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The President’s Science & Technology Awards (PSTA) are the highest honours accorded to research scientists and engineers in Singapore. These national awards are given annually to individuals or teams for their outstanding and invaluable contributions to the research and development of science and technology in Singapore.

Congratulations once again to our 2014 winners!

**President’s Science & Technology Medal**

The President’s Science and Technology Medal (PSTM) is awarded to outstanding individuals who have made distinguished, sustained and exceptional contributions and played a strategic role in the development of Singapore through the promotion and management of R&D.

**PSTM 2014 Winner:**
**Professor John Eu-Li Wong**  
National University Health System

“For his outstanding contributions to the scientific research and biomedical science communities, and his visionary leadership in healthcare and medical sciences in Singapore”

**President’s Science Award**

The President’s Science Award (PSA) is presented to research scientists and engineers in Singapore who have made outstanding contributions in basic research leading to the discovery of new knowledge or the pioneering development of scientific or engineering techniques and methods.

**PSA 2014 Winner:**
**Professor Loh Kian Ping**  
National University of Singapore

“For his outstanding research on graphene chemistry”

**President’s Technology Award**

The President’s Technology Award (PTA) gives recognition to research scientists and engineers in Singapore who have made outstanding contributions to research & development resulting in significant technology with industrial applications.

**PTA 2014 Team Winner:**
**Professor Wong Tien Yin**  
Singapore Eye Research Institute  
**Professor Wynne Hsu**  
**Professor Lee Mong Li**  
National University of Singapore

“For their outstanding contributions to the development of technology in ocular image analysis for the screening and evaluation of significant clinical problems in eye and vascular diseases”

**PTA 2014 Team Winner:**
**Professor Subbu Venkatraman**  
Associate Professor Tina Wong  
**Professor Freddy Boey**  
Nanyang Technological University

“For their innovative application of nanostructures and novel drug delivery approach to combat blindness from glaucoma”

For more information please visit us at:  
[www.a-star.edu.sg/psta](http://www.a-star.edu.sg/psta)
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ON THE COVER
A scalable platform for growing heart muscle cells developed by A*STAR researchers may lead to repair of damaged heart cells [p25]
The Young Scientist Awards (YSA) recognise young researchers, aged 35 years and below, who are actively engaged in R&D in Singapore, and who have shown great potential to be world-class researchers in their fields of expertise. This award is organised by the Singapore National Academy of Science (SNAS) and supported by A*STAR.

Young Scientist Award 2014 Winners

Assistant Professor Melissa Jane Fullwood
Cancer Science Institute of Singapore
“For her research on chromatin interactions in cancer”

Assistant Professor Wang Qijie
Nanyang Technological University
“For his research on fundamental studies and applications in mid-infrared and far-infrared photonics and optoelectronics”

Assistant Professor Zhang Baile
Nanyang Technological University
“For his research on fundamental as well as practical invisibility cloaking from light and heat”

For more information please contact:
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Assistant Prof R. Subramaniam
Tel: 67903854 | Email: subramaniam.r@nie.edu.sg
The Non-Volatile Memories division at the A*STAR Data Storage Institute (DSI), led by Franck Ernult (center), is developing two non-volatile memory systems that could replace existing technologies for memory and storage.

You know that feeling. For the last few hours, you have been drafting a critical report that is due today. You have been surprisingly lucid and efficient in your writing and are anticipating the imminent relief that will accompany sending the report off. Then, to your horror, the screen freezes, leaving you with no choice but to shut your machine down.

This scenario inspires terror because of the type of memory embedded in your computer. Dynamic random access memory (DRAM) is a volatile memory technology, meaning it requires power to maintain the information stored inside it — hence the irretrievable loss when the power source is cut. Despite this inconvenience, the high speed and endurance achieved by volatile memory systems to date make them the primary choice for temporary data storage on personal computers. When you save a document, the information is then ‘written’ onto a more permanent but slower non-volatile storage option such as a hard disk drive that makes your files magically reappear on start-up.

The only non-volatile memory contender currently on the market is flash, which is limited to smartphones, tablets and cameras. But researchers at the A*STAR Data Storage Institute (DSI) are contributing to the emergence of two non-volatile memory systems — spin-transfer torque magnetic random-access memory (STT-MRAM) and resistive random-access memory (RRAM) — that could replace both DRAM and flash. These systems are also being integrated into large storage and computing architectures that may blur conventional distinctions between memory and storage.

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Challenging memory

Computers store and process information as binary digits, or bits, represented as one of only two values — true or false, yes or no, plus or minus, on or off, one or zero. STT-MRAM and RRAM both speak the same binary language.

In STT-MRAM systems, each unit or ‘device’ is made of two small magnets, whose orientations can be changed by passing a current through the device. When both magnets face the same direction, the resistance in the device is low, but the resistance significantly increases when the magnets are made to face in opposite directions. Information can be stored in the device by assigning a binary value of one or zero to the high and low resistance levels. RRAM uses oxides instead of magnets to establish the binary code. The resistance in the oxide can be decreased by a factor of a 1,000 by applying a voltage to the device or by reversing the direction of the voltage. Information is thus also expressed as binary, as either a high- or low-resistance unit.

Comparing the processing efficiencies of the two systems with flash requires a bit of multiplication. Both RRAM and STT-MRAM offer speeds 1,000 times faster than flash — speeds measured in nanoseconds instead of microseconds. Moreover, whereas flash can write and erase data between 10,000–100,000 times before becoming unreliable, RRAM can endure one billion write cycles, and STT-MRAM has an almost infinite endurance. The two systems have caught the eye of the semiconductor industry, being acknowledged by the International Technology Roadmap for Semiconductors as the most promising challengers to mainstream memory and storage technologies.

“STT-MRAM shares the same performance, speed and endurance as DRAM, and on top of that, it is non-volatile so you could save a tremendous amount of energy with it,” says Franck Ernult, manager of the Non-Volatile Memories division at the DSI. And while STT-MRAM could potentially replace volatile DRAM, RRAM is predicted to replace non-volatile flash.

Researchers at the A*STAR Data Storage Institute are developing non-volatile memory systems that could prevent off-power memory loss

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Researchers at the DSI engage in a wide range of activities related to non-volatile memory technologies, from device physics to the design and fabrication of integrated circuits.

The DSI is working closely with industry to address remaining barriers to the commercialization of these technologies.

**Bit by bit**
At the level of a bit, the performance of STT-MRAM is on par with that of volatile systems, making it ideal for use as memory. But it has been difficult to reproduce that singular victory across the billions of bits required to process information in a high-capacity memory chip. The DSI has therefore partnered with a US-based manufacturer of semiconductor devices to ensure stable and uniform performance at larger scales. The DSI team is also developing techniques to reduce the power consumption involved in writing and erasing data in STT-MRAM devices.

RRAM is more suitable as a technology for storage and is relatively simple to manufacture, but packing as many devices as possible onto a tiny chip without degrading its performance can be challenging. Researchers at the DSI are conceptualizing high-density stacking options, including setting stacks on top of each other. “Flash is already moving toward the integration of three-dimensional architectures, which require different device specifications than those currently used by the industry,” notes Ernult. “We are taking RRAM in a similar direction so as to maintain its competitive edge against flash technology.”

But the real concern with RRAM is that researchers are still debating its working mechanism, which makes it problematic to rely on. The DSI, in collaboration with the University of Cambridge in the UK, is therefore studying the process of writing and erasing data in RRAM systems to eventually improve control over them. The focus thus far has been on the materials used in the active oxide layer and surrounding electrode, through which current is injected into the device. “We have discovered that the electrode is quite important and that the choice of material for this electrode needs to be carefully selected so that it matches the performance of the oxide,” explains Ernult.

**Faster booting**
The performance specifications achieved by Ernult’s division are being incorporated into designs and technologies developed by the DSI’s Data Center Technologies division, with the ultimate goal of constructing data centers based on non-volatile memory systems. “The unrelenting proliferation of cloud computing has created an unprecedented amount of data and will place tremendous stress on data centers,” explains division manager, Khai Leong Yong. “Emerging non-volatile memory devices will enable data to be processed at much higher speeds than existing systems.”

Data centers today are designed to separate ‘main memory’, based on faster yet temporary volatile technologies, from ‘secondary storage’, based on slower yet persistent non-volatile technologies. This separation requires data to be retrieved and copied from storage to memory at every boot-up — a costly and time-consuming process. “New non-volatile memory devices, however, can serve as main memory, thus creating persistent memory systems that retain data even without power and support fast rebooting,” adds Yong.

The DSI is developing the hardware and software for such ‘active storage’ facilities that show a nine-fold improvement in data transactional performance over standard storage systems using DRAM. The division is also making headway in the area of security by enhancing the encryption and decryption of information stored in data centers.

“We take a holistic approach at the DSI, from device-level research into material structure, fabrication, signal processing and integrated circuit design, to system-level integration of non-volatile memory devices into new storage and computing architectures and systems,” says Yong. The state-of-the-art equipment and close industry ties at the DSI ensure that researchers solve problems that are relevant and applicable, and that may even spare you the agony of losing your hard-earned work.
When Nobel Laureate Sydney Brenner established the A*STAR Molecular Engineering Laboratory five years ago, his aim was to bring together a small team of young, high-caliber researchers from disparate disciplines to catalyze game-changing advances in science and technology. His venture has now borne fruit with the laboratory’s development of fluorescent molecular rotors — used for almost 20 years to measure physicochemical properties such as viscosity — to probe the molecular interactions between proteins involved in cancer.

This new tool for cancer researchers makes it possible to directly observe the disruption of target protein–protein reactions, taking much of the labor and guesswork out of early drug development. “Interdisciplinary research is the key to making truly revolutionary advances in science,” notes Brenner. “This molecular rotor strategy provides such an example, with great potential for many applications, particularly for drug discovery.”

Joining the dots
Fluorescent molecular rotors belong to a class of naturally twisted compounds that emit fluorescent light when untwisted. For decades, researchers have known that this unwinding can occur when the rotors are placed in highly viscous micro-environments, making them useful for probing properties such as viscosity and the dynamics of polymerization. Yet despite knowledge of their unique properties, the use of fluorescent molecular rotors for biological research has been limited.

Brenner’s idea was to see if rotors could be used to assay biological interactions, explains Yin Nah Teo, who led the research team on this project. “His initial thought was that perhaps we could use molecular rotors for detecting interactions between biomolecules. So we established a research team to investigate whether environmental effects through interaction with proteins could also result in the same change in fluorescence properties.”

The idea led Brenner and Teo to consider the types of protein interactions that could benefit most from such a molecular probe. A natural target, given A*STAR’s world-leading expertise in the area, was the protein known as p53, a ‘tumor-suppressor’ that plays a critical role in cancer development.

“Protein–protein interactions are the key mechanisms of cell signaling pathways in living systems,” explains Teo. “We formed a multidisciplinary project, in close collaboration with Farid Ghadessy and the A*STAR p53 Laboratory, looking at potential uses of the rotors for specific interactions involving this important protein.”

The A*STAR p53 Laboratory, headed by chief scientist Sir David Lane, is a world-leading center for p53-based cancer research. In about half of all cases of cancer in humans, the p53 gene is mutated, resulting in the production of a faulty p53 protein. And in cancer patients with an apparently normal p53 gene, the p53 pathway is believed to be faulty. One of p53’s normal functions is to catalyze DNA repair in damaged or mutated cells, thereby suppressing tumor formation. Faulty p53, or a fault in the protein’s functional pathway, undermines this protective process.

With insights gained from Ghadessy’s team at the A*STAR p53 Laboratory, the interaction between p53 and one of its regulators, the protein Mdm2, was selected as the target for the molecular probe. “Farid Ghadessy is an expert in p53 and Mdm2, and had a lot of ideas about how we could use this probe, such as in drug screening.”

Mdm2 inhibits the overproduction of p53 in healthy individuals. High levels of Mdm2, however, can result in a loss of p53 activity. Studying the interactions between p53 and Mdm2 is one of the most active areas of research at the A*STAR p53 Laboratory, providing a clear target for Teo’s molecular rotor project.

“The team we pulled together was very multidisciplinary, involving chemists and biologists from the A*STAR Molecular Engineering Laboratory and the...”

Features & Innovations

A*STAR p53 Laboratory, as well as bioinformaticians from the A*STAR Bioinformatics Institute,” says Teo.

Finding the right parts
Adapting the fluorescent molecular rotors for the detection of specific protein–protein interactions meant finding ways to bind or ‘conjugate’ the rotors to peptide sequences on the target proteins so that rotor rotation would be restricted only when the proteins underwent specific interactions with each other — something that had never been attempted before.

“It took several months to synthesize different versions of the rotors,” explains Teo. “We tested multiple molecular rotor designs, methods of conjugation and peptide sequences in search of a probe design that would give the best change in fluorescence signal. Min Yen Lee in our research team was instrumental in realizing these changes.”

Eventually, the research team was able to design and synthesize a molecular rotor that binds specifically to a p53 peptide and fluoresces only when it interacts with Mdm2. “Our results show that biomolecular interactions indeed restrict intramolecular rotation, resulting in fluorescence,” says Teo. “The rotor provides a direct readout through fluorescence intensity for studying the interactions of proteins with small molecules, peptides or other proteins.”

By applying the newly designed rotor in a rapid, high-throughput assay, Walter Goh at the A*STAR p53 Laboratory was able to quickly screen a large library of protein fragments for molecules that inhibit the interactions between p53 and Mdm2. In doing so, the researchers discovered 15 potential inhibitors. Seven of these could not be detected by standard techniques, highlighting the specific sensitivity of the rotor-based assay.

A world of potential
Brenner is understandably proud of the team’s achievement with the fluorescent molecular rotor strategy and the success of such a strong multidisciplinary and inter-laboratory project. “Through our skills in chemistry and by enlisting the support of groups outside the A*STAR Molecular Engineering Laboratory, Yin Nah Teo has been able to develop a very useful assay,” says Brenner.

“Unlike lower-throughput methods, our simple turn-on fluorescent probes provide rapid and sensitive readout,” notes Teo. “High-throughput screening for small molecules that bind to therapeutically important proteins is therefore one of the most promising applications of our work.”

The fluorescent molecular rotor strategy represents an invaluable new research tool that could significantly accelerate cancer research by allowing specific protein interactions to be explored in unprecedented depth. The strategy is not limited, however, to specific protein–protein interactions or for sole use within the A*STAR p53 Laboratory — it is also broadly applicable to many possible molecular interactions, making it a very exciting development indeed. “This work exemplifies how the translational convergence of chemistry and biology is giving rise to exciting new systems with wide-ranging applications,” says Ghadessy.

Turning up the heat on hydrogels

Researchers at A*STAR have developed temperature-sensitive gels that can be used for a wide range of biomedical and cosmetic applications

One day in 2005, polymer chemist and undergraduate student Xian Jun Loh was sprinkling organic compounds into a container of water to see whether they would dissolve. The polymer proved to be soluble, but something unexpected happened when Xian Jun heated the mixture in an oven set to body temperature — the clear, odorless liquid formed into a gel. “It looked and felt like hair gel,” recalls Xian Jun, who was used to seeing water-based solutions solidify and freeze when cooled, but not when heated. He immediately proceeded to repeat the experiment. “I had to make sure it wasn’t just a one-off accident.”

Much to his relief, the result wasn’t a fluke. Xian Jun now heads Research Planning and co-manages the Consumer Care Technology Programme at the A*STAR Institute of Materials Research and Engineering (IMRE), but is still based in the same lab

Temperature-sensitive gels that solidify when heated can be used to continuously deliver drugs to the body for up to three months.
where he made his initial discovery. At IMRE, Xian Jun has access to state-of-the-art facilities for developing and testing new gels as well as the expertise of like-minded polymer chemists. The institute has given him the space to really test the versatility of his polymers.

Making it biodegradable
Researchers have been studying and clinically testing thermogels for controlled drug delivery for over 30 years now. Earlier gels typically consisted of linked blocks of homogenous polymer chains, such as a polymeric string of ethylene oxide monomers connected to another polymeric string of propylene oxide monomers.

But these substances were not biodegradable and required much higher polymer quantities — about a fifth of the formulation — to be mixed with water. This meant introducing more foreign substances to the body, which raised concerns over unknown side effects. Some studies found that rats and rabbits had higher lipid and cholesterol concentrations following these therapies. Furthermore, the gels dissolved back to liquid within hours, making them unsuitable for long-term delivery.

Xian Jun resolved many of these problems by introducing biodegradable linkages into the thermogelling polymer chain. “The idea was somewhat bio-inspired,” he explains. All the proteins in our bodies are made of amino acids. Each amino acid unit is connected to its neighbors by stable amidic bonds and further packed into a compact three-dimensional structure via reversible hydrogen bonds. Xian Jun identified that urethane bonds had a similar structure to these naturally occurring polyamide linkages with a comparably high presence of hydrogen bonding. He decided to use urethane linkages to assemble the synthetic monomers in a way that mimicked naturally occurring systems.

This resulted in polymers that broke down into smaller units and could be flushed out of circulation weeks or even six months after being introduced to the body. The tighter packing meant that less polymer is needed to turn water into gel — from 20 per cent of the mixture down to 2 per cent. Once solidified, the coherent structure released trapped proteins at a sustained rate for an unprecedented three months. “No other thermogel can release proteins for such a long time,” says Xian Jun.

Cancer therapy
Xian Jun is now conducting research to test the polymer’s efficacy for cancer drug delivery. His team first examined the polymer in environments designed to simulate the dynamic conditions in the human body. They infused a known anticancer drug into a polymer formulation and then affixed the gel onto a semipermeable membrane in a constantly replenishing solution. The gel lay just above a persistent colony of cells derived from cervical cancer, known as HeLa cells. For two weeks straight, the drug permeated into the solution through tiny pores in the gel, like the slow and steady trickle in a drip chamber. During this time, the researchers observed reduced proliferation of the HeLa cells. Further studies on tumors in a mouse model showed similar impedance to growth.

Localized injection of the gel offers a more direct route for delivering drugs to a tumor than ingesting medication, which involves circulating drugs throughout the body. The gel-based treatment could eventually lead to reduced drug doses and limit the side effects of chemotherapy for cancer patients.

Since this novel carrier can deliver small-molecule drugs, proteins, lysosomes and even insulin, it has the potential to improve the lives of many patients. Its long active lifetime may allow diabetics to forgo daily insulin injections and instead just have them four times a year. Furthermore, sustained and localized delivery of pain medication could provide constant relief to patients. Xian Jun foresees working closely with drug companies to introduce their tried and tested drugs in more effective ways.

Clearer care
In addition to administering drugs, the thermogel could also be used for tissue repair and engineering. One approach would be to use the gel as a fibrous mat or scaffold for growing thin sheets of cells. Once matured, the cells could then be implanted onto various parts of the body with the polymer eventually degrading into nonexistence. But since the field of scaffold engineering is crowded with other promising alternatives (such as hydrogels, carbon nanotubes and fibrin mesh), Xian Jun sees greater potential for biodegradable thermogels as cell encapsulation materials. In this scenario, researchers would grow and maintain living cells in the polymer matrix itself, which could then be used to seal injured tissue and caulk perforations through direct injection. So far, Xian Jun has proved that cells can survive in the gel, but he has yet to find a way of squeezing the encapsulated cells through a tiny needle without rupturing their membranes.

While these thermogels are extremely promising, it may be some time before they find their way into actual patient treatments. In the meantime, Xian Jun is collaborating with a cosmetics manufacturer to develop gels with even lower polymer concentrations that can trap and slowly release active anti-aging ingredients. The company is keen on moving away from the traditional tinted and turbid creams toward a product that is transparent and slightly viscous. “Our material fits the bill, and it is aesthetically pleasing,” says Xian Jun, who expects the gels to make a public appearance within a year. “These thermogels are proving to be really special materials.”

Making micro-sense

Researchers at the A*STAR Institute of Microelectronics are developing MEMS sensors and platforms to help build a hyper-attentive world of objects

Ever wonder how your smartphone got so smart? It stores pictures right-side up, knows where the North Star is, and always brings you home. This spatial acumen originates from micrometer-sized devices known as microelectromechanical systems (MEMS) that sense velocity, acceleration and magnetic field strength. Demand for MEMS has boomed recently, with MEMS sensors finding their way into satellites, cars, gaming consoles, cameras and, of course, mobile phones.

“MEMS technology is re-invigorating the somewhat stagnant semiconductor industry,” says Alex Gu, director of the Sensors, Actuators and Microsystems Programme at the A*STAR Institute of Microelectronics (IME). “Produced using the same set of tools and processes as the conventional semiconductor industry, MEMS support the integration of multiple sensing functionalities onto a very small footprint at a very affordable price.” MEMS facilitate the intercommunication of objects envisioned by ‘the Internet of things’, where light bulbs connect with smartphones and traffic lights with approaching vehicles.

Gu and colleagues at IME are realizing this vision through developing advanced MEMS devices that monitor the environment, harvest vibrational energy and keep time. They are also contributing to the three-dimensional consolidation of stand-alone MEMS devices onto one shared platform with integrated circuits, paving the way for large-scale deployment.

Gases such as carbon dioxide, hydrocarbons and ketones are critical indicators for environmental monitoring. They absorb light at specific wavelengths in the mid-infrared region. Optical detectors read the absorption signatures of gases in the atmosphere but are too bulky, expensive or specialized. Gu’s team is developing a MEMS gas-sensing platform that is small enough to fit in a mobile phone and that can be tuned over a wide wavelength range, allowing it to monitor various gases associated with environmental and health risks. Sensor lifetimes, however, are limited by battery capacity — a common problem for wireless sensor nodes. To overcome this issue, MEMS vibration energy harvesters convert otherwise wasted ambient vibrational energy into usable electrical energy to sustain the sensors. IME researchers are expanding the frequency range of existing harvesters to double their efficiency (see top left image).

Timing devices are essential for synchronizing multiple components in electronic systems and tracking the chronology of events as simple as mouse swerves and clicks. The market is currently dominated by devices based on quartz crystals cut to different sizes and shapes to achieve appropriate frequencies. But time is running out for this technology. Gu’s team is developing compact MEMS timing devices that have customizable frequencies and superior performance. Furthermore, they are much cheaper to manufacture than quartz-based devices. “MEMS are fabricated using batch processing techniques. We could produce over 60,000 timing devices on an 8-inch silicon wafer for a few cents per device.”
Giving rehabilitation a hand

A new A*STAR collaboration offers an ideal environment to bridge the brain–computer divide for rehabilitation therapy

The concept of controlling objects by thought alone was once considered the exclusive domain of science fiction. Yet in a little over a decade, scientists have realized the wildest fantasies of futurists by developing brain–computer interface (BCI) technology and have taken the first steps toward alleviating the suffering of motor- and cognition-impaired patients. BCI technology received a further boost in September 2014 with the launch of a USD 100 million center dedicated to cutting-edge research in rehabilitation medicine.

The Rehabilitation Research Institute of Singapore (RRIS; pronounced ‘rise’) is a joint venture between A*STAR, Nanyang Technological University and the National Healthcare Group. It exploits their combined technological, academic and clinical expertise to address three main areas: stroke rehabilitation, aging and psychosocial rehabilitation. “The development of innovative technologies in these areas is vital for providing better and more affordable care to patients,” notes Tan Geok Leng, executive director of A*STAR’s Science and Engineering Research Council.

BCI technology noninvasively captures neural signals from patients to address two key needs — direct communication with an external device (for example, a computer cursor or potentially a replacement limb, such as a robotic arm) and rehabilitation of brain activity for those with motor- or neural-impairments. The latter approach is of great interest to Cuntai Guan and colleagues at the A*STAR Institute for Infocomm Research.

BCI is only one of many technologies supported through the RRIS collaboration. A*STAR’s other research institutes (including the Institute of Microelectronics, Singapore Institute for Clinical Sciences, Singapore Immunology Network, Singapore Bioimaging Consortium and the Genome Institute of Singapore) will provide strong research and development capabilities in the areas of wearable electronics, immunosenescence, nutritional science and neuroimaging. “RRIS will accelerate the adoption and commercialization of A*STAR technologies by industry,” says Tan, “to make Singapore a top regional hub in rehabilitation science and medicine.”

