CIIL
Center for Infection & Immunity of Lille

NEWSLETTER

#7 | July 2022

The Newsletter is a tool that helps to communicate on the scientific activities of the CIIL. It is also an excellent tool to present the men and women who contribute to the scientific production of our unit. In this issue as well as in the following ones, we have decided to present the profiles of researchers of the CIIL. We start with the presentation of Alexandre Grassart who is creating a new team with the financial support of an ATIP/AVENIR grant. We also present the profiles of Françoise Jacob-Dubuisson, Philip Supply and Anne Tsicopoulos, three researchers who have been actively participating to the scientific project of the CIIL since its creation.

Jean Dubuisson

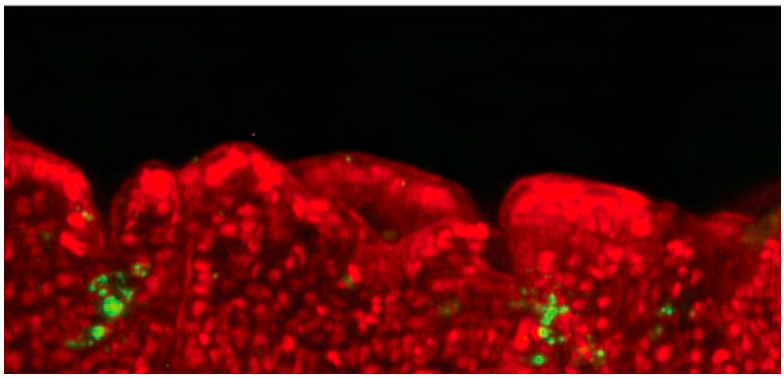
Profiles of our researchers

Alexandre GRASSART

The ATIP-AVENIR 2021 laureate (CNRS-INSERM)

At the interface of cell biology, microbiology and bio-engineering, my research aims at understanding how host-microbe interactions are impacted by mechanical forces. After a PhD in Cellular and Molecular Biology at the Institut Pasteur in Paris in the laboratory of Professor Alice Dautry-Varsat, I joined in 2010 the laboratory of Professor David Drubin at the University of California Berkeley (USA). During this period, I studied endocytosis, a fundamental process controlling the interactions of the cell with its environment. My work contributed to the field in two main aspects. First, I identified the first molecular markers of a poorly characterized endocytic portal that is essential in the immune response as it is used by many cytokine receptors such as interleukin-2. Second, I revealed a new model of organization and stoichiometry of key endocytic factors controlling the physical maturation and scission of endocytic vesicle from the plasma membrane of the cell. Then, I expended my field of investigation to host-pathogen interactions through the lens of biophysics by joining the lab of Professor Philippe Sansonetti at the Institut Pasteur of Paris in 2014. While there is increasing evidence that tension at the membrane level plays a key role on the biology of the cell, the role of physical cues on pathogens infectivity at the interface of their cellular host remained poorly understood at the time. Using Shigella as an entero-invasive pathogen model, my work revealed that the 3D organization of the intestinal tissue plays a critical role in the infection of this bacterium. More importantly, I identified that the application of stretching forces on the intestinal epithelium, mimicking the physiological movements associated to peristalsis, has a significant impact on Shigella infectivity. This discovery reinforced the growing importance of mechanobiology in our understanding of host-pathogen interactions. In 2020, I joined the Institut Pasteur of Shanghai as a Professor at the Chinese Academy of Sciences, where I established the Bioengineering and Microbiology Laboratory. During this interlude, I was honored to be awarded an ATIP-Avenir funding (CNRS-INSERM), thanks to which I established my new team at the Center of Infection and Immunity of Lille (CIIL) in spring 2022.

The main project of my team aims at deciphering the underlying molecular mechanism exploited by Shigella to take advantage of physical forces and invade the intestinal barrier. Although it is known that motile Shigella propagate across the intestinal barrier by cell to cell passage by an intracellularly remodeling of the plasma membrane leading to the generation of “bacterial protrusion”, the mechanisms supporting the formation, elongation and scission of these structures remain poorly understood. What are the host molecular factors involved? Do the biomechanics of the cellular host regulate these events ? How do physical forces influence this stage of infection? In parallel to the study of these fundamental questions, our group is also devoting an important effort to the development of new technologies based on microfabrication and microfluidics such as “organ on chip”. These biomimetic microfluidic systems aim at



