Architecture, Function and Dynamics in Life Sciences: ArchiFun a MSCA Cofund International Doctoral Programme (DP)

1st Call for application: deadline June 1st 2024; selection in june-july 2024; recruitment October 1st 2024

ArchiFun is an international, interdisciplinary and inter-sectoral DP centred on **Horizon Europe** Work Programme's **Destination 5**: Unlocking the full potential of new tools, technologies and digital solutions for a healthy society. With the support of the Marie Skłodowska-Curie Actions Cofund, ArchiFun has the goal of providing a shared, highly qualified training for a new generation of **researchers in life sciences**, by equipping them for academic, public or private career paths. ArchiFun will explore the relationships between architecture, function and dynamics across all levels, spanning from atoms up to cells, organisms and ecosystems.
Each recruited **doctoral fellow** will also have the opportunity to build a **personal career development plan**, by choosing among the offers of secondments in the implementing and associated partners, as well as the dedicated training schools organised by ArchiFun in the period 2025-2029. More information online: https://cofund-archifun.univ-lyon1.fr

At the **University Claude Bernard Lyon 1**, the following research projects are proposed:

Doctoral School ED340 – BMIC

Project 1: The Role of Interactions with Dendritic Cells in Sustaining NK Cell Functions in Tumor Conditions
Project 2: Elucidating the specific neuronal vulnerability in Friedreich ataxia preclinical models
Project 3: Peripheral determinants of viral encephalitis: towards predictive biomarkers and limited neurovirulence
Project 4: Study of the tropism of a new variant of TDP-43 (G376V-TDP-43) responsible for distal myopathy but not an ALS

Doctoral School ED 206 – Chimie

Project 1: Characterisation and in vitro validation of biopolymers for the treatment of iron overload
Project 2: Structural and functional investigation of a molecular regulator involved in bacterial silver resistance using NMR
Project 3: Vaccine design using synthetic outer membrane vesicles incorporating defined carbohydrate antigens and immunostimulants

Doctoral School ED 160 – EEA

Project 1: Contribute to the development and optimization of a signal processing and AI algorithm for microwave imaging in the early detection of breast cancer

Doctoral School ED 476 – NSCo

Project 1: SchizApathy: Unveiling the Neuro-Computational bases of Apathy in Schizophrenia
Project 2: PEPsy project

Doctoral School ED 52 – PHAST

Project 1: Investigation at the molecular level of the interaction of fluorescent probes with early protein aggregates
Project 2: Development of Laser-generated Surface Acoustic Wave Immuno-sensors

Doctoral School ED 341 – E2M2

Project 1:The hexokinase phosphorylation landscape: new regulation of an "old" enzyme

Project 2: Regulation and impact of post-transcriptional modifications of *Staphylococcus aureus* tRNAs
Project 3: Stress gradients and the emergence of treatment resistance in infection and cancer

1 Doctoral Fellow per Doctoral school will be recruited in 2024.

At **University of Granada**, the following research projects are proposed:
Project 1: Efficacy of targeted nanoparticles coated with hybrid cell membranes against pancreatic cancer heterogeneity
Project 2: Explainable AI-powered multimodal data integration for healthcare
Project 3: Biomimetic Magnetic Hydrogels for Translational Research
Project 4: Unravelling amyloid aggregation with novel luminescent probes for super-resolution microscopy

2 Doctoral Fellows will be recruited in 2024

At the **Technical University Munich**, the following research projects are proposed:
Project 1: Structural and functional characterization of the disease-linked inner mitochondrial membrane protein MPV17
Project 2: Molecular insights into the misfolding pathways of antibody light chain proteins
Project 3: Structure, interactions and dynamics of an RBM17 complex linked to alternative splicing regulation

3 Doctoral Fellows will be recruited

At the **National Institute of Chemistry**, the following research project is proposed:
Project 1: Host-pathogen interactions in Plants: Characterization of NLPs, a new family of pore-forming proteins

1 Doctoral Fellow will be recruited

The Role of Interactions with Dendritic Cells in Sustaining NK Cell Functions in Tumor Conditions

Natural killer (NK) cells are innate cytotoxic lymphocytes. They play a crucial role in anti-tumor immunity, especially against hematological malignancies and metastasis.
NK cells display several effector functions including granule-dependent cytotoxicity and secretion of inflammatory cytokines (TNF-α and IFN-γ) and chemokines (CCL3-5 and XCL1). Target cell recognition by NK cells is finely regulated through a wide range of activating versus inhibitory receptors. NK cell activation and effector functions are triggered when activating signals overcome inhibitory ones, the latter being provided upon recognition of MHC-I molecules. Mechanistically, this involves opposing actions of kinases and phosphatases on common substrates.

When they get activated, NK cells are capable of killing multiple targets through the release of their cytotoxic granules filled with granzymes and perforins, and through their expression of FasL, binding to its death receptor Fas on target cells.
Tumor cells are sensitive to NK cells. Indeed, they may lose MHC-I expression as a result of genomic instability combined with CD8-T cell mediated editing. Nonetheless, NK cells become dysfunctional, refractory to activation during cancer development, limiting their anti-tumor effectiveness. This phenomenon involves both tumor-induced immune suppression and tumor-induced exhaustion resulting from persistent stimulation through activating receptors. However, despite intense research efforts, the molecular mechanisms of NK cell dysfunction remain poorly understood, which clearly limits our ability to design efficient NK cell-based cancer therapies.

A major limitation to the exploration of the NK cell exhaustion mechanisms is the access to a sufficient number of exhausted NK cells in a physiological cancer model. NK cells are indeed often scarce in the tumor bed, and their isolation is complicated in solid tumors. To circumvent this issue, we developed a hematological tumor model based on the RMA T cell lymphoma line; this model grows in the spleen, the most important NK cell reservoir in the mouse body. Since previous papers linked exhaustion and activation, we modified the RMA line using Crispr/Cas9-mediated genome editing to knock out MHC-I expression and we expressed the strong NKG2D ligand Rae1-β by means of a lentiviral vector. As expected, MHC-I-/- RMA cells expressing Rae1-β are highly susceptible to NK cell mediated killing, both in vitro and in vivo, while parental cells are not. NK cell dysfunction is only induced in the tumor model that activates NK cells in a tumor mass-dependent way. Surprisingly, NK cells expressing immune checkpoints (ICPs) demonstrate heightened reactivity compared to those that do not express them.

We recently discovered that dysfunctional NK cells have reduced mTOR activity upon exposure to physiologic IL-15 concentration. This reduced IL-15 dependent mTOR activity could explain at least in part their poor reactivity since IL-15 is a central cytokine in NK cell development and activation, and mTOR inhibition has a profoundly negative effect on NK cell reactivity. mTOR is the catalytic subunit of two distinct complexes: mTORC1 and mTORC2. Studies in cell lines have shown that mTORC1 is a coincidence detector for two classes of input signals: 1) metabolic cues and 2) growth factors such as cytokines. We previously reported that mTOR was strongly activated after exposure of NK cells to high concentrations of IL-15, whereas low doses of IL-15 triggered only phosphorylation of the

transcription factor STAT5. More recently, we found that IL-18 and IL-15 synergized to induce strong mTOR activity, leading to enhanced NK cell proliferation while other innate cytokines (IFN-I, IL-12) had no effect. Since IL-15 and IL-18 are primarily produced by dendritic cells (DCs) and monocytes/macrophages respectively, this suggests the contribution of different myeloid types in NK cell mTOR activation.

We therefore postulate that the reactivity of NK cells in the tumor context depends on their cyclical stimulation by cytokines such as IL-15 produced by DCs, which contributes to maintain high mTOR activity. Other cytokines produced by DCs or other myeloid cells (monocytes, macrophages) could also positively contribute to this regulation. In our model, NK cell exhaustion would result from a loss of signal reception from DCs, in favor of tumor growth. ICPs may also play a role in regulating interactions with DCs or tumors, or, analogous to inhibitory NK cell receptors, they could preserve NK cell reactivity upon chronic interaction with their ligands on myeloid cells.

To test these hypotheses, we propose four work packages (WPs) aimed at:

1) Investigating the role of different types of DCs and the cytokines they produce in maintaining NK cell anti-tumor reactivity in different mouse tumor models.
2) Characterizing the function, transcriptome, and localization of distinct NK cell subpopulations defined by ICP expression in the spleen.
3) Defining the proteomic signature of exhausted NK cells and that dependent on mTOR.
4) Designing and testing strategies to target DCs to enhance the anti-tumor functions of NK cells.

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Main ArchiFun theme involved:

- ☐ Host-pathogen interactions;
- ☐ Mechanisms of bacterial resistance and cancer onsets;
- ☐ Neurodegenerative and autoimmune diseases;
- ☑ Translational research in prevalent diseases;
- ☐ Physiology and ecology;
- ☐ Neurosciences and cognition.

Elucidating the specific neuronal vulnerability in Friedreich ataxia preclinical models

Neurodegenerative diseases (NDs) remain an ever-increasing, incurable burden on our society, wherein specific neurons within defined regions of the central nervous system degenerate. To date, the mechanisms underlying the major NDs are poorly understood. One such group of neurodegenerative diseases is Hereditary Ataxias, caused by mutations in >60 genes, leading to the degeneration of cerebellar neurons and motor related circuits.

In our lab, we focus on Friedreich Ataxia (FA), an inherited early-onset ataxia with symptoms onset during adolescence, caused by a GAA repeat expansion within the frataxin gene, leading to low levels of frataxin (FXN). The disease onset and severity correlate to the residual levels of FXN, which inversely correlates with the length of the GAA expansion. FXN is a ubiquitous nuclear encoded mitochondrial protein involved in iron-sulfur (Fe-S) cluster biogenesis, an essential process in all cells that provide co-factors for the function of numerous proteins involved in key cellular pathways, such as central metabolism, protein translation and DNA metabolism. With disease progression, patients develop progressive ataxia of the limbs, associated with cerebellar dysarthria, sensory loss, and abnormal pyramidal signs, due to degeneration of proprioceptive neurons located in the dorsal root ganglia (DRG), a key component of the sensory system, the corticospinal tract, as well as the spinocerebellar tract, becoming wheelchair-bound within 10–15 years after onset. Interestingly, spinal motoneurons (MNs) are not particularly affected by the degenerating process until end stage of the disease unlike DRG sensory neurons (SNs). While much progress has been made in understanding the pathogenesis of FA through the development of animal and cell models, there are still many unanswered questions regarding the specific neuronal vulnerability, which might represent a breakthrough in the development of new therapies for patients.