But most importantly, says Guan, it is about helping people. “We have seen people’s lives being changed through our work, which is very inspiring.”
Master strokes lead to high-precision, long-range actuators

In 2003, A*STAR research engineer Daniel Teo Tat Joo made a shrewd observation while developing high-precision mechanical stages with large ranges of motion. Stages are mechanical devices that are used, for example, to position and move slides under a microscope. To perform high-precision movements, they use actuators, which convert energy into motion. But Teo observed that no actuators had been developed that were capable of both nanometer-resolution positioning and millimeter-scale movement, or stroke. “This was a crucial gap in precision engineering,” Teo explains.

This discovery inspired Teo to develop an actuator with a high positioning accuracy and a millimeter stroke range that could be used in cutting-edge medical and manufacturing equipment. Within a year of working at the A*STAR Singapore Institute of Manufacturing Technology (SIMTech), Teo came up with the idea for a new class of nanopositioning actuators — flexure-based linear actuator, or FELA for short. “FELA is a marriage between an electromagnetic driving module, which is governed by Lorentz-force actuation, and a flexure-based bearing,” explains Teo. In 2004, he took up a SIMTech-funded PhD scholarship to conduct fundamental and theoretical research to enhance the performance of this patented technology and ensure that FELA realized the triumvirate of controllability, motion repeatability and reliability.

To achieve this goal, Teo needed to develop a system capable of performing constant and predictable movements without unexpected jerks. He first designed a new magnetic circuit with a constant magnetic field to ensure that the force generated by the electromagnetic driving module varied linearly with the current over the entire stroke range.

Another challenge was that flexure-based bearings behave nonlinearly (and highly uncontrollably) because they use elastic bending to generate large movements, or deflections. To overcome this problem, Teo constructed a theoretical model that helped him design a flexure-based bearing with a linear stiffness that was capable of generating large deflections.

He developed a prototype in 2005, which boasted a positioning resolution of 10 nanometers and a traveling range of 5 millimeters. This prototype was then successfully integrated as an essential component of a high-precision manipulator for facilitating movement in a process known as ultraviolet nanoimprint lithography.

Soon FELA was in demand for various other applications, including automated needle insertion and as components in alignment systems for optical fiber transceivers. “Its simple and low-cost control architecture, as well as its maintenance-free and inexpensive bearings, make FELA a cost-effective solution for a range of industries,” explains Teo.

In 2014, FELA was recognized as a significant global innovation by the R&D 100 Awards, sometimes dubbed ‘the Oscars of invention’; past winners include the compact disc, ATM and halogen lamp. “[FELA] offers a new generation of cost-effective, high-precision systems for the precision engineering industry,” notes Lim Ser Yong, executive director of SIMTech. The award is “a reflection of SIMTech’s research strategy to address challenges and issues faced by industry.”

Teo’s ambitions for FELA do not end there — he is actively trying to refine its precision to subnanometer and picometer levels and expand its stroke range to the meter range. Many issues need to be overcome to achieve this, such as magnetic field interference and environmental disturbances. However, Teo is looking forward to the challenge. “With many uncertainties and perhaps also some new phenomena, I am very excited about what I can discover and present to the research community in the future,” he says.
Immunology:

A signal to kill

*Crystal structure reveals how minor variations make receptor proteins activate or inhibit natural killer cells*

The ribbon-shaped crystal structure of the activating killer-cell immunoglobulin-like receptor, KIR2DS2 (blue), bound to its human leukocyte antigen target, HLA-A*11:01 (green).

Natural killer (NK) cells are white blood cells that can detect and destroy abnormal cells, including cancer cells or cells infected by viruses. A*STAR researchers have now resolved a longstanding puzzle concerning the receptor proteins that NK cells use to distinguish between normal and abnormal cells. Their work suggests ways in which NK cells could be modified to improve their response to a range of diseases.

Killer-cell immunoglobulin-like receptors (KIRs) found on the surface of NK cells bind to proteins called human leukocyte antigens (HLAs) on the surface of blood cells. This KIR–HLA interaction allows NK cells to recognize abnormal or infected blood cells. Each NK cell carries both activating KIRs and inhibiting KIRs. “These complementary KIRs provide a yin–yang balance of signals to the NK cell that determine whether it should kill the target cell or just leave it alone,” says Ee Chee Ren from the A*STAR Singapore Immunology Network.

The activating and inhibiting KIR proteins are very similar: they differ only at three or four amino acids, suggesting that they have evolved from a common gene. This similarity also raises the question of how such small variations in structure allow these receptor proteins to trigger opposite effects on the NK cells.

A clear way to understand the binding between the KIRs and their HLA targets is to resolve the three-dimensional structure of crystals of the complexes bound together using X-ray crystallography. To date, this has only been achieved for inhibiting KIRs. Now, Ren and co-workers at A*STAR have managed to identify the HLA molecule that binds to a specific activating KIR, known as KIR2DS2.

Ren’s team has also determined the first detailed structure of crystals of the activating KIR bound to its HLA partner, HLA-A*11:01 (see image). “The structure shows precisely how the activating KIR recognizes and binds to a different HLA than its related inhibiting KIR, and also reveals the three amino acids that differ between the two KIRs,” explains Ren. However, only one of them — a tyrosine residue — seems to matter.

The researchers validated the conclusions from their crystallography studies by studying the binding of the activating KIRs to live cells.

Having clarified the different ligand-binding characteristics of activating and inhibiting receptors, the researchers now plan to investigate the detailed binding mechanism that activates the cell-killing activity of NK cells. This could advance the use of these cells in therapeutic applications, for example by enhancing their cell-killing effects against specific types of diseased cells.

Developmental biology: Poised for reproduction

The DNA of stem cells that mature into sperm stands ready to express factors required for reproduction and early development

The transition from immature reproductive cell to newly fertilized egg can involve many alterations in gene expression. An international team co-led by researchers at A*STAR has shown that at each stage of development reproductive cells may reach a ‘crossroad’ where they can ‘choose’ whether to express the gene that will allow them to progress to the next developmental ‘junction’, or not.

Led by Ernesto Guccione from the A*STAR Institute of Molecular and Cell Biology in Singapore and Brad Cairns from the University of Utah, the team analyzed the regulatory marks on DNA and histones that occur during the different stages of male germ cell development. They found that many genes involved in sperm formation and early development contain both activating and inhibitory regulatory marks. These may provide a ‘stop-or-go’ mechanism for reproductive cells at each stage of maturation, giving them the green light to develop to the next stage.

The formation of mature sperm cells begins when primordial germ cells (PGCs) transform into adult germline stem cells (AGSCs). The AGSCs mature into sperm by undergoing specialized chromosome-reducing divisions called meiosis. Once a mature sperm joins with an egg cell, the organism begins its developmental program.

Epigenetics is the study of changes in gene expression caused by modifications of genomic DNA or its supporting histone proteins. For example, adding methyl groups to DNA is thought to suppress expression of targeted genes, whereas adding methyl groups or acetyl groups to particular amino acids on histone proteins can either activate or inhibit gene expression. In many stem cell populations, histone proteins tend to have both activating and inhibitory regulatory marks near genes related to development: a state called ‘bivalency’. The researchers looked at AGSCs and assessed the extent of changes in DNA methylation or in histone bivalency during the transitions from stem cells to mature sperm.

The team found that the DNA of genes required for meiosis tend to be methylated in PGCs, but not in AGSCs. The DNA of both AGSCs and mature sperm contained low levels of methylation as well as bivalent histones near genes whose expression are required to initiate and control embryonic development.

“Surprisingly, genes primarily involved in the latest stages of sperm maturation are actively transcribed from promoters bearing both repressive marks such as DNA methylation and activating marks on histones,” Guccione points out.

The findings show how epigenetic regulation of gene expression is tightly controlled across the different stages of sperm formation.

Targeting key cells for a dengue virus infection model

Modulating the expression of a protein that protects against viral infection allows researchers to study dengue vaccines in mice

Dengue virus infects hundreds of millions of people living in tropical countries every year. Transmitted via mosquito bites, the virus typically causes fever, but may also lead to potentially fatal organ failure. The development of mouse models of dengue virus infection could hasten an effective response to the disease, for which no specific treatments or licensed vaccines currently exist.

“We thought ablating interferon signaling in one or the other cell type could be sufficient to make mice susceptible to infection by the dengue virus.”

Now, an international team of researchers led by Katja Fink at the A*STAR Singapore Immunology Network has investigated small alterations in the normal mouse immune system to allow for the study of dengue virus infection, treatment and prevention.

Mice with a normal immune system are unable to be infected by dengue virus, which means that they cannot be used as model systems to understand dengue infection in humans. On the other hand, mice lacking normal interferon responses, which are generally required to fight viral infection, can be infected with the virus. But these mice do not mount a normal immune response, which does not make them very useful for learning about how the immune system reacts to dengue virus infection. Fink and colleagues reasoned that it would be highly beneficial to the field to have a mouse model that could be both infected with the virus and exhibit as normal an immune response as possible.

“Dendritic cells and macrophages are immune-cell types that had been previously shown in humans and mice to be potential targets for dengue virus infection, and both are key mediators of the immune response,” explains Fink. “Therefore, we thought ablating interferon signaling in one or the other cell type could be sufficient to make mice susceptible to infection by the dengue virus,” she says.

Fink and team found that mice lacking interferon signaling in T cells, another type of immune cell, could not be infected, whereas those lacking interferon signaling in dendritic cells or macrophages were killed by a dengue virus infection. However, the researchers further showed that mice lacking interferon signaling in either dendritic cells or macrophages could also mount an immune response to the infection, including the activation of T cells that could target the virus (see image). The immune system of these mice could also generate an effective neutralizing antibody response to a dengue vaccine candidate.

The development of these mouse models of dengue virus infection could allow for rapid progress in research into potential dengue virus treatments or vaccines that could be efficacious in humans.

Protein engineering:

Fighting inflammation in multiple mammals

An antibody that blocks immune signaling offers a promising research tool and potential therapeutic agent

Through a combination of natural immune selection and targeted protein engineering, A*STAR researchers have generated an antibody that efficiently blocks a disease-related inflammatory pathway in both human cells and mouse experimental models.

The signaling protein interleukin-1β (IL-1β) helps marshal a rapid immune counterattack in the event of infection or injury. However, excess IL-1β activity can result in damaging inflammation, contributing to disease states ranging from gout and diabetes to various autoimmune disorders. IL-1β inhibitors are an active area of drug discovery, and Cheng-I Wang and colleagues at the ASTAR Singapore Immunology Network recently set out to generate an IL-1β-specific antibody that might prove useful for tackling inflammatory disease.

The mammalian immune system has evolved to generate antibodies that can bind to foreign molecules with remarkable affinity and specificity: scientists have streamlined this by developing experimental methods that offer a simpler means for obtaining such antibodies. Wang and colleagues took such an approach, using protein engineering to obtain a promising antibody that proved capable of binding and inhibiting both human and mouse IL-1β.

The researchers focused on a small number of amino acids in the antibody that were likely to contribute to binding, and generated a library of antibody variants with random substitutions at these sites. By screening this library, Wang and colleagues obtained antibodies with remarkably improved performance relative to the starting molecule. “We were surprised to see the extent of the improvement,” he says. “We obtained clones that showed 20- to 50-fold better affinity.”

The best of these antibody variants, P2D7, proved 11 times more effective at inhibiting IL-1β than canakinumab, a commercially available anti-inflammatory drug. Canakinumab and P2D7 bind the same protein but recognize distinct targets on that molecule. Furthermore, P2D7 binds to the mouse and monkey versions of the IL-1β protein with high affinity, whereas canakinumab does not. “This will make it possible for us to use this antibody in various in vivo disease models,” says Wang. As a proof of concept, the researchers used mouse models to show that P2D7 could counter the symptoms of arthritis (see image) and peritonitis, or even increase survival in animals injected with human myeloma cells.

This demonstrates the clear value of P2D7 as a research tool, but Wang’s team is also exploring the clinical potential of this antibody. “Together with a pharmaceutical company, we are planning to study the applications of P2D7 in disease areas that are less explored, in which IL-1β may also play a role,” he says.

Chikungunya fever can cause severe and long-lasting joint pain, with several epidemics affecting multiple continents in the past decade. The illness is caused by chikungunya virus (CHIKV), but there is no effective vaccine or drug against it. Now, research led by Lisa Ng from the A*STAR Singapore Immunology Network provides details of how the immune system responds to CHIKV — findings that could support the development of vaccines and therapies.

One problem in developing therapies against CHIKV is that most animal studies tell us little about the disease in humans. To address this, a group led by Pierre Roques at the French Alternative Energies and Atomic Energy Commission have developed a model in macaque monkeys, which are biologically very similar to humans. Ng and colleagues conducted experiments using these macaques, in line with European directives for good animal practice.

“Macaques are susceptible to experimental CHIKV infections and serve as an effective non-human primate model for detailed studies,” explains Ng. “The close evolutionary relationship between macaques and humans allows researchers to predict the effects of the disease in humans based on observations in macaques.”

Ng and her team analyzed antibodies taken from the blood of macaques infected with CHIKV at 16 days and 180 days after infection. They found that the antibodies did not just tag the virus — so that immune cells could recognize and destroy it — but also could disable the virus on their own.

They also identified several specific sites on CHIKV proteins to which the antibodies bound. A number of these sites had not been previously identified, but two of them matched sites that are also recognized by human CHIKV antibodies (see image). Both sites are at one end of a structural CHIKV protein called E2 glycoprotein.

The researchers also looked at how the immune systems of the macaques responded to two different strains of CHIKV with slightly different versions of E2 glycoprotein. Macaques infected with a strain called IMT, isolated from La Reunion Island in the Indian Ocean, had a stronger antibody response than animals infected with a strain isolated from Singapore called SGP11. Their findings correspond with observations in humans infected with the different CHIKV strains.

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“These findings highlight the similarity of anti-CHIKV antibody responses in humans and macaques and also emphasize the importance of E2 glycoprotein in vaccine formulation,” says Ng. “Our study provides critical knowledge to improve understanding of CHIKV infection and immunity, vaccine design and preclinical studies.”

The introduction of foreign DNA into human cells through a process known as ‘transfection’ allows scientists to study gene expression in the laboratory and enables clinicians to treat genetic diseases. The methods commonly used for this procedure work for most cell types, except when it comes to B cells — a group of infection-fighting white blood cells in the immune system that have proven extremely difficult to transfec

transfect without the use of viruses. Viruses, however, pose a number of safety issues.

A team led by scientists at the A*STAR Bioprocessing Technology Institute and the A*STAR Institute of High Performance Computing has now developed a non-viral strategy to deliver DNA into this intractable cell type. By optimizing a technique termed sonoporation, the researchers managed to introduce genes into B cells with high rates of success.

“Our work is the first to demonstrate the use of sonoporation as an alternative, non-viral method for stable and highly efficient transfection of recalcitrant B cell lines,” says biomedical engineer and study leader Andre Boon-Hwa Choo.

Sonoporation combines ultrasonic sound frequencies and tiny gas-filled bubbles to generate transient pores in the cell membrane through which DNA can travel. Choo and his colleagues tweaked the acoustic energy levels and microbubble concentrations to deliver a circular piece of DNA that they could track visually in a trio of human B cell lines.

In one cell line, for example, the researchers achieved around 43 per cent transfection efficiency through sonoporation, compared to just 3 per cent with a conventional transfection method called lipofection (see image). Through further selection techniques, the researchers enriched the population of transfected B cells to more than 70 per cent. They achieved similarly impressive results with the two other B cell lines.

According to Charlene Li Ling Yong, co-first-author of the study along with Dave Siak-Wei Ow, the sonoporation-based transfection technique can now be used in the laboratory to better understand how B cells regulate immune responses against pathogens. “It allows scientists to elucidate the biological pathways of immune responses,” says Yong.

Numerous clinical research teams are also pursuing B-cell-based gene therapies to induce tolerance against autoimmune diseases. The method described in the current study could come in particularly handy for treatments in the human body — without any of the adverse effects of viral-mediated gene therapy. “Sonoporation has the potential to be applied in vivo,” Ow says. “It offers a safer and noninvasive alternative to existing gene therapies.”

In an investigation of a specific group of immune cells, researchers at A*STAR identified a limited repertoire of receptors that recognize bacterial targets. This finding led to the further discovery of a previously undetected population of these cells, indicating that they have wider functions than previously thought.

The research team, led by Lucia Mori from the A*STAR Singapore Immunology Network, focused on a population of T cells (which are themselves a type of white blood cell) called mucosal-associated invariant T (MAIT) cells. MAIT cells are abundant in organs such as the gut, liver and kidney, as well as in the blood, and are activated by a wide variety of bacteria.

Like all T cells, MAIT cells express proteins called T-cell receptors (TCRs) that recognize microbes (see image). TCRs are made up of α and β chains that vary to make every TCR unique. Each MAIT cell expresses just one type of TCR. Mori and her team investigated the variety of TCRs expressed by MAIT cells in healthy individuals using a powerful technique called RNA deep sequencing, which enabled more detailed analysis of TCRs than ever before.

The team of Singaporean and Swiss scientists found that the repertoire of TCRs in MAIT cells was extremely limited; this is in contrast with all other types of T cells, which express a much larger variety of TCRs.

“This means that a few original cells that are characterized by a particular TCR β signature clonally expand under stimulation by bacterial products,” explains Mori. “These cells were possibly selected as the most efficient at this process, so persist in the body to be continuously restimulated.”

Mori and her team also discovered a second population of MAIT cells that had not been previously identified. Similar to the previously described population of MAIT cells, this population has distinct TCR α chains, but the two populations are distributed differently between organs in the body, suggesting that they have different roles.

“MAIT cells monitor intestinal microbial flora and are the best candidates for detecting changes in the gut microbiome induced by pathological changes,” says Mori. “Their presence in other organs opens up intriguing areas of research.”

The group now hopes to build on this work and gain further insight into the functions of MAIT cells.

“We are investigating a large set of bacteria for their capacity to stimulate all MAIT cell populations and are looking for additional microbial products that can stimulate these cells,” says Mori.

A labeling compound identified at A*STAR that specifically marks neuronal stem cells is not only a useful research tool, but could also assist clinical efforts to repair neurological damage in patients.

Even as adults, we retain reservoirs of neural stem cells that can develop into mature replacements for dead or damaged neurons. However, these reserves are relatively small, and insufficient for repairing severe injuries to the brain or spinal cord. Larger numbers of these stem cells could potentially be grown in culture dishes, but to do so researchers would need to be able to separate them from mature, fully developed neurons that are ineffective for tissue repair.

Young-Tae Chang’s group at the A*STAR Singapore Bioimaging Consortium is looking for a way “to find and isolate neural stem cells using fluorescent dyes, to then grow them in larger numbers to treat neuronal damage or neurodegenerative diseases.”

Chang and Sohail Ahmed of the A*STAR Institute of Medical Biology recently succeeded in identifying such a dye. One of the challenges in cultivating neural stem cells is that although some will divide ‘symmetrically’ to yield two new stem cells, others divide ‘asymmetrically’ to produce one stem cell and one mature neuron or glial cell.

Reliably identifying true stem cells with existing dyes has proved challenging to date. “These dyes just diffuse out into both cells,” says Chang. He and Ahmed therefore screened a large library of fluorescent compounds in search of a dye that consistently remains stem-cell-specific.

One molecule, which the researchers named CDy5, proved particularly promising. Cultured neural stem cells gradually form structures called neurospheres, composed of both stem cells and neural cells of various stages of maturity. After labeling neurospheres with CDy5, Chang and Ahmed separated out the brightly labeled cells from the dimly labeled ones. Strikingly, cells that were strongly labeled by CDy5 were ten times more likely to form neurospheres (see image). Experiments with single cells showed that this dye remained stem-cell-specific even during asymmetric division, and the researchers subsequently learned that CDy5 forms a strong chemical bond with a protein that is exclusively active in neural stem cells.

Chang intends to use CDy5 to identify culture conditions that either help stem cells maintain their identity or prompt their development into mature nervous tissue. He is also keen to make this tool available to other groups. “I will distribute CDy5 to whoever is interested in using the probe and am excited to see what kinds of new applications or discoveries result,” he says.

Alzheimer’s disease:

Brain arteries in a dish

Smooth muscle cells designed to mimic blood vessels in the brain may help to understand Alzheimer’s

A technique to grow cells very similar to smooth muscle cells found in brain arteries, will make it easier to study the contribution of blood vessel disease to neurodegenerative conditions, a new study shows.

Smooth muscle cells are an important component of blood vessel walls, but surprisingly, they are organ-specific: for instance, smooth muscle cells in brain arteries differ from those in the coronary arteries.

The detailed attributes of these cells can be important in disease processes. Therefore to study diseases such as Alzheimer’s disease (AD) or vascular dementia, the cells used in the laboratory need to closely match the cells in the living brain.

“It is very difficult to get access to brain tissue or specimens for research,” says Christine Cheung from the A*STAR Institute of Molecular and Cell Biology. “So our lab’s focus is to create organ-specific blood vessel cells.”

Starting with human stem cells, Cheung’s team set out to make brain vascular smooth muscle cells. First they made a chemical cocktail that would drive the stem cells to become embryonic tissue that develops into the brain. “From there we were able to make the brain-specific smooth muscle cells,” says Cheung.

The team then used these cells to study a process important in AD: the accumulation of a toxic substance known as amyloid-β that is mainly produced by nerve cells. Unlike their counterparts in other organs, healthy smooth muscle cells in brain arteries can remove and break down amyloid-β. Cheung suspects this is an important mechanism for lowering the ‘amyloid burden’ in the brain.

Her team showed that their newly created cells function well: they seem to process amyloid-β in the same way as cells naturally found in human brain arteries. And just like brain smooth muscle cells, their ability to remove amyloid-β, is markedly reduced in conditions of oxygen deprivation. So in this low-oxygen state, the cells provide a model for AD.

Cheung seized the opportunity to use the cells to test potential AD therapies. “We needed to create a good, robust and consistent measurement technique that we could use to assess existing pharmaceutical and nutritional compounds,” she explains.

The proof-of-concept high-throughput assay developed by the team did just this: it can simultaneously test nearly 100 different compounds on oxygen-deprived cells to see whether amyloid processing is improved.

Cheung says that, to date, little attention has been paid to blood vessel pathology in Alzheimer’s. These newly derived cells, however, will help clarify the role of blood vessels in the disease.

Cancer biology: Tumor-causing crosstalk

Signals that pass between genetically abnormal epithelial cells and their genetically normal support cells drive tumor formation in a fruit fly cancer model

Genetically abnormal epithelial cells can interact with genetically normal support cells to help each other proliferate and to drive tumor formation, shows a study by A*STAR researchers using a fruit fly model1. Inhibition of the signals that pass between these cells has the potential to block the growth and development of solid tumors.

“This study identified the signals secreted from the epithelial cells that are driving tumors in this model.”

In addition, the team found that the genetic elimination of one cell type reduced the proliferation of the other cell type. These findings suggest that crosstalk between genetically abnormal epithelial cells and genetically normal mesenchymal cells could play a role in tumorigenesis, and that blocking this interaction could reduce tumor growth.

Perlecan is a known secreted cofactor for Wg and Dpp signaling. Cohen and his colleagues found that perlecan is secreted by the genetically modified epithelial cells and that it accumulates on the mesenchymal cells. Reducing the expression of perlecan could prevent tumor formation caused by EGFR overexpression in the fly imaginal disc.

“This study identified the signals secreted from the epithelial cells that are driving tumors in this model,” explains Cohen. “Our next goal is to identify the signal that comes back from the mesenchymal cells.”
People who have had an organ transplant or have autoimmune diseases are more likely to become ill. Research into a key cellular signaling system suggests this may be partly due to previously unknown effects of treatment drugs, and it also reveals broader insights into how immunity is controlled.

“Our findings may help explain why patients treated with immunosuppressive drugs are more susceptible to infections,” says Jan Fric of Paola Castagnoli’s research group at the A*STAR Singapore Immunology Network, “however, any clinical implications need to be checked through further study.”

The team of A*STAR researchers and co-workers in Singapore and Austria investigated the calcium and calcineurin–NFAT signaling pathway in myeloid cells and their progenitors. NFATs are a family of transcription factors — proteins that bind to DNA and regulate specific genes. They control the continuous renewal of various types of blood cells that are derived from progenitor cells formed by the bone marrow. NFATs underpin a healthy immune system, including the manufacture of T-cells, which help to regulate the immune system and kill infected or diseased cells.

