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Abstract book

Posters

AN INTERDISCIPLINARY APPROACH TO OPTIMIZE APTAMER-MEDIATED SIRNA DELIVERY FOR LIVER CANCER CELLS

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Résumé

The tumor microenvironment significantly influences cancer development, progression, and therapeutic resistance. We are interested in targeting in a secreted molecule found to be highly overexpressed in a large proportion of hepatocellular carcinoma (HCC), suggesting it plays a critical role in tumor biology. Our project aims to inhibit its expression, using small interfering RNAs (siRNAs). They are a powerful molecule for gene silencing, but their clinical application is limited by challenges such as instability in biological fluids and poor cellular uptake. To overcome these barriers, this study focuses on developing a targeted delivery system using aptamer-based approaches, which offer high specificity and low immunogenicity. Aptamers are short, single-stranded DNA or RNA molecules that can fold into defined three-dimensional structures to bind specific cellular targets with high affinity. Aptamers targeting cell-surface receptors are also capable of cellular internalization, making them promising carriers for the delivery of therapeutic molecules like siRNAs. Our objective is to evaluate the potential of siRNA delivery thanks to aptamers that target a cell surface marker overexpressed in HCC, using two strategies:

- Aptamer–siRNA Conjugates (AsiCs) – Direct chemical linkage of siRNA to the aptamer
- Aptamer-Functionalized Nanoliposomes (AsiNLs) – Liposomes encapsulating siRNA and decorated with surface aptamers

Our preliminary data provide information on the stability of the aptamer and of the AsiC in different buffers and medium. To facilitate the delivery of siRNA, various prototypes of Neutral and cationic liposomes, decorated with aptamers have been formulated, exhibiting an average size of approximately 100 nm with variety in zeta range conferring to the composition. Currently, the project is progressing, the formulations of interest possess low toxicity

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and we are focusing on optimizing the delivery of AsiNL into the cells.

We next plan to optimize siRNA cytosolic delivery thanks to photochemical and pharmacological approaches. Hence, Liposomes emerges as a promising concomitant delivery system to enhance the internalization and bioavailability of siRNA guided by aptamer towards specific HCC cell-surface receptors.

Mots-Clés: Hepatocellular carcinoma, Aptamer, siRNA, Liposomes

Targeting antimicrobial compounds in boswellia resin described in a tuberculosis remedy from arab medieval pharmacopeia

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Résumé

Antimicrobial resistance (AMR) has emerged as a major public health threat in the 21st century, being associated with an estimated 4.95 million deaths in 2019. To address AMR, the World Health Organization has published a bacterial priority pathogens list, in which rifampicin resistant *Mycobacterium tuberculosis* is classified as 'critical' - the highest priority - due to the lack of effective treatments and the increasing emergence of resistance to existing antibiotics (1). In response to this growing threat, the discovery of new antimycobacterial drugs has become crucial.

Ethnopharmacognosy focuses on traditional medicines to identify bioactive molecules that have not yet been described. This traditional knowledge can provide valuable resources for current research (2). A consortium of scientists, including historians, microbiologists, chemists, and bioinformaticians, has developed a database from Arab Medieval Pharmacopoeia to search for potential active ingredients (3). This work led to the identification of a remedy used in the 9th Century to treat scrofula, a skin and mucous membrane infections caused by *M. tuberculosis*. The remedy consists of 8 plants and 2 metals, including resins of *Boswellia* genus, also called frankincense. Frankincense, which are rich in terpenoids compounds and known for its anti-inflammatory properties, has been described in Ibn Sina's Canon of Medicine to treat tuberculosis (4).

In this research, we studied *Boswellia* resins antibacterial activity and targeted potentially active molecules by bio-guided fractionation and Features Based-Molecular Networking. After fractionation of the frankincense ethyl acetate extract, two fractions showed antibacterial activity against *M. tuberculosis* H37Rv (MIC value $<$ 200 μg/mL) while three showed antibacterial activity against Gram positive *Staphylococcus aureus* HG001 (MIC value $<$ 100

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μg/mL), *Staphylococcus epidermis* ATCC14990 (MIC value $<$ 50 μg/mL) and *Bacillus subtilis* ATCC23857 (MIC value $<$ 20 μg/mL). To determine which molecular families are present in these fractions, a dereplicative approach based on HPLC-HRMS/MS data was performed, confirming the presence of sesqui and diterpenes. In addition, pharmacophoric deconvolution - an innovative differential analysis of 2D NMR spectra correlated with antibacterial activity - is currently underway to specifically identify the pharmacophores from these fractions and accelerate their isolation and their structure identification.

Mots-Clés: Boswellia, Antibacterial, Tuberculosis, Molecular Networking

Cellular Hypoxia Imaging with Phosphorescent Sensors

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Résumé

Brain tumors are currently the most frequently diagnosed type of cancer in children and young adults. These tumors are characterized by a hypoxic heart, where oxygen levels can go to below 0.5%, while normal brain oxygen levels (physioxia) are around 5% O2. There is an emerging need to develop 3D in vitro models to reproduce the full complexity of the initial tumor. In actual state of the art, the few studies using patient-derived tumoroids do not study the oxygen level heterogeneity which is a crucial point to reproduce as faithfully as possible a patient- derived model.
To validate the presence of hypoxic heart in our *in vitro*, 3D tumoroid models derived from brain tumor, we aimed to use oxygen sensitive nanosensors developed in our lab. To this end, tumoroids are cultured in a rigid matrix mixed with nanosensors, to detect oxygen levels by direct fluorescence microscopy imaging. For this purpose, we use two types of luminescent oxygen-sensitive nanosensors based on polymeric nanomaterials (nanorods and nanoparticles). They contain energy-donating fluorescent dye (cyanine or rhodamine) combined with O2-sensitive acceptors phosphorescent dye (platinum porphyrin), to obtain two colors ratiometric luminescent systems involving a Förster resonance energy transfer (FRET) to amplify the oxygen sensitivity while minimizing the impact on oxygen levels. Nanoparticles are formulated by nanoprecipitation and nanorods are obtained by sonicating nanofibers produced by electrospinning.

First, we optimized the formulation of the nanoparticles by variying the loading in donor and acceptor. This led us to determine an optimal acceptor/donor ratio of 0.01 for two pairs of dyes, with dye loading up to 50wt% with respect to the polymer. Based on these results, we produced the corresponding nanorods with the same compositions. The resulting nanoparticles are, ranging in diameter from 30 to 50 nm, and nanorods obtained have a typical of 400-600 nm and a length around 5 μm. Then, we introduced our nanosensors in

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the matrix. Imaging of different tumoroid showed that the nanoparticles localized inside the tumoroid and penetrated the cells. However, nanorods, which have a larger structure than nanoparticles at around 100 nm, do not tend to penetrate tumoroids, but accumulate all around them, regardless of tumoroid size. Moreover, nanomaterials do not inhibit cell proliferation, enabling them to be validated as biological tools for use in living models.
Nanoparticles compared to nanorods seem more promising for studying hypoxia in these 3D models. Varying the amount of oxygen in the cultures is necessary to determine if it is possible to recreate an oxygen gradient in these in vitro brain tumor models. Further observations are planned, notably by testing different percentages of matrix to vary its rigidity.

Mots-Clés: Tumoroid, Hypoxia, Phosphorescence, Nanomaterials, FRET

An Easy and Efficient Method to Detect Concatemers in rAAV-Mediated Knock-In Projects Using Stuffer DNA

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Résumé

Introduction:
Genome editing with the CRISPR/Cas9 system is a powerful tool for creating animal models. However, achieving precise knock-ins often faces inefficiencies, particularly due to concatemerization of donor DNA during integration. Recombinant adeno-associated viruses (rAAV) have emerged as a promising solution for efficient donor DNA delivery, offering improvements in knock-in workflows.

Aims:
This study aimed to optimize an rAAV-based knock-in protocol for rodents and establish a robust method to detect and characterize concatemers using PCR targeting "stuffer" DNA sequences.

