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Posters

A story of a frog, a fungus and some bacteria: Microbiome mediated colonisation resistance via siderophore production

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Résumé

Batrachochytrium dendrobatidis (*Bd*) is a devastating fungal pathogen that has led to catastrophic declines in amphibian populations worldwide. Responsible for the disease chytridiomycosis, *Bd* threatens up to 50% of all amphibian species worldwide leading to the single biggest extinction crisis in vertebrates. Chytridiomycosis is a fungal disease, where *Bd* zoospores colonise amphibian's skin, leading to skin thickening, disrupted skin permeability, and electrolyte imbalances, ultimately leading to cardiac arrest and death. Understanding the mechanisms involved in host-pathogen interactions is essential for developing effective strategies to combat this epidemic. One of the main host defence mechanisms against invading pathogens is nutritional immunity, wherein the host restricts essential nutrients, such as metals, to impede pathogen growth. The cornerstone of nutritional immunity is the host's ability to restrict the availability of essential metals, such as iron, zinc, and manganese, which are vital for the growth, virulence and survival of pathogens. By limiting the access to these nutrients, the host aims to inhibit the proliferation of invading pathogens. Metal restriction, by the secretion of metal binding proteins such as transferrin, is known to play a crucial role during infection, as it can significantly impact the virulence and survival of pathogens. In addition to nutritional immunity, the skin microbiome plays a pivotal role in shaping the outcome of chytridiomycosis. The skin of amphibians harbours diverse microbial communities, which have been shown to provide protection against *Bd* infection through various mechanisms, including the production of anti-fungal metabolites. While the importance of the skin microbiome is evident, the effects of host-induced metal starvation on these microbial communities have not been explored. It is plausible that metal restriction may influence the composition and functionality of the skin microbiome, potentially altering the production of anti-fungal metabolites and overall protective capacity against *Bd*.

Preliminary results from our research have hinted at the potential role of the skin microbiome in aiding the host during *Bd* infection. Specifically, our data suggest that under iron deficiency, the skin microbiome may contribute to host defence by producing metabolites, iron-chelating molecules called siderophores, with potent anti-fungal properties. These anti-fungal metabolites are produced in metal limiting conditions similar to those encountered during infection suggesting that these metabolites may act in synchrony with host-induced

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nutritional immunity. Exploring the effect of these specific metabolites and understanding how their production affects pathogenesis could open up novel therapeutic avenues for managing chytridiomycosis. Our collaborator Dr. Kieran Bates, has isolated several bacterial strains from mid-wife toads' skin and we have begun to build a minimal bacterial community representing the most abundant bacterial phylogroups of the frog's skin. Furthermore, we have developed a colorimetric high-throughput screen allowing the rapid and efficient identification of siderophore producing strains. This allowed us to show that most isolates tested so far, produce high amounts of siderophores under iron limitation. Additionally, while siderophore production required iron deprivation, we could also show that environmental factors such as pH and carbon source could highly influence siderophore production. Finally, we have tested a multitude of strains baring or not the ability to produce siderophores, for their inhibitory effect of the growth of the fungal pathogen *Bd*. These inhibition experiments revealed that 1. *Bd* is unable to exploit any of the tested siderophores, 2. *Bd* lacks competitive iron uptake systems; 3. nor it is equipped with systems able to degrade these metabolites.

Mots-Clés: siderophores, microbiome, iron, fungal pathogen, Bd, nutritional immunity, colonisation resistance

ADME-Tox department– Illkirch

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Résumé

The drug research process is time-consuming and tedious. It requires numerous studies and rearrangement of the molecules under development. Evaluation of ADME-Tox properties (Absorption, Distribution, Metabolism, Excretion and Toxicity) must be conducted during the exploration, optimization and preclinical development phases. These properties can be use to characterize, select compounds and predict their behavior after administration in humans. Our service is part of the Strasbourg Integrative Biological Chemistry Platform (PCBIS), located at ESBS, and provides services in the field of ADME-Tox. The service has been working with academic and non-academic laboratories (corporations, start-ups and private companies) since 2008. Our test catalog includes all aspects of the ADME-Tox properties of small drug molecules. The activity of the service is carried out according to the quality rules put in place in the laboratory (ISO 9001 and NF X50-900 certification).

Mots-Clés: ADME, toxicity

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Aptamer-based targeted therapeutic approach in sepsis-induced disseminated intravascular coagulation

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Résumé

Sepsis is a major public health concern because of its prevalence which is approximatively at 50 million cases per year in the world and its acute mortality recaching 20% of all global deaths. According to the Third International Consensus, sepsis is defined as "a life-threatening organ dysfunction caused by a dysregulated host response to infection". Septic shock is the most severe form of infection which in 30% of cases can lead to disseminated intravascular coagulation (DIC) with a mortality rate of 60%. Nowadays, there is no specific treatment. DIC is associated with dysregulation of immunothrombosis. Immunothrombosis refers to all the interactions between immunity and coagulation, especially in the response to pathogens. This dysregulation leads to an excess of coagulation and a deficiency of fibrinolysis.
We hypothesise that a treatment against DIC targeting both the procoagulant response and restoring fibrinolytic insufficiency in patients with septic DIC might limit microthrombi formation and subsequent organ dysfunction and mortality. Our research project aims to develop an innovative strategy based on bifunctional nucleic acid aptamers. These are short oligonucleotide sequences with high affinity and specificity for their targets and with many advantages over antibodies such as the chemical synthesis, high reproducibility, easy modifications, and less restrictive storage conditions. The bifunctional aptamer is composed of:

