed to infection by the *Plasmodium* parasite. Spread by the *Anopheles* mosquito, the parasite claims more than 400,000 lives around the world each year.

After entering the bloodstream of a human host via a mosquito bite, the *Plasmodium* parasite takes up residence in liver cells, or hepatocytes, where they multiply before being released into the bloodstream of their human host. The parasites multiply further in the bloodstream and are taken up by mosquitoes for the next phase of their life cycle.

Some parasites will become dormant in the liver, where they can persist for weeks, months or even years. These dormant parasites, responsible for malaria relapse, are known as hypnozoites, and little is known about their biology, in part due to the lack of appropriate models to study them outside a living host.

“A majority of the existing two-dimensional liver models are physiologically-limited, short-term assays of less than two weeks, which are unable to sustain the liver-specific functions required for the proper development of the malaria parasite,” said Pablo Bifani, a Principal

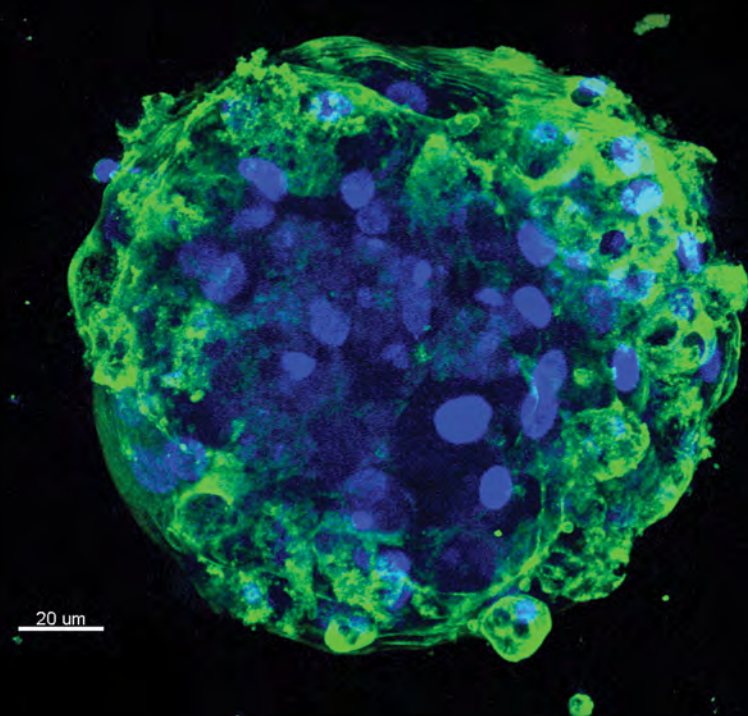
Investigator at A*STAR’s Singapore Immunology Network (SIgN).

Together with SIgN colleague Adeline Chua and international collaborators, Bifani created liver organoids by seeding hepatocytes onto a biologically compatible biomaterial, called 3D Cellusponge. The liver spheroids mimic the liver microenvironment, allowing for a longer-term investigation of the life cycle of the *Plasmodium* parasite outside a living host.

By infecting the liver organoids with human-associated *Plasmodium* strains, the researchers were able to recapitulate the life cycle of relapsing malaria in a petri dish. Importantly, the spheroids could be maintained for more than three weeks, and the parasites that multiplied within the liver spheroids during that time remained capable of re-infecting red blood cells.

To validate their liver organoid model, the researchers used a drug called KDU691, which was previously shown to prevent malaria relapse in monkeys when administered at the time of infection, but which was ineffective if administered after an infection had been established. Bifani’s team reported similar results with their liver organoid model, suggesting that the liver organoid model may be a useful tool for screening antimalarial drugs and predicting drug efficacy *in vivo*.

Going forward, Bifani and colleagues plan to use their liver organoid model to identify genes involved in hypnozoite formation and reactivation from dormancy. “This [knowledge] will shed light on the liver-stage biology of the parasite, allowing for the development of predictable assays that can be used to screen compounds in the search for new antimalarial drugs,” he said. ★



LEFT

Three-dimensional liver organoid models provide a more physiologically relevant environment for drug screening.

1. Chua A. C. Y., Ananthanarayanan, A., Ong, J. J. Y., Wong, J. Y., Yip, A. *et al.* Hepatic spheroids used as an in vitro model to study malaria relapse. *Biomaterials* **216**, 119221 (2019).

MICROBIOLOGY

Solving the puzzle of human gut microbiomes

A*STAR scientists have devised an algorithm for accurately assembling genomes, paving the way for in-depth analysis of microbial communities in the human gut.

WHY THIS MATTERS

- Bacterial communities in the gut—collectively known as the gut microbiome—are known to affect the physiological functions of the body.
- Characterizing the gut microbiome remains a challenge due to the lack of available tools to accurately piece together bacterial genomes.

Regardless of one's standard of personal hygiene, bacteria coat every inch of our bodies and even live inside us. These microbes play important roles in maintaining health, and imbalances in their populations can result in disease.

Scientists interested in studying microbial communities, or microbiomes, often rely on a technique called metagenomics, in which bacteria are obtained from their native environment and processed for DNA sequencing. This is especially useful for studying gut microbiomes since some gut bacteria are difficult to grow in the lab.

However, metagenomic studies come with limitations. Sequencing short DNA fragments from a community of bacteria comprising hundreds of different species means that DNA fragments, or reads, need to be accurately assembled, much like the pieces of a very complex jigsaw puzzle.

"Current metagenomics assemblers only provide fragmented assemblies when there are multiple strains of the same species in the microbiome. Microbiome studies are then limited by the resolution of genetic analysis and the ability to understand microbial functions in communities harboring hundreds of bacterial species," said Denis Bertrand, a Staff Scientist at A*STAR's Genomic Institute of Singapore (GIS).

Together with collaborators across Singapore, including clinicians from Tan Tock Seng Hospital, and colleagues in Croatia, Bertrand sought to increase the accuracy of genome assembly in metagenomics studies using long Nanopore reads.

Assessing 197 stool samples from ongoing clinical studies, the team devised a method to analyze a majority of the samples and obtain high-quality data for long-read sequencing. By combining this data with accurate short reads, the researchers developed a hybrid assembly algorithm, OPERA-MS, which allowed them to assemble individual genomes of strains in the bacterial community from billions of DNA sequences.

"We found that OPERA-MS provides up to ten times more complete genomes compared to methods based on short-reads, and at least five times more accurate genomes than other approaches that rely on long reads," Bertrand said. When used to analyze the gut microbiomes of 28 antibiotic-treated patients, OPERA-MS facilitated the discovery of gene combinations responsible for resistance to several antibiotic classes.

"These assemblies serve as valuable references for studying the evolution of antibiotic-resistant microbes in the gut. We can now distinguish between antibiotic-resistant strains and those that are benign residents, allowing us to track the spread of both infectious and beneficial bacteria in their natural ecosystem," Niranjan Nagarajan, Associate Director and Senior Group Leader at GIS, explained.

As part of the integrated Omics research program at A*STAR, the team plans to use OPERA-MS to generate an Asian gut bacterial reference genome and identify microbiome variations between and within ethnic groups. In collaboration with Hong Kong-based Civet Bioscience, OPERA-MS will also be used to monitor the microbiome of patients who have undergone fecal microbiota transplantation. ★

IMPACT

With the OPERA-MS algorithm, researchers will be able to obtain accurate genome assemblies of bacteria in the gut to shed light on health and disease.

LEFT

Like a complex jigsaw puzzle, assembling bacterial genomes from short DNA sequences presents a significant challenge to genomics.

1. Bertrand, D., Shaw, J., Kalathiyappan, M., Ng, A. H. Q., Kumar, M. S. *et al.* Hybrid metagenomic assembly enables high-resolution analysis of resistance determinants and mobile elements in human microbiomes. *Nature Biotechnology* **37**, 937-944 (2019).

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BIOSENSORS

Protein-protein interactions made visible

A*STAR researchers have turned a bacterial protein into a biosensor that can be used to visualize interactions between proteins.

Rather than function in isolation, proteins are part of a larger network of interactions necessary for life. By mapping out protein-protein interactions in biological systems, researchers can obtain a better understanding of health and disease.

However, given the small size of protein molecules and their often-transient binding to one another, scientists have had difficulty directly observing protein-protein interactions. Researchers led by Farid Ghadessy, a Group Leader at the p53 Laboratory, A*STAR, have found a way to simplify the detection and observation of protein-protein interactions by reverse-engineering an enzyme from nature.

In collaboration with Robert Robinson at the Institute of Molecular and Cell Biology (IMCB), A*STAR, and colleagues in Thailand, Ghadessy modified CueO, a copper-oxidizing enzyme from the bacterium *Escherichia coli*, into a protein sensor. “We decided to use CueO because it is stable across a wide range of temperatures, its structure is well

known, and the reaction it catalyzes is easily observed,” he explained.

The next step involved Ghadessy’s team grafting fragments of the tumor suppressor protein p53 onto CueO, which allowed them to gain insights into how another protein, MDM2, interacts with p53. “Elevated levels of MDM2 can indicate the presence of certain cancers,” Ghadessy said.

When MDM2 binds to the fragments of p53 engrafted onto CueO, the enzymatic activity of CueO was triggered, resulting in the conversion of a colorless compound into a colored product. Importantly, the color intensity increased proportionately with the concentration of MDM2.

“We discovered that our biosensors were sensitive in the micromolar range, and that we could use them to detect protein-protein interactions in a single test tube with easy visual indication, without the need for purification or washing steps,” said Ghadessy. In addition, the researchers showed that their CueO-based biosensor specifically detects interactions between MDM2 and p53—no color change occurred in the presence of inhibitors that prevent MDM2 from binding to p53.

To further demonstrate the customizability of their CueO-based biosensor, the researchers engrafted an antibody onto CueO, showing that CueO-mediated color change only occurred when the antibody binds to a viral protein. “In the future, we plan to make other biosensors based on the CueO enzyme, and we expect these biosensors to be very helpful in addressing important structural questions about the cancer-related proteins we study,” said Ghadessy. ★

ABOVE

Biosensors can be used to detect transient protein-protein interactions and provide structural information about proteins.

1. Sana, B., Chee, S. M. Q., Wongsantichon, J., Raghavan, S., Robinson, R. C. *et al.* Development and structural characterization of an engineered multi-copper oxidase reporter of protein-protein interactions, *Journal of Biological Chemistry* **294**, 7002-7012 (2019).

Photo credit: Kenneth Eward, Biografix / Science Photo Library

CANCER BIOLOGY

Detecting illegal assemblies in cancer cells

A new method to measure crucial protein-protein interactions in cancer cells may have implications for drug design and discovery.

The second leading cause of death globally, cancer is a disease characterized by uncontrolled cell division. To develop new and better treatments for cancer, researchers are delving deep into the signaling pathways that drive rogue behavior in cancer cells.

At the crossroads of several of these signaling pathways sits a protein complex called eIF4F, which controls whether a cell divides or not. Because the eIF4F complex requires three sub-components—eIF4A, eIF4E and eIF4G—coming together to work, scientists have considered targeting the assembly process of eIF4F to treat

cancer. For that to happen, scientists need a clear method to monitor the interactions among the sub-components of eIF4F.

“Conventional approaches to study eIF4E:eIF4G interaction are experimentally demanding, tedious and can only be done in dead cells,” said Christopher Brown, a Principal Investigator at the p53 Laboratory, A*STAR. “Our method, the NanoLuc-based protein fragment complementation assay or protein-protein interaction assay, can be performed in live cells with high throughput.”

Put simply, when eIF4E and eIF4G interact in living cancer cells, luminescent

or fluorescent signals are generated. If a compound successfully prevents eIF4E from complexing with eIF4G, the luminescent or fluorescent signal is lost, granting the researchers the ability to identify upstream or downstream factors that perturb eIF4E:eIF4G interaction.

Using this assay, the researchers validated that eIF4F complex assembly is regulated by another protein named 4EBP1. Meanwhile, 4EBP1 is controlled by two upstream signaling pathways named RAS/ERK and PI3K/AKT/mTOR.

When the researchers used small molecule drugs to inhibit the RAS/ERK and PI3K/AKT/mTOR pathways concurrently in cancer cells, eIF4F complex assembly was blocked, and the cells were less resistant to treatment.

“Using our technique to monitor eIF4E:eIF4G interaction in living cells, we aim to drive the discovery of new modalities against ‘difficult drug targets’ within cells, such as the eIF4F complex and KRAS,” said Brown. Additionally, his team is developing methods to probe the interfaces between proteins and other biomolecules such as DNA, which will allow druggable sites in proteins to be identified.

“More importantly, we are developing target-agnostic assays to measure the permeability of macrocyclic compounds directly. We hope that this type of assay will allow us to understand the parameters required for the uptake of macrocyclic compounds into cells,” Brown added. The findings from this ongoing study may have implications for cancer drug design, by screening for anticancer drugs that show optimal uptake into tumors. ★

LEFT

Fluorescent probes in living cells can reveal the underlying biology of cancer, which could lead to the discovery of better anticancer drugs.

1. Frosi, Y., Usher, R., Lian, D. T. G., Lane, D. P., Brown, C. J. Monitoring flux in signaling pathways through measurements of 4EBP1-mediated eIF4F complex assembly. *BMC Biology* **17**, 40 (2019).

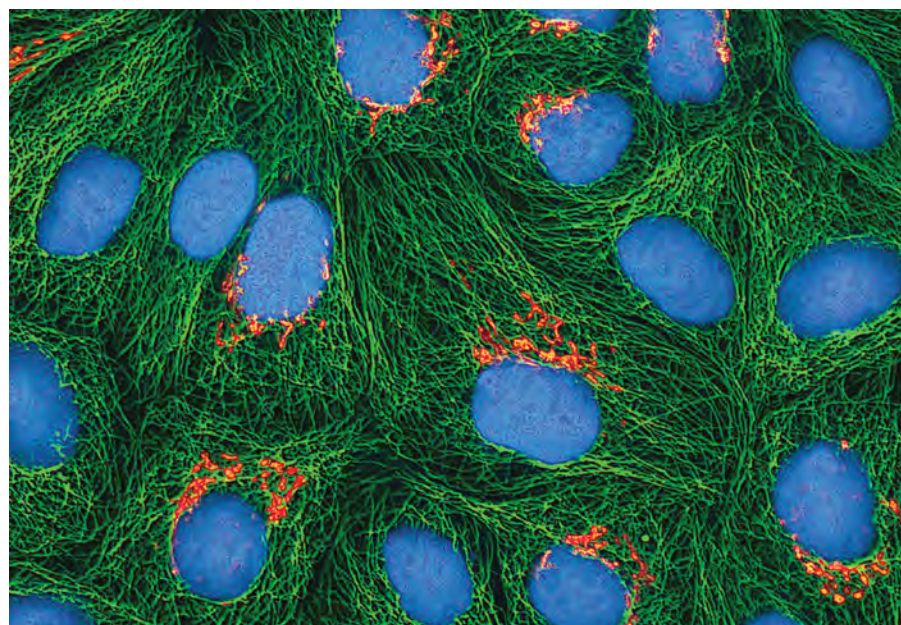


Photo credit: skeeze / Pixabay

CANCER BIOLOGY

A stabilizing strategy to keep cancer cells in check

Bortezomib, a drug that inhibits the ‘waste disposal system’ in animal cells, could be used to suppress cell division in cancer.

Floor the accelerator and a car speeds up; step on the brakes and it comes to a halt. In cells, an analogous system of ‘accelerators’ and ‘brakes’ exists to regulate cell division. One protein in particular—called p53—has often been described as the ‘brakes’ of mammalian cell division, making sure that cells suffering from irreversible DNA damage do not survive in the body.

Unsurprisingly, the loss of p53 can result in diseases of uncontrolled cell division—cancer is a case in point. Compounds that directly or indirectly stabilize p53 in the cell, leading to elevated p53 levels, are therefore attractive as anticancer drugs. One strategy to stabilize proteins in cells is by blocking

the activity of the proteasome—the cellular machinery responsible for getting rid of proteins tagged for degradation.

Researchers led by A*STAR Chief Scientist Sir David Lane thus hypothesized that a proteasome inhibitor called bortezomib could be effective against solid tumors. Bortezomib has already been approved for the clinical treatment of certain blood cancers, but its efficacy against solid tumors remained unclear.

Studying bortezomib in a popular mouse model of benign intestinal tumors (APC^{min/+} mice) and in mice engrafted with human colorectal cancer cells (known as xenograft models), the team found

that bortezomib was highly effective in controlling tumor growth without toxicity. “We found that tumors in the intestines of bortezomib-treated APC^{min/+} mice show marked upregulation of p53, p21 and BAX,” said Lane, referring to cellular proteins that arrest cell division and promote cell death.

The researchers further showed that bortezomib caused p53 to enter the nucleus of the cell, where DNA resides. There, p53 activates the expression of genes that function collectively to suppress cell division.

