amU Microbiology, bioenergies and biotechnology institute
Aix Marseille Université

IM2B Scientific day

November 19th, 2024

Presentation of IM2B awarded projects

Poster sessions

Networking

Campus Joseph Aiguier
Marseille
Amphithéâtre Desnuelle

amidex Aix Marseille Université | FRANCE 2030

IM2B Scientific day

8:30 - 9:00 WELCOMING COFFEE

9:00 - 9:30 **INTRODUCTION**
Eric Cascales, Christophe Laloi, Deborah Byrne

9:30 - 10:15 SESSION 1
Moderators: Bérengère Ize (LISM) & Sonia Longhi (AFMB)

9:30 - 9:45 Understanding interactions between the mitochondrial porin VDAC2 and the pro-apoptotic protein BAX - **Varun Ravishankar** (LISM & BIP)

9:45 - 10:00 Structural and functional studies of the SARS-CoV2 replication complex and its inhibitors - **Ashleigh Shannon** (AFMB)

10:00 - 10:15 Cytotoxic Cu(II) complexes in the context of cancer: insights from the cells - **Olga Iranzo** (iSm2), Pierre Dorlet (BIP), and Régine Lebrun (IMM)

10:15 - 10:35 FLASH TALKS
Giulia Panzironi (M2P2), Gerlind Sulzenbacher (AFMB), IGEM, Isabelle Imbert (LISM)

10:35 - 11:15 COFFEE BREAK and POSTER SESSION

11:15 - 12:00 SESSION 2
Moderators: Katia Duquesne (iSm2) & Christophe Laloi (BIAM)

11:15 - 11:30 Molecular and structural basis of the mechanism of type IVa pili machinery activation in *Myxococcus xanthus* - **Camille Herrou** (LISM & LCB)

11:30 - 11:45 OM-PSG: Oxidation of Methionine in Proteostasis, Survival, and Growth - **Maxence Vincent** (LCB)

11:45 - 12:00 The importance of NAD metabolism to drought and senescence in *Arabidopsis thaliana* - **Elias Feitosa Araujo** (BIAM)

12:00 - 12:15 ExploGT: exploring the sweeter aspect of flagella structure - Vincent Lombard (AFMB), **François Alberto** (LCB) & Gerlind Sulzenbacher (AFMB)

12:15 - 12:35 FLASH TALKS
Julius Martinkus (LISM), Olivier Bornet (IMM), James Sturgis (LISM), Denis Ptchelkine (AFMB)

12:35 - 14:15 LUNCH and POSTER SESSION

Moderators: Laurent Aussel (LCB) et Eric Cascales (LISM)

14:15 – 15:00 Keynote lecture: CPJ IM2B
Untangling Environment-Microbiota Interactions: from basic principles to emergent properties - **Sophie Tronnet** (Umea university & iSm2)

15:00 – 15:15 Mathematical modeling of the ecology and evolution of host-associated microbiomes - **Florence Bansept** (LCB)

15:15 – 16:00 COFFEE BREAK and POSTER SESSION

16:00 – 16:45 SESSION 3
Moderators: Muriel Masi (MCT) & Pierre Rousselot-Pailley (iSm2)

16:00 – 16:15 Stereochemical Tuning of Nickel-Based HER Electrocatalyst - **Jana Mehrez** (iSm2)

16:15 – 16:30 Plant-associated microbiota: Genetics in the rhizosphere - **Johannes Stuttmann** (BIAM)

16:30 – 16:45 Monitoring the localization and dynamics of infection by filamentous phages at the cellular level- **Laetitia Houot** (LISM) and Tam Mignot (LCB)

16:45 **CONCLUDING REMARKS**
Marie-Thérèse Guidici-Orticoni

TALKS - ABSTRACTS

SESSION 1

9:30 - 9:45

Varun Ravishankar (LISM & BIP) **PhD**

Understanding Interactions Between the Mitochondrial Porin VDAC2 and the Pro-apoptotic protein Bax

V. Ravishankar (a, b), L.M.A González (c), V. Prima (a), M. Queralt-Martin (c), G. Gerbaud (b), L. Bergdoll (a)
a Laboratoire d'Ingénierie des Systèmes Macromoléculaires, LISM, CNRS, UMR7255, Marseille, France
b Bioénergétique et Ingénierie des Protéines, BIP, CNRS, UMR7281, Marseille, France
c Laboratory of Molecular Biophysics, Physics Department, University Jaume I, Castellón, Spain

The Voltage Dependent Anion Channel (VDAC) is the most abundant ion and metabolite transporter in the mitochondrion and maintains mitochondrial homeostasis. VDACs are involved in numerous cellular pathways ranging from glycolysis to steroidogenesis. As of recent, the role of VDACs in modulating apoptosis, a form of programmed cell death in eukaryotes has been increasingly demonstrated, specifically with the pro-apoptotic molecules Bak and Bax. Upon induction of apoptosis, Bax form large pores in the mitochondrial outer membrane triggering cell death. Though the conformations of Bax are well known, the mechanism of recruitment of Bax to the mitochondrion remains unclear. We study VDAC2 and Bax in vitro to test interactions at a molecular level. We show for the first time, a complex formation between the two proteins and monitor the conformational changes using different biochemical and biophysical techniques.

Keywords : VDAC2, Mitochondria, apoptosis, Bax, nanodiscs

9:45 - 10:00

Ashleigh Shannon (AFMB) **Newcomer**

Structural and functional studies of the SARS-CoV2 replication complex and its inhibitors

Ashleigh Shannon 1, Aurélie Chazot 1, Camille Falcou 1, Véronique Fattorini 1, Adel Moussa 2, Steven Good 2, Jean-Pierre Sommadossi 2, Francois Ferron 1, Karine Alvarez 1, Bruno Canard 1
1 AFMB, CNRS, Aix-Marseille University, UMR 7257, Case 925, 163 Avenue de Luminy, 13288 Marseille Cedex 09, France
2 Atea Pharmaceuticals, Inc.; 225 Franklin St., Suite 2100, Boston, MA 02110 USA.

Nucleoside/tide analogues (NAs) have long been used in the fight against viral diseases, and now present a promising option for the treatment of COVID-19. Once activated to the 5'-triphosphate state, NAs act by targeting the viral RNA-dependent RNA-polymerase (RdRp) for incorporation into the viral RNA genome. Incorporated analogues can either 'kill' (terminate) synthesis, or 'corrupt' (genetically or chemically) the RNA. However, the use of NAs against coronaviruses has been complicated by the presence of a virally encoded exonuclease domain (nsp14) with proofreading and repair capacities. Here I will discuss our work on promising NA candidates, and how small chemical modifications of these NAs can impact their mechanism of action to combat CoV-specific features.

