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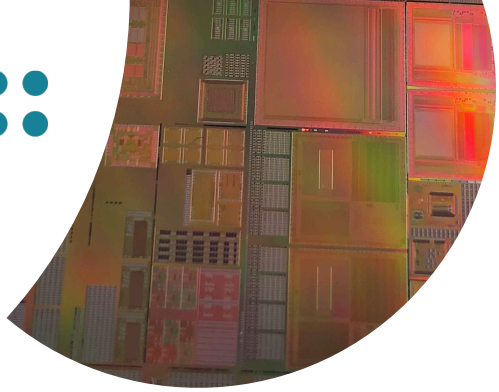

Scientific Newsletter

FALL 2021

Where does the shape of the Romanesco cauliflower come from?

The golden ratio and the ***Fibonacci sequence*** are constants found in many fields, some of them very far from mathematics. Thus, they appear in nature, within many biological forms; the branching of trees, the arrangement of leaves on a stem, the inflorescence of a cauliflower, the arrangement of pine cone scales, or the shell of snails. But the plant for which the spirals that follow the Fibonacci sequence are most apparent is undoubtedly the romanesco cauliflower. However, like the majority of eaten plants, cabbages have been domesticated through artificial selection, which has resulted in changes in their appearance, size or growing area. They are the great champions of shape changes; the same species has thus given the surprising Romanesco cabbage (*Photo*) with the most singular plant forms and whose formation constituted a mystery.

Romanesco cabbage resembles broccoli with its apple green color and cauliflower with its compact shape. Its flower buds are arranged in regular spirals and its geometric shape (called fractal) is very particular and decorative. The mystery of its formation has just been solved by IRIG researchers [***collaboration***]. Thanks to work combining mathematical modelling and plant biology, the scientists were able to determine that cauliflowers, and Romanescos in particular, are in fact buds that are designed to become flowers but which never reach their goal. Instead, they develop into stems, which in turn continue trying to produce flowers. The cauliflower is born from this chain reaction, resulting in a succession of stems upon stems. This study shows that the brief incursion of buds into a flowering state profoundly affects their functioning and allows them, unlike normal stems, to grow without leaves and to multiply almost infinitely. The atypical shape of the Romanesco is explained by the fact that its stems produce buds more and more rapidly (whereas the production rate is constant in other cauliflowers). This acceleration gives each floret a pyramidal appearance, making the fractal aspect of the structure clear.

The study highlights how the selection of mutations in plants during the process of domestication has changed their shape, sometimes drastically, into the fruits and vegetables on our shelves.

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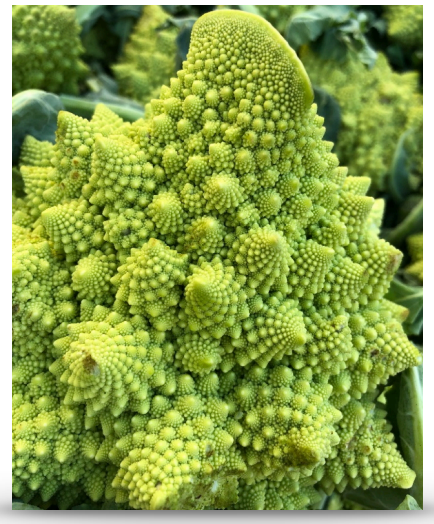

Photo of a Romanesco.
© Nathanael Prunet

Contact: François Parcy
LPCV
Cell & Plant Physiology Laboratory

Collaboration. Scientists from the Laboratoire physiologie cellulaire & végétale (CNRS-CEA-INRAE-Université Grenoble Alpes) and the laboratoire reproduction et développement des plantes (CNRS-ENS Lyon-INRAE) participated. Several foreign universities were also involved in the study: the Polytechnic University of Valencia, Spain; the University of Milan, Italy; the University of California, USA; the University of Nottingham, UK; and the National Autonomous University of Mexico.

In mathematics, the ***Fibonacci sequence*** is a sequence of integers in which each successive term is the sum of the two preceding terms, and which begins with 0 and then 1. Thus, the first ten terms that compose it are 0, 1, 1, 2, 3, 5, 8, 13, 21 and 34. This simple logic sequence is considered as the very first mathematical model in population dynamics.