Methods:
We developed 10 new animal lines (4 rat and 6 mouse) using an optimized protocol inspired by Mizuno et al. (2018). Zygotes were electroporated with CRISPR/Cas9 ribonucleoproteins and incubated for 5-6 hours in an rAAV donor DNA preparation. Embryos were then washed and re-implanted into foster females on the same day. To detect concatemers, we included a "stuffer" sequence -non-essential 'junk' DNA- at one end of the donor DNA, positioned between ITRs. Detection of this sequence via PCR indicates that homologous recombination did not occur correctly, suggesting the presence of concatemes at the target locus. Comprehensive allele characterization was performed using 5' and 3' PCR, Sanger sequencing, and droplet digital PCR copy counting (ddPCR).

Results:

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At least two positive founders per project were obtained with fewer than 80 embryos, demonstrating high efficiency. The inclusion of stuffer DNA enabled effective detection of concatemerized donor DNA through PCR. In one example, a 3764 bp knock-in in Sprague Dawley rats achieved 100% knock-in efficiency, with concatemerization easily identified using this approach. Other examples will be shown to highlight the success and potential of this screening approach.

Conclusions:

The use of stuffer DNA in rAAV constructs simplifies concatemer detection, improving the reliability of knock-in generation. This optimized workflow aligns with the 3Rs principles by reducing animal use and refining experimental methods.

Mots-Clés: targeted transgenesis, AAV, CRISPR/Cas9, mouse, rat, concatemers

Development of innovate flow cytometry methodologies applied to the field of microbiology

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Résumé

Introduction and objectives:
Flow cytometry has evolved significantly over the past two decades. The miniaturization of optical systems (lasers, optical benches), fluidics (capillaries) and a sample loader (with High-Throughput Screening, HTS) have made this technology increasingly accessible to a broad range of users. What once required a dedicated room with complex pumping systems and specific currents is now condensed into simple, compact benchtop cytometers.

The increase in the number and power of lasers, now commonly found in most cytometers on the market, has significantly multiplied the number of detection parameters while greatly enhancing 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.

In this context, our eBioCyt UPS1401 platform, a "Unité Propre de Service" of the University of Strasbourg, has been developing fluorescence detection assays linked to different bacteria strains (Gram positive & negative, cocci or bacilli) since 2013 (1).

More recently, we have contributed to the development of measurements of the differential binding of fluorescent probes to several bacterial strains, as well as measurements of the effect of naturally occurring substances on bacterial toxicity.

Materials and Methods:

Bacteria were incubated with various innovative probes, combining Nile Red with different sites of the antimicrobial peptide Ubi29-41. Fluorescence intensity measurements were carried out using flow cytometry. Additionally, bacteria were incubated for 24 hours with different compounds, followed by staining with propidium iodide. Samples were analyzed using capillary flow cytometry (Guava EasyCyte Plus, Merck Millipore).

Results, discussion and conclusion:

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This work illustrates various applications, such as measuring the differential binding of fluorescent probes depending on the bacterial strain. Indeed, the synthesized probes showed varying levels of fluorescence labelling depending on the bacteria. The peptide probe UNR-1, incorporating the fluorophore as a minimalist non-natural amino acid (Alared), demonstrated the highest efficiency (2; 3).

This type of study could provide valuable alternatives to conventional bacterial infection diagnostic tests, being highly efficient and compatible with HTS methodologies. The research also demonstrated the possibility of generating dose-response curves for various molecules with bacteriotoxic activity. The development of advanced software has enabled rapid and highly accessible analyses for a wide community of scientific users.

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

Characterization of clonal heterogeneity in Chronic Lymphocytic Leukemia

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Résumé

Chronic lymphocytic leukemia (CLL) is the most common adult leukemia in adult and results from the abnormal proliferation of mature B cells that accumulate in the blood, bone marrow and secondary lymphoid organs.
Recently, **next-generation sequencing (NGS)** has shown that some patients exhibit **clonal heterogeneity** when the **VDJ genes** encoding the variable region of the B-cell receptor are analysed. This heterogeneity is characterised by the presence of major and minor clones in the global leukemic mass but not considered in treatment, which targets only major clones, leaving a selective advantage to minor clones, which may be more aggressive and lethal to patients.
The aim of our study is to better characterise the clonal heterogeneity in the global CLL leukemic mass, by using a new **bio-informatics** approach to obtain the full-length VDJ sequence in order to analyse the clone's abundance and the mutational profile.

Mots-Clés: CLL, Heterogeneity, Immune repertoire, Bioinformatics, VDJ sequences

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Optimization of acetylcholinesterase immobilization monitored with a rapid and specific colorimetric technique

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Résumé

The goal of this work was to optimize the conditions for acetylcholinesterase immobilization on different stationary phases and to monitor the immobilization reaction using a rapid technique. This procedure will enable the construction of an immobilized enzyme reactor for use in enzyme inhibitor screening. For this study, amino silica (NUCLEODUR 100 - 5μm NH2) and a monolithic disk (CIM, 7.9mm x 2.1mm, aldehyde-coated) were used. The enzyme immobilization was carried out *in batch* and the reaction was monitored by evaluating the activity of the residual acetylcholinesterase in solution using Ellman's assay. The influence of the enzyme-to-stationary phase ratio was tested. It was shown that the grafting reaction occurred rapidly on both types of media. It was also demonstrated that a greater amount of acetylcholinesterase could be immobilized on the CIM disk before saturation

Mots-Clés: Acetylcholinesterase, Immobilization, immobilized enzyme reactor, inhibitor screening

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Polyethyleneimine-based carbon dots for siRNA delivery

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Résumé

Carbon dots (CDs) are spherical carbon nanoparticles (NPs) that exhibit advantageous properties, including small size ($<$ 10 nm), solubility and stability in aqueous media, and photobleaching-resistant intrinsic fluorescence. These properties make them attractive for bioimaging and drug delivery applications. Small interfering RNAs (siRNA) are double-stranded non-coding RNAs (21-24 bp) that can silence any gene in a specific manner by operating within the RNA interference pathway. These nucleic acids stand as promising therapeutic tools, but their clinical use is limited by several major obstacles. Due to plasma enzymes, renal clearance or macrophage phagocytosis, their presence in the bloodstream and tissues is only transient. Furthermore, their size and negative charge prevent their spontaneous passage across the plasma membrane. As well, once internalised in cell by endocytosis, siRNAs tend to remain sequestered in endosomal compartments, where they can be degraded before reaching the cytoplasm. Consequently, the use of vectors to protect siRNAs, facilitate their delivery into cells and promote their endosomal escape is a potentially fruitful avenue for siRNA therapies. In the present work, we investigated the siRNA delivery potential of a library of 7 CDs produced from citric acid and polyethyleneimine (PEI) of varying molecular weight under different pyrolysis conditions (pH, temperature, activation mode). We characterised the size, charge and fluorescence properties of the various CDs, assessed their capacity to complex siRNA, and investigated their transfection activity and safety in two cell lines expressing the green fluorescent protein (GFP) and/or luciferase (Luc). All the CDs were able to complex siRNA. Two of them, made from PEI 600 or PEI 1200, demonstrated a potent silencing activity ($>$ 70%) with a low toxicity (viability $>$ 80%), as assessed by measurements of the luciferase activity (bioluminescence) in the U87 GFP/Luc and A549 Luc cell lines, and the cell viability (MTT assay). The silencing activity was confirmed by showing a decrease in GFP fluorescence in U87 GFP/Luc cells, by confocal microscopy. To further improve the transfection efficiency of these CD, the next step will be to functionalise them with a photosensitiser (PS), capable of producing singlet oxygen under light irradiation. Light irradiation of complexes made of PS-doped CDs will promote decomplexation of the siRNA payload and its release into the cytosol, by cleavage of a oxygen-sensitive linker, releasing the cationic charges on the NPs, and by destabilization the endo/lysosomal membrane, respectively.

