

## How photons disappear?

Photons are grains of light that propagate in the vacuum with a well-defined energy. When they interact with matter, they can be transformed into photons of different energy. Thus, two photons can merge into a new photon whose energy is the sum of the energies of the initial photons. It is the non-linearity of the interaction between light and matter that makes this process possible. Conversely, can a photon spontaneously disappear, producing photons of smaller energy, when it travels through a crystal? While this process is classically forbidden, quantum physics allows it. It has even been used to produce pairs of entangled photons. However, this spontaneous fission of a visible or infrared photon is extremely inefficient: it only concerns a few photons per million.

In arrays of Josephson junctions between superconducting islands, the oscillations of the superconducting phase difference propagate like optical photons, but at microwave frequencies. The advantage of these arrays is that one can introduce an extremely strong nonlinearity for these photons by reducing the size of the junctions. A small junction in series with a homogeneous chain of large junctions acts as a qubit coupled to a transmission line. The nonlinearity comes from the modulation of the qubit energy by the electric charge in its environment. This modulation is attributed to "quantum slips" of the phase difference at the small junction. Since the qubit energy depends on the charge, the quantum phase slips couple a quasi-resonant photon with the low-energy photons. With a researcher from Yale University, IRIG scientists have established that this coupling becomes particularly efficient to break a resonant photon into a quasi-resonant photon accompanied by many low-energy photons.

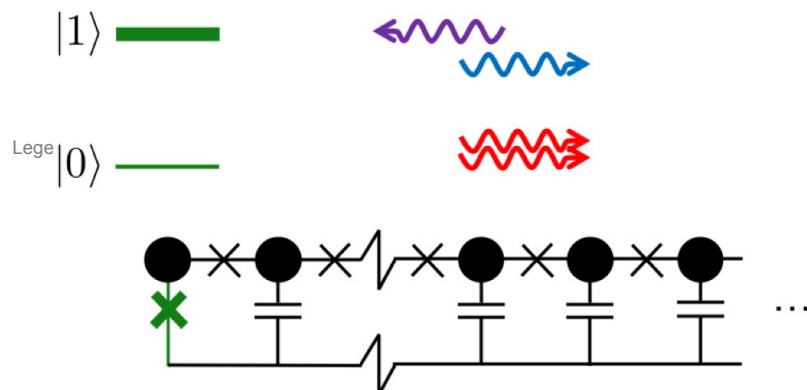
Photon lifetime has been measured in several superconducting circuits at the University of Maryland. The theory, which attributes the disappearance of resonant photons to quantum phase slips, allowed those

researchers to interpret the results for circuits characterized by a transmission line's impedance greater than the resistance quantum. Outside this regime, other processes compete with quantum phase slips. To date, there is no theory to account for this. From this point of view, the experiments have realized a "quantum simulation" of a quantum impurity problem: the qubit coupled to its environment. Microwave photonics appears to be a promising field to study other complex quantum systems.

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When a superconducting qubit (green) resonates with a microwave photon (purple) propagating in a transmission line (black), it can break it very efficiently into a quasi-resonant photon (blue) and several low energy photons (red).

# Three-dimensional architectures of phytoplankton for energy metabolism

Phytoplankton play an essential role in sustaining life on Earth. By converting CO<sub>2</sub>, sunlight and nutrients into biomass and oxygen, the single-celled organisms that make up phytoplankton are responsible for about 50% of **primary production**. They contribute to food chains and to the biological pump, a major component of the marine carbon cycle that allows the fixation of CO<sub>2</sub> in the oceans by sedimentation of microalgae. Understanding the cellular basis of phytoplankton response to environmental changes could inspire promising developments in biotechnology.

To date, the morphological characteristics of phytoplankton have been visualized primarily by light microscopy, three-dimensional (3D) confocal microscopy, and two-dimensional (2D) electron microscopy studies. However, these studies provide images with insufficient resolution to reveal cellular ultrastructure, and 2D electron microscopy cannot provide a complete volumetric description of these cells and their organelles.

A collaboration between several IRIG laboratories (LPCV, IBS, MEM) allowed the 3D reconstruction of the cellular architecture of eukaryotic cells representative of the main phytoplankton families (Figure), using focused ion beam scanning electron microscopy (FIB-SEM)<sup>[1]</sup>. The FIB-SEM images constitute a unique resource to identify the properties of subcellular architectures that have been maintained during the evolution of plankton. These include, the conservation of the volume occupied by the main organelles, and the preservation of the volumetric relationships and proximity between the energy-producing organelles mitochondria and plastids.

By revealing how an intimate interaction between cellular structures and physiological responses allows cells to adapt to various environmental conditions, these results shed light on the cellular basis of the flexibility of phytoplankton energy metabolism. Thanks to the combination of many expertises, researchers have shown the existence of a close link between the mitochondria-plastid interactions and the capacity to produce of algal biomass in mixotrophy (i.e. the synergy between respiration and photosynthesis) in the extremophilic microalga *Galdieria sulphuraria* [2], the model diatom *Phaeodactylum tricornutum* [3] and the oleaginous microalga *Microchloropsis gaditana* [1, 4] were characterized.

