Issue 5
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SINGAPORE

A*STAR Research

NANOTECHNOLOGY

A SLICK APPROACH TO CLEANING UP OIL SPILLS

A MILLION CELLS, 2 YEARS:

BIG DATA TACKLES ASIAN GENOMES

- Ghostly measurements through quantum wizardry
- Avoiding adverse reactions to medication
- Tofu to make us too full for more

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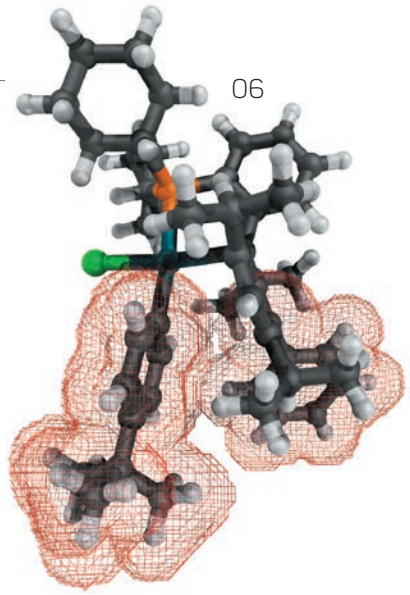

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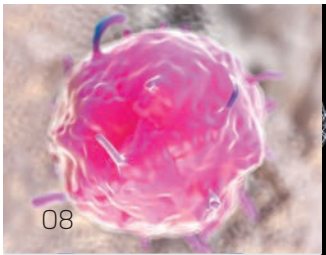
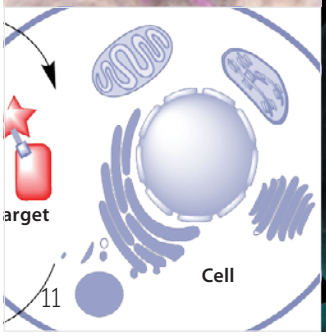
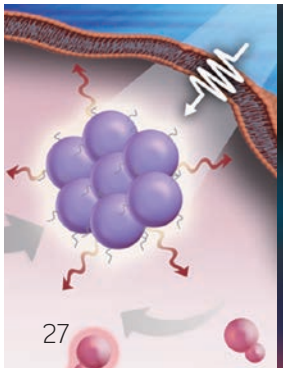
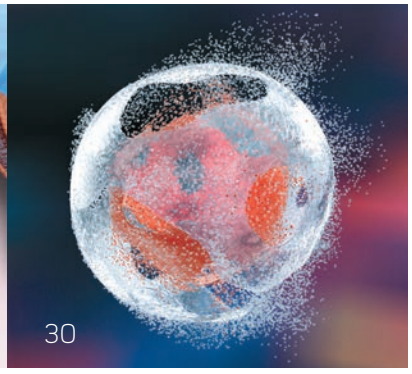
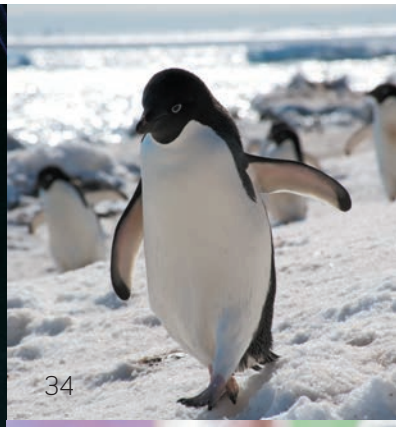
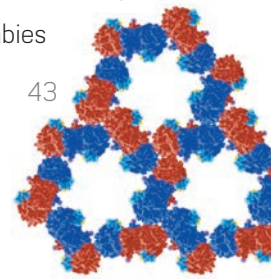


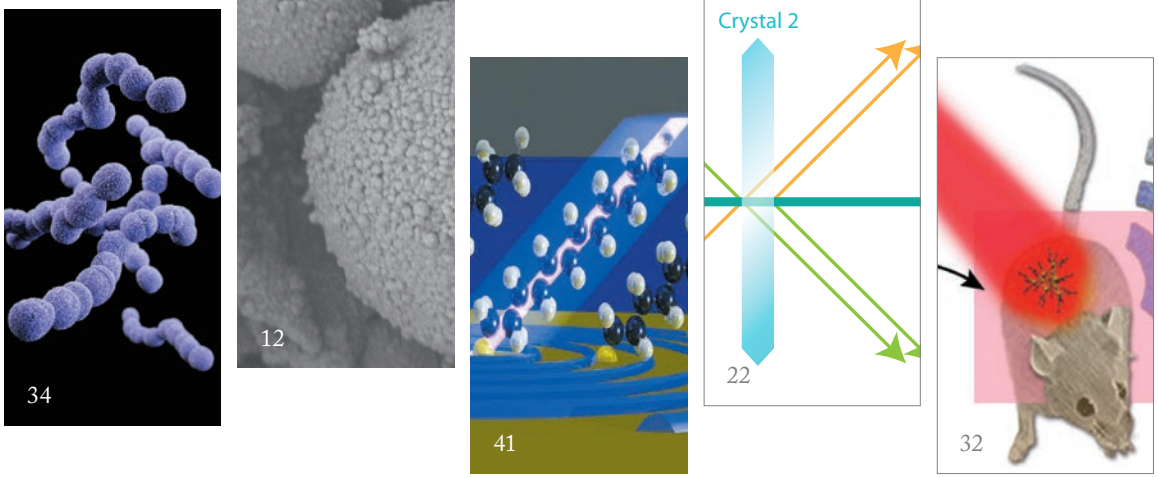












[NOTES FROM THE EDITORS]

*Editorial board member, Evan Newell, introduces the latest issue of A*STAR Research*

Welcome to the first edition of *A*STAR Research* for 2017. This issue showcases the most exciting work from A*STAR researchers published on the website between October and December of 2016.

As usual, our researchers produced lots of great papers, which made selecting which ones to highlight in *A*STAR Research* a difficult task. So please do let us know if you have any thoughts about the balance of articles, and be sure to keep up to date with *A*STAR Research* by regularly visiting the website.

This issue features Singapore's strategy for tacking the exponentially increasing volume of genomics data though a large collaborative project called c-BIG (p. 34), which spans several A*STAR research institutes including the GIS, I²R, IHPC and the BII. Too much data seems like a good problem to have, but it is still a problem that needs innovative solutions and that is what c-BIG promises to deliver. Our other feature, on page 12, shows how small things, namely nanomaterials developed by IBN researchers, can take on big problems such as oil spills and the provision of clean water.

It is impossible to introduce all the highlights here, but we'd like to point to a few themes, including the very strange properties of light. On page 41, Nikodem Tomczak and colleagues show us new ways to create tiny, but well-behaved sources of light. Even stranger, Dmitry Kalashnikov and his team, show us that light's 'spooky' quantum properties, that even Einstein couldn't quite wrap his head around, can now be harnessed for practical applications such as environmental monitoring (p. 22).

On the biomedical side, several new and improved methods for imaging tissues and cells are described, including improved bioacoustic imaging of animals (p. 32), probes for biofilms (p. 26) and a method for measuring temperature at a subcellular level (p. 29).

As an immunologist, I am also happy to introduce several interesting immunology-related studies, including one from Anis Larbi (p. 47) and colleagues that shows us that there is a relationship between the immune system and frailty in elderly Singaporeans.

As usual, this is just a cross-section of the issue; we hope that you enjoy perusing the rest of its offerings.

COVER IMAGE

Nanotechnology provides the tools to clean up oil spills [p.12]
© TongRo Images Inc/Getty

Iron-tellurium conducts electricity best when in a disordered amorphous phase.

Materials science:

DISORDERLY CONDUCT

AN ANALYSIS OF ELECTRON BEHAVIOR EXPLAINS WHY THE PHASE-CHANGE MATERIAL IRON-TELLURIUM BEST CONDUCTS ELECTRICITY IN ITS DISORDERED AMORPHOUS PHASE

Solid materials whose atoms are arranged in a well-ordered crystalline structure are usually better conductors of electricity than randomly structured, or amorphous, solids. Recently, however, A*STAR researchers found that iron–tellurium (FeTe) breaks this rule, displaying higher conductivity, and optical reflectivity, in the amorphous phase.

A recent study, published in the journal *Acta Materialia*, describes their efforts to understand why FeTe's behavior is counterintuitive to expectations[1].

FeTe is a phase-change material, with the ability to rapidly switch its state from crystalline to amorphous and back again when it is heated or cooled, a property which makes it useful for data storage and memory applications. Conventional phase-change materials such as germanium–antimony–tellurium (GST), commonly used in rewritable DVDs, display higher optical reflectivity and electrical conductivity in their crystalline state because the highly ordered structuring of atoms in the crystal results in more electron vacancies, or holes, that act as charge carriers.

"FeTe behaves differently from other phase-change materials," explains Kewu Bai at the A*STAR Institute of High Performance Computing, who worked on the project with scientists from the National University of Singapore. "We hypothesized that these unusual characteristics may be connected with the behavior of 'lone-pair' electrons. This refers to a pair of electrons from any one atom that are not involved in the bonding of materials."

The team prepared thin films of FeTe at room temperature to produce amorphous structures, and at 220 degrees Celsius to acquire crystalline samples, and showed that the films could be flipped between the two states using a fast pulsing laser. They analyzed the molecular structure of the different films using X-ray spectroscopy, electron microscopy and first-principles calculations to investigate these unusual properties of FeTe.

The researchers confirmed the existence of lone-pair electrons in both the amorphous

and crystalline phases. In the crystalline phase, where Te and Fe atoms were strongly bonded in a regular lattice, electrons were engaged in strong hybridization, meaning their orbitals overlapped and caused their electrons to localize. Thus, lone-pair electrons were incorporated as part of the integral structure.

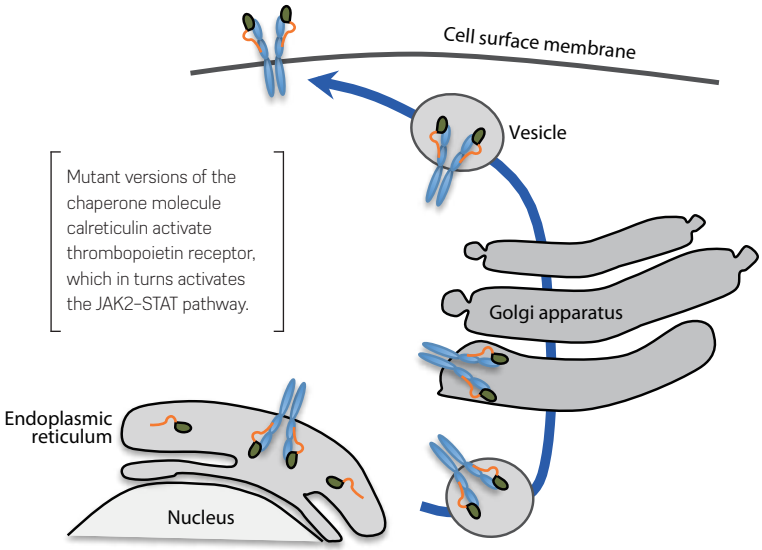
In contrast, when FeTe entered its amorphous phase, some Te atoms were orientated so that their lone-pair electrons delocalized from the atoms, resulting in holes that acted as charge carriers.

"We are hopeful that FeTe could prove to be useful material for phase-change memory," says Bai. "It could also act as an effective thermoelectric material, generating electric current in response to temperature."

1. Ho, H. W., Branicio, P. S., Song, W. D., Bai, K., Tan, T. L. *et al.* Unravelling the anomalous electrical and optical phase-change characteristics in FeTe. *Acta Materialia* **112**, 67–76 (2016).

Myeloproliferative neoplasms:

NOT ALL CHAPERONES ARE TO BE TRUSTED



Mutant versions of the chaperone molecule calreticulin activate thrombopoietin receptor, which in turns activates the JAK2–STAT pathway.

MUTANT VERSIONS OF A 'HOUSEKEEPING GENE' ARE SHOWN TO ACTIVATE A PATHWAY THAT LEADS TO OVERPRODUCTION OF CERTAIN KINDS OF BLOOD CELLS

Myeloproliferative neoplasms (MPNs) are blood cancers that cause the bone marrow to produce too many red or white blood cells, or platelets, leading to various complications. There is no known cure for most MPNs.

In 2013, scientists discovered a link between some forms of MPNs and mutations in a housekeeping gene known as *CALR*, which codes for calreticulin — a 'chaperone molecule' that promotes folding of proteins.

Stefan Constantinescu of the Ludwig Institute for Cancer Research in Belgium says the 2013 breakthrough was the first known example of a chaperone turning into an oncogenic activator of cell proliferation, but that molecular mechanisms underlying this connection were unknown.

"To learn how to treat MPNs, we first need to know what cell signaling or survival pathways are active in diseased cells," explains Choong Meng Ling of the A*STAR Experimental Therapeutics Centre.

Studies by Constantinescu, Ling and co-workers have implicated an abnormal interaction between CALR mutants and the receptor for the hormone thrombopoietin (MPL/TpoR), which regulates the production of blood platelets, in the process that drives some MPNs[1]. The work involved an international collaboration with Robert Kralovics's group, in Vienna, Austria and William Vainchenker's group in Villejuif, France.

One of CALR's roles is the folding and processing of the thrombopoietin receptor before it is transported to the cell surface. The researchers discovered that the mutant CALRs incorrectly fold the thrombopoietin receptor into active receptors, both in the cell and at its surface. This persistently activates the JAK2–STAT pathway (see image), which the team had previously shown is activated in some MPNs.

The scientists then employed a drug combination study approach known as the Chou–Talalay method. "This approach allowed us to kill two birds with one stone: it helped us to simultaneously identify the cell survival pathways downstream of mutant CALRs and the drugs that could be used

to block these pathways synergistically," says Choong.

The study has important treatment implications. "The JAK2 inhibitor, ruxolitinib, is the drug of choice for treatment of MPNs," explains Choong. "But since JAK2 is a common signaling molecule involved in diverse functional pathways, blocking its activity would produce many side-effects. We found that MEK/ERK inhibitors could work synergistically with the JAK2 inhibitor (ruxolitinib), which would allow us to achieve the same therapeutic goal using lower quantities of the inhibitors, thus reducing side-effects."

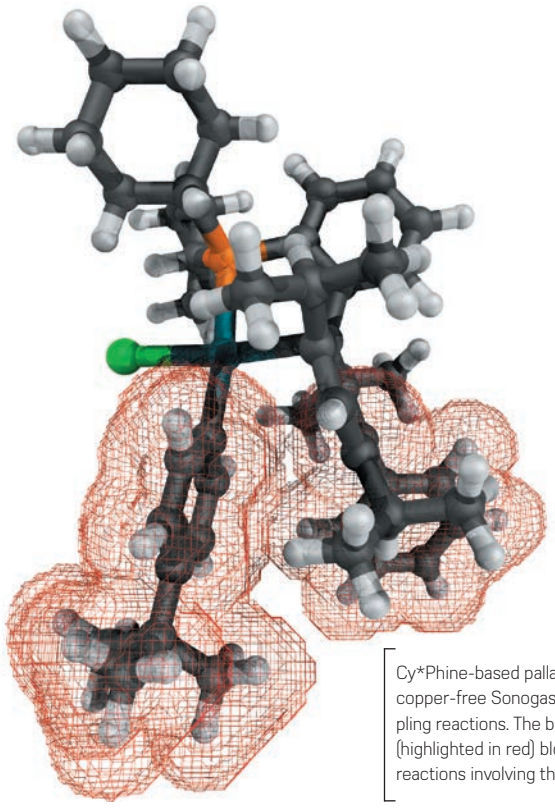
The researchers intend to search for ways to prevent the abnormal interaction between CALR mutants and the thrombopoietin receptor. "Now we know how CALR mutants affect thrombopoietin receptor signaling, we can look for ways to block the interactions between CALR and the thrombopoietin receptor," notes Choong.

1. Chachoua, I., Pecquet, C., El-Khoury, M., Nivarthi, H., Albu, R.-I. *et al.* Thrombopoietin receptor activation by myeloproliferative neoplasm associated calreticulin mutants. *Blood* **127**, 1325–1335 (2016).

Catalysis:

THE POWER OF THREE

INSIGHT INTO THE ROLE OF A THREE-RING LIGAND MAY IMPROVE PALLADIUM-CATALYZED REACTIONS USED FOR SYNTHESIZING ORGANIC PRODUCTS



Cy*Phine-based palladium catalyst for copper-free Sonogashira cross-coupling reactions. The benzene rings (highlighted in red) block potential side reactions involving the ligand.

Palladium-catalyzed organic reactions, such as Sonogashira cross-coupling, may be made more efficient and substrate-tolerant as a result of new findings at A*STAR.

Having previously developed a catalyst that outperforms existing state-of-the art analogs, the researchers now show how a catalyst structure sparks unprecedented activity[1].

Sonogashira cross-coupling — which assembles molecules by joining terminal alkynes to chlorinated aromatic compounds, while keeping the triple bond intact — is used for synthesizing organic products destined to become pharmaceuticals and molecular electronics. Typically, the assembling relies on a copper salt that increases the catalytic productivity by assisting the alkyne addition to the catalyst. However, this salt also promotes the formation of by-products that require complex and time-consuming purification.

Yee Hwee Lim and co-workers from the A*STAR Institute of Chemical and Engineering Sciences and Singapore Bioimaging Consortium have solved this issue by creating a palladium complex, without copper, that can still catalyze Sonogashira cross-coupling. Lim explains that the less metal used, the better it is for downstream purification stages. "To use this catalyst in an industrial process, we want to decrease the metal content," she adds.

As well as being more efficient, the copper-free system can be applied to a broader range of substrates than its commercially available analogs. This performance enhancement hinges on the Cy*Phine ligand, which consists of a phosphine-type molecule bearing three interconnected benzene rings. However, its underlying mechanism remained unclear.

With Adrian Matthew Mak from the A*STAR Institute of High Performance Computing, Lim's team has computationally identified the main steps of the catalytic cycle. They discovered that the rate limiting step is where the alkyne binds to the catalyst–aromatic substrate complex.

When the catalytic activities achieved via Cy*Phine and a two-ring ligand were further compared, the team showed that during this alkyne addition, the third benzene ring blocked unwanted reactions (see image on page 6).

"Without the third ring, a part of the catalyst could actually go through these unproductive pathways" says Lim "and this reduces the efficiency of the main catalytic cycle." Explaining that side reactions proceed depending on their energy difference from the next catalytic step, she adds that, in contrast to the Cy*Phine-based catalyst, "these two energies are quite similar for the two-ring system."

The Cy*Phine-based catalyst has been commercialized by Aspira Scientific for the past year. The team has applied the catalyst to different reactions and observed enhancement effects. Now, says Lim, "we are planning to look more closely at how it is affecting the catalytic cycle".

1. Mak, A. M., Lim, Y. H., Jong, H., Yang, Y., Johannes, C. W. *et al.* Mechanistic insights and implications of dearomative rearrangement in copper-free Sonogashira cross-coupling catalyzed by Pd-Cy*Phine. *Organometallics* **35,** 1036–1045 (2016).

Nutrition:

TOFU TO MAKE US TOO FULL FOR MORE

A COMMON FOOD ADDITIVE COULD BE COMBINED WITH TOFU TO CONTROL APPETITE AND CALORIE CONSUMPTION IN ASIAN DIETS

Tofu is a popular part of the Asian diet

The food additive polydextrose could be put into tofu to reduce hunger pangs and stimulate weight loss in people of Chinese ethnicity, according to new research from A*STAR[1].

Polydextrose — a large molecule comprised of approximately 12 smaller glucose molecules — has a distinct arrangement that is very difficult for the human digestive system to break apart. This has led to its widespread use in food across Europe and North America to promote a feeling of fullness without contributing significantly to the calorie count. There have, however, been few studies on the use of polydextrose in people of Asian origin. "This is significant, because the metabolic responses of Asians to various dietary factors are markedly different to those of Europeans," says Christiani Henry of the A*STAR Singapore Institute for Clinical Sciences.

Henry's team, along with co-workers from other Singaporean institutions, studied the response of people of Chinese ethnicity to eating soybean curd supplemented with polydextrose[1]. Twenty-seven healthy men ate one of four tofu mixtures on different days. Their response to each test mixture was monitored throughout the course of the study. The four options consisted of low-protein or high-protein liquid soybean curd, taken with or without added polydextrose. These mixtures were eaten alongside other controlled food provided during each test day, with the total amount of food consumed to be decided by each participant.

The most significant dietary finding was that the subjects ate fewer calories when consuming the low-protein mixture with polydextrose than they did with the low-protein bean curd on its own. The researchers also used blood sampling to investigate the role played by two gut hormones known to be involved in controlling hunger and satiety and they used ultrasound scans to estimate the rate at which the subjects' stomachs emptied after the test meals. Taken together, the results suggest that polydextrose may be a useful additive for soybean curd products aimed to help weight control in the Asian market, given the popularity of tofu in Asian food.

"We are also exploring whether polydextrose can be used in solid food to elicit a similar response to that seen in soybean curd," says Henry. The researchers have established contact with a local business, with a view to translating their research findings into a manufactured product. The appearance of weight control tofu on supermarket shelves may be just a matter of time.

1. Soong, Y. Y., Lim, W. X., Leow, M. K. S., Siow., P. C., Teh, A. L. & Henry, C. J. Combination of soya protein and polydextrose reduces energy intake and glycaemic response via modulation of gastric emptying rate, ghrelin and glucagon-like peptide-1 in Chinese. *British Journal of Nutrition* **115,** 2130–2137 (2016).