(i) an anti-phosphatidylserine aptamer to bind fibrinolytic mesenchymal stem cell-derived microvesicles, aiming to restore local fibrinolytic activity ;

(ii) an aptamer directed against thrombin as a vector to guide microvesicles near the microthrombi and to reduce excessive coagulation activity.
Our current results indicate that aptamers targeting thrombin and phosphatidylserine have a stability in culture medium and Foetal Serum Bovine which depends on aptamers. We will then assess the stability of bifunctional aptamers and evaluate the *in vitro* efficiency of our strategy. The inhibition of coagulation will be determined from coagulation curve and fibrin quantity measured by ELISA. On the other hand, the plasmin generation and ELISA quantifications of fibrin degradation products and D-dimers will be used to evaluate the restoration of fibrinolysis. Afterwards, we will determine the efficacy and the tolerance of our strategy on a murine model of septic DIC.

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Mots-Clés: sepsis, disseminated intravascular coagulation, aptamer, microvesicle

Biocidal Components of Essential Oils for Datura Weed Control

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Résumé

Datura (*Datura stramonium* L., 1753) is an annual weed of the solanaceae family, notorious for its high tropane alkaloid content, including atropine, hyoscyamine, and scopolamine, rendering it highly toxic. In addition, this invasive plant has the capacity to proliferate a lot, particularly due to the very high resistance of its seeds. This poses significant risks to both human food and animal fodder due to potential contamination. Traditional weed management methods like mechanical weeding have little effect on datura and are often impractical, while chemical control raises concerns regarding weed resistance and environmental impact. In light of this, biocontrol using natural organisms and products, detrimental to the target plant, emerges as a promising alternative. Experimentation involving the application of various essential oils at different concentrations on young datura seedlings (4-6 leaf stage) revealed promising biocidal effects, notably with mountain savory, oregano, and clove oils, even at low concentrations. While the chemical compositions of these essential oils are partly understood, the specific components responsible for their biocidal properties have to be identified. Thus, analytical methods using GC-FID and GC-MS will be developed to highlight the active compounds and data will be compared with databases and commercial standards. Subsequently, the bioherbicidal activity of the identified compounds, alone or in synergy, will be measured to determine their phytotoxicity on datura. In conclusion, this research aims to uncover the biocidal components of essential oils for effective datura weed control, with potential implications for sustainable weed management practices.

Mots-Clés: essential oils, weed control, D. stramonium

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Bioherbicide: Evaluation of the antigerminative potential of Hypholoma fasciculare

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Résumé

Synthetic pesticides have been used against weeds since the mid-19s century. However, health and environmental concerns have grown in the last decades. In this context, CambaP team has carried out studies aiming at identifying new biocontrol products from plant and fungal origins in order to offer a more effective alternative to the synthetic herbicides. In this way, numerous plants and fungi were harvested and tested for their phytotoxic activities using a new technique recently developed in the laboratory and adapted to 96-well plates (1). This first screening step allowed highlighting the herbicidal potential of the higher fungi, *Hypholoma fasciculare*. Thus, the anti-germinative and growth inhibition activity of this mushroom was demonstrated for the first time.
The goal of the present work is to identify the active phytotoxic compounds of *H. fasciculare* aqueous extract. An original approach consisting in coupling an HPLC separation and the phytotoxic activity test was implemented, allowing a bio-guided fractionation at the analytical scale. An optimisation of the HPLC-DAD analytical conditions was performed and pointed out several fractions of interest. The active fractions were further analysed using a HPLC-ESI-HRMS method to formulate identification hypotheses. Among the identified molecules, some have already been described in the literature for their anti-germinative activities, and some have not. In order to better characterise the active compounds, higher scale fractionation of the aqueous extract was performed using a preparative HPLC device to obtain larger quantities of products. Again, several active fractions were detected and are currently analysed. Thanks to additional HRMS and NMRs analyses, the identification of the compounds underlying *H. fasciculare* activities is expected.
(1) Flieller; Riffault-Vallois; Bergaentzlé; Ennahar. Fast and Reproducible 96-Well Plate-Based Method for the Evaluation of the Antigerminative Potential of Plant Extracts and Phytotoxic Compounds. *J. Agric. Food Chem.* **2022**, *70* (25), 7842–7850. https://doi.org/10.1021/acs.jafc.2c02911.

