Journées du Campus d'Illkirch 2023

Posters

Acidic conditions increase the benefit of hydroxamate siderophore exploitation for the bacterial pathogen Salmonella enterica

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Résumé

Infectious diseases remain one of the leading causes of death worldwide. Due to high microbial adaptation capacities, our solutions for disease control and prevention are continually challenged. One of the most important bacterial adaptations in infectious environment concerns **the supply of iron**. This metal is a nutritional resource essential for almost all living organisms including pathogenic microorganisms. A common way for bacteria to acquire this nutrient is through **the secretion of siderophores**, small organic molecules that scavenge iron and deliver it to the bacteria via specific receptors. While many bacteria use their own siderophores to acquire iron, some also have the ability **to exploit those produced by other microorganisms,** present in their environment. This is the case of the pathogenic bacterium *Salmonella enterica*, which can exploit at least three exogenous siderophores (exo-siderophores) in addition to produce its own siderophores to acquire iron. Although the nature of the three exo-siderophores that *S. enterica* can exploit to acquire iron has been identified, little is known about the environmental conditions favoring an ecological advantage of exo-siderophore exploitation. In this study, the goal is to assess whether acidity of the intestinal environment may influence the exploitation of exo-siderophores in *S. enterica*. In an interesting way, we found that acidic growth conditions increase the growth benefit associated to exo-siderophores exploitation more strongly than at neutral pH in *S. enterica*. Understanding how, when and where siderophores can be exploited by pathogens will give us important insights into pathogen biology and will be crucial for the design of pathogen control strategies.

Mots-Clés: infectious diseases, iron, siderophores, Salmonella, ecological advantage, acidity, intestine

*Intervenant

ANCHOR microscopy for single-particle tracking in live cell

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Abstract

Over the past decades, 3C techniques have been widely used with success to probe the spatial organization of the genome, from the chromatin loop scale to the entire genome. However, these approaches give an averaged view of the chromatin organization; especially, highly dynamical structures could potentially be screened by this averaging process.
Then, as a complementary approach to the 3C techniques, we propose to study the diffusion properties of specific loci of chromatin at the single-cell level.

Keywords: chromatin dynamics, enhancer, promoter looping, single cell

*Speaker

Aptamers as innovative tools for active siRNA delivery in tumor cells

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Résumé

Known for several years as specific and efficient molecules for the inhibition of gene expression, siRNAs offer great therapeutic promise in oncology. However, their use for therapeutic applications is challenging: due to their negative charge, their instability and their size, they fail to enter cells unassisted (*Alshaer et al, 2021*). To overcome this problem, innovative active delivery strategies based on conjugates that combine siRNA and ligands for active cell targeting can be used. These innovative strategies have already been proven therapeutically efficient with the GalNac targeting strategy (*Springer et al, 2018*).
In the lab, we are interested in aptamers as innovative ligands for cell targeting. They are single-stranded DNA or RNA oligonucleotides referring to chemical antibodies with high affinity and selectivity for their target (*Zhou et al, 2016*). Nevertheless, their use for siRNA delivery is still under studied. When the aptamers' targets are membrane proteins expressed at the cell surface and internalized, their binding aptamers can be escorted inside cells with their associated siRNA. Our project aims at deciphering and optimizing the delivery of siRNAs mediated by nucleic acid aptamers in glioblastoma cells, the most aggressive tumor of the central nervous system.

During my internship, the design of our chimera molecule has been performed. It will consist in two parts:

(i) a targeting part: a 2' fluoro modified RNA aptamer that targets a cell surface receptor. We will use an aptamer targeting the cell-surface receptor EGFR, overexpressed on the surface of glioblastoma cells, internalized by endocytosis (*Ivaska et al, 2011*).

(ii) a therapeutic part: a siRNA that will allow cell death.

