RESEARCH REPORT 2019
Luxembourg Centre for Systems Biomedicine

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INTRODUCTION

The Luxembourg Centre for Systems Biomedicine (LCSB) was created within the Health Technologies Initiative from the Government of Luxembourg as one of the research priorities of the University of Luxembourg in 2009. The LCSB is an interdisciplinary centre of the University, independent of the faculties. It combines experimental and computational approaches to analyse complex biological systems and disease processes.

The LCSB focuses on neurodegenerative diseases, particularly on Parkinson’s disease (PD), with a parallel approach to investigating other chronic diseases for their common mechanisms with PD. Its strategy is based on an interdisciplinary integrated systems approach, with core competences in the area of functional genomics and genetics, metabolomics, imaging, bioinformatics and computational biology, thus combining experimental molecular and cellular neurobiology with computational approaches. Through the establishment of research groups led by clinical scientists, the LCSB also bridges to the clinic. In addition to patients and family studies, technological platforms and models on different scales, covering in silico, in vitro and in vivo models, are the essential elements of the LCSB research strategy. Performing systems biomedicine on this multi-scale level is key to gaining insight into human biology and disease.

The integration of the LCSB in both the Luxembourg research landscape and the international systems biomedicine community is a driving force of the centre. The LCSB is forming strategic partnerships with internationally outstanding research institutions in order to create a powerful research network. Among those partners for the technological and systems biology approach are the Gladstone Institutes in San Francisco, the Institute for Systems Biology (ISB) in Seattle, the Systems Biology Institute (SBI) in Tokyo, the RIKEN Centre for Integrative Medical Sciences in Yokohama, and the EMBL in Europe. In addition, high-level clinical partnerships have been formed with the Paracelsus-Elena-Clinic in Kassel, the University of Oxford, University of Tübingen and Radboud University Nijmegen, who excel through their unique patient cohorts in PD.

On a national level, the LCSB forms the Personalised Medicine Consortium (PMC), together with the Centre Hospitalier de Luxembourg (CHL), the Integrated BioBank of Luxembourg (IBBL), the Luxembourg Institute of Health (LIH) and the Laboratoire National de Santé (LNS). Within the PMC, they complement each other in the field of biomedicine from basic research to translational research into the clinic, creating an excellent basis to obtain competitiveness and critical mass in health care research.

LCSB is also building bridges between basic research and industry. Collaborations have been initiated with various players, from small and medium enterprises to large global companies. The goal of these collaborations is to drive an output and product-oriented research in a win-win situation.
RESEARCH

Objectives
The LCSB sees itself as a basic research centre bridging discovery and clinical application. As such, the major focus is on the analysis of complex biological systems and disease processes. With the focus on systems biomedicine, the research strategy is based on the integration of four major elements: patients, experiments, computation, and technology. Major biomedical questions drive the LCSB’s current and future research agenda. This strategy requires a highly interdisciplinary research environment, with a strong collaboration amongst computer scientists, engineers, mathematicians, physicists, biologists and clinical scientists. The LCSB has also an important role in crossing disciplines of different faculties within the University. It is the LCSB’s guiding conviction that the major future challenges of the society will be based at the interface of disciplines and that their solutions will require major skills in analysing complex systems. The long-term objective of the LCSB is thus to grow into a centre of complex systems analysis by developing tools and getting insight into the general principles, which might apply also to other domains such as material sciences, social sciences, and finance.

Vision
- Understand the mechanisms of complex biological systems and disease processes.
- Enable new ways to cure or prevent human diseases based on the mechanistic understanding of complex biological systems and disease processes.

Mission
- Carry out fundamental research in the field of systems biology and biomedicine, including the development of new models and technologies.
- Analyse the mechanisms of disease pathogenesis, with a special focus on Parkinson’s disease and other neurological diseases.
- Identify and validate new targets for disease prevention and intervention.
- Explore opportunities for knowledge transfer from basic research into industrial applications.
- Contribute to science and society in Luxembourg.

The LCSB is collaborating with worldwide leading institutions, as well as regional and national partners. The integration of multiple disciplines and different ways of thinking is only possible in an interdisciplinary centre such as LCSB. We have developed a culture of collaborative research and our philosophy is one of „curiosity driven research“. Leading themes are the transfer from basic research towards actual applications for patients, the building of additional bridges across disciplines to connect with other national partners, as well as the industry by creating spin-offs and valorising research results.

