École Doctorale
des Sciences de la Vie
et de la Santé
STRASBOURG

Université
de Strasbourg

Doctoral School DAYS 2025

European Doctoral College of the University of Strasbourg, France

May 19-20, 2025

Abstract Book

Powered by:

Long Talks

Characterization of clonal heterogeneity in chronic lymphocytic leukemia

Research area: ESBS UMR7242 - CHRU Strasbourg

BOUTKHIL Loubna, RIMELEN Valerie, VALLAT Laurent,

Chronic lymphocytic leukaemia (CLL) is the most common adult leukemia 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. This heterogeneity is not taken into account 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

---

Keywords : **CLL, Heterogeneity, Immune repertoire, Bioinformatics, VDJ sequences**

Glucocorticoids and Muscle Regeneration: A Double-Edged Sword?

Research area: Cellular and molecular biology

Emilia Calvano, Sirine Souali-Crespo

Skeletal muscle has a remarkable capacity to **regenerate** after injury, a process primarily driven by **satellite cells**, the muscle-resident stem cells that activate, proliferate, and differentiate to restore damaged tissue. This tightly regulated process involves a complex interplay between muscle cells, the immune system, and hormones.

Among these hormones, **glucocorticoids** play a central role. They are widely prescribed to manage chronic inflammatory conditions, such as asthma and inflammatory myopathies, by **reducing inflammation**, which can improve patient mobility. However, their long-term use is limited by significant side effects, including muscle atrophy. Despite their widespread use, the specific impact of glucocorticoids on muscle regeneration remains poorly understood, and it is still unclear whether their effects on this process are **beneficial** or **detrimental**.

The aim of this PhD project is to better understand how glucocorticoids influence muscle regeneration by examining their effects on muscle tissue repair and their direct impact on satellite cell function. Using a murine model of acute muscle injury, I induce damage to the muscle and assess the effects of glucocorticoid treatment on the regenerative process post-injury. Ultimately, this research aims to inform the clinical use of glucocorticoids, particularly for patients with muscle injuries or chronic inflammatory diseases. Importantly, this work will focus on satellite cells, which express the **glucocorticoid receptor**, to dissect cell-autonomous responses. It will also explore how glucocorticoid exposure reshapes the broader muscle **microenvironment**, including inflammatory and stromal components, critical for effective regeneration.

Keywords : Skeletal muscle regeneration, Satellite cells, Glucocorticoids, Inflammation, Glucocorticoid receptor

Implication of the atypical cadherin Mucdhl in inflammatory bowel disease (IBD)

Research area : Cellular and molecular biology

[Yassine ESSALKI[1]], [Simon PESCHARD[1]], [Isabelle GROSS[1]] [Nadia JESSEL[1]]

[1] INSERM, Strasbourg University, UMR 1260, CRBS 67000 Strasbourg, France

---

Chronic inflammatory bowel diseases (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), are characterized by persistent inflammation of the gastrointestinal tract, leading to tissue damage. These conditions involve intermittent inflammatory flare-ups, causing abdominal pain, weight loss, diarrhea, and potentially severe complications. A key factor in IBD pathophysiology is the disruption of the intestinal barrier, which increases intestinal permeability and exacerbates inflammation. This disruption often precedes mucosal damage, as deregulations of intestinal epithelial cell adhesion proteins (ex: E-cadherin) are observed in IBD patients even in the absence of inflammation.

Our laboratory focuses on MUCDHL, an atypical cadherin involved in intermicrovillous adhesion that stabilizes the intestinal brush border. Decreased MUCDHL expression in IBD patients correlates with reduced microvillus size. Furthermore, unpublished results indicate that genetic inactivation of Mucdhl in mice increases the severity of colitis induced by dextran sulfate sodium (DSS), suggesting that MUCDHL loss contributes to IBD. The aim of my thesis is to elucidate how MUCDHL loss promotes IBD development and progression at the cellular and molecular levels, to propose new therapeutic approaches.

Keywords : **[IBD], [inflammation], [barrier], [permeability], [MUCDHL]**

How early life stress alters the brain: the sex specific role of oxytocin in memory and emotion

Research area : [Neuroscience]

[H. Illouz], [E. Tanche], [Y. Menger], [O. Schaack], [V. Lelièvre], [P. Poisbeau]

Early-life stresses (ELS) are adverse events affecting nervous system development and later-life functioning. They increase the risk of psychiatric disorders, chronic pain, and affective comorbidities. The hippocampus, critical for memory and emotional regulation, is particularly sensitive to such adversity. Using the neonatal maternal separation (NMS) model, we examined sex-specific behavioral and molecular alterations, focusing on hippocampal function, neuroinflammation, and oxytocinergic signaling pathways.

NMS induces mechanical and thermal hypersensitivity and anxiety-like behaviors in both male and female rats. However, spatial memory deficits emerge exclusively in males, correlating with a pro-inflammatory response in the dorsal CA1 hippocampal subregion. Molecular analyses highlight male- and dorsal CA1-specific mRNA overexpressions, suggesting disrupted chloride homeostasis, alongside alterations in oxytocin (OT) and oxytocin receptor (OTR) signaling. These results underscore a pronounced sex difference in hippocampal vulnerability, with males showing greater susceptibility to spatial memory impairments and neuroinflammation.

To explore the organizational role of OT during ELS, daily intraperitoneal OT injections were administered during the NMS period (P2-P12). Remarkably, OT treatment fully rescued the altered behavioral phenotype, confirming OT's crucial involvement in mitigating NMS's long-term consequences. This suggests that NMS animals experience a transient OT deficit or reduced OTR affinity during this critical developmental window, leading to persistent disturbances.

These findings underscore the critical role of OT in mitigating early-life stress effects and highlight a sex-specific vulnerability to NMS. This work emphasizes the necessity of considering sex as a critical factor in understanding and addressing the long-term impacts of ELS, offering new therapeutic avenues targeting OT pathways to alter neurodevelopmental outcomes.

Keywords: **[Early life stress], [Oxytocin system], [Nociception], [Hippocampus], [Sex differences]**

Lipid nanoparticles as efficient therapy vectors for skeletal muscle pathologies

Research area: Translational research

Jacqueline Ji[1], Eva Lipkow[1], Corine Crucifix[1], Nicolas Anton[2], Jocelyn Laporte[1]

[1] Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), Illkirch-Graffenstaden, France, [2] Laboratoire de Conception et Application de Molécules Bioactives, Strasbourg, France

---

Adeno-associated viruses (AAVs) are the most widely used gene therapy vector for the treatment of genetic muscle disorders. However, AAV-based systems face significant limitations such as immunogenic responses, particularly in the liver, and restricted DNA packaging capacity. Gene therapy vectors that simultaneously 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 engineered for skeletal muscle targeting. LNPs present an attractive alternative to viral vectors due to their higher payload capacity, compatibility with repeated administration, and reduced immunogenicity. To enhance muscle-specific delivery, we rationally designed LNPs functionalized with MyomP1, a peptide derived from Myomerger, and encapsulated either DNA or mRNA encoding luciferase reporter genes. In vitro studies in murine C2C12 myoblasts and myotubes, as well as in 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 the context of mRNA delivery.

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

Keywords: **Gene therapy, Lipid nanoparticle, muscle, nucleic acid delivery**

Discovery of novel defluorinases using droplet-based microfluidics

Research area: Molecular Biology

Radi Khodr (IBMC), Enrico Bocconetti (IPCB), Stéphane Vuilleumier (IPCB), and Michaël Ryckelynck (IBMC)

Per- and Polyfluoroalkyl substances (PFAS) are a very diverse class of recalcitrant compounds (also known as forever chemicals) which are essentially of anthropic origin. Industrially produced PFAS are used for a wide range of applications, and now represent ubiquitous contaminants in the environment. A main feature of PFAS is their extensive persistence conferred by their halogenation; the carbon-fluorine bond being the most stable covalent single bond known. Today, there is a growing need to decontaminate the environment of these pollutants. Our goal is to discover PFAS-degrading enzymes either through environmental sampling or via directed evolution.

Complementarily to current physical and chemical remediation processes, enzymatic degradation represents an attractive approach for PFAS remediation. However, enzymatic degradation of the C–F bonds of such compounds haven't been fully assessed. Naturally, the ability for a biological system (e.g., bacteria) to break down "foreign" environmental pollutants relies on the inherent contact with such a substance. This proves to be rather challenging when dealing with synthetic pollutants like PFAS.

Biodegradation of fluorinated compounds entails the fact that the molecules in question must break down the carbon-fluorine bonds in the various PFAS forms, releasing fluoride ions. Therefore, monitoring fluoride production could allow monitoring of enzyme activity. In this view, our team developed "FluorMango" biosensor, a fluorogenic structure switching aptamer specifically emitting fluorescence in the presence of fluoride and is compatible with droplet-based microfluidic ultrahigh-throughput screening platforms. This allows for rapid screening of novel defluorinases that could be adapted to downstream characterization and industrial applications.

Based on this approach, two main discovery pipelines are explored:
a) An in vivo approach in which populations of bacteria (either E. coli expressing gene libraries or environmental samples collected on PFAS-contaminated sites) are encapsulated in droplets (with a PFAS substrate and the biosensor) and screened for their degradation capacity.
b) An in vitro approach in which a mutant gene library prepared from a known gene, are individualized in droplets where they will be amplified prior to being expressed in a cell-free system and screened as above for the capacity of encoded proteins to degrade the PFAS substrates.

Keywords: **PFAS, enzymes, bioremediation, aptamers, microfluidics**

The mRNA decay factor Pat1 mediates a global control of poly(A) tail length that is suppressed by a ribosome mutation

Research area : Molecular Genetics

Lucie Labeauvie, Claudine Gaudon-Plesse, Jackson Peter, Hélène Zuber, Bertrand Séraphin

---

Eukaryotic mRNAs are key actors of gene expression; thus, their levels need to be finely tuned. Post-transcriptionally, levels of mRNAs are governed by their turnover in the cytoplasm. Critical steps of mRNA decay encompass deadenylation, decapping, and exonucleolytic mRNA body digestion, that constitutes the 5' -> 3' pathway.
The conserved decapping regulator Pat1 appears to impinge on all those processes. Despite evidence that Pat1 is a conserved and central actor of the 5' -> 3' mRNA decay pathway, some studies have reported that only a fraction of the transcriptome is impacted by its deletion. Moreover, Pat1 was described both as a translational repressor of oligoadenylated mRNAs and as a global translation initiation enhancer. Altogether, published data fail to provide definitive clues about the role of Pat1 in cellular mRNA control.

To elucidate Pat1's function, we performed multi-omic and genetic analyses of a Δpat1 mutant in the yeast *Saccharomyces cerevisiae*. Transcriptome and proteome analyses revealed that the expression of only a subset of genes is affected by Pat1's inactivation. In contrast, a FLEP-seq analysis, allowing the sizing of the poly(A)-tail of each transcript in the cells by Nanopore sequencing, revealed that the absence of Pat1 impacts the length of the poly(A)-tail at the transcriptome level. Unexpectedly, we have observed that the growth defects caused by Pat1's inactivation are partially suppressed by a mutation in a ribosomal protein. This suppressor mutation also partly rescued the abnormal poly(A)-tail length of Δpat1 cells. Moreover, we observed that polysome profiles are altered in the absence of Pat1 and further modulated by the suppressor.
Altogether, our analyses are consistent with the model where the activation of decapping, that becomes rate-limiting, is the primary defect in the absence of Pat1. Our study supports the conclusion that Pat1 is a general mRNA decay factor even if its absence results in transcript-specific changes in mRNA levels. Altered mRNA decay in Pat1 deficient cells, and consequent translational impairments, can both be partly rescued by altered ribosomes.