A bacterial toxin guided by a human protein

Pseudomonas aeruginosa is an ***opportunistic pathogenic bacterium*** causing nosocomial acute infections, as well as fatal chronic infections in cystic fibrosis patients. Clinical isolates are frequently multi-resistant to antibiotics, which complicates the management of infected patients. *P. aeruginosa* possesses an arsenal of virulence factors, the most active of which is an ***injectisome*** that injects toxins directly into target cells. ExoU is the most harmful toxin injected by this system. Its necrotizing action is linked to its phospholipase activity (*Figure*) that causes the rupture of the plasma membrane of the host cell, and results in severe lesions in infected tissues.

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To carry out their biological activity, bacterial toxins often hijack molecules or mechanisms of the host cell. IRIG researchers used a genetic screen employing CRISPR-Cas9 technology to search for genes that might be involved in ExoU toxicity. Only one such gene was identified! This gene encodes the human DNAJC5 protein, which is known to play a central role in the secretion of some cytoplasmic proteins *via* an unconventional vesicular transport system (***MAPS***). The researchers demonstrated that DNAJC5 guides the toxin to the plasma membrane of the host cell, where ExoU can exert its toxic activity (*Figure*). They showed that cells deficient in DNAJC5, or Drosophila in which the DNAJC5 gene ***orthologue*** was down regulated, are largely resistant to ExoU toxicity.

The transportation system provided by the DNAJC5 protein is thus the Achilles heel of *Pseudomonas aeruginosa*'s ExoU toxin. This discovery could be used to prevent the devastating action of ExoU in acute *P. aeruginosa* infections.

An ***opportunistic pathogenic bacterium*** is able to cause disease as a result of a reduction in the body's defenses.

The ***injectisome***, or type III secretion system (T3SS), is a virulence system present in some pathogens that allows them to inject toxins into the cells they infect.

MAPS is a recently discovered secretory system for misfolded cytosolic proteins.

Ortholog describes similar genes present in two different species. These genes originate from the same ancestral gene and have retained identical structure and function throughout evolution.

An ***endosome*** is a small spherical structure (a vesicle) that traffics in the cytoplasm.

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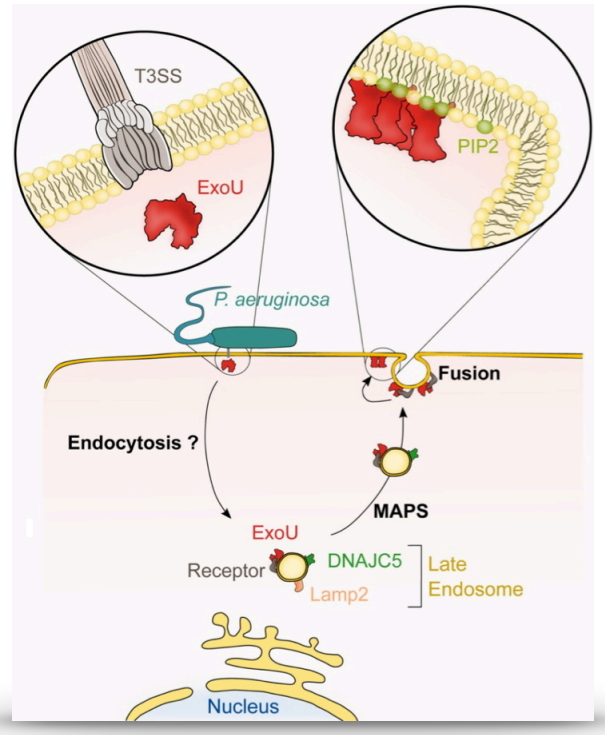

Vesicular trafficking of ExoU in the infected cell.
After being injected into the host cell by *P. aeruginosa* type III secretion system (T3SS; also called ***injectisome***), ExoU is taken up by late ***endosomes*** that are involved in a specific secretion system called ***MAPS***. In this system, the vesicles are transported to the cellular periphery and fuse to the plasma membrane. ExoU ends up on the inner side of the plasma membrane, where it interacts with the phospholipid PIP2, which modifies ExoU structure and triggers its phospholipase activity.

The Achilles' heel of pathogenic bacteria: Their cell wall formation mechanism

The cell wall plays an essential role in bacterial survival. For decades, the biosynthetic mechanism of its central component, the peptidoglycan, has been exploited as the target for beta-lactam antibiotics (penicillins and cephalosporins). The peptidoglycan forms a three-dimensional structure, comparable to a "fisherman's net" that surrounds most bacteria. It is not only essential for cell stability, but also for the proper execution of the different stages of the cell cycle, such as division and elongation of the wall. Many proteins that participate in the peptidoglycan biosynthesis machinery are essential for bacterial survival. Despite the widespread of resistance to antibiotics in clinics and hospitals worldwide, the bacterial cell wall biosynthesis process remains a target of choice for the possible development of new antibacterial agents.