Mots-Clés: Carbon Dot, Nanoparticle, Vector, siRNA delivery, Gene silencing

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Polythiophene based Carbon Dots for siRNA delivery and PDT: Application to Atopic Dermatitis

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Résumé

This research project aims to synthesize photosensitive "Carbon Dots" (CDs) for the delivery of therapeutic small-interfering RNA (siRNA) in the treatment of Atopic Dermatitis (AD). The objective is to provide a non-invasive alternative to currently available treatments, which are often costly, require frequent dose administration, and are commonly associated with undesirable side-effect.(1)

The proposed strategy involves delivering siRNA into the cells to regulate the immune response. However, using siRNA poses certain challenges: it struggles to enter cells and is rapidly degraded by the nucleases. To overcome these limitations, siRNA can be delivered using nanoparticles.(2) The use of nanoparticles allows to both protect the siRNA from the nuclease and facilitate cellular uptake. For this research project, it was decided to work on carbon-based nanoparticles called "Carbon Dots" (CDs), specifically on **polythiophene based CDs**. These kinds of nanoparticles offer several advantages, including high stability, biocompatibility, low toxicity and their good solubility in aqueous solution.(3) Additionally, they often exhibit very interesting photoluminescence properties.

The CDs used in this project are synthesized via a monowave pyrolysis of cationic polythiophene polymers. The polymers, obtained from aromatic building blocks, contain both ammonium functional groups and amine functional groups, allowing further post-functionalization. The pyrolysis is carried out in an acidic aqueous solution. Both the polymers and the resulting CDs are characterized using Dynamic Light Scattering (DLS) to determine their size and their zeta potential (charge). The aim is to obtain small (below 100 nm) and positive particles (around 20 mV). The photophysical properties of the particles are also analyzed, including absorbance, emission and excitation spectra, as well as their fluorescence quantum yield and their singlet oxygen quantum yield. The particles should be able to produce singlet oxygen, as similarly structured CDs are already used in Photo-Dynamic Therapy (PDT) applications.(4) Singlet oxygen production is crucial, as it enables the siRNA-CD complex to escape endosomal vesicles upon light irradiation, mimicking the PDT mechanism. Additionally, a possible functionalization of the amine function on the polymer/CDs is the addition of singlet oxygen sensible cationic linker. These linkers are inspired by the reactivity of β-amino-acrylate with singlet oxygen.(5) Their presence is a key feature, as they facilitate siRNA release upon singlet oxygen generation, thereby increasing cytosolic siRNA concentration.

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Mots-Clés: Carbon Dots : Polythiophene : Atopic Dermatitis : siRNA : Photodynamic therapy

Magnesium catalyzed (3+2) cycloaddition of donor-acceptor cyclopropanes and (N,S)-heterodienes to access functionalized thiolanes

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Résumé

Heterocycles are widely present in both therapeutics and natural products, representing more than 85% of bioactive compounds.(1) Even though nitrogen is still the most common heteroatom, other heteroatoms-especially sulfur-are increasingly gaining prominence. Thanks to its rich chemistry and valuable biophysical properties, such as enhanced bioavailability and lipophilicity, sulfur is becoming more prevalent in modern drug design.(2) Nowadays, sulfur can be found in diverse cyclic or acyclic structures and with various oxidation states. Saturated cyclic scaffolds, such as thiolane, are present in natural bioactive compounds (or their synthetic analogs) such as biotin, tetronothiodin, Nuphar sesquiterpene thioalkaloids, or salacinol.(3) However, the rapid and efficient synthesis of such functionalized compounds remains a challenging task, and the development of new synthetic routes is necessary.

Here, we report the synthesis of a large variety of highly functionalized thiolanes **4** (up to 84 % yield) through a (3+2) cycloaddition involving donor–acceptor cyclopropanes (DACs) **2** and (*N,S*)-heterodienes **3.** DACs **2** were generated through a Rh(II)-catalyzed cyclopropanation of a diazo compound **1** with an alkene, while (*N,S*)-heterodienes **3** were obtained after condensation of an acetal derived from dimethylformamide on a thioamide. DACs are known to be more reactive than classical cyclopropane and are activated in our case by a magnesium-catalyst, to act as 1,3-dipoles. After optimization of the reaction conditions, the reaction scope was extended to various DACs and heterodienes, and we successfully synthesized a family of 13 polysubstituted thiolanes with good diastereoselectivity (ranging from 80/20 to 100/0). The chemical stability and reactivity of the amidine moiety is still ongoing.

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Mots-Clés: Thiolanes, (3+2), cycloaddition, Diazo compounds, Magnesium, Scope

*Intervenant

Enhanced drug delivery via antibody–drug conjugates and siRNA-linked nucleic-acid aptamers targeting EGFR in glioblastoma cells

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Résumé

Active targeting in drug delivery is based on the binding of ligands to receptors present on the surface of targeted cells in order to promote the internalization of ligand conjugated drugs. The most well-known conjugates are antibody-drug conjugates (ADCs), which combine the specificity of monoclonal antibodies with the cytotoxic of chemotherapeutic drugs. In addition to antibodies, nucleic acid aptamers, known as single stranded DNA or RNA oligonucleotides referring to chemical antibodies with high affinity and selectivity for their target (*Zhou et al, 2016*), are promising to deliver conjugated drugs or therapeutics nucleic acids such as siRNA by active targeting delivery (*Mercier et al, 2017*).
In our team, we are interested in developing an aptamer-siRNA chimera called AsiC targeting EGFR (epidermal growth factor receptor), a receptor, internalized by endocytosis (*Ivaska et al, 2011*) and often overexpressed in glioblastoma cells, the most aggressive tumour of the central nervous system. Our AsiC consists of a targeting part: a 2' fluoro modified RNA aptamer E07 targeting EGFR (Cruz da Silva, Foppolo et al, 2022) and a therapeutic part: a siRNA for gene silencing. The therapeutic efficacy of such conjugates not only depends on target specificity but also on efficient internalization into tumor cells. However, so far, no therapeutic approach to enhance endocytosis of conjugates is available.

In recent studies, we showed that gefitinib, a tyrosine kinase inhibitor directed against the EGFR, induces a massive, non-physiological endocytosis of EGFR, known as gefitinib-mediated endocytosis (GME), in different glioblastoma cell lines (*Blandin et al, 2021 ; Cruz Da Silva et al, 2021).* We thus hypothesized that besides promoting endocytosis of EGFR,

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gefitinib could also promote endocytosis of its ligands. In this study, we proved by quantitative fluorescence bioimaging, that gefitinib is indeed able to strengthen the endocytosis of fluorophore-conjugated EGFR-specific antibodies and aptamers. We also showed that the GME potentiates the toxicity of an ADC and the efficacy of an AsiC. Our results suggest the development of a new therapeutic combination with gefitinib, to potentiate the delivery of ADC, AsiC and likely other conjugates targeting EGFR in glioblastoma, while limiting side effects on non-targeted cells. Our results have been submitted for publication and are already available online as a preprint (https://www.biorxiv.org/content/10.1101/2024.10.22.617611v1).

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Mots-Clés: antibody, drug conjugates, bioimaging, epidermal growth factor, gefitinib, glioblastoma, endocytosis, nucleic acid aptamers

Common medicinal plants with topical use : a metabolomic and proteomic approach targeting cutaneous microbiota

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Résumé

The skin microbiota is essential for skin health. Some bacteria, like Staphylococcus epidermidis or Staphylococcus hominis, help maintain the cutaneous barrier and reduce inflammation after skin wound (1). Dysbiosis can lead to dermatological inflammatory conditions, like dermatitis. That shows the importance of maintaining the homeostasis (2). The effect of dermal products prepared with medicinal plants on microbiota is not fully investigated and do not cover growth, metabolomic and proteomic aspects of skin microbiota after topical application. To gain a comprehensive understanding, special attention will be given to how plant molecules metabolized by the microbiota interact with the skin and other bacteria (3). In this project, we screen the effects of twenty traditional medicinal plants all already sourced such as Aloe vera, Arnica montana, Calendula officinalis, or Hamamelis virginiana. First, on skin bacteria and human healthy keratinocyte lines viability assays, then on more complex in vitro models of skin microbiota (4). Alongside we perform extractions and chemical characterization of extracts using dereplication tools and molecular networking. Bioguided screening is performed on bacterial viability and growth by broth microdilution assay to determine MIC (Minimum Inhibitory Concentration) values and to target right fraction to purify allowing to find specific molecules responsible of bioactivity. Stress levels will also be measured by an innovative fluorescent solvatochromic probe method that can help us monitor changes in the bacterial cell envelope in response to stress (5) at sub-MIC concentrations. Proteomic analysis of bacterial metabolism will be used to detect changes between treated and untreated conditions, as bacterial metabolites can also affect the skin in response to external compounds.
This work could help create safer, more effective skin treatments and cosmetic products that respect both the skin and its natural microbial balance.