RNA sequencing:

FINE-TUNING PROTEIN SYNTHESIS

THE RATE OF PROTEIN PRODUCTION IN DIFFERENTIATING STEM CELLS IS CONTROLLED BY MORE COMPLEX FACTORS THAN PREVIOUSLY THOUGHT

Tweaks in the sequence of messenger RNAs (mRNAs) can influence their rate of protein production, A*STAR researchers have shown[1]. This process is important for how embryonic stems cells (ESCs) differentiate into other tissue types.

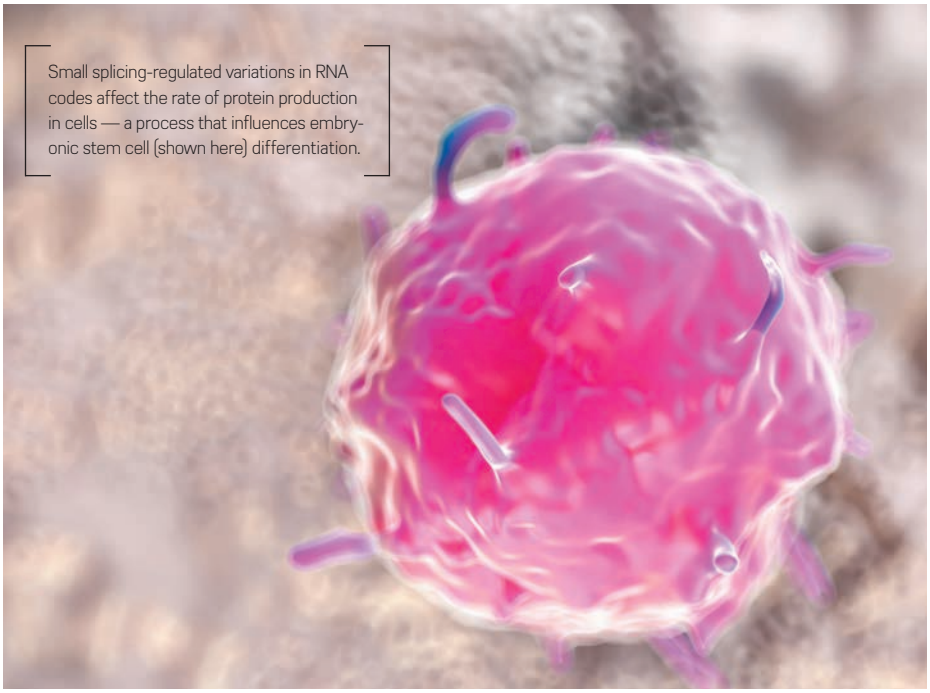
ESCs are cells in an embryo that can develop into any tissue in the body. Their differentiation into other cell types is controlled by protein production through regulating the expression of the genes that encode the proteins.

Ribosomes are molecular machines that translate mRNA sequences into proteins. A single gene can produce many different mRNA variants through an editing process called RNA splicing. Many of these mRNA variants produce similar but different protein variants. mRNAs contain a coding region that has an 'untranslated region' (UTR) on either end that regulates protein synthesis.

"We set out to determine how changes in the mRNA UTR sequences after splicing influence the rate at which mRNA variants are translated into proteins," explains Leah Vardy from the A*STAR Institute of Medical Biology.

By comparing the rate at which mRNA splice variants were translated into proteins in ESCs and neural precursor cells (NPCs), a cell type into which ESCs can differentiate, Vardy's team found that small changes in the mRNA sequence generated during splicing influence the rate of protein production of these variants. This made a big difference to their respective protein levels.

"We already knew that UTRs controlled the rate of translation, but have now shown that different splice variants within the same cell can also be translationally regulated through variations in their UTRs," says Vardy.



Small splicing-regulated variations in RNA codes affect the rate of protein production in cells — a process that influences embryonic stem cell (shown here) differentiation.

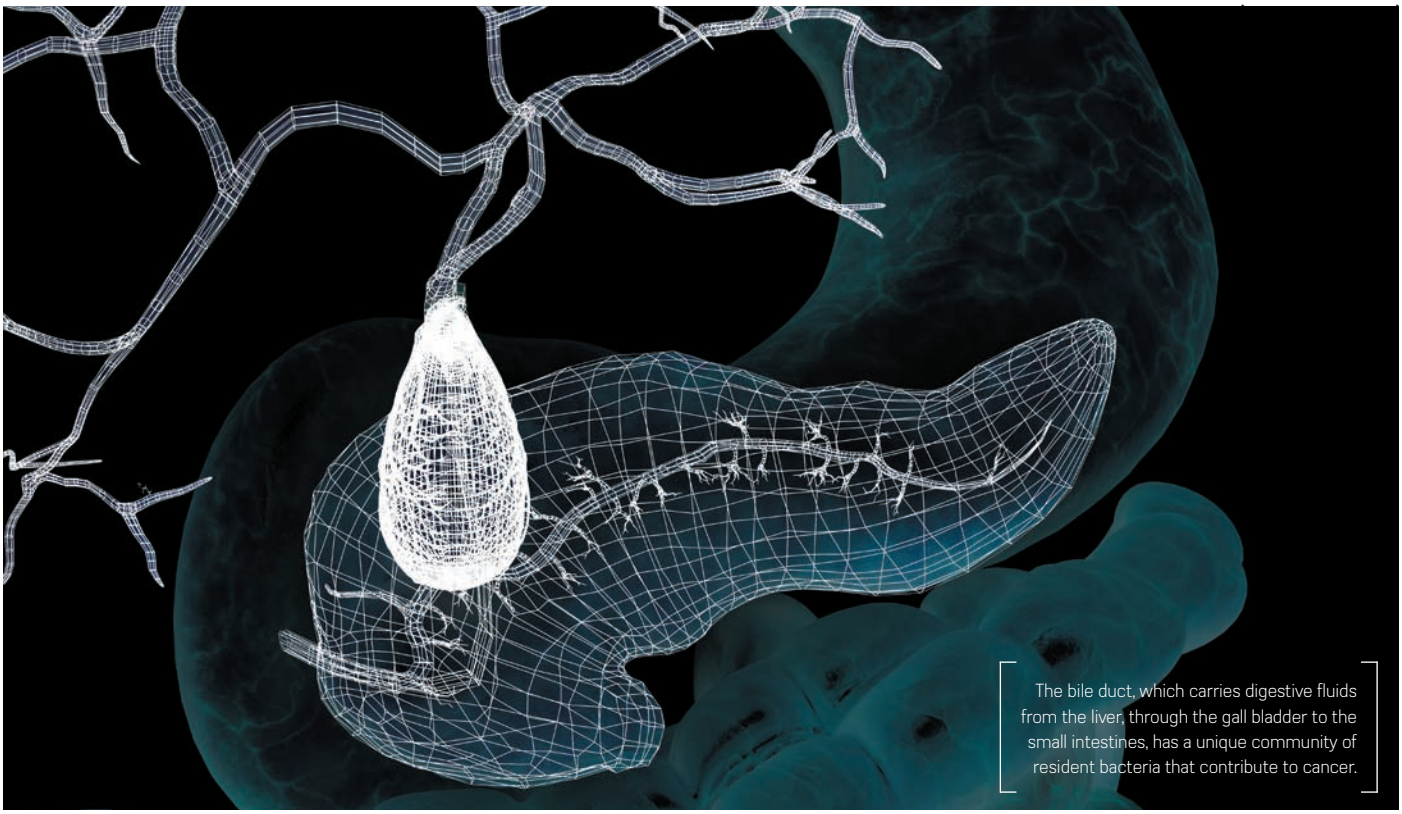
The researchers used RNA sequencing to determine the translation rates of different variants based on the numbers of ribosomes attached to mRNAs. Those with a high load of ribosomes were considered more highly translated.

The team found, for example, 31 different genes that showed variant-specific changes in translation rates in ESCs and NPCs. They also found that, in ESCs, 10 per cent of mRNAs with multiple variants had different translation rates for each variant. The different translation rates correlated with differences in the UTR sequences, which the researchers believe are behind the variation in translation rates.

"These findings confirm an added level of complexity where different splice variants from the same gene can be translated into proteins at very different rates within the same cell," says Vardy. "This shows that splicing also controls the rate of protein production from specific variants, and not just protein sequence."

The team next plans to identify some of the key regulatory sequences within UTRs to determine how they regulate the translation rate.

1. Wong, Q. W., Vaz, C., Lee, Q. Y., Zhao, T. Y., Luo, R. *et al.* Embryonic stem cells exhibit mRNA isoform specific translational regulation. *PLoS One* **11**, e0143235 (2016).



The bile duct, which carries digestive fluids from the liver, through the gall bladder to the small intestines, has a unique community of resident bacteria that contribute to cancer.

Digestive microbes:

BILE DUCT BACTERIA POSE CANCER RISK

THE MIX OF MICROBES LIVING IN THE BILE DUCT COULD PLAY A ROLE IN A RARE BUT LETHAL FORM OF CANCER

From the stomach, to the gut and the breast — scientists have shown a link between cancer and the microbial communities living in various organs of our body. Now, researchers at A*STAR have made a new association between the microbes in the bile duct and fatal tumor formation[1].

"We don't fully understand the risk factors or environmental causes for many cancers," says Niranjan Nagarajan at the A*STAR Genome Institute of Singapore, who led the study. "Microbiome research allows us to figure out whether these cancers might have a microbial basis, and potentially intervene to prevent or reduce the risk."

Bile duct cancer, or cholangiocarcinoma, affects only about 1 in every 100,000 individuals worldwide, but incidence and mortality rates have increased in Southeast Asia, and in Thailand they are as high as 85 in 100,000. A major risk factor associated with cholangiocarcinoma in the region is infection from the liver fluke parasite found in raw fish.

Nagarajan and colleagues wanted to know if the little-studied bile duct microbiome also contributed to the disease. Using a technique known as 16S rRNA sequencing, they studied the bacterial composition in samples of healthy bile duct tissue and adjacent tumors taken from 60 patients, including those who had been infected with liver flukes.

Overall, they found that the bile duct microbiome was dominated by exotic species not typically seen in the human body. "The diversity was remarkable," says Nagarajan. "Bile duct tissue has a unique microbial signature even compared to nearby organs like the liver."

Comparative analyses presented more surprises. In samples not infected with liver fluke, tumor tissue had significantly higher levels of *Stenotrophomonas* bacteria. Several *Stenotrophomonas* species are known to trigger inflammation, which is a common mechanism by which bacteria cause cancer.

The researchers could not, however, clearly distinguish between normal and tumor tissue in samples infected by liver fluke. But they did notice that, compared to the parasite-free subset, the infected samples had an abundance of intestinal *Bifidobacteriaceae*, *Enterobacteriaceae* and *Enterococcaceae*. Further computational analysis revealed that these bacteria metabolize ammonia and bile acids, which are known to promote colorectal cancer.

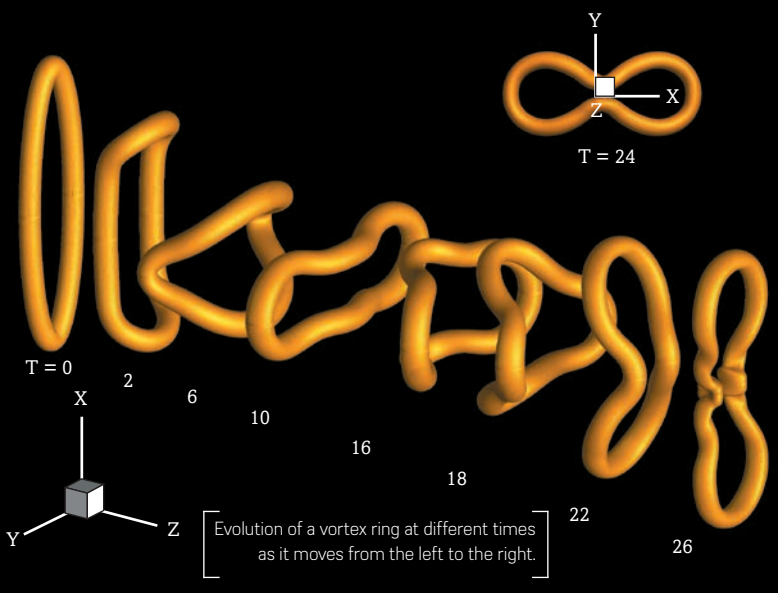
The researchers plan to conduct further genomic analyses on a much larger sample size and study these processes in cell culture experiments. "We want to grow human bile duct cell lines, expose them to the various bacteria and see how they respond," says Nagarajan. "Even if it does not lead to the initiation of the cancer, bacteria could be accelerating the process of tumor formation."

1. Chng, K. R., Chan, S. H., Ng, A. H. Q, Li, C., Jusakul, A. *et al.* Tissue microbiome profiling identifies an enrichment of specific enteric bacteria in *Opisthorchis viverrini* associated cholangiocarcinoma. *EBioMedicine* **8,** 195–202 (2016).

Fluid dynamics:

VISUALIZING VORTICES

PREVIOUSLY TOO COMPLEX TO TACKLE, COMPUTER SIMULATIONS CAN NOW SHOW HOW ELLIPTICAL VORTEX RINGS FORM



Evolution of a vortex ring at different times as it moves from the left to the right.

Understanding how smoke rings form and dissipate could lead to technology allowing airplanes to soar more efficiently and blood to flow more freely through the human heart.

Smoke rings and the airflows surrounding airplanes are two examples of vortex rings, which occur when a fast-moving fluid — liquid or air — flows within a slower moving environment. As this faster fluid slows down at the fringes, whirls are created that start to develop into stable vortex rings.

Vortex rings were previously considered to be too complicated to solve. But researchers at A*STAR have now developed a computational model to simulate the motion of an elliptical vortex ring in a slow-moving fluid in a range of different situations, allowing for a more realistic description of vortex rings[1].

Vortex rings present issues in fields such as engineering as they can hamper fluid flow. For example, they can increase the fuel consumption of a car by reducing its aerodynamic efficiency. Understanding the formation of vortex rings under different circumstances is therefore important to improve the mass flow in fluidic devices and around moving vehicles. Previous studies of vortex rings focused mainly on perfectly circular structures, as they are much easier to mathematically model than the more common and realistic elliptical ones, explains Cheng Ming from the A*STAR Institute of High Performance Computing (IHPC).

This challenge did not deter Cheng and the IHPC team. To investigate elliptical vortex rings, the researchers used a simulation technique called the lattice Boltzmann method, which divides space into a lattice of coordinates and calculates the particle flow for each set of coordinates individually. This approach is often used to model large and complex systems, such as those in weather forecasts.

The simulations allow for a detailed study of the evolution of vortex rings as they move through space, and the shape changes that

the rings undergo (see image on page 10). For example, the computer model can determine the aspect ratio at which a vortex ring breaks into sub-rings. It can also show how, after encountering a flow of mass against it, the ring is deformed and tilts as it moves further downstream. Effects like this are often the cause of aerodynamic turbulence affecting vehicles and airplanes.

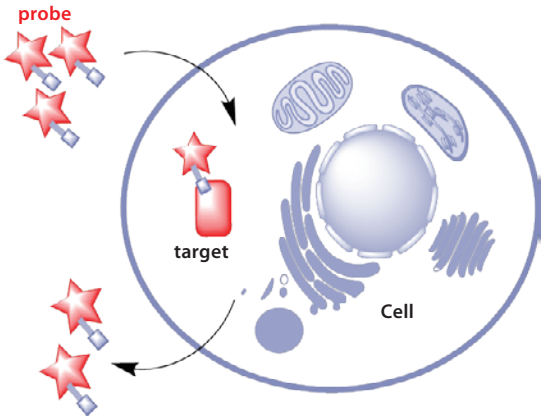
"This study not only fills the gap in the current knowledge of the dynamics of an elliptic vortex ring," says Cheng, "but also addresses the issue of whether an elliptic ring undergoes vortex stretching and compression during axis-switching, which is important for various applications."

These applications can include biomedical devices to improve blood flow in the heart.

1. Cheng, M., Lou, J. & Lim, T. T. Evolution of an elliptic vortex ring in a viscous fluid. *Physics of Fluids* **28**, 037104 (2016).

Fluorescence imaging:

MATCHING PROBES TO TARGETS



A new model developed by A*STAR researchers allows probes to be designed that can enter cells easily and label target molecules only. This will enable improved imaging of cellular processes.

PROBES THAT CAN EASILY ENTER CELLS TO LABEL TARGET MOLECULES ONLY CAN BE READILY MADE THANKS TO A NEW MODEL

A new model developed by A*STAR researchers will assist the production of tailor-made probes for imaging specific molecules inside cells. This method promises to improve the imaging of living cells and hence uncover more secrets about how they operate.

Small chemicals that latch onto specific biomolecules inside living cells and emit light when irradiated by laser light — known as fluorescent probes — are widely used to explore the roles and functions of biomolecules in cells.

However, this highly popular imaging technique suffers from two significant problems. First, probes often attach to other biomolecules besides the target ones, which gives rise to a background signal that can obscure the signal from the target biomolecule. Second, some probes struggle to cross cell membranes, making them hard to smuggle into live cells.

Now, Young-Tae Chang of the A*STAR Singapore Bioimaging Consortium and his co-workers have developed a predictive model that can overcome both problems. This model can be used to develop designer probes that are highly specific to single biomolecules and can cross cell membranes with ease (see image).

The team investigated more than a thousand probes and statistically analyzed the results. They discovered that the behavior of probes inside cells is mainly determined by just three properties: their solubilities in water and fatty substances (known as lipids) and also a parameter that indicates the charged surface area of a molecule.

Furthermore, the researchers identified the optimal values of these parameters for specific situations. Chang explains: "For example, if we know that probes with high hydrophilicity may not be able to cross a cell membrane, we can adjust the hydrophilicity to the ideal value given by the model."

The team demonstrated this approach by using probes to specifically label various organelles, such as mitochondria, lysosome and the Golgi apparatus. They also used probes to label proteins in cells.

Chang is excited about this potential of the method. "Using cell-permeable, background-free probes will allow a far better imaging for exploring dynamic processes of intracellular biomolecules in their native environment, especially in the fields of chemistry, biology and medicine."

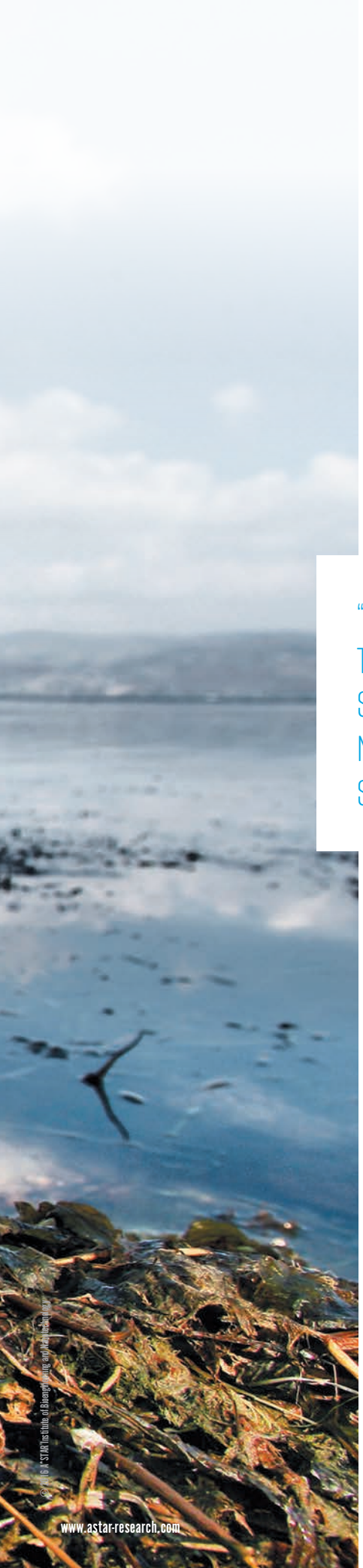
The team plans to extend their technique. "A long journey has just started," says Chang. "We aim to expand the system for performing multicolor intracellular labeling to examine subcellular structures with precise detail in a complex biological environment. We also dream of developing a toolbox of probes with various functional groups for extensive applications."

1. Alamudi, S. H., Satapathy, R., Kim, J., Su, D., Ren, H. *et al.* Development of background-free tame fluorescent probes for intracellular live cell imaging. *Nature Communications* **7**, 11964 (2016).

NANOTECHNOLOGY

PROVIDING THE TOOLS TO CLEAN UP OIL SPILLS

*A*STAR researchers are using the power of nanoscience to clean the Earth's waters*



Oceanic oil spills are tough to clean up. They dye feathers a syrupy sepia and tan fish eggs a toxic tint. The more turbulent the waters, the farther the slick spreads, with inky droplets descending into the briny deep.

Now technology may be able to succeed where hard-working volunteers have failed in the past. Researchers at the A*STAR Institute of Bioengineering and Nanotechnology (IBN) are using nanoscience to turn an oil spill into a floating mass of brown jelly that can be scooped up before it can make its way into the food chain.

Yugen Zhang and Huaqiang Zeng at the A*STAR Institute of Bioengineering and Nanotechnology.