Contact: Andréa Dessen
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One of the proteins that plays a central role in the formation of the bacterial wall is MreC, which is believed to form a platform for the stabilization of other proteins involved in cell wall elongation. The work conducted by IRIG researchers shows how MreC is able to self-associate and organize itself into different polymers, such as filaments, and even tubes. The scientists studied MreC from three different pathogenic bacteria: *Escherichia coli*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. The protein produced by the latter bacterium, a nosocomial pathogen that causes serious infections, was chosen for structural studies. Structures obtained using cryo-electron microscopy images (data collected at the Glacios microscope at the IBS) as well as with X-ray crystallography (atomic resolution data collected at the LNLS synchrotron in Campinas, Brazil), revealed key regions that lead to the formation of tubular forms of MreC. Any disruption of this polymerized form, such as those generated by the introduction of mutations, impacted not only the ability of MreC to produce polymers *in vitro*, but also the production of MreC itself in the bacterial cell, a phenomenon that the researchers verified by experiments carried out directly in strains of *P. aeruginosa*.

The interaction surfaces between the different building blocks of MreC polymers could thus represent a target for the development of totally novel inhibitors that could prove to be candidates for future antibiotics.

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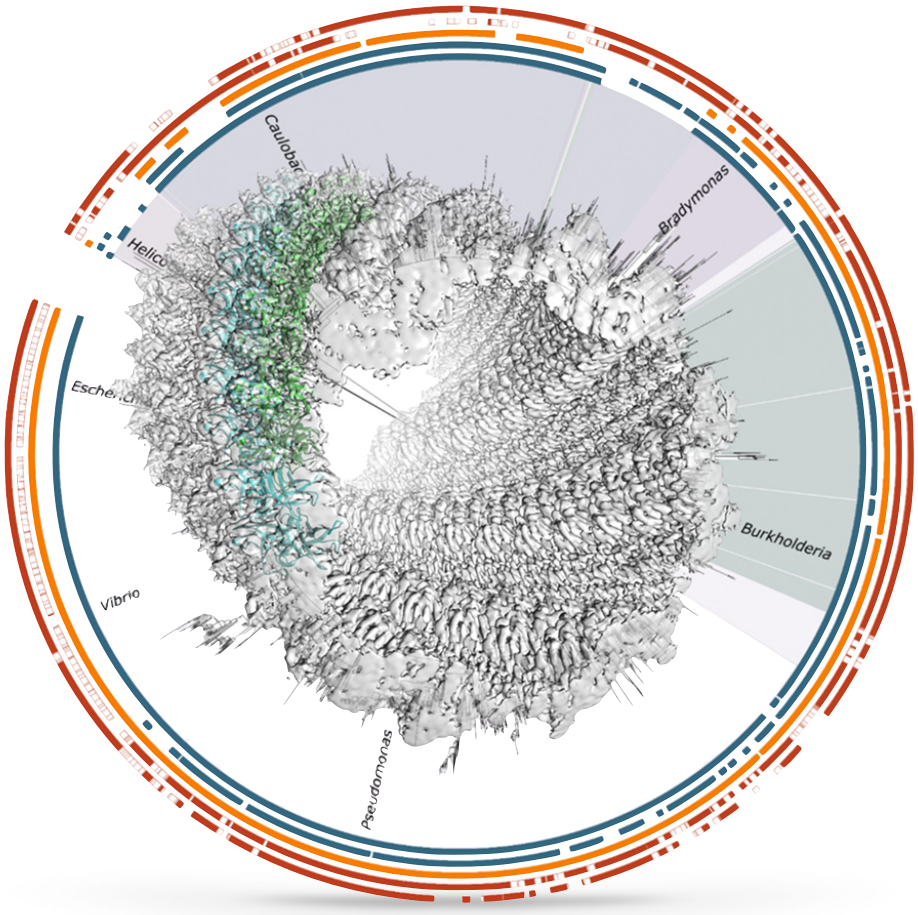

Overlay of the cryo-EM map of MreC on a phylogenetic tree of MreC variants present in Proteobacteria. The circular tree indicates the very high conservation of amino acids shown to be essential for the stability of the MreC structure. One of the six antiparallel filaments that make up the MreC tube is shown in blue and green.