Research strategy
Diseases can be perceived as perturbations of networks, where genetic defects or environmental influences lead to a disturbance that cannot be compensated. Mathematical models are therefore used to describe a network on the molecular level that reflects the observations on a cellular or organism level. To gather data for a computational hypothesis generation and for the subsequent validation, the LCSB engages in a variety of different models and reaches out to the patient. We interpret “multi-scale modelling”, a concept from physics and engineering in biomedicine: from computer to cells to animals to individuals to families and back. Based on different model systems, we produce feedback loops of hypothesis generation and validation, thereby jumping scales from in vitro models to full in vivo organisms like zebrafish or mouse, up to the human being and family studies of inherited diseases. For complex diseases, a multi-scale approach can give new insights and allows a transfer between different disease models, thus requiring a systems approach to unravel the underlying mechanisms. This research theme is a guidance allowing our research groups to unite in one common research goal.
Currently, we are witnessing an explosion in the amount of data derived from biological experiments and clinical research. Furthermore, it will be very difficult for individual institutes to maintain the fast and expensive cycles of sustaining top infrastructure necessary for genomic, proteomic or bioinformatics analysis of biological data. Hence, there is a need for computational platforms provided to the scientific community by dedicated research centres. The goal of the LCSB is to offer bioinformatic, medical informatics, and computational expertise as well as access to high-performance computing and large data storage to other research institutes within Luxembourg and beyond.

Mathematical and computational models to understand human diseases
Biology is becoming an extremely data-rich research discipline. In addition, to allow efficient means of data acquisition, data storage and high-performance computing, the development of suitable algorithms and mathematical descriptions is becoming a critical and essential component in future research efforts. To this end, the LCSB directs major efforts towards the development of computational models, such as models of the human microbiome or models of stem cell differentiation.

State-of-the-art infrastructure
As a shift from academia to the commercial sector can be observed in many molecular technologies, LCSB carefully monitors their development and availability. The LCSB invests in those technologies that are of strategic importance for its research and that give it a competitive edge. Infrastructure and services can be used by external partners on a fee-for-service basis.

Interdisciplinarity
Research at the LCSB relies heavily on the integration of different technologies, models, disciplines, and expertise spanning from mathematical theory to medical needs in the clinic. We strive to be an interdisciplinary centre where the whole is more than the sum of the parts – we do not only pursue research in different areas but tackle questions that can only be answered in a collaborative approach involving different disciplines. Establishing a broad interdisciplinary spectrum of expertise is an explicit goal of the LCSB, and is reflected by the various professional backgrounds of the different Principal Investigators (PIs) as well as the composition of their research groups.

Bridging basic and clinical research
The integration of medicine into systems approaches is important for both parties: On one hand, medical observations in daily care can give valuable new directions to research; on the other hand, a close interaction between basic and clinical research helps to feed research results back into the clinic. The LCSB supports the training of clinical scientists and provides opportunities for medical doctors to combine research activities with patient care.
Research focus

All research programmes at the LCSB focus on biomedical questions. The application of advanced research tools, such as mathematical and computational modelling, metabolomics and imaging, as well as the development of new methodologies, aim at new findings in biology while at the same time also aim at further development of the technologies and methods applied. While each research group pursues also its own research lines, all groups of the centre contribute to the central research theme: neurodegenerative diseases with a special focus on Parkinson’s disease (PD). This research subject was chosen based on its potential in terms of scientific novelty, medical relevance and innovation combined with the requirement for an interdisciplinary approach.

Although genetic as well as environmental factors are known to contribute to the pathogenesis of neurodegenerative diseases, a thorough understanding of the underlying disease-causing mechanisms is still missing. To elucidate mechanisms that play a role in disease origin and progression, a systems approach is needed. Therefore, the LCSB aims to look at the pathophysiological hallmarks of neurodegenerative diseases, such as mitochondrial dysfunction and neuroinflammation, from a systems’ point of view. Besides the genetic factors underlying diseases, the LCSB is also interested in understanding the influence of gene-environment interaction. Here, the primary focus is put on understanding the role of the gut microbiome. Furthermore, the hallmarks and mechanisms of neurodegenerative diseases most likely overlap with those of other diseases and several of them might be present simultaneously in elderly patients. Clinical phenotypes alone seem insufficient to provide an understanding of the pathophysiological mechanisms. A comparative and integrated analysis of diseases across the traditional clinical boundaries (e.g. neurodegenerative diseases versus metabolic diseases) may lead to a redefinition of clinical phenotypes and to new approaches in the treatment of neurodegeneration. Therefore, research efforts at the LCSB are also directed towards identifying the underlying networks of such co-morbidities.

Figure: Research programmes at LCSB.
RESEARCH GROUPS

Being an interdisciplinary centre, the LCSB was founded as an entity separate from the faculties. The establishment of an autonomous structure allows a more flexible operation than is normally possible in the traditional setup of a university faculty. From a governance point of view, the LCSB chose research groups as primary unit of organisation. The groups are accompanied by research support and management structures that help them to be competitive in the international research community. Each group is led by a Principal Investigator (PI). The LCSB currently has 14 research groups, covering three areas: computational, experimental, and clinically-oriented translational groups. Even though listed distinctly below, many of the groups reach across over several disciplines, being not solely experimental or computational.