Mots-Clés: Cutaneous microbiota, homeostasis, traditional plants, dereplication, molecular networking, metabolomic, proteomic

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Aptamer mediated selective and modulable siRNA delivery

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Résumé

Active targeting delivery, the combination of a biomolecule with a targeting ligand (Paunovska et al., 2022), is a promising strategy to deliver different kinds of drugs, in particular small interfering RNAs (siRNAs). Indeed, one major issue about siRNAs is their poor cell penetration when they are unassisted due to their negative charge, their size and their hydrophilicity (Alshaer et al., 2021). Therefore, conjugating this biomolecule with a targeting ligand would make it possible to overcome these barriers. Aptamers appear to be interesting candidates since they are single stranded DNA or RNA with a high affinity and selectivity to a specific target (Zhou et al., 2016), that can be a cell-surface receptor (Cruz Da Silva et al., 2022; Fechter et al., 2019; Mercier et al., 2017). In this study, we aim to develop selective and modulable structures associating a siRNA with one or more aptamers: an Aptamer-siRNA chimera (AsiC) and a nanotrain, to improve siRNA delivery.
First, we would like to create an AsiC combining one RNA or DNA aptamer with a siRNA. In our previous studies, we used an AsiC composed of a siRNA and an RNA aptamer, linked thanks to an RNA/RNA sticky bridge. In this study, we compared the hybridization of this AsiC with a novel one composed of the same siRNA, and the same RNA aptamer, but extended with a DNA sequence complementary to that of the siRNA to form an AsiC with an RNA/DNA sticky bridge. Our results show that the RNA/DNA binding is as efficient as the RNA/RNA sticky bridge. Second, we designed an innovative multifunctional and modulable nanotrain, inspired by the works of Cao and his team (Cao et al., 2023). Our nanotrain will be composed of one siRNA (the locomotive, also called the 'therapeutic part'), associated with one to three homo- or hetero-valent aptamers (the boxcars, also called the 'targeting parts'). The various elements will have hybridization sequences that will enable controlled self-assembly. Thanks to their versatile nature, the number, position and type of aptamers (DNA or RNA) could be easily changed. So far, we have designed RNA boxcar sequences and predicted their secondary structures using prediction softwares.

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Our preliminary results of RNA/RNA and RNA/DNA AsiC are encouraging and confirm the feasibility to combine DNA et RNA aptamers in nanotrains, new siRNA active delivery tools with great potential thanks to their selectivity and versatility. As perspectives, we wish (1) to deepen the characterization of RNA/DNA AsiC (stability and functional cell assays), and (2) characterize their intracellular traffic by bioimaging thanks to environment-sensitive fluorescent probes developed by our collaborators.

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Mots-Clés: siRNA, Aptamer, Aptamer, siRNA chimeras, Nanotrain, Active delivery strategy

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

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Résumé

Fluorogenic dimers with polarity-sensitive folding are powerful imaging probes able to switch on their fluorescence only after interacting with their targets, making them promising tools for live cell imaging.(1) Our team previously reported the first near-infrared fluorogenic dimers derived from cyanine 5.5 dyes for the optical detection of G protein-coupled receptors.(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, resulting in residual background fluorescence in complex biological media.
In the search for improved probes, we synthesized and studied 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. 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.

The optimized asymmetric fluorogenic dimer was grafted to carbetocin, an agonist of the oxytocin receptor, for the imaging 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

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Targeted Release of Drugs using Photolabile Groups and Photoactivatable Nanoparticles : Application to Neovascular Diseases of the Retina

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Résumé

Neovascular diseases of the retina represent a leading cause of blindness. They are characterized by an extensive proliferation of new permeable blood vessels, also called angiogenesis. The leakage of the permeable blood vessels can cause inflammation and at term affects the vision. Angiogenesis is the final common pathway of many retinal diseases such as age-related macular degeneration (AMD) and diabetic retinopathy (1),(2). Current therapies for the treatment of retinal pathologies are mainly based on strategies able to block the progress of neovascularization by inhibiting VEGF (vascular endothelial growth factor), a key player in pathological neovascularization (2). However, VEGF also has neuroprotective and neurotrophic effects on the retina (3). Consequently, the VEGF inhibition needs to be carefully balanced and targeted to the pathological regions.
Therefore, we want to introduce a new strategy based on a light activated version of VEGF inhibitor. The main advantage of light triggered therapy should be to precisely control a drug's activity, to minimize side effects and to increase the therapeutic efficiency. Unfortunately, light-based therapies for eye treatment are already described (2),(4) but are often invasive or not specific to the targeted cells, inducing undesirable side effects.

We propose the development of a novel therapeutic strategy for pathological neovascularization, characterized by superior spatial resolution. The idea is to administer an inactive, but light-activatable NPs into the bloodstream and subsequent photoactivation using light will liberate the VEGF inhibitor and potentially restrict anti-angiogenesis treatment to pathological regions.

In this project, we aim to develop light-activatable liposomes based on the concept of light induced permeabilization (5). The idea is to work with coumarin photolabile protecting group (PPG) to cage different lipid analogs. Two distinct strategies will be developed in parallel. In the first one, a blue sensitive coumarin derivative is going to be coupled with dioleoylphosphatidylethanolamine (DOPE), a phospholipid known to induce liposome destabilization (6). After liposomal formulation of the caged phospholipid analog and irradiation, it should lead to a liposomal permeation. The second strategy is a new concept, where a blue sensitive coumarin will be coupled to different fatty acids. Upon light irradiation, the

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resulting amphiphilic byproduct is expected to destabilize the liposomal membrane, leading to a permeation. To enhance the versatility of our lipid mimics, we propose to use a copper-catalyzed azide–alkyne cycloaddition (CuAAC) reaction to couple diverse hydrophilic moieties.

At term, the most promising candidate of this project should lead to a general application of light induced VEGFR inhibitor, allowing the incorporation of various drug types during liposome formulation.

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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

*Intervenant

From Invader to Solution: Exploring Himalayan Balsam as a Source of New Bioherbicides

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Résumé

According to data from the Food and Agriculture Organization of the United Nations, the total pesticide use in agriculture was estimated at 3.7 million tonnes of active ingredients in 2022, double the amount used in 1990. Numerous studies have demonstrated a link between excessive pesticide use and environmental degradation, resource depletion and adverse effects on Human health. This escalating concern has drawn the attention of governments worldwide.
In response, European countries are actively implementing new measures and best practices to reduce pesticide use by 50% by 2030, in line with the goals of the European Green Deal. Within this context, our research aims to contribute to this objective by developing environmentally friendly alternatives, such as bioherbicides. These biocontrol products, composed of natural components, are less likely to cause environmental damage than conventional pesticides, due to their natural occurrence.

This work is part of an ongoing research project focused on invasive alien plant species abundantly present in the Grand Est region of France, with the goal of exploring their phytochemical diversity for potential phytotoxic effects. A preliminary screening on *Lepidium sativum* L. seeds highlighted high anti-germinative activity of crude extracts from *Impatiens glandulifera* Royle's leaves. The results presented here show the implementation of an innovative, in-house developed method combining HPLC-microfractionation with anti-germinative testing in 96-well microplates, as part of a bioguided fractionation approach to further investigate the ethanolic leaf extract.
Among the obtained microfractions, two exhibited an outstanding activity. Phytochemical analysis using HPLC-UV-HRMS suggests the presence of naturally occuring naphthoquinone glycosides in these fractions. Method optimization is currently underway to purify these naturally occurring compounds in sufficient quantities for further experiments.