This study shows that the physiological responses of phytoplankton are based on specific characteristics of

their main organelles (plastids and mitochondria), opening up perspectives of exploitation in the field of algal biomass production upstream of biotechnology applications.

**Primary production** is the rate at which energy is converted into organic substances by terrestrial and aquatic photosynthetic (photoautotrophic) organisms.

**These expertises** involved cryo-electron tomography performed at the MEM laboratory and at the IBS, photophysiology, lipidomics and metabolic engineering performed at the LPCV, proteomics at the Biosanté laboratory, metabolomics at the Heinrich Heine University of Düsseldorf in Germany and at the University of Liège in Belgium, and finally metabolic modelling at Oxford Brookes University in the United Kingdom.

These studies were carried out thanks to the ANR « MoMix » funding, the European Community (ERC AdG "Chloromito") and through collaborations with the Fermentalg [3] company and the Total petrochemical group [4].

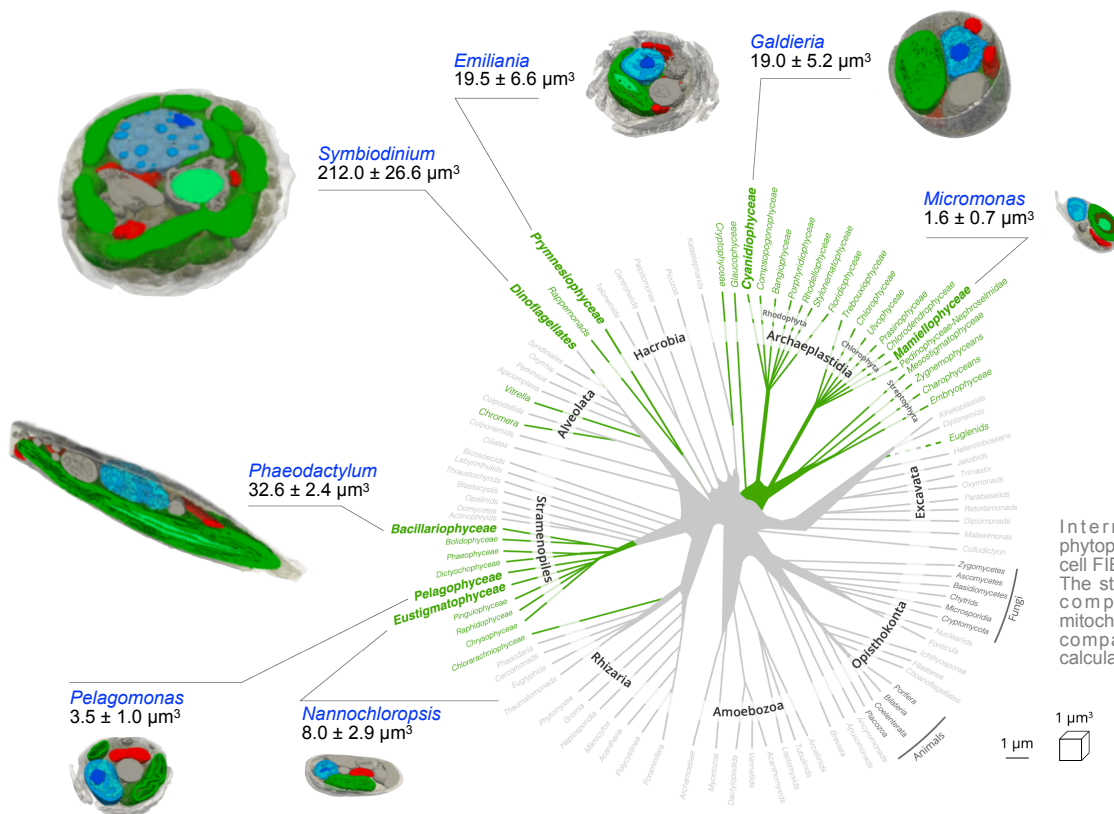
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Internal cellular architecture of phytoplankton cells obtained from whole-cell FIB-SEM images. The structures reveal the main subcellular compartments: plastids (green), mitochondria (red), nucleus (blue), other compartments (grey), and allow to calculate their volume.

## A successful fusion for a key intermediate en route to the nitrogenase active site

Some bacteria are able to fix atmospheric nitrogen and convert it into ammonia at room temperature, a reaction of major importance for plants (nitrogen cycle). The enzyme responsible for this reduction is nitrogenase, a metalloprotein that uses two metal centers: P-cluster and FeMo-co. The first one is an atypical  $[\text{Fe}_8\text{S}_7]$  cluster, which allows the electron transfer to the FeMo-co. The latter is the active site itself and corresponds to an organometallic  $[\text{MoFe}_7\text{S}_9\text{C}(\text{R})\text{-homocitrate}]$  center. While the reaction mechanism of nitrogenase is still the subject of intense debate, the processes responsible for the FeMo-co production and insertion into the enzyme are far from being deciphered and are also sources of intense controversy.