**Computational focus**
- Bioinformatics Core
- Biomedical Data Science
- Computational Biology
- Environmental Cheminformatics
- Systems Control

**Experimental focus**
- Developmental & Cellular Biology
- Eco-Systems Biology
- Enzymology & Metabolism
- Integrative Cell Signalling
- Molecular & Functional Neurobiology

**Translational focus**
- Clinical & Experimental Neuroscience
- Interventional Neuroscience
- Neuropathology
- Medical Translational Research

Overlapping projects, joint personnel, and regular discussions amongst PIs ensure a close cooperation between the different groups and build bridges from artificial intelligence over experimental biology to clinical studies.
Bioinformatics Core group

Overview
The Bioinformatics Core group has a central role in the LCSB, as it enables the researchers to efficiently manage, analyse and interpret their data. The group ensures efficient data flow within and between experimental, theoretical-computational and medical oriented groups. The focus is on the development and deployment of (clinical) data management and storage systems as well as cost- and time-efficient automated data analysis pipelines in close collaboration with computer scientists and biologists covering a broad spectrum of disciplines. To this end, the research in the Bioinformatics Core evolves around better accessibility and interpretation of the ever increasing but often ‘messy’ data. The group develops algorithms for data mining and visualisation and works on methodologies to make data FAIR: Findable, Accessible, Interoperable and Reusable. Due to its developments around data operations and knowledge generation, the Bioinformatics Core serves as an integrator in LCSB, which is reflected by its important role in LCSB’s cross-sectional projects like NCER-PD and the Parkinson’s Disease (PD) map (see Flagship projects, pages 20-22). In its function as Luxembourg’s ELIXIR node, the group provides services also for the other biomedical research stakeholders in Luxembourg and serves as an international data hub.

Key projects
ELIXIR Luxembourg Node: ELIXIR-LU
ELIXIR, the European infrastructure for life science information, aims to provide long-term access to bioinformatics tools and biological data. ELIXIR-LU (see Flagship projects, page 21) aims to “give life” to data for translational medicine, the combination between the clinical and experimental environment. This means to optimise value gained from such data by improving usability and sustainability. On the national level, support in standardising and electronic capture of clinical data is provided together with hosting and analysis pipelines. Internationally, we host translational medicine data and give support for the curation and standardisation of data sets to improve the reusability and value of the data for the research community. Rather than a project, ELIXIR is a pan-European initiative on governmental level that links the countries in their aim of long-term sustainability of bioinformatics resources. ELIXIR-LU is endorsed by the Ministry of Higher Education and Research in Luxembourg.

Making life science data FAIR: FAIRplus
Wide sharing of knowledge and data drives the progression of science and FAIRification and reuse of existing data creates the opportunity to build the large aggregated cohorts needed to detect rare signals and manage the many confounding factors in translational research. FAIRplus is a project funded within the Innovative Medicines Initiative (IMI) and its main goal is to develop the guidelines, tools and metrics needed to make data FAIR, aiming to deliver a catalogue of high quality FAIRified IMI datasets. FAIRplus comprises a coalition of Europe’s leading experts in data interoperability, standards, pre-clinical to clinical translation and long-term sustainable data repositories, with decades of experience in making data available to a wide scientific community for reuse.

Data integration across diseases and disciplines: SYSCID, SysMedPD, AdaML, BIOMAP, Smart4Health
The core facility provides data management and analysis for several international consortia. The data analysed in these projects cover imaging, mobile/sensor, genomic, epigenomic, and metagenomics data as well a multitude of physiological factors combined with in vivo and in vitro data from model systems. The group takes care of the standardisation of data, integration across species, platforms and data types for analysis as well as the visualisation in disease maps. The work includes building semantic data models and the use of machine learning and data mining approaches to gain maximum knowledge from the data. The projects are funded through Horizon 2020 and the Michael J. Fox Foundation (MJFF).
Text and data mining: PD map
The goal of text mining tasks is to analyse a wide scope of scientific publications to detect factors involved in physiological and pathological states. Using statistical and natural language processing techniques, we aim at gaining insight into causal relations between molecular and phenotypic components, enabling better understanding of individual diseases as well as the comparison between them. This information then feeds into the disease map as knowledge base and allows the mapping of own results on the publicly available literature. These methods are also helping to establish a more automated curation of clinical data and to make existing clinical data usable for computational analyses by applying text mining based on artificial intelligence methods to understand clinical records and convert them to standardised expressions.

