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- Biomimetic Materials
- Biomaterials and Multiscale Technologies for Cell and Tissue Engineering
- Drug, Protein and Gene Delivery
- Macromolecular Antimicrobial Agents
- Nanosystems for Drug Screening
- Green Chemistry and Sustainable Technologies

INVITED SPEAKERS

- Kazunari Akiyoshi, Kyoto University, Japan
- Ehud Gazit, Tel Aviv University, Israel
- James Hedrick, IBM Almaden Research Center, USA
- Sangyong Jon, Korea Advanced Institute of Science and Technology, South Korea
- Kenichi Kuroda, University of Michigan, USA
- Ming Ta Michael Lee, RIKEN, Japan
- Yingfu Li, McMaster University, Canada
- Yi Lu, University of Illinois at Urbana-Champaign, USA
- Sylvain Martel, Polytechnique Montréal, Canada
- Andres Martinez, California Polytechnic State University, USA
- Scott T. Phillips, The Pennsylvania State University, USA
- Chao-Nan (Miles) Qian, Sun Yat-sen University Cancer Center, China
- Jianhua Qin, Dalian Institute of Chemical Physics, China
- Gregory N. Tew, University of Massachusetts Amherst, USA
- Gordon Wallace, University of Wollongong, Australia
- Leslie Yeo, RMIT University, Australia
- Evelyn Yim, National University of Singapore, Singapore
- Tao Zhang, Dalian Institute of Chemical Physics, China

REGISTRATION FEES*

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IBN-is Student Forum Only: Dec 9, 2014 SGD 53.50

IMPORTANT DATES

- Early Bird Registration Deadline: Oct 1, 2014
- Online Registration Deadline: Nov 15, 2014

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Research at A*STAR is helping to ensure a sustainable future for the aviation industry.

Sustainable ways to keep us flying

Multidisciplinary research at A*STAR is producing new technologies to improve safety and efficiency in the aviation industry

The global aviation industry continues to expand, with over 3 billion people expected to fly commercially in 2014, along with 38 million metric tons of cargo. This activity will have a huge impact on the environment and requires vast resources. In order to make air travel a sustainable option for future generations, the industry needs to perform continual research into technologies that reduce both economic and environmental costs.

The A*STAR Aerospace Programme was set up to combine the efforts of A*STAR researchers who are striving to provide a sustainable future for aviation. Since 2007, scientists from several A*STAR research institutes have worked together on over 50 multidisciplinary projects to pioneer manufacturing techniques, safety inspection devices and analytical methods to improve flight management. To highlight this success, their work was showcased earlier this year at the Singapore Airshow 2014.

Finding faults faster

Aircraft will always experience wear and tear, and A*STAR researchers are developing fast, cost-effective methods to identify parts in need of repair. Crucially, these methods aim to be non-destructive, which means that fewer parts need to be removed or replaced during inspection, saving both time and money.

One major problem for the industry is the infiltration of water into the aircraft body, especially in the lightweight honeycomb structures found in the tail and wings. Dmitry Isakov from the A*STAR Singapore Institute of Manufacturing Technology (SIMTech) is leading efforts to help technicians spot exactly where the water is.

“Water always finds the easiest way to get in, which is around discontinuities such as joints and bolts,” says Isakov. “Once inside, the water expands and contracts as it freezes and melts, damaging structures, causing corrosion and increasing the aircraft’s weight.”

To identify areas where water has entered, technicians currently heat the aircraft surface and then use thermal cameras to observe its cooling. Regions that cool too quickly indicate the presence of water below the surface. The method requires two engineers, is slow and cannot distinguish between water and excess sealant left behind after repairs.

Isakov has developed an alternative approach. “When a vacuum is created around a bolt head, water can boil even at room temperature,” he explains. Boiling consumes heat, causing the material around the water to cool down by as much as several degrees. “Water detection using my vacuum method requires just one technician, is fast and highly sensitive, and there is no ambiguity with the sealant,” Isakov adds.

Building the future of flight

In addition to improving fault detection, researchers at A*STAR are providing new methods for repairing damaged aircraft parts or manufacturing new designs. One promising technology under development is laser-aided additive manufacturing (LAAM) (see “Laying the groundwork for a manufacturing revolution”). This method, an example of three-dimensional printing technology, uses a high-energy laser beam to deposit materials in precisely controlled geometric structures or to fill in cracks.
The ‘bottom-up’ approach is less wasteful than traditional methods that involve cutting components out of larger chunks of material.

“LAAM can repair or fabricate parts with excellent mechanical properties and resistance to wear and corrosion,” says Guijun Bi, a leading researcher at SIMTech. Bi is adapting LAAM techniques to build and repair structures using so-called superalloys, which maintain their strength even under the extreme conditions of a working jet engine.

The precise control provided by LAAM is enabling Bi and his co-workers to re-use components that would previously have been impossible or very costly to repair. The ‘remanufacturing’ of components is central to the aims of the A*STAR Aerospace Programme and provides clear environmental benefits through reducing resource consumption.

Keeping problems at bay

Other high-tech maintenance tools being developed at SIMTech include a system that uses electromagnetic waves to detect slight variations in the thickness or composition of components that may be the result of corrosion. Further monitoring can be provided by using piezoelectric sensors that ‘hear’ structural failures in real time, with the added benefit of turning the mechanical energy of the aircraft body into electricity.

The lifetime of aircraft components can be greatly extended by applying advanced water- and ice-repellent — ‘superhydrophobic’ — coatings developed at SIMTech that protect parts from condensation, corrosion and mold, as well as improving aerodynamics to reduce fuel consumption. Researchers are also pioneering the use of lasers, instead of expensive corrosive chemicals, to strip damaged coatings from turbines, allowing the parts to be re-used.

Improving the human factor

Aside from the development of inspection and remanufacturing tools, diverse expertise in computer simulation at A*STAR is providing novel ways to improve the experience of airline customers and staff.

Anyone who has flown will have heard the cabin crew asking passengers to switch off their electronic devices during take-off and landing because stray signals can interfere with flight systems. Now, researchers at the A*STAR Institute for Infocomm Research (I²R) have written simulations that identify the best way to arrange electronics on the aircraft for minimizing interference, enabling airlines to provide customers with wireless services without compromising safety. Another useful software developed at the I²R, named ‘Super De-haze’, provides pilots and air-traffic controllers with clearer imagery by removing the effects of haze, fog and smoke.

Finally, the complex challenge of airport management is being tackled by I²R researchers who have developed a flight prediction algorithm that captures the interactions of weather and flight congestion. Their algorithm won first prize in the GE Flight Quest 2013 challenge, estimating flight arrival times that were 40 per cent more accurate than current industry estimates. By applying similar models, airlines could achieve more efficient operation of gates and runways.

Flying ahead of the competition

The A*STAR Aerospace Programme is a prime example of the benefits of multidisciplinary research. The program is attracting interest from aviation giants such as Airbus and Boeing, and is set to ensure that Singapore maintains its competitive edge in the global aerospace marketplace.

More importantly, by improving aircraft design, maintenance, fuel economy and customer satisfaction, A*STAR is leading the way toward a more sustainable and environmentally friendly business model for aviation. Only through such long-sighted efforts will future generations continue to enjoy the many benefits of air travel.
Randomly arranged items usually have poor optical properties. The rough — or random — surface of a frosted-glass window, for example, obscures the view of an object. The optical industry therefore expends considerable effort reducing any surface irregularities in optical devices to avoid the uncontrollable scattering of light characteristic of random structures.

But now, a research group led by Ying Zhang from the A*STAR Singapore Institute of Manufacturing Technology (SIMTech) has made good use of randomness by studying how random structures can improve the performance of lasers. Together with a team led by Qijie Wang at Nanyang Technological University in Singapore, the group has demonstrated the world’s first electrically pumped mid-infrared random laser, which operates at a 10-micrometer wavelength. The laser is as bright as conventional diode lasers but produces less-speckled images.

Light waves from a conventional laser oscillate in perfect synchronicity, across both time and space. Perfect alignment of the light waves at different times and different locations across the beam profile is known as temporal and spatial coherence, respectively. When a laser illuminates a surface, a speckled pattern is typically visible, which indicates spatial coherence. The speckles result from the laser beam reflecting from different parts of the surface. Because the waves are in sync, they create spatial interference effects in the eye of an observer. This distortion is undesirable, particularly in biomedical imaging applications conducted in the infrared region of the spectrum.

Random lasers are the solution to this type of distortion, says Zhang. “Random lasers show the same high temporal coherence as that of other lasers but have a lower spatial coherence,” he explains. “High temporal coherence gives the desirable brightness, but it is the low spatial coherence that removes the speckles caused by interferences.”

To realize a random laser in the mid-infrared spectrum, Zhang and co-workers used a semiconductor quantum cascade laser into which they had drilled a random pattern of nanoholes. At a sufficiently high density, these holes prevent the formation of a regular laser pattern within the semiconductor (see image).

Instead, the pattern of a random laser forms, with low spatial coherence.

Employing a quantum cascade laser to realize the random lasers allows for the polarization of the laser light perpendicular to the laser surface. This propagation minimizes losses owing to the air-hole structure.

The research team’s wafer-fabrication competencies enabled them to drill holes deep enough into the laser chip, with sufficiently smooth side walls to minimize losses in the laser itself. By introducing these perfections and overcoming a number of other practical hurdles, Zhang and his colleagues succeeded in making the lasers efficient enough to provide lasing during electrical operation.

Nevertheless, notes Hou Kun Liang of SIMTech, who invented the mid-infrared random laser, more work is needed to bring random lasers to market. “We are working on a random laser that operates at room temperature. And in the long-term, we plan to extend random lasers from the infrared to even longer wavelengths, where they can penetrate materials and be used for inspection of various polymer packaging, quality-control of printed electronics, biomedical imaging and other applications.”
As a newborn takes its first breath, microbes that line the mother’s birth canal are already making their way into the infant’s gut. And with the baby’s first taste of breast milk, many more will settle in. This process of accommodating a friendly assortment of bacteria in the digestive tract — collectively referred to as the gut microbiota — is essential for healthy development and helps the infant to digest foods, synthesize vitamins and enzymes and fight pathogenic invasion.

Understanding the factors that affect the development of microbial populations in babies is the aim of a new partnership between the A*STAR Genome Institute of Singapore (GIS) and Nutricia Research, part of the international food company Danone. The investigation will involve a clinical and genomic study of the gut microbiome, which is the sum of the genomes of all the microbes that reside in the gut. “This partnership is combining the genomic expertise of the GIS, who have developed high-throughput approaches to monitoring the microbiome, with the early life nutrition and clinical expertise of Nutricia Research to investigate the health benefits of bacterial supplements,” says Martin Hibberd, associate director at the GIS.

The researchers hope their findings could lead to improved infant nutrition for better health in early, and even adult, life.

**Complex and resilient**

A harmonious relationship with the bacteria that share our digestive tracts is increasingly being recognized as essential for good health.

“Early-life perturbations of the developing gut microbiota can have an impact on the immune, metabolic and neurological systems, and potentially lead to long-term, adverse health outcomes,” says Kaouther Ben Amor, senior team leader for gut microbiology and physiology at Nutricia Research. Studies have shown that a rift in the relationship between microbes and the body can contribute to diseases in humans such as allergy, asthma, obesity, diabetes, inflammatory bowel syndrome and even autism. But while previous focus lay on identifying the patterns of microbial colonization associated with disease states, the new endeavor should help to define what a healthy bacterial population looks like — filling a gap in our current knowledge.

“A healthy microbiome has not yet been characterized in full,” explains Hibberd. “We do know, though, that it is complex and in the study we will be seeking to associate this with healthy outcomes.” Researchers have determined that a healthy bacterial community is not only diverse but also ecologically stable, which means that it can maintain its community structure in the face of stress or rebound to the default state following a disruption, adds Ben Amor.

**Milk for health**

Understanding the dynamics of the gut’s microbial ecosystem and the factors that drive its development can be used to help optimize health in later life. Of particular importance is nutrition, in which the infant’s mother plays a lead role. “Human milk is normally the first dietary exposure in infancy, and it is considered the best nutrition for growth and healthy development of the newborn,” says Ben Amor.

Human milk contains two groups of compounds that are considered beneficial for health — prebiotics and ‘good’ bacteria that are also found in probiotic supplements.

Prebiotics are compounds that stimulate the growth of specific good bacteria in the large intestine. These include the nondigestible carbohydrates known as oligosaccharides found in human milk. Probiotics are live bacteria, such as species of the lactic-acid-producing *Lactobacillus* and *Bifidobacterium*, which help maintain microbial balance in the gut.

Researchers believe that a better understanding of the contribution of prebiotics, probiotics and a combination of the two — synbiotics — in fostering normal bacterial populations could be used to improve the design of nutritional products that supplement or complement human milk.

Other factors also have an effect on the development of an infant gut microbiome, says Hibberd. Antibiotics, for example, may change the pattern of bacterial colonization. Without exposure to the bacterial populations in the mother’s birth canal, the gut microbiota of cesarean babies more closely resembles the microorganisms found on the surface of the mother’s skin. Given the increasing rates of cesarean deliveries worldwide, including in Asia, more research is needed to investigate the implications for health of the surgical delivery of babies.

**Feeding bacteria**

The GIS–Nutricia Research partnership hopes to bring new insights to these nutritional aspects of...
Improving drug delivery for breast cancer treatment

A nontoxic hydrogel developed by the A*STAR Institute of Bioengineering and Nanotechnology and IBM Research offers a new way forward for breast cancer therapeutics

Breast cancer is the most common form of invasive cancer affecting women worldwide. Treatment usually involves surgery followed by a course of either chemotherapy, radiotherapy or hormone therapy designed to reduce the risk of the cancer’s recurrence. In recent years, however, targeted drugs have increasingly become an effective option to fight the disease.

One such drug is Herceptin, also known as trastuzumab, a monoclonal antibody that can slow or even halt tumor growth in patients with human epidermal growth factor receptor 2 (HER2)-positive breast cancer — a particularly fast-growing form of the disease that affects one in four patients. Typically, the drug is administered at a clinic through an intravenous drip, a process that can take up to 90 minutes. A major drawback of this method of delivery is that without frequent follow-up doses, Herceptin loses its effectiveness. Therefore, patients are commonly required to visit the clinic on a weekly basis.

Now, a team of researchers from the A*STAR Institute of Bioengineering and Nanotechnology (IBN) and IBM Research led by Yi Yan Yang and James Hedrick has developed a more efficient way of delivering Herceptin to breast cancer cells. The approach promises to improve the effectiveness of treatment for the HER2-positive form of the disease. Drawing on IBN’s expertise in the development

A team of researchers led by Martin Hibberd (left), associate director at the A*STAR Genome Institute of Singapore, is partnering with Nutricia Research in a clinical and genomic study of the infant microbiome.
of novel biomaterials, the researchers recognized the advantages of using a hydrogel — comprising 96 per cent water and a uniquely designed polymer — as a carrier for Herceptin in the body.

The hydrogel is nontoxic, biodegradable and can be injected under the skin without causing an inflammatory response. By varying the composition and concentration of the polymer, the researchers were able to fine-tune the hydrogel’s properties to ensure a slow and sustained release of the anticancer drug, thus increasing the efficiency of delivering the drug to the target site. Correspondingly, use of the hydrogel for drug delivery has the potential to reduce the frequency at which patients need to be injected with Herceptin from once a week to once every four weeks.

Recently, the researchers conducted studies in mice, which confirmed that their Herceptin-loaded hydrogel delivers the drug efficiently, before it degrades within 6 weeks. They found that 4 weeks after injection, tumors in the mice had decreased in size by as much as 77 per cent. The researchers say that their next goal will be to conduct clinical trials in humans, in conjunction with industrial partners.

Other teams at IBN are also exploring the innovative use of hydrogels for controlled-release drug delivery to tackle different types of cancer (see “Greater anticancer potency with less risk”). The institute has always cultivated a multi-pronged approach toward cancer research, notes Jackie Y. Ying, professor and executive director at IBN. “Our multidisciplinary research teams are working with various industrial, clinical and academic partners to develop new materials and tools to improve cancer diagnosis and treatment,” she adds. “This latest breakthrough with our long-term partner IBM Research promises more efficient administration of anti-cancer drugs and more effective treatment of breast cancer, which we hope will benefit breast cancer patients worldwide.”

Shoddy sorting disrupts memory-making signals

Every year, roughly 1 in 1,000 children worldwide are born with Down’s syndrome. This developmental disorder, associated with potentially severe intellectual and learning disabilities among other characteristics, is caused by the presence of a third copy of chromosome 21, resulting in abnormal activity levels for the more than 300 genes on this chromosome. Scientists have had difficulty identifying the core genes responsible for the disorder, but Wanjin Hong’s team at the A*STAR Institute of Molecular and Cell Biology in Singapore have identified a gene with an important role in brain signaling that is one possible culprit⁴.

Hong and colleagues focused their attention on sorting nexin 27 (SNX27), one of a family of proteins that coordinate movement of other proteins to different compartments of the cell. Previously, the team showed that SNX27 contains distinctive structural features that suggested it may be active at neuronal synapses³. In addition, mice lacking this protein displayed severe developmental and neurological abnormalities, including measurable cognitive defects.

“SNX27-knockout mice demonstrated behavioral characteristics that make them good candidates for research on learning and memory,” explains Li Shen Loo, a research scientist in Hong’s laboratory. Intriguingly, this study also revealed that lower levels of SNX27 were also apparent in brain tissue from patients with Down’s syndrome, and showed that SNX27 production is reduced by one of the genes present on chromosome 21.

Loo and Hong decided to examine brain structure and function in SNX27-deficient mice. They observed considerable fluid accumulation within the mutant mouse brain and underdevelopment of the dentate gyrus — a part of the brain responsible for learning.
and memory. These alterations made further analysis tricky, according to Loo.

“The knockout brains were too soft and watery to characterize with traditional techniques,” she says, “but I was able to investigate the mechanism of memory impairment in these mice using live cell imaging techniques.” By examining different combinations of fluorescently labeled proteins in neurons from wild-type and SNX27-deficient mice, Loo and her colleagues could directly observe the real-time behavior of individual synapses in the presence and absence of SNX27.

Those experiments confirmed that SNX27 is predominantly found within dendritic spines (see image), the part of the neuron that receives incoming signals at the synapse. SNX27 specifically resides within structures called recycling endosomes, which help to shuttle neurotransmitter receptors and other proteins between the cellular interior and the cell surface.

When the researchers experimentally simulated long-term potentiation, the neuronal activation process associated with memory building, they observed that SNX27-bearing endosomes moved from the cell interior to the surface. These endosomes also contained GluA1, a component of the receptor for the neurotransmitter glutamate. In the absence of SNX27, GluA1 is no longer efficiently transported to the surface of the dendritic spines — in fact, it becomes susceptible to degradation. Since glutamate–GluA1 signaling is critical for long-term potentiation, SNX27 could play a major role in enabling transmission of memory-related signals.

“These results could account for the learning deficits observed in Down’s syndrome,” notes Loo, who sees a potential therapeutic opportunity. “Since Down’s syndrome patients produce less SNX27, re-introducing the protein may restore memory function,” she says.

Setting sequencing free

Researchers at the A*STAR Bioinformatics Institute develop a pioneering mobile application for portable analysis of DNA sequences

A*STAR molecular cell biologist Samuel Gan was in the midst of an exasperating work trip in Shanghai, China. While he was away from his office, Gan’s email inbox was filling up with DNA sequencing files that needed his urgent attention.

Gan’s research group in Singapore is engaged in therapeutic antibody production and had engineered DNA molecules known as plasmids to transmit protein-encoding information into cells. Before the plasmids could be introduced into cells, however, their DNA sequences had to be determined and verified. But with no way to interpret these sequences on his smartphone, Gan was unable to instruct his team back home to begin the next stage of producing antibodies.

“I asked myself: ‘Why can’t I use my phone to do something so simple?’” explains Gan, team leader of the Antibody and Product Development Laboratory at the A*STAR Bioinformatics Institute (BII). “I was frustrated.”

Knowing there had to be a way to unlock the processing power contained in his smartphone to decode the sequencing files, Gan posed this challenge to a research officer in his team, Phi Vu Nguyen. Within three months, Nguyen had developed ‘DNAApp’ — the first mobile application, or app, for viewing and analyzing DNA sequencing files on an Android mobile device. And since its April 2014 launch, close to 700 scientists in over 11 countries have already downloaded Android and iOS versions of the app.

Decoding DNA

DNA sequencing methods allow scientists to determine the precise order of the four basic units, or ‘bases’, of DNA — adenine (A), guanine (G), cytosine (C) and thymine (T) — in a sample. These methods have been used to sequence DNA strands of varying lengths from individual genes to the 3 billion base pairs of the human genome. By analyzing this genetic information, researchers can identify sequence mutations to improve our understanding of the genetic causes of a range of diseases, with the added possibility of finding treatments.

Conventional sequencing technology employs automated machines to record the information contained in genetic material as raw data in the ‘Abi’ file format. “Anyone who works on gene sequences will be familiar with these files,” explains Gan. Until now, researchers have relied on conversion programs designed for personal computers to translate this data into more intelligible sequences of As, Cs, Gs and Ts, which has prevented them from completing sequence analysis while away from...
With DNAApp on the scene, scientists can now undertake this task wherever they may be.

**Mobile revolution**

In Gan’s eyes, mobile applications are the next frontier in helping scientists keep up with the increasing demands of speedier science, especially in the field of gene sequencing. “Fifty to sixty years ago, a researcher may have been able to publish a paper in the journal *Nature* after cloning a particular restriction enzyme,” notes Gan. “Today, even sequencing a whole genome may not generate sufficient data for publication in a high-impact journal.”

The formerly labor-intensive and time-consuming work of sequencing a gene can now be completed in a day at an industrial scale and without human intervention. Next-generation sequencing technologies promise complete sequences of five human genomes within a week, compared to the decade it took to decipher the first human genome. “Whole-genome sequencing is now part of the norm,” adds Gan. “Our standards have gone up and we need to move along with those standards.”

Until recently, however, mobile apps had not taken advantage of the processing power and tools built into smartphones in a way that really contributed to research. “Many existing apps are simply textbooks pasted into an application,” says Gan. “DNAApp could transform the way that scientists view their phones as a tool for increasing productivity.”

**Screening sequences**

DNAApp lets users work through DNA sequencing tasks with a few simple gestures — allowing them to easily visualize sequences and assess the quality of their samples. Researchers can copy, cut and paste sections of genetic code, and even search, locate and jump to sections of interest in the sequence.

Another useful function of DNAApp is the ability to convert a DNA sequence into its ‘reverse complement’, replacing each base in a sequence with its natural partner — A with T, and G with C. This feature is especially useful when initial sequencing is performed on the DNA strand that pairs with the strand of interest.

In addition, DNAApp’s ‘translation’ feature can interpret the sequence of bases as a chain of corresponding amino acids, enabling a deeper investigation of the effects of mutations, for example to determine the association between small changes in the DNA sequences that encode viral proteins and drug resistance.

Users are already finding DNAApp to be an essential tool. “It has released me from being stuck in front of a computer or a laptop,” explains Gan. “I can do sequencing analysis on the go: on the bus, on the train — basically anywhere.”

Feedback from those who have downloaded the app has been extremely positive and in recognition of the impact that the app is making, DNAApp has been accepted for publication by leading journal *Bioinformatics*, following the addition of extra features and the development of a comprehensive user guide. “It has been rewarding to create something that makes the life of a researcher easier and helps to move their research forward,” says Gan.

**Advanced automation**

Gan and Nguyen plan to develop further apps that they hope will make the life of the experimental scientist easier as well as establish the smartphone as a convenient tool for the lab. Frank Eisenhaber, executive director of the BII, has pledged his continued support for these endeavors, stating that: “We will continue to develop creative ideas for useful and efficient tools and techniques in computational biology for applications in the life science field.”

Smartphones, of course, take up only a slice of the innovation potential in the broader field of computational biology, points out Gan. His dream is to have robots perform experiments for him, so that he can concentrate his efforts on the theoretical — rather than the operational — aspects of his research. “And with advanced automation already built into a lot of laboratory equipment, that reality may not be too far off,” he says.
Life on the big screen

A prize-winning microscopy image of a developing mouse sperm cell taken by A*STAR scientists lights up Times Square in New York

Science and show business may sound like an unusual combination, but advances in technology mean that scientists can now capture dramatic images of their research that easily match the glamor of Broadway. A striking image captured by Graham Wright and Henning Horn from the A*STAR Institute of Medical Biology (IMB) in Singapore during their ground-breaking investigations into fertility was the regional winner in the microscopy category of the 2013 GE Healthcare Life Sciences Cell Imaging Competition. Fittingly, together with the other prize-winning images, the image was recently displayed on a large, high-resolution screen in New York’s iconic Times Square.