Protein degraders drug delivery in Multiple Myeloma

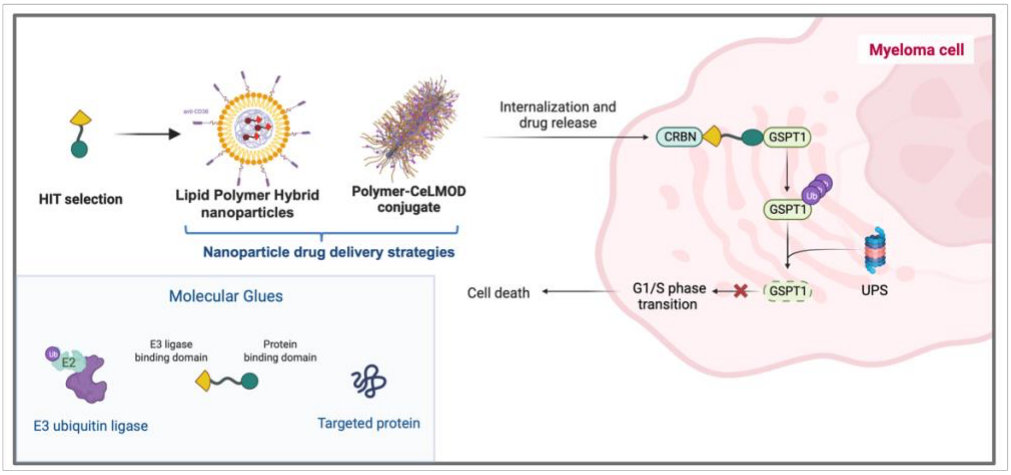
Research area : Oncology

Pedro Lopez Navarro[1], Charline Keller[3], Mainak Banerjee[1, 2], Christine Carapito[3], Alexandre Detappe[1], Loïc Charbonniere[2]

1 : Nanotranslational Laboratory, Institut de Cancerologie de Strasbourg Europe (ICANS). Laboratoire labellisé Ligue contre le Cancer.
2 : Equipe Synthèse Pour l'Analyse (SynPA), Institut Pluridisciplinaire Hubert Curien (UMR 7178), CNRS Université de Strasbourg.
3 : Laboratoire de Spectrométrie de Masse BioOrganique, Institut Pluridisciplinaire Hubert Curien (UMR 7178), CNRS, Université de Strasbourg.

Multiple Myeloma (MM) is the second most prevalent hematological cancer, characterized by frequent relapses and the development of resistance to existing therapies. Proteolysis-Targeting Chimeras (PROTACs) and molecular glues (MGs), represent emerging therapeutic strategies that selectively degrade disease-associated proteins. Among these, Cereblon E3 Ligase Modulating Drugs (CELMoDs) targeting GSPT1 have shown significant efficacy in MM treatment. However, their clinical application is limited by potential toxicity and restricted biodistribution. To overcome these challenges, we have developed a Bottlebrush Polymer-CELMoD nanocarrier targeting GSPT1.

We synthesized a library of novel CELMoDs using a Lenalidomide-based scaffold. Through drug screening in cancer cell lines, we identified a lead compound based on its potent activity and its ability to induce targeted protein degradation in vitro. Proteomic analysis using mass spectrometry was performed to comprehensively characterize the compound's selectivity, degradation efficiency, and downstream effects in Myeloma cells. In vivo studies showed that the Bottlebrush Polymer-CELMoD nanocarrier improved pharmacokinetics, with prolonged circulation and enhanced tumor accumulation, highlighting its therapeutic potential in MM.



Keywords : **Multiple Myeloma, Molecular Glue, GSPT1, Nanoparticle, Proteomics**

Assessing the role of loop extrusion in enhancer-promoter communication dynamics

Research area : Chromatin Dynamics

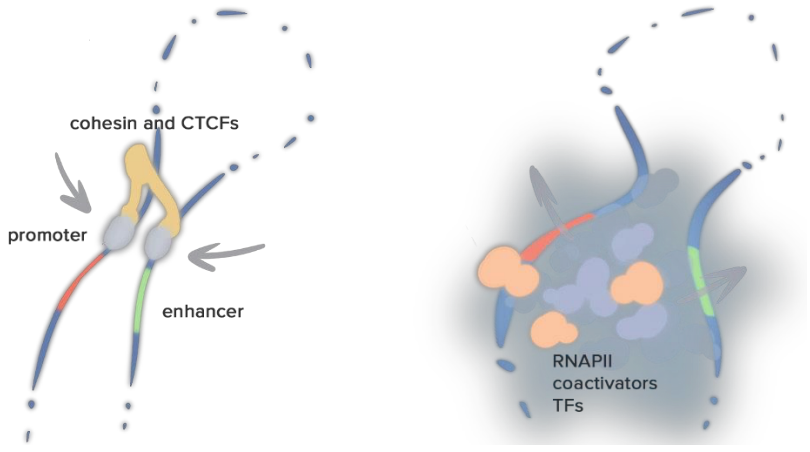
Mariia Nazarova, Cathie Erb, Bastien Molcrette, Thomas Sexton at al.

Recent advances in the field showed that enhancer-promoter communication is not linearly dependent on physical proximity, and the mechanism also seem to be affected by locus specifics, underlining the requirement of systematic and context-specific approach to identify the principles governing their dynamics. This project studies the enhancer switch of the *Sox2* gene during differentiation from embryonic stem cells to neural progenitors, where *Sox2* regulation shifts from one distal enhancer (SCR, ~100 kb apart) to an even more distant one (DNE, ~ 400 kb apart). That model coupled with various perturbations allows us to investigate the relationship between epigenetic context, cohesin loop extrusion and enhancer-mediated transcription activation.

In our lab, we study enhancer-promoter communication through dynamic analysis, as we have found that chromatin mobility can sensitively reflect the transcriptional state. We use ANCHOR(*parS*/ParB) loci labeling and MS2/MCP-based nascent transcript reporting to track enhancer-promoter dynamics in real time. Upon induced cohesin degradation, we observed that while the transcriptionally active state remains largely unchanged, the OFF state of both loci becomes significantly more relaxed. This suggests that (1) transcriptional machinery dominates over architectural proteins during transcription and (2) cohesin plays a role in maintaining chromatin architecture specifically between the bursts, potentially by occupying these loci more frequently.

Future work will determine whether the extent of reliance on cohesin loop extrusion for enhancer activation alters with increased genomic distance, and to investigate which factors may mediate the apparent competition between transcriptional regulators and architectural factors. Overall, this project will provide insights into the balance between structural and transcriptional regulation of enhancer-promoter communication, particularly in the context of differentiation and changing chromatin architecture.

Keywords : enhancers, promoters, transcription, loop extrusion



Pol IV function in viral disease severity

Research area: Virology

Zoi Pentheroudaki, Christophe Himber, Pierre Videau, Olivier Zekri, Christophe Ritzenthaler, Todd Blevins

---

Plants have evolved sophisticated defense mechanisms against viral infections, with RNA interference (RNAi) providing a potent sequence-specific antiviral response. RNA virus replication generates double-stranded RNAs (dsRNAs) that are processed by plant Dicer-like (DCL) enzymes into 21-22 nt small interfering RNAs (siRNAs), which guide translational repression or cleavage of viral transcripts in the cytoplasm. Intriguingly, the nuclear 24 nt siRNA pathway mediated by RNA polymerase IV (Pol IV) is required for a recovery phenotype following certain viral infections in *Arabidopsis thaliana*, suggesting a function in modulating disease symptoms. Pol IV is an enzyme primarily known for its role in RNA-directed DNA methylation and transposon silencing. However, the mechanism by which Pol IV contributes to viral tolerance remains unclear. In this study, we investigate the role of Pol IV in viral disease severity using two distinct plant models that are susceptible to infection by *grapevine fan leaf virus* (GFLV). GFLV is a major pathogen in viticulture, responsible for the economically damaging fanleaf disease.

To explore Pol IV's function, we used a reverse genetic approach and CRISPR/Cas9 editing to generate Pol IV knockout lines in *Nicotiana benthamiana* and grapevine (*Vitis spp.*). In *N. benthamiana*, northern blot analysis confirmed the loss of 24 nt siRNA production, indicating a loss of Pol IV function. Upon infection with GFLV, *pol IV* mutants displayed characteristic mosaic symptoms and initial results suggested a higher viral titer compared to wild-type plants. Similarly, we successfully generated and validated Pol IV knockouts in grapevine. To further explore its role during infection, we plan to conduct *N. benthamiana* and grapevine grafting experiments using infected scions on *pol IV* mutant rootstocks of the corresponding species. Viral load will be quantified using ELISA and RT-qPCR, while next-generation sequencing will assess siRNA movement and epigenetic changes.

Preliminary data indicate that Pol IV is involved in limiting GFLV accumulation, as *N. benthamiana* Pol IV mutants displayed clear viral symptoms seven days post-infection, whereas wild-type plants remained asymptomatic. Understanding this mechanism could offer novel insights into plant antiviral defense strategies and unravel the role of Pol IV in viral disease severity through its effect on viral accumulation.

Keywords: Pol IV, Virus, CRISPR/Cas9, RdDM, Epigenetics

[IN IMMUNE-MEDIATED NECROTISING MYOPATHY, ANTI-HMGCR ANTIBODIES INHIBIT HMGCR ACTIVIY AND LEAD TO MYOPATHIC LIPID DROPLETS ACCUMULATION IN MYOFIBRES]

Research area: [Inflammation]

[Giulia Quiring], [Mustapha Oulad-Abdelghani], [Béatrices Lannes], [Yves Allenbach], [Olivier Benveniste], [Olivier Boyer], [Aleksandra Nadej-Pakleza], [Bernard Geny], [Margherita Giannini], [Alain Meyer]

Anti-HMGCR antibodies are a biomarker of immune-mediated necrotizing myopathy (IMNM), a subtype of inflammatory myopathies (IM) characterized by weakness and myofiber necrosis of unknown mechanism. These antibodies are internalized into myofibres and may disrupt HMGCR function. However, no direct evidence of this hypothesis has been reported to date.
The aim of this study was to investigate whether anti-HMGCR antibodies interfere with HMGCR activity and have a myopathic effect.

Anti-HMGCR and control antibodies were obtained from rabbits and plasmapheresis eluates from IM patients (anti-HMGCR n=5, and anti-SRP n=2). HMGCR activity was assessed by spectrophotometry in the presence of autoantibodies or pravastatin. Human antibodies were electroporated into human myotubes, followed by H&E and oil red-O staining. Thirty-four IM patients (anti-HMGCR: n=10; other IM: n=24) and 3 no-NMD controls were included. Muscle biopsies were stained for IgG and lipid droplets, scored (0–4) by blinded myopathologists.