*Intervenant

A few preliminary studies have been conducted. We showed that the aptamer is stable over 48h proving its stability in the cell medium. The selectivity of the EGFR-targeted aptamer was shown by fluorescence confocal microscopy on glioblastoma cells overexpressing EGFR. Bioimaging studies have also shown a better internalization of EGFR in the presence of the tyrosine kinase inhibitor, gefitinib, known to favor EGFR internalization (*Blandin et al, 2021*). Preliminary assays to study siRNA directed against luciferase in cells expressing luciferase showed a decrease in luciferase expression in the presence of siRNA.
In the perspective of the project, a pH-sensitive fluorescent aptamer-siRNA conjugate will be synthetised in order to follow the trafficking of this complex in the intracellular compartments (early and late endosomes). Improvement of the delivery will be performed by pharmacological and photochemical approaches.

Mots-Clés: Aptamers, siRNA, EGFR, Glioblastoma, Gefitinib

Blocking GABAα5 to reduce cognitive impairments in Down Syndrome

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Résumé

Down Syndrome (DS) is one of the major causes of genetic intellectual disability, with a prevalence of 1/800 births worldwide. DS patients show cognitive impairments notably memory (working, spatial and long-term), cognitive flexibility, and behavioral inhibition. In addition to comorbidities such as epilepsy, these deficits are among the factors that restrict the patients' autonomy most.
DS has been linked to an over-inhibition in the central nervous system. GABA receptor blockers showed encouraging results as a therapeutic strategy; however, they can provoke seizures that could be potentialized by DS comorbidities. A more specific target to reduce cognitive impairments has been suggested by a significant increase of performance in memory tasks in mice invalidated for the GABAα5 receptor compared to wild-type littermates.

GABAα5 receptors are localized in the apical dendrite of pyramidal cells and specifically activated by Martinotti cells. These cells are present in the somatosensory and frontal cortices and in the hippocampus. Given the function associated with these regions, Martinotti cells can be involved in the intellectual disability displayed by DS patients.
In order to assess the role of GABAα5 in DS, we will evaluate the efficiency of GABAα5 receptor modulators on the cognitive performances of the DS mouse model (Dp(16)1Yey) in tasks challenging spatial memory, working memory, learning and cognitive flexibility. As we hypothesize that alpha5 modulators act mainly through reducing the action of Martinotti cells, we aimed at testing this path of action more specifically. For this purpose, we focus on one of the major genes related to intellectual disability in DS, namely *Dyrk1a*, that also has been targeted to enhance cognition in rodent models. Therefore, we will examine more specifically the contribution of Martinotti cells to DS cognitive phenotype by reducing to a normal level the expression of *Dyrk1a* specifically in these cells in the same mouse model.

Mots-Clés: Down Syndrome, DYRK1A, GABA receptors, Martinotti Cell, intellectual disability

*Intervenant

Characterization of the first animal model of Microphthalmia Syndromic 12

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Résumé

Microphthalmia Syndromic 12 (MCOPS12) is a rare syndrome with severe neurologic symptoms which result from *de novo* point mutations in Retinoic Acid Receptor (RAR) β. Several of these mutations have been described with the most frequent corresponding to p.R387C gain of function mutation. Mechanisms and progression of neurological symptoms are not clear, but RARβ-dependent pathways such as calcium and dopaminergic signaling are potentially involved. To study the disease onset and progression we have used a newly generated mouse model of MCOPS12 carrying the p.R394C mutation, homologous to the p.R387C found in patients. Like patients, mice carrying this mutation displayed microphthalmia and an evolution of motor and cognitive symptoms from birth to adult stage. Obtained data allowed to identify the time points of such deficits onset which define windows of opportunity for preventive treatments that will target dysregulated pathways and potentially delay or prevent the disease onset.

Mots-Clés: Microphthalmia Syndromic 12, Retinoic Acid Receptor β, Phenotypying characterization, Mouse model

*Intervenant

Cortical over-inhibition in Down syndrome

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Résumé

Down syndrome (DS), caused by a trisomy of chromosome 21 (T21), is the most common genetic developmental disorder, with an incidence of 1 in 800 live births. It's characterized by cognitive deficits including intellectual disability and memory deficits.
DS patients show a disturbance in the excitatory/inhibitory balance of cortical circuit towards more inhibition. Amongst genes located on chromosome 21, *DYRK1A* has received increased interest as a key contributing in neuronal development and timing of neuronal progenitor cell migration and differentiation of excitatory neurons. However, no study has looked at DYRK1A impact on the development of GABAergic inhibitory neurons.

