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Scientific Newsletter

FALL 2022

Acknowledgements

In the spirit of the Institute, the IRIG scientific letter once again brings together magnificent presentations of achievements in a wide range of disciplines and themes. Over the past few years, I have enjoyed working on the editing of this letter as it has been one of the tools that has allowed me to follow the evolution of your research projects and to be delighted by them. As I am about to leave you, I would like to thank those of you who have contributed to this important communication exercise, and I hope that this letter will continue to share scientific information within the Institute while contributing to the influence of IRIG.

Jérôme Garin, Head of IRIG from January 2019 to September 2022

Proteogenomics: On the correct use of databases

Proteogenomics aims to use ***personalized*** in addition to the canonical ***databases*** of the species to better characterize the proteins of samples analyzed by mass spectrometry. To avoid the multiplication of ***sequence variants*** in these databases, which would then become too large and ambiguous, researchers have proposed to reduce these databases to the ***transcripts*** expressed in the biological sample analyzed. However, it appears that this reduction in database size artificially increases the confidence of peptide identification. Therefore, what biostatistical method can be considered to restore the results' reliability?

Contact: Thomas Burger
Biosanté
Biology and Biotechnology for Health Laboratory

Reference databases are essential for mass spectrometry based identification. Using them, the digital tool generates theoretical mass spectra, as to propose a list of amino acid sequences and their matching probabilities with the experimental mass spectra. Then, to validate the resulting identifications, it is necessary to estimate both the proportions of true matches (between a sequence and a spectrum) and the so-called "false discovery rate" (resulting from random matches devoid of biochemical ground). To do so, the most classical method is to include "decoy" sequences in the database, which are biologically irrelevant, as to subsequently count the number of experimental spectra matching them.

Researchers at IRIG have shown that the smaller the database (because of its transcriptome informed-pruning) the more the false discovery rate is underestimated by the decoy count. They explain that the increase of identification sensitivity on a transcriptome-informed reduced database is in fact a statistical artefact: with a smaller database, fewer decoys are generated, which reduces the probability that some of them are sufficiently realistic to mimic identification errors. Because the false discovery rate is critical to the reliability of the downstream biological conclusions, the Irig researchers propose alternative statistical methods to control for it, and show these methods are less sensitive to the database size.

This calls into question the sensitivity increment induced by transcriptome-informed reduced databases, but the approach remains interesting as it facilitates the identification of ambiguous proteins by reducing the proportion of sequence homologies between different proteins. These results, implemented in data processing routines, contribute to the future of computational proteogenomics, and show a good example of interdisciplinary cooperation

Proteogenomics combines proteomics (identification and quantification of all the proteins in a sample) with genomic/transcriptomic approaches. While genomics studies the DNA sequences of living beings, transcriptomics identifies and quantifies ***transcripts***, *i.e.* RNAs resulting from the transcription of DNA. Transcriptomics makes it possible to estimate the level of expression of genes, whereas genomics does not.

Personalized database: from genomic and/or transcriptomic knowledge specific to the pathology studied, or even directly derived from the genome and/or transcriptome of each patient.

Sequence variants: the sequence of a gene changes from one individual to another.

REFERENCE

Fancello L and Burger T. An analysis of proteogenomics and how and when transcriptome-informed reduction of protein databases can enhance eukaryotic proteomics. *Genome Biology*, 2022

Nanoplatelets free of toxic heavy metals for optoelectronics

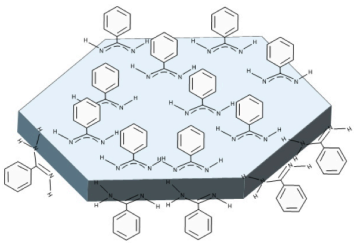
Nanomaterials research is interested in colloidal semiconductor nanocrystals for their optoelectronic properties that strongly depend on their composition, size and morphology. In particular, colloidal nanoplatelets have recently emerged as a new class of quantum well nanomaterials with novel optical properties when compared to ***quantum dots***. However, the difficulty of synthesis and structural control of these nanocrystals remains a challenge.

Contact: Gaël De Paëpe
MEM
Modeling and Exploration of Materials

In collaboration with the Institute of Physical Chemistry of the Polish Academy of Sciences at Warsaw, Poland, researchers at IRIG present a new approach to produce ZnO nanoplatelets with controlled thickness (from 3.2 to 7.5 nm) and durable colloidal stability. These Zn-based nanoplatelets are an attractive alternative, free of toxic heavy metals, to conventional cadmium chalcogenide-based colloidal 2D semiconductor nanostructures.