To test whether anti-HMGCR antibodies inhibit the function of their target, HMGCR enzymatic activity was assessed in vitro in the presence of anti-HMGCR, control antibodies and pravastatin. A dose-dependent inhibition of HMGCR activity, similar to pravastatin, was observed with rabbit and human anti-HMGCR, but not with control IgG or anti-SRP plasma.
To test whether anti-HMGCR internalization in myofibres exerts a myopathic effect, human myotubes were electroporated with human purified anti-HMGCR, control IgG or pravastatin. Antibody presence in the cytoplasm 4 days after electroporation was confirmed. H&E staining showed necrotic human myotubes after electroporation with purified anti-HMGCR and pravastatin, but not with control IgG. Oil red-O staining revealed a lipid droplet accumulation in human myotubes electroporated with purified anti-HMGCR and pravastatin, but not with control IgG; This finding was also observed in patients. Lipid droplet accumulation score was ten-fold higher in anti-HMGCR IMNM patients compared to other IM and no NMD patients (3.2 ± 0.6 vs. 0.3 ± 0.5; vs. 0.3 ± 0.6, respectively, $p=0.0001$). A score ≥ 2 was a hallmark of anti-HMGCR IMNM.

Together, these data demonstrate that anti-HMGCR antibodies inhibit HMGCR activity leading to accumulation of myopathic lipid droplets in myofibres. These findings could have potential implications for both the diagnosis and treatment of IMNM.

Chronic pain is linked to a neural archetype that optimizes learning from punishments

Research area: Clinical and cognitive neuroscience

Francesco Scarlatti, Ludovic Dormegny-Jeanjean, Roman Schefzik, Emanuel Schwarz, Martin Löffler, Jack R. Foucher, Herta Flor

Chronic pain is a leading cause of disability, yet its underlying susceptibility traits remain unclear. Disorders like chronic pain may stem from extreme neural types, or archetypes, optimized for specific cognitive functions and reflected in patterns of resting-state networks. We examined a sample from the general population (n = 892) and three clinical samples with subacute back pain (n = 76), chronic back pain (n = 30), and treatment-resistant depression (n = 24). Using the sample from the general population, we found three neural archetypes that optimize different cognitive strategies. Clinical pain samples, compared to the sample from the general population, mapped close to an archetype that optimize learning from punishments (Archetype P). We independently replicated these archetypes in the clinical pain samples, revealing an association between Archetype P and pain severity. These findings suggest a neural-cognitive trait underlying chronic pain susceptibility.

Keywords: Chronic pain; Instrumental learning; Punishments; Resting-state; Functional magnetic resonance imaging

Blitz Talks

The co-inhibitory receptor BTLA: Towards a better understanding of altered BTLA expression in lupus cell subsets

Research area: Immunology

Mélanie SAYAH, Hélène Dumortier, Fanny Monneaux.

Co-inhibitory receptors are essential for maintaining immune homeostasis and preventing autoimmune diseases such as systemic lupus erythematosus (SLE). SLE is characterized by the production of autoantibodies directed against nuclear components, leading to immune complex deposition in vital organs, which can be life-threatening. B and T lymphocyte attenuator (BTLA) is a co-inhibitory receptor expressed on various immune cells. BTLA-deficient lupus mice show increased disease severity, highlighting a protective role for BTLA in immune regulation and in the context of SLE.

Our team's previous work has demonstrated impaired functionality of BTLA signaling in lupus $CD4^+$ T cells (Sawaf *et al.*, 2018), altered BTLA expression in activated regulatory T (aTreg) cells (Aubergeon *et al.,* 2021), and in double negative memory (DN) B cells (Aubergeon *et al*., 2024) from lupus patients. More recently, we showed that administrating an agonist anti-BTLA antibody to lupus-prone NZB/W mice had therapeutic effects (Gherardi *et al*., 2025), suggesting BTLA as a promising target for the development of similar strategies in humans. However, effective therapeutic targeting of BTLA in SLE remains challenging due to its dysregulated expression: BTLA is highly expressed on aTreg cells, which should be preserved, whereas it is poorly expressed or non-functional on pathogenic DN B cells and $CD4^+$ T cells, which are key disease effectors.

Therefore, restoring BTLA expression and functionality in relevant immune subsets is crucial. The aim of my project is to decipher the molecular and cellular mechanisms regulating BTLA expression. We will explore: i) the role of HVEM, BTLA's ligand, in both its membrane and soluble forms, on BTLA expression; ii) the influence of lupus-associated cytokines on BTLA expression, including blocking antibody experiments and correlation studies; and iii) the contribution of specific microRNA to BTLA regulation in key cell subsets. This project may pave the way for a three-step therapeutic strategy: first, evaluating BTLA expression and function in each patient; second, correcting BTLA alterations with tailored treatments; and finally, therapeutically targeting BTLA in SLE patients using an agonist anti-BTLA antibody.

Keywords: Autoimmunity, systemic lupus erythematosus, inhibitory receptors, B and T lymphocyte attenuator.

Piezo1 is expressed and functional in bone marrow endothelial cells

Research area : Mechanobiology

Marine Devaux[1], *Noémie Brassard-Jollive*[1], *Laurie Ruch*[1], *Josiane Weber*[1], *Thiebault Lequeu*[1], *Clara Bache*[1], *Manuel Koch*[2], *Nathalie Brouard*[1], *Rémi Peyronnet*[2], *Catherine Léon*[1]

[1]*UMR_S1255, INSERM, Etablissement Français du Sang Grand-Est, Université de Strasbourg, Strasbourg, France*

[2]*Institute for Experimental Cardiovascular Medicine, University Heart Center Freiburg Bad Krozingen, and Faculty of Medicine, University of Freiburg, Freiburg, Germany*

Platelets are crucial for haemostasis. They are produced in the bone marrow by megakaryocytes (MK), giant cells which, once mature, are located along the sinusoids vessels. MK extend podosomes that push on bone marrow endothelial cells (BMEC) to create transendothelial pores, so pro-platelets can enter the blood stream. In this process, a complex set of mechanical forces is at play. We aim to understand the role of BMEC stretch activated channels (SAC) during MK transendothelial passage.

Primary cultures of BMEC were isolated by enzymatic digestion from Wild Type (WT) mice and SAC activity was assessed by patch clamp experiments using the cell-attached configuration. BMEC plasma membrane was stretched by applying increasing negative pressure pulses via the patch pipette. Our results show the presence of stretch-induced currents at the BMEC plasma membrane. Single channel analysis suggests the presence of cation non-selective SAC. Nanoindentation measurements on WT BMEC showed decreased stiffness in the presence of Gadolinium (SAC inhibitor). RT-qPCR was performed on WT BMEC to identify SAC expression level. We identified Piezo1 as the most expressed. Using Piezo1 Knock-in (Ki) mice we observed increased stretch-induced compared to WT BMEC.

Our results show that BMEC express functional SAC at their plasma membrane, which modulate cell stiffness, among which Piezo1 is functional. Further investigations will assess Piezo1 involvement in BMEC response to MK-mediated mechanical cues and overall transmigration.

Keywords : **Piezo1, stiffness, endothelial cell, bone marrow, patch clamp**

[Dissecting the Promoter-Enhancer landscape in TNBC cancer stem cells to understand their role therapy resistance.]

Research area : [Cancer,genomics]

[Karan Joshi], [Dr. Thomas Sexton], [Cathie Erb]...

Triple Negative Breast cancer is 20% of all the breast cancer incidences in humans. Their classification is by their exclusion from the other major forms of breast cancers which have predominant markers (namely: ER[estrogen receptor], PR[progesterone receptor], HER2+[human epidermal growth factor receptor 2]). So naturally they are extremely heterogenous in their dynamics. It is the most aggressive form of breast cancer, and is often detected at an advanced stage. Its prognosis is very bleak, and the probability of recurrence of this type is very high. They are also difficult to design targeted therapies for (due to the absence of the usual receptor targets). Often cancers are considered enhanceropathies as cancers often target or use enhancer dysregulation as a mode to either metastasize or escape therapy altogether. This is primarily due to the existence of cancer stem cells.
This project aims to unravel the Promoter-enhancer landscape of cancer stem cells to understand the underlying framework of their role in tumor survival and therapy resistance. To do so we're combining various levels of genomic and epigenomic techniques like Promoter-Capture Hi-C, CUT&TAG and Single-Cell multi-omics to identify the key enhancers and their downstream targets involved in maintaining stemness in cancer and also how they change in response to epigenetic drugs (BETinhibitors). Overall the project will provide insight in which enhancers might be crucial for the tumor to maintain itself and allow us to design more precise targeted therapies against them

---

Keywords : **[Cancer], [Therapy resistance], [Epigenomics].**

Study of the impact of pervasive transcription in *Staphylococcus aureus* lacking RNA-binding proteins involved in its control, after 1000 generations of evolution

Research area : Microbiology, molecular biology and evolution biology

Théo Markezic, Isabelle Caldelari, Alexandre Smirnov

Bacterial transcription relies on three main components: the core RNA polymerase (RNAP), a sigma (σ) factor, and a DNA template with defined promoter and terminator sequences. Transcription begins at the promoter, typically an AT-rich region. However, because genomes naturally contain many AT-rich areas, some of these can inadvertently act as cryptic promoters, leading to **pervasive transcription** that initiates outside of canonical gene promoters.

This phenomenon can be costly or harmful. It wastes energy by producing non-functional RNAs and frequently occurs in antisense orientation, which can repress sense gene expression. It also promotes R-loop formation (RNA-DNA hybrids), increasing the chances of collisions with replication machinery and compromising genome stability. Furthermore, excessive transcription alters DNA supercoiling, which can interfere with proper transcription and replication. To control these effects, bacteria use factors like **Rho**, which terminates transcription of unwanted RNAs, and **RNase III**, which degrades RNA duplexes post-transcriptionally.

Given its costs, the persistence of pervasive transcription across evolution is puzzling. Why hasn't it been eliminated? One hypothesis is that, despite its drawbacks, it might serve as an evolutionary engine by producing novel RNAs that could gain function over time. This raises two fundamental questions:

1. Does pervasive transcription contribute to evolutionary innovation?
2. If we stop repressing pervasive transcription, how will organisms adapt to this global transcriptomic disruption?

To explore this, we performed an **experimental evolution** study using ***Staphylococcus aureus***. We evolved 18 populations over 1,000 generations, including wild-type strains, Δ*rho* mutants and *rnc*⁻ mutants. This setup allows us to assess how pervasive transcription shapes bacterial growth, energy use, genome integrity, and RNA networks over time. The phenotypic characterization of these evolved populations is currently ongoing.