However, Lane and colleagues noted that bortezomib also stabilized p53 in non-cancerous cells of the intestine which are responsible for renewing the intestinal lining. The intestinal lining is shed regularly, so its replacement by intestinal stem cells and another group of dividing cells known as transit amplifying cells is crucial.

“Elevation of p53 and its stabilization by bortezomib may therefore result in gastrointestinal side effects,” said Yuezhen Xue, a Research Scientist in Lane’s lab and the first author of the paper. However, bortezomib was shown to have a short functional half-life in the body, and intestinal stem cells convert between fast-dividing and quiescent states, so a well-timed dose of bortezomib could avoid persistent damage to gastrointestinal tissues, she added.

The researchers intend to study the effects of bortezomib in xenograft models of other types of cancer. They are using the CRISPR/Cas9 gene editing system to create multiple p53-positive and p53-null isogenic cancer cells to establish their xenograft tumors, which will allow them to further explore cancer therapy strategies based on p53 status. ★

ABOVE

Small molecule inhibitors that interfere with the protein degradation machinery in cancer cells could be useful as therapeutics.

1. Xue, Y., Barker, N., Hoon, S., He, P., Thakur, T. *et al.* Bortezomib stabilizes and activates p53 in proliferative compartments of both normal and tumor tissues in vivo. *Cancer Research* **18**, 3744 (2019).

CANCER BIOLOGY

Sweet serendipity unveils anticancer strategy

A better understanding of the way sugar units are attached to the surface proteins of cancer cells may help to improve targeted cancer treatments.

Often maligned for its role in diabetes, sugar is critical for cells to signal to one another and their environment. These sugar units are typically attached to proteins on the surface of cells via the activity of enzymes.

The locations, lengths and sequences of sugar units on a protein can affect how it is recognized by certain drugs. Hence, the 'glycosylation pattern' of proteins has implications for the efficacy of targeted therapy, especially in the context of cancer.

"Currently, antibody-based cancer therapeutics are focused on targeting the correctly glycosylated protein that is overexpressed in cancer cells compared to normal cells," said Sir David Lane, Chief Scientist of A*STAR. But because these correctly glycosylated proteins may also be expressed in normal cells, the treatment may result in side effects.

"If we are able to target the aberrantly glycosylated proteins that are only expressed on neoplastic cells but not the normal tissues, we would gain access to significant enhancements in drug specificities and drug concentrations, without the fear of off-target toxicities associated with the use of higher drug doses," Lane explained.

In a study published in *Oncogene*, his team was able to generate an antibody—called 6E6—that distinguishes between the glycosylation patterns on the RON receptor, a protein often expressed by aggressive cancers.

Lane recalls the serendipitous nature of the discovery. His team was originally

"Our research highlights the importance of looking into glycosylation changes in neoplastic cells compared to normal cells."

disappointed that 6E6 did not bind well to correctly glycosylated RON expressed by cancer cells grown in a petri dish.

However, when the researchers injected 6E6 into mice engrafted with human cancer cells, they observed much stronger binding. It turned out that the tumors in mice were expressing much

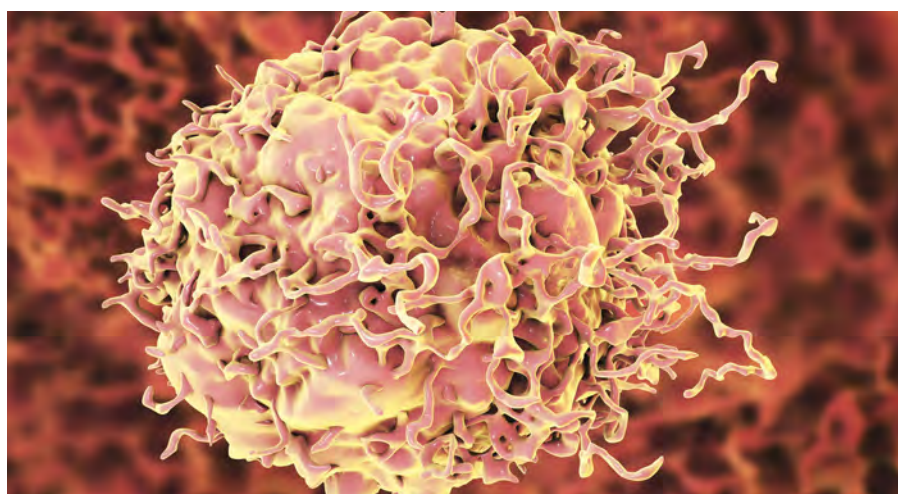
higher levels of unglycosylated RON, which 6E6 is sensitive to.

Importantly, tumor growth in mice treated with 6E6 was inhibited by almost 80 percent compared to a control group, indicating that 6E6 has therapeutic potential. The researchers are currently investigating the mechanism of this therapeutic effect, and their preliminary data suggests that 6E6 recruits a subset of immune cells known as natural killer cells to cause cell death in the tumors.

The researchers have also mapped the binding interactions between 6E6 and unglycosylated RON. They found that 6E6 recognizes and binds to a sequence of three amino acids in the alpha chain of RON; these amino acids form a loop constrained by a chemical bond known as a disulfide link.

"Our research highlights the importance of looking into glycosylation changes in neoplastic cells compared to normal cells," said Xin Yu Koh, a Postdoctoral Research Fellow in Lane's lab and the first author of the study. Going forward, the team aims to test their antibodies in more advanced preclinical animal models and explore combinational therapies. ★

1. Koh, X. Y., Koh, X. H., Hwang, L. A., Ferrer, F. J., Rahmat, S. A. B. *et al.* Therapeutic anti-cancer activity of antibodies targeting disulfide bond constrained epitopes on unglycosylated RON receptor tyrosine kinase. *Oncogene* (2019).



Cancer cells may add sugar chains to their proteins differently from normal cells.

IMMUNOLOGY

The rediscovered origin of an immune cell subtype

By mapping the development trajectory of immune precursor cells, A*STAR scientists showed that plasmacytoid dendritic cells were incorrectly classified in the immune ‘family tree.’

A person’s history can reveal a lot about their character, skills and inclinations, and this information is often used by employers to assess candidates for a professional role. Analogously, the origins of immune cells give researchers and clinicians clues about how each type of immune cell functions in the body.

“If you want to understand how cells ‘work’ you need to understand how they develop to begin with,” said Florent Ginhoux, Senior Principal Investigator at the Singapore Immunology Network (SIgN).

Focusing on a group of immune cells known as dendritic cells (DCs), his team has discovered that two subtypes

of DCs—plasmacytoid and conventional DCs— which were previously thought to be derived from a common progenitor, in fact arise from distinct lineages.

Plasmacytoid DCs are among the first cells to recognize pathogens and will immediately secrete molecules that not only alarm other immune cells to clear the infection, but also directly kill pathogens and infected cells. Conventional DCs, in contrast, are more specialized in directly activating and priming T cells to orchestrate a very powerful and specific immune response.

Ginhoux’s team thoroughly mapped out the development trajectories of DCs using a combination of cell sorting,

RNA sequencing and computational techniques. Their data revealed that a protein called Ly6D found on the surface of early lymphoid progenitors identifies cells that will give rise to plasmacytoid DCs and not to conventional DCs. The surface protein CD115 was also useful for classifying immune progenitors, especially the conventional DCs.

“We confirmed that CD115⁺ common DC progenitor cells follow a myeloid trajectory that we identified in our 2015 publication. On the other hand, CD115[−] progenitor cells, which were previously thought to be common DC progenitors, actually belong to a completely different family of immune cells—the lymphoid family,” Ginhoux explained, adding that these findings challenge researchers to re-evaluate the way the lymphoid family of immune cells is organized.

He emphasized that a thorough understanding of the developmental trajectories of conventional and plasmacytoid DCs will facilitate further immunological studies on initial responses to infections, autoimmune disorders and cancer.

“There are still a lot of open questions surrounding DCs with regards to their tissue specificity, tissue-specific functions, activities during early life and aging, and so on. One of the main interests in the field is to exploit DC function, especially their antigen presentation capacity, as a target for novel vaccination and therapy strategies. Targeting DC progenitor cells could enhance those strategies,” he said. ★

LEFT

Dendritic cells play an important role in the immune response to infections, autoimmune disorders and cancer.

1. Dress, R. J., Dutertre, C. A., Giladi, A., Schlitzer, A., Low, I. *et al.* Plasmacytoid dendritic cells develop from Ly6D⁺ lymphoid progenitors distinct from the myeloid lineage. *Nature Immunology* **20**(7), 852-864 (2019).

IMMUNOLOGY

Enhancing the immune response to fungal infections

A*STAR scientists have found a way to boost the activity of neutrophils, a subset of immune cells, against disease-causing fungi.

According to the Global Action Fund for Fungal Infections, more than 300 million people around the world suffer from serious fungal infections. While several antifungal drugs are available in clinics and hospitals, their efficacy is declining due to the advent of drug-resistant fungal strains.

“Drug resistance among pathogenic fungi is surfacing at an accelerated rate. An example is the emergence of the superbug *Candida auris* that, in recent times, has caused infections in hospitals worldwide,” warned Kong-Peng Lam, Executive Director at A*STAR’s Bioprocessing Technology Institute (BTI). Because of the urgent need for new ideas and ways to combat fungal infection, Lam and his team began exploring how the body naturally protects itself against disease-causing fungi.

The researchers focused their attention specifically on neutrophils, an abundant type of white blood cell that forms the first line of defense in antifungal immunity. The team discovered that the loss of a protein called Dok3 within neutrophils boosted the neutrophils’ antifungal capabilities.

“Using a technique called co-immunoprecipitation, which shows

binding between different proteins, we identified that Dok3 bridges the interaction between two other proteins—PP1 and Card9,” said Jia-Tong Loh, a Research Fellow in Lam’s lab and the lead author of the study. When close together, PP1 inhibits the function of Card9, resulting in the suppression of neutrophil responses to fungal infection. Therefore, when Dok3 is deleted, the antifungal activity of neutrophils is restored.

Lam’s team further showed that the genetic deletion of Dok3 enhanced survival rates in mice with systemic *Candida* infections. This was attributed to a stronger neutrophil presence in the brains and kidneys of Dok3-deleted mice compared to normal mice used as a control group, which resulted in better control of infection in Dok3-deleted mice.

“Now that we know Dok3 suppresses antifungal defenses, we can potentially remove the brakes on antifungal immunity. Implementing an immune-based approach can reduce fungicide use in the clinics, slow down the evolution of fungi resistance and improve the clinical outcomes of

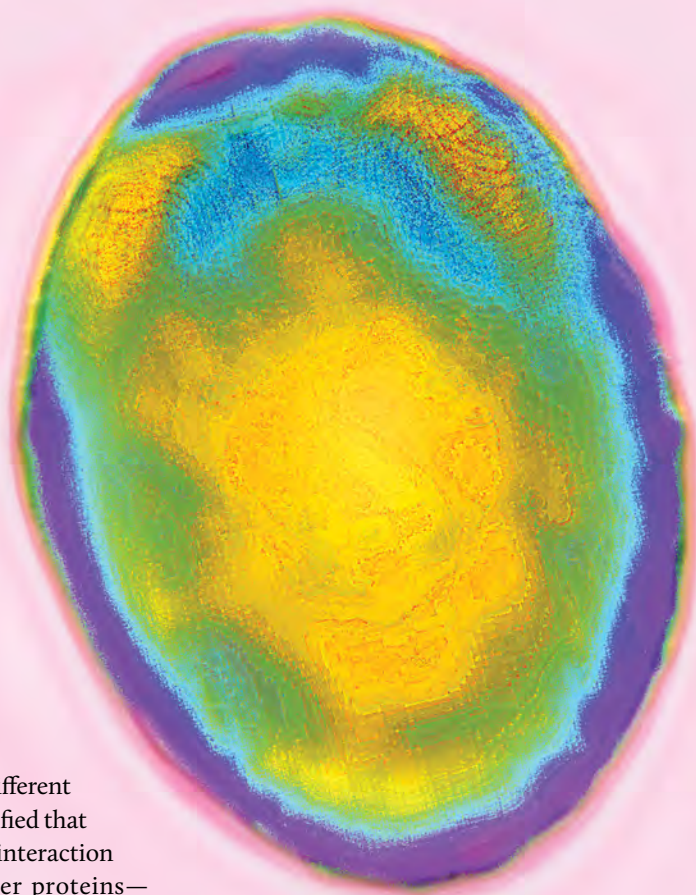
fungal-infected patients,” said Lam, who recommended a two-pronged approach—antifungal drugs to kill the pathogen, coupled with drug compounds that target Dok3 to enhance immune-mediated clearance of infection.

In collaboration with scientists from the Institute of Molecular and Cell Biology (IMCB), Lam’s team plans to screen for potential drugs that disrupt Dok3-Card9 binding. If successful, this discovery may lead to a novel immune-based therapy for life-threatening fungal infections. ★

ABOVE

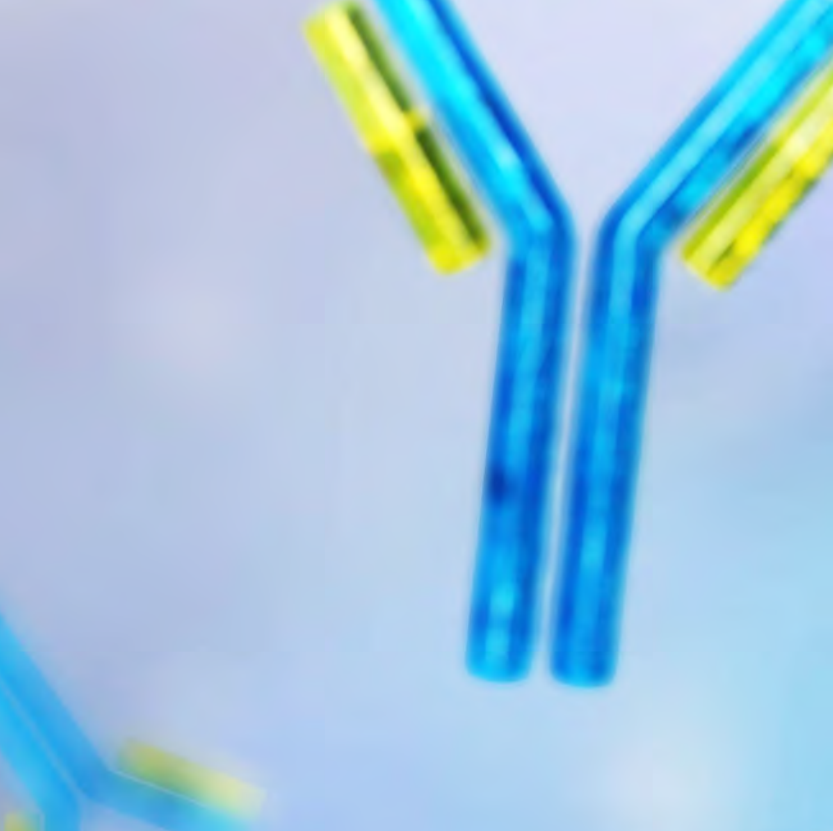
Candida auris is a fungus that presents a serious global health threat. It is resistant to most antifungal drugs and has caused severe illness in hospitalized patients.

1. Loh, J. T., Xu, S., Huo, J. X., Kim, S. S., Wang, Y. *et al.* Dok3-protein phosphatase 1 interaction attenuates Card9 signaling and neutrophil dependent antifungal immunity. *The Journal of Clinical Investigation* **129**, 2717–2729 (2019).



***DEFYING
CONVENTION
IN***

***CANCER
DRUG
DISCOVERY***



In fact, Zeng's research has shown that an intracellular protein, PRL3, can be targeted by an antibody for cancer therapy. She has since founded Intra-ImmuSG, an A*STAR spin-off company, to test the efficacy of PRL3-zumab, a first-in-class humanized monoclonal antibody, in patients suffering from a range of solid tumors. The drug has been approved by the US FDA's Investigational New Drug program for Phase II clinical trials on patients in the US with any solid cancers.

Describing the process of bringing a scientific discovery from bench to bedside as “a duty and a calling,” Zeng is optimistic about the trial results. She tells *A*STAR Research* how the careful use of animal models, self-belief and perseverance have brought her to the brink of pioneering a new class of anticancer therapies.

Q: WHY DID YOU CHOOSE TO FOCUS ON PRL3 AS A TARGET FOR CANCER TREATMENT?

My research on PRL3 began more than two decades ago, when I first identified and characterized the *PRL3* gene in 1998. The gene is also known as *PTP4A3* and encodes a phosphatase—an enzyme that removes a chemical tag (a phosphate group) from other proteins inside cells.