Genomic Analysis: NCER-PD, MJFF LRRK2, Epi25K consortium, MechEPI
The Bioinformatics Core uses whole exome and genome sequencing (WES and WGS) to detect rare coding variants in genomes in order to study complex diseases in the context of family pedigree and case/control studies. The studies include mainly neurological diseases like Parkinson’s disease, epilepsy, or Alzheimer’s disease. Using state-of-the-art genomic analysis pipelines, the group identifies disease-associated genetic variants. The role of these prioritised candidate variants is then validated by the experimental groups applying animal and cell models. The validation projects are funded through the Luxembourg National Research Fund (FNR) and MJFF.

Responsible and Reproducible Research (R3)
The aim of the R3 initiative at the LCSB is to raise research quality and increase the overall reproducibility of scientific results. This ambitious goal is achieved through state-of-the-art infrastructure, GDPR compliant data processes and data handling methods, as well as platforms and tools for high-quality scientific computing code. The classical research publication workflow is standardised by structuring data, capturing lab protocols and experimental methods in electronic lab notebooks, source code versioning, workflow management, and freezing of project states via virtualisation technologies. The R3 program is built on three pillars: the R3 Pathfinder, the R3 School, and the R3 Accelerator. Based on individual consultations, the R3 Pathfinder programme helps the individual groups of LCSB to follow best practices. The R3 School comprises courses on data protection, version control, and computer basics. The R3 Accelerator aims at more mature projects with strong R3 components that can be further boosted in their level of quality and reproducibility. The R3 Clinic, where scientists and researchers receive hands-on support to push the boundaries of R3 with their individual projects, links all three facets.

Key publications
2. Schubert, J. et al. (2014) Mutations in STX1B, encoding a presynaptic protein, cause fever associated epilepsy syndromes. Nature Genetics, 46(12), 1327-32; doi:10.1038/ng.3130
Key projects

Gender differences in neurodegenerative diseases (GenderND project)

In both PD and AD, gender differences have been observed in the incidence and phenotypic manifestations of the disorder. Although these differences may result from diverse behaviors and lifestyles, previous studies suggest that the underlying causes are more complex and disease-specific, and involve hormonal and genetic influences. In the GenderND project, we investigate whether specific genetic factors contribute to the observed gender differences in neurodegenerative disorders.

For AD, we are using animal models and molecular data from human biospecimens to study a candidate gene derived from the statistical analysis of multiple large-scale omics datasets from AD case/control studies. The corresponding sex-linked gene, ubiquitin-specific peptidase 9 (USP9), has a gender-biased activity in the human brain and encodes an enzyme reported to regulate the phosphorylation of MAPT, a protein thought to play a central role in AD pathogenesis. We had previously shown that the knockdown of USP9 results in a decreased MAPT gene expression in zebrafish embryos and in a human cell culture model, and that gender differences in MAPT and USP9 alterations also occur in two mouse models for AD. More recently, we have started to use single-cell sequencing of brain tissue from AD mouse models in order to investigate gender-dimorphic disease-associated changes across different brain tissues and cell types. These analyses are conducted in two mouse models, the Tg276 model, which expresses human Alzheimer amyloid-beta (Abeta) precursor protein carrying an AD-associated mutation, and the THY-Tau22 model, which does not develop any motor deficits, but displays the characteristic cognition deficits for AD. For both models, we are studying the two main affected brain tissues in AD, the hippocampus and the cortex.

For PD, an initial analysis of gender-linked genes using an integrated analysis of omics datasets from PD case/control studies revealed multiple candidate disease genes and pathways with gender-specific changes in PD. These candidates are currently further investigated using analyses of PD-related changes in the surrounding molecular interaction network. The resulting information on disease-linked gender differences in the brain transcriptome can provide new insights to support the development of patient-tailored diagnostic and therapeutic approaches for the studied neurodegenerative disorders.
Multi-dimensional stratification of PD patients for personalized interventions (PDStrat project)

Current therapeutic approaches for PD help alleviate some of the major symptoms, but can neither halt nor slow the progression of the disease. As part of a multicenter collaborative research project, led by Prof. Peter Heutink from the German Centre for Neurodegenerative Diseases (DZNE), we are investigating new approaches for personalised interventions against PD, hypothesizing that previous clinical trials have failed for three main reasons: First, selection of potential drug targets is rarely based on robust biological evidence. Second, the disease process begins decades before clinical symptoms are observed and clinical trials on patients are therefore likely too late to reverse the neurodegeneration. Third, participants have been selected largely ignoring their underlying disease biology, phenotypic variation and their genetic risk profile, resulting in a very heterogeneous population of cases with very different progression of disease. Therefore, we aim to develop a novel concept for disease-mechanism based disease onset and progression prediction, and subsequent target discovery and patient stratification for Parkinson’s disease. Our goals are to identify novel targets based on biological evidence with matching precision cohorts for clinical trials that will allow for personalised therapeutic interventions based on genetic and genomic risk profiles and clinical subtypes. To reach our goal, we are using a systems biology approach by generating multi-dimensional clinical, genetic/genomic and biological risk and progression profiles for patients and at risk individuals and integrating these data in network models for target discovery and prediction and progression models for patient stratification. Within the project consortium, the Biomedical Data Science group is responsible for the machine learning and network analyses of omics data and clinical records. As part of our initial work, we have implemented new machine learning methods to improve the prediction of dichotomized clinical outcomes by exploiting information from continuous outcome predictions and correlation patterns between different clinical outcomes. The project is funded by the Luxembourg National Research Fund (FNR) as part of the multilateral ERACoSysMed JTC2 2017 call.

