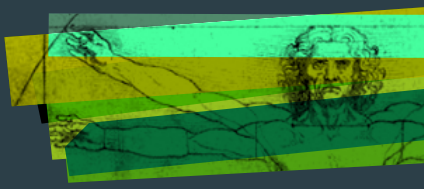
LE STUDIUM

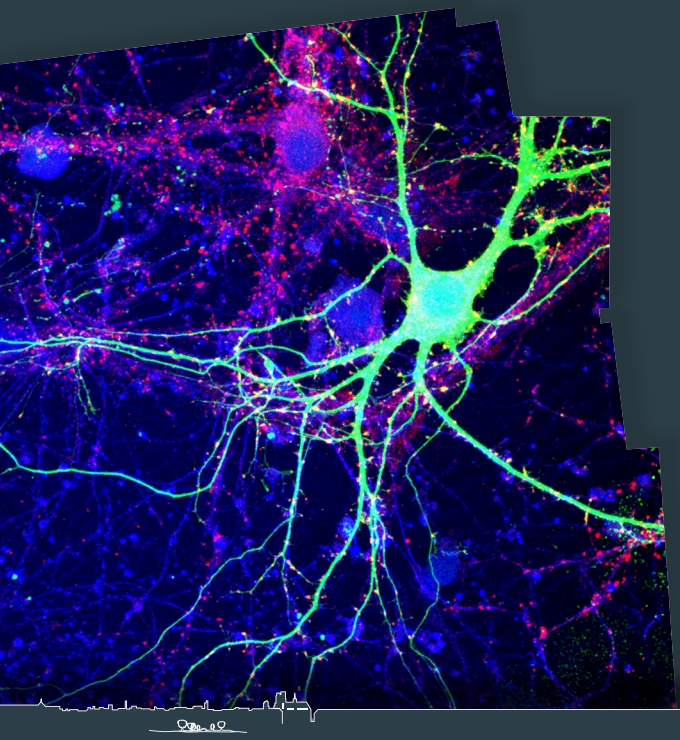
CONFERENCES

VIRTUAL MEETING | 2022



24-25 May 2022

RNA therapeutics and Neuroscience



LOCATION

VIRTUAL MEETING

CONVENORS

Dr Kathia Zaleta

LE STUDIUM / MARIE SKŁODOWSKA-CURIE RESEARCH FELLOW

FROM University of California San Diego (UCSD) - USA

IN RESIDENCE AT Imaging and Brain Unit (iBrain), INSERM / University of Tours - FR

Prof. Patrick Vourc'h

Imaging and Brain laboratory Unit (iBrain), INSERM / University of Tours - FR

LE STUDIUM Loire Valley Institute for Advanced Studies

L'Europe s'engage en région Centre-Val de Loire

ORLÉANS MÉTROPOLE

Inserm

Université de TOURS

BIOPHARMACEUTICALS

CHRU

FHU GenoMeds

LE STUDIUM

CONFERENCES

VIRTUAL MEETING | 24-25 MAY 2022

ABSTRACTS

RNA therapeutics and Neuroscience

CONVENORS

Dr Kathia Zaleta
LE STUDIUM / MARIE SKŁODOWSKA-CURIE RESEARCH FELLOW
FROM: University of California San Diego (UCSD) - USA
IN RESIDENCE AT: Imaging and Brain Unit (iBrain), INSERM / University of Tours - FR

Prof. Patrick Vourc'h
Imaging and Brain Unit (iBrain), INSERM / University of Tours - FR

ORGANIZING COMMITTEE

Sophie Gabillet, General Secretary

Dr Aurélien Montagu, Scientific Relations Manager

Maurine Villiers, Communication & Events Manager

LE STUDIUM Loire Valley Institute for Advanced Studies • Région Centre-Val de Loire • FR

EDITO

Created in 1996 on the CNRS campus in Orleans La Source, LE STUDIUM has evolved to become the multidisciplinary Loire Valley Institute for Advanced Studies (IAS), operating in the Centre-Val de Loire region of France. LE STUDIUM has its headquarters in the city centre of Orleans in a newly renovated 17th century building. The amazing facilities are shared with the University of Orleans. In 2014 new developments and programmes linked to the smart specialisation of the Centre-Val de Loire region came to strengthen existing IAS collaborative relationships with the local and the international community of researchers, developers and innovators.

LE STUDIUM IAS offers to internationally competitive senior research scientists the opportunity to discover and work in one of the IAS's affiliate laboratories from the University of Tours, the University of Orleans, National Institute of Applied Sciences (INSA) Centre Val de Loire and ESAD Orléans, as well as of nationally accredited research institutions located in the region Centre-Val de Loire (BRGM, CEA, CNRS, INSERM, INRAE). Our goal is to develop and nurture trans-disciplinary approaches as innovative tools for addressing some of the key scientific, socio-economic and cultural questions of the 21st century. We also encourage researchers' interactions with industry via the IAS's links with Poles of Competitiveness, Clusters, Technopoles, and Chambers of Commerce etc.

LE STUDIUM has attracted near two hundred and fifty experienced researchers coming from 47 countries for long-term residencies. In addition to their contribution in their host laboratories, researchers participate in the scientific life of the IAS through attendance at monthly interdisciplinary meetings called LE STUDIUM THURSDAYS. Their presentations and debates enrich the regional scientific community at large (researchers of public and private laboratories, PhD students, research stakeholders' representatives, etc...)