A novel enzymatic process for CO_2 reduction

The quest for carbon neutrality requires the development of technologies for the transformation of CO_2 and its derivatives into commodity products from low-carbon energy. In this context, the design of new efficient and environmentally friendly catalytic processes for CO_2 reduction is at the heart of societal concerns. In the field of catalysis for sustainable chemistry, biocatalysts have the advantage of being highly selective and efficient under mild conditions (ambient temperature and pressure, aqueous solvents). However, despite their benefits, the cost of large-scale production of enzymes and their lack of stability still limit the development of such approaches.

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This is especially true for complex redox metalloenzymes, such as Carbon Monoxide Dehydrogenase (CODH), which remains to date the most efficient catalyst for the activation of small molecules like CO and CO_2. CODH has an active site that is unique in biology. It is composed of a multi-metallic $NiFe_4S_4$ center whose biosynthesis involves several molecular steps, requiring a specific multi-protein machinery which is still poorly understood. Over the last few years, IRIG researchers have studied in detail the ***chaperone proteins*** involved in this mechanism. These studies have allowed them to develop a heterologous CODH production system in which the gene of the carboxydotrophic bacterium *Rhodospirillum rubrum* (capable of using CO as a source of carbon and energy) is expressed in *Escherichia coli*. This process produces, in a single purification step, an enzyme that is as stable and active as a natural CODH. The researchers went further by immobilizing this recombinant CODH on functionalized carbon nanotubes [in collaboration with the DCM-BEA of UGA Grenoble], which allowed them to develop a bioelectro-catalytic system that is stable for several hours to achieve the inter-conversion of CO_2 to CO (*Figure*), reaching current densities of 4.2 $mA.cm^{-2}$ for CO_2 reduction and 1.5 $mA.cm^{-2}$ for CO oxidation.

Compared to existing electrochemical CO_2 reduction processes based on molecular catalysts, this enzymatic process achieves similar performances while bringing the advantages of operating in a reversible manner, under mild conditions and with very low overpotentials. These results allow to consider their use for CO_2 reduction as well as for CO oxidation, in the context of synthesis gas purification processes for the production of various chemicals or fuels.

Biocatalysis is the use of natural catalysts, such as enzymes, in an organic synthesis reaction.

A ***chaperone protein*** is a protein whose function is to assist other proteins in their maturation.

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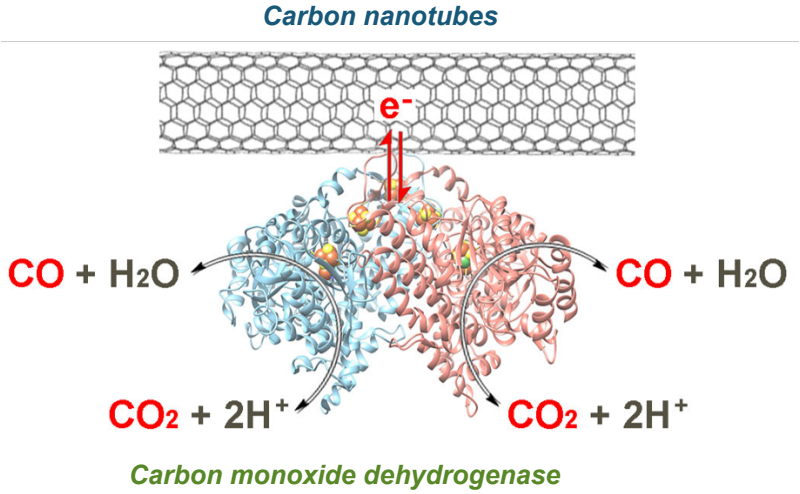

Interconversion of CO_2 to CO catalyzed by carbon monoxide dehydrogenase grafted onto carbon nanotubes.

Optics accelerates the use of phages as an alternative to antibiotics

The growing number of antibiotic-resistant bacterial infections (antibiotic resistance), a consequence of the overuse of antibiotics, has been identified by the WHO as one of the main public health threats by 2050. Phage therapy, based on the use of bacterial viruses (bacteriophages), is generating considerable interest in this context. However, in order to be implemented, it requires the identification of the active phage on a given bacterium in order to destroy it. This assessment is currently based on visual detection of lysis plaques formed by bacteriophages on Petri dishes covered with bacteria (*Figure*). This visual inspection, both manual and excessively time-consuming (12-24h), limits the possibilities of applying phage therapy to the patient.