“The image is of a mouse sperm cell, also known as a spermatocyte, highlighted with three fluorescent labels that show DNA (blue), KASH5 protein (green) and the SCP3 protein (red), which is required for the pairing of chromosomes,” explains Wright. “This image was a particularly striking example when we captured it — the orientation of the proteins we were studying and the two sperm cells stained in blue on both sides made it aesthetically pleasing.”

The image was the result of a collaboration between Wright, head of the IMB Microscopy Unit (IMU), Horn, a senior research fellow, and the research teams of Colin Stewart and Brian Burke, also of the IMB, who discovered that the KASH5 protein is vital for successful chromosomal movements during meiosis — the division of cells necessary for successful sexual reproduction. Sperm and eggs need accurate chromosome pairing if they are to mature correctly, so without chromosomal activity guided by the KASH5 protein, fertility is adversely affected.

The researchers collected the image on a GE DeltaVision OMX microscope, which enables biological samples to be imaged in super-resolution in three dimensions. Wright and Horn spent time perfecting their sample preparation and honing the settings on the microscope to acquire their high-resolution prize-winning image. “The increased resolution we were able to achieve allowed us to visualize chromosome pairing events in spermatocytes.”

“Paired chromosomes — the paired red lines in the image — are not resolved by conventional light microscopy techniques,” explains Horn. “The ability to determine whether chromosomes are paired or not was critical for understanding the function of KASH5 in meiosis, so images such as this really can change how we understand diseases and problems such as infertility.”

At the IMB, research is focused on understanding a number of human diseases and medical conditions and providing improved treatments. Researchers have direct access to state-of-the-art equipment, including high-end microscopes, through core technology platforms such as the IMU.

By gaining further insight into the processes behind meiosis using these advanced microscopy techniques, Wright and Horn hope that their work will shed light on a range of biological processes, including those underpinning human fertility problems.

“Microscopy is usually used to visualize and analyze how proteins, cells and tissues are organized and how they behave,” states Wright. “But with fluorescent dyes and live-cell imaging techniques, we can find where proteins are located and follow what they do over time. This can give us excellent insights into the function of a protein and what goes wrong with it in the diseases we study.”

Collaborating to boost the microfluidics industry

The A*STAR Singapore Institute of Manufacturing Technology establishes three new industry collaborations to further innovation in microfluidics

Diagnostics, pharmaceutical development and drug delivery require manipulation of microscopic volumes of liquid, and microfluidics is the science of producing devices to do this. In a move that will speed up the growth and development of the microfluidics industry, the A*STAR Singapore Institute of Manufacturing Technology (SIMTech) has recently sealed three new collaborations with global companies, forming partnerships that will lead to novel and cost-effective microfluidic solutions.

The market for polymer-based microfluidic devices is growing, with its value expected to reach US$2.7 billion by 2018. SIMTech has already demonstrated its commitment to the industry by establishing the SIMTech Microfluidics Foundry (SMF) in 2011.

“The SMF spearheads innovations in microfluidics manufacturing technology and provides design, prototyping and production services for microfluidics development and applications,” says SIMTech’s director of research programmes, Wang Zhiping. “Through various collaborations with industry, SMF nurtures and grows the microfluidics industry by supporting the business and research community.”

The three new collaborations reflect the diversity in SIMTech’s microfluidics expertise. The first collaboration is with specialist manufacturer InziGn, in which SIMTech will transfer manufacturing technology to the company, allowing mass production of microfluidic devices.

“InziGn has 30 years of experience in precision tool making, particularly plastic injection mold fabrication,” says Steven Lau, InziGn director of product development. “This expertise in high-volume and high-quality medical manufacturing is well positioned for mass production of microfluidic devices.” The agreement will allow the SMF to continue supporting the development of novel devices while InziGn manufactures those that are ready to enter mass production for global markets.

The second collaboration sees the licensing of SIMTech microfluidics technology to Singapore-based biotechnology company Austrianova, which encapsulates living cells in microdroplets of nonreactive polymers. Austrianova’s ‘Cell-in-a-Box’ technology allows the isolation, protection, storage and transportation of living cells and has wide-ranging applications, from healthcare, to agriculture and environmental protection. Following the agreement, Austrianova will use SIMTech microfluidics-based droplet generation technology to make the cell encapsulation process more efficient.

“SIMTech’s droplet generator is made from a disposable polymer chip,” explains Austrianova CEO Brian Salmons, “meaning that sterilization is not required and the downtime of the manufacturing line is reduced.”

The final agreement initiates a research collaboration between SIMTech and UK-based diagnostics company QuantuMDx. “We are leveraging SIMTech’s microfluidics expertise to develop portable point-of-care assay cartridges for use as part of our handheld molecular diagnostics (MDx) device,” explains QuantuMDx co-founder and chief scientific officer, Jonathon O’Halloran. The assay cartridges being developed will provide microfluidics handling of samples, on-chip sample preparation, polymerase chain reaction capabilities and detection modules.

“The MDx device will be able to diagnose disease and detect drug resistance in less than 15 minutes, for just a few dollars, by the patient’s side,” says O’Halloran. Under the agreement, multiple cartridges will be developed for the universal device to test for diseases such as drug-resistant malaria, tuberculosis and sexually transmitted diseases, as well as to perform tumor profiling and companion diagnostics.

“The device will be particularly useful in areas lacking the healthcare infrastructure necessary for traditional laboratories, though the device’s low cost and rapid turnaround times will also benefit developed healthcare systems,” he notes.
Singapore — a knowledge-based economy known for its business efficiency and global competitiveness — is fast becoming the information and communications technology (ICT) capital of the world. In 2013, the World Economic Forum ranked Singapore as the most ‘network-ready’ country in Asia, well ahead of its neighbors Taiwan, South Korea and Hong Kong.

In view of rapid growth in Singapore’s infocommunications sector, the A*STAR Institute for Infocomm Research (I²R) has partnered with key players in Singapore’s ICT economy to found REACH@I²R (REsearch And Commercialization Hub), a cluster of joint laboratories designed to nurture technological innovation that meets the needs of multiple industries.

“REACH@I²R brings together a diversity of scientific capabilities to address the specific needs of local and international companies,” says Lee Shiang Long, executive director of I²R. More than 130 research and development personnel are already working in the joint laboratories and their expertise is helping to accelerate co-innovations with industry partners in finance, energy, telecommunications, healthcare, transportation, media and entertainment. “REACH@I²R truly epitomizes our concept of marrying research with industry,” Lee affirms.

To ensure the greatest impact, Lee is leading REACH@I²R with a system approach that harnesses I²R’s diverse range of scientific capabilities and is supported by several key initiatives, including the creation of a vibrant ‘ecosystem’ for ICT research that incorporates multinational and globally competitive companies, as well as small and medium enterprises. REACH@I²R focuses on fostering ICT innovation that brings value to Singapore’s economy, setting up the necessary infrastructure and processes for tighter integration with industry, as well as working with local government to ensure sustained investment in ICT in Singapore.

Already, the REACH@I²R cluster has produced several original ICT solutions. In the field of healthcare, these include a system for assessing visual acuity, performing augmented-reality laser surgery and detecting eye diseases, co-developed with Singapore’s National Healthcare Group and Tan Tock Seng Hospital, as well as technologies for detecting myopia, glaucoma and age-related macular degeneration, in collaboration with Japanese optical equipment manufacturer Topcon.

REACH@I²R is also making progress in the area of communications, including the development of energy-efficient networks for high-capacity communications, in conjunction with Singapore’s ST Electronics. In the area of human language and speech technologies, REACH@I²R laboratories are creating software for voice authentication, speech recognition, real-time interpretation and automated dialog replacement. One such example is a voice recognition software for unlocking smartphones, developed with Chinese web services company Baidu.

In the automotive industry, in conjunction with the Chinese automobile and new-energy company BYD, REACH@I²R is developing autonomous technologies for driving vehicles remotely, activating brakes and steering wheels. In the energy sector, smart grids for the production and distribution of electricity with significantly improved connectivity, security, stability and intelligence are the focus of a REACH@I²R collaboration with energy utility group Singapore Power.

Singapore’s positioning as an attractive gateway for companies looking to grow internationally has made A*STAR a partner of choice for the co-creation of knowledge and innovation for the global market. “We have many companies that are proactively investing in joint labs at REACH@I²R. They have faith in our capabilities and decided to co-innovate with us on a sustained basis to create a competitive edge for themselves,” notes Lee. “Embracing research and development in this way is set to positively impact the infocommunications ecosystem in Singapore.”
Technology on the catwalk

Researchers at A*STAR find innovative ways of incorporating their technologies into wearable fabrics and electronics

Summer days bring thoughts of beach picnics, outdoor barbecues and pool parties. Yet it only takes the buzz of one tiny mosquito to dampen the fun.

But what if your outfit was equipped to release the scent of lemongrass? A quick rub of your hands over the fabric and the pests would be kept at bay by the odor they so despise. This proposal was just one of many wearable technologies caught walking down the runway earlier this year at a showcase organized by A*STAR’s technology transfer arm, ETPL (Exploit Technologies Pte Ltd), during the Startup Asia Singapore 2014 conference.

To realize this bug-repelling design concept, Loh Xian Jun at the A*STAR Institute of Materials Research and Engineering (IMRE) developed a sprayable mixture of a fragrance oil and a polymer that emits its scent at a sustained rate. The process is based on the same microencapsulation technology used to control the release of drugs.

Rather than tackle mosquitoes, the mixture was used at the showcase to enhance the sensory experience of several elegant dresses designed by students at Nanyang Technological University in Singapore, under the guidance of visiting assistant professor Galina Mihaleva. Among the other wearable technologies enriching the fashion collection were luminescent silks, printed electronic materials that switch from opaque to transparent in response to changes in temperature, and sensor beads that darken under extended exposure to ultraviolet light.

The purpose of the technology showcase, entitled Next-to-the-Skin, was to bridge the gap between research and product development, explains assistant vice president at ETPL, Radiana Soh. “We sought to create a platform for scientists, fashion and industrial designers, and industry professionals on which they could engage and ideate at the early stages of commercializing a technology.”

By introducing the concept of ‘design thinking’ in the early stages of the technology transfer process, ETPL was able to offer A*STAR researchers a new perspective on developing products that address consumer needs while providing innovators with a better understanding of the technologies available to them.

“The process was a game-changing experience for me,” reflects researcher Santiranjan Shannigrahi from the IMRE, who created the ultraviolet-sensitive beads. “I would never have thought that our materials could find a use in such applications.”

The success of this new way of thinking was apparent in the host of prototypes developed by A*STAR researchers in the month leading up to the final exhibit.

For example, Serene Ng Lay Geok at the A*STAR Data Storage Institute invented SleepPro, a smart sleeping pillow that uses patented software developed by the A*STAR Institute for Infocomm Research to monitor the sleeper’s heart rate, breathing and sleep patterns. The comfortable headrest can be used by elderly or sick patients to alert caregivers of any abnormal behavior.

Another prototype presented at the showcase was MKool, a cooling pad designed by Shah Kwok Wei, a researcher at the IMRE. The pad combines heat-conductive nanofibers and a phase-change material that stores heat by changing its physical state to keep the body’s temperature at a comfortable level. Nanosilver ions, also incorporated into the fabric, confer the pad with antibacterial and odor-killing properties.

Beyond highlighting the potential for technological innovation at A*STAR, Next-to-the-Skin also emphasized the commercial viability of prototypes such as MKool, notes Soh. “We hope to accelerate the process of bringing our products to market by creating an ecosystem for early-stage collaboration through events such as this.”
Research Highlights

CELL BIOLOGY & IMMUNOLOGY
**The secretions of stem cells**

Tiny vesicles secreted by mesenchymal stem cells can modulate the immune system and prevent the rejection of grafted tissue

Mesenchymal stem cells (MSCs) can be extracted from many different types of tissues and are currently used in clinical trials for a range of conditions, including autoimmune diseases. Now, a team of researchers led by Sai Kiang Lim at the A*STAR Institute of Medical Biology in Singapore has demonstrated that small vesicles secreted by MSCs, called exosomes, can exert anti-inflammatory effects on immune cells in tissue culture and also in mice that have received skin transplants.

Exosomes contain a variety of proteins and other factors from their originating cells. Previous studies have shown that MSC-derived exosomes protect heart tissue, prompting Lim and colleagues to investigate whether MSC exosomes could have an effect on immune-cell function. The researchers isolated MSC exosomes but found that they did not have a direct effect on the proliferation of lymphocytes — a type of white blood cell — taken from the spleen. However, because lymphocyte function is steered by monocytes, another type of white blood cell, the researchers decided to further investigate whether MSC exosomes could activate monocytes instead. They discovered that MSC exosomes reduced the expression of pro-inflammatory factors and increased the expression of anti-inflammatory factors in monocytes.

Toll-like receptors on immune cells are proteins that initiate the immune response following activation by many different factors, including one found in MSC exosomes called fibronectin 1 (FN1). By blocking FN1 with an antibody, the researchers were able to reduce the ability of MSC exosomes to activate monocytes.

When Lim and colleagues exposed the exosome-treated monocytes to developing T cells, a type of lymphocyte, the cells matured into regulatory T (T<sub>reg</sub>) cells — a cell type that suppresses the immune system. The findings suggest that MSC exosomes probably act directly on monocytes, which can then modulate lymphocyte maturation and function.

As a result of their immunosuppressive properties, T<sub>reg</sub> cells can help to prevent the rejection of skin grafts by the immune system. To test whether MSC exosomes could facilitate the process, the researchers grafted skin onto mice, and then treated some of the grafted mice with MSC exosomes. Rejection of the grafted skin was delayed by a few days in the exosome-treated mice compared to normal mice, probably due to the higher levels of T<sub>reg</sub> cells in the exosome-treated mice.

“Our findings suggest that MSC exosomes could be used to alleviate diseases that have a dysfunctional immune component, such as lupus, psoriasis and sepsis,” explains Lim. “A*STAR is currently funding the clinical development of MSC exosomes,” he says.

Developmental biology:

Helping cultured stem cells get back to nature

Cultured human embryonic stem cells given the ‘3iL’ treatment more closely resemble natural stem cells from the developing embryo

Human embryonic stem cells (hESCs) have the ability to both convert into any cell type in the human body and to proliferate indefinitely in the laboratory. However, cultured hESCs, which are plucked from the developing embryo and then grown in vitro, often display a number of biological differences when compared to the pluripotent epiblast cells in the early embryo from which they originated.

“We found that pluripotent human embryonic stem cells can be transformed into a state that is closer to how they appear in real life,” says Huck Hui Ng, executive director of the GIS, who led the research. “This new cell state has the potential to improve many applications of hESCs, such as disease modeling.”

Now, a team of researchers at the A*STAR Genome Institute of Singapore (GIS) has developed a way to make hESCs more closely resemble true epiblast cells.

“We found that pluripotent human embryonic stem cells can be transformed into a state that is closer to how they appear in real life,” says Huck Hui Ng, executive director of the GIS, who led the research. “This new cell state has the potential to improve many applications of hESCs, such as disease modeling.”

To induce the more native state, Ng and his colleagues screened 11 small molecules to find those that promoted the expression of NANOG, a gene involved with self-renewal. The researchers identified a cocktail of three chemicals, plus the growth factor LIF (3iL), that nearly doubled NANOG expression levels in the hESCs while maintaining these cells in a state of perpetual growth (see image). They called the resulting stem cells ‘3iL’ hESCs.

The chemically treated and non-treated hESCs expressed similar levels of a handful of genes that underlie pluripotency, the ability to both self-renew and to develop into any cell type. But only the 3iL stem cells had a gene expression profile that closely matched that of epiblast cells taken straight from early embryos. Study author Jonathan Göke suggests that laboratory culturing could have rewired certain regulatory networks in conventionally grown hESCs. “The 3iL treatment potentially resets this process,” he says.

“Culture conditions have been optimized to support self-renewal, and these culture conditions are very different from the complex signaling and communication system of embryos,” notes Yun Shen Chan, another author on the study. “Therefore, it seems like the culture conditions used to grow hESCs might partly explain the differences between lab-cultured hESCs and in vivo epiblast cells.”

The researchers also explored whether the 3iL recipe could be applied to induced pluripotent stem (iPS) cells, which are adult cells reprogrammed into an embryonic-like state. “A full comparison of 3iL iPS cells with ‘standard’ iPS cells still has to be done,” Chan says, “but our preliminary results indicate that 3iL could result in iPS cells of higher quality.”

Bioanalysis:

Focusing on the sweet spot

A novel targeted mass spectrometry technique uncovers an elusive sugar modification in stem cells

Recently, O-linked N-acetylglucosamine (O-GlcNAc), a sugar ring that reversibly modifies proteins inside the cell nucleus and cytoplasm in a process known as O-GlcNAcylation, has been revealed to be a key regulator of cell signaling in the body. Researchers believe that cellular changes induced by O-GlcNAc may be linked to chronic diseases, such as diabetes and cancer. Characterizing such dynamic relationships, however, is challenging.

Normally, O-GlcNAc detection relies on ‘shotgun’ mass spectrometry (MS), which breaks proteins into sequence-specific peptide chains and measures their molecular weight. But as O-GlcNAcylated peptides are present in such small amounts, building up concentrations sufficient for detection requires time-consuming and tedious enrichment methods.

Now, Julien Maury and Andre Choo from the A*STAR Bioprocessing Technology Institute in Singapore and co-workers have developed a simple way to detect native O-GlcNAcylated proteins — even at 10,000-fold dilution — with a targeted MS technique known as multiple reaction monitoring (MRM)¹.

Instead of scrutinizing all possible peptides from a protein system, MRM-MS filters out masses that correspond to an expected precursor ion. Then, the joined-up precursor peptide and selected fragment ions — termed ‘transition couples’ — are monitored to build up a quantitative assay of the protein structure.

Choo explains that by focusing on specific O-GlcNAc-modified targets, MRM-MS enhances detection limits without extensive labeling or enrichment techniques. Furthermore, he notes that the method’s ability to spot sugar modifications in complex peptide mixtures could greatly simplify cell bioanalysis.

After programming the MRM-MS system to spot standard O-GlcNAcylated peptides, the team turned their attention to the enzyme glycogen synthase kinase-3 beta (GSK-3β), which competes with O-GlcNAc for binding sites on proteins and is linked to numerous high-profile diseases. Although unconfirmed, GSK-3β may itself be modified and regulated by O-GlcNAc.

To investigate O-GlcNAcylated GSK-3β peptides, the researchers used gel electrophoresis to extract GSK-3β from proteins derived from human embryonic stem cells (hESCs). Subsequent MRM-MS analysis of the sample revealed the presence of a novel O-GlcNAcylated GSK-3β peptide with three potential binding sites — a modification with potential significance for the enzyme’s autoinhibition mechanism. Using their technique, the researchers could also quantify changes in O-GlcNAcylated GSK-3β peptide levels following hESC drug treatment.

Choo and Maury anticipate that their work could help to detect and quantify O-GlcNAcylated peptides in samples where protein amounts are scarce, such as mouse brains. “Additionally, scientists want to know the dynamic variations in O-GlcNAc following drug treatment and cell differentiation studies,” they say. “Quantification by MRM-MS is exactly suited to these sort of investigations.”

Protein samples ready for mass spectrometry (MS); multiple reaction monitoring MS provides a reliable means to detect protein modification and may shed light on the cellular mechanisms behind chronic diseases.

Embryonic stem cells (ESCs) have the potential to form more than 200 distinct cell types in the human body. Although ESCs can differentiate into any specialized tissue, scientists are still unsure of how to coax these pluripotent cells to reliably form a desired cell type without producing a mix of contaminating cell lineages.

“We can precisely differentiate embryonic stem cells into a pure population of a given lineage of interest.”

A research team led by Bing Lim from the A*STAR Genome Institute of Singapore has now developed a new method for directing ESCs into highly pure populations of liver cells or pancreas cells. These untainted populations of organ-specific cells could form the basis of future therapies or be used as platforms upon which to screen drugs.

“Heterogeneous mixtures of cell types are unsuitable for transplantation or other therapeutic purposes,” says Kyle Loh, a previous member of Lim’s lab now based at the Stanford University School of Medicine in the United States. “We can precisely differentiate embryonic stem cells into a pure population of a given lineage of interest,” he explains.

Lay Teng Ang of the liver research program in Lim’s lab sought a way to guide human ESCs to differentiate reliably into endoderm — the cell type that gives rise to organs including the lungs, liver and intestines. To do this, Ang systematically perturbed developmental signals at four consecutive steps of endoderm formation, searching for molecules that could produce a single, desired cell type as well as block the induction of unwanted alternatives. Along the way, the researchers generated what Lim calls “a roadmap for endoderm differentiation.”

Their strategy showed that a variety of growth factors and signaling proteins initially help transform ESCs into endoderm progenitor cells. However, the researchers had to inhibit these same molecules within 24 hours to prevent the cells from turning into another tissue type known as mesoderm.

Knowledge of this timing and the downstream signaling dynamics eventually allowed Lim’s team to differentiate the ESCs into pure populations of liver and pancreas cells, while excluding other lineages at each developmental branch point. The researchers also noted the endodermal enhancers existed in a surprising diversity of ‘pre-enhancer’ states as uncommitted cells prior to activation: they also documented the ‘permissive’ chromatin marks that provide ESCs with their flexible developmental capacity.

“We first needed to understand the signals that control stem cell differentiation — and thus what controls lineage splitting,” Loh says. “Then we could unilaterally repress differentiation of ESCs toward other cell types and instead drive stem cells exclusively toward a uniform population of desired stem cells.”

Stem cells: The fat source makes the difference

**Stem cells derived from different types of fat express different cell-surface markers**

Mesenchymal stem cells (MSCs) have a natural ability to differentiate into various cell types, such as muscle, cartilage and bone. They can be classified according to their source and include adipose-derived stem cells (ASCs) and bone-marrow-derived stem cells (BMSCs). ASCs, in particular, hold tremendous potential for tissue engineering and regenerative medicine because of their relatively high abundance and ease of isolation.

Shigeki Sugii at the A*STAR Singapore Bioimaging Consortium and co-workers have now isolated ASCs from two different sources of fat: subcutaneous fat found underneath the skin and visceral fat from inside the abdominal cavity. The team showed that ASCs derived from subcutaneous fat express cell-surface markers that differ from those derived from visceral fat. The finding has implications for determining the origins of ASCs and the roles of their different subtypes in metabolism-related conditions, such as obesity, and diseases such as soft-tissue tumors.

Stem cells, like all other cells, express molecules at their surface that are recognized by the body's immune system. Like a fingerprint, the expression profile of these cell-surface markers is unique to each stem cell type. Scientists already know that MSCs express the cell-surface markers CD73, CD90 and CD105 but not CD14, CD19, CD34 and CD45. Recent studies have also shown that while ASCs express CD36 but not CD106, the opposite is true for BMSCs. Thus, MSCs derived from different tissues express different cell-surface markers, providing a valuable tool for determining the origins of MSCs.

Sugii and co-workers therefore proposed that ASCs derived from different types of fat may also express different cell-surface markers. To investigate this, they extracted subcutaneous and visceral fat from 12 obese patients, as well as normal and obese mice. After isolating ASCs from the fat, the team analyzed the expression profiles of over 240 cell-surface markers for each sample. Their analysis revealed a high level of CD10 expression in ASCs derived from subcutaneous fat compared to ASCs derived from visceral fat. In addition, they detected a high level of CD200 expression in ASCs derived from visceral fat compared to those derived from subcutaneous fat.

"Our results suggest that CD10 and CD200 are markers of high and low adipogenic capacities," says Sugii. "Therefore, CD10 and CD200 are biomarkers as well as indicators of adipogenic potentials for use in high-throughput drug-screening systems."

The immunoglobulin E (IgE) antibody released by the immune system is a pivotal defense against gut parasites and toxins. However, the same antibody when misdirected can also cause allergic responses to food or substances in the environment. An international team led by researchers at the A*STAR Singapore Immunology Network has now discovered a regulatory mechanism that keeps IgE levels in check.

A better understanding of the process of IgE production and how it can be subverted should enable scientists to develop the next generation of allergy treatments. “If we know the mechanism, potentially we could intervene therapeutically to prevent the development of severe allergic and anaphylactic reactions,” says A*STAR immunologist Maria Curotto de Lafaille, who led the research.

A type of white blood cell known as a B cell can change the antibodies that it produces through a process known as immunoglobulin class switching. B cells that produce IgE can arise through one of two distinct switching mechanisms. Either the B cells go through a sequential process that involves another type of immunoglobulin, IgG, as an intermediary, or they take a direct route to IgE generation. The sequential process creates IgE with high affinity for antigens — the substances that can trigger an immune response and can cause severe allergic reactions. In contrast, the direct switching pathway produces IgE antibodies that bind their antigens weakly. These low-affinity antibodies can compete with the problematic IgE to prevent anaphylaxis.