For the period 2015-2021, LE STUDIUM has operated with an award from the European Commission, a programme supporting the mobility of international researchers, the Marie Skłodowska-Curie Actions (MSCA) COFUND programme. For the period 2022-2025, LE STUDIUM has joined the FIAS Programme (French Institute for Advanced Study) along side five other institutes in France, also supported by the MSCA Actions. Since 2013, LE STUDIUM is also an official partner of the Ambition Research and Development programmes initiated by the Centre-Val de Loire Regional Council to support the smart specialisation strategy (S3) around priority domains: biopharmaceuticals, renewable energies, cosmetics, environmental metrology, digital twins, materials, forestry and natural and cultural heritage. Furthermore our current collaboration with the ATHENA European University Consortium allows us to welcome fellows from ten European partners universities. New programmes are currently designed to include all major societal challenges.

Researchers are also invited and supported by the IAS to organise, during their residency and in collaboration with their host laboratory, a two-day LE STUDIUM CONFERENCE. It provides them with the opportunity to invite internationally renowned researchers to a cross-disciplinary conference, on a topical issue, to examine progress, discuss future studies and strategies to stimulate advances and practical applications in the chosen field. The invited participants are expected to attend for the duration of the conference and contribute to the intellectual exchange. Past experience has shown that these conditions facilitate the development or extension of existing collaborations and enable the creation of productive new research networks.

The present LE STUDIUM CONFERENCE named "*RNA therapeutics and Neuroscience*" is the 119th in a series started at the end of 2010 listed at the end of this booklet.

We thank you for your participation and wish you an interesting and intellectually stimulating conference. Also, we hope that scientific exchanges and interactions taking place during this conference will bring opportunities to start a productive professional relationship with presenting research laboratories and LE STUDIUM Loire Valley Institute for Advanced Studies.

Yves-Michel GINOT

Chairman
LE STUDIUM

INTRODUCTION

The RNA & NEUROSCIENCE meeting aims to bring together graduate students, academic and industry scientists working in the fields of RNA and the Central Nervous System (CNS) to present their latest research results, ideas, developments, biological medical applications and discuss the current concepts and the challenges that we are facing for future research.

RNA therapeutics is a novel technology that provides a unique treatment for a variety of diseases. While it has been a success for the COVID19 pandemic, the RNA community still has the challenge on delivering some of these RNA molecules in other parts of the body (e.g. brain and cardiac tissue) and better specificity to have a full therapeutic effect. Therefore, this meeting will explore the novel RNA therapeutics that are been currently developped and their mechanisms to cure a variety of diseases.

Additionally, the complexity of the brain provides a unique challenge to find therapeutics to cure neurodevelopmental disorders and neurodegenerative diseases such as Alzheimer's and ALS. These diseases are characterized by the lack of gene regulation causing protein imbalance at the synapses and therefore cell death. Thus, in this meeting we will also explore the mechanisms that cause these diseases and the novel therapeutics that are currently being developed for their cure.

This international conference is organised in the framework of the BIOPHARMACEUTICALS ARD CVL Programme.

PROGRAMME

TUESDAY 24TH MAY (14:00-18:50 GMT+2 PARIS)

14:00 Official Opening by **Sophie Gabillet** (General Secretary of LE STUDIUM Loire Valley Institute for Advanced Studies), **Dr Kathia Zaleta** & **Prof. Patrick Vourc'h**

SESSION 1 : RNA REGULATION

Chairman: Dr Frédéric Laumonnier

14:30 **Prof. Antonella Riccio -** RNA metabolism in developing neurons

15:10 **Dr Ana Cristina Calvo -** Peripheral RNA Biomarkers in Amyotrophic Lateral Sclerosis

15:50 **Dr Débora Lanznaster -** Combined Metabolomics and targeted-transcriptomics analysis in the muscle of early-stage ALS patients

16:30 *Coffee break*

SESSION 2: RNA DELIVERY

Chairman: Prof Chantal Pichon & Dr Fédérico Perche

16:50 **Dr Horacio Cabral -** RNA/Polymer-Based Supramolecular Approaches for mRNA Delivery

17:30 **Prof. Steven Dowdy -** Delivery of RNA Therapeutics: The Great Endosomal Escape!

18:10 **Director Jason Potter -** What matters for making highly expressed mRNA.

18:50 *End of the conference day*

WEDNESDAY 25TH MAY (14:00-19:00 GMT+2 PARIS)

SESSION 3: RNA THERAPEUTICS - PART 1

Chairman: Prof. Patrick Vourc'h

14:00 **Prof. Jernej Ule -** How do protein-RNA condensates form and contribute to disease?

14:40 **Dr Liliane Massade -** Squalenoyl siRNA PMP22 nanoparticles, a potent therapy for Charcot-Marie-Tooth disease type 1A

15:20 **Dr Jean-René Martin -** The snoRNA jouvence, a new versatile tool to protect against the deleterious effects of ageing, as well as inversely, for the treatment of cancer.