Beyond an original synthetic procedure, based on the controlled hydrolysis of organometallic complexes by H_2O, this work reveals the particular role of the chosen benzamidine ligands. Using ***dynamic nuclear polarization***-enhanced solid-state NMR, the researchers increased the intensity of the ^{15}N NMR signals of the surface ligands without resorting to ^{15}N isotopic enrichment. This approach allowed them to reveal the original role of the benzamidine ligands in stabilizing the surface of these nanomaterials, since they can bind to both polar (basal planes) and non-polar (lateral surfaces) facets of the nanocrystals. This bimodal stabilization, where the synthetic ligands behave as both ***X- and L-type ligands***, allows obtaining hexagonal nanoplatelets.

Further study of the interactions and arrangement of surface ligands is underway. This fundamental information on the organic-inorganic interfaces of ZnO nanoplatelets will facilitate the design of new stable and size-controlled colloidal suspensions.



ZnO nanoplatelets and its organic–inorganic interfaces.

Optoelectronics is the study of electronic components that emit or interact with light.

Quantum dot: semiconductor nanoparticle with specific optical and electronic properties due to their nanometric size.

Dynamic Nuclear Polarization or DNP: the polarization of the unpaired electrons is transferred to the nucleus in order to better observe the atoms by NMR.. The sensitivity is improved to obtain information on the nature of the bonds at the interfaces, here between the nanoplatelets and the ligands.

X- or L-type ligand: X-ligand and the metal provides one electron each; L donates two electrons to the metal.

REFERENCE

Terlecki M, Badoni S, Leszczyński MK, Gierlotka S, Justyniak I, Okuno H, Wolska-Pietkiewicz M, Lee D, De Paëpe G and Lewiński J. ZnO Nanoplatelets with controlled thickness: Atomic insight into facet-specific bimodal ligand binding using DNP NMR. *Advanced Functional Materials*, 2021

Are tattoos really inert?

Tattooing is a practice that traces back to the Stone Age, and consists in injecting non-biodegradable and persistent pigments into the dermis. This practice has been democratized since the 1970's and the number of followers has not stopped growing with a younger and younger public. Today, approximately 100 million people are tattooed in Europe. Motivations can be cultural (fashion phenomenon) or medical, *e.g.* to conceal scars, breast reconstruction and targeting of areas to be treated by radiotherapy. The current fashion tends to increase both the surface of tattooed skin and the number of colors used. This exposes the skin to a large variety of substances present in the pigments which can be mineral (based on metallic particles) or more recently, organic (based on dyes resulting from petrochemistry).

Contacts: Thierry Rabilloud and Bastien Dalzon
CBM
Chemistry and Biology of Metals Laboratory