The biosynthesis of FeMo-co requires the action of a dozen accessory proteins gathered in the NIF (Nitrogen Fixation) assembly machinery. The NifB protein is the key enzyme in this process because it is responsible for the fusion of two  $[\text{Fe}_4\text{S}_4]$  centers and the insertion of a carbide ion and a sulfide ion to produce a  $[\text{Fe}_8\text{S}_9\text{C}]$  precursor called NifB-co. IRIG researchers are studying the catalytic mechanisms of metalloenzymes containing transition metal, but also the mechanisms of synthesis and insertion of these metal sites into the enzymes in which they are to be inserted. In 2020, in collaboration with the Universidad Politécnica of Madrid, they published the first crystal structure of the NifB protein, representing an early state of the NifB-co synthesis reaction. This work was quickly followed by another publication by a team of American researchers reporting a crystal structure with all metal centers present. Unfortunately, this team poorly modeled their crystallographic data and was therefore unable to correctly identify the active site content.

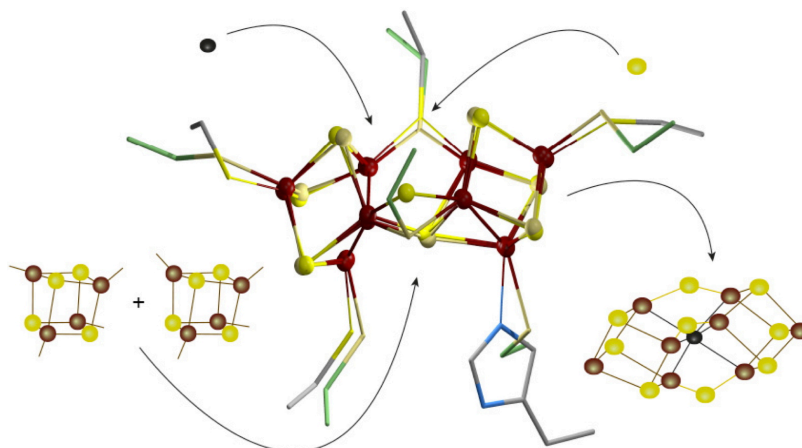
By revisiting these crystallographic data, the IRIG researchers were able to highlight the unexpected presence of a  $[\text{Fe}_8\text{S}_8]$  center resulting from the fusion of  $[\text{Fe}_4\text{S}_4]$  centers. This crystal structure allows to redefine the order of the reaction steps, showing that the fusion of the FeS centers must take place before the insertion of the carbide ion. The peculiar coordination of this intermediate clearly highlights the role of the protein matrix in the well-orchestration of the NifB-co biosynthetic steps, thus unveiling the mechanism of the enzyme.

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The K-cluster: a new intermediate in the synthesis of NifB-co, with a three-dimensional structure and coordination similar to that of the P-cluster. Schematic view of the different events allowing the fusion of two  $[\text{Fe}_4\text{S}_4]$  centers (left) leading to the K-cluster (in the center and superimposed on the crystal structure of the nitrogenase P-cluster), then the arrival of the carbide ion and the sulfide ion to produce NifB-co (right).

# Well Plate Maker: A user-friendly app to limit batch effects in large-scale biomedical studies

With the ever-increasing throughput of instrumental pipelines used in biomedical research, it has become possible to analyze hundreds of samples, covering various biological conditions and **confounding factors**. Although these large-scale approaches promise unprecedented statistical power, they pose new challenges in terms of experimental design. In particular, it becomes necessary to avoid batch effects and limit technical biases through various randomization designs.

Many factors can influence results in clinical research, particularly biases in sample distribution before biochemical preparation. To simplify the tedious task of distributing assays across multiple **well plates** (Picture) while addressing the aforementioned constraints, researchers at IRIG have developed an easy-to-use application, **Well Plate Maker** (WPM).

Several tools already exist to work on plate maps (e.g. sample coordinates in a 96-well plate). However, WPM differs in two ways. First, the researchers designed WPM to include a graphical interface, making it user-friendly, even without programming skills. Second, WPM can be used upstream of the experiment to optimize the design of the plate maps for which it is indeed necessary to optimize the distribution of samples on the preparation plates, to limit bias (Figure). To do so, the user can specify a certain number of constraints, such as group membership, spatial or neighborhood constraints, size and number of plates. Moreover, in the case of multiple plates, samples are distributed evenly among the different plates according to the total number of samples and the size of the groups, thus avoiding "plate effects". Overall, WPM simplifies block randomization to limit other types of batch effects.

Thanks to its large range of options, WPM can be adapted to a wide range of applications (omics analysis, screening...), making it possible to easily design well plate maps, which can then be printed for wet-lab implementation. For example, Well Plate Maker is used at IRIG to optimally distribute plasma samples from clinical cohorts, prior to their biochemical preparation and proteomic analysis by mass spectrometry.

In statistics, a **confounding factor**, or confounding variable, is a random variable that influences both the dependent and explanatory variables.