In studies with live mice and cultured human cells, the researchers uncovered new links in complex networks of signaling and control. These allow stem cells in the bone marrow to develop into different types of mature myeloid cells of the blood and immune system.

Fric explains that the team’s findings also improve our understanding of how immunity is controlled. Specifically, the team reports that calcineurin, a protein that is activated by calcium ions, can activate the NFAT transcription factor system. This in turn inhibits the proliferation of granulocyte-monocyte progenitor (GMP) cells. Conversely, inhibiting the calcium and calcineurin–NFAT signaling pathway can enhance the proliferation of GMP cells. The GMP cells develop into myeloid cells, which are the first line of defense against infections.

The research also shows that a cell-surface receptor known as Flt3-L can activate the calcium and calcineurin–NFAT signaling pathway, and it identifies various genes that are involved.

“The calcineurin–NFAT pathway is an important target of many drug therapies,” Fric explains, “and we now need to further investigate the influence of such therapies on the development of myeloid cells in immunosuppressed patients.”

Fric points out, however, that the immunosuppressive drugs concerned are quite successful and so caution is needed before changing clinical practice. “But our findings may provide evidence that could help to control infections better in treated and immunosuppressed patients,” he concludes.

Cancer:  
Protein induces self-destruction in cancer cells

Uncovering the role of a particular protein in the progression of ovarian cancer may assist both prognosis and targeted treatment

The role of a phosphatase protein in promoting the self-destruction of healthy cells and the progression of ovarian cancer has been identified by A*STAR researchers. Known to be overexpressed in cancer cells, the protein, which is called PTP4A3, could be useful as a biomarker for disease prognosis.

In order to grow and spread, cancer cells actively exploit some of the body’s natural cellular processes. One example of such a process is the self-destruction and recycling of long-lived or damaged cells in the body. This process is known as autophagy and is necessary for maintaining healthy cells.

Autophagy is carried out by organelles known as autophagosomes, whose job is to ‘dismantle’ cells and transport the unwanted cellular material to be recycled into new membranes and proteins. Autophagosomes go through a maturation process during autophagy, which allows them to carry out their role effectively.

Through performing a series of experiments investigating the role of PTP4A3 in cancer progression, Qi Zeng and co-workers at the A*STAR Institute of Molecular and Cell Biology in Singapore, together with other scientists in Singapore and the United Kingdom, have uncovered links between the overexpression of PTP4A3 and the acceleration of autophagosome maturation that occurs during autophagy.

“Our experiments on the expression of PTP4A3 in ovarian cancer cells led us to discover that the protein exploits the autophagy pathway in order to promote cancer cell growth,” says Zeng.

The researchers found that PTP4A3 accumulates in autophagosomes, accelerating their maturation process. This in turn encourages the destruction of healthy cells and allows the cancer to take hold (see image). Unexpectedly, Zeng and her team also discovered that PTP4A3 itself then submits to autophagy at a later stage. In this way, PTP4A3 appears to act as a ‘fine tuner’, restoring balance to the autophagic system after the cancer has progressed.

Following analysis of a large set of data taken from ovarian cancer patients, the team found that high levels of PTP4A3 together with autophagy genes were significantly correlated with poor prognosis in human ovarian cancer patients. “This means that PTP4A3 levels could potentially act as a useful biomarker for prognosis in the future,” explains Zeng.

In addition, the researchers found that the inhibition of autophagy reduced PTP4A3-driven cancer cell growth in their experiments.

“This study suggests that autophagy could be used as a potential target for arresting PTP4A3-driven tumor progression in patients with high expression of both PTP4A3 and autophagy genes,” says Zeng.

Immunoology:
Pairing progeny with their proper precursors

Proof that CD14+ immune cells in the skin are derived from monocytes in the blood helps to understand their function in regulating immunity

Dendritic cells and macrophages are immune cells that orchestrate diverse immune functions within many body tissues, including the skin. New work by A*STAR researchers and colleagues shows that CD14+ cells in the skin — long classified as dendritic cells — are actually macrophages derived from blood monocytes, which means they operate differently within the human immune system.

Dendritic cells and macrophages are broadly similar: both take up antigens and present the antigen to so-called naive T lymphocytes that have not been previously exposed to other antigens. But skin macrophages, which are derived from blood monocytes, present antigens to so-called memory T lymphocytes within the skin that are already tuned to respond to one particular antigen.

Immunologists had initially identified CD14+ cells within the skin as dendritic cells. This was partially due to their ability to migrate out of chunks of skin that are placed in a cell culture dish. However, CD14+ cells are only weakly able to stimulate naive T lymphocytes, calling into question their classification as dendritic cells, since activation of naive T lymphocytes is a key characteristic of dendritic cells. In addition, recent gene expression analyses by Florent Ginhoux and Naomi McGovern from the A*STAR Singapore Immunology Network and colleagues demonstrated that CD14+ cells express many of the same genes as monocytes within the blood, which suggested that skin CD14+ cells may actually be monocyte-derived macrophages.

The researchers therefore examined what would happen to CD14+ cells in skin in humans whose blood monocytes were being ablated as preparation for a hematopoietic stem cell transplant. They found that as monocytes were depleted in the blood, CD14+ cells in the skin were lost. During the recovery period, monocyte numbers in the blood rebounded, followed by a restoration of CD14+ cells in the skin. These findings suggest that, in humans, CD14+ cells in the skin are derived from monocytes.

The researchers found further evidence that skin CD14+ cells better stimulate memory T lymphocytes than naive T lymphocytes, indicating that CD14+ cells behave more like macrophages than dendritic cells.

“Understanding the biology of monocyte-derived cells within human skin will aid in development of new therapies for inflammatory skin conditions, including psoriasis and eczema,” says Ginhoux.

The ability to grow human heart muscle cells in bulk could help routine replacement of heart cells damaged during a heart attack and may also improve testing of pharmaceutical drugs on heart cells, shows A*STAR research.1

Cardiovascular diseases such as heart attacks and strokes are the world’s leading causes of death. During a heart attack some of the heart muscle cells become starved of blood and die. These cells are rarely replaced due to the heart’s limited regenerative ability, which means that patients have to live with the limitations of a damaged heart.

Steve Oh led a team of researchers from the A*STAR Bioprocessing Technology Institute in designing a prototype ‘platform’ that enables heart muscle cells, or cardiomyocytes, to be produced from human pluripotent stem cells. Oh believes that this platform could be scaled up to industrial production levels.2

“The key is to be able to produce these cells in bulk,” says Oh. “Eventually we will be able to produce trillions of cells, but our current work involves developing the prototype process that is generating hundreds of millions of cells per batch.”

Traditionally, tissue culture is grown on a flat plate, but Oh’s team used ‘microcarriers’ — tiny polystyrene spheres that are electrically charged and coated with a protein — as the base for their culture (see image).

“Stem cells like to grow as clusters,” says Oh. “These microcarriers enable them to do that while suspended in a spinner flask, which is better for bulk production,” says Oh.

Researchers typically agitate stem cell cultures to help the cells receive nutrients and prevent them from clumping. Oh’s team conducted extensive research into the optimal levels of agitation required to increase the number of cells produced. They showed that too much agitation in the first few days of culture growth is detrimental.3

The team developed two effective protocols: one using stirring and another employing a rocking motion. “Both approaches have potential for industrial scale production,” says Oh.

They also created an automated video analysis technique to efficiently quantify the cardiomyocytes produced using their process.4 The technique was used to visualize the newly grown heart cells as they regularly contract or beat — just as cardiomyocytes do in a living heart.

“Before the video analysis system, we had to depend on experienced eyes to identify beating microcarrier—cell aggregates,” says Oh.

The next step for the group is to scale up production to more commercially useful levels. The process currently uses a 50 milliliter flask of microcarriers and stem cells, but, says Oh, “We need to take it to a one liter controlled bioreactor.”

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A new cellular labeling strategy gives researchers an efficient tool for studying the development of tissue that could help prevent the onset of obesity and cardiovascular disease.\(^1\)

Most people think about fat in terms of the white adipose tissue that stores the body’s excess energy, and which steadily — and visibly — accumulates as one becomes out of shape or obese. However, there is another type of fat tissue that can prevent rather than promote weight gain. “Brown adipose tissue not only stores fats, but also has the ability to burn fats to release energy as heat,” explains Bin Liu of the A*STAR Institute of Materials Research and Engineering.

Liu sees this tissue as a promising target for anti-obesity drugs, and her group set about designing a fluorescent molecule that could help scientists visualize the development of brown adipose cells. These cells can be characterized based on the number and organization of their mitochondria, the organelles that drive cellular metabolism. However, existing mitochondrial dyes tend to absorb each other’s fluorescence at high concentrations, resulting in a weaker overall signal as they accumulate.

In collaboration with Hong Kong University of Science and Technology researcher Ben Zhong Tang, Liu’s team devised a fluorescent dye that exhibits ‘aggregation-induced emission’. “This means that the probe does not emit fluorescence in dilute solutions,” explains Liu, “but it becomes highly fluorescent when it accumulates in mitochondria, without any self-quenching effects.”

After 20 minutes of treatment with their AIE-MitoGreen-1 probe, Liu’s group achieved bright labeling of mitochondria in brown adipose cells that lasted for more than a day. This labeling approach also left cultured cells largely unharmed, whereas only 10 per cent of cells survived prolonged treatment with a commercially available mitochondrial dye. The researchers subsequently used AIE-MitoGreen-1 to monitor the development of brown adipose tissue from precursor cells, observing changes in cell shape and mitochondrial organization over seven days (see image).

Since the basic stages of brown adipose development are well characterized, this probe could help identify treatments that stimulate or impede this process. “We hope to use our probe to monitor the activity of brown adipose cells in response to various stimuli, such as drug intervention or temperature changes,” says Liu. Her group aims to further improve their probe so that it shines longer and brighter. Ultimately, she hopes to develop variants that fluoresce at near-infrared wavelengths, which can be detected deeper within living tissue. “We would apply these probes to long-term monitoring of brown adipose cells in animal models.”

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Research Highlights

CHEMISTRY & MATERIALS
Optical materials: Dynamic duo to fight crime and cancer

Miniature two-color barcodes have the potential to combat forgery and track cancerous cells

Tiny rod-like single crystals that act as miniature dual-color barcodes have been synthesized by A*STAR researchers and co-workers. The researchers have demonstrated the potential of these barcodes for two very different applications: anti-counterfeiting measures and cell tracking.

So-called lanthanide-doped upconversion materials are highly promising for applications against crime and cancer as they have adjustable morphologies and tunable output wavelengths — they can also be fabricated by inexpensive processes that are easily scaled up. To date, single-crystal nanocrystals made from these materials have been impractical as multicolor barcodes because their tiny size makes them too small to be observed using conventional optical microscopes.

Xiaogang Liu at the A*STAR Institute of Materials Research and Engineering and co-workers based in Singapore, China and Australia overcame this problem by synthesizing different-colored microscale rods that have red, green or blue tips (see image). These rods are made of a lanthanide-doped upconversion material, NaYF₄, and are sufficiently long that their colored tips can be readily resolved using a standard microscope.

As a first step, the researchers controlled the lengths of the NaYF₄ microrods by varying the doping concentration of gadolinium. They then adjusted the color of the microrods by varying the doping concentrations of ytterbium (Yb³⁺) and erbium (Er³⁺) ions. The colored tips were simply a different phase of the same material as the microrods and were fabricated using a minor variation of the same procedure. Different combinations of microrod and tip colors were made by adjusting the doping concentrations of the microrods and tips.

Liu and team demonstrated the potential of these miniature barcodes for anti-forgery measures by producing two transparent security inks: one that contained dual-color microrods with green centers and red tips and a control ink that contained green microrods. When illuminated by an infrared laser beam and viewed by conventional microscope, printed patterns produced using the two inks are essentially indistinguishable under low magnification and have practically identical spectral properties. However, when observed under high magnification, the red tips of the microrods in the non-control ink are clearly visible, allowing the two inks to be easily distinguished from each other.

The researchers also point out that since the microrods can be internalized by cancer cells, it should be possible to use them as optical probes for imaging tumors. Liu notes that unlike conventional ‘top-down’ techniques, which have low yields and are expensive, the use of NaYF₄ microrods with tips separated by spacers has the potential to “provide a ‘bottom-up’ solution for gram-scale production of microsized barcodes.”

Self-cleaning surfaces:

The importance of a single groove

An innovative algorithm exposes the energy pathways that cause super-repellent surfaces to stop working

‘Superhydrophobic’ surfaces, such as anti-icing or self-cleaning windows, are remarkably effective at repelling water molecules. However, they may suddenly — and dramatically — lose their superhydrophobic features. A*STAR researchers have now identified a cause for the widespread ‘wetting transition’ by pinpointing how infiltration of a single microscopic groove can cause such an event1.

Weiqing Ren from the A*STAR Institute of High Performance Computing and the National University of Singapore used a ‘climbing string’ computational technique to model a micropatterned surface that uses microfabricated pillars to trap air pockets and so repel water molecules.

When a water droplet contacts a superhydrophobic interface, it immediately beads up and forms a near-perfect sphere. Under conditions of thermal or vibrational stress, however, the water droplet collapses and fully wets the substrate. This transition occurs when enough work is supplied to cross a bottleneck, known as an energy barrier, connecting the wet and dry states.

Identifying where energy barriers occur on micropatterned surfaces could dramatically improve their manufacture. A promising way to study this problem is by using computer models of ‘minimum energy paths’ (MEPs), which are intermediate structures during the transition between two states. Currently, most algorithms are designed to only identify the points in a system where energy minimums occur; the unstable nature of energy barriers makes them trickier to spot.

Ren’s method strings together the wet and dry minimum energy states through a smooth curve. An algorithm then seeks out MEPs available for the transition by shifting the string’s endpoint to higher and higher energies. This changes the string shape and eventually a ‘saddle point’ emerges when the physical forces acting on the curve reach a steady state. The shape of the saddle point corresponds to the energy barrier.

“Unlike other techniques, the climbing string method gives direct control over the energy of the evolving endpoint — guaranteeing that the computed saddle point is directly connected to the particular energy minimum,” says Ren.

Simulating a superhydrophobic grid of microscopic pillars with the climbing string algorithm revealed the mechanisms of wetting in striking detail (see image). The critical saddle point proved to be the entry of a small quantity of liquid into a single groove between micropillars. Crossing this barrier enabled the liquid to propagate laterally across the surface in a stepwise fashion, often nucleating from a central point before zipping along the grooves and filling them.

“By numerically studying energy landscapes, we now have a quantitative basis for designing optimized patterned surfaces in engineered systems,” says Ren.

Fluorescence-based detection of pesticides and other environmentally harmful chemicals is limited by the ability of current methods to reliably and selectively sense specific chemical species. A*STAR researchers have now developed a co-precipitation process that removes excess reagents to improve the efficiency of fluorescent sensors.

The fluorescence properties of protein-protected gold clusters make them useful for detecting and sensing various chemical species, such as hydrogen peroxide and mercury. However, the detection sensitivity is hampered by any free protein molecules that remain in the cluster solution, as these proteins may reduce the fluorescence or interact with the chemical species under detection. Commonly used methods for isolating protein-protected gold clusters (for example, ultracentrifugation, chromatography and dialysis) are often blighted by practical problems such as solubility issues or insufficient separation if the protein is too large or similar in size to the protected metal clusters.

Ming-Yong Han, Yong-Wei Zhang and colleagues at the A*STAR Institute of Materials Research and Engineering, the A*STAR Institute of High Performance Computing and the National University of Singapore have discovered a simple way to remove excess bovine serum albumin (BSA) from a solution of BSA-protected gold (Au$_{25}$) clusters following modification of the clusters.

Their separation method involves the co-precipitation of Au$_{25}$ clusters and zinc hydroxide in a basic solution, followed by centrifugation and removal of the supernatant, which contains the free BSA (see image). When re-dispersed in buffer solution, the precipitate forms a transparent solution of BSA-protected gold clusters.

Han’s team proposes that the co-precipitation process involves the binding of hydroxide ions with the surface Au(I) ions of the clusters and the subsequent interaction between zinc ions and hydroxide ions, resulting in zinc hydroxide being precipitated.

The mechanism is also effective using copper (II), cadmium (II) and lead (II) ions in strong, basic solutions and leads to the formation of the corresponding metal hydroxides.

“Once purified, the BSA-protected clusters are highly sensitive in detecting hydrogen peroxide and mercury ions and prove to be a visually selective detection method for four different pesticides,” says Han.

In the future, the team intends to investigate the use of the surface-binding interactions to grow gold nanoparticles from the clusters. “We also hope to use the purified clusters to develop new fluorescent sensors that have a high sensitivity and selectivity,” explains Han. “Moreover, we plan to extend the purification method to other clusters, such as platinum and silver, and study their atomic structure and potential for enhanced performance in sensing and detection applications.”

**Chemical detection:**

**A purer solution**

A separation method that isolates protein-protected gold clusters enables improved sensing of toxic mercury compounds and pesticides

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Reliable batch-to-batch formation is crucial for crystalline, active pharmaceutical ingredients as two different polymorphs of the same drug may function very differently in the body. Sendhil Poornachary from the A*STAR Institute of Chemical and Engineering Sciences and colleagues now report that the surface chemistry of modified glass substrates can influence the nucleation and formation of specific polymorphs of the drug, carbamazepine, within a certain concentration range of supersaturated solutions\(^1\).

The isolation of a specific polymorph is usually achieved by controlling homogeneous crystal nucleation. However, heterogeneous nucleation, which relies on the presence of a nucleating surface, is more ‘thermodynamically favorable’, meaning less energy is required. The most common heterogeneous method is to add seed crystals to a solution, but problems such as inconsistencies in seed crystal properties and cross-nucleation between crystal polymorphs may result in unwanted polymorphs.

Poornachary and team show that modified glass surfaces can selectively nucleate two different polymorphic forms of carbamazepine, an anticonvulsant and mood-stabilizing drug. “This concept of template-induced crystallization shows promise for improving batch-to-batch reproducibility with respect to the crystal form obtained,” says Poornachary.

The researchers produced cyano-, mercapto- and fluoro-functionalized glass vials by altering the interior surface using a silanization method, and then observed crystallization of the drug from supersaturated ethanol solutions. The two polymorphs were easily distinguished; either needle-shaped crystals corresponding to the metastable form II or tetrahedral-shaped crystals of the more energetically stable form III (see image).

The researchers found that at an initial drug concentration of 60 milligrams per milliliter, the metastable form II crystals were preferentially formed on the cyano-surface with no evidence of the crystals transforming to the more stable polymorph even after 24 hours. The mercapto- and fluoro-surfaces, however, preferentially nucleated form III with a small amount of metastable form II. In control vials, both polymorphs crystallized in tandem and transformed to the stable form within 24 hours. The time taken for the first crystals to appear was similar for all three modified surfaces, and significantly faster than in the control vials.

“We plan to improve our fundamental understanding of this template-induced crystallization process through molecular modeling and simulation,” says Poornachary.

The researchers envisage that better knowledge of the molecular packing arrangements in the crystal polymorphs — along with an enhanced understanding of crystal nucleation — may enable the design of template substrates with specific chemistries, eventually improving polymorphic selectivity for a variety of active pharmaceutical ingredients.

“We are also exploring scale-up of the template-induced crystallization approach using functionalized seed crystals and template particles,” adds Poornachary.

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Sustainable chemistry:
From bygones to nylons

An efficient catalytic process converts sugary biomass into a renewable feedstock for polymer production

The environmental impact of synthesizing adipic acid, an important precursor of nylon polymers, can be dramatically reduced by a chemical technique developed by Yugen Zhang and co-workers from the A*STAR Institute of Bioengineering and Nanotechnology.

The researchers found that an oxygen–rhenium catalyst complex transforms bio-based compounds derived from straw waste and other agricultural material into adipic acid with higher yields and lower emissions than conventional processes.1

Producing bulk chemicals from renewable sources is a key objective for manufacturers seeking to reduce their dependence on petroleum-based raw materials. However, typical compounds produced by biorefining are quite different from current feedstocks. Many are made up of oxygen-rich sugar rings — mixtures that are thermally unstable and difficult to manipulate into new molecules. Finding ways to catalytically remove oxygen atoms from sugars, sugar alcohols and sugar acids is a critical challenge, says Zhang.

Recently, chemists have begun using a reaction known as deoxydehydration (DODH) to realize this goal. This technique uses oxygen–rhenium catalysts to remove neighboring hydroxyl (OH) groups from a hydrocarbon starting material and convert it into a double-bonded alkene — a compound more amenable to synthetic processing. But, until now, only sugar alcohols with multiple OH groups have been successfully converted by DODH reactions.

Zhang and his team examined whether mucic acid, a molecule that can be synthesized in large quantities from galactose sugar rings, would respond to DODH techniques. They dissolved the mucic acid in boiling alcohol and then added a pinch of the special oxygen–rhenium catalyst. This reaction worked better than expected, stripping off the sugar acid’s OH groups with almost perfect efficiency. Zhang notes that this high reactivity can be traced to the two terminal carboxylic acid groups that activate mucic acid’s internal carbon–OH bonds.

After synthesizing the double-bonded muconic acid derivative, the researchers transformed it into adipic acid by using a platinum–carbon catalyst to hydrogenate the alkene positions — a simple chemical trick with only negligible byproducts. Further experiments revealed that the two-step mucic-to-adipic-acid bioconversion could be carried out in a single reaction pot with an overall yield of 99 per cent — a significant boost over the 60 per cent yields that standard protocols give.

The catalytic route also eliminates the nitrous oxide pollutants commonly released when petrochemicals are turned into nylon. “This highly efficient and green route for bio-adipic acid production should help draw more academic and industrial efforts to renewable feedstocks,” says Zhang.

A*STAR researchers are helping to advance the development of hydrogen-powered cars by producing innovative materials that could make on-board hydrogen generators a reality. Hydrogen is a renewable resource with the potential to power everything from households to cars, but its use is currently limited by a lack of green and practical production methods.

Current approaches to generating hydrogen as a power source are anything but environmentally friendly. Obtaining hydrogen through steam reforming and electrolysis of water — the splitting of water into hydrogen and oxygen by applying an electric current — requires high energy input and fossil fuels. In contrast, the process of ethanol steam reforming (ESR) uses ethanol derived from renewable biomass to produce hydrogen and other products.

One drawback of ESR, however, is that it requires high reaction temperatures to proceed and therefore a catalyst is needed to spur on the reaction. Another downside of ESR is that it often produces carbon monoxide as a byproduct, which is toxic and can also lead to poisoning of hydrogen fuel cells.

Luwei Chen, Armando Borgna and colleagues at the A*STAR Institute of Chemical and Engineering Sciences have developed an iron-promoted rhodium-based catalyst on a calcium-modified aluminum oxide support for ESR. This catalyst enables hydrogen to be generated more efficiently with less environmental damage as the reaction can occur at temperatures as low as 350 degrees Celsius and produce almost no carbon monoxide as a byproduct. The presence of iron oxide enables carbon monoxide to be converted into carbon dioxide and hydrogen via a reaction known as the water–gas shift reaction. Thus, the iron promotion effect on the rhodium-based catalyst is the key to removing carbon monoxide — something that is exceedingly difficult to achieve on rhodium alone.

Additional benefits of ESR are the commercial advantages stemming from the catalyst being quite stable and having a long active lifetime. This means that the catalyst will permit long cycle lengths, minimize the regeneration frequency and reduce the operational downtime for on-board steam reformers. Chen explains that these factors are “essential for maintaining profitable operations in reforming units. Similarly, a stable catalyst would reduce the operating cost for an on-board reformer.”

Chen notes that the catalyst will enable “better operational flexibility in terms of economics and on-board reformer size (since carbon monoxide purification units can be removed),” which she says will “make a significant impact in the design of efficient and simple on-board reactors.” Hence, this research is promising for advancing the realization of small-scale on-board reformers for hydrogen-powered cars.

Hollow, crystalline particles of lithium iron phosphate can enhance the performance of lithium-ion batteries by enabling an easier flow of lithium ions, A*STAR researchers have found. Lithium-ion batteries are ubiquitous in cell phones, laptops and other portable electronic devices, and are increasingly used in electric cars. During charging, lithium ions inside the battery leave the positive cathode, travel through a liquid electrolyte and enter the negative anode (often made of carbon). This flow of ions reverses during discharging.