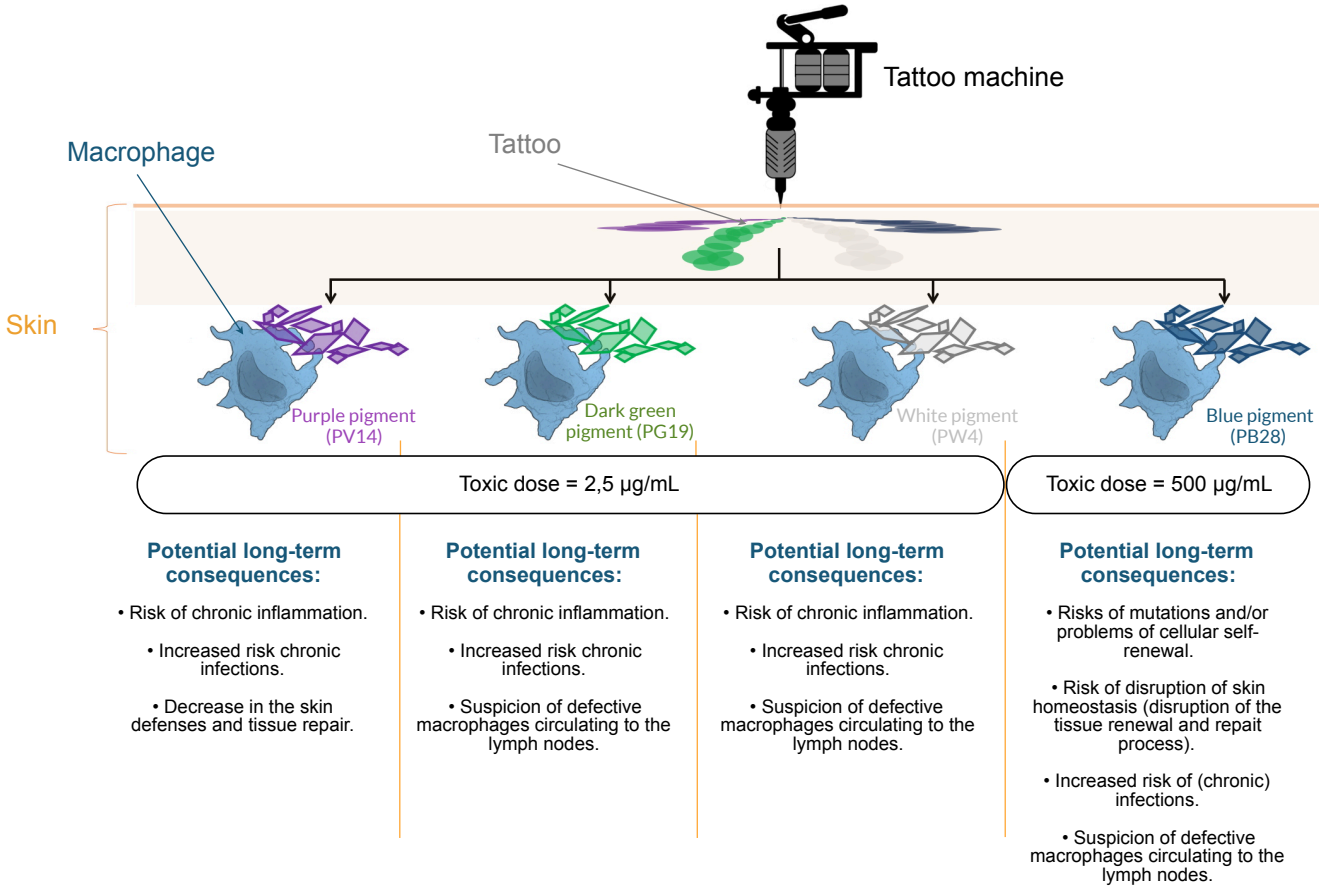
Short-term effects of tattooing such as transient inflammation are very frequent and widely documented. However, the question arises of possible long-term effects, *i.e.* several years after the tattooing. Indeed, the skin is far from being inert and can respond to these exogenous substances which, in fact, evolve during the lifetime. Currently, it is still difficult to evaluate the link between tattoos and chronic diseases, from simple dermatosis to auto-immune diseases and a possible greater sensitivity to cancers. Only clinical cases inform us about a possible relation of cause and effect, without really determining the associated mechanisms.

IRIG researchers propose to evaluate the effects of different pigments used as tattoo ink on a cell type present in the dermis and responsible for the persistence of the tattoo: macrophages. Macrophages are immune cells that play a key role in the inflammatory response and the proper functioning of the skin. In the case of tattooing, macrophages internalize the pigment particles and immobilize them at the injection site, which induces the long-term permanence of the tattoos. How do some of these pigments used in the composition of inks act on macrophages? How do these cells respond to this new stress and to the ingestion of a large quantity of particles that are not naturally present in the skin? What are the long term effects since the tattoo is present for a lifetime? These are the questions that IRIG researchers seeked to answer by developing a long-term cell culture model of macrophages.

To this purpose, the pigments were first characterized by evaluating their size, their shape, their capacity to dissolve in a biological environment. These characteristics may influence their effects. Then the toxic dose of each pigment tested was determined on macrophages. Finally, the perturbations on the macrophage functions and the mechanisms involved in the possible toxicity were evaluated. All the tests were performed just after exposure to the pigments and several days later in order to investigate the persistence of the effects over time. The researchers were able to conclude that the short-term functional and delayed effects were more or less important from one pigment (material) to another.

REFERENCE

Devcic J, Dussol M, Collin-Faure V, Pérard J, Fenel D, Schoehn G, Carrière M, Rabilloud T and Dalzon B. Immediate and sustained effects of cobalt and zinc-containing pigments on macrophages. *Frontiers in Immunology*, 2022



Possible effects of cobalt pigments.