Back then, I had overexpressed the gene in mammalian cells, which caused those cells to undergo very drastic changes in morphology and behavior—some cells became five to ten times larger than usual and developed multiple nuclei (normal cells only have one nucleus containing the cell's genome). The membranes of those cells also became very ‘spiky’ with protrusions known as pseudopodia. I called those cells ‘monster cells.’

Later, in 2001, Professor Bert Vogelstein's group from Johns Hopkins University in the US demonstrated a tight association between *PRL3* and cancer—specifically, they observed high expression of *PRL3* in metastatic colon cancers, but not in benign or normal colon tissues. Professor Vogelstein is a renowned cancer researcher and I learned a lot from him.

Since 2001, my research lab and many others worldwide have thoroughly characterized PRL3 as a cancer protein (oncoprotein) that is overexpressed and associated with multiple human cancer types.

Just because a molecular target resides inside a cancer cell does not mean that it cannot be targeted by antibodies, says Qi Zeng, founder of A*STAR spin-off company Intra-ImmuSG.

In 1997, the US Food and Drug Administration (FDA) approved the first antibody drug—rituximab—for the treatment of non-Hodgkin's lymphoma, the most common form of blood cancer in adults. Rituximab didn't just improve patient outcomes with few side effects; it represented a novel approach to developing cancer therapies: find a molecular target displayed on the surface of a cancer cell, then engineer an antibody against it.

Since then, many antibody-based cancer therapies have been developed against external-facing or extracellular molecules on cancer cells. But what about intracellular targets—surely cancer cells are as different from normal cells on the inside as they are on the outside? Qi Zeng, a Research Director at A*STAR's Institute of Molecular and Cell Biology (IMCB), and an Adjunct Professor at the National University of Singapore's Yong Loo Lin School of Medicine, has answered that question with a definitive yes.

Photo credit: uistart77777 / Shutterstock

Q: MOST ANTIBODY-BASED THERAPIES TARGET EXTRACELLULAR PROTEINS. WHY DID YOU CHOOSE TO DEVELOP AN ANTIBODY TARGETING PRL3 WHICH IS FOUND INSIDE CELLS?

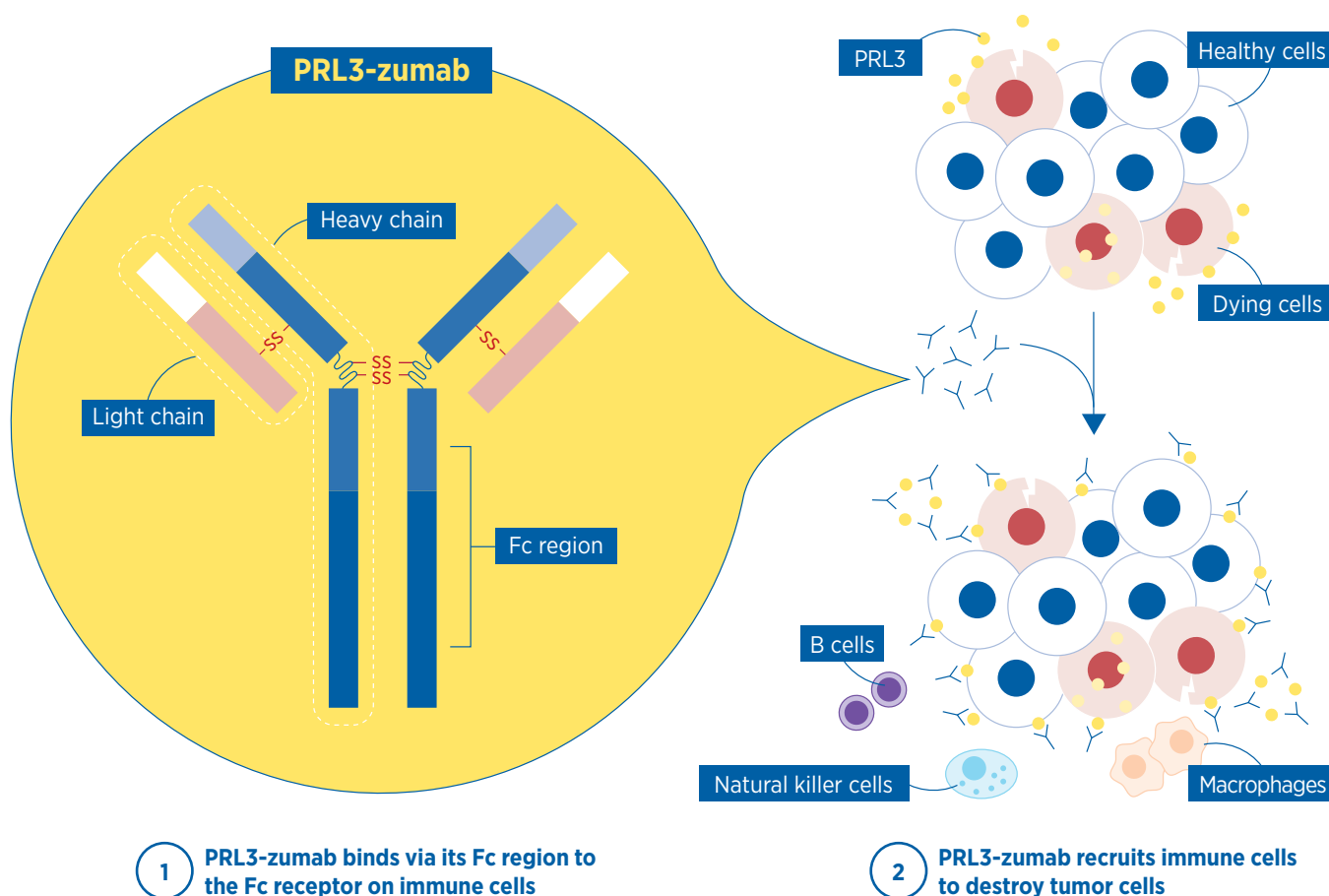
The development of antibody therapies has focused mainly on targeting a few extracellular proteins—those secreted by cells or found on the surface of cells. This is because antibodies are generally believed to be too large to enter cells. What this means is there remains a largely untapped pool of intracellular therapeutic targets, such as phosphatases, kinases and transcription factors.

Since 2008, my lab has presented evidence suggesting that intracellular proteins highly expressed in cancer cells can be targeted by monoclonal antibody-based or vaccination immunotherapies. Although our results repeatedly proved the concept, people were still skeptical. But that did not deter me from further testing my approach.

After developing an antibody called PRL3-zumab, we demonstrated its mechanism of action against an intracellular target. When cancer cells are stressed or dying, PRL3 gets turned ‘inside-out’—it becomes exposed on the surface of cancer cells, or released into the tumor microenvironment, making it available for antibody binding.

We tested PRL3-zumab in multiple mouse models of cancer and showed that PRL3-zumab specifically bound to PRL3 to trigger an immune response against tumors. This, in turn, results in a ‘kill-and-leak’ domino effect that causes tumors to shrink by as much as 90 percent. These results have been published in *Nature Communications*.

We have used more than seven different mouse models for more than ten years to test this unconventional cancer immunotherapy, and the anticancer effects of PRL3-zumab have been consistent against tumors that express PRL3. Here, I want to emphasize the importance of using the right mouse models for the testing of anticancer compounds—an animal model, if used correctly, gives very clinical-like insights on the efficacy of treatment.



Q: WHAT ARE THE ADVANTAGES OF USING PRL3-ZUMAB INSTEAD OF A SMALL MOLECULE DRUG THAT TARGETS PRL3?

The main advantage of PRL3-zumab is that it binds very specifically to PRL3, which is specifically expressed in cancer cells but not in normal cells. Researchers usually use small molecule drugs to target intracellular PRL3, but by virtue of their small size, they travel everywhere in the body and get taken up by many different organs, which can lead to undesirable side effects.

In 2016, the Health Sciences Authority (HSA) of Singapore recognized the novelty of our treatment approach and the potential of our preclinical results. This paved the way for us to conduct first-in-man trials in Singapore using PRL3-zumab for targeted antibody therapy of cancer. I am very grateful to HSA and many local clinicians who continue to support this home-grown project.

Working with a team of oncologists in the department of hematology-oncology at the National University Cancer Institute, Singapore, we completed a Phase I clinical trial in 2018 to evaluate the safety of PRL3-zumab in 23 cancer patients. We concluded that the drug is very safe and showed some early efficacy—there is no dose-limiting toxicity.

Q: WHAT WERE SOME OF THE CHALLENGES YOU FACED IN STARTING INTRA-IMMUSG?

The foremost difficulty was getting people to trust and have confidence in PRL3-zumab, to believe in the therapeutic potential of an antibody that targets an intracellular protein. But I think the years of research and the promising findings surrounding the use of PRL3-zumab in various cancer models speak for themselves.

Another major challenge was producing fresh PRL3-zumab for clinical trials. The production of the drug is very expensive, yet we provide the drug for free and cover most of the medical costs to patients enrolled in clinical trials.

In 2015, A*ccelerate Technologies Pte Ltd, the commercialization arm of A*STAR, encouraged me to spin off Intra-ImmuSG to further develop

PRL3-zumab for clinical use. When that happened, I was very lucky to have received the backing of an angel investor to finance my R&D and clinical trials. A*STAR still supports Intra-ImmuSG in terms of maintaining the patent portfolio family for PRL3-zumab, with worldwide coverage.

Currently, we face challenges in recruiting patients for clinical trials. Patients eligible for PRL3-zumab treatment must have run out of all standard-of-care treatments. By then, the patients' immune system is very weak and their response to treatment may not be as ideal as we hope for. Nevertheless, I am very excited to see that the drug has been found to be safe and has shown early signs of efficacy.

Q: GOING FORWARD, WHAT ARE YOUR PLANS FOR INTRA-IMMUSG?

We have an ongoing HSA-approved Phase II clinical trial at the National Cancer Center Singapore where we are testing the efficacy of PRL3-zumab in late-stage cancer patients with solid tumors. So far, we have enrolled one liver cancer patient, and our treatment has stabilized the patient's acute condition, so we're optimistic. We are also carrying out a Phase Ib extension clinical trial with the National University Hospital, Singapore, to further validate the safety of PRL3-zumab in patients suffering from leukemia.

Having said that, there is a sense of urgency to recruit more patients because our patent on PRL3-zumab will eventually expire. Hence, I am very delighted that we have just received approval under the US FDA's Investigational New Drug program to carry out Phase II clinical trials in the US on patients with any type of solid tumors.

We are also applying to the National Medical Products Administration, China, and the drug administrations of other countries to run clinical trials. This way, we may be able to speed up the patient recruitment process.

Finally, in addition to PRL3-zumab, we have developed a pipeline of drug/vaccine candidates for further testing and validation. We are awaiting a new era of cancer immunotherapy in the near future. ★

COMPUTATIONAL FLUID DYNAMICS

Making waves in marine engineering

Computational fluid dynamics models and 3D printing are changing the way artificial ocean basins are designed.

Being able to predict how boats respond to a variety of ocean conditions determines if multimillion-dollar vessels will sink or swim. To reliably mimic specific flow conditions in the ocean, researchers typically rely on artificial ocean basins—facilities equipped with wave and current generation systems.

“Artificial ocean basins use multiple-channel inlets to send smooth, fast flows of water into the test area, where researchers can test how boats and marine structures will respond,” said My Ha Dao, a Group Leader at the Institute of High Performance

Computing (IHPC), A*STAR. “However, as slower, wall-hugging layers of water emerge from the inlets, they combine into slow layers of reduced flow that can propagate throughout the test area and affect experiments adversely.”

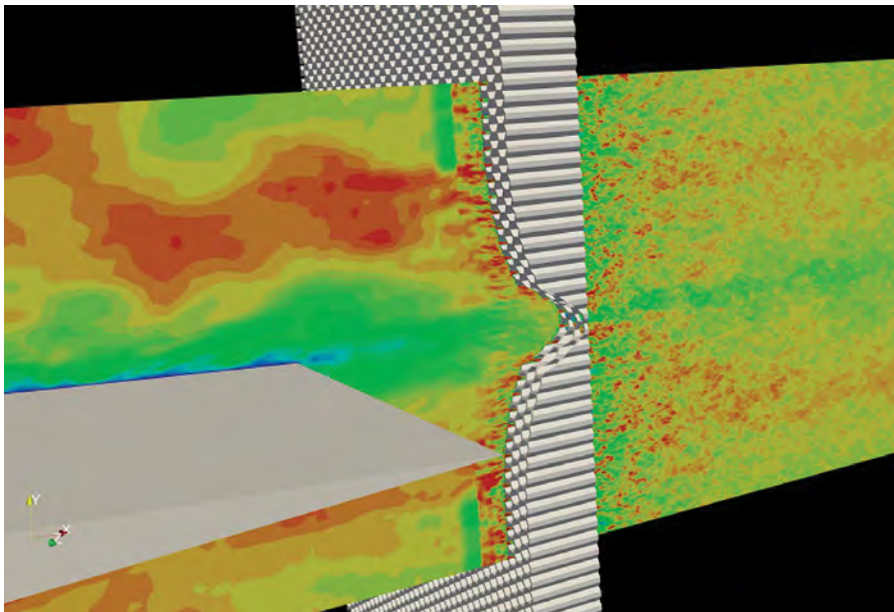
To maintain the velocity of water entering the artificial ocean basin, Dao’s team proposed using a ‘barrier’ resembling a honeycomb. The barrier was designed using computational fluid dynamics for modeling and predicting how turbulent flows of air or water will move around obstacles or through channels.

After optimizing for parameters such as the radii of arcs and the dimensions controlling honeycomb shape, Dao and his team eventually settled on a honeycomb with 4 mm-diameter holes in it. They also demonstrated, via simulation, that the structure would reduce deficit and fluctuations in the velocity distribution of water downstream of the inlet.

Seeking to validate their model, the researchers 3D-printed their prototype honeycomb and tested it in the lab. “We were able to verify the results of our computational fluid dynamics models; the 3D-printed honeycomb mitigated the velocity deficit while improving the uniformity of velocity of water flowing through our system,” Dao said, adding that the velocity variation decreased from 7-8 percent without the honeycomb to just 1-2 percent with the honeycomb in place.

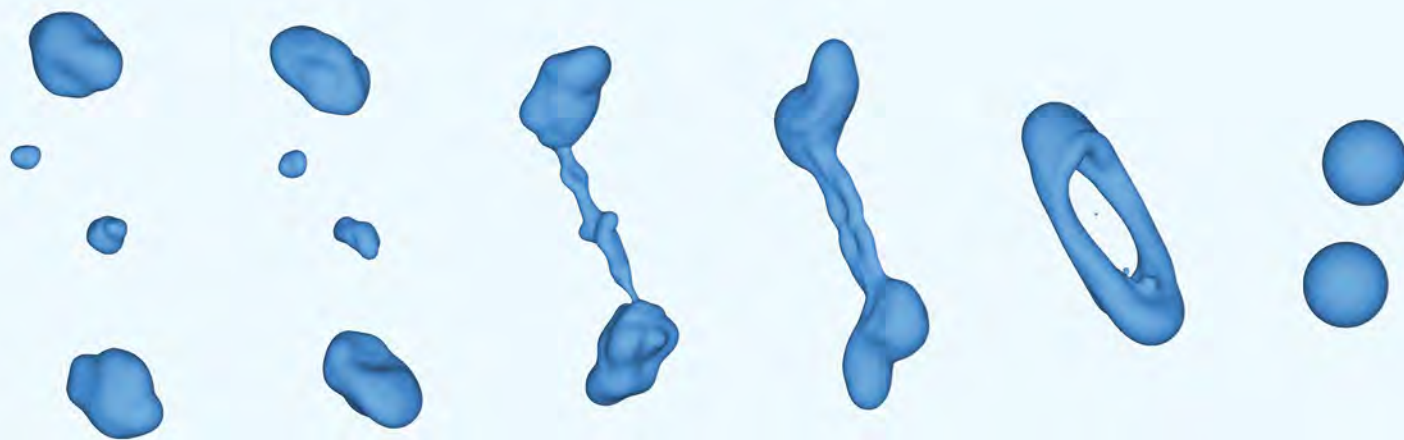
The methodology developed by Dao is currently being used to design honeycomb shapes for the inlets of the deep-water basin at the Technology Centre for Offshore and Marine Singapore (TCOMS), a joint R&D center by A*STAR and the National University of Singapore.

Going forward, Dao intends to use his technique to solve more difficult problems that have a flow element to them. “In principle, our method can be used to cover more complex geometries, such as corners where horizontal and vertical walls meet. In addition, the shaped honeycomb method can be used for more than simply smoothing out flow profiles—it could also be used to produce flow profiles of any desired shape to further expand the capabilities of artificial ocean basins.” ★



Computational fluid dynamics simulation of a proposed honeycomb barrier for use in artificial ocean basins.