But conventional cathode materials have serious drawbacks: lithium cobalt oxide, for example, is toxic and expensive, and while lithium iron phosphate is cheaper and nontoxic, it suffers from low electrical conductivity.

Jackie Ying of the A*STAR Institute of Bioengineering and Nanotechnology in Singapore and colleagues have now developed a form of lithium iron phosphate particles that act as a cathode for better performance. The diamond-shaped particles, which look like miniature melon seeds, are hollow, and have a crystalline structure that is easier for lithium ions to enter (see image).

The team shaped their particles using tetra(ethylene glycol) (TEG), which contains oxygen atoms that bind to iron atoms in the growing crystal. This suppresses the growth of the crystal along one of its axes, ultimately giving lithium ions a shorter distance to travel as they diffuse into the material. Since the particles are hollow, the lithium ions may interact with both outer and inner surfaces of the cathode material, thus increasing the reaction area.

The team made particles that were about 3.5 micrometers long and 1.5 micrometers wide by mixing solutions of lithium dihydrogen phosphate and iron sulfate with TEG, adding lithium hydroxide, and then heating the mixture at 180 degrees Celsius for 10 hours.

Scanning electron microscopy and transmission electron microscopy images showed that the particles were hollow, with thin walls. X-ray diffraction experiments confirmed that crystal growth had been constrained as expected. When tested in a battery, these particles had a comparable capacity and better stability than commercial lithium iron phosphate nanoparticles.

The team found that using more TEG and higher temperatures produced smaller particles that were less likely to be hollow. By adding lithium hydroxide before iron sulfate, they could create smaller particles — less than one micrometer in length — that were still hollow, yielding higher discharge voltages. “Our submicro plates have higher capacity and comparable stability compared to commercial nanomaterials,” says Ying.

Her team is developing other cathode materials, such as lithium manganese orthosilicate, that also have hollow structures and an even higher electrical capacity.

A more sustainable approach to a bond-forming reaction extensively used in the pharmaceutical and fine-chemical industries has been developed by an international research team led by A*STAR. The team used the solvent-free, catalytic reaction to produce high yields of a wide range of amides, including the antidepressant moclobemide and other drug-like molecules.

“Amide groups are widely found in pharmaceuticals,” explains Anqi Chen from the A*STAR Institute of Chemical and Engineering Sciences who led this research with the National University of Singapore (NUS). Examples include moclobemide, the cholesterol-lowering drug Lipitor, the anticancer drug Velcade and the anti-HIV drug Isentress. Current routes used to synthesize amide-containing molecules are expensive and generate lots of waste, prompting the need for cheaper, greener approaches.

An attractive alternative to conventional methods is transamidation — the catalytic reaction of a primary amide with an amine to make a secondary or tertiary amide. One of its advantages is that ammonia is the only byproduct. A range of organocatalysts and metal catalysts have recently been trialed for reactions of this type.

Now, Chen and his team have demonstrated that the solid-state catalyst mesoporous niobium oxide is suitable for transamidation in the absence of any solvent. “Compared with other reported transamidation catalysts, the niobium catalyst has the advantages of a broad substrate scope, a good functional group tolerance and high yields of amide products,” says Chen.

“The catalyst can also be conveniently recovered after the reaction and reused several times without appreciable loss of activity,” explains Chen.

The team’s collaborators at NUS had previously developed the catalyst for other reaction types. The catalyst is simple and inexpensive to synthesize and does not require any noxious solvents. It has a highly regular spherical structure with a diameter of approximately 500 nanometers and an acidity comparable to that of sulfuric acid. “The catalyst can also be conveniently recovered after the reaction and reused several times without appreciable loss of activity,” says Chen.

The catalyst can also be conveniently recovered after the reaction and reused several times without appreciable loss of activity.

The team tested the niobium oxide catalyst in reactions of a wide range of primary amides with various primary and secondary amines. The corresponding amides were all obtained in high yields. To further showcase the utility of the catalyst in this type of reaction, the team used it to synthesize the antidepressant moclobemide and other drug-like molecules in gram quantities with excellent yields.

The only downside is that the reactions require a relatively high temperature (around 150 degrees Celsius). “The promising results from this work should promote the development of more efficient catalysts that allow this valuable transformation to be carried out at lower temperatures to facilitate its application,” says Chen.

Nanoparticles: Shaken, not stirred, is best for cancer imaging

Tiny conjugated polyelectrolyte-nanoparticle probes produced by ultrasonication prove superior to commercial products

James Bond liked his martini to be ‘shaken not stirred’, and now A*STAR researchers have found that shaking, rather than stirring, also produces better nanoparticles for bioimaging — with important implications for spying on cancer.

Fluorescent probes currently used for bioimaging (for example, cadmium selenide quantum dots) fluoresce brightly enough to show up on detectors but may be toxic and thus unsuitable for use in the body. Now, Bin Liu and her colleagues from the A*STAR Institute of Materials Research and Engineering have successfully fabricated nanoparticle probes that are biocompatible and also have a high specificity and photostability. Furthermore, these new probes have excellent performance in the far-red to near-infrared region of the electromagnetic spectrum, which is of particular interest for cancer imaging.

The team’s method is elegant in its simplicity — it improves the optical properties of the probes by just varying the size and shape of the nanoparticles¹. “This allows us to circumvent complicated molecular design and synthesis processes,” explains Liu. “It provides a facile but efficient method for developing highly far-red–near-infrared fluorescent probes.”

The researchers produced the nanoparticles in water by two methods — stirring and ultrasonication (that is, ‘shaking’ at very high frequencies). Ultrasonication yielded nanoparticles with average sizes of 4 nanometers, which is considerably smaller than their stirred counterparts. These nanoparticles were also much brighter, having a quantum yield of 26 per cent in water — more than five times brighter than the nanoparticles produced by stirring.

Liu explains that ultrasonication produces polymer chains that are closer together, resulting in “compact structures that can effectively prevent water invasion and thus suppress quenching, yielding enhanced fluorescence.”

The researchers then tested the behavior of the nanoparticles produced by sonication in a biological setting to determine whether they would be effective probes for a specific biological target. They chose streptavidin, a protein that has a high affinity for epithelial cell adhesion molecule (EpCAM) — a common biomarker for various cancers. After conjugating streptavidin to the surfaces of the nanoparticles, the researchers investigated the nanoparticles’ effectiveness as an extracellular probe for EpCAM by employing MCF-7 breast cancer cells as a model cell line (see image). The nanoparticles exhibited an excellent photostability and a much higher fluorescence than a commercially available probe (Cy3-SA).

Liu notes that by switching streptavidin with another protein, the same nanoparticles could be used to target other biomarkers. “This will lead to a new generation of fluorescent probes for image-guided therapy,” she says.

The ability to ‘thread’ a molecular ligand through a metal–organic framework (MOF) to alter the pore size of the material — and yet allow the MOF to retain its crystallinity and principal structural features — has been demonstrated in a new study by A*STAR1.

MOFs are three-dimensional, coordination networks comprising metal ions and organic molecules and usually are crystalline, porous materials with many applications including storage of gases such as hydrogen and carbon dioxide. While ‘threaded’ MOFs have been synthesized in the past, they remain challenging to easily and reliably produce.

Inclusion of molecular ligands creates a flexible, interpenetrated MOF — similar to stitching a thread through fabric to make a new pattern. Use of bridging ligands of varying lengths potentially could lead to materials with many different properties in terms of gas adsorption, gas separation and catalysis.

Now, Andy Hor and colleagues at the A*STAR Institute of Materials Research and Engineering and the National University of Singapore show how solvate molecules adhering to the surface of the channels on a cadmium-based coordination polymer can be replaced with nitrogen-containing ligands that form a bridge between two metal ions of the MOF. These dipyridyl ligands of lengths varying from 0.28 to 1.10 nanometers are then threaded through the pores of the framework to form flexible MOF structures with different porosities (see image).

A surprise for the researchers came when long dipyridyl ligands that were expected to cause structural collapse of the framework were accommodated by slippage of two-dimensional layers within the structure. “Our observation that within these crystals, two side-by-side layers can slip or slide across to create space for guests suggests that these MOFs are actually smarter than we thought because they can respond to external stimuli without losing their crystallinity,” says Hor.

The researchers used the solvent diethylformamide (DEF), rather than the less bulky dimethylformamide solvate, to create cadmium-based double layers with large enough channels to permit the dipyridyl ligands to thread through. They also replaced other DEF solvates within the structure with water to minimize congestion.

“We hope to apply a similar approach to other MOFs — using a range of metals and organic molecules — and to test the boundaries for creating adaptable three-dimensional materials,” says Hor. “We could introduce different functional organic moieties to the present MOF and create materials with magnetic, electronic and photonic functionalities.”

Also, the dynamic nature of these MOFs makes them attractive candidates for selective gas adsorption materials.

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Organic chemistry:

Ruthenium catalyst goes with the flow

A green route to key molecular building blocks delivers a continuous stream of products

An efficient catalyst has opened up an environmentally benign route to a family of molecular building blocks found in many pharmaceuticals and agrochemicals, a study shows.

Molecular building blocks known as substituted amines contain a nitrogen atom bonded to at least two carbon atoms. They are often made by reacting nitrogen-containing amines with carbon-based molecules bearing a halogen atom such as chlorine, but this process tends to produce significant amounts of toxic waste.

Cleaner synthesis processes use a catalyst to connect the carbon chain of an alcohol molecule to the amine. But these catalysts, which contain metals such as ruthenium and iridium, usually dissolve in solution with the reactants. This makes it difficult to separate them from the products once the reaction is completed, wasting precious catalyst and increasing processing costs.

Balamurugan Ramalingam and colleagues at the A*STAR Institute of Chemical and Engineering Sciences have now developed a ruthenium catalyst that does not dissolve in solution, potentially making this reaction greener and more efficient.

The team used linker molecules containing phosphorus atoms to attach the ruthenium compound \([\text{Ru}(p\text{-cymene})_2\text{Cl}_2]\) to tiny polystyrene beads or granules of silica. These particles are easily filtered from the reaction mixture.

The researchers optimized the catalyst’s activity by testing different types of linker and varying the amount of ruthenium compound on each particle. They then used the best catalyst to join together a wide range of amines and alcohols, producing various substituted amines in good yields.

The catalyst could be recycled over five reactions without much loss in activity, and very little ruthenium leached from the solid particles into solution. Ramalingam’s team then exploited the catalyst to produce a drug molecule called piribedil (used to treat Parkinson’s disease) in almost 100 per cent yield.

The catalyst beads can also be packed into hollow columns (see image) so that reagents flow over them to deliver a stream of products. Such continuous-flow systems are increasingly used to make pharmaceuticals or other high-value chemicals, as a more efficient and sustainable alternative to conventional ‘batch-by-batch’ processes.

The scientists slowly pumped an amine and an alcohol through the loaded column at a temperature of 120 degrees Celsius. This delivered a continuous flow of product in 60–70 per cent yields for 21 hours, with virtually no loss of ruthenium.

“In principle, the reaction could be scaled up to production scale, and the complete conversion could be achieved by recycling the reagents,” says Ramalingam. The team is now using the catalyst to make amine-based polymers.

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A*STAR researchers have devised a way to destabilize gold nanoclusters so that they form tiny atomic nuclei that then grow together into perfectly proportioned, 12-sided dodecahedron crystals. These unique polyhedra have energy-rich surfaces that can boost the catalytic efficiency of important chemical reactions and serve as potential adsorption sites for targeted sensor devices.

“We observed a four-fold enhancement in catalytic ability for our dodecahedra compared to gold nanoparticles during the reduction of 4-nitrophenol to 4-aminophenol.”

Typically, gold nanoclusters are prepared by chemically reducing a gold–sulfur precursor in the presence of an organic stabilizing agent. This procedure creates a symmetric core of gold atoms protected by a thin layer of surface groups known as thiolates. Researchers have developed many techniques for varying the size of the nanoclusters to tune their chemical and physical properties. But destabilizing gold-thiolate bonds to enable further transformations into polyhedral crystals has proved more challenging.

To tackle this issue, an interdisciplinary team led by Yong-Wei Zhang from the A*STAR Institute of High Performance Computing and Ming-Yong Han from the A*STAR Institute of Materials Research and Engineering in Singapore investigated strategies to destabilize gold clusters by oxidizing the surface-protecting thiolates. While promising, this approach has its risks: previous attempts using ozone destabilization agents produced uncontrolled aggregation of gold atoms into macroscopic precipitates.

The researchers examined if switching to a milder hydrogen peroxide destabilization agent would give more favorable results. They first synthesized a solution of 25-atom gold clusters stabilized by outer layers of bovine serum albumin (BSA). When hydrogen peroxide was added to the mixture, the team’s mass spectrometry instruments showed that covalent gold–sulfur bonds slowly ruptured.

This peroxide-based destabilization initially produced smaller 11-atom gold clusters. But after sitting for nearly a week at room temperature, these clusters transformed into remarkable dodecahedron shapes (see image).

High-resolution scanning electron microscopy revealed that every facet on the dodecahedra had identical crystallographic orientations — a rare distribution of gold atoms known as a [110] facet. Density functional theory calculations initiated by co-author Guijian Guan showed that these unusual structures arise when amino acids liberated from BSA during the destabilization reaction attach to the nanoparticles and promote growth in every crystal direction except the [110] orientation.

Guan explains that because [110] facets have the highest surface energies among standard gold facets, they present strong attractive forces to incoming molecules — a phenomenon that improves the catalytic capacity due to a stronger binding affinity to target molecules. “For example, we observed a four-fold enhancement in catalytic ability for our dodecahedra compared to gold nanoparticles during the reduction of 4-nitrophenol to 4-aminophenol,” he notes.
A ‘one-step’ coating that blocks protein growth and kills surface-bound bacteria on silicone may significantly reduce infections from medical devices such as catheters, finds a study led by A*STAR Institute of Bioengineering and Nanotechnology researchers. Yi Yan Yang and international co-workers accomplished this with a synthetic technique that combines biomimetic surface adhesion and antimicrobial capabilities into a brush-like polymer film.

Implanting foreign materials into biological environments inevitably leads to accumulation, or ‘fouling’, of surfaces with pathogenic biomolecules. To block this film growth, researchers from A*STAR are experimenting with coatings known as ‘polymer brushes’ — arrays of macromolecular chains that bind to surfaces and modify properties such as bioadhesion.

One popular coating is called poly(ethylene) glycol (PEG), a soft, water-soluble material often used in drug delivery. Chemists can easily modify the structure of PEG for grafting onto device surfaces, where it provides a strong physical barrier against biomolecule adsorption. However, as PEG has no antimicrobial abilities, with time bacteria can overcome the PEG films and grow onto the surface.

Yang and co-workers are working to improve polymer brushes using biodegradable materials called aliphatic polycarbonates. Recently, they developed a ‘living’ ring-opening polymerization that can attach antimicrobial molecular units and PEG chains to the polycarbonate backbone with high precision. Experiments revealed that catheters coated with this polycarbonate–PEG film eradicated *Staphylococcus* bacteria and had excellent blood compatibility. However, it failed to prevent fouling from one of the most dangerous pathogens in hospitals — *Escherichia coli* bacteria.

Now, the researchers have developed a polymer brush with broad-spectrum antibacterial capabilities and a simplified coating process. They added three key components to the polycarbonate backbone: effective antimicrobial cations, PEG chains for antifouling, and dopamine groups that stick to silicone rubber in a manner similar to adhesive proteins found in mussel shells. Linking the three components into a single polymer, however, required chemical ingenuity in the form of co-interacting, metal-free catalysts.

“The amounts of co-catalysts we used were crucial,” says Yang. “PEG is a large molecule, and we had to add the catalysts in a stepwise manner to ensure a complete reaction.”

The polymer’s dopamine groups allowed attachment to catheter surfaces through a single-step dipping procedure. Imaging tests showed that the film killed both *Staphylococcus* and *E. coli* bacteria upon contact, and prevented these pathogens from establishing fouling layers (see image). Yang notes that these findings, in combination with the coating’s stability under simulated blood flow, indicate this approach’s potential for preventing infection in intravascular catheters.

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Metal-free catalysis generates brush-like polymer coatings that prevent build-up of toxic fouling films on medical devices

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A straightforward and effective process for coating silver, gold and platinum nanoparticles with functionalized silica shells at room temperature has been developed by A*STAR (see image). Crucially, unlike conventional methods for producing silica-coated metal nanoparticles, this process is based on water and does not employ alcohol, making it both cost-effective and environmentally friendly.

Silica-coated noble metal nanoparticles have attracted great interest because they can be used as catalysts as well as in calorimetric and optical applications. They are typically produced using silane precursors, but these are generally insoluble in water. Consequently, alcohol has to be added to water to facilitate the hydrolysis of these precursors, increasing the cost of production and making the process less green.

Now, a team led by Ming-Yong Han and Shah Kwok Wai at the A*STAR Institute of Materials Research and Engineering has devised an alcohol-free method for producing silica-coated noble metal nanoparticles.

To do this, the team took a commonly used precursor, tetramethoxysilane (Si(OCH₃)₄), and substituted a polar group (mercaptopropyl) for a methoxy group (O–CH₃), which resulted in a water-soluble precursor. Then, to enable this precursor to bind directly with the metal nanoparticle surfaces, they functionalized it with a thiol group (–SH).

This process has many advantages. It is straightforward to implement, efficient, universal and easily scalable. Furthermore, since the thickness of the silica shell increases with coating time, shell thickness can be readily controlled up to several tens of nanometers.

By slightly modifying the process, Han and colleagues could also produce nanoparticles that have a high activity for an extremely sensitive spectroscopic technique known as surface-enhanced Raman scattering (SERS) and are promising for highly sensitive detection in analytic and biological applications. SERS is based on the hugely enhanced Raman signal generated when a Raman-active compound is adsorbed on a metal surface. The researchers prepared the fluorescence-free SERS-active nanoparticles by sandwiching Raman-active molecules between the noble metal nanoparticle and the silica shell.

“The simplicity of the silica coating process means it has great potential for coating and protecting the surfaces of various kinds of metal nanoparticles,” explains Han. “Furthermore, the resulting highly negatively charged and SERS-active metal nanoparticles with thiol-functionalized silica shells and surface-protective features are very promising for various applications involving aqueous solutions.”

In particular, Han notes, this water-based route to facile, efficient and functional silica coating of metal nanoparticles at room temperature could be extended to coat metal oxide nanoparticles for green building applications.

Nanoparticles:
Alcohol-free zone

Noble metal nanoparticles can be coated with silica by a simple, environmentally friendly process that does not employ alcohol.

A new catalyst could have dramatic environmental benefits if it can live up to its potential, suggests research from Singapore. A*STAR researchers have produced a catalyst with gold-nanoparticle antennas that can improve water quality in daylight and also generate hydrogen as a green energy source.

This water purification technology was developed by He-Kuan Luo, Andy Hor and colleagues from the A*STAR Institute of Materials Research and Engineering (IMRE). “Any innovative and benign technology that can remove or destroy organic pollutants from water under ambient conditions is highly welcome,” explains Hor, who is executive director of the IMRE and also affiliated with the National University of Singapore.

Photocatalytic materials harness sunlight to create electrical charges, which provide the energy needed to drive chemical reactions in molecules attached to the catalyst’s surface. In addition to decomposing harmful molecules in water, photocatalysts are used to split water into its components of oxygen and hydrogen; hydrogen can then be employed as a green energy source.

Hor and his team set out to improve an existing catalyst. Oxygen-based compounds such as strontium titanate (SrTiO$_3$) look promising, as they are robust and stable materials and are suitable for use in water. One of the team’s innovations was to enhance its catalytic activity by adding small quantities of the metal lanthanum, which provides additional usable electrical charges.

Catalysts also need to capture a sufficient amount of sunlight to catalyze chemical reactions. So to enable the photocatalyst to harvest more light, the scientists attached gold nanoparticles to the lanthanum-doped SrTiO$_3$ microspheres (see image). These gold nanoparticles are enriched with electrons and hence act as antennas, concentrating light to accelerate the catalytic reaction.

The porous structure of the microspheres results in a large surface area, as it provides more binding space for organic molecules to dock to. A single gram of the material has a surface area of about 100 square meters. “The large surface area plays a critical role in achieving good photocatalytic activity,” comments Luo.

To demonstrate the efficiency of these catalysts, the researchers studied how they decomposed the dye rhodamine B in water. Within four hours of exposure to visible light, 92 per cent of the dye was gone, which is much faster than conventional catalysts that lack gold nanoparticles.

These microparticles can also be used for water splitting, says Luo. The team showed that the microparticles with gold nanoparticles performed better in water-splitting experiments than those without, further highlighting the versatility and effectiveness of these microspheres.

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Pentagonal nanorods have a unique morphology that confers interesting compositional and shape-dependent properties — including excellent stability and high catalytic activity — that make them excellent candidates for industrial catalysts. Now, researchers in Singapore have developed a simple chemical process to grow uniform pentagonal nanorods composed of gold and copper\(^1\). These new materials readily catalyze the direct alkylation of an amine with an alcohol, rendering them useful in the fields of materials chemistry and nanotechnology.

“We successfully synthesized gold–copper pentagonal nanorods with controlled size and composition by a seed-mediated growth route,” explains lead researcher Jackie Ying from the A*STAR Institute of Bioengineering and Nanotechnology. The ‘seeds’ are multiple crystals of elongated gold decahedrons, joined together by shared faces — an arrangement known as multiply twinning.

To create the nanorods, the team placed the gold seeds in a solution containing a copper precursor and applied heat — a process that produced nearly uniform pentagonal nanorods (see image). Ying’s team showed that they could control the length of these nanorods by changing the amount of gold seeds added to the copper precursor. Adding a 1:1 ratio of gold to copper produced nanorods approximately 24 nanometers long. The diameter of the nanorods remained the same, however, regardless of the ratio of metals used.

The ability to control the size and composition of the nanorods means it is easier to control the properties of the bimetallic gold–copper nanoparticles compared to nanoparticles made of just one metal, Ying explains.

Next, the team evaluated the catalytic activity of these gold–copper nanorods in a carbon–nitrogen-bond-forming reaction — the direct alkylation of an amine using an alcohol. “This hydrogen-borrowing strategy is an attractive synthetic method for the C–N bond formation as it is an environmentally friendly process that produces only water as a byproduct,” says Ying.

The nanorods were examined as catalysts for this reaction using the model substrates p-toluene sulphonamide and benzyl alcohol. “Our heterogeneous catalyst showed higher catalytic activity toward the C–N coupling reaction and better recyclability compared to commercially available catalysts,” Ying says.

Beyond catalysis, Ying predicts these new materials could be useful in electronics, chemical sensing and even biomedicine. Her team now plans to use the nanorods as seeds themselves to synthesize nanoparticles comprised of a gold–copper core surrounded by a shell of another material, such as platinum, for energy applications.

\(^1\) Yang, J., Chng, L. L., Yang, X., Chen X., Ying, J. Y., Multiply-twinned intermetallic AuCu pentagonal nanorods. Chemical Communications 50, 1141–1143 (2014).
Cancer:

Lighting up tumors

A*STAR researchers have developed a hybrid metal–polymer nanoparticle that lights up in the acidic environment surrounding tumor cells. Nonspecific probes that can identify any kind of tumor are extremely useful for monitoring the location and spread of cancer and the effects of treatment, as well as aiding initial diagnosis.

Cancerous tumors typically have lower than normal pH levels, which correspond to increased acidity both inside the cells and within the extracellular microenvironment surrounding the cells. This simple difference between tumor cells and normal cells has led several research groups to develop probes that can detect the low pH of tumors using optical imaging, magnetic resonance and positron emission tomography.

Most of these probes, however, target the intracellular pH, which requires the probes to enter the cells in order to work. A greater challenge has been to detect the difference in extracellular pH between healthy tissue and tumor tissue as the pH difference is smaller. Success would mean that the probes are not required to enter the cells.

“We now plan to check further for any toxicity issues and assess the biological distribution and pharmacological profile of the probes before hopefully moving on to clinical trials.”

“We aim is to address the challenge of illuminating tumors universally,” says Bin Liu from the A*STAR Institute of Materials Research and Engineering. Liu’s team, together with colleagues from the National University of Singapore, based their new probe on polymers that self-assemble on gold nanoparticles. The resulting hybrid structure is not fluorescent at normal physiological pH values: instead acidic conditions similar to those around tumor cells of approximately pH 6.5 alter chemical groups on the surface of the probes and switch on their fluorescence.