Keywords : Nucleoside analogues, polymerase, exonuclease, viral replication

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10:00 - 10:15

Olga Iranzo (iSm2), Pierre Dorlet (BIP), and Régine Lebrun (IMM) **Interdisciplinary**

Cytotoxic Cu(II) complexes in the context of cancer: insights from the cells

Alejandro Blanco 1,2, Mathieu Eder 1,2, Hery Dinah Ratovonindrina 1,3, Marc Maresca 1, Cendrine Nicoletti 1, Laetitia Shintu 1, Régine Lebrun 3, Pierre Dorlet 2, Olga Iranzo 1.
1. Aix Marseille Université, CNRS, Centrale Méditerranée, iSm2, UMR CNRS 7313.
2. Aix-Marseille Université, CNRS, BIP, IMM, UMR CNRS 7281.
3. IMM, CNRS FR3479, Proteomic Facility, Marseille Proteomique

Cancer is a major health problem and important efforts are devoted to find out novel and effective treatments. Despite the progresses achieved in targeted- and immune-therapies, reality shows that in a clinical setting at least one potent cytotoxic compound is required in the therapeutic regimen. Metal-based chemotherapeutics (e.g. cisplatin, carboplatin and oxaliplatin) have shown high clinical relevance becoming first-line treatment for several cancer types. However, the non-physiological nature of the metal often leads to undesired side-effects. This situation has awaked a quest for the development of transition biometal-based therapeutics.
In this project we are developing cytotoxic redox-active Cu complexes and deciphering their location and mechanisms of action inside cells to improve their therapeutic efficiency.

Keywords : Cancer, Copper Complexes, Reactive Oxygen Species, Cytotoxicity

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SESSION 2

11:15 - 11:30

Camille Herrou (LISM & LCB) **PhD**

Molecular and structural basis of the mechanism of type IVa pili machinery activation in *Myxococcus xanthus*

Camille Herrou 1,2, Sébastien Lhospice 2, Agrim Gupta 1, Tâm Mignot 2, Romain Mercier 2, Latifa Elantak 1
1. Laboratoire d'Ingénierie des Système Macromoléculaires, CNRS-UMR7255, France
2. Laboratoire de Chimie bactérienne, CNRS-UMR7283, France

In bacteria, twitching motility is driven by multiprotein membrane nanomachines called Type IV pili (T4P) that extend and retract pilin fibres to promote cell movement. More precisely, the extension/retraction of pilin fibres is mediated by actions of cytoplasmic ATPases that assemble single membrane pilins into a fibre. While the molecular mechanism of pilin assembly by T4P machine (T4PM) is now well characterised, what triggers and coordinates multiple T4PM remains poorly understood. In our model, *Myxococcus xanthus*, the protein SgmX has been described as the master regulator of T4PM. SgmX activates preassembled T4PM by an unknown mechanism. Combining NMR spectroscopy and fluorescence microscopy we investigated the interactions between SgmX and the T4PM components. From our results, we were able to draw a model for T4PM activation that results of sequential protein interactions orchestrated by *SgmX*.

Keywords : Type IV pili, motility, nanomachine

11:30- 11:45

Maxence Vincent (LCB) **Newcomer**

OM-PSG: Oxidation of Methionine in Proteostasis, Survival and Growth

During bacterial infections, immune cells generate reactive oxidants to damage pathogens in a process known as the respiratory burst. While certain oxidants have been extensively studied, others remain poorly understood due to their highly reactive nature, which complicates direct observation ex vivo. Here, we introduce a novel microfluidic platform that enables the continuous, real-time synthesis of short-lived oxidants. By coupling this approach with single-cell measurements of bacterial physiology, we reveal previously unrecognized bacterial phenotypes, offering new insights into adaptive mechanisms that bacteria employ under oxidative stress. Our findings provide a fresh perspective on bacterial survival strategies at the host-pathogen interface, advancing our understanding of microbial resilience in response to host-induced oxidative stress.

Keywords : Oxidative stress, Bacteria, Neutrophil, Microfludics, Single-cell

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11:45- 12:00

Elias Feitosa Araujo (BIAM) **Newcomer**

The importance of NAD metabolism to drought and senescence in *Arabidopsis thaliana*

The restriction of water availability causes drought stress, directly affecting plant physiology, growth, development, and productivity. Plant responses to drought stress involve a complex interplay of various pathways, and research has demonstrated that autophagy plays a key role in mediating these responses because Arabidopsis, tomato, and wheat mutant plants deficient in autophagy have reduced drought tolerance. Recently, it was demonstrated an evolutionarily conserved role of autophagy, from yeast to humans, in maintaining the levels of NAD+ during stress to sustain survival. Although these pioneering studies have highlighted the crucial interplay between NAD and autophagy in various eukaryotes and its implications for survival under stress, it remains uncertain whether the Autophagy-NAD axis is conserved in plants. The current project aims to explore the conservation of the Autophagy-NAD axis in plants, its regulatory mechanisms, and its effects on responses to drought stress.

Keywords : NAD, autophagy, senescence, drought

12:00- 12:15

Vincent Lombard (AFMB), **François Alberto (LCB)** & Gerlind Sulzenbacher (AFMB) **Interdisciplinary**

ExploGT: exploring the sweeter aspect of flagella structure

Magnetotactic bacteria (MTBs) are microaerophilic aquatic bacteria that can swim (using their flagella) toward their optimal development zone, the oxic-anoxic transition zone (OATZ). For that, they are helped by magnetotaxis (detection of the Earth's magnetic field) and chemotaxis (detection of the oxygen gradient). These three components (magnetotaxis, chemotaxis, and swimming) define an unique property of MTBs: the magneto-aerotaxis. We demonstrated that the flagellin (the protein that forms the flagellar filament) of MTBs is glycosylated, and that this glycosylation is crucial for the set-up of the filament and therefore for the motility. The synergy of our respective expertise in bacterial genetics, protein crystallography, and glycobiology bioinformatics has allowed us to identify three new types of bacterial glycosyltransferases found in MTBs, as well as in many other bacterial species, and to demonstrate their essential roles in the mechanism of forming a functional flagellum.

Keywords : Magnetotactic bacteria, motility, flagella structure, glycosylation, glycosyltransferases

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KEYNOTE LECTURE

14:15 - 15:00

Sophie Tronnet (Umeå university & iSm2) **CPJ IM2B**

Untangling Environment-Microbiota Interactions: from basic principles to emergent properties

In animal and plant cells extracellular ATP (eATP) functions as signaling molecule and regulates the immune response. During inflammation intestinal bacteria are exposed to increasing concentrations of eATP originating from the mucosa. Whether bacteria respond to eATP is unclear. Here we show that commensal *Escherichia coli* responds to eATP at physiologically relevant concentrations by modifying its transcriptional and metabolic landscape. Combined genome-scale modelling highlighted the global metabolic modifications. Moreover, several gut pathogens respond to eATP by regulating the expression of selected virulence factors. Our results indicate that eATP is a signaling molecule in bacteria that promotes inter-kingdom communication in the gut by modulating the intestinal microbiota and pathogens.