The origin, functional properties and population dynamics of IgE-producing cells had been poorly understood. To track the dynamics of IgE production in a living system, Curotto de Lafaille and her colleagues created a new mouse strain that fluoresces whenever and wherever IgE antibodies are produced. Using the mouse model, the researchers showed that IgE-producing cells that undergo the direct switching pathway at germinal centers — sites in the lymphatic system where B cells differentiate and mature to enhance their antigen binding — tend to get stuck in that developmental state and end up ‘failing to thrive’. “These cells have a lot of defects and they end up dying,” says Curotto de Lafaille.

The failure of the IgE cells to directly produce antibodies with strong binding ability imposes a strong constraint on IgE-based immune responses. “If you easily make so many high-affinity IgE cells, you’d be constantly in danger of life-threatening reactions like anaphylaxis,” Curotto de Lafaille explains. “That’s why there’s an evolutionary pressure to limit this process.”

The binding of hormones to their receptors plays a key role in the development of many organs of the body. The apelin receptor is expressed in the developing embryo, coming online many hours prior to its presently known ligand, apelin. Organisms that lack apelin have less severe developmental defects than those lacking the receptor, leading scientists to believe that there is an alternative ligand for the apelin receptor that is expressed during the very early stages of development.

Now, Bruno Reversade and colleagues at the A*STAR Institute of Medical Biology and the A*STAR Institute of Molecular and Cell Biology in Singapore have identified the hormone ELABELA as an apelin-receptor ligand that is present at the earliest stages of development. The team’s findings suggest that ELABELA plays a key role in the maturation of the endoderm and the creation of the heart.

Early embryonic development is characterized by the development of three germ layers: the ectoderm, which forms skin and nervous tissue; the mesoderm, which forms the cardiovascular system; and the endoderm, which forms the inside of the gastrointestinal tract.

When the researchers generated zebrafish embryos lacking ELABELA expression, they found reduced levels of early endodermal markers in the early stage embryos. If they allowed the embryos to grow to later developmental stages, Reversade and colleagues found that the zebrafish hearts either did not develop or were highly abnormal. These findings suggest that ELABELA is required for the maturation of endodermal cells that drive the development of nearby mesodermal cells, which in turn go on to form the heart.

As the abnormal heart phenotype in ELABELA-deficient zebrafish embryos was similar to that of apelin-receptor-deficient embryos, the researchers decided to investigate if ELABELA acts by binding to the apelin receptor (see image).

They discovered that ELABELA and the apelin receptor are expressed at the same time and similar location in the early embryo. Expressing the apelin receptor in cell lines that did not normally express it allowed binding of ELABELA to the receptor at the cell surface, says Reversade. “These results suggest that ELABELA is the earliest ligand to bind to the apelin receptor through the role it plays in driving the primary stages of the development of the embryo.”

ELABELA’s importance to heart formation could also apply to the development of other organ systems. “ELABELA is expressed in kidneys and prostate in humans, but its role there remains unknown for now,” notes Reversade.

The ELABELA hormone has been identified as an important apelin-receptor ligand in early heart development. Wild-type zebrafish embryos have normal cardiac development (top), while embryos lacking the apelin receptor (center) develop similar cardiac deformities to embryos lacking ELABELA (bottom).

Newly identified hormone ELABELA regulates the generation of the embryo’s endoderm and is required for the normal formation of the heart.

Fishing for new therapeutics

A zebrafish-based screening method reveals compounds that could help contain the effects of immunological disorders

Immune cells known as neutrophils are recruited by chemical signals released from sites of injury and infection. Their primary purpose is to attack pathogens and recruit additional immune defenses, but these cells can also inflict tissue damage through prolonged activation, contributing to serious diseases such as inflammatory bowel disease and chronic obstructive pulmonary disorder.

Philip Ingham and colleagues at the A*STAR Institute of Molecular and Cell Biology in Singapore have developed a fish-based screening technique that can rapidly identify compounds to potentially control such excessive neutrophil activation.1 Previously, Ingham collaborated with Stephen Renshaw’s team at the University of Sheffield, United Kingdom, to generate a genetically modified zebrafish strain in which neutrophils are selectively labeled through production of a fluorescent protein.2

The zebrafish model allowed for easy observation of neutrophil behavior in the transparent living zebrafish embryos and larvae. “We showed that neutrophil migration could be blocked by exposing fish larvae to known migration inhibitors,” explains Ingham. Following on these findings, Ingham and Renshaw adapted the model to screen a library of fungal extracts and identify new molecules with similar migration-inhibiting effects.

“Most chemical screens in zebrafish use pure compounds of known chemical composition,” says Ingham. “We were able to discover compounds with specific biological effects by screening fairly complex mixtures found in natural extracts.”

Starting with over 1,000 extracts produced by Singapore-based company MerLion Pharmaceuticals, the researchers identified two extracts that inhibited neutrophil recruitment in zebrafish following tail injury. Fractionation of the two revealed the active ingredients responsible for the effect. Since one of the molecules had previously been linked to toxic effects, Ingham and colleagues focused on the other molecule, PF1052.

Notably, they found that PF1052 specifically blocks neutrophil migration to zebrafish tail wounds without affecting other immune cells (see image). They also found that the extract appears to block formation of the structures that coordinate cellular movement. It does this by interfering with the process of cellular polarization — the mechanism by which cells establish their relative orientation. Interestingly, PF1052 does not affect polarization via established mechanisms, which, Ingham suggests, shows there is a novel route to controlling the inflammatory response.

Although the research effort was primarily intended as a proof-of-concept study, Ingham believes that it opens up wider possibilities and that the effects of PF1052 merit further investigation. “The next step would be to test PF1052 in an in vivo mammalian inflammation model to verify its efficacy and at the same time assess its potential toxicity,” he says.


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Cell biology:

Detailing the development of red blood cells

Understanding the diversity of immature red blood cells in greater detail could help protect against Asia’s most common form of malaria

Red blood cells are released into the blood stream in their immature form — reticulocytes — from the bone marrow where they develop. Reticulocytes are important markers for certain blood disorders and infectious diseases but their maturation has been poorly understood. Now, an international research team led by Laurent Renia from the A*STAR Singapore Immunology Network has characterized, in fine detail, the properties of reticulocytes at different stages of maturation.

Studies in the 1930s identified distinct subtypes of reticulocytes but only provided basic descriptions of their different maturation stages. According to Renia, these early studies have mostly been forgotten, and more detail is needed to better understand the reticulocyte maturation process.

“The quantity and type of reticulocytes released into the circulation provide important information for the diagnosis and prognosis of certain diseases,” explains Renia. “Despite the significance of reticulocytes, limited information is available about their biology and it is incorrectly assumed that reticulocytes are a fairly uniform type of blood cell.”

Aided by recent advances in fluorescent staining, Renia and his team set out to describe the properties of reticulocytes at different stages of maturation by studying cells from the reticulocyte-rich blood of the human umbilical cord. They separated the reticulocytes into four subpopulations according to the level of their expression of a specific surface protein, which decreases as the cells mature.

“This helped us to fully characterize the chemical and biomechanical differences of these reticulocyte stages,” says Renia. They found that as reticulocytes mature, the outer membrane and internal structures are reorganized, creating cells that are smaller, less rigid and have the characteristic concave shape of red blood cells (see image). They also revealed changes in expression levels of specific proteins and the attenuation of metabolic pathways.

“We uncovered an incredible level of reticulocyte heterogeneity characterized by significant chemical, biophysical and metabolic modifications,” says Renia. “No-one else has characterized reticulocytes on such a fine scale.”

The differences the team noted demonstrate that reticulocyte maturation is a continual process, meaning that reticulocytes should not be grouped together as one cell type. In addition, the finding hints that the maturation process could have direct clinical applications.

“Reticulocytes are important in the study of vivax or ‘relapsing’ malaria, the most common form of malaria in Asia, because the Plasmodium vivax parasite specifically targets reticulocytes,” he says. “We hope our findings will help to develop vaccines that block the blood stage life cycle of vivax malaria parasites.”

City life can be an assault on the senses — quite literally, in the case of allergies. The steady increase in global urbanization is mirrored by a growing prevalence of allergy-associated respiratory problems. Potential triggers include insects, mold, pollen and animal hair, but now A*STAR researchers have uncovered a single culprit with a disproportionate role in allergy onset in tropical urban settings.

Allergies arise when the body mounts an immune response to a foreign molecule that it mistakenly perceives as a threat. Whenever the body encounters that trigger — for example, a pollen grain — it produces large numbers of antibodies against the trigger. The resulting inflammatory response can cause symptoms including asthma and rhinitis. Olaf Rotzschke and colleagues from the Singapore Immunology Network and De Yun Wang of the National University of Singapore began their study by surveying the antibody responses of 206 volunteers to a dozen common allergic triggers. Remarkably, the great majority of these Singapore-born individuals responded to one particular antigen: the tiny dust mites found in many homes (see image). This trend remained clear even after the researchers expanded their cohort to look at a larger group of individuals. “According to our study, 80 per cent of Singaporeans respond to the mite, with roughly 40 per cent developing allergic rhinitis and 15 per cent developing asthma,” says Rotzschke. “Globally these are among the highest figures reported so far.”

The allergic reaction appears to be a consequence of the Singaporean urban environment. When the researchers examined newly arrived Chinese immigrants, fewer than 30 per cent mounted a strong antibody response against dust mites; in contrast, for immigrants who had lived in Singapore eight years or longer, the response rate was indistinguishable from lifelong Singapore residents. “This phenomenon of gradual acquisition of an allergic reaction has been shown in other countries for other allergens as well,” explains Rotzschke. However, in temperate regions in Western countries, the most common allergic trigger is pollen, suggesting that this dust-mite-associated sensitization may be more characteristic of tropical cities like Singapore.

By identifying a single target, the findings could be used to provide relief to large numbers of allergy sufferers. But the existence of such a large population with a shared, strong response to a single antigen has broader implications for research as well, notes Rotzschke. “We are currently planning a functional analysis of immune pathways in combination with genome-wide genetic studies to better characterize the molecular and genetic basis of allergies,” he says.

Stem cells:

Animal-free reprogramming improves safety

Reprogramming of adult cells in animal-free conditions provides a safer culture system for therapeutic stem cells

Human stem cells produced through genetic reprogramming are beset by safety concerns because current techniques alter the DNA of the stem cells and use material from animals to grow them. Now, A*STAR researchers have developed an efficient approach that produces safe, patient-specific human stem cells.

Human induced pluripotent stem cells have the potential to treat a number of diseases without the ethical issues associated with embryonic stem cells. Pluripotent stem cells can be produced from adult cells by introducing genes that reprogram them. Typically, the stem cells are grown on a layer of mouse cells in solutions (known as media) that contain animal proteins — and therefore, potentially may also carry disease. For such stem cells to be safe for use in humans, they need to be grown in ‘xeno-free’ conditions, which are devoid of material from other animals.

Andrew Wan and Hong Fang Lu at the A*STAR Institute of Bioengineering and Nanotechnology in Singapore and colleagues set out to develop a new xeno-free system. The researchers carried out the genetic reprogramming of cells on an artificially produced protein substrate rather than mouse cells. They also used media that contained no animal components. The result was more efficient reprogramming than seen with conventional approaches.

“A xeno-free system will eliminate the risk of disease transmission from other species, which is important for regulatory approval,” explains Wan. “Yet there have been few studies on cell reprogramming under totally xeno-free conditions.”

The researchers went one step further by addressing the problem of cells acquiring alterations to their DNA during reprogramming.

“Incorporation of transgenes into the genome of the cell poses another safety issue, risking unwanted genetic alterations,” explains Lu. “In our work, the transgenes were introduced to initiate the reprogramming, but after this they were removed from the cell, leading to transgene-free stem cells.”

The researchers demonstrated that after genetic reprogramming and the removal of the added genes, the stem cells could still develop into different cell types. They were even able to induce them to form dopaminergic neurons, the type that degenerates in Parkinson’s disease. The conditions in which the stem cells were grown mean that they are suitable for clinical use and can be derived from a patient’s own cells, ensuring complete compatibility.

“Regulatory approval for clinical application of stem cells largely depends on the conditions in which the stem cells are derived,” says Wan. “We present a workable protocol for the reprogramming of fibroblasts to stem cells that minimizes any potential safety risks.”

The human body maintains a healthy layer of skin thanks to a population of stem cells that reside in the epidermis. Previously, the signals responsible for regulating these so-called ‘interfollicular epidermal stem cells’ (IFESCs) were unclear, but now scientists in Singapore and the United States have shown that these cells secrete proteins in the Wnt signaling pathway to control their own balance between renewal and differentiation.

"The mechanism of stem cell function that we describe is novel to the skin and, as far as we know, also unprecedented in the entire stem cell field," says Xinhong Lim of the A*STAR Institute of Medical Biology, who led the study. “Skin stem cells themselves can be a source of stem cell self-renewing signals. This expands the paradigm for how mammalian stem cells are regulated.”

Lim and his colleagues focused on Wnt proteins because of their well-established roles in stem cell maintenance and hair growth. To determine if IFESCs respond to Wnt signals, the researchers created transgenic mice in which they could visually track the fate of cells producing Axin2, a protein triggered by Wnt activity.

They discovered that Axin2 was expressed by cells that contribute to wound healing, found in the deepest layer of the epidermis (see image). Through detailed molecular analysis, the team confirmed that these cells were IFESCs. In addition, they noted that the same stem cells also expressed several Wnt family genes themselves. Together, these findings indicate that IFESCs are both the source and the target of Wnt signals.

The researchers concluded that the self-renewal of IFESCs is maintained by an autocrine or self-stimulating loop of Wnt production. “The stem cells, rather than a separate stem cell niche, regulate epidermal thickness and regeneration,” Lim says.

These results led the team to question how the IFESCs could escape this regulatory loop and commence the differentiation process. Seeking answers, Lim and his colleagues tracked the expression patterns of Wnt inhibitors in the skin.

They found that while the stem cells also secreted Wnt inhibitors, these signals localized to the cells situated above the stem cell compartment, reinforcing differentiation there. “By producing both an autocrine Wnt signal and a paracrine Wnt inhibitor,” Lim explains, “the stem cells in the skin act as the organizing center for the tissue.”

The team’s discovery could lead to new therapies that manipulate Wnt signaling to improve wound healing. According to Lim, it could advance skin stem cell culture protocols, helping scientists to grow skin grafts more efficiently.

The upper respiratory tract is the first line of defense against air pollutants, including allergens, bacteria and environmental toxicants. Finger-like protrusions called cilia on the surface of the human mucous membrane, or epithelium, sway back and forth when irritated. This coordinated ‘beating’ movement of the cilia helps to remove foreign materials and is an important protective mechanism.

Wei Wang and Zhi Ping Wang at the A*STAR Singapore Institute of Manufacturing Technology, De Yun Wang at the National University of Singapore and co-workers have now developed the first microfluidic device that enables the direct observation of cilia and their beating frequency on a polyester membrane. The artificial system is used to observe the effects of air pollutants on cells in the upper airway.

The researchers constructed their microfluidic device using glass and a transparent, moldable polymer to ensure a clear view of the cilia and their activity. A membrane — designed to support the cultivation and differentiation of human nasal epithelial stem cells — was inserted into a small chamber on the device and fresh or contaminated air was fed through a tiny channel.

Five weeks after seeding the membrane with human nasal epithelial stem cells the researchers could observe the formation of beating cilia. The cilia beating frequency varied across samples but the difference was typically within a few hertz.

When fresh air was passed into the chamber, the researchers observed a 3 per cent drop in cilia beating frequency relative to the baseline value. In contrast, when air mixed with 0.5 milligrams per cubic meter of formaldehyde was passed through the chamber, they observed a 7.4 per cent increase in cilia beating frequency relative to the baseline value.

Further increasing the formaldehyde concentration to 1 milligram per cubic meter led to a dramatic increase — up to 136.4 per cent — in cilia beating frequency relative to the baseline value. At even higher formaldehyde concentrations of 3.0 milligrams per cubic meter, however, the researchers observed an unexpected decrease in cilia beating frequency, possibly due to irreversible cell damage caused by the formaldehyde.

By enabling the observation of cilia beating frequency, the experimental model described in this work provides more realism for clinical applications. The device can be used to directly test for toxicity and toxic mechanisms, screen for drugs that reduce irritation, and assess the level of risks associated with a particular air pollutant. “The technology has applications in chemical analysis, environmental monitoring, medical diagnostics and cellular studies,” says Wang.

Data storage:

Ensuring solid-state drives are up to scratch

A data buffering scheme improves the performance of solid-state drives in large-scale, data-intensive applications

Solid-state drives (SSDs) store digital information using electronic circuits. The power efficiency of SSDs and their ability to read and write data quickly means that they are becoming the primary storage device in computers. A major drawback of SSDs, however, is the limited number of times that data can be stored and deleted — an aspect that hinders the use of these devices for data-intensive applications known as data-center environments.

Qingsong Wei and co-workers at the A*STAR Data Storage Institute and National University of Singapore have developed a scheme for writing data to SSDs that could circumvent these problems to make solid-state drives useful for an even broader range of applications.

SSDs divide their storage space into distinct areas called blocks. A computer can either save large files across consecutive blocks — a process known as sequential writing — or write smaller files in blocks scattered throughout the device — so-called random writing.

The researchers conducted an intensive workload study of the distribution of read and write request sizes over ten real enterprise workload traces supplied by the Storage Network Industry Association. They found that the highest traffic was from small, random requests of less than 64 kilobytes in size.

Generally, random writing is much slower — by as much as four times — than sequential writing. One way around this bottleneck is to use part of the memory as a ‘buffer’. The buffer briefly stores data as it comes into the drive, which then enables sequential writing at a later time. Current buffer management approaches improve sequential writing but only at low buffer usage, wasting expensive buffer space.

Wei’s team helped to solve this problem through an alternative approach that categorizes the data in the buffer by its popularity, which reflects how frequently the data is likely to be needed. The scheme retains popular blocks in the buffer, rather than deleting them, and sequentially writes less popular blocks to the SSD.

“Our buffer management scheme can increase sequential writing with high buffer utilization, thus improving performance and extending the lifetime of the SSD,” says Wei.

The researchers tested the approach and demonstrated that the so-called popularity-aware buffer management scheme, or PAB, can achieve an improvement in performance of up to 72 per cent and triple the device lifetime compared to existing schemes. “Our method reduces the cost of SSDs by improving buffer utilization and is easy to implement,” explains Wei.

“Our next step will be to design smarter SSDs by integrating these same ideas with emerging non-volatile memory.”

The shrinking dimensions and decreased power consumption of modern electronic gadgets have created opportunities for energy harvesting processes that tap into free, green energy from the environment. Vibration harvesters, for example, produce small amounts of electricity from everyday mechanical disturbances such as wind currents, traffic noise or footsteps.

Now, Kui Yao and co-workers from the A*STAR Institute of Materials Research and Engineering in Singapore have discovered a way to give lightweight polymer vibration harvesters a hundredfold boost in energy output — a finding that may help to eliminate manual battery recharging in microsensors and mobile devices.

Many vibration harvesters contain piezoelectric substances that create an electric voltage when mechanically bent. By fabricating piezoelectric materials into cantilevers that resemble a diving board, these devices can oscillate from ambient vibrations and generate electricity. Researchers often use piezoelectric ceramics because they impart large amounts of electrical charges; however, the brittleness of ceramics makes them unsuitable for prolonged and large vibrational movements.

Yao and co-workers investigated a plastic-based piezoelectric material, polyvinylidene fluoride (PVDF), which is low cost and readily undergoes mechanical strain. To make efficient vibration harvesters from PVDF, researchers must stack the polymer in multiple layers, improving the output current and reducing the electrical impedance that is inherent to piezoelectric materials. But when too many thin piezoelectric layers are stacked, the cantilever can become too stiff for bending-mode vibrational harvesting.

To optimize piezoelectric harvesting with plastic films, the team deployed an analytical approach. Developing a mathematical model of a multilayered polymer cantilever coated with metal electrodes, the researchers systematically calculated how different material parameters affected the energy output.

Their simulations revealed some often-ignored factors “such as the thinness of electrode coatings and the material’s electrical parameters,” says Yao. “These can have a dramatic effect on the electricity generated by bending multilayer polymers.”

One key parameter identified was the need to match the electrical impedance with an optimum load resistance. The researchers’ analysis showed that the energy output of a 22-layered piezoelectric structure could be from 5 to 400 times higher than a single-layer piezoelectric polymer of similar dimensions.

The team then tested the feasibility of their analytical results by fabricating a PVDF-based vibrational harvester on a flexible aluminum substrate. They used scalable dip-coating procedures to build up polymer multilayers and ensured thin metal electrode coatings with physical vapor deposition techniques.

“Our experimental results are promising and show that, for many practical applications, piezoelectric polymer multilayers may enable harvested energy to replace batteries,” notes Yao.

Ferroelectric materials have an intrinsic electrical polarization caused by a small shift in the position of some of their atoms that occurs below a critical point called the Curie temperature. This polarization can be switched by an external electric field, an effect exploited in some computer memory devices.

By explaining the origin of puzzling polarization patterns previously seen in a ferroelectric material called barium titanate, Rajeev Ahluwalia and Nathaniel Ng at the A*STAR Institute of High Performance Computing in Singapore and colleagues have stumbled on a way to ‘write’ polarization patterns in nanoscale ferroelectric materials.

Ferroelectric crystals contain a patchwork of nanoscale ‘domains’, each with a different intrinsic polarization. While an understanding of how these domains form would help to develop reliable applications for ferroelectric materials, two different imaging techniques previously revealed contradictory results about the domains in barium titanate. Ahluwalia’s team therefore set out to solve this puzzle.

One technique — transmission electron microscopy (TEM) — which uses a beam of electrons to probe a crystal’s properties, suggests that the domains comprise long strips arranged in four quadrants, where the net polarization in each quadrant points inward or outward from the surface. The other technique — piezoresponsive force microscopy (PFM) — also reveals a quadrant formation, but the polarizations are parallel to the surface so that the overall polarization of the crystal forms a closed loop (see image).

Ahluwalia and his colleagues hypothesized that the TEM’s electron beam changes the polarization pattern in the sample. PFM, in contrast, uses a sharp tip to detect deformations in the material caused by a localized electric field.

The scientists developed a theoretical model, which revealed that an increase in electron density in the crystal produced the same polarization pattern that they observed with TEM. They also calculated that the radial electric field created by an electron beam could generate other distinctive features of this pattern.

Under normal conditions, an electron beam might not alter the domains. But if the beam is strong enough to heat the sample above the Curie temperature, the material loses its intrinsic polarization. As it cools, the radial electric field induced by the electron beam shapes how the domains reform.

The team’s discovery serves as a warning that electron beam techniques could alter the very domains that researchers are seeking to measure. However, electron beams could be used to deliberately alter polarization patterns in ferroelectric materials, something that is potentially useful for the next generation of memory devices with higher storage densities, says Ahluwalia.

The direct approach to microcavities

A robust micrometer-scale structure for trapping light enhances optical interactions in advanced photonic devices

Trapping light into a small volume is a useful way of amplifying optical effects. Optical cavities, for example, can enhance the interaction between light and matter. Incorporating these tiny structures into actual devices is difficult however, because they are easily broken or can become optically misaligned.

Xia Yu at the A*STAR Singapore Institute of Manufacturing Technology and co-workers have now developed an optical-fiber-based structure that harnesses the potential of light trapped in a microcavity. The novel design also provides a robust route to advanced devices for filtering and sensing light.

Yu and colleagues melted silica glass to form a sphere with a diameter of 182 micrometers. They then patterned the end of an optical fiber with a gold grating and held it close to the microsphere. The grating coupled light propagating along the fiber into the sphere (see image). Light with the right wavelength traveled around in circles within the sphere, trapped by the smooth silica–air interface. This confined light is known as a whispering-gallery resonant mode.

The A*STAR team investigated the properties of their structure by measuring the amount of light at each wavelength that managed to escape from the cavity back into the fiber. The typical wavelength-dependent response of a microsphere is a sharp, symmetric peak centered on the resonant wavelength of the cavity.

Instead, the researchers observed an asymmetric spectral peak, which they recognized as a clear signature of the so-called Fano effect, indicating strong interaction or interference between the whispering-gallery mode and the light in the fiber directly reflected back from the grating.

“This interfering effect makes Fano resonances especially sensitive to changes in either of the participating systems: a slight perturbation results in dramatic alteration in the optical characteristics,” says Yu. “An obvious application of Fano resonance is for use in ultra-sensitive detection.”

In previous investigations of the optical Fano effect, researchers inserted (and extracted) light into the cavity through the side of an optical fiber — an approach that proved unstable and inefficient. The method used by Yu and colleagues of directly inserting light into the cavity through the end of the fiber proved far more robust, making the technology a plausible platform for cheap and compact optical-resonator-based photonic devices.