**Well plates:** These are plates allowing to analyze simultaneously up to 96 different samples, contained in wells.

**Well Plate Maker** is implemented in R and available on **Bioconductor** under the Artistic 2.0 open source license. In addition to the classical scripting, it can be used *via* a graphical interface, developed with Shiny technology.

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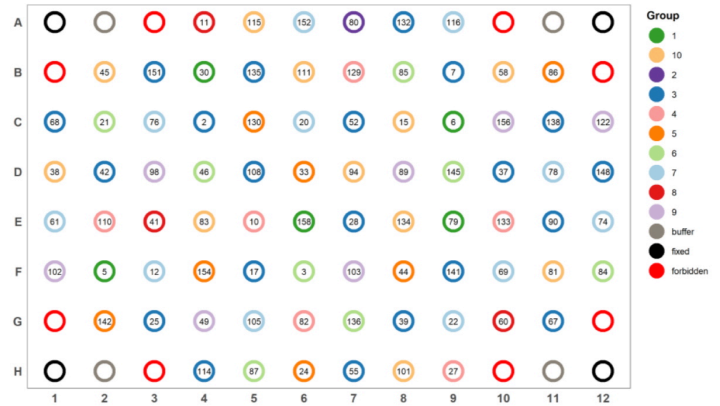
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96-well plate



Representation of a map of well plates generated by WPM.



# Morphology of chemically stabilized proton exchange membranes for fuel cell

The proton exchange membrane fuel cell, or PEMFC, converts chemical energy into electricity for the next generation of clean vehicles. Among the many attractive features of PEMFCs, manufacturers value high power density and fast start-up. This revolution requires the replacement of the current perfluorinated sulfonated membrane in the heart of the device (Nafion® type), which no longer meets requirements: the challenge is to develop low cost membranes, associated to low environmental impact and high mechanical properties beyond 80-100°C. However, alternative membranes, such as sPEEK, have two major drawbacks compared to perfluorinated membranes: their lifetime is shorter and their performances are lower. This issue is currently tackled by IRIG researchers with the objective to develop high performance membranes enabling the widespread implementation of PEMFCs.

sPEEK is an attractive polyaromatic membrane for high operating temperatures thanks to its excellent thermomechanical properties. However, it remains too sensitive to chemical oxidation during fuel cell operation. This sensitivity leads to a drastic degradation of its lifetime (a few hundred hours, compared to tens of thousands of hours for Nafion®). It is therefore useless in its pristine form but a good candidate to quickly assess stabilization strategies. Thus, IRIG researchers have employed a strategy that has been little explored until now. It consists in chemically stabilize the membranes by means of hybridization process. It has been applied successfully to sPEEK membranes [1]. A sol-gel phase was developed by self-condensation of MPTMS within the sPEEK host membrane in order to protect it from oxidizing species generated during fuel cell operation. This is a sacrificial stabilizing process where the SH thiol groups, carried by the sol-gel phase, can be oxidized until the formation of SO<sub>3</sub>H sulfonic acid groups. This disruptive strategy has allowed to design a new generation of hybrid membranes endowed with self-protective and self-improving *operando* capabilities. The researchers explored the structure-properties interplay of these hybridized sPEEK membranes, as the morphology (size, interaction/dispersion, connectivity) and localization (polar/apolar regions) of the sol-gel phase inside the host membrane are parameters expected to be crucial for ionic conductivity, gas permeability, chemical stability and mechanical properties of the hybrid membranes [2]. The nanostructure was explored at dimensional scales ranging from a few to a hundred of nanometers, using a combination of AFM/3D FIB-SEM (real space) and small angle neutron scattering (SANS)/wide angle X-ray scattering (WAXS) (reciprocal space) characterization techniques. The AFM modulus images showed that the sol-gel phase is distributed into large spherical domains

whose size varies from 100nm to 200nm depending on the sol-gel content. Contrast variation SANS (Laue-Langevin Institute in Grenoble) allowed two important observations: on the one hand, the ion channels of the sPEEK are not obstructed (preserved ionic conduction) because they are only compressed by the insertion of the sol-gel phase; on the other hand, this phase has a hierarchical structure, like aggregates of elementary particles (Figure 1). These observations show that the sol-gel nanoparticles grow in the so-called interbundle areas of the host sPEEK membrane (Figure 2).

This multidisciplinary and multiscale experimental study addressed the question of the ideal distribution and localization of the sol-gel phase for an efficient chemical stabilization. The perspectives of this work consist in exploring the hybridization by a regenerative stabilizing sol-gel phase, a very promising strategy to increase the lifetime of the membranes.

**sPEEK:** sulfonated polyether ether ketone. A possible alternative to Nafion® for proton exchange membrane fuel cells due to its many properties, including low cost and low gas permeability, availability, good proton conductivity, high thermal stability but poor chemical stability.