Mots-Clés: Impatiens glandulifera Royle, phytotoxicity, biohercides, bioguided microfractionation, invasive plants

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BIN1 gene replacement reverts BIN1-related centronuclear myopathy

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Résumé

Centronuclear myopathies (CNM) are severe genetic disorders characterized by generalized muscle weakness associated with organelle mispositioning in myofibers. Most CNM cases are caused by mutations in proteins involved in membrane remodeling, including amphiphysin 2 (BIN1). There is no treatment and the pathological mechanisms are not understood. Here, we aimed to cure the *Bin1*-CNM mouse model (*Bin1*mck-/-) via an adeno-associated virus (AAV)-based gene replacement strategy. Early systemic exogenous BIN1 expression efficiently prevented disease progression. Moreover, BIN1 expression after disease onset reverted all disease signs four weeks after treatment, including motor defects, muscle weakness, muscle and myofibers hypotrophy, kyphosis, nuclei and mitochondria misposition, and altered T-tubules network. We then validated the most efficient construct combining a myotropic AAV serotype with the muscle BIN1 isoform. The rescue correlated with normalization of autophagy and excitation-contraction coupling markers. Cellular and in vivo investigations revealed that different BIN1 natural isoforms shared similar beneficial effects. Artificial constructs coding for separated protein domains rescued different CNM hallmarks. Only the muscle-specific BIN1 isoform combined the different cellular functions of BIN1 on membrane tubulation and dynamin (DNM2) regulation necessary for a full rescue. Overall, this study validates BIN1 gene replacement as a promising strategy to cure BIN1-related centronuclear myopathy.

Mots-Clés: congenital myopathy, myotubular myopathy, amphiphysin, BIN1, BAR domain, endocytosis, membrane remodeling, gene therapy, adeno, associated virus

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Revealing Altered T-Tubule Networks in Mtm1 Knockout Mice at 7 weeks through 3D myofibers Ultrastructural Imaging

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Résumé

Centronuclear myopathies (CNMs) are congenital disorders characterised by muscle weakness and the abnormal centralisation of nuclei within muscle fibers. The main genes associated with CNM include MTM1, DNM2, BIN1, RYR1, and SPEG. To date, no effective treatment is available. Despite extensive research, a limited understanding of the ultrastructural organisation of myofibers hampers our comprehension of CNM pathogenesis and the identification of potential therapeutic targets.
To overcome this limitation, we employed Focused Ion Beam Scanning Electron Microscopy (FIB-SEM) imaging of skeletal muscle tissue from wild-type (WT) and Mtm1 knockout (KO) mice at different developmental stages (2 and 7 weeks). This approach provided high-resolution images, and we created a pipeline to segment mitochondria, transverse tubules and Z-disks, enabling the reconstruction of 3D skeletal muscle models.

The 3D reconstruction revealed a well-organised architecture of WT myofibers with regular sarcomeric alignments and an even distribution and shape of T-tubules and mitochondria, whereas for the Mtm1 KO mice, mitochondria and Z-disks seem normal, but T-tubules networks seems misoriented and more ramified. Furthermore, at 7 weeks of age, Mtm1 KO muscle displayed misoriented and ramified T-tubules, but also we noticed a variation in the Z-disks' orientation and sacomeres delimitations. No major structural anomalies in mitochondria were visible, but further investigations are currently ongoing. To quantify the structural aberrations, we will next perform computational morphometry, and we plan to cross our findings with transcriptomic and proteomic data obtained at the same ages.
Overall, FIB-SEM analyses on Mtm1 KO muscle samples revealed the progressive appearance of architectural anomalies of the myofibers and especially of T-tubules, taking a central role in excitation-contraction coupling (ECC). Concomitantly, Mtm1 KO mice are normal at 2 weeks and exhibit severe muscle weakness at 7 weeks. T-tubule misalignment occurs as the first detectable anomaly, suggesting that it may serve as an early disease marker and potentially as principal therapeutic target.

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Mots-Clés: Centronuclear Myopathies, Myotubularin 1, Ultrastructural Imaging, Image Processing, Skeletal muscle, Quantification, 3D Reconstruction.

Machine-learning algorithm applied to magnetic localization

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Résumé

The application of machine learning algorithms is explored to enhance magnetic localization systems, particularly for foot shape measurement. Traditional methods, such as plaster casting are long and prone to error (1). On the other hand, optical 3D scanners, have limitations, especially for patients with significant foot deformations due to occlusions and manual corrections required by orthotists. Magnetic localization offers a promising alternative as it does not require a line of sight (2), making it suitable for various applications, including indoor navigation (3), surgical tracking (4), and motion capture (5).
The primary objective of this project was to assess the performance of machine learning algorithms, specifically Random Forest (RF) and K-Nearest Neighbors (KNN), in improving the accuracy of magnetic localization. Machine learning models can handle non-linearity and noise (6-8).

The experimental setup involved a magnetic field generator with four planar coils and a magnetic field camera featuring 64 magneto-resistive sensors (9). Systematic scanning with the camera produced 4992 data points, each associated with the magnetic field vector at specific locations in a $40\times40\times40$ cm^3 volume. The camera's position was controlled with high precision using rulers and Lego bricks, ensuring accurate measurements.

In addition to experimental data, simulated data were generated using the Python Radia library, which relies on the Biot-Savart Law (10). This simulation produced 400,221 data points, that were divided into training (80%) and testing (20%) datasets, with the experimental data serving as an additional test set to evaluate the algorithms' performance. RandomForestRegressor and KNeighborsRegressor modules from scikit-learn (v1.5.1) library (11) were implemented and trained with the simulation train dataset

Random Forest outperformed K-Nearest Neighbors in terms of accuracy. RF's ensemble approach effectively captured nonlinear relationships, resulting in a mean absolute error

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(MAE) of 0.71 mm on simulated data, compared to 1.48 mm for KNN. However, when tested on experimental data, RF's MAE increased to 1.16 mm, indicating a discrepancy between simulated and experimental datasets.

The spatial distribution of errors showed that most errors occurred at the edges and corners of the volume of interest. This is due to the limited number of neighbors available for averaging in these regions. Training in a larger simulation volume reduced edge-related errors in the prediction zone. This adjustment improved the overall accuracy of the localization system.

Despite these improvements, the precision of the algorithms was limited by the discrepancy between simulated and real magnetic field strengths. This discrepancy was particularly noticeable near the coils, where the magnetic field gradient is steep, leading to higher errors in distance calculations.

While Random Forest showed promise in enhancing magnetic localization accuracy, our study highlighted the need for further research to bridge the gap between simulated and experimental data. Future work could involve increasing the number of experimental measurements or refining simulation models to better match real-world conditions These findings support advances in medical and industrial magnetic localization. By addressing the challenges identified in this study, we can further advance the precision and reliability of magnetic localization technologies.

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Mots-Clés: magnetic localization, random forest, gradient boost, machine learning

Synthetic lipopeptides to fight against multiresistant fungal infections

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Résumé

As invasive fungal infections (IFI) are increasingly common in the nosocomial setting (> 1.6 million patients die annually of IFI(1)), the WHO published in 2022 a fungal priority pathogen list, counting as a first step to prioritize fungal pathogens and promote a research that until then was somehow left behind.
Echinocandins (ECs), as part of the more recent antifungals, are cyclic lipopeptides (LPs) which non-competitively inhibit the β-(1,3)-D-glucan synthase (GS)(2) (found exclusively in fungi), leading to the loss of the fungal cell wall integrity(3). Their specificity gives them the advantage of being well-tolerated, but despite their evident potential only 4 ECs are currently on the market and some examples of resistant strains have already been identified(4).

The aim of the project Fungipep is to identify new drug candidates inhibiting the GS with the objective of skirting emerging resistance encountered with currently available ECs. Another important aspect of this project is to elucidate the inhibition mechanisms used by these original lipopeptides as the recognition site of ECs on the FKS1 catalytic unit of GS has never been totally confirmed.