Another possible application for the technology is as an optical switch. “A good switching device must be fast,” explains Yu. “Therefore, the next step in our research will be to attempt to control the speed of the whispering-gallery mode Fano resonance.”

Bioanalysis:

Microbeads are easily fixed

A passive method for sorting and fixing microbeads of different sizes could lead to cheaper and more functional biological assays

Biological assays are an integral part of the researcher’s toolkit in the fields of biomolecular chemistry and genomics. Microfluidic microbead systems, which consist of arrays of beads coated with an assay-specific reagent, have revolutionized biological assay technology by allowing the high-throughput detection of target molecules from small sample volumes. Fabrication of the microbead systems, however, requires great care and various ancillary devices.

Chee Chung Wong and colleagues from the A*STAR Institute of Microelectronics have now developed a passive and robust method for manufacturing sorted arrays of multiple microbead types.

The preparation of microbead systems conventionally involves the use of a pump to introduce a bead-carrying fluid into a microfluidic circuit. The beads then adsorb to the walls of the microchannels with little control over position or sorting. The resultant microbead-coated channels can be used for targeted molecule detection, but the beads can be easily dislodged by flow.

Recognizing the limitations of conventional systems, Wong and his colleagues set out to develop a passive, pumpless method for preparing more robust microbead arrays. “There are no pumpless bead sorting strategies currently available,” notes Wong. As a result, “we had to research and study three-dimensional trap architectures that could efficiently perform size-based bead sorting.”

The researchers used semiconductor fabrication technologies to create a trap architecture consisting of a top surface with larger micrometer-sized holes and an underlying diffusion gap. When a drop of fluid containing microbeads is placed on the top surface, the beads become trapped in the micrometer-sized holes while the fluid is free to flow through the diffusion layer and out of the array. This structure has the advantage of allowing beads of different sizes to be trapped and permanently fixed in different parts of the device as the fluid evaporates (see image).

“We studied how a droplet of liquid evaporates and how this affects the flow field,” says Wong. “Based on simulations and experiments, we were able to optimize our microtrap architecture for efficient size-based sorting of a range of different bead sizes.”

The researchers expect their fabrication method to alleviate ease-of-use issues associated with current bead sorting assays and also to significantly speed-up throughput by allowing multiple molecular targets to be detected in one device. “The additional dimension of bead size would directly increase the number of analytes that can be detected,” says Wong. “They could increase from two, for a conventional two-color system, to six for a system with three bead sizes in different trap regions.”

Electronic transistors, which act as miniature switches for controlling the flow of electrical current, underpin modern-day microelectronics and computers. State-of-the-art microprocessor chips contain several billion transistors that switch signals flowing in electrical wires and interconnects (see image). With increasing data-processing speeds and shrinking chip sizes, however, wires and interconnects waste considerable energy as heat.

One alternative is to replace electrical interconnects with energy-efficient optical interconnects that carry data using light signals. However, a practical analogue of the transistor for optical interconnects does not yet exist. Hence, Vivek Krishnamurthy from the A*STAR Data Storage Institute and co-workers in Singapore and the United States are developing a practical ‘photonic transistor’ for optical interconnects that can control light signals in a similar manner to electronic transistors.

The researchers’ latest photonic transistor design is based on prevalent semiconductor technology and offers attractive attributes of high switching gain, low switching power and high operating speed1.

Importantly, the research team’s design enables a switching gain of greater or equal to 2, which means the output signal is more than double the strength of the input signal. Hence, the transistor can be cascaded: the output signal from one photonic transistor is sufficiently strong so that it can be split to feed several others. Known as ‘fan-out’, this functionality means the design can become a building block to be scaled up to form larger circuits with many such switching elements connected together for all-optical processing on an optical interconnect platform for data- and telecommunications. Furthermore, Krishnamurthy says that the design consumes 10–20 times less power than the conventional all-optical switching technologies and can operate at very fast speeds.

The team’s design consists of a circuit of coupled silicon waveguides that guide infrared light with a wavelength of 1.5 micrometers. Some of the waveguides feature an optically active material, such as an indium gallium arsenide semiconductor, that can amplify or absorb signal light depending on whether or not it is optically excited. During operation, the intensity of a short-wavelength routing beam is used to control the strength of an output beam by altering the amount of absorption and gain in the circuit.

The researchers are now working to experimentally realize their optical transistor. “We are realizing it on a silicon chip so that it will be compatible with current microelectronic industry standards to enable commercial deployment,” explains Krishnamurthy. “Once we experimentally verify the prototype, we could further integrate it into large-scale optical switching systems for optical interconnects.”
Nanotechnology:
Finding the right mix

Computer simulations indicate that mixing silicon with other materials improves the diversity of nanoscale electronic devices.

The semiconductor silicon lies at the heart of the current revolution in electronics and computing. In particular, it can produce compact integrated circuits when processed by modern techniques capable of fabricating structures just a few nanometers in size.

Ng and Tan used state-of-the-art computer simulations to assess the structural stability and electronic properties of silicon-based nanowires. As their name suggests, nanowires are just a few nanometers wide but can be up to a millimeter long. They exhibit unusual electronic properties because their small width confines the motion of electrons across the wire.

The properties of silicon nanowires are well established, but there is considerable scope to expand their applicability. Scientists anticipate they could realize a more diverse range of characteristics by partially replacing silicon with other elements that are in the same column as silicon in the periodic table. There are many potential materials — including carbon, germanium and tin — each of which can be combined with silicon in any ratio to form an alloy.

Consequently, the total number of possible alloys is immense. The researchers thus undertook a comprehensive search of all these silicon-based alloys to determine which are atomically stable and which have the best properties for nanowire devices.

Ng and Tan employed three mathematical techniques (namely, density functional theory, the cluster expansion method and the Monte Carlo method) to simulate different atomic arrangements in nanowires.

"Instead of evaluating all possible alloy structures, our multiscaled simulation approach enabled rapid large-scale comparison of different combinations of alloy structures and selected the thermodynamically stable ones," explained Ng.

The most stable germanium–silicon and tin–silicon nanowires were found to be those in which the silicon atoms are concentrated around the edge of the wire and the other atomic species are at the core. Conversely, an optimum carbon–silicon nanowire exhibited an ordered arrangement of the atomic species (see image).

Once they had identified the optimum atomic arrangement, Ng and Tan calculated the energy bandgap — a critical parameter for determining the electronic properties of semiconductors.

"Next, we plan to improve the bandgap prediction for silicon-based nanowires and develop our approach to address more complicated nanosystems for energy applications," says Ng.

Now, Man-Fai Ng and Teck Leong Tan at the A*STAR Institute of High Performance Computing in Singapore have shown that mixing silicon with similar materials can open the door to the fabrication of nanoscale devices with a diverse array of properties that have a wider range of applications.

"Next, we plan to improve the bandgap prediction for silicon-based nanowires and develop our approach to address more complicated nanosystems for energy applications."

Metamaterials: Making it big

The use of a fabrication technique borrowed from the semiconductor industry brings metamaterial applications a step closer to reality

Metamaterials are engineered to interact with light and sound waves in ways that natural materials cannot. They thus have the potential to be used in exciting new applications, such as invisibility cloaks, high-resolution lenses, efficient and compact antennas, and highly sensitive sensors.

While the theory of this interaction is relatively well understood, it has been challenging to fabricate metamaterials that are large enough to be practical. Now, Yi Zhou and colleagues at the A*STAR Data Storage Institute in Singapore have demonstrated a promising new fabrication technique that can produce large areas of an important class of metamaterial, known as fishnet metamaterials.1

Most optical metamaterials consist of tiny repeated metallic structures. When light of a particular frequency falls on them, it establishes oscillating fields inside each structure. These fields can resonate with each other and thereby produce desirable collective behavior. Fishnet metamaterials usually have several vertically stacked repeat units spread out over much larger lateral dimensions. Because they are structured both vertically and laterally, they are called three-dimensional materials.

Fishnet metamaterials are usually made in one of two ways. They can be fabricated by carefully patterning individual films and then stacking these films on top of each other. However, this multilayer process is difficult, as it requires careful alignment of the films.

The second approach is to pattern a sacrificial substrate and then deposit repeated layers onto it. This ‘pattern-first’ process suffers from its own difficulties, the most important of which is that the total thickness of the final fishnet material is typically limited to tens of nanometers or less. This restricts the kind of resonances that can be achieved and, in turn, the functionality of the final film.

Zhou and colleagues were able to increase the total thickness of pattern-first fishnet films to around 300 nanometers, allowing five bilayers of film to be deposited and resulting in a strong characteristic resonance and pronounced metamaterial behavior. To achieve this, they adopted a technique called trilayer lift-off, which is commonly used in industry but seldom applied in research laboratories. It involves patterning a sacrificial layer of a photoresist resting on a layer of silicon dioxide under which lies a second photoresist layer.

“This technique will help researchers design large-area three-dimensional nanodevices more easily, and help bring the science of metamaterials to reality.”

By alternating the patterning and etching steps, the A*STAR team could achieve a film thickness greatly exceeding the size of the lateral patterns etched into the film. “This technique will help researchers design large-area three-dimensional nanodevices more easily,” says Zhou, “and help bring the science of metamaterials to reality.”

Magnetic refrigeration is attracting attention as an efficient way to chill sensitive scientific instruments. This refrigeration method exploits the magnetocaloric effect, in which an external magnetic field controls the temperature of a magnetic material. Effective magnetic refrigerants are often difficult to prepare, but now Andy Hor of the A*STAR Institute of Materials Research and Engineering and the National University of Singapore and his colleagues have created a powerful magnetic refrigerant that is easy to make in the lab1.

Compounds with a large magnetocaloric effect typically contain atoms with many unpaired electrons, each of which generates its own tiny magnetic moment. During magnetic refrigeration, an external magnetic field forces these atomic magnetic moments to line up in the same direction. As the magnetism of the atoms becomes more ordered (which reduces the entropy of the system), the material’s temperature rises.

Once the heat has been removed by a flowing liquid or gas, the external magnetic field is reduced. This allows the atomic magnetic moments to become disordered again, cooling the material so that it can be used to draw heat from an instrument, before repeating the cycle.

Magnetic refrigerants commonly use the gadolinium(III) ion (Gd³⁺), because it has seven unpaired electrons. Most gadolinium complexes are made under harsh conditions or take a very long time to form, which limits their wider application. In contrast, the magnetic refrigerant developed by Hor and colleagues is remarkably easy to make.

The researchers simply mixed gadolinium acetate, nickel acetate and an organic molecule called 2-(hydroxymethyl)pyridine in an organic solvent at room temperature. After 12 hours, these chemicals had assembled themselves into an aggregate containing a cube-like structure of atoms at its heart (see image).

The team measured how an external magnetic field affected this ‘cubane’ material as the temperature dropped. Below about 50 K, they found that the material’s magnetization increased sharply, suggesting that it could be an effective magnetic refrigerant below this temperature.

The scientists then tested the effects of varying the external magnetic field at very low temperatures. They found that at 4.5 K, a large external field caused an entropy change that was close to the theoretical maximum for the system — and larger than most other magnetic refrigerants under similar conditions.

According to the team, the magnetocaloric effect of magnetic refrigerants has typically been enhanced by creating ever-larger clusters of metal atoms. In contrast, their cubane shows that much simpler aggregates, prepared under straightforward conditions, are promising as magnetic refrigerants.

Materials:

**Cubic cluster chills out**

Gadolinium-based material that can be cooled by varying a magnetic field may be useful for cooling low-temperature sensors

![Magnetic refrigerant](image)

The magnetic refrigerant contains a cubic structure made of two gadolinium ions (pink), two nickel ions (green) and four oxygen atoms (red), surrounded by 2-(hydroxymethyl)pyridine molecules.

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Plasmonic devices — such as superlenses, hyperlenses and plasmonic waveguides — have exciting potential for research and commercial applications because they permit optical lithography, imaging and waveguiding to be performed at resolutions below the diffraction limit of light. These devices often require low-loss ultrathin metal films, which are difficult to fabricate using current deposition techniques. Researchers have investigated processes such as seed layer deposition and thermal annealing to reduce the surface roughness and grain-boundary density of these films. To date, however, these processes have not been hugely successful.

Now, Ee Jin Teo and colleagues at the A*STAR Institute of Materials Research and Engineering, Singapore, the University of Hyogo, Japan, and the National University of Singapore have used gas cluster ion beam (GCIB) processing to smooth ultrathin metal films and thereby enhance their properties. A GCIB consists of thousands of gas molecules that are weakly bound by van der Waals forces. Such a beam is able to smooth out surface irregularities and reduce film thickness with nanometer precision. This processing significantly enhances surface plasmon resonance and propagation, and enables the fabrication of ultrathin films with extremely low electrical resistivity and optical loss. Unlike monomer ion beams used in conventional ion-beam milling and plasma etching, a cluster of nitrogen gas molecules with an energy of 20 kiloelectron volts impinging on a silver film can deliver a high energy density to a relatively small volume: yet the cluster penetrates to a depth of only a few nanometers. The impact of the beam on the film causes silver atoms in surface peaks to scatter sideways toward valleys, voids and grain boundaries. As well as producing a smoother surface, this processing triples the grain width through the redeposition of atoms at grain boundaries.

The team’s GCIB treatment resulted in up to a four-fold improvement in the electrical and optical properties of films of a thickness of 12 nanometers. “The unique characteristics of GCIB irradiation meant that in a single irradiation step we could reduce scattering losses due to surface roughness, grain boundaries and voids,” notes Teo.

The research team also used the technique to smooth the top surface and sidewalls of lithographically patterned silver-stripe waveguides, increasing the propagation lengths of surface plasmons in these waveguides.

“In the future, we intend to use this technique to improve the color purity of plasmonic color filters or reflectors, and also to increase the patterned area of superlens nanolithography,” says Teo. “Such developments will bring plasmonic research a step closer to commercialization.”

Natural surfaces that repel water, such as lotus leaves or butterfly wings, often have a rough, microscale texture that traps air beneath the liquid droplet. By mimicking these biological structures, researchers have developed ‘superhydrophobic’ coatings that are highly resistant to wetting. One trick unknown to nature, however, is the ability to repel hydrocarbon-based oils that have much lower surface tension than water and tend to spread out rather than bead up.

Jia Min Chin and co-workers from the A*STAR Institute of Materials Research and Engineering and A*STAR Institute of Bioengineering and Nanotechnology in Singapore have now discovered a simple procedure to synthesize ‘omniphobic’ interfaces that repel both oil and water using intricate, mushroom-shaped, metal–organic crystal frameworks.

Recent efforts toward omniphobic surfaces have focused on producing reentrant microscale textures, which have curved shapes that inherently retain air pockets. These structures prevent oil from wetting the surface and stabilize the beaded droplet state. Currently, complicated and labor-intensive lithographic fabrication techniques are needed to generate such textures.

Chin and co-workers investigated a ‘bottom-up’ strategy to synthesize omniphobic films using metal–organic frameworks (MOFs) — compounds that connect metal ions into multidimensional structures using hydrocarbon-based linkages. Previous studies have shown that an aluminum-containing MOF, known as NH₃-MIL-53(Al), can controllably form micro- and nanoscale rods and needles. The team suspected that suitable synthetic conditions could yield spontaneous needle growth upward from a substrate, forming a micro-rough surface with numerous trapped air pockets.

To achieve this, the researchers mixed their MOF precursor with an aluminum oxide membrane and applied ‘hydrothermal’ high temperature–high pressure aqueous reaction conditions. This resulted in perpendicularly aligned needles on both sides of the membrane. Next, the team faced the challenge of transforming the needles into curved textures suitable for repelling oil. After many attempts, they spotted an important clue — the modified membranes ‘floated’ on top of aqueous surfaces due to their superhydrophobic nature.

Chin and her team exploited this floating effect by suspending the microneedle-covered membrane in an aqueous solution of the MOF precursor. Additional MOF growth occurred only on the wetted tips of the needles, expanding the crystalline stems into mushroom-like caps (see image). By controlling the reaction time to generate a targeted cap size, the researchers’ omniphobic surface successfully repelled long-chain hydrocarbon oils.

Chin notes that this benchtop, chemical process produces results previously limited to facilities with expensive, high-tech equipment. “Our aim was to develop simple techniques for fabricating interesting structures which are accessible to scientists around the world,” she says.

Antimicrobials: Polycarbonates to tackle multidrug resistance

Polymers that can be fine-tuned for optimal effect could help fight multidrug-resistant infections

The rise of drug-resistant microbes is a major challenge facing medicine. The World Health Organization’s 2014 report on global surveillance of antimicrobial resistance warns of the very real possibility of the twenty-first century becoming “a post-antibiotic era — in which common infections and minor injuries can kill.” In the face of this threat, researchers worldwide are exploring approaches to find new compounds that combine selective antimicrobial efficacy with low toxicity toward mammalian cells.

Yi Yan Yang at the A*STAR Institute of Bioengineering and Nanotechnology in Singapore and co-workers have now created a range of large polycarbonate molecules that are potent antimicrobials and are tolerated well by rat red blood cells, suggesting that they could prove similarly effective in humans. Crucially, by subtly varying the composition of the polycarbonate molecules, the researchers could fine-tune the selectivity and activity of these candidate drugs.

Antimicrobial polycarbonates are long-chain polymers made by linking small monomer molecules. Each monomer contains two components: one that is hydrophobic and physically inserts into the cell membrane of bacteria and fungi; and one that carries a positively charged group that is attracted to the negative charge on the surface of microbial cells.

By carefully tinkering with the hydrophobic and hydrophilic balance between the components of these new monomers, the researchers were able to create polymers that adhere to microbial cells and disrupt their cell membranes, thereby killing the cells (see image).

The polycarbonates developed by the researchers have proven highly effective against a variety of clinically isolated multidrug-resistant bacteria and fungi. A further benefit is that the molecules are biodegradable, which means that, when used in clinical situations, they should take effect and then degrade naturally. This attribute provides a crucial advantage over other synthetic alternatives that persist and cause undesirable side effects.

Using scanning electron microscopy, the researchers showed that the molecules work by breaking open the microbial cell membrane — a mode of action they believe reduces the likelihood of microbes becoming resistant to the polycarbonates.

The ability to explore different compositions within the monomers may allow further enhancements, according to the researchers. “By carefully controlling the structure and the ratio of the two components, we can enhance dramatically the selectivity of the polymers toward a broad range of pathogenic microbes,” says Yang. The researchers will now study the in vivo efficacy of the optimal polymer using intravenous injection into mice infected with methicillin-resistant Staphylococcus aureus (MRSA) bacteria that have developed resistance to a broad class of antibiotics.

Dye-sensitized solar cells (DSSCs) rely on dyes that absorb light to mobilize a current of electrons and are a promising source of clean energy. Jishan Wu at the A*STAR Institute of Materials Research and Engineering and colleagues in Singapore have now developed zinc porphyrin dyes that harvest light in both the visible and near-infrared parts of the spectrum. Their research suggests that chemical modification of these dyes could enhance the energy output of DSSCs.

DSSCs are easier and cheaper to manufacture than conventional silicon solar cells, but they currently have a lower efficiency. Ruthenium-based dyes have been traditionally used in DSSCs, but in 2011 researchers developed a more efficient dye based on a zinc atom surrounded by a ring-shaped molecule called a porphyrin. Solar cells containing these dyes absorbed more infrared light than YD2-o-C8 and had efficiencies of up to 10.5 per cent, matching the performance of a YD2-o-C8 cell under the same testing conditions (see image).

Theoretical calculations indicate that connecting the porphyrin and perylene sections of these dyes by a carbon–carbon triple bond, which acts as an electron-rich linker, improved the flow of electrons between them. This bond also reduced the light energy needed to excite electrons in the molecule, boosting the dye’s ability to harvest infrared light.

Adding bulky chemical groups to the dyes also improved their solubility and prevented them from aggregating — something that tends to reduce the efficiency of DSSCs.

However, both WW-5 and WW-6 are slightly less efficient than YD2-o-C8 at converting visible light into electricity, and they also produce a lower voltage. “We are now trying to solve this problem through modifications based on the chemical structure of WW-5 and WW-6,” says Wu.

Comparing the results from more perylene–porphyrin dyes should indicate ways to overcome these hurdles, and may even extend light absorption further into the infrared. “The top priority is to improve the power conversion efficiency,” says Wu. “Our target is to push the efficiency to more than 13 per cent in the near future.”

Increasing the cost-effectiveness of photovoltaic devices is critical to making these renewable energy sources competitive with traditional fossil fuels. One possibility is to use hybrid solar cells that combine silicon nanowires with low-cost, photoresponsive polymers. The high surface area and confined nature of nanowires allows them to trap significant amounts of light for solar cell operations. Unfortunately, these thin, needle-like structures are very fragile and tend to stick together when the wires become too long.

Now, findings by Xincai Wang from the A*STAR Singapore Institute of Manufacturing Technology and co-workers from Nanyang Technological University could turn the tables on silicon nanowires by improving the manufacturing of silicon ‘nanoholes’ — narrow cavities carved into silicon wafers that have enhanced mechanical and light-harvesting capabilities. Nanoholes are particularly effective at capturing light because photons can ricochet many times inside these openings until absorption occurs. Yet a practical understanding of how to fabricate these tiny structures is still lacking. One significant problem, notes Wang, is control of the initial stages of nanohole formation — a crucial period that can often induce defects into the solar cell.

Instead of traditional time-consuming lithography, the researchers identified a rapid, ‘maskless’ approach to producing nanoholes using silver nanoparticles. First, they deposited a nanometer-thin layer of silver onto a silicon wafer, which they toughened by annealing it using a rapid-burst ultraviolet laser. Careful optimization of this procedure yielded regular arrays of silver nanospheres on top of the silicon surface, with sphere size and distribution controlled by the laser annealing conditions.

Next, the nanosphere–silicon complex was immersed into a solution of hydrogen peroxide and hydrofluoric acid — a mixture that eats away at silicon atoms directly underneath the catalytic silver nanospheres. Subsequent removal of the silver particles with acid produced the final, nanohole-infused silicon surface (see image).

The team analyzed the solar cell activity of their nanohole interfaces by coating them with a semiconducting polymer and metal electrodes. Their experiments revealed a remarkable dependence on nanohole depth: cavities deeper than one micrometer showed sharp drops in power conversion efficiency from a maximum of 8.3 per cent due to light scattering off of rougher surfaces and higher series resistance effects.

“Our simple process for making hybrid silicon nanohole devices can successfully reduce the fabrication costs which impede the solar cell industry,” says Wang. “In addition, this approach can be easily transferred to silicon thin films to develop thin-film silicon—polymer hybrid solar cells with even higher efficiency.”
Plants and herbs containing phthalide molecules — organic compounds that fuse aromatic benzene rings with cyclic, oxygen-bearing hydrocarbons known as lactones — have been used since ancient times as natural medicines to treat diseases such as cardiovascular ailments. Chemists have recently discovered that phthalides can act as valuable ‘scaffolds’ in the construction of pharmaceuticals with complex structures and more potent capabilities. Unfortunately, phthalides are normally produced through multistep synthetic pathways that can significantly increase manufacturing costs.

Jayasree Seayad and co-workers from the A*STAR Institute of Chemical and Engineering Sciences in Singapore have now developed a novel catalytic process that can generate numerous phthalide compounds in a single step from readily available feedstocks — a ‘green’ chemistry approach that simultaneously boosts synthetic efficiency and reduces production of environmentally hazardous waste.

One way that chemists are trying to improve the effectiveness of their reactions is with carbon–hydrogen (C–H) activation. Typically, C–H bonds are inert and require harsh conditions to cleave. By contrast, C–H activation reactions can transform these bonds into new functional groups under very mild conditions using catalytically active transition metals such as rhodium complexes.

Seayad and her team anticipated that benzaldehyde — a common benzene derivative bearing a simple carbonyl side chain known as an aldehyde — could serve as ideal reagents for phthalide synthesis because of their structural similarity. To achieve this goal, however, the researchers had to find a way to selectively activate the benzaldehyde C–H bond located beside the aldehyde chain. This is a challenge, notes Seayad, because aldehyde groups are poor at ‘directing’ the transition metal catalyst to this site.

To overcome this obstacle, the team turned to a combination of rhodium and organic amine catalysts (see image). The amine catalyst first activates benzaldehyde by replacing its oxygen atom with nitrogen, forming a functional group known as an imine. The imine bonds rapidly to the rhodium catalyst and positions it for favorable C–H activation. Then, a second benzaldehyde molecule couples with the activated complex to generate the desired phthalide in high yields. Performing this reaction under oxidative conditions ensures easy regeneration of the rhodium–amine system for numerous catalytic cycles.