Cross section of an intestinal epithelium cultured in an organ-on-a-chip system. Shigella bacteria are localized in the intestinal crypts (Green). The nucleus and actin cytoskeleton of enterocytes were labeled with DAPI and phalloidin-Alexa647 (Red)

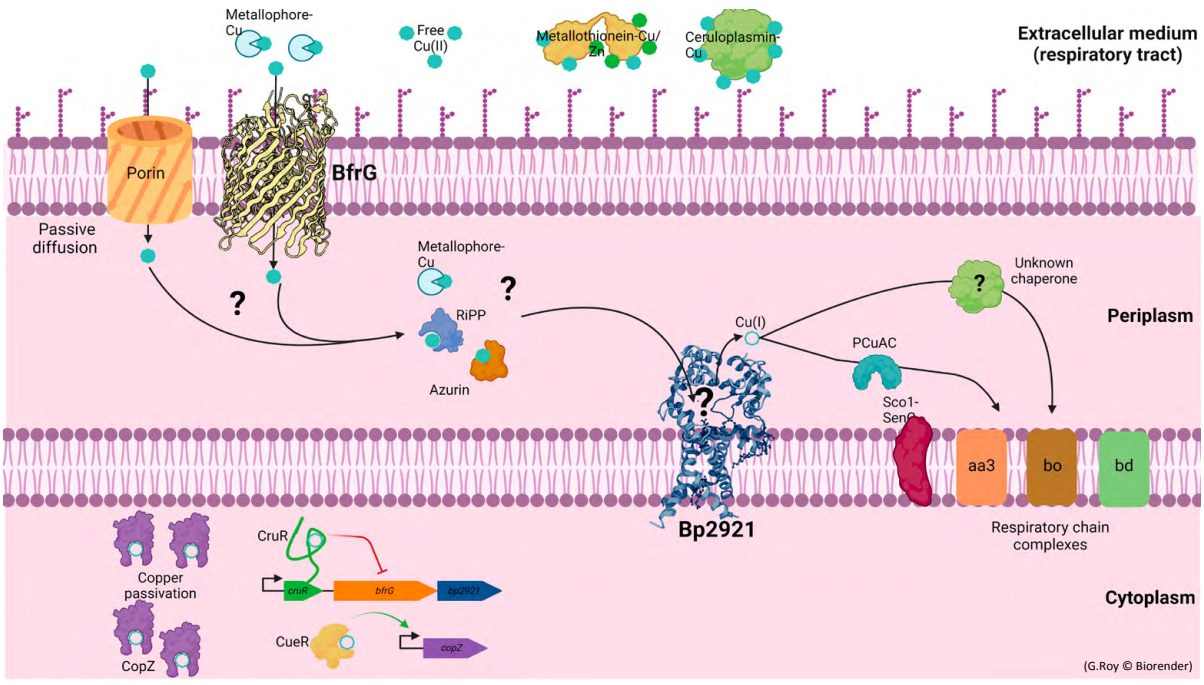
improving the physico-chemical microenvironment such as physiological reconstruction of tissue topology, bio-mimetic of mechanical forces, oxygenation tension reconstruction or co-culture of intestinal microbiota in vitro. In the long-term, we wish to further develop these emerging technologies in order to provide the research community with clinically relevant solutions in a user friendly system.