16:00 **Prof. Krzystof Sobczak -** Compounds which alleviate the pleiotropic toxicity of RNA harboring expanded CGG repeats in the Fragile X–associated syndrome

16:40 *Coffee break*

SESSION 4: RNA THERAPEUTICS - PART 2

Chairman: Dr Kathia Zaleta

17:00 **Prof. Stefano Gustincich -** Antisense long non-coding SINEUP RNAs: from molecular mechanism to therapeutics

17:40 **Prof. Michelle L. Hastings -** Antisense Oligonucleotides for the Treatment of Disease

18:20 **Dr Lori L. Isom -** Dancing to a different tune: TANGO offers a precision medicine approach to treating Dravet syndrome

19:00 *Conclusion*

ARD CVL
BIOPHARMACEUTICALS

A drug is any substance or composition presented as having properties for treating, preventing or diagnosing disease in humans or animals. Whereas biopharmaceuticals in the strict sense of the term, are molecules that have the characteristic of being produced from living organisms or their cellular components. These molecules are intermediate between chemical drugs and organisms' intrinsic biologics. By definition, a biopharmaceutical is any drug whose active substance is a therapeutic macromolecule produced by living organisms. Biopharmaceuticals are overwhelmingly protein-based, mainly represented by non-living vaccines, therapeutic antibodies, enzymes, protein hormones and growth factors. As proteins, their injection is today mandatory by injection.

The proportion of biopharmaceuticals in the drug market has dramatically increased over the past decade. The Centre-Val de Loire region is at the cutting edge of research in the pharmaceutical sector with strong capabilities of multidisciplinary regional research teams. Since 2013, the development of biopharmaceuticals is a regional priority with effective budget lines to support research and to facilitate innovative inter-sectorial industrial development and partnerships for socioeconomic development.

The Biopharmaceuticals programme aims to:

- Develop a flagship research and development pole on biopharmaceuticals.
- Configure the biopharmaceuticals field by inter-sectorial development and innovation in the pharmacy/health sectors through start-ups, SMEs including established local and regional based multinational companies.
- Promote the transfer of technologies/competences to existing and new businesses.
- Support the development of new competences for the sector.

The Biopharmaceuticals Programme is driven by the University of Tours and mobilises actors in the pharmaceutical sector, from fundamental research to production, to develop the tomorrow's biopharmaceutical treatments and production centres in the Centre-Val de Loire region. A number of innovative projects including academic and industrial partnerships covering a wide spectrum of biological molecules and domains receive funding to bring immediate outcomes: vaccines, therapeutic antibodies, nucleic acids, lipoproteins, bio- production of medicines...

BIOPHARMACEUTICALS
Innovation Dynamics in Health
IN REGION CENTRE-VAL DE LOIRE

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CONVENORS

SPEAKERS

CONVENORS

Dr Kathia Zaleta

LE STUDIUM / Marie Skłodowska-Curie Research Fellow

University of California San Diego (UCSD)

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Kathia Zaleta, PhD majored in Pharmaceutical Chemistry and Biology from the University of Veracruz, Mexico with the undergraduate thesis 'Synthesis and Purification of 2-Selenil-4,6-Dimethyl-1,3,2-Dioxaphosphorines'. Later, she completed her master and doctoral research studies at the University of Nebraska-Lincoln in collaboration with CINVESTAV-IPN where she investigated the catalytic mechanisms of the modular polyketide synthases (PKS) and Non-ribosomal peptide synthetases (NRPS) to allow chemical reprogramming for the synthesis of novel natural products with potential drug activity. She carried out her first postdoctoral studies at Stanford University, where she was introduced to the RNA therapeutics field by developing an RNA therapy to treat patients with the cardiovascular disease Hypertrophic Cardiomyopathy (HCM) which is caused by single nucleotide variants (SNVs). Dr Zaleta continued her academic career at the University of California San Diego (UCSD) where she was part of the NIH 4D Nucleome consortium and worked in technology development methods to map RNA-genome and Protein-protein interactions to understand nuclear organization in space and time. In 2020, she was awarded the Le Studium/Marie Sklodowska-Curie fellow award with the research proposal: Protein translation enhancement therapy for the treatment of neurodegenerative diseases; and in the same year her project was awarded with a research grant by the French ARSLA association. Currently, she is an R&D Scientist at Thermo Fisher Scientific in California.

Prof. Patrick Vourc'h

Imaging and Brain laboratory Unit (iBrain), INSERM / University of Tours

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Prof. Patrick Vourc'h is professor in biochemistry and molecular biology at the faculty of medicine of Tours, and CHRU (Hospital) of Tours, assessor (research) of the Dean of the Faculty of Medicine and President of the Biomedical Research and Public Health Committee (CRBSP, CHRU Tours). As the head of the platform of genomics of the University of Tours and of the platform of molecular biology of the CHRU of Tours, he has access to high throughput sequencing, transcriptomics. He has regional, national and international collaborations with the Montpellier Neuroscience Institute, National Polytechnic Institute in Mexico City, Utrecht University, European Consortium Strength and Mine on ALS. He is a member of the team "Neurogenomics and neuronal physiopathology" of the iBRAIN research Unitof the University of Tours, Inserm, and is part of the Centre for ALS (CHRU of Tours) where Prof. Vourc'h is particularly involved in molecular diagnosis for patients with ALS and ALS-FTD (dementia). HIs team is also member of the Labex MabImprove (Laboratory of Excellence, monoclonal antibodies) Tours-Montpellier.