The researchers found that this strategy enabled both identical and modified benzaldehydes to couple into phthalides, opening the way for chemists to produce a diverse range of these bioactive molecules with ease. “This discovery will lead to simplified synthesis of phthalide intermediates from readily available aldehydes while avoiding multistep pathways,” says Seayad.

**Thermoresponsive polymers:**

**Positive progress on antifouling**

*Heat-responsive polymers that do not breakdown in water may lead to new antifouling coatings and enhanced oil recovery*

Thanks to the positively and negatively charged units in their monomers, zwitterionic polymers have a high affinity for water — a property known as hydrophilicity. This property helps prevent fouling, namely the build-up of contaminants. Current zwitterionic polymers are not effective in water as they use monomers such as commercially available acrylamide and methacrylates that tend to decompose and lose their electrostatic characteristics when wet.

To solve this issue, a team led by Vivek Vasantha from the A*STAR Institute of Chemical and Engineering Sciences in Singapore has now developed zwitterionic polymers based on water-stable monomers that incorporate nitrogen-containing derivatives known as imidazoles. The team introduced the zwitterions to readily accessible, hydrophobic polystyrene to boost its hydrophilicity in water by forming a hydration layer through electrostatic interactions and hydrogen bonding.

To synthesize the monomers, Vasantha’s team reacted styrene precursors with positively charged imidazoles before attaching the negatively charged sulfonate functional groups. The monomers produced polymers with intact zwitterionic properties, meaning that they retained their positive and negative charges.

These new imidazole-based polymers exhibited some novel solubility characteristics: unlike their conventional water-soluble counterparts, they swelled in water and dissolved only in highly concentrated brine. These differences stem from dipole–dipole interactions and the more hydrophobic nature of the new polymers compared to acrylamide and methacrylate.

With high tolerances to salt, pH and temperature, these polymers became increasingly viscous when subjected to higher shear forces in brine. This characteristic — similar to ‘silly putty’, which is malleable in one’s hands but is unchanged when hit with a hammer — makes the polymers attractive for enhanced oil recovery and marine antifouling coatings.

Another advantage of the new polymers is their reversible phase change: between 5 °C and 95 °C, the polymers formed gels that become clear fluids when heated above the so-called critical temperature in brine and that revert to their stable cloudy state on cooling.

“This phase transition results from the disruption of the equilibrium between salt, water and zwitterionic species,” says Vasantha. The polymer chains expand on heating and collapse below the critical temperature. The researchers could control the critical temperature by simply varying either the brine or polymer concentration. For example, the transition occurred at 20 °C at a low polymer concentration but at 40 °C at a higher polymer concentration.

“We are currently designing new zwitterionic polymers and copolymers with salt- and heat-responsive behavior for a wide range of applications, such as enhanced oil recovery, low-temperature protein separation and antifouling,” says Vasantha.

Microfluidics: Mixing through oscillations

A tiny device produces oscillatory flows that enhance the mixing of viscous fluids for chemical reactions

Devices that manipulate very small volumes of fluids are applied in diverse fields, including printer technology, DNA processing and cooling systems for electronics. For some processes involving fluids, such as mixing, it is useful to generate oscillating flows, but this can be difficult for particularly viscous fluids. Now, A*STAR researchers have developed a microfluidic oscillator that produces oscillations even in very viscous fluids.

“In miniaturized fluidic devices, the viscous force of the fluid dominates the flow, and mixing becomes a challenging task,” says Huanming Xia from the A*STAR Singapore Institute of Manufacturing Technology (SIMTech), who led the study with co-workers at SIMTech and the A*STAR Institute of High Performance Computing. “The microfluidic oscillator is a part of our continuous effort to solve this problem.”

Microfluidic valves and pumps have diaphragms, which are usually made from soft materials, such as rubber, and are operated via external forces. Yet the tiny device, less than 4 millimeters in size, developed by Xia’s team does not need external control. Instead, when the diaphragm is placed in a fluid flow, it responds elastically by wiggling up and down to make the device oscillate automatically (see image). To adapt the design for use with very viscous fluids, the researchers replaced the rubber diaphragm with one made from copper and beryllium foil.

“Flow-induced vibrations are usually related to flow instabilities and analyzed using a spring–mass model,” explains Xia. The transition from laminar flow to oscillatory flow in their new oscillator was counterintuitive, because increased pressure led to reduced flow rates. The team recognized that this behavior was similar to ‘negative differential resistance’ — a well-established concept that describes certain electric circuits in which an increased voltage leads to a lower current.

Xia’s team is currently developing a complete mathematical model of their device using negative resistance and other concepts ‘borrowed’ from electric circuit theory. This should assist them to optimize the device design for practical applications; for example, the enhanced mixing of viscous fluids enabled by the device can intensify and control chemical reactions.

Research Highlights

GENETICS & DISEASE
Found in the brain, the MeCP2 protein plays a crucial role in the control of hormones that regulate appetite and metabolism. Deletion of MeCP2 may increase cravings for fatty foods and lead to obesity.

Obesity: The power of proteins to battle the bulge

**Improved understanding of the proteins that help the body to control appetite may be useful for the treatment of obesity**

The main cause of weight gain, and ultimately obesity, is an energy imbalance in the body triggered by increased food intake, often coupled with reduced energy expenditure. Two hormones called leptin and α-MSH (α-melanocyte-stimulating hormone) regulate the so-called ‘energy homeostasis’ in the body by influencing the brain to control appetite, metabolism and behavior.

The hormone leptin acts on at least two sets of neurons in the brain, including a group called the pro-opiomelanocortin (POMC) neurons. Scientists know that impaired POMC regulation leads to leptin resistance and obesity, but are unclear about the exact mechanism by which this occurs.

Now, Weiping Han and colleagues at the A*STAR Singapore Bioimaging Consortium and A*STAR Institute of Molecular and Cell Biology, together with co-workers from Hungary and the United States, have shown that deleting a protein called methyl-CpG-binding protein 2 (MeCP2) can promote weight gain and obesity in mice.

The team previously investigated the onset of leptin resistance during a high fat diet. "We examined whether epigenetic changes, such as DNA methylation, could alter the gene expression of the critical regulators of energy homeostasis," notes Han. "POMC neurons are regulated by MeCP2, so we chose to investigate what would happen if this relationship was disrupted."

The team bred genetically altered mice that had MeCP2 deleted specifically in the POMC neurons in the hypothalamus region of the brain. Their growth and feeding behavior on a high fat diet were then compared to their littermate control group with fully functioning MeCP2. After eight months, the MeCP2-knockout mice showed higher levels of leptin circulating in the blood, alongside increased body weight and fat levels.

Under normal physiological conditions, MeCP2, along with other factors, allows increased expression of POMC. "This occurs in response to cues from hormones such as leptin," explains Han. "POMC is then processed to generate α-MSH, which is released from POMC neurons to control appetite and feeding behavior."

The researchers found that without MeCP2 to increase POMC expression in response to rising leptin levels, not enough α-MSH was released to stem the appetite. This led to all of the MeCP2-knockout mice becoming overweight.

"Knowing that increased DNA methylation in POMC promoters may lead to leptin resistance and weight gain provides new options for weight management," states Han. "Targeting epigenetic modification and regulation might be a viable approach to treat obesity."

Hoping to better understand the onset of obesity, the team is now researching other proteins that might affect the neurons and hormonal pathways in the brain.

Flu vaccine enables rapid response

Influenza pandemics, such as the 2009 H1N1 ‘swine flu’ outbreak, pose a serious risk to the global population. Vaccination is one route to protection but current manufacturing methods for vaccines limit the volume and speed of production. Now, an international team of researchers, including A*STAR’s Program in Translational Research on Infectious Disease, Experimental Therapeutics Centre and Singapore Immunology Network, has developed a more efficient production process

In 2009, it took five months for the vaccine against the H1N1 virus to become available, and the number of doses made was sufficient for only a fraction of the global population. Both shortfalls were down to the conventional vaccine production process and so the research team wanted to develop a more efficient technique.

“The licensed H1N1 flu vaccine is made by growing the influenza virus in chicken eggs,” explains David Skibinski, the study’s lead author. In comparison, “our vaccine is produced by expressing the main surface antigen of influenza in bacteria.”

The researchers attached the antigen — part of a protein from the surface of the H1N1 virus — to harmless virus-like particles and injected them into mice (see image). Compared with the mice that were inoculated with the licensed vaccine, animals inoculated with the new vaccine produced a similar number of effective antibodies against the H1N1 virus, which demonstrated that the new production method provided immunity as effectively as the existing vaccine.

There was, however, an extra advantage: mice that were inoculated with the new vaccine produced more T cells than mice that were inoculated with the licensed vaccine. T cells play an important role in protecting the body against influenza, reduce the severity of the disease, and provide protection against different strains of the virus.

Skibinski says the new way of producing the influenza vaccine could improve the global response to future pandemics by shortening the time between the emergence of a virus and production of a functional vaccine, thus enabling individuals to be vaccinated before they become infected. “Greater cost-efficiency and yields would also enable many smaller or developing nations to manufacture their own vaccine,” adds Skibinski.

Having shown the new vaccine’s effectiveness in mice, the research team’s next step was to see if the findings could be transferred to humans. So far, the results are positive, reports John Connolly, the study’s senior author. “A clinical trial in humans under the direction of the A*STAR D3 unit has demonstrated that the vaccine is safe, well tolerated and induces antibody responses comparable to those of approved seasonal influenza vaccines.”
The synthesis of proteins by translation of RNA relies on a protein complex called eIF4F. As formation of this complex is frequently unregulated in cancers, the ability to restore its regulation could pave the way to develop new cancer therapies. A Singapore-based team headed by Christopher Brown and David Lane from the A*STAR p53 Laboratory and Dilraj Lama at the A*STAR Bioinformatics Institute have used computer modeling to design molecules that may be able to do just that.

Regulation of eIF4F involves disrupting an interaction between two proteins in the complex, eIF4E and eIF4G. Brown and colleagues set out to mimic this disruption with artificial peptides, or small protein fragments, that bind to eIF4E in the same way as eIF4G. To do this, they controlled the peptide structures using an approach called peptide stapling.

In the context of a whole protein, peptides usually form sections of localized structure, but when these peptides are removed from a protein they become linear and highly flexible, explains Brown. “A peptide staple is a chemical modification that can link two points of a peptide together and reintroduce the structure.”

The research team designed different stapled peptides and ran computer simulations to reveal their structures, both when bound to eIF4E and when free in solution. They also synthesized the peptides and experimentally characterized their structures (see image).

Combining the information obtained by both experimentation and simulation showed where there was potential for improvement. “This meant we could design new stapled peptides, which could then be further characterized through experiments and also through computer simulations,” says Brown. “The new designs altered individual amino acids. The aim of these alterations was to either optimize the peptide when bound to eIF4E or, when in solution, to make it look more like the bound structure.”

Using the optimization process, the team produced two stapled peptides that interacted strongly with eIF4E. This required modification so that their structures would stay the same in solution as when bound to eIF4E.

“We developed a process to design highly potent inhibitors of an interaction relevant to disease,” says Brown. “Our next step is to further understand how and why certain stapled peptides are biologically active whilst others are not.”

Although these stapled peptides are not yet ready for use in treatment, Brown hopes this will soon change. “In the future, we envision developing the eIF4E stapled interacting peptides into biologically active molecules and testing them in relevant disease models.”

Pathogenic fungi like *Candida albicans* can cause oral, skin, nail and genital infections. While exposure to pathogenic fungi is generally not life-threatening, it can be deadly to immunocompromised patients with AIDS or cancer. A variety of antifungal medications, such as triazoles and polyenes, are currently used for treating fungal infections. The range of these antifungal medications, however, is extremely limited, with some fungal species developing resistance to these drugs.

Yi Yan Yang at the A*STAR Institute of Bioengineering and Nanotechnology in Singapore and co-workers, in collaboration with IBM Almaden Research Center in the United States, have discovered four cationic terephthalamide-bisurea compounds with strong antifungal activity, excellent microbial selectivity and low host toxicity. These small molecules can self-assemble into fibers, bind fungal membranes and rupture the cells of a variety of fungal species. The results may expand the possibilities of medication for battling drug-resistant fungal species.

Conformational analysis revealed that the terephthalamide-bisurea compounds have a Z-shaped structure: the terephthalamide sits in the middle, urea groups on both sides of the terephthalamide, and cationic charges at both ends. These small molecules can self-assemble into fibers, bind fungal membranes and rupture the cells of a variety of fungal species. The results may expand the possibilities of medication for battling drug-resistant fungal species.

When dissolved in water, the terephthalamide-bisurea compounds aggregate to form fibers with lengths ranging from a few hundred nanometers to several micrometers. Some of the compounds form fibers with high flexibility and others with high rigidity.

The researchers evaluated the antifungal activity of their terephthalamide-bisurea compounds against *C. albicans*. They found that all of the cationic compounds effectively inhibited fungal growth, even when the fungal concentration increased from $10^2$ to $10^5$ colony-forming units per milliliter.

The researchers believe that the potent antifungal activity is largely due to the formation of fibers with extremely small diameters in the order of 5 to 10 nanometers, which facilitates the rupture of fungal membranes. “This is particularly important because the fungal membrane of *C. albicans* is multilayered and has low negative charges,” explains Yang. “It also helps explain why cationic terephthalamide-bisurea compounds could easily penetrate the fungal membrane.”

The terephthalamide-bisurea compounds also eradicated clinically isolated drug-resistant *C. albicans*. The compounds prevent the development of drug resistance by rupturing the fungal membrane of *C. albicans* and disrupting the biofilm (see image). Additionally, cytotoxicity tests showed that the cationic terephthalamide-bisurea compounds exhibit low toxicity toward mammalian cells and in a mouse model, revealing that the compounds “are relatively safe for preventing and treating fungal infections,” says Yang.
Genomics:
The significance of silence

An enzyme that silences DNA activity may be crucially involved in health and cancer

Cancers can develop when the complex molecular networks that control the activity of DNA are disrupted. Researchers from the A*STAR Institute of Molecular and Cell Biology in Singapore, led by Dmitry Bulavin, have studied Wip1, an enzyme central to these molecular networks that may both help to keep cells healthy but also become part of the problem when things go wrong. The team’s findings suggest that this enzyme, and its associated signaling pathways, could be a target for the development of new drugs to combat some types of cancer. They may also explain why some cancers are resistant to drug therapy.

Bulavin and co-workers uncovered the role of the Wip1 enzyme when examining the deactivation or ‘silencing’ of regions of DNA by DNA methylation. DNA methylation occurs when methyl (CH₃) groups are added to the cytosine bases of DNA. This process is known to be vital for the normal control of gene activity as cells and organisms develop. Changes in DNA methylation patterns, however, are also involved in the development of many diseases, including cancer.

Wip1 is known to influence the activity of other molecules by removing phosphate (PO₄³⁻) groups. However, enzymes rarely act alone to regulate gene activity — they are components of complex networks of interactions. To clarify the Wip1 enzyme’s role in health and disease, the team looked at its direct and indirect interactions with several other molecules already implicated in cancer, including DNA itself.

Working in mice and with human cancer cells, the researchers examined the effects of creating Wip1 deficiency, as well as stimulating the overexpression of the gene that makes Wip1. They found varied effects on more than a thousand genes, including increases and decreases in DNA silencing when the normal activities of Wip1 were disrupted. Taken together, the results suggest that the Wip1 enzyme plays an important role in controlling DNA methylation in tightly coiled — and often inactive — regions of DNA known as ‘heterochromatin’. The enzyme’s DNA-silencing effects are mediated through interaction with two other proteins involved in the onset of cancer, known as ATM and BRCA1.

The team’s results may be particularly relevant to mutations in primary breast cancers. “Ultimately, cancer develops and evolves as a result of mutations that contribute to these processes, but the phenomenon is very poorly understood,” says Bulavin. “We show Wip1 is critical to maintaining the integrity of the genome, which provides new avenues to understanding cancer evolution and the mechanisms responsible for developing anticancer drug resistance.”

Reprogramming a cell to express a foreign gene is relatively straightforward. However, reprogramming a cell to express multiple foreign genes is far more complex, especially if trying to exert control over the interplay between these genes. Now, researchers led by Yuansheng Yang of the A*STAR Bioprocessing Technology Institute, Singapore, have developed a strategy to finely control the relative activity of multiple genes in parallel.

“Our strategy can be used to critically impact the biological activity, serum half-life and immunogenicity of therapeutic proteins.”

Some of the complexity of reprogramming lies in balancing proportions: many proteins consist of multiple component subunits, and for the complex to assemble properly, each subunit must be manufactured in specific proportions. Yang’s strategy was to manipulate an RNA sequence known as an internal ribosome entry site (IRES). Most messenger RNA (mRNA) molecules represent the product of a single gene that gets translated into a single protein, but some viruses produce mRNAs that contain protein-coding regions from multiple genes separated by an IRES. This makes the IRES a powerful scientific tool for multigene expression. “As genes linked by an IRES are translated independently, we can adjust their relative expression by varying the strength of the IRES,” explains Yang. “However, the number of naturally available IRESs is limited and can only provide a narrow range of gene expression.” Yang’s team decided to expand their options by generating a library of IRESs with a variety of sequence mutations. Then, they assessed how these variants affected relative protein production when inserted between two genes.

To test the different IRES constructs, the researchers produced antibodies — immune proteins formed of large ‘heavy-chain’ and smaller ‘light-chain’ protein components. The 24 variants they generated varied considerably in relative gene activity, ranging from a 12 to 96 per cent reduction in the production of light-chain proteins relative to heavy-chain proteins.

These differences had a profound effect on mature antibody assembly. For instance, producing the two components in equal proportions resulted in high levels of antibody production but also yielded many undesirable byproducts. However, an IRES that increased relative light-chain to heavy-chain production by 50 per cent virtually eliminated those byproducts without a meaningful impact on total antibody production.

A United States biotech company has already expressed interest in licensing this strategy for improved antibody production. Meanwhile, Yang’s group has additional applications in mind. For example, these sequences could help manipulate glycosylation pathways, a crucial mechanism for functional modification of proteins that depends on finely choreographed activity from multiple enzymes. “This can critically impact the biological activity, serum half-life and immunogenicity of therapeutic proteins,” says Yang.

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Scientists have known for decades that the protein p53 is critical for safeguarding against genetic damage and the onset of cancer. Many tumors exhibit mutations that undermine p53’s ability to regulate the genes responsible for activating DNA repair and halting cell division. While p53 also appears to be an important controller of other cellular processes, identifying the additional genes that p53 targets remains challenging.

Now, Ee Chee Ren and colleagues at the A*STAR Singapore Immunology Network have developed an assay that could accelerate identification of the genes that p53 regulates. The interaction between p53 protein (top) and the p53 response element DNA sequence of a target gene (DNA helix). Yellow arrows indicate the positions of several amino acid sequence variations affecting the DNA-binding region of p53.

Prior searches for p53 target genes were informed by only limited knowledge of the interaction between p53 and DNA; these studies compared changes in gene expression in the presence or absence of normal, or ‘wild-type’, p53, but without validating the genes that appeared to be affected or their physical association with p53. However, Ren and colleagues were able to use p53RE-defining sequence elements that they had previously identified to design a bioluminescence-based assay capable of accurately determining whether genes are subject to p53-mediated regulation.

Ren’s team assembled a panel of 16 p53 proteins with slight structural differences by combing a database of DNA sequences from cancer patients and identifying sequence changes that affected the amino-acid makeup of p53. This allowed them to distinguish variants that exhibited similar p53RE binding and gene activation to wild-type p53 from those that were nonfunctional.

Importantly, they found that similarly classified p53 variants exhibited the same DNA binding and gene activation profiles as each other, enabling the effects of p53 to be determined from multiple, parallel experiments. “We developed an efficient screening approach that can examine putative p53 target genes based on the signature obtained with these 16 variants, instead of relying on only one test with just the wild-type p53,” explains Ren.

The team’s assay proved far more efficient at accurately identifying known p53 target genes than standard experimental techniques, confirming its effectiveness as a discovery tool. The researchers demonstrated this by using their panel to identify nearly 600 new target genes of p53 that affect diverse cellular functions. “Our results show that the reach of p53 is far wider than previously believed, and that it participates in many normal physiological processes of the cell,” says Ren.

A computer-based model of cell particle dynamics shows that the lopsided torque produced by forces within a cell may explain the previously puzzling motion and shape of rotating cell pairs. The model provides novel insights that challenge current thinking about the causes of developmental abnormalities and of cancer and other diseases linked to disrupted cell rotation.

A crucial feature of the model of key particle motions, developed by Fong Yew Leong at the A*STAR Institute of High Performance Computing in Singapore, is that it spontaneously produces cells with the same shape and rotational characteristics as real cells.

During the development of multicellular organisms, cells rotate around each other in a coordinated manner. This rotation does not occur in some cancer cells, which implies that disrupting normal cell movement may influence disease as well as development.

Isolated cells growing on flat surfaces tend not to rotate. However, two joined cells can rotate spontaneously and continuously, and will often develop a sigmoidal or 'S-shaped' interface that resembles the yin and yang symbol (see image). As the cells rotate, they appear to 'moonwalk' around one another; each cell moves in the opposite direction to its protrusion into the other cell and in the reverse manner to cells moving on their own.

Leong modeled the cells in two dimensions as an assembly of cytoplasm particles surrounded by a cell membrane. As cell movement is driven by the interaction of actin and myosin protein filaments in the cell’s cytoskeleton, Leong designed the model to include the formation and degradation of actin and myosin chains attached to the inner cell membrane.

The simulation showed the interaction of actin and myosin within the cell — known as ‘undirected actomyosin forcing’ — which is powerful enough to generate the shape and movement of the cells. Crucially, as Leong explains, “forces that are angled toward the cell membrane lead to an unbalanced torque that rotates the cell.” He also considers the spontaneous emergence of the torque, due to the tilted forces, to be the most significant insight provided by his model.

“The next step is to develop a three-dimensional model that explains cell cluster rotation \textit{in vivo}, rather than just on a two-dimensional surface,” says Leong. He hopes future iterations of the rather simple current model will help explain the real-life complexity of the movements of multiple cells and ultimately advance approaches to addressing developmental abnormalities and diseases linked to the disruption of cell movement.

Genomic differences between individuals can change the physical organization of RNA transcripts, affecting their expression

The information contained within a messenger RNA (mRNA) transcript goes beyond the protein recipe embedded in its sequence. mRNAs consist of single strands of nucleotides that can pair with each other in the same way as double-stranded DNA molecules. The resulting ‘secondary structures’ help determine when and how the encoded protein gets produced.

Researchers led by Yue Wan of the A*STAR Genome Institute of Singapore and Howard Chang of Stanford University in the United States have applied a powerful experimental technique to build a detailed map of secondary structures for the entire human mRNA 'transcriptome'.

Their Parallel Analysis of RNA Structure (PARS) method entails isolating the total mRNA content of a biological sample, then treating it with two different enzymes that selectively cut single- or double-stranded RNA segments. By using sequencing technology to map these cut sites, the researchers could chart the secondary structure of each mRNA transcript.

The analysis uncovered some interesting general features of mRNA structure. For example, protein-coding regions had less structure than noncoding regulatory sequences, particularly in segments involved in splicing — an enzymatic process that expands the number of proteins encoded by a single transcript. Additionally, nearly 10 per cent of the mRNAs the team examined assumed multiple secondary structure arrangements, suggesting that ‘switching’ between conformations plays an important regulatory role.

The researchers also investigated instances where mRNA structure is affected by differences in the genomic DNA sequence from which it was transcribed. “There is extensive genetic variation between individuals,” explains Wan. “To understand the extent to which this causes structural alterations in humans, we performed a genome-wide analysis in a family of three individuals.” This showed that seemingly minor differences can have a considerable impact: roughly 15 per cent of the single-nucleotide sequence variations between individuals caused structural changes in an mRNA (see image).

Although other researchers predicted that genome sequence differences would have such an effect, Wan’s findings represent the first direct demonstration of the extent of this phenomenon. “We identified over 1,900 nucleotide variants that cause structural changes in the human transcriptome — far more than anybody else has discovered previously,” he says.

In several instances, the researchers found evidence that these variant-associated changes may impact gene regulation, including protein production, and therefore contribute to certain disease states. “This work was done on healthy individuals, but our findings suggest that some mutations may cause disease by altering gene regulation,” says Wan. “Future work could compare diseased with normal tissues to identify and characterize structure-changing mutations.”

Being able to view tumor blood vessels without surgery or potent dyes can improve our understanding of the environment in which a tumor grows. Now, a team of researchers, including Chang-Tong Yang from the A*STAR Singapore Bioimaging Consortium, have developed a contrast agent that selectively labels the tumor blood vessel network for use in noninvasive tumor imaging studies.