The thesis project objective is to elucidate the specific neuronal vulnerability in FA, with two complementary aims:

Aim 1: To investigate the molecular differences underlying MNs and SNs vulnerability, we will take advantage of induced pluripotent stem cells (iPSCs) derived from FA patients with different GAA expansion sizes together with isogenic controls (where the expansion has been corrected using CRISPR/Cas9 system) and healthy controls. We will differentiate the iPSCs into spinal MNs and SNs. First, we will characterize the different cell lines in terms of expression markers of mature neurons, survival in culture, morphological analysis such as complexity of axonal breanching, soma size, electrophysiological properties using patch clamp, and mitochondrial activity. Finally, we will use omics techniques such as proteomics to identify new pathways that might be relevant for understanding neuronal vulnerability and the molecular signature that makes one type of neuron more vulnerable than the other in FA. With this approach, we will be able to identify specific pathways that can be relevant for the disease progression and that can be pharmacologically targeted.

Aim 2: This aim will be devoted to the characterization of MNs and SNs in a recently generated mouse model, FxnG127V KI (a point mutation previously found in FA patients), that presents low levels of frataxin in all cells types, recapitulating a scenario similar to FA-affected individuals. The phenotype in MNs and SNs in the FxnG127V KI mouse model has never been characterized before. Nevertheless, preliminary data from our team, show mitochondrial impairments and synaptic connectivity alteration in the cerebellum (another

major affected region in FA) that also reflect behavioural impairment. Therefore, we will characterize both dorsal root ganglia (DRG) and lower MNs longitudinally from pre-symptomatic to symptomatic stages of the disease from a morphological point of view, investigating mitochondria morphology and acitivity using enzymatic assay, synapses connectivity, and related activity markers. This deep characterization will allow us to identify the entry point to validate our human proteomics result in a more complex system and to have biomarkers that can be used as read out of a successful modulation of a specific pathway. To this aim the putative candiate pathway will be modulate via gene therapy using adeno-associated viruses (AAVs) or pharmacological approach in order to validate the relevance of the selected molecule in ameliorating FA pathophysiology both in vivo and in vitro.
This ambitious project tackles, for the first time, the relationship between vulnerable and resilient neurons in the context of FA. Previously, the absence of suitable disease models posed a significant obstacle to research progress. By integrating human iPSC models with a newly developed mouse FA rodent model, we aim to address a crucial question that is fundamental to FA research.

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Proposed collaboration within ArchiFun network:
University of Padova Italy

Proposed list of secondments:
Prof. Elena Ziviani, Department of Biology, University of Padova, Padova, Italy

Main ArchiFun theme involved:

- ☐ Host-pathogen interactions;
- ☐ Mechanisms of bacterial resistance and cancer onsets;
- ☑ Neurodegenerative and autoimmune diseases;
- ☐ Translational research in prevalent diseases;
- ☐ Physiology and ecology;
- ☐ Neurosciences and cognition.

PERIPHERAL DETERMINANTS OF VIRAL ENCEPHALITIS: TOWARDS PREDICTIVE BIOMARKERS AND LIMITED NEUROVIRULENCE

The Covid-19 pandemic was a reminder of the threat posed to human health by the burden of RNA viruses. RNA viruses include both emerging viruses such as Nipah virus (NiV) and West Nile virus (WNV), and long-established viruses for which the number of cases is rising again today, such as dengue virus (DENV) or measles virus (MeV). Measles virus (MeV) and Nipah virus (NiV) are enveloped negative-strand RNA viruses of the Paramyxoviridae family, both of which infect the respiratory system early in the course of infection. NiV usually reaches the central nervous system (CNS) and induces an acute, fatal neurological disease, and relapses can occur in patients who have survived. In rare cases, MeV virus also infects the CNS, causing fatal neurological disease, sometimes even years after primary infection: the high mutation rate of RNA viruses allows neuroinvasive variants to emerge, even if CNS cells do not express any MeV receptors. Dengue virus (DENV) and West Nile Virus (WNV) are two enveloped positive-strand RNA viruses within the Flaviviridae family. DENV causes different neurologic complications, such as viral encephalitis when the virus reaches the CNS or encephalopathy that involves acute liver failure. WNV is notably responsible for encephalitis manifested by extrapyramidal disorders, tremor, parkinsonism, and a case fatality rate between 10% and 30%.

In the first task of this PhD project we will focus on the early peripheral events determining the neurovirulence of viral infections. In subtask 1.1 we will take advantage of hamster and human organotypic lung and liver cultures well mastered in the laboratory, to **identify predictive biomarkers of neurovirulence** by RNA sequencing and RT-qPCR of infected tissue, and assay of several molecules in culture media, by comparing the pathogenesis of neurovirulent and non-neurovirulent viral strains. As neuroinvasive leukotropic viruses can use white-blood cells to disseminate in the body through the blood flow, we want to figure out in subtask 1.2 what the **differences of peripheral resident macrophages susceptibility and cell-mediated transport are**, when infected by neurovirulent or non-neurovirulent viral strains. In subtask 1.3 we will focus on the **infection of the liver that can induce major changes in the organism homeostasis**. **We wonder to what extent it consequently participates in the associated neurological injuries**, by altering the BBB integrity or over-activating microglia for instance, or in the enlargement of the virus tropism through the acquisition of a new molecular phenotype of the viral envelope including molecule involved in liver-brain exchanges after budding.

The second task of this PhD project will focus on the CNS response upon infection and in subtask 2.1 we will investigate the cellular tropism of different viral strains, neurovirulent or not, using IF and FACS methods developed and mastered by the team. As several neurovirulent viruses target immune cells, we will compare the antiviral and proviral roles of microglia, as well as the consequences of microglia infection/activation on the brain parenchyma in subtask 2.2. Our final question will be to decipher the functional consequences of different CNS viral infections in subtask 2.3, and how the use of neuromodulatory therapies could restore a physiological phenotype. We will adapt **GCaMP** protocols for brain slices from the literature to hamster organotypic cerebral cultures, whether whole sagittal brain cultures or cerebellum cultures. We will then use them to assess the **functional consequences of infection on neuronal electrical activity**, comparing neurovirulent to non-neurovirulent strains. We will then be able to study the spatial correlation between infected foci and local or global electrical activity disturbances. As epileptic seizures are common complications of viral encephalitis, this tool could help us to model and study its pathogenesis, and we could use it as a **platform to assess the therapeutic potential of anti-epileptic drugs or other neuromodulators to correct the electrophysiological phenotype** of infected brains.

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Main ArchiFun theme involved:

- ☐ Host-pathogen interactions;
- ☐ Mechanisms of bacterial resistance and cancer onsets;
- ☑ Neurodegenerative and autoimmune diseases;
- ☐ Translational research in prevalent diseases;
- ☐ Physiology and ecology;
- ☐ Neurosciences and cognition.

Study of the tropism of a new variant of TDP-43 (G376V-TDP-43) responsible for distal myopathy but not an ALS

Amyotrophic Lateral Sclerosis (ALS) and ALS associated with Fronto Temporal Lobar Degeneration (ALS-FTLD) are fatal neurodegenerative disorders characterized by progressive muscular paralysis reflecting degeneration of upper and lower motor neurons (MNs) in the primary motor cortex, corticospinal tracts, brainstem and spinal cord. The deterioration of the patient's conditions is irreversible and death occurs with 1-5 years after the onset for 70% of affected patients, often by respiratory failure. Approximately 10% of ALS cases are considered "familial" (fALS), or transmitted within families, while the remaining cases are considered "sporadic" (sALS), or presenting without a clear familial history. Although more than 30 potentially causative or disease-modifying genes have been identified, pathogenic variants in *SOD1*, *C9ORF72*, *FUS/TLS*, and *TARDBP*, account for over 50% of the familial cases, most frequently with disease causing variants in other genes being relatively uncommon. Many of the identified variant genes were found to be involved in RNA or protein homeostasis cellular processes.

Although less common than sALS, fALS has played an outsize role in our understanding of disease mechanisms through the discovery of ALS-causing mutations within families and subsequent experimental perturbations of these mutant genes. Because fALS occurs within the same family across multiple generations, genetic approaches can be used to pinpoint the mutated gene that tracks with people who developed ALS and away from those who did not. Like several neurodegenerative disorders, ALS and ALS-FTLD are associated with the accumulation of misfolded proteins both inside and outside of neuronal and glial cells. TDP-43 (reviewed in Smethurst *et al.*, Neuropathol&Appl Neurobiol 2015) is the major component of Tau an α-synuclein negative but Ubiquitin positive pathological inclusions found in the brains of patients with ALS and FTLD (Arai *et al*., BBRC 2006; Neumann *et al.*, Science 2006). TDP-43 pathological inclusions are observed in more than 95% of ALS and ALS-FTLD affected patients making this protein a key component in the pathology that it is essential to study and characterize.

We recently identified a new missense variant of TDP-43 (G376V-TDP-43) into the C-terminal prion-like domain of the protein in two French families affected by an autosomal dominant distal myopathy but not fulfilling diagnostic criteria for ALS (Zibold *et al.,* Brain 2023). Patients from both families presented with progressive weakness and atrophy of distal muscles, starting in their 5th-7th decade. Muscle biopsies revealed a degenerative myopathy characterized by accumulation of rimmed (autophagic) vacuoles, disruption of sarcomere integrity and severe myofibrillar disorganization. Variant pathogenicity was supported by functional studies. The G376V mutant increased the formation of cytoplasmic TDP-43 condensates in cell culture models, promoted assembly into high molecular weight oligomers and aggregates in vitro, and altered morphology of TDP-43 condensates arising from phase separation. Moreover, the variant led to the formation of cytoplasmic TDP-43 condensates in patient-derived myoblasts and induced abnormal mRNA splicing in patient muscle tissue. Strikingly, changing the same glycine residue into an aspartic acid (G376D) causes an ALS with a rapid progression. Comparisons of G376V and G376D in different cellular models as well as in vitro using recombinant proteins revealed that the G376V variant was more prone to form insoluble TDP-43 condensates compared to both the G376D variant and the wild type TDP-43, while the G376D variant had only a very minor effect.

This is the first time that two different substitutions altering the same amino acid position in TDP-43, primary drive an inherited disease in two separated directions ie muscular disorder versus a fatal ALS. In this context, we propose to investigate at the cellular and transcriptomic levels (mRNA expression and splicing variants characterizations) if the G376V TDP-43 variant displays a muscle tropism and can impact this specific tissue compared to the G736D variant. For this purpose, we will explore the functional consequences of the expression of both variants (G376V and G376D) into MNs and muscle cells differentiated from CRISPR Cas9 modified-iPSC but also from iPSC derived from patients to evaluate the imprinting impact of the familial genetic background. Interestingly, two additional French families suffering of distal myopathy (unpublished) were recently identified with the same G376V TDP-43 variant. Analyses will also be conducted in patient muscle biopsies from the different families to characterize the impact of this mutation.
Because we cannot exclude that additional modifier genes could attenuate the phenotype associated with the G376V-TDP-43 variant into our families, we also propose to determine through whole exome analyses, if candidate modifier genes can be highlighted in different patients from the different identified families.
Overall, this project will give important informations on molecular and cellular properties of TDP-43 through an original experimental approach that will consist in starting from patients with a distal myopathy associated with a TDP-43 mutation to better characterize the role of TDP-43 in the ALS pathology.
Understanding why the G376V-TDP-43 is associated with a less deleterious disease is of utmost importance to identify potential therapeutical targets to fight against ALS.