Integrative machine learning analysis of neuroimaging, omics and clinical data for PD (MultimodalPD)

The reliable diagnosis of PD is still a challenge, even in the motor stage. While brain neuroimaging can facilitate the diagnostic process, limitations in the robustness of predictive features extracted from the data and the cost and time requirements associated with imaging procedures restrict their practical utility. Using blood omics data as an additional information source may provide both improved diagnostic models and/or an initial filter to decide on whether to apply classical imaging approaches.

We have therefore started to conduct combined neuroimaging and omics analyses on PD patients and unaffected controls to investigate the potential of integrative machine learning approaches for improving diagnostic predictions and providing new pathophysiological insights. In our first study of 60 PD patients and 15 healthy age- and gender-matched controls, using FDG and FDOPA PET brain neuroimaging and blood GC-MS metabolomics, we could show that metabolomics data can significantly improve the diagnostic discrimination power. Moreover, the metabolomic signatures also revealed interesting disease-inherent changes in cellular processes, including oxidative stress response and inflammation. We are currently extending these analyses from idiopathic PD patients to subjects with PD-associated genomic variants in the key susceptibility gene GBA, in order to better understand the molecular alterations in distinct familial and sporadic forms of PD.

Key publications

Clinical & Experimental Neuroscience group

Overview
The group is bridging the gap between integrated clinical research and basic science, to help citizens with effective prevention, diagnosis and therapy in Luxembourg and beyond. One major research focus, supported by the FNR PEARL Excellence Programme for Research in Luxembourg, is the clarification of communication processes in specific cells leading to neurodegeneration in Parkinson’s disease (PD). The group has identified novel gene mutations related to familial PD and deciphered genetic variants in candidate sporadic PD-associated genes. The group studies the functional consequences of identified mutations and the molecular signaling cascades in the pathogenesis of PD.

Key projects
I. Fundamental Research
Functional analysis of PD-associated genes using High Content Screening
The substantial expertise of the group in phenotypic assay development adds to the unique automation platform to discover neuroprotective compounds by repurposing of already approved drugs. Automated workflows allow expansion of all cell types and neuronal differentiation of induced pluripotent stem cells (iPSC) and small molecule neuronal precursor cells (smNPCs), as well as preparation of assay plates with these cells for high throughput screenings.

The CEN Group studies several different genes relevant for PD:

DJ-1 for mechanism-based genetic and pharmacological treatment of PD
One of the causes of familial PD is homozygous loss-of-function mutations of DJ-1. DJ-1 is a protein encoded by the gene PARK7, with broad biological functions including effects on mitochondrial and lysosomal homeostasis. Patient-derived iPSCs are differentiated into smNPCs and then into midbrain-specific dopaminergic (mDA) neurons to study the effect of DJ-1 loss-of-function in PD. Cellular phenotypes are used for library screens to detect compounds that rescue PD phenotypes.

Pathological role of α-synuclein encoding gene (SNCA) in PD
Genome-Wide Association Studies (GWAS) suggest the SNCA locus as a major risk factor in idiopathic PD. The study involves differentiation of isogenic A30P PD-derived iPSCs and a non-PD familial control, into mDA neurons to study the link between α-synuclein and mitochondrial dynamics and the cells’ functionality. Related ongoing studies on neuronal autophagy and α-synuclein investigate how the interplay of mDA neurons and astrocytes can lead to the spread of PD.

GBA mutation as a genetic modifier of familial PD
Mutations in the GBA gene are the most common genetic risk factors for developing PD. The study obtained fibroblasts from a PD patient carrier of a GBA mutation alongside with a PARK2 mutation to explore if GBA could modify the expression of PD on a familial PD background. These fibroblasts were reprogrammed into iPSC, which were CRISPR-Cas9 edited for GBA and differentiated into mDA neurons. Protein expression, lysosomal activity, α-synuclein metabolism and mitochondrial morphology and function can then be studied.