1. Zheng, Y., Le, Q. T., Dao, M. H., Magee, A. R. Optimization of Honeycomb Shape for Mitigation of Flow Deficit Behind a Separation Wall. *Applied Ocean Research* 91, 101869 (2019).



COMPUTATIONAL FLUID DYNAMICS

Flow simulations made simple and stable

Factoring in the conservation of mass makes simulations of two-phase flows more stable and accurate.

Gargling mouthwash and building a hydroelectric dam might seem like completely unrelated activities, yet they have one thing in common—both involve the flow of gas and liquid, in what is known as a two-phase flow system. By understanding how two-phase flow works in different contexts, researchers can better understand natural phenomena and solve engineering problems. Common examples of two-phase flow systems include bubbles, fountains and waves on the sea.

Although 3D simulations are useful for investigating two-phase flows, the accuracy of these simulations may sometimes miss the mark. Pao-Hsiung Chiu, a Scientist at A*STAR's Institute of High-Performance Computing (IHPC), has therefore proposed adapting a mathematical strategy used for

modeling crystal growth to simulate two-phase flow systems.

Known as the Allen-Cahn equation-based phase-field method, this approach addresses concerns over the conservation of mass, a parameter that, if left to fluctuate, can introduce instability into two-phase flow models. "When this method is applied, mass conservation should be strictly satisfied due to the physical nature of the incompressible two-phase flows," said Chiu, adding that the method is also easier to implement numerically.

Apart from improving the stability and accuracy of simulations, Chiu also highlighted how his proposed technique supports the simulation of complex processes such as jet pinching-off, bubble merging or bursting, droplet splashing

or collision, and even dam-breaking. Bubble dynamics, for one, are important in spaceflight applications. In space, rapid changes in pressure can result in cavitation, where the high energy generated by rapidly collapsing bubbles in liquids causes wear and tear in machinery, including rocket engines.

"Many problems of practical importance and scientific significance involve the tracking of the interface between different phases, where surface tension needs to be taken into account," noted Chiu.

Beyond refining the simulation of two-phase flows, Chiu is also interested in accurately modeling capillary forces in fluid systems. Capillary force can be seen in action when paint is taken up with a paintbrush, for instance.

"When investigating microscale problems, capillary force is dominant," said Chiu. "My future work will focus on developing a surface force model which can better represent capillary force." ★

BOTTOM

Computational models that simulate two-phase flows can help to explain natural phenomena like bubble merging and bursting.

1. Chiu, P. A coupled phase field framework for solving incompressible two-phase flows. *Journal of Computational Physics* **392**, 115-140 (2019).

PHOTONICS

Taming ultraviolet light with silicon

A*STAR scientists are paving the way for miniaturized UV spectral filters made from hybrid silicon and aluminum nanostructures.

WHY THIS MATTERS

- Non-metals that exhibit plasmon resonances under ultraviolet light are rare.

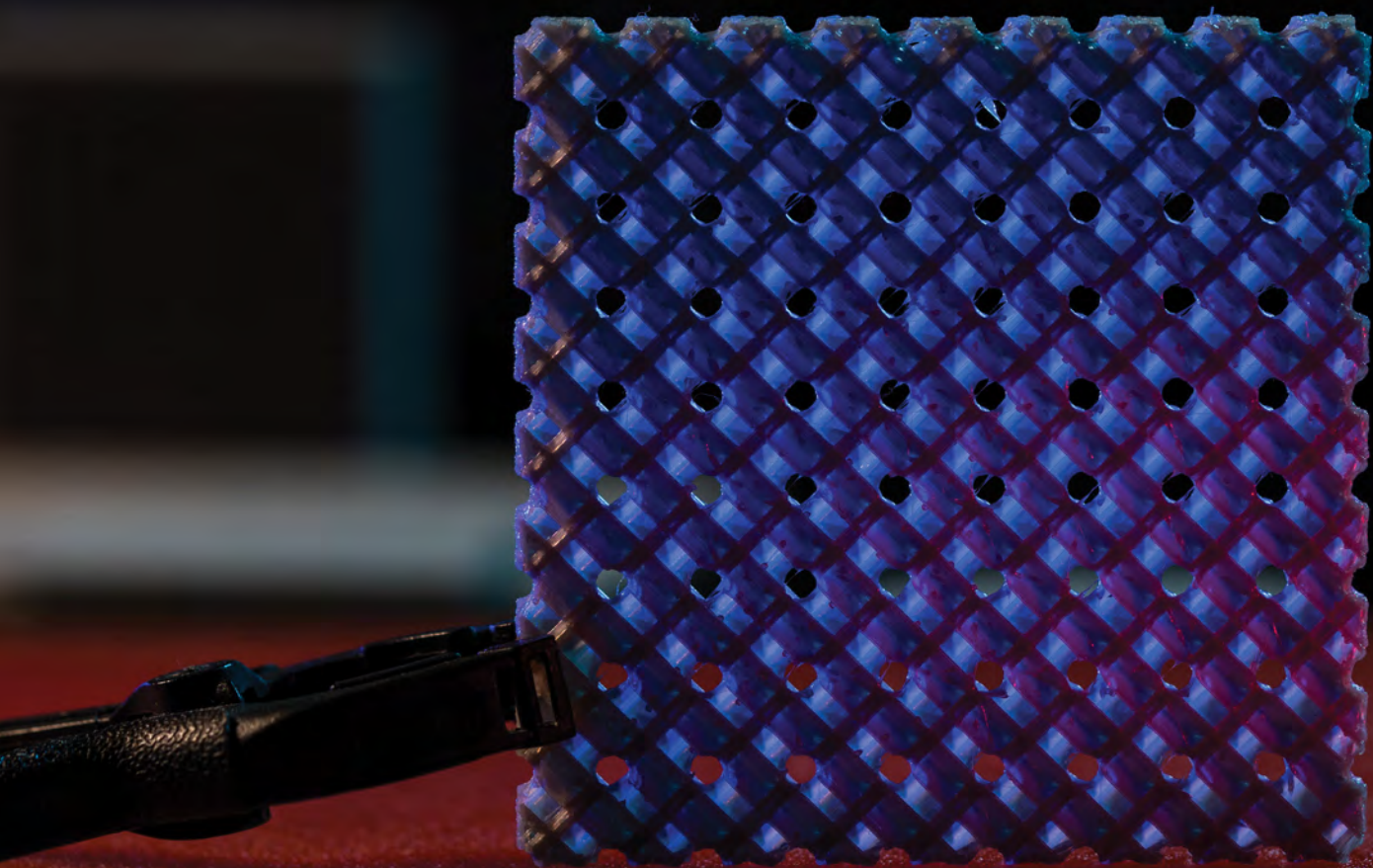


Photo credit: science photo / Shutterstock

“We are made of star stuff,” declared astronomer Carl Sagan in the science-themed television series *Cosmos: A Personal Voyage*. He wasn’t just waxing lyrical—scientists have used a technique called spectroscopy to reveal that elements such as carbon, hydrogen, nitrogen and oxygen, which are all essential to life, were indeed created by earlier generations of stars.

Spectroscopy typically involves shining a beam of electromagnetic radiation on an object and observing how it responds to the radiation. The spectral response reveals information about the object’s structure and properties. In certain materials, typically metals with free electrons, exposure to radiation can cause these collective charges to oscillate in what is known as plasmons, which affect the optical properties of the material. Non-metals that exhibit plasmon resonances under ultraviolet (UV) radiation, however, are rare.

A team of nanoscientists led by Joel Yang, a Senior Scientist at A*STAR’s Institute of Materials Research and Engineering (IMRE), investigated the possibility of developing miniaturized UV spectral filters based on silicon nanostructures. The team, including collaborators in China and Denmark, first tested silicon nanodisks and nanoholes, both in isolation and in pairs.

“Our experiments showed that silicon, despite being a non-metal, exhibits plasmonic resonances like a metal, specifically in the UV spectrum,” explained Zhaogang Dong, the study’s lead author, adding that this has implications for UV spectral filters and paves the way for plasmon-enhanced silicon photodetectors.

The researchers also explored the potential improvement in plasmonic resonances if silicon and aluminum were combined. “Both silicon and aluminum are complementary metal-oxide-semiconductor-compatible materials. In addition, aluminum is a known ‘good’ plasmonic metal in the UV spectrum, making it a useful benchmark for

comparing silicon’s plasmonic properties to industry standards,” Dong said.

To test whether silicon and aluminum function synergistically, the researchers ran computer-aided simulations of silicon-aluminum nanoantennae. They then fabricated the nanoantennae to experimentally record the extent of plasmonic resonance under UV light exposure, with the aim of validating the results from their simulations.

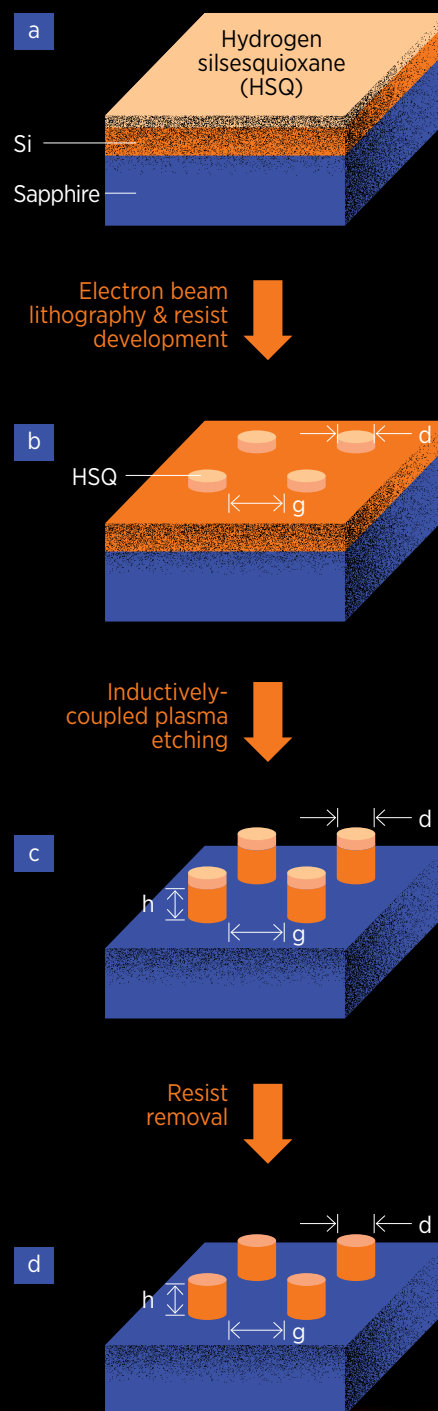
Their results indicate that, on its own, silicon exhibits plasmonic resonance comparable to that of aluminum. Furthermore, the researchers noted that hybrid silicon-aluminum nanostructures could provide improved resonances for UV-related applications such as examining the geometric properties of molecules, or splitting water with UV light to generate hydrogen fuel.

“Importantly, the hybrid silicon-aluminum nanostructures could be potentially used for creating miniaturized spectrometers in the UV region,” suggested Dong. “We would like to explore nanostructured silicon for UV opto-electronic devices that rely on this localized plasmon resonance characteristic,” he concluded. ★

IMPACT

The ability to manipulate ultraviolet light using hybrid silicon-aluminum nanoantennae could pave the way for hypersensitive photodetectors or efficient hydrogen fuel cell catalysts.

1. Dong, Z., Wang, T., Chi, X., Ho, J., Tserkezis, C. *et al.* Ultraviolet Interband Plasmonics With Si Nanostructures. *Nano Letters* **19**, 8040-8048 (2019).



ABOVE

Fabrication process of Si nanostructures on top of the sapphire substrate. (a) Hydrogen silsesquioxane (HSQ) resist is spin coated onto the 130-nm-thick Si film. (b) Electron beam exposure to fabricate the HSQ resist mask. (c) Inductively-coupled plasma (ICP) etching of Si etching by using Cl_2 gas. (d) HSQ resist removal by hydrofluoric (HF) acid (5%) for 30 seconds.

CYBERSECURITY

Keeping eavesdroppers in the dark

A*STAR researchers have created a communication protocol to secure information exchange between a base station and Internet of things nodes.



As the Internet of Things (IoT) gains attraction, wireless devices will face the dual challenge of obtaining power while keeping wireless communications private.

“Most of these devices will be small and thus have limited energy storage; harvesting power wirelessly will be key to avoiding the need to frequently replace batteries,” explained Roohollah Rezaei, a Graduate Student at A*STAR’s Institute for Infocomm Research (I²R). “In addition, since these devices will transmit information wirelessly, their over-the-air communications are inherently insecure and can be picked up by eavesdroppers.”

Although conventional cryptographic methods can be used to encrypt messages that are transmitted wirelessly, Rezaei was keen to create physical layer security that would rely on the communication protocol itself to prevent eavesdropping. “Unlike the usual cryptographic algorithms, physical layer security would not require additional structures for distributing and managing secret keys,” he said.

Together with collaborators in China and Iran, Rezaei’s team devised a communication protocol that creates a large difference between the communication rate of legitimate IoT nodes and eavesdroppers.

In effect, their protocol raises the rate at which information can be transmitted securely from IoT nodes to a base station where information processing occurs. The network therefore achieves a higher secrecy rate.

To validate the security of their protocol, the researchers constructed a model network in which several IoT nodes, including possible eavesdropper nodes, transmit information wirelessly to a base station. Each node harvests power from the base station before its transmission time, then uses that power to transmit its information during an allocated time slot.

“Using our model, we showed that the base station running our protocol assumes that none of the IoT nodes can be trusted—at each time slot, one of the nodes transmits information to the base station while the remaining nodes are considered as potential eavesdroppers,” Rezaei said. During this stage, artificial noise is generated to blind non-transmitting nodes, so even if one of them has been compromised by a hacker (i.e., it becomes an eavesdropper), the information being transmitted remains secure.

“Having worked out the right signal structure, the second stage consists of

allocating time slots so that the total communication throughput between the base station and the nodes is maximized,” he added.

The researchers demonstrated that their two-stage process outperformed common algorithms in securing wireless communications. Moving forward, Rezaei’s team plans to explore whether their technique allows the detection of other types of eavesdroppers—especially passive eavesdroppers which are typically more difficult for base stations to identify.

“We are also considering more sophisticated bandwidth schemes that allow more nodes to transmit their information at the same time, to see if we can overcome signal interference and maintain the same level of secrecy,” he concluded. ★

ABOVE

Data transmitted wirelessly by Internet of Things (IoT) devices is inherently insecure and can be picked up by eavesdroppers.

1. Rezaei, R., Sun, S., Kang, X., Guan, Y. L., Pakravan, M. R. Secrecy Throughput Maximization for Full-Duplex Wireless Powered Communication Networks. *IEEE 2019 International Conference on Communications*.

MACHINE LEARNING

Building better superalloys with AI

Machine learning could pave the way for the creation of novel alloys for a range of practical applications.

In Marvel's *Iron Man* movie series, protagonist Tony Stark relies heavily on the artificial intelligence JARVIS for his superhero needs. Not the least of JARVIS' abilities is designing and constructing Iron Man's impressive suit of armor. Accomplishing such a task would require a deep knowledge of the physical properties of metals and metallic alloys, an incredible feat given the vast number of permutations of alloy compositions.

Taking us one step closer to a real-life JARVIS, researchers at A*STAR's Institute of High Performance Computing (IHPC), together with scientists in the US and Russia, have developed a machine learning model for determining the structure-

property relationship in multi-principal element alloys (MPEAs).

"The emergence of high-entropy alloys and, more generally, MPEAs, is a paradigm shift in conventional alloy design," said Mehdi Jafary-Zadeh, a Scientist at IHPC. However, he noted that it is often difficult to assess local lattice distortion—the displacement of atoms away from their ideal positions within an organized structure, or lattice—in MPEAs. Local lattice distortion affects the physical and mechanical properties of the resultant alloys.

