Scientific Newsletter

AUTUMN 2023

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At the front page of IRIG

Fluorescent proteins switch in the cold

Biological molecules can be observed at the nanoscale with fluorescence nanoscopie. Single-molecule localization microscopy (SMLM) improves the resolution of fluorescence microscopy normally limited by diffraction. One challenge is to work at cryogenic temperature to preserve the native structure of the investigated samples, like in cryo electron microscopy. The "magic" of SMLM lies in the properties of the employed fluorophores used to label the biological target. Those fluorophores are able to efficiently switch between a fluorescent on-state and a nonfluorescent off-state. However photoswitching is killed at very low temperature.

Contact: **Dominique Bourgeois**
IBS
Institut de Biologie Structurale

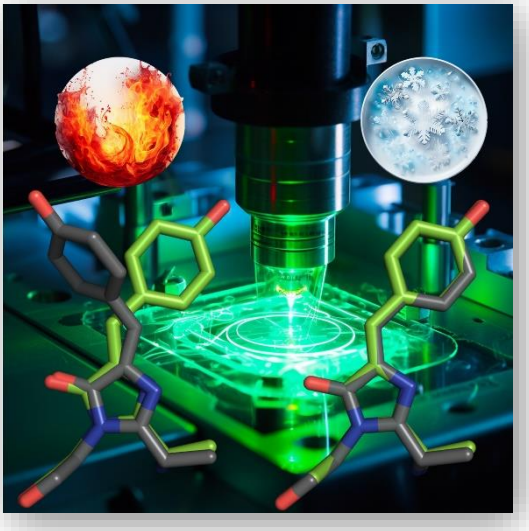

Figure: Conformational changes of the rsEGFP2 chromophore upon switching at room temperature (left) or at cryogenic temperature (right). The fluorescence on-state is shown in green and the nonfluorescent off-state in grey. An artistic view of a cryo-SMLM microscope is shown in the background. © Virgile Adam / IBS.

In response, researchers at IRIG, in collaboration with the University of Göttingen in Germany, investigated the cryo-switching properties of rsEGFP2, a fluorescent protein, combining X-ray crystallography with optical spectroscopy and SMLM using a dedicated microscope operating at cryogenic temperature. They found that rsEGFP2 still switches at - 170 °C, based on a photophysical mechanism different from that observed at room temperature. Whereas at room temperature switching is based on *cis-trans* isomerization of the chromophore, a large conformational change, the data suggest that cryo-switching involves the formation of a so-called "radical states" without any substantial conformational change (see **figure**).

Moreover, the researchers found that the fraction of rsEGFP2 molecules that can efficiently cryo-switch before photo destruction (photobleaching) is enhanced by about 30% using weak UV illumination at 355 nm instead of 405 nm as classically used for SMLM at room temperature. Thus 355 nm light substantially improves the effective labeling density in cryo-SMLM.

The study opens the door to obtaining crisper cryo-nanoscopy images. Applying the optimized UV illumination to real biological samples is now the goal, notably in the context of cryo-correlative studies between SMLM and Focused Ion Beam for Scanning Electron Microscopy and electron tomography, a top challenge for today's integrated structural cell biology.

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Photophysical Studies at Cryogenic Temperature Reveal a Novel Photoswitching Mechanism of rsEGFP2
Journal of the American Chemical Society 2023

Electron gases for magnetization control

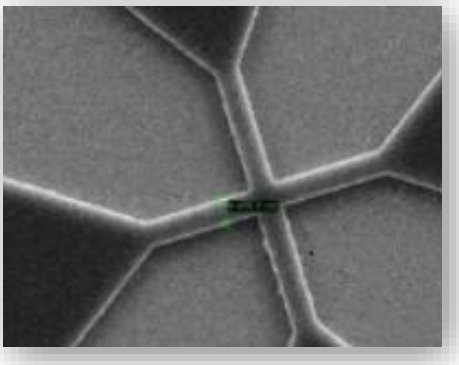
New generations of memories are developed for logic and artificial intelligence devices with very low energy consumption. This responds to environmental concerns, and to the fact that the energy consumption of chips and the resulting overheating limits their performance. One possible approach is to develop spintronic devices that use currents to control the direction of magnetization.

