SCIENTIFIC NewsLetter

INTERDISCIPLINARY RESEARCH INSTITUTE OF GRENOBLE

July 2019 N°04

Olfactory biosensors

Volatile organic compounds (VOCs) are the main components of odors and are abundant in our environment. In recent decades, their monitoring has become a concern, particularly in air quality control, industrial manufacturing processes, public safety, health, etc. To meet this growing need, sensors inspired by the biological nose, such as olfactory biosensors and electronic noses, are being developed.

Contact: Yanxia Hou-Broutin
SyMMES
Molecular Systems and nanoMaterials for Energy and Health Laboratory
UMR 5819 - CEA - CNRS - UGA

In order to improve the performance of these sensors, particularly sensitivity and selectivity, two families of proteins from the animal olfactory system are of particular interest to researchers: olfactory receptors and odor-binding proteins (OBP). The latter are ideal candidates for such applications. They are stable to temperature and pH variations, to some organic solvents, they are soluble, therefore easy to produce and purify, and their binding properties to their ligands are modifiable by mutagenesis.

In collaboration with the Centre des Sciences du Goût et de l'Alimentation (CSGA) in Dijon, researchers from the IRIG's Molecular System and Nanomaterials for Energy and Health Laboratory developed sensitive olfactory biosensors using rat OBPs. After designing and immobilizing several variants of these proteins on the chip, they demonstrated their ability to bind and recognize ***ß-ionone***, ***hexanal*** and ***hexanoic acid***.

The obtained olfactory biosensors have very low detection limits in both concentration and molecular weight. Such a good sensitivity is thanks to the fact that the binding of VOCs to the active sites of these OBPs would induce a change in the conformation of these proteins. This change would result in a variation in the local refractive index to which surface plasmon resonance imaging (SPRi), used here as a transducer, is extremely sensitive. Other advantages of these biosensors are their high selectivity, especially at relatively low VOC concentrations, and high repeatability, as well as good stability with a lifetime of up to two months.

Further work could pave the way for the design and use of new custom-made olfactory proteins to specifically target a wider range of VOCs with a high societal impact.

β-ionone is an aroma compound found in a variety of essential oils, contributing significantly to the odor of raspberries. ***Hexanal*** is found in wine. It comes from the skin of the grape. Depending on its maturity, it is responsible for the more or less herbaceous notes that are sometimes found in the wine. ***Hexanoic acid*** gives a fatty and cheesy odor.

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Hurot C, Brenet S, Buhot A, Barou E, Belloir C, Briand L and Hou Y. Highly sensitive olfactory biosensors for the detection of volatile organic T compounds by surface plasmon resonance imaging. *Biosensors and Bioelectronics*, 2019

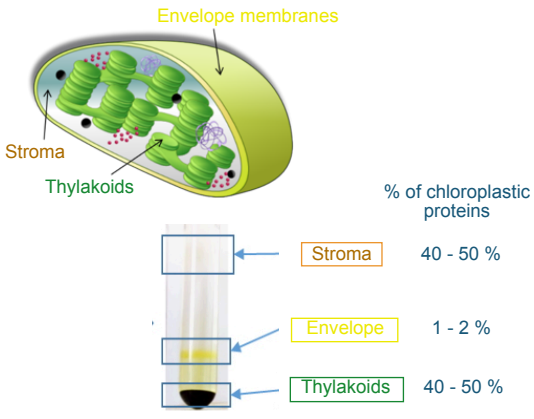
Towards a better understanding of the regulation of chloroplast biogenesis and functions

Cells from higher plants and algae contain a compartment specific to the plant kingdom: the chloroplast. This organelle is the place of oxygen production and of synthesis of vitamins, amino acids, fatty acids, lipids, starch... *i.e.* most compounds essential for our diet and sources of bioenergy. To synthesize these molecules, the chloroplast only needs CO_2, water, minerals and some metals. The energy used to catalyze all these reactions derives from the conversion of solar energy into chemical energy through photosynthesis. However, the mechanisms that regulate dynamic exchanges between the chloroplast and other cell compartments still remain poorly understood.