Mots-Clés: Bioherbicide, anti, germinative activity, fungi, Hypholoma fasciculare

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Dereplication analysis of an antiplasmodial extract from Combretum aculeatum

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Résumé

Malaria pose significant challenges to public health (WHO, 2023). Current therapeutic options for this disease are limited, often associated with side effects, low efficiency, and the recent proliferation and prevalence of resistant strains of *Plasmodium falciparum*, parasite responsible of the disease. Thus, there is a need to explore new treatment possibilities. This study aimed to analyze the chemical composition of an antiplasmodial extract (in vitro IC50: 3.1 μg/mL against *P. falciparum*, SI: 24) obtained from *Combretum aculeatum*, a medicinal plant from Niger's traditional pharmacopoeia. For this purpose, a molecular-networking-guided method for the accelerated discovery of new compounds has been applied. HPLC-PDA-HRMS/MS molecular-networking-based dereplication strategy highlighted the presence of known antiplasmodial flavone *C*-glycosides, including vitexin, iso-vitexin, orientin and iso-orientin in addition with triterpenoids and lipids derivatives by comparison of their MS/MS fragmentation spectra (Kazuno *et al.*, 2005). Additional HPLC-DAD quantification of the flavones *C*-glycosides (5.7-9.8 mg of equivalent vitexin/ per g of extract) suggest that other compounds and/or synergistic effects may play a role in the antiplasmodial activity of the extract of *C. aculeatum*. The results of the present study confirm the relevance to explore new treatments possibilities against diseases with low efficiency therapeutic options, such as malaria. Moreover, traditional pharmacopoeia seems to be a good source for those new therapeutic options.

Mots-Clés: Medicinal plants, malaria, molecular networking, LC, MS/MS

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Development of a vaccinal approach based on the delivery of messenger RNA

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Résumé

Until recently, the use of messenger RNA (mRNA) for vaccination wasn't widely considered as a viable approach. However, considerable progress in this method, particularly through the utilization of modified mRNA, has garnered substantial interest and investment both academically and industrially. This renewed in attention has been primarily due to the development of mRNA vaccines, notably the ones targeting SARS-CoV-2. Additionally, various other RNA vaccines have entered clinical trials, and several other are in preclinical development (against pathogens and cancer).
The attractiveness of mRNA technology extends beyond its ability to efficiently trigger the synthesis of specific proteins or peptide antigens. Indeed, mRNA has several advantages: *i*- it does not require nuclear localization or transcription prior to translation of the gene of interest and, *ii*- there is a negligible risk of genomic integration; *iii*- in addition, mRNA is interesting because a relatively small dose is needed to produce many copies of protein and several antigens can be put onto a unique mRNA.

Unfortunately, mRNA is a polyanionic molecule that does not cross cell membrane barriers. Hence, the key to unlocking the full potential of mRNA lies in the development of safe and efficient agents that can package, deliver, and release mRNA intracellularly.

Successful vaccine must allow an optimal presentation of the antigen (Ag). Therefore, vaccination with mRNA must allow both the expression of the Ag by antigen presenting cells (APCs) and the induction of APC maturation since the APC are the most important cells for the induction of an anti-tumor immunity.

Our team has a large panel of synthetic vectors able of delivering efficiently plasmid DNA or siRNAs into cells, like cell penetrating peptide (CPP). CPPs are short peptides (8 to 40 amino acids) capable of crossing tissue and cell membranes, and they are used to transport a wide variety of biologically active cargoes into cells. Due to their versatility and membrane-crossing capacities, CPPs represent interesting candidates to be investigated in regards of the synthesis of novel vaccine vectors in cancer vaccination.

Among the CPPs, the cationic amphipathic histidine-rich LAH4 family stood out for its ability to transport a variety of cargoes, including DNA and siRNA. The LAH4 family consists of cationic amphiphilic peptides able to fold in α-helical secondary structures and

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contains four histidines allowing the modulation of their interactions with membranes in a pH-dependent manner.
Here, we focused our work on LAH4 peptides (and in a more detailed manner on LAH4 ; KKALLALALHHLAHLALHLALALKKA), and their ability to transfect dendritic cells (DC) with mRNA encoding either for luciferase or GFP (green fluorescent protein). We also tested the ability of our DC, after their transfection with LAH4 complexed with an mRNA encoding Ovalbumine (OVA), to activate the lymphocyte T CD8+ by making a co-culture of our DC with the hybridoma B3Z cells. We were able to measure the metabolic activity of beta-galactosidase, which correlates with IL-2 production, and IL-2 production itself correlates with LT proliferation and activation.

Mots-Clés: mRNA, vaccination, dendritic cells, LAH4, cancer, immunology, cell penetrating peptide

Development of an automated system in a complex environment to study the influence of the group on the personality of mice

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Résumé

Social interactions play a crucial role in shaping personality traits. These individual traits can influence vulnerability to certain pathologies, physiological responses to stress, anxiety, or addiction, and can lead to risky behaviors that ultimately determine chances of survival. These behavioral variations could explain the differences observed in the progression of some diseases, as well as in reaction to treatments.
However, many behavioral studies are conducted in standardized tests over a few minutes and the social life is not taken into consideration. In these conditions, the range of behaviors observed is voluntary limited.