Assessing the extent of the blood vessel network within a tumor enables doctors to determine how far along the cancer has progressed and to predict whether a tumor will respond to intravenous chemotherapy drugs through modeling the drug’s potential path and absorbance. While noninvasive imaging techniques can help visualize all blood vessels in patients, visualizing the blood vessels of a tumor requires a contrast agent that can selectively label the tumor vessels. Such substances concentrate within tumors by binding to molecules that are more prevalent in tumors than in normal tissues.

The researchers developed a contrast agent called Gd(DO3A-Lys) that has an optimal half-life, meaning it persists in the blood long enough to be useful as an imaging aid. Contrast agents that strongly bind blood albumin increase the visibility of large blood vessels and are already in clinical use, explains research co-leader Edward George Robins. However, Gd(DO3A-Lys) only weakly binds albumin, enabling the contrast agent to specifically target and visualize areas that contain tumors and their microvasculature.

Contrast agents eventually leave the body when filtered through the kidneys. Promisingly, the researchers were able to show that Gd(DO3A-Lys) has low toxicity to kidney cells growing in culture. In addition, mice given a single dose of Gd(DO3A-Lys) showed no kidney toxicity at both low and high doses, although the high-dose treatment did result in some damage to the liver — the organ responsible for breaking down drugs.

After administering Gd(DO3A-Lys) to tumor-bearing mice, Yang and colleagues used contrast-enhanced magnetic resonance imaging (CE-MRI) to demonstrate that the contrast agent selectively localized to the site of the tumor. Importantly, as only a minimal amount leaked from the blood vessels into the tumor itself, Gd(DO3A-Lys) appeared to be retained within the tumor’s blood vessel network, allowing the fine structures of the tumor vessels to be clearly imaged.

While the team’s results are encouraging, clinical use of Gd(DO3A-Lys) is still a long way off. Further study is needed to assess the sensitivity of Gd(DO3A-Lys) to detect changes in the tumor vasculature and potential interactions with tumor treatments such as anti-angiogenesis drugs.

Dysfunctions in cilia, tiny hair-like structures that protrude from the surface of cells, are responsible for a number of human diseases. However the genes involved in making cilia have remained largely elusive. In the first comprehensive analysis of its kind, researchers from the A*STAR Institute of Molecular and Cell Biology in Singapore have identified hundreds of genes involved in the proper formation of a particular type of cilia that help to remove mucus and dirt from the lungs.1

“This collection of genes will be invaluable for our understanding of how cilia are made,” says Sudipto Roy, a developmental biologist and senior author of the study. “More importantly, it will greatly facilitate the diagnosis of cilia-based diseases.”

Cilia come in two forms: primary, or non-motile, cilia that serve as environmental sensors for cells throughout the body, and motile cilia, which constantly beat to sweep debris from the middle ear and respiratory tract (see image). Mutations that affect the function of motile cilia play a role in genetic disorders like primary ciliary dyskinesia, which affects the lungs and other sites where such cilia are present.

Previously, Roy and colleagues found that a transcription factor called forkhead box protein J1 (Foxj1) is the master regulator of motile cilia development.2 The researchers demonstrated that Foxj1 controls the expression of numerous genes involved in the production of these moving, microscopic protrusions. However, they were unsure as to exactly which genes were implicated in the process of making functional motile cilia.

Roy’s team, guided by senior research fellow Semil Choksi, therefore decided to perform a systematic analysis of all the genes in the zebrafish, a common model of cilia development. They discovered more than 500 genes with mammalian counterparts that are activated by Foxj1, the majority of which had not previously been associated with cilia production.

The researchers randomly selected 50 of these genes for functional studies. They artificially boosted expression levels of each gene by injecting lab-made RNA into zebrafish embryos, and found that around 30% of the proteins encoded by the genes localized to the motile cilia. The researchers also inactivated each of these 50 genes in turn using morpholinos, a standard knockdown tool. In this way, they showed that more than 60% of the genes were needed for the proper differentiation or functioning of motile cilia.

According to Choksi, the team’s collection of cilia-associated genes is set to help researchers identify previously unknown mutations behind cilia disorders in patients — and ultimately, perhaps, new therapies.

A new metal carbonyl-based assay has potential as a clinical diagnostic tool and offers several advantages over current monitoring of sugars in urine samples.

Medical diagnostics: Sweet sensing

A highly sensitive and specific biological assay requires only a tiny sample volume to monitor glucose levels in patients with diabetes

Diabetes is a major health problem that affects 371 million people worldwide, and a high blood glucose level is one of many complications associated with diabetes and pre-diabetes. Malini Olivo at the A*STAR Singapore Bioimaging Consortium and co-workers have now developed a highly sensitive and specific surface-enhanced Raman scattering (SERS)-based assay to detect glucose in urine.

SERS-based assays represent a simple and convenient method to monitor glucose levels in urine. The technology uses organometallic compounds as ‘sugar receptors’ for sensing glucose and a gold–silver substrate for enhancing signals from these receptors. Most such sugar receptors, however, have a low detection limit and specificity. They often require samples to be purified prior to testing and may not be able to distinguish glucose from similar sugars such as fructose and galactose. As a result, SERS-based assays are not yet an accessible and dependable solution for monitoring glucose levels in patients with diabetes.

Searching for a more effective test, Olivo and team developed an assay which uses tricosmium carbonyl conjugates as sugar receptors and an in-house fabricated material — bimetallic film over nanospheres (BMFON) — as the signal-enhancing substrate. They found that the metal carbonyl conjugates selectively bind simple sugars and produce a unique absorption peak for glucose at 2111 centimeters−1, which lies in the so-called ‘silent’ mid-infrared region of the SERS spectrum between 1800–2200 centimeters−1. The intensity of the peak varies with glucose concentration and can be used to measure glucose quantities. In addition, BMFON enhances the signal intensity to extend the limit of detection to 0.1 millimoles of glucose per liter to cover a range that includes the physiological glucose concentration of 5 millimoles per liter.

Using their newly developed assay, the researchers could determine the glucose level in urine samples spiked with a standard glucose solution. The new assay has three key advantages: only a low sample volume is required; there is no need for the sample to be purified; and the assay’s sugar receptors do not need to be conjugated to SERS-active nanoparticles.

“Our work is unique in that we used a metal carbonyl probe to access an uncluttered region in the SERS spectrum, which most other organic probes cannot provide,” says Olivo. “By coupling this probe to a SERS-based assay, we achieved a high selectivity and sensitivity in the detection of glucose over other sugars.”

“These advantages should mean that this concept for a glucose assay can be developed into a clinical diagnostic tool,” says Olivo.

Neural probes: Slimmed down for a better fit

A thinner probe array that uses a silicon-based microstructure could underpin safer neural implants

Neural probe arrays are expected to significantly benefit the lives of amputees and people affected by spinal cord injuries or severe neuromotor diseases. By providing a direct route of communication between the brain and artificial limbs, these arrays record and stimulate neurons in the cerebral cortex.

The need for neural probe arrays that are compact, reliable and deliver high performance has prompted researchers to use microfabrication techniques to manufacture probe arrays. Now, a team led by Ming-Yuan Cheng from the A*STAR Institute of Microelectronics, Singapore, has developed a three-dimensional probe array for chronic and long-term implantation in the brain. This array is compact enough to freely float along with the brain when implanted on the cortex.

The neural probe array needs to be implanted in the subarachnoid space of the brain, a narrow region of 1–2.5 millimeters in depth that lies between the pia mater and dura mater brain meninges. “A high-profile array may touch the skull and damage the tissue when relative micromotions occur between the brain and the probes,” explains Cheng. To avoid this problem, the array should be as thin as possible.

Existing approaches produce low-profile arrays using microscopic electrodes machined from a silicon substrate. These approaches, however, restrict the maximum probe length to the thickness of the substrate and the number of recording electrodes. Other methods generate three-dimensional arrays from silicon-supported two-dimensional probes. Complex and expensive to fabricate, these arrays are too bulky because the silicon support also incorporates the application-specific integrated circuit (ASIC) chip for neural recording.

Cheng and colleagues fabricated two-dimensional probes and inserted them into a thin slotted silicon platform for assembly (see image). To produce a three-dimensional probe array, they joined this assembly to the recording chip. Instead of being aligned, however, the team found that the contacts of the probe electrodes and recording chip were orthogonally arranged with respect to each other, resulting in mismatched planes.

“To solve this issue, the team manufactured a silicon-based connector, or interposer, that electrically linked these components,” says Cheng. “This innovative microassembly effectively controls the final height of the array to within 750 micrometers.”

Compared with commercial neural probes, the new array exhibited competitive electrical properties, including electrode impedance. Moreover, biocompatibility tests showed that the presence of array components did not rupture cell membranes or suppress cell growth. The team is currently refining their approach to integrate the array with a wireless recording chip and make the assembly fully implantable.

Cataracts are the main cause of visual impairment worldwide and account for more than 50 per cent of blindness in developing countries. Diagnosis of the condition generally involves a labor-intensive method of ophthalmic examination and grading by comparing the individual’s lens against a set of standard reference photographs (see image).

Now, Yanwu Xu and co-workers from the A*STAR Institute for Infocomm Research, together with researchers in Singapore and China, have developed a system to grade the severity of cataracts automatically using slit-lamp photographs. The technique offers potential for improved clinical management of cataracts and may be extended to other eye conditions in the future.

Cataracts are categorized as nuclear, cortical or posterior, according to their location and appearance. The researchers studied nuclear cataracts, the most common type of cataract, which involve clouding, or opacification, of the central part of the lens. They analyzed a database of 5378 images taken using slit-lamp illumination, the standard method for examining and photographing cataracts.

From this analysis, the research team developed a computer-based system in which each image of a lens is divided into three sections — nucleus, anterior cortex and posterior cortex — and visual features are extracted from a grid of overlapping patches for analysis. The software uses a statistical process, known as group sparsity regression, to determine each cataract’s grade on a standard scoring system. The distinguishing aspect of the group sparsity regression step is that it selects just a few groups of features of intensity and texture in each image, which reduces the influence of random noise and increases the speed of computation.

While not the first attempt to grade cataracts automatically, the researchers found that their system outperforms existing methods by more closely matching the results of manual grading by experienced clinicians. “Manual assessments can be subjective, time-consuming and costly,” says Xu. “Our automated system offers the prospect of greater objectivity and efficiency in diagnosing and monitoring this major condition in huge numbers of people. In addition to the initial diagnosis it could also help doctors to monitor the progression and treatment of cataracts.”

The researchers now hope to refine their software and to explore the possibility of applying it to other eye conditions such as pathological myopia, glaucoma and age-related macular degeneration. Their ultimate aim is to develop a more comprehensive commercialized system.

“The computation is not complex,” explains Xu. “It could form an ocular screening platform based on cloud computing technology that could also provide a remote service.”

Biofuels: Catalytic upgrade

New catalysts to remove oxygenated compounds from bio-derived oils may lead to better and cheaper renewable biofuels

Dwindling crude oil reserves, accompanied by rising prices and environmental concerns, have led to increased interest in the use of renewable fuels. Biofuels produced from waste agricultural or forestry material are particularly desirable because they avoid diverting resources from the production of food crops.

“We hope to develop the selectivity even further so that the catalyst becomes useful for developing fine chemicals as well as fuels.”

Oils produced by high-temperature treatment of these waste materials, however, contain a large amount of oxygenated compounds that result in undesirable properties such as high viscosity and corrosiveness. Now, Jie Chang, Armando Borgna and co-workers from the A*STAR Institute of Chemical and Engineering Sciences in Singapore describe a series of catalysts that might be used to upgrade these oils by removing the undesirable oxygen-containing functional groups. Using the compound guaiacol as a model for oxygenated bio-derived oils, the researchers found that the most promising catalysts for guaiacol deoxygenation are comprised of molybdenum metal on a carbon support.

The diversity of sources of waste biomass means that there is great variability in the content of the bio-oils produced by the initial heat treatment, which is itself the subject of much research. Guaiacol provides, in a single and easily available compound, the types of oxygen-containing functional groups that typically need to be removed.

Catalysts for the related process of desulfurization are widely used in petroleum refineries to produce cleaner fuels, but they are not optimized for deoxygenation. “The desulfurization catalysts are well developed and understood because of extensive research into the mechanisms by which they work,” explains Chang. “We are using guaiacol as a model compound to develop a similar level of understanding for deoxygenation.”

The best catalysts identified by the researchers show complete conversion of guaiacol and over 80 per cent selectivity to the desired hydrocarbon products within minutes.

Chang and co-workers undertook a detailed study of the structure of the catalysts before and during the reaction, as well as of the catalysts that were deactivated. They also attempted to identify the reaction process — in particular, the types of oxygen-containing functional groups that react first and whether this affects the performance of the catalyst.

While catalyst selectivity is critical, other factors such as the activity and stability of the catalyst will prove equally important because of their impact on the economics of the overall process. “There is a long way to go before this complete ‘biomass-to-fuel’ process can become commercial,” says Chang. “Also, we hope to develop the selectivity even further so that it becomes useful for developing fine chemicals as well as fuels.”

Biomimetic materials:

Keeping in contact

Transparent polymeric films with near-uniform, continuous nanoprotrusions show high water pinning abilities

A*STAR researchers have used nanoimprinting methods to make patterned polymeric films with surface topography inspired by that of a rose petal, producing a range of transparent films with high water pinning forces.

A surface to which a water droplet adheres, even when it is turned upside down, is described as having strong water pinning characteristics. A rose petal and a lotus leaf are both superhydrophobic, yet dissimilarities in their water pinning properties cause a water droplet to stick to a rose petal but roll off a lotus leaf. The two leaf types differ in their micro- and nanoscale surface topography and it is these topographical details that alter the water pinning force. The researchers found that the water pinning forces on these continuously patterned surfaces were much greater than on non-patterned surfaces and surfaces composed of isolated nanopillared structures or nanoscale gratings. They could then achieve high water pinning forces by patterning the nanoprotrusions onto polymeric films with a range of different non-patterned hydrophobicities, including polycarbonate, poly(methyl methacrylate) and polydimethylsiloxane (see image).

The imprinted surfaces developed by Jaslyn Law and colleagues at the A*STAR Institute of Materials Research and Engineering and the Singapore University of Technology and Design have uniformly distributed patterns of nanoscale protrusions that are either conical or parabolic in shape. The researchers found that the water pinning forces on these continuously patterned surfaces were much greater than on non-patterned surfaces and surfaces composed of isolated nanopillared structures or nanoscale gratings. They could then achieve high water pinning forces by patterning the nanoprotrusions onto polymeric films with a range of different non-patterned hydrophobicities, including polycarbonate, poly(methyl methacrylate) and polydimethylsiloxane (see image).

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In contrast, nanoimprinting methods are capable of fabricating versatile and large-scale surfaces, and can be combined with roll-to-roll techniques, hence potentially enabling more commercial applications.

The patterned polycarbonate surfaces were also shown to reduce the ‘coffee-ring’ effect: the unevenly deposited film left behind upon the evaporation of a solute-laden droplet. This mitigation of the coffee-ring effect may assist microfluidic technologies and, more generally, the patterned surfaces could be used in arid regions for dew collection or in anti-drip applications such as in greenhouses.

Cancer drugs can be modified to specifically target tumor sites to help personalize cancer treatment. And while it is relatively easy to determine if the drugs have been delivered to the correct location, it is more difficult to monitor their therapeutic success. Now, a team led by Bin Liu from the A*STAR Institute of Materials Research and Engineering in Singapore, in collaboration with Ben Zhong Tang at Hong Kong University of Science and Technology, has developed an anticancer drug with an inbuilt mechanism that shows if it is working.

Platinum-based drugs are effective against many cancers, killing cells by triggering cellular suicide, or apoptosis. These drugs can, however, have severe side effects. Nontoxic forms can be modified, as a type of prodrug, to convert to their toxic form only after entering the targeted tumor cells, so as not to harm noncancerous cells.

Liu and colleagues went one step further by modifying a platinum-based prodrug to not only target tumor cells effectively, but also show whether or not it was having the desired effect. According to Liu, this added feature could be crucial for improving cancer treatment.

“Early evaluation of a patient’s response to a specific cancer therapy is important in clinical applications because it can minimize the duration of ineffective courses,” explains Liu. “The effectiveness of cancer treatment is commonly evaluated using magnetic resonance imaging to measure the tumor size, but this is unsatisfactory as the change in size is not obvious at the early stages of therapy.”

In their new system, Liu and her team included an apoptosis sensor that is released when the prodrug converts to its toxic form inside the targeted tumor cells. The toxic form triggers cell apoptosis and activates an enzyme called caspase 3, which then cleaves the apoptosis sensor and causes it to fluoresce green. This provides a visual signal that the drug is killing the cells.

Liu and her colleagues tested the mechanism by treating cultured cancer cells with the modified platinum prodrug. They observed a gradual increase in fluorescence in the cancer cells, reaching a maximum level of fluorescence six hours after treatment. Noncancerous cells were not affected in the same way, further proving the effectiveness of their targeting mechanism.

Such noninvasive and real-time imaging of drug-induced apoptosis could be used to evaluate the therapeutic response to a specific anticancer drug at an early stage, explains Liu. “Our system can simultaneously deliver the therapeutic drugs and noninvasively evaluate the therapeutic responses in situ.”

Drug delivery:

A ‘click’ toward localized chemotherapy

‘Click’ chemistry produces a hydrogel with less toxicity and greater tissue localization in a mouse cancer model

Platinum-based chemotherapy drugs are commonly used to treat a wide variety of solid tumors, including cancers affecting the breast, colon and lung. However, only a small amount of these anticancer drugs typically reaches the target organ system. This inefficiency not only reduces the efficacy of the drug; it also leads to severe side effects, ranging from nausea to kidney toxicity or deafness.

Charlotte Hauser and her co-workers at the A*STAR Institute of Bioengineering and Nanotechnology in Singapore have now developed a platform for the localized and sustained release of platinum-based anticancer therapeutics that overcomes many of these limitations.

The research team’s novel gel formulation — a combination of specialized peptides and the drug oxaliplatin — led to dramatic growth inhibition when injected directly into the breast tumors of mice. In addition, the gel treatment produced a better tolerance profile than standard oxaliplatin drug therapy.

“Compared to the free drug, our injectable drug-loaded conjugate is just as effective in inhibiting tumor growth, but with lower systemic toxicity and higher localization in the target tissue,” says Hauser.

Hauser’s team started with a unique class of ultrashort peptides that have the ability to spontaneously self-assemble and form hydrogels. The researchers attached oxaliplatin to the peptides using a technique known as ‘click’ chemistry, which enables the synthesis of complex molecules through the joining of multiple attached parts (see image). The resulting oxaliplatin-peptide hydrogels proved highly lethal against two human cell lines derived from cervical cancer and colon cancer tissues, respectively. Laboratory analyses showed that the hydrogels bound to the DNA of the cancer cells, arresting their replication cycle — just as free oxaliplatin does. Furthermore, the whole construct was biocompatible and generally non-immunogenic.

The researchers compared their hydrogel head-to-head with unmodified oxaliplatin in a breast cancer mouse model by injecting the drugs locally into the mouse tumors. Where the drug was delivered as a hydrogel, they documented higher rates of drug accumulation in the tumor — but lower levels of drug toxicity in the kidneys and livers — compared to the free oxaliplatin control. As a result, the mice given the hydrogel maintained more body weight, which can be used as a surrogate measure of their overall health.

“This combination product could serve as a tissue replacement device for the controlled release of important drugs that require localized and injectable treatments,” says Hauser. “We are exploring if this general approach can be utilized to attach a variety of other bioactive molecules.”

In order to determine the best treatment for patients affected by cancer, it is crucial for physicians to identify how the disease is spreading via lymph nodes in the body — a process known as metastasis. Progression of the disease is currently monitored by dissecting lymph nodes during surgery and subsequently performing biopsies. However, using a more sensitive and accurate method that is less invasive based on optical imaging technologies to visualize disease progression in situ could further improve cancer patient diagnoses and limit the time that they are required to spend in surgery.

“We also hope to develop macrophage probes with near-infrared for better depth penetration, as well as subset-specific agents capable of differentiating between ‘good’ and ‘bad’ macrophages in many different diseases.”

Now, Young-Tae Chang and co-workers at the A*STAR Singapore Bioimaging Consortium, together with Jung Sun Yoo and researchers at the National University of Singapore, have developed a novel fluorescent probe that is capable of infiltrating lymph nodes and highlighting cancer progression. The probe could potentially be used to provide information for making rapid diagnoses during surgery.

“Immune cells in lymph nodes, such as macrophages, have a novel role in disease progression,” explains Yoo. “We wanted to find a macrophage-targeting fluorescent probe that could distinguish metastasized lymph nodes from inflamed or normal lymph nodes.”

The team sifted through many libraries of fluorochromes — small nontoxic organic molecules suitable for intraoperative imaging — while searching for those that could specifically stain macrophages. Flow cytometry and testing with human blood samples allowed the researchers to select the best fit for their fluorescent probe.

“We then used the probe for in vivo mouse imaging,” explains Yoo. “This tested whether the probe could pinpoint the sentinel lymph node — the first node to drain a tumor and potentially initiate cancer spreading through the body.” Following injection, the team’s probe immediately accumulated in the sentinel lymph node of the mouse, with bright signals in inflamed nodes and less bright signals in metastasized nodes (see image).

The new probe has several potential biomedical applications. Researchers could use it to investigate the behavior of macrophages in lymph nodes in vivo. Surgeons could obtain real-time information on the infiltration of tumors and how far the disease has spread while a patient is still in surgery, eliminating the need for pre-operative biopsies and minimizing the potential for further surgical procedures.

“We also hope to develop macrophage probes with near-infrared for better depth penetration, as well as subset-specific agents capable of differentiating between ‘good’ and ‘bad’ macrophages in many different diseases,” states Yoo. “Subtype-specific fluorochromes will have a high impact on the future of clinical imaging.”
Regenerative medicine:

Finding the sweet spot for cartilage formation

A synthetic scaffold with fine-tuned physical properties helps cartilage regrowth at injury sites

Joint injuries often fail to mend properly when not given assistance. In particular, cartilage exhibits a poor capacity for self-repair. It is possible to stimulate regeneration by implanting synthetic scaffolds loaded with cartilage-forming cells (known as chondrocytes) at the site of an injury. However, the quality of the repair depends on the extent to which healthy cartilage can form on a given scaffold.

“Our findings highlight the importance of incorporating considerations of hydrogel stiffness into the design of scaffolds intended for cartilage tissue engineering.”

Now, researchers led by Moto-ichi Kurisawa of the A*STAR Institute of Bioengineering and Nanotechnology in Singapore have devised an optimized formula to encourage proper regrowth of cartilage. Kurisawa’s group has worked extensively with polymers known as hydrogels (see image), whose stiffness strongly depends on the conditions under which they are synthesized. This property enabled the researchers to explore the extent to which the hydrogel stiffness affects cartilage formation. “Research indicates that cells that have adhered to a solid substrate are able to sense mechanical stimuli, and that this can affect many important physiological processes in a way similar to biochemical signals,” says Kurisawa.

Ideally, joints should be enriched in smooth hyaline cartilage rather than tough and dense fibrocartilage, and the researchers suspected that stiffer hydrogel scaffolds might promote increased fibrocartilage formation. To test this idea, they synthesized three different kinds of gelatin–hydroxyphenylpropionic acid (Gtn–HPA) hydrogels, which they classified as having low, medium or high stiffness. Chondrocytes generally survived well and proliferated when seeded onto all three scaffolds, but they produced cartilages of considerably different qualities.

Chondrocytes cultivated on Gtn–HPA-high produced the highest density of cartilage, but this tended to be primarily fibrous in nature, whereas cells grown on Gtn–HPA-low produced only low levels of cartilage. However, the medium-stiffness scaffold proved well suited to the production of hyaline cartilage, facilitating efficient tissue repair after implantation in a rabbit model of joint injury. After four weeks, these animals showed robust hyaline cartilage growth; in contrast, recipients of low- and high-stiffness scaffolds exhibited only limited repair or dense fibrous tissue formation, respectively. Importantly, the Gtn–HPA-medium scaffold also readily dissolved after implantation.

“Our findings highlight the importance of incorporating considerations of hydrogel stiffness into the design of scaffolds intended for cartilage tissue engineering,” says Kurisawa. Based on the optimal ‘middle ground’ identified in this study, he and his colleagues are now hopeful that they can demonstrate the same level of safety and efficacy in large animal models as the next step toward moving to the clinic.

Monoclonal antibodies represent the largest and fastest-growing segment of international biopharma. While these therapeutic agents are a boon for global healthcare, productivity constraints pose a serious challenge for manufacturers seeking to make sufficient amounts for therapeutic applications. Now, A*STAR researchers have developed a high-capacity method to purify monoclonal antibodies that uses magnetic nanoparticles and also introduces new operating conditions.