**MPTMS:** 3-(mercaptopropyl)trimethoxysilane.

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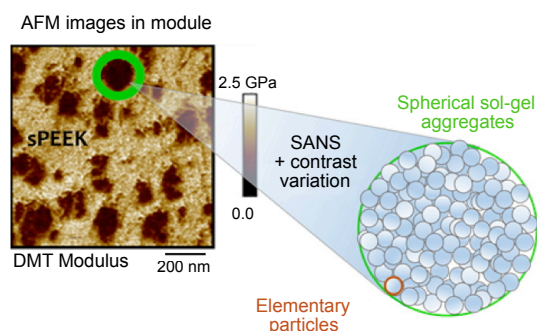


Figure 1. AFM modulus image of a hybrid membrane containing about 30% of sol-gel phase (top), a spherical sol-gel aggregate of spherical elementary particles.

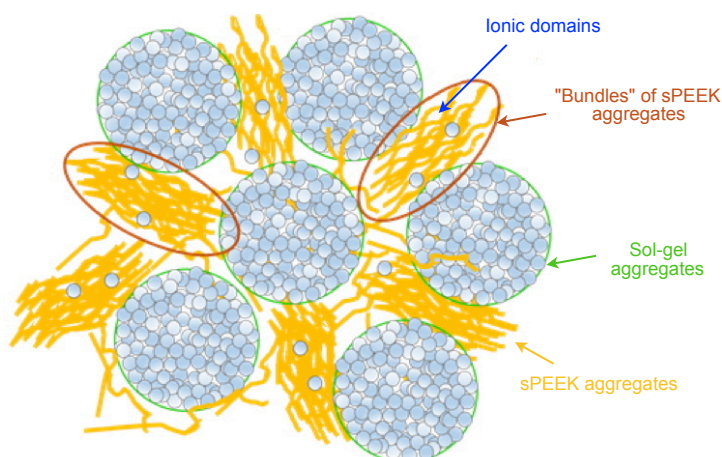


Figure 2. Multiscale structural model of hybrid membranes.

# The enzymology of tRNAs modifications: A biochemist's treasure trove

RNA biosynthesis involves many biochemical events that are essential for its maturation. From an enzymatic point of view, the nucleoside modification phenomenon is certainly one of the most remarkable of these events. It allows a multitude of structural changes in the canonical bases adenosine, guanosine, cytosine and uridine. A number of these modifications, which result from complex processes (hyper-modifications), have been characterized and are mainly located at positions 34 and 37 of transfer RNAs (tRNAs) in the so-called "anticodon" loop (Figure). They are particularly interesting because they are known to optimize the decoding function of messenger RNA by directly controlling codon-anticodon interactions. Enzymes catalyzing nucleoside modifications are fundamental targets for research, as they allow the study of protein-RNA interactions and are implicated in some diseases. Despite the wealth of genetic, physiological and biochemical data on the tRNA-modifying enzymes, very little structural data are available in the literature.

For several years, IRIG researchers have been studying the enzymes involved in the biosynthetic pathway leading to the formation of the **hyper-modified nucleoside**  $ms^2i^oA37$ . This hyper-modification results from a sequence of enzymatic reactions successively catalyzed by the enzymes **MiaA**, **MiaB** and **MiaE**, the last two of which belong to the class of metallo-enzymes. By combining biochemical, spectroscopic and crystallographic methods, IRIG researchers have identified the structural elements of MiaE that allow the docking of the enzyme with tRNA and the channeling of molecular oxygen, both of which are essential primo-processes that initiate the hydroxylation transformation.

The crystallographic structure of the *Pseudomonas putida* MiaE enzyme (pp-MiaE) has been obtained by the researchers. This structure is in the form of a single, compact domain that consists essentially of a bundle of four antiparallel  $\alpha$ -helices housing a catalytic center with two iron atoms. These two metal ions are coordinated by histidines and glutamates, and bridged by an oxygen atom characterized as " $\mu$ -oxo" by spectroscopy. Two functional channels were identified. The first was mapped using krypton atoms (oxygen substitute) introduced under pressure into Pp-MiaE crystals. This channel of hydrophobic in nature, is undoubtedly the diffusion route of molecular oxygen to the active site for catalysis. The second channel, polar by nature, is interpreted as a large cavity to stereoselectively accommodate the anti-codon part of the tRNA and to position the adenine base 37 close to the active site to facilitate the hydroxylation of its isopentenyl moiety. A structural model of the MiaE-tRNA complex was

simulated based on the crystallographic structures, the biochemical and spectroscopic results. This model reveals that a non-canonical flexible loop of the MiaE enzyme, is likely involved in tRNA recognition and docking processes.

Together, these results pave the way for understanding the mechanisms involved in enzyme-substrate recognition in tRNA modification reactions.

This **hyper-modified nucleoside** is 2-methylthio-N<sup>6</sup>-(cis-hydroxy)isopentenyl-adenosine ( $ms^2i^oA37$ ) which is present at position 37 adjacent to the anticodon of some tRNAs.

**MiaA** allows the addition of the isopentenyl group on the N<sup>6</sup> exocyclic nitrogen of adenosine, converting tRNA-A37 to tRNA- $i^oA37$ .