Keywords : extracellular ATP, Intestinal bacteria, Inter-kingdom communication, Inflammation

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15:00 - 15:15

Florence Bansept (LCB) **Newcomer**

Mathematical modeling of the ecology and evolution of host-associated microbiomes

In the group, we use mathematical models that combine analytical and numerical techniques with stochastic simulations to study microbial communities, and in particular those that live in association with hosts, a.k.a. microbiomes. We also seek and develop experimental collaborations – contact us if interested! With our models, we aim at addressing a wide range of related questions, like: How do hosts and microbes co-evolve? How is the gut microbiome diversity maintained in the host?What are the control mechanisms that the host can exert on its microbiome at minimal costs? How can within-host microbial dynamics impact an infectious spread at the scale of the host population? Can microbiome modeling provide valuable insights for clinical applications or to public health decision makers? In my presentation, I will give an overview of our past achievements and the projects that currently keep us awake at night.

Keywords : Mathematical modeling, Ecology, Evolution, Microbial Communities, Population Dynamics

SESSION 3

16:00 - 16:15

Jana Mehrez (iSm2) **PhD - EJD**

Stereochemical Tuning of Nickel-Based HER Electrocatalyst

Jana Mehrez, Michael Papadakis, Renaud Hardré, Maylis Orio

Numerous challenges for the upcoming decades arise from the worldwide reliance on fossil fuels as energy carriers and raw materials for industrial products. The resources being finite, there is a great interest of the research community to find alternative sources. Hydrogen is regarded as an "ideal" fuel due to its abundance and considering it only emits water vapor as side-product during its combustion. The new challenge is to generate hydrogen from renewable and sustainable resources. A first series of nickel catalysts was designed in our lab from chemical tuning of the redox-active thiosemicarbazone (TSC) ligand. Different substituents were placed in para position of the phenyl ring of the TSC ligand. Another series also was created to enhance the ligand influence playing with substituent positioning.[2] The resulting electrocatalytic performances of the complexes indicated that both chemical nature and positioning of the ligand substituent indeed influences the electrochemical and catalytic behavior of the system. To get further insight into these observations, we are now investigating a new series of nickel based electro-catalysts with non-polar substituents. The resulting complexes are evaluated for their capability to mediate electrocatalytic hydrogen evolution with the aim to establish clear structure-property relationships.

Keywords : Hydrogen Evolution Reaction , Electrochemistry, Electrocatalysis , Energy , Nickel Catalyst

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16:15 - 16:30

Johannes Stuttmann (BIAM) **Newcomer**

Plant-associated microbiota: Genetics in the rhizosphere

Plants release significant amounts, up to 20 %, of photosynthetically-fixed carbon into the rhizosphere, the thin layer of soil directly surrounding plant roots and influenced by these. Accordingly, the rhizosphere represents an attractive energy-rich environment colonized by diverse pro- and eukaryotic microorganisms. Although some of these microorganisms may be pathogenic, the root microbiota overall provide important services to their host plants, such as protection from biotic and abiotic stresses and nutrient solubilization. We are interested how microbe-microbe interactions and plant genotype shape root-associated microbial communities. Further, we are interested in genetic determinants and mechanisms underpinning plant-beneficial effects. Current efforts for leveraging rapid genetic modification of diverse environmental bacteria as well as future objectives will be presented.

Keywords : Plant-growth promoting rhizobacteria, microbiome, genetic engineering, plant

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16:30 - 16:45

Laetitia Houot (LISM) and Tam Mignot (LCB) **Interdisciplinary**

Monitoring the localization and dynamics of infection by filamentous phages at the cellular level

Callypso PELLEGRI 1, Romane GUARINO 1, Thierry DOAN 1, Flora HONORE 2, Tâm MIGNOT 2 and Laetitia HOUOT 1
1 LISM (Laboratoire d'Ingénierie des Systèmes Macromoléculaires)
2 LCB (Laboratoire de Chimie Bactérienne)

Filamentous phages are viruses that behave like bacterial symbionts that are an underestimated component of microbiota. They notably improve antibiotic resistance and virulence in human and plant pathogens. Yet, the infection process remains poorly understood at the molecular level, and the dynamic parameters of the process are unknown, as real-time visualization of viral infection was not yet available. In this project, we combined our expertise in the biology of filamentous phages and single cell fluorescent microscopy to decipher the dynamic of the infection process for the fd/*E. coli* couple. We validated individual fluorescent labeling strategies on the different macromolecular structures involved : the phage capsid, the pilus and the phage DNA and started the monitoring their dynamics during filamentous phage infection at the cell level. We expect these tools to be helpful for structural and biochemical characterization of key infection steps, and to open up prospects for future studies of other filamentous phages associated with pathogens

Keywords : Filamentous phages, infection, dynamics, single cell microscopy

FLASH TALKS & POSTERS - ABSTRACTS

TRAINING PROJECTS

Elsa Garcin (IGS)

VR4LIFE: Virtual reality for chemistry, biochemistry and structural biology education

Virtual reality (VR), Artificial Intelligence (AI), and Augmented Reality (AR) are shaking up the world and transforming the way we interact with these technologies. Jobs in VR are therefore attracting more and more young graduates. VR technologies are also changing the way we learn and teach and have the potential to shift from a culture of lectures (teacher centric) to one of learning by doing (student centric). Numerous studies have shown that students learn better and retain more information when they can touch, manipulate, and interact. Using these tools will enable students to fully engage in their learning and validate their competencies.
The VR4LIFE project aims to implement innovative pedagogies ("learning by doing", collaborative project-based learning, participative workshops) to put students in situations that bring them closer to professional life, train them in cutting-edge technologies, and familiarize them with virtual reality. In addition, the use of immersive technology will foster their engagement and understanding of complex subjects.

Keywords : Pedagogical initiatives, Virtual reality, Biochemistry, Structural Biology, immersive teaching

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IGEM Flash talk - session 1

DUCHEMIN Lisa (President of iGEM Aix Marseille University), OLIVIER Alice (Treasurer of iGEM Aix Marseille University), MENOU Robin (Secretary of iGEM Aix Marseille University), HOAREAU Romain (part of iGEM Aix Marseille University), VERDU Lila (part of iGEM Aix Marseille University), PERRIN Thibault (part of iGEM Aix Marseille University), CHARBONNIER Alice (part of iGEM Aix Marseille University)

Bac'Attack : an innovative take on plant protection against viruses

Our project aims to develop a new method to combat plant viruses, particularly targeting BYDV, the cause of barley yellow dwarf virus. BYDV is spread by aphids and severely impacts cereal crops in our region of Provence as well as globally. We intend to implement an approach based on synthetic biology, genetically modifying a bacterium to selectively detect and eliminate the aphids that transmit the virus, without resorting to pesticides. This method would help reduce economic and environmental damage while preserving ecosystems. If our project is successful, we hope to extend this innovative approach to other viral plant diseases transmitted by insects.

Keywords : plant viruses, bacteria, synthetic biology, aphid, iGEM

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Isabelle Imbert & Christophe Bordi (LISM) Flash talk - session 1

BioProduction Educational Platform (PPBioProd)

Biomanufacturing is a set of processes which, through the use of living (micro)-organisms leads to the generation of a biomolecule of interest (e.g. RNA, enzymes, antibodies, viruses...) for a wide range of applications (e.g. agri-food, pharmaceuticals, biofuels...). Accelerating, reinforcing and developing French biomanufacturing is one of the major challenges of national policy, and will involve the creation of many new jobs. The BioProduction Educational Platform (PPBioProd) is part of this strategy, and aims to enable students and trainees to implement and optimize bioprocesses leading to the production of pure and functional proteins.
In addition, PPBioProd relies on (i) an excellent range of existing practical work focused on protein biochemistry, and (ii) a local network of biotech companies. PPBioProd will thus enable a transformation of current teaching practices, through the use of cutting-edge equipment used in research laboratories and industrial settings, and thus meet the needs of industry in this field.
The target audience ranges from Bachelor 2 to Masters students, and even beyond (PhD students, vocational training...).