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Main ArchiFun theme involved: Neurodegenerative and autoimmune diseases

Characterisation and in vitro validation of biopolymers for the treatment of iron overload

Iron metabolism is precisely regulated by a number of proteins that ensure its absorption, circulation, storage and recycling. When iron homeostasis is disrupted, various pathologies can occur, including iron deficiency anaemia and haemochromatosis resulting from iron overload. While haemochromatosis is a well-known chronic disease characterised by excessive iron levels, acute dysregulation of iron homeostasis has also been linked to a number of other pathologies, including sepsis stroke, acute kidney injury. Iron levels have even been proposed as an indicator of outcome in intensive care patients (Figure 1)

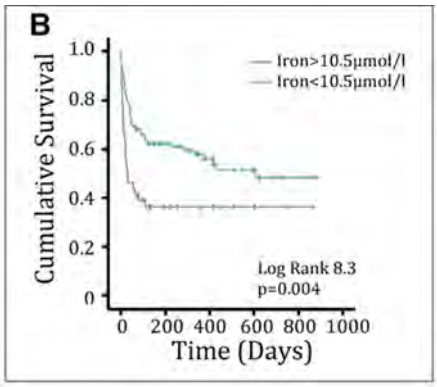

Figure 1: Survival and iron levels on admission of intensive care patient

ILM (François Lux, Fennec Team) has developed a biopolymer composed of chitosan functionalised by various chelates, including DOTAGA (M. Natuzzi et al., Sci. Rep., 2021). Chitosan@DOTAGA has been associated with a haemodialysis system and has been approved by the French and Spanish authorities in clinical trials for the treatment of copper overload in Wilson's disease (NCT05917327). In parallel, ISA (Agnès Hagège, TechSep team) specialises in the development of analytical techniques to study chelation in biological media and to determine the size of nano-objects. A unique tool based on Taylor dispersion analysis coupled to ICP/MS has been developed to monitor the fate of nano-objects in media as complex as serum: biodegradation, association with proteins, etc. (L. Labied et al., Anal. Chem. 2021; L. Labied et al. Anal. Chim. Acta, 2021; A. Degasperi et al, Talanta, 2022).

Such chitosan-based biopolymers are currently being studied for the treatment of iron overload. A clinical trial in acute or chronic liver failure has been submitted to the French agency in October 2023. In this context, the characterisation of these biopolymers and the study of their iron chelation in competition with various biomolecules, including albumin, transferrin and small ligands such as citrate or haem, compared with traditional chelating agents such as deferiprone, is of prime importance. The use of such a coupling will enable unique characterisation of their chelation capacity, both in buffers and in biological fluids such as serum.

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Main ArchiFun theme involved:

- ☐ Host-pathogen interactions;
- ☐ Mechanisms of bacterial resistance and cancer onsets;
- ☐ Neurodegenerative and autoimmune diseases;
- ☑ Translational research in prevalent diseases;
- ☐ Physiology and ecology;
- ☐ Neurosciences and cognition.

Structural and functional investigation of a molecular regulator involved in bacterial silver resistance using NMR

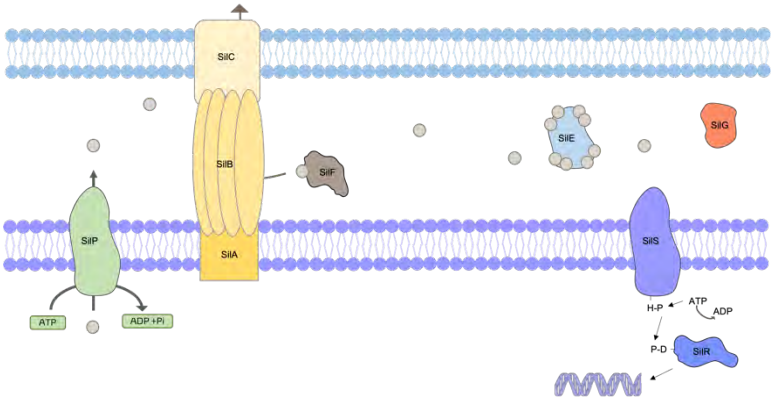

Figure 1 : Composition of the sil system

Like werewolves and vampires, bacteria have a weakness: silver. The antimicrobial properties of this precious metal have extensively been used for thousands of years. Despite this long-standing history and its demonstrated activity against Gram-negative bacteria, the complete bactericidal mode of action of silver remains unclear. Nevertheless, silver misuse can damage the cells and a note of caution is mandatory about its potential toxicity. To counteract the toxic effect of silver, Gram-negative bacteria have developed different resistance mechanisms, including the efficient efflux of the metal out of the cell. The first silver-resistant plasmid pMG101 was isolated from *Salmonella* strain after the death of patients in the burn ward at the Massachusetts General Hospital. The silver-resistant gene cluster is composed of nine genes: a chemiosmotic efflux pump (SilCBA), an ATPase efflux pump (SilP), a responder and membrane sensor performing two-component transcription regulation (SilRS) and three periplasmic silver-binding proteins SilE, SilF and SilG.

Our group is interested into the understanding of the complete mechanism of silver ions eviction through the efflux pump system *sil*. Until now, we tried to decipher how the interplay between SilB, SilF and SilE proteins contribute to the silver efflux pump mechanism. The next challenge will be the understanding of the role of SilG. This small protein was the last discovered and the only homologous protein is found in the *cop* system, namely CopG (identity 44%). The role of CopG is to interconvert Cu(I) and Cu(II) to minimize toxic effects and facilitate export by the efflux pump. In our case, we wonder if SilG has also a role of an oxidoreductase for Ag(I). The structures in the free and complexed with silver ions forms must be resolved and we would like to understand the interaction with the other periplasmic proteins. To reach our goal, we first produce and purify the different periplasmic proteins and then, we combine biophysical methods, namely Nuclear Magnetic Resonance (NMR), Circular Dichroism (CD) and Small Angle X-Ray Scaterring (SAXS) with hybrid molecular dynamics to completely describe the mechanism of the eviction of silver ions through the efflux pump.

The project will be hosted by the analytical science institute located in Lyon/Villeurbanne (France). This new institute comprise around 150 researchers and is among the largest analytical science center in Europe. The thesis project will be developed inside the Biosys group and will mainly make use of NMR and will benefit from the expertise of the group members. A part of the project will be dedicated to the production of isotope labeled proteins.

Hiring profile - The successful candidate should have completed (or in stage of completion) M.Sc. degree either in biochemistry, structural biology, biology, physical chemistry or related fields. Willingness to learn NMR will be strongly appreciated.

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group web-site: http://nmrbiolchem.univ-lyon1.fr/

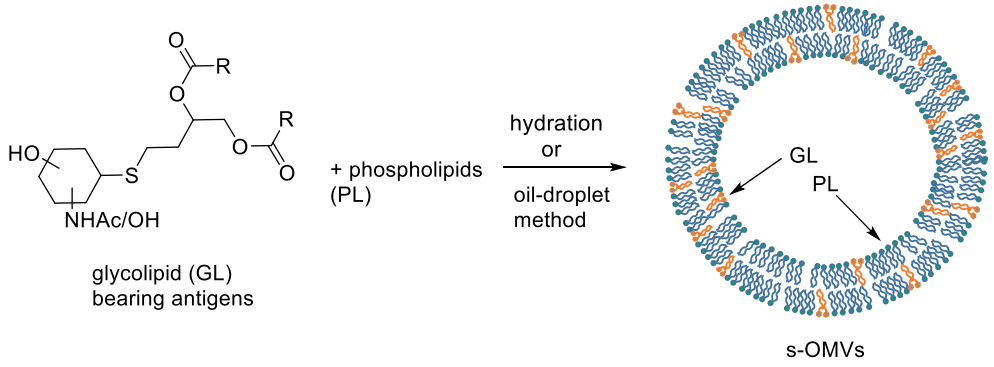
Main ArchiFun theme involved:

- ☐ Host-pathogen interactions;
- ☑ Mechanisms of bacterial resistance and cancer onsets;
- ☐ Neurodegenerative and autoimmune diseases;
- ☐ Translational research in prevalent diseases;
- ☐ Physiology and ecology;
- ☐ Neurosciences and cognition.

Vaccine design using synthetic outer membrane vesicles incorporating defined carbohydrate antigens and immunostimulants

Nature-inspired synthetic outer membrane vesicles (s-OMVs) will be used as vaccines or vaccine carriers. Bacterial infections and cancer represent two major causes of death worldwide. In most cases known therapies such as antibiotics, chemo- or radiotherapy are insufficient and vaccination becomes a vital alternative. The control of the composition of a vaccine is one of the main issues of their preparation. Synthetic vaccines are today of great interest and some are in early clinical stage. However, their formulation often still remains ill-defined, which slows their possible commercialization and introduces variability in individual responses. Fully synthetic vaccines based on synthetic OMVs incorporating defined, synthetic carbohydrate antigens and adjuvants, are more feasible in terms of preparation and formulation representing a significant improvement on commercially available vaccines.

A recent study reported the chemical synthesis of s-OMV prototypes combining phospholipids and synthetic glycolipids bearing the appropriate carbohydrate to promote biological activity as the stimulation of immune system[1]. Activation of an immune response involves extremely complex mechanisms and can be achieved mainly as passive or active mechanisms[2]. The key steps in immune stimulation are (1) recognition of the antigenic structure by the B-lymphocyte antigen receptor (BCRs); (2) activation of T cells by presentation of bacterial substructures on the MHC type II by the T-cell receptor (TCR); (3) activation of cellular (innate) immunity pathways by adjuvants bearing pathogen-associated molecular patterns (e.g. TLR4).



Several fully synthetic carbohydrate antigens, T-cell activators, and PAMP-associated adjuvants will be synthesized in Peter Goekjian's group using fluorous-tag methodology [3] and flow chemistry to streamline the synthesis process. We are particularly interested in extending the role of fluorous tags beyond isolating synthetic intermediates to increasing immunogenicity and monitoring s-OMV incorporation.

1. a) Fayolle, D.; Berthet, N.; Doumeche, B.; Renaudet, O.; Strazewski, P.; Fiore, M. Towards the preparation of synthetic outer membrane vesicle models with micromolar affinity to wheat germ agglutinin using a dialkyl thioglycoside. *Beilstein J. Org. Chem.* **2019**, *15*, 937–946, doi:10.3762/bjoc.15.90; b) Chieffo, C.; Comte A.; Strazewski P.; Fiore M. Synthetic Outer Membrane Vesicles Bearing Tn Antigen. Eur. J. Org. Chem. 2023, e202300820; doi.org/10.1002/ejoc.202300820

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Main ArchiFun theme involved:

- [x] Host-pathogen interactions;
- [ ] Mechanisms of bacterial resistance and cancer onsets;
- [ ] Neurodegenerative and autoimmune diseases;
- [x] Translational research in prevalent diseases;
- [ ] Physiology and ecology;
- [ ] Neurosciences and cognition.