"NANOSCIENCE MAKES IT POSSIBLE TO TAILOR THE ESSENTIAL STRUCTURES OF MATERIALS AT THE NANOMETER SCALE TO ACHIEVE SPECIFIC PROPERTIES"

"Nanoscience makes it possible to tailor the essential structures of materials at the nanometer scale to achieve specific properties," says chemist Yugen Zhang at IBN, who is developing some of the technologies. "Structures and materials in the nanometer size range often take on distinctive properties that are not seen in other size ranges," adds Huaqiang Zeng, another chemist at IBN.

JELLY SLICK

There are many approaches to cleaning an oil spill, but none are completely effective. Fresh, thick grease can be set ablaze or contained by floating barriers for skimmers to scoop out. The slick can also be inefficiently hardened, messily absorbed, hazardously dispersed, or slowly consumed by oil-grazing bacteria. All of these are deficient on a large scale, especially in rough waters.

Organic molecules with special gelling abilities offer a cheap, simple and environmentally friendly alternative for cleaning up the mess. Zeng has developed several such molecules that turn crude oil into jelly within minutes.

To create his 'supergelators', Zeng designed the molecules to associate with each other without forming physical bonds. When sprayed on contaminated seawater, the molecules immediately bundle into long fibers between 40 and 800 nanometers wide. These threads create a web that traps the interspersed oil in a giant blob that floats on the water's surface. The gunk can then be swiftly sieved out of the ocean. Valuable crude oil can later be reclaimed using a common technique employed by petroleum refineries called fractional distillation.

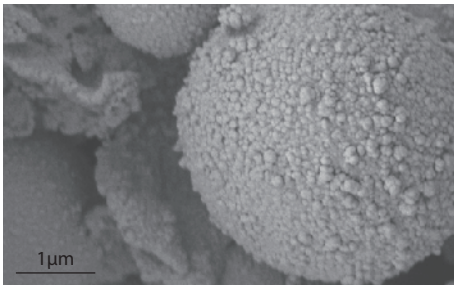
Zeng tested the supergelators on four types of crude oil with different densities, viscosities and sulfur levels in a small round dish. The results were impressive. "The supergelators solidified both freshly spilled crude oil and highly weathered crude oil 37 to 60 times their own weight," says Zeng. The materials used to produce these organic molecules are cheap and non-toxic, which make them a commercially viable solution for managing accidents out at sea. Zeng hopes to work with industrial partners to test the nanomolecules on a much larger scale.

UNSALTY WATER

Scientists at IBN are also using nanoscience to remove salt from seawater and heavy metals from contaminated water.

With dwindling global fresh and ground water reserves, many countries are looking to desalination as a viable source of drinking water. Desalination is expected to meet 30 per cent of the water demand of Singapore by 2060, which will mean tripling the country's current desalination capacity. But desalination demands huge energy consumption and reverse osmosis, the mainstream technology it depends on, has

"IN WATER, ALL OF THESE IONS ARE HIGHLY HYDRATED, ATTACHED TO LOTS OF WATER MOLECULES, WHICH MAKES THEM TOO LARGE TO GO THROUGH THE CHANNELS"



Porous nanoparticles can remove toxic heavy metals from contaminated water to trace amounts within seconds.

a relatively high cost. Reverse osmosis works by using extreme pressures to squeeze water molecules through tightly knit membranes.

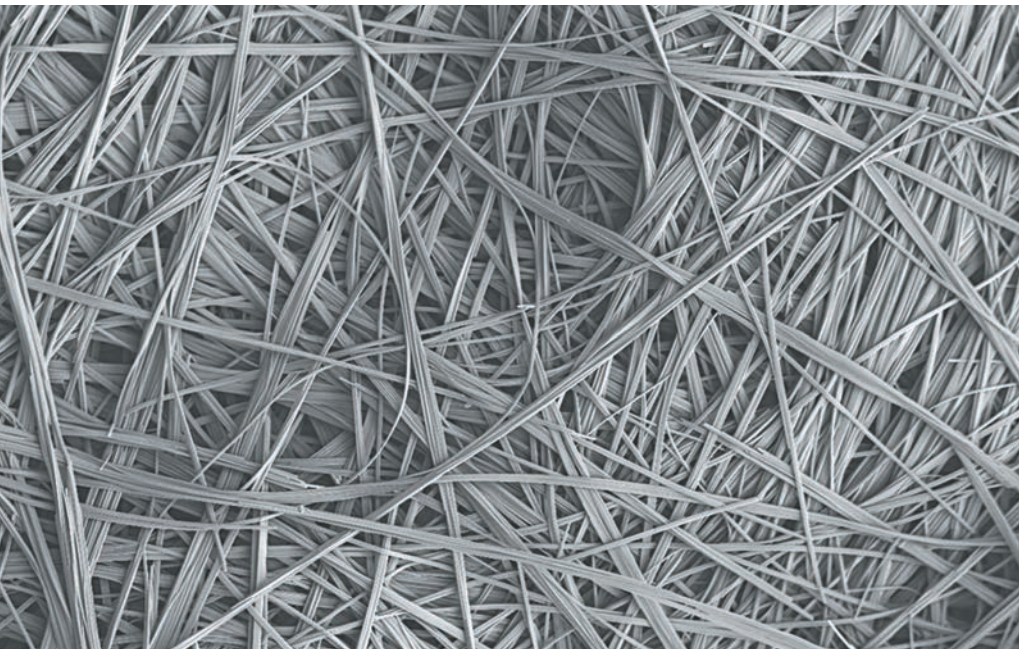
An emerging alternative solution mimics the way proteins embedded in cell membranes, known as aquaporins, channel water in and out. Some research groups have even created membranes made of fatty lipid molecules that can accommodate natural aquaporins. Zeng has developed a cheaper and more resilient replacement.

His building blocks consist of helical noodles with sticky ends that connect to form long spirals. Water molecules can flow through the 0.3 nanometer openings at the center of the spirals, but all the other positively and negatively charged ions that make up saltwater are too bulky to pass. These include sodium, potassium, calcium, magnesium, chlorine and sulfur oxide. "In water, all of these ions are highly hydrated, attached to lots of water molecules, which makes them too large to go through the channels," says Zeng.

The technology could lead to global savings of up to US$5 billion a year, says Zeng, but only after several more years of testing and tweaking the lipid membrane's compatibility and stability with the nanospirals. "This is a major focus in my group right now," he says. "We want to get this done, so that we can reduce the cost of water desalination to an acceptable level."

STICK AND NON-STICK

Nanomaterials also offer a low-cost, effective and sustainable way to filter out toxic metals from drinking water.



As the nanofibers form, they trap crude oil in a tangled net that floats above the water.

Heavy metal levels in drinking water are stringently regulated due to the severe damage the substances can cause to health, even at very low concentrations. The World Health Organization requires that levels of lead, for example, remain below ten parts per billion (ppb). Treating water to these standards is expensive and extremely difficult.

Zhang has developed an organic substance filled with pores that can trap and remove toxic metals from water to less than 1 ppb. Each pore is 10–20 nanometers wide and packed with compounds, known as amines that stick to the metals.

By exploiting the fact that amines lose their grip over the metals in acidic conditions, the valuable and limited resource can be recovered by industry, and the polymers reused.

The secret behind the success of Zhang's polymers is the large surface area covered by the pores, which translates into more opportunities to interact with and trap the metals. "Other materials have a surface area of about 100 square meters per gram, but ours is 1,000 square meters per gram," says Zhang. "It is 10 times higher."

Zhang tested his nanoporous polymers on water contaminated with lead. He sprinkled a powdered version of the polymer into a slightly alkaline liquid containing close to 100 ppb of lead. Within seconds, lead levels reduced to below 0.2 ppb. Similar results were observed for cadmium, copper and palladium. Washing the polymers in acid released up to 93 per cent of the lead.

With many companies keen to scale these technologies for real-world applications, it will not be long before nanoscience treats the Earth for its many maladies.

The widely tunable all-fiber laser developed by researchers at A*STAR can produce infrared laser beams at an unprecedented range of wavelengths. The compact, inexpensive design could have multiple applications in medical and military fields.

Lasers:

TUNING INTO MORE COMPACT LASER DESIGNS

A LASER DEVELOPED AT A*STAR CAN PRODUCE INFRARED BEAMS OVER AN UNPRECEDENTED RANGE OF WAVELENGTHS

A*STAR scientists have developed a unique fast-pulsing fiber laser that has the widest wavelength output to date[1]. This type of laser could replace several fixed-wavelength lasers and form the basis of compact devices useful for a range of medical and military applications.

The team developed an all-fiber laser, constructed similarly to a fiber-optic cable. The key component is a glass tube, whose core is doped with atoms that act as a gain medium — a material from which energy is transferred to boost the output power of the laser — through which light particles, or photons, travel. The doping atoms are selected according to the specific wavelengths of light that they will absorb, store and then release, creating an efficient, controllable output beam.

"To date, most tunable all-fiber pulsed lasers achieve a maximum tuning range of about 50 nanometers," says Xia Yu from the A*STAR Singapore Institute of Manufacturing Technology, who worked on the project with her team and her collaborator Qijie Wang from Nanyang Technological University. "We have achieved a widely tunable laser in the mid-infrared wavelength band, with a range of 136 nanometers (from 1,842 to 1,978 nanometers). We used thulium as the doping atom; this generates a laser that operates in the eye-safe range, meaning it could have medical and military applications."

The researchers combined two techniques to create their laser and ensure the output was tunable. They used nonlinear polarization evolution, a filtering effect that picks out pulses of light at the desired wavelength and

channels them into the output beam. This simultaneously ensures that the output can be adjusted to a specific wavelength while generating ultrafast pulsed light. They also used bidirectional pumping — injecting energy into the gain medium from both ends of the fiber — to ensure a high optical power over as wide a range of wavelengths as possible. The gain occurs when thulium ions are excited to higher-energy states; they then release more photons when they return to lower-energy states.

"This is the state-of-the-art, widely tunable all-fiber laser with pulsed output at this wavelength," says Yu. "We have shown that every parameter, from the pumping scheme to the use of nonlinear polarization evolution, is critical to the final output."

Yu's team believe that their simple, inexpensive and compact laser could one day be used in combination with high-power amplifiers to generate other forms of laser, including extreme ultraviolet and soft X-ray beams.

1. Yan. Z, Sun, B., Li, X., Luo, J., Shum, P. P. *et al.* Widely-tunable Tm-doped mode-locked all-fiber laser. *Scientific Reports* **6**, 27245 (2016).

Drug delivery:

POLYMER SCAFFOLDS BUILD A BETTER PILL TO SWALLOW

NANOPARTICLE DRUGS CAN MAKE IT EASIER FOR MEDICATIONS TO REACH THEIR TARGETS

Nanoscale, cross-linked polymer scaffolds (pictured) can help deliver a surprisingly high amount of drugs with poor water solubility to aqueous targets.

The huge doses of drugs required to combat cancer could be reduced thanks to the work of A*STAR researchers, and the drugs themselves may become more effective. The researchers have developed a polymeric 'scaffold' that helps drugs that often have trouble entering the bloodstream, such as anti-cancer agents, form highly stable nanoparticles with improved bioavailability[1].

Many medications that target tumor cells are made from water-repelling hydrocarbon molecules, which require extra processing or high doses rates to enter aqueous biological environments. A safer alternative is to 'nanosize' pharmaceuticals into 10 to 1,000 nanometer particles using either mechanical grinding or special crystallization techniques. These extra-small medications easily slip into water and are effective against tumors, but it is hard to prevent them from agglomerating into larger precipitates with less potency.

Ulrike Wais and Alexander Jackson from the A*STAR Institute of Chemical and Engineering Sciences and Haifei Zhang at the University of Liverpool have developed a way to lessen agglomeration problems by using poly(ethylene glycol) and acrylamide (PEG-PNIPAM) — biocompatible polymers that are highly water soluble and can stabilize water-repelling molecules because they have similar surfactant-like hydrocarbon chains.

The team synthesized PEG-PNIPAM into 'hyperbranched' spheres that are reinforced with short carbon cross-linking molecules. They then mixed the spheres with test drug compounds such as ibuprofen and blended them together to create an emulsion between the water-repelling and water-attracting components.

> **"THE DRUGS AND POLYMER SPHERES HAD INTEGRATED INTO A POROUS, SCAFFOLD-LIKE STRUCTURE."**

The next step required a way to freeze-dry the emulsion so it could be pulverized into nanoparticles, but this involved solving a tricky processing problem. "If phase separation occurs before the sample is completely frozen, drug crystals form that are neither nanosized nor stabilized against agglomeration by the scaffold," explains Wais.

The researchers prevented phase separation during freeze-drying by ensuring the emulsification was extremely uniform before spraying it as tiny droplets into a pool of liquid nitrogen. Dynamic light scattering and

scanning electron microscopy analysis of the solidified emulsion revealed that the drugs and polymer spheres had integrated into a porous, scaffold-like structure.

After mechanically grinding the freeze-dried emulsion into drug nanostructures, the researchers found their open framework made it simple to dissolve them into water. Furthermore, the drugs could be transformed into nanoparticles with yields of 100 per cent using surprisingly low levels of PEG-PNIPAM spheres.

"The polymer structure and level of branching directly affect drug nanoparticle stabilization. This method gives us a way to investigate it systematically," says Jackson. He notes that this method is synthetically straightforward and could be applied to a wide range of pharmaceuticals.

1. Wais, U., Jackson, A. W., Zuo, Y., Xiang, Y., He, T. & Zhang, H. Drug nanoparticles by emulsion-freeze-drying via the employment of branched block copolymer nanoparticles. *Journal of Controlled Release* **222**, 141–150 (2016).

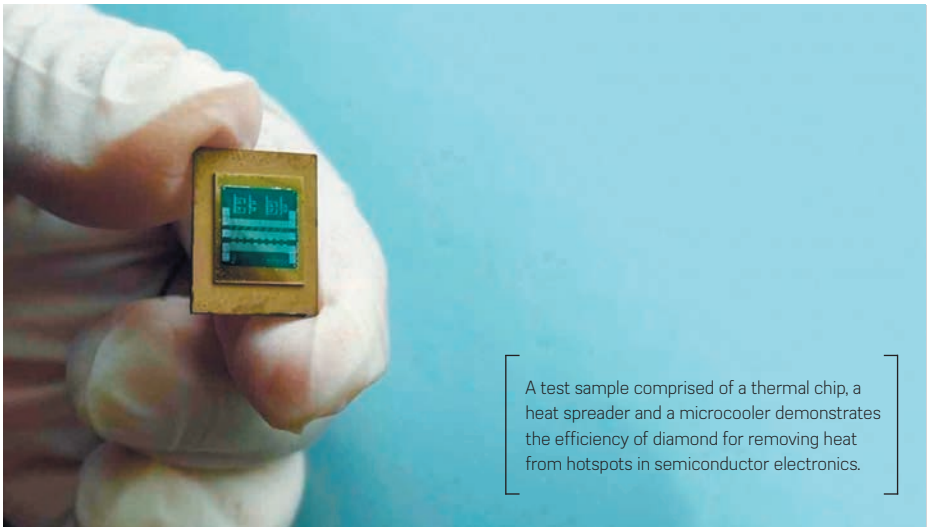
Electronics:

DIAMONDS MAKE A DEVICE COOLER

A LAYER OF DIAMOND CAN PREVENT HIGH-POWER ELECTRONIC DEVICES FROM OVERHEATING

Powerful electronic components can get very hot. When many components are combined into a single semiconductor chip, heating can become a real problem. An overheating electronic component wastes energy and is at risk of behaving unpredictably or failing altogether. Consequently, thermal management is a vital design consideration.

This becomes particularly important in devices made from gallium nitride. "Gallium nitride is capable of handling high voltages, and can enable higher power capability and very large bandwidth," says Yong Han from the A*STAR Institute of Microelectronics. "But in a gallium nitride transistor chip, the heat concentrates on tiny areas, forming several hotspots." This exacerbates the heating problem.



A test sample comprised of a thermal chip, a heat spreader and a microcooler demonstrates the efficiency of diamond for removing heat from hotspots in semiconductor electronics.

Han and co-workers demonstrate both experimentally and numerically that a layer of diamond can spread heat and improve the thermal performance of gallium nitride devices.

The researchers created a thermal test chip that contained eight tiny hotspots, each 0.45 by 0.3 millimeters in size, to generate the heat created in actual devices. They bonded this chip to a layer of high-quality diamond fabricated using a technique called chemical vapor deposition. The diamond heat spreader and test chip were connected using a thermal compression bonding process. This was then connected to a microcooler, a device consisting of a series of micrometer-wide channels and a microjet impingement array. Water impinges on the heat source wall, and then passes through the microchannels to remove the heat and keep the structure cool.

Han and the team tried their device by generating 10–120 watts of heating power in test chips of 100 and 200-micrometer thickness. To dissipate the heating power, the diamond heat spreading layer and microcooler helped maintain the structure at a temperature below 160 degrees Celsius. In fact, the maximum chip temperature was 27.3 per cent lower than another device using copper as the heat spreading layer, and over 40 per cent lower than in a device with no spreading layer.

The experimental results were further confirmed by thermal simulations. The simulations also indicated that the performance

could be improved further by increasing the thickness of the diamond layer, and that good bonding quality between the gallium nitride chip and the diamond heat spreader was crucial to obtain the best performance.

"We next hope to develop a novel microfluid cooler of higher and more uniform cooling capability, and to achieve thermal management using a diamond layer of high thermal conductivity near an electronic gate," says Han.

1. Han, Y. Lau, B. L., Tang, G. & Zhang, X. Thermal management of hotspots using diamond heat spreader on Si microcooler for GaN devices. *IEEE Transactions on Components, Packaging and Manufacturing Technology* **5,** 1740–1746 (2015).

Nutrition:

SOLID START INFLUENCED BY CULTURE

HOW SINGAPOREAN INFANTS OF DIFFERENT BACKGROUNDS ARE INTRODUCED TO FOOD

The timing and approach by which infants are introduced to solid food varies according to their cultural background, a Singaporean study suggests. A*STAR researcher Toh Jia Ying says health practitioners should be aware of these differences when offering advice to parents about the transition to solid food.

These insights come from a large-scale ongoing study of mothers and infants called Growing Up in Singapore Towards healthy Outcomes (GUSTO), a collaboration between Singapore's National University Health System (NUHS), KK Women's and Children's Hospital (KKH) and the A*STAR Singapore Institute for Clinical Sciences. The large cohort of mothers and infants being followed in the GUSTO study allowed close monitoring of how infants of Chinese, Malay or Indian ethnicity were weaned off of breast milk or formula and introduced to food.[1]

The study showed greater independent self-feeding by Malay infants, perhaps reflecting a cultural propensity for eating with hands. In the Chinese group, there was a wider use of probiotics. Babies of Indian ethnicity were more likely to be given dietary supplements, have oil and seasonings added to their foods, and consume more sweetened drinks from the bottle. In general, most infants had some exposure to sweetened drinks by 12 months of age, but Toh notes that it is not advisable to feed infants sweetened beverages at a young age.

The introduction to solid food can form lifelong feeding habits.

© Tang Ming Tung/Moment/Getty

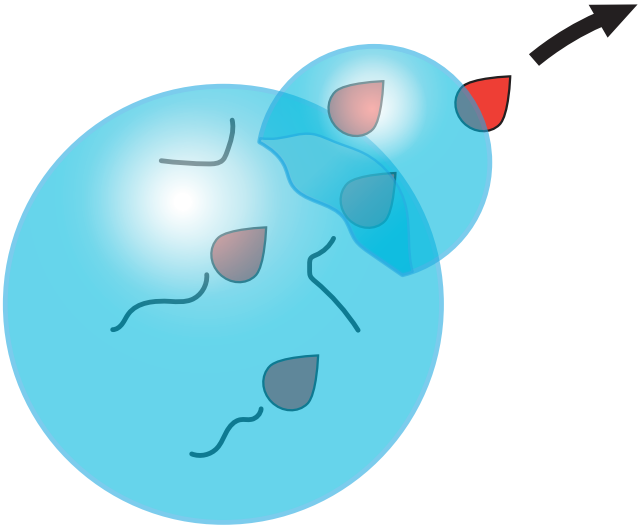
Toh says that a key finding across all ethnic groups was that a significant number of infants — a third of the cohort — were still given blended food at 12 months. The team recommends that by then, children should be given solid foods in bite-sized pieces, as this encourages children to chew, promoting the development of jaw muscles.

"The research indicates that certain cultural traditions are still widely practiced in modern cosmopolitan Singapore," says Toh. These cultural differences in infant feeding practices have not been well studied in the past, a gap that the multi-ethnic GUSTO study is particularly well-placed to address. Toh says that findings from the study will assist healthcare professionals in forming everyday guidance for parents and caregivers through the research team's links with Singapore's Health Promotion Board and Ministry of Health.

1. Toh, J. Y., Yip, G., Han, W. M., Fok, D., Low, Y-L. *et al.* Infant feeding practices in a multi-ethnic Asian cohort: The GUSTO study. *Nutrients* **8,** 293 (2016).

Polymers:

NANOSCALE FACTORIES BUILT TO ORDER



Understanding how radical fragments (red droplets) react with surrounding water molecules to create hydroxyl radicals, while leaving behind hydrophobic residues (grey), can help green chemistry researchers.

PERFORMING CHEMICAL REACTIONS INSIDE TINY DROPLETS CAN HELP MANUFACTURERS DEVELOP GREENER PROCESSES FOR COATING DRUGS

An A*STAR-led discovery could lead to improvements in the way drugs are delivered to the right parts of the body by uncovering the mechanisms that help oil, water, and free radicals mix in tiny droplets[1,2].