Keywords: Pervasive trasncritpion, Rho, RNase III, experimental evolution, *Staphylococcus aureus*

The function of m6A mRNA modification during meiosis in yeast

Research area: Cellular and Molecular Biology

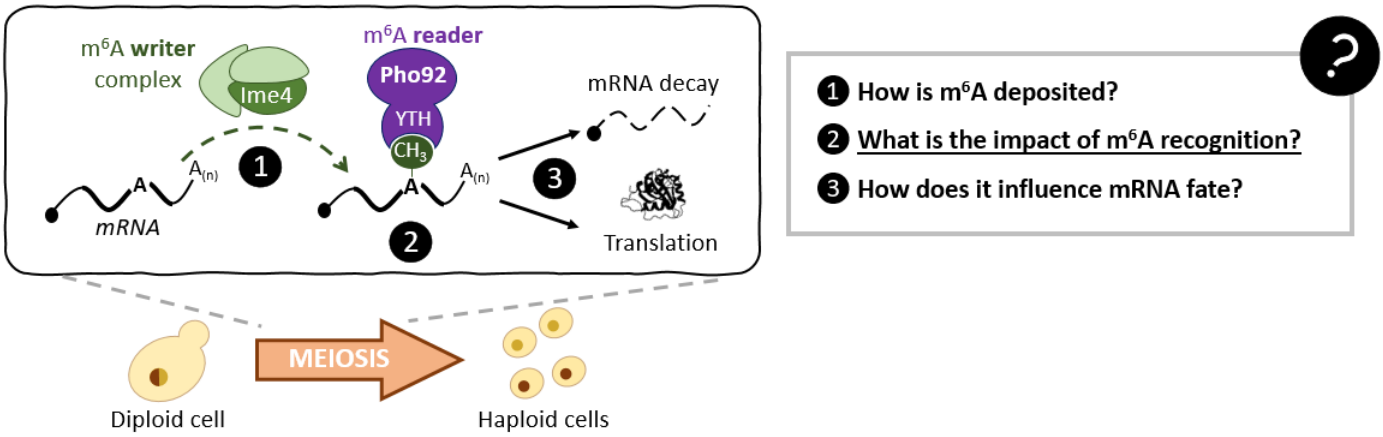
Lina Sène, 2nd year PhD Student, Séraphin's team (IGBMC)

Most eukaryotic messenger RNAs (mRNAs) transcribed by polymerase II are post-transcriptionally modified with addition of a 5' cap as well as a 3' poly(A) tail, and splicing of introns. Some internal A residues are also methylated to form N6-methyladenosine (m6A) contributing to the regulation of gene expression and beyond to the control of development or pathologies. This modification plays crucial roles in regulating mRNA fate—affecting splicing, stability, export, translation, and degradation. Despite its importance, the molecular mechanisms through which m6A exerts its functions remain incompletely understood, largely due to the limited number of functionally validated m6A sites.

In the model organism *Saccharomyces cerevisiae*, m6A formation occurs only during meiosis and is catalyzed by the MIS "writer" complex, which includes the methyltransferase Ime4 (ortholog of METTL3). A previous study from our team identified Pho92, a YTH domain-containing "reader" protein (homologous to YTHDF1), that binds specific m6A sites during meiosis. This interaction influences downstream mRNA processes, most likely involving translation regulation and mRNA decay. Notably, disruption of a single methylated adenosine was shown to delay meiotic progression, underlining the functional importance of m6A in this context.

My PhD project aims to investigate the role of m6A modifications during yeast meiosis. Specifically, I am focusing on understanding how m6A impacts the stability and translation of mRNAs and how Pho92 mediates these effects via specific interaction with other proteins during meiosis. Through a combination of genetic, molecular, and biochemical approaches, this research contributes to deciphering the biological significance of m6A and the broader landscape of epitranscriptomic regulation in a well-defined and tractable model system.

Keywords : **Methylation, mRNA, Regulation, Meiosis, Yeast**



A novel ELISA for Universal Viral Detection: Rapid and Cost-Effective dsRNA Quantification

Research area : Molecular Biology

Thanos Xhurxhi, *Hélène Scheer, Daniel Clesse, Subhankar Sahu, Emilie Vantard and Christophe Ritzenthaler*

Viral replication universally produces double-stranded RNA (dsRNA), making its detection a powerful approach for broad-spectrum diagnostics. It is also the most common contaminant in mRNA vaccine production. Conventional antibody-based ELISAs for dsRNA can be expensive, laborious, and difficult to bioengineer and produce. To address these challenges, we developed a flexible sandwich ELISA platform using recombinant dsRNA-binding proteins as both capture and detection agents. Two binding ligands were compared: the Flock House virus B2 domain and a novel double stranded binding protein (dRBP). Each ligand was genetically fused to either alkaline phosphatase (ALP) for a colorimetric readout or NanoLuciferase (nLuc) for bioluminescent detection.

In assays employing B2 as both capture and detection moieties (B2/B2 ELISA), we achieved high sensitivity in the low-nanogram range and excellent specificity for dsRNA, with minimal to no response for other nucleic acids. To further enhance performance, we tested the dRBP fusions to serve as detection agents in a hybrid B2/dRBP ELISA. Although the ALP-dRBP combination was unsuccessful, the nLuc-dRBP assay outperformed B2/B2-based formats, delivering a sub-nanogram per milliliter detection limit and robust quantification of natural and synthetic dsRNA analogs with high specificity.

We tested these ELISAs on real-life infected samples with clarified crude leaf extracts. Both B2- and dRBP-based assays clearly distinguished infected from healthy tissue in *Nicotiana benthamiana* leaf samples infected by a plethora of viruses like, Turnip mosaic virus and Tomato bushy stunt virus amongst others.

Our dsRNA binding protein ELISAs offer a rapid, cost-effective, and RNA extraction-free method for universal dsRNA quantification, outperforming commercially available kits in sensitivity and cost-efficiency. They hold promise for applications in plant virology, vaccine production quality control, and point-of-care pathogen surveillance. Future work will integrate these assays with sequencing workflows to enable simultaneous dsRNA quantification and strain identification.

Keywords: **dsRNA detection, ELISA, B2 FHV, viral diagnostics, plant virology, mRNA vaccines**

Consequences of a STING gain-of-function on the Phenotype and Tolerance of B lymphocytes

Research area : Immunology

Grégoire Hopsomer (PhD student, supervised by Pr. Pauline Soulas-Sprauel)

STING (Stimulator of Interferon Genes) is a central adaptor of cytosolic DNA sensing pathways, constitutively activated by gain-of-function (GOF) mutations found in a monogenic autoinflammatory disease designated as SAVI (STING-Associated Vasculopathy with onset in Infancy). While the role of STING in innate immune responses is well established, its impact on B cell biology remains largely unexplored.

Using a knock-in mouse model harboring the heterozygous STING GOF mutation V154M, we are currently investigating how chronic STING activation affects B cell phenotype. Preliminary results reveal a marked B cell lymphopenia, accompanied by a hyperactivated phenotype. To better characterize this profile, we are analyzing how STING GOF alters signaling downstream of key B cell receptors, including BCR, CD40, and TLRs, through in vitro stimulation assays and molecular approaches.

Given the frequent association of type I interferonopathies with autoantibody production, we also plan to explore whether STING GOF contributes to a breakdown in peripheral B cell tolerance.

This project will help define the role of STING in the regulation of B cell fate and the maintenance of immune homeostasis.

Keywords : **Autoimmunity, B cell, STING, Interferonopathies**

[Development of functional nucleic acids in non-conventional solvents using droplet microfluidics]

Research area: Molecular Biology

[Emma Tixier], [PhD. Magali Frugier], [Prof. Michael Ryckelynck]

Aptamers are small nucleic acids typically developed in vitro by SELEX to specifically recognize all types of molecules. Compared to antibodies, aptamers are highly stable (especially in DNA or modified nucleotides), inexpensive to synthesize in large quantities and non-immunogenic. In addition, several aptamers can be combined to create tools with complex functions. My team has a strong expertise in the design of RNA aptamers capable of forming a fluorescent complex with a fluorogen, so that their functionality can be easily measured by fluorometry, so they can be used for biosensing or cell imaging.

To date, such studies have been carried out only in aqueous media. However, although nucleic acids are soluble and functional in aqueous media, they are also particularly sensitive to pH, nucleases and salts. To overcome these drawbacks, we are exploring the development of aptamers in ionic liquids. Indeed, the unique physicochemical properties of ionic liquids, such as their structuring effect and tunability, make them suitable solvents and stabilizing media in chemical synthesis, electrochemistry, and even biotechnology.

By combining ionic liquids with droplet-based microfluidics, a high-throughput technology that allows *in vitro* compartmentalization and screening of millions of molecules, we hope to accelerate the identification of robust aptamers that remain functional in conditions of high salinity and complexity, such as environmental and biological media.

Keywords: **[Aptamers], [Ionic Liquids], [Droplet microfluidics], [Directed evolution], [Fluorescence]**

Antigen targeting to dendritic cells using functionalized plant-derived virus like particles

Research area: Immunology

Anaëlle Utard, Vianney Poignavent, Hélène Dumortier

In the context of cancer, therapeutic vaccines aim to educate the immune system to recognize and eliminate cancer cells by targeting specific tumor antigens at their surface. Dendritic cells are the immune response initiating cells thanks to their ability to capture and present antigens to specific T lymphocytes, thus activating them. In particular, conventional dendritic cells type 1 (cDC1) play a critical role in pathogen defense and immunological tumor rejection, indeed they are specialized in performing antigen cross-presentation, thereby linking innate and adaptative immunity. They are therefore a relevant cell target for antitumoral vaccine development.

My thesis project thus aims to validate the use of virus-like particles (VLP) as an antitumoral therapeutic vaccination platform targeting cDC1.

For that purpose, non-infectious self-assembling virus-like particles are produced in plants using the capsid protein of the Grapevine Fanleaf Virus (VLP_{GFLV}). The VLPs' inner cavity contains the model antigen ovalbumin (OVA), while the outer surface is functionalized with a nanobody coupled to an anti-Clec9a antibody so that the VLPs are addressed to cDC1 which specifically express the C-type lectin. We evaluated *in vivo* the ability of Clec9A-targeted $^{OVA}VLP_{GFLV}$ to promote specific lysis of target cells and assessed the endogenic OVA-specific CD8 T lymphocyte activation.

We demonstrated that addressing $^{OVA}VLP_{GFLV}$ to cDC1 efficiently promotes T lymphocyte activation resulting, *in vivo,* in an efficient antigen-specific cytotoxic T lymphocyte response. Notably, we found that $^{OVA}VLP_{GFLV}$ do not present self-adjuvanting properties, thus the co-administration of a DC maturation agent (poly I:C) is required. We also showed that $^{OVA}VLP_{GFLV}$ prophylactic vaccination delays the growth of B16-OVA melanoma tumor. Finally, we will assess whether $^{OVA}VLP_{GFLV}$ therapeutic vaccination is efficient against the same tumor model, using a multiple injections schedule and extend this proof-of-concept to other tumor models using more physiologically relevant antigens than OVA.

To conclude, our results demonstrate that the use of cDC1-targeting antigen-containing VLP_{GFLV} is a promising strategy to generate specific and cytotoxic anti-tumoral immunity.

Keywords: virus-like particles, dendritic cells, therapeutic vaccine, cancer

Multi-omics study of intracellular transport defects impacting focal adhesion in myotubular myopathy in mice

Research area : Translational Research

Supriya Priyadarshani SWAIN[1], David REISS[1], Alice MONCHEAUX[1], Nadege DIEDHIOU[1], Sarah DJEDDI[1], Jacqueline JI[1], Marie GORET[1], Jocelyn LAPORTE[1]

[1] Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), Illkirch-Graffenstaden, France

---

X-linked myotubular myopathy (XLMTM) is rare and a severe form of centronuclear myopathy (CNM) caused by the loss-of-function mutations in *Myotubularin 1* (*MTM1).* Previous studies have reported significant impairments in focal adhesion dynamics and integrin intracellular localization in 8 weeks *Mtm1*$^{-/y}$ mice. However, the underlying causes of these defects and their progression across different disease stages remain poorly understood.