Observations conducted in more naturalistic settings over extended periods reveal a broader and more ethologically relevant repertoire of behaviors, reflecting evolutionarily conserved phenotypes with translational value. To investigate the influence of the group on the individuals, we must keep mice in groups in order to look at their social life, and to measure individual behaviors outside the group. From those data, we will be able to identify behavioral profiles.

We have developed an automated behavioral measurement system within a complex environment where animals live in groups and can isolate themselves in a test module to perform individual cognitive tests.

We measure cognitive abilities of individuals with the Delayed Non-Matching To Position learning and memory task. In this setup, mice must nose poke at an operant hole located on an automatic feeder upon a light stimulus and then recall the position of the initial stimulus to nose poke on the opposite side to receive a pellet. The delay between the stimuli increases as the animal progresses through different phases of the test.

A home-made Python-based software controls the system's various devices, ensuring automated execution of experiments and real-time collection of behavioral data and events. This system enables experiments to be conducted over extended periods, up to several weeks, without any human intervention required besides food and water provisioning. Automation offers numerous advantages, including time savings, enhanced reproducibility, and improved animal welfare.

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We have successfully conducted four weeks long experiments with groups of four animals demonstrating the functionality of the system. We detected circadian rhythm of activity in the test modules, individual preferences towards one of the module. The learning curve built for each animal showed differencies in cognitive performance.
This proof of concept provides insights into the diverse strategies adopted by the animals and allows the detection of distinct behavioral profiles. This system is going to be combined with the "Live Mouse Tracker" system to correlate the individual cognitive data with the group's social dynamics in order to understand how social interactions contribute to the individuation process.

Mots-Clés: behaviour, individuation, personality, automated environment

Development of miniaturized microbiological assays using capillary cytometry

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Résumé

Flow cytometry has continued to progress over the last two decades. The miniaturization of optical systems (lasers, optical benches), fluid rheology (capillaries) and a built-in autosampler for 96-well plates (High-Throughput Screening) have made this technology accessible to the greatest number of users. What used to require a special space with complex pumping systems and specific currents is now reduced to simple and compact benchtop cytometers. The increase in the number and power of lasers, which often equip most cytometers currently on the market, has considerably increased the number of reading parameters, but has also greatly increased sensitivity thresholds.

Initially reserved for the acquisition of fluorescence associated with eukaryotic cells, the detection of smaller particles such as bacteria has opened up new possibilities for cytometry in the field of microbiology. Rapid analysis and quantitative characterization of micro-organisms are attracting growing interest in many areas of microbiology.

t's in this context that our eBioCyt UPS1401 platform, a "Unité Propre de Service" of the University of Strasbourg, has been developing fluorescence detection tests associated with different bacteria strains (Gram positive & negative, cocci or bacilli) since 2013(1).

More recently, we have participated in the development of measurements of the differential binding of fluorescent probes to several bacterial strains, as well as measurements of the effect of naturally occuring substances on bacterial toxicity.
1. Carré G, Benhamida D, Peluso J, Muller CD, Lett MC, Gies JP, et al. On the use of capillary cytometry for assessing the bactericidal effect of TiO2. Identification and involvement of reactive oxygen species. Photochem Photobiol Sci. avr 2013;12(4):610-20.

Mots-Clés: Flow cytometry, microbiology, bacteria, toxicity, fluorescent probes

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High-throughput screening by fluorescence polarization to discover specific inhibitor of Peroxiredoxin-2 (PRDX2)

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Résumé

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Hepatocellular carcinoma (HCC) is a major public health burdens for which we have only unsatisfactory treatment options. Major etiologies are chronical infections with hepatitis B and C viruses (HBV, HCV), alcoholic liver disease (ALD), nonalcoholic fatty liver disease (NAFLD) and its more severe form, the nonalcoholic steatohepatitis (NASH). Lifestyle changes, physical inactivity, obesity, and diabetes have resulted in a steady increase in the incidence of HCC. The discovery of new therapeutic targets is therefore essential. The combination of a cell-based system predicting the progression of liver disease and HCC risk, with transcriptomic analyses of liver tissue from HCC patients allowed the identification of the protein peroxiredoxin 2 (PRDX2) as a key player in the development of chronic liver disease and carcinogenesis. Therefore, PRDX2 is a highly relevant therapeutic target to prevent HCC development.

In this regard, we decided to recombinantly produce and purify the human PRDX2 protein in order to perform a high-throughput screening (HTS) by fluorescence polarization (FP) intended at discovering small molecule inhibitors. PRDX2 production was performed

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with the yeast *Pichia pastoris* operated in the secretion mode. After optimizing several production and purification parameters, a robust procedure was set up, leading to the repeated delivery of pure and homogenous PRDX2 lots at 30 μM

Each batch of protein was validated by measuring the redox activity of PRDX2 in a colorimetric assay (DO measurement at 600nm). Indeed, the xylenol orange enable to quantify the oxidation of ferrous ions (Fe2+) to ferric ions (Fe3+) by hydrogen peroxide, changing from orange to purple. But, in presence of the protein, the hydrogen peroxide is reduced by PRDX2, which prevents the formation of ferric ions and so, prevents the xylenol color change.