Contact: **Jean-Philippe Attané**
SPINTEC
Spintronics and Component Technology

Researchers at IRIG have used Ta/CoFeB/MgO thin-film stacks possessing a two-dimensional electron gas, which offer key advantages for integration into spintronic technologies. The conductivity of the electron gas can be modulated using an electric field applied across the $SrTiO_3$ substrate, with two switchable and remanent high- and low-resistivity states of the device. The resistance contrast is over 1000%. Researchers then measured the spin-orbit torques acting on magnetization in the Hall cross, and found that the effects on magnetization are different for the high- and low-resistivity states.

This non-volatile electrical control has the potential to create a new generation of devices for memory and logic applications, and for artificial intelligence.

Fundings: ANR Contrabass, Institut Universitaire de France, ERC Fresco, European projects FET-OPEN Tocha and ITN Spear, and Upstream Technological Platform PTA.



Scanning microscopy image: Hall cross nanostructure used for measurements. Branch widths range from 200 nm to 2 µm.

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Non-volatile electric control of spin-orbit torques in an oxide two-dimensional electron gas
Nature Communications 2023

Migratory cells infiltrate tissues

Cellular mechanical effects are classically achieved by the contraction of actin filaments thanks to molecular motors, the myosins. However, actin alone does not allow cells to pass through such small interstices. Researchers at IRIG, in collaboration with teams from CNRS and Utrecht University in Holland, have revealed the vital role played by microtubules in achieving such a morphological transformation (**figure**).

Contact: **Manuel Théry**
LPCV
Cell & Plant Physiology

Since 2013, as they have developed their studies on the shape and division of stem cells, researchers have discovered how sensitive microtubules are to the pressure forces experienced by the cell in the interstices. The scientists have cleverly developed a device based on a kind of plastic film in which living cells are grafted: by stretching the film, it becomes possible to apply precise deformations to the cells. Forces are applied with perfectly controlled speed, frequency, direction and pressure-relaxation. In this way, microtubules, which are generally highly dynamic, are stabilized when the cells are compressed into the interstices, enabling them to progress. In addition, the researchers identified the molecular mechanism of microtubule stabilization: proteins associated with the growing ends of microtubules ensure their rapid elongation by repositioning themselves in the deformed zones.

These studies reveal new fundamental properties of microtubules and demonstrate their involvement in cell migration in response to the mechanical and spatial constraints of the environment. These processes could well be involved in tumor invasion or tissue exploration by immune cells. The mechanism identified could lead to the development of a new molecular strategy to control cell migration.

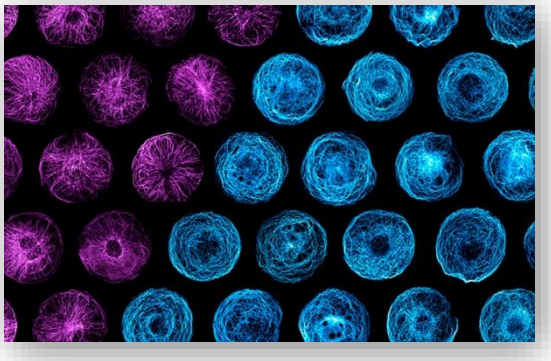

Figure: microtubule network. architecture

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Compressive forces stabilise microtubules in living cells
Nature Materials 2023

Helical dislocations give an additional twist to graphene

Magic angle twisted bilayer graphene has emerged in the past decade as a platform for non-conventional superconductivity. This behavior arises in the presence of a localized flat band as the twist angle approaches a well-defined value referred to as the magic angle, shown in a pioneering theoretical work. This theoretical prediction was later confirmed by experiments, giving more details on the superconducting behavior of magic angle twisted bilayer graphene. Of particular interest is the observed symmetry breaking between hole and electron doping that is characteristic of this type of superconductivity.