Tunneling effect in SAM radical enzymes

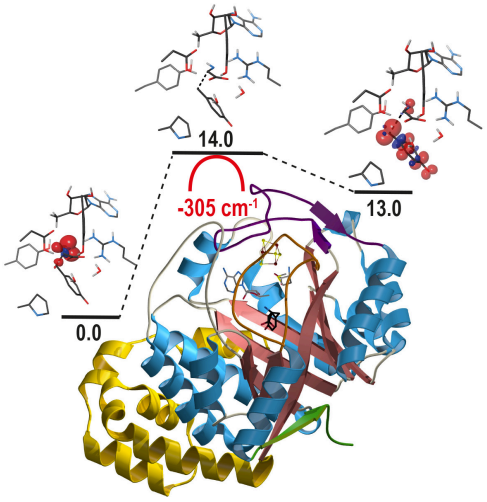
Transition metals play a key role in protein function. Thanks to their unique properties enzymes can afford reactions otherwise impossible. Hence, the so-called SAM Radical proteins form a superfamily of metalloenzymes that use a [4Fe-4S]$^{+}$ active center to cleave S-adenosyl-L-methionine (SAM) and generate a 5′-deoxyadenosyl radical. This high-energy radical enriches the panoply of possible chemical reactions, leading to the use of "SAM Radical" enzymes for cofactor biosynthesis, peptide modification, or even antibiotic synthesis.

Contact: Yvain Nicolet
IBS
Institut de Biologie Structurale

Because of their potential, metalloenzymes can be considered as biotechnological tools. Thus, some research aims at designing new artificial enzymes. This research focuses notably on understanding how the protein backbone controls the the high-energy intermediates that are involved in catalysis.

The work carried out by the IRIG researchers is part of this context. These researchers studied ThiH and NosL, two SAM radical proteins. These enzymes have the same primary structure as well as similar substrate binding modes. Therefore, comparing how they break alternative C-C bonds should lead to a better understanding of the mechanisms involved. The crystal structure of ThiH highlighted an unusual protonation state of its substrate L-tyrosine. Moreover, the quantum modeling of the reactions has revealed a ***tunneling effect*** lowering the activation barrier of the reaction. Finally, subtle structural changes between the two enzymes affect this activation, leading to the difference in specificity of the reaction and thus to different C-C bond breaks.

Tunneling effect: A quantum phenomenon, which cannot be explained by classical mechanics. It allows a quantum object to pass a potential barrier even if its energy is lower than the minimum energy required to overcome this barrier.



Structure of the ThiH protein under the energy profile of the reaction.

REFERENCE

Amara P, Saragaglia C, Mouesca JM, Martin L and Nicolet Y. L-tyrosine-bound ThiH structure reveals C-C bond break differences within radical SAM aromatic amino acid lyases. *Nature Communications*, 2022

Vertical STT MRAM does not fear temperature

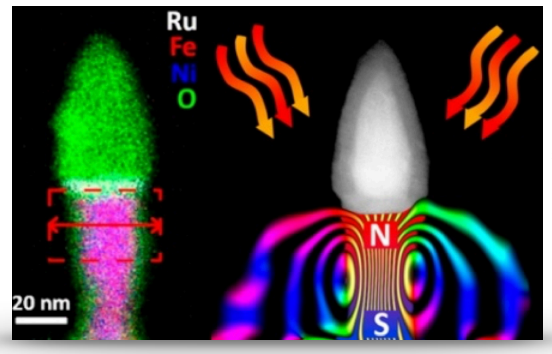
Magnetic RAM (MRAM) memory is booming. It is non-volatile, energy-efficient, fast and non-degradable, whereas flash memory is limited to a few thousand writes. MRAM is already used as a cache memory or in some low-power or fast applications, such as microcontrollers. Its wider use would require further improvements in thermal stability, *i.e.* the retention of data over time, even at high temperatures.

Contact: Olivier Fruchart
Spintec
Spintronics and Component Technology

The highly efficient Spin Transfer Torque (STT) MRAM could replace, *e.g.* computer's DRAM. To do this, it would be necessary to be able to increase the density of the magnetic elements, which requires miniaturisation. However, the layers of ultra-thin and small-size MRAMs elements are very sensitive to thermal heating, which causes them to lose stored data.
Since 2018, researcher at IRIG [***collaboration***] have been developing a new type of STT-MRAM based on magnetic bits with a vertical shape, providing resistance against thermal effects. They have designed such a nanopillar with 20nm diameter, comprising the storage layer. The magnetization state of the nanopillar was monitored with the technique of electron holography. The outcome directly demonstrates this claim: magnetization is nearly insensitive to temperature, remaining largely homogeneous, vertical and symmetric. Consequently, the nanopillar remains unaffected by temperature thanks to the magnetostatic shape effect and its large volume.

The results confirm directly that vertical STT-MRAM is a viable route to lift limitations of the current MRAM technology, opening new markets for these memories such as in the automotive industry, or for high-density DRAM with low power consumption.