At present, therapeutic antibodies are generally purified by a technique known as protein A affinity chromatography. The process yields a high purification factor — typically 99 per cent — but it is slow, thereby creating a severe productivity bottleneck. The process is largely hindered by the low capacity of protein A, which binds monoclonal antibodies at an average rate of 50 grams per liter of protein A chromatography media. The overall purification process requires unpurified antibodies to pass through columns packed with the media in multiple cycles that can take up to a week.

A research team led by Pete Gagnon and co-workers from the A*STAR Bioprocessing Technology Institute in Singapore have developed an alternative method with 1,000 times the capacity of protein A. The technique involves the use of polyethylene glycol, which causes the antibodies to be deposited on the surface of starch-coated magnetic nanoparticles (see image). The particles are collected in a magnetic field, undeposited contaminants are washed away and the purified antibodies recovered by removing the polyethylene glycol.

"The high capacity of our nanoparticle method makes it much faster than column chromatography," explains Gagnon. "Instead of the pharmaceutical industry norm of five to eight cycles, the new process requires only one cycle, which takes just a few hours." This reduction dramatically increases the productivity of the new approach over traditional methods.

The new method also required the research team to develop new operating conditions. Polyethylene glycol has been used for decades to process antibodies, but it has never achieved the level of purity needed for clinical therapeutics. The team discovered that by elevating the salt concentration, they could reduce contaminant levels from about 250,000 parts per million to 500: the same level achieved by protein A. A single follow-on polishing step using a multimodal chromatography column further purified the antibodies to clinical quality standards.

Gagnon notes the high potential for adoption of the new technology by industry. In addition to solving the longstanding problem of productivity for monoclonal antibodies, the nanoparticle approach can be applied to many other therapeutic proteins and also to viral vaccines.
Erythropoietin (EPO) — a key regulator of red blood cell production — is widely used for treating certain cancers and anemia induced by chronic kidney disease. However, the time that EPO resides in the bloodstream depends on the extent to which it is modified by sugar chains containing sialic acid. This modification, known as sialylation, prevents liver cells from taking up and destroying glycosylated EPO (see image). EPO fully modified with sugars containing sialic acid persists in the bloodstream for about one hour — five times longer than unsialylated EPO.

Since as much as 80% of manufactured EPO is discarded because it is insufficiently sialylated, optimizing the sialylation of EPO may reduce the expense and dosing requirements of EPO therapy.

Previously, a team led by Zhiwei Song of the A*STAR Bioprocessing Technology Institute in Singapore increased the sialylation level of EPO in mammalian cells deficient in the enzyme N-acetylgalactosaminyltransferase (GnT I) by introducing a functional GnT I gene to the cell line. EPO sialylation in the genetically modified cells was almost 25% more than that obtained from wild-type cells when cultured under laboratory conditions.

The same group has now improved on the capacity of that line by using a process called methotrexate amplification to boost yields of sialylated EPO by an additional 2.5-fold. Moreover, the increased yields can be achieved under industrially relevant conditions.

The process involves inactivating the gene that normally makes dihydrofolate reductase (DHFR) in the GnT I-deficient cell line, and then expressing a segment of foreign DNA that encodes EPO, DHFR and a foreign GnT I enzyme. Because methotrexate inhibits DHFR, which is essential for cell survival, treatment of this modified cell line with increasing concentrations of methotrexate selects cells that have multiple copies of the DHFR gene on the foreign DNA segment. As these cells also have multiple copies of the adjacent EPO and GnT I genes, the process also boosts levels of fully sialylated EPO.

The researchers tested the sialic acid content of the EPO produced from the best line and found that it was over 60% higher than the amount produced by an existing industrial line. Although the yields obtained are still not high enough for manufacturing industrially relevant levels of EPO, Song is confident that additional rounds of methotrexate amplification and modification of the medium and other culture conditions could further increase yields. “Optimal glycosylation is an important consideration for the regulatory use of many protein drugs,” he adds. “So our strategy might find applications beyond EPO alone.”

Predicted structure of glycosylated human erythropoietin (EPO), with the sialylated sugar chains shown in purple and pink.

Artificial intelligence: 
Quick to recognize

Neural networks that function like the human visual cortex may help realize faster, more reliable pattern recognition

Despite decades of research, scientists have yet to create an artificial neural network capable of rivaling the speed and accuracy of the human visual cortex. Now, Haizhou Li and Huajin Tang at the A*STAR Institute for Info-comm Research and co-workers in Singapore propose using a spiking neural network (SNN) to solve real-world pattern recognition problems. Artificial neural networks capable of such pattern recognition could have broad applications in biometrics, data mining and image analysis.

Humans are remarkably good at deciphering handwritten text and spotting familiar faces in a crowd. This ability stems from the visual cortex — a dedicated area at the rear of the brain that is used to recognize patterns, such as letters, numbers and facial features. This area contains a complex network of neurons that work in parallel to encode visual information, learn spatiotemporal patterns and classify objects based on prior knowledge or statistical information extracted from patterns.

Like the human visual cortex, SNNs encode visual information in the form of spikes by firing electrical pulses down their 'neurons'. The researchers showed that an SNN employing suitable learning algorithms could recognize handwritten numbers from the Mixed National Institute of Standards and Technology (MNIST) database with a performance comparable to that of support vector machines — the current benchmark for pattern recognition methods.

Their SNN has a feedforward architecture and consists of three types of neurons: encoding, learning and readout neurons. Although the learning neurons are fully capable of discriminating patterns in an unsupervised manner, the researchers sped things up by incorporating supervised learning algorithms in the computation so that the learning neurons could respond to changes faster.

The researchers tested the performance of the SNN by challenging it with images from the MNIST, which contains 60,000 training images and 10,000 testing images of handwritten numbers (ranging from zero to nine). After several training iterations, the SNN could recognize all the numbers in the database. The accuracy of the SNN was high (around 94 per cent) for training images and moderate (around 79 per cent) for testing images. Compared with support vector machines, the encoding and learning processes of the SNN were fast for training images and moderately fast for testing images.

“We utilized biologically plausible mechanisms to build a cognitive system that is capable of effective and efficient cognitive computations,” says Tang. “Together with other related works, this paper paves the way for constructing a general structure of spiking neural systems for cognitive computation.”

Cancer biology:

Exercising restraint to stall tumor growth

*Regulatory mechanisms that help ‘trap’ an enzyme may prevent the production of cancer-promoting molecules*

Many proteins undergo an assembly-line-style process of glycosylation as they travel from a cellular structure called the endoplasmic reticulum (ER) to the Golgi apparatus and on through its various compartments, after which they are released. Disruptions in this process can contribute to a variety of diseases. Now researchers from A*STAR have identified a regulatory mechanism that prevents the production of glycosylated proteins that potentially promote cancerous growth.

The glycosylation process — executed by various enzymes in a stepwise fashion — attaches complex sugar molecules onto proteins, which can fundamentally alter the destination and function of these proteins in the cell. Many tumors produce proteins that are known as ‘Tn antigens’: these proteins have been decorated with a single sugar molecule by enzymes from the N-acetylgalactosaminyltransferase (GalNAc-T) family. GalNAc-T enzymes normally reside within the Golgi, but Frederic Bard and colleagues at the A*STAR Institute of Molecular and Cell Biology in Singapore previously showed that these enzymes relocate to the ER in certain tumors. Here they target resident proteins for modification and thus contribute to Tn production.

To better understand why this happens, Bard’s team screened more than 900 regulatory proteins that affect Tn levels. Remarkably, their experiments uncovered a dozen different proteins that appear to ensure that GalNAc-T enzymes are retained within the Golgi instead of being delivered to the ER. “We were surprised by the number of regulators that we could identify,” says Bard. One of these, a signaling protein called extracellular signal regulated kinase 8 (ERK8), appeared to be particularly important for restricting Tn accumulation to the Golgi.

When the researchers inhibited production of ERK8, they observed a clear redistribution of GalNAc-Ts from the Golgi to the ER, indicating that this protein normally puts the brakes on such ‘backward’ traffic (see image). “After knocking down ERK8, the relocation pathway responds like a compressed spring that has just been released,” says Bard. The subsequent production of Tn antigens stimulates the active cell migration observed in aggressive cancers, and ERK8 similarly appears to act as a critical check against this behavior in cultured cancer cells.

The researchers also noted unusually low ERK8 levels in tumor tissue taken from breast and lung cancer patients. Bard’s team is now working with animal models to determine the importance of this protein as a safeguard against cancerous growth. “We are building a mouse model where we can experimentally remove ERK8 and observe the effects in healthy and cancer tissues,” says Bard. “We predict that loss of ERK8 will promote tumor invasiveness.”

Research Highlights

ENGINEERING & NANOTECHNOLOGY
Photonics: 

Silicon helps light go through the right channels

Improved design of lasers on optoelectronic chips will advance optical communications

When it comes to data transmission, light is superior to electronics. An ability to transmit data in parallel by utilizing multiple light wavelengths allows optical fibers to carry more information than electrical cables. Computers are currently based on electronics, but they would benefit from employing optical signals. However, for this to become a reality, it needs to be implemented on a small scale and result in low power consumption.

Now, Vivek Krishnamurthy from the A*STAR Data Storage Institute in Singapore and his colleagues have designed a laser on a microelectronic chip that has a lower power consumption and a higher efficiency.1

“By developing lasers on silicon, we can combine the electronic data processing capability of the microelectronic chip with the high energy efficiency of optical communications over distances ranging from a few micrometers within a chip to hundreds of meters in data centers,” says Krishnamurthy.

The processing speed of the microelectronic chip is limited by its power consumption; most of the power is consumed by the connecting electrical wires and links. Optical links, on the other hand, consume practically no energy but are limited by the power consumption of the light source, which is often a laser. For optical links to be feasible on a small scale, the electrical power consumption of lasers must be reduced, yet still be able to generate sufficient optical energy for transmission.

Lasers cannot be made from silicon as it is a poor light emitter. Instead, lasers are fabricated by bonding an active material based on indium phosphide — a good light emitter — to a thin silicon film. However, because silicon is better for carrying optical signals, the light from the laser needs to be routed through the silicon chip via optical channels. This requires fabricating optical channels in silicon outside the laser region.

Generating light efficiently in the active medium and efficiently routing it via the silicon layer simultaneously reduces the electrical current required and increases the power generated. Calculations show that this silicon-based design will have a three to four times higher light generation efficiency than competing schemes.

This high efficiency makes the silicon-based laser design promising for making optical chips, which, says Krishnamurthy, is the next step for the project team. “We have begun the experimental demonstration of the laser,” he says. “Our plan is to integrate this laser onto our silicon platform and develop a fully functional photonic system for applications, for example, in data communications and storage.”

Energy:

Self-powered wireless light detectors

A low-power photodetection system can harness enough energy to power an autonomous sensor and monitoring network

Light detectors are used extensively in daily life as brightness sensors and as receivers for remote control devices in electrical gadgets, for example. However, operating these detectors requires electrical energy, which limits their versatility.

Now, Kui Yao and colleagues from the A*STAR Institute of Materials Research and Engineering in Singapore have developed a photodetector that can harvest just small quantities of detected light to generate enough energy to power a sensing signal transmission through a radio-frequency transmitter.

While the energy contained in a beam of light can be converted into electricity, this energy is not usually sufficient to continuously power an electrical circuit. Even the use of batteries to power a circuit is impractical in many circumstances, explains Yao. “Use of photosensors may take place under extremely harsh conditions intolerable to batteries, or involve environmental monitoring network systems where it may be too expensive or unrealistic to maintain batteries for each sensor.”

Operating an electrical circuit under low-power circumstances requires a buildup of energy, which must be generated by the photodetector. However, commonly used photodetector materials, which are based on semiconductors, lose too much energy for this to occur. “Conventional photodetectors can’t accumulate the minute photovoltaic energy and then harness it to drive a load in a sustainable manner,” explains Yao.

To overcome such energy losses, Yao and colleagues developed photodetectors made from ferroelectric compounds. These insulating materials can separate electrical charges as well as store them with low losses. Ferroelectric detectors can also generate a larger electrical voltage than semiconductors, making it easier for them to power other electrical components.

The researchers connected their ferroelectric detector to a specially designed electrical circuit, which is mechanically opened and closed by a switch in the form of a piezoelectric cantilever. Any generated electricity is temporarily stored in the ferroelectric detector and a capacitor. Once the electrical charge of the capacitor is sufficiently high, the cantilever changes its shape and closes the electrical circuit. This activates a commercial radio transmitter.

So far, the team’s main challenge in developing the device has been to minimize electrical losses. Remarkably, Yao and his team have shown that almost 70 per cent of the accumulated electrical charge can be retrieved from the capacitor — even ten minutes after the light source has been switched off. This advantage provides the team’s device with the potential for use in a wide range of applications, such as wireless optical sensors and monitoring networks.

Wireless transmission at microwave frequencies is important for high-data-rate transmission applications, such as mobile phone networks, satellite links and remote imaging. Now, Xianshu Luo and colleagues from the A*STAR Institute of Microelectronics in Singapore have investigated different designs of silicon modulators that enable fast data conversion from electrical to optical signals.

A key component in a microwave photonic network is the modulator, which converts an electrical signal into an optical signal. “The performance of the microwave photonic system relies on the quality of this conversion, which is determined by factors such as loss, noise and signal distortion,” explains Luo. As the modulator acts a bridge between optical components and silicon-based electronics, it should be fabricated on a silicon chip.

The researchers built their modulators according to standard specifications used for semiconductor electronics. A typical modulator consists of two small channels for light — so-called waveguides — etched into a silicon chip (see image). Light is fed into a waveguide on the chip, which then splits into two; modulation occurs when these two beams are reunited. If the light passing through one channel is delayed slightly compared to that in the other channel, the signals from both beams will either cancel each other out or reinforce each other. This property is used to generate the ‘0’ and ‘1’ signals for digital transmission.

In silicon modulators, light transmission in one waveguide is delayed by applying a radio signal, which results in electrical charges either being added to or removed from the material surrounding the waveguide. This addition or subtraction of charge modifies the optical properties of silicon.

Modulators based on the addition or removal of electrical charges have different attributes. While the initial injection of electrical charge carriers — charges that are free to move — is fast in modulators based on the addition of charges, the carrier recombination takes time, which slows down the overall speed. Modulators that have electrical carriers removed, reducing the nonlinear optical effects, experience less noise in the modulated signals.

The different characteristics of the two types of modulators mean that they are suited to different applications, and the researchers’ experiments are helping to inform this choice. Both designs are capable of fast speeds, with the devices under test having an operation bandwidth of about 10 gigahertz, according to Luo. “More recently we have demonstrated similar modulators with even larger bandwidths of up to 28 gigahertz, which means that they can work at even faster rates of data transmission,” he says.

Photonics: Enabling next-generation wireless networks

A modulator that converts an electrical signal into an optical signal could enable faster wireless data transmission
In the eighteenth century, scientists faced a conundrum: is light a wave or a particle? One of the strongest pieces of evidence to support the ‘wave view’ — the landmark double-slit experiment — was reported in 1804 by the scientist Thomas Young. Young passed coherent light through two closely spaced slits and observed a set of interference fringes, a result that occurs with wave phenomena like sound or water. This observation became the basis for the modern wave theory of light.

Two hundred years later, Arseniy Kuznetsov and co-workers from the A*STAR Data Storage Institute, together with collaborators in Australia, Singapore, the United Kingdom and Russia, have performed an experiment analogous to Young’s experiments but using nanoscale objects. The team studied the light scattering in the visible and near-infrared wavelength regions from a cluster of two or three closely spaced gold plasmonic nanoparticles. They observed interference and resonance effects that resemble those seen in Young’s experiments.

In particular, while studying a trimer system consisting of three discrete metallic nanodisks of about 145 nanometers in diameter and 60 nanometers thick, the team found evidence for the presence of near-field, subwavelength-sized optical vortices and the circulation of electromagnetic energy (see image). This finding is very similar to what occurs to the energy flow pattern in a Young-type experiment performed with three slits.

One of the key issues in nanoplasmonics is the interaction between metallic nanoparticles at the nanoscale. “Even if the separation between two or multiple non-periodically arranged nanoparticles is of the order of wavelength, their interaction can be strong enough to change their scattering and absorption properties,” notes Kuznetsov. “This can be explained by the peculiarities of the Poynting vector (energy) flow around the nanoparticles and formation of optical vortices, which produce a pattern of field lines similar to Young’s classic experiment.”

The team’s findings, says Kuznetsov, not only expand our fundamental understanding of how light interacts with nanoclusters of metallic particles, but have both theoretical and practical applications. “They may also prove useful for applications such as improved solar cells and plasmonic biosensors.” However, their most remarkable application, he suggests, may be in the emerging area of nanoantennas.

In the future, the team is aiming to study the resonance properties and interactions of nanoparticles made from nonmetallic materials. In particular, they plan to investigate high-refractive index dielectric materials such as silicon, which, unlike metallic particles, do not suffer from high optical losses.

Example of the energy flow and optical vortices found around closely spaced gold nanoparticles. The effects resemble the field lines seen in Young’s slit experiments.

Electronics:

More speed, less interference

A semi-analytic model can compute electromagnetic interference on an electronic circuit board ten times faster than existing commercial software

As electronic components on electronic circuit boards continue to shrink, problems of electromagnetic compatibility are arising. Such problems include unwanted ‘noise’ effects due to electromagnetic interference and susceptibility. “Electromagnetic interference is a critical problem for the electronics industry,” explains Xian-Ke Gao from the A*STAR Institute of High Performance Computing in Singapore. “Engineers are keen to understand how the electronic circuits react. However, it is difficult to measure such effects experimentally, because disassembling the device would affect the physical testing.”

To address this problem, the electronics industry has developed a suite of computer modeling tools, but these are cumbersome and require a lot of computing power. Now, Gao and colleagues have developed a computer model that is able to solve such problems more than ten times faster than existing models.

Fairly coarse models are typically used to model electromagnetic interference effects on electronic circuit boards (see image). To do this, the device is divided into a grid of small cubes, and the electromagnetic fields to and from each cube are modeled individually. This approach requires a lot of computing power, especially if the grid size is small, but it has the advantage that it is flexible and can be adapted to various geometries. Except for interference effects, the same computer models can be applied to calculate electromagnetic fields for a range of electrical devices other than circuit boards.

A more targeted and efficient approach is required to measure interference effects. Researchers use mathematical equations to describe the electrical currents in a conducting wire. The physics of these transmission-line equations are well understood and, once adapted to the unique properties of circuit boards, are far easier to solve by a computer algorithm than the other, coarser modeling.

The first tests of the software package developed by the A*STAR researchers, which is based on the transmission-line equations, reliably solved a number of standard problems for electronic circuits. Compared to commercial models, the new software achieved very good agreement, especially for the main region of interest — frequencies below one gigahertz.

Speed, however, is the key advantage of using the software. Whereas commercial software requires more than two hours of computing on a regular laptop, the A*STAR software package needed less than ten minutes for the same task, explains Gao. “Our computational problem-solving kit can shorten electromagnetic interference trouble-shooting in the product design phase and therefore translates into time and cost savings for the industry.”


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Labeling biomolecules with light-emitting nanoparticles is a powerful technique for observing cell movement and signaling under realistic, \textit{in vivo} conditions. The small size of these probes, however, often limits their optical capabilities. In particular, many nanoparticles have trouble producing high-energy light with wavelengths in the violet to ultraviolet range, which can trigger critical biological reactions.

Now, an international team led by Xiaogang Liu from the A*STAR Institute of Materials Research and Engineering and the National University of Singapore has discovered a novel class of rare-earth nanocrystals that preserve excited energy inside their atomic framework, resulting in unusually intense violet emissions\textsuperscript{1}. Nanocrystals selectively infused, or ‘doped’, with rare-earth ions have attracted the attention of researchers, because of their low toxicity and ability to convert low-energy laser light into violet-colored luminescence emissions — a process known as photon upconversion. Efforts to improve the intensity of these emissions have focused on ytterbium (Yb) rare-earth dopants, as they are easily excitable with standard lasers. Unfortunately, elevated amounts of Yb dopants can rapidly diminish, or ‘quench’, the generated light.

This quenching probably arises from the long-range migration of laser-excited energy states from Yb and toward defects in the nanocrystal. Most rare-earth nanocrystals have relatively uniform dopant distributions, but Liu and co-workers considered that a different crystal arrangement — clustering dopants into multi-atom arrays separated by large distances — could produce localized excited states that do not undergo migratory quenching.

The team screened numerous nanocrystals with different symmetries before discovering a material that met their criteria: a potassium fluoride crystal doped with Yb and europium rare earths (KYb\textsubscript{2}F\textsubscript{7}:Eu). Experiments revealed that the isolated Yb ‘energy clusters’ inside this pill-shaped nanocrystal (see image) enabled substantially higher dopant concentrations than usual — Yb accounted for up to 98 per cent of the crystal’s mass — and helped initiate multiphoton upconversion that yielded violet light with an intensity eight times higher than previously seen.

The researchers then explored the biological applications of their nanocrystals by using them to detect alkaline phosphatases, enzymes that frequently indicate bone and liver diseases. When the team brought the nanocrystals close to an alkaline phosphate-catalyzed reaction, they saw the violet emissions diminish in direct proportion to a chemical indicator produced by the enzyme. This approach enables swift and sensitive detection of this critical biomolecule at microscale concentration levels.

“We believe that the fundamental aspects of these findings — that crystal structures can greatly influence luminescence properties — could allow upconversion nanocrystals to eventually outperform conventional fluorescent biomarkers,” says Liu.
Modulators are key components within optical fiber networks and serve to transfer information from an electrical current to a signal suitable for optical fibers. They function by turning a light beam on and off quickly and the faster they can do this, the more data that can be transmitted.

An increase in data traffic creates a need for a reduction in the cost and size of optical components. An improved low-loss design for modulators, suitable for silicon computer chips, has been developed by Soon Thor Lim and colleagues from the A*STAR Institute of High Performance Computing, the A*STAR Institute of Microelectronics and collaborators from Fujikura Ltd.

Existing optical modulators are based on lithium niobate, a material that is expensive and unsuitable for silicon chips. While silicon offers an inexpensive alternative, it can only be used with the addition of other elements that can create positive and negative movable electrical charges. Modulation requires the movable charges to be channeled in and out of the device by an alternating electrical voltage, which controls both the speed of light through the chip and the data rate. Light that passes through this region and crosses light that passes through a neutral silicon region creates interference effects in the optical beam that switches the light on and off.

Previous modulator designs contained charged regions that were relatively large, with the drawback that they increased light absorption in the chip. However, in the team’s proposed design, this area is reduced so that less of the laser beam passes through the charged region (see image).

After computer modeling the performance of the modulator, the team fabricated their device on a silicon chip that has light channels only 220 nanometers high and 550 nanometers wide. Compared to designs with large charged areas, these modulators reduced optical losses by up to 28 per cent and operated at faster speeds of 10 gigabits per second.

“Our device has a speed and optical losses comparable to existing technology such as lithium niobate,” says Lim. “One reason for this high performance is because we used highly accurate computer codes developed in-house.”

Successfully demonstrating the device also highlights how modeling software can reduce the required number of experiments, Lim adds. “Simulation and analysis helps to visualize the physical behavior of these cutting-edge optical devices. This can identify potential problems and circumvent the need for costly multiple design iterations ultimately accelerating the speed to market.”

The two plots show the distribution of electrical charges in different modulator designs. Compared to a previous design (top), the modulator improved by computer modeling (bottom) contains fewer free electrical charges, as depicted by lighter shades of blue (positive charge) and yellow (negative charge).

Grid computing is a powerful form of distributed computing wherein a network of loosely coupled and geographically separated computers, typically of different computational powers, work together to perform data-intensive calculations. The technology uses numerical simulations to help investigate a variety of challenging scientific problems, including the subatomic world revealed by particle accelerators like the Large Hadron Collider.

The current trend in the industry is to build grid-computing systems that can run not just one but multiple large-scale, numerical simulations. While most systems can guarantee good performance, this is usually accompanied by significant cost and large storage requirements. To optimize the cost–performance relationship of large-scale grid-computing systems, scientists must overcome several issues — one of which is the efficient allocation of computational resources, known as scheduling.

Rubing Duan and Xiaorong Li at the A*STAR Institute of High Performance Computing in Singapore and co-workers have now developed a scheme to address the scheduling problem in two large-scale applications: the ASTRO program from the field of cosmology, which simulates the movements and interactions of galaxy clusters, and the WIEK2k program from the field of theoretical chemistry, which calculates the electronic structure of solids. The researchers’ new scheme relies on three game-theory-based scheduling algorithms: one to minimize the execution time; one to reduce the economic cost; and one to limit the storage requirements.