Cellular endophenotypes of VPS35 mutation in PD
The VPS35 D620N mutation has been identified as an autosomal-dominant cause of familial PD. The VPS35 protein is part of the retromer complex and is implicated in the sorting and moving of proteins from endosomes to the trans-Golgi network. This project aims to study the phenotype of mDA neurons derived from patients.
Disease modifiers in LRRK2 PD
Mutations in the LRRK2 gene are the most common cause of familial PD. Together with the Bioinformatics Core and the Gladstone Institute, the project analysed several families with a history of LRRK2 PD cases and identified variants segregating with age of onset. The current phase focuses on characterising candidates and PD associated phenotypes in cultures of mDA neurons from iPSCs derived from LRRK2 PD patients. This project is funded by the Michael J. Fox Foundation.

The relevance of Miro1 for mitochondrial dynamics in PD
The outer mitochondrial membrane protein Miro1 acts as a sensor for cytosolic calcium levels and is involved in the regulation of ER-mitochondrial contact sites and calcium homeostasis. These, in turn, are important for the initiation of autophagy. In collaboration with Prof. Anne Grünewald (LCSB, page 36-37), we analyse the effect of mutations in Miro1 on calcium homeostasis and mitophagy. The data collected shows that mutations of Miro1 interfere with the regulation of ER-mitochondrial contact sites and with calcium homeostasis, which subsequently leads to dysregulation of autophagy pathways.

II. Clinical Research
National Centre of Excellence in Research on Parkinson’s Disease (NCER-PD)
NCER-PD aims to improve diagnosis and stratification of PD by developing novel disease biomarkers. PD patients and healthy subjects are recruited in Luxembourg and the Greater Region. This project is funded by the Luxembourg National Research Fund (FNR), and further information can be found in LCSB’s flagship projects, on page 20.

Treatment of gait disturbances in PD
The team participates in a multicentre clinical study on the implementation of novel deep brain stimulation to treat therapy-resistant gait-freezing in advanced PD patients. An innovative concept, targeting the substantia nigra pars reticulata in the brain, is used in a clinical study, supported by Medtronic, comprising of ten centres in Germany and Luxembourg.

III. Integrated Care Concepts
The group is developing innovative patient care concepts for PD and other conditions. One of these concepts is ParkinsonNet Luxembourg, which brings healthcare professionals together and facilitates Parkinson-specific specialisation, interdisciplinary collaboration and exchange of knowledge. This interaction aims that every PD patient in Luxembourg receives the best possible care (www.parkinsonnet.lu). Moreover, the group leads the Programme Démence Prévention (pdp), launched by the Luxembourgish Ministry of Health. pdp aims to implement a programme employing a personalised lifestyle intervention to prevent, or at least delay, the development of dementia in a target population, defined by a mild cognitive impairment.

Key publications
Computational Biology group

Overview
The main goal of the Computational Biology Group is to understand how molecular networks (e.g. gene regulatory, protein-protein interactions and signalling networks) mediate cellular processes involved in cellular differentiation and reprogramming. The group develops mathematical and computational approaches using multiple sources of biological information (e.g. transcriptomics, epigenomics, proteomics) in order to build network-based models that consider key molecular characteristics underlying these cellular processes. These models, which range from single-cell to cell population levels, aim to address relevant questions in the field of stem cell research and regenerative medicine including the study of iPSC disease models, increasing efficiency and fidelity of cellular differentiation and reprogramming, and modelling cell-environment interactions within the context of tissue regeneration. The development of these models is essential in order to gain a better understanding of cellular differentiation and reprogramming, which will enable researchers to design novel strategies to guide stem cell experimental research and regenerative medicine approaches. In addition, the group also studies how perturbations of molecular networks give rise to phenotypes resulting in human disease. These perturbations, which range from the complete loss of a gene product to the specific perturbation of a single molecular interaction, may arise from genetic variations, epigenetic modifications, and genome-environment interactions. In particular, we use gene regulatory network-based models in order to address a number of relevant issues, such as the identification of molecular signatures of disease states and their master regulators. These might serve as novel drug targets or diagnostic biomarkers. Thus, diseases can be diagnosed, treated and prevented by understanding and intervening in the networks that underlie health and illness.

Figure: Molecular networks in health and disease.

Key projects
Network-based approach to design new strategies for cellular reprogramming: clinical applications
The goal of this project is to build a computational platform that relies on gene regulatory network models to develop strategies for identifying optimal reprogramming determinants triggering transitions between specific cell types. The platform is based on Boolean network modelling framework to simulate cell state transitions. Importantly, it is applicable for closely related cell sub-types with similar transcriptional profiles. This platform will be applied to projects relevant to cell therapies and regenerative medicine. For example, in collaboration with Prof. Michele De Luca at the Centre for Regenerative Medicine in Modena, Italy, we intend to predict reprogramming determinants to derive corneal limbus stem cells from cultured epithelial keratinocytes. The derived experimental protocol will be further optimized and used for treating patients who have lost their corneal limbus stem cells by injury or burn.