Collaboration with the Electronics and Information Technology Laboratory of CEA-Grenoble (CEA-Leti), the Upstream Technological Platform (PTA), and the Platform for Nanocharacterisation (PFNC).



Left, vertical MRAM based on an iron-nickel nanopillar (pink). Right, magnetization inside the nanopillar, and the resulting stray field around it, both quite insentitive to temperature

REFERENCE

Almeida TP, Lequeux S, Palomino A, Sousa RC, Fruchart O, Prejbeanu IL, Dieny B, Masseboeuf A and Cooper D. Quantitative visualization of thermally enhanced perpendicular shape anisotropy STT-MRAM nanopillars. *Nano Letters*, 2022

A new evolutionary theory to explain the origin of oxygenation of the atmosphere

About 4 billion years ago, different forms of life appeared on Earth in an environment deprived of dioxygen (O_2). They are at the origin of two of the three major "domains of life" still present on our planet, the "Archaea" and the "Bacteria" (*Figure 1*, ❶).

Contact: Éric Maréchal
LPCV
Cell & Plant Physiology Laboratory

"Oxygenic photosynthesis" is a type of photosynthetic process that captures atmospheric CO_2 by combining hydrogen atoms obtained from ambient water, leading to the production of dioxygen. This release of O_2 had a major impact on the Earth, promoting the so-called "great oxygenation event" (GOE) about 2.4 billion years ago, the first step toward dioxygen loading of the Earth's atmosphere (*Figure 1*, ❸). Through reconstruction of protein evolution (molecular phylogeny) and examination of fossil rocks, it is estimated that oxygenic photosynthesis appeared in Bacteria at least 3 Billion years ago (*Figure 1*, ❷), at the root of the group of present-day cyanobacteria. In these primitive cyanobacteria (proto-cyanobacteria), thylakoids (intracellular membranes specialized for photosynthesis) were not yet present; photosystems (protein complexes capturing light energy) were inserted directly into cell-limiting membranes.

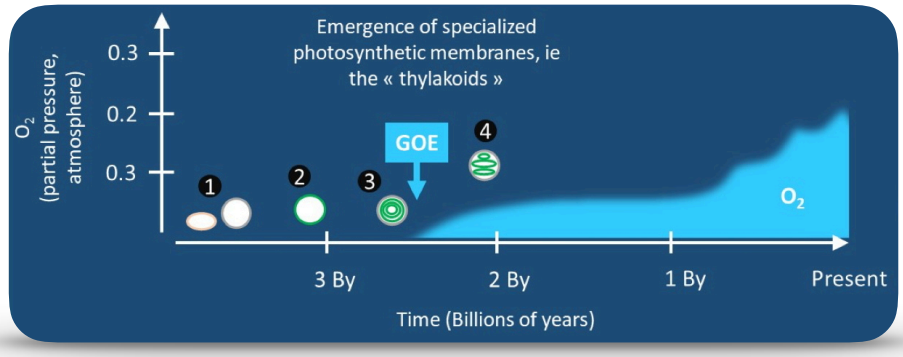


Figure 1: Impact of photosynthetic organisms on the Earth's atmosphere. The appearance of primitive cell architectures is indicated, and photosynthetic membranes are schematized in green. "GOE": great oxygenation event or great oxidation event (appearance of dioxygen in the atmosphere).

At IRIG, researchers evaluated the role of the emergence of thylakoids inside proto-cyanobacteria cells. They showed that these additional membranes increased the photosynthetic surface by introducing a multiplier effect consistent with a major impact on atmospheric oxygenation. However, should it be cyanobacteria or chloroplasts derived from them in the "Eukarya" domain of life, there is no known protein causing the "budding" of such intracellular membrane structures from peripheral membranes. The researchers then explored an alternative pathway that does not involve membrane budding but a simple transition between a phase comprising lipids that do not self-organize into membranes, but into a phase known as inverted hexagonal ("Hexagonal II" or *HexII* phase), and a phase of stacked membranes ("Lamellar" or *Lm* phase) (*Figure 2*).

The acquisition of the biosynthesis pathway of the sulfolipid sulfoquinovosyldiacylglycerol correlates with the emergence of thylakoids, allowing a sufficient supply of anionic Lm lipids to trigger a *HexII* ➛ *Lm* phase transition. With this non-vesicular lipid phase transition, a framework is also available to reexamine the role of companion proteins in nonconcentric thylakoid biogenesis (*Figure 1*, ❹). Consistently, non-vesicular biogenesis of thylakoids has been recently observed in present-day cyanobacteria.