Contribute to the development and optimization of a signal processing and AI algorithm for microwave imaging in the early detection of breast cancer

Breast cancer is the most frequently diagnosed cancer in women worldwide, particularly in developed countries, where it accounts for 33% of all female cancer cases and contributes to 14% of cancer-related deaths in women. Early detection significantly improves patient survival rates, while reducing treatment time and associated costs.

The primary objective of this study is to develop innovative methodologies for the direct and indirect detection of breast cancer by exploiting the dielectric properties of tissues. Pathological conditions alter the electrical characteristics of biological tissues when exposed to electromagnetic waves, resulting in distinct "dielectric signatures". The use of microwave (MW) imaging technology, combined with flexible microstrip antennas, offers a cost-effective, non-invasive alternative to traditional diagnostic methods such as X-ray mammography, ultrasound and MRI.

In addition, this research focuses on advanced signal processing techniques and the integration of artificial intelligence (AI) for image reconstruction. Signal processing plays a crucial role in extracting relevant information from microwave signals, reducing noise and improving imaging accuracy. Advanced algorithms promise increased resolution and image quality, enabling precise localization and characterization of breast anomalies.

However, this undertaking is not without its scientific challenges. The project faces several major hurdles, not least the development and refinement of signal processing algorithms specifically designed for microwave imaging systems. These algorithms aim to improve image quality, resolution and accuracy of anomaly detection. In addition, state-of-the-art deep learning techniques and artificial neural networks are being explored to streamline image reconstruction and improve the accuracy of anomaly detection in microwave breast imaging.

Innovative strategies for pre-processing and denoising microwave signals are also being investigated to increase the signal-to-noise ratio and improve overall image fidelity. Efficient algorithms for the localization and characterization of breast tumors using microwave data are being developed, with AI playing a key role in obtaining reliable results.

In addition, the project aims to evaluate algorithmic performance through in-depth simulations and real-life measurements, taking into account parameters such as sensitivity, specificity and computational efficiency.

This research aims to revolutionize breast cancer detection by exploiting the dielectric properties of tissue, advancing signal processing methodologies and integrating AI for image reconstruction. By overcoming scientific challenges, we can expect greater diagnostic accuracy, helping to improve patient outcomes and increase healthcare efficiency.

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Main ArchiFun theme involved:

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- ☐ Mechanisms of bacterial resistance and cancer onsets;
- ☐ Neurodegenerative and autoimmune diseases;
- ☑ Translational research in prevalent diseases;
- ☐ Physiology and ecology;
- ☐ Neurosciences and cognition.

SchizApathy: Unveiling the Neuro-Computational bases of Apathy in Schizophrenia

SchizApathy is inter-disciplinary and inter-sectorial, covering system neuroscience, computational neuroscience and clinical sciences, and involves an international collaboration between two labs with complementary expertise.

SchizApathy's rationale. Apathy, marked by a reduced engagement in effortful, reward-driven actions, exhibits a high prevalence in the majority of psychiatric and neurological disorder, including schizophrenia (47%), depression (38%), Parkinson's Disease (40%), and Alzheimer's Disease (49%)[1]. Even when not the core symptom of the disorder, apathy significantly diminishes patients' quality of life and adversely affects their functional outcomes[2]. However, current research on apathy faces two significant challenges. First, subjective reporting of symptoms in psychiatry and neurology does not capture the distinct neural and computational mechanisms underlying apathy across disorders[3,4]. Recent neuroimaging and computational modeling studies reveal that apathy may stem from alterations in either effort processing, reward processing, or a combination of both in different populations[5,6,p1]. This highlights the need for developing neuro-computational biomarkers that offer objective insights into the putative causes of apathy in specific disorders. Secondly, there is a lack of effective therapeutic approaches to improve engagement in effort and alleviate apathy in patients, as many treatments fail to target the specific neuro-computational alterations mentioned above, leading to mixed clinical outcomes[7]. Importantly, these two challenges are particularly prominent in schizophrenia, a disorder where apathy emerges as a significant manifestation of negative symptoms. Recently, Dr. Knolle and her collaborators showed that schizophrenia is associated with an alteration of reward processing in reinforcement learning tasks, quantified through computational modeling of behavior[p2,p3] and neuroimaging data[p4,p5]. SchizApathy will test the hypothesis that schizophrenia involves an alteration of reward processing extending beyond reinforcement learning, affecting effort-based decision-making and contributing to the emergence of apathy in this population.

SchizApathy's objectives. WP1, conducted at TUM, aims to identify neuro-computational biomarkers of apathy in schizophrenia, focusing on potential reward processing alterations. 40 patients will be classified as apathetic or non-apathetic based on the Apathy Evaluation Scale. They will complete an effort-based decision-making (EBDM) task, an incentive motivation (MID) task, and a reinforcement learning (Go/NoGo) task. In addition, multimodal neuroimaging (structural MRI, DTI and rs-fMRI) will be employed to quantify alterations in morphometry, and in structural and functional connectivity within effort and reward processing networks. Computational modeling parameters of effort and reward processing will be associated with potential neurobiological alterations and compared between the apathetic and non-apathetic groups[2,p1]. WP2, conducted at UCBL, aims to test the role of the ventral striatum, a key node of the reward system, in effort-based decision-making among apathetic patients, investigated through assessing the impact of non-invasive brain stimulation on effort engagement. 20 apathetic patients will undergo temporal interference stimulation (TIS) of the ventral striatum[8], a non-invasive electrical stimulation approach previously employed by Dr. Derosiere and his former PhD student (P. Vassiliadis)[p6]. E-field modeling and fMRI will quantify the impact of TIS on striatal activityp[6]. Two separate sessions will involve TIS during the EBDM task using an intermittent theta burst stimulation protocol increasing striatal activity[9] and a sham stimulation, enabling the measurement of the stimulation's impact on the decision to engage in effort, as well as on effort and reward processing through computational modeling of behavior[2].

Proposed working plan: Months 1-5 (UCBL): Develop task battery and computational models for WP1 and 2. Punctual secondments at TUM for code development on reinforcement learning tasks. Months 6-18 (TUM): Conduct data acquisition and analysis for WP1. Months 19-36 (UCBL): Conduct data acquisition and analysis for WP2 and thesis writing. PhD supervisor Dr. Derosiere has successfully supervised 19 Master and 2 PhD students, including through international co-supervision programs, on topics related to effort-based decision-making[p1], reward processing[p7,p8] and brain stimulation[p9,p10]. He holds the Habilitation to Direct Research (HDR) and currently co-supervises 1 PhD student (current supervision rate: 50%).

[pX] Personal contributions

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The PhD student working on SchizApathy will be supervised by Dr. Gerard Derosiere (PhD, HDR), INSERM researcher at UCBL in France. The project will involve a tight collaboration with Dr. Franziska Knolle (PhD), group leader at TUM in Germany, where the student will realize secondments.

Main ArchiFun theme involved:

- ☐ Host-pathogen interactions;
- ☐ Mechanisms of bacterial resistance and cancer onsets;
- ☑ Neurodegenerative and autoimmune diseases;
- ☑ Translational research in prevalent diseases;
- ☐ Physiology and ecology;
- ☑ Neurosciences and cognition.

PEPSY Project

Background and rationale

One of the key objectives of modern psychiatry is to identify objective biomarkers that can help improve the diagnosis and prognosis of patients. This objective is more broadly embedded in the promise of 5P medicine: predictive, preventive, personalized, purpose-driven (and participatory). In the context of schizophrenia -one of the most disabling psychiatric disorders- prospective studies have shown that the first few months following a first episode of psychosis (FEP) constitute a crucial time window for optimizing the therapeutic strategy and improving long-term prognosis. Specifically, around 40% of patients will experience treatment failure, i.e. absence of symptomatic remission at 3 months, and these patients will have a considerably increased risk of developing life-long treatment-resistant schizophrenia (at 5 years, at 10 years). Identifying the non-responders early would enable adapting the first-line management of these patients, and consequently deliver precision care in order to preserve long-term prognosis.

In recent years, the combination of functional magnetic resonance imaging (fMRI) and machine-learning methods has made some progress towards the identification of biomarkers capable of predicting individual patient trajectories. Here we aim to use one of the most recent developments in this burgeoning field, that associates naturalistic fMRI (i.e. movie watching in the scanner) and functional connectivity mapping, that has shown great promise in predicting patient evolution, e.g. relapse in substance addiction, but has not yet been applied to schizophrenia.

Aim

In this project, we aim to leverage state-of-the-art analyses techniques applied to naturalistic fMRI, in order to identify functional brain connectivity markers predictive of the absence of remission at 3 months after an FEP. Specifically, we will use connectome-based modeling and dynamic independent component analysis (ICA). In addition, we will follow the evolution of these markers over the first three months and assess their relationship with the clinical and prognostic measures at 6 months and 1 year.

Methodology

FEP patients will be recruited across 4 early intervention centers in France (Lyon, Saint-Etienne, Clermont-Ferrand, Grenoble), before treatment initiation. Patients will be recruited via the extensive network collaborating with these early intervention centers. Initial assessments will include clinical and biological measures (standard workup and toxicology) renewed at 3, 6 and 12 months, in addition to neurocognitive evaluation and naturalistic fMRI, renewed at 3 months.

Experimental design: a training sample will be selected randomly (80% of patients) to build the model predicting the absence of remission, while a validation sample (20% of patients) will be used to assess the model's performance (sensitivity and specificity). After acquisition, fMRI data will be centralized and analyzed at a single site (Lyon). The dynamic ICA analyses will be performed in close collaboration with the group of Prof Fabio Sambataro (University of Padova, Italy), who's a world-leading expert on this approach.

Feasibility

- Long-time collaboration with the 4 early intervention centers for FEP (previous projects PRESTO and PRIMO), ensuring recruitment of patients within the timeframe of a PhD

- Joint supervision by a psychiatrist specializing in schizophrenia (E. Fakra) and a neuroscientist expert in neuroimaging (G. Sescousse), with a history of joint supervision and publications
- Collaborations with expert on machine learning: Institut Pasteur, Paris (V. Guillemot)
- Collaboration with expert on dynamic ICA: University of Padova (F. Sambataro)
- Funding for data acquisition (PHRC) and ethical approval already obtained

Implications

Early assessment of the likelihood of remission would open new avenues for optimal management of FEP, e.g. by prioritizing therapeutic strategies that are currently only offered as second- or third-line treatments. These therapeutic adjustments should have beneficial long-term consequences for schizophrenia, i.e. fewer patients developing a chronic disease, better prognosis and, consequently, a reduction in the considerable direct and indirect costs associated with this illness.