Emulsion polymerization is an emerging technology used to produce enormous chain-like molecules called polymers inside oil-filled drops suspended in water. This approach enables producers of goods such as latex paints to do away with traditional oil-based solvents, which helps them meet stricter environmental controls. Recently, researchers have discovered that 'mini-emulsions', in which droplets are shrunk to nanoscale sizes using powerful blenders and stabilized with fatty molecules, can produce nanoparticles for applications including controlled drug release.

> "THE NANOREACTORS GROW COMPLETELY INDEPENDENTLY, AND WE CAN ACHIEVE VERY HIGH REACTION RATES."

Alex van Herk from the A*STAR Institute of Chemical and Engineering Sciences explains that in mini-emulsions, each droplet can be regarded as a 'nanoreactor' — a segregated system where all the ingredients for polymerization are present in one spot. Once a highly reactive free radical enters the drop, the small molecules inside link into chains. "The nanoreactors grow completely independently, and we can achieve very high reaction rates," he says.

This polymerization only works when one free radical enters a nanoreactor. However, the molecules that generate free radicals, known as initiators, generally produce them in pairs. To better understand these radical movements, van Herk and colleagues from the Netherlands and the United Kingdom investigated the effects of using initiators that either repelled or attracted water molecules.

Typical initiators are water-soluble and researchers propose that they create pairs of free radicals in water where one of the free radicals enters the nanoreactor and starts the polymerization. However, when the initiator is a water-repelling molecule, such as lauroyl peroxide, theory predicts the chemical reaction will be hindered because the two radicals in a confined space would easily recombine and the polymerization process would not start.

Surprisingly, the A*STAR-led team found mini-emulsion polymerization proceeded rapidly and completely using lauroyl peroxide initiators. To explain this discrepancy, the team deduced that a free radical must leave by an alternative mechanism, known as chain transfer, which transforms one of the water molecules surrounding the nanoreactor into a hydroxyl radical compound. The remaining radical produces latex nanoparticles that correspond one-to-one with the initial droplet size — a benefit for manufacturers seeking to predict morphologies with exact specifications.

"Industry is only modestly adopting mini-emulsion polymerization, partly because its mechanism is not fully understood and controllable yet," says van Herk. "These findings give us a better edge to design and produce special nanoparticle morphologies such as low-cost nanocapsules."

1. Jansen, T. G. T., Meuldijk, J., Lovell, P. A. & van Herk, A. M. On the miniemulsion polymerization of very hydrophobic monomers initiated by a completely water-insoluble initiator: thermodynamics, kinetics, and mechanism. *Journal of Polymer Science Part A: Polymer Chemistry* **54**, 2731–2745 (2016).
2. Jansen, T. G. T., Meuldijk, J., Lovell, P. A. & van Herk, A. M. On the reaction characteristics of miniemulsion polymerization with aqueous phase initiation — Experiments and modeling. *Macromolecular Reaction Engineering* **9**, 19–31 (2015).

A national pharmacogenomics testing program could help reduce the thousands of extra days Singaporeans stay in hospital each year due to adverse reactions to medication.

Pharmacogenomics:

AVOIDING ADVERSE REACTIONS TO MEDICATION

SINGAPOREANS SPEND 48,000 EXTRA DAYS A YEAR IN HOSPITAL DUE TO ADVERSE DRUG REACTIONS

About one in every ten patients is admitted to Singapore's biggest acute-care hospital due to adverse reactions to medication, according to a study led by a team of researchers at A*STAR and Singapore General Hospital.

The team investigated the prevalence of serious medication side-effects among 1,000 patients admitted to Singapore General Hospital. They found a much higher incidence of adverse drug reactions (ADRs) than the 3 to 9 per cent reported by other researchers in previous studies.

"The headline finding was that about 12 per cent of patients admitted to hospital had a severe side-effect to a medication — this included 8 per cent where ADRs were the main cause of admission," says Liam Brunham from the A*STAR Translational Laboratory in Genetic Medicine and the National University of Singapore, one of the authors of the study.

The most common culprits for ADRs in the study were cardiovascular drugs, anti-coagulants, anti-platelets and chemotherapeutic drugs. Reactions included gastrointestinal symptoms, such as diarrhea; electrolyte abnormalities that can cause a life-threatening abnormal heart rhythm; and bleeding.

On average, the patients with ADRs were in hospital for four days, compared to a three-day stay for other patients. Eleven of these patients were admitted to high care, some of whom were left permanently disabled or dead. Two of the deaths were attributed to the drug reactions.

"If we extrapolate our finding to the entire country of Singapore, this amounts to an extra 48,000 days in hospitals each year

simply because patients have had a side-effect to medications that a doctor has prescribed them," says Brunham. This shows how considerable the problem is in terms of cost, morbidity and mortality.

Brunham's group and collaborators in the Surveillance and Pharmacogenomics Initiative for Adverse Drug Reactions (SAPhIRE) program and the A*STAR Genomics Institute of Singapore are doing a follow-up study on the cost benefits of implementing a national pharmacogenomics testing program. This program would allow doctors to analyze a patient's genetic makeup to predict if he or she is likely to have an adverse reaction to particular medications.

Alexandre Chan of the National University of Singapore, and principal investigator for the study, says that a national pharmacogenomics testing program would "reduce costs, prevent unnecessary admissions and reduce the morbidity associated with drug-induced adverse drug reactions."

1. Chan, S. L., Ang, X., Sani, L. L., Ng H. Y., Winther M. D. *et al*. Prevalence and characteristics of adverse drug reactions at admission to hospital: a prospective observational study. *British Journal of Clinical Pharmacology* **82**, 1636–1646 (2016).

Hard disks:

APPLYING HEAT TO STAY ON TRACK

A CLEVER ELECTRO-THERMAL ACTUATOR AND LEVER SYSTEM KEEPS HARD DRIVE RECORDING HEADS ON TRACK

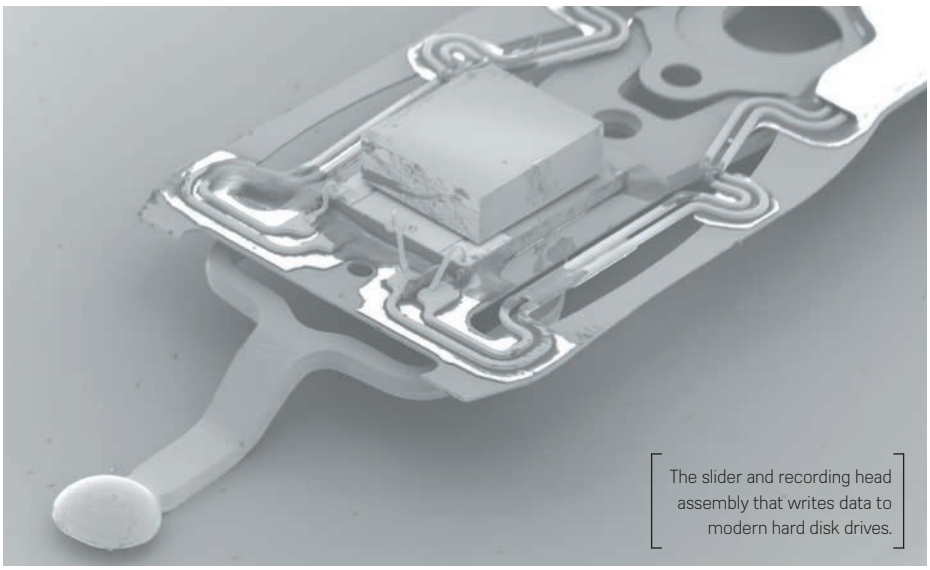
As the density of data stored on a hard drive gets close to multiple terabytes per square centimeter, the precision of the internal components is becoming increasingly significant. A*STAR researchers have designed a system that achieves a new milestone in micro-positioning, and it could become the next industry standard[1].

Like a 21st century update of the gramophone record, hard disk drives consist of thin magnetic platters with data bits arranged on concentric tracks. The data bits are read and written by a magnetic recording head that floats a few nanometers above the platter surface at the end of a 'slider' arm, while the platter spins at high speed beneath it.

But unlike an old record, a modern disk drive contains up to a million tracks per inch and track widths as narrow as 25 nanometers. And that density is increasing every year. This means future disk drives will require extraordinarily precise head positioning and responsiveness to meet performance expectations.

"The actuator mechanism will need to be able to position the recording head with a precision of just one or two nanometers," explains Jiaping Yang from the A*STAR Data Storage Institute. "The actuator schemes commonly used today can offer a fast response, but will have difficulty achieving the positioning accuracy needed for future high-density drives."

Yang and his colleagues from A*STAR and Nanyang Technological University in Singapore have been investigating the possibility of using an electrically activated thermal expansion element to control the position of the recording head at the end of the slider. The latter provides the larger-scale movement needed to navigate across multiple tracks.



The slider and recording head assembly that writes data to modern hard disk drives.

Their electrothermal element is called a thermal unimorph and it consists of a comb-like set of silicon teeth interlaced with polymer expanders. Yang says: "Silicon has high thermal conductivity but small thermal expansion, while the polymer expander has a large thermal expansion coefficient, but low thermal conductivity. When we resistively heat the element by applying electricity to the silicon, the polymer expands, causing the silicon comb to bend."

Although the thermal unimorph can be controlled with nanometer precision, its range of motion was previously too limited to be of practical use. Yang and his team overcame this limitation by adding a rotary lever action that magnified the stroke length by six times.

"We are now exploring possible approaches to improve actuation speed performance, such as designing a more efficient heat path, investigating new thermally active materials, and further miniaturization of the actuator footprint," says Yang.

1. Lau, G. K., Yang, J., Tan, C. P. & Chong, N. B. An electro-thermally activated rotary micro-positioner for slider-level dual-stage positioning in hard disk drives. *Journal of Micromechanics and Microengineering* **26**, 035016 (2016).

Spectroscopy:

GHOSTLY MEASUREMENTS

A QUANTUM EFFECT ALLOWS INFRARED MEASUREMENTS TO BE PERFORMED BY DETECTING VISIBLE LIGHT, BRINGING OPPORTUNITIES FOR CHEAPER, BETTER PERFORMANCE SPECTROSCOPY

By weaving some quantum wizardry, A*STAR researchers have achieved something that appears to be a contradiction in terms — using visible light to perform spectroscopy at infrared wavelengths[1]. Even more mysterious is that the visible light does not even pass through the sample being measured.

Infrared spectroscopy is widely used by chemists to identify chemicals from their unique 'fingerprints' in the infrared region. However, infrared-light sources, elements and detectors tend to have inferior performances and be more expensive than their visible-light counterparts.

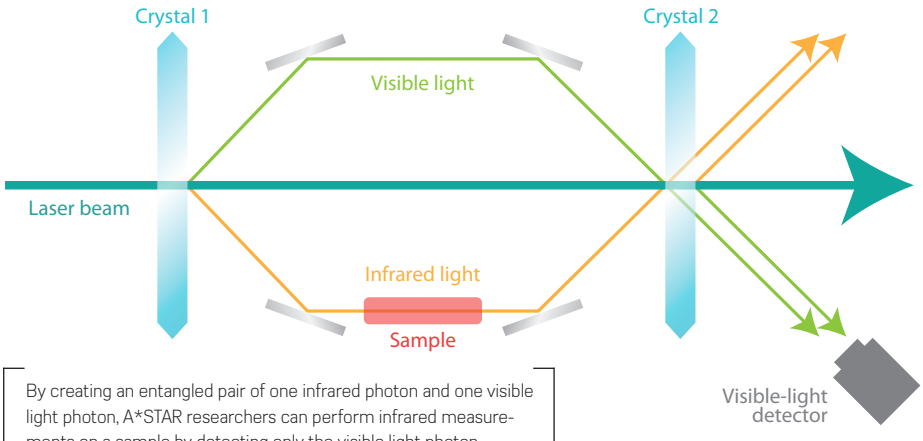
Now, Dmitry Kalashnikov at A*STAR Data Storage Institute and his co-workers have hit on a way to overcome this problem and realize the best of both worlds — using visible light to perform measurements in the infrared region.

They achieved this by exploiting a quantum effect known as entanglement. In this phenomenon, two quantum particles (in this case, particles of light known as photons) are so intimately connected that changing the quantum state of one particle simultaneously alters the state of the other particle, even when the two particles are separated in space. This is the "spooky action at a distance" that Einstein famously objected to.

Kalashnikov and his team used a special crystal to create a pair of entangled photons, a visible one and an infrared one (see image). The infrared photon passed through a sample, whereas the optical one did not. The two photons then crossed at a second crystal and the visible photon was detected. Since any changes that the sample induced in the infrared photon were reflected in the visible photon, the team could infer information about the sample's infrared properties by measuring only the visible photon.

The researchers demonstrated the potential of this technique by using it to measure the presence and concentration of carbon dioxide in samples of air.

"We are confident that this method will find a broad variety of practical applications, for example in environmental monitoring and health diagnostics," says Kalashnikov.



By creating an entangled pair of one infrared photon and one visible light photon, A*STAR researchers can perform infrared measurements on a sample by detecting only the visible light photon.

"This study demonstrates that quantum optics is moving out of the realm of purely fundamental science," he adds. "We are seeing a rise of practical applications in different fields, including cryptography, metrology, imaging and sensing. Our work is another example of this trend."

The team intends to extend the technique to longer wavelengths in the terahertz and far-infrared ranges. They are also considering integrating the system on single platform, which would make it easier to implement.

1. Kalashnikov, D. A., Paterova, A. V., Kulik, S. P. & Krivitsky, L. A. Infrared spectroscopy with visible light. *Nature Photonics* **10,** 98–101 (2016).
2. Wolf, J-P. & Silberberg, Y. Quantum optics: Spooky spectroscopy. *Nature Photonics* **10,** 77–79 (2016).

Biomedical materials:

SHARPSHOOTING NANOPARTICLES HIT THE TARGET

LAYERED NANOCAPSULES SELECTIVELY DELIVER DRUGS TO EXACTLY WHERE THEY ARE NEEDED

Magnetic, pH-responsive nanoparticles deliver the antitumor drug doxorubicin (fluorescing red in this image) to cancer cells

A nanoparticle-based drug delivery system that can sense and respond to different conditions in the body, as well as to an externally applied magnetic field, could enhance doctors' ability to target drugs to specific sites of disease.

A*STAR researchers created the multifunctional nanocapsules by wrapping magnetic iron oxide nanoparticles inside a biocompatible polymer coat that could be tuned to respond to acidity or temperature[1]. The team has already shown that the nanoparticles can selectively deliver the toxic antitumor drug doxorubicin to cancer cells.

Some previous nanoparticle drug delivery systems have incorporated magnetic field responsiveness, and others have shown pH or temperature responsiveness. The nanoparticles developed by Chaobin He, Zibiao Li and their colleagues at the A*STAR Institute of Materials Research and Engineering are unusual in that they combine multiple stimuli-responsive behaviors into a single nanoparticle.

The team made their nanoparticles by coating iron oxide particles with silica, then attaching the biocompatible poly(lactide) (PLA) polymer via a process known as stereocomplexation. The PLA polymer strands self-assemble themselves around the iron core, forming a flexible shell that can be loaded with drug molecules.

The iron oxide core allows doctors to physically target the encapsulated drug to specific sites in the body using an external magnetic field, explains Zibiao Li, a member of the team. "This characteristic of stimuli-responsive nanocarriers is especially important in cancer therapy to prevent the serious side effects of chemotherapy," he says. By selectively delivering chemotherapy drugs to a tumor, the drug's harmful effect on healthy cells can be minimized.

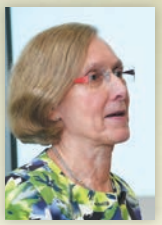
The researchers further enhanced the selective delivery of their nanocapsules by coating them with newly designed PLA copolymers that can respond to changes in pH or temperature. A polymer named PLA-PDMAEMA, for example, swells up in acidic conditions, loosening its grip on its cargo of drugs as it expands. As tumor cells are typically more acidic environments than healthy cells, these nanoparticle should selectively release their drugs within cancer cells.

When the researchers loaded their PLA-PDMAEMA coated nanoparticles with the anticancer drug doxorubicin, they showed that the drug was released significantly faster under acidic conditions. Initial tests with breast cancer cells confirmed the capsules were taken up by the cells and able to release their cargo to kill the cells.

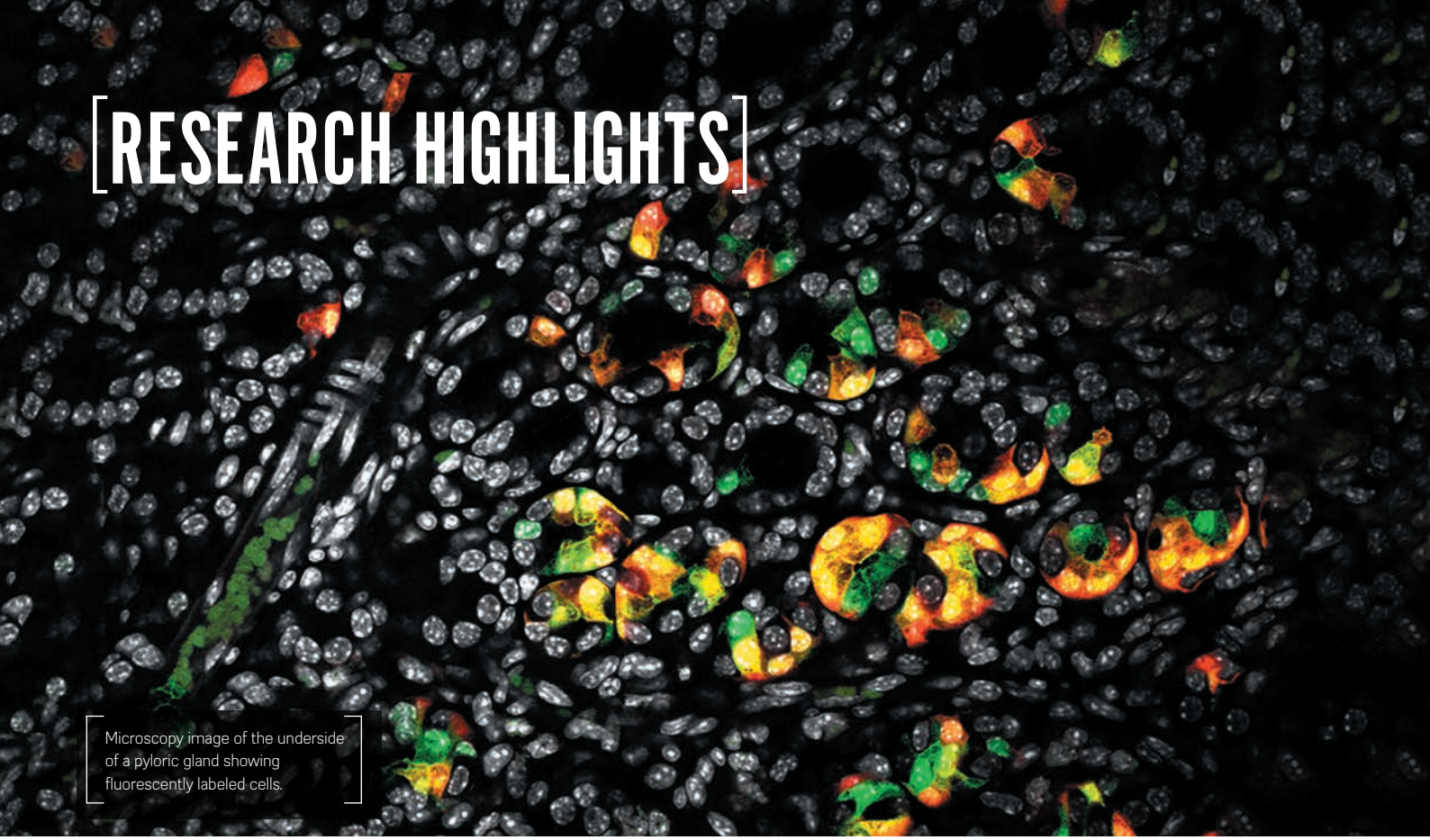
The next step will be to optimize the size of the nanocapsules size, before testing them in animal models. "Further exploration of using these nanoparticles for combined drug delivery and bioimaging are also in progress," Li says.

1. Li, Z., Yuan, D., Jin, G., Tan, B. H., & He, C. Facile layer-by-layer self-assembly toward enantiomeric poly(lactide) stereocomplex coated magnetite nanocarrier for highly tunable drug deliveries. *ACS Applied Materials & Interfaces* **8,** 1842–1853 (2016).

[THOUGHT LEADERS]



The inaugural Conference on Research Integrity, jointly organized by A*STAR, NUS, NTU and SUTD, took place on 22 November in the Biopolis. The conference aimed to engage the Singaporean research community on the topic of research integrity and to foster discussion on common principles and standards related to research. Singapore's research leaders, including A*STAR Managing Director Raj Thampuran (pictured below with microphone) gathered to share their thoughts and listened to eminent keynote speakers including Mary Ritter, Emeritus Professor at Imperial College, London (rightmost inset), Sir David Lane, Chief Scientist at A*STAR (leftmost inset) and (not pictured) Ms Jana Christopher, former data integrity analyst for EMBO press.



Microscopy image of the underside of a pyloric gland showing fluorescently labeled cells.