Keywords : Bioproduction, proteins, chromatographic purification, quality control, professional integration

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Jens Krarup (BIAM) & **Julius Martinkus (LISM)** Flash talk - session 2

Development of a High Throughput Platform for NANOparticles SCreening Against ANtibiotic-Resistant Bacteria (NANOSCAN)

Antibiotic resistance is a major global health threat, reducing the effectiveness of conventional antibiotics and leading to longer illnesses, higher healthcare costs, and increased mortality rates. To address this, researchers are exploring nanoparticles with unique properties to overcome resistant bacteria. However, despite extensive research, there is still a need for an efficient, low-cost, and accessible screening process. This proposal aims to develop a high-throughput screening platform to evaluate the efficacy of nanoparticles against antibiotic-resistant bacteria that cause human infections. The platform will integrate automation and standardization for rapid, systematic assessment of nanoparticle libraries, using generic equipment and open-source software. This interdisciplinary effort will accelerate the translation of nanoparticle research into clinical applications.

Keywords : Resistance, Bacteria, Nanoparticles, Antibiotics, Screening

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James Sturgis (LISM) Flash talk - session 2

Molecular Scale Biophysics Summer School

James Sturgis (LISM), Elsa Garcin (IGS), Deborah Byrne (IMM), Marlene Martinho (BIP) and others .

The molecular scale biophysics summer school has been organized for the last 2 years with the support of IM2B, CIVIS and MOSBRI. The summer school brings students from multiple european universities, at Masters and PhD level, in the CIVIS consortium to Marseille for a week of practical work using multiple biophysical methods that we have available locally. This summer school is part of a blended intensive programme (BIP) teaching programme developped in the Structural Biochemistry masters programme (Master BSG). This year we had students from Rome, Bucharest, Athens and Stockholm attending the school and teachers from Stockholm and Rome to reinforce the local team.

CORE FACILITIES

Olivier Bornet (IMM) Flash talk - session 2

IMM NMR platform for the study of biological molecules

NMR is one of the essential experimental techniques for researchers in structural biology, enzymology, and molecular chemistry, enabling them to characterize the structure of a wide range of molecules (from metabolites to macromolecular complexes) using the magnetic properties of atoms. The NMR platform at the Institut de Microbiologie de la Méditerranée (IMM FR 3479) is equipped with a 600 MHz NMR spectrometer for liquid samples, combined with a cryoprobe, providing research teams in Aix-Marseille with the highest-performance NMR equipment in the southern region (in terms of sensitivity and resolution). Recently, the IMM NMR platform has been upgraded with the acquisition of a new generation electronic console, allowing access to new methodologies. Applications include: structural characterization of macromolecules; study of biological molecules "in-cell"; in-situ monitoring of biological molecules in complex mixtures; identification and structural characterization of new natural molecules.

Keywords : NMR, proteins, metabolites, interactions, structure

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Marielle Bauzan, **Deborah Byrne (IMM)**
Biomass & Protein Engineering Core Facility, IMM Institute of Microbiology of the Mediterranean /CNRS-AMU

Hands on bioprocess engineering training for undergraduates

The Global Technology Forum organized by the OECD (Organisation for Economic Co-operation and Development) in 2024 highlighted synthetic biology as an emerging technology of great importance. What does this mean? Policies will change and investors will invest! This means that in the near future, there will be funding and subsidies for SMEs in the circular bioeconomy. These companies will require highly trained students. Students that are trained in interdisciplinary projects from the model to the function. But, they will also need training in bioprocess & protein engineering. The Biomass core facility at IMM has acquired funding through IM2B from AMU/AMidex to provide training for future students from Masters to PhD. Training in bioprocesses, is taught almost exclusively in a theoretical and conceptual way because of the lack of suitable equipment & experienced engineers. We hope to fill this gap by training undergraduates with the aim of increasing their employability in biotechnology with our newly acquired bioreactors.

Keywords : Protein engineering, Bioprocess, protein purification, bioreactors, Training

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Yann Denis (IMM) & Christophe Bordi (LISM)

Plateforme Transcriptome

The IMM "Transcriptome platform" offers a full range of genomics and transcriptomics services for microbiology. Since its creation, the platform has constantly expanded its expertise in genomics, developing and adapting protocols in order to offer a range of services for a wide panel of microorganisms. This platform and the services it offers are open to all laboratories in both the public and private sectors.

Keywords : Genomic, Transcriptomic, Real Time quantitative PCR, Digital PCR, NGS

Marseille Protéomique team (IMM)

Update on Marseille Protéomique – IMM

Marseille Proteomique (MaP) is a three-site proteomics facility (CRCM, MCT, IMM) which offers, with its cutting-edge mass spectrometry equipment, a wide panel of complementary analyses for protein identification and characterization, biomolecule quantification, biomarker discovery, binding imaging studies and structural proteomics. This poster focuses on MaP-IMM expertise which encompasses basic research on proteins from all living kingdoms for studying broaden aspects such as enzymatic activities, growth, energetic regulations, virulence, signalling pathways …related to environmental and health concerns. MaP-IMM provides: N-ter Edman sequencing; large scale protein identification and quantification; interactomics; detection and quantification of chemical or post-translational modifications of proteins; global mass analysis of intact proteins, protein-protein (oligomerization), protein-ligand, protein-metal interactions using MALDI-ToF or Electrospray Mass spectrometry.

Keywords : Quantitative Proteomics, Interactomics, Intact proteins, Chemical or Post-translational modifications

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Bertrand Légeret, Marie Bertrand, Stéphan Cuine, Florian Veillet, Damien Sorigue, Fred Beisson, Gilles Peltier, **Yonghua Li-Beisson (BIAM)**

HelioBiotec : A microalgae platform for energy production, green chemistry and the environment

The HelioBiotec partnership research platform was created in 2009 and Its ambition is to serve as an anchor for the establishment of a scientific and technological cluster of excellence in bioenergy in the PACA region. HelioBiotec's investments have focused on the acquisition of equipment to develop ambitious scientific projects aimed at removing the biological barriers limiting the deployment of industrial applications for the production of 3rd generation biofuels, and synthons for green chemistry. Cutting-edge equipment has been acquired for high-throughput screening of microalgae strains (culture chambers, transplanting robot, flow cytometers, fluorescence imaging, etc.), characterization of their productivity (fleet of instrumented photobioreactors, on-line gas analysis) and their biochemical and physiological characteristics (fluorescence microscopy, biochemical and biophysical analyses). Particular emphasis has been placed on the development of lipidomics through the acquisition of various high-performance instruments (HPTLC, GC-MS, UPLC-MS-qTOF, etc.), as lipids represent a prime target for applications in biofuels, nutrition and nutraceuticals.
HélioBiotec has been labelled by the European IBISBA network of platforms, and is a member of the IBISBA European infrastructure project for industrial biotechnology.