After validating the switching mechanism in pH-controlled solutions, the researchers tested the probes using cultured cells and also in tumor-bearing mice illuminated under bright light. Twenty-four hours after injection into the mice, obvious and clear fluorescence was seen only from tumor-bearing tissue, using either whole-body imaging or examination of removed organs (see image). The ability to observe the fluorescence of tumors using noninvasive whole-body examination of living mice indicates the potential of the nanoprobes for use in clinical situations with human patients.

“Our probes have so far proved to be biocompatible, which will be crucial for biomedical applications,” says Liu. “We now plan to check further for any toxicity issues and assess the biological distribution and pharmacological profile of the probes before hopefully moving on to clinical trials,” she adds. This is the latest of several recent advances in nanoscale medical technology from Liu’s group.

References:
Research Highlights

GENETICS & DISEASE
Three bacterial strains capable of degrading a prolific toxic compound present in water and soils have been successfully isolated using gene sequencing

Researchers have successfully isolated three strains of *Dehalococcoides* — bacteria capable of degrading polychlorinated biphenyls (PCBs) — as well as characterizing the three strains to pinpoint the exact genes responsible for breaking down PCBs.

PCBs are toxic compounds that were used as coolant fluids in industry until, following heavy pollution of water and soils, they were banned in most countries by the late 1970s. Certain bacteria can be used to biodegrade harmful substances such as PCBs that have been released into the environment.

Researchers have known for almost 20 years that bacteria such as strains of *Dehalococcoides* can degrade PCBs, but these particular bacteria have proved difficult and cumbersome to isolate. The strains are found in large communities of interacting bacteria, as it is difficult to single out information stemming from the target bacterium.

Following on from this analysis, the team was able to isolate suitable *Dehalococcoides* strains. The team then enriched the bacterial strains by growing them on a substrate made from tetrachloroethene, which encouraged the growth of *Dehalococcoides*. This method of culturing pure, highly efficient bacteria could be replicated on a larger scale to clean up PCBs in the environment.

The researchers hope that methods established in their work will serve as a template for isolating other challenging bacteria. “Our next step is to develop computational methods to mine the genomic data and understand how bacteria in the community interact. This will help to engineer bacteria that grow better on these substrates,” states Nagarajan.

Mobile pieces of DNA can jump around in the human genome and exert powerful regulatory effects on neighboring genes at new insertion sites. The most common mobile genetic elements are known as LINE-1 retrotransposons and comprise almost 20 per cent of all our DNA. Yet most genome-wide studies have underestimated the full impact of these important genetic regulators.

To correct this, a team led by William Burkholder at the A*STAR Institute of Molecular and Cell Biology in Singapore took a fresh look at the effects of LINE-1 retrotransposons on gene function and disease susceptibility. When they did this, the researchers found evidence that a subset of LINE-1 elements seems to have exerted a beneficial effect on recent human evolution.

Alexandre Kuhn from the Burkholder laboratory and other colleagues performed a comprehensive survey of LINE-1 retrotransposons in the genomes of 20 individuals of Asian descent. The research team, which included scientists from Singapore and the United States, found that, on average, an individual has more than 800 LINE-1 retrotransposons. Despite their high prevalence, these important genetic elements have not been adequately accounted for in standard arrays designed for genome-wide analyses of human disease or natural variation.

The researchers showed that this oversight, rather than originating from a technical error, was due to a limitation of the most commonly used methodologies. Specifically, they found that the DNA letter differences, known as single-nucleotide polymorphisms (SNPs), measured on the arrays are not closely linked to nearby LINE-1 elements, and hence the standard arrays failed to ‘tag’ them.

In order to tag LINE-1 retrotransposons to neighboring SNPs, Burkholder’s team integrated LINE-1 information with whole-genome sequencing data collected by the 1000 Genomes Project — a massive international effort to create a detailed map of human genetic variation. They found that most LINE-1 elements could indeed be tightly associated with SNPs. According to Kuhn, this means “that genome-wide association studies of their phenotypic effects could be conducted based on SNP genotyping in large human populations.”

To prove the point, the researchers ran the new analysis and then studied the genetic sequences surrounding the mobile genetic elements. In this way, they found evidence for recent positive selection, meaning that some of the LINE-1 retrotransposons conferred an evolutionary advantage when they jumped into new spots in the genome. This could be seen in the unusually long blocks of DNA surrounding several LINE-1 retrotransposons — an indication that the genetic elements and their flanking sequences had swept through the population.

A*STAR researchers show that changes in histone modifications accelerate a type of stomach tumor known as gastric adenocarcinoma—a cancer that occurs more frequently in Asian populations than Western populations.

While cancer cells typically contain numerous genomic mutations that alter genetic activity responsible for proliferation and migration, other ‘epigenetic’ mechanisms are also at play. For example, histone proteins that support chromosomal DNA (see image) can undergo chemical modifications that may either boost or restrain gene expression.

The research team led by Patrick Tan of the A*STAR Genome Institute of Singapore used a technique known as nanoscale chromatin immunoprecipitation sequencing (nano-ChIP-seq) as a starting point to investigate gastric adenocarcinoma. This technique, which was developed at A*STAR, allows researchers to specifically analyze chromatin modifications occurring at different genomic sites, making it possible to predict the genes that are most likely to be active or inactive in a given cell.

By comparing nano-ChIP-seq analyses of gastric adenocarcinoma and normal stomach tissue, Tan and colleagues identified hundreds of genomic sites with distinct differences in histone modification. Although many of these differences were observed at known promoters — sequences that regulate the activity of nearby genes — many more were found at cryptic promoters, which had not yet been described.

These unconventional promoters seem to profoundly alter gene expression. In several instances, the researchers determined that cryptic promoters contribute to the production of abnormal protein variants. Analysis of patient tumor samples suggested that these changes may directly influence the patient’s health.

“Patients whose stomach cancers expressed such altered genes experienced very poor clinical outcomes, which indicates that cryptic promoters may functionally contribute to aggressive disease,” says Tan.

The sequence elements contained within promoters act as a ‘landing platform’ for various transcription factor proteins that directly govern gene expression. Tan and colleagues found that many of the cryptic, cancer-associated promoters were bound by transcription factors that form part of the polycomb repressor complex 2 (PRC2), a transcription factor assembly that contributes to histone modification. Importantly, PRC2 is also highly active in embryonic stem cells, which remain biochemically locked into an ‘immature’ developmental program.

“This suggests that stomach cancers may ‘regress’ toward a more primitive state, exhibiting properties similar to cells during very early stages of development,” says Tan.

Although this study only gives initial data for one particular cancer, Tan believes the platform has the potential to provide a general strategy for profiling epigenetic abnormalities, which could potentially inform the diagnosis and treatment of other tumor types.

Like fossils buried beneath a modern landscape, the human genome is littered with sequences that originated from ancient viral DNA insertion events. Scientists have long assumed that these ‘transposable elements’ are, like fossils, biologically inactive and primarily interesting as a window into evolutionary history. However, researchers at the A*STAR Genome Institute of Singapore have now uncovered evidence that some of these sequences play a prominent role in early embryonic development.

Ng and Bourque set out to clarify the role of this RNA by performing experiments in which they selectively depleted it from stem cells. hESCs are actively maintained in a so-called ‘pluripotent’ state, from which they are capable of developing into any cell type in the human body (see image). In the absence of HERV-H RNA, hESCs rapidly lost their pluripotency; the researchers noted that the loss of HERV-H expression considerably altered the activity of many genes associated with cell development and proliferation.

Closer analysis revealed that the HERV-H RNA binds to multiple protein partners, including a protein called OCT4, which plays a role in establishing and maintaining stem cell pluripotency. Ng and Bourque’s team determined that the resulting RNA-protein complexes may assemble at specific sites within the HERV-H genomic element. These sequences in turn act as ‘enhancers’, which help coordinate the activation of other pluripotency-related genes residing in both adjacent and remote regions of the genome.

According to the study’s lead author, Xinyi Lu, a postdoctoral researcher in Ng’s laboratory, the emergence of the regulatory activities executed by HERV-H could represent an important step in the evolution of our early ancestors. “HERV-H first integrated into the primate genome around 45 million years ago and is only found in the primate genome,” says Lu, “and so it may contribute to some of the differences between primates and other mammals.”

In future work, Ng and colleagues hope to extend their investigation of HERV-H function to identify other possible protein partners and perhaps other mechanisms by which these ancient sequences exert their influence.
Parasitology:

Genes of carcinogenic liver fluke revealed

The sequencing of a harmful Asian liver fluke’s genome brings hope of better treatments

The tiny liver fluke, *Opisthorchis viverrini* causes damage out of all proportion to its size. Consumed as cysts within raw fish by people in Thailand, Laos and Cambodia, it causes the tropical disease, Opisthorchiasis, putting its victims at risk of cancer. Despite affecting millions of people in Asia, no vaccine exists and there is only one drug available for use. Now, A*STAR researchers have sequenced its genome, shedding light on how it copes with its strange life cycle, and suggesting new approaches to treatment.

*O. viverrini*, also known as OV, has a long life cycle that begins with eggs infecting freshwater snails, explains Niranjan Nagarajan of the A*STAR Genome Institute of Singapore. “Infected snails release OV larvae into the water and they encyst themselves in freshwater fish,” says Nagarajan. “Eating uncooked infected fish can lead to infections in people.” Its presence can also cause fibrosis, inflammation and the deadly cancer, cholangiocarcinoma.

Using genetic material from 25 flukes, Nagarajan worked with a large international team to produce a ‘draft’ genome for the organism that reveals genes that serve OV well in its peculiar niche.

The fluke makes its home in the supposedly ‘inhospitable’ human bile duct, says Nagarajan, which is not an easy place to live. “It contains bile acids and other toxins, and the level of oxygen is variable and often low,” he explains.

The researchers found genes for a repertoire of antioxidants that were needed to cope with bile acids and other toxins found in bile. And OV has genes that enable it to dine on bile lipids, with enzymes that break down the fat droplets known as micelles.

“Strikingly we also identified a massively expanded family of 25 lipid-binding proteins,” the authors said.

The fluke hemoglobin has an unusually high affinity for oxygen, and this helps OV to survive at these low-oxygen levels, adds Nagarajan.

On a more sinister note, they found that OV can digest the epithelial cells that line the bile duct itself, known as cholangiocytes.

Several mechanisms probably come into play to cause cholangiocarcinoma, says Nagarajan, and OV will have a role in this.

“OV-induced inflammation, damage to the biliary tract due to the fluke’s attachment or feeding, and its secretion of proteins that can induce cell proliferation, could all increase risk of cancer,” says Nagarajan.

He hopes that knowledge gained from the genome will lead to new strategies to combat the fluke, for example by reducing its ability to survive in bile.

Genetic testing:

Colorful nanoprobe tests make a simple test

**Gold nanoparticles linked to single-stranded DNA create a simple but versatile genetic testing kit**

Tests for identifying genetic variations among individuals, which can be used to develop precisely targeted drug therapies, are a current focus in the emerging field of pharmacogenomics. A*STAR researchers have now developed and patented a customized and elegant nanoprobe for assessing sensitivity to the drug warfarin.

To develop the nanoprobe, Jackie Ying at the A*STAR Institute of Bioengineering and Nanotechnology and co-workers in Singapore, Taiwan and Japan devised a relatively simple procedure that uses standard laboratory equipment and can be easily adapted for other genetic tests.

“Our method is faster, more cost-effective and more accurate than existing alternatives,” says Ying.

Ying’s method detects genetic variations known as single-nucleotide polymorphisms (SNPs) that differ in only a single-nucleotide building block of DNA. In the case of warfarin — the most frequently prescribed anticoagulant — there are SNP differences in specific parts of the genome that indicate whether a patient will tolerate the drug or suffer serious side effects.

The researchers used gold nanoparticles attached to short sections of DNA that bind to specific complementary sequences of DNA through the base pairing that holds together double-stranded DNA. These nanoprobe were exposed to fragments of DNA that had been cut out and amplified from a patient’s genome.

The nanoprobe are initially pink due to surface plasmonic effects involving ripples of electric charge. When analyzed, if the probes do not bind to the DNA fragments, they aggregate and become colorless on exposure to a salt solution. If they do bind to the target, they will not aggregate but will remain pink until heated to a ‘melting temperature’ at which the base pairing is disrupted and the DNA strands of the probe and the genome fragments separate. For cases of partial complementarity — in which the fragments are mismatched by a single nucleotide — the melting temperature is lowered by an amount depending on the level of mismatch. This allows SNPs to be detected through their different melting temperatures.

The resulting color change is easily visible to the human eye but can also be evaluated automatically (see image). The system can also distinguish between homozygous genotypes (where a person carries the same SNP on each member of a pair of chromosomes) and heterozygous genotypes (where a person carries different SNPs on each chromosome).

“The patented warfarin test kit is available for commercialization or licensing,” says Ying. “We have developed and are validating assay kits for several other applications in pathogen detection, pharmacogenomics and genetic disease screening.”

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Malaria:

Resolving a sticky situation

Malaria-infected cells may latch onto healthy blood cells for protection rather than to aid transmission

The distinctive ‘clumping’ of blood cells that blocks vessels and causes tissue damage in malaria-infected patients is the focus of a multinational collaboration, which includes A*STAR researchers.

‘If we identify the parasite protein at the surface of the red blood cells, we can try to develop a compound to block this interaction.’

When malaria parasites are released into a host following a mosquito bite, they make their way into red blood cells (RBCs). In some cases, infected RBCs stick to uninfected RBCs — a phenomenon known as ‘rosetting’ (see image) leading to the formation of structures called rosettes. Studies of Plasmodium falciparum, the species responsible for the most malaria cases worldwide, suggest that rosetting either assists with the infection of new cells or acts as a shield against immune detection. The process can also contribute to certain complications associated with malaria.

Researchers led by Bruce Russell of the National University of Singapore recently set out to establish the function of rosetting in Plasmodium vivax, a less-well-understood species that nevertheless is responsible for almost 350 million cases of malaria per year. Laurent Renia, a collaborator on the study from the A*STAR Singapore Immunology Network, notes that the study of P. vivax relative to P. falciparum has been limited by the lack of robust experimental culture models. “P. vivax grows exclusively in reticulocytes, the young form of RBCs, which represent only 1 to 2 per cent of circulating RBCs in a normal person,” he says. “In contrast, P. falciparum grows in normocytes, which are mature RBCs.”

The researchers employed recently developed techniques for working with patient blood samples to analyze rosetting in P.-vivax-infected RBCs. They determined that cells infected by P. vivax and P. falciparum form rosettes with similar frequencies. Surprisingly, most P. vivax rosettes incorporated normocytes rather than reticulocytes, even though the latter cells are the preferred target of this species. “This mechanism was thought to facilitate the invasion of uninfected RBCs by reducing the distance between the parasite and the target cell,” says Renia. “Instead, rosetting could help the parasite to escape the host immune system.”

The researchers also learned details about how rosettes form. By using antibodies to block specific proteins on the surface of RBCs, they determined that a molecule called glycophorin C contributes to P.-vivax-mediated rosetting. Indeed, rosetting was rarely detected with RBCs lacking glycophorin C.

The other side of the puzzle remains unclear, however — the P. falciparum protein responsible for rosetting is absent in P. vivax, and Renia is now keen to uncover this missing molecule. “If we identify the parasite protein at the surface of the red blood cells, we can try to develop a compound to block this interaction,” he says.

A computer program that cross-references disease symptoms with DNA sequencing data can detect the faulty genes responsible for rare disorders with greater accuracy than other methods. Developed by scientists at A*STAR, the software could provide a valuable tool for clinicians hoping to offer a genetic diagnosis for patients — especially children — with mysterious ailments.

“An early diagnosis for these children could give them a head start in their therapeutic treatment and help improve the quality of their lives,” says Pauline Ng, a bioinformatics researcher at the A*STAR Genome Institute of Singapore who led the study.

Phen-Gen, the researchers describe as “the first algorithm to integrate patient symptoms for genome-wide predictions.” The researchers also created a web portal (phen-gen.org) to support clinicians and researchers, including those with limited computational experience.

In computer simulations to test the accuracy of Phen-Gen, the software identified the causal gene in 88 per cent of cases. This was a 19–58 per cent improvement over simulations run on existing prediction algorithms.

The researchers further evaluated the efficacy of Phen-Gen with real patient data taken from people with intellectual disabilities and no known cause of their disorders. Phen-Gen ranked the causal gene at the top of the list in 8 of 11 patients, and within the top five genetic suspects in all patients. What’s more, Phen-Gen ran its analysis faster than other methods, yielding results in just 15 to 30 minutes.

Ng and her team are now working with clinicians in Singapore and internationally to elucidate the genetic cause of undiagnosed disorders. Many independent groups are also starting to incorporate the software into their own evaluations. “We are happy to report that over the past two months more than 60 users have downloaded the software,” says Ng.

Scientists first identified a gene called ‘mutated in colorectal cancer’ ($Mcc$) in 1991, yet many questions remain about the gene’s function in cancer formation and normal tissue development. Using zebrafish as a model laboratory system, researchers from A*STAR have now discovered that $Mcc$ plays a critical role in the cellular alignment of the early embryo.

“Our work revealed how $Mcc$ participates in the complex cellular movements that establish the vertebrate body plan in the zebrafish embryo,” says Ray Dunn, a cell and developmental biologist at the A*STAR Institute of Medical Biology in Singapore who led the research.

The zebrafish ($Danio rerio$) is a powerful model system for the study of vertebrate development. The tropical freshwater minnow has small, transparent embryos that allow scientists to watch molecular processes in the laboratory in exquisite detail — all in real time.

Dunn and his colleagues thus decided to isolate and study the zebrafish version of the $Mcc$ gene to better evaluate its expression and functions. Using bioinformatic and genetic techniques, they pinpointed the zebrafish $Mcc$ gene near the end of chromosome 5. (Zebrafish have 25 chromosomes, two more than humans.) The researchers silenced the $Mcc$ gene in developing zebrafish and observed multiple morphological defects in the embryos.

Several complex cellular rearrangements shape the primordial body plan during the gastrulation phase of development in all vertebrates, including humans. One crucial step involves the polarization of cells, which is coordinated by members of the Wnt signaling pathway. Through a series of detailed laboratory experiments, Dunn’s team showed that, in the zebrafish embryos, the protein encoded by the $Mcc$ gene acts downstream of various Wnt proteins to regulate the developmental process that narrows the fish body and elongates it from head to tail.

In one such experiment, for example, the researchers demonstrated that overexpression of the $Mcc$ protein can reverse the developmental ills caused by depletion of two Wnt proteins, Wnt5b and Vangl2. Furthermore, increased $Mcc$ activity can overturn problems caused by the ablation of a Wnt5b receptor protein called Ror2. Biochemical studies also revealed a direct physical interaction between $Mcc$ and a specific part of the Vangl2 protein. Dunn and his colleagues repeated many of the same experiments in the African clawed frog ($Xenopus laevis$) and observed similar expression patterns and molecular interactions.

“Our identification of $Mcc$ as an intracellular component of non-canonical Wnt signaling is entirely novel and unexpected,” Dunn says. The team’s next step will be establishing a better understanding of $Mcc$’s role in colorectal cancer and in hepatocellular carcinoma, a common form of liver cancer.
A double blast to ward off pneumonia

Despite advances in vaccination and antimicrobial therapy, community-acquired pneumonia remains a leading cause of morbidity and mortality, even in highly developed countries. Desmond Heng, Reginald Tan and co-workers at the A*STAR Institute of Chemical and Engineering Sciences have now developed a dry powder inhalation formulation to treat bacterial infections associated with this disease.

Community-acquired pneumonia, a type of lung inflammation contracted outside of a hospital or nursing-home setting, is most often caused by infections with bacteria, such as Klebsiella pneumoniae, Pseudomonas aeruginosa and Staphylococcus aureus. The condition affects people of all ages, but is particularly prevalent among infants, the elderly and patients with chronic obstructive pulmonary disease.

The formulation developed by the team contains two important ingredients: ciprofloxacin hydrochloride (CIP), an antibiotic commonly used to eliminate pathogenic bacteria, and beclomethasone dipropionate (BP), a corticosteroid commonly used to inhibit inflammatory responses.

The novelty of this formulation lies in the combination of the two drugs. Previous clinical trials have evaluated the efficacy, safety and tolerability of CIP on human patients. The inhalation of BP is known to be an effective treatment for patients with non-asthmatic chronic airflow obstruction. Yet no one had put these two together to check the feasibility of combining CIP and BP as an inhalable dry powder formulation for direct concomitant delivery to the lungs.

The researchers employed a state-of-the-art spray drier to prepare the CIP–BP dry powder and ensure uniformity, fineness and overall quality. They characterized the CIP–BP dry powder using field-emission scanning electron microscopy and showed that the majority of particles had a diameter of approximately 2.36 micrometers — small enough to be breathed in. X-ray diffraction analysis revealed only a single diffuse, broad peak, suggesting that the CIP–BP dry powder is highly amorphous.

“What’s important is that the CIP–BP dry powder exhibits superior aerosol performance and excellent antimicrobial activities,” explains Heng. “Our follow-up microbial assays show that a concentration as low as one microgram per milliliter is enough to inhibit three of the bacteria known to cause this type of pneumonia.”

“We found that it is feasible to package the CIP–BP dry powder in an inhaler that can treat bacterial infections associated with community-acquired pneumonia,” adds Heng.

Dry powder inhalers exhibit several advantages over traditional drug delivery methods, including improved formulation stability, enhanced delivery efficiency, excellent portability and ease of use. The delivery of CIP and BP via dry powder inhalers may become a novel and useful strategy for treating patients with community-acquired pneumonia.

A new web tool allows researchers to obtain higher protein yields for a range of life sciences applications including biosensing and drug manufacturing. Designed by scientists at A*STAR, the program provides users with a simple and flexible interface for optimizing the sequence of synthetic genes for improved expression of desired proteins.

Through millions of years of natural selection, genes have evolved to allow for optimal protein expression. This includes a preference for certain DNA triplets, called codons, over others because of the relative abundance of the molecules involved in protein production.

As a consequence, when biologists take genes from one organism and insert them into another to create synthetic genetic circuits, the gene sequences are often poorly adapted for the protein machinery of the new host. That is why scientists must go through a process known as codon optimization to tailor the DNA specifically to the new host organism. Most existing tools for codon optimization have fairly limited design criteria, however, and their rigid algorithms often fail to find the best sequence for a particular application.

Dong-Yup Lee, together with colleagues Ju Xin Chin and Bevan Kai-Sheng Chung from the A*STAR Bioprocessing Technology Institute in Singapore, sought a more adaptable solution for codon optimization. They designed ‘Codon Optimization OnLine’, or COOL, a user-friendly platform that incorporates customizable design parameters to find the optimal gene sequence. The tool also supports a wide range of visualization capabilities, which means researchers can graphically compare the quality of various optimized DNA sequences.

“COOL is the first web application that provides a full suite of optimization options as part of a multi-objective framework,” says Lee. “We developed novel computational algorithms for codon optimization and implemented it into COOL so that any scientists in the bioengineering, biotechnology and synthetic biology fields can easily use it for their gene design.” In his own lab, Lee says that he has designed several gene sequences using COOL and “obtained very good results.”

The program provides suggested codon usage and codon pair patterns that correspond to efficient expression for four hosts — these include two bacterial species (Escherichia coli and Lactococcus lactis) and two yeast species (Pichia pastoris and Saccharomyces cerevisiae) — all of which are commonly used in synthetic biology. Researchers can also input reference gene sets manually and Lee’s team is currently extending the built-in expression systems to include mammalian and plant cells.

Ethnicity plays a significant role in the likelihood of developing certain diseases, such as diabetes. South Asians, for example, are known to be more insulin resistant than other Asians, and scientists have long believed that this is because South Asians tend to accumulate fat around the waist more than other Asian ethnicities. Now, a team of researchers from Singapore, including scientists at A*STAR, have established that problems with muscle insulin signaling pathways, rather than how fat is distributed, may explain these differences in insulin resistance.

Generally, when people become more obese, they become more insulin resistant. E. Shyong Tai and co-workers at the National University of Singapore, in collaboration with scientists at three A*STAR facilities, show that insulin resistance in South Asians is less affected by obesity and fat distribution than in people of Chinese and Malay origin.