Contact: **Pascal Pochet**
MEM
Modeling and Exploration of Materials

Interestingly, this critical symmetry breaking is absent in a simple single-particle model of a flat twisted bilayer graphene structure containing pure screw van-der-Waals dislocation segments between the moiré spots. In a series of two papers [1, 2] the IRIG and UIUC team showed that to recover the requisite symmetry breaking while keeping the physics simple, one needs only to add a dislocation edge loop to the initial screw dislocation (purple lines on figure 1a). As a result, a mixed dislocation associated with a helical topology is stabilized. The helicity of the core is genuinely 3D [2] and gives rise to an out-of-plane displacement as highlighted on figure 1b.

This topology drives the Fermi level away from the charge neutrality point, causing partially filled flat bands in magic-angle [1]. This deformation also causes a charge redistribution in the twisted bilayer, causing a particular broken symmetry (central inset in Figure 1b) typically observed in high temperature superconductors and reported in magic-angle twisted bilayer graphene. The findings of the team also explain several exciting experimental observations in magic-angle, including striped charge order and evolution of the vibrational spectra, neither of which can be explained in terms of the conventional deformation mode with pure screw dislocations.

The understanding of moiré physics deriving from dislocation concepts makes it possible to rationalize these and many other observed features within a well-established framework. Additional findings may yet emerge from this dislocation model for the moiré structure in twisted bilayer graphene. Indeed, while solitons provide one simple framework for understanding moiré patterns, there is no comparable concept of helical solitons… but helical dislocations have been observed in materials for more than seven decades!

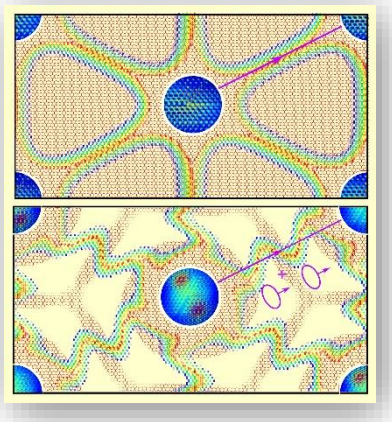

Figure 1: Top views of a magic angle twisted bilayer graphene super-cell.
The red honeycomb lattice is the upper graphene layer shadowed by a plain yellow plane positioned at the average mid-distance between the two graphene layers. The color dots highlight the core of the helical dislocation [2] as revealed by the bond convolution between the two layers. The central moiré of the cell, is overlaid by orbital projected density map close to the Fermi level as calculated from a tight binding Hamiltonian [1] for each case. (a) with the pure screw dislocation (purple line), the moiré spot is homogeneous (b) with the helical dislocation, that results from the reconstruction of the screw segment with purple edge loops, the moiré spot presents the broken symmetry due to half filled bands.

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Corrugation-driven symmetry breaking in magic-angle twisted bilayer graphene
Communications Physics 2022

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Helical dislocation in twisted bilayer graphene
Extreme Mechanics Letters 2023

Bacteriophages as diagnostic tools

Bacteriophages are viruses that parasitize bacteria. Bacteriophages are parasitic viruses that replicate exclusively in their host, with remarkable specificity. For almost a century, phage suspensions have been used therapeutically in certain Eastern European countries (Russia and Georgia) as an antibiotic treatment. Today, phages are incorporated into some food packaging or wound dressings to reduce the proliferation of bacteria such as *Escherichia coli*, *Listeria* and *Salmonella*.