Development of computational strategies for efficient cell conversions based on single cell RNA-sequencing
In our previous work, we developed a computational platform for identifying core transcription factors (TFs) that define the identity of each subpopulation of cells in a given single-cell RNA-seq dataset. The method predictions were validated experimentally for the conversion of human neuroepithelial cells into dopaminergic neuron progenitor cells. However, within the transcriptional core, identification of most efficient combinations of TFs (sub-cores) remains a problem. In the current project, we develop a computational method for identifying such sub-cores of TFs that can more efficiently induce cellular subpopulation conversions. Our approach will combine gene regulatory network inference with information theoretic measures of synergy and redundancy to identify sub-cores in the entire transcriptional core that determines subpopulation...
identity. This project is carried out in collaboration with Prof. Deepak Srivastava’s lab at the Gladstone Institutes, where our predictions for efficient conversions of cardiac right ventricular cells into left counterparts will be validated. The outcome of this project will be essential for designing strategies to treat patients with cardiovascular diseases.

Computational approach to directing cellular reprogramming into multiple cell types

Current reprogramming protocols are able to reprogram one cell type into another. However, derivation of in vitro tissues consisting of multiple cell types from a single source of cells is a challenge and is of clinical interest. In this project we aim to develop computational methods that predict reprogramming factors that can convert one cell type into multiple cell types in a controlled way. Our previous work for identifying core TFs in each subpopulation of cells from single-cell RNA-seq data will be extended, so that it can predict an optimal set of TFs that could simultaneously induce multiple reprogramming from one cell type. The experimental validation will be performed in collaboration with Prof. Ernest Arenas’ lab at Karolinska Institute. Experiments on cultured astrocyte cell colonies will be conducted to perturb sets of TFs that have been predicted to induce cellular conversions into different proportions of neural stem cells and neurons. The outcome of this project will be used for transplantation therapies for neurodegenerative diseases, such as Parkinson’s disease. This project is supported by the Luxembourg National Research Fund (FNR).

Computational models for advances in regenerative medicine

To conceive innovative approaches in regenerative medicine, knowledge exchange by creating synergies between experimental biologists and computational experts is essential. Therefore, we recently established a satellite research group at the Center for Cooperative Research in Biosciences (CIC bioGUNE), a multidisciplinary research institute focusing on personalised medicine and drug discovery. In this framework, we will concentrate together with Sascha Jung, former postdoc at the LCSB, on stem cell research and regenerative medicine, with particular emphasis on cellular conversion for cell therapy and tissue engineering. The goal of the collaboration with Prof. María Luz Martínez Chantar is to design a computational model of liver cirrhosis, the leading liver disease in Europe, that predicts cellular conversion factors for reverting the disease phenotype. A pre-clinical validation of these factors will then be conducted in living mouse models. The project in collaboration with CIC bioGUNE principal investigator Ashwin Woodhoo aims to identify the gene regulatory network, which gives Schwann Cells - the glial cells in peripheral nerves - their ability to repair injured nerves and understand how manipulations of this network could boost nerve regeneration. By focusing on the implementation of mathematical and computational models within these two projects in the field of liver and neurological diseases, we intend to better understand regeneration.

Key publications

Developmental & Cellular Biology group

Overview
In recent years, it has been accepted that PD is a disorder which is not only characterised by a loss of dopaminergic neurons in the **substantia nigra**, but also has a strong neuro-developmental aspect. The aim of our research is to understand, model and treat PD. Particularly, we are interested in elucidating how developmental processes contribute to the susceptibility to suffer from PD. Human stem cells, either neural stem cells or pluripotent stem cells, are in the centre of all of our research approaches. We use these cells to generate advanced *in vitro* disease models, including three-dimensional brain organoids (so called “mini brains”), which shall help us to understand the cellular and molecular processes underlying disease onset and progression. By further developing these models, we will at least partially be able to replace animal experiments and to take an additional step in the direction of personalised medicine. Concerning the molecular processes we are particularly interested in linking the molecular function of PD-associated proteins with cell cycle progression, protein aggregation and mitochondrial/lysosomal function. Additionally to PD, we are also working on Battens disease/Neuronal Ceroid-Lipofuscinosis (NCL), which is a childhood neurodegenerative disease. Multiple projects are funded by the FNR and the European Commission.

Key projects

Is Parkinson's disease a neuro-developmental disorder?
PD is leading to a variety of motor and non-motor symptoms. Interestingly, non-motor symptoms often appear a decade or more before the first signs of motor symptoms; some are remarkably similar to those observed in cases of impaired neurogenesis and several PD-related genes have been shown to play a role in embryonic or adult neurogenesis. Indeed, animal models deficients in the genes *Nurr1*, *Pitx3*, *SNCA*, and *PINK1* display deregulated embryonic neurogenesis and *LRRK2* and *VPS35* have been implicated in neuronal development-related processes such as Wnt/β-catenin signalling and neurite outgrowth. Finally, the roles of PD related genes, *SNCA*, *LRRK2*, *VPS35*, *Parkin*, *PINK1*, and *DJ-1* have been studied in NSCs, progenitor cells and iPSCs, demonstrating a role for some of these genes in stem/progenitor cell proliferation and maintenance. Together, these studies strongly suggest a link between deregulated neurogenesis and the onset and progression of PD and present strong evidence that, in addition to a neurodegenerative disorder, PD can also be regarded as a developmental disorder.