Françoise JACOB-DUBUISSON

CNRS senior researcher

After a PhD thesis in Biochemistry at the University of Liège in Belgium, where I characterized a β-lactamase of Streptomyces, I spent 3 years at the Washington University in St Louis, USA. My post-doc was focused on the assembly of pili in uropathogenic Escherichia coli. Since I joined the CNRS, I have worked on the whooping cough agent Bordetella pertussis. Bacteria have always fascinated me, as they recapitulate in a single cell all the mechanisms that define life. They feed themselves, reproduce, undergo developmental programs, associate with one another, perceive signals, communicate between them and with their environment, adapt and evolve. As a biochemist my main interest is to understand mechanisms at the molecular level. B. pertussis is a nice model to study both bacterial pathogenesis and physiology. Fitness is essential for bacteria to thrive in their specific niches, i.e., the human respiratory tract in the case of B. pertussis. I have first tried to elucidate the biogenesis of the adhesin FHA, a major virulence factor of B. pertussis produced and secreted in industrial amounts by this bacterium, and also a main antigen of current acellular vaccines. We have discovered that this protein secretion pathway is widespread among bacteria, and we have called it TPS for 'Two-Partner secretion'. The second partner in this system is the membrane protein FhaC, which mediates the transport of FHA without known sources of energy. We have tried and are still trying to describe how its structure, which we solved some years ago, determines its transport function. Our recent results indicate that the dynamics of this transporter is essential to its activity. I have also studied for some years BvgS, the protein that mediates signal transduction to regulate the expression of the numerous virulence factors of B. pertussis. We have characterized the structure and the dynamics of the various domains that compose this complex protein, leading us to propose a mechanism of signaling which should apply to the family of sensor-kinases of which BvgS is a model. More recently I have become interested in copper homeostasis in B. pertussis. With a few other transition metals, copper is one of the oligo-elements necessary for life, as a co-factor of redox enzymes and electron transporter. These metals are toxic in excess, and phagocytic cells take advantage of this toxicity to kill microorganisms. We have found out that B. pertussis has very limited defenses against copper, most likely because it is overwhelmingly extracellular and lives in a very predictable environment given its restricted ecological niche. To the contrary, this pathogen has developed an original copper acquisition system, which is not characterized although it is found in a number of bacterial species, and we are currently studying this pathway. To control copper homeostasis B. pertussis has developed sophisticated mechanisms of regulation, which we have begun to decipher. Finally, we have identified in B. pertussis a BvgS-regulated operon coding for a founding member of a new family of natural products linked to copper. To characterize these systems, we have turned to another organism as a model, Caulobacter crescentus.

Science is a collective endeavor, and these projects have been or are being performed in collaboration with biophysicists and their techniques of X-ray crystallography, nuclear magnetic or electron paramagnetic resonance, mass spectrometry, and more recently cryo-electron microscopy. Of course, I do not forget all the PhD students and post-docs whom I have had the chance to supervise and who have been key to move these projects forward! I really appreciate doing science in France and at the CNRS, because I have had the opportunity to choose my way of being a scientist, coordinating projects with collaborators in various disciplines while affording some time at the bench.



AnneTSICOPOULOS

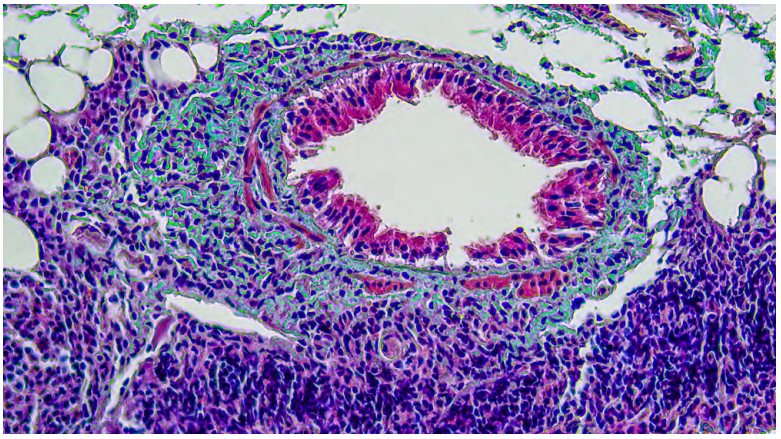
INSERM senior researcher

J'I studied Medicine at Lille University and was certified in respiratory medicine and in Allergy in 1987. Meanwhile, I started my scientific career in Pr André Capron's laboratory (Center for immunology and biology of parasites), at the Pasteur institute of Lille under the supervision of Michel Joseph and then of André Bernard Tonnel. In 1990, I went as a post doc in London in the laboratory of Pr AB Kay at the National Heart and Lung institute and at the Brompton Hospital, where I spent 2 years and half. When I got back, I was appointed CR1 at Inserm (1992) and I integrated the « Contrat Jeune Formation » headed by Pr AB Tonnel, which was later upgraded to an Inserm Unit in 1994. In 2002, I was appointed DR2 at Inserm, and I took the head of the Pulmonary Immunity team in 2010, in the context of the creation of the CIIL. During these years, I assumed different scientific functions, in particular as member of IPL scientific council, of ANR committees, of Fondation du souffle scientific council, of the Inserm commission of clinical research, as president of the scientific council of the French Society for Allergy, and as scientific advisor in allergy for the ITMO IHP (Immunology Hematology Pneumology, head Paul Henri Romeo), then for the ITMO I3M (Immunology inflammation infectiology microbiology, head Jean François Delfraissy and then Yasdan Yasdanpanah).