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Suggestion for secondment

We believe that a secondment in the lab of Prof Fabio Sambataro at the University of Padova would be highly beneficial for the project and the student. Firstly, Prof Sambataro is a recognized authority in the use of advanced methods for analyzing functional brain connectivity —the very methodology crucial to the success of this project. Their extensive expertise in this field is underscored by numerous publications, in particular in the domain of schizophrenia. Secondly, our labs share a history of collaboration, albeit without forging strong ties as of yet. Building upon our past engagements, this collaboration represents an excellent opportunity to strengthen the bonds between our research teams. By combining our respective strengths, we aim to create a synergy that will not only enhance the quality of the proposed project but also foster long-term partnerships between our labs. This collaboration with Prof Sambataro is poised to yield innovative outcomes, capitalizing on our collective knowledge and skills in the field of schizophrenia.
NB: Given the meticulous consideration given to the proposed secondment and the absence of comparable alternatives in terms of relevance, we put forth only one recommendation.

The hexokinase phosphorylation landscape: new regulation of an “old” enzyme

1- State of the art

Glucose is the preferred carbon and energy source for most organisms and largely impact many regulatory pathways in living cells. Although it is the most abundant monosaccharide on Earth, extracellular glucose concentrations fluctuate, and most cells must adapt to these variations to survive. The remarkable capacity of eukaryotic cells to optimize their metabolism in response to glucose availability requires glucose sensing systems and sophisticated signal transduction pathways. Glucose catabolism involves multiple cellular enzymatic processes starting with glycolysis which is one of the most conserved biological pathways in life kingdom. The first step of glycolysis is the irreversible phosphorylation of intracellular glucose in glucose-6-phosphate by the hexokinases/glucokinases enzymes. This step is essential for cells and the hexokinase activity is submitted to multiple regulations to efficiently metabolize glucose according to its extracellular availability. In mammals, hexokinase disfunctions cause several pathologies such as hereditary diabetes, hyper-insulinemia and the Warburg effect in cancer cells. In addition to their well-known role as glucose phosphorylating enzymes, hexokinases are also involved in many other cellular functions such as insulin secretion, apoptosis, and longevity.

Similarly to the Warburg effect in cancer cells, the yeast *Saccharomyces cerevisiae* produces energy through glycolysis and fermentation rather than oxidative phosphorylation, even in the presence of oxygen. This metabolic treat depends on a molecular process known as glucose repression. The hexokinase Hxk2 plays a pivotal role during glucose repression by feeding glycolysis with glucose-6-P and as a nuclear transcriptional regulator inhibiting the expression of genes involved in alternative carbon source metabolism. Understanding what are the molecular mechanisms that control hexokinases functions is then crucial to better appreciate their cellular role in physiological and pathological situation.

Among the molecular mechanisms known to control its activity, the phosphorylation of hexokinase is particularly important. In *S. cerevisiae*, glucose dependent phosphorylation of Hxk2 at serine 15 controls its transcriptional function during glucose repression through the regulation of its nucleo-cytoplasmic distribution and its interaction with different transcriptional factors. Recent phosphoproteomic studies in yeast have shown that Hxk2 is phosphorylated on several other residues suggesting a high degree of regulation by phosphorylation. **However, the physiological consequences of Hxk2 phosphorylation on its functions and on yeast cellular physiology remains largely unknown.**

We propose to characterize the role of hexokinase phosphorylation on its functions and on cell adaption to glucose variation. Yeasts will be used as experimental models since they display a high degree of conservation, and efficient genetic, molecular, and genomic tools to study glucose signaling and metabolism.

2- Strategy

Unbiased identification of Hxk2 phosphorylation sites: to identified Hxk2 phosphorylation sites a Hxk2 phospho-mapping strategy will be followed. After GFP-trap purification followed by proteolytic cleavage, Hxk2 phospho-peptides will be enriched by iMAC and/or TiO2 and sequenced by MS/MS analysis. The effect of carbon source on Hxk2 phosphorylation will be then addressed via the same strategy after glucose starvation of the cells. This might allow to identify differentially phosphorylated residues in Hxk2 in response to carbon source.

Functional characterization of Hxk2 phosphorylation sites: Each Hxk2 phosphorylated serine, threonine or tyrosine identified will then be mutated to alanine (non-phosphorylatable) an aspartic/glutamic acid (phosphomimetic residues) via a CRISPR/Cas9. Each mutant will be then analyzed for hexose-kinase activity by enzymatic assays and glucose dependent growth. The influence of these phosphorylation events on Hxk2 regulatory function will be addressed by analyzing in each mutant 1/ the expression of

glucose repressed genes (transcriptional reporter systems) and 2/ the ability of mutated Hxk2 to interact with the transcriptional repressor Mig1 (Co-IP) in response to carbon sources. This might allow to understand if Hxk2 phosphorylation at these different residues promotes or inhibits hexokinase functions. These mutants would be further characterized by a structural approach to correlate the effect of Hxk2 phosphorylation on its structure and on its activities.

Hxk2 phosphorylation and signaling pathways: It is important to identify the different kinases phosphorylating Hxk2 at the identified residues and what are the signals controlling their activity toward Hxk2. To isolate the kinases phosphorylating Hxk2 a targeted proximity-dependent biotynylation labelling strategy is currently developed in the lab. Our preliminary results indicate that several protein kinases might interact with Hxk2. Theses identified kinases will be tested for Hxk2 phosphorylation by kinases assays and by analyzing the effect of their belonging signaling networks on Hxk2 *in vivo* functions during glucose repression.

3 - Expected Results

By identifying Hxk2 phosphorylation sites, the belonging kinases and the consequence on hexokinase functions this project will help to better understand the complex mechanisms of Hxk2 regulation necessary for yeast cells to adapt to carbon sources. Regarding the high degree of identity between yeast and mammalian hexokinases, the conservation of theses regulatory mechanisms might be later addressed in mammals, helping to better understand the role of hexokinase in physiological and pathological situation such as diabetes or during the Warburg effect in cancer cells.

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Proposed collaboration within ArchiFun network (not mandatory at this stage):

Proposed list of secondments (not mandatory, but recommended if known already):

Main ArchiFun theme involved:

Mechanisms of cancer onset

Physiology

Regulation and impact of post-transcriptional modifications of *Staphylococcus aureus* tRNAs

Synopsis/Abstract

Staphylococcus aureus is a commensal bacterium in humans, but it is also a major pathogen responsible for numerous nosocomial and community infections. Its infectious success in various tissue environments is associated with a wide range of virulence and stress response factors that enable it to develop complex invasion and immune escape strategies. The expression of virulence factors is finely regulated by various molecular mechanisms such as transcription factors, two-component systems and regulatory RNAs.

However, a new translational regulation mechanism involves post-transcriptional modifications of RNAs (Antoine et al. 2021). These modifications, produced by specific enzymes, are present on all types of RNA and can modulate translation efficiency by influencing RNA structure, codon usage bias, interaction with other molecules or the efficiency of ribosome action. In collaboration with Stefano Marzi's group (IBMC, Strasbourg), we have identified 57 modification enzymes, 37 of which affect *S. aureus* transfer RNAs. We were able to show that the genes encoding these modification enzymes are extremely well conserved within the *S. aureus* specie and are therefore part of the core genome. We therefore hypothesized that these **post-transcriptional modifications of tRNAs could play an essential role in an infectious context and finely regulate the translation of bacterial factors**.

The thesis project will be divided into three work axes:

Axis 1: Regulation of enzymes and post-transcriptional modifications of tRNAs

Although the genes encoding tRNA modification enzymes are highly conserved, the control of the expression of these genes remains to be explored. We have put forward the hypothesis that these genes could be induced in response to stress and could be essential in an infectious context.

To explore this hypothesis, several avenues of study will be considered:

- The transcriptional expression of the various modifying enzymes will be analyzed by RT-qPCR on *S. aureus* cultures subjected to various stresses related to the infectious context: antibiotic stress, oxidative stress, iron deficiency, acidic pH, in contact with blood or tissue.....
- In parallel, we will seek to decipher the regulatory network involved in the expression of these modification enzymes using the method described by Weiss et al. 2022. To do this, we will use the Nebraska library of *S. aureus* mutants (available in the laboratory) and select all mutants deficient in transcriptional regulators and two-component systems. We will analyze the expression of modification enzyme genes in these different mutants.

Axis 2: Determining the bacterial factors whose expression is altered by tRNA modifications.

To understand how tRNA modifications can influence the virulence and physiology of *S. aureus*, we will seek to identify the bacterial factors affected by these modifications. To this end, 'clean' mutants deficient in the modifying enzymes will be created. Transcriptomic (RNAseq) and proteomic analyses will be carried out and comparisons made. To identify the factors impacted at the translational level, we will look for factors that are differentially expressed in proteomics but whose transcriptional level is not altered.

Axis 3: Deciphering the mechanisms of translational regulation.

The modulation of virulence factor expression by tRNA modifications can be caused by several mechanisms, such as an improvement in the translational efficiency of codon-enriched genes whose decoding is facilitated by these modified tRNAs (Aubee et al., 2016) or a reduction in translation fidelity due to the stimulation of reading frame shifting (Fleming et al., 2022). To determine the mechanisms by which enzymes indirectly regulate translation, several approaches are planned:

- Decoding efficiency will be compared using reporter gene into which we will introduce the target codons of the modified tRNAs. The translation efficiency of wild-type and deficient strains for the modifying enzymes will be compared.
- Ribosome profiling will be carried out to quantify the translation efficiency of each mRNA and to determine the rate of translation.

- Quantification of the reading frame shift will be evaluate by constructing a pair of reporter genes under the same promoter, separated by an intergenic region enriched in the codons studied and possessing a stop codon.

All the data generated by this thesis project will enable us to **understand the role and importance of post-transcriptional modifications of *S. aureus* tRNAs during the infectious process**, and thus to **describe a new molecular mechanism for regulating virulence**.

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Proposed collaboration within ArchiFun network (not mandatory at this stage):

Proposed list of secondments (not mandatory, but recommended if known already):

Main ArchiFun theme involved:

Host-pathogen interactions.

Mechanisms of bacterial resistance.

Stress gradients and the emergence of drug resistance in infection and cancer

Synopsis/Abstract
Cancer and antimicrobial resistance are among the greatest health threats of our time. Treatment failure in bacterial infection and cancer may result from the acquired resistance to drug therapy of the causative agents, namely bacterial or cancer cells. Both situations have interesting commonalities including the higher likelihood of progressive, sequential acquisition and accumulation of resistance mutations compared to the one-step appearance of high-level resistance; or the involvement of detoxification strategies in which cells cooperate to reduce local toxicity for the common good of the community.