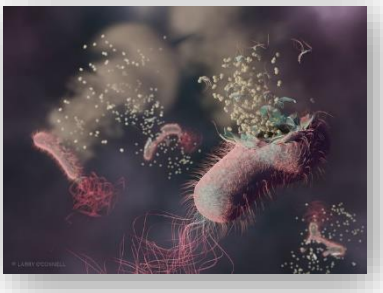
More recently, bacteriophages have been used as a biosensing element for diagnostic purposes. However, they need to be produced in large quantities, and then immobilized on a sensor. To this end, scientists at IRIG, in collaboration with LETI/DTBS, have developed a phage purification method to obtain active viral particles in large numbers. They also tested various chemical modifications of the bacteriophages to physically immobilize them on the gold surface of a biosensor, while maintaining their infectivity. The bio-active devices thus obtained show the highest phage density ever described in the literature.

Several biosensors have been produced with different bacteriophages to demonstrate their specificity with respect to their host bacteria.

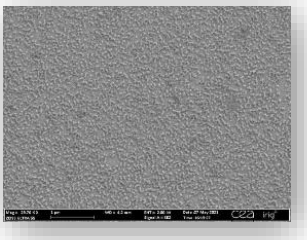
These results could lead to miniaturized devices, functionalized with different bacteriophages, enabling the sensitivity of a pathogenic bacterium to a specific virus to be tested more rapidly, in just a few hours.

And since we are faced with a growing number of antibiotic-resistant strains, phages represent a serious alternative as an antibiotic treatment, provided they can be produced on a large scale, and in a characterized and reproducible manner for therapeutic purposes.

Contact: **Yoann Roupioz**
SyMMES
Molecular Systems and nanoMaterials for Energy and Health



Artist view showing bacteria blasting after bacteriophage infection
© Larry O C'onnell



Scanning electronic microscopy image of bacteriophages on the gold-coated surface.

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Approaching the Geometric Limit of Bacteriophage Conjugation to Gold: Synergy of Purification with Covalent and Physisorption Strategies
ACS Biomaterials Science & Engineering 2023

New cryogenic exchangers for HL-LHC at CERN

The European Organization for Nuclear Research (CERN) in Geneva has decided to intensify the **luminosity** of the Large Hadron Collider in order to increase the number and energy of **hadron collisions**. To achieve this, the magnetic field intensity will be increased around the collision zones using new superconducting magnets cooled in a pressurized **superfluid helium** bath with heat exchangers to evacuate the energy flux.

Researchers at IRIG first designed the 1.8 K (near absolute zero) cooling system for the 27 km of superconducting magnets installed in the LHC. The challenge was to design a new exchanger for extracting 70 W of thermal power from the future four D2 superconducting magnets close to the collision zone where the detectors are installed, and limiting the temperature up to 220 mK at 1.8 K (respectively 55 mK at 2 K). Another major demand is that these new exchangers have to be compact (dimensions under 0.5 m) to fit into the restricted space of the LHC.

In France, IRIG is the only research institute having a cryogenic station with a helium refrigerator capable of extracting up to 400 W at 1.8 K: this unique test bench was used to characterize the performances of the prototype exchanger for the HL-LHC D2 magnets (figures 1-2).

Thanks to these results, the researchers must now supply the final D2 magnet exchangers, incorporating the specific mechanical interfaces and long-term reliability required for the HL-LHC. One of these exchangers, selected at random, has just passed all thermal performance tests at the DSBT's 1.8 K test station. The exchanger's performances in extracting 70 W of heat are in line with the predictions of the DSBT designing code, and well above CERN's requirements, since it has a temperature rise margin close to twice that corresponding to the specifications (figure 3).

The exchangers can now be delivered to CERN and integrated into the D2 magnets for final installation in the LHC. These world-first results combine an integrated design with modeling that takes into account mechanical interfaces and the specific heat law of superfluid helium. These studies contribute to the IRIG's ranking as one of the best cryogenic laboratories in the world and its helium test station shows remarkable adaptability to meet the challenges of accelerators, fusion reactors and quantum computer farms.