We hypothesize that a dysregulation of DYRK1A driving developmental alterations of inhibitory circuits is responsible for defective synaptic integration, network desynchronization and thus phenotypic characteristics in DS.
In this project, we will use a mouse model of T21 to investigate the development of interneurons in terms of their proliferation, migration, differentiation and maturation in order to highlight the impacted mechanism(s). We will then use a conditional inactivation approach to normalize *Dyrk1a* gene dosage in GABAergic neuron precursors or post-mitotic GABAergic neurons of our trisomic mouse model, to see if the impacted mechanisms as well as the associated cognitive deficits can be rescued.

Mots-Clés: DYRK1A, Down syndrome, GABAergic interneurons, Cortical development, Cognitive impairment, Developmental disease

*Intervenant

Development and validation of miniaturized spheroid assays

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Résumé

3D cellular models, such as spheroids, offer an intermediate level of complexity between single-layer cell culture and the use of animal models, reproducing in vitro organization of a micro-tumor. These models have been shown to be more predictive of treatment response than single-layer cell culture models.
Here we describe the development and validation of miniaturized (96-well) spheroid assays for quantification of spheroid growth and evaluation of cytotoxic effects of compounds.

Mots-Clés: spheroid, 3D cellular model

*Intervenant

Development of a targeted demethylation approach for a tumor suppressor gene in gastric cancer

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Résumé

In eukaryotes, DNA methylation is a key element of epigenetic regulation of gene expression and is involved in fundamental processes such as embryonic development and carcinogenesis. DNA methylation is stable through cell divisions but it is also reversible by TET proteins action. In a pathological context, tumor cells show hypermethylation of tumor suppressor gene promoters, leading to inhibition of the expression of these genes. But because of its reversible nature, DNA methylation is a target of choice in epigenetic therapies of cancer. Current DNA methylation inhibitors induce global non-specific demethylation, which can lead to many adverse effects and can conversely re-express oncogenic genes. Therefore, a strategy of choice is to develop technologies for the targeted epigenetic reactivation of specific genes. The *SLFN11* gene is described in the literature as a tumor suppressor gene involved in chemotherapy resistance and frequently hypermethylated in gastric cancer. It is therefore an interesting candidate to validate a targeted demethylation approach. Because this gene is still poorly understood, we are first testing the re-expression of *SLFN11* in a gastric adenocarcinoma cell line model (AGS line) in response to demethylation. Two pharmacological inhibitors of DNA methylation (5-azadC and GSK-3484862) are tested, thus establishing our proof of concept. In parallel, experiments have been initiated to build by molecular cloning a biotechnology tool, based on the use of the dCas9-Suntag-TET1 system described in the literature by *Morita et al. (2016)*, to induce targeted demethylation of *SLFN11* in gastric cancer cells. This tool shows promising preliminary results in AGS gastric cancer cells but improvements are needed. We are currently optimizing the tool to improve the transfection efficiency and modulate the quantities of TET1 transfected in the cells. In the future, we will test other delivery methods (mRNA or ribonucleoprotein particles) and test the impact of targeted epigenetic reactivation of *SLFN11* on chemotherapy resistance in cellular models of gastric cancer. This project will pave the way towards the optimization of strategies of targeted epigenome correction with a therapeutic aim in cancer.