Stem cells:

TRACKING STEM CELLS IN THE STOMACH LINING

STEM CELL BEHAVIOR REVEALED BY A COMBINATION OF MODELING AND MICROSCOPY

The renewal of cells in a healthy stomach is being studied by A*STAR researchers through a multidisciplinary approach that combines cell lineage tracing experiments and mathematical modeling[1]. The models provide a valuable baseline for studying gastric diseases, and the approach can be used to investigate the developmental dynamics of other organs.

Nick Barker's team at the A*STAR Institute of Medical Biology studied the development of flask-shaped pockets in the lining of the stomach's pyloric region, known as pyloric glands, which secrete gastrin and mucus into the stomach. Within each gland is a dividing pool of stem cells that contributes to two essential processes. Some of the daughter cells remain undifferentiated and slowly replace other stem cells within the gland, while others migrate upwards and differentiate to renew the stomach lining relatively rapidly. The researchers used stochastic models to study these two processes, using observations of fluorescently labeled cells to fit the models.

Since stem cells divide and replace other stem cells, eventually all of the cells in a gland will have descended from a single stem cell. To investigate this process, the team extended an earlier model that assumed the process starts with a single stem cell, rather than a pool. By building a model without this assumption, the team discovered that only a few of the stem cells are in a position for their descendants to effectively expand to the entire gland.

The researchers also quantified the renewal of the stomach lining by stem cell proliferation and differentiation.

By integrating stochastic models and experiments they showed that the processes of proliferation and differentiation are tightly coupled; stem cells normally divide at the same rate as their daughters differentiate, ensuring that the pool remains the same size.

The team also applied this approach to quantify stem cell proliferation and differentiation under conditions that resembled disease,

when these processes become uncoupled.

These models will help understand gastric diseases as well as normal pyloric gland development. "For instance, we can examine whether gastric cancer is the result of an imbalance between stem cell proliferation and differentiation or is associated with a slower replacement of stem cells, meaning that faulty stem cells are not replaced early enough" says Carmen Pin, a visiting researcher from the Institute of Food Research in the UK who undertook the study with Barker and Marc Leushacke from the A*STAR team.

"The same approach could be used to study the dynamics of any population of cells which can be genetically labeled and traced in other organs," they added.

1. Leushacke, M., Barker, N. & Pin, C. Quantifying Lgr5-positive stem cell behaviour in the pyloric epithelium. *Scientific Reports* **6**, 21923 (2016).

Bacterial biofilms:

A PROBE FOR BIOFILMS UNVEILS BACTERIA

THE FIRST PROBE FOR BACTERIAL BIOFILMS OPENS THE DOOR FOR NEW WAYS TO COMBAT ANTIBACTERIAL RESISTANCE

The key to overcoming antibacterial resistance could lie in our ability to detect biofilms — a structure formed by bacterial communities that protects them from anti-bacterial drugs. Scientists at A*STAR have developed the first fluorescent probe to detect biofilms in a living animal model of corneal infection, allowing for access to the bacteria for treatment.

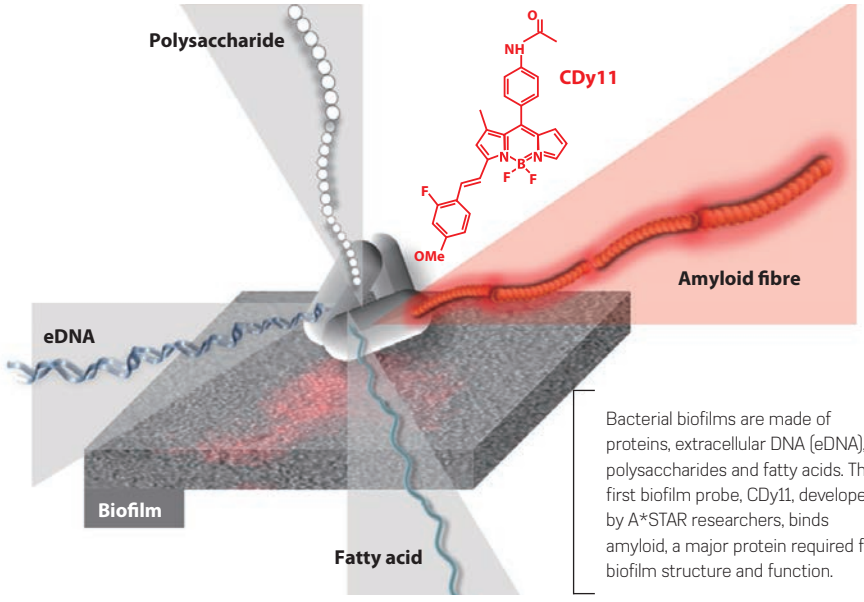
Bacterial infections have traditionally been treated using antibiotics. "When bacteria are isolated, they are relatively easy to detect and kill using antibiotics. Once they get together, however, they form a protective structure around their community — a so-called biofilm," says Young-Tae Chang, team leader from the A*STAR Singapore Bioimaging Consortium.

These biofilms — thick substances made of extracellular DNA, proteins, polysaccharides and fatty acids — are a large contributing factor for antibiotic resistance. "It is hard for antibiotics to penetrate the biofilm to reach the bacteria and treat them," explains Chang.

To access biofilm-covered bacteria, scientists first need to be able to detect the protective structure. Chang and his team discovered a biofilm probe using a technique previously developed in his lab, called the diversity-oriented fluorescence library approach (DOFLA).

DOFLA uniquely generates small fluorescent molecules for use as probes by creating simple fluorescent scaffolds that can be modified upon binding to target molecules. DOFL compounds are generated without prior knowledge of a target, which overcomes the limitations of target-oriented approaches, where the applicability of such compounds in complex biological systems is often not guaranteed.

Chang and his team screened their 10,000-member molecular library for compounds that bind amyloid, a major scaffolding protein in biofilm. They identified the probe, named CDy11 (compound of designation yellow 11), by screening the compounds in high- versus low-amyloid expressing strains of the



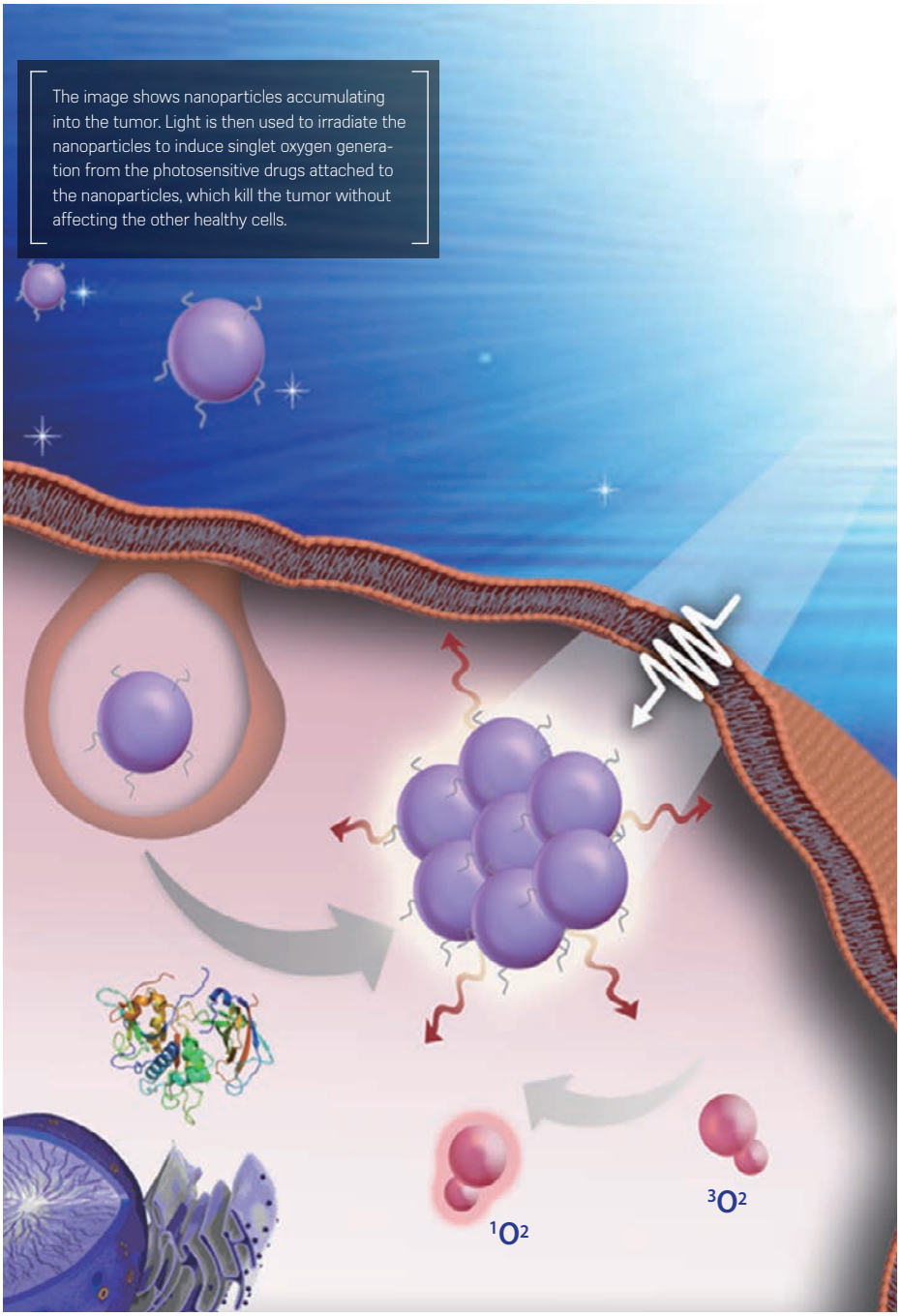
Bacterial biofilms are made of proteins, extracellular DNA (eDNA), polysaccharides and fatty acids. The first biofilm probe, CDy11, developed by A*STAR researchers, binds amyloid, a major protein required for biofilm structure and function.

Pseudomonas aeruginosa bacteria, which has advanced antibiotic resistance mechanisms.

Chang explains that CDy11 can be used to show where bacteria are hiding and, therefore, which sites to treat. Since amyloid is such a fundamental structure in biofilms, he predicts that CDy11 may have broad applicability. His team has already confirmed that CDy11 can detect biofilms of several strains of bacteria.

"Currently, there is no direct method to detect or visualize biofilms, so diagnosis is also extremely difficult. Our CDy11 is the first probe to solve the problem," notes Chang. His team is currently identifying probes to mark other biofilm components and widen the toolbox for biofilm detection. ■

1. Kim, J.-Y, Sahu, S., Yau, Y.-H., Wang, X., Shochat, S. G. *et al.* Detection of pathogenic biofilms with bacterial amyloid targeting fluorescent probe, CDy11. *Journal of the American Chemical Society* **138**, 402–407 (2016).



The image shows nanoparticles accumulating into the tumor. Light is then used to irradiate the nanoparticles to induce singlet oxygen generation from the photosensitive drugs attached to the nanoparticles, which kill the tumor without affecting the other healthy cells.

Nanomaterials:

SMART MATERIAL HUNTS CANCERS

THE PURSUIT OF MICROSCOPIC DRUGS THAT CAN BE TRACKED AS THEY FIGHT CANCER IS A STEP CLOSER

Microscopic drug molecules could soon be sent into the body to fight disease and their journey tracked using photoacoustic imaging, after researchers developed a smart material that can locate and image cancer sites inside tissues[1].

A team from the A*STAR Singapore Bioimaging Consortium and Nanyang Technological University (NTU) has developed a 'nanophotonics platform' that measures changes in the local tissue environment at the site of a tumor or cancer, by measuring enzyme reactions specific to the cancer.

This nanophotonics platform includes a promising compound for increasing the

contrast of photoacoustic images, which allows imaging of tissue *in vivo*.

"Nanomaterials have been recognized as promising platforms for the battle against many urgent health concerns including cancer, cardiovascular and neurodegenerative diseases," say lead researchers Malini Olivo from A*STAR and Xing Bengang from NTU.

"However, a critical challenge remains in designing targeted nanoplatforms that are capable of selectively localizing at the specific diseases; in particular, tumor sites for early-stage diagnosis and effective treatment," explains Olivo, who says their new work addresses this challenge.

"These developments have the potential to improve diagnostics and allow for the development of therapies that can be delivered at the cell level, leading to fewer side-effects," says Olivo.

Previously direct targeting of diseased cells had used ligands (or molecules) to bind nanoparticles to a cell with the complementary receptor.

However, Olivo says the inability of the ligand to differentiate between normal and tumor cells was a flaw in the strategy. A key to the latest innovation is that the nanophotonics platform is adapted to respond to a tumor-specific enzyme and then accumulate at that site.

The accumulation of the nanophotonics platform improves the effectiveness of light treatments that kill cancer cells, such as photodynamic therapy and laser irradiation, and opens the possibility of inhibiting tumor growth through injection of nanoscale smart drugs.

Olivo says nanostructures offer great potential in biomedical applications due to properties such as tunable chemical composition, flexible morphology, high surface area, and multivalent binding ability.

Nanostructures also have the potential to penetrate pores in the lining of blood and lymphatic vessel walls allowing the nanostructures to more effectively target and accumulate into the diseased region.

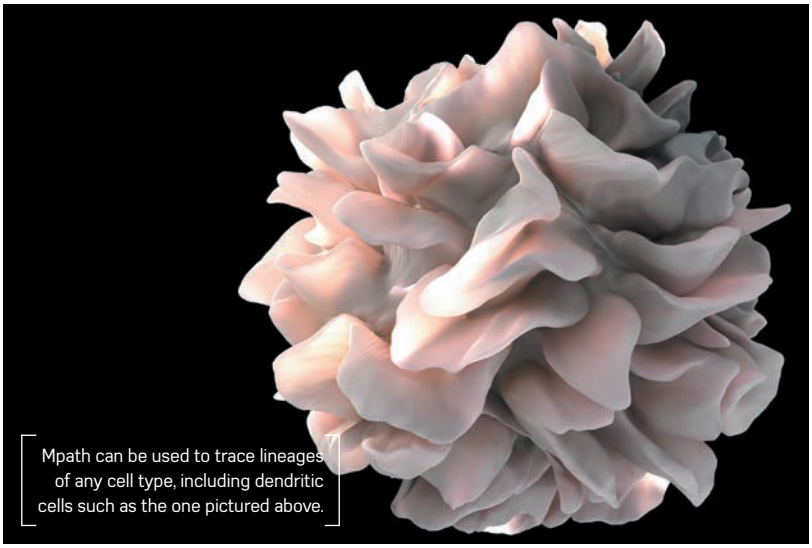
Olivo says their approach could be expanded into other areas of nanomedicine, opening "new doors for selective and precise theranostics in future clinical applications".

1. Ai, X. Ho, C., Aw, J., Attia, A. B. E., Mu, J. *et al. In vivo* covalent cross-linking of photon-converted rare-earth nanostructures for tumour localization and theranostics. *Nature Communications* **7,** 10432 (2016).

Bioinformatics:

THE PATH TO CELL FATE

ALGORITHM CAN TRACE LINEAGE TRAJECTORIES THROUGH SINGLE-CELL GENE EXPRESSION DATA



Mpath can be used to trace lineages of any cell type, including dendritic cells such as the one pictured above.

A new algorithm created at A*STAR could reveal new insights into disease state and offer a way to see how drugs are working. The tool, dubbed Mpath allows scientists to track the trajectories of different cell lineages on the basis of their gene activity at the individual cell level.

"For the academic research community, Mpath can be used to infer pathways and key regulators of cellular development and differentiation," says Jinmiao Chen, a computational biologist at the A*STAR Singapore Immunology Network who led the algorithm's development. "And for pharmaceutical companies, when a drug is tested with different dosages or at different time points, Mpath can be used to map the kinetics of drug response."

The latest RNA-sequencing technologies allows researchers to analyze gene expression profiles with unprecedented single-cell resolution. But it was still a challenge to connect the dots between which individual cells gave rise to one another. Enter Mpath.

Mpath takes in gene expression information to infer the progression of cells from their progenitor state. It does not require data on huge numbers of cells or at different time points, rather it works with single-cell data from a variety of different sequencing technologies, and can construct both linear and branching differentiation pathways.

To demonstrate the algorithm's usefulness, Chen and her colleagues used Mpath to trace the development of mouse dendritic cells, a type of white blood cell involved in mounting immune responses against pathogens. By analyzing single-cell RNA, the tool exposed the timing of the branching event from precursor cells into the two functionally distinct lineages of dendritic cell and uncovered

two novel regulators of cell fate — all of which was validated with wet lab experiments.

The researchers also considered the process of human muscle cell development, demonstrating the intricate crosstalk between muscle and non-muscle cell lineages and revealing seven waves of gene expression changes during differentiation. According to Chen, "Mpath can be used to trace lineages of any cell type." For example, one could extend the method to study "how cancer cells have progressed from benign to malignant, as well as which genes are driving the progression," she says.

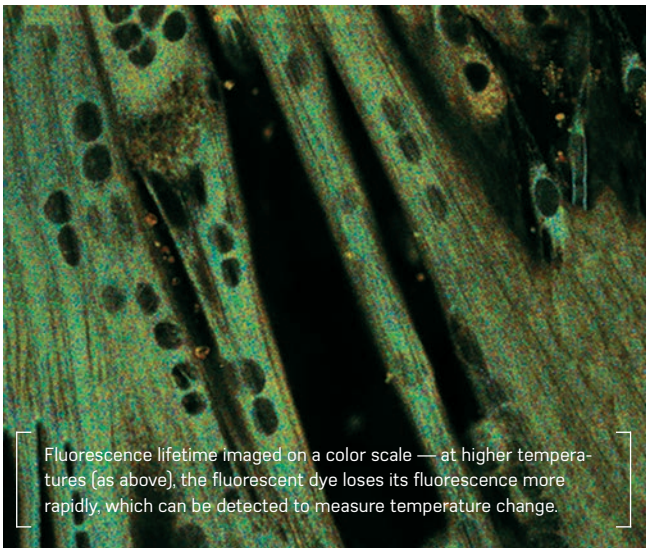
Chen and her colleagues are now working on an enhanced version of the algorithm called Mtree, which would allow the simultaneous analysis of two or more independent development pathways, such as in the brain where different immune lineages are distinct from other tissues. ■

1. Chen, J., Schlitzer, A., Chakarov, S., Ginhoux, F. & Poidinger, M. Mpath maps multi-branching single-cell trajectories revealing progenitor cell progression during development. *Nature Communications* **7**, 11988 (2016).

Fluorescence imaging:

CELLULAR THERMOMETER

LIGHT-EMITTING DYES CAN MEASURE HEAT GENERATION WITHIN LIVING CELLS WITH SUBCELLULAR ACCURACY



Fluorescence lifetime imaged on a color scale — at higher temperatures (as above), the fluorescent dye loses its fluorescence more rapidly, which can be detected to measure temperature change.

A technique that uses fluorescent dyes to measure the temperature inside living cells is helping to reveal the mechanism by which living organisms generate heat.

An international team co-led by A*STAR researchers have shown that the dyes, which adjust their light emission in response to temperature, can be used to measure heat generation within muscle cells. The technique has already resolved one controversy over the amount of heat such cells can generate.

Warm-blooded animals must produce a significant amount of heat to maintain their temperature. Muscle tissue is one source, generating heat through shivering. But muscle cells can also heat up via a chemical process called non-shivering thermogenesis (NST). This process is less well understood, and even the estimates of the amount of heat generated this way by the body can differ wildly. Some tests have suggested they could raise their temperature by one degree Celsius via NST, whereas an estimate calculated from a mass of cells suggested a mere 10^{-5} degree Celsius per cell.

Birgitte Lane and Hideki Itoh at the A*STAR Institute of Medical Biology, along with collaborators in Japan, have unraveled these results using temperature-responsive dyes developed at the Singapore Bioimaging Consortium.

The team used ER thermo yellow, a dye that sticks to the structure in muscle cells thought to be the site of NST, the sarcoplasmic reticulum. The dye fluoresces after being exposed to light, and the length of time it remains illuminated varies with temperature.

Using A*STAR's analytical fluorescence microscopy facility, the researchers could measured the fluorescent lifetime of the dye with sub-nanosecond accuracy. When they dosed muscle cells with caffeine, they detected a temperature increase of around 1.6 degrees Celsius using ER thermo yellow. When they repeated the experiment using another dye that diffuses throughout the cell, no such temperature increase was observed, confirming the heat was generated at the sarcoplasmic reticulum.

"Using this approach allows us to look at heat generation much more accurately within cells, so we can see which cell types have this capability and where in the cell the heat generation is taking place. We can then start to dissect the mechanism," says Lane.

The result confirms that specialized cells within muscle tissue are able to raise their temperature by around 1 degree Celsius via NST. This new methodology could now be used for medical applications such as screening drug for conditions like obesity or heat regulation disorders like malignant hypothermia. ■

1. Itoh, H., Arai, S., Sudhaharan, T., Lee, S.-C., Chang, Y.-T., *et al.* Direct organelle thermometry with fluorescence lifetime imaging microscopy in single myotubes. *Chemical Communications* **52**, 4458–4461 (2016).

Rupturing a cell releases tens of thousands of proteins, which can be hard to track unless you have already tagged proteins of interest.