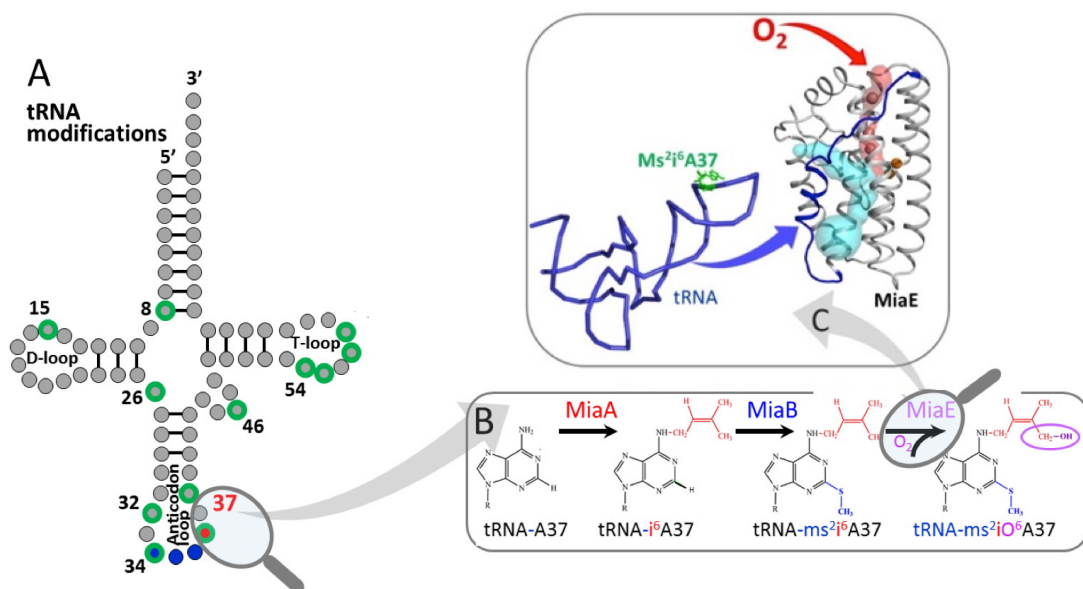
**MiaB** is an enzyme of the Radical-SAM family that catalyzes the insertion of the thiomethyl group at position 2, converting tRNA- $i^oA37$  to tRNA- $ms^2i^oA37$ .

**MiaE** belongs to the family of proteins with a non-heme iron center. This family consists of extremely diverse enzymes, identified in all kingdoms of life, which catalyze an impressive number of chemical oxidations.

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A - Typical modifications of tRNAs (in green), including those at position 37 on the anticodon loop.

B - Details of the A37 hyper-modifications of tRNA catalyzed successively by the MiaA, MiaB and MiaE enzymes.

C - Structure of the MiaE enzyme, the oxygen channel is in red, the cavity housing the tRNA is in light blue. The tRNA is in blue and the modified base is in green.

# Spintronics relies on the Janus SPTSe biface

A new two-sided *Janus* SPTSe material has been designed by IRIG researchers for potential applications in magnetic and ferroelectric memories. This two-dimensional material is a member of the family of transition metal dichalcogenides (S, Se or Te atoms), which are sought after for their electronic, photonic, catalytic or magnetic properties, which are closely linked to the details of their 2D structure.

The ultimate goal of this research is to intensify the *Rashba spin-orbit coupling* in TMDC, which generates a spin accumulation from a simple charge current flowing in the material (Figure 1). The accumulated spins diffuse to generate a spin current that can be absorbed by a magnetic layer in contact with the TMDC, causing the magnetization of this layer to reverse. Thanks to the Rashba spin-orbit coupling, the application of an electric current allows to orientate the magnetization of the magnetic layer and thus to write an information bit "0" or "1". The stronger the Rashba coupling, the more intense the spin current generated and the easier it is to reverse the magnetization of the layer, thus limiting the energy consumption of this type of magnetic memory. Intrinsically, the TMDC PtSe<sub>2</sub> has a strong spin-orbit interaction (thanks to the heavy platinum atoms). However, there was a major problem in the choice of this material because of its vertical symmetric structure that limits the Rashba coupling. Indeed, SePtSe is composed of two identical layers of chalcogen Se enveloping a central layer of metal Pt (Figure 2, bottom left). IRIG researchers have succeeded in removing this obstacle by substituting the Se atoms in the top layer, by means of controlled sulfurization under H<sub>2</sub>S atmosphere, to make an asymmetric two-dimensional material, called Janus S/Pt/Se (Figure 2, bottom right). This new material shows a strong Rashba coupling.