SPEAKERS

Prof. Horacio Cabral

The University of Tokyo

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Horacio Cabral is an Associate Professor in the Department of Bioengineering, The University of Tokyo. He received his Ph.D. in Materials Engineering from The University of Tokyo in 2007. Dr. Cabral was an Assistant Professor at the Graduate School of Medicine, The University of Tokyo until 2010. Dr. Cabral has made major influential achievements in the development of clinically translatable polymeric nanocarriers for drug and gene delivery, accomplishing seminal contributions in understanding the effect of nanocarrier design on activity. Dr. Cabral has published more than 100 articles (h-index: 49) and has filed more than 30 patents. Dr. Cabral serves as associate editor of *Science and Technology of Advanced Materials* (STAM), *MedComm*, *Nanomaterials*, and *Macromolecular Bioscience*.

RNA/Polymer-Based Supramolecular Approaches for mRNA Delivery

Messenger RNA (mRNA) therapeutics are attracting much attention, particularly after the approval of two mRNA vaccine formulations for COVID-19. However, as a therapeutic modality, mRNA still has issues of poor bioavailability, showing rapid enzymatic degradation in physiological environments and the tendency to induce uncontrollable inflammatory responses. Supramolecular approaches for mRNA delivery are a realistic strategy for improving its bioavailability, reducing immunogenicity and enhancing the translational activity. Among supramolecular mRNA formulations, polymeric micelles, *i.e.*, core-shell nano-structures self-assembled by polyion complexation between catiomers and mRNA in aqueous conditions, can effectively reduce enzymatic degradation of mRNA in biological milieu through precise control of the polymer design. mRNA-loaded polymeric micelles can improve the intracellular delivery of mRNA toward safe and efficient mRNA delivery to various cells and organs. Moreover, polymeric micelles can be combined with orthogonal supramolecular approaches using complementary RNA oligonucleotides, which allows installing protective and functional moieties to mRNA, or crosslinking various mRNA strands, by engineered hybridization. Herein, I will present our recent efforts to apply polymer- and RNA-based supramolecular approaches for effectively transporting mRNA therapeutics to target cells *in vivo*.

Dr Ana Cristina Calvo

LAGENBIO (Laboratory of Genetics and Biochemistry), Faculty of Veterinary-Research Institute of Health of Aragon (IISA), CIBERNED, University of Zaragoza

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Dr. Ana Cristina Calvo, PhD in Biochemistry, Molecular and Cellular Biology in the University of Zaragoza (2003). Dr. Calvo is Full Professor of Genetics and she is a member of the LAGENBIO (Teragen and Regeneragen) Group of the Research Institute of Health in Aragon. Her research interests are focused on the identification of prognostic, diagnostic and predictive biomarkers of ALS that she has been investigated during the last 18 years. Dr. Calvo has wide expertise in preclinical and translational studies in ALS. Her h index is 18 (Scopus) and she is coauthor of 39 articles. She has participated in 21 projects and industry contracts and has 3 patents. She is lately interested in the role of non-coding RNA species and the intestinal microbial community in ALS.

Peripheral RNA Biomarkers in Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease of unknown origin that causes progressive muscle paralysis and motor neuron death. The need of reliable biomarkers of ALS that can be accurately monitoring along disease progression is an increasing field of research. Previous studies in our research group are focused on the analysis of the transcriptional expression of mRNA of target genes related to calcium and glucose metabolism, oxidative stress, inflammation (pro- and anti-inflammatory cytokines, NLRP3 inflammasome), neuromuscular junction dismantlement and muscle damage and reinnervation in serial muscle biopsies and blood samples of transgenic SOD1G93A mice. Among all these mRNA targets, we have validated the transcriptional collagen type XIX (COL19A1) levels in muscle and blood samples of sporadic and familial ALS patients in a multicentre study, suggesting a compensatory effect of this collagen to ameliorate the disease progression under neurodegenerative conditions specific to ALS. In order to improve the predictive capacity of this potential prognostic biomarker, we are recently investigating circular RNA targets that could be closely related to this collagen and to the neurodegenerative progression and longevity in transgenic SOD1G93A mice. These findings could provide not only a better understanding of the disease but also a panel of biomarkers that could be easily validated in ALS patients.

Prof. Steven Dowdy

UCSD School of Medicine

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Steven Dowdy, PhD, is a Professor of Cellular & Molecular Medicine at UCSD School of Medicine. He received his PhD in molecular genetics from the University of California Irvine and was a Damon Runyon Postdoctoral Fellow at the Whitehead Institute at MIT. In 1994, Dr. Dowdy joined Washington University School of Medicine as an Assistant Professor where he was also an Investigator of the Howard Hughes Medical Institute (1994-2012). He moved his lab to UCSD in 2001. His early research focused on understanding the molecular basis of G1 cell cycle deregulation during oncogenesis by the RB and p16 tumor suppressor genes, and cyclin:Cdk complexes. Later work has focused on the synthetic chemistry, development and delivery of RNA therapeutics. His current work is focused on addressing the rate-limiting delivery endosomal escape step of all RNA therapeutics.

Delivery of RNA Therapeutics: The Great Endosomal Escape!

All macromolecular therapeutics, including ASOs, siRNAs, peptides, proteins, CRISPR, mRNA and non-viral DNA vectors, are taken up into cells by endocytosis. However, <1% to none of the endocytosed therapeutic cargo escapes from the endosome into the cytoplasm and nucleus of the cell. Thus, for all RNA therapeutics, endosomal escape remains the rate-limiting delivery step that prevents their effective use to treat cancer, pandemic influenza, and heart disease. Our research is focused on addressing this problem by developing new chemistry to synthesize novel universal endosomal escape domains (uEEDs) with the goal of enhancing endosomal escape of by 10-fold in the absence of toxicity.