Cell biology:

PLAYING TAG WITH PROTEINS

PROXIMITY LABELING REVEALS THE KEY COMPONENTS OF A STRUCTURE THAT GIVES CELLS THEIR SENSE OF PLACE

The protein complexes cells use to attach to the local biological matrix do more than hold cells in place — they help the cell sense what tissue they are in and what cell type they should be. However, the unstable nature of these 'focal adhesion' protein complexes makes them difficult to study. Researchers at A*STAR have built a working model of the focal adhesion by using a molecular tagging technique to precisely identify all the proteins involved[1].

With an average cell containing 10,000–15,000 proteins, it is difficult to ascertain how specific proteins come together to perform certain functions. Paxillin is a well-known focal adhesion protein, but a "bewildering array" of other proteins have been proposed to make up the adhesion complex, says Ed Manser from the A*STAR Institute of Molecular and Cell Biology, who led the work.

> "YOU CAN 'TAG' A PROTEIN SUCH AS PAXILLIN AND GRAB IT ONCE THE CELL HAS BEEN BROKEN, IN THE HOPE OF FINDING ITS CELLULAR PARTNERS."

"The problem is that proteomic methods are largely a smash and grab activity," Manser explains. "You can 'tag' a protein such as paxillin and grab it once the cell has been broken, in the hope of finding its cellular partners." His team takes a more careful approach: "we tag all the local proteins before we break the cell."

The 'BioID' tagging technique used by the team[2], also developed at A*STAR, involves producing a cell line in which paxillin is fused to an enzyme that can add biotin to proteins within approximately 20 nanometers: an average protein is about five nanometers wide. These newly biotinylated proteins are easily identified among the cellular rubble inside the ruptured cell.

The researchers combined BioID with stable isotope labeling to calculate the relative enrichment of proteins relative to paxillin and kindlin-2: they used this to infer their location within the focal adhesion structure.

Previous studies had proposed hundreds of proteins make up focal adhesions. The IMCB team identified just 35 proteins involved in the

process and suggest fewer than 50 distinct focal adhesion proteins. "We also confirmed that only seven of these proteins directly bind to paxillin, which is a credible number," Manser says.

The experiments also turned up a few surprises. Paxillin was previously thought to sit on the cell surface or 'plasma membrane' — but the lack of tagged local membrane proteins indicated it lies some distance away.

"The excitement is to develop a proteomic technique that can actually give you much better resolution than optical super-resolution methods," Manser says. Studies of other protein complexes are already underway. ■

1. Dong, J.-M., Tay, F. P.-L., Swa, H. L.-F., Gunaratne, J., Leung, T. *et al*. Proximity biotinylation provides insight into the molecular composition of focal adhesions at the nanometer scale. *Science Signaling* **9**, rs4 (2016).
2. Roux, K. J., Kim, D. I., Raida, M. & Burke, B. A promiscuous biotin ligase fusion protein identifies proximal and interacting proteins in mammalian cells. *The Journal of Cell Biology* **196**, 801–810 (2012).

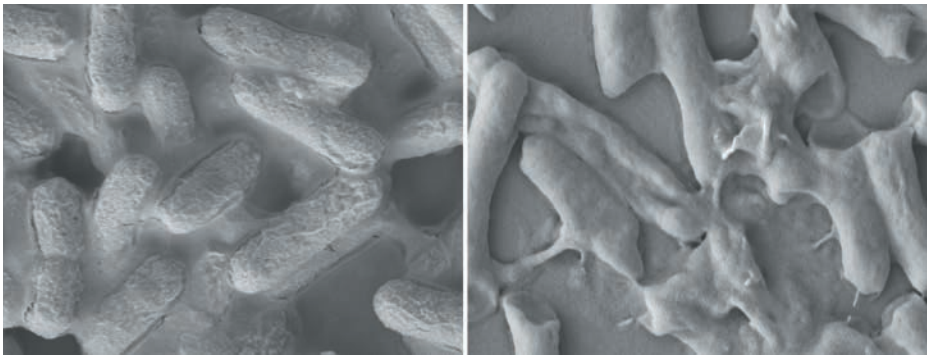
Antimicrobial materials:

KILLING BACTERIA IN SECONDS

A SYNTHETIC MATERIAL THAT KILLS COMMON BACTERIA IN SECONDS COULD HAVE FAR-REACHING APPLICATIONS IN HEALTHCARE AND DOMESTIC SETTINGS

Killing bacteria quickly and efficiently is key to tackling the spread of infections, but the recent increase in drug-resistant bacteria has made this task particularly challenging. Now, A*STAR researchers have developed a synthetic molecule capable of killing bacteria such as *Escherichia coli* in seconds, far more rapidly than any antimicrobial product, such as hand wash or surface spray, currently on the market[1].

The challenge for scientists is to create antimicrobial agents capable of killing bacteria efficiently and effectively and yet are safe for humans. Synthetic oligomers — tiny complexes that consist of a few selected molecules bound together — can be structurally engineered to exhibit certain behavior and have proven to be promising antimicrobial candidates.



A*STAR researchers have developed a synthetic molecular complex capable of destroying common bacteria in seconds. Above are *E. coli* cells before (left) and after (right) treatment with the new material, which works by penetrating and destroying the cells' membranes.

> "WE'VE BEEN WORKING ON NOVEL ANTIMICROBIAL MATERIALS FOR SIX YEARS."

"We've been working on novel antimicrobial materials for six years," says Yugen Zhang at the A*STAR Institute of Bioengineering and Nanotechnology, who led the project in collaboration with scientists from Nanyang Technological University. "We had considerable success with our previous designs, but we wanted to further improve the speed at which our oligomers could destroy bacteria effectively."

Based on prior designs, the team constructed seven new materials and tested their ability to destroy four common pathogens, including *E. coli*. First, they tested the materials for safety on mammalian cells. They then trialed the oligomers' antimicrobial activity, and analyzed how their different structures affected their performance in killing bacteria. The researchers identified one particular material, which exhibited superior efficacy compared to their other oligomer designs, and to existing antimicrobials.

"When I first saw the results from our material, I simply couldn't believe it," says Zhang. "It killed 99.7 per cent of *E. coli* in 30 seconds; an unprecedented result. We knew that the material's physical properties played a significant role in these results, so we investigated how the oligomer interacted with the bacteria using computer-aided molecular simulations."

The team created the material using positively charged molecules linked together in a chain to attract negatively charged bacteria cells. It has a unique structure with one 'tail' at each end of the chain, and they found that, once the bacterium is 'caught', these tails act like drills that penetrate and destroy the bacterial cell membranes. When the cell membranes are ruptured, the bacteria die instantly.

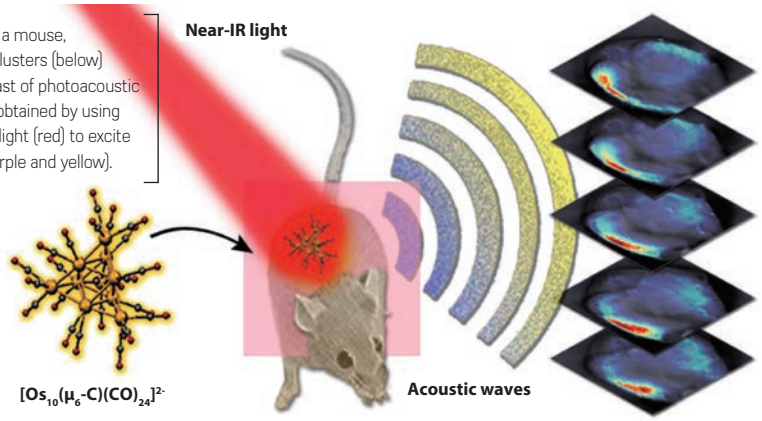
Crucially, Zhang's team also found that the oligomer is self-gelling in alcohol. This property will make the material easy to use in products such as hand wash and surface spray.

1. Riduan, S. N., Yuan, Y., Zhou, F., Leong, J., Su, H. *et al*. Ultrafast killing and self-gelling antimicrobial imidazolium oligomers. *Small* **12,** 1928–1934 (2016).

Photoacoustic imaging:

BOOSTING THE CONTRAST

THE CONTRAST OF PHOTOACOUSTIC IMAGES MAY BENEFIT FROM A PROMISING COMPOUND WITH LOW TOXICITY AND HIGH STABILITY



When injected into a mouse, osmium carbonyl clusters (below) enhance the contrast of photoacoustic images, which are obtained by using near-infrared laser light (red) to excite acoustic waves (purple and yellow).

A compound for enhancing the contrast of photoacoustic imaging — an emerging imaging modality that involves 'listening' to the sound generated by laser light — has been developed by A*STAR researchers[1].

Photoacoustic imaging is an intriguing way to capture a picture of biological tissue in the body. Researchers shine ultrashort pulses of near-infrared laser light onto the region to be imaged. Tissue absorbs the light, causing it to heat up and expand and the expansion generates sound waves that are picked up by an ultrasound detector and used to generate an image.

Since it does not use ionizing radiation, photoacoustic imaging is safer than X-ray imaging and combines the advantages of optical imaging (good contrast) with those of ultrasound imaging (high spatial resolution and tissue penetration). Currently, it is mainly used in research laboratories, but it has several potential clinical applications.

Compounds known as contrast agents are used to boost the contrast of photoacoustic images. While metal carbonyl clusters — molecules with metal atoms at their centers and limbs of carbon monoxide — have high photoacoustic contrasts, the contrast peaks at wavelengths that are too low to be useful for photoacoustic imaging.

Now, Malini Olivo at the A*STAR Singapore Bioimaging Consortium and co-workers have discovered a way to shift the optical absorption of metal carbonyl clusters to longer wavelengths. They found that using metal cores that have high nuclearity pushes the optical contrast into the near-infrared range (680 to 1,000 nanometers), which is so important for photoacoustic imaging.

When they injected osmium carbonyl clusters into the bloodstream of mice, they observed up to a four-fold enhancement in the photoacoustic signal from certain tissues, compared to that obtained with metal carbonyl clusters that have a low nuclearity.

"We demonstrated the potential of high-nuclearity carbonyl clusters of ruthenium and osmium as photoacoustic contrast agents in whole-body preclinical imaging," says Olivo. "The clusters exhibit low toxicity, high stability and superior photoacoustic stability compared to the clinically approved near-infrared dye indocyanine green."

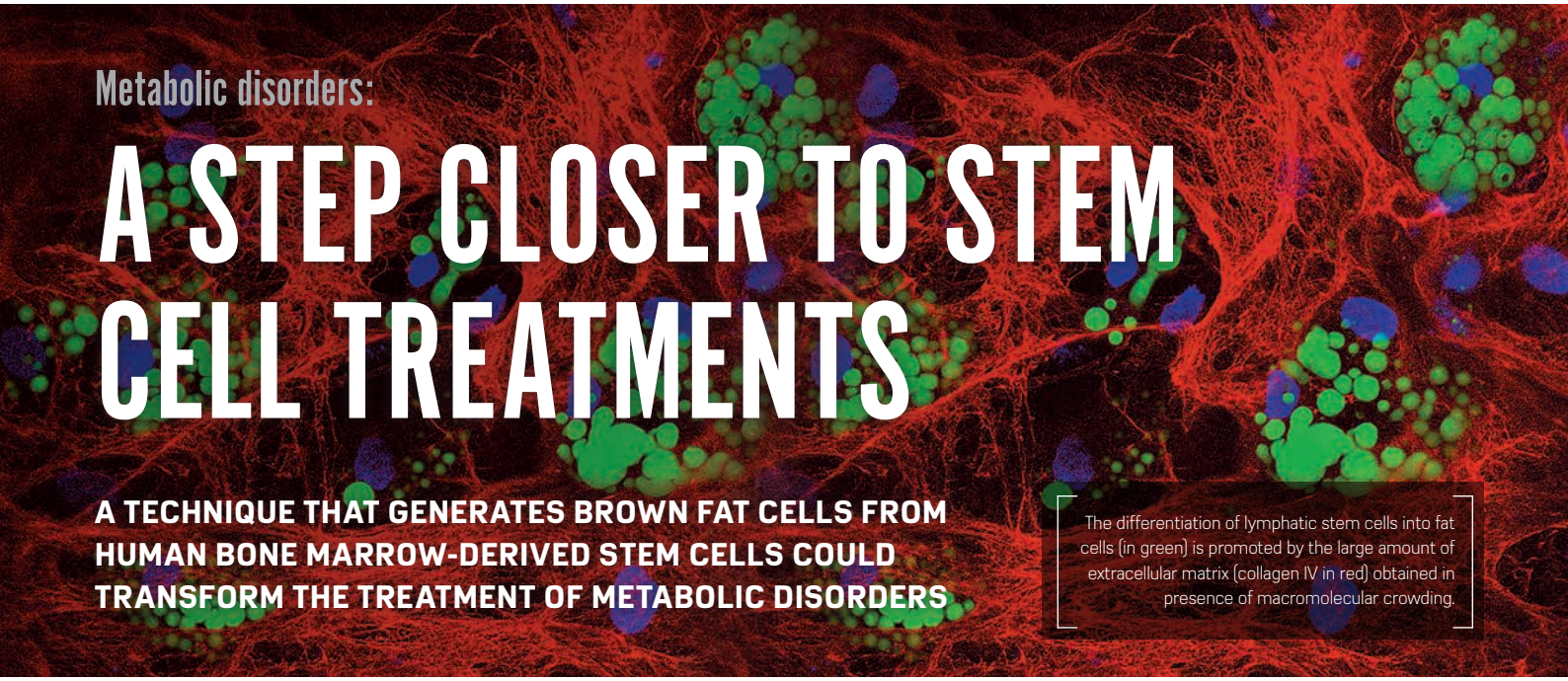
More broadly, the study emphasizes a neglected class of compounds. "This work highlights the potential biological applications of organometallic complexes, which have not been well explored," says Olivo.

"Metal-based therapeutic and imaging agents are becoming increasingly important.

The *in vivo* evaluation of this class of clusters provides insights into the toxicity of such compounds, which should help to reduce the stigma associated with heavy-metal toxicity."

The team intends to improve the biocompatibility of the clusters and also functionalize the clusters with certain ligands to enable targeted imaging.

1. Lam, Z., Balasundaram, G., Kong, K. V., Chor, B. Y., Goh, D. *et al.* High nuclearity carbonyl clusters as near-IR contrast agents for photoacoustic in vivo imaging. *Journal of Materials Chemistry B* **4**, 3886–3891 (2016).



Metabolic disorders:

A STEP CLOSER TO STEM CELL TREATMENTS

A TECHNIQUE THAT GENERATES BROWN FAT CELLS FROM HUMAN BONE MARROW-DERIVED STEM CELLS COULD TRANSFORM THE TREATMENT OF METABOLIC DISORDERS

The differentiation of lymphatic stem cells into fat cells (in green) is promoted by the large amount of extracellular matrix (collagen IV in red) obtained in presence of macromolecular crowding.

A renewable source of brown fat cells, and a simple method for generating them in cell cultures, has been discovered by A*STAR researchers and could help with the development of personalized therapies for metabolic diseases.

There are two main types of fat cells, or adipocytes, in the body: white adipocytes that store energy and brown adipocytes that burn energy and generate heat. Babies have an abundance of brown adipocytes as they help infants to keep warm, but recently scientists have discovered the cells also exist in small quantities in adults. By encouraging the body to use, rather than store, excess energy, brown adipocytes could prove useful in the engineered modulation of energy consumption and the regulation of blood sugar levels — two mechanisms that can fail in metabolic disorders such as obesity and diabetes.

Until now, there had been no safe, reliable method of generating large quantities of brown adipocytes to explore their therapeutic potential. A new technique, developed by Michael Raghunath, Cedric Badowski and co-workers at the A*STAR Institute of Medical Biology together with scientists at the National University of Singapore, hinges on unlocking the potential of mesenchymal stem cells taken from human bone marrow (bmMSCs) to differentiate into brown adipocytes.

> "WE WANTED TO DETERMINE WHICH FAT CELLS WERE PRESENT IN ADULT BONE MARROW, BECAUSE THIS HADN'T BEEN FULLY INVESTIGATED BEFORE."

"We wanted to determine which fat cells were present in adult bone marrow, because this hadn't been fully investigated before," explains Michelle Lee, a researcher on the project. "To our surprise, we found brown adipocytes present, and discovered that the bone marrow environment could trigger bmMSC differentiation into brown fat cells. We decided to emulate that environment in the laboratory."

When stem cells are taken out of the body and placed in cultures, they lose the crowded, intricate microenvironment that enables them to generate a protective extracellular matrix and function correctly. The team used a technique called 'macromolecular crowding' to mimic the microenvironment and encourage bmMSC differentiation. By adding extra molecules to bulk out the culture, Lee explains, there is an increase in reaction mechanisms within the culture that allows the cells to secrete and remodel the extracellular matrix.

Using cutting-edge microscopes to visualize the processes, the researchers found that macromolecular crowding created a cocoon of collagen around the stem cells, greatly enhancing matrix signaling and stimulating large quantities of viable, fully functional brown adipocytes from bmMSCs (see image).

"Once the potential for this technology is fully explored, we could transform the treatment of metabolic disorders and provide considerable benefits for society," says Badowski.

1. Lee, M. H., Goralczyk, A. G., Kriszt, R., Ang, X. M., Badowski, C. *et al.* ECM microenvironment unlocks brown adipogenic potential of adult human bone marrow-derived MSCs. *Scientific Reports* **6**, 21173 (2016).

BIG DATA DETECTIVES

A*STAR researchers are bringing big data genome analytics to Singapore

The cost of sequencing a human genome has plummeted over the past 15 years from one hundred million dollars to one thousand. Every seven months, the rate of sequencing doubles, with projections that by 2025 the genome of every known and catalogued species on Earth will have been spelled out, sometimes several times over.

"Genome analytics is getting more and more powerful and is pervading all aspects of science and biomedicine, both on the research side and on the clinical side," says Shyam Prabhakar, a group leader at the A*STAR Genome Institute of Singapore (GIS). But, the resources needed to compute and store millions of gigabytes of data are enormous. Scaling up the analysis is not as straightforward as just hooking up more computer processors and hiring more staff.

"A common misconception is that big data genomics just involves pouring money down a pit, turning a crank, and waiting for the answers to come out. It is a much more expert-driven exercise," says Prabhakar. "When you go from ten samples to a hundred samples to a hundred thousand samples, suddenly, even the simplest problems become awfully complicated."

"In theory, more data means more information and more knowledge. But in practice, we tend to get overwhelmed by the data and don't know where to look," says Niranjan Nagarajan, also at the GIS. "We have to invest in building systems that can cope with the data."

Prabhakar and Nagarajan recently joined together with colleagues from the GIS and other A*STAR institutes to launch a major initiative to establish infrastructure for big data genome analytics in Singapore. The Centre for Big Data and Integrative Genomics (c-BIG) is a joint collaboration between four institutes at A*STAR, including the GIS, the Bioinformatics Institute (BII), the Institute for Infocomm Research (I^2R) and the Institute of High Performance Computing (IHPC), and was formally launched at a symposium on 10 November 2016.

c-BIG includes projects designed to catalog genetic variation in Singapore, predict a cancer patient's response to drugs, prevent the

Computing, analyzing and storing millions of gigabytes of genomic data calls for large-scale collaboration.

next viral pandemic or track down the source of a bacterial outbreak. Ultimately, data-driven genomics could lead to more precise diagnoses and treatments in the clinic.

SINGAPORE SEQUENCED

Most genomic analyses rely on a reference genome, a representative assembly of the entire set of nucleotides in the genome of a particular species.

For humans, the six-billion-letter reference has since 2001 been maintained and regularly updated by a small international consortium of scientists. The entire genome of Asian and African individuals has been published, however the current genomic literature is substantially skewed to represent Caucasian males.

c-BIG is supporting the Platinum Genome Project to construct reference genomes for the Chinese, Malay and Indian populations in Singapore. These genomes will be assembled using sophisticated algorithms that patch together two types of sequencing information — long, but error-prone, DNA fragments containing up to 10,000 base pairs, and concise, precise 150-nucleotide strands. Combining the two formats will offer an extremely accurate representation of genomes in the region, to inform a growing field of medical genomics better tailored to local needs.

"We want to do our analysis locally, and not have to borrow insights from other populations. Everything in Singapore is different — from the diets, to the lifestyles, genomes, healthcare systems and compliance to drug regimens," says Prabhakar.

A larger c-BIG project called SG10K is building a database of 10,000 home-grown genomes to understand the genetic diversity within Singapore. Every section of these genomes will be read and re-read a dozen times. To achieve this, computational engineers at A*STAR, together with several research institutes in Singapore, will need to invent new algorithms. "We are taking the algorithm that many people in the world use

The A*STAR Centre for Big Data and Integrative Genomics is building reference genomes for the Chinese, Malay and Indian populations in Singapore.

to analyse hundreds of genomes, and scaling it up with innovative improvements to analyse 10,000 genomes efficiently," says Chaolong Wang, a computational geneticist at the GIS in charge of data analytics for SG10K.

CELL SCALE

Every human being is born with a unique set of DNA. And every cell in their body typically contains an identical copy of this genetic barcode. So what differentiates brain cells from fat cells? How can we account for variation among cells the way we do for variation among people? The answer, for some, is big data.

Mixed into the cellular soup are noodles of RNA that provide a snapshot of the genes being expressed. Every cell has a unique RNA expression profile, which researchers at c-BIG are beginning to characterize through the Cellular Human Bodymap (CellHuB) project. They have already sequenced every tiny snip of RNA, collectively known as the transcriptome, present in 10,000 cells and, working up to a rate of 3,000 cells a day on average, plan to profile a million cells in two years.