Contact: **Bernard Rousset**
DSBT
Low Temperature Systems Department

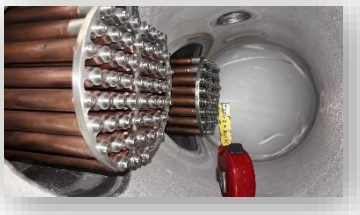

Figure 1: Inside view of heat exchanger with copper tube bundles.

Figure 2: Outside view of the heat exchanger before testing at 1.8 K.

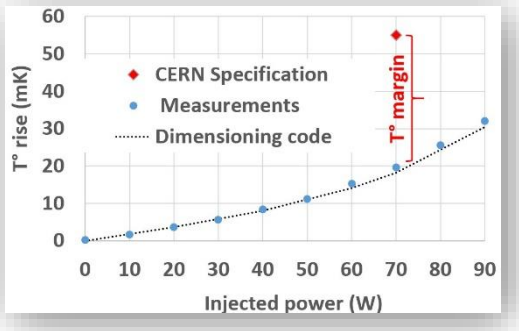

Figure 3: Thermal performance curves at 2 K - 1.3 bar with a good agreement between measurements and predictive code.

- **Luminosity**: in particle physics, luminosity corresponds to the number of collisions.
- **Hadron collision**: The LHC collides protons at energies up to 14 TeV.
- **D2 Superconducting magnet**: These magnets will be installed in the HL-LHC to recombine particles beams in the interaction zones on either side of the interaction points (collisions)
- **Superfluid helium**: Specific state of liquid helium below a transition temperature close to 2.2K

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Performance assessment of multi-tube He II/He II heat exchanger using a single tube sample
Cryogenics 2023

Tamoxifen activated CreERT2 recombinase is toxic for young mice

Mice are widely used as a model to study human diseases or to decipher gene activity or function *in vivo*.

Tamoxifen is frequently used in research using genetically modified mice, as it induces the transfer of the CreERT2 recombinase to the cell nucleus, which can then suppress the target genes of interest.

Researchers at IRIG administered tamoxifen to CreERT2 young mice. Ten days later, they observed morbidity and mortality: the young mice stopped gaining weight and showed hematological defects with severe anemia and disorganization of the bone marrow vascular bed (see **figure**).

These results show that activation of CreERT2 by tamoxifen significantly reduces cell proliferation in the bone marrow and spleen. This is due to a toxic side effect of the CreERT2 recombinase. This discovery should allow a better use of these models, improving the accuracy of the interpretation of the observed phenotype, saving time and resources.

The results of the study carried out by the researchers show the need to include CreERT2 controls injected with tamoxifen, without targeting the gene of interest, in experimental designs.

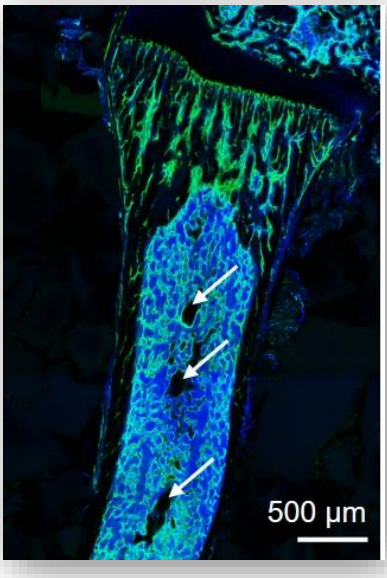

Figure: disorganization of the bone marrow in the femur of a tamoxifen-injected CreERT2 young mouse (see arrows). Confocal microscopy: cell nuclei (blue) and blood vessels (green).