This theory, which proposes a plausible answer to the emergence of thylakoids and GOE, has been selected to be part of the *Darwin Reviews of Journal of Experimental Botany*.

REFERENCE

Guéguen N and Maréchal E. Origin of cyanobacterial thylakoids *via* a non-vesicular glycolipid phase transition and their impact on the Great Oxygenation Event. *Journal of Experimental Botany*, 2022

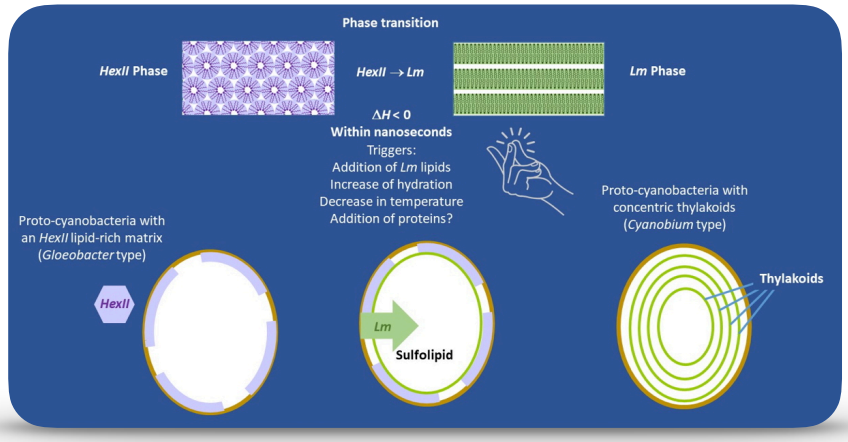


Figure 2: An addition of Lm lipids (lamellar phase) to HexII lipids (hexagonal phase II) can generate membrane multilayers via a non-vesicular phase transition (thylakoid origin).

The light on a new biomarker detection technique

Blood biomarkers are molecules that signal the presence of a disease, for example, with high sensitivity and specificity. Their detection is of utmost importance to better detect predictive or early signs of certain diseases. Several techniques exist but the quantification of a target such as a biomarker present at very low concentrations in a complex sample (blood, urine, polluted water, etc.) remains a challenge.

Contact: Arnaud Buhot
SyMMES
Molecular Systems and nanoMaterials for Energy and Health laboratory

Different techniques have been developed, including ELISA (Enzyme Linked ImmunoSorbent Assay) which uses two different antibodies and an enzymatic amplification. However, ELISA is sometimes limited or ineffective when the target is present at very low concentrations. While enzymatic amplification is linear in time, exponential amplifications based on oligonucleotides (short strands of synthetic DNA or RNA) have been developed and are the basis of the ***PCR*** technique. Two variants of ELISA exist:

- **immuno-PCR** where the enzymatic amplification of ELISA is replaced by an exponential amplification of DNA by PCR while retaining the recognition of the biomarker by antibodies, and
- **apta-PCR** where the secondary antibody is substituted by an ***aptamer*** to which a short nucleotide sequence is grafted for PCR amplification. However, immuno-PCR requires two antibodies specific to the target of interest and the development of a DNA-conjugated antibody, which makes it complicated and costly. The use of aptamers as a substitute for antibodies makes apta-PCR very interesting, especially in complex environments. Nevertheless, these two variants have the disadvantages of PCR amplification, namely the need to work at several temperatures and a high sensitivity to inhibitors present in the samples to be studied. Loop-mediated isothermal amplification, or LAMP, represents an interesting alternative. It is one of the most specific and sensitive methods among the isothermal amplifications, it is also less sensitive to inhibitors present in biological samples and has the advantage of being performed at constant temperature.

Researchers at IRIG [***collaboration***] have demonstrated the proof of concept of an **apta-LAMP** assay using thrombin (a protein involved in blood clotting) as a biomarker. They used two aptamers described to bind to two different epitopes of this protein to form a stable sandwich. The test developed is based on a DNA amplification using a dumbbell structure (Patent) requiring only 2 primers instead of 4 or 6. In an original way, the researchers introduced an aptamer sequence at different positions of the dumbbell structure in order to analyze the impact on the amplification and recognition of thrombin. The amplification of these different alters was validated in less than 30 minutes and the quantitative detection of thrombin was achieved with a detection limit of 100pM and a quantification range of 3 orders of magnitude corresponding to physiological conditions.