Contacts: Emmanuel Picard and Emmanuel Hadji
Pheliqs
Quantum Photonics, Electronics and Engineering

An original approach has been proposed by researchers from Grenoble's IRIG, the Department of Technologies for Biology and Health (DTBS), the Microelectronics Technology Laboratory, and Swiss researchers [a ***collaboration*** between photonics researchers, biophysicists and microbiologists] to develop and test optical systems to verify phage-bacteria matching very quickly. The chosen strategy is to develop a lensless imaging system around a large format CMOS optical sensor (*Figure*). The analysis of lysis plaques is based on the use of an algorithm developed in-house, which allows to reconstruct the images diffracted by the bacteria in order to determine in real time the number and the kinetics of the lysis plaques. These measurements provide information on the nature and efficiency of phages against bacteria and allow to determine their titer, to study their morphologies and their growth kinetics. This new lens-free imaging approach has several advantages. Firstly, in the absence of lenses, the resolution and the field of view of the image are only limited by the pixel pitch and the size of the sensor. However, the current technologies drawn by the digital imaging sector allow the realization of very large sensors with very small pixels (typically several tens of million pixels on a sensor of 24 by 36 mm). Consequently, a much larger field of view than that of conventional optical microscopy is available for monitoring phage-bacteria interaction. Moreover, since the pixel pitch of current sensors is only a few microns, lens-free imaging allows to resolve structures of a few tens of microns, such as nascent bacterial micro-colonies, and thus to save time in the analysis process.

Using this technique, the researchers report that they determined the susceptibility of *Staphylococcus aureus* to different phage after only 3 hours and the infectious titer after 8 hours and 20 minutes. These times are much shorter than the 12 to 24 hours usually required for naked-eye observation and counting of lysis plaques. In addition, continuous monitoring of the samples allowed the study of plate growth kinetics and confirmation of the correlation between bacterial density and phage diffusion in the agar layer. Finally, with the resolution of 4.3 µm (*Figure*), the researchers were able to detect phage-resistant *Klebsiella pneumoniae* bacterial microcolonies within the boundaries of the lysis plates, showing that their prototype is also a suitable device for monitoring phage resistance.
This first proof of concept of a lens-free imaging system, implemented as a compact and economical device, opens a promising avenue for phage therapy. Several national and international programs are underway to validate its applications. Future work will also focus on the development of more refined algorithms allowing the morphological classification of plaques (and therefore phages) according to their growth rate but also their morphotype.

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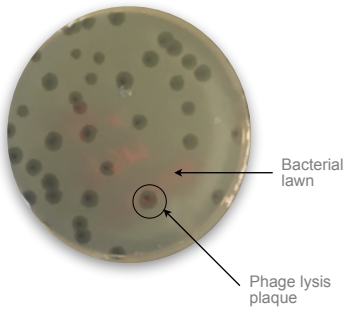
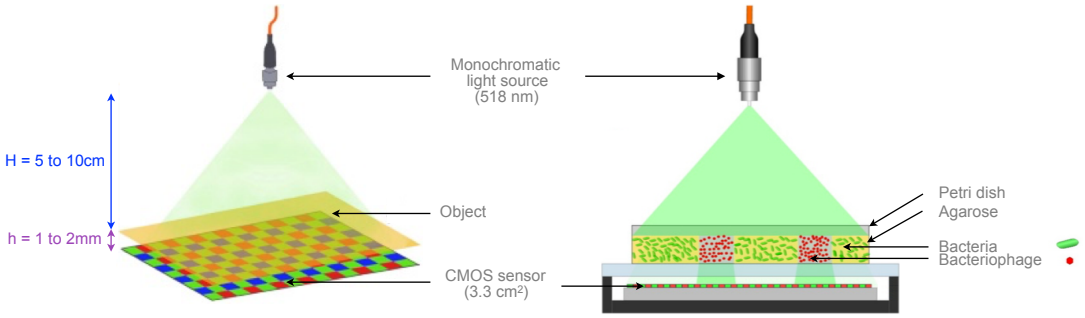
Collaboration: Quantum Photonics, Electronics and Engineering (IRIG, CEA-Grenoble, France); Department of Microtechnologies for Biology and Health (Leti-DTBS, CEA-Grenoble, France); LTM–Micro and Nanotechnologies for Health (CNRS, France); Department of Fundamental Microbiology (University of Lausanne, Switzerland).