Keywords : Mi(a)croalgaes, Lipids, hydrocarbon, photobioreactors; Lipidomics

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Vincent Lombard (1), Elodie Drula (1,2), Marie-Line Garron (1), Matthieu Boulinguiez (1), Pedro M Coutinho (1), Bernard Henrissat (1,3) and Nicolas Terrapon (1)
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2 Biodiversité et Biotechnologie Fongiques, INRAE, Marseille, France
3 Technical University of Denmark, DTU Bioengineering, Kgs Lyngby, Denmark

The Carbohydrate-Active EnZYme database: platform

Thirty years have passed since the emergence of the classification of carbohydrate-active enzymes into sequence-based families, which became the CAZy database and website (www.cazy.org). Thanks to its expertise and manual curation, the CAZy database has been the world reference for CAZyme analysis and classification for 25 years. The three main tasks of the CAZy curators are (i) to semi-manually annotate the modularity of the sequences released daily from the Genbank database, (ii) to create new families based on literature discoveries, and (iii) to capture any additional functional characterisation informing about the diversity of specificity in each family. In an era of large-scale sequencing and high-throughput biology, our team now provides a bioinformatics service platform for the analysis of '-omics' data to identify CAZymes and propose a collaborative interpretation of the data. We offer a range of analysis options, from automated analysis to manual curation. Our annotation approach is designed to provide users with high quality results based on the latest CAZy families.

Keywords : CAZy Classification, Enzyme, Carbohydrate, Metagenomics, Platform

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Denis Ptchelkine (AFMB) Flash talk - session 2

NEW ELECTRON MICROSCOPE AT AFMB

Recent technological advances in cryo-electron microscopy (cryo-EM) led to the development of new high performance electron microscopes. Cryo-EM can address a wide range of biological questions such as molecular basis of immune response, mechanisms of enzymatic reactions, identify key determinants of receptor-ligand recognition, structures of different virus forms and their interaction with host factors, structural aspects of immune synapse.
AFMB has received its new 200kV TEM on its site at the Luminy campus in Marseille. The microscope has the most modern configuration and is equipped with X-FEG electron gun, autoloader (12 grids), an energy filter and a last generation fast camera. This very high-tech equipment is used for efficient cryo-EM grids screening, high resolution data collection in SPA mode and tilt series acquisition (cryo tomography) on thin lamella (100-200nm thickness). We also offer training program for students and scientists who wish to develop their skills in electron microscopy. Research teams in Marseille and the region South benefit from direct on-site access to high resolution TEM that helps them to develop projects for which cryo-EM analysis is essential.

Keywords : Cryo-EM, high resolution, biological macromolecules, tomography

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Gerlind Sulzenbacher (AFMB) Flash talk - session 1

The Marseille Integrative Structural Biology Platform, PBSIM, AFMB

The PBSIM team: Renaud Vincentelli, Claire Debarnot, Anaïs Gaubert, Maria Mate, Véronique Roig-Zamboni, Denis Ptchelkine, Gerlind Sulzenbacher

The Marseille Integrative Structural Biology Platform (PBSIM, https://www.afmb.univ-mrs.fr/en/facility/structural-biology/) welcomes scientists interested in structural biology and beyond. PBSIM is hosted at the AFMB laboratory and offers state-of-the-art equipment accessible to a broad scientific community from both academia and industry. PBSIM is recognized by the IBiSA, AMU and FRISBI labels.
The facility covers all the steps from cloning to protein structure determination. It gathers six nodes; 1) HTP cloning, expression in E. coli and HTP purification, 2) a service for the selection and production of nanobodies, 3) a service for the expression of recombinant proteins in eukaryotic cells. These services are directly linked to the platforms dedicated to biophysical characterization (4) and the determination of 3D structures by crystallography (5) or cryo-electron microscopy (6).
The PBSIM mission is 1) to set up and give access to users to the latest equipment and expertise in integrative structural biology in collaboration with strategic partners; 2) to implement new methods according to the community needs; 3) to bring together a scientific community aiming to develop correlative microscopy approaches; 4) and most importantly, to train users and students to acquire expertise in new integrative structural biology techniques.

Keywords : protein expression, biophysics, X-ray crystallography, electron microscopy, nanobodies

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INTER-LABORATORY RESEARCH PROJECTS

Josiane Azizi (MIO,LISM), Hélène Gaussier (MIO) & Matthieu Nouailler (LISM) **PhD**

Enzymatic and Structural Characterization of iron oxidizing proteins from seafloor iron-rich microbial mats

Zetaproteobacteria are chemolithoautotrophic iron oxidizing bacteria that retain energy from iron oxidation at neutral pH under oxic conditions. Present in marine environments and specifically associated to iron rich hydrothermal vents ; they are subjected to a certain hydrostatic pressure and are known as piezophiles. This research aims to study the iron oxidizing process of these microorganisms focusing on the marine bacterium *Ghiorsea bivora* by first verifying the presence of an iron oxidizing activity in vitro through biochemical assays, identifying the proteins responsible for this activity by mass spectrometry and finally studying their activity and structure under atmospheric and high pressure condition. As a first step, the presence of an iron oxidizing activity was verified by the appearance of rust in the presence of iron under microaerophilic conditions. Next, we managed to produce *Ghiorsea* outer membrane porin, cytochrome 2, in Ecoli which is, based on metagenomics studies, responsible for the iron oxidation.

Keywords : Iron rich mats, outer membrane porin, iron oxidizing proteins, zetaproteobacteria , cytochromes

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Alejandro Blanco (iSm2, BIP)

Understanding the Anticancer Properties of Cu(II) Complexes based on Phen-Histidine ligands

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Cancer is one of the main causes of death worldwide. Since the discovery of the anticancer effect of Cisplatin, the bioinorganic chemistry community has intensified the research on metal complexes with anticancer properties in order to overcome the different drawbacks of Cisplatin and the rest of platinum based metallodrugs, being the main one their high toxicity, which leads to undesired side-effects. Copper has become a real alternative to these complexes since it is a very versatile biometal. Two copper complexes based on phenanthroline and histidine containing ligands were synthesized and characterized by different methods such as potentiometry, spectroscopy, mass spectrometry, electrochemistry and DFT calculations. They show high stability in aqueous solution and are redox active (Cu2+/Cu+). DNA cleavage studies reveal low redox based nuclease activity and the cytotoxic studies using MCF7 and A2780 cancer cell lines reveal moderate IC50 values. To further understand their properties and optimize their cytotoxic effect, Cu uptake, ROS production and in cellulo EPR spectroscopy studies were carried out. These data will be presented and correlated with their cytotoxic activity.