The development of the FP-HTS assay required the screening of the fluorescent libraries (1280 lissamine-labelled molecules from UMR 7200). Then, the fluorescent molecule selected and validated to bind the protein, was used as a probe for the screening of different chemical libraries (Prestwick©, CNE, Strasbourg and Sherbrooke) for a total of more than 10 900 molecules. Following this primary screening performed at 10 μM, 524 hits were released from all the libraries and then, were tested at two concentrations (1 and 10 μM). At the end of this second step, 175 hits were confirmed. Then, a post-screening analysis was carried out based on chemical structure and powder availability, for selected the compounds to test in a dose-response experiment. At this time, 46 hits were validated in dose-response assay (77 have not yet been tested).

Finally, the hits validated in FP-assay were tested on the activity of PRDX2 (test described above) in order to select the inhibitors of PRDX2. At this time, two molecules appear to reduce activity as much as conoidin A (= reference inhibitor of PRDX2).

The next step will consist firstly to test the toxicity of the inhibitors of PRDX2 on liver cell lines and primary hepatocytes, then to look their effect on HCC development in our cell-based model *.

* Crouchet E, Bandiera S, Fujiwara N, et al. A human liver cell-based system modeling a clinical prognostic liver signature for therapeutic discovery. Nat Commun. 2021;12(1):5525. Published 2021 Sep 17. doi:10.1038/s41467-021-25468-9
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Lipid coated UpConverting NanoParticles for Single Particle Tracking

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Résumé

Single particle tracking (SPT) is a powerful technique for real-time microscopic visualization of the movement of individual biomolecules within or on the surface of living cells. (1) However, SPT often suffers from the suboptimal performance of the photon-emitting labels used to tag the biomolecules of interest. For example, fluorescent dyes have poor photostability, while quantum dots suffer from blinking that hampers track acquisition and interpretation. Upconverting nanoparticles (UCNPs) have recently emerged as a promising antiStokes luminescence label for SPT. UCNPs are nanoparticles (_˜5-100 nm) of fluoride or oxide materials, doped with lanthanide ions. The doping allows the particles to exhibit anti-stokes emission of visible light upon excitation in the near-infrared region. (2) Unlike two-photon absorption of organic dyes or quantum dots, UCNPs have very efficient and steady upconversion. UCNPs also exhibit remarkable photostability, with no drop in emission nor blinking after hours of illumination.
Recently we demonstrated targeted SPT of FcϵRI receptors on living rat basophil leukaemia RBL-2H3 cells using UCNP based labels. (3) Thanks to their particular photophysics UCNPs appear as an interesting alternative label to organic dyes and quantum dots in particular for SPT studies in complex biological samples over a long period of time. In order to promote the use of UCNPs in single-molecule microscopy, several challenges still need to be overcome. In this context, we will present a recently developed lipid based protective surface coating (4) that allows (i) to disperse UCNPs in aqueous environment, (ii) to maintain their monodispersity and (iii) stability in cell culture media, and (iv) to reduce to a minimum of their unspecific binding to cell membranes while allowing their functionalization with a targeting agent.

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Mots-Clés: Upconversion nanoparticles, phospholipids, surface chemistry, single particle tracking, single molecule microscopy

MALDI-ToF mass spectrometry: a versatile tool for analyzing biomolecules, polymers and microorganisms

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Résumé

Matrix Assisted Laser Desorption Ionization (MALDI) is a soft ionization method used in mass spectroscopy to analyze sensitive macromolecules prone to fragmentation under other ionization conditions, such as peptides, proteins, oligonucleotides, lipids, or synthetic polymers.
Possible biochemical applications include but are not limited to identity check of synthetic peptides and oligonucleotides, reaction monitoring, controls of amino acid substitutions or post-translational modifications, and protein-ligand complexes studies.

When applied to microbiology, MALDI-ToF (time-of-flight) mass spectrometry can also draw unique proteomic fingerprints of microorganisms, which, by comparison with the MALDI Biotyper® (MBT) database, may allow identification down to the species level within a few minutes. This database currently contains over 4200 species, and may be locally completed with in-house research results.
The PACSI service of the Plateforme de Chimie Biologique Intégrative de Strasbourg (PCBIS – UAR 3286) was entrusted with the MALDI mass spectrometer of the Faculty of Pharmacy of the University of Strasbourg. As a result, this method is now available to the wider scientific community between two practical courses.