Main discoveries : https://orcid.org/0000-0002-1579-2763

My initial project focused on the mechanisms involved in allergen specific immunotherapy by using the model of Hymenoptera venom allergy, for which efficiency of desensitization is about of 95%. The results showed that platelets were involved as IgE-dependent effector cells, and were under the influence of T cell suppressor factors induced by immunotherapy. During my post doc, I participated in the princeps discovery of the first description of a Th2 profile in bronchoalveolar lavages from asthmatic patients, which drove a series of animal and human studies, leading to anti Th2 therapies currently used in severe asthma patients. The next part of the project consisted in the development of a humanised model of immunodeficient SCID mice grafted with human skin and autologous mononuclear cells from allergic patients, allowing to test different therapeutic approaches preclinically, such as chemokine receptors inhibitors. Mechanisms involved in Th2 exacerbation by pollutants (diesel particles) and obesity were also deciphered, as well as was described the first chemokine able to attract regulatory T cells (CCL18). Finally, we have recently demonstrated that house dust mite allergen can directly activate the innate receptor NOD1 and contribute to the aggravation of eosinophilic T2 asthma (Th2 type). These data have led to the publication of over 100 papers.

Objectives of the current project If to date, T2 asthma has adapted therapies, non T2 neutrophilic asthma is not targeted by biotherapies. In this context, our project is to decipher the mechanisms involved in non T2 asthma, through original models of neutrophilic asthma, through translational studies on samples from non T2 severe asthma patients, and through genetically modified mice, in order to define new therapeutic targets, focused in particular on bronchial remodelling.



Microphotography of a lung section from a neutrophilic asthma model exhibiting a strong bronchial remodelling visualised by collagen fibres staining by Masson's trichrome (in green).

Philip SUPPLY

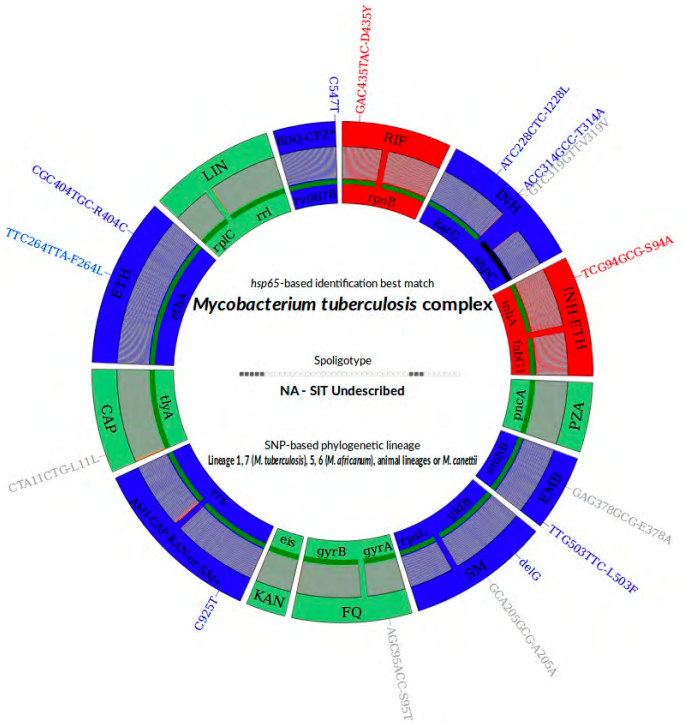
CNRS senior researcher

I completed a Master's degree in bioengineering and then a thesis at the University of Louvain in Belgium on the enzymatic properties and cross-genetic complementation of proton pumps in the yeast Saccharomyces cerevisae, a model organism for eukaryotes. I then joined Camille Locht's team at the IPL, at the time a young INSERM Team, to work on a subject of more direct biomedical interest, offering closer possibilities for developing applications. Thanks to a European Marie Curie grant, I began my post-doctorate there in 1994, then was recruited as a CNRS Research Fellow in 1996, and appointed Research Director since 2008. Our team is led by Nathalie Mielcarek since 2020.