Keywords : Cancer, metallodrugs, copper, ROS, cytotoxicity

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Lucia Bidondo (BBF)

Discovering thioredoxin targets in *Neurospora crassa* during growth on cellulose

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Reactive oxygen species (ROS) are important signaling molecules, notably through their capacity of oxidizing proteins and modifying their activity or subcellular localization. Cys can be oxidized into disulfide bonds (S-S) having potentially regulatory function in enzymes or transcription factors. Thioredoxins (Trx) are small thiol oxidoreductases that play a pivotal role in ROS response by reducing numerous S-S in cells. In the saprotrophic ascomycete *Neurospora crassa*, an increased production of ROS occurs during cellulose degradation. Here, we aim to identify the proteins that are potentially redox regulated during cellulose degradation in Neurospora crassa. Our approach consists of isolating the Trx interacting proteins from *N. crassa* grown on cellulose by using affinity chromatography with an immobilized “bait-catching” Trx mutant. We identified 1 938 proteins among which key metabolic enzymes and signaling proteins regulating the production of the cellulose-degrading enzymes.

Keywords : Affinity chromatography, *Neurospora crassa*, protein oxidation, thioredoxin

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Barbara Cardoso Domingues (MCT)

Antimicrobial Activity of Degradable Synthetic Copolymers

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Antimicrobial-resistant ESKAPEE pathogens represent a global threat to human health. Among these, *Acinetobacter baumannii* is particularly concerning due to a lack of effective chemical therapies, making it a significant threat in healthcare settings. Cationic synthetic amphiphilic antibacterial copolymers (sACs) appear as a promising class of antimicrobial compounds due to their high antibacterial activity, low toxicity, and minimal risk of inducing resistance. As part of the COPOTIC project, a library of degradable sACs was synthetized and screened for antimicrobial and antibiofilm activities to find the more efficient antibacterial compounds with the minimal cytotoxicity. The results show that sACs are highly effective against *A. baumannii*, including multidrug-resistant clinical isolates, and lose their activity when degraded. These results highlight their potential for future therapeutic applications against this critical pathogen.

Keywords : ESKAPEE, Antibiotic resistance, Synthetic amphiphilic copolymers, *Acinetobacter baumannii*

Maeva Cunha (AFMB)

Intrinsically Disordered Regions in Hox proteins : unravelling their role in conformational properties, liquid-liquid phase separation and gene regulation

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This project aims to understand how transcription factors (TFs), such as Hox proteins, regulate gene expression and achieve functional specificity. Hox proteins, highly conserved throughout evolution, share similar DNA-binding motifs yet are able to specifically modulate the expression of certain genes. The study focuses on two Drosophila Hox proteins, Ubx and AbdA which are close paralogs of the family. In particular Ubx contains a large intrinsically disordered N-terminal domain (NTD). The hypothesis is that these TFs use liquid-liquid phase separation (LLPS) for nuclear sub-compartmentalization, contributing to their specificity. Biochemical and biophysical approaches are underway to characterize the conformational properties of the proteins and to assess the ability of the NTD to undergo LLPS, which could uncover a key regulatory mechanism driving Hox protein specificity.

Keywords : Intrinsically Disordered Proteins, LLPS, Hox paradox, Transcription Factors

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Giulia Panzironi (M2P2 & BBF) Flash talk - session 1

Screening of enzymatic activities for doxycycline bioremediation

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Antibiotics (ABs) are heavily used in human and animal treatments, with usage expected to rise by 200% between 2015 and 2030, if no measures are taken. Frequently detected in wastewater, they are only partially removed in treatment plants, contributing to environmental accumulation and AB resistance, a critical health threat projected to cause up to 106 deaths annually by 2050. Effective degradation of ABs in wastewater is essential, and enzyme-based processes offer a sustainable alternative to synthetic chemical treatments. Funded by the EU-PRIMA project "FUNZYbio," our study screens the ability of six enzymes to transform doxycycline using a novel high-throughput protocol for UV/Vis spectrophotometry and high-performance liquid chromatography (HPLC). Additionally, a high-throughput antibiogram assay was developed to evaluate the residual antimicrobial activity of the transformed AB. These methods enable rapid, efficient screening of enzyme activities, reducing experimental time, volumes and reagents.

Keywords : Antibiotic bioremediation, Biocatalyst, Antimicrobial activity, UV/Vis spectroscopy, High-throughput

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Mrunal Patil (MCT)

Microscopic Insights: Unlocking the Antimicrobial Potential of 5-Fluorouracil Derivatives

The escalating prevalence of antibiotic resistance underscores the urgent need for innovative therapies targeting multidrug-resistant microorganisms. Previous research has highlighted 5-fluorouracil (5-FU) as a promising candidate due to its intrinsic antibacterial properties; however, its significant toxicity restricts its application in antibacterial treatment. This study aims to provide microscopic evidence of the robust antibacterial activity exhibited by a tri-hexylphosphonium derivative of 5-fluorouracil. Utilizing electron microscopy, we elucidate the mechanism by which these phosphonium-based 5-FU derivatives cause marked septal damage and cytosolic alterations in *Staphylococcus aureus*, while also inducing plasmolysis in *Escherichia coli*. Further analysis revealed that compound (6c) generated significant alterations in membrane permeabilization and depolarization in S. aureus and E. coli cells at the MIC concentration. Additionally, 6c's non-haemolytic activity indicates its potential as a therapeutic option for treating multidrug-resistant bacterial infections.

Keywords : 5-fluorouracil, phosphonium derivatives, antimicrobial, microscopy

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Lara Pelzer (LISM)

The Tol-Pal system: Interactions and role during cell division in *Escherichia coli*

Lara Pelzer (1,2), Maximilien Rouzaud (1,2), Marlène Marthino (2,3), Callypso Pelligri (1,2), Laetitia Houot (1,2), Denis Duché (1), Christophe Bernard (1,2)
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The Tol-Pal system is a macrocomplex composed of five main proteins that straddles the three layers of cell envelope in Gram negative bacteria [1]. TolA, TolQ and TolR forming the inner membrane complex, TolB in the periplasm and Pal anchored to the outer membrane. During cell division, Pal accumulates to the septum thanks to the other proteins of the system and help the step of cell constriction [2,3]. My project is focused on the identification of the system's interaction network using a microscopy approach based on a localization assay. So far, we were able to identify several potential targets for the recruitment of the system that still need to be confirmed. In addition, we are trying to characterize the dynamics of TolA in complex with its different interactants through electron paramagnetic resonance. This technique will not only give us information on the dynamics but also on the structure of the second domain of TolA.

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Keywords : Membrane proteins, Microscopy, EPR

Gwénola Simon (MIO), Corinne Valette (MIO), Laurie Casalot (MIO) and Marianne Guiral (BIP)

Genetic-tool development in *Aquifex aeolicus*

The hyperthermophilic *Aquifex aeolicus* bacterium has been isolated from shallow underwater volcanic hot springs. It belongs to the Aquificales family, chemolithoautotrophs growing only in the presence of inorganic sulfur compounds, hydrogen and CO2 as carbon source. These microaerophilic microorganisms, considered to be primary in the trophic chain, play a key role in the biogeochemical cycling of sulfur and carbon in marine hydrothermal ecosystems. Aquificales can fix CO2 via the reverse tricarboxylic acid cycle (rTCA). While this mechanism has been proposed for CO2 assimilation in various anaerobic or microaerophilic bacteria, it has been little studied. In *A. aeolicus*, although certain respiratory enzymes involved in sulfur and hydrogen metabolism have been characterized and several bioenergetic pathways proposed, this complex energy metabolism is still only partially understood. This study is based on genome analysis and is the fruit of biochemical and physiological work. A detailed understanding of the involved mechanisms requires genetic approaches. Various experiments have been carried out to develop the necessary genetic tools.