“In 2011, we realized that insulin resistance is more complex than differences in obesity levels,” explains Tai. “However, only recently could we take measurements on a large-enough scale to properly understand the mechanisms.”

Tai and his team investigated 264 healthy adult males comprising 101 Chinese, 82 Malays and 81 South Asians. They measured the distribution of body fat in all the individuals, as well as the levels of proteins linked to glucose metabolism in muscle cells in the Chinese and South Asian groups.

Although obesity was associated with increased insulin resistance in all three ethnic groups, the team found that the effects were far more pronounced in Chinese and Malays. More surprisingly, lean South Asians were also found to be insulin resistant.

“The levels of some proteins involved in glucose metabolism in the skeletal muscles of South Asians were lower than in their Chinese counterparts.”

The body naturally regulates blood sugar levels by releasing the hormone insulin. When blood sugar goes up in a healthy person, insulin is released to stimulate organs such as the muscles to absorb glucose and either use it or store it as a future source of energy. In individuals with insulin resistance, this process does not work efficiently, meaning that the body has to release higher levels of insulin to lower glucose levels.

Further research into the muscle samples taken from South Asians may help the team identify novel pathways relevant to insulin resistance. This could lead to new ways of preventing or treating type II diabetes.

Singaporean researchers have developed a genetic test that can reliably identify different subtypes of a specific kind of kidney cancer. The test has the potential to improve treatment, as it can assess a patient’s prognosis and likely response to therapy.

Clear cell renal cell carcinoma (ccRCC) is the most common type of kidney cancer (see image). The prognosis for patients with ccRCC who undergo surgery to remove a tumor is difficult to predict, however, since outcomes vary. The benefit of tyrosine kinase inhibitor (TKI) therapy — the most frequently used medication administered for ccRCC — also varies between patients. Evidence suggests that different outcomes could result from different subtypes of the disease; in which case, a test that identifies markers of these subtypes could improve treatment.

Min-Han Tan and co-workers from the A*STAR Institute of Bioengineering and Nanotechnology, Singapore General Hospital and National Cancer Centre Singapore aimed to develop a test that could identify genetic markers of different ccRCC subtypes. To look for relevant genes, they turned to genetic material preserved from 279 patients who had undergone surgery for ccRCC in the past 15 years.

“We used microarrays to broadly survey all transcribed genes within the tissue sample,” explains Tan. “We employed statistical methods to filter likely candidates, before using a separate method (quantitative real-time polymerase chain reaction) to determine which candidates had the best performance.”

The team identified eight genes whose expression levels differed between two subtypes of ccRCC, allowing them to develop an assay based on these eight genes to test patients. They validated the test by using it on other groups of patients; the expression profiles of the eight genes proved to be a reliable predictor of a good or poor prognosis. Analysis of a subset of patients with ccRCC who received TKI therapy as well as surgery also showed that the assay can reliably predict whether patients will benefit from TKI therapy.

“Their expression profiles have been validated as a reliable predictor of a good or poor prognosis,” says Tan. “On a prognostic level, it allows an accessible way to determine good and poor outcomes in patients undergoing surgery. On a predictive level for tyrosine kinase inhibitors, ours is the first study to associate biological subtypes with drug response.”

Tan says that the assay could be used to improve surveillance strategies for patients with ccRCC, especially those with a poor prognosis, but that clinical trials are needed to determine the value of the assay in relation to various therapies.

A study of women in Singapore has revealed a possible link between depression in mid-to-late pregnancy and levels of folate, also known as folic acid or vitamin B9.

"Depression affects as many as 12 per cent of women during pregnancy and 7 per cent just after birth," explains study leader Mary Chong of the A*STAR Singapore Institute for Clinical Sciences. “We were keen to find out if nutrition during pregnancy could play a role in optimizing a mother’s mental health, and in this study we focused on folate and vitamin B12.”

Previous studies in groups such as the elderly and adolescents have suggested possible links between depression and low levels of these B vitamins, explains Chong. Limited studies in women both during and shortly after pregnancy, however, had not demonstrated any link.

Chong worked with a large team of researchers in Singapore and collaborators from the United Kingdom, New Zealand and Canada as part of the Growing Up in Singapore Towards healthy Outcomes (GUSTO) study group. Women participating in the study completed a questionnaire known as the Edinburgh Postnatal Depression Scale, between the twenty-sixth and twenty-eighth week of pregnancy, and then again three months after giving birth. Their responses were matched against measurements of plasma folate and vitamin B12 taken during pregnancy.

Low folate levels were found to be associated with depression during pregnancy, but not with postnatal depression. There was no association between depression and vitamin B12.

Chong emphasizes that finding an association between low folate levels and antenatal depression does not indicate any direction of causation. She notes, however, that folate is required for the synthesis of neurochemicals that are known to regulate moods and behavior, and so low folate levels may in part cause depression.

It is also possible that depression results in poor nutritional habits or causes some of the depressed women to neglect taking their recommended vitamin supplementation, leading to folate insufficiency. Such lack of adherence to prenatal supplementation recommendations has been demonstrated in previous studies by other researchers.

Chong’s group plans to begin a new study in January 2015 that will start by measuring folate and vitamin B12 levels in women before conception. It will then follow, both during and after pregnancy, the prevalence of depression in those women who conceive. “This will enable us to further understand the direction of causality in this relationship and when to intervene, so as to help support mothers’ mental well-being,” says Chong.

Cancer biology:

Hippo competition provides cancer clue

A protein on which two molecular pathways converge could be the key to new cancer therapies

Two molecular pathways — one that causes cancer and one that protects against it — compete to control cellular levels of one protein, according to a new study by A*STAR researchers and colleagues. The finding highlights a central role for the protein in cancer, and could lead to new therapies.

The Ras and Hippo pathways converge on the Yes-associated protein (YAP) and regulate its stability. The Ras pathway increases the stability of YAP by controlling the expression of proteins called suppressors of cytokine signaling (SOCS). YAP in turn targets the AREG gene and triggers the production or Amphiregulin proteins that increase the ability of normal cells to transform into cancer cells.

The next stage in our research is to identify druggable targets that regulate YAP activity in desirable ways."

The Hippo pathway regulates the activity and levels of a protein called Yes-associated protein (YAP), and is known to protect against cancer, making it a potential target for cancer drugs. Now, work led by Xin Hong and Hung Nguyen in Stephen Cohen’s research group at the A*STAR Institute of Molecular and Cell Biology has shown that the Ras pathway, which is known to have a role in the development of tumors, competes with the Hippo pathway to regulate the activity and levels of YAP.

Previous work by the same international research team showed that reducing cellular levels of proteins called suppressors of cytokine signaling 5 (SOCS5) enhanced the ability of the Ras protein to transform normal cells into cancer cells. Building on these findings, the researchers directly manipulated levels of SOCS proteins in cultured human epithelial cells, and showed that depletion of these proteins had the same effect as activation of Ras: that is, increased activity and levels of YAP that led to an increased ability of normal cells to transform into cancer cells.

The team went on to show that SOCS6 normally forms a complex with YAP that is targeted for degradation. Depleting the SOCS proteins therefore prevents formation of this complex and reduces the degradation of YAP, which leads to increased cellular levels of the protein. This process is in contrast to the Hippo pathway, which encourages tagging of YAP for degradation, thereby preventing its levels in cells from increasing.

“Ras pathway activity promotes YAP stability, acting in opposition to the Hippo pathway, which promotes YAP turnover,” summarizes senior investigator Cohen. “It seems that all roads lead to YAP.”

“Our study also suggested that amplification or activation of YAP could act as a backup mechanism to support tumor growth if Ras activity is shut off,” adds Hong.

“This suggests that YAP activity might contribute to drug resistance when Ras inhibitors are applied to cancer cells.”

The position of YAP at the interface of two pathways that are important in cancer makes it a prime target for new therapies.

“The next stage in our research is to identify druggable targets that regulate YAP activity in desirable ways,” concludes Cohen.

Data storage:

Seed first, heat later for better writing

A new technique for heat-assisted magnetic recording media promises improved writeability for next-generation hard drives

Heat-assisted magnetic recording (HAMR) is a new process that realizes the three goals of magnetic recording — readability, writeability and stability. A*STAR researchers have now succeeded in improving its writeability by employing a thermal design that permits a higher density recording.

HAMR magnetically records data using a laser to locally heat the area being written. Careful control of the thermal spot size on the medium and the thermal gradient during writing allows more information to be written in a smaller area. The recording medium’s thermal profile is influenced by its physical and chemical properties, such as its optical characteristics, microstructure and layer structure, which impact the recording performance and density.

Jiang Feng Hu and his team from the A*STAR Data Storage Institute wanted to better control the thermal profile. The three layers making up the write layer — the heat-sink layer, underlayer and top layer — must support high thermal gradients. In addition, the top layer should be crystalline with controllable microstructural features. An L1₀-ordered iron–platinum alloy film is a popular top layer as it exhibits a high magnetic anisotropy.

However, choosing a suitable heat-sink layer is challenging. Copper-based materials are attractive due to their high thermal conductivity, but a mismatch between the structures of the crystalline layer and the underlying magnesium oxide limits the growth of the L1₀ phase. Although this mismatch can be corrected by inserting a layer between the heat sink and the underlayer, doing so reduces the thermal performance of HAMR media — “This will produce a smaller thermal gradient and media signal-to-noise ratio (SNR),” explains Hu. This is problematic as a high SNR is a critical measure of recording-media performance.

Hu’s team focused on a technical solution called the ‘seed-then-heat-sink approach’ and corresponding media design. As this design does not require an additional layer, it attains a large thermal gradient and a higher media SNR. A textured copper nitride film is used as a seed layer to induce an orientation of magnesium oxide that promotes L1₀-ordered iron–platinum film growth. The subsequent deposition of the iron–platinum alloy film, as a high-temperature process, decomposes copper nitrate into copper, which provides a suitable heat-sink layer.

Hu notes this approach enables a large thermal gradient during the writing process. “This large thermal gradient is critical to the iron–platinum-based medium for HAMR application, especially for HAMR media with smaller grains to support the ultrahigh areal density that HAMR technology is targeting,” says Hu.

Quantum effects bridge the gap

Quantum effects in nanometer-scale metallic structures provide a platform for combining molecular electronics and plasmonics

Plasmonic devices combine the ‘super speed’ of optics with the ‘super small’ of microelectronics. These devices exhibit quantum effects and show promise as possible ultrafast circuit elements, but current material processing limits this potential. Now, a team of Singapore-based researchers has used a new physical process, known as quantum plasmonic tunneling, to demonstrate the possibility of practical quantum plasmonic devices.

Tunneling is an intriguing aspect of quantum mechanics whereby a particle is able to pass through a classically insurmountable barrier. Theoretically, quantum plasmonic tunneling is only noticeable when plasmonic components are very closely spaced — within half a nanometer or less. However, researchers from the A*STAR Institute of Materials Research and Engineering, the A*STAR Institute of High Performance Computing and the National University of Singapore were able to observe quantum effects between materials spaced more than one nanometer apart.

They investigated the tunneling of electrons across a gap between two nanoscale cubes of silver coated with a monolayer of molecules. High-resolution transmission electron microscopy showed that these nanocubes self-assembled into pairs. The separation, and hence the tunneling distance, between the nanoparticles could be controlled by the choice of surface molecule — between 0.5 and 1.3 nanometers in the cases tested.

The monolayer of molecules had another function — to provide molecular electronic control over the frequency of the oscillating tunnel current, which could be tuned between 140 and 245 terahertz (1 terahertz = 10^{12} hertz), as was shown by monochromated electron energy-loss spectroscopy.

Theoretical predictions, supported by experimental results, confirmed the nature of the plasmon-assisted tunnel currents between the silver cubes. “We show that it is possible to shine light onto a small system of two closely spaced silver cubes (see image) and generate a tunnel current that oscillates very rapidly between these silver electrodes,” explains A*STAR researcher Michel Bosman. “The oscillation is several orders of magnitude faster than typical clock speeds in microprocessors, which currently operate in the gigahertz (= 10^9 hertz) regime.” At the same time, the results also demonstrate the possibility of terahertz molecular electronics.

Two factors contributed to the success of the experiments. First, the nanocubes had atomically flat surfaces, maximizing the tunneling surface area between the two nanoparticles. Second, the molecule-filled gap increased the rate of tunneling, making it possible to measure plasmon-assisted quantum tunneling.

“We will now use different molecules in the tunnel gap to find out how far the tunnel currents can be carried, and in what range we can tune the oscillation frequency,” says Bosman.

As the sensitivity of plasmonic sensors reaches new heights, so does the challenge of using tiny sample volumes. Dmitry Kalashnikov and co-workers at the A*STAR Data Storage Institute in Singapore have devised an innovative way to improve sensor accuracy by exploiting the unique properties of quantum optics.

Some new sensors based on plasmonic nanostructures can detect responses from samples that contain only about 100,000 molecules. While increased sensitivity is a boon, the low volumes of the sample mean that the light used to probe such tiny amounts can induce unwanted changes in them. Therefore, it is necessary to probe samples using very low light levels — even down to the ultimate limit of single photons. However, conventional techniques cannot cope with such small amounts of light: the signal strength from a sample becomes low compared with the background noise, rendering accurate detection impossible.

To address this problem, Kalashnikov and his team created a pair of ‘frequency-entangled’ photons by passing laser light through a nonlinear crystal. The frequencies of these two photons add up to that of the photon that initially hit the crystal. Thus, by measuring the frequency of one photon, the researchers could easily calculate the frequency of the other one.

To set up the measurement system, the researchers sent one of the photons to a plasmonic sensor — a hexagonal array of gold nanoparticles — and detected it with a highly sensitive light detector (see image), while using a spectrometer to measure the frequency of the other photon. By measuring the number of photon pairs that are simultaneously spotted by the two light detectors, they could determine the dependence of the transmittance of the sensor on the frequency.

To investigate the robustness of the measurements to background noise, the researchers then artificially introduced noise to the measurement of the photon that passed through the spectrometer. With this, they found that their technique could detect a tiny change in the refractive index (approximately 1 per cent) of a liquid sample placed immediately in front of the plasmonic sensor — even in the presence of severe noise up to 70 times stronger than the signal.

When they performed the same measurement using conventional spectroscopy, they found that the noise completely swamped the signal.

“The trick is that both photons reach the detectors at the same moment, whereas noise is completely random, making it highly unlikely to cause both detectors to fire simultaneously,” Kalashnikov explains.

The extremely low photon levels of the technique make it suitable for measuring photosensitive compounds and ultralow concentrations of other substances.

Data storage:

**A clearer view of a hot technique**

*A multifunctional testing instrument helps improve production of laser-heated hard disk drives with enhanced storage capacities*

Today’s hard disk drives can hold terabytes of digital data, but manufacturers are having trouble squeezing more storage capacity into these devices using conventional procedures. Now, a new technique that promises to solve this impasse — heat-assisted magnetic recording (HAMR) — can be integrated more efficiently into future hard drives thanks to an analytical tool developed by A*STAR researchers.

Data-storing ‘bits’ inside hard disk drives have to be turned on and off with magnetic fields. But as bit sizes diminish to improve storage density, the recording heads need stronger and stronger fields to resolve individual magnetic grains. Eventually, impractically large fields are required to read and write data.

The HAMR approach uses a small laser mounted on the disk recording head to heat up the magnetic material before writing to it. The increase in temperature reduces the magnetic field intensity necessary for data storage and consequently, smaller bit sizes can be used. Rapid cooling of the magnetic grains ensures the stability of the freshly recorded data.

Researchers are confident that the HAMR technique can lead to 20-terabyte hard drives within a few years if some specific challenges can be overcome. One current problem is that accurately testing the temperature-dependent recording in localized regions is difficult. Typical analytical methods have to heat up relatively large sample volumes, a time-consuming process that can irreversibly damage HAMR media.

Hongzhi Yang, with a team from the A*STAR Data Storage Institute and the National University of Singapore designed an improved ‘pump–probe’ laser device to scrutinize HAMR devices. The instrument uses an initial intense beam to heat up a localized region of the magnetic disk. Then, a weaker laser probes the heated region for the micro-magneto-optic Kerr effect (µ-MOKE), a phenomenon that can gauge a material’s magnetization state. By repeating these measurements with different heating beam conditions, the researchers obtained detailed data on HAMR writing, reading and magnetic states from specific microscopic spots on the hard drive surface — information currently unavailable through other techniques.

“The challenge in developing this testing instrument was integrating the complex optical and mechanical components to achieve good signal-to-noise ratios and uniform temperature distribution in the media during heating,” says Yang. “But compared to traditional bulk-heating techniques, our method is much faster, allows full disk measurement and avoids annealing effects.”

The team is confident that this instrument can be incorporated into disk drive manufacturing plants as HAMR captures a larger share of magnetic recording technology.

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Laser processing: Making waves with lasers

Laser processing produces deep ripples in silicon over a wide area — something that could enhance solar cell efficiency

A*STAR scientists have produced a uniform nanoscale ripple pattern over a wide area on a silicon surface by scanning a femtosecond laser beam across it. Given that a rippled surface is much less reflective than a smooth surface, this simple innovation could enhance the efficiency of solar cells by boosting their ability to harvest more sunlight.

The use of lasers to produce periodic surface structures is currently an area of intense research. Laser processing has the important advantage that it heats only the surface of a material, leaving underlying structures unaffected. However, many laser processing methods are limited: they can process only small areas and shallow ripples.

Now, Xincai Wang and co-workers from A*STAR’s Singapore Institute of Manufacturing Technology and Nanyang Technological University have successfully addressed these limitations. They demonstrate the potential of their technique by using it to produce a uniform ripple pattern on a silicon substrate (see image) over a large area of 30 millimeters by 30 millimeters, with an average ripple depth of 300 nanometers — about three times greater than that of other techniques.

“This increase in depth can substantially reduce light reflection and improve the light-trapping ability of the ripple structure,” Wang notes. “Hence, if the structure is used in photovoltaic devices, more light will be trapped within the structure, thereby enhancing the device efficiency.”

Straightforward and inexpensive, the technique simply involves using a cylindrical lens to widen a femtosecond laser beam to a width of 50 micrometers and then scanning the beam across the surface.

As the energy of the laser’s photons exceeds the bandgap of silicon, the photons excite electrons from the valence band to the conduction band. Such electrons would usually relax by transferring their energy to the atomic lattice, thereby heating it. However, the extremely short pulse durations mean that instead they generate an electron wave on the surface. This in turn produces a light wave, which interferes with the incoming laser beam. The silicon is removed in locations where the incoming and outgoing light waves constructively interfere with each other, giving rise to the valleys in the ripple pattern.

The researchers found that on rippling the average reflectance of a silicon surface dropped from 39.7 per cent to 12.5 per cent, which meant light absorption was enhanced by 41 per cent as a result of strong scattering by the ripple structure. This effect could be exploited to manage photon behavior in solar cells and light-emitting diodes.

Nanoplasmonics: Making a tiny rainbow

By varying the size and spacing of aluminum nanodisks, researchers generate images that contain over 300 colors and are not much wider than a human hair

A scheme for greatly increasing the number of colors that can be produced by arrays of tiny aluminum nanodisks has been demonstrated by A*STAR scientists.

Conventional pigments produce colors by selectively absorbing light of different wavelengths — for example, red ink appears red because it absorbs strongly in the blue and green spectral regions. A similar effect can be realized at a much smaller scale by using arrays of metallic nanostructures, since light of certain wavelengths excites collective oscillations of free electrons, known as plasmon resonances, in such structures.

An advantage of using metal nanostructures rather than inks is that it is possible to enhance the resolution of color images by a hundred fold. This enhanced resolution, at the diffraction limit of light, is critical for data storage, digital imaging and security applications. Aluminum — because of its low cost and good stability — is a particularly attractive material to use.

Joel Yang and Shawn Tan at the A*STAR Institute of Materials Research and Engineering and co-workers used an electron beam to form arrays of approximately 100-nanometer-tall pillars. They then deposited a thin aluminum layer on top of the pillars and in the gaps between them. In these arrays, each pixel was an 800-nanometer-long square containing four aluminum nanodisks.

The plasmon resonance wavelength varies sensitively with the dimensions of the nanostructures. Consequently, by varying the diameter of the four aluminum nanodisks in a pixel (all four nanodisks having the same diameter), the scientists were able to produce about 15 distinct colors — a good start, but hardly enough to faithfully reproduce full-color images.

By allowing two pairs of diametrically opposite nanodisks to have different diameters from each other, then varying the two diameters enabled them to increase this number to over 100. Finally, they generated over 300 colors by varying both the nanodisk diameter (but keeping all four diameters within a pixel the same) and the spacing between adjacent nanodisks in a pixel (see image). “This method is analogous to half-toning used in ink-based printing and results in a broad color gamut,” comments Yang.

The researchers demonstrated the effectiveness of their extended palette using a Monet painting. They reproduced the image using both a limited and extended palette, with a much better color reproduction from the extended palette. Amazingly, they shrank the image from 80 centimeters to a mere 300 micrometers — a 2,600-fold reduction in size.

“The use of a more cost-effective metal has the potential to move this technology closer to adoption,” Tan notes.

Quantum optics:

The seeing power of frogs

A quantum light source proves that light-sensitive cells in frog eyes can detect single photons

Miniature light detectors in frog eyes known as retinal rod cells are directly and unambiguously shown to detect single photons of light — an astounding sensitivity considering that a humble 60-watt light bulb spews out a staggering $10^{20}$ photons per second. Using a specially developed light source that generates single photons, a new A*STAR study finds that a rod cell has an almost one-in-three chance of detecting an incoming photon.

Scientists have known for some time that rod cells are sensitive to single photons. This was inferred based on statistical modeling in studies that used classical light sources such as lamps, lasers and light-emitting diodes that generate a statistical distribution of photons. In contrast, the light source developed by Leonid Krivitsky and co-workers at the A*STAR Data Storage Institute and A*STAR Institute of Medical Biology is a truly single-photon source and hence eliminates the need to statistically analyze measurement results, thus enhancing measurement accuracy.

“Our method is both direct and universal,” notes Krivitsky, “as it is not based on any particular statistical model of the cell response and thus does not involve any indirect assumptions.”

In the developed light source, a nonlinear optical crystal is irradiated with light from an ultraviolet laser. Most photons pass directly through the crystal, but approximately one in a million is split into two visible-light photons having twice the wavelength (532 nanometers) of the original photon (266 nanometers). One of these two photons is detected by a photodiode and used to trigger an acousto-optical modulator, causing it to divert the second photon to a tapered optical fiber directed at a pipette containing a rod cell from a frog’s eye (see image). Any signal produced by the rod cell is then detected.

Racing against the clock, since rod cells lose their viability after one to two hours, the researchers measured ten rods cells taken from ten different frogs. They found an average quantum efficiency of approximately 30 per cent — very close to that of human rod cells estimated from behavioral experiments. Krivitsky notes that rod-cell efficiency is comparable to the quantum efficiencies of state-of-the-art man-made single-photon detectors such as photomultipliers (40 per cent) and avalanche photodiodes (50 per cent); remarkably, rod cells occupy an area of only 5 by 50 micrometers and contain their own power supply.

The new light source could be further used to investigate how the quantum efficiency varies with wavelength, since it is easy to vary the wavelength of the generated single photons.

Three-dimensional (3D) integration of various materials on top of bulk silicon could be the best answer for cost-effectively marrying optical devices with electronics. A*STAR researchers have used this approach to create a photodetector system for optical communications on a silicon chip.

As computers become increasingly powerful, there is a need to find elegant ways to combine electronics and high-speed optical interconnect technology to meet the growing demand for ever faster data processing and communication. “We believe that, in the future of on-chip and chip-to-chip communication, opto-electric integrated circuits on silicon will be a key technology to realize high-speed, low-power and low-cost chips,” explains Junfeng Song from the A*STAR Institute of Microelectronics.

To date, most attempts to make hybrid electronic-optical silicon chips have relied on silicon-on-insulator (SOI) technology in which an insulating layer of silicon dioxide is formed on a silicon wafer. While this approach works well, it has the disadvantage of being very expensive — SOI wafers cost about ten times more than bulk silicon wafers. SOI wafers also suffer from poor thermal conductivity, making it difficult to dissipate heat from devices.