Phage lysis plaque.
To test the sensitivity of a bacterium to a bacteriophage, the two organisms are co-cultured on the surface of an agar-coated Petri dish where a bacterial lawn forms. If the bacterium is sensitive to the bacteriophage, it is killed (lysis) and a phage lysis plaque is formed on the surface of the agar plate as the lysis progresses.
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Custom prototype without lens.
The device is composed of a detection system and an illumination module. The sensing system consists of a 22.3 × 14.9 mm² APS-C (Advanced Photo System type-C) CMOS (Complementary Metal Oxide Semiconductor) sensor from a "consumer" digital camera. The sensor consists of a 5344 × 3516 pixel matrix with a pitch of 4.3 µm. The Petri dish on which the bacteriophages are located is placed directly on the sensor and illuminated from above by a monochromatic green LED (518 nm) coupled to a 200 µm diameter multimode optical fiber (Thorlabs M72L02). The images are acquired directly and converted to .tiff files. They are then processed using two different algorithms; the first processes the entire image area to detect plaques, while the second processes only a cropped subimage of each plaque to calculate the growth rate.
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Real-time monitoring of graphene growth on liquid copper

The synthesis of large, defect-free two-dimensional materials is a major challenge toward industrial applications (photovoltaics, semiconductors, etc.). Chemical vapor deposition (CVD) is to date the most promising method to produce large, high quality graphene sheets. However, polycrystalline copper generates defects in the graphene layer, and after cooling, the layer forms domains and wrinkles that significantly degrade its quality. The researchers are working to develop a new process on liquid metal catalysts under CVD growth conditions.

Contact: Gilles Renaud
MEM
Modeling and Exploration of Materials

Liquid metal catalysts, *e.g.* molten copper, have been employed for the fast growth of uniform graphene film of the highest quality, while using temperature, pressure and flow conditions that are comparable to those used with solid catalysts. However, the lack of in situ techniques led to empirical growth recipes. Thus, an in situ multiscale monitoring coupled with a real time control of the growth parameters is necessary for efficient synthesis. This allows to control *operando* the morphology, at large scale from the atom to the millimeter, to organize the interactions of the graphene crystals and to optimize the obtained film. Until now, there were significant hurdles against the realization of *in situ* monitoring techniques including heat and evaporation of the liquid metal, curve and dynamic of the liquid surface, and the presence of reactive CVD gas at close to atmospheric pressure.

IRIG researchers [***collaboration***] have succeeded in monitoring the growth of graphene on liquid copper *via* four complementary methods (*Figure 1*) applied *in situ* and in real time: synchrotron X-ray diffraction and X-ray reflectivity, Raman spectroscopy and radiation mode optical microscopy (*Figure 2*). Synchrotron X-rays confirm the superior crystallinity of the produced, monolayer-thick graphene. Real-time monitoring allows to control the size, shape and purity of the crystals and to optimize the growth rate. Finally, the experimental observations associated with a modelization allowed to understand the growth mechanisms.

This process proves to be reliable and opens the way to the rapid production (*Figure 3*) of defect-free single-crystal graphene on surfaces of several square centimeters, suitable for various electronic applications. This real-time monitoring and control methodology is also useful for the scientific study of other 2D materials.

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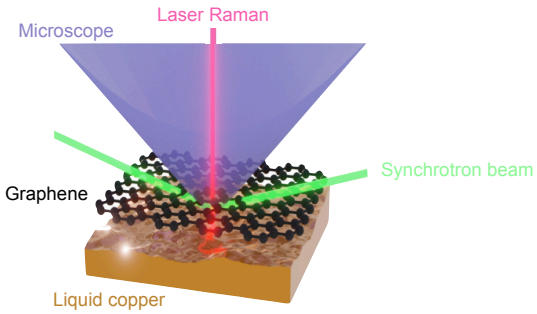

Figure 1: Configuration of four complementary in situ, operando and real time methods: synchrotron X-ray diffraction and reflectivity, Raman spectroscopy, and radiation-mode optical microscopy, applied to a graphene layer grown on liquid copper.
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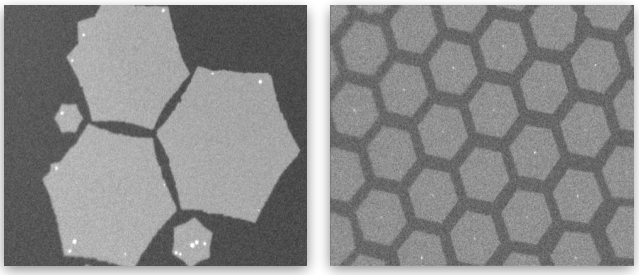