Prof. Stefano Gustincich

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Stefano Gustincich is Deputy Director for the "Technologies for Life Science (LifeTech)" and Director of the "Central RNA Lab" of the Italian Institute of Technology (IIT), Genoa, Italy and Director of e "Centro per la Medicina Personalizzata Preventiva e Predittiva – CMP^{3}vda" in Aosta (IIT), Italy.
He is Full Professor in Applied Biology on leave of absence from the International School of Advanced Advanced Studies (SISSA), Trieste, Italy.
After obtaining a PhD in Molecular Genetics & Biotechnology from SISSA, he was awarded a "long-term EMBO fellowship" at the Department of Neurobiology of Harvard Medical School, Boston (USA) where in 1998 he became "Instructor in Neurobiology". In 2003 he won the "career development award" of the Giovanni Armenise-Harvard Foundation and in 2011 the National Award for Innovation in Biotechnology.
His lab studies the role of non-coding DNA in brain functioning and neurodegenerative diseases.
He recently discovered the SINEUPs, a new functional class of antisense long noncoding RNAs able to increase translation of their sense gene.
Since the beginning of his scientific career, he published 125 peer-reviewed research articles and reviews with a total IF of 1108, an average IF of 8.2, a *h* Index of 50 and more than 17.200 citations. He is the co-founder of TranSINE Therapeutics, a start-up based in Cambridge, UK.

Antisense long non-coding SINEUP RNAs: from molecular mechanism to therapeutics

Natural SINEUPs are antisense long non-coding RNAs that enhance translation of sense mRNAs. Their activity depends on the combination of two domains: the overlapping region, or binding domain (BD), dictates SINEUP specificity, while an embedded inverted SINEB2 element acts as effector domain (ED) to UP-regulate mRNA translation. Their modular structure can be employed to artificially engineer their BDs and design synthetic SINEUPs to specifically enhance translation of virtually any target gene of interest.

By unveiling new cues on the molecular mechanism of SINEUP activity, I will present previously undescribed natural members of this functional class of non-coding RNAs.

By taking advantage of the design of synthetic SINEUPs, I will discuss representative examples of rescuing pathological phenotypes in patients' derived cells and in mouse models of disease.

Prof. Michelle Hastings

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Dr. Hastings, PhD, is a Professor and Director of the Center for Genetic Diseases at the Chicago Medical School at Rosalind Franklin University of Medicine and Science. She received her PhD from Marquette University and was a post-doctoral fellow at Cold Spring Harbor Laboratory. Her research focuses on understanding the molecular mechanisms of disease and discovering new therapeutic approaches that modulate pre-mRNA splicing. Her work has resulted in the discovery of effective means of targeting splicing with antisense molecules for the potential treatment of diseases including Batten disease, Usher syndrome, cystic fibrosis, Alzheimer's and Parkinson's disease.

Antisense Oligonucleotides for the Treatment of Disease

Antisense oligonucleotides (ASOs) have proven to be an effective therapeutic platform for the treatment of disease. These short, single-stranded, modified nucleotides function by base-pairing with the complementary sequence of an RNA and modulating gene expression in a manner that is dependent on ASO design and targeting site. We have devised a number of approaches to alter splicing with ASOs to correct or improve gene expression and pathology in disease models. One of our approaches is under development for the treatment of CLN3 Batten disease, a fatal, pediatric lysosomal storage disease caused by mutations in a gene encoding the lysosomal membrane protein CLN3. The most common mutation associated with CLN3 Batten is a deletion of exons 7 and 8 ($CLN3^{\Delta ex78}$), which disrupts the mRNA open reading frame. We devised a therapeutic strategy for treating CLN3 Batten disease using an ASO that alters splicing *CLN3* splicing to correct the open reading frame of the mutated transcript. Treatment of $CLN3^{\Delta ex78}$ neonatal mice with this ASO resulted in the desired splicing effect throughout the central nervous system, improved motor coordination, reduced histopathological features of the disease in the brain and extended life in a severe mouse model of the disease. Our results demonstrate that ASO-mediated reading frame correction is a promising therapeutic approach for CLN3 Batten disease.

Dr Lori Isom

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Dr. Isom is the Maurice H. Seevers Professor and Chair of the Dept of Pharmacology at the Univ of MI Medical School. Her research focuses on voltage-gated sodium channel function and the roles of sodium channel gene variants in developmental and epileptic encephalopathy, including Dravet syndrome. Dr. Isom collaborated with Stoke Therapeutics to develop the first antisense oligonucleotide precision therapeutic for Dravet syndrome, now in clinical trials. Dr. Isom is Co-PI of the NINDS-funded EpiMVP Center Without Walls. She co-chairs the Dravet Syndrome Foundation Scientific Advisory Board and served on the Board of the AES. She has received awards for research and mentoring, including a NINDS Javits R37 MERIT award. She is a Fellow of the AAAS, a Fellow of ASPET, and a Fellow of AES. Dr. Isom was elected to the National Academy of Medicine in 2021.