The team instead decided to explore the use of conventional bulk silicon wafers, which are a natural platform for microelectronics, but then fabricate optical devices in layers integrated on top of the wafers, resulting in a 3D design.

Song and co-workers demonstrated this concept by fabricating an integrated photodetector system. A germanium detector was built directly on top of a silicon wafer (see image) and fed with an optical waveguide and grating coupler formed in silicon nitride. The researchers tested the detector and found it was capable of handling data at speeds of 10 gigabits per second per wavelength channel. The team is confident that this can be pushed to much higher speeds.

“In the current device, the three-decibel bandwidth is small but by employing an electronic amplifier we can already get 20 gigabits per second rather easily,” explains Song. “I don’t think that the data rate has any physical limit, so it should be possible to achieve 50 gigabits per second or higher.”

According to Song, the next challenge is to make more sophisticated integrated systems featuring more optical devices and more electronics. Possibilities include adding optical modulators, variable optical attenuators, optical switches, electronic amplifiers and electronic drivers to the chip circuitry. Other plans are to experiment with using alternative materials on top of the silicon, such as aluminum nitride, which has electro-optic properties and could bring new functionality.


A germanium photodetector integrated on a silicon bulk wafer.
Concerns over toxicity have constrained the clinical application of photoacoustic imaging — a new experimental technique used to detect tumors lurking among healthy cells. A*STAR researchers from the Singapore Bioimaging Consortium and an international team have now discovered how to improve both the safety and the tumor-locating efficacy of photoacoustic imaging using ‘photosensitizer’ contrast agents.

Unlike typical optical measurements, photoacoustic imaging can probe deep into tissue by using ultrasonic waves generated by laser light pulses. This hybrid method produces strikingly clear pictures of complex biosystems, such as vascular tumor networks in organs and tissues. However, clinical applications of photoacoustic imaging have been limited by safety concerns surrounding ‘contrast agents’ — light-absorbing molecules injected into cells to enhance imaging resolution.

Photosensitizers are molecules that play key roles in a cancer treatment known as photodynamic therapy. These nontoxic substances, which are normally based on conjugated aromatic ring systems, specifically bind to malignant cells while leaving healthy tissue untouched. Exposing a photosensitizer to laser light causes it to produce excited-state oxygen molecules, which react with diseased cells to kill them. The photosensitizer is then rapidly eliminated from the target tissue.

Photosensitizers have often been overlooked as diagnostic imaging aids because, after laser excitation, their fluorescence output is not penetrating enough for deep-tissue imaging. But Malini Olivo and his team realized that the poor fluorescence efficiency of excited-state photosensitizers could prompt them to relax though thermal conversions instead — conditions that might favor production of acoustic pressure waves for photoacoustic imaging. To achieve this, the researchers needed to find photosensitizers that can absorb light at the near-infrared wavelengths used for deep-tissue imaging.

After testing different photosensitizers, the team selected a promising substance known as zinc phthalocyanine (ZnPc) to inject into living mice with tumors. Experiments confirmed their hypothesis: the photosensitizers produced strong photoacoustic signals as they passed through vital organs and zeroed in on the tumor cells. Within ten minutes of injection, signals from the ZnPc contrast agents illuminated the precise position of diseased tissue (see image). The ZnPc cleared harmlessly from the mice within one day, resulting in a low overall toxicity.

“Many photosensitizers have already been widely studied in clinical trials, and so using them in photoacoustic imaging offers great potential as an alternative diagnostic technique,” says Olivo. “Combining the preferential tumor uptake and high photodynamic therapy efficacy of photosensitizers with photoacoustic imaging can help achieve long-term monitoring of cancer progression and therapy under in vivo conditions.”

Bioimaging: Targeting tumors with sound and vision

Dual-action chemical agents improve a high-resolution and noninvasive way to detect cancer

A chemical probe that selectively and safely illuminates tumor cells can significantly boost the capabilities of an alternative bioimaging technique (red; concentration of probe one hour after injection).

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Biotechnology:

Using fungi to catch algae

Fungal waste biomass from biotechnology applications could be used to harvest microalgae for fuel and chemical production

Waste biomass from fungal fermentation processes could be used to bind to and harvest microalgae being used in other biotechnology applications. A*STAR researchers have successfully demonstrated this procedure with fungal mycelium — the main vegetative part of a fungus such as the tangled mass of underground fibers beneath sprouting mushrooms.

Suitable fungal biomass might be obtained cheaply or perhaps even freely to offer a sustainable and environmentally sound method for harvesting microalgae. The potential uses of microalgae include burning their biomass as fuel or turning them into mini-factories for making biodiesel or specific chemicals including lipids, sugars or drugs.

“The lack of an economic and effective method for harvesting microalgae is one of the bottlenecks limiting their commercial use in biotechnology,” explains Mahabubur Talukder of the A*STAR Institute of Chemical and Engineering Sciences.

Microalgae can be cultured in a broth and existing methods for harvesting them include centrifugation or a precipitation process called flocculation using chemical treatments. All current methods however suffer significant drawbacks, explains Talukder. For instance, centrifugation is too expensive to be used for low value uses of microalgae, such as biofuel. Similarly inadequate, chemical flocculation contaminates the harvested microalgae with toxic metal salts, causing difficulties in further processing or extraction of desired products.

The A*STAR team knew that less toxic natural materials such as starch could be used to precipitate and collect some freshwater microalgae, but this is not suitable for marine microalgae due to undesirable effects of the salty solutions. What is needed is a nontoxic and preferably natural and widely available material that can bind to, immobilize and precipitate both freshwater and marine microalgae. This led the researchers to investigate fungal mycelium, which they found was not only effective but could also add value by contributing to the total biomass in the combined and harvested material.

The team screened several varieties of fungi with varying results, in some cases achieving a harvesting efficiency of 97 per cent after several hours of mechanical mixing with four times the mass of wet mycelium\textsuperscript{1}. Detailed analysis indicated that the key to the binding and immobilizing effect is a simple ionic attraction between the differing electric charges on the surface of the microalgae and the fungal mycelium.

“The next step is to find a collaborator or industrial partner willing to invest in and further explore the invention and commercialize it,” says Talukder, as his focus turns from the laboratory toward the challenges of scale-up and industrial production.

Healing of burn wounds requires that dead tissue is removed and new skin tissue is regenerated, while ensuring that the wound is closed rapidly to minimize the risk of infection and scar formation. A*STAR researchers now report non-immunogenic and non-cytotoxic hydrogels composed of nanofibrous peptide assemblies, which expedite wound closure and promote epithelial and dermal regeneration almost twice as fast as existing wound dressings.

Current methods of burn care, which include gauzes, hydrogels, hydrocolloids, foams and films made from natural or synthetic materials, are in need of improvement. Mepitel, for example, is a silicone-coated polymeric net, which maintains gaseous permeability to the wound but not the hydrated environment required for healing.

Charlotte Hauser and colleagues at the A*STAR Institute of Bioengineering and Nanotechnology in Singapore show that the nanofibrous hydrogels, composed of assemblies of short and easy-to-synthesize peptides, effectively promote in vivo wound healing. The transparency of the hydrogel allows the wound to be observed during the recovery process.

“Our ultrashort peptide-based hydrogel virtually fulfills all the criteria essential for accelerated wound healing,” says Hauser. “The hydrogel can provide a moist environment as a consequence of its extremely high water retention capacity of up to 99.9 per cent, which, to our knowledge, is unmatched by currently available wound dressings.”

This moisture-rich environment stimulates the removal of dead tissue in a process termed autolytic debridement. Encouragingly, the hydrogel-treated wounds showed autolytic debridement on day 8, whereas for Mepitel-treated wounds it was not observed until day 10. The knock-on effect of this enhanced tissue removal is that the hydrogel-treated wounds close faster. By day 14, the researchers noted almost complete regeneration of the epidermal layer for the hydrogel-treated group and also observed the presence of precursors for the formation of hair follicles.

Hauser and colleagues aim to investigate if other therapies could be combined with the hydrogels, such as the addition of compounds— for example anti-infectious drugs — to further speed up wound closure.

Other applications are also possible, adds Hauser. “We plan to probe the healing properties of the hydrogel in chronic skin wounds such as bed sores and diabetic ulcers.”

“Considering the long shelf-life stability of sealed peptides stored at room temperature, we are particularly keen to develop ‘just-add-water’ formulations,” says Hauser. “This would involve the addition of a fixed volume of clean water to the peptide powder at the point of application, which would greatly reduce transportation costs and potentially revolutionize emergency medicine for burn wounds in war zones and developing countries.”

A fast and accurate urine test for bladder cancer developed by A*STAR researchers has the potential to replace the currently used invasive physical probe.

Cystoscopy — a clinical procedure that uses a narrow, tubular optical instrument called a cystoscope to view inside the bladder — is currently the gold standard for detecting cancer in this organ. However, the technique is not favored by most patients because it is invasive, expensive and time consuming.

Malini Olivo at the A*STAR Singapore Bioimaging Consortium and co-workers have now developed a rapid immunoassay to detect and quantify alpha-1 antitrypsin (A1AT), a recently discovered urinary antigen and a potential biomarker for bladder cancer. The new tool could be used as a high-throughput screening platform to identify patients at risk of developing the urologic condition.

“Our device is extremely versatile because, in theory, the osmium carbonyl clusters can be swapped with other metal carbonyl species to account for different needs and purposes.”

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The immunoassay employs two advanced technologies, namely surface-enhanced Raman scattering (SERS), a powerful spectroscopic technique for detecting analytes at low concentrations, and bimetallic film over nanoparticles, a planar substrate for enhancing SERS signals. Together these technologies help to overcome interfering signals from the matrix background such as proteins in urine. The bimetallic film over nanoparticles is also coated with osmium carbonyl clusters to which target-seeking antibodies can be conjugated for assaying A1AT (see image).

The researchers first tested the immunoassay on a series of standard solutions containing A1AT antigens at various concentrations in the range 10 to 1,000 nanograms per milliliter. They observed a ‘fingerprint’ of A1AT antigens — a spectral change in the 1,850 to 2,130 cm\(^{-1}\) region that increases with concentration.

The scientists then tried the immunoassay on urine samples from nine patients. They found significantly elevated levels of A1AT in bladder cancer patients. There was also a marked difference in the A1AT concentrations of cancer and non-cancer patients, which suggests that the technique is highly discriminative, specific and accurate. Importantly, only tiny amounts of sample were required: A1AT concentrations could be quantified using as little as ten microliters of urine.

Compared to conventional immunoassays, the SERS-based bioassay has two practical advantages: the low-volume sample requires no purification prior to testing and the device has a simple design.

With further developments, the device may help save the lives of millions of would-be patients.

“We have developed a smart SERS biosensor for the rapid screening of bladder cancer,” says Olivo. “Our device is extremely versatile because, in theory, the osmium carbonyl clusters can be swapped with other metal carbonyl species to account for different needs and purposes.”

Surface condensation:

Extremely repellent surfaces

A computational technique reveals how tiny pillars affect the condensation of vapor onto a surface

A computational technique to analyze how water vapor condenses on a surface patterned with an array of tiny pillars has been co-developed by an A*STAR researcher. Calculations carried out using this technique reveal that water droplets preferentially form either on top of the pillars or in the gaps between them, depending on factors such as the height and spacing of the pillars.

Surfaces that strongly repel water, known as superhydrophobic surfaces, are important for many industrial applications as well as self-cleaning, defrosting and anti-icing surfaces. Scientists have discovered that inherently water repellent surfaces can be made much more water repellent by patterning them with micro- or nanoscale structures.

On such surfaces, water droplets can either be suspended across neighboring protrusions or impaled between them. The transition between these two states has previously been explored experimentally and theoretically. Furthermore, the effect of microstructures on vapor condensation has been studied experimentally, but there have been few computational studies of how droplets initially form by condensation from vapor.

Now, Weiqing Ren from the A*STAR Institute of High Performance Computing and Yunzhi Li of the National University of Singapore have systematically analyzed how micropillars on a hydrophobic surface affect the condensation of water vapor. To do this, they used a powerful computational technique known as the string method, which Ren developed in a previous study.

Ren and Li used the technique to investigate the effect of parameters such as the height and spacing of the micropillars and the supersaturation and intrinsic wettability of the surface on the condensation process. They discovered that both the pathway and configuration of the initial nucleus from which droplets form — known as the critical nucleus — depends on the geometry of the surface patterns. In particular, the scientists found that for tall, closely spaced pillars on a surface with a low supersaturation and low wettability, the critical nucleus prefers the suspended state, whereas for the opposite case it prefers the impaled state. By generating a phase diagram, they could determine the critical values of the geometrical parameters at which the configuration of the critical nucleus changes from the suspended state to the impaled state.

These results provide “insights into the effect of surface structure on condensation,” explains Ren, “and a quantitative basis for designing surfaces optimized to either inhibit or enhance condensation in engineered systems.”

In the future, the researchers intend to study how fluid flow affects nucleation and the wetting transition on patterned surfaces.

The characterization of individual components in an unknown crystalline powder mixture is a challenge that has eluded scientists for many years. Now, A*STAR researchers have for the first time invented a methodology to accurately determine the crystal structures present in such mixtures. Powder X-ray diffraction (PXRD) is a powerful tool used to determine the structure of crystal-line solids. Every solid has its own unique crystal structure which, when hit by X-rays, produces a unique diffraction pattern—a ‘fingerprint’ from which the solid can then be identified and characterized through computational analysis. However, traditional PXRD works best with pure single-component powders; mixed powders of unknown solids are far more difficult to analyze because the diffraction patterns overlap and are difficult to separate. Another complication is that individual solids can produce slightly different diffraction patterns depending on how the crystals are shaped and orientated in the powder samples.

Marc Garland and co-workers at the A*STAR Institute of Chemical and Engineering Sciences in Singapore have developed a new methodology, the PXRD-BTEM-Rietveld method, which combines two existing techniques to determine the individual crystal structures in a powder mixture.

Garland and his team first used PXRD to obtain diffraction datasets from pre-prepared mixtures of several different powders. They then used their own algorithm, called band-target entropy minimization (BTEM), to sift through the entire dataset, looking for the simplest underlying patterns and to untangle overlapping diffraction patterns. “BTEM is a blind separation technique,” explains Garland. “By searching for the simplest patterns—those with the smoothest profiles and the least signal disorder—we obtain accurate estimates of each pure component’s diffraction pattern.” Garnald and his team then used computational structure determination, including so-called Rietveld refinement, to obtain the crystal structures for each solid. This allowed the researchers to characterize the unknown components in the mixtures.

“One example of an application for our new technique could be investigating polymorphism in pharmaceuticals,” says Garland. “Each polymorphic pharmaceutical solid has a unique diffraction pattern resulting from its crystal structure, and it is incredibly important to the pharmaceutical industry to identify these from mixtures.” The researchers plan to further refine their methodology, and hope to eliminate the problem of measuring irregularities due to crystal orientation.

Nanosystems that are ‘thera-nostic’ — they combine both therapeutic and diagnostic functions — present an exciting new opportunity for delivering drugs to specific cells and identifying sites of disease. Bin Liu of the A*STAR Institute of Materials Research and Engineering and colleagues at the National University of Singapore have created nanoparticles with two distinct anticancer functions and an imaging function, all stimulated on demand by a single light source. The nanoparticles also include the cell-targeting property essential for treating and imaging in the correct locations.

The system is built around a polyethylene-glycol-based polymer that carries a small peptide component that allows it to bind preferentially to specific cell types. The polymer itself serves as a photosensitizer that can be stimulated by light to release reactive oxygen species (ROS). It also carries the chemotherapy drug doxorubicin in a prodrug form.

"We are now attempting to use near-infrared laser light to improve the tissue penetration and move toward on-demand cancer therapy."

The natural fluorescence of the polymer assists with diagnosis and monitoring of therapy as it shows where nanoparticles have accumulated. The ROS generated by light stimulation have a direct ‘photodynamic’ therapeutic activity, which destroys the targeted cells. The ROS additionally break the link between the polymer and the doxorubicin. Thus, cancer cells can be subjected to a two-pronged attack from the ROS therapy and the chemotherapy drug that is released within them (see image).

“This is the first nanoplatform that can offer on-demand and imaging-guided photodynamic therapy and chemotherapy with triggered drug release through one light switch,” explains Liu, emphasizing the significance of the system.

The researchers demonstrated the power of their platform by applying it to a mixture of cultured cancer cells, some of which overexpressed a surface protein that could bind to the targeting peptide on the nanoparticles. Fluorescence imaging indicated that the nanoparticles were taken up by the target cells and that ROS and doxorubicin were released within these cells — all at significantly higher levels than in cells used as controls. The doxorubicin that was released in the cell cytoplasm readily entered the nucleus — its site of activity. Crucially, the combined therapy had a greater cytotoxic effect than any one therapy alone.

“The white light used in this work does not penetrate tissue sufficiently for in vivo applications,” Liu explains, “but we are now attempting to use near-infrared laser light to improve the tissue penetration and move toward on-demand cancer therapy.”

She also suggests that with a few modifications, the system may be suitable for the diagnosis and treatment of other pathological processes including inflammation and HIV infection.

Device longevity can be improved through use of computer models that optimize the friction properties of diamond-like coatings used in hard disk drives.

Surface engineering:

Diamonds in the crush

Theoretical simulations reveal how nanoscale lubricating systems can ease friction between surfaces coated with diamond-like carbon

Diamond-like-carbon (DLC) coatings are an innovative technology, exhibiting the twin properties of mechanical toughness and ultralow friction. These features, which are desirable in abrasive environments, have led to the widespread adoption of DLC films in microelectromechanical systems, such as hard disk drives. But because these coatings contain amorphous carbon atoms that produce rough, nanoscale textures, it is difficult to optimize their friction properties using classical theories designed for macroscopic objects.

By performing atom-level simulations of nanoscale friction, Ling Dai and co-workers from the A*STAR Institute of High Performance Computing in Singapore have now uncovered critical clues for designing better systems to lubricate and protect DLC coatings.

Perfluoropolyether (PFPE) is a Teflon-like polymer that is commonly sandwiched between DLC-coated substrates to reduce friction and protect against damage. Understanding the friction mechanisms between these ultrathin films is tricky; these materials have contrasting hard and soft mechanical properties, and the sandwich arrangement obscures any direct observation of atomic structure and activity.

To better understand how nanoscale lubrication works in microdevices, the researchers constructed an atomic DLC–PFPE–DLC triple layer using a three-dimensional computer modeling program. They set one DLC slab as a substrate and the other as a ‘slider’. They then used molecular dynamics techniques to simulate how the lube film responds when the slider moves. However, it was challenging to describe the atomic interactions in this complex material, and so Dai’s team developed hybrid computations that combined several potential energy expressions to replicate the many-body forces in this system.

Simulating frictional motions at different speeds and PFPE film thicknesses revealed that the lubricating film behaves as a solid — the polymer retained its shape without deforming from internal shearing. However, the lubricating film displayed two distinct and competitive modes of motion at an interface: a ‘stick–slide’ action that produced jerky, stepwise displacements, and a continuous motion that caused the film to slide with fluctuating velocities. The team’s analysis showed that these two types of motions switched on or off depending on adhesion factors, such as thermal vibrations and the interfacial roughness.

After mapping the local friction forces along the sliding interfaces, the researchers discovered a way to link the law describing macroscopic friction to the nanoscale using a simple mathematical modification — a finding with practical importance for the surface engineering of DLC coatings.

“Because our model closely resembles the materials used in industrial applications, this work can serve as a guide for future experimental developments,” says Dai.

A*STAR researchers have developed an anticipatory alarm system based on dynamic models of industrial processes using concepts similar to extreme weather forecasting.

A large industrial plant such as an oil refinery contains many interdependent units. In such a complex system, many things could potentially go wrong, which explains why engineers need sophisticated alarms to help them deal with abnormal situations. Having too many alarms, however, is almost as problematic as having none — especially if all of the alarms go off at the same time.

Arief Adhitya and co-workers at the A*STAR Institute of Chemical and Engineering Sciences in Singapore and the National University of Singapore have developed a system that provides accurate short-term predictions of the state of the machinery in a plant, thus enabling operators to take action before alarms are triggered1.

“With so many interacting units, a fault can trigger a domino effect, setting off a large number of alarms within a short time, known as an alarm flood. This can confuse and overwhelm an operator, who might then activate the emergency shutdown, which leads to a costly loss of production,” says Adhitya. “Recent studies reveal that operators who are able to predict the evolution of the state of the plant are best able to cope with alarm floods.”

Industrial alarm systems monitor large numbers of process variables — such as the temperature or pressure in boilers — and activate alarms if those variables go outside defined ‘safe’ ranges. Previous methods of dealing with alarm floods have included dynamic adjustments of alarm limits and screening of alarms to remove false or duplicate alarms. Adhitya and co-workers went further. They combined detailed models of the industrial processes with historical data relating to machine behavior to estimate the rates of change of process variables. With this additional information, operators can assess when each variable is likely to trigger its alarm and can take evasive action.

The researchers tested their system with a case study of a depropanizer plant, which separates hydrocarbons of different sizes in an oil refinery. They simulated several faults, including loss of cooling water and fouling of the condenser, and found that their system predicted all the alarms successfully.

More importantly, the added information provided by their system reduced the diagnosis time for operators by around 35 seconds. The team is hopeful that their system could improve the efficiency of many different processes within and outside the oil industry.


Operators managing a complex industrial plant can greatly benefit from models that predict when problems may arise.
Computer hard drives store data by writing magnetic information onto their surfaces. In the future, magnetic effects may also be used to improve active memory in computers, potentially eliminating the need to ‘boot up’ a computer. One way to achieve this is through a memory technology known as STT-MRAM that utilizes information stored in a pair of thin magnetic layers. By performing calculations, Chee Kwan Gan and colleagues from the A*STAR Institute of High Performance Computing have proposed ways to improve STT-MRAM memory through identifying design options for achieving faster switching speeds, and hence faster data write times. 

In STT-MRAM devices, the relative orientation of the magnetic fields in the two thin layers determines the electrical resistance experienced by a current flowing through the device. If the magnetizations of both layers are aligned in the same direction, then the electrical resistance will be lower than when the layers have different magnetic alignments. Switching the device between different magnetic states — which corresponds to writing information into the memory — is achieved by electrons whose magnetic property, the spin, is aligned in one direction. Collectively, these electrons are able to change the direction of the magnetization in one of the layers. The time it takes to switch the magnetic direction depends on several factors, including the initial relative orientation of the magnetic fields in the two layers. The magnetization of the switched layer can follow various complex paths during the switching process (see image).

In previous experiments, the switching process was found to depend on two parameters. Using their computational model, the researchers could focus on one parameter — the less-studied ‘field-like’ term — that accounts for the relative orientation of the magnetic fields in both layers. The strength of this term depends on various factors, such as the device geometry and the materials used.

The calculations by the researchers show that, for devices with a strong field-like term, there is greater potential to reduce switching times than for devices in which the field-like term is negligible. Gan explains that this discovery will assist the development of improved STT-MRAM devices. “Our findings will motivate experimentalists to fabricate devices with strong field-like terms,” says Gan.

Furthermore, a better understanding of the origin of the field-like term is needed, adds Gan. “Although the effect of the field-like term has been confirmed experimentally and investigated in this work through simulations, it is important to understand its physical origins in order to improve material design.”

Three-dimensional (3D) movies, which require viewers to wear stereoscopic glasses, have become very popular in recent years. However, the 3D effect produced by the glasses cannot provide perfect depth cues. Furthermore, it is not possible to move one’s head and observe that objects appear different from different angles — a real-life effect known as motion parallax. Now, A*STAR researchers have developed a new way of generating high-resolution, full-color, 3D videos that uses holographic technology.

Holograms are considered to be truly 3D, because they allow the viewer to see different perspectives of a reconstructed 3D object from different angles and locations (see image). Like a photograph, a hologram contains information about the size, shape and color of an object. Where holograms differ from photographs is that they are created using lasers, which can produce the complex light interference patterns, including spatial data, required to re-create a complete 3D object.

However, generating high-resolution, moving holograms to replace current 3D imaging technology has proved difficult. To enhance the resolution of their holographic videos, Xuewu Xu and colleagues at the A*STAR Data Storage Institute in Singapore used an array of spatial light modulators (SLMs).