My research focuses on the conditions of emergence, evolution and genomics of Mycobacterium tuberculosis, the deadliest bacterial infectious agent and the leading contributor to mortality due to antimicrobials. I use the acquired knowledge to develop new molecular diagnostic and surveillance tools in collaboration with the Genoscreen R&D team, with whom I am collaborating since more than 15 years. At the origin of this work, I discovered a new repetitive genetic element, dispersed in the genome of the bacterium. With my group, we characterized the inter-strain variation of these molecular markers, called MIRU-VNTRs and analogous to minisatellite regions of eukaryotic genomes, then multiplexed their analysis for genotyping, epidemiological tracing and analysis of the structure of large-scale bacterial populations. I led the standardization of this approach with an international consortium, then developed with Genoscreen corresponding kit systems, used in more than 40

countries. I co-founded an online database (www.miru-vntrplus.org), allowing the analysis and cataloguing of genotypes identified worldwide. This approach has been adopted globally – for example by the US CDC, where I gave two trainings in Atlanta, and the European CDC – as the standard for molecular surveillance of tuberculosis for almost a decade. The advent of "Next Generation Sequencing" (NGS) technologies allowed us to complement these screenings with whole genome sequencing (WGS) for exhaustive molecular analyses. Using comparative genomics, I identified exceptional clinical isolates, originating from East Africa, representing different ancestral lineages of the pathogen. With my group, we showed that some of these strains exhibit a lower virulence/persistence in infection models, suggesting that they represent extant reflections of the ancestral pool from which M. tuberculosis emerged, by a gain in mechanisms of pathogenicity. Two of these mechanisms have been revealed in collaboration, associated with a change in cell morphotype due to intrachromosomal recombination, and to an increased capacity for resistance to different types of stress encountered during infection, respectively. With 30 teams from the five continents, we retraced the evolutionary history of a main, more recent lineage of the pathogen, called Beijing, and showed that the epidemic expansion of 2 major multi-resistant clones of this lineage coincided with the collapse of the public health system in the former USSR. With another global consortium led by the University of Oxford, we demonstrated the diagnostic potential of WGS for prediction of drug resistance, based on the analysis of 10,000 genomes, including more than 1,400 obtained in Lille. However, as WGS is not routinely feasible on clinical samples without culture (which takes at least 1-2 weeks for M. tuberculosis), I developed with Genoscreen a new diagnostic NGS-based test, called Deeplex®-MycTB, directly applicable to such samples. This test is based on the amplification in a single multiplex of 18 main resistance targets, plus speciation and genotyping targets, followed by deep sequencing. Used as a kit coupled with a web app for fully automated analysis, it can detect mutations of resistance to 13 antibiotic classes simultaneously, including those now defining extensive drug resistance – a unique capability to date. We were able to show its high degree of accuracy compared to standard phenotypic tests and WGS performed after culture. This test has already been adopted by the WHO for several drug resistance surveys, as well as in more than thirty countries. In particular, it allowed us to reveal an outbreak of multi-drug resistant tuberculosis undetected by standard diagnostics in South Africa, and to discover a new ancestral lineage of the pathogen in the African Great Lakes region (Figure).

My current projects aim to better define the bacterial molecular mechanisms favoring the development of antibiotic resistance, as well as to define the ecology of the ancestral pool from which M. tuberculosis emerged. In parallel, with Genoscreen, I am notably develping another test by NGS, for the detection of resistance and genotyping of Mycobacterium leprae - which cannot be cultivated in vitro -, which can be applied directly to biopsies from patients affected by leprosy.



Deeplex-MycTB results identifying a MDR-TB strain from Rwanda with an atypical genotypic profile. Subsequent WGS using Ilumina and Pacbio sequencing showed that this strain represents a previously unknown sister clade of the Mycobacterium tuberculosis complex (from Ngabonziza et al., Nature Communications, 2020). Target gene regions are grouped within sectors in a circular map according to the anti-tuberculous drug resistance with which they are associated. The two sectors in red indicate regions where rifampicin and isoniazid resistance-associated mutations were detected. The multiple sectors in blue refer to regions where as yet unknown mutations were detected, whereas sectors in green indicate regions where no mutation or only mutations not associated with resistance (shown in grey around the map) were detected. Information in the center indicates results of species identification and genotyping of the strain, based on detection of CRIPR spacers (spoligotype) and phylogenetic SNPs.