"It is a formidable data analytics challenge," says Prabhakar. Such single-cell analysis, he explains, could be used to compare healthy states with diseased states, or even to classify and diagnose patients more precisely.

"The big data, precision-medicine dream is to build a database on a national scale," says Prabhakar — one that includes genome sequence data, RNA extracts, patient medical records, and other demographic intelligence. "Once the database is large enough, we will start to see patterns."

To really succeed in that endeavor, the GIS has to work closely with the high performance computing experts at the IHPC and the hard-core data scientists at I^{2}R. "We are the muscle behind the analytics," says bioinformatician Feng Mengling.

MIX AND MATCH

While researchers at the GIS are more familiar with interpreting genomic sequences, researchers at I^{2}R have extensive experience in extracting and analyzing other heterogeneous clinical information.

In 2014 Feng and his team at I^{2}R developed a tool to assess the benefits of red blood cell transfusions. When to administer such interventions for patients in intensive care has been a subject of controversy. Their statistical evaluation was based on clinical reports of close to 40,000 individuals admitted to hospitals in the United States between 2001 and 2008. Feng's work found that blood transfusions doubled the chance of survival for older, sicker patients, but halved survival rates in younger, healthier patients.

Feng is also working with several hospitals in Singapore to help them anticipate when a patient might need to undergo intubation. "It is a small operation but still requires a bit of prep time. We are developing a deep learning predictive model that will give clinical care staff 12 hours to prepare for the procedure," says Feng, referring to a type of artificial intelligence that enables computers to learn by recognizing patterns. Many of these systems have been inspired by the neural networks in the human brain.

Under c-BIG, A*STAR plans to collaborate with the academic medical centers in Singapore to integrate clinical and genomic data for the first time to form one large pool of information that scientists and clinicians can dip into. "Data analytics can offer physicians the evidence needed to make more effective decisions, which will benefit their patients," says Feng.

Meanwhile, researchers at the IHPC are developing high-performance artificial intelligence tools and a collaborative platform needed to power c-BIG. "We are excited to contribute our expertise and technologies in high-performance computing and artificial intelligence to efficiently and intelligently analyse the vast trove of medical and genomic data," says computer scientist Rick Goh Siow Mong at the IHPC. "Through this program, we hope to advance the study of how specific medicine can be administered based on the detected variability in an individual — this is no mean feat."

UNWELCOME SUPERBUGS

In the time it takes to sequence a single human, machines can sequence a hundred bacterial genomes. "Bacteria are cheaper to sequence, which means that we can collect thousands or even millions of genomes to get a really fine-grained view of how bacteria evolve in an environment," says Nagarajan, who is doing exactly that as part of a joint project between c-BIG and the GIS Efficient Rapid Microbial Sequencing (GERMS) platform known as Resistance and Outbreak Tracking in Singapore (ROUTES).

Nagarajan is building a tool that can study the diversity and evolution of the hundreds of trillions of bacteria residing inside the human gut. More specifically, he is looking at how some 'superbugs' become resistant to a last-resort class of antibiotics known as carbapenems. Killing almost half of the patients they infect, these superbugs are spreading fast, from New York to Israel, Greece and further east, but they have yet to cause serious trouble in the Singaporean stomach.

Nagarajan wants to find out how these bacteria are transmitted between individuals, what conditions make them feel more or less welcome in the gut, and how their presence affects the gut environment. His research could even lead to potential remedies, whether it be a bacteria that the new residents find repulsive or one that can kill them. "There is an arms race within bacteria, and a lot of groups are searching the genetic information of microbial communities for potential antibiotics."

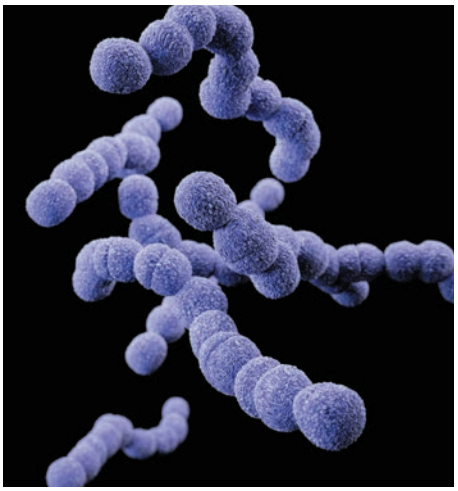
A FISHY INFECTION

In early 2015, a mysterious outbreak of group B streptococcus appeared in Singapore. Streptococcus typically lines the intestines and urinary tracts of one-third of healthy adults, and is mostly considered harmless. The only doctors who really worry about the disease are obstetricians, because of the risk it poses to newborns. But here were strong and fit adults succumbing to the infection, arriving at emergency wards feeling feverish and confused, with severe headaches.

Between January and July, 238 streptococcus cases were detected in Singapore, compared to an annual average of 150. Government officials soon traced these cases to a popular dish of raw freshwater fish called yusheng, which is served with rice porridge at hawker stalls. "Group B streptococcus was not known to be a food-borne illness until this outbreak," says Swaine Chen at the National University of Singapore and GIS.

Chen and his colleagues used genomic sleuthing to further investigate the source of the outbreak. "We found that more than 90 per cent of the group B streptococcus cases during that period were infected by the exact same strain," he says. "It was a total slam dunk."

Genomics offers a level of precision that other detective tools cannot, and has become common practice in outbreak surveillance. It was used to assess the 2014 Ebola epidemic, and is regularly used to monitor listeria and salmonella contamination in food. As part of ROUTES, Chen is trying to increase the scale and speed of such analyses for almost any bacteria.



Courtesy of CDC/ James Archer

Data detectives confirmed the food source of a spike in group B streptococcus infections in Singapore in 2015.

© Simon Bottomley/DigitalVision/Getty

Influenza virus sequencing has shown that even penguins can catch the flu.

Driving the initiative is the proliferation of genomic bacterial data. He now has access to 2,000 strains of group B streptococcus, with close to 50,000 strains of *Escherichia coli* and 130,000 strains of *Streptococcus pneumoniae* becoming publically available in the next year. Once the system is in place, Chen's team could analyse the raw bacterial data of an emerging outbreak within six hours, instead of the week it took them in 2015. Eventually, the system will be so easy to use that physicians could do the detective work themselves. "We want clinicians to be able to manage the deluge of data without needing to know much about the low-level processing."

PENGUIN FLU

What Chen is doing for bacteria, Sebastian Maurer-Stroh at the BII is doing for viruses. In the midst of the swine flu pandemic in 2009, he developed FluSurver, a public online tool for analyzing sequences of the influenza virus to identify mutations and determine how those changes affect the structure of the virus.

The World Health Organization's national influenza centers based in 113 countries use FluSurver for their surveillance, connected to the virus database of the Global Initiative for Sharing All Influenza Data (GISAID), which amounts to up to 20,000 sequences of influenza analyzed every year. The tool has been used to spot where and when new mutations emerge in distant reaches of the world, including antiviral resistant swine flu variants in Singapore and Australia, as well as highly pathogenic strains of the avian influenza virus in Mexican chicken farms and in live poultry markets in eastern China. In 2014, a large collaborative team including Maurer-Stroh confirmed for the first time that even penguins catch the flu, and that their flu type is not dangerous to humans.

Beyond FluSurver, the BII has applied genomic analysis tools to outbreaks of norovirus, adenovirus, dengue, hepatitis C, and even the Zika virus in Singapore. In 2016 for example, the BII team, together with the National Public Health Laboratory of the Ministry of Health, rapidly characterized the local Zika strain linked to a large cluster of cases in Singapore. Under c-BIG, Maurer-Stroh also plans to work with colleagues at the I^2R to map the genetic diversity of dengue against the movement of commuters through the public transport system.

Eventually, c-BIG expects to take such analyses into our everyday lives. "In the future, genomics will be just as ubiquitous as computing — everyone will have sequencers in their hands, their homes and various devices, essentially serving as sensors for life," says Nagarajan. "We need to develop algorithms and robust systems that can aggregate data, make inferences and provide useful information about our environment in real-time."

Fat cells that build up around internal organs have higher levels of retinoic acid — a molecule derived from vitamin A — than fat cells in the fat layer beneath the skin.

Obesity:

FAT CELL GENETICS COULD BE KEY TO FIGHTING OBESITY

THE SECRET TO PREVENTING HARMFUL FAT BUILDING UP AROUND INTERNAL ORGANS MAY LIE IN THE GENETICS OF FAT STEM CELLS

A molecular mechanism that influences the development of fat cells has been identified by A*STAR researchers[1]. The discovery suggests that an excessive amount of vitamin A could have a role in obesity, and the work could lead to new strategies to ameliorate the risks of fat depositing around internal organs.

Humans have at least two types of fat: subcutaneous fat that sits in a layer beneath the skin, and visceral fat that collects around internal organs. The two fat cell types develop differently and have different properties. Subcutaneous fat is relatively harmless, but visceral fat is considered 'bad' or of 'low quality', and an excess of it leads to visceral obesity, which is associated with metabolic diseases such as diabetes.

"The contributions of subcutaneous and visceral fat to pathology and physiology are known to be different, but the molecular mechanisms of these differences were unclear," explains Shigeki Sugii from the A*STAR Singapore Bioimaging Consortium. "By understanding the molecular basis, it is possible to explore therapeutic targeting to improve the quality of visceral fat."

Sugii and colleagues started from the assumption that the distinct properties of subcutaneous and visceral fat cells arise from inherent differences in the stem cells that they develop from. To look for these differences, they analyzed the gene expression in human fat-derived stem cells from subcutaneous and visceral fat. Their analysis revealed gene expression differences that ultimately led to high levels of retinoic acid in visceral fat cells. Further experiments in cultured fat stem cells showed that retinoic

acid inhibits proper development of these cells into mature, functional fat cells. Visceral fat cells are compromised in their proper fat functions, though they can expand in size by gobbling up excessive lipids. The researchers went on to demonstrate that the high levels of retinoic acid were due to its upregulation by a developmental factor called WT1, and that blocking WT1 or retinoic acid signaling reversed the developmental defect in visceral fat stem cells.

"Our findings imply that the developmental origin of visceral fat is different from that of subcutaneous fat, and that this difference results in retinoic-acid-mediated differences in fat cell quality," says Sugii.

Owing to the relationship between retinoic acid and visceral obesity, Sugii says that his team needs to now look closely at whether excessive intake of vitamin A increases the risk of visceral obesity. He also suggests that their findings could lead to new treatments to prevent visceral obesity and its associated complications, such as metabolic diseases.

"A potential therapeutic approach that targets the retinoic acid signaling pathway would be worth testing for counteracting visceral obesity in animal models," says Sugii.

1. Takeda, K., Sriram, S., Chan, X. H. D., Ong, W. K., Yeo, C. R. *et al.* Retinoic acid mediates visceral-specific adipogenic defects of human adipose-derived stem cells. *Diabetes* **65**, 1164–1178 (2016).

Materials science:

UNDERSTANDING HOW FLAT PHOSPHORUS GROWS

MODELING THE GROWTH OF TINY FLAKES OF A TWO-DIMENSIONAL FORM OF PHOSPHORUS COULD HELP RESEARCHERS ONE DAY PRODUCE BETTER ELECTRONICS

The door to developing superior electronic devices, such as flexible circuits, has been nudged open by A*STAR researchers' modeling of possible methods to manufacture one of the crucial ingredients.

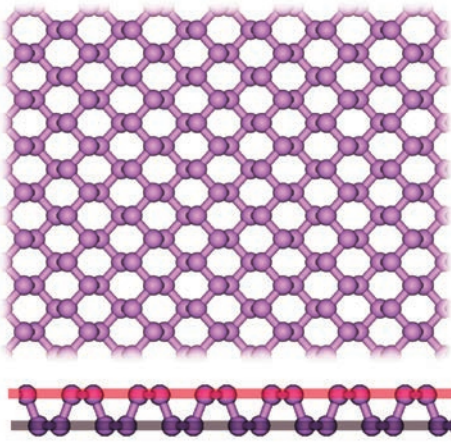
Phosphorene is a two-dimensional (2D) form of the element phosphorus. Despite having electronic properties superior to other 2D materials such as graphene (2D carbon) and silicene (2D silicon), phosphorene's potential for application in high-performance devices has been limited by how difficult it is to reliably produce commercially viable quantities of it in large, thin, high-quality nanosheet form.

At present, phosphorene can only be obtained by mechanical and chemical exfoliation of black phosphorus, which is costly and produces low yields of uneven films. Other 2D materials such as graphene and molybdenum disulfide can be directly grown using chemical vapor deposition and physical vapor deposition, but no such methods exist for growing phosphorene.

The new model developed by Junfeng Gao and colleagues from the A*STAR Institute of High Performance Computing will make it possible for researchers to tackle this challenging technical problem by choosing the best process conditions for the growth of large-size, high-quality phosphorene directly on a surface[1].

Gao and the team tried to find the best way to grow high-quality single layers of phophorene directly on a surface by modeling the effect of different substrates on the growth of a phosphorene flake containing just 27 atoms.

"The stability of the growing nanoflake is highly sensitive to the substrate and crucial to its continued growth," explains Gao. "If the interaction strength is too weak, the substrate causes the flake to buckle; but if the interaction is too strong, the inner bonds



Black phosphorene (depicted above) nanoflakes are only dynamically stable on a moderate interaction substrate, leading to continuous growth of single layer phosphorene.

between the phosphorene atoms will break and an alloy may form."

The researchers compared the effect of two different substrates on the growth of the phosphorene nanoflake — a copper substrate, commonly used for growing graphene, which bonds with the phosphorene through strong chemical processes, and a hexagonal hydrogen boron nitride (h-BN) substrate that couples with the phosphorene via weak van der Waals bonds.

The copper substrate caused the nanoflake to break, whereas the h-BN was unable to stabilize its flat structure. By boosting the strength of the bonding between the nanoflake and the h-BN substrate, their simulations showed that the 2D growth of the phosphorene was maintained. "Our work is the first attempt to explore the direct growth of phosphorene and provides guidance in the search for suitable substrates," says Gao.

1. Gao, J., Zhang, G., Zhang, Y.-W. The critical role of substrate in stabilizing phosphorene nanoflake: A theoretical exploration. *Journal of the American Chemical Society* **138**, 4763–4771 (2016).

Cyber security:

WEAKNESS OF 2G MOBILE PHONE NETWORKS REVEALED

A FAST AND RELATIVELY SIMPLE ATTACK ON SECOND GENERATION DIGITAL MOBILE PHONE COMMUNICATIONS HIGHLIGHTS THE NEED TO UPDATE SECURITY ON OLDER MOBILE NETWORKS

A*STAR researchers have shown that the 2G GSM encryption scheme can be hacked within seconds and urgently needs to be updated.

The encryption scheme used for second generation (2G) mobile phone data can be hacked within seconds by exploiting weaknesses and using common hardware, A*STAR researchers show. The ease of the attack shows an urgent need for the 2G Global System for Mobile Communications (GSM) encryption scheme to be updated.

GSM was first deployed 25 years ago and has since become the global standard for mobile communications, used in nearly every country and comprising more than 90 per cent of the global user base.

"GSM uses an encryption scheme called the A5/1 stream cipher to protect data," explains Jiqiang Lu from the A*STAR Institute for Infocomm Research. "A5/1 uses a 64-bit secret key and a complex keystream generator to make it resistant to elementary attacks such as exhaustive key searches and dictionary attacks."

Any encryption scheme can be hacked given sufficient time and data, so security engineers usually try to create an encryption scheme that would demand an unfeasible amount of time to crack. But, as GSM gets older, weaknesses in the A5/1 cipher and advances in technology have rendered GSM communications susceptible to attack.

Straightforward 'brute force' attacks by guessing the secret key from the data stream are still intensively time consuming, and although A5/1 was reported to have been successfully attacked in 2010, the details of the attack were kept secret. By exploiting weaknesses in the A5/1 cipher, Lu and his colleagues have now demonstrated the first real-time attack using a relatively small amount of data.

"We used a rainbow table, which is constructed iteratively offline as a set of chains relating the secret key to the cipher output," says Lu. "When an output is received during an attack, the attacker identifies the relevant chain in the rainbow table and regenerates it, which gives a result that is very likely to be the secret key of the cipher."

Using two specific exploits, Lu's team was able to reduce the effective complexity of the key to a level that allowed a rainbow table to be constructed in 55 days using consumer computer hardware, making possible a successful online attack, in most cases within just nine seconds.

"GSM is still widely used in telecommunications, but its A5/1 encryption system is now very insecure," says Lu. "Our results show that GSM's 64-bit key encryption is no longer sufficient and should be upgraded to a stronger scheme as a matter of urgency."

1. Lu, J., Li, Z. & Henricksen, M. Time–memory trade-off attack on the GSM A5/1 stream cipher using commodity GPGPU. Applied Cryptography and Network Security (2015). 13th International Conference, ACNS 2015, New York, NY, USA, June 2–5, 2015, in *Lecture Notes in Computer Science* **9092**, 350–369.

Plasmonics:

LIGHTING THE WAY TO MINIATURE DEVICES

ELECTROMAGNETIC WAVES CREATED ON A LAYER OF ORGANIC MOLECULES COULD PROVIDE THE PERFECT ON-CHIP LIGHT SOURCE FOR FUTURE QUANTUM COMMUNICATION SYSTEMS

A team of scientists including A*STAR researchers has captured tiny flashes of light from an ultrathin layer of organic molecules sandwiched between two electrodes that could replace lasers and LEDs as signal sources for future miniature, ultrafast quantum computing and light-based communication systems[1].

To investigate electromagnetic waves called plasmons, which skim along the interface between two materials, Nikodem Tomczak from the A*STAR Institute of Materials Research and Engineering and colleagues collaborated with Christian A. Nijhuis from the National University of Singapore to construct a junction consisting of a layer of thiol molecules on a metal electrode and liquid gallium-indium alloy as a top electrode.

The team created plasmons by applying a voltage across the thiol layer. Although thiol is an insulator, the layer was thin enough for electrons to quantum tunnel between the electrodes, exciting plasmons on the thiol layer's surface in the process. The plasmons then decayed into photons, tiny pulses of light that Tomczak and his colleagues were able to detect.

"We were surprised that the light did not come from the whole junction, but instead just from very small spots that blink at different frequencies," said Tomczak.

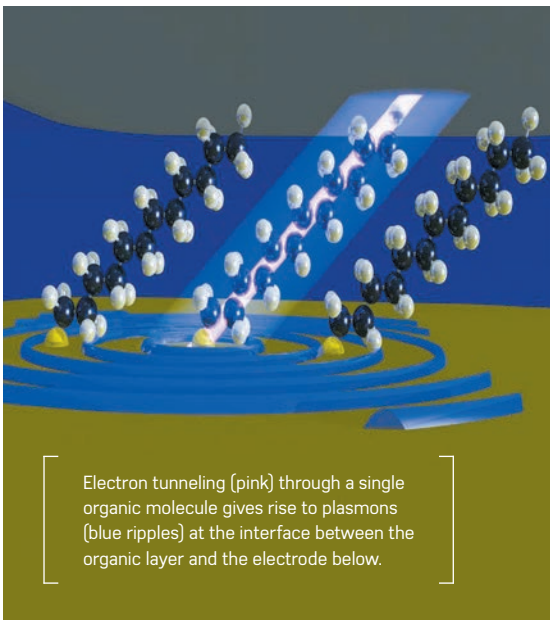
The team found that the light generated by the plasmons was polarized, and that both the polarization and the wavelength of the light varied with the voltage applied across the junction and the molecules used to form the organic layer.

"The spots are diffraction-limited, polarized and their blinking follows power-law statistics," said Tomczak. "We need further experiments to confirm, but it is very similar to emission from other single photon sources, such as quantum dots or nanodiamonds."

Further evidence that the light is from plasmons decaying into a single photon came from Chu Hong Son and his team at the A*STAR Institute of High Performance Computing who modeled the spots as the product of the smallest possible source, a single dipole emitter, and achieved results consistent with the experimental observations.

Tomczak believes the layers can be scaled down to junctions built from a single molecule, opening up the potential to integrate plasmonic light sources on to silicon-based circuits, replacing large external light sources such as a laser.

The team also explored one-molecule thick layers of carbon chains terminated with a metallic ferrocene group developed by the group of Christian A. Nijhuis. Because this compound is asymmetric, it allows tunneling in one direction, effectively acting as a diode.



Electron tunneling (pink) through a single organic molecule gives rise to plasmons (blue ripples) at the interface between the organic layer and the electrode below.

"By keeping the same architecture and tuning the chemistry of the monolayer you can create a range of different devices," said Tomczak.

1. Du, W., Wang, T., Chu, H.-S., Wu, L., Liu, R. *et al.* On-chip molecular electronic plasmon sources based on self-assembled monolayer tunnel junctions. *Nature Photonics* **10**, 274–280 (2016).

Malignant lymphoma cells attack the body instead of protecting it.

Cancer genetics:

FACING OFF A DEADLY MUTATION

A GENE MUTATION THAT MAKES THE BODY TURN LIFE-SAVING IMMUNE CELLS INTO A RARE AND DANGEROUS CANCER HAS BEEN IDENTIFIED FOR THE FIRST TIME

The cause of a rare and highly aggressive form of non-Hodgkin lymphoma that attacks the face has been discovered by an international genome-scanning research project.

The team found that people with a mutation in a gene that helps the body recognize foreign invaders had almost twice the risk of developing the cancer known as extranodal natural killer T-cell lymphoma (NKTCL).