To reach these high goals, a first part of the project was focused on the development of a SPPS methodology (sometimes associated to click-like bioconjugate chemistry), to access unprecedented EC analogues. For now, around 40 original LPs were generated and all of them were tested on various strains of fungi, making it possible to quickly evaluate their bioactivity and adjust their structure accordingly.

The synthetic routes developed during this project were also exploited to synthesize innovative molecular tools like LPs carrying a fluorophore. These will later be used in fluorescence microscopy to try to confirm the binding site of ECs by FRET experiments (in association with a GS fusion protein that we have designed and are currently bioproducing).

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Mots-Clés: lipopeptides, invasive fungal infections, antifungals, β, (1, 3), D, glucan synthase

Targeted Exploration of Bioinspired Cascade Reactions: A One-Pot Total Synthesis of Nesteretal A

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Résumé

Nesteretal A, a representative of a novel class of cage-like metabolites, was isolated in 2019 by Yang *et al.* from the coral-derived actinomycete *Nesterenkonia halobia*.(1) This natural product exhibits an intriguing complex highly oxygenated structure including four intricated cycles and seven stereocenters, six of which are quaternary. The biosynthetic pathway proposed by the authors involving diacetyl as a plausible and unique precursor makes nesteretal A an interesting and challenging synthetic target for bioinspired total synthesis. From diacetyl, by a succession of self-aldolizations/hemiacetalizations catalyzed by (*S*)-proline mimicking an aldolase-type mechanism, we performed an expeditious one-pot total synthesis of nesteretal A.(2) In a context where atom, step, and redox economies are important, this single operation from costless diacetyl clearly competes with classical multistep total syntheses.(3, 4)

Versatility in the diacetyl auto-assembly prompted us to explore the "bioinspired metabolomes" generated "in the flask" using chemoinformatic tools such as MetWork.(5) This powerful *in silico* metabolization tool, based on a reaction toolbox and MS/MS spectra prediction, allowed us to illuminate the hypothetic biosynthetic pathway leading to nesteretal A, along with a wide chemical space including nesteretal A-like cage molecules. Among them, *iso*-nesteretal, a potentially not yet discovered natural product, was anticipated, targeted, and isolated.

This work falls within the scope of a trend where chemoinformatics and natural products chemistry are becoming closely linked(6) with an innovative and concrete application in total synthesis.

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Mots-Clés: total synthesis, natural products, chemoinformatics, cheminformatics, mass spectrometry, biosynthesis, biomimetic

New antimalarial flavones active against artemisinin-resistant strains of P. falciparum: a chemical-biology approach to target discovery

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Résumé

Malaria is one of the most predominant parasitic diseases in the world and especially in tropical and developing countries. Accordingly, to the last WHO report, malaria affects 263 million people annually, with 608 000 deaths primarily from *Plasmodium falciparum*. Climate changes and emergence of resistance to artemisinin, the most potent antimalarial drug used today, urge for the development of new drug candidates to treat malaria. Preventive treatment such as insecticides and vaccines (MosquirixTM) show still insufficient efficacy.
In a previous ethnopharmacological approach followed by pharmacomodulation, the laboratory identified a new class of antiplasmodial synthetic flavones. These molecules showed an unmet *in vitro* target candidate profile: *in vitro*activity on resistant *P. falciparum* strains, no cross resistance with reference drugs, rapidity of action on all stages of the intraerythrocytic cycle, inability to select resistant parasites, putative efficacy on hepatic stages. Physicochemical and pharmacokinetic parameters must be improved for an increased *in vivo* activity. Moreover, the molecular target(s) of these flavone-based compounds to date are unknown.

In this work we aim to apply a chemical-biology approach to answer these questions. We synthetize flavone-fluorophore conjugate as tools to identify the subcellular localization of the lead compound in the parasite and to pursue proteomic studies to identify the parasitic proteins with which flavones could interact. Three aspects have been explored: 1/the synthesis of the flavone moiety; 2/ the synthesis of appropriate linkers; 3/the coupling with a fluorophore by using Sonogashira cross-coupling reaction.

Then, incubation of the synthetized probe with *P. falciparum*-infected blood cells is envisioned as well as the application of confocal microscopy coupled with chemoproteomic studies to identify the molecular target.

Mots-Clés: Malaria, synthetic flavones, chemo, biology, flavone, fluorophore conjugates, metal, catalyzed cross, coupling reactions

*Intervenant

Measuring the membrane order of the inner leaflet in the HIV-1 Gag assembly in the plasma membrane

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Résumé

HIV-1 is one of the viruses responsible for AIDS (acquired immunodeficiency syndrome) and remains a major global public health threat, with approximately 40 million people infected. HIV-1 has a lipid envelope acquired during budding from the host plasma membrane (PM). Several lipidomics studies have reported that the specific set of lipids, such as sphingomyelin (SM), cholesterol (Chol), phosphatidylinositol-(4,5)-bisphosphate (PI(4,5)P2), and phosphatidylserine (PS), are enriched in virions. In the late phase of the viral replication cycle, viral Gag proteins are recruited to PI(4,5)P2 in the inner leaflet of the PM, forming multimeric assemblies with an inherent membrane curvature as a platform for virus assembly. In the PM, lipids are asymmetrically distributed between the outer and inner leaflet. SM is mainly found in the outer leaflet, whereas PI(4,5)P2 and PS are found in the inner leaflet. Saturated lipids, such as SM, together with Chol, tend to form tightly packed (liquid-ordered, Lo) domains called "lipid rafts", creating lateral heterogeneity in the outer leaflet. On the other hand, most inner leaflet lipids are (poly)unsaturated and form more loosely packed (liquid-disordered, Ld) domains.

A major question in HIV-1 assembly is how Gag proteins in the inner leaflet enrich outer leaflet lipids, such as SM, into virions without direct contact. Our recent results showed that Gag brings SM-rich and Chol-rich domains into close proximity in a multimerization- and membrane curvature-dependent manner(1). Our findings further raise the question of how Gag proteins communicate with outer leaflet lipids to enrich them. In vitro experiments have suggested that Gag prefers Ld domains for membrane binding. The Lo domain in one leaflet has also been shown to induce the formation of the Lo domain in the other leaflet, which is composed of Ld lipids. However, the physical properties (lipid order, polarity, and viscosity) around the Gag are largely unknown. Therefore, it is essential to determine the physical properties of the inner leaflet in Gag assemblies to address the issue of the lipid enrichment by Gag.

In this study, we tried to quantitatively measure membrane order in the inner leaflet of Gag assemblies using recently developed environment-sensitive dyes NR-Halo(2) and advanced microscopy.
We first established labeling conditions for the Halo-tagged Gag protein (Gag-Halo) with NR-Halo dyes in the cell. We then performed ratiometric imaging and fluorescence lifetime imaging microscopy (FLIM) of HeLa cells expressing wild-type Gag (Gag WT) and

*Intervenant

the multimerization-deficient Gag-WM mutant, in addition to control proteins that prefer liquid-ordered (Lo) and liquid-disordered (Ld) domains. Gag-Halo conjugated with NR12-Halo (Gag-Halo-NR12) reported that Gag WT showed a higher membrane order than Gag-WM and Ld domain control. In our previous study, Gag mutants defective in the membrane curvature formation (Gag-P99A and Gag-EE) were less effective in enrichment of lipid domains than Gag WT(1). When we measured membrane orders in the cells expressing these mutant assemblies, they showed higher membrane orders than Gag-WM and comparable to Gag WT. These results suggest that Gag multimerization increases membrane order in the inner leaflet of Gag assemblies.

Lipid nanoparticles as efficient therapy vectors for skeletal muscle pathologies

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Résumé

Research area: Translational research
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Rare muscle disorders, such as congenital myopathies, are life-threatening diseases for which no treatment is currently available. The most utilised gene therapy vectors for treating these genetic muscle disorders are adeno-associated viruses (AAVs). However, recombinant adeno-associated vectors (rAAV) face several limitations, including restricted transgene capacity (below 2.5 kb), immunogenic responses due to preexisting immunity, and particularly liver toxicity resulting from the accumulation of rAAV in the liver, which triggers a strong immune response. To date, gene therapy vectors that achieve high efficiency, muscle specificity, and safety have not yet been developed.