The researchers performed calculations wherein they stopped the competition for resources when the iteration reached the upper limit of optimization. They compared their simulation results with those from related algorithms — namely, Minimum Execution Time, Minimum Completion Time, Opportunistic Load Balancing, Max-min, Min-min and Sufferage. The new approach showed improvements in terms of speed, cost, scheduling results and fairness.

Furthermore, the researchers found that the execution time improved as the scale of the experiment increased. In one case, their approach delivered results within 0.3 seconds while other algorithms needed several hours.

Nonetheless, the researchers note that their algorithms may not be suited for applications that are highly heterogeneous.

Prior to this study, only a handful of work had considered the optimization of multiple metrics. However, Duan, Li and colleagues were able to present a model approach to reducing the execution time, economic cost and storage requirements in grid-computing systems, especially when used in large-scale applications.

“Our game-theory-based scheduling algorithms possess great potential for large-scale applications,” says Duan. “We are looking into how the algorithms adapt to other metrics, such as memory, security, resource availability, network bandwidth and multiple virtual organizations.”

Scheduling algorithms based on game theory can make better use of computational resources to reduce the costs of grid computing

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Electric vehicles are becoming more popular due to their environmental credentials and relatively low running costs. However, most existing electric vehicles need to be recharged every 100 to 150 kilometers, with each recharge potentially exposing information related to a customer’s payment and location. Now, researchers at A*STAR have described a new system that would allow quick and easy money transfers at electric vehicle charging stations, without jeopardizing customer privacy.

“Cybersecurity is an important factor for payment systems, but it is often ignored by users or administrators until the system is being attacked,” says researcher Joseph Liu from the A*STAR Institute for Infocomm Research in Singapore. “No one should have their daily habits or behavior traced without their consent.”

The recharging of electric vehicles presents unique challenges for privacy, not least because some cars with solar panels are able to sell electricity back to the grid, meaning payments flow in both directions. Without tight security, payment companies or hackers could monitor where and when cars are charged, gaining insight into people’s lifestyles that could be exploited for targeted spam marketing.

“Some popular electronic payment systems like credit cards do not provide any privacy, while other systems like prepaid cash cards may not be suitable for large payments, or are not insured against card loss,” says Liu.

“Cash is anonymous, but requires expensive machines to keep cash stores secure from thieves.”

The new system developed by Liu and co-workers is based on an in-car unit that resembles a smartphone or tablet and, along with a range of security benefits, allows two-way anonymous payments for recharging. Users can instantly shut down their accounts and retrieve unused credit. Also, if their car is stolen they can revoke the location privacy to help police trace the car. In the event of a dispute between a user and a supplier, either party can submit the claims to an independent judging authority for investigation.

The researchers tested their system by simulating three different types of attack: a hacker trying to track the transactions of an honest user, a user trying to underpay for services, and a supplier trying to slander an honest user. The system proved robust against all three attacks.

The team has now implemented a prototype of their secure charging system. They will install the tamper-proof in-car units on a fleet of 100 new electric vehicles that will arrive in Singapore later this year, thanks to collaboration with the Chinese carmaker BYD Auto.

An electronic payment system developed at A*STAR will protect the privacy of customers recharging their electric vehicles.

A visual saliency technique that can detect and extract relevant information from both still and moving images has many applications for computer image processing. Such a technique can be used to detect motion, distinguish different objects and improve the quality of specific parts of an image through selective compression.

Shijian Lu and colleagues from the A*STAR Institute for Infocomm Research in Singapore have developed a robust and efficient method for capturing such salient information from images and movies. They found that the key lies in the distribution of brightness and color between pairs of pixels.

Digital images are encoded as pixels, or points in an image. To detect an object (for example, a person standing in the foreground), brightness variations between neighboring pixels could be compared. However, considering just individual pixels can be deceiving, as the context is important when seeking to distinguish between important details and unimportant background information.

The technique developed by the A*STAR researchers hence involves counting the pixels in an image based on their color and then plotting the distribution. This makes not only the distribution of colors apparent, but also the frequency at which pairs or neighboring pixels appear. A low frequency of pixel pairs with a certain difference in color indicates a region of high interest, as it denotes clear boundaries between objects. In this way, the salient features can be easily identified; for example, not only large areas of contrast in a photograph, such as a yellow school bus in front of a neutral background, but also contrasts in smaller areas, such as a person wearing a safety vest (see image).

“Our model has great potential for predicting the point in an image that will attract the human eye,” comments Lu. “Apart from generic object detection, it can be applied to tasks such as guiding robots or to the smart design of web pages and advertisements.”

The next step for the researchers will be to apply this scheme to detecting motion in videos, which follows similar rules as identifying relevant information in still photographs. Moreover, Lu says that their algorithm enables more complex approaches to image analysis.

“Combining our bottom-up modeling algorithm with a top-down visual search could solve many challenging computer vision problems — such as anomaly detection or target search — in a more robust, efficient and cognitive manner.”

Analyzing pixel correlations in photographs outperforms current methods of extracting relevant information for image analysis.

“A*STAR Institute for Infocomm Research

Image processing:
Focusing on what’s relevant

Saliency map (right) of an image (left), which illustrates how a computer model is able to identify salient information such as the high-visibility vest from an image based on statistical analysis.

Antireflection coatings are familiar from their use in everyday optical devices, such as glasses and lenses. They can increase the amount of light that passes through optical instruments by reducing the fraction of light reflected (and hence lost) at surfaces. Antireflection coatings have applications beyond visible light: for instance, in the infrared and terahertz regimes they are useful for chemical sensing and imaging applications, such as those employed at airport security checks.

Now, Jing Hua Teng from the A*STAR Institute of Materials Research and Engineering and colleagues from the A*STAR Institute of Microelectronics and Osaka University, Japan, have developed ultrathin antireflection coatings for terahertz waves that can be applied to almost any surface. “Their fabrication is very straightforward, as it takes only one step of photolithography, metal deposition and lift-off,” explains Teng.

Antireflection coatings are usually based on interference effects, which requires them to be at least as thick as the wavelength of light. This is practical for visible light, with wavelengths in the range of hundreds of nanometers. However, it is a serious limitation for infrared and terahertz radiation, which has much longer wavelengths of the order of hundreds of microns. Moreover, as these coatings are often functional only over narrow frequency ranges, they do not operate across the broad ranges needed for terahertz sensing applications.

The research team developed antireflection coatings based on metamaterials, which are metallic structures that are much smaller than the wavelength used. These structures completely alter the optical properties of a material in a predetermined way, enabling the generation of a much broader range of optical effects than those that occur naturally. One application of the unusual optical effects they produce is invisibility cloaks.

In the new design for metamaterial surfaces developed by the researchers, thin strips of chromium are fabricated on a silicon surface to form a grating (see image). Silicon, being flexible, is a typical material for terahertz optics. When terahertz light passes through the stripes and into the silicon, its phase is changed in the same way as for the much thicker coatings based on interference effects; this suppresses surface reflection.

These metasurfaces have the advantage that they can function across an unprecedentedly wide frequency range, namely 0.06 to 3 terahertz. The flexibility of the coatings for other wavelengths and applications also enhances their commercial appeal, comments Teng. “The beauty of this method is that it is very flexible and can be easily adapted to other metals and substrates.”

Computer numerically controlled (CNC) milling machines — used in high-tech industries such as aeronautical manufacturing — operate continuously to maximize the production efficiency. However, this requires careful monitoring for any tool deterioration. Omid Geramifard and co-workers from the Singapore Institute of Manufacturing Technology at A*STAR have now developed an improved algorithm for diagnosing tool-wear problems before they occur.

“With more accurate, data-driven diagnostics and prediction, more efficient tool usage can occur for assured quality of the produced workpiece.”

CNC milling machines cut and shave metal materials into precisely specified structures. To ensure they can operate 24 hours a day without any unnecessary downtime, the milling tools are carefully monitored with unobtrusive sensors and analytical computer models. The algorithm developed by Geramifard’s team uses a sophisticated, multicomponent model that narrows down tool sensor data to the most effective group for analysis — an innovation that boosts its predictive capabilities while maintaining its computational efficiency.

As it is hard to estimate when tools will fail by simply using physical models of actual milling machines, researchers tend to use data-driven models that analyze historical tool-wear patterns. One such method, known as the hidden Markov model (HMM), hypothesizes that a tool’s condition depends only on its past behavior, a simplification known as the Markov assumption. ‘Hidden’ states are then introduced to account for degradation factors that cannot be inspected directly. Using probability equations to relate observable data to the hidden states, HMMs can calculate how tools change over time.

Geramifard and co-workers improved current models by developing a multimode HMM-based approach that captures and analyzes several different tool-wear parameters. They ‘trained’ each mode of the HMM with selected segments of experimental data, and then combined the multiple outputs using weighting schemes. However, running simultaneous HMMs requires intense computational power, which significantly slows the CNC machine monitoring process.

To overcome this problem, the researchers devised a ‘windowing’ technique that reduces the computational cost by performing HMM calculations over a short time frame — selected through a cross-validation process in the training phase — instead of over the full observation sequence. When the team used their multimodal approach to predict tool wear in a real CNC milling machine, they found their approach outperformed conventional techniques when appropriate window lengths were adopted. This breakthrough in accuracy was realized by removing unnecessary connections to old observations.

“This model opens the path to more effective stochastic modeling of tool wear and degradation,” says Geramifard. “With more accurate, data-driven diagnostics and prediction, more efficient tool usage can occur for assured quality of the produced workpiece.”

Nano-optics: Getting the most out of tiny lasers

An off-center waveguide enables light to be efficiently extracted from nanoscale lasers

Semiconductor optical devices are becoming increasingly commonplace. For example, light-emitting diodes, as they become more power efficient, are rapidly replacing conventional light bulbs. Lasers too are now found in every barcode scanner and compact-disc reader.

“Our scheme, based on directly joining a waveguide, enhances light extraction by splitting the plasmon mode.”

When designing these devices, a crucial consideration is how best to get the light generated within the solid material out into the real world. Chee-Wei Lee at the A*STAR Data Storage Institute, Singapore, and international colleagues have now proposed a light-extraction scheme that is capable of transferring over half the light created by a submicrometer-scale laser into a waveguide.

Plasmonic lasers are the smallest lasers created to date — they can even be smaller than the wavelength of the light they emit. This counterintuitive property results from plasmons, which are hybrid electron–light particles created by coupling light with electrons in a metal.

Lee and his team considered the simplest plasmonic laser: a ring of a light-emitting semiconductor coated with a thin silver layer. Light can travel round and round inside the ring, which provides the optical cavity required in most laser devices. What is more, this tiny laser can be bonded onto a silicon substrate to make it compatible with compact photonics-on-a-chip technology. Lee and his team used computer simulations to demonstrate that high extraction efficiency is obtained when a waveguide (a light-carrying submicrometer-wide semiconductor strip) is directly connected to the side of the laser.

The team used a numerical simulation technique called finite-difference time-domain to study the performance of waveguides of different widths connected at different points on the laser. Their models revealed that the optimal structure is an asymmetric one. When the extraction waveguide is displaced from the center of the ring — so that the waveguide is flush with the edge of the cavity — it produces a peak out-coupling efficiency of 56 per cent (see image). “Our scheme, based on directly joining a waveguide, enhances light extraction by splitting the plasmon mode,” explains Lee.

Scientists have previously extracted light from plasmonic lasers by running a waveguide extremely close to, but not touching, the cavity ring. Light can leak across the gap between the laser and the waveguide through an effect called evanescent coupling. But this approach requires precise control over the gap size and the optical properties of the material in the gap. The method developed by the team, however, can be implemented using much simpler device fabrication. “We are now in the process of actually realizing such a device,” says Lee.

The ability to detect tiny quantities of molecules is important for chemical sensing as well as biological and medical diagnostics. In particular, some of the most challenging and advanced applications involve rare compounds for which only a few molecules may be present at a time. The most promising devices for achieving ultrahigh-precision detection are nanoscale sensors, where molecules are placed in tiny gaps between small gold plates. But this method is effective only if the molecules are positioned accurately within the gaps. Now, Jinghua Teng from the A*STAR Institute of Materials Research and Engineering, Singapore, and colleagues from the National University of Singapore have developed a sensor where molecules are efficiently guided and placed into position¹.

The electronic resonances occurring in gold nanostructures are like very powerful antennas, able to amplify radiation from small molecules in their vicinity. This permits even the detection of single molecules. In order for the signal to be picked up by the antennas, however, the molecules need to be precisely located within electromagnetic ‘hot spots’ (see image). “We approached this challenge and developed a method to selectively bind the molecules to the electromagnetic hot spots in the nanoantenna structure for maximum effect,” explains Teng.

The researchers needed to prepare the device surface such that the molecules bind only to the desired areas between the gold plates — not on them. They achieved this by depositing a thin titanium film between the gold plates. The titanium oxidizes in air, forming stable titanium dioxide, which is insulating and has very different properties to the gold plates. The researchers then covered the surface with various organic solutions that selectively prevent proteins and other molecules from binding to the gold while attracting the molecules of interest to the titanium pad. In initial tests, signals from molecules attached to the titanium in the hot spot showed a six times higher sensitivity than those randomly attached across the device.

The next step will be to increase the sensor sensitivity to the ultimate limit, explains Teng. “People have been dreaming of and working toward single-molecule sensing. This work is part of these efforts. It provides a way to selectively bind biomolecules to the hot spots and proves that it can enhance the molecular sensitivity and reduce the amount of sample required.” Further improvements in device design will however be required, adds Teng. “Moving forward, we would like to further push the sensitivity by optimizing the structure and try multi-agent sensing in one chip.”

Microscopes are conventionally used to image tiny features. However, their resolution is inherently limited by the wavelength of light. This limitation means that they can resolve only structures larger than a few hundred nanometers.

Now, Leonid Krivitsky and Boris Luk’yanchuk at the A*STAR Data Storage Institute in Singapore and co-workers have demonstrated an alternative optical approach capable of mapping surfaces at resolutions below 100 nanometers. 1

Diffraction is the tendency for all waves, including light, to spread out when they pass near an object or through a gap. This effect means that optical imaging systems cannot resolve objects smaller than roughly half the wavelength of the illuminating light. Thus, for red light with a wavelength of about 600 nanometers, the resolution will be approximately 300 nanometers.

Luk’yanchuk and his colleagues previously showed that a micrometer-scale transparent bead placed on a surface can circumvent this so-called diffraction limit. They demonstrated that light passing through the bead, when collected by a conventional microscope, can create an image of the surface beneath it with a resolution of 50 nanometers. However, generating a complete two-dimensional map requires scanning the bead across the surface — not easy to perform in a controlled way when the sphere is only 6 micrometers across. “We have now improved this superresolution technique by developing a method to controllably move the imaging microspheres,” says Krivitsky.

Krivitsky and his team accomplished such spatial scanning using a tiny pipette with a tip just 1 or 2 micrometers wide. Computer simulations confirmed that the presence of the pipette would not adversely affect the superresolution capability of the microspheres. To fasten the pipette to the bead, they sucked the air out from within its cavity (see image).

The team then connected the other end of the pipette to a mechanical stage, which could move in steps as small as 20 nanometers. Importantly, the vacuum inside the pipette created a bond tight enough to ensure that the bead did not disconnect as it was dragged across a surface. The researchers demonstrated the effectiveness of their system by successfully imaging trial samples with features as small as 75 nanometers.

While other techniques, such as near-field scanning microscopy, can perform sub-diffraction-limit imaging, they require very expensive systems. “The real advantages of our technique are its simplicity and its price,” says Krivitsky. “The idea could be applied to a variety of superresolution applications such as sample inspection, microfabrication and bioimaging.”

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Laptops have the advantages of being more versatile and portable than their desktop counterparts. But these attributes impose considerable demands on the electronic components in a laptop — particularly the hard drive. The magnetic disk inside a hard drive rotates at a rate of several thousand revolutions a minute. At the same time, a read/write head moves only a few nanometers above the disk surface to access information on the disk. At such high speeds, large vibrations can permanently damage the hard drive.

To help reduce hard drive failures, Jianqiang Mou and colleagues from the A*STAR Data Storage Institute in Singapore have now developed a computer model that can predict and minimize the effects of vibrations on the hard drive and ultimately help to improve laptop design.

Current designs of many laptops actually compound the problems caused by vibrations. For example, to provide protection from external impact and accidents, laptops are often encased in special housings intended to absorb accidental drops and other shocks. Such laptop designs can actually be counterproductive if not done properly, explains Mou. “The commercial notebook computer industry rarely understands how chassis design can substantially affect the performance of the hard drive. Some notebook computers are designed with vibration sources, for example the loud speaker, located close to the hard drive.”

To get back to the fundamentals of laptop design, the researchers developed a theoretical framework that models the propagation of vibrations from various components in a laptop, such as the speakers, to the hard drive. Underpinning this framework are mathematical equations that describe the transmission of vibrations in laptops, and these equations form the input for a computer model applied to specific laptop designs.

The results of the researchers’ calculations can be used to inform general laptop design strategies. For example, often very stiff materials are used for laptop cases to provide enhanced mechanical strength. However, stiff materials tend to transmit high-frequency vibrations more strongly than flexible materials, and it is difficult for hard drives to compensate for these frequencies. Softer materials are preferable as they suppress higher frequency vibrations, leaving only slower vibrations which are easier for hard drives to compensate.

“Our study provides an effective approach for computer and hard drive makers to optimize the chassis design and component mounting.”

"Our study provides an effective approach for computer and hard drive makers to optimize the chassis design and component mounting,” adds Mou. “Furthermore, the methodology presented in our paper can be applied for analysis and optimal design of other computer chassis, such as servers in data centers.”

Two-dimensional sheets of electronic materials, such as graphene, show promise for practical nanoelectronics applications, including transparent electronic circuits used in electronic displays. Molybdenum disulfide (MoS\(_2\)) is of particular interest because, unlike metallic graphene, it is semiconducting, like silicon — the semiconductor that underpins today’s computer technology.

Now, Yongqing Cai from the A*STAR Institute of High Performance Computing in Singapore, with colleagues from China and the United States, has calculated that, by adding hydrogen to a MoS\(_2\) surface, regions of the surface can be converted into metallic ‘roads’. These roads can transport electrical charges between different areas of a MoS\(_2\) nanosheet, enabling the fabrication of integrated electronic circuits.

Computer chips require both semiconductors and metals. Semiconductors (typically silicon) are the basis for electronic components such as transistors, whereas metals (generally copper or gold) are used for wires that transport electrical charges around a chip. One advantage of using two-dimensional sheets such as MoS\(_2\) is that semiconductors and metals can be integrated on the same sheet, facilitating the development of nanoscale computer chips.

For this to become a reality, the semiconducting properties of a MoS\(_2\) sheet need to be modified to enable some areas of the sheet to become metallic and hence electrically conducting. Cai dubs these regions ‘nanoroads’. “The design of conductive nanoroads on two-dimensional nanosheets — in a way that doesn’t compromise their structural integrity — is critical for transporting electrical charges and to create reliable, highly conducting channels for nanoelectronics applications,” explains Cai.

MoS\(_2\) has to be modified before it can conduct electricity, since it requires additional atoms to be able to transport electrical charges. The researchers simulated the effects of adding hydrogen atoms to the surface of a MoS\(_2\) sheet and found that MoS\(_2\) will become metallic in areas where hydrogen atoms bond to its surface. They showed that adding lines or chains of hydrogen atoms to the surface created metallic strips. The researchers’ calculations reveal that these strips, or nanoroads, are reliable electrical conductors, and, importantly, they do not damage the structure of the underlying sheets.

In terms of practical implementation, the technology already exists for depositing hydrogen on semiconductor nanosheets: hydrogen has been deposited on other two-dimensional sheets, including graphene. Before MoS\(_2\) sheets can be used to produce components such as transistors, a method for producing electron-deficient regions needs to be developed. Once this practical challenge has been addressed, the way will be open to successfully using MoS\(_2\) in integrated electronic applications.

Cybersecurity: Cracks emerge in the cloud

A systematic analysis reveals that cloud storage services have security weaknesses that can inadvertently leak users’ data

As individual computer users increasingly access the Internet from different smartphones, tablets and laptops, many are choosing to use online cloud services to store and synchronize their digital content. Cloud storage allows consumers to retrieve their data from any location using any device and can provide critical backups in the case of hard disk failure. But while people are usually vigilant about enacting security measures on personal computers, they often neglect to consider how safe their files are in the cloud.

Now, findings from a team led by Jianying Zhou of the A*STAR Institute for Infocomm Research in Singapore promise to improve the security of popular online services and better protect users by revealing hidden flaws associated with an important cloud storage feature — the ability to share files with friends, co-workers or the public.

Sharing content is an attractive way to let far-flung colleagues view and collaborate on projects without using email attachments, which often have strict file size limitations. Data sharing can be either public, with no access controls; private, in which the cloud service provider authenticates sharing through login controls; or ‘secret’ uniform resource locator (URL) sharing where people without an account on the cloud service can access data by following a specific web link.

The A*STAR-led researchers analyzed the security of three well-known cloud service providers — Dropbox, Google Drive and Microsoft SkyDrive — and found that all three had vulnerabilities many users might encounter. They uncovered several risks related to the sharing of secret URLs. Because URLs are saved in various network-based servers, browser histories and Internet bookmarks, frequent opportunities exist for third parties to access private data. Furthermore, the URL recipient may send the link to others without the data owner’s consent.

Another danger lies in the practice of URL shortening — reducing long web addresses to brief alphanumeric sequences for easier sharing on mobile devices. Although the original URL may point to a privately shared file, shortening changes this address into plain text unprotected by encryption. Zhou also notes that because short URLs have very limited lengths, they are susceptible to brute-force attacks that can dig out supposedly secret files.

Zhou explains that the root cause of cloud security problems lies in the need to balance usability with privacy protection. “Users should be careful when they share files in the cloud because no system is perfectly secure. The cloud industry, meanwhile, needs to constantly raise the bar against new attacks while keeping the service as functional as possible.”

In 2009, the global number of city dwellers surpassed that of rural dwellers. Understanding how cities evolve is vital to a world that will continue to urbanize. Now, researchers at A*STAR have developed a computer model that can reconstruct cities — building them from the ‘bottom up’ — to investigate the fundamental mechanisms that underpin and govern city growth.

Modeling dynamic urban growth presents many challenges due to the complexity of city systems and the technical and data requirements of model building. Christopher Monterola and co-workers at the A*STAR Institute of High Performance Computing, together with scientists in the United Kingdom, have built a model based on a so-called cellular automation system, which uses a minimalist approach to simulate city growth and support planning for sustainable cities.

“The overarching vision of our team is to capture the form, structure and dynamics of different cities to better understand, manage, design and evaluate urban systems,” explains Monterola. “Essentially, we are trying to generate the recipe for a sustainable, smart city.”

The model works by taking a simple set of rules — for example, land value and physical constraints to building such as water bodies and parklands — and defining the probable land use of each cell, or unit of land, according to the information provided by its neighboring cells. The model then takes into account the different land-use sectors — industrial, business and residential — and determines their ‘range of influence’, to decide how far a certain type of land use will spread within a certain radius. For each simulation, the team set the model center at the original marketplace, or central business district, of a real city, and let the model ‘grow’ the city from there.

The team validated their results using high-resolution data from various cities, including Singapore, Toronto and Las Vegas. Their model replicated, fairly accurately, the land-use patterns of the actual cities (see image).

“Our results suggest that there are some generic rules that a growing city follows as it evolves,” Monterola states. “We found that there was an effective and stable cluster size for business, residential and industrial areas in all the cities studied, and their size ratios are remarkably regular. Hence, sustainability concepts must be somehow anchored on accepting this innate evolution, and policies need to be planned around such constraints.”

The team plans to further develop their urban growth model, for example by investigating the limitations on individual city transport systems and working on ways to make cities run more efficiently and sustainably.

The Agency for Science, Technology and Research (A*STAR) is Singapore’s lead government agency dedicated to fostering world-class scientific research and talent for a vibrant knowledge-based economy.

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

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

- Bioinformatics Institute (BII)
- Bioprocessing Technology Institute (BTI)
- Clinical Imaging Research Centre (CIRC)
- Data Storage Institute (DSI)
- Experimental Therapeutics Centre (ETC)
- Genome Institute of Singapore (GIS)
- Institute of Bioengineering and Nanotechnology (IBN)
- Institute of Chemical and Engineering Sciences (ICES)
- Institute of High Performance Computing (IHPC)
- Institute for Infocomm Research (I2R)
- Institute of Materials Research and Engineering (IMRE)
- Institute of Medical Biology (IMB)
- Institute of Microelectronics (IME)
- Institute of Molecular and Cell Biology (IMCB)
- National Metrology Centre (NMC)
- Singapore Bioimaging Consortium (SBIC)
- Singapore Institute for Clinical Sciences (SICS)
- Singapore Institute of Manufacturing Technology (SIMTech)
- Singapore Immunology Network (SIgN)