To address this, we performed transcriptomic and proteomic analyses on the *Mtm1*$^{-/y}$ mouse model at pre-symptomatic (E18.5), early (2w) and late (7w) developmental stages. Our results reveal that the pathways related to intracellular transportation, integrin activation and recycling, vesicle trafficking, and extracellular matrix (ECM) organization were consistently altered. An early upregulation of caveolin-dependent endocytosis and ECM components along with impaired fast recycling of integrins, were observed. These findings were confirmed by measuring the mRNA and protein expression. This ultimately led to the intracellular accumulation of active β1-integrin at the late disease stage. In silico analyses further indicated that these defects occur at the early endosomal level due to the absence of MTM1 and not at the late endosomal stage. Additionally, in-vitro studies have validated the overexpression of slow recycling transporter and caveolins during the later stages of disease progression.

Overall, these findings suggest that major defects in various intracellular transport systems have impact on the dysregulation of focal adhesion and cytoskeleton dynamics. This highlights intracellular transporters as a strong promising therapeutic targets for restoring cellular homeostatis at early disease stages in *Mtm1KO* mouse models.

Keywords : *Myotubularin 1 (MTM1), Intracellular transport, Integrin signalling, Extracellular Matrix (ECM), Multi-omics*

Posters

Characterization of a new key factor in the STING pathway in Drosophila

Research area : [Drosophila Innate Immunity]

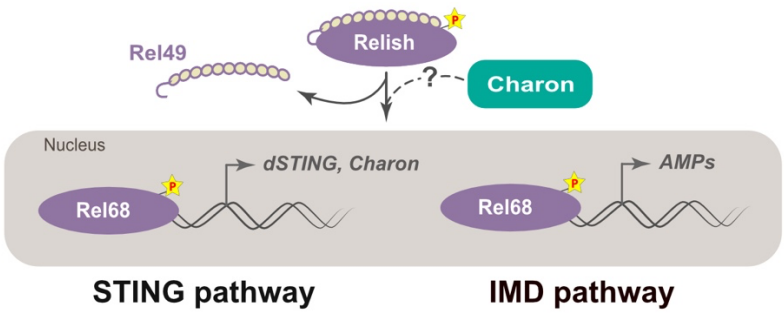
[Taïma Lorentzen]

A constant arms race between host and virus has shaped the innate immunity system of all living organisms, resulting in a diverse range of anti-viral responses. However, some pathways are conserved across evolution, such as the STING pathway, which was caracterised in humans as a pathway regulating the NF-kB response and IFN responses (Ablasser and Chen, 2019). The STING pathway was recently characterized in Drosophila (Cai et al., 2020, 2023) (Goto et al., 2018) , as a pathway activated by the detection of cGAMP, syntesized upon viral RNA detection. My lab has recently shown that the STING pathway actually shares a lot of common factors with an anti-bacterial innate immune pathway, the Immunodeficency (IMD) pathway. Indeed, both pathways are activated by different molecules, but converge towards activation and cleavage of the Relish Transcription factor. Once cleaved, Rel68 translocates to the nucleus and initiates either the transcription of the STING regulated genes, or antimicrobial peptides.

To uncover the mechanism behind this difference in transcriptional responses, I chose to work on the Charon protein, as it's coded by a gene regulated by the STING pathway. Furthermore, Charon has been reported to interact with Relish in two papers that have opposing conclusions concerning Charon's regulatory role within the IMD pathway.

To study Charon's regulatory role, I used dual luciferase assays in Drosophila cells to follow STING and IMD pathway activity. My results show that Charon is actually necessary for the STING pathway and dispensable for the IMD pathway. I have also been able to show that Charon does act downstream of Rel68, making it a key factor of STING pathway. In addition, I have been establishing a map of Charon's interactants, hoping to understand the mechanism by which it can regulate the STING pathway.

Keywords : [STING], [IMD], [regulation], [Charon], [NF-KB]



Piezo1 is expressed and functional in bone marrow endothelial cells

Research area : Mechanobiology

Marine Devaux[1], *Noémie Brassard-Jollive*[1], *Laurie Ruch*[1], *Josiane Weber*[1], *Thiebault Lequeu*[1], *Clara Bache*[1], *Manuel Koch*[2], *Nathalie Brouard*[1], *Rémi Peyronnet*[2], *Catherine Léon*[1]

[1]*UMR_S1255, INSERM, Etablissement Français du Sang Grand-Est, Université de Strasbourg, Strasbourg, France*

[2]*Institute for Experimental Cardiovascular Medicine, University Heart Center Freiburg Bad Krozingen, and Faculty of Medicine, University of Freiburg, Freiburg, Germany*

Platelets are crucial for haemostasis. They are produced in the bone marrow by megakaryocytes (MK), giant cells which, once mature, are located along the sinusoids vessels. MK extend podosomes that push on bone marrow endothelial cells (BMEC) to create transendothelial pores, so pro-platelets can enter the blood stream. In this process, a complex set of mechanical forces is at play. We aim to understand the role of BMEC stretch activated channels (SAC) during MK transendothelial passage.

Primary cultures of BMEC were isolated by enzymatic digestion from Wild Type (WT) mice and SAC activity was assessed by patch clamp experiments using the cell-attached configuration. BMEC plasma membrane was stretched by applying increasing negative pressure pulses via the patch pipette. Our results show the presence of stretch-induced currents at the BMEC plasma membrane. Single channel analysis suggests the presence of cation non-selective SAC. Nanoindentation measurements on WT BMEC showed decreased stiffness in the presence of Gadolinium (SAC inhibitor). RT-qPCR was performed on WT BMEC to identify SAC expression level. We identified Piezo1 as the most expressed. Using Piezo1 Knock-in (Ki) mice we observed increased stretch-induced compared to WT BMEC.

Our results show that BMEC express functional SAC at their plasma membrane, which modulate cell stiffness, among which Piezo1 is functional. Further investigations will assess Piezo1 involvement in BMEC response to MK-mediated mechanical cues and overall transmigration.

Keywords : **Piezo1, stiffness, endothelial cell, bone marrow, patch clamp**

[Dissecting the Promoter-Enhancer landscape in TNBC cancer stem cells to understand their role therapy resistance.]

Research area : [Cancer,genomics]

[Karan Joshi], [Dr. Thomas Sexton], [Cathie Erb]...

Triple Negative Breast cancer is 20% of all the breast cancer incidences in humans. Their classification is by their exclusion from the other major forms of breast cancers which have predominant markers (namely: ER[estrogen receptor], PR[progesterone receptor], HER2+[human epidermal growth factor receptor 2]). So naturally they are extremely heterogenous in their dynamics. It is the most aggressive form of breast cancer, and is often detected at an advanced stage. Its prognosis is very bleak, and the probability of recurrence of this type is very high. They are also difficult to design targeted therapies for (due to the absence of the usual receptor targets). Often cancers are considered enhanceropathies as cancers often target or use enhancer dysregulation as a mode to either metastasize or escape therapy altogether. This is primarily due to the existence of cancer stem cells.
This project aims to unravel the Promoter-enhancer landscape of cancer stem cells to understand the underlying framework of their role in tumor survival and therapy resistance. To do so we're combining various levels of genomic and epigenomic techniques like Promoter-Capture Hi-C, CUT&TAG and Single-Cell multi-omics to identify the key enhancers and their downstream targets involved in maintaining stemness in cancer and also how they change in response to epigenetic drugs (BETinhibitors). Overall the project will provide insight in which enhancers might be crucial for the tumor to maintain itself and allow us to design more precise targeted therapies against them

---

Keywords : **[Cancer], [Therapy resistance], [Epigenomics].**

Study of the impact of pervasive transcription in *Staphylococcus aureus* lacking RNA-binding proteins involved in its control, after 1000 generations of evolution

Research area : Microbiology, molecular biology and evolution biology

Théo Markezic, Isabelle Caldelari, Alexandre Smirnov

Bacterial transcription relies on three main components: the core RNA polymerase (RNAP), a sigma (σ) factor, and a DNA template with defined promoter and terminator sequences. Transcription begins at the promoter, typically an AT-rich region. However, because genomes naturally contain many AT-rich areas, some of these can inadvertently act as cryptic promoters, leading to **pervasive transcription** that initiates outside of canonical gene promoters.

This phenomenon can be costly or harmful. It wastes energy by producing non-functional RNAs and frequently occurs in antisense orientation, which can repress sense gene expression. It also promotes R-loop formation (RNA-DNA hybrids), increasing the chances of collisions with replication machinery and compromising genome stability. Furthermore, excessive transcription alters DNA supercoiling, which can interfere with proper transcription and replication. To control these effects, bacteria use factors like **Rho**, which terminates transcription of unwanted RNAs, and **RNase III**, which degrades RNA duplexes post-transcriptionally.

Given its costs, the persistence of pervasive transcription across evolution is puzzling. Why hasn't it been eliminated? One hypothesis is that, despite its drawbacks, it might serve as an evolutionary engine by producing novel RNAs that could gain function over time. This raises two fundamental questions:

1. Does pervasive transcription contribute to evolutionary innovation?
2. If we stop repressing pervasive transcription, how will organisms adapt to this global transcriptomic disruption?

To explore this, we performed an **experimental evolution** study using ***Staphylococcus aureus***. We evolved 18 populations over 1,000 generations, including wild-type strains, Δ*rho* mutants and *rnc*⁻ mutants. This setup allows us to assess how pervasive transcription shapes bacterial growth, energy use, genome integrity, and RNA networks over time. The phenotypic characterization of these evolved populations is currently ongoing.

Keywords: Pervasive trasncritpion, Rho, RNase III, experimental evolution, *Staphylococcus aureus*

A story of a frog, a fungus and some bacteria:

Microbiome mediated colonisation resistance via siderophore production

Research area : Microbiology

Christos Paschalidis[1], *Kieran Bates*[2] *and Olivier Cunrath*[1]

[1] ESBS, CNRS, Université de Strasbourg, France

[2] Queen Mary University of London, United Kingdom

Batrachochytrium dendrobatidis (Bd) is a devastating fungal pathogen that has led to catastrophic declines in amphibian populations worldwide. Responsible for the disease chytridiomycosis, *Bd* threatens up to 50% of all amphibian species worldwide leading to the single biggest extinction crisis in vertebrates. *Bd* zoospores colonise amphibian's skin, leading to skin thickening, disrupted skin permeability, and electrolyte imbalances, ultimately leading to cardiac arrest and death. Understanding the mechanisms involved in host-pathogen interactions is essential for developing effective strategies to combat this epidemic. One of the main host defense mechanisms against invading pathogens is nutritional immunity, wherein the host restricts essential nutrients, such as metals, to impede pathogen growth. In addition to nutritional immunity, the skin microbiome plays a pivotal role in shaping the outcome of infection. The skin of amphibians harbors diverse microbial communities, which have been shown to also protect against Bd, notably by producing antifungal peptides. While the importance of the skin microbiome is evident, it remains unclear how host-driven metal starvation affects these microbial communities. It is plausible that metal restriction by the host may influence the composition and functionality of the skin microbiome, potentially altering the overall protective capacity against *Bd*.

Our research suggests that under iron deficiency, the skin microbiome may contribute to host defense by producing its own iron-chelating molecules called siderophores. These metabolites, with potent anti-fungal properties, are produced in metal limiting conditions similar to those encountered during infection suggesting that they may act in synchrony with host-induced nutritional immunity. With our collaborator Dr. Kieran Bates, we constructed a minimal bacterial community representing the most abundant bacterial phylogroups of the amphibian's skin. Furthermore, we have developed a high-throughput pipeline to rapidly identify siderophore producing strains. This allowed us to show that most isolates tested so far, produce high amounts of siderophores under iron limitation. Finally, we have tested a multitude of strains for their inhibitory effect on the growth of *Bd*. These growth inhibition assays revealed that 1. *Bd* is unable to exploit any of the tested siderophores, 2. *Bd* lacks competitive iron uptake systems; 3. nor it is equipped with systems able to degrade these metabolites. Exploring the effect of these specific metabolites and understanding how their production affects pathogenesis could open novel avenues for managing chytridiomycosis.