Keywords : Polyextremophile organisms, genetic tools

RESEARCH PROJECTS WITH INTERNATIONAL PARTNER

Roseline Assiah Yao, Sara Vujakovic, Delphine Chaduli, Djihane Damoo, Sacha Grisel, Mireille Haon, Juliet Nilsson, Matthias Kretschmer, Jean-Guy Berrin, James Kronstad and Bastien Bissaro (BBF)

Deciphering the biological function of the unique Lytic Polysaccharide Monooxygenase (LPMO) from the plant pathogen *Ustilago maydis*

Fungi exhibit different lifestyles in nature, in close contact with their hosts. These interactions require enzymatic modifications of the fungal cell wall (FCW), which is composed of structural polymers such as glucans and chitin (1). These enzymatic modifications are carried out by carbohydrate-active enzymes (CAZymes; 2). While the functions of some synthesis and hydrolytic CAZymes on FCW polysaccharides has been investigated (3,4), the function of oxidative CAZymes remain largely unknown. Here, using the plant pathogenic fungus *Ustilago maydis* as a biological model, we studied the biological role of its unique lytic polysaccharide monooxygenase (LPMO; 5), which we have previously shown to act on FCW chitin (6). To this end, we used wet enzymology to probe the enzyme substrate specificity, confocal microscopy to determine the enzyme cellular localization, and reverse genetics (using CRISPR-Cas9) to investigate the LPMO involvement in fungal growth and plant infection.

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(4) Rizzi YS, Happel P, Lenz S, Urs MJ, Bonin M, Cord-Landwehr S, Singh R, Moerschbacher BM, Kahmann R. 2021. Chitosan and Chitin Deacetylase Activity Are Necessary for Development and Virulence of Ustilago maydis. mBio 12:e03419-20.
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Yao RA, Reyre J-L, Tamburrini KC, Haon M, Tranquet O, Nalubothula A, Mukherjee S, Le Gall S, Grisel S, Longhi S, Madhuprakash J, Bissaro B, Berrin J-G. 2023. The Ustilago maydis AA10 LPMO is active on fungal cell wall chitin. Applied and Environmental Microbiology 89:e00573-23.

Keywords : Filamentous fungi; fungal cell wall; pathogenesis; CRISPR-cas9; CAZymes.

Frédéric Carrière (BIP)

The yellow mullet fish oil from the Banc d'Arguin Imrâguens in Mauritania: An example of polyunsaturated fatty acids transfer from diatoms to the fish within the alimentary chain

Mohamed Vall Sidi Boune (1), Mohamed Ahmed Sidi Cheikh (2), Mamadou Abdoul Ba (2), Nathalie Barouh (3), Bertrand Legeret (4), Sidi Mohamed Ould Souvi (5), Mohamed Vadel Deida (1), Hélène Launay (6) and Frédéric Carrière (6)
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The Banc d'Arguin National Park (PNBA) in Mauritania is listed by the UNESCO World Heritage. It is characterized by an exceptionnal marine biodiversity with numerous endemic species and it provides a major site of reproduction for western Africa fish. The Imrâguens form fisherman communities established at PNBA, who live upon fishing the yellow mullet (*Mugil cephalus)* during its migration and derived products.
The analysis of the fish oil produced by Imrâguens from mullet heads is rich in omega 3 polyunsaturated fatty acids (37.7 % of total fatty acids). The main fatty acid is eicosapentaenoic fatty acid (EPA ; 20.18 ± 0.01 %). This fatty acid is particularly abundant in diatoms, that contribute to 20-30% of mullet feeding. The identification of 16:4n-1 also provide a good trophic marker for yellow mullet feeding on diatoms. The digestive lipases potentially involved in the mobilization of these fatty from diatoms were identified from the analysis of *Mugil cephalus* genome. Genes encoding four lipases homologous to pancreatic carboxylester hydrolase were identified. These later could be involved in the lipolysis of galactolipids, the main lipids present in diatom photosynthetic membranes which are rich in EPA.

Keywords : omega-3 fatty acids; galactolipase; grey mullet; microbial lipids; *Mugil cephalus*

Lucía Gandarias, Sandy Payan, Damien Faivre & Sandra Prévéral (BIAM)

Using genetic engineering to obtain immunotherapy-mediating magnetic nanoparticles synthesized by magnetotactic bacteria

In this study we create a synergy between the power of immunotherapy and the potential of magnetic nanoparticles to mediate in cancer therapies. For this, we take advantage of the ability of magnetotactic bacteria to synthesize magnetosomes, magnetite magnetic nanoparticles surrounded by a proteolipidic bilayer that can be functionalized using genetic engineering tools. Here, we functionalize the magnetosomes with an immunotherapy-mediating protein that attaches to the tyrosine-kinase receptor HER2, overexpressed in certain types of cancer such as HER2-positive breast cancer. When the immunotherapy-mediating proteins attach to their receptor, they trigger a cascade that causes the apoptosis of HER2-positive cells. Moreover, the functionalized magnetosomes can be used as a specific probe for magnetic resonance imaging, or as heating agents for magnetic hyperthermia or photothermia.

Keywords : Immunotherapy, genetic engineering, magnetic nanoparticles, magnetotactic bacteria, cancer treatment

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RESEARCH PROJECTS WITH A COMPANY

Thelma Barnetche, Qiujuan Shen, Agnès Amouric, Elise Courvoisier-Dezord, Yasmina Mekmouche, Alexandre Ciaccafava, Thierry Tron (iSm2)

Vibrational probes for redox enzymes exploration

Redox enzymes are efficient biocatalysts, and understanding their catalytic mechanisms is crucial for creating bio-inspired catalysts. While bioelectrochemical methods reveal enzyme activity, spectroscopic techniques are needed for structural insights. This project uses SEIRA spectroscopy (Surface-Enhanced InfraRed Absorption) to study redox enzymes, focusing first on laccase, a multicopper oxidase involved in oxygen reduction. Traditional methods using SEIRA and electrochemistry face limitations due to the lack of intrinsic protein markers preventing precise and localised studies. To overcome this, genetic engineering is used to introduce vibrational markers at key structural points which respond differently to environmental factors, enabling detailed analysis of enzyme reactions, orientation, and structural changes during activity. This improves enzyme study accuracy by providing electrode immobilization points and vibrational markers for SEIRA analysis.