Mots-Clés: MALDI mass spectrometry, polymers, oligonucleotides, peptides, proteins, Biotyper, bacteria and yeast identification

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METABOLIC EVALUATION OF THE RELATIONSHIP BETWEEN MACROPHAGES AND OSTEOSARCOMA TUMOR CELLS

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Résumé

Osteosarcoma is considered as the first cause of bone cancer affecting predominantly adolescents and young adults. This cancer is thought resulting from a modulated balance between bone-producing osteoblastic cells and bone-destroying osteoclastic cells. It remains up to date a challenge to treat insofar as some patients' disease progresses, develops resistance and metastasizes despite first line strategy. It is therefore necessary to understand

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the full range of cellular and molecular mechanisms to understand tumor cells interplay with their microenvironment. In fact, tumor cellular microenvironment seems to play a role in the growth of these malignant osteoblastic cells and favors the expression of genes involved in the proliferation, migration, and metabolic adaptation of this cancer. By the past, multiple works including ours showed beyond the identification of hypoxic areas within the diagnostic biopsy sample, giant osteoclastic cells associated to infiltration of macrophages expressing CD68+ and/or CD163+ markers. Those microenvironmental cells are modulated the tumoral balance between pro-inflammatory and pro-tumoral markers modulating the osteosarcoma growth and invasion. These macrophagic cells have also been correlated with poor prognosis and increased risk of relapse in osteosarcomas when associated to hypoxic modulation. Based on this preclinical work, our team hypothesized that the presence of these biphenotypic macrophages and the hypoxia modulation would lead to alter metabolic pathways within tumor cells. Then, the objective of this study was to differentiate macrophages expressing these different biomarkers and culture them in presence of patient-derived osteosarcoma cells and be able to study their relationship at the level of tumor escape as well as at the metabolic level.To investigate this objective, we started by differentiating monocytes into macrophages CD68+, CD163+ and presenting both positivity (e.g., CD68+/CD163) using various stimulatory factors. Thereafter, these immune cells were co-cultured in 3D spheroids with several established osteosarcoma patient-derived cell lines (PDCL). This co-culture models enabled us to study cell phenotypic characteristics, their proliferation and migration under normoxic or hypoxic conditions. To differentiate both cells in those experiments, we were using nanoparticle cell labeling. Finally, we studied in those 3D co-cultures metabolites' quantification using HRMAS NMR (High Resolution with Magic Angle Rotation), as well as at spatial level using non-targeted MALDI mass spectrometry. Based on different stimulation factors, two types of macrophages were derived from blood monocytes expressing CD68 or both CD68+/CD163+ membrane markers. By co-culturing metastatic osteosarcoma tumor cells with biphenotypic macrophages, we were able to evidence that macrophages were accelerating a rapid tumor cell invasion in normoxia, but also in hypoxia. The HRMAS NMR study highlighted major metabolic switches in tumor cells varying differently in both oxygen concentrations but also in presence of macrophages. Combining hypoxia and a high concentration of macrophages in close vicinity to the osteosarcoma cells was favoring significantly the anaerobic glycolytic pathway, glutaminolysis and a methionine salvage pathway. The non-targeted MALDI spectrometry approach identified two significant compounds. One was distinguished in normoxic conditions and was a witness of proinflammatory tumor cell profile in the center of the 3D co-culture models. Another one was evidenced in hypoxic conditions heterogeneously distributed from the periphery to the center of the spheroid and correlated to sphingolipid metabolism. In addition to recreating in 3D models the macrophagic microenvironment present in osteosarcomas, we were able to link macrophages' presence, oxygen modulation and tumor escape and invasion probably through metabolic pathway modulation.

Mots-Clés: osteosarcoma, immunity, macrophages, cell invasion, metabolism

Metal-catalyzed amide reduction for the synthesis of non-natural basic amino acids and their use in SPPS

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Résumé

In the peptide field, the repertoire of basic amino acids is inherently limited to just three natural variants. This constraint restricts the spatial exploration of basic side chains, posing challenges in designing peptides with tailored interactions and selectivity. However, the synthesis of non-natural basic amino acids offers a promising avenue to overcome this limitation, facilitating the creation of diverse peptidomimetics. These novel amino acids not only expand the chemical diversity available for peptide design but also hold the potential to improve interactions and selectivity with targeted proteins.
Our work reports an efficient and selective Ruthenium-catalyzed amide reduction allowing the rapid production at the gram-scale of original non-natural amino acids exhibiting a large diversity of basic tertiary amines on their side-chain.
These new amino acids show a particular reactivity in classical coupling reaction condition, as the basicity of the side chain is efficient to intramolecularly deprotect the Fmoc group, leading to a second coupling reaction.

Mots-Clés: non naturals amino acids, peptides synthesis, coupling réaction, Metal, catalyzed amide reduction

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Optical control of Piezo1 channels

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Résumé

Sensing mechanical forces in the environment is vital for organism's survival. In 2010, the discovery of Piezo ion channels in vertebrates showed that they are the molecular sensors of mechanical sensitivity, the least known sense. Activation of Piezo channels requires sophisticated and non-specific methods of mechanical stimulation of the cell. The development of alternative activation methods able to specifically and rapidly activate these channels in vivo is challenging and therefore needs new technologies. Using biomolecular engineering combined with patch-clamp electrophysiology, we have developed an opto-chemical technology that makes the mouse Piezo1 channel sensitive to light. By covalently tethering an azobenzene-based photoswitch to a cysteine introduced by site-directed mutagenesis, we showed that light irradiation at 365 nm rapidly opened the pore. The reprogramming of this channel allows, in the absence of mechanical stimulus, to rapidly modulate its activity by light, without changing its mechanical sensitivity. Furthermore, this tool could provide a basis for understanding the mechanism of Piezo channels.