Dancing to a different tune: TANGO offers a precision medicine approach to treating Dravet syndrome

Dravet syndrome is an intractable developmental and epileptic encephalopathy caused largely by *de novo* variants in *SCN1A* resulting in haploinsufficiency of the voltage-gated sodium channel α subunit NaV1.1. We employed Targeted Augmentation of Nuclear Gene Output (TANGO) technology, which modulates naturally occurring, non-productive splicing events to increase target gene and protein expression and ameliorate disease phenotype. We identified antisense oligonucleotides (ASOs) that specifically increase the expression of productive *Scn1a* transcript in human and mouse cell lines, as well as in mouse brain. We showed that a single intracerebroventricular dose of a lead ASO at postnatal day 2 or 14 significantly reduced the incidence of electrographic seizures and sudden unexpected death in epilepsy (SUDEP) in the F1:129S-$Scn1a^{+/-}$ x C57BL/6J mouse model of Dravet syndrome. Increased levels of productive Scn1a transcript and NaV1.1 protein were confirmed in brains of treated mice. Our results suggest that TANGO may provide a unique, gene-specific approach for the treatment of Dravet syndrome.

Dr Débora Lanznaster

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Dr Débora Lanznaster is a post-doctoral researcher in the UMR1253 iBrain (INSERM/University of Tours). She received her PhD in Neuroscience from the Federal University of Santa Catarina in 2016. Her research is focused on neurodegenerative diseases, such as amyotrophic lateral sclerosis. Dr. Lanznaster investigates the physiology of TDP-43 aggregates and their toxic role in the neurodegeneration observed in ALS. She uses cell culture and animal models of ALS, and develops research on samples and data from ALS patients. Dr. Lanznaster is also interested in the identification of biomarkers for diagnosis and prognosis of neurodegenerative diseases, as well as the investigation of therapeutic options to improve the treatment of ALS patients.

Combined Metabolomics and targeted-transcriptomics analysis in the muscle of early-stage ALS patients.

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by the progressive loss of motor neurons, leading to paralysis and death of patients after 3-5 years of symptoms' onset. While skeletal muscle is severely attained in ALS patients, few studies analysed the pathological and metabolic alterations taking place in the muscle. By applying a broad metabolomics analysis, we demonstrated a very discriminant metabolomic profile of the muscle of early-stage ALS patients compared to controls. Further analysis of the most discriminant metabolites highlighted a major impact in several metabolic pathways such as amino acids metabolism, biosynthesis and degradation, aminoacyl-tRNA biosynthesis and the metabolism of glyoxylate and dicarboxylate. Multivariate analysis showed that muscle metabolome was associated with weight variation, while C10-carnitine levels were associated with survival. A targeted transcriptomics approach revealed increased levels of two major genes involved in the antioxidant response, *SOD3* and *GLRX2*. Our broad metabolomics analysis combined with targeted transcriptomics revealed pathological alterations in the muscle of early-stage ALS patients that could be applied in the clinic as biomarkers for diagnosis and prognosis. Furthermore, these alterations represent new targets for the development of therapies to improve the clinical management of ALS patients.

Dr Jean-René Martin

CNRS/University Paris-Saclay, Institut des Neurosciences Paris-Saclay (NeuroPSI, UMR-9197)

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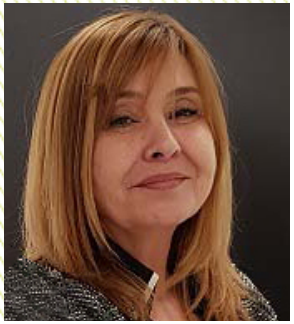
Jean-René Martin received his Doctorat in Veterinary Medicine from Montréal University (UdM), Québec, Canada, in 1984. After two years of teaching Farm Animal Practice at UdM, he moved to Pasteur Institute, Paris, France, where he obtained a Ph.D. in 1993, in genetic and molecular biology in Drosophila. He then worked as Post-Doc, studying Neuro-Ethology in Drosophila, in Würzburg University, Germany, until end of 1999. In 2000, he started his own lab in Orsay, France, and since 2006, he is Research Director, CNRS, at the Neurosciences Institute Paris-Saclay, France, where he is studying the mechanisms of ageing, longevity, and neurodegeneration. In the last few years, he has identified a new small nucleolar RNA (snoRNA) named jouvence, first in Drosophila and thereafter in human.

The snoRNA jouvence, a new versatile tool to protect against the deleterious effects of ageing, as well as inversely, for the treatment of cancer.

We have recently identified a new Small Nucleolar RNA (snoRNA) named jouvence (youth in English) in *Drosophila*. Jouvence, required in the epithelium of the gut, is involved in longevity and neurodegeneration/neuroprotection (Soulé et al., Nat. *Comm*., 2020). As the snoRNAs are well conserved through evolution, we have identified its homologue in human. Briefly, we have shown, in human culture cells, that the overexpression of jouvence increases the proliferation of the cells. A transcriptomic analysis (RNA-Seq) has revealed that several genes involved in the EMT (Epithelial to Mesenchymal Transition) pathway are deregulated, suggesting a dedifferentiation process, compatible to a rejuvenation of the cells. Inversely, its knockdown by siRNA decreases the cells proliferation. Here, a RNA-Seq analysis has revealed that the majority of the gene involved in the ribogenesis and in the spliceosome are importantly depleted. This last effect has been demonstrated in several cancerous cell lines, as HCT116 and Caco-2 (human colon adenocarcinoma), MCF7 (breast cancer), A549 (lung cancer), and U87 (glioblastoma) attesting of its broad efficacy (El-Khoury et al, *BMC-Genomics*, 2020). In this context, jouvence could represent a new and original target, first through its overexpression, to protect against deleterious effect of ageing, while secondly and inversely, though its knockdown, to fight against cancers. Therefore, jouvence could represents a new therapeutic approach (sno-Therapy).