Mots-Clés: DNA methylation, cancer, genome editing, tumor suppressor genes, SLFN11, dCas9/Suntag

*Intervenant

Development of Mechanochemical reaction conditions for Buchwald-Hartwig amination reaction

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Abstract

Transition metal catalysed coupling reactions play an important role in chemical synthesis, facilitating diverse bond formations. These reactions are frequently used in medicinal chemistry(1); an important example is the Buchwald-Hartwig amination reaction, which, through Palladium catalysis, allows the formation of an aromatic carbon-nitrogen bond between amines and aryl halides, a moiety that is frequently used in drug development(2). However effective, these reactions necessitate the use of hazardous and toxic solvents, making them costly to the environment and to the health of workers, and making the use of these reactions in scale up pharmaceutical manufacturing disadvantageous. The industry, since 20 years, have made it a priority to make their production green by the minimization, replacement, recycling or removal of said solvents(3).
Mechanochemistry(4), is the use of mechanical energy generated through milling or grinding for chemical transformation. It is a tool that has experienced a significant comeback thanks to its applicability to green chemistry(5), as the demand for cleaner and safer reaction conditions have grown, especially in chemical and research and development based pharmaceutical industries. Through avoiding the use of bulk solvents, solvent-free mechanochemical reactions provide cleaner synthesis methods for chemists, safer reaction conditions for the environment and profitable conditions for industrial applications.

Although the use of various trans-metal catalysed reactions under mechanochemical reaction conditions have been thoroughly investigated, few published research can be found for Buchwald-Hartwig aminations (6–8). The most notable example(9) uses liquid assisted grinding to prohibit the aggregation of the Palladium catalyst in the reactor, yet the agent used, a cyclic diene, is not at all adapted to green chemistry.

In our lab, we are interested in using this relatively new technology for developing the right conditions for solvent-free Buchwald-Hartwig amination reactions. Starting from a model reaction, we investigated the technique of "Design of Experiments (DoE) ", an applied statistical approach (10) to reaction optimization that allows the variation of multiple factors simultaneously. This approach enables the evaluation of a large number of reaction parameters in a relatively small number of experiments.

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Keywords: Buchwald, Hartwig amination, Green chemistry, Mechanochemistry, Design of Experiments

DYRK1A and neuronal morphology

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Résumé

Down Syndrome and DYRK1A Syndrome are two genetic disorders causing intellectual disability (ID). In both afflictions, the dosage of the Dual-specificity serine threonine Kinase 1A (DYRK1A) is modified, disturbing proper development and functioning of the central nervous system. Considering the implication of DYRK1A in many cellular signaling pathways, including cytoskeleton rearrangement, synaptic plasticity and activity-dependent transcription, an altered neuronal morphology could be one of the causes of neuronal malfunctioning and, to a further extent, ID.
In this study, we assessed the impact of Dyrk1a gene dosage on neuronal morphology in the hippocampus, a key region for learning and memory. We focused more specifically on the main glutamatergic neurons of the input and output of this structure, namely the granule cells of the Dentate Gyrus (DG) and the pyramidal neurons of the Cornu Ammonis 1 (CA1). Mouse models with either a single (Dyrk1a+/-) or three (TgDyrk1a) functional copies of the gene were used. Neuronal morphology was determined through classical Golgi-Cox staining coupled with confocal imaging and followed by 3D neuronal arborization reconstruction and analysis using Single Neurite Tracer in Fiji.
Our results indicate that Dyrk1a under-expression severely alters neuronal morphology in the DG and CA1, both in size and complexity. However, in opposition to what could have been expected from the reported increase in hippocampal size, Dyrk1a overexpression had a very limited impact on hippocampal arborizations, especially in CA1.

Mots-Clés: DYRK1A, hippocampus, neuronal mophology, Golgi staining, confocal imaging

*Intervenant

Fast and robust single particle reconstruction in 3D fluorescence microscopy

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Résumé

Single particle reconstruction has recently emerged in 3D fluorescence microscopy as a powerful technique to improve the axial resolution and the degree of fluorescent labeling. It is based on the reconstruction of an average volume of a biological particle from the acquisition of multiple views with unknown poses. Current methods are limited either by template bias, restriction to 2D data, high computational cost or lack of robustness to low fluorescent labeling. In this work, we propose a single particle reconstruction method dedicated to convolutional models in 3D fluorescence microscopy that overcomes these issues. We address the joint reconstruction and estimation of the poses of the particles, which translates into a challenging non-convex optimization problem. Our approach is based on a multilevel reformulation of this problem, and the development of efficient optimization techniques at each level. We demonstrate on synthetic data that our method outperforms the standard approaches in terms of resolution and reconstruction error, while achieving a low computational cost. We also perform successful reconstruction on real datasets of centrioles to show the potential of our method in concrete applications.