Stress gradients are an additional common point of cancer and antimicrobial resistance. In solid tumors, stress gradients are found at increasing distances from blood vessels that supply both anticancer drugs and resources including oxygen. Similar gradients arise in tissue infections, with decreasing antimicrobial drug concentrations farther from the circulation, or biofilms in which drugs poorly penetrate deeper layers. Larger-scale stress gradients also encountered in the environment as bacteria migrate at the interface of drug-polluted and drug-free settings. Stress gradients have been demonstrated in microfluidic experiments to facilitate the fixation of resistance mutations both in bacterial populations under antimicrobial stress [Zhang 2011], and in cancer cell populations under anticancer stress [Baym 2016]. However, infection and cancer research are most often conducted separately and we lack a comprehensive understanding of the influence of stress gradients in the emergence and diffusion of stress resistance. We hypothesize that a systematic comparative study of antimicrobial and anticancer resistance emergence under stress gradient situations may benefit both fields.

Our objective is to construct a correspondence mapping between the phenomena involved in the evolution of solid tumor, tissue infection, and microbial communities in dynamic environments involving stress gradients. Based on this mapping, we will identify common points to develop a foundational model of stress gradient adaptation that may inform both microbial and cancer research. We will focus on key parameters that can apply to solid tumor, tissue infection and environmental adaptation: cell mobility, stress intensity, resource gradient and population diversity (both of bacteria and of cancer cells with varying phenotypes).

To conduct the comparison in a unified eco-evolutionary framework, we will rely on stochastic population modelling using *msevol*, a multiscale ecosystem modelling framework under continuous development at the Centre International de Recherche en Infectiologie (CIRI) of Lyon, France. The msevol framework implements a graph-based representation of the nested organization of resistance genes and cells structured in metapopulations (i.e. common environment with shared resources and stress) that are interconnected trough migration and submitted to spatio-temporal stress fluctuations. Biological entities (e.g. plasmids, cells, or patients) are graph nodes viewed as containers whose properties (e.g. basal growth for cells) are modulated by their content (e.g. resistance gene inducing a fitness cost) and their enclosing container (e.g. growth limited by the microhabitat occupancy and carrying capacity). Biological events are implemented as graph rewriting rules that modify node and edge multiplicities while avoiding redundancy of identical containers, that are represented as a single graph node with a multiplicity property. Compared with current ecosystem modelling paradigms such

as differential equations, agent-based, or membrane computing models, the msevol paradigm provides the required scalability and flexibility to examine complex multi-scale interactions in large populations.

The initial phase of the project will involve defining and implementing, utilizing the msevol formalism, a select number of pivotal biological events and parameters. These will be carefully chosen to capture the essential ecological phenomena driving the emergence and dissemination of resistance, while ensuring that the model remains manageable and tractable. Such rules (including logistic cell growth, natural and drug-induced cell death, plasmid transfer and loss, and patch-to-patch cell diffusion) have already been designed for the case of antimicrobial resistance. They will be adapted to model cancer cell dynamics as closely as possible to bacterial cells for comparison purposes.

Next, the effect of spatial structuring will be investigated using diverse patch organizations, varying in terms of migration intensity and stress. Base structures, such as linear 1-D or hexagonal-2D grid subject to stress gradients, could be used to calibrate the models based on published in vitro experiments in comparable systems [Zhang 2011, Baym 2016], then extended to more complex and natural organizations such as tissue, biofilms, or aquatic environments.

Finally, the msevol formalism will be extended to investigate the effect of spatial structuration on the sharing of public goods (e.g., beta-lactamase enzymes or diffusible growth factors), providing an alternative mechanism for a "cheating" cell to gain resistance without acquiring a costly trait. Incorporating evolutionary game theory, which is increasingly utilized in cancer modeling [Renton and Page, 2021], into msevol could complement the experimental studies aimed at understanding the detoxification process in biofilms or tissues [Amanatidou et al, 2019; Tai et al, 2022].

This research project is at the interface of ecology and medicine. The PhD candidate will have the opportunity to join a striving research environment at the Centre International de Recherche en Infectiologie of Lyon, tightly linked with the Lyon University Hospitals. Candidates with experience in mathematics, computer science or quantitative ecology will be considered favorably.

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Proposed collaboration within ArchiFun network:
Randall Centre for Cell & Molecular Biophysics: cell motility, cancer biology

Proposed list of secondments:
Randall Centre for Cell & Molecular Biophysics: modelling of cancer cell motility and tissue structure based on cell imaging approaches

Main ArchiFun theme involved:

Mechanisms of bacterial resistance and cancer onsets;

Investigation at the molecular level of the interaction of fluorescent probes with early protein aggregates

Protein aggregation is ubiquitous in many neurodegenerative diseases. Different types of fibrils may be formed in relation with each pathology. However, fibril formation mechanisms are overall still poorly understood. The first steps of aggregation involves the emergence of ill-folded proteins and the formation of the early oligomers. These early steps are particularly difficult to investigate. Optical methods involving fluorescent probes like thioflavin T or congo red A are broadly used to detect and monitor the appearance of protein aggregates: the optical properties of these fluorophores are dramatically affected upon binding to β-sheet rich structures such as fibrils. The increase of fluorescence is generally interpreted as an increase of the fibrils concentration, thus allowing to monitor the aggregation kinetics. Promising methods for the early diagnosis of neurodegenerative diseases build on this very principle.[1]

Several issues nevertheless limit the specificity and reproducibility of fluorophore-based methods. In particular, the connexion between the fibrils morphology and their chromophore binding affinity remains an open question.[2] Moreover, it is not clear whether the fluorophore properties depend on the fibril development stage (early oligomers vs. advanced fibrils).

We propose to investigate the interaction between fluorophores and small oligomers at the molecular level through an original approach based on ion mobility and mass spectrometry (IMS-MS) coupled to action spectroscopy. The interest of IMS-MS in the present context is to isolate the different oligomers present in the heterogeneous aggregation medium in order to study them separately. Namely, MS allows precise determination of the stoichiometry of the oligomers and the exact number of chromophores attached to it. In addition, IMS provides information in the conformational properties of the oligomers.[3] As fluorescence is difficult to measure in a mass spectrometer, the optical properties of the oligomer-fluorophore complexes will be probed by action-spectroscopy: **the originality of our approach consists in performing photofragmentation measurements on the oligomer-chromophore complexes in the mass spectrometer in order to characterize their optical properties in relation with their size (MS) and shape (IMS).**

As a case study, model peptide sequences displaying well-known aggregation behaviour will be investigated. Beyond the stoichiometry (from MS), and the geometry (from IMS) of the oligomer-chromophore complexes, their stability will also be investigated from collision-induced activation in the gas phase. Variable-temperature electrospray experiments are also planned in order to investigate their stability in solution. The PhD candidate will have access to high-level IMS-MS and MS instruments coupled to different laser sources available at iLM.

Hiring profile – Master degree in Physics/Chemistry with strong background in mass spectrometry and/or spectroscopy, skills for team working and a taste for experimental works! Basic knowledge in mass spectrometry and minimal programming skills would be appreciated. Fluency in English is mandatory.

Conditions and benefits – Duration of the PhD program – **36 months full time work contract** (37.5h/week)**, starting October 2024**. **Attractive salary. Funding includes travel to secondments.** Additional benefits: 47 days paid holiday leave, sick leave, parental leave, unemployment insurance.

(1) Atarashi, R. *et al.* Ultrasensitive Human Prion Detection in Cerebrospinal Fluid by Real-Time Quaking-Induced

Conversion. *Nat. Med.* **2011**, *17* (2), 175–178. https://doi.org/10.1038/nm.2294.
(2) De Giorgi, F. *et al.* Novel Self-Replicating α-Synuclein Polymorphs That Escape ThT Monitoring Can Spontaneously Emerge and Acutely Spread in Neurons. *Sci. Adv.* **2020**, *6* (40). https://doi.org/10.1126/sciadv.abc4364.
(3) Bleiholder, C. *et al.* T. Ion Mobility Spectrometry Reveals the Mechanism of Amyloid Formation of Aβ(25-35) and Its Modulation by Inhibitors at the Molecular Level: Epigallocatechin Gallate and Scyllo-Inositol. *J. Am. Chem. Soc.* **2013**, *135* (45), 16926–16937. https://doi.org/10.1021/ja406197f.

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Possible secondments within the ArchiFun consortium include complementary characterization of the oligomers by mass photometry at Institut Pasteur.

Main ArchiFun theme involved:

- ☐ Host-pathogen interactions;
- ☐ Mechanisms of bacterial resistance and cancer onsets;
- ☑ Neurodegenerative and autoimmune diseases;
- ☐ Translational research in prevalent diseases;
- ☐ Physiology and ecology;
- ☐ Neurosciences and cognition.

Development of Laser-generated Surface Acoustic Wave Immuno-sensors

Synopsis

Surface Acoustic Wave (SAW) devices have emerged as promising candidates for the advancement of rapid, low-cost, lab-on-chip point-of-care biosensors (*Zida et al. 2021*). These biosensing devices offer potential for early disease diagnosis and biomarker monitoring due to their sensitivity in detecting small variations in mechanical properties (i.e. changes in mass, density, rigidity, viscosity) resulting from cellular processes such as division, differentiation, communication and death, as well as subcellular events like DNA replication, protein folding, and organelle biogenesis. However, existing SAW biosensors typically rely on bulky piezoelectric substrates with interdigitated electrodes, which often lack biocompatibility and operate at fixed acoustic frequencies, limiting their sensitivity and agility. Additionally, integrating and interfacing such devices with other biomedical and microfluidic systems poses significant challenges due to their unwieldy electronic settings.

To address these limitations, opto-acoustic techniques present an alternative approach. These techniques utilize laser light to generate and probe high frequency ultrasonic waves (*Chen 2016*). In this project, we propose to leverage opto-acoustic schemes to design and develop a laser-based SAW biosensor operating over a wide frequency spectrum, ranging from tens of MHz up to a GHz. We will use laser-induced diffraction gratings to excite and probe these high-frequency SAWs remotely (*Vega-Flick et al. 2015*), without the need of interdigitated piezoelectric transducers. This biosensor aims to enable fast and efficient detection of cellular and biomolecular processes, including specific antibody-antigen binding events, serving as a proof of concept. Since the sensing mechanism of SAW devices relies primarily on mass-loading to detect binding events, resolution can be limited by the low mass of molecular antibodies. To enhance signal detection and render our sensor more sensitive, we propose to use functionalized biocompatible and biodegradable micro-droplets (*Montel et al. 2015*) as signal amplifiers. Droplets functionalized with antibodies will bind specifically the antigen and enhance the mass-loading by several orders of magnitude. The adhesion of the droplet on the antigen-covered surface will additionally allow us to measure the antigen-antibody binding energy. Finally, we will leverage our droplet-assisted sensitive SAW immuno-sensor to detect typical autoantibodies associated with autoimmune disorders (*Schlichtiger et al. 2012*), such as those found in rheumatoid arthritis, type I diabetes, and systemic lupus erythematosus.