This method, which does not require the use of antibodies, can be integrated into a portable medical device and allows the quantification of various biomarkers with a high specificity and sensitivity, from clinical samples, in situations suitable for a so-called bedside analysis. Finally, it opens perspectives in the field of diagnostics, especially for its application to cardiac diseases requiring a rapid diagnosis. It is for this reason that the **Auvergne Rhône-Alpes region** supports this research via the **DEDICATE project**.

Collaboration: Microfluidic Systems and Bioengineering Lab of the Technologies for Healthcare and Biology Division, (DTBS) at the Electronics and Information Technology Laboratory of CEA-Grenoble (CEA-Leti).

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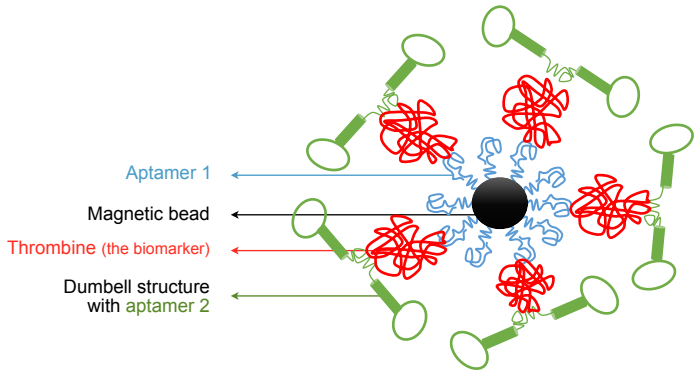
Aubret M, Savonnet M, Laurent P, Roupioz Y, Cubizolles M and Buhot A. Development of an innovative quantification assay based on aptamer sandwich and isothermal dumbbell exponential amplification. *Analytical Chemistry*, 2022
Patent: **Savonnet M, Buhot A, Cubizolles M, Roupioz Y**. Method for detecting and optionally quantifying an analyte with a double stem-loop oligonucleotide and said oligonucleotide. FR3108124A1

PCR (*Polymerase Chain Reaction)*: A DNA polymerization chain reaction that results in its exponential amplification.

Aptamer: A synthetic oligonucleotide, DNA or RNA, capable of binding a specific ligand thanks to its three-dimensional structure. Their selectivity and ligand binding properties allow aptamers to be compared to antibodies.



The sample in which the presence of a biomarker is searched for (here the thrombin protein) is first brought into contact with magnetic beads on which a first aptamer presenting an affinity with thrombin has been grafted.
The dumbbell structure comprising the second aptamer is then added and will form a sandwich with thrombin if it is present in the sample to be analyzed.
A LAMP amplification in the presence of 2 primers will then be performed and the target quantified.

On-chip optical nano-tweezers towards culture-less fast bacterial state and viability assessment

Bacteria are essential to humans. For instance, they participate to food digestion through the intestinal microbiota. Some are pathogenic and must of course stay outside the human body, a function provided primarily by the skin. In case of infection by inhalation/ingestion or by wound, taking an antibiotic was for a long time the absolute remedy. However, recent studies show that due to excessive use of antibiotics, a number of bacteria have gradually mutated and become resistant to antibiotics. Current WHO projections even envisage that this could become one of the leading causes of death by 2050, with several million non-curable infections per year. In this context, knowing how to identify a bacterium or test its reaction to a stress such as that caused by an antibiotic, could make it possible to greatly limit the far too widespread use of antibiotics. This could therefore contribute to reduce the dramatic phenomenon of bacterial resistance.

Contact: Emmanuel Hadji Pheliqs
Quantum Photonics, Electronics and Engineering

Building upon their knowledge in the field of nanophotonics, the IRIG researchers [***collaboration***] have imagined being able to use light to capture a bacterium on a silicon chip. It should be remembered that a force-like effect of the light, called radiation pressure, was first identified by Arthur Ashkin in the early 1970s, when he attempted to trap atoms with lasers. This led him, some fifteen years later, to propose the concept of "optical tweezers" and thus the idea that one could manipulate micro-objects (dielectric spheres of micrometric size) trapped at the waist (*i.e.* the narrowest point of the beam) of a tightly focused laser by the so-called gradient force effect. The discovery of this principle earned him the award of the Nobel Prize in Physics in 2018.

In their approach, the consortium's researchers took advantage of their ability to localize light within a microstructure fabricated on a silicon chip. It is indeed a kind of "light box", in scientific terms an optical nanocavity, in which the light is localized, somehow bouncing between its walls. They then observed that once trapped by the light from the nanocavity, the bacteria interacted with it by modulating the resonance frequency of the nanocavity. Everything then happens as if, like a tuning fork, the sound emitted, here the resonance frequency of the cavity, was modified by the environment surrounding the tuning fork. This gave rise to the idea for researchers to try to test, or determine, the state of a bacterium through its interaction with light within the optical trap provided by the device.