Mots-Clés: Piezo channels, Photoswitch, Chemical optogenetic, Azobenzene

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Optimization and delivery of a targeted reactivation system for a tumor suppressor gene in gastric cancer cells

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Résumé

The aim of my project is to develop a targeted demethylation system for the *SLFN11* tumor suppressor gene in gastric cancer. This aggressive cancer is usually detected late, due to a lack of clinical signs before it reaches an advanced stage, making surgical resection impossible. Treatment is essentially based on chemotherapy. However, resistance to chemotherapy agents has been observed in patients. *SLFN11* gene codes for a helicase that inhibits DNA repair and promotes cell death in response to DNA damage, such as those induced by chemotherapy agents. In resistant cancers, several studies show that this gene is repressed, unlike in patients responding to chemotherapy. It therefore appears to be one of the best predictors of response to treatment. This loss of expression is thought to be due to cytosine hypermethylation of the S*LFN11* promoter and It's been described that *SLFN11* is hypermethylated in 30% gastric cancer. Demethylating agents, like Decitabine, are used to reactivate this gene. However, these treatments lead to global hypomethylation of the genome, resulting in high toxicity and oncogene reactivation. A targeted demethylation approach to *SLFN11* appears to be the strategy of choice.
In my project, AGS (gastric adenocarcinoma) cells are used as a study model. These cells exhibit hypermethylation of the *SLFN11* promoter. We have discovered that in these cells, different epigenetic mechanisms are involved in reactivating the gene. Indeed, demethylation of its promoter is necessary, but only in the context of a permissive environment enable by chromatin acetylation.Because this understanding of gene mechanics is found with general inhibitors, we want to be specific in this reactivation. Thus, the main objective of this project is to develop an epigenetic tool for targeted reactivation of *SLFN11* in AGS cells.
We chose a lentivirus-based viral system, with the aim of adding an aptamer system to recruit an acetylating agent. Indeed, a lentiviral system allows the transduction of more genetic material and offers greater stability than conventional transfection methods.Different gRNAs are currently being tested, to cover different regions of the *SLFN11* promoter, in combinaison with various chromatin activators (acetylation). Based on these results, we will be able to select the best combination to generate the corresponding messenger RNA sequence and deliver it into gastric cancer tumoroid models.

Mots-Clés: DNA methylation, epigenetics, cancer, genome editing, tumor suppressor genes, SLFN11

*Intervenant

PCBIS : from research tools to drug candidates

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Résumé

The "Platform of Chemistry and Integrative Biology of Strasbourg" (PCBIS) is a high-throughput screening platform created in 1997.
By making specific and innovative technologies available, PCBIS gives researchers and engineers in both the academic and private sectors access to tools for discovering bioactive molecules, the precursors of future medicines based on advances in our knowledge of living organisms.

The platform has implemented a quality management system that enabled it to obtain ISO 9001 international certification and NF X50-900 certification.

PCBIS has a regional, national and European remit and is a member of the ChemBioFrance national research infrastructure.
The platform comprises several departments : chemical libraries, compound analysis (PACSI), target libraries, membrane and soluble protein production (IMPRESS), assay development and screening platform, ADME.

Mots-Clés: PCBIS, platform, screening

*Intervenant

Rational design of cyanine-based fluorogenic dimers for background-free imaging of GPCRs in living cells

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Résumé

Fluorogenic probes are optical imaging tools which can swap their fluorescence properties in response to a change in their microenvironment They are able to switch on their fluorescence only after interacting with their targets, thus leading to high signal-to-noise ratio in wash-free conditions, making them powerful probes for live-cell bioimaging.(1) Among this variety of probes, we focused our interest on dimeric turn-on probes which remain in a non-fluorescent H-aggregated state in polar environment (extracellular media) but exhibit a strong fluorescence upon disruption of the aggregate in apolar environment (cell membranes or hydrophobic receptor pockets).
Our team previously reported the first near-infrared fluorogenic dimers derived from cyanine 5.5 dyes for the optical detection of GPCRs.(2) However, due to their hydrophobic character, these dimers are prone to form non-specific interactions with circulating proteins such as albumin and with the lipid bilayer of the cell membrane, which can generate a residual background noise, low contrast images and false-positive response. In the search for improved probes, we synthesized and studied the properties of a series of less hydrophobic cyanine 5 dimers. By modulating the chemical structure of the cyanine units and after evaluation of various parameters, we selected the novel asymmetric cyanine 5.25-based fluorogenic dimer able to form intramolecular H-aggregates and self-quenched in aqueous media. Moreover, this optimal probe enabled to significantly reduce the non-specific interactions with bovine serum albumin and lipid bilayers (membrane mimics) as compared to the first generation of cyanine 5.5 dimers.