Mots-Clés: Fluorescence microscopy, inverse problem, optimization

*Intervenant

ICS ES cell service: Derivation, culture and genetic modification of embryonic stem cells

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Résumé

Despite recent advances in CRISPR/cas9 technologies, embryonic stem cells remain the gold standard to produce complex mouse models. Even if presented by many as outdated, ES cell approaches are still the only way to obtain, in a defined timing and efficient manner, complex alleles (Birling et al., 2021). Moreover, ES cells with the help of CRISPR, allow now to obtain alleles that were not possible to obtain before (or needed the screening of thousands of clones) and guaranty the success of the gene targeting.
At PHENOMIN ICS, the ES cell service is in charge of the different culture steps to generate mutant ES clones by homologous or illegitimate recombination. We therefore work in close collaboration with the genetic engineering service. Once validated clones have been identified, we prepared them for injection, which is done by the microinjection service.
We will present as well the other activities of the ICS ES cell service, for instance derivation of embryonic stem cells from mouse lines.

Mots-Clés: emryonic stem cells, ESC, mouse model

*Intervenant

Insights into interactions between the CCR4-NOT deadenylase and the Nascent polypeptide Associated Complex (NAC): a link between translation and mRNA degradation?

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Résumé

mRNA decay contributes to the regulation of gene expression by modulating transcript levels. In eukaryotes, this process initiates by the shortening of the 3' polyA tail (called deadenylation), a regulated step that not only sets mRNA decay rate but also blocks further translation initiation. The CCR4-NOT complex is the main eukaryotic deadenylase. Data from large scale analyses and biochemical results from the team revealed an interaction between Caf130, a subunit of the yeast CCR4-NOT complex, and Btt1, a subunit of the nascent polypeptide associated complex (NAC). The latter assembly is involved in the folding of nascent polypeptides at the ribosome exit tunnel. To investigate the role of the CCR4-NOT / NAC interaction, we determined the interactome of Caf130 by tandem-affinity purification and mass spectrometry. This confirmed earlier results and revealed the presence of a new partner, Yjr011C, a protein of unknown function. The latter was biochemically validated to be a relevant partner. Two-hybrid assays further substantiated interactions of Caf130 with Btt1, Not1 and Yjr011C. This assay was used with truncated and chimeric constructs to map interaction domains. This preliminary interaction map needs to be further refined to better understand the underlying functions.

Mots-Clés: CCR4, NOT complex, NAC complex, ribosome, RNA decay, translation, yeast

*Intervenant

Interdisciplinary approach for specific delivery of active molecules to infectious sites

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Résumé

In the last decade, research on medicinal plants has increasingly turned to historical medicinal-botanical texts, both to study the dissemination of traditional pharmacopoeias and to identify leads for new drugs (Pitchon et al., 2022). These texts show a synergistic approach of healthcare, notably in the anti-infective field, by combining active principles acting on the pathogen and on the host (Abdallah et al., 2022). Nowadays, exploiting the potential around these new drug leads requires the development of strategies to deliver these molecules to the infectious site in order to optimize their efficacy and synergy.
Aptamers are short single-stranded nucleic acid molecules that can specifically bind to their targets such as proteins, small organic compounds, metal ions, and even whole cells with high affinity and specificity. These, so called, chemical antibodies, have gained much attention as molecular probes for bioimaging due to their unique properties such as small size, stability, low toxicity, and ease of chemical modification (Elskens et al., 2020). Aptamers have for example been designed to bind bacteria like *Staphylococcus aureus* (Moon et al., 2015), *Mycobacterium tuberculosis* (Aimaiti et al., 2015), *Escherichia coli* (Marton et al., 2016), *Pseudomonas aeruginosa*(Wang et al., 2011)...