“SLMs are devices used in current two-dimensional projectors to alter light waves and generate projections,” explains Xu. “In a 3D holographic display, SLMs are used to display hologram pixels and create 3D objects by light diffraction. Each SLM in our system can display up to 1.89 billion hologram pixels every second, but this resolution is not high enough for a seamless large video display.”

To address this challenge, Xu and his team divided every frame of their hologram video into 288 sub-holograms. They then streamed the sub-holograms through 24 high-speed SLMs stacked together in an array. This technique was combined with optical scan tiling, which uses a scanning mirror to combine the signals from the SLMs, thus filling in any gaps in the physical tiling array. Finally, the researchers sped up the full-color video playback using powerful graphics processing units. This combination of technologies produced one high-resolution, full-parallax moving hologram displaying 45 billion pixels per second.

“We increased the resolution of the holographic display system by 24 times,” states Xu. “The full-color 3D holographic video plays at a rate of 60 frames per second, so it appears seamless to the human eye.”

Potential applications of the new technique include 3D entertainment and medical imaging. However, new SLM devices with a smaller pixel size, higher resolution and faster frame rate are required before large-scale 3D holographic video displays can become a reality.
3D manufacturing:

Elements of successful connections

Element-by-element tracking of laser processing reveals how metallic alloys reorganize during microscale laser melting processes

High-power lasers that can selectively cut and join metallic products are becoming increasingly important in today’s manufacturing industry. Now, Yingchun Guan from the A*STAR Singapore Institute of Manufacturing Technology and her co-workers have developed a technique that reveals exactly how molten elements vaporize and move about inside a laser-generated surface ‘plume’—findings that can advance additive manufacturing techniques used to print three-dimensional (3D) objects.

Researchers investigating the feasibility of 3D-printed implant materials often turn to magnesium–aluminum (Mg–Al) alloys because they are lightweight, tough and biocompatible. Recently, the A*STAR team demonstrated that laser surface melting of these alloys enhances their corrosion resistance as a result of a notable enhancement in the surface concentration of aluminum. It is difficult, however, to make the link between the initial alloy composition and the final product after laser processing, as many complex interactions occur in the cloud-like plume of laser-generated vapor particles.

Guan and her team designed a new experimental setup that can quantify which molten alloy elements are ejected into the laser plume. They positioned a thin silicon substrate perpendicular to a Mg–Al-based alloy a few millimeters from the laser firing point. Laser pulses then generated a plume that deposited onto the silicon surface. When the researchers used a scanning electron microscope (SEM) to examine the deposits, they saw clear evidence of a phase explosion—a mixture of liquid and vaporized particles thrown out by the laser impact. These liquid deposits rendered many sections of the silicon wafer unsuitable for quantitative analysis. But by combining the element-identifying capability of the SEM with time-of-flight mass spectrometry, the team produced ‘mass-resolved images’ that reconstructed the distribution of gaseous secondary ions in the plume.

The mass-resolved images revealed that Mg ions were evenly dispersed at high concentrations inside the plume. In contrast, the population of Al ions rises in the middle of the near-field region close to the laser firing point. Analysis showed that the Al species in the plume ‘fly’ further than those of Mg because of their higher transport rates in the hot near-field region.

Guan notes that the site-specific analytic capabilities of this technique should give researchers finer control over selective surface vaporization of alloying elements for enhanced, high-tech applications. “Our chemical analysis of the transport rates and distribution of vaporized species in the plume offers improved understanding of critical laser processes, including those used in additive manufacturing,” she says.

Urban planning:

Helping trains take the strain

An agent-based model that analyzes commuters’ travel data will improve the Singapore rail experience

The introduction of smartcard ticketing for Singapore’s public transport system has enabled A*STAR researchers to provide valuable predictive data on potential train overloading. This will enable system planners to address critical bottlenecks as the system stretches to accommodate an expanding population1,2.

Over one million commuters — roughly 20 per cent of Singapore’s population — use the mass rapid transit (MRT) system every day. With the population slated to increase by 26 per cent by 2030, this growth needs to be managed in a way that prevents system delays and overcrowding. A suboptimal transport system could lead to dissatisfied customers and higher economic costs.

To conduct their investigation, Christopher Monterola and colleagues at the A*STAR Institute of High Performance Computing used a modeling technique known as an agent-based model (ABM), which identifies key individual influencers, or ‘agents’, in a complex system and models them in a relatively ‘natural’ way. The team chose three tractable agents: the commuters, the train and the station. Unlike other transportation models, the ABM can consider interactions between agents.

The team examined two main problems that lead to travel delays: overloading and overcrowding. By varying the train’s loading capacity (the maximum number of commuters a train can accommodate at a given time), the team identified a threshold capacity: beyond this tipping point even a few additional commuters produce a cascade of delays. Similarly, more passengers waiting on crowded platforms in popular routes may also significantly increase delays and extend travel times.

Prior to its use for scenario planning, the model was experimentally validated using a week of Singapore smartcard data, which corresponds to 14 million journeys. The data collected for each journey included the anonymized smartcard ID, journey ID, date, origin and destination stations, ‘tap-in’ and ‘tap-out’ times, and the distance traveled.

The model can be used to assist MRT system planners in alleviating strains on a system should it become overloaded through the provision of real-time information on threshold capacities and ‘bottleneck’ stations.

Monterola says his team is passionate about finding ways to improve the robustness and efficiency of the MRT system. “This work is scientifically challenging, but more importantly, it is socially relevant,” he explains.

Other transportation systems could also use the model, which can be “augmented to work with real-time data, to enable a live stream view of all commuter movement in a city,” says Monterola. The team is currently working with behavioral scientists to interpret the influence of these system variables on commuter satisfaction — perhaps ultimately even at the individual level.

One challenge in producing strong, elastic and hard-wearing nanocomposites is obtaining an even distribution of the nanoparticles in the metal matrix. Now, researchers at A*STAR have used a process known as friction stir processing (see image) to produce an evenly distributed mix of nano-sized aluminum oxide ($\text{Al}_2\text{O}_3$) particles in aluminum. Their technique is a viable new method for manufacturing nanocomposites and has exciting potential for the car, space and defense industries.

“Current powder metalurgy or liquid processing methods fail to achieve uniform processing,” says research leader Junfeng Guo, who is from the A*STAR Singapore Institute of Manufacturing Technology.

Guo’s team drilled hundreds of 1-millimeter-diameter holes into the surface of a thin sheet of an aluminum alloy. They then injected a slurry of aluminum oxide nanoparticles into the holes and heated the sheet in an oven. After cooling the sheet, the team plunged a rotating tool into it — this is the friction stir processing step. The friction generated between the tool and the sheet caused the material to plasticize. The tool was moved around to ensure that the entire sheet was plasticized.

Placing the nanoparticles in the sheet prior to the friction stir processing step significantly increased the concentration of nanoparticles in the composite. “It also reduced the amount of airborne particles produced during powder placement and friction stir processing,” explains Guo.

The team used scanning electron microscopy to check two key properties that influence the strength of nanocomposites. They first demonstrated that the nanoparticles were uniformly dispersed, which means the material has no weak points. They also found that the grains or crystals of the aluminum matrix that recrystallized after being plasticized were extremely small; smaller aluminum matrix grains can flow past each other more smoothly than larger particles, enhancing the strength of the material.

By measuring the grain size after performing friction stir processing with and without the $\text{Al}_2\text{O}_3$ nanoparticles, the team showed that the nanoparticles contributed to the reduction in grain size.

The best nanoparticle distribution and smallest aluminum alloy grains were obtained after passing the rotating tool through the sheet four times. The team then demonstrated that the composite made in this way had significantly improved hardness and tensile strength compared to untreated aluminum alloy sheets.

“We plan to continue this research to further improve the mechanical and thermal properties as well as the wear resistance of the nanocomposites.”

Nanoparticles: Taking self-assembly to the limits

Gold nanoparticles smaller than 10 nanometers spontaneously self-organize in entirely new ways when trapped inside channel-like templates. A new study shows that this feature could facilitate easier nanoscale manufacturing of biosensors and plasmonic devices with intricate, high-density surface structures. Generating surface patterns at scales of 10 nanometers and below is difficult with current technology. An international team, led by Joel Yang from the A*STAR Institute of Materials Research and Engineering in Singapore, is helping to circumvent this limitation using a technique known as ‘directed self-assembly of nanoparticles’ (DSA-n).

This approach takes spherical nanoparticles that spontaneously organize into ordered, two-dimensional films when inserted into lithographically defined templates. The templates impose geometric constraints that force the films to organize into specific nanoscale patterns. Most patterns produced by DSA-n, however, are simple periodic arrangements. To broaden this technique’s capabilities, researchers are exploring ‘structure transitions’ that occur when ordered states break down.

Using electron-beam lithography techniques, the team carved out an array of inward tapering trenches designed to fit one to three rows of gold nanoparticles. After depositing a monolayer of 8-nanometer particles in the template, they used scanning electron microscopy to identify any emergent width-dependent patterns. Between periodically ordered rows, the researchers saw clear evidence of transition state zones — regions where the tiny spheres buckle out of alignment and gradually take on new, triangular packing patterns.

Researchers identify new ways of patterning gold nanoparticles with sub-10-nanometer resolution based on ‘structure transitions’ that occur when ordered states break down.

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After analyzing the transition states with computational Monte Carlo simulations, Yang and co-workers identified several dominant recurrent patterns with different geometries from typical DSA-n depositions. Because the conditions needed to generate these patterns can be predicted mathematically, the team is confident these findings can have practical surface engineering applications.

“The success of DSA-n depends on the positioning accuracy of the particles,” says Yang. “By exploiting the rich set of structural geometries that exist between ordered states, we can design templates that guide particles into complex periodic and nonperiodic structures.”

Materials science:

A weld of difference

Higher welding speed improves the quality of welds between dissimilar alloys

Simple changes in technique can yield big improvements, say A*STAR researchers investigating how welding speed and the placement of materials affect the quality of welds between dissimilar alloys. They discovered that the tensile strength increases with increasing welding speed and becomes even higher when the softer alloy is placed on the advancing side of the weld.

Friction stir welding is a relatively new technique for joining flat sheets of metals and alloys together. It is most suited for binding aluminum and aluminum-based alloys — materials that are traditionally difficult or impossible to weld. In addition, it has many advantages over conventional welding methods, including better weld appearance, enhanced mechanical properties, improved safety and lower setup costs.

First demonstrated in 1991, friction stir welding is now widely applied across a diverse range of operations in various industries, including the automotive, aerospace and electronics industries. A recent surge of research interest has focused on how to best apply friction stir welding on dissimilar alloys; in particular, how temperature, stress and material flows affect the quality of welds between AA6061 and AA7075 aluminum alloys — structural materials widely used in the construction of wheel spacers, aircraft fuselages and robotic casings.

Junfeng Guo and his team from the A*STAR Singapore Institute of Manufacturing Technology took up the challenge of examining the mechanical properties and microstructures of welds between AA6061 and AA7075 for five different welding settings. They found that a higher welding speed resulted in better welds because less friction heat was generated. The resulting lower temperature meant that the melted materials had little time to recrystallize and consequently their grain size became finer. Similarly, the placement of AA6061 aluminum alloy on the advancing side resulted in better welds because less friction heat was generated.

The researchers examined the ‘onion rings’ — characteristic patterns formed during friction stir welding (see image) — on both the advancing and retreating sides of the weld. They found that multiple vortices formed in the recrystallized zone when the softer A6061 aluminum alloy was placed on the advancing side of the weld. These vortices enhanced material mixing and hence improved the mechanical properties of the weld.

The findings have important implications for materials science and the development of the next-generation friction stir welding machines. “Joining of dissimilar materials is a challenge frequently faced in industries,” says Guo. “Friction stir welding enables the welding of dissimilar materials where fusion welding would have been inappropriate.”

A mathematical model can improve the accuracy and repeatability of a positioning system by learning to anticipate tiny errors in its movements, show A*STAR researchers.

Micromanipulation systems are used to control objects’ positions with exquisite precision and play a vital role in applications such as telescopes and laser communication. Most rely on feedback sensors to reach the desired position, but these sensors introduce a time lag that can reduce the accuracy in applications requiring rapid responses. Although analytic forward models (AFMs) can be used to predict when positioning errors might occur and compensate for them in advance, they must be extremely accurate and uniquely tailored to a particular micromanipulation system.

Now, Yan Wu of the A*STAR Institute for Infocomm Research in Singapore, in collaboration with colleagues from the Harbin Institute of Technology in China, has developed a system that combines both approaches. The team created a machine learning algorithm that can improve the accuracy of its analytic control model based on sensor feedback\(^1\).

Their enhanced analytic forward model (EAFM) combines a simple AFM with a ‘heteroscedastic Gaussian process (HGP)’ algorithm, which compensates for any residual difference between the AFM’s output and the desired position.

The team built a tip–tilt micromanipulation system that uses four piezoelectric drivers to change the position of its platform. These drivers can move the platform up to 32 micrometers, and position it with an accuracy of 10 nanometers. A capacitance gauge located next to the platform can measure its position to within one nanometer (see image).

The researchers trained the HGP algorithm by running 125 random control signals through their micromanipulation system. It learned to make probabilistic predictions that could compensate for errors in the AFM’s output.

The team then tested the system with 30 different control signals, which were intended to move the platform by up to 28 micrometers. In every case, the EAFM system achieved smaller positioning errors than the AFM alone. And in trials of continuous movement, where the platform had to hit a series of four different points over a brief time, the EAFM outperformed the AFM in all but one of ten tests.

“All the experiments demonstrated that the AFM has errors with a very large variance (between 1 and 8 micrometers), whereas the EAFM keeps the errors at around 1 micrometer or less,” says Wu. “We are now putting this micromanipulator platform into a laser communication system, while investigating methods to further reduce the steady-state errors.”

Data-intense multimedia applications are stretching cellular network capacities to their limits, but A*STAR researchers have developed a strategy to ease this burden using ‘data offloading’. By using high-level computational algorithms to investigate data transfer between cellular base stations and ‘complementary’ setups such as home Wi-Fi systems, the team identified optimal ways to satisfy user demands across multiple, heterogeneous networks.

Wi-Fi networks and small, low-power femtocell and picocell cellular antennas are inexpensive, simple to install and highly compatible with existing smartphones and tablets. For these reasons, mobile operators consider data offloading to these complementary networks as a more feasible way to expand capacity than installing obtrusive infrastructure or bidding for new frequency spectra.

Chin Keong Ho and Sumei Sun from the A*STAR Institute for Infocomm Research in Singapore and colleagues probed one of the biggest obstacles for implementing this sharing technique: deciding when and how much data to offload from the primary network. “Many parameters, such as user requirements and cellular coverage, can affect the real-time performance of the base station,” says Ho. “The dynamics of network and user traffic make optimal offloading decisions very challenging.”

Ho notes that the loads, or demand for cellular service, of networks using data offloading are coupled through complex, nonlinear relationships. For example, increasing the load on one base station can produce interference with another base station. To maintain the same quality of service, the second base station may have to increase its load or power — subtle changes that can ripple through the combined Wi-Fi and cellular networks.

To resolve these problems, the researchers developed a simple but accurate model to describe a network of base stations that can interfere with each other and a series of complementary cells that can accept excess data. They then employed advanced mathematical tools to produce a load-coupled equation that characterized and optimized the data-sharing network in detail.

As a result, the team could suggest potential strategies. “One interesting finding is that for certain networks, it is impossible to satisfy user demands no matter how large the powers of the base stations,” says Ho. “Consequently, data offloading is the only means to serve the users — a finding that highlights the fundamental importance of this approach.”

The researchers believe that their load-coupling model could find practical use by determining the optimal number of small cells or Wi-Fi access points in an offloading system. Furthermore, their equations could ‘future-proof’ mobile networks by analyzing performance degradation as user requirements inevitably change.

Mathematical analysis reveals how to maximize access to mobile networks by seamlessly ‘offloading’ traffic to smaller Wi-Fi and cellular systems.

Using Wi-Fi access points to deliver content to mobile devices can significantly boost the speed and capacity of wireless networks.

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A concept that balances large-scale installations of low-cost and low-power antennas to boost cellular coverage in difficult environments will also provide better connectivity to more users. Developed by A*STAR, this new architecture for wireless communications can help service providers meet growing demands for increased network capacity and improved energy efficiency.

Jingon Joung, Yeow Chia and Sumei Sun from the A*STAR Institute for Infocomm Research in Singapore sought to combine two state-of-the-art wireless technologies into a novel type of antenna system. The first technology, known as large-scale multiple-input multiple-output (L-MIMO), uses numerous ‘co-located’ antennas to significantly reduce relative noise levels inside devices. The second, called distributed-antenna systems (DAS), replaces conventional high-power antennas with strategically placed compact nodes that can split up and transmit signals more efficiently due to improved line-of-sight pathways.

The team’s strategy, known as large-scale distributed-antenna systems (L-DAS), seeks to implement DAS with a massive installation base, as seen with MIMO antennas (see image). To realize this goal, however, required a way to evaluate the costs and benefits associated with this innovative infrastructure — simply increasing the number of antenna nodes does not automatically improve wireless network efficiency.

Using a complex computer simulator, the researchers quantified the performances of multi-user L-DAS networks by evaluating their energy efficiencies (that is, the number of bits decoded per joule). According to Joung, modeling energy efficiency is challenging because L-DAS antennas communicate in two ways — wirelessly or through fiber-optic cables — and each channel has different and often proprietary power requirements.

“Another challenge is implementing real-world parameters in the L-DAS network simulator,” says Joung. “Many of these parameters have a large dynamic range, from a few quadrillionths of a watt to tens of watts, which can cause precision issues with the computer simulation.”

At first glance, the original ‘naïve’ L-DAS setup seemed to have a greater energy consumption than the L-MIMO system with co-located antennas. However, the team identified four key attributes that could dramatically enhance the L-DAS energy efficiency: proper antenna selection, clustering of antennas, pre-coding to improve channel quality, and computerized power control. With these improvements, the L-DAS network outperformed both L-MIMO and DAS technologies.

The group is now looking to the future. “Heterogeneous network (HetNet) architectures that can seamlessly support different 2G, 3G, 4G or WLAN networks are strong candidates for future communication networks,” says Joung. “Because L-DAS architecture can be applied to many HetNet applications, this work can help ensure a gentle and smooth replacement of real-life networks with HetNet.”
Manufacturing:

Closing the loop for greener production

A technique to analyze production processes highlights the financial benefits of recycling

A method developed by A*STAR for analyzing the financial benefits of incorporating the recycling and reuse of materials in manufacturing processes is expected to encourage more companies to adopt environmentally friendly production practices. Increasing global consumerism is leading to a waste time bomb. All too often, products that reach the end of their useful life are either incinerated, releasing harmful byproducts into the atmosphere, or dumped in landfill sites, which are rapidly becoming full.

Both individuals and multinational companies could take greater responsibility for reducing waste by recycling. "Manufacturers are facing ever increasing pressure due to resource scarcity and environmental legislation," says Jonathan Sze Choong Low from the A*STAR Singapore Institute of Manufacturing Technology. "Dealing with it by just reducing resource consumption will not hold up anymore."

Now, Low and his co-workers have extended the traditional linear production chain that links the manufacturer to the supplier to the consumer so that it leads back to the manufacturer once the product is no longer of use — creating a so-called closed-loop supply chain.

A crucial link in a closed-loop supply chain is the process of reinserting recovered parts and materials back into the original manufacturing process — known as a closed-loop production system. Such integrated reuse of resources clearly benefits the environment, but businesses need to be certain that a production system is financially viable before they will adopt it. Low and his colleagues have now created a cost-modeling technique that simplifies this analysis.

Their procedure considers the product as a collection of recyclable subunits. In contrast, previous models considered the product as a whole, but this approach is complicated since different components have different closed loops.

“The product structure-based integrated life cycle analysis, or PSILA, technique we have developed allows users to break down a complex closed-loop production system into smaller and simpler subsystems to be modeled,” explains Low. “This breakdown provides users with the deep analysis and insights needed to understand how and why the system works and what to look out for if it is implemented.”

Low and the team validated the usefulness of PSILA by applying it to the life cycle of a flat-panel display. They were able to simulate the economic performance of a closed-loop production process for this common consumer product and show that such a process is financially beneficial.

“Our next step is to include environmental considerations, such as carbon emissions, into the PSILA technique,” says Low.

A new mapping tool makes preparing for natural disasters and responding to their aftermath easier than ever. Researchers from the A*STAR Institute of High Performance Computing in Singapore have developed a computer model that analyzes networks of interconnected roads to predict the speediest routes for rescuers to take using real-time data uploaded by aid workers on the ground.

The hours and days following disasters such as typhoons or earthquakes are a critical time for relief operations. However, efforts to reach victims and distribute supplies are often hindered by infrastructure challenges. For example, when Typhoon Haiyan — one of the strongest cyclones on record — devastated the central Philippine city of Tacloban in 2013, survivors were stranded for weeks in hard-to-reach areas (see image).

This catastrophe motivated Christopher Monterola and co-workers at A*STAR to tackle the problem of disaster relief using the tools of ‘network science’. These techniques aim to quantify relationships in complex networks by graphing the connections, or ‘edges’, between individual objects known as ‘nodes’. Mathematical analysis of these parameters can reveal important properties such as the size and strength of connections between particular nodes.

The researchers developed a procedure that automatically transforms street maps into a network of nodes (road intersections) and edges (road segments). Powerful algorithms then calculate the minimum time needed and best route for rescuers to traverse between any two nodes. Built-in flexibility enables continuous updating of the input map data using crowd-sourced sites such as the Humanitarian OpenStreetMap. Furthermore, municipalities can use this tool to model infrastructure destruction scenarios before they occur.

The team tested how parameters such as the flow of goods to and from relief centers evolved in two different model networks: a grid lattice common in cities and a ‘scale-free’ road network that represents a mix of urban hubs and rural spokes. These investigations revealed that traditional, idealized models are inherently different from actual roads found in cities such as Tacloban. According to Monterola, this means that the conventional assumptions used in planning may not apply, and that reliable, high-resolution data are needed to quantify the robustness and accessibility of road structures for relief efforts.

“This work can be crucial in formulating contingency plans for disaster relief operations,” says Monterola. “It shows that a network-science-based tool, driven by actual data, can guide logistics planning in areas hit by calamities. Specifically, it allows for fast yet accurate humanitarian logistics planning even in the absence of complete information about the extent of damage.”

Electric vehicles:

Security for both real and cyber worlds

A combined cyber and physical security system will protect customers charging electric vehicles on smart electricity grids

As electricity grids become more sophisticated, grid administrators can collect instantaneous data on consumer and supplier behavior. The ‘smart grid’ then learns to improve the reliability, costs and sustainability of electricity distribution. However, smart grids present new security challenges, especially for mobile systems such as electric vehicles (EVs), which can be attacked both electronically and physically.

Now, Jianying Zhou and Aldar Chan at the A*STAR Institute for Infocomm Research have developed the first automatic security system that protects EVs from combined cyber–physical attacks.

“Most existing authentication systems merely apply cybersecurity schemes directly to the smart grid, leaving gaps in the protection,” explains Zhou. “The problem is especially serious for EVs, because the charging infrastructure is publicly open. Anyone could plug in an EV, even if it is stolen.”

A particular danger is the so-called substitution attack, whereby a criminal can ‘digitally imitate’ an EV, plugging in their own device while the EV owner pays for the electricity. Chan and Zhou demonstrated a successful substitution attack on an existing EV charging station. “We plugged in kettles and hair dryers; it could be anything that draws current,” says Zhou.

After proving that this security loophole existed, the researchers worked to improve the classic ‘challenge-response’ protocol for online security. “Instead of using a single challenge — which is a random number used to test if a user really is who he claims to be — we used one challenge sent through the wireless cyber path and another challenge through a physical path or the charging cable,” says Zhou. “This ensures that the EV is connected physically to the right spot in the power grid, and that it is a real EV meeting existing EV standards.”

Perhaps inevitably, Chan and Zhou found that they could not achieve physical authentication using software alone. They had to design a new onboard hardware mechanism that binds an EV to its digital identity. However, they discovered a way to embed the challenge number in one of the signaling lines of the charging cable, so that existing charging stations will not need to be modified.

The researchers believe that their new security system could protect other components in the power grid, such as relays and transformers, as well as cardless ATMs. "With more research we could devise systems to ensure that the person withdrawing cash actually has digital authorization," says Zhou.

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