As part of this, the doctoral candidate will i) design and fabricate an all-optical SAW sensor using numerical tools and microfabrication techniques, ii) characterize the performance and sensitivity of the sensor and identify its optimal parameters and configuration using optoacoustic setups and analytical/numerical analysis, iii) perform the

biochemical protocols needed for the functionalization of the sensor surface and signal amplifiers, and iv) measure and analyze the immuno-sensing performance of the biosensor.
The proposed doctoral thesis, which aims at developing novel SAW biosensors for the next generation micro/nano diagnostics technologies, is highly interdisciplinary, and draws upon concepts from applied physics, mechanical and materials engineering, physical chemistry, as well as immunology and life sciences. Throughout this project, the doctoral candidate will be part of the Biophysics team at Institut Lumière Matière (ILM). The identification and preparation of autoantibodies for the evaluation of the sensor's performance as well as the detection of autoimmune disorders will be developed in collaboration with immunologists at the Centre International de Recherche en Infectiologie (CIRI). We also envision connecting with ArchiFun's industrial partners, such as NanoTemperTech, NovAlix, and Fida Biosystems, whose expertise in biosensing and diagnostics could facilitate the translation of these novel technologies into commercial devices. We anticipate that these collaborations, along with the thesis's interdisciplinary nature, will contribute significantly to the doctoral candidate's research profile, laying a solid foundation for their future professional career.

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Proposed partners for secondments:
NanoTemperTech, NovAlix and Fida Biosystems

Main ArchiFun theme involved:

Neurodegenerative and autoimmune diseases;

Efficacy of targeted nanoparticles coated with hybrid cell membranes against pancreatic cancer heterogeneity

Pancreatic cancer (PC) presents formidable challenges due to its aggressive nature, poor prognosis, and limited treatment options. Conventional anticancer therapies struggle against its heterogeneity and the protective tumor microenvironment (TME), including tumor cells (TCs), cancer stem cells (CSCs), and cancer-associated fibroblasts (CAFs). Enrichment in CSCs subpopulations contributes to aggressiveness and treatment resistance, highlighting the potential of CSCs targeting. The dynamic interplay of tumor and immune cells, notably tumor-infiltrating lymphocytes (TILs), is pivotal in cancer progression. TILs, having recognize multiple cancer cell targets, ideal for targeted therapy. Moreover, platelets, typically involved in hemostasis, adjust their function in response to tumor signals, becoming tumor-educated platelets (TEP), thus offering a promising intervention target

The main objective of this project is the development of complex new biomimetic hybrid cell membrane (CM)-based nanoparticles (NPs) loaded with anticancer therapeutic agents (drugs and miRNAs) to target proliferative TCs, CSCs and the TME of PC in two *in vitro* (based on 3D bioprinted biomimetic hydrogels) and *in vivo* (patient-derived xenograft organoids) models. This general objective will be achieved through the development of the following specific objectives: Objective 1. Synthesis, physico-chemical, colloidal and ultrastructural (cryoEM) of MA-SLNPs and SFNPs with the chemotherapeutics encapsulated. Isolation, characterization (flow cytometry, and CM extraction of CSCs, CAFs (primary cell cultures from surgical samples), Platelet and TILs (tumor tissue) from PC patients (male and female) and/or established cell lines. Phenotypic, metabolomics and proteomics characterization of the hybrid CM will be done in accordance with methodologies developed by the research group and using bioinformatics. Also, NPs will be functionalized with 4 miRNA mimics and their correct incorporation will be evaluated. Objective 2. *In vitro* targeting effects of NPs and hybrid CM-based NPs loaded with therapeutic agents on 2D and 3D-bioprinted hydrogels that mimic TME and based on natural biomaterials such as decellularized extracellular matrices (dECMs) obtained from tumor stromal cells (CAFs), fibrinogen and thrombin, and including 3D patient-derived organoids (PDO) and patient-derived xenograft organoids (PDXOs) (male and female). The hydrogels will be physicochemical and mechanical characterized to analyse changes in the viscoelastic properties. Objective 3. *In vivo* systemic toxicity, bioavailability, biodistribution and targeted anti-tumor effectiveness of biomimetic CM-based NPs in humanized PDX (male and female mice) to simulate patient immune-tumor interactions realistically.

This multidisciplinary approach, leveraging nanomedicine, omics and biofabrication methods, holds significant potential in enhancing outcomes for PC patients with poor prognosis. Collaborations with internationally recognized centers of excellence will contribute to the advancement of knowledge in this critical area and will improve translational research and personalized oncology.

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- University of Padua
- University of Claude Bernard Lyon

Main ArchiFun theme involved:

- ☐ Host-pathogen interactions;
- ☐ Mechanisms of bacterial resistance and cancer onsets;
- ☐ Neurodegenerative and autoimmune diseases;
- ☑ **Translational research in prevalent diseases**;
- ☐ Physiology and ecology;
- ☐ Neurosciences and cognition.

Explainable AI-powered multimodal data integration for healthcare

Despite numerous efforts in the field of complex diseases to apply the findings of genetic association studies (GWAS) in clinical practice, replication of these findings in diverse populations often shows weaknesses or inconsistencies. This is due to the diversity of symptoms, degrees of severity and responses to treatment that individuals with the same complex disease present, despite sharing the same diagnosis. Until now, the classification of these patients into subtypes has been mainly based on a single approach, either genomics or clinical features, which has been insufficient to fully understand the triggers of the disease, limiting the application of personalised treatments.

In this context, there has been a growing interest in biomedicine in combining machine learning (ML) and artificial intelligence (AI) strategies to address complex diseases. This involves the integration of diverse knowledge domains, such as genetic data, clinical data, questionnaires, sociological data, environmental data and medical images, into a single learning system. However, very few of these systems have focused on unsupervised learning approaches, which generate new knowledge rather than simply reproducing existing knowledge. In addition, most current machine learning models are black boxes that lack a focus on interpretability and do not address the uncertainty inherent in biomedical systems.

To address this challenge, the method known as Phenotype to Genotype Many-to-Many Relationship Analysis (PGMRA), an approach based on machine learning and optimisation, has been developed to discover unsupervised relationships between genetic and phenotypic variables. In this project, we propose to expand this approach to a multimodal context in which genetic data and medical images are integrated. This marks a significant advance in jointly harnessing genetics and medical imaging for a more complete understanding of complex diseases.

Our hypothesis is that improved and personalised treatments for complex diseases can only be achieved with a multifaceted (multiview) and temporal study of the individual's trajectory. The multifaceted view is based on the study of multiple and diverse phenotypic measures called "phenome", the collective characterisation and quantification of groups of biological molecules that give rise to the structure, function and dynamics of an organism or organisms called "omics data", and the phenomic-multiomic associations resulting from the combination of these knowledge domains that will define the architecture of the disease. Moreover, the aggregation of multiple images (functional, structural, volumetric MRI) complemented with neuromodulation tools used in neurosciences (EEG, FNIRS, TMS), will allow to resolve the multifaceted description of the individual, and consequently, to determine a distributed architecture (subtypes) of the disease. Also, the longitudinal evolution of a complex disease architecture under different environmental conditions defines a person's risk trajectories and will help to define the dynamics of that architecture. ML and AI techniques applied to these trajectories will allow predicting person-centred risks and recommending the best treatments and decisions to prevent diseases.

The goal of this proposal is to provide a machine learning (ML) and artificial intelligence (AI) framework to encode information from multiple omics and medical images into structural data and systematically combine that information to provide multifaceted descriptions of complex objects or phenotypes. The ultimate goal is to unveil the genotypic-phenotypic architecture of complex diseases such as Alzheimer's or leukaemia through the integration of omics and neuroscience.

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- Technische Universität München

Main ArchiFun theme involved:

- ☐ Host-pathogen interactions;
- ☐ Mechanisms of bacterial resistance and cancer onsets;
- ☐ Neurodegenerative and autoimmune diseases;
- ☐ **Translational research in prevalent diseases; x**
- ☐ Physiology and ecology;
- ☐ Neurosciences and cognition.

Biomimetic Magnetic Hydrogels for Translational Research

The use of magnetic nanomaterials in Translational Research is a hot topic today due to its multiple applications in biomedicine and valuable contributions in terms of improving people's quality of life. The goals posed by Tissue Engineering (functional vascular networks, interfaces, structural hierarchy and functional characteristics) are perhaps the most complex and ambitious scientific challenges for the next generation of biomaterials. This translates into the need to establish gradients or subcompartments of composition, temporal changes, and use of cells to drive tissue and organ morphogenesis. In this scenario, the manufacture of hybrid and biomimetic biomaterials, made up of biopolymers, magnetic nanomaterials and cells, which can be distributed and structured in real time and remotely using external magnetic fields, allows the manufacture of biomaterials that serve as cellular support for the regeneration of tissues. Following this approach, the interest behind magnetic biomaterials lies in the possibility of adjusting their structural, mechanical and electrical properties ad hoc and with minimally invasive interventions, in a reversible manner, through the application of external magnetic fields.

The main idea of this project is the creation of a conceptually new type of bioactive materials sensitive to magnetic fields capable of being directly manipulated in situ in real time. This is a multidisciplinary and interdisciplinary topic of great importance at the intersection of applied physics and biology of potential interest in several aspects within System Biology including biofabrication, 3D bioprinting and organs-on-a-chip technology.

Magnetic hydrogels will be fabricated using an unprecedented homemade device that is capable to generate high frequency fields (up to 4 kHz) to promote the formation of time-averaged magnetic driven scaffolds for directed cell growth and differentiation. For the first time the resulting structures will be frozen within a living hydrogel under a confocal magneto-rheomicroscope. An exhaustive physicochemical and mechanical characterization (rheology) will be carried out. Next, biological assays will follow in the design of advanced medical tools and therapies. We anticipate synergy within Archifun consortium in Mechanical characterization of biomaterials and viscometry (with ESRF, IP & BIFI-UZ) and microfluidics (with NTT & FIDABIO).

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Proposed collaboration within ArchiFun network (not mandatory at this stage):

TBD Newtonian

Proposed list of secondments (not mandatory, but recommended if known already):

TBD

Main ArchiFun theme involved:

- ☐ Host-pathogen interactions;
- ☐ Mechanisms of bacterial resistance and cancer onsets;
- ☐ Neurodegenerative and autoimmune diseases;
- ☑ **Translational research in prevalent diseases**;
- ☐ Physiology and ecology;
- ☐ Neurosciences and cognition.