To perform *in vitro* imaging studies, the optimized asymmetric fluorogenic dimer was grafted to carbetocin, an agonist of the oxytocin receptor, for the specific visualisation of this receptor at the cell surface under no-wash conditions. Herein, we report that the optimal cyanine 5.25 conjugate displays a significant improvement of the signal-to-noise ratio compared to the previous generation of dimeric cyanine 5.5 probes. It enables visualization of the oxytocin receptor without any washing step and without any fluorescent background even in the cell growth medium in presence of serum protein.
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Mots-Clés: fluorescent probes, fluorogenic dimers, peptides, GPCR, bioimaging

Remodeling the immune microenvironment with microRNAs to sensitize head and neck squamous cell carcinomas to immunotherapies

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Résumé

Head and Neck Squamous Cell Carcinoma (HNSCC) are highly heterogeneous cancers with a high relapse potential. Patients with locoregional and metastatic recurrence (LR/M) HNSCC have a poor prognosis, with a median survival not exceeding than 15 months. They are treated with PD-1 immunotherapy ; Pembrolizumab or Nivolumab. However, immunotherapy is only effective in 15-20% of patients. With this in mind, we now need 1- to find biomarkers capable of identifying patients who could benefit from this treatment, and 2- to develop strategies to potentiate the effects of immunotherapy by targeting the immune microenvironment. MicroRNAs (miRs) are small non-coding RNAs that would be good candidates. Our team recently showed that a disappearance of miR-30a-3p and miR-30e-3p in HNSCC was associated with poor prognosis and tumor relapses (Conrad et al., 2023). By targeting members of the TGF-β family, miRs were able to attenuate both survival and motility of HNSCC cells and tumoroids. Secretomes of miRs-transfected HNSCC cells were able to polarize and activate M1-type macrophages which exert stronger phagocytic activities toward tumor cells (Conrad et al., 2023). Going further, we report here that miRs are involved in the remodeling of HNSCC immune microenvironment. Their overexpression leads to the upregulation of PD-L1 and the secretion of proinflammatory and chemoattractive cytokines like CXCL10, CCL5 and CCL20 from HNSCC cells (Conrad et al., in preparation). This participate in the attraction and infiltration of macrophages and LTCD8+ into HNSCC spheroids which lead in immune cell-induced cytotoxicity. In conclusion, by restoring anti-tumour immunity, miR-30-3p could potentiate the effects of immune checkpoint inhibitors in HNSCC.

Mots-Clés: Head and Neck Cancers, microRNAs, immunomodulation, immunotherapies

*Intervenant

Synthesis of 2,3-benzoxazepin-4-one as an original CyclON through a double SN1 pathway

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Résumé

Benzodiazepines represents one of the most and highly explored class of seven membered heterocycles containing two rings nitrogen with a significant interest in pharmaceutical industry. These molecules are considered as a "privileged structures" in medicinal chemistry due to their ability to provide ligands for various receptors.1,2 In this project, we are focusing on the development of 2,3-benzoxazepin-4-one as a bioisoster of 2,3- benzodiazepin-4-one in terms of biological interactions (Donor and acceptor bonds), and this by replacing the imine bond of 2,3-benzodiazepin-4-one by a sp3 carbon-oxygen bond. This resulting scaffold is original and has not been previously described in the literature. It belongs the family of two rings (N-O) named CyclON. The 2,3-benzoxaepin-4-one CyclON is obtained in 4 steps via a double SN1 mechanism using 3-isochromanone as starting reagent.
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Mots-Clés: Benzoxazepinone, CyclON, heterocycle, Diversity, SN1

*Intervenant

Synthesis of lipid analogues carrying blue light sensitive photoremovable group and luminol derivative: toward application of anti-cancerous drug release on nanoparticle using CRET assisted photolysis.

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Résumé

Smart Drug Delivery Systems using internal sources of light are highly sought after. In this context, luminol and its derivatives appear as promising candidates: they emit light within 420 – 460 nm in oxidative environment, especially in presence of H2O2(1). This one happens to be overproduced in inflammatory tissues, such as cancer tissues(2). This report describes the advances on the synthesis of lipid analogs carrying respectively a blue light sensitive coumarin photoremovable protecting group (PPG) and a luminol derivative (L-012), that can further lead to nanoparticles exploiting L-012 chemiluminescence to selectively release an anticancer drug in cancer tissues through Chemiluminescence Resonance Energy Transfer (CRET) assisted photolysis.
Inside cancer cells, L-012 is oxidized in presence of overproduced hydrogen peroxide, resulting in emission of light at lmax = 460 nm. This energy is transferred on a nearby coumarin PPG-Drug system through CRET to finally release the drug. L-012' and PPG' represent respectively L-012 and PPG sub-products after photolysis.

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Mots-Clés: Smart Drug Delivery Systems, photolysis, chemoluminescence resonance energy transfer, multi, steps synthesis, nanoparticles, cancer therapy