To overcome these challenges, we developed a novel lipid nanoparticle (LNP)-based delivery system specifically designed to target skeletal muscle. Since the success of the COVID-19 mRNA (messenger RNA) vaccine, LNPs hold substantial promise for expanding the landscape of mRNA-based therapy to gene therapy applications. LNPs have demonstrated a higher payload capacity, compatibility with repeated administration, and reduced immunogenicity compared to viral vectors. Moreover, we rationally designed LNPs to be muscle-specific by conjugating MyomP1, a peptide from the muscle-fusogenic protein Myomerger, onto the nanoparticle's surface. We encapsulated either pADN or mRNA encoding luciferase reporter genes. In vitro studies in murine C2C12 myoblasts, myotubes, and human myoblasts demonstrated that MyomP1-modified LNPs achieved a 10-fold increase in transduction efficiency compared to unmodified LNPs. In vivo, studies further demonstrated that MyomP1-functionalized LNPs significantly enhanced muscle transduction when delivering DNA cargo, whereas the same modification induced a liver-detargeting effect in mRNA delivery.

These findings highlight the versatility and safety of LNP-based gene delivery and suggest that MyomP1-engineered LNPs possess significant potential to enhance therapeutic outcomes for patients with rare muscle diseases, providing a promising alternative to traditional viral gene therapy platforms.

*Intervenant

Mots-Clés: gene therapy, lipid nanoparticle, peptide, muscle, nucleic acid delivery

Development of an Online Bioanalytical Device for Acetylcholinesterase Inhibitors Screening

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Résumé

Alzheimer's disease (AD) is a chronic and progressive brain disorder that is responsible for 75% of all cases of dementia. Numerous studies indicate that limiting the hydrolysis of acetylcholine (ACh) through acetylcholinesterase (AChE) inhibitors is an important way of treating Alzheimer's disease. However, the traditional methods for screening AChE inhibitors have the disadvantage including being time-consuming, expensive, labor-intensive and unstable. In this study, a device for Online AChE inhibition evaluation by high-temperature liquid chromatography-mass (HTLC) spectrometry hyphenated with an immobilized enzyme reactor (IMER) was developed. HTLC is a green analytical tool that can be applied to achieve rapid separation of compounds with fewer organic solvents. Firstly, 3 known AChE inhibitors (galanthamine, huperzine A, and tacrine) were selected. A separation method based on HTLC was established. AChE inhibitors were rapidly separated in 7.5 minutes with 10% EtOH solvent by utilizing a combination of gradients of temperature and flow rate. Then, an IMER was connected to HTLC. The AChE inhibitory activities were evaluated and compared based on the acetylcholine peak areas (mass signals) obtained after chromatographic separation and elution through the IMER. Finally, this bioanalytical device will be applied to natural plant extracts, which are known to contain AChE inhibitors. This device can be used to qualitatively compare the anticholinesterase activities of AChE inhibitors and may also aid in the discovery of new inhibitors.

Mots-Clés: Alzheimer's disease, High, temperature liquid chromatography, Acetylcholinesterase inhibitor, Green analytical chemistry

*Intervenant

Diversity-Oriented Transformation of Doyle-Kirmse Products in Flow

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Résumé

Diversity-Oriented Synthesis {1} (DOS) and cascade rearrangement reactions represent powerful strategies that, when combined, can significantly accelerate drug discovery by providing a rapid access to a large variety of complex compounds from simple precursors. The Doyle-Kirmse (2,3)-sigmatropic rearrangement represents a good example of such a strategy, yielding complex compounds with various points of diversification starting from readily available thioethers and diazo compounds.{2} However, despite the high interest for this reaction only few post-Doyle-Kirmse reactions have been developed, especially when an allene is obtained as the —2,3) rearrangement product.{3}

In this context, a modular and divergent approach toward thiophene-derived structures has been developed using flow chemistry involving cascade reactions starting from a unique precursor, obtained through a rhodium-catalysed rearrangement. The corresponding allenes were heated at high temperatures in *tert*-amyl alcohol to trigger multiple cascade sequences yielding three different benzothiophene derivatives, depending on the conditions used and substrate structures. Overall, this strategy allowed a swift and direct access to complex molecular structures containing *S*-heterocyclic scaffolds of interest in drug discovery.{4}

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Mots-Clés: Flow chemistry, Diazo compounds, Doyle, Kirmse reaction

A novel screening method for ALOX5 inhibitors based on TLC plate and enzymatic assay

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Résumé

In 2021, according to the "Assurance Maladie", almost 4 million French people were suffering from chronic inflammatory diseases, representing a major public health challenge(1). Arachidonate 5-lipoxygenase (ALOX5) plays a central role in the pathogenesis of several of these diseases, including asthma, rheumatoid arthritis and Crohn's disease. ALOX5 catalyses the production of leukotrienes, the pro-inflammatory lipid mediators that trigger the inflammatory response responsible for various pathologies. This involvement makes ALOX5 a prime therapeutic target for the development of new anti-inflammatory drugs. At present, Zileuton is the only drug approved to specifically inhibit this enzyme, and only in the United States(2). Available ALOX5 inhibitor screening assays rely mainly on expensive commercial enzyme kits using chromogenic or fluorogenic reactions, which are not suitable for complex plant extract screening.
In contrast, enzymatic assays coupling thin-layer chromatography (TLC) separation with *in situ* enzymatic reactions have been developed for the screening of enzyme inhibitors such as cyclooxygenase 2(3), tyrosinase and acetylcholinesterase, offering direct and rapid visualization of enzyme inhibition. TLC assays coupled with an *in situ* enzyme assay are also suitable for screening complex plant extracts(4).

Here, we present the development of a simple, rapid and cost-effective enzymatic assay on TLC plates for detecting ALOX5 inhibitors. The test is based on the oxidation of a colourless reagent during the enzyme conversion of arachidonic acid to leukotriene, generating an electrophilic intermediate that will complex with a second reagent to form a grey-coloured compound. The grey colouration indicates the enzyme activity, while zones of inhibition appear as white spots on the grey background. The method was developed and validated using three positive inhibitors: zileuton, nordihydroguaiaretic acid, and piperine, as well as two negative controls: caffeine and salicylic acid(5). This TLC-based enzymatic assay provides an efficient and accessible alternative for the preliminary screening of ALOX5 inhibitors, facilitating the discovery of new anti-inflammatory agents, particularly from complex plant extracts.

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Mots-Clés: Arachidonate 5, lipoxygenase, thin layer chromatography, in situ enzyme assays, inhibitor screening, complex plant extract.

Antimicrobial Potential of Fomitopsis pinicola: From Bioactivity Screening to Compound Identification

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Résumé

Antibiotic resistance is one of the most pressing global health threats, with an increasing number of pathogens becoming resistant to conventional treatments.It was reported that by 2050, about 10 million of deaths will be attributed to antibioresistance(1). Macromycetes are currently considered as valuable sources of diverse secondary metabolites including antimicrobial compounds (2)(3). This research work focuses on the macromycete Fomitopsis pinicola, a wood-rotting basidiomycete widely distributed across the Northern Hemisphere and known for its chemical diversity, such as lanostane-type triterpenoids(4). The aim of this study is to explore the antimicrobial potential of F. pinicola through bioautography-guided fractionation. Among the three extracts obtained from F. pinicola, ethyl acetate crude extract was the most promising and was tested against Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis, and Candida albicans using two complementary methods, TLC-bioautography method and the spot-on-lawn method. Preparative HPLC fractionation of the ethyl acetate crude extract, followed by antimicrobial activity screening led to several bioactive fractions. The active fractions are currently under investigation in other to purify the most promising ones and carry on their chemical characterization using HPLC-HRMS and NMR analyses. Among the active compounds observed during HPLC analysis, one of them is already isolated, purified and the structure is under elucidation. These preliminary findings support the potential of F. pinicola as a new source of antimicrobial compounds. More antimicrobial compounds are expected from this work as well as their respective antimicrobial activities.