Jafary-Zadeh's group thus invented a machine learning method which they call moment tensor potential (MTP). Instead of training their model using data on the

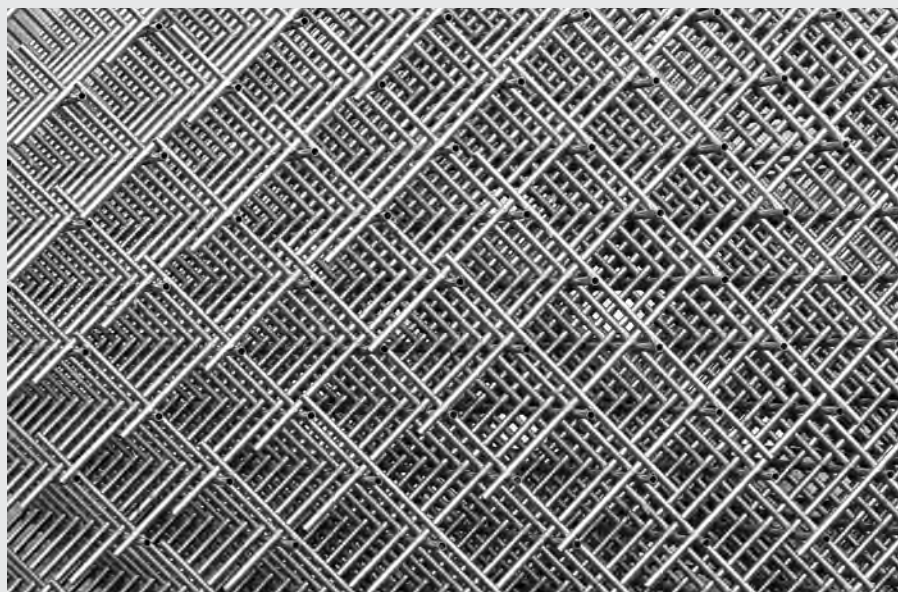
lattice or bulk properties of alloys from experiments or theoretical calculations, the researchers relied on energy, force and stress data obtained from quantum mechanical calculations.

They trained their model on a dataset of 414 different atomic configurations, opting for a 'learning-on-the-fly' scheme which reduces the number of quantum mechanical calculations needed to obtain a readout on local lattice distortions. This allowed them to increase the efficiency of the model without sacrificing accuracy.

"Using our MTP model, we calculated the elastic moduli of single-crystal and polycrystalline MPEAs comprising cobalt, iron and nickel," Jafary-Zadeh said. "Our results were in excellent agreement with theoretical and experimental data, indicating that our method allows us to reliably and effectively explore the dynamic processes such as solidification, plastic deformation and failure mechanisms in MPEAs."

Going forward, Jafary-Zadeh thinks that the integration of computational techniques with state-of-the-art experiments will be extremely beneficial for the discovery and development of novel alloys with diverse applications.

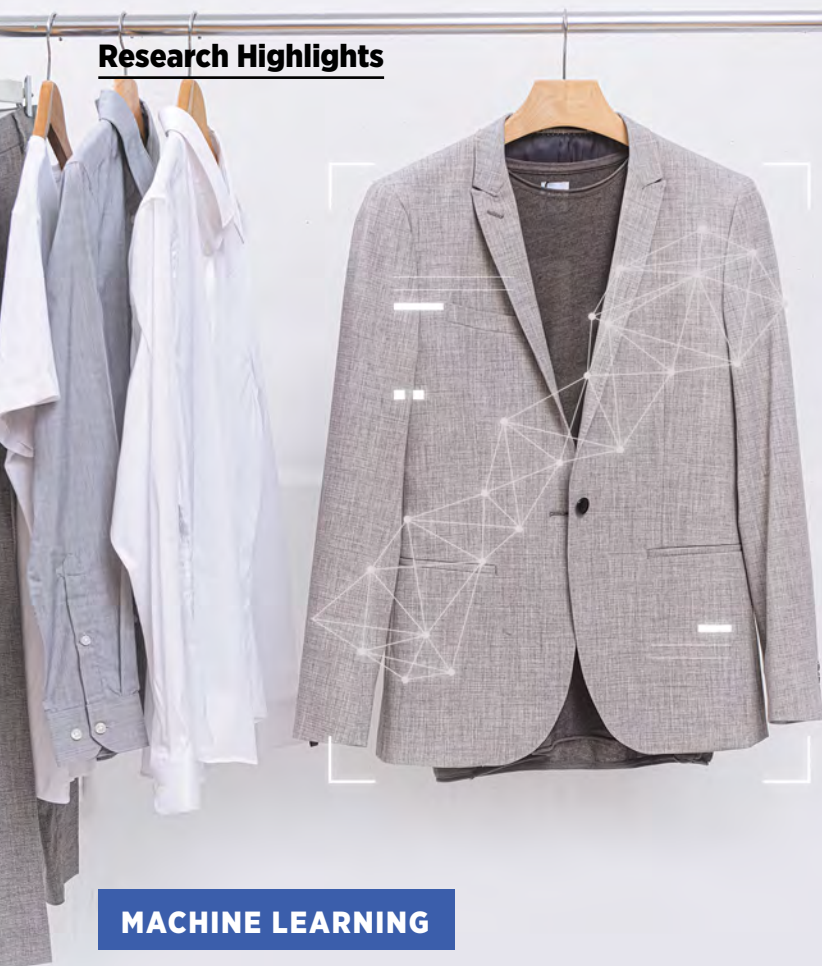
"We would also like to further extend our machine learning model for interatomic potential by including relevant alloying elements. This will enable the design of a new generation of high-entropy superalloys (alloys for high temperature applications), or those with ultra-high magnetic saturation and electrical resistivity," he said. ★



LEFT

Depending on the types and proportions of metals used, the resultant properties of alloys can differ greatly.

1. Jafary-Zadeh, M., Khoo, K. H., Laskowski, R., Branicio, P. S., Shapeev, A. V. Applying a machine learning interatomic potential to unravel the effects of local lattice distortion on the elastic properties of multi-principal element alloys. *Journal of Alloys and Compounds* **803**, 1054-1062 (2019).



MACHINE LEARNING

Neural networks in vogue

Machine learning algorithms can now generate realistic images of clothing and classify them accurately.

Imagine chancing upon a nice blouse while shopping online and wondering what it would look like if it were black instead of blue, or with different buttons on it. In the future, one need not guess how variants of the blouse might look like—a sophisticated computer program developed by Kenan Emir Ak, a Scientist at A*STAR's Institute for Infocomm Research (I²R), could generate images of alternative forms of the blouse in a matter of seconds.

“Computer-automated image generation has taken a massive leap forward with the development of generative adversarial networks (GANs), allowing us to envision previously impossible tasks, such as automated generation of a fashion

image with an arbitrary set of fashion attributes,” Ak explained.

A GAN consists of two neural networks—a generator and a discriminator—competing against each other. While the generator tries to produce realistic samples, the discriminator attempts to distinguish the generated (or faked) samples from real ones. As both networks are trained together, the generator produces increasingly realistic samples over time.

Using large databases of fashion images collected from shopping websites, Ak had a rich source of images to start with. However, his team encountered one important technical hurdle—in addition to looking realistic, the auto-generated images had to

accurately reflect specific attributes, such as long sleeves, or a different color. As more and more attributes are specified, conventional GANs struggle to simultaneously manipulate image attributes while determining whether an image is real or fake.

“As such, we added a neural network to detect the regions of interest for a given attribute, such as a person's arms in ‘sleeveless’ images,” Ak said. “Then, we assigned an additional discriminator network to focus strictly on those regions of the image. With both discriminators in place, the generator is forced to create images that are not only realistic overall, but also have realistic attribute manipulations in specific regions.”

The researchers called their model Attribute Manipulation Generative Adversarial Network, or AMGAN for short. Applying AMGAN to two large image datasets—DeepFashion and Shopping100k—the researchers showed that their technique outperformed other GANs by some 14 percent overall in terms of classification accuracy and realistic manipulation of attributes in fashion images.

Beyond generating, manipulating and classifying images, Ak is optimistic that AMGAN can be used to deal with complex 3D objects and be scaled to different domains other than fashion.

“Currently, AMGAN uses concrete attribute values, such as a specific collar design or color patch, as input for image manipulation, which means that achieving a specific ‘look’ might require many separate steps and become very tedious,” he said. “We are exploring the possibility that a simple textual description such as ‘retro style’ or ‘country fashion’ could be used to aggregate multiple manipulations into a simple, one-step task.” ★

ABOVE

Over time, generative adversarial networks can be trained to produce increasingly realistic images.

1. Ak, K. E., Lim, J. H., Tham, J. Y., and Kassim, Ashraf A. Attribute Manipulation Generative Adversarial Networks for Fashion Images. *The IEEE International Conference on Computer Vision (ICCV)* (2019), 10541-10550.

MACHINE LEARNING

Helping machines get the plot

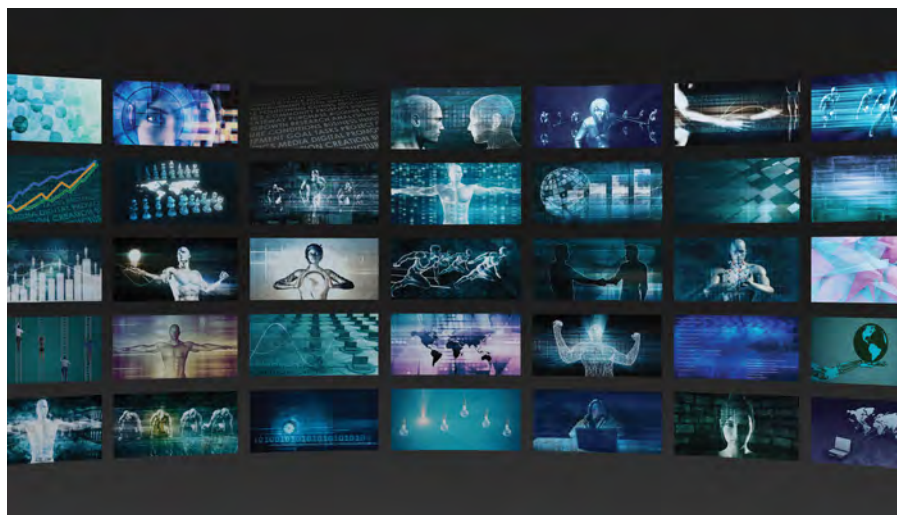
A*STAR scientists have devised a learning framework to enable machines to integrate visual, auditory and text data.

In the 1994 American comedy-drama *Forrest Gump*, the titular character played by Tom Hanks decides to undertake a three-year marathon. For someone who has watched the movie, the motivation for Forrest's impulse is clear: upset that his love interest, Jenny, has left him, he decides to go for a run one morning and just keeps running.

But ask a machine “Why did Forrest Gump embark on his three-year marathon?” and it will probably be stumped. The context and plotlines of the movie can only be inferred by combining visual and text information present in the video—no easy feat for a machine.

“Current machine learning algorithms do not effectively integrate different types, or modalities, of information,” said Chuan Sheng Foo, a Scientist at A*STAR's Institute for Infocomm Research (I²R).

To overcome this problem, Foo's team developed a machine learning framework that processes individual frames in videos as images, combines that data with subtitle texts, then uses that collective information to answer questions based on movie clips. Called the Holistic Multi-modal Memory Network (HMMN) framework, the technique involves the use of a bank of questions—and their answers—in the early stage of training the information-processing algorithms.



The HMMN machine learning framework was able to provide accurate answers to movie trivia.

“Our framework is more effective at leveraging the available information in videos to answer questions.”

“The use of answers at the start of the inference process, before the answer prediction stage, helps identify relevant cues in the multi-modal data,” said Foo. This is akin to a student taking a reading comprehension test and being able to focus on the parts of the passage that matter.

The HMMN framework was evaluated for accuracy in answering questions from two benchmark video datasets (MovieQA and TVQA) comprising video clips and subtitles from 140 movies and six popular American TV shows. More than 100,000 questions were used for training, with another 15,000 used for validation and testing the framework.

“HMMN outperformed competing methods on MovieQA datasets and produced more accurate answers upon combination with the state-of-the-art system on TVQA. This indicates that our framework is more effective at leveraging the available information in videos to answer questions,” said Foo.

He added that HMMN could be useful for interactive exploration and querying of complex multi-modal databases. For example, HMMN could help to find related videos about performing maintenance on factory machinery, or respond to queries about broadcast video.

Moving ahead, the team is exploring how contextual information, such as knowledge graphs describing relationships between words and spatial relationships between images, can be incorporated into their model to enhance the reasoning of textual and visual semantics. ★

1. Wang, A., Luu, A.T., Foo, C.S., Zhu, H., Tay, Y. et al. Holistic Multi-modal Memory Network for Movie Question Answering. *IEEE Transactions on Image Processing* (2019).

SUSTAINABILITY

The cost of clean water

A*STAR researchers have found a metric by which to measure the environmental impacts of NEWater and tap water production in Singapore.

WHY THIS MATTERS

- Singapore's water demand is approximately 430 million gallons a day.
- Desalination of seawater and the recycling of used water help buffer against potential water supply shocks, but the environmental cost of these technologies and processes are not well accounted for.

There is a Chinese proverb that goes, "When drinking water, remember the source." In Singapore, where clean water can be obtained at the turn of a faucet, it is easy to take for granted how much resources are needed to ensure our water security.

Additionally, although Singapore has advanced water purification and recycling technologies allowing for the economical production of clean water for day-to-day consumption, the environmental costs associated with the use of such technologies remain poorly understood.

Photo credit: Lipskiy / Shutterstock

“Water recycling in industries can help to reduce overall water consumption. However, as water recycling can be resource-intensive, the environmental impacts of using recycled water versus that of public water supply should be considered to prevent unintended trade-offs,” explained Ms. Cadence Hsien, a Research Engineer at A*STAR’s Singapore Institute of Manufacturing Technology (SIMTech). “To be able to make this comparison for informed decision making, we first need to quantify the environmental impact of water supply in Singapore.”

Hsien and colleagues thus conducted a life cycle assessment of Singapore’s national water sources, including water derived from desalinated seawater, treated stormwater and recycled water, better known as NEWater. As Singapore’s water supply system is complex and comprises numerous components, such as rivers, reservoirs, desalination plants and water distribution networks, the group decided to begin the life cycle assessment by first evaluating the environmental impact of each activity involved in the water supply.

The group used eight indicators selected from the ReCiPe method to measure environmental impact. They reported that the production of NEWater and tap water had similar impacts on particulate matter generation, fossil fuel depletion, photochemical oxidation and human toxicity. This is because NEWater production and desalination involve the use of similar chemicals and materials in the maintenance of the membrane and reverse osmosis processes.

However, NEWater production had a greater direct impact on ozone depletion than tap water production, owing to the emission of nitrous oxide from the water reclamation process. The researchers thus

highlighted that better removal of biological nutrients in the water reclamation plants could reduce the environmental impact of NEWater production.

They also noted that some treated water from water reclamation is not further purified into NEWater and ends up being discharged into the sea. This represents a form of waste and inefficiency, which could be weeded out from the water supply chain.

“Our study quantifies the environmental impact of producing tap water and NEWater in Singapore using life cycle assessment. These results can be used to support decisions on water use and water recycling. Additionally, the insights provided by the life cycle assessment points to potential areas where the Singapore water system can be improved in terms of environmental impact,” Hsien concluded. ★

IMPACT

With a detailed assessment of the environmental costs associated with meeting Singapore’s water needs, policymakers can then take targeted measures to address inefficiencies in water treatment processes.

BACKGROUND

Singapore obtains its water from ‘four national taps’: imported water, water from local catchment, desalination and NEWater.

1. Hsien, C., Low, J. S. C., Chan, S., Tan, W. H. Life cycle assessment of water supply in Singapore—A water-scarce urban city with multiple water sources. *Resources, Conservation and Recycling* **151**, 104476 (2019).

DRUG MANUFACTURING

Getting drug molecules to fall in line

By controlling the crystallization of pharmaceutical compounds using template surfaces, high quality drugs may be manufactured more cheaply and easily.

Snowflakes and hailstones are both made of water, yet they differ greatly in terms of their shape, size and other physical properties. The differences arise due to the way water freezes in the atmosphere through a process called crystal formation.

Interestingly enough, similar processes take place in drug manufacturing. By controlling how active pharmaceutical ingredients (APIs) crystallize, drug companies can influence the bioavailability and efficacy of the drug in the patient's body. However, precise manipulation of API crystallization remains a challenge.

A technique known as template-induced nucleation could be the answer to this crystallization conundrum, said Sendhil Poornachary, a Scientist at the Institute of Chemical and Engineering Sciences (ICES). Poornachary is the corresponding author on a study with Reginald Tan, a Principal Scientist at ICES; they collaborated with scientists at Imperial College London and the Indian Institute of Technology Patna.

Fundamentally, template-induced nucleation involves the use of specialized

This knowledge will ultimately prove useful in intensifying the process of drug formulation and finding applications for new drugs.

surfaces that influence the way individual API molecules aggregate en route to crystal formation. As crystals begin to form—or nucleate—on a template surface, the surface can favor physical and chemical interactions that control crystal structure, resulting in different solid forms known as polymorphs.

Crystal polymorphs of a molecule typically exhibit different physical properties, despite being chemically identical, due to their different crystal lattice arrangements. Different polymorphs of a drug crystal can dissolve at different rates or concentrations, affecting how the

human body responds to the drug. They can also have different physical properties—such as density, shape or melting point—which must be considered during the pill manufacturing process.

“In our review, we describe how epitaxy, topography and surface chemistry affect template-induced crystal nucleation, with a specific focus on API crystallization,” Poornachary added.