Contact: Norbert Rolland
LPCV
Cell & Plant Physiology Laboratory
UMR 5168 - CEA - CNRS - Inra - UGA

Current estimates suggest that the chloroplast contains about 3500 different proteins catalyzing above-cited reactions. Variations in environmental conditions (light, temperature, etc.) perceived by plants require constant adaptations of the chloroplast composition to optimize its functioning. However, the vast majority of these 3500 proteins must be imported into the chloroplast by mechanisms that, for some of them, remain unknown. These mechanisms are themselves catalyzed by yet unidentified proteins located in two biological membranes (the envelope) that surround the chloroplast. As these envelope proteins are very rare at the chloroplast and plant cell scales, their identification required the use of cell fractionation (to enrich them, *Figure*) and proteomics approaches (to identify them). During this study, researchers from the Cell & Plant Physiology Laboratory and the Large Scale Biology Laboratory combined biochemical, imaging, mass spectrometry, bioinformatics and statistical approaches to reveal previously unidentified protein components present in these membranes.

This study sheds new light on the composition of the chloroplast envelope membranes, and opens new perspectives in understanding mechanisms that regulate the dynamics of chloroplast biogenesis and functions. Efforts to characterize the role of novel proteins identified during these approaches are performed in the two above-cited IRIG's laboratories.



Fractionation of the chloroplast and relative abundance of proteins present in each sub-compartment.

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MRAM integration into standard microelectronics processes

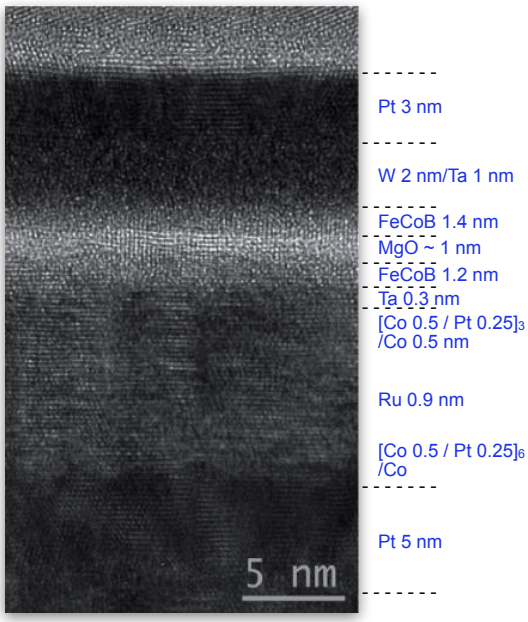
MRAM is a memory type that uses the orientation of magnetization as the elementary unit of information, the bit. In the latest generation of MRAMs, magnetization is oriented perpendicular to the plane of the material layers, deposited in sequence to form a material stack and create a magnetic tunnel junction (MTJ). The properties of these layers are improved by annealing after deposition. However, the maximum annealing temperature of such a junction is limited to 300°C, while some manufacturing processes in the microelectronics industry require annealing temperatures of 400°C. Is it possible to remove this limitation and extend the application field of MRAMs?

Contact: Ricardo Sousa
Spintec
Spintronics and Component Technology laboratory
UMR 8191 CEA - CNRS - UGA - G-INP

At the heart of MRAM is a perpendicular magnetic tunnel junction based on a stack of CoFeB/MgO/CoFeB (*Figure*) for which researchers at IRIG's Spintronics and Component Technology laboratory (Spintec) are at the forefront. In order to improve its magnetic and electrical properties, a so-called annealing step after deposition is necessary. ***During this annealing***, the boron atoms of the CoFeB layers migrate and allow crystallization of the junction. To limit the diffusion of boron into other parts of the MTJ, tantalum (Ta) with higher affinity for boron is used as a protective capping layer. During this annealing, Ta captures part of the iron atoms of the MTJ, which degrades the MRAM. This undesirable capture appears above 300°C.

Spintec researchers had the idea of replacing Ta with tungsten with better refractory properties. They then observed that after annealing at 400°C tungsten had a lower tendency to capture iron. The layers then remain more homogeneous, which improves the properties observed at their interfaces. It becomes even possible to increase the annealing temperature up to 450°C while significantly improving the magnetic performance of the active part of the junction. Integrating MRAMs into standard microelectronics processes is becoming a reality.

During this annealing, tantalum tends to absorb boron from the tunnel barrier. It also absorbs some of the iron from the magnetic electrode and changes its chemical composition. The MRAM's magnetoresistance and information retention time are then degraded. Tungsten, on the other hand, captures less iron during annealing, leading to improved properties after annealing.