Keywords : **Siderophores, skin microbiome, Bd, amphibians, infection, metals, iron**

The function of m6A mRNA modification during meiosis in yeast

Research area: Cellular and Molecular Biology

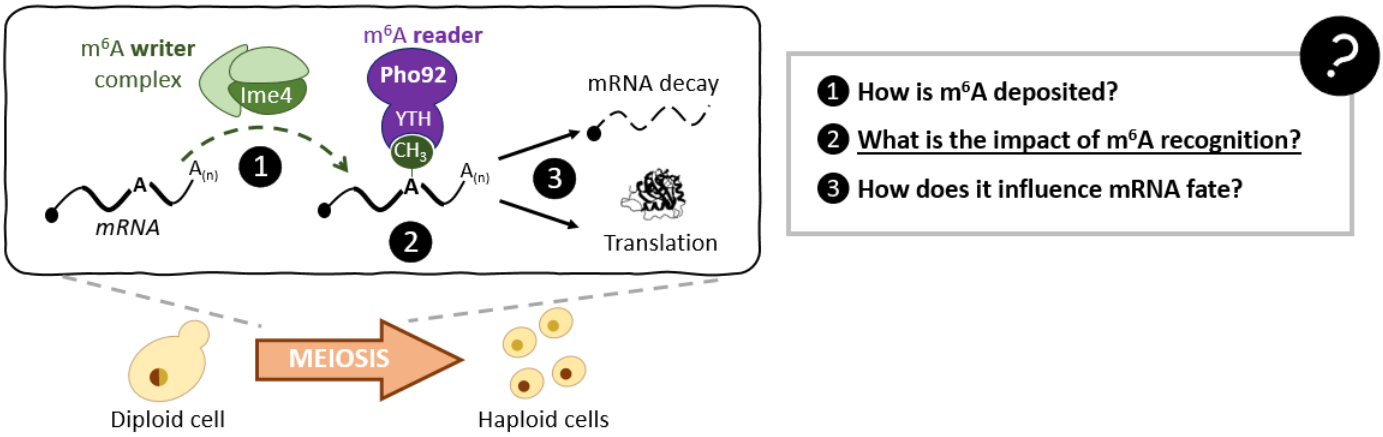
Lina Sène, 2nd year PhD Student, Séraphin's team (IGBMC)

Most eukaryotic messenger RNAs (mRNAs) transcribed by polymerase II are post-transcriptionally modified with addition of a 5' cap as well as a 3' poly(A) tail, and splicing of introns. Some internal A residues are also methylated to form N6-methyladenosine (m6A) contributing to the regulation of gene expression and beyond to the control of development or pathologies. This modification plays crucial roles in regulating mRNA fate—affecting splicing, stability, export, translation, and degradation. Despite its importance, the molecular mechanisms through which m6A exerts its functions remain incompletely understood, largely due to the limited number of functionally validated m6A sites.

In the model organism *Saccharomyces cerevisiae*, m6A formation occurs only during meiosis and is catalyzed by the MIS "writer" complex, which includes the methyltransferase Ime4 (ortholog of METTL3). A previous study from our team identified Pho92, a YTH domain-containing "reader" protein (homologous to YTHDF1), that binds specific m6A sites during meiosis. This interaction influences downstream mRNA processes, most likely involving translation regulation and mRNA decay. Notably, disruption of a single methylated adenosine was shown to delay meiotic progression, underlining the functional importance of m6A in this context.

My PhD project aims to investigate the role of m6A modifications during yeast meiosis. Specifically, I am focusing on understanding how m6A impacts the stability and translation of mRNAs and how Pho92 mediates these effects via specific interaction with other proteins during meiosis. Through a combination of genetic, molecular, and biochemical approaches, this research contributes to deciphering the biological significance of m6A and the broader landscape of epitranscriptomic regulation in a well-defined and tractable model system.

Keywords : **Methylation, mRNA, Regulation, Meiosis, Yeast**



A novel ELISA for Universal Viral Detection: Rapid and Cost-Effective dsRNA Quantification

Research area : Molecular Biology

Thanos Xhurxhi, *Hélène Scheer, Daniel Clesse, Subhankar Sahu, Emilie Vantard and Christophe Ritzenthaler*

Viral replication universally produces double-stranded RNA (dsRNA), making its detection a powerful approach for broad-spectrum diagnostics. It is also the most common contaminant in mRNA vaccine production. Conventional antibody-based ELISAs for dsRNA can be expensive, laborious, and difficult to bioengineer and produce. To address these challenges, we developed a flexible sandwich ELISA platform using recombinant dsRNA-binding proteins as both capture and detection agents. Two binding ligands were compared: the Flock House virus B2 domain and a novel double stranded binding protein (dRBP). Each ligand was genetically fused to either alkaline phosphatase (ALP) for a colorimetric readout or NanoLuciferase (nLuc) for bioluminescent detection.

In assays employing B2 as both capture and detection moieties (B2/B2 ELISA), we achieved high sensitivity in the low-nanogram range and excellent specificity for dsRNA, with minimal to no response for other nucleic acids. To further enhance performance, we tested the dRBP fusions to serve as detection agents in a hybrid B2/dRBP ELISA. Although the ALP-dRBP combination was unsuccessful, the nLuc-dRBP assay outperformed B2/B2-based formats, delivering a sub-nanogram per milliliter detection limit and robust quantification of natural and synthetic dsRNA analogs with high specificity.

We tested these ELISAs on real-life infected samples with clarified crude leaf extracts. Both B2- and dRBP-based assays clearly distinguished infected from healthy tissue in *Nicotiana benthamiana* leaf samples infected by a plethora of viruses like, Turnip mosaic virus and Tomato bushy stunt virus amongst others.

Our dsRNA binding protein ELISAs offer a rapid, cost-effective, and RNA extraction-free method for universal dsRNA quantification, outperforming commercially available kits in sensitivity and cost-efficiency. They hold promise for applications in plant virology, vaccine production quality control, and point-of-care pathogen surveillance. Future work will integrate these assays with sequencing workflows to enable simultaneous dsRNA quantification and strain identification.

Keywords: **dsRNA detection, ELISA, B2 FHV, viral diagnostics, plant virology, mRNA vaccines**

Synergistic Effects of Transcriptional Inhibitors with PARP Inhibitors in Cancer

Research area : Cancer Biology

Clara Capelli, Max Cigrang, Julian Obid, Maguelone Nogaret, Frédéric Coin (Genome Expression and Repair Team, Institute of Genetics and Molecular and Cellular Biology, University of Strasbourg)

Synthetic lethality is a mechanism where a single genetic event is compatible with cell survival, but the simultaneous occurrence of multiple genetic events leads to cell death. This principle can be leveraged in DNA damage repair pathways to selectively eliminate cancer cells with specific genetic vulnerabilities. Genome integrity is safeguarded against endogenous and exogenous DNA-damaging agents through various mechanisms, depending on the type of damage and the cell cycle stage. One of these pathways, homologous recombination repair (HRR), operates to repair double-stranded breaks with high fidelity [1]. The deficiency of this mechanism is exploited in cancer therapy using PARP inhibitors (PARPi). Several PARPi are used to treat BRCA1/2-mutated ovarian, breast, and pancreatic cancers. These cancers lack HRR due to BRCA mutations and are therefore highly sensitive to PARPi, which completely inhibit single-strand repair. This leads to synthetic lethality and, consequently, apoptosis of cancer cells. However, the effectiveness of this treatment is limited by BRCA mutation status [2]. To overcome this limitation, the concept of 'BRCAness' emerged in the early 21st century. It refers to a set of approximately 250 genes whose deficiency results in a phenocopy of BRCA1/2 loss-of-function mutations [3]. Thus, PARPi can be used independently of BRCA1/2 status. Certain transcriptional inhibitors act as DNA-binding agents and have shown therapeutic potential across multiple cancer types. These compounds could potentially induce a BRCAness phenotype and be used to trigger synthetic lethality. The aim of this project is to test whether transcriptional inhibitors can induce a BRCAness state and if their combination with PARPi can trigger synthetic lethality in BRCA-proficient cancer cells.

[1] Rose, M. *et al.* (2020) 'PARP Inhibitors: Clinical Relevance, Mechanisms of Action and Tumor Resistance', *Frontiers in Cell and Developmental Biology*, 8.
[2] Murai, J. and Pommier, Y. (2023) 'BRCAness, Homologous Recombination Deficiencies, and Synthetic Lethality', *Cancer Research*, 83(8), pp. 1173–1174.
[3] Lord, C.J. and Ashworth, A. (2016) 'BRCAness revisited', *Nature Reviews. Cancer*, 16(2), pp. 110–120.

Keywords : synthetic lethality, transcriptional inhibitors, PARP inhibitors, homologous recombination, BRCAness.

Deciphering the mode of action and regulation of Cohesin using a simplified parasite complex

Research area: Cellular and Molecular Biology

Deleurence Eva, Christophe Romier, Marie-Laure Diebold

Eukaryotic Cohesin is a ring-shaped complex essential for sister chromatids cohesion, 3D genome organization and DNA repair. Cohesin activity relies on an ATPase cycle that triggers conformational changes required for DNA loop extrusion and DNA tethering by Cohesin. This cycle is regulated by multiple auxiliary subunits, which modulate Cohesin's structural transitions and functions. Understanding the molecular mechanisms that govern Cohesin activity remains a major challenge due to the large size and flexibility of this complex.

To overcome these limitations, I use the Cohesin complex from the microsporidian parasite *Encephalitozoon cuniculi* (ec) that encodes shorter proteins. This also applies to ecCohesin subunits, making this complex more amenable to biochemical and structural characterization. My project aims to reconstitute the ecCohesin complex to investigate its ATPase-driven conformational dynamics and to decipher how its regulatory subunits modulate its activity.

Using biochemical, biophysical and structural analyses, including gel filtration, mass photometry and negative-stain electron microscopy, I have revealed that the ecCohesin core complex forms mono-, di-, and trimeric assemblies and adopts multiple ATP-dependent conformations. I also showed that the ecScc3 regulatory subunit binds directly to the ecCohesin core complex and significantly enhances its ATP hydrolysis rates. This minimal system therefore offers mechanistic insight into Cohesin's dynamic behavior and establishes a tractable platform for future structural and functional studies.

Keywords : **Cohesin, Microsporidia, ATPase cycle, Conformational dynamics, Regulatory subunits**

Astrocytic regulation (transcriptome/ epigenome) during memory formation: impact of Tau pathology

Research area : [Neurosciences]

Johanne Gambi[1], Isabel Paiva[1], Iris Grgurina[1], Brigitte Cosquer[1], Damien Plassard[2], Stéphanie LeGras[2], Charles Decraene[1], Aminé Isik[1], Jean-Christophe Cassel[1], Luc Buée[3], Karine Merienne[1], David Blum[3], Anne-Laurence Boutillier[1]

Alzheimer's disease (AD), is characterized by aggregation of Aß peptides and phosphorylated Tau protein, early forming in the hippocampus and spreading to the rest of the brain, causing progressive neuronal loss, affection of executive functions and memory impairments. Growing evidence shows that Tau neuronal pathology plays a key role in the development of cognitive deficits. However, the mechanisms leading to changes in synaptic plasticity and resulting in AD symptoms remain undefined, as most studies refer to autonomous neuronal dysfunction. Astrocytic role in AD has recently been described but still needs to be explored at several levels (molecular, cellular and systemic). Indeed, astrocytes appear as crucial players in long-term memory processes and neuronal plasticity; of note, during AD, astrocytes become reactive, which alters their physiological functions, contributing to the chronic neuroinflammatory environment and network dysfunctions.