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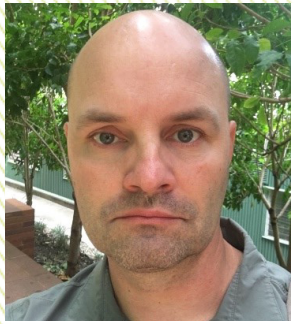
Liliane Massade, DR1 CNRS, is the head of "The "Targeted therapy for peripheral neuropathies" team (https://dhns-inserm.fr/equipe3-2/) of the INSERM UMR 1195 and a part of LaBEX Nanosaclay. She has a large expertise in cellular and molecular biology and in the pharmacology of siRNA. Before focusing her research on neuroscience, she worked on defining the involvement of transcription factors in breast and thyroid carcinogenesis. Since 2008, she is working on the introduction of targeted therapies for cancers and monogenic neuropathies. Her research is supported by grants from the ANR, Nanosaclay, ARC, ERC, SATT, LaBEX Nanosaclay, ... She published more than 70 articles (e.g. Comm. Biol, Cancer Res., Oncogene, J Control Release, PNAS, ...) and has 2 patents.

Squalenoyl siRNA PMP22 nanoparticles, a potent therapy for Charcot-Marie-Tooth disease type 1A

Charcot-Marie-Tooth disease type 1A (CMT1A), caused by a duplication in chromosome 17, results in peripheral myelin protein 22 (Pmp22) over-expression and axon demyelination. The diagnosis of CMT1A is based on decreased nerve conduction velocity (NCV) and compound muscle action potential (CMAP), with progressive muscle weakness and impaired sensations. Here, we provide a new therapy for CMT1A, based on the normalization of PMP22 expression by specific siRNA conjugated to squalene nanoparticles (siRNA PMP22-SQ NPs). Their administration resulted in the normalization of Pmp22 protein levels, reversed neuropathy scores and restored locomotor activity in two transgenic mouse models of CMT1A. Moreover, NCV and CMAP were significantly improved. Pathological studies demonstrated the regeneration of myelinated axons and myelin compaction. The normalization of sciatic nerve Krox20, Sox10 and neurofilament levels reflected the regeneration of both myelin and axons. Importantly, the positive effects of siRNA PMP22-SQ NPs lasted for as long as three weeks, and their renewed administration again resulted in full functional recovery. Beyond CMT1A, our findings can be considered as a potent therapeutic strategy for dominantly inherited peripheral neuropathies. They provide the proof of concept for a new precision therapy based on the normalization of disease gene expression by siRNA.



Director Jason Potter

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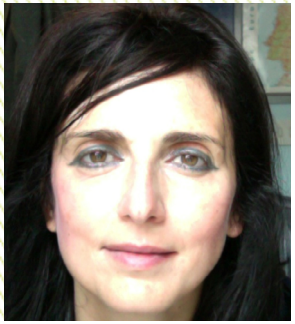
Jason Potter leads the Cell Biology Genome Editing R&D team in the Life Sciences Solutions Group of Thermo Fisher Scientific in Carlsbad, CA. His group is focused on developing tools for the entire genome editing workflow, including generation of editing nucleases in mRNA or protein formats, mRNA IVT systems, and developing new LNP delivery formulations. His group develops workflows for primary cells such as T, NK and iPSC; bioproduction lines such as CHO and HEK293; and mouse models.

What matters for making highly expressed mRNA.

The need for high quality mRNA for therapeutic applications has grown substantially over the last few years due to the efficacy of the COVID mRNA vaccines. The expanding mRNA applications generally use custom mRNA made by IVT systems. Here I will discuss what factors matter for generating mRNA that can be expressed at high levels in mammalian cells. This will include how to optimize yields, capping efficiency, and transcript purity including the removal of immunogenic byproducts. By controlling the quality of mRNA transcripts we have been able to increase expression levels in cells and mice by over 10 fold.



Prof. Antonella Riccio

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I was awarded an MD PhD degree from Catholic University Medical School in Rome and spent several years as a postdoctoral fellow in the Department of Neuroscience of the Johns Hopkins University School of Medicine in Baltimore (MD). I moved at UCL in 2005 where I became professor of Neuroscience. The research in my lab focuses on understanding how gene expression is regulated in neurons and we use cutting-edge techniques to study epigenetic, transcriptional and post-transcriptional mechanisms responsible for regulating neuronal development.

RNA metabolism in developing neurons

Neurons are cells with a complex morphology, which maintain their cellular structure through the compartmentalized expression of proteins essential for growth and plasticity. Asymmetric localization of RNA is an evolutionarily conserved mechanism that allows spatial restriction of protein synthesis to specific cellular compartments. Incorrect processing and delivery of mRNA causes developmental defects and severe human neurological disorders. In neurons, mRNA transcripts are transported to both dendrites and axons where they are rapidly translated in response to stimuli. The talk will explore how transcripts localized in sympathetic neuron axons are transported, processed, and translated in response to neurotrophins. Special emphasis will be given to the nature of the 3'UTRs of targeted axons and to the presence of unique elements that may determine their fate. I will also discuss our findings indicating that the 3'UTR of localized transcripts undergo axonal cleavage and remodelling, thereby generating mRNA isoforms expressing a shorter 3'UTR, which are rapidly translated, and axonally cleaved RNA fragments with yet unknown function.