An epitaxial relationship exists when the molecular arrangement of the template surface resembles the structure of a developing crystal, promoting further crystallization. Meanwhile, topography, which includes nanoscale pores and engineered patterns, can selectively induce nucleation of a specific polymorph if certain geometric features are matching. Some strong chemical interactions, such as hydrogen bonds or van der Waals forces, can even pull drug molecules into exact arrangements, again favoring particular polymorphs, he explained.

“We extensively describe how functionalized glass can control the crystallization of carbamazepine, a model pharmaceutical drug. We also explored the solute concentrations and temperature ranges where the template effect is most prominent and can be practically useful,” he said.

The team further used computational molecular modeling to provide insights into the mechanisms underlying template-induced crystallization. “This knowledge will ultimately prove useful in intensifying the process of drug formulation and finding applications for new drugs,” Poornachary said. ★

ABOVE

The crystallization of active pharmaceutical compounds affects the bioavailability and efficacy of drugs.

-
1. Parambil, J. V., Poornachary, S. K., Heng, J. Y. Y., Tan, R. B. H. Template-induced nucleation for controlling crystal polymorphism: from molecular mechanisms to applications in pharmaceutical processing. *CrystEngComm* **21**, 4122-4135 (2019).

MECHANICAL ENGINEERING

Stronger by design

Customizing the size, shape and orientation of 3D-printed lattices can make structures stronger while requiring less material.

If you can imagine it, you can print it—this is the promise of additive manufacturing, more commonly known as 3D printing, which allows individuals and organizations to design a wide range of structures and produce them cheaply and reproducibly.

“Lattices are perhaps the ultimate application of 3D additive manufacturing because they are intricate structures that can utilize very little material to fill a volume while still providing structural rigidity,” said Stefanie Feih, a Senior Scientist who leads the Polymer Processing Group at A*STAR’s Singapore Institute of Manufacturing Technology (SIMTech).

She added that lattices also absorb impact energy well, providing insulation

from vibration and noise. Moreover, they can be used to provide effective thermal insulation, or maximize heat transfer, thanks to their high surface-area-to-volume ratio.

“The conventional state-of-the-art approach in lattice design is to simply repeat a unit cell over and over throughout a given volume, to give a uniform array of struts,” Feih explained.

Recognizing an opportunity to expand the possibilities of lattice design by modifying the dimensions of each repeating unit cell, Feih’s team, with collaborators at the National University of Singapore, devised a computational approach to vary the spacing, length and

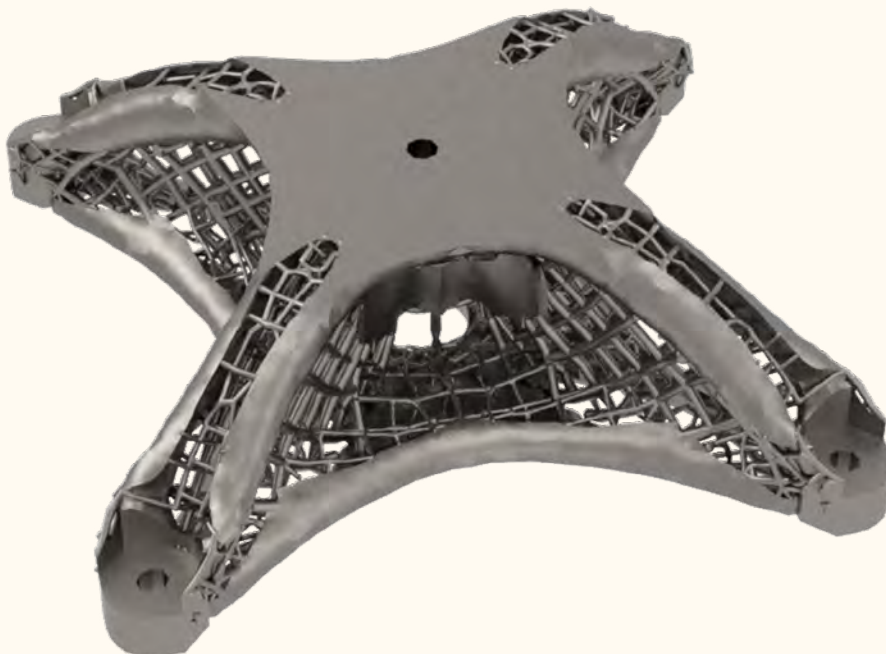
orientation of lattice beams throughout a structure. This allowed the researchers to generate what is known as graded lattice infill arrangements, which can be optimized for strength.

“Our novel design approach integrates data about how force is aligned throughout a structure,” said Stephen Daynes, a Scientist at SIMTech and the lead author of the study. “By creating an interconnected network where the beams are aligned with the paths experiencing the most strain, we were able to improve the structural performance of the lattice using the same amount of material distributed more efficiently.”

Daynes and the team validated their methodology by designing two common engineering components—a floor beam and a spider bracket. “Our method resulted in a floor beam that is 3.5 times stiffer than a commercially optimized square cell lattice infill of the same mass,” said Daynes.

Meanwhile, for the spider bracket, the researchers designed a structure which had less overhang, thereby removing the need for support materials during production. The tradeoff? Only a small reduction in stiffness. The researchers have filed a US patent application for their lattice design approach.

Daynes highlighted that their technique could also be used to minimize buckling or yielding in 3D-printed lattice structures. “We also believe that these lattices can have favorable heat transfer properties since they have a much larger surface area than equivalent solid components. We are exploring how best to take advantage of this property,” he said. ★


LEFT

3D-printed lattices designed with strain distribution in mind display enhanced structural performance.

1. Daynes, S., Feih, S., Lu, W. F., Wei, J. Design concepts for generating optimized lattice structures aligned with strain trajectories. *Computer Methods in Applied Mechanics and Engineering* **354**, 689-705 (2019).

NANOTECHNOLOGY

Getting deep insights into tumors

An imaging technique that distinguishes tumor-associated blood vessels from normal ones could pave the way for better clinical management of tumors.

More than just a traitorous lump of cells, tumors are clever instigators of rebellion in the body, encouraging the growth of a network of blood vessels around them to maintain a supply of essential nutrients and oxygen. By understanding of how blood vessels sprout around tumors—a process known as angiogenesis—scientists can develop drugs to cut off the tumor's lifeline and improve patient outcomes.

To observe the architecture of blood vessels in tumors, researchers led by

Lai Guan Ng, a Principal Investigator at A*STAR's Singapore Immunology Network (SIgN), and Bin Liu, a Professor at the National University of Singapore, devised an imaging tool that not only allows for clear visualization of blood vessels in deep brain tissue, but also helps distinguish abnormal tumor blood vessels from normal ones. The research was carried out by Shaowei Wang, a Researcher from the National University of Singapore.

A major consideration for deep tissue imaging is the wavelength of light used—not all wavelengths penetrate deeply into biological tissue. “Traditional two-photon microscopy usually uses laser light in the near-infrared (NIR)-I region (700-950 nm) as the excitation light source, which limits the imaging depth to around 500 μm ,” Ng explained. “Compared to NIR-I light, NIR-II light (1,000-1,700 nm) can penetrate much deeper to excite a fluorescent probe inside living tissues.”

Getting the right fluorescent probe was the second hurdle the team needed to overcome. Traditional NIR probes, which have poor solubility in water, suffer from aggregation-induced quenching—their fluorescence is largely reduced when they clump together in an aqueous biological environment. Hence, the researchers used a combination of nanotechnology and engineering to create fluorophores that emit a strong fluorescence when clumped together.

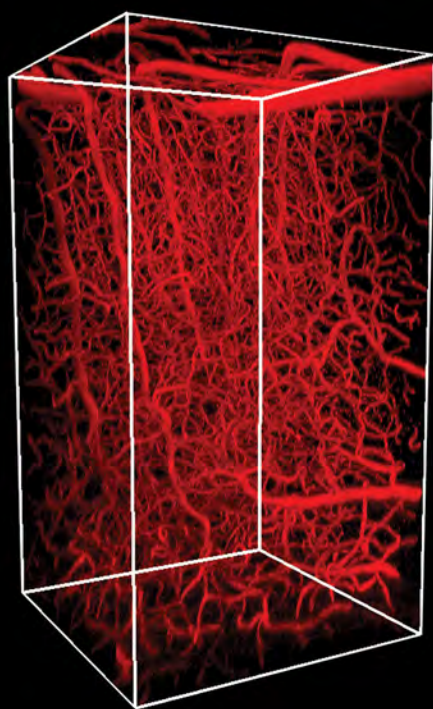
“We fabricated small nanoparticles comprising thousands of fluorophore molecules aggregated together for live tissue imaging. This is known as aggregation-induced emission,” said Wang.

Tested in mice, the researchers showed that their nanoparticles excited by NIR-II light could illuminate blood vessels in the brain with great clarity to a depth of around 1 mm—twice as deep as what has been possible by conventional imaging methods. The nanoparticles also differentiated normal brain vessels from tumor vessels; the intensity of the fluorescence was higher in tumor vessels.

“[The brighter fluorescence in tumors] is attributed to the unique leaky structure of tumor vasculature, which allows the fluorophores to stick to the vessel wall and form bright aggregates,” said Ng.

Compared to fluorophores presently approved by the FDA for clinical imaging, the nanoparticles in this study have higher stability in water, good biocompatibility and a longer circulating time in the blood. The high-resolution imaging afforded by the nanoparticles has the potential to improve the detection of residual tumor tissue after surgical excision of the bulk tumor.

The team is already thinking of ways to improve the performance and function of their fluorescent probe. “The imaging depth can be improved by using light with longer wavelengths. Additionally, antibody or peptides can be attached to the surface of our nanoparticles, giving them specific tumor-targeting capabilities,” said Wang. ★



LEFT

Blood vessels associated with tumors appear more tortuous than normal blood vessels.

1. Wang, S., Liu, J., Goh, C. C., Ng, L. G., Liu, B. NIR-II-Excited Intravital Two-Photon Microscopy Distinguishes Deep Cerebral and Tumor Vasculatures with an Ultrabright NIR-I AIE Luminogen. *Advanced Materials* **31**, e1904447 (2019).

STRUCTURAL BIOLOGY

How bacteria move their DNA around

A*STAR researchers have figured out the detailed structure of a type of protein filament responsible for tethering and moving DNA in bacteria cells.

Better known for producing botox—a compound associated with wrinkle removal in cosmetic clinics—the bacteria *Clostridium botulinum* is also studied by scientists seeking to understand fundamental biological processes such as bacterial cell division. As a bacteria cell divides, it needs to properly segregate DNA into the resultant daughter cells, a process carried out by strands of proteins.

Earlier research has shown that these protein filaments are encoded by the bacteria's DNA (also called a plasmid), and that the proteins differ among bacteria species. In this study, researchers led by Robert Robinson, a Research Director at A*STAR's Institute of Molecular and Cell Biology (IMCB), zoomed in on the filamentous protein ParM in *C. botulinum*, seeking to understand its structure and function.

Collaborating with scientists in Japan, Robinson's group used a technique called cryogenic electron microscopy (cryoEM) to obtain high resolution (4.2 Angstrom) structural data on ParM, which revealed that it generally persists as a 35 μm -long filament, with a diameter of 26 nm.

Importantly, the researchers observed that ParM is a complex of 15 loosely associated, left-handed helical, single

strands. A cross-section of the 15 strands reveals a central strand surrounded by an intermediate layer of six interacting twisted helical strands running antiparallel to the central strand. The remaining eight strands form an outermost layer, running antiparallel to the six intermediate strands.

Robinson remarked that the result was “surprising” because *Escherichia coli*, another common model bacteria, makes use of only a pair of two-stranded ParM filaments to segregate its DNA. Hence, the 15-strand configuration of ParM in *C. botulinum* is likely to allow for greater generation of force and motion.

“Our findings tell us that many filament designs are used in moving plasmids. This is likely selected for during evolution, since two types of plasmid existing in the same cell will require two different segregation systems to faithfully maintain both plasmids,” he explained.

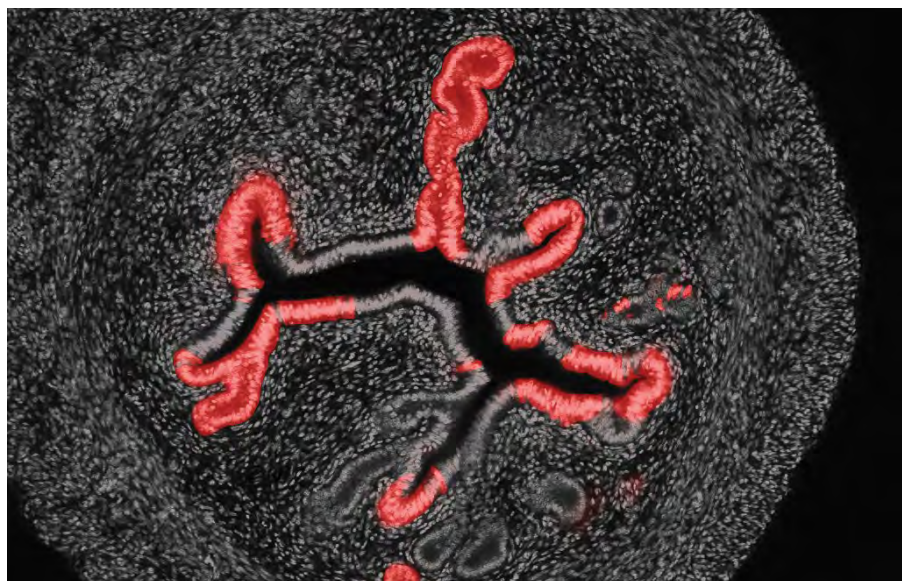
Robinson added that mutations affecting the structure of ParM could also influence plasmid propagation. Learning about the mechanisms controlling plasmid segregation could, in turn, shed light on how bacteria acquire certain traits, such as antibiotic resistance and toxin production. ★



BACKGROUND

In dividing bacterial cells, DNA is segregated into two daughter cells with the help of protein filaments.

1. Koh, F., Narita, A., Lee, L. J., Tanaka, K., Tan, Y. Z. *et al.* The structure of a 15-stranded actin-like filament from *Clostridium botulinum*. *Nature Communications* **10**, 2856 (2019)



ORGAN DEVELOPMENT

Bringing the womb to birth

A*STAR scientists have identified stem cells required for successful embryo implantation during pregnancy.

Despite the availability of fertility drugs and procedures such as *in vitro* fertilization, a successful pregnancy still depends on the proper development and maintenance of the uterus. Should abnormalities occur in the uterine lining, a woman may still experience difficulty conceiving a child.

“The glands and epithelial cells that line the inner surface of the uterus are essential for embryo implantation in the adult female. However, their neonatal source was unknown,” said Nick Barker, a Research Director at A*STAR’s Institute of Medical Biology (IMB). His team, collaborating with colleagues in Singapore and Japan, therefore decided to perform a series of cell-tracking experiments in mice to identify the cells that eventually make up the uterine lining.

The researchers noted that the uterine lining of prepubertal female mice contained

stem cells expressing the protein Lgr5. Using female mouse embryos genetically engineered so that the cells fluoresced whenever Lgr5 is expressed, Barker’s team showed that Lgr5-containing stem cells already exist in female mouse embryos.

The expression of Lgr5 in the reproductive tract of female mice increased dramatically in the first two weeks after birth, before gradually declining to low levels post-puberty. Importantly, the Lgr5-expressing cells eventually generate the epithelial lining of not just the uterus, but also that of the ovaries and upper vagina.

These findings were further confirmed by harvesting Lgr5-expressing uterine cells from two-week old mice and establishing a 3D culture system that mimics the structure and function of the female reproductive tract. Collectively, the results show that Lgr5-expressing cells are indispensable

for the normal development of the female reproductive tract.

“Our *ex vivo* organoid cultures revealed that Lgr5-expressing cells initially function as stem cells for both the uterine epithelia and the uterine glands, but then later function exclusively as gland stem cells as the uterus matures. These glands secrete key hormones later in adulthood that are essential for successful embryo implantation during pregnancy,” Barker explained.

Additionally, the team discovered that uterine cells which do not express Lgr5 secrete molecules called Wnt ligands to regulate the development and organization of Lgr5-expressing cells. The Wnt signaling pathway is known to control cell proliferation and regeneration, so Barker and colleagues are interested in exploring how the dysregulation of Wnt signaling could contribute to cancer formation in the female reproductive tract.

“Understanding the cause of uterine disorders in adults, including cancer, where stem cells have a role, could lead to development of novel therapeutics for those conditions,” Barker concluded. ★

“Understanding the cause of uterine disorders in adults, including cancer, where stem cells have a role, could lead to development of novel therapeutics for those conditions.”

ABOVE

Cells that make up the lining of the uterus play an important role in allowing embryo implantation and subsequent fetal development.