Using a fluorescent original aptamer targeting *Pseudomonas aeruginosa*, we recently demonstrated by confocal microscopy how this aptamer provides highly sensitive and selective detection of *P. aeruginosa*. Moreover, this aptamer allows the differentiation of *P. aeruginosa* from *S. aureus* in a pool of bacteria, whether they are dead or alive.
In this project, this aptamer will be anchored onto nanoliposomes to try to selectively target *P. aeruginosa* and deliver encapsulated anti-infective compounds to the infectious site.

*Intervenant

Our further research will involve: i) the formulation and physico-chemical characterization of maleimide-functionalized liposomes made of phospholipids and cholesterol, and filled with active isolated ingredients identified from the medieval arabic pharmacopoeia; ii) the conjugation of the aptamer onto the liposome via a thiol-maleimide Michael addition and iii) the evaluation of the selective delivery of anti-infective drugs to *Pseudomonas strains* in a complex medium.

Mots-Clés: aptamers, infectious, bacteria, confocal, microscopy

Isolation and characterisation of anti-inflammatory activity of natural compounds from traditional Chilean medicine

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Résumé

In the framework of the search for new anti-inflammatory agents, the laboratory is dedicated to the discovery of new bioactive compounds from several medicinal plants from the Atacama People's traditional medicine. In a first step, cytotoxic (cell viability) and anti-inflammatory (production of pro-inflammatory cytokines) activities were assessed of extracts obtained from classical maceration procedures from selected medicinal plants species. The less polar extracts from *Fabiana* species (*F. squamata* and *F. denudata*, Solanaceae) showed the more promising *in vitro* results, inhibiting the production of pro-inflammatory cytokines (82% and 89% for *F. squamata* and 61% and 77% for *F. denudata* of IL-1β and IL-10 inhibition respectively at 50 μg/mL, final concentration) by LPS-stimulated peripheral blood mononuclear cells (PBMC) with no effect on cell viability. To explore the chemical composition of these two extracts, a molecular networking approach was applied, based on HPLC-PDA-HRMS/MS data, identifying polymethoxyflavonoids and sesquiterpenes as major compounds. Bio-guided fractionation of the two extracts is been performed, evaluating the production of IL-10 by LPS-stimulated PBMC at 5 and 50 μg/mL. The purification of compounds from more active fractions is in progress to identify the bioactive compounds and explore their anti-inflammatory activity in *in vitro*/*in vivo* models.

Mots-Clés: pharmacognosy, natural compounds

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Long-range Energy Transfer Between Dye-loaded Nanoparticles: Observation and Amplified Detection of Nucleic Acids

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Résumé

Förster resonance energy transfer (FRET) is essential in optical materials for light-harvesting, photovoltaics and biosensing, but its operating range is fundamentally limited by the Förster radius of ∼5 nm. Here, FRET between fluorescent organic nanoparticles (NPs) is studied for the first time in order to break this limit. The donor and acceptor NPs are built from charged hydrophobic polymers loaded with cationic dyes and bulky hydrophobic counterions. Their surface is functionalized with DNA in order to control surface-to-surface distance. It is found that the FRET efficiency does not follow the canonic Förster law, reaching 0.70 and 0.45 values for NP-NP distance of 15 and 20 nm, respectively. This corresponds to the FRET efficiency decay as power four of the surface-to-surface NP-NP distance. Based on this long-distance FRET, a DNA nanoprobe is developed, where a target DNA fragment, encoding cancer marker survivin, brings together donor and acceptor NPs at ∼15 nm distance. In this nanoprobe, a single molecular recognition results in unprecedented color switch for > 5000 dyes, yielding a simple and fast assay with 18 attomoles limit of detection. Breaking the Förster distance limit for ultrabright NPs opens the route to advanced optical nanomaterials for amplified FRET-based sensing of biomolecules.