Unravelling amyloid aggregation with novel luminescent probes for super-resolution microscopy

Alzheimer's disease (AD) is the most common neurodegenerative disease, affecting 1 in 10 individuals over the age of 65, and 1 in 3 individuals over the age of 85. A key distinctive feature of AD is the presence in the brain of fibrillar deposits of proteins, known as amyloid plaques. Amyloid plaques are extracellular deposits of a small peptide of about 4 kDa called β-amyloid peptide (Aβ), which arises from the sequential proteolysis of amyloid precursor protein (APP) by secretase enzymes. However, clinical studies have shown that it is not the generation of mature amyloid plaques, but rather the levels of soluble Aβ aggregates that correlate with the severity of the disease and the extent of synaptic loss. In fact, numerous studies have reported different types of Aβ aggregates with varying toxicity, and it has been suggested that as the size of the aggregates increases, their toxicity decreases. Moreover, recent neuroimaging research indicates a spatiotemporal evolution in the accumulation of Aβ, which occurs in the early stages of AD. Given this background, the development of new sensors is necessary to implement new strategies for early diagnosis and monitoring of personalized treatments.
Thus, this Doctoral Thesis project will undertake the development of new luminescent sensors with improved properties for the study of amyloid aggregation using advanced techniques of multidimensional fluorescence microscopy and super-resolution nanoscopy. These new visualization tools will allow addressing different aspects of the molecular mechanism of the early steps in the pathogenesis of AD and its cytotoxicity, facilitating the surpassing of the current knowledge frontier in the field and tackling new pharmacological objectives as well as more precise therapies in future AD treatments. The Doctoral Thesis project is inherently multidisciplinary, incorporating collaborations with internationally recognized centers of excellence, which will result in the achievement of a quality doctorate, a significant milestone for the hired fellow's research career initiation.

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Proposed list of secondments (not mandatory, but recommended if known already):

Main ArchiFun theme involved:

Neurodegenerative and autoimmune diseases;

Translational research in prevalent diseases;

STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF THE DISEASE-LINKED INNER MITOCHONDRIAL MEMBRANE PROTEIN MPV17

The membrane protein MPV17 localized in the inner mitochondrial membrane is one of the causes of the so-called "mitochondrial DNA depletion" syndrome (MDDS), i.e. the reduction of the amount of DNA in the mitochondrial matrix. Since the essential components of the respiratory chain are encoded on mitochondrial DNA, mutations in the MPV17 gene lead to severe liver damage and hepatocerebral dysfunction, and thus usually to death in early infancy. The function and mechanistic details of MPV17 are relatively poorly understood, limiting therapies to the treatment of symptoms. In this project, we aim to better understand the molecular causes of MPV17-related diseases. We will first elucidate whether MPV17 is a transporter of metabolites, e.g. precursors of nucleic acids. For this purpose, the direct binding of metabolites to MPV17, but also the effect of deletion of MPV17 on the mitochondrial metabolome will be investigated. A potential role of MPV17 as a voltage-gated proton channel will also be investigated, which would lead to a reduction of the membrane potential when the respiratory chain is overloaded, thus reducing the formation of reactive oxygen species (ROS) and damage to the cell, including the mitochondrial DNA. MPV17 has been also associated with the stabilization of mitochondrial cristae, which is directly linked to respiratory chain efficiency. To investigate this potential function, we will perform cryo-electron tomography studies using MPV17-knockout cells. With the high spatial resolution this provides an accurate picture of the location of MPV17 and its potential involvement in the cristae. MPV17 appears to be activated by oxidative conditions. Therefore, we will perform our functional studies under high ROS stress conditions, which may lead to oxidation of the cysteine residues in MPV17, inducing structural changes. In the second part of the study, the three-dimensional structure of MPV17 will be obtained by NMR spectroscopy and cryo-electron microscopy. The monomer at reducing conditions will be studied by NMR spectroscopy, and the oligomer populated at oxidative conditions will be studied by cryo-electron microscopy to better understand the mechanism of MPV17 activation. With the obtained structural information, the influence of mutations on MPV17 function can be better understood, which is the basis for a specific therapy of MPV17-induced MDDS.

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Main ArchiFun theme involved:

Mechanisms of bacterial resistance and cancer onsets;

Neurodegenerative and autoimmune diseases;

Molecular insights into the misfolding pathways of antibody light chain proteins

Even though LC misfolding in systemic AL amyloidosis has been studied for 50 years, many structural aspects are not well understood and the misfolding pathways remain elusive. In the first funding period, we could establish and apply methods to investigate the structure and aggregation kinetics of amyloid fibrils derived from lambda-light chains and resolve the influence of selected point mutations in the misfolding process. We will combine NMR experiments and MD simulations to provide molecular insights into the misfolding pathways of ex vivo amyloid fibrils derived from lambda- and kappa-light chains. The work program will address the following aims: (i) Characterize three crucial steps in misfolding: Dimerization, local unfolding of the light chain, and formation of oligomeric intermediates (ii) Characterization of the stability and dynamics of AL fibrils. (iii) Modulation of amyloid structure by cellular components. (iv) Comparison of the aggregation behavior of lambda- and kappa-light chains. In summary, this project aims to resolve the structural dynamics of all species along the misfolding pathways from monomer to fibrils and to investigate the influence of mutations in the misfolding process. We expect that our combined NMR/MD simulation approach will yield molecular insights into the mechanism of light chain misfolding.

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Main ArchiFun theme involved:

Neurodegenerative and autoimmune diseases;

Neurosciences and cognition.

STRUCTURE, INTERACTIONS AND DYNAMICS OF AN RBM17 COMPLEX LINKED TO ALTERNATIVE SPLICING REGULATION

The CHERP, RBM17(SPF45) and SR140 proteins have been recently shown to form a stable complex that plays a key role in cell-cycle progression through a specific alternative splicing program [1]. This project combines NMR and integrative structural biology to study the structure, dynamics and molecular interactions of the CHERP/RBM17/SR140 complex, its interactions with additional proteins and the molecular effects of phosphorylation of its components linked alternative splicing. The three core proteins comprise extensive intrinsically disordered regions, that are expected to mediate protein-protein interactions within the complex and with additional factors, associated to the spliceosome. An integrative approach, centered around the use of solution-state NMR spectroscopy combined with complementary techniques, e.g. SAXS/SANS, X-ray crystallography and cryo-EM will be employed.

We have previously shown that the RBM17 UHM domain mediates key interactions for 3' splice site recognition and recently found that RBM17 plays a crucial role in the splicing of short introns with short poly-pyrimidine-tracts. This process is further modulated by an interaction of the RBM17 UHM with a ULM in SAP30BP, a component of the activated spliceosome [2]. We will combine NMR, crystallography and cryo-EM to study the assembly and protein-protein and protein-RNA interactions of the complex. Alphafold will enable initial modelling of the predicted globular domains (UHM, RRM, CID, SURP), while NMR and will be crucial for characterizing the role of the extended disordered regions. Phosphorylation of RBM17 is expected to modulate the function of the CHERP/RBM17/SR140 complex, with a potential link to DNA damage response. We will employ biochemical experiments and NMR to determine the effects of phosphorylation on the structure and dynamics of the complex (using phosphomimicking mutations and *in vitro* phosphorylation).

We will test if the complex binds RNA (motifs at the 3' splice site), and study interactions with U2 snRNP components and further factors (SAB30BP) that have been linked to mediate the splicing activity of the CHERP/RBM17/SR140 complex.

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Proposed collaboration within ArchiFun network (not mandatory at this stage): -

[1] a) Al-Ayoubi et al . (2012) Mitogen-activated protein kinase phosphorylation of splicing factor 45 (SPF45) regulates SPF45 alternative splicing site utilization, proliferation, and cell adhesion. Mol Cell Biol *32*, 2880-2893; doi: 10.1128/MCB.06327-11 b) De Maio A., et al (2018) RBM17 Interacts with U2SURP and CHERP to Regulate Expression and Splicing of RNA-Processing Proteins. Cell Rep *25*, 726-736 e727; doi: 10.1016/j.celrep.2018.09.041. c) Martin E., et al . (2021) Alternative splicing regulation of cell-cycle genes by SPF45/SR140/CHERP complex controls cell proliferation. RNA *27*, 1557-1576; doi: 10.1261/rna.078935.121

[2] a) Corsini L., et al (2007) U2AF-homology motif interactions are required for alternative splicing regulation by SPF45. Nat Struct Mol Biol *14*, 620-629; doi: 10.1038/nsmb1260. b) Fukumura K., et al. (2021) SPF45/RBM17-dependent, but not U2AF-dependent, splicing in a distinct subset of human short introns. Nat Commun *12*, 4910; doi: 10.1038/s41467-021-24879-y. c) Fukumura, et al (2023). SAP30BP Interacts with RBM17/SPF45 to Promote Splicing in a Subset of Human Short Introns. Cell Reports 42 (12): 113534. doi:10.1016/j.celrep.2023.113534.

Proposed list of secondments (not mandatory, but recommended if known already):

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- Randall at King's College London

Main ArchiFun theme involved:

- Intrinsically disordered proteins
- Macromolecular complexes
- Small angle scattering

Host-pathogen interactions in Plants: Characterization of NLPs, a new family of pore-forming proteins

Necrosis- and ethylene-inducing 1-like proteins (NLPs) are important virulence factors of plant-associated microorganisms such as bacteria, fungi and oomycetes (*1, 2*). NLPs were shown to exhibit cytotoxicity towards plant cells and hence promote infections and toxic effects. NLPs damage plant lipid membranes through a multi-step mechanism that involves binding to plant sphingolipids, glycosyl inositol phosphoceramides (GIPC) (*3*), oligomerisation at the plant membrane level and, finally, disrupting the lipid membrane by forming small, transient pores. Membrane damage by pore formation used by NLPs is different from other families of pore forming toxins and is adapted to plant target membranes (*4*). Although basic steps of membrane-damage mechanism of NLPs are known, crucial details on understanding on molecular assemblies formed at the surface of lipid membrane are still missing. We are investigating this process using structural biology, biophysical and biochemical approaches to gain unique insights into NLPs membrane damaging mechanism. The aim of the project will be to characterize NLPs pore with structural biology approaches. Understanding molecular damage induced by NLPs at the molecular level will enable the development of strategies to inhibit NLP activity and improve crop's health.

The doctoral fellow will be trained in protein production and purification as well as in biochemical and biophysical approaches to study molecular assemblies of NLPs formed at the surface of lipid membranes. The doctoral fellow will prepare recombinant NLPs and use model membranes, such as liposomes and lipid nanodiscs, to image any oligomers composed of NLPs by cryo-electron microscopy.

The National Institute of Chemistry is fully equipped with state of the art instruments for carrying out the research project (https://www.ki.si/en/about-the-institute/research-infrastructure/), including a Glacios 200 kV cryoTEM. The Institute is part of the MOSBRI project, where the doctoral fellow can perform secondments, whenever other complementary techniques be needed to complete the characterization of the NLP pores.

References:

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3. T. Lenarcic *et al.*, Eudicot plant-specific sphingolipids determine host selectivity of microbial NLP cytolysins. *Science* **358**, 1431-1434 (2017).
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Proposed collaboration within ArchiFun network:

Main ArchiFun theme involved:

x Host-pathogen interactions;

Mechanisms of bacterial resistance and cancer onsets;

Neurodegenerative and autoimmune diseases;

Translational research in prevalent diseases;

Physiology and ecology;

Neurosciences and cognition.