We use PD patients’ specific iPSCs to generate neural stem cells and dopaminergic neurons. Importantly we not only include patients with mutations in classically know PD associated genes (e.g. *LRRK2*, *VPS35*, *Pink1*, *Parkin*, *ATP13A2*, and *SNCA*) but also patients with novel mutations as well as idiopathic patients. As controls iPSCs from healthy individuals are used. In the thereby derived neural stem cells and dopaminergic neurons we define PD-associated cellular- and molecular phenotypes (e.g. neurite complexity, neuronal differentiation, cell death, stress resistance, mitochondria activity). Additionally, we use these cells to establish co-culture systems mimicking the complex cellular composition in the intact brain. Finally, these cells represent excellent platforms for mechanistic studies and for the identification of new genetic modifiers or small molecule drugs addressing PD-associated motor and non-motor symptoms. Currently we are using these models to test novel small molecule compounds that potentially find applications in future PD therapies.

Genetic engineering in human induced pluripotent stem cells for *in vitro* disease modelling
Personalised PD patient-specific iPSCs have the advantage of carrying the disease inducing mutations. However, they have the drawback that, aside from the PD-associated mutation the genetic backgrounds of the individuals are extremely...
diverse. Therefore, it is not easy to compare phenotypes observed with cells from different patients. Nevertheless, they are valuable, because only these patient specific cells fully represent the conditions that lead to the disease. However, in order to overcome these limitations, we additionally make use of isogenic iPSCs carrying defined PD-specific mutations. Technically, these mutations are introduced in human iPSC by using the CRISPR/Cas system. The comparison of personalised cells with isogenic cells will allow us to deduce phenotypes and mechanisms that are disease and mutation specific. Additionally, this approach probably gives us the opportunity to define unifying themes that are common to PD.

Human brain organoids and brain-on-a-chip technology
The human brain is an immensely complex structure, therefore the investigation of its development as well as modelling of disease processes in lower animal models and traditional cell cultures have clear limitations. We use human iPSCs to generate several millimetre large, three-dimensional human brain organoids, collectively called mini-brains. In many aspects, these organoids resemble the developing human brain. They contain all relevant cell types (neurons, astrocytes and oligodendrocytes), have functional synapses as well as axon myelination. Most importantly they show a spatial organisation and asymmetry that resembles the developing human brain. We are currently working on making these models even more predictive for the actual human brain, by including the immune cells of the brain (microglia) as well as vasculature. A particular feature of our mini-brains is their specificity for the human midbrain, which makes them highly relevant for PD. To achieve a higher degree of automation and parallelisation, we cultivate iPSC-derived midbrain-specific organoids in 3D microfluidics devices. With both approaches (microfluidics-based and free floating brain organoids) we are currently developing more advanced protocols and models to obtain complex brain organoids. This will allow us to study brain development as well as neurodegenerative processes in complex human systems.

From organoids to assembloids
PD is a complex disease, not only affecting single brain regions (like the midbrain), in contrast PD is affecting the complete brain or even the complete body. By assembling organoids representing either different brain regions, e.g. the midbrain and the striatum, or different parts of the body, e.g. the brain and the intestine, we develop more complex and representative in vitro disease models. These connected assemblies of different organoids are called assembloids. Interestingly, all different organoids can be derived from a single stem cell culture from any individual. Hence they are truly personalised models.

Key publications

Principal Investigator
Jens C. Schwamborn
Start: June 2013

STAFF
Postdocs
Silvia Bolognin
Gemma Gómez Giró
Graham Robertson
Claudia Saraiva
Project Manager
Matthieu Gobin
PhD candidates
Ugne Dubonyte
Jennifer Modamio Chamarro
Anna Monzel
Nathasia Muwanigwa
Isabel Rosety
Sònia Sabaté Soler
Semra Smajic (jointly with Grünewald lab)

Technical assistants
Thea van Wüllen
Kyriaki Barmpa
Eco-Systems Biology group

Overview
The main goal of the Eco-Systems Biology Group is to develop and apply molecular systems biology approaches to obtain unprecedented understandings of mixed microbial communities (e.g. gastrointestinal microbiota), their interactions with the environment (e.g. the human host), and how certain microbial community compositions lead to certain outcomes (e.g. pathogenesis). Changes to the human microbiome (microbial dysbiosis) have been linked to a number of human