In order to form the Janus SPTSe, the TMDC PtSe<sub>2</sub> material is first heated to 370°C to generate vacancies in the top layer. This layer is then exposed to a controlled temperature of 350°C under H<sub>2</sub>S atmosphere to fill these gaps and obtain the substitution of Se by S. Upon cooling, the new 2D compound reorders. It is obviously necessary to master perfectly the steps of formation of the layers of S, Pt, and Se atoms in order to exploit at best the properties of the Janus SPTSe from the spintronics point of view. These steps have been studied thanks to the specific means of characterization that exist within IRIG. The formation steps of the Janus compound have been methodically characterized *in situ* and *operando* by grazing incidence synchrotron X-ray diffraction at the ESRF. The two-sided atomic composition of Janus has been verified *ex situ* by angle-resolved X-ray photoemission spectroscopy. This Janus compound is a first experimental realization exhibiting strong Rashba spin-orbit coupling that could improve the writing efficiency of spin-orbit torque memories (*SOT-MRAM*) and limit power consumption.

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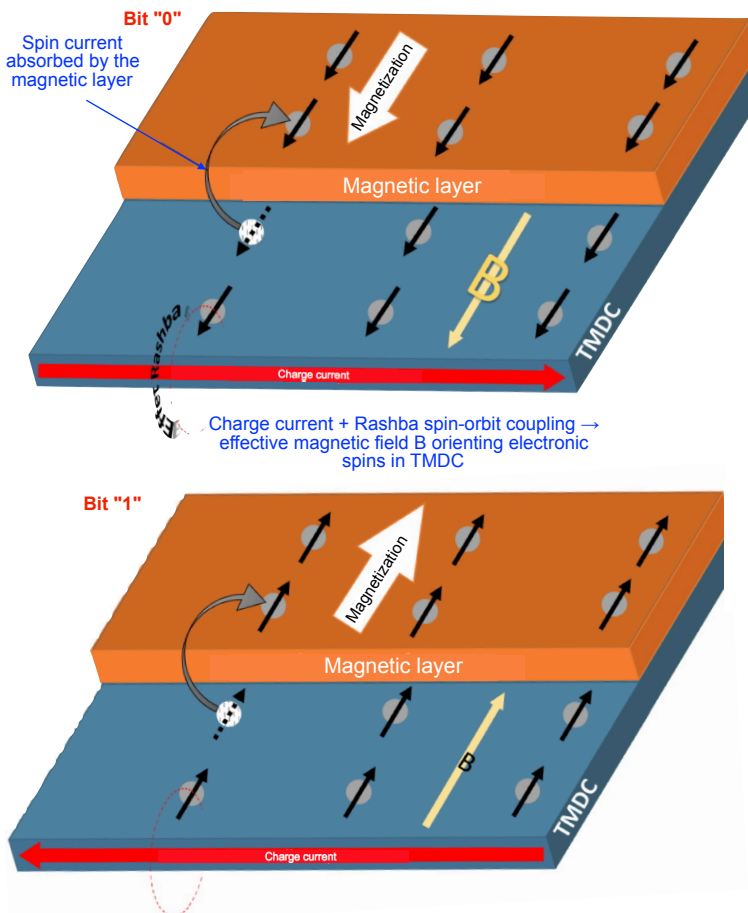


Figure 1. Effect of Rashba spin-orbit coupling in a TMDC layer and writing of the magnetic layer.

**Rashba spin-orbit coupling.** This relativistic effect, in which an electric field is felt as a magnetic field by the spin-orbit coupling, allows to generate a spin current from a charge current. It allows to sort dynamically the spins (up and down) in the material. For example, in SPTSe, the symmetry breaking with respect to the plane of platinum atoms creates a vertical electric dipole. The electric field thus created, coupled to the platinum which is a heavy element, generates a strong Rashba spin-orbit coupling. In this way, such a monolayer could be used in a magnetic memory of the *SOT-MRAM* (Spin-Orbit Torque Magnetic Random-Access Memory) type for writing the magnetic storage layer.



**Janus.** The reference to the two-faced Greek God Janus is due to the typical structure of the material in which the two chalcogen layers (S and Se) around the Pt layer have a different chemical nature.

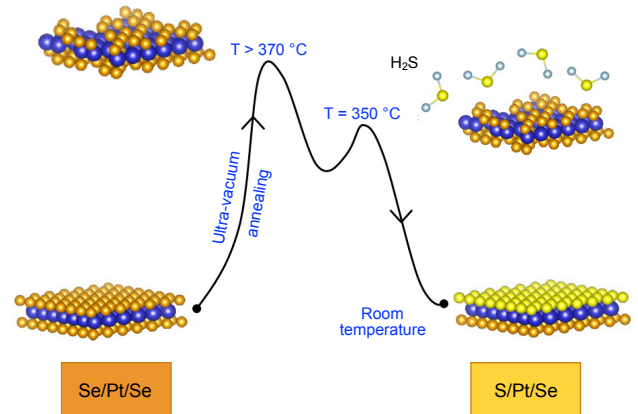


Figure 2. Pictorial scheme for the transformation of PtSe<sub>2</sub> into SPTSe. Atoms: Pt blue, Se orange, S yellow.