1. Seishima, R., Leung, C., Yada, S., Murad, K. B. A., Tan, L. T. *et al.* Neonatal Wnt-dependent Lgr5 positive stem cells are essential for uterine gland development. *Nature Communications* **10**, 5378 (2019).

MATERIALS SCIENCE

Measuring how wet a surface can get

A modified form of atomic force microscopy can be used to better characterize liquid-repellent surfaces.

Look closely at any seafaring vessel and you might notice algae, plants and small animals like mussels attached to its hull. These hitchhikers cause a problem known as biofouling, compromising a ship's performance (due to the additional weight that lowers the ship's fuel efficiency) and affecting the vessel's structural integrity.

Because the root cause of biofouling is long-term contact with water, tuning the wettability of the ship's hull is key. However, characterizing the wetting properties of a material or surface is technically challenging, said Dan Daniel, a Research Scientist at A*STAR's Institute of Materials Research and Engineering (IMRE).

"The conventional approach of studying wetting properties involves placing a droplet on the surface and looking at the angle it makes with the surface. While this is easy to do, it is also crude and does not yield much information about surface wettability," he explained.

To obtain a more thorough understanding of surface wettability, Daniel and his colleagues used a modified form of atomic force microscopy (AFM). Conventional AFM involves the use of a solid silicon-based AFM tip to 'sweep over' a surface and quantify the atomic-level interactions between the tip and the surface. In this study, the researchers used a

40-wt% glycerin–water droplet of diameter 20–50 μm in place of the solid tip to glean insights into how a surface interacts with liquid at the atomic scale.

Applying their technique, Daniel's team was able to measure the adhesion force and energy required to detach a water droplet from a surface, reporting that they were able to record forces with nanonewton precision. The researchers were also able to capture the rapid detachment of water droplets with millisecond resolution.

"Our technique can be used to objectively assess the effectiveness of various surface coatings and optimize their future design," Daniel suggested. "I plan to also use this technique to quantify the forces required to extract crude oil from rocks under different conditions. The findings from such a study will have important implications for oil extraction and recovery," he said. ★

BACKGROUND

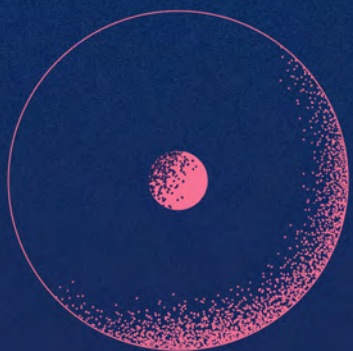
Marine biofouling can be reduced by coating ship hulls with superhydrophobic materials.

1. Daniel, D., Lay C. L., Sng, A., Jun Lee, C. J., Jin Neo, D. C. *et al.* Mapping micrometer-scale wetting properties of superhydrophobic surfaces. *Proceedings of the National Academy of Sciences of the United States of America* **116**, 25008–25012 (2019).



RETHINKING BREAST CANCER RISK





To help clinicians formulate better screening programs for breast cancer, A*STAR's Jingmei Li is developing a risk scoring system to detect the disease in its early stages.

In October each year, pink ribbons become more prominently displayed on clothing and street corners, the symbol of an international movement to raise awareness about breast cancer. The debilitating disease, which impacts some 2.1 million women each year according to the World Health Organization, is not untreatable, but must be diagnosed early to increase the chances of survival.

Predictive models based on genetics can also help clinicians identify women most at risk of developing breast cancer so that appropriate screening methods and intervals can be recommended. However, many current methods of breast cancer risk assessments rely on the reporting of family history and the identification of rare gene variants. This may limit the accuracy of prediction and restrict the overall impact of breast cancer screening programs.

Jingmei Li, a Senior Research Scientist at A*STAR's Genome Institute of Singapore (GIS), wants to expand the toolkit for calculating an individual's breast cancer risk with a risk scoring system that accounts for the cumulative effects of common gene variants. In this interview with *A*STAR Research*, the 2017 Young Scientist Award winner shares how she became interested in breast cancer research and explains the importance of leveraging genetics to peek into the future of women's health.

Q: WHY DID YOU CHOOSE BREAST CANCER AS A RESEARCH TOPIC?

While I was a graduate student at the Karolinska Institute, Sweden, I was drawn to a group of brilliant clinicians and researchers who were really passionate about the work they were doing for breast cancer patients. I felt that they went above and beyond the science and genuinely cared about their patients. The energy was positively contagious!

I found even deeper motivation when I shadowed a breast cancer surgeon in Singapore as he met with women who had been screened for breast cancer. It was only for a day, but I experienced first-hand the anxiety of the patients as they came in for their consultation with the surgeon, not knowing what diagnosis they would be given. I had to fight back my own tears when the lucky ones cried with relief upon hearing that they did not have breast cancer.

Q: WHY DO YOU THINK IT IS IMPORTANT TO STUDY BREAST CANCER IN THE ASIAN CONTEXT?

The bulk of genetic knowledge on breast cancer available today is derived from studies performed on participants of European descent. Less than 14 percent of such studies involved Asians. The frequencies of disease-associated SNPs can vary greatly between populations; simply transplanting the findings from European-based population genetics studies to the Asian context could, therefore, lead to inaccurate risk estimates and limited clinical utility.

Apart from genetic susceptibility to disease, Asian women are substantially different from women of European ancestry in nearly all aspects of life, including lifestyle, reproductive profile, cultural and religious beliefs related to health, socioeconomic status and drug metabolism and response. Major differences are also known to exist within ethnic subgroups.

Among the three main ethnic groups in Singapore, Chinese women are at higher risk of developing breast cancer in comparison to their Malay and Indian counterparts. However, Chinese breast cancer patients were less likely to die from breast cancer compared to Malays and Indians.

Q: WHAT IS THE KEY PROBLEM YOU ARE TRYING TO SOLVE AS A GENETICIST STUDYING BREAST CANCER?

My work focuses on the prevention or early detection of breast cancer. A lot of money and attention has gone towards finding treatments for the disease, but not enough has been said and done about preventing cancer in the first place.

Current measurements for genetic risk rely on family history, not genetics. This means that a woman is considered to be at higher risk only when she has family members who have had breast cancer. However, as family sizes shrink and families become more distant, retrieving an accurate family history is becoming more difficult. A person's genome, on the other hand, can be more informative about the person's medical history and, aptly, medical future as well.

In my projects, I scrutinize DNA to look for genetic differences that determine who is likely to get breast cancer and who is not. My aim is to discover novel susceptibility markers and mechanisms relating to breast cancer development and progression. Knowing what makes the time bomb of breast cancer tick will ultimately be helpful in stratifying the population according to the likelihood of getting the disease, so that resources can be reallocated to diagnosing high-risk individuals.

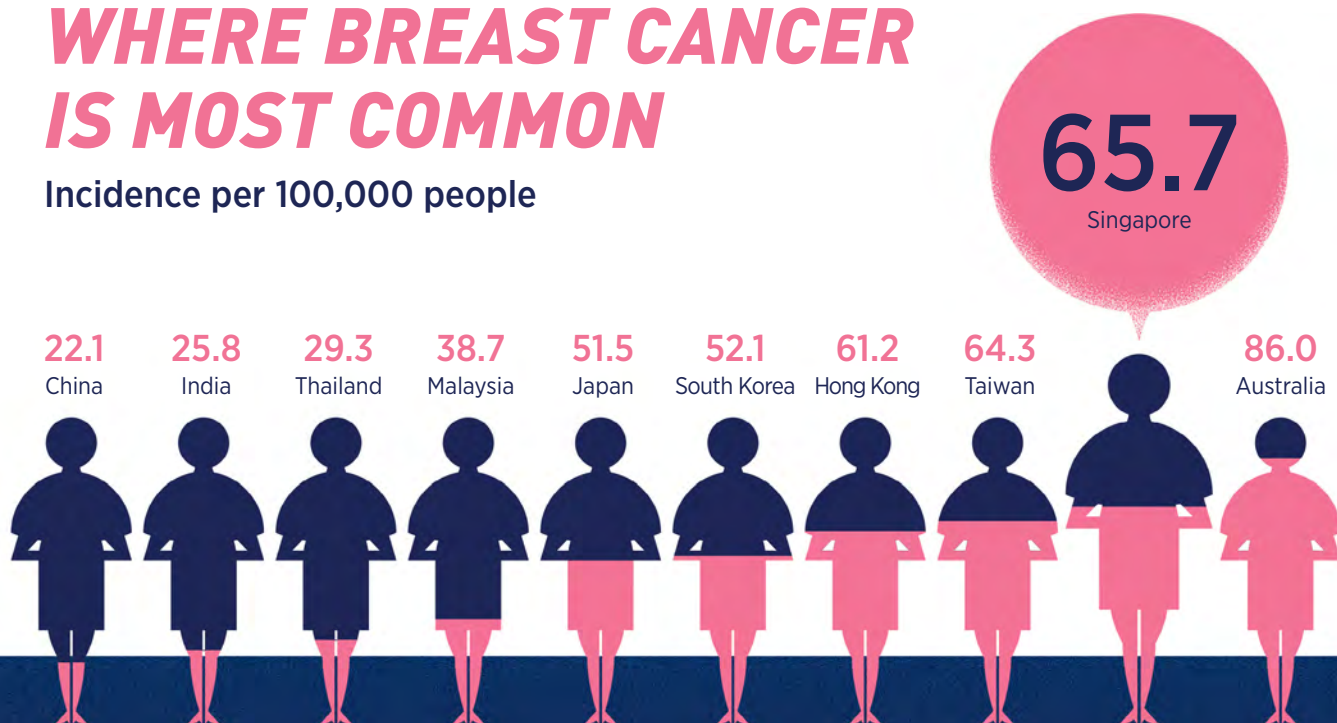
Q: HOW ARE YOU DEVELOPING BETTER GENETIC SCREENS FOR ASSESSING BREAST CANCER RISK?

Over the past decade, I have been deeply involved in identifying common genetic variants, or single nucleotide polymorphisms (SNPs; pronounced as 'snips'), that are linked to breast cancer. Individual SNPs contribute only a marginal effect to breast cancer risk, but collectively, they provide tangible and actionable insight for grouping women according to their predisposition to breast cancer. In total, the breast cancer research community has found 313 of such SNPs to date.

A woman who knows if she has a high genetic risk of developing breast cancer in her lifetime can make lifestyle changes to prevent cancer. More importantly, she can make conscious and informed choices about when and whether to go for mammography, which has been shown to save lives through the early detection of breast cancer. The survival rate for women whose breast tumors were discovered early is almost 100 percent.

WHERE BREAST CANCER IS MOST COMMON

Incidence per 100,000 people



Source: Economist Intelligence Unit

Q: GOING FORWARD, WHAT OTHER RELATED RESEARCH QUESTIONS WILL YOU BE PURSUING?

The heritability of breast cancer (due to shared genetics) is only estimated to be approximately 30 percent. If a disease is only 30 percent genetic, then it can never be 100 percent predicted. Much remains to be explained as genetic events do not completely clarify clinical behavior and outcomes.

One of the areas that I think will become a significant focus for breast cancer research is epigenetics—the modification of gene expression independent of changes to the genetic code itself. For example, DNA methylation is a key regulator for many developmental processes and has been shown to regulate gene activity by switching genes ‘on’ or ‘off.’

DNA methylation is also a dynamic process and may be modified by lifestyle or the environment; methyl groups can be added or removed from DNA in response to factors such as smoking, diet, alcohol and exercise. Therefore, DNA methylation may play a role in bridging the genetic and environmental risk factors for breast cancer.

So far, methylation markers have been identified in blood-based DNA and shown to vary according to age, body mass index, alcohol and tobacco consumption and so on. By going beyond genetics and incorporating epigenetics into the calculation of breast cancer risk, I believe that we can improve our prediction models for the disease.

Q: APART FROM MORE RESEARCH, HOW ELSE SHOULD WE BE ADDRESSING BREAST CANCER?

I think there is room for improvement in terms of how the fruits of scientific discovery are conveyed to the general public. I agree with Sir Mark Walport, former government chief scientific advisor to the UK, who said, “Science is not finished until it’s communicated.”

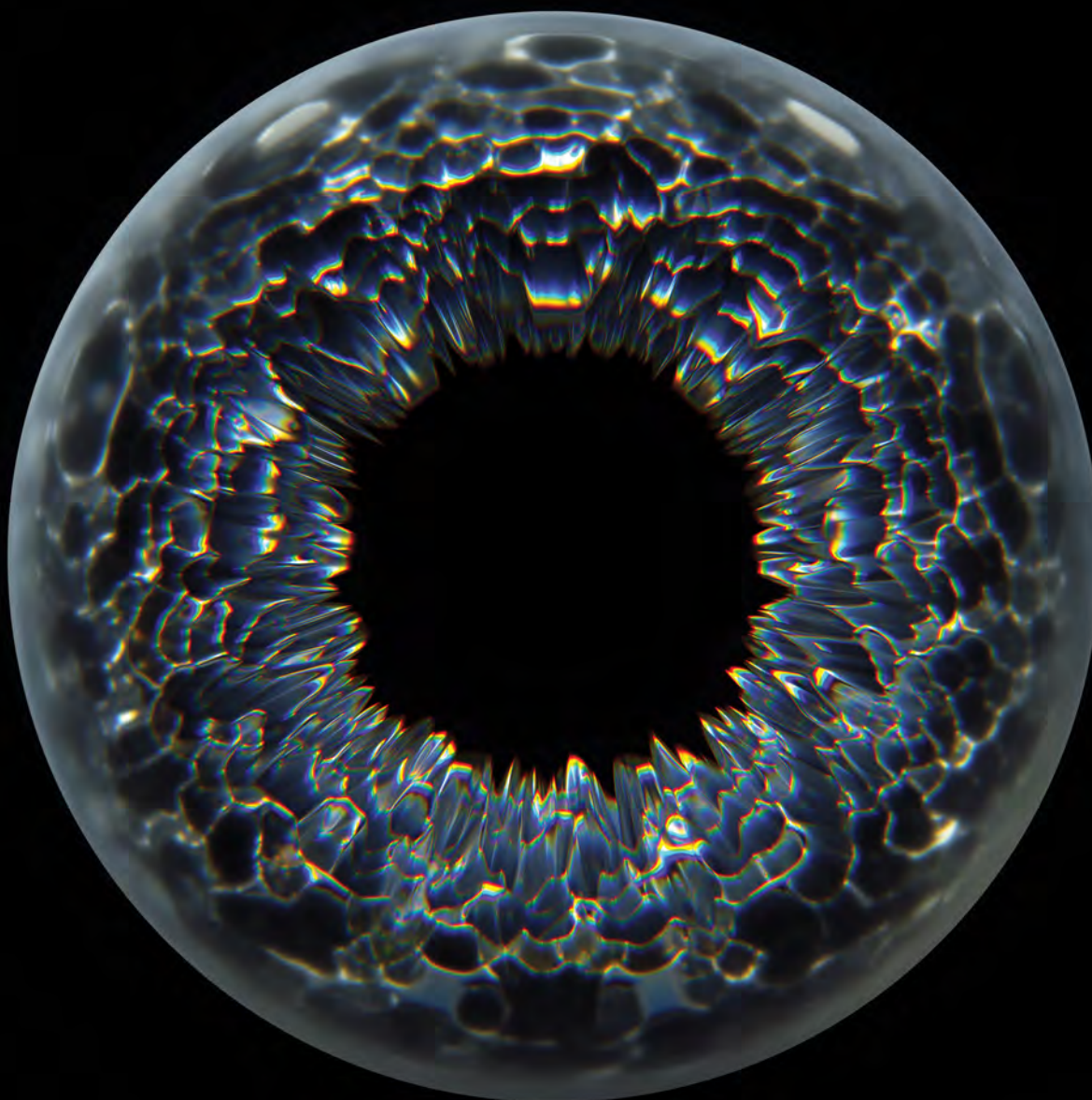
There is evidence that risk-based screening for breast cancer saves lives, but many women are still largely ignorant about what they can and should do to detect the disease in its early stages. Other factors like cultural influences may also result in women putting off going to the doctor.

Hence, the bigger challenge in breast cancer research is how to communicate relevant findings to the public and patients in a way that spurs them to take personal action. After all, knowledge without action is not power, but impotence. ★

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1. Li, J., Ugalde-Morales, E., Wen, W. X., Decker, B., Eriksson, M. *et al.* Differential Burden of Rare and Common Variants on Tumor Characteristics, Survival, and Mode of Detection in Breast Cancer. *Cancer Research* **78**(21), 6329–6338 (2018).

Dr. Jingmei Li
Senior Research Scientist,
Genome Institute of
Singapore, A*STAR





MATERIALS SCIENCE

A vision for better eye treatments

Researchers at A*STAR have invented a biodegradable thermogel that mimics the clear, gelatinous substance called vitreous in the eye.