Mots-Clés: Fomitopsis pinicola, antimicrobial activity, bioautography, triterpenes, natural products

*Intervenant

Fluorescent probes for the detection of bacteria in body fluids

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Résumé

Rapid diagnosis of bacterial infections is a major challenge in the fight against antimicrobial resistance, particularly to enable targeted antibiotic administration and to avoid antibiotic overuse. Current diagnostic methods are slow as they rely on bacterial culture (1), leading to the widespread use of broad-spectrum antibiotics, which promotes resistance development and increases patient mortality. To address this critical challenge, we are developing an innovative strategy for bacterial detection in body fluids based on enzyme-activatable "turn-on" fluorescent probes. These probes consist of two Nile Red fluorophores linked by a peptide substrate specific to an exoenzyme from Staphylococcus species. In aqueous environments, the fluorescence of the probes is quenched due to the formation of non-fluorescent H-aggregates (2). Upon enzymatic cleavage, the fluorophores are separated, leading to the fluorescence "turn on". The validation of the concept was performed with a probe cleavable by trypsin, followed by the synthesis of four probes targeting glutamyl endopeptidase V8, a Staphylococcus-specific enzyme. The fluorogenic properties of the probes were assessed in various solvents and biological media, including bovine serum. Although promising fluorescence properties were observed, enzymatic and bacterial assays require further optimization. This approach may ultimately enable faster and more specific diagnosis, helping to limit the misuse of antibiotics and contributing to the fight against bacterial resistance.

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Mots-Clés: Nile Red, peptide, probes, bacteria, staphylococcus, enzyme, trypsin, glutamyl endopeptidase

*Intervenant

Towards new functionalized 1,4-thiazepines through metallocarbene-mediated ring expansion

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Résumé

The pharmaceutical industry is currently faced with the need for molecular diversity and structural originality to discover new drug candidates. Heterocycles play a key role in the structure of bioactive molecules.(1) Among them, mixed sulfur and nitrogen (N,S)-heterocycles, especially 5- and 6-membered rings, have demonstrated their interest in medicinal chemistry and pharmaceutical industry.(2) Medium-sized rings are however under-represented due to the difficulty of synthesis. In this context, the development of new synthetic routes is crucial to access complex and original (N,S)-heterocyclic structures efficiently and in a minimum of steps.
Here we report a ring expansion of 6-membered (N,S)-rings leading to new diversified 7-membered (N,S)-heterocycles. Starting from 1,3-dihydrothiazine precursors **1** obtained via a three-component reaction developed by our group,(3) the ring expansion proceeds via their reaction with a metallocarbene to yield 1,4-thiazepines **3**. The metallocarbene is generated in situ by decomposition of the diazo compound 2 in the presence of a rhodium(II) complex. After optimisation, the scope of the reaction was investigated by varying both the 1,3-dihydrothiazine **1** and the diazo partner **2**. Finally, the reactivity of the 1,4-thiazepines was explored and allowed to obtain new (N,S)-heterocycle **4**, amidothioether **5** and aminothioether **6**.

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Mots-Clés: methodology, sulfur, diazo, heterocycle, metallocarbene

*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, screening, platform

*Intervenant

Derivation, culture and genetic modification of mouse embryonic stem cells (mESC) at PHENOMIN-ICS.

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Résumé

Introduction
Organoid-based experiments are invaluable for studying organ development and disease modeling. These experiments rely on high-quality starting materials, such as mouse embryonic stem cells (mESCs). Our team specializes in deriving mESC lines from any genetic background, including wild-type, transgenic, and mutant mice. Additionally, we offer custom generation of genetically engineered alleles, including fully humanized alleles, tailored to meet specific research needs.
Aims
We aim to provide researchers with custom mESC lines derived from live or frozen embryos, sperm, or mice, while also creating 'à la carte' genetically engineered mESCs using CRISPR-assisted or traditional approaches.

Methods
Our standardized protocol enables the derivation of mESCs from diverse sources and genetic backgrounds. For cases where no mouse line harboring the desired mutation exists, we modify our proprietary wild-type mESCs to introduce custom alleles. Our team employs advanced genetic engineering tools, including CRISPR and traditional mESC techniques, to generate precise modifications, such as large-scale humanizations.
Quality control measures ensure the integrity of the derived lines, including Southern blotting, long-range PCR, ddPCR for chromosomal anomalies, Giemsa karyotyping, and genotyping.

Results
We have achieved consistent success in all mESC derivation attempts. CRISPR technology has enabled the generation of alleles previously considered challenging or unfeasible, such as complex genetic modifications and large-scale humanizations. Our proprietary mESC lines exhibit a high transmission rate of over 80% per injected clone, supporting efficient in vitro differentiation and downstream applications.
Conclusions
Our expertise in deriving high-quality mESC lines and engineering complex genetic modifications positions us as a valuable resource for researchers in organoid-based experiments and genetic studies. We are committed to providing tailored solutions to advance your research. For inquiries, contact us at mutagenesis@igbmc.fr.

*Intervenant

Mots-Clés: mESC, mouse embryonic stem cells, in vitro model

Stereoselective β-C-aryl glycosylation through Ni-catalysed cross-coupling

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Résumé

C-aryl glycosides are an important class of natural products with various biological activities against cancer or as hypoglycemic agents.(1) *Pseudomonas aeruginosa* (PA) is an opportunistic bacterium leading to chronic infections and has now developed antibioresistance, becoming a major threat for human health. Different strategies have been designed to tackle PA infection using multivalent glycoclusters in an antiadhesive strategy.(2) In the same context, we are investigating the synthesis of *C*-aryl β-glycosides as potential ligands of lectins for anti-infectious applications. Very few and recent methodologies propose highly β-stereoselective *C*-glycosylation and using Ni(0) catalysis.(3) We study and report here a robust and β-stereoselective reaction towards *C*-aryl glycosides in the galactose series, from commercially available and bench stable starting materials. *C*-Aryl glycosides in the glucose, fucose and mannose series have also been synthesized to expand the scope of the reaction and will be soon evaluated in biological assays. DFT calculations have been conducted to elucidate the mechanism and rationalize the β-stereoselectivity of the *C*-glycosylation.

Mots-Clés: C, aryl glycoside, catalysis, nickel, methodology

*Intervenant

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 fungal pathogen responsible for chytridiomycosis, a disease that has caused catastrophic declines in amphibian populations worldwide by infecting amphibians' skin. This infection disrupts skin permeability and electrolyte balance, leading to cardiac arrest and death. One of the host's primary defence strategies is nutritional immunity-limiting access to essential metals like iron, zinc, and manganese-to inhibit pathogen growth and virulence.
In parallel, the amphibian skin microbiome plays a crucial role in chytridiomycosis resistance by producing antifungal metabolites. However, the impact of host-driven metal restriction on these microbial communities remains largely unexplored. Our preliminary data suggest that iron limitation triggers skin bacteria to produce siderophores, specialized iron-chelating molecules with strong antifungal properties. This points to a potentially important synergy whereby host nutritional immunity and skin commensals' siderophore production act together to reduce iron bioavailability, critically limiting *Bd*'s access to this essential micronutrient.

To investigate this interaction, we isolated bacterial strains from the skin of *Alytes obstetricans* and assembled a minimal bacterial community representing the most abundant bacterial phylogroups. Using a high-throughput screening method, we found that most isolates, tested so far, produce siderophores under iron limitation, and that environmental factors such as pH and carbon source significantly influence siderophore production.

Importantly, our functional assays revealed that Bd: 1) cannot exploit any of the tested siderophores, 2) lacks competitive iron acquisition systems, and 3) cannot degrade siderophores. This suggests that microbial siderophores effectively reinforce host-imposed iron limitation, creating an environment hostile to Bd.
Together, these findings reveal a novel mechanism by which the amphibian skin microbiome complements nutritional immunity, enhancing protection against Bd. Understanding this interplay could pave the way for microbiome-based strategies to mitigate chytridiomycosis and aid amphibian conservation.

Mots-Clés: Siderophores, skin microbiome, Bd, amphibians, infection, metals, iron

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