Study co-author, Chiea Chuen Khor, a medical doctor and group leader at the A*STAR Genome Institute of Singapore, said natural killer T cells normally played a crucial role in rapidly responding to both tumor cells and viruses.

These cells destroy pathogens before they take hold in the body by recognizing tiny molecules on their surface and launching an attack.

"When people have this mutation, instead of destroying pathogens, some of the natural killer T cells can betray the body and turn malignant," he says. "These cancerous cells can eat away at tissues in the face".

If it is not discovered early, about half of all sufferers die within five years.

"Our study suggests that the way in which the person's immune cells initiate their immune response is critical," Khor says. "Initiating too weak an immune response encourages the tumor cells to proliferate and causes the tumor to embed and grow."

Khor's team examined 514 people with the cancers and more than 5,800 unaffected people across parts of China, Hong Kong and Singapore. They found a mutation in a gene called *HLA-DPB1* increased the likelihood of the cancer by 84 per cent.

"*HLA-DPB* codes for the critical role of presenting foreign antigens to the immune system to initiate its response," Khor says. "When *HLA* genes are involved, it is a delicate balance between killing invading germs and tumor cells, and avoiding the friendly fire of autoimmunity."

People with a mutation in *HLA-DPB* also appear more susceptible to Beryllium disease, in which the immune system attacks the lungs.

Previous research has linked NKTCL with infection with Epstein-Barr virus, which Khor says is usually relatively harmless in the body.

It is still not known why Epstein-Barr could trigger NKTCL, although it has also been linked to the development of another type of nasal cancer.

"Normally our immune system is good, strong, and is able to control the virus well. However, sometimes, when the immune system is weaker, the virus is not so well controlled. In that light, perhaps the virus could have malignant transformative potential," Khor says.

1. Li, Z., Xia, Y., Feng, L., Chen, J., Li, H. *et al.* Genetic risk of extranodal natural killer T-cell lymphoma: a genome-wide association study. *The Lancet Oncology* **17**, 1240–1247 (2016).

Structural biology:

X-RAY CRYSTAL STRUCTURE OF A HUMAN PROTEIN COMPLEX

SOLVING THE 3D STRUCTURE OF A NEWLY OBSERVED PROTEIN COMPLEX IN MAMMALIAN CELLS PAVES THE WAY FOR INTERESTING APPLICATIONS

The first three-dimensional (3D) structure of a human protein complex within intact mammalian cells has been obtained directly by A*STAR scientists[1]. It could provide new opportunities in structural biology, in developing cellular sensors and in validating anti-cancer drugs that target a specific protein.

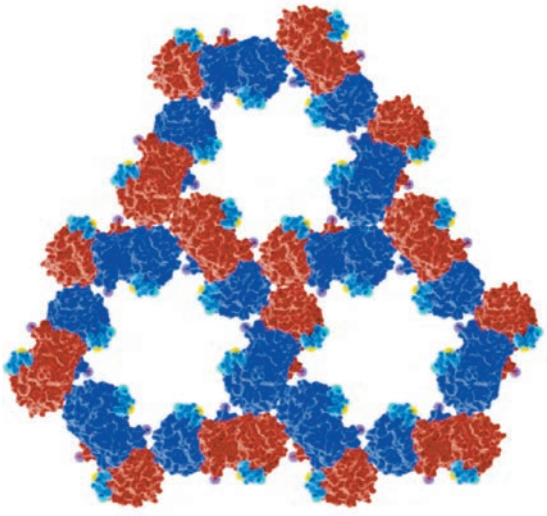
The complex generates a highly ordered honeycomb shape made of two proteins: PAK4 (p21-activated kinase 4) and its newly discovered inhibitor Inka1. PAK4 is essential for several cellular processes occurring in the junctions between cells, and when malfunctioning, plays a role in cancer metastasis.

"We discovered that Inka1 is the natural inhibitor of PAK4, and we observed by chance that when mammalian cells are engineered to make both these proteins, PAK4 spontaneously forms long crystals," reveals Ed Manser, from the A*STAR Institute of Molecular Biology and Cell Biology. The group were the first to discover the PAK kinases.

Creating highly ordered arrangements of proteins, known as protein 'crystals', is the first step to determine their 3D structure through X-ray analysis. Since it is very rare for these crystals to form inside a cell, all protein 3D structures obtained so far have been crystallized artificially, from highly pure and homogeneous protein samples. In this study, however, the atomic structure can be determined without removing the crystals from the cells.

Teaming up with Robert Robinson's group, and using an X-ray microbeam to shoot at these tiny intracellular crystals, the scientists were able to achieve a high structural resolution of 0.295 nanometers — comparable to the one obtained with PAK4 complexes crystallized outside of the cell.

The research team also took advantage of the organization of this crystal. The PAK4 forms a hexagonal frame, similar to wax cells in a honeycomb. This hexagonal scaffolding has internal channels that can host other proteins. In the first instance, the research team fused Inka1 with a well-known fluorescent protein called GFP, allowing them to follow crystal growth with much



The organization of proteins PAK4 (red and blue) and Inka1 (light blue) as determined from crystals formed inside cells.

more precision. Then, it turned out that almost any protein small enough, when fused to Inka1, can be crystallized inside the PAK4 honeycomb.

The team also monitored where and how quickly these crystals grew inside human cells. In most cases the crystals reached the full length of the cell cytoplasm — between 50 and 100 micrometers — before pausing. "Surprisingly cells tolerate the foreign crystal growing inside them and remain alive for days. Current spinoffs of this system include introducing environmentally responsive proteins into the crystals, which can make incredibly bright sensors," concludes Manser.

1. Baskaran, Y., Ang, K. C., Anekal, P. V., Chan, W. L., Grimes, J. M., Manser, E. & Robinson, R. C. An *in cellulo*-derived structure of PAK4 in complex with its inhibitor Inka1. *Nature Communications* **6**, 8681 (2015).

Nutrition:

PROTEIN IN PREGNANCY FOR HEALTHIER BABIES

THE GLOBAL CHILDHOOD OBESITY EPIDEMIC COULD BE TACKLED BY EXPECTANT MOTHERS ADJUSTING THEIR DIET

Research in Singapore finds that babies have lower abdominal fat when their mothers' diet is relatively protein-rich and low in carbohydrate and fat during pregnancy. A*STAR researcher Mary Chong says this revelation may offer a new strategy for very early intervention to tackle the growing problem of obesity in childhood and later life.

Diet in pregnancy could reduce child obesity.

This research is part of a large scale ongoing study of mothers and infants called 'Growing Up in Singapore Towards healthy Outcomes (GUSTO)', a collaboration between Singapore's National University Health System (NUHS), KK Women's and Children's Hospital (KKH) and the A*STAR Singapore Institute for Clinical Sciences.

The dietary choices of 320 pregnant women were recorded around the 27th week of pregnancy, followed by MRI scans to detect the abdominal fat levels of their babies two weeks after birth[1]. These scans reveal details of the distribution of abdominal fat, which is more revealing than merely assessing total fat in the body. The women consuming most protein were still eating amounts of protein within the normal range recommended by dietary guidelines. The effect on their babies' abdominal fat levels was particularly noticeable in boys, and was only linked to

high consumption of animal protein, not plant protein.

"Childhood obesity and metabolic diseases have reached epidemic levels globally," says Ling-Wei Chen, joint first author of the research paper, together with Mya-Thway Tint, both at Singapore's Yong Loo Lin School of Medicine. He adds that Asians are at higher risk of metabolic diseases than Caucasians of similar BMI levels. The study's focus on mothers of Asian origin therefore makes it especially relevant for planning effective dietary guidance throughout the region.

One strength of the GUSTO study is that it can tease out differences related to different cultural backgrounds. The beneficial influence of high protein diets, for example, was stronger in Chinese and Indians than in Malays. "This may be due to inherent differences in body composition, or dietary pattern, among these groups," says Yung Seng Lee, also of the A*STAR group.

The researchers are now monitoring the children through their early years to detect longer- term effects. They have already performed new MRI scans in the children's fifth year, with further scans planned between 12 and 14. "Our early results may provide invaluable information for offering better nutritional guidance to pregnant women and those planning a pregnancy, but we need to continue to track the growth of these children to confirm this" says Lee. ■

1. Chen, L-W., Tint, M-T., Fortier, M. V., Aris, I. M., Bernard, J. W. *et al.* Maternal macronutrient intake during pregnancy is associated with neonatal abdominal adiposity: The growing up in Singapore towards healthy outcomes (GUSTO) study. *The Journal of Nutrition* **146**, 1571–1579 (2016).

Monocytes have a one-step pathway for inflammasome activation.

Immunology:

ONE-STEP IMMUNE ACTIVATION

HOW WHITE BLOOD CELLS JUMP INTO ACTION IN RESPONSE TO FOREIGN MICROBES

Pro-inflammatory molecules in the blood are essential for fighting off microbial invaders. But too much of these immune-signaling factors, and the body can go into septic shock. A team from the A*STAR Singapore Immunology Network has now elucidated the mechanism by which bacterial pathogens can rapidly trigger the processing of a key pro-inflammatory protein into its active form[1].

This discovery "offers new drug targets for acute and chronic inflammatory disorders, including sepsis," says Alessandra Mortellaro, the A*STAR immunologist who led the research.

The body's immune system reacts to microbial infection through a group of white blood cells that release immune-signaling molecules known as cytokines, including one called interleukin-1β (IL-1β). Yet, this cytokine occurs first in a biologically inactive form, and must be processed by caspase-1, an enzyme which itself is activated by a multi protein complex called the inflammasome.

In most types of immune cells, revving up the inflammasome requires a priming stimulus followed by a second activation signal. But in monocytes — the key mediators of early responses to infection — inflammasome activation happens in a distinctive one-step pathway following exposure to a component of the bacterial wall, known as a lipopolysaccharide (LPS). How this occurs, however, was poorly understood until Mortellaro and her colleagues found out.

The researchers isolated monocytes from human blood, cultured the cells in their laboratory, and then added LPS derived from the bacterium *Escherichia coli*. They noticed that two enzymes, caspase-4 and -5, seemed to be expressed in the cells following LPS exposure, and this led to rapid processing of the caspase-5.

Mortellaro and her team then blocked the activity of these enzymes to show not only that caspase-4 and -5 are essential for cytokine release, but that some of the products of caspase-5 processing and other key molecular

players are needed for the one-step activation to work, with no additional cues besides LPS.

Notably, all of the experiments were conducted with human monocytes, not mouse cells as most previous studies had done, and as Mortellaro notes: "There are considerable differences between human and mouse immunity."

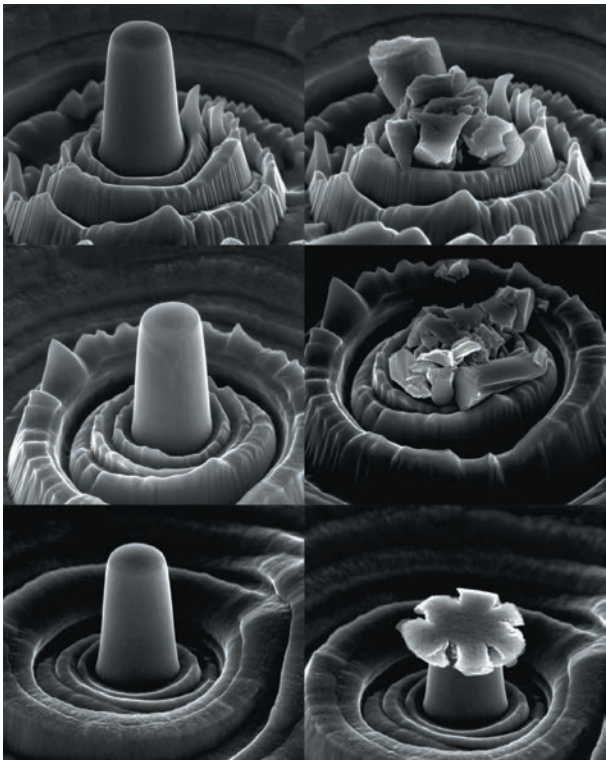
Since septic shock results from the flood of cytokines like IL-1β that can follow an infection, Mortellaro hopes that blocking the one-step activation pathway she teased apart could have therapeutic value in patients with sepsis. And there could be applications in other disease contexts, too. "It would be interesting to investigate this pathway in a context of metabolic and chronic inflammatory diseases," Mortellaro says.

1. Viganò, E., Diamond, C. E., Spreafico, R., Balachander, A., Sobota, R. M. & Mortellaro, A. Human caspase-4 and caspase-5 regulate the one-step non-canonical inflammasome activation in monocytes. *Nature Communications* **6**, 8761 (2015).

Materials:

STRESSED OUT COATINGS

FINE-GRAINED MICROSTRUCTURE COULD TOUGHEN PROTECTIVE COATINGS



Scanning electron microscope images show pristine (left) and compressed (right) micropillars of (top) chromium nitride, (middle) chromium aluminum nitride, and (bottom) chromium aluminum nitride / silicon nitride nanocomposite.

Hard materials like chromium nitride are used as wear and corrosion protection coatings in a wide range of applications, including metal cutting. Now, A*STAR researchers have discovered exactly how such materials behave when used in high-stress situations, paving the way to producing even better coatings[1].

One way to improve a material's resistance to wear is to increase its hardness. This depends mainly on the force it can withstand before it starts to permanently deform. In most crystalline materials, this deformation occurs when defects, known as dislocations, start to move through a material's crystal structure.

Currently used coating materials are very brittle, with a toughness only a little more than that of window glass. Also, previous research has shown that it is very difficult to break crystals that are extremely small. So Shiyu Liu of the A*STAR Singapore Institute of Manufacturing Technology and co-workers have used this effect to study how coatings based on chromium nitride might deform.

The researchers first made microscopic pillars of the material, roughly 380 nanometers across. Then they compressed them using a diamond flat punch in a scanning electron microscope at temperatures up to 500 degrees Celsius, and studied their responses (see image).

They found that if the chromium-nitride-based coatings are made with very fine grains, each roughly 10 nanometers across, with each grain separated by a thin grain boundary phase, the force required to deform such materials increased dramatically. Indeed, deformation began at stresses very much higher than expected, and close to the theoretical maximum value from calculations. Liu's team has shown that this increase happened when the grains became so small that they did not contain dislocations, so that the applied forces had to be sufficiently large to form new dislocations within the grains.

It had long been thought that the thin grain boundary phase would be the main factor in determining the material's properties. However, the researchers have shown this was not the case, providing a way to reliably make a hard material.

The results show that the formation of a fine-grained microstructure could provide a

ceramic coating with enhanced hardness and fracture toughness. "This could be a viable approach for the development of super-hard and tough protective coatings for high-temperature and high-pressure applications," says Liu.

The team plans to use the results in advanced manufacturing and engineering applications, such as protective coatings in high-speed machining tools for titanium and nickel-based alloys.

1. Liu, S., Wheeler, J. M., Michler, J., Zeng, X. T. & Clegg, W. J. Plastic flow at the theoretical yield stress in ceramic films. *Scripta Materialia* **117**, 24–27 (2016).
2. Liu, S., Raghavan, R., Zeng, X. T., Michler, J. & Clegg, W. J. Compressive deformation and failure of CrAlN/Si_3N_4 nanocomposite coatings. *Applied Physics Letters* **104**, 081919 (2014).

Aging:

SEARCHING FOR A CURE FOR FRAILTY

AN IN-DEPTH ANALYSIS OF BLOOD CELLS REVEALS SIGNS OF AGING

"Doctors can't give you drugs just because you complain of being old," says Anis Larbi, who is trying to revolutionize the way elderly people are cared for. Larbi and his team at the A*STAR Singapore Immunology Network (SIgN) are looking at aging as a complex process, including the appearance of symptoms as with disease, the first of which is frailty.

Along with collaborators in Singapore, China and Canada, Larbi's team is the first to identify a link between inflammation, immunity and the signs of physical frailty[1]. "By determining the biological signature of frailty, we can start thinking of possible medical interventions."

Frailty is a condition associated with aging that limits a person's mobility and increases their risk of falling, hospitalization and death. "Frailty per se will not kill you," says Larbi. "But it will affect your quality of life." Unlike most diseases for which there are underlying causes, scientists have typically described frailty in purely symptomatic terms. Larbi and his co-workers wanted to understand whether an 'immunological frailty' is also present in the general state of malaise.

A*STAR researchers are looking for biological markers of physical frailty.

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The researchers recruited around 100 Singaporean adults aged 55 or older from the Singapore Longitudinal Aging Study. They were assessed for their level of frailty based on two established models — the Fried frailty status, which looks at five physical symptoms from slowness to weight loss, and the Rockwood Frailty Index, which measures a broader range of dysfunctions. Blood samples were analyzed for markers of inflammation and signs of aging in immune cells.

Of the multiple biomarkers simultaneously tested using multiplex technology at the Immunomonitoring Platform at SIgN, eight were found to be either positively or negatively associated with frailty. Two of the biomarkers are linked to interleukin-6, an inflammatory cytokine which is associated with many age-related diseases.

At the cellular level, frailer people had higher levels of two types of white blood cells — poorly functioning, exhausted B cells, and inflammatory $CD14^+CD16^+$ monocytes. Cytotoxic T cells in these individuals "looked older", expressing lower levels of a protein called CD28, which is essential for their activation, proliferation, differentiation and overall survival.

The researchers are conducting similar analyses on elderly populations in other regions. "We want to know if these indicators are specific to Asia, or if there is a universal signature for frailty," says Larbi. Further molecular studies could ultimately lead to methods for detecting frailty early in life and identifying pathways to slow down debilitating physical symptoms.

1. Lu, Y., Tan, C. T. Y., Nyunt, M. S. Z., Mok, E. W. H., Camous, X. *et al.* Inflammatory and immune markers associated with physical frailty syndrome: findings from Singapore longitudinal aging studies. *Oncotarget* **7**, 28783–28795 (2016).

[VOICES FROM A*STAR]

www.research.a-star.edu.sg/blog

*Voices from A*STAR* is a monthly blog published on the *A*STAR Research* website. It features a personal account of the challenges and rewards of a life in science by A*STAR researchers from a range of disciplines. Staff interested in contributing to the *Voices from A*STAR* blog are encouraged to contact the Managing Editor.

Russell Hewitt

Scientist II, ICES

"Chemists are now trying to make processes green as early as possible both to improve throughput and hasten the development toward commercial production. Ultimately this delivers the chemicals we need, in quantities that we can use, with a holistic view on safety for workers, the public and the environment."

Sandhya Sriram

Programme Management Officer, SBIC

"Scientists have a responsibility to work with the media to deliver the 'right' science to the public and make sure it reaches them in a timely fashion."

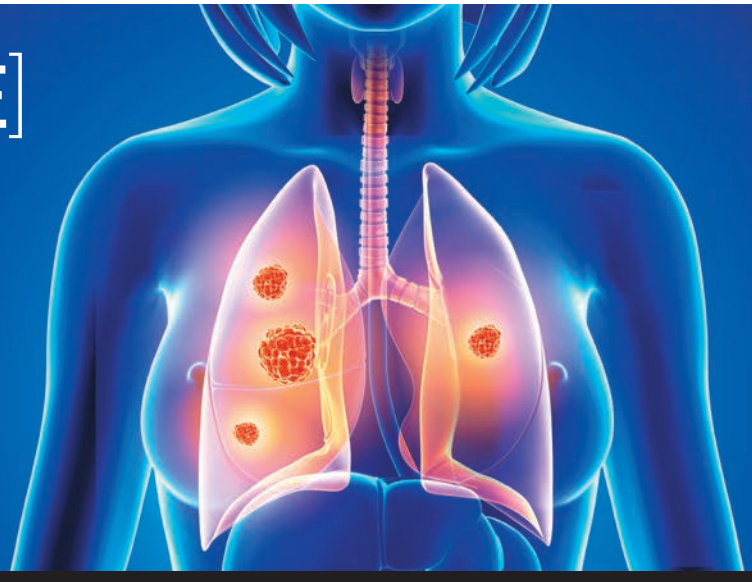
Flora Teoh

PhD Student, SIgN

"By learning more about how chemotherapy interacts with microbes and the consequences of such interactions, we may eventually be able to prevent such opportunistic infections in cancer patients, anticipate drug resistance and assist physicians in selection of therapy, ultimately improving the outcome of cancer treatments and the life expectancy of cancer patients."

[NEXT ISSUE]

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



Meta-analysis:

ANXIETY OVER ANXIETY RESEARCH

Researchers find evidence of shelved negative results in preclinical studies of anxiety

Optoelectronics:

MIX AND MATCH LASERS

Combining silicon with an optically active material enables tiny lasers compatible with industrial fabrication techniques

Cancer:

SMALL RNAS OFFER BIG HOPE FOR LUNG CANCER TREATMENT

A pair of microRNAs implicated in the spread of lung cancer could lead to new diagnostics and therapies

Agency for
Science, Technology
and Research

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

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

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

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Bioprocessing Technology Institute (BTI)
Clinical Imaging Research Centre (CIRC)
Data Storage Institute (DSI)
Experimental Therapeutics Centre (ETC)
Genome Institute of Singapore (GIS)
Institute of Bioengineering and Nanotechnology (IBN)
Institute of Chemical and Engineering Sciences (ICES)
Institute of High Performance Computing (IHPC)
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Institute of Microelectronics (IME)
Institute of Molecular and Cell Biology (IMCB)
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