This is actually the demonstration they have just recently made and which was the subject of a publication in the *Small* journal. The researchers successively optically trapped bacteria which they subjected to an external stress, carried out for the experiment by immersion in a bath of increasing temperature. For each trapping, they observed the interaction of the bacteria with the optical nanocavities and were thus able to determine the threshold of non-viability, which could be described as the death of these bacteria, with an almost instantaneous response time. This amounts to saying that as soon as the bacterium is within the optical trap, ones know its "healthy" state. This instantaneous reading is of fundamental interest because it becomes therefore no longer necessary, as is the case in current bacteriological analyses, to wait 1 to 2 days for the bacteria sampled to develop on a culture dish in order to be able to test their sensitivity to an antibiotic.

The next step on the agenda is to go from heat stress to that suffered in the presence of an antibiotic, an experiment on which the researchers are currently working. They also aim to show that the same approach could be applied to the interaction of a bacteriophage (a bacterial-destroying virus) with a bacterium, thus allowing a wider and easier use of the latter when no more antibiotics have an effect.

Collaboration with the Microelectronics Technologies Laboratory (LTM, CNRS) and the Imaging Systems for Life Laboratory (LSIV)of the Technologies for Healthcare and Biology Division (DTBS) at the Electronics and Information Technology Laboratory of CEA-Grenoble (CEA-Leti).

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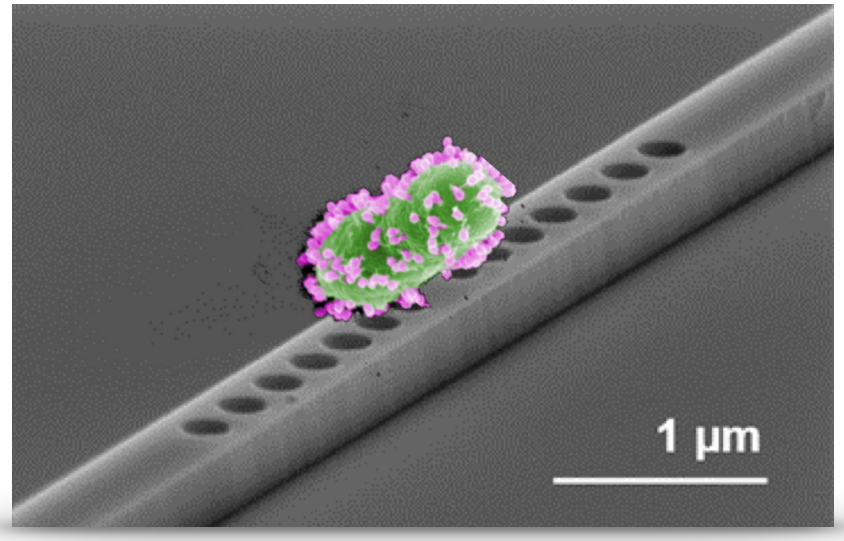
Tardif M, Picard E, Gaude V, Jager JB, Peyrade D, Hadji E, Marcoux PR. On-chip optical nano-tweezers for culture-less fast bacterial viability assessment. *Small*, 2021



Representation of a bacterium trapped on an optical nanocavity.

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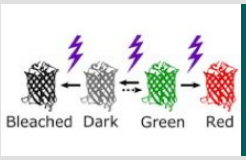

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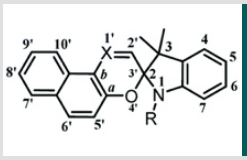

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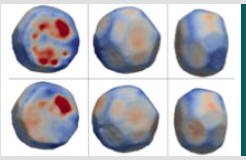

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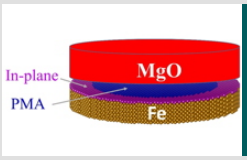

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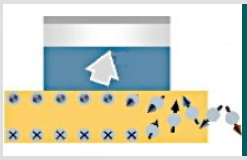

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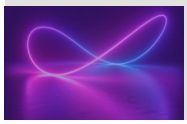

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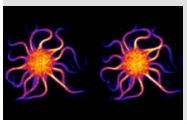

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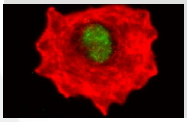

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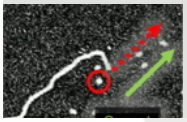

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