The research trainees

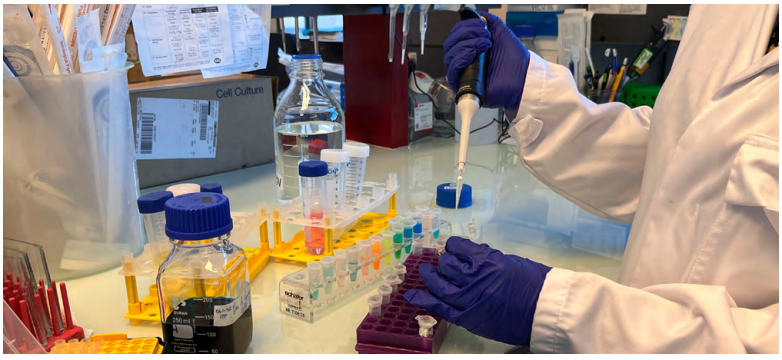
Since 2009, many labs from Lille covering numerous scientific fields participate in the “Apprentis Chercheurs” project developed in partnership with the non-profit organization “L’Arbre des Connaissances” and several middle- and high-schools from the northern (59) district.

This scheme involves about 14-16 pupils yearly (a pair formed with one 8th grade pupil from middle-school plus one 2nd year from high-school) who join a lab for about eight half-days internships from January till June. This enables them on one hand to discover the Research behind the scene and the different categories of workers in a lab. On the other hand, they have the opportunity to experiment for real with a small scientific project and present their work during the annual Apprentis Chercheurs seminar.

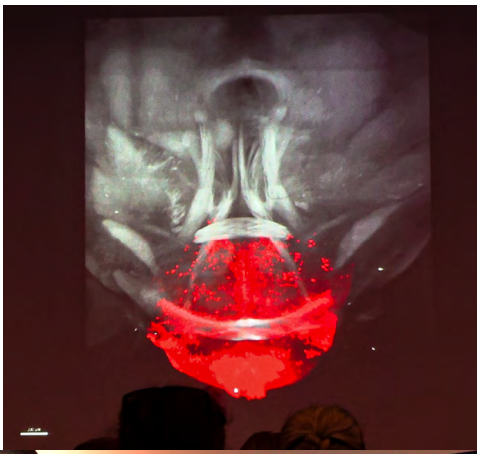
Since the beginning of this scheme in Lille, the CIIL is a major player. From 2009 to 2018, Frank Lafont (DR, CNRS) and then Ghaffar Muharram (Ass. Prof, U. Lille) are the local coordinators. And two to three teams from CIIL are participating yearly in this operation of promoting science toward younger generations.

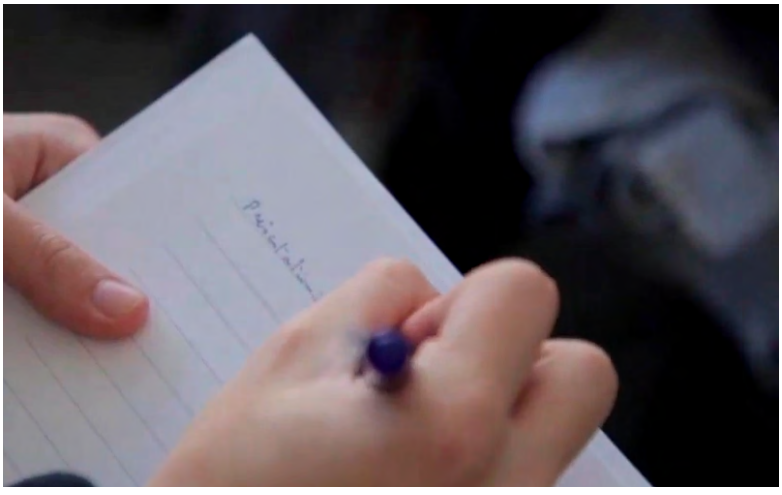
Everybody : PhD students, Post-docs, Technicians, Researchers, Professors can participate. The one and only criteria is your motivation.

So please join us, if you want to share this very enriching experience.