Keywords : Spectroelectrochemistry; Redox enzymes; Catalysis; Orientation; Immobilisation

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Juliet F. Nilsson (BBF)

Why filamentous fungi encode dozens of LPMOs? *Podospora anserina* as a case study

Juliet F. Nilsson (1), Mireille Haon (1), Sacha Grisel (1), Simon Arragain (2), Jean-Guy Berrin (1) and Bastien Bissaro (1)

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Lytic Polysaccharide Monooxygenases (LPMOs), known for their "boosting effect" on the enzymatic hydrolysis of recalcitrant polysaccharides such as chitin [1] and cellulose [2], play a pivotal role in the global carbon cycle during biomass decay in nature. However, LPMOs are far from being completely understood from a physiological perspective [3]. Alike many filamentous fungi, the coprophilous fungus Podospora anserina encodes 41 LPMOs, 33 of which belong to the Auxiliary Activity 9 family (PaAA9s). The reasons for this multiplicity still remain enigmatic despite several studies [4-6]. Here, combining in-silico and biochemical characterizations, we carried out a comprehensive comparative analysis of the 33 PaAA9s. The study revealed striking differences among the PaAA9s, suggesting distinct roles during plant biomass decay and hypothetical non-conventional, emerging physiological functionalities.

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Keywords : LPMO, *Podospora anserina,* cellulose, functional multiplicity

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OTHER RESEARCH PROJECTS

Letitia Leydet, **Agnès Amouric**, Anna Marie Bastide, Arianna Pelissou, Hugo Stephant, Mireille Attolini, Pierre Rousselot-Pailley, Gilles Iacazio & Katia Duquesne (iSm2)

Construction of a new screening platform for Terpene synthases using the Terpene Mini Path

Terpenes are natural compounds with a wide range of interests in the flavour industry, food and medicine. Access to terpenes by natural means remains a challenge. Thanks to the terpene mini-pathway, we offer a very efficient biotechnological alternative since in only two enzymatic steps we access the universal precursors of all terpenes (1). Then we implemented this simplified pathway with i) a prenyl transferase to convert the universal precursors into C10, C15 or C20 linear diphosphates and ii) a terpene synthase which catalyze the cyclization of these linear molecules into terpenes (2). 18 putative fungal sesquiterpene synthases genes recently annotated in the genome of the Polyporales *Leiotrametes menziesii* (3) have been cloned and overexpressed in order to test in vivo and in vitro each purified enzyme with the implemented TMP. The characterization is ongoing to identify the produced terpenes. Therefore, our biosynthetic pathway offers an easy method to synthesize different terpenes backbones and to discover and characterize new terpene synthases.

1) Couillaud et al. ACS Omega (2019), 2) Couillaud et al. ACS Omega (2022), 3) Hage et al. Microbial Genomics (2023)

Keywords : terpenes, mini-path, biocatalysis, biodiversity

Emilie Gachon (BIAM)

Magnetosensing in magnetotactic bacteria

Magnetotactic bacteria (MTB) are microorganisms in aquatic environments that biomineralize magnetic nanoparticles, called magnetosomes, which form chains inside the cells. These chains provide a magnetic moment, enabling MTB to align with geomagnetic field lines and efficiently navigate toward microaerobic conditions. It is thought that MTB only passively align along magnetic field lines. We studied the SS-5 strain, tracking its movement with and without magnetic fields. Our findings reveal that SS-5 performs magnetosensing, swimming faster in a physiological magnetic field, a change that is independent of the illumination wavelength and disappears when the magnetic backbone of the cell, is disrupted. This suggests that SS-5's magnetosensing relies on mechanical signal transduction along the magnetosome chain rather than a cryptochrome-based mechanism, marking the first evidence of active MTB adaptation to magnetic fields.

Keywords : Sensing, Magneto-mechanical transduction, Microbial biophysics

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Pierre Leroux (MCT)

In vitro antibacterial evaluation of a chemical library of heterocyclic synthons. Interest of thienopyrimidine scaffold against *Staphylococcus aureus*

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In vitro antibacterial screening of a chemical library of 74 heterocyclic synthons with a wide variety of structures against ESKAPE-E pathogens revealed several hits (> 50% inhibition) using both manual and automated protocols: 15 hits for *Staphylococcus aureus*, 2 for *Acinetobacter baumannii*, and 1 for *Escherichia coli*. Structural analysis of the identified hits highlighted a recurrent scaffold as one hit was a 6-phenylthieno[2,3-d]pyrimidine (MIC for *S. aureus* 6.1 ± 2.4 µM; *A. baumanni*i 17.1 ± 4.9 µM; *E. coli* 26.0 ± 11.3 µM) and four hits were 6-phenylthieno[3,2-d]pyrimidines (6.8 ± 2.7 µM ≤ MIC S. aureus ≤ 10.7 ± 5.3 µM). Kinetic studies on S. aureus showed that most thienopyrimidine hits maintained complete inhibition between 18 and 24 hours of incubation, suggesting a bactericidal profile. New compounds derived from a pharmacomodulation study will help refine structure-activity relationship (SAR) hypotheses, which will be addressed in a forthcoming publication.

Keywords : *Staphylococcus aureus*; In vitro screening; Thienopyrimidine; Structure-Activity Relationships; MIC

Antoine Reho (BBF)

Are some of the GH5s from the maize pathogen *Ustilago maydis* active against its own cell wall ?

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Fungal cell walls (FCW) are mainly composed of chitin and β-1,3-glucans [1]. Strikingly, very little is known on the scope of fungal carbohydrate-active enzymes (CAZymes) active on FCW polysaccharides [2, 3]. Using the maize fungal pathogen *Ustilago maydis* as model, we have recently shown that several of its oxidative (AA10, AA3_2) and hydrolytic (GH16) CAZymes can cleave or modify FCW components. Here, we focused on members of the GH5_9 subfamily, some of which were previously identified in the *U. maydis* secretome [4], and whose members are suspected to be active against FCW β-1,3-glucans [1, 5]. We first carried out in silico analyses, then, after heterologous expression of *Um*GH5_9A in the yeast *Pichia pastoris*, we proceeded to characterize it biochemically. To unravel its biological function, we used CRISPR-Cas9 to generate *ΔUm*GH5_9A deletion mutants, and initiated their phenotyping, analyzing notably the fungal growth and alteration of the *U. maydis* FCW.

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Keywords : Filamentous fungi, *Ustilago maydis*, Fungal cell wall, GH5, β-Glucans

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Fine-tuning natural antimicrobial peptides from the rumen microbiome to enhance activity

In the context of the threat of antimicrobial resistance, AMPs have proven to be good candidates.
In previous work, a family of antimicrobial peptides (Lynronne family) was identified and isolated These peptides are good candidates both in terms of their antimicrobial activities and their low toxicity (Oyama et al., npj Biofilms Microbiomes 2017).
The objective of our work is to modify the residues of this family of AMPs to further improve their antimicrobial activities while reducing their toxicity against human cellswe designed, synthesized, and purified Lynronnes analogues containing the same number but different cationic residues.
Currently, we are carrying out antimicrobial tests by the cascade dilution method (Minimum Inhibitory Concentration (MIC) assay) on ESKAPE bacteria. Toxicity tests on human cells will be also performed to evaluate their therapeutic potential.

Keywords : Antimicrobials peptides, SPPS, microbiology, toxicity