Contact: **Claire Bouvard**
Biosanté
Biology and Biotechnologies for Health

REFERENCE

Rossi M, Salomon A, Chaumontel N, Molet J, Bailly S, Tillet E and Bouvard C
Probing Warning regarding hematological toxicity of tamoxifen activated CreERT2 in young Rosa26CreERT2 mice
Scientific Reports 2023

Understanding the metabolism of silver nanoparticles in the liver using a 3D hepatocyte model

Silver nanoparticles (Ag) are massively used for their antibacterial properties due to the release of silver in ionic form. Studies have estimated that we ingest between 1 and 80 µg of Ag per day. Moreover, medical devices such as catheters or burn dressings release silver nanoparticles into the bloodstream. To measure their impact on our health, researchers study the metabolism of silver nanoparticles in the liver. However, conventional human cell models do not allow for exhaustive in vitro study of nanoparticles. On the other hand, animal models bring little insight into the cellular and molecular mechanisms involved.

Researchers at IRIG and ESRF Synchrotron in Grenoble, made use of an original 3D hepatocyte cellular model [1]. This bridges the gap between cellular and animal studies, by allowing for the observation *in vitro* of phenomena occurring *in vivo* that standard 2D cellular models cannot reproduce, such as the excretion of transformed nanomaterials in the bile. By combining several characterization techniques, such as X-ray fluorescence nano-imaging, X-ray absorption spectroscopy and electron microscopy, the researchers studied the behavior of nanoparticles in the liver [2]. Hepatocytes, the major of liver cells, were exposed to silver nanoparticles on the one hand, and to a silver salt on the other, in order to simulate injection or ingestion, respectively. X-ray and 3D electron microscopy images revealed silver diffusion into various intracellular compartments (cytosol, nuclei, ...); the images also showed silver accumulation in vacuoles (Figure A white arrow), and excretion into bile-collecting ducts (Figure B white arrow). Speciation analyses revealed the formation of both organic silver-sulfur AgS species, as previously observed in 2D cell cultures [3] and the formation of inorganic Ag_2S species not observed previously.

Using a pseudo-organ as cellular model and a customized combination of elemental and structural nano-imaging and atomic spectroscopy, this study reveals subcellular and molecular mechanisms linked to the human toxicity of a broadly used nanomaterial in the liver. These results allow us to better understand the effects on our health, and contribute to the development of safer nanomaterials.

Contacts: **Aurelien Deniaud** and **Giulia Veronesi**
LCBM
Chemistry and Biology of Metals laboratory

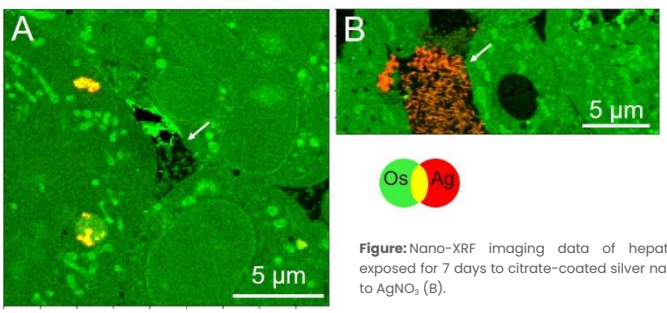

Figure: Nano-XRF imaging data of hepatocyte spheroids exposed for 7 days to citrate-coated silver nanoparticles (A) or to $AgNO_3$ (B).

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Biomaterials Science 2023
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Deciphering silver nanoparticle fate in liver up to biliary excretion using HepG2/C3A spheroids in scenarios mimicking different exposure pathways
Environmental Science: Nano 2023
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Nuclear translocation of silver ions and hepatocyte nuclear receptor impairment upon exposure to silver nanoparticles
Environmental Science: Nano 2020

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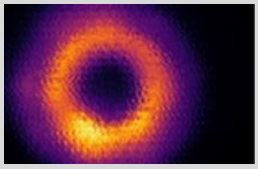

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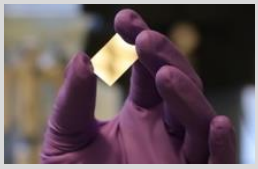

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