Material stack and thickness in nm of the different layers of materials used in magnetic tunnel junctions after annealing at 425°C.

REFERENCE

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When crack front makes waves

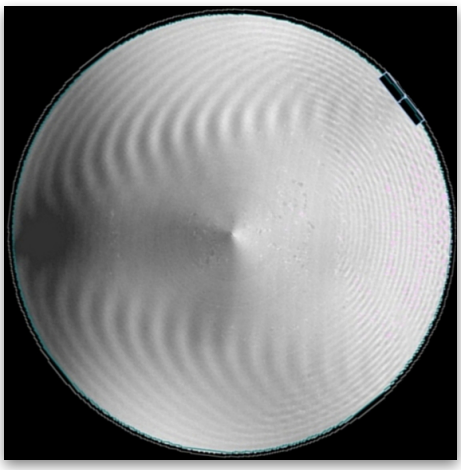
Silicon-on-Insulator (SOI) substrates used for the manufacture of high performance / low power electronic components include a monocrystalline silicon film obtained by fracture of a bulk silicon wafer. To precisely control this fracture plane, a thin layer of tiny cavities is created at the desired depth by implanting hydrogen ions into the wafer (***SmartCut***™). The surface thus revealed by the fracture has an alternation of rough and smooth textures. However, these inhomogeneities may impact the performances of the circuit that will be engraved on this surface.

Contact: François Rieutord
MEM
Modeling and Exploration of Materials laboratory
UMR CEA - UGA

A team from the IRIG's Modeling and Exploration of Materials laboratory, in collaboration with researchers from the DRT and Soitec (a world leader in the production of innovative semiconductor materials) have deciphered the mechanism behind the patterns observed on the surface of silicon wafers when manufacturing SOI substrates.

On the wafer images taken after fracturation, wave-like patterns looking like the wake of a boat put the researchers on the sound waves track. They therefore measured the speed of propagation of the crack generated by the cleavage (a few km/s) using an infrared optical device and analysed the acoustic waves emitted with piezoelectric sensors. These self-emitted acoustic waves propagate from one side of the fracture front to the other and are reflected at the end of the silicon wafer, which behaves like a filter or a resonator. The system selects only certain acoustic (flexural) waves whose velocities are in phase with that of the fracture. These bending waves produce periodic deformations of the silicon wafer assembly, which deflects the fracture from its nominal trajectory, resulting in observed variations in roughness. A simple model makes it possible to check that the periodicity of the patterns agrees with what is experimentally observed.

This discovery will allow further improvements in this process. More widely, it will help to understand the dynamics of the brittle fracture of materials.



Wave patterns observed on SOI wafer images after fracturing.

Smart Cut™ is a generic thin film transfer process used for the manufacture of silicon-on-insulator substrates on an industrial scale invented by Michel Bruel at CEA-Leti. The implantation of light ions in an oxidized silicon substrate leads to the formation of a weakened zone buried within the crystal.

REFERENCE

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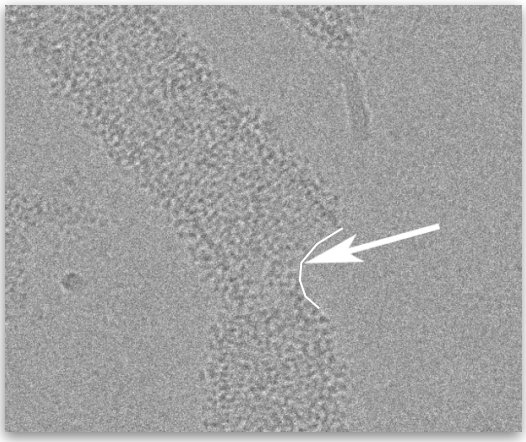
How does an enveloped virus emerge from a cell?

Many viruses (HIV, measles, influenza, etc.) have a lipid envelope that protects their genetic information. The viral particle forms a lipid vesicle at the plasma membrane of the infected cell carrying the genetic information of the virus before it escapes by cleaving the newly formed viral membrane envelope from the host cell membrane, a process called membrane fission. Would it be possible to observe this phenomenon in order to observe membrane fission?