Prof. Krzystof Sobczak

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Krzysztof Sobczak – professor of Adam Mickiewicz University (AMU) in Poznan (Poland). He got PhD degree and postdoctoral degree in chemistry from the Institute of Bioorganic Chemistry PAS. He was awarded the title of professor of biology in 2019. Since 2019, he is the director of the Institute of Molecular Biology and Biotechnology of AMU. In 2005-2009 he completed a postdoctoral fellowship at the University of Rochester (USA). In 2010, he founded a new research group, whose interests focus on the elucidation of the molecular pathomechanisms of human genetic diseases caused by the expansion of trinucleotide repeats and the development of therapeutic strategies targeting mutant RNA. He is the author of over 70 original articles which were cited ~4,000 times (ORCID: 0000-0001-8352-9812).

Compounds which alleviate the pleiotropic toxicity of RNA harboring expanded CGG repeats in the Fragile X–associated syndrome

Fragile X-associated tremor/ataxia syndrome (FXTAS) is an incurable neurodegenerative disorder caused by expansion of CGG repeats in the FMR1 5'UTR. The RNA containing expanded CGG repeats ($rCGG^{exp}$) causes cell damage by interaction with complementary DNA, forming R-loop structures, sequestration of nuclear proteins involved in RNA metabolism and initiation of noncanonical translation of polyglycine-containing protein (FMRpolyG), which forms nuclear insoluble inclusions. During the lecture we will discuss the therapeutic potential of short antisense oligonucleotide steric blockers (ASOs) and small compounds targeting directly the $rCGG^{exp}$. In nuclei of FXTAS cells ASOs affect R-loop formation and correct miRNA biogenesis and alternative splicing, indicating that nuclear proteins are released from toxic sequestration. In cytoplasm, ASOs significantly decrease the biosynthesis and accumulation of FMRpolyG. Delivery of ASO into a brain of FXTAS mouse model reduces formation of inclusions, improves motor behavior and corrects gene expression profile with marginal signs of toxicity after a few weeks from a treatment. We also identified small compounds, CMBLs, which bind to RNA structure formed by $rCGG^{exp}$ and attenuates translation of toxic FMRpolyG and formation of nuclear inclusions in FXTAS cells. Our results indicate that CMBL4c can reduce FMRpolyG-mediated cytotoxicity and apoptosis. Importantly, its therapeutic potential is also observed once the inclusions are already formed.

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I graduated at The Rockefeller University and started my research group in 2006, which is currently located at The Francis Crick Institute in London. We study the structure, function and evolution of ribonucleoprotein complexes (RNPs) and their roles in neurodegenerative diseases. We developed iCLIP (individual-nucleotide resolution UV crosslinking and immunoprecipitation), a method that identifies in vivo protein-RNA interactions in a transcriptome-wide manner. In recent years, we addressed the mechanisms controlling alternative splicing, characterised the function of several RNA-binding proteins that are implicated in motor neuron disease, and revealed how mutations can lead to disease or evolution by modifying protein-RNA interactions or mRNA structure (more at www.ulelab.info).

How do protein-RNA condensates form and contribute to disease?

Mutations in many genes encoding RNA-binding proteins (RBPs) cause neurologic diseases, and especially the amyotrophic lateral sclerosis (ALS). These mutations tend to be located within intrinsically disordered regions (IDRs) of RBPs. To understand how these mutations act, we developed crosslinking and Immunoprecipitation (CLIP) to obtain transcriptome-wide maps of in vivo protein-RNA interactions. I will also describe our recent technical advances that helped us to use CLIP in a quantitative manner and to gain broader insights from analyses of public data. We developed an improved individual nucleotide resolution CLIP protocol (iiCLIP), which produces highly sensitive and specific data, and thus enables quantitative comparisons of interactions across conditions (Lee et al., 2021). Finally, I will exemplify how these advances helped with studies of mutations in the IDR of TDP-43, a protein that is a central factor in ALS and several other neurodegenerative diseases. CLIP enabled us to disentangle the principles driving the condensation of TDP-43 on cellular RNAs, which showed that the IDR selectively fine-tunes the RNA binding properties and functions of the protein (Hallegger et al., 2021). I'll also discuss how insights into the specificity of RBP condensation open new doors for understanding of protein-RNA complexes as mediators of signalling, disease and evolution.

Reference:

Hallegger, M., Chakrabarti, A.M., Lee, F.C.Y., Lee, B.L., Amalietti, A.G., Odeh, H.M., Copley, K.E., Rubien, J.D., Portz, B., Kuret, K., et al. (2021). TDP-43 condensation properties specify its RNA-binding and regulatory repertoire. Cell 184, 4680–4696.e22.

Lee, F.C.Y., Chakrabarti, A.M., Hänel, H., Monzón-Casanova, E., Hallegger, M., Militti, C., Capraro, F., Sadée, C., Toolan-Kerr, P., Wilkins, O., et al. (2021). An improved iCLIP protocol. bioRxiv, doi.org/10.1101/2021.08.27.457890

POSTERS

- **Network Based Approach for Targeting Human Kinases commonly associated with ALS and Other diseases**

Fatima Khatoon[1], Vijay Kumar[1*]

[1] Amity Institute of Neuropsychology and Neurosciences, Amity University, Noida

- **Extensive network-based approach to identify the potential biomarkers of Amyotrophic Lateral Sclerosis**

Kartikay Prasad[1], Vijay Kumar*

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Email: vkumar33@amity.edu

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