Figure 2: Radiation-mode optical microscopy of self-organised hexagonal graphene flakes on liquid copper (Image scale: ~1 mm).
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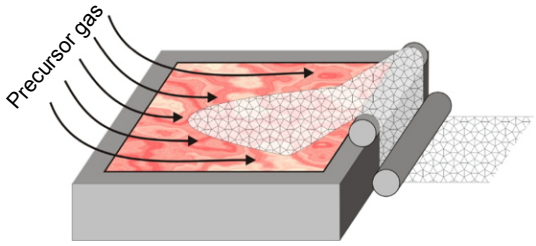

Figure 3: Squetch of a new scaled-up reactor capable of growing large graphene flakes on liquid metal catalysts.
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Fantastic voyage with neutrons inside batteries

With climate change and pollution placing increasing strain on the environment, scientists are working to develop sustainable energy solutions that reduce our dependency on fossil fuels. Among the most prominent clean-energy technologies are batteries, fuel cells and electrolysers, Neutron scattering is emerging as a powerful research tool for the development of such devices.

Contact: Sandrine Lyonnard
SyMMES
Molecular Systems and nanoMaterials for Energy and Health laboratory

Fuel cells and electrolysers have several components in common, one of which is the Polymer Electrolyte Membrane (PEM) – a semi-permeable material that acts as both an electrolyte (selectively conducting ions) and a separator (preventing undesirable side-reactions, electronic conduction and gas diffusion). PEMs are also used for ion-selective separation and considered key for new generations of "all-solid state" batteries. Critical issues that are currently being investigated address improvements in durability and performance efficiency combined with lowered production costs.

To this end, neutron scattering techniques, such as reflectivity, inelastic and quasi-elastic scattering, small and wide angle scattering, are enabling IRIGs scientists [***collaboration***] to study the multi-scale structure and ion transport mechanisms within the membrane. Such information is invaluable for the design of advanced membranes formulation towards improved properties. Neutron tools are powerful to study the structure and dynamics of PEMs over a range of length- and time-scales, because of their high sensitivity to H-containing material, possibility to perform isotopic contrast based experiments, as well as high penetration through metallic containers and ceramics, therefore allowing to probe fuel cell devices under realistic operation conditions.

The intrinsic complexity of membranes represents a challenge for the prediction and optimization of their functions. However, the suite of neutron scattering techniques allow to disentangle the multiple complex processes occurring within the membrane as a function of key parameters, e.g. hydration and temperature. In particular, the state and distribution of water can be quantified in situ and correlated to fuel cell performance in actual operational conditions. This is critical, as membrane drying or water flooding are major causes of loss in performance and stability.

Thanks to new instrumentation, brighter neutron sources, and innovative *operando* experiments on real electrochemical systems, neutron scattering techniques are emerging as a powerful research tool for the development of clean-energy devices.

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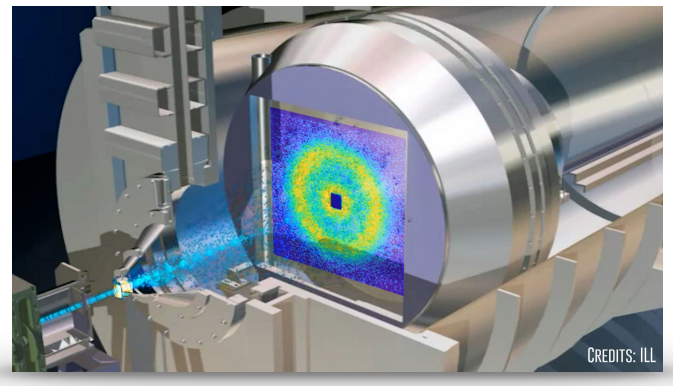
Collaboration : Department of Chemistry, University College London, United Kingdom
ISIS Neutron and Muon Source, United Kingdom
Laboratoire Léon Brillouin (CEA-CNRS), Université Paris-Saclay, France
CEA-Liten (CEA-UGA), France