Contact: Christophe Caillat
IBS
Institut de Biologie Structurale
UMR 5075 - CEA - CNRS - UGA

When a virus emerges from a cell, complex membrane remodeling, called budding is induced. The final step of budding includes the separation of the newly formed membrane-enveloped virus from the intact plasma membrane. This is catalyzed by spiral filaments made of proteins from the ESCRT machinery (*Endosomal Sorting Complex Required for Transport* ESCRT-III), which constrict the membrane to the point of fission with the help of the energy-providing ATPase VPS4B.

IRIG researchers have developed an *in vitro* system to reconstitute ESCRT-III filaments. They then used both atomic force microscopy to monitor the evolution of the diameter of the spiral tubes in real time and electron microscopy to image filament constriction at higher resolution. This revealed that the details of VPS4B action at two steps: first, VPS4B reduces gradually the diameter of the filaments (constriction); secondly, VPS4B cleaves the filaments asymmetrically into two ends, with one end taking the form of a "dome-like" structure.
Thus, cleavage and dome formation have been suggested to constrain the membrane such that it ultimately leads to the release of the virus from the host cell.



Structure of spiral tubes formed by ESCRT-III proteins. The arrow shows where a tube is cut, with one of the two sides in the shape of a dome. Electron microscope image. © CEA

REFERENCE

Maity S, Caillat C, Miguet N, Sulbaran G, Effantin G, Schoehn G, Roos WH and Weissenhorn W. VPS4 triggers constriction and cleavage of ESCRT-III helical filaments. *Science Advances*, 2019

Novel therapeutic targets for prostate cancer

MicroRNAs (miRNAs or mIRs) are very short single-stranded ribonucleic acids (usually 21 to 24 nucleotides), capable of inhibiting the expression of a target gene by pairing to a complementary sequence of its messenger RNA leading to its translational repression or degradation. Research on mIRs has highlighted their multiple roles in regulating (negatively and sometimes positively) gene expression. Aberrant expression of mIRs is also reported to be involved in many diseases, and mIRs-based therapies are currently under study.

Contact: Xavier Gidrol
BGE
Large Scale Biology laboratory
UMR 1038 - CEA - Inserm - UGA

In a close collaboration with Dr. Charlotte Bevan's team at Imperial College of London, and more specifically Dr. Claire Fletcher who spent 6 months in this team, researchers at the IRIG's Large Scale Biology laboratory have participated in the characterization of several miRs that play a role in prostate carcinogenesis.
Androgens and their receptor (AR) play a major role in prostate cancer. In order to systematically identify miRs that may modulate AR activity in prostate cancers, a cell line expressing the AR gene fused to luciferase (a luminescent protein that tracks AR gene expression in cells) was transfected in the presence of a collection of inhibitors of all known miRs in the human genome. Seventy-eight inhibitors led to modulation of AR expression. Of these, three significantly reduced the transcription of the AR gene as well asmRNA and protein levels.

In summary, this study identified miRs that modulate AR activity in prostate cancers, including hormone castration-resistant prostate cancer. These observations are based on new mechanisms that could open up new therapeutic avenues.

The results of this study in detail:

Of the 78 miR inhibitors, miR-346, miR-361-3p and miR-197 inhibitors significantly reduced RNA gene transcription, mRNA and protein levels, while inducing apoptosis and inhibiting proliferation, epithelial-mesenchymal transition, migration and invasion of transfected prostate cells. Conversely, the corresponding miR mimics increased AR activity through a new anti-dogmatic mechanism uncovered by this study, direct association with the 3' UTR region of the AR gene and stabilization of transcription. Analysis of the miR targets identified by AGO-PAR-CLIP revealed roles in DNA replication and repair, cell cycle, signal transduction and immune function. As expected, silencing these targets, including tumor suppressors ARHGDIA and TAGLN2, phenocopied miR effects, demonstrating physiological relevance.

Transfection is the process of gene transfer, *i.e.* the introduction of exogenous genetic material into eukaryotic cells.

REFERENCE

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The Laboratories

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Publishing Director
Jérôme Garin

—

Editor and electronic format
Pascal Martinez
Pascal.Martinez@cea.fr

—

Editorial Board
Christophe Caillat, Yanxia Hou-Broutin, Xavier Gidrol, François Rieutord, Norbert Rolland, Ricardo Sousa

irig.cea.fr

Interdisciplinary Research Institute of Grenoble
CEA-Grenoble
17 avenue des Martyrs | 38054 Grenoble cedex 9

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