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Nucleic acid hybridization at the interface of fluorescent nanoparticle probes

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Abstract

The significance of RNA biomarkers has been proven repeatedly in recent years; they are characteristic for many diseases such as cancer, and can be used for monitoring normal and pathological processes, as well as the progression of the disease and/or treatment (1,2,3). For the detection of such biomarkers, our team has introduced nanoprobes for nucleic acids based on dye-loaded polymeric nanoparticles (NPs) as light-harvesting nanoantennas (4). Their core contains > 1000 encapsulated dyes and the shell consists of nucleic acids serving as recognition units (5,6). The detection takes place by hybridization between the nucleic acids grafted on the NP surface and the biomarker with the complementary sequence. Thus, it is important to understand the hybridization process at the particle interface and compare it with the hybridization of the single strands. Moreover, the multivalency of the NP has some effect on the hybridization. To elucidate this, we studied the DNA hybridization at the NPs interface using Förster Resonance Energy Transfer (FRET). The surface architecture of DNA functionalized NPs was varied using different linkers and density of the capture sequence. We studied different parameters such as hybridization kinetics, thermal stability of the duplexes and sensitivity to mutations. The obtained results will guide the development and optimization of nanoprobes for ultrasensitive detection of DNA/RNA biomarkers.

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Keywords: fluorescent nanoparticles, FRET, RNA biomarkers, cancer detection

PCBIS: Chemical libraries, biological models, technological tools and early ADMETox

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Résumé

From assay development to lead characterization PCBIS proposes its expertise to contribute to the development of drug candidates:
- Assay development, miniaturization, automation and validation

- Screening of chemical libraries and extracts

- Early ADMETox evaluation to characterize compounds

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last publications:

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Mots-Clés: Chemical biology, screening, ADME, Fluorescence, drug

Quantitative Assay for Diacylglycerol Kinase Kappa with Mass Spectrometry and Parallel Reaction Monitoring.

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Résumé

Liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) is now a robust and accurate method for the quantification of peptides and proteins in biological samples. Here we describe the development for quantification of mouse Diacylglycerol Kinase Kappa (DGKK), a membrane protein involved in lipid metabolism and second messenger production, but which is not correctly detected with antibody-based methods. The assay uses an Exploris Q-Orbitrap system with a specific acquisition setup called Parallel Reaction Monitoring (PRM) and stable isotope labeled peptides (SIL). The result is an absolute quantification in fmole/μl or pg/μl.

In practice, proteins are extracted with a protocol common to bottom-up proteomics, digested with trypsin and peptides are identified and quantified on the basis of MS and MS/MS ion chromatograms. To obtain absolute quantification, peptides of interest are also synthesized with stable isotopes on K (13C615N2-lysine; +8 Da) or R (13C615N4-arginine; +10Da). and added to the samples. These synthetic peptides are used for standard curve construction and determination of analytical parameters such as specificity, accuracy, reproducibility. They are also added to each sample for quality control and standardization purposes.

With a detection limit of less than 1 fmole/μl, this assay allows the detection and quantification of mouse DKGG in Hela cell engineered to express the protein. In mouse brain, DGKK is not detected, confirming a very low level of expression. Improvement in sensibility and protein extraction are still under investigation and will be discussed.

Mots-Clés: analytical chemistry, mass spectrometry, Parallel Reaction monitoring, Diacyl glycerol Kinase Kappa