In this work, we study astrocytic contribution to AD through their transcriptomic and epigenomic response i) to memory processes and ii) to pathology, in the hippocampus of both WT mice and an AD-like mouse model with tauopathy, the THY-Tau22 mice. Hippocampal astrocytes were isolated using magnetic beads directed against the ACSA-2 protein, in 3- and 9- month-old control WT and THY-Tau22 mice, that have been subjected or not (Home Cage) to spatial learning in the Morris Water Maze. RNA-seq analyses performed after learning showed significant up-regulation of extracellular matrix (ECM) and focal adhesion -associated genes (547 genes) in WT mice. Surprisingly, these pathways were down-regulated (135 genes) in pathological Tau mice. This led us to hypothesize that astrocytic-driven ECM remodeling is important at synapses during acquisition of spatial memory. Further, we validated astrocytic dysregulation of several genes involved in ECM composition and interaction by in situ hybridization (RNAscope). Epigenomic analyses were performed in Home Cage mice using the Cut&Tag-seq technology with two active histone marks (H3K27ac and H3K4me3). They are currently being analyzed to see the chromatin accessibility of the genes involved in these pathways in WT and tauopathic conditions. Our data underlines a remodeling of ECM composition during learning processes in the hippocampus of WT mice, these interactions being altered in a Tauopathic mouse model.

Keywords : **[Astrocytes], [Transcriptomics], [Epigenetics], [Tauopathy], [Memory]**

THE HIV-1 SILENCER BCL11B/CTIP2 REGULATES THE TLR3-MEDIATED CELLULAR RESPONSE TO VIRAL INFECTIONS IN MICROGLIAL CELLS.

*Muhammad Kashif [b], Solene Fenninger [b], Marco De Rovere [a], Clementine Wallet [b], Christian Schwartz [b], Olivier Rohr [b], Thomas Loustau [b].

Research area: Molecular Virology

Human Immunodeficiency Virus (HIV) continues to be a significant global health concern, affecting more than 40.4 million people. Its most severe stage, Acquired Immunodeficiency Syndrome (AIDS), severely weakens the immune system, making individuals vulnerable to infections and cancers. While antiretroviral therapy (ART) effectively controls the virus, it cannot eliminate it. Once treatment stops, HIV rapidly rebounds from latent reservoirs, making viral latency the key obstacle to a definitive cure (Marban et al., 2005). Our research focuses on CTIP2/BCL11b, a 6-zinc finger transcription factor involved in establishing and maintaining HIV-1 post integrative latency in microglial cells, the main latent reservoir in the CNS. We showed that CTIP2 promotes heterochromatin formation at the viral promoter, silencing gene expression (Cherrier et al., 2013). Additionally, we identified a novel interaction between CTIP2 and the 7SK ribonucleoprotein complex, which sequesters P-TEFb in an inactive form, preventing HIV-1 transcription and reservoir reactivation (3). HIV-1 evades innate immune responses by suppressing TLR3-IFN signalling, which inhibits antiviral factors such as ISGs, HIV restricting miRNAs, and interferons (Liu et al., 2020). Paraspeckle components play a key role in immune defense by forming the HEXIM1-DNA-PK-paraspeckle complex (HDP-RNP). Using Poly (I:C) which is a synthetic analogue of a double-stranded RNA (dsRNA) and recognized by endosomal Toll-like receptor 3 (TLR3) (Liu et al., 2020) mimicking HIV-1 infection in our case, we showed that Poly (I:C) promotes CTIP2 gene and protein expression having a correlation with Paraspeckles, Neat1 and Interferon induction with respect to TLR3 mediated cellular response. Using CLIP-seq and quantitative LC-MS/MS mass spectrometry, we showed that CTIP2 interacts with multiple molecular complexes, including paraspeckle and speckle components, as well RNAs involved in mRNA splicing and degradation(Sobhy et al., 2024). According to our investigation, the CTIP2 overexpression leads to the downregulation of paraspeckle components, Neat1, and interferons, forming a negative feedback loop that enhances viral latency. Given CTIP2's role in silencing viral transcription and modulating innate immunity, it represents a promising target for latency-reversing strategies. Investigating HIV-1 latency mechanisms is crucial for identifying new latency-reversing agents, overcoming a major barrier to virus eradication, and advancing curative approaches for HIV-1 infection.

.

Keywords: HIV-1 Latency, BCL11b/CTIP2, Paraspeckle components, RNA processing, TLR3-IFN signalling

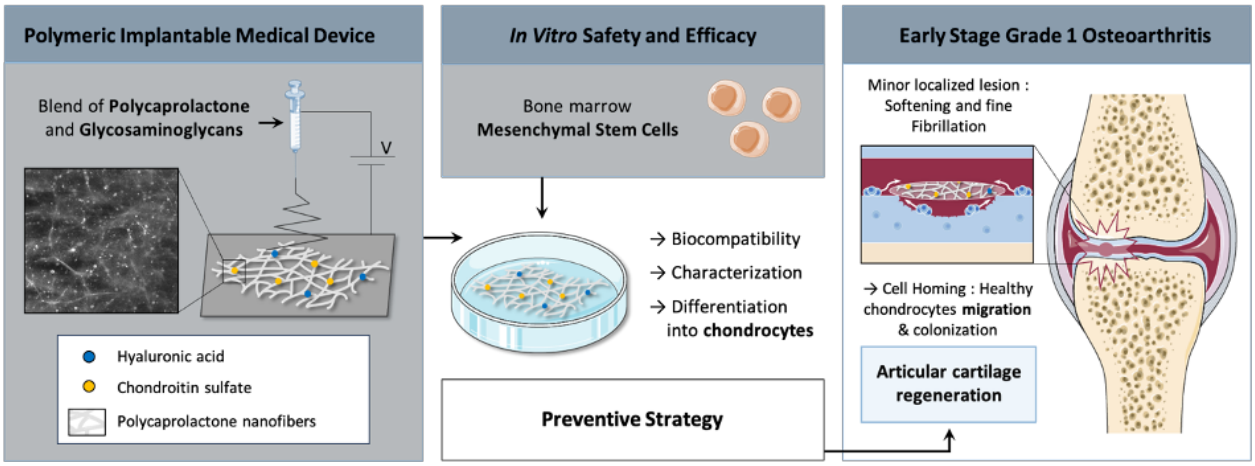
[Early osteoarthritis prevention through a cell-homing implantable medical device]

Research area : [Regenerative Medicine]

[Morgane Meyer], [Rana Smaida], [Jacky Hua], [Nadia Jessel], [Florence Fiorretti]

Regenerative medicine holds tremendous potential in addressing the complex challenges associated with osteoarthritis, a widespread degenerative condition affecting millions worldwide. Current treatments fail to effectively regenerate damaged cartilage, emphasizing the need for innovative interventions during the early stages of the disease. Our work aims to tackle this challenge by developing a novel strategy in the form of an implantable medical device to prevent osteoarthritis at early stages with reduced, localized lesions. The implantable medical device is made of an electrospun nanofibrous scaffold made of poly(ε-caprolactone) supplemented with essential components of the cartilaginous extracellular matrix, including chondroitin sulfate and hyaluronic acid, to attract chondrocytes from surrounding healthy tissue through a cell-homing effect. A comprehensive validation was conducted to assess its physicochemical properties, biocompatibility, and efficacy. Our findings demonstrate the suitable physicochemical properties, biocompatibility, and *in vitro* efficacy of the developed polymeric implantable medical device. These innovative implantable medical devices hold promise for advancing the treatment of early-stage osteoarthritis and eventually enhancing the quality of life for patients struggling with this challenging condition.

Keywords : **[regenerative medicine], [polymeric medical device], [osteoarthritis], [cartilage], [stem cells]**



SUCROSE BINGEING IN MICE: CONSEQUENCES ON MOTIVATION AND BRAIN ACTIVITY

Research area : Neurosciences

H. Odent , A.-S. Aubry , K. Herbeaux , A. Isik , J. Tyrode , V. Mathis , M.C. Olmstead , R. Bourdy , K. Befort

Food intake is tightly regulated by homeostatic and hedonic mechanisms. The arcuate nucleus of the hypothalamus (ARC) is a key integrator of homeostatic control, balancing caloric intake and energy expenditure. The reward system, particularly the mesocorticolimbic dopaminergic pathway, governs hedonic regulation, driving food intake for pleasure, particularly in response to palatable foods (i.e. sugar/fat). Yet, in modern countries, the constant accessibility to palatable food has led to food-related disorders characterized by difficulties in limiting and controlling food intake. Among these eating disorders, binge eating disorder (BED) is the most prevalent. BED is characterized by an overconsumption of palatable food within a short period, leading to physical and psychological consequences. Preclinical studies on animal models of BED have revealed functional alterations in reward-related brain regions, notably the ventral tegmental area (VTA), which plays a key role in motivational processes. Recently, we found alterations in the reinforcement properties of rewards such as ethanol and sucrose following binge sucrose intake. Based on these observations, we hypothesized that neuroadaptations, within the mesocorticolimbic pathway could underlie the altered reward processes observed in BED. To investigate behavioral and cellular adaptations associated with BED, we used our sucrose bingeing model of intermittent access (4h/day) to a 17% sucrose solution in a two-bottle choice paradigm for 8 weeks. Binge-like behavior was defined as excessive sucrose consumption within the first hour of access. We then assessed the long-term impact of sucrose bingeing on motivation and neural activity in brain structures involved in the homeostatic and reward-related aspects of food intake. Motivation for sucrose was evaluated using an operant progressive ratio schedule and then cFos immunostaining was performed to assess neural activity.
Our results, in male C57Bl6/J mice, indicate that although sucrose bingeing did not alter motivation, it led to an increased neural recruitment in the VTA and the ARC. These findings highlight potential long-lasting neuroadaptations in both homeostatic and hedonic regions of food intake, following intermittent sucrose exposure, and provides a framework to investigate how BED might remodel brain-feeding circuits. More studies are required, particularly in female mice, to improve our knowledge of long-term consequences of BED.

Keywords : *binge eating disorder, sucrose, reward system, motivation, brain activity*

[ribosomal DNA (rDNA) damage repair mechanism]

Research area : [____Cellular and Molecular Biology___]

[Speaker], [Priyanka Pundir], [Dr. Sandrine Morlot]…

The genome of a cell inevitably experiences double-strand breaks (DSBs). These DSBs could arise due to clash of machineries, mutations and even due to Homologous recombination (HR). Although HR is responsible for extensive and accurate repair of DSBs, however uncontrolled HR often results in chromosome translocation, loss of heterozygosity and deletion of repetitive sequences. These repercussions of uncontrolled HR are often seen in regions of genome which are present in multicopy repeats. One such multicopy region of the genome (150-200 copies in yeast) is the ribosomal DNA (rDNA), located in the nucleolus, well separated from the rest of the nucleus.