# Reducing the environmental impact of perpendicular magnetic memories

The information and communication technologies sector has been growing rapidly for three decades, driven by strong consumer demand and remarkable technological progress. At the same time, for several years now, societal demand for controlled growth that respects the environment has emerged and has become an integral part of the public debate. How can these two aspirations be reconciled, beyond imprecatory formulas? At IRIG, where researchers are working on future generations of memories for quantum technologies or future processors for the Internet of Things, a research team has asked itself the following question: what is the environmental impact of the memories it develops?

IRIG researchers have carried out a comprehensive review of the sustainability of perpendicular magnetised memories, a low-power storage technology. Supply-critical materials such as cobalt (Co), ruthenium (Ru), tantalum (Ta) and platinum (Pt) are used to various degrees in their composition. Taking into account the quantity of material used per device, platinum, 80% produced by South Africa and Russia, presents the greatest risk (geostrategic, environmental, economic, etc.). However, according to the study published by IRIG researchers, the economic and environmental cost of a memory is dominated by the silicon substrate. A memory contains 127g of silicon, compared to 3.4mg of platinum.

However, it is interesting, in order to reduce any dependence, to substitute the platinum used in the Co/Pt **multilayer** with a Co/Ni multilayer in the reference layer of a magnetic random access memory (MRAM). Nickel is not a rare metal and therefore does not present any risks in its supply. The researchers' results (*Figure*) show that it is possible to change the composition of the

**reference layer** [Co 0,5nm/Pt 0,25nm]<sub>xN</sub> by a layer of composition [Co 0,18nm/Ni 0,55nm]<sub>xN</sub> while retaining the same magnetic properties and thermal stability. Furthermore, the substitution of platinum for nickel does not require the use of other critical materials. A reduction in the global warming potential of the reference layer by a factor of 3 can therefore be achieved.

A wider use of MRAMs by the microelectronics industry can therefore be envisaged in a sustainable way by replacing platinum with nickel and potentially reducing the availability risks.

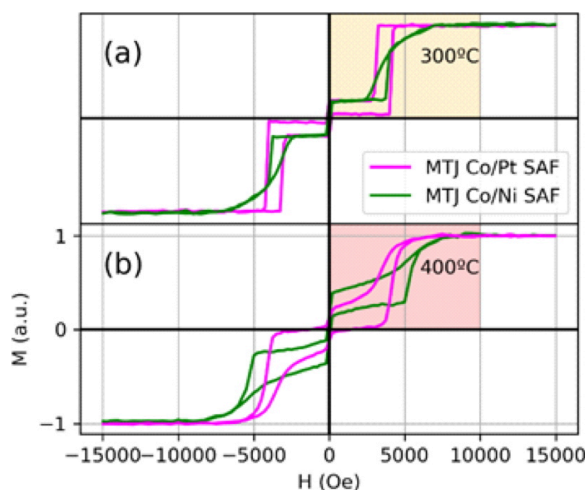
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A **multilayer** is a layer that is a repetition of a basic pattern. For example [Pt/Co]<sub>N</sub> where N is the number of repetitions of Pt/Co.


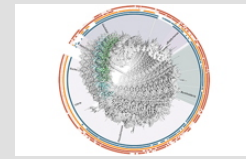
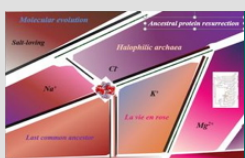
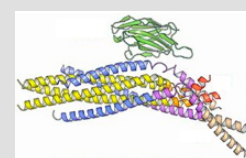
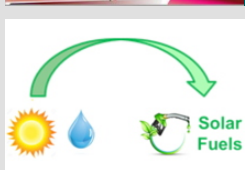
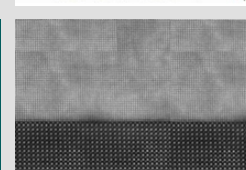

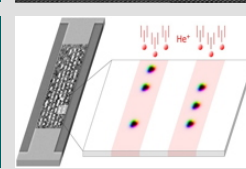
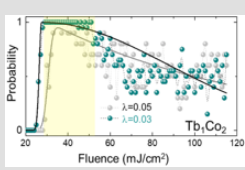
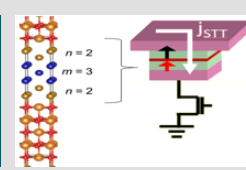
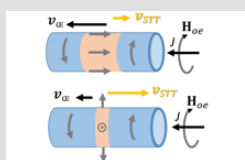
Schematically, a magnetic memory consists of two magnetic layers separated by a non-magnetic layer. One magnetic layer, called the **reference layer**, has a fixed magnetisation. The orientation of the other magnetic layer is free. When the magnetization of both layers is in the same direction the memory is in the "zero" state. When the magnetization of the free layer is turned in the opposite direction then the memory is in the "one" state.



Substituting the Co/Pt multilayer with a Co/Ni multilayer in the reference layer of an MRAM does not lead to a degradation of the thermal stability.


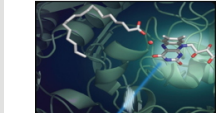

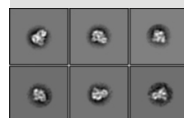




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