*Intervenant

RFamide peptides and their receptors in the modulation of pain

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Résumé

There are two main types of pain: acute pain, which is intense but short-lived, and chronic pain, which is long-lasting and disabling. Worldwide, 30% of adults suffer from chronic pain. 70% of them are unable to relieve their pain causing major social and economic repercussions. During the 1990s, the use of opioids for pain management began to increase, particularly in the United States. Today, opioid abuse is growing exponentially with the Covid-19 crisis and remains the leading cause of preventable death in the United States, ahead of motor vehicle accidents with nearly 500,000 deaths since 2000. The analgesic effects of opiates are mainly due to the activation of mu-opioid receptors. Their chronic use results in the development of pain hypersensitivity, analgesic tolerance and dependence, the most commonly observed side effects of opioids. In the laboratory, we are particularly interested in studying the involvement of GPCRs from the RFamide family in the modulation of nociception and adaptations associated with chronic opioid administration. To this purpose, we evaluated the activity of central administration of different RFamide peptides, RFRP3, NPFF and QRFP-26, on nociceptive threshold and/or morphine-induced analgesia in wild-type and NPFFR1, NPFFR2 and QRFPR KO mice. Our data show that (i) QRFP-26-induced hyperalgesia was present in WT, NPFFR1 and NPFFR2 KO animals but not in QRFPR KO mice. (ii) RFRP-3-induced hyperalgesia was present in WT and NPFFR1 KO mice but absent in NPFFR2 and QRFPR KO animals. (iii) NPFF anti-morphine activity was present in all genotypes although it was significantly reduced in QRFPRa KO animals. As expected, these data confirm that QRFP-26 is the endogenous ligand of QRFPR. Unexpectedly, RFRP3-induced hyperalgesia seems to be mediated by both NPFFR2 and QRFPRa while NPFF anti-morphine activity is partially mediated by QRFPRa. Our results indicate that *in vivo* it is difficult to associate pain-modulating activity of RFRP3 and NPFF to their cognate receptors. Further studies will be needed to fully explain these unexpected data.

Mots-Clés: Pain, Morphine analgesia, RFamide Neuropeptides

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Siderophore production by the human gut microbiome.

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Résumé

Siderophore production by the human gut microbiome.
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he human gut microbiome is a complex microbial community playing an important role in host health (1). Being a dynamic system, changes in the abundance of different nutrients can affect the delicate balances between microbes. Nutrient metals, like iron, zinc, or manganese, are essential to all living organisms and are a key factor in bacterial competition. Additionally, the human body tightly regulates iron levels, for example, in the gut to prevent the growth of potentially harmful bacteria (2),(3). This creates a competitive environment where bacterial species that can effectively scavenge iron have a selective advantage over those that cannot. This "scavenging" is made possible by metallophores such as siderophore in the case of iron-chelation. Siderophores are small organic molecules with an extremely strong affinity for iron. While siderophore production is a widespread behavior observed in the majority of bacteria in nature (4), the production of siderophores by the gut commensal microbiome is unknown. These metallophores can have important implications on human health, as they can shape the composition and function of the whole gut microbiome (5). Here we assess, for the first time, the diversity and abundance of siderophores produced by common intestinal bacteria. We are developing an identification pipeline applicable on various gut microorganisms and examine whether a phylogenetic pattern emerges among the siderophore producers of the gut microbiome.

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Mots-Clés: microbiome, gut, siderophores, commensals, bacteria, iron, metals, phylogenesis, diversity

Structural prediction and mass-guided isolation of new potentially bioactive compounds from ammoniacum (Ferula communis)

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Résumé

The recent proliferation and prevalence of antimicrobial multi-resistant infections has prompted the development of other strategies and alternatives to urgently combat this global threat. For this purpose, past mastering of remedies formulation appears as a wealth of resources for present research. In particular, Arab Medieval Pharmacopeias (SC1) (AMP) were explored by our interdisciplinary team gathering researchers from biology, chemistry, humanities and informatics sciences. One remedy from the Ibn Al-Kindi Pharmacopeia (9th Century) which combines plant-based products and metal was reproduced and biological activity was tested. Ammoniacum, one of the five ingredients, showed antimicrobial activity against Gram + cutaneous bacteria.

This present study aimed to further explore this gum-resin from *Ferula communis* using a molecular-networking-guided method for the accelerated discovery of new compounds. HPLC-PDA-HRMS/MS molecular-networking-based dereplication strategy highlighted the presence of known sesquiterpene coumarins (SC) among potential new derivatives by comparison of their MS/MS fragmentation spectra. By this approach, a new hydroxycinnamic-ferulenol derivative was predicted in the extract. Mass-guided isolation followed by structural characterization allowed us to corroborate the predicted structure of this new SC compound. This targeted isolation led to a total of two known SC exhibiting antimicrobial activity and two new SC structures. The results of the present study confirm the interest attached to *Ferula communis'* gum exploration in the discovery of new structures not described yet.

Mots-Clés: Natural Products, Molecular networking, HPLC, PDA, HRMS/MS, Targeting isolation, Antimicrobial

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