WHY THIS MATTERS

- During eye surgery, the natural gel-like substance in the eyeball, called vitreous, is often lost.
- Existing vitreous substitutes cause side effects like poor vision and may require further surgery for removal.

Our eyeballs are round thanks to vitreous, a clear, gel-like substance that maintains the shape of the eye while allowing nutrients to circulate within it. As we age, vitreous may become increasingly liquefied, leading to shear stress in the eye and potential retinal detachment.

To treat disorders such as retinal detachment, the vitreous often has to be removed and replaced post-operatively by temporary substitutes such as an inert gas or silicone oil, which serve as a tamponade or packing agent. However, limitations of current replacement materials include poor vision, the need to adopt an uncomfortable head-down sleeping position for up to two weeks, restrictions on air travel and the need for additional surgeries to remove the vitreous substitutes.

Seeking to overcome such constraints, researchers led by Xinyi Su at A*STAR's Institute of Molecular and Cell Biology (IMCB) and Xian Jun Loh at the Institute of Materials Research and Engineering (IMRE), together with colleagues at the National University of Singapore and the Singapore Eye Research Institute,

developed a thermogel that could serve as a long-term vitreous substitute. The team used a polymer called EPC which is transparent at the body temperature of 37°C and has a similar refractive index to that of natural vitreous.

"This thermogel consists of low amounts of polymer which helps to improve the biocompatibility of the material, making it useful for biomedical applications," said Loh.

"We demonstrated that a hydrogel comprising seven-weight-percent EPC (EPC-7%) is biocompatible in rabbit surgical models for up to six months. We also showed that it can function as an internal packing agent for retinal detachment repair surgery in non-human primates for up to one year," added Su.

Being biodegradable, EPC-7% also has an edge over silicone oil as it does not require subsequent surgical removal. Importantly, when the hydrogel is broken down, it facilitates the formation of a vitreous-like body similar in protein composition to native vitreous in the eye.

The team is moving forward to commercialize the EPC-7% gel under the

trade name Vitreogel, and intends to further test its application in humans. They are also exploring the use of Vitreogel as a sustained drug delivery platform for biologics to the posterior segment of the eye and as a scaffold to facilitate stem cell transplantation for retinal disease such as age-related macular degeneration.

"We have recently started a spin-off company called Vitreogel Innovations. We hope to obtain regulatory approval from the US Food and Drug Administration and CE marking in Europe before bringing Vitreogel to clinical trials within the next five years," said Su. ★

IMPACT

The biodegradable hydrogel is not only suitable as a long-term vitreous substitute, it could also be used as a medium for drug delivery and stem cell-based therapy in the eye.

LEFT

During surgery to correct eye disorders such as retinal detachment, the gel-like substance that maintains the shape of the eyeball is often removed and must be replaced with suitable substitutes.

1. Liu, Z., Liow, S. S., Lai, S. L., Alli-Shaik, A., Holder, G. E. *et al.* Retinal-detachment repair and vitreous-likebody reformation via a thermogelling polymer endotamponade. *Nature Biomedical Engineering* **3**, 598–610 (2019).

MATERIALS SCIENCE

Brushing oil away

A thin film of water forms over surfaces coated with charged polymers, resulting in super-repellent materials with self-cleaning properties.

Inspired by nature, scientists have created oil-repellent coatings by mimicking the nanosized structures found on fish scales and clam shells. These coatings have found their way into anti-smudge mobile phone screens, the linings of pipes carrying crude oil, and even the surfaces of medical implants, which cannot have bacteria adhere to them lest infections ensue.

Another way to repel oil is to coat surfaces with a polyzwitterionic brush—a layer of polymers bearing equal numbers of positive and negative charges. The polyzwitterionic brush is thought to repel oil by retaining a thin film of water on the coated surface, which means

that the surface remains oil-proof even underwater.

“The existence of such a hydration film has been postulated, but never experimentally verified,” said Nikodem Tomczak, a Scientist at A*STAR’s Institute of Materials Research and Engineering (IMRE).

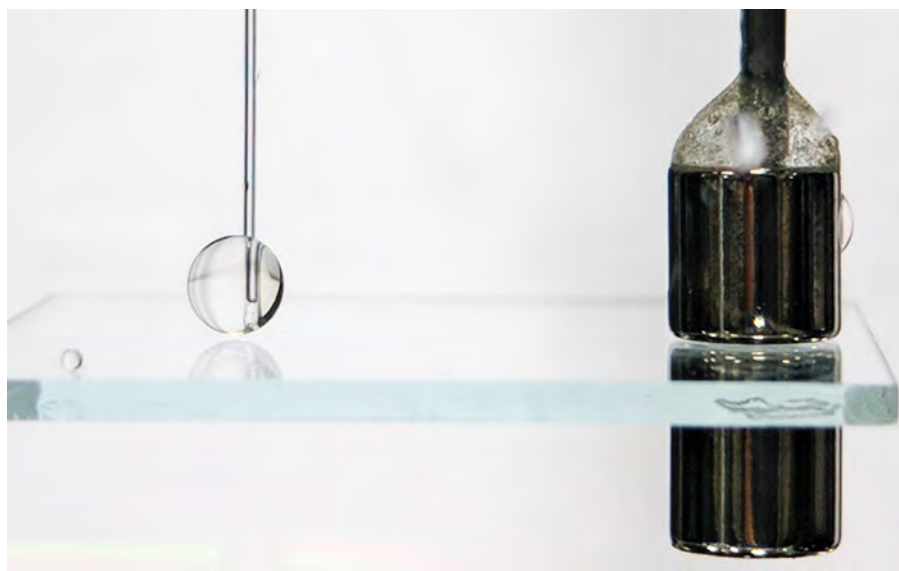
To investigate whether polyzwitterionic brush surfaces indeed create a hydration film that protects surfaces against oil staining, Tomczak’s team coated a glass surface with a polyzwitterionic material—poly(sulfobetaine methacrylate), or PSBMA for short—before immersing the material in water.

Dan Daniel, also a Scientist at IMRE and a co-author of the study, developed a method called reflection interference contrast microscopy to observe the formation of a very thin water film measuring 100 nm in thickness over the PSBMA-coated surface. He also customized an instrument for quantifying the frictional forces required to move an oil droplet along the coated surface.

“The oil droplet interacted with the substrate with ultralow adhesion, allowing the droplet to slide across the surface with almost no friction, like a car tire hydroplaning over a wet road,” Daniel said.

Compared to nanostructured oil-repellent surfaces, polyzwitterionic brush surfaces that induce hydration film formation reduce the frictional force between oil droplets and the surface by about a thousand times. By further exploring how frictional force correlates with hydration film thickness, the researchers aim to further improve the performance of their polyzwitterionic brush surfaces. They will also enhance the sensitivity of the equipment used to study the hydration film.

“The aim of understanding the physics behind these materials is to formulate a design principle to create self-cleaning, super-repellent coatings. Such coatings have important uses, such as in the prevention of fouling (the accumulation of marine organisms and oil) on underwater surfaces and in water purification membranes,” said Tomczak. ★



This oil droplet is almost completely repelled by a surface coated with a polyzwitterionic brush.

1. Daniel, D., Chia, A. Y. T., Moh, L. C. H., Liu, R., Koh, X. Q. *et al.* Hydration lubrication of polyzwitterionic brushes leads to nearly friction- and adhesion-free droplet motion. *Communications Physics*. **2**, 105 (2019).

MATERIALS SCIENCE

Plant polymer helps joints heal

By mixing a substance normally found in wood with a biodegradable plastic, A*STAR scientists have developed a nanofiber that promotes cartilage repair.

A common definitive solution for stiff door hinges and degenerated knee joints is to have them replaced. In the latter case, however, knee joint replacement entails a great deal of pain and a long road to recovery.

When the cartilage that serves as a shock absorber in our joints gets degraded by oxidative stress caused by inflammation, friction between the adjacent bones causes persistent pain. This is often diagnosed as osteoarthritis.

“Currently, effective treatments for osteoarthritis are lacking as cartilage does not self-regenerate,” said Dan Kai, a Scientist at A*STAR’s Institute of Materials Research and Engineering (IMRE). In a collaborative effort, the team led by Li Zheng from Guangxi Medical University in China developed a novel nanofibrous biomaterial made of lignin, commonly found in wood, that promotes cartilage regeneration.

Capitalizing on the strong intrinsic antioxidant activity of lignin, the team modified the surface of lignin by polymerizing it with an FDA-approved polymer, poly (ϵ -caprolactone) (PCL), creating a soft and flexible biomaterial with mechanical properties like cartilage tissue.

To determine the effectiveness of the antioxidant properties of PCL-lignin nanofibers, the team treated human chondrocytes, which are cells that make

up joint cartilage, with hydrogen peroxide and measured the amount of cell death with or without PCL-lignin. They found the survival rate of cells grown on PCL-lignin to be twice of those grown without the nanofiber.

At the molecular level, the better survival rate was attributed to PCL-lignin activating antioxidant enzymes through an intracellular process called autophagy. Simultaneously, autophagy facilitates the removal of biomolecules damaged by oxidative stress, thereby suppressing the accumulation of potentially toxic substances inside the cells.

“Compared to commonly used antioxidants such as vitamin C or E, our lignin-based biomaterials have more stability and their antioxidant effects last longer,” said Kai.

When tested in a rabbit model of osteoarthritis, the nanofiber gradually released lignin into the joint space, exhibited anti-inflammatory effects and repaired the damaged cartilage tissue over a period of four weeks.

“Osteoarthritis management mainly focuses on pain relief and reducing inflammation through non-steroidal anti-inflammatory drugs and steroids. Our nanofiber now provides a new approach for treatment as it can reduce inflammation and promote cartilage regeneration,” added Kai.

Meanwhile, the team is planning to develop new lignin-based biomaterials such as gels that can be used during minimally invasive surgeries.

“To further demonstrate the function and safety of these biomaterials, we will be carrying out more animal studies and pre-clinical trials before transitioning into human clinical trials,” he said. ★

ABOVE

The nanofiber was developed from lignin for its strong antioxidant activity.

1. Liang, R., Zhao, J., Li, B., Cai, P., Loh, X. J. *et al.* Implantable and degradable antioxidant poly(ϵ -caprolactone)-lignin nanofiber membrane for effective osteoarthritis treatment. *Biomaterials* **230**, 119601 (2020).

MATERIALS SCIENCE

MXimizing the power of alloys

A*STAR researchers have developed a computational technique that allows them to predict the properties of MXene alloys.

Over the past few decades, our television screens and computer monitors have transformed from big, bulky items into slender touch-screen devices. This 'slimming' effect has been made possible by the discovery of electrically conductive materials like indium tin oxide (ITO) that are transparent and thin, making them suitable for liquid crystal displays and light-emitting diodes.

Now, researchers at A*STAR and the National University of Singapore are exploring atomically thin materials with properties that supersede commercial ITO transparent conductors. A family of materials that shows promise is MXenes, thin layers of any combination of transition metals, which could pave the way for even thinner displays with greater functionality.

However, given the wide range of transition metals available, finding the ideal mix of metals or alloys with the desired

properties remains problematic. Teck Leong Tan, a Research Scientist at A*STAR's Institute of High Performance Computing (IHPC), thinks that a computational approach will be useful to predict how novel MXene alloys will behave.

Leveraging the computational resources at IHPC, his team developed a technique that allows them to model what happens when a transition metal is substituted in a MXene alloy. "Since the titanium (Ti)-containing Ti_2CO_2 MXene has already been identified as a semiconductor, we wondered if alloying it with elements such as vanadium (V), niobium (Nb) or tantalum (Ta) would give it transparent conducting capabilities," said Tan.

By combining the computational modeling methods of density functional theory, cluster expansion and Monte Carlo calculations, the researchers were able to simulate the effects of substituting Ti in

Ti_2CO_2 with V, Nb or Ta. They were also able to predict the molecular configurations of the proposed alloy combinations at varying temperatures.

They found that MXenes with the chemical formula $\text{Ti}_{2(1-x)}\text{V}_{2x}\text{CO}_2$, where Ti is replaced by V in specific proportions within the material, would exhibit high electrical conductivities of more than 10^3 Siemens per centimeter, comparable to commercial ITO conductors and outperforming Ti_2CO_2 and V_2CO_2 . Their model also indicated that $\text{Ti}_{2(1-x)}\text{V}_{2x}\text{CO}_2$ is likely to have high optical transparency of more than 90 percent.

As the current findings are computational predictions, the team aims to verify their discovery with experimental results—by actually fabricating $\text{Ti}_{2(1-x)}\text{V}_{2x}\text{CO}_2$ MXenes and testing their properties. They also intend to apply their computational technique to other combinations of MXene alloys.

"Computationally, there are still many different MXenes for exploration as alloys. We expect that there could be more MXene alloys with interesting properties that are waiting to be discovered," said Tan. ★

ABOVE

MXenes, thin layers of any combination of transition metals, could be useful in liquid crystal displays and light-emitting diodes.

1. Wong, Z. M., Tan, T. L., Tieu, A. J. K., Yang, S. W., Xu, G. Q. Computational Discovery of Transparent Conducting In-Plane Ordered MXene (i MXene) Alloys. *Chemistry of Materials* **31**, 4124-4132 (2019).

MATERIALS SCIENCE

Catalyzing the future of clean energy

An improved design for nickel hydroxide catalysts could reduce costs and improve the efficiency of hydrogen fuel and oxygen generation.

In the face of climate change, our need to switch to clean energy sources is more pressing than ever. However, many of these energy sources, such as solar and wind energy, face a key issue with intermittency, as the energy they supply is dependent on the time of day and weather. A potential solution to offset intermittency is electrochemical water splitting, which generates hydrogen fuel and oxygen.

However, electrochemical water splitting also comes with its challenges. Besides being slow, the process is also costly due to the use of noble metals such as ruthenium and iridium as electrocatalysts. Switching to cheaper metals such as nickel would help to reduce costs associated with electrochemical water splitting. While scientists have been working on nickel hydroxide as an alternative for more than two decades, its performance as a catalyst has not been up

to par with industry standards thus far.

Seeking to boost the effectiveness of nickel hydroxide catalysts, researchers at A*STAR's Institute of High Performance Computing (IHPC), joined by others from the Institute of Chemical and Engineering Sciences (ICES) and the National University of Singapore, have employed a combination of theoretical and experimental methods to optimize catalyst design.

"The oxygen evolution reaction (OER) is the bottleneck in electrochemical water splitting due to the sluggish kinetics arising from the oxygen double bond formation and multiple electron transfer reactions," explained Zhigen Yu, a Senior Scientist at IHPC and one of the study's authors. Hence, nickel hydroxide's catalytic action in OER is crucial for water splitting efficiency.

"Based on our density functional theory (DFT) simulations, we found that

reducing nickel coordination to four from six dramatically reduces the OER overpotential to 0.36 V," explained Yu. The reduction in overpotential indicates that less energy is required to kickstart OER, thereby increasing reaction rate and catalytic efficiency.

To achieve the chemical configuration needed for reduced overpotential, the group designed a nickel hydroxide catalyst in the form of nanoribbons. Using X-ray absorption spectroscopy and scanning transmission electron microscopy, they confirmed that the nickel hydroxide nanoribbons were able to provide stable four-coordinated nickel sites, which resulted in improved OER and water splitting efficiency. The group also found that the overpotential was a mere 162 mV, one of the best performances among nickel hydroxide catalysts to date.

"For the first time, we present a novel alternating nickel 4-/6-coordinated nickel hydroxide that is successfully stabilized by introducing tensile strain," Yu said. Apart from replacing noble metals in OER catalysts, the group's methodology could reveal novel ways to reduce OER overpotential, which will make the switch to clean energy easier than before. ★

BACKGROUND

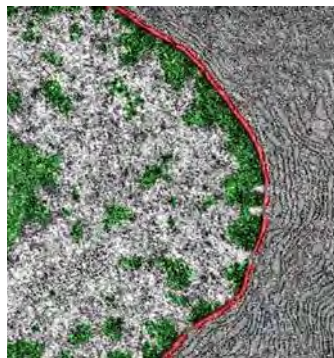
Efficient catalysts are essential for the economical production of renewable fuels.

1. Wang, X.P., Wu, H.J., Xi, S.B., Lee, W.S.V., Zhang, J., *et al.* Strain stabilized nickel hydroxide nanoribbons for efficient water splitting. *Energy and Environmental Sciences*. (2020).



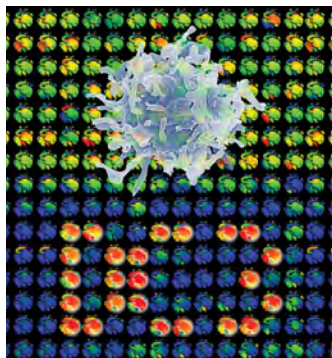
NEXT ISSUE

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



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