Artist illustration for neutrons passing through fuel cell that give a SANS scattering image, instrument D22 at I'ILL.
Illustration taken from the video "*Neutrons an inevitable tool for your research on fuel cell*".
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Heavy-ion irradiation effects on last MRAM generation

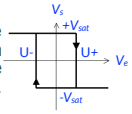
The space environment exposes electronic systems to harsh conditions where they are subjected to wide temperature variations and, more importantly, to incessant bombardment by high-energy particles. These particles can cause considerable damage to microelectronic components such as semiconductor memories and catastrophically alter data. Magnetic Random Access Memories (MRAM) are known to be almost insensitive to radiation effects. At IRIG, physicists are working on MRAMs whose dimensions can be reduced and which could therefore be interesting candidates for high-density storage and to replace memories currently used by the space industry.

Contact: Gregory Di Pendina
Spintec
Spintronics and Component Technology

The most common semi-conductor memories work by trapping and untrapping electrical charges. In space (geostationary satellite, distant space missions...) these components are subjected to an intense bombardment of very high energy particles (electrons, protons, gamma rays,...). Some of these particles, often the most energetic, can pass through the shielding devices and damage memories or disrupt their operations. Indeed, a highly energetic particle is capable of "untrapping" a charge, or on the contrary to create one, which has for effect to modify the information in a punctual or definitive way.

MRAMs store information using the relative orientation of the magnetic magnetizations of two ferromagnetic layers. In the absence of a charge, MRAM memories are not susceptible to such a perturbation. IRIG researchers are studying different types of MRAMs. They have studied the robustness to irradiation of Magnetic Tunnel Junctions (MTJ) under radiations for memories that can be used for high-density MRAM memories (over 1 Gb of memory capacity), which involve the most advanced manufacturing processes they have developed. The irradiation experiments were performed in the cyclotron resources at the Université Catholique de Louvain (UCL). The irradiation of the memories with $^{124}Xe^{35+}$ heavy ions of 995 MeV (the most energetic ions available in Leuven, representative of the particle energy that can impact the circuits) shows an insignificant sensitivity of the MTJs to these particles and no modification of their electrical properties. Moreover, the researchers observed that the magnetoresistance properties were slightly improved after irradiation. However, modifications of some magnetic properties can be observed, in particular the reduction of the coercive field and a shift of the ***hysteresis*** cycle. These last changes could lead to a degradation of the memory usage, especially in terms of stability. The causes of these degradations seem to be the consequence of heating effects induced by the irradiation (energy deposited by the particles).

Hysteresis is the property of a system whose evolution does not follow the same path depending on whether an external cause increases or decreases.



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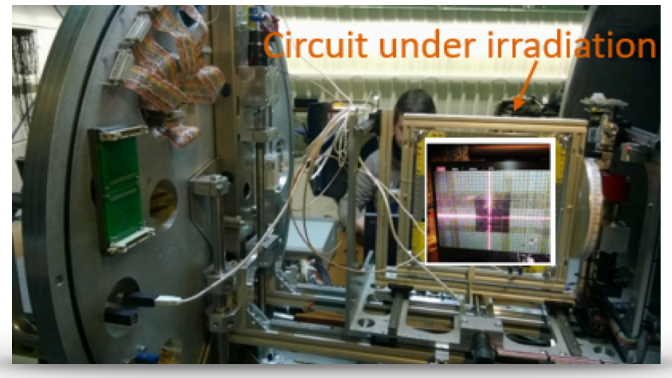
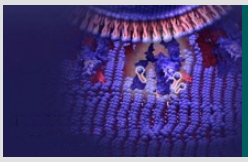


Irradiation chamber at the Leuven cyclotron. The inserted image shows the ion beam (purple cross) on the circuit during irradiation.

Other scientific news of the IRIG laboratories



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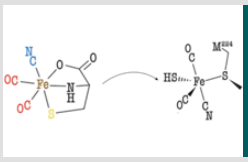

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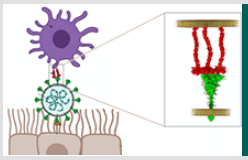

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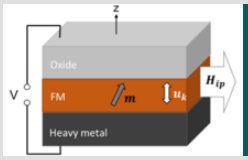

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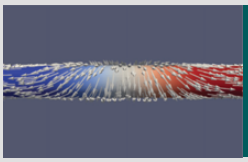

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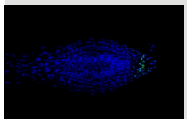

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