Despite its unique location, structural arrangement, and susceptibility to DSBs, the rDNA region successfully contributes towards the extensive demand of rRNA production, which itself accounts towards 60% of total RNA produced in a cell. The same rRNA later contributes towards steady protein production by ribosome biogenesis. . The complexity and seamless repair of this region alone makes the repair process especially interesting to study.

In our research, we aim to elucidate the mechanism of DSB repair by employing a combination of imaging and proteomic techniques. This approach enables us to track and characterize the repair process, shedding light on the molecular mechanisms involved in maintaining genomic stability. We have used Saccharomyces cerevisiae as our model organism.

Our preliminary results, in agreement with previous research, show that damaged rDNA exits the nucleolus upon DSB induction. Our results also indicated towards transcription repression being one of the steps involved during DSB repair

---

Keywords : **[__DSB___], [_DNA damage____], [__rDNA___], [DSB repair_____], [_____]**

Impact of the Loss of the Cohesin Subunits on the Early Development of the Zebrafish Larvae

Research area: Cellular and Molecular Biology

Rigal Jules, Christophe Romier, Christelle Golzio, Marie-Laure Diebold

The Cohesin complex is a crucial protein complex composed of three core subunits: SMC1A, SMC3, and RAD21. It plays a vital role in maintaining chromosome cohesion throughout the cell cycle, ensuring proper mitosis and meiosis. Additionally, Cohesin is involved in the formation of DNA loops, which are essential for example in transcription regulation and immunoglobulin gene recombination.

The zebrafish model is particularly relevant for studying the Cohesin complex because it exhibits developmental defects similar to those found in Cornelia de Lange syndrome (CdLS), a rare disorder caused by disruptions in the Cohesin complex. These similarities make zebrafish a great model for studying cohesinopathies and understanding the molecular mechanisms underlying these conditions.

In our study, we used zebrafish lines heterozygous for nonsense mutations in Cohesin subunits and observed significant developmental anomalies in homozygous offspring, including mitotic defects and increased cell death events. These mutant lines allowed us to compare the impact of losing different Cohesin subunits, providing insights into their specific roles in development. These findings highlight the essential role of Cohesin in zebrafish development and validate this model for studying cohesinopathies. Furthermore, these results enable us to determine when to use this model for Cohesin complex studies, which we will continue with phenotypic rescue experiments involving injections of mutant and wild-type mRNA into zebrafish eggs to better understand the molecular mechanisms underlying the observed developmental anomalies.

Keywords : **Cohesin, Zebrafish, Neurodevelopmental disorder, Mitosis, Cellular death**

Autoreactive B cells in Antiphospholipid Syndrome

Research area : Immunology

Julien Rottura, Yannick Dieudonné, Sabine Depauw, Maud Villeneuve-Adessi, Stéphane Giorgiutti, Anne-Sophie Korganow & Vincent Gies

Primary antiphospholipid syndrome (PAPS) is a rare autoimmune disorder characterized by prothrombotic autoantibodies (antiphospholipid antibodies, aPL) targeting phospholipids or their cofactors. B cells are known to play a critical role in PAPS pathogenesis, particularly through the production of aPL. However, the disturbances in B cell biology and the nature of aPL-producing clones in this disease remain poorly understood. We recently demonstrated that aPL-specific B cells are present in the natural repertoire and exhibit polyreactivity. PAPS B cells show dysregulation of the mTORC1 and MYC pathways, potentially explaining the survival and maturation of aPL-B cells in PAPS. To gain further insight in PAPS pathophysiology, we now focused specifically on B cells reactive to the domain I of β2-glycoprotein 1 (DmI), as anti-DmI antibodies are strongly associated with APS pathogenicity (i.e. thrombosis occurrence), making them ideal candidates. Using tetramerized DmI antigens, we identified DmI-specific B cells via flow cytometry in both healthy donors (HD) and PAPS patients. These cells are enriched in the unswitched memory compartments in PAPS B cells. Interestingly, in HD but not in PAPS, DmI-specific B cells are eliminated during the transition from the naive to the switched memory compartment, further supporting our previously published findings. Single cell RNA-sequencing together with B cell receptor sequencing of these B cells are now ongoing to better elucidate the mechanisms underlying the survival and maturation of pathogenic autoreactive B cells in PAPS. Altogether, our work will provide new insights into PAPS and B cell tolerance breakdown in autoimmune diseases, potentially highlighting novel therapeutic targets.

Keywords : autoimmune disease, B cells, flow cytometry

The multiple-hit hypothesis for neurodevelopmental disease in the DOHaD context: What can we learn from early-stressed vasoactive intestinal peptide's (VIP) deficient mice?

Research area : [___neurosciences____]

Edith Tanché, Marlene Salgado-Ferrer, Yannick Menger, Vincent Lelièvre

---

The Developmental Origin of Health and Disease, or DOHaD, explores the potential impact of early life experiences on individual vulnerability towards future diseases. The multiple-hit hypothesis emerged in this context to try to explain how an additive combination of early-life events: genetic predisposition, prenatal adversity, and postnatal adversity (Bale, 2014) may be sufficient to explain pathologies such as epilepsy, schizophrenia, or autism spectrum disorders.
The vasoactive intestinal peptide (VIP) is a highly–conserved, pleitropic peptide that possesses neurotrophic and anti-inflammatory properties. In rodents, a surge of VIP released during pregnancy is essential for proper neurogenesis and growth, and its abolition leads to microcephaly (Gressens et al., 1993-1994). Constitutional deletion of VIP causes increased vulnerability towards systemic inflammation (Hamidi et al., 2006), whilst chronic stress has been shown to modulate VIP secretion (Gavalda et al., 1993). Considering that these two stressors are capable of triggering neurodevelopmental disease when performed during pregnancy (Han et al., 2021), VIP appears like a legitimate candidate for genetic vulnerability in a multiple-hit model.
To model the multi-hit paradigm in mice we chose to perform maternal restraint stress on VIP-deficient females to combine genetic susceptibility and psychosocial stress known for its inflammatory and societal dimension.
We first assessed the effect of stress on pregnant mice by measuring their corticosterone levels. Then we quantified placental genes expression to detect possible functional alterations susceptible to induce intra-uterine growth retardation and affect brain outgrowth. After their birth, brains of stressed and non-stressed mice were morphologically compared, revealing that the breadth of microcephaly correlated with the combination of stressors (VIP deficiency and prenatal stress). Both results were consistent with the establishment of a multiple-hit model. Finally, we aimed at correlating structural brain abnormalities with behavioural alterations focusing on specific structures such as cortex and amygdala.
These studies will shed light on the pivotal role of placenta in the multiple-hit hypothesis and offer a better understanding of the limits and extents of our mouse model in the context of DOHaD, considering polymorphisms in genes encoding VIP and its receptors have been already detected and associated with human pathologies.

Keywords : **DOHaD, early-life stress, neurodevelopment, placenta, behaviour**

[Optionally, you can attach a relative scheme/graphical representation to fill up the remaining page. DO NOT SURPASS 1 PAGE !]

Interactions Between Claustrum Activity and Cortical Dynamics During the Sleep-Wake Cycle

Research area : Neurosciences

Flora Thellier, Coline Portet, Karin Herbeaux, Antoine Valera, Jesse Jackson, Yaroslav Sych, Romain Goutagny

---

The claustrum is a small, elongated subcortical nucleus that is remarkably conserved across evolution and characterized by its widespread projections to the entire cortical mantle. This extensive connectivity suggests a role in high-order cognitive functions, including attention and awareness. Surprisingly, in head-fixed experiments, we recently showed that claustrum activity strongly correlates with cortical brain states: it is more active during highly synchronized cortical state (slow-wave sleep) than during desynchronized ones (wakefulness or REM sleep). Further, we have shown that stimulating the Claustrum specifically during Slow-wave sleep following learning increases memory consolidation. Does the claustrum contribute to the memory consolidation network activated during sleep? Do these findings conflict with previous studies emphasizing its role in attention? To address these questions, we recorded claustrum activity using fiber photometry alongside local field potentials (LFPs) in various cortical areas known to be important for memory consolidation (the Prefrontal and Retrosplenial cortices and the dorsal Subiculum, a key output structure of the hippocampal formation) across different vigilance state before, during and after the sampling phase of a novel-object location task. Our preliminary results (n=4 mice) confirm that claustrum activity is higher during slow-wave sleep than during wakefulness or REM sleep. Consistently, we did not detect claustral activity during task performance. To better quantify the relationship between claustrum activity and cortical dynamics, we employed two metrics: theta/delta ratio as an index of cortical synchronization and the slope of the power spectrum as an index of cortical excitation/inhibition (E/I) balance. Confirming and extending our previous results, we showed that, in freely moving mice, Claustrum activity was positively related to cortical synchronization and decreased E/I balance. We are currently investigating the bidirectional relationship between claustrum activity and cortical dynamics.

Keywords : Claustrum, Local Field Potentials (LFP), Fiber Photometry, Slow-Wave Sleep

Fluorescence-based microviscosity mapping of cellular condensates

Research area : Liquid-Liquid Phase Separation

Avantika Avantika[1], Chloé Frey[1], Carla Faivre [2], Lucie Quirin [1], Mayeul Collot [2], Nicolas Anton[3], Pierre Hener [4],Pascal Didier[1], Halina Anton[1]

[1]Laboratoire De Bioimagerie et Pathologies, UMR 7021 CNRS, Université de Strasbourg, Faculté de Pharmacie, 74 route du Rhin, 67401 Illkirch, France, [2] Laboratoire de ChémoBiologie Synthétique et Thérapeutique UMR 7199 CNRS,[3] Inserm 1260 Nanomédicine Régénerative, [4] Plateforme "Imagerie in vitro", Institut des Neurosciences Cellulaires et Intégratives

Phase-separated biomolecular condensates play a crucial role in organizing cellular biochemical reactions. These liquid-like droplets exhibit a range of physical states, from liquid to gel-like and glass-like phases. Their material properties directly affect their physiological functions, hence characterizing the microviscosity of these assemblies provides key information for understanding their biological role. Techniques commonly employed to probe viscosity in biomolecular condensates include microrheology (Lai et al., 2009) and Fluorescence Recovery After Photobleaching (FRAP) (Ishikawa-Ankerhold et al., 2012). However, the former is limited to “in vitro” experiments and the latter only provides an average viscosity of the condensate. In this context, molecular rotor-based viscosity sensing has emerged as a powerful approach for mapping microscale viscosity (Haidekker et al., 2004; Haidekker and Theodorakis, 2007; Kuimova et al., 2008). BODIPY-based molecular rotors are known for their high sensitivity to viscosity and high quantum efficiency. In our study, we employ BODIPY-based molecular rotors to measure the microviscosity in cellular condensates using fluorescence lifetime imaging microscopy (FLIM). We show that the nucleolus exhibits different viscosities in different sub-compartments. Furthermore, we show that the inhibition of rRNA transcription results in a more viscous nucleolus. Additionally, we compare the viscosities of the nucleolus with the stress granules, a cytoplasmic biomolecular condensates. Altogether the presented data show that the use of molecular rotors combined with FLIM enables high-resolution, dynamic viscosity measurements in physiological conditions, providing new insights into the material properties of cellular biomolecular condensates.

Keywords: Liquid-Liquid Phase Separation, Fluorescence Lifetime Imaging Microscopy (FLIM), nucleolus, molecular rotors.