Ghaffar MUHARRAM
coordinator of the tree of knowledge





As we close the science season at the center, we would like to look back on an important event of this year. After those long months where we had all been deprived of contacts, we wanted to organize a festive moment where science, sharing of experiences, art and the joy of meeting each other would be on the program. The very first CIIL Gala evening took place on Tuesday April 26, 2022 at the Gare Saint Sauveur in Lille. During the plenary lecture, Dr. Delphine Grynberg (MCU, Senior Lecturer at the University of Lille) led us to reflect on empathy and the conditions that foster it, and presented her results on the consequences of empathy in different clinical situations. The following session, organized by the Association des Jeunes Chercheurs du CIIL (AJCC), allowed former doctoral students of the center to share their professional path with great generosity. We thus discovered the career paths of Dr. Vianney Souplet (Innobiochips, Lille), Dr. Emmanuel Hermann (University of Lille) and Dr. Karine Petitprez (Haute Autorité de Santé, Paris) who have followed paths as different as they are rich, in business creation, teaching/research or clinical project management. After these first two sessions organized in the Gare Saint Sauveur cinema, the setting and atmosphere changed: painting, dance duo and troupe, stand up and music were on the program for the CIIL's got Talent session! And there was plenty of talent on the Bistrot St So stage! No less than 12 artists (for one night or not!) performed under warm and hearty applause! Enough to launch the dance party that followed! The atmosphere was festive and relaxed and everyone was visibly happy to enjoy this moment of relaxation .... **«to be done again» claimed many participants!»**

Thank you to all those who helped us prepare this event, thank you to all the speakers, thank you to all the artists, thank you to the AJCC for organizing the Alumni and CIIL's got Talent sessions ... and thank you all for your participation!

Have a great summer and see you soon for more scientific and convivial events!

The scientific animation team

The CIIL's team of scientific animators (from the left to the right)
Muriel PICHAVANT, Patricia DE NADAI, Christine PIERROT,

The news in brief ...

The award winner

Congratulations to Philip Supply who received the **Gardner Middlebrook Lifetime Achievement Award 2022**. Togehter with Genoscreen, he developed the first diagnostic test by targeted deep sequencing for the extanded detection of multi- and ultra-resistance to antibiotics used against tuberculosis

Newcomer in the CIIL

Welcome to Sabrina PECQUEUR who joined the CIIL to ensure the financial management of the IBL building in replacement of Régine Blanchet, who will leave at the end of this year to follow personal projects after a beautiful career of good and loyal services.

Departure from the CIIL

Jonathan Carlier, the head of the technical service of the IBL building, will leave the region for personal projects. To ensure his replacement, an offer has been published on the CNRS job portal and at APEC.

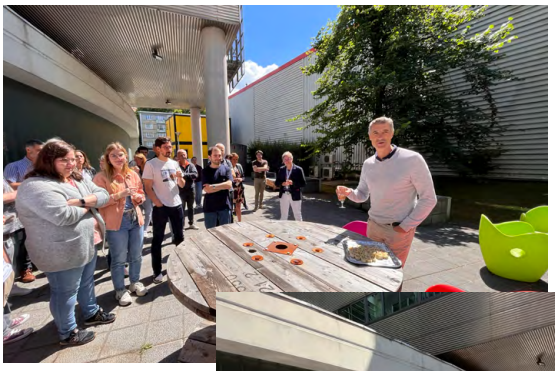
Construction work on the IPL campus

As you could read it on the website immobilier.pasteur-lille.fr , the campus of the Institut Pasteur de Lille will be affected by construction works which will affect the IBL building. Indeed, the construction of a new building on 24 Bd Louis XIV will be preceded by the demolition of older infrastructures including the Lévy building. In this context, it has been proposed to the occupants of the Cedilla to temporally move to the offices of the 3rd floor left empty at the end of August by the teams working on cancer. The occupants of the Cedilla will reintegrate their office at the end of October, before the arrival of the parasitology teams of the Emile Roux building.

Enjoy your summer !

Farwell party from the cancer teams

In August, the teams working on cancer will leave the IBL building. This departure of the campus of the Institut Pasteur de Lille will be the end of a chapter for all of us. Research on cancer has been at the heart of the IBL project brought by Dominique Stéhelin. These teams were the first to move into the building in 1996 and some of our teams followed suit. We shared almost 25 years a life together inside the IBL. This is a nostalgic moment for all of us. On this occasion, the teams of the Unit Canther as well as the Unit OncoTHAI invited us for a drink and we thank them very much for this last moment of shared conviviality. We wish them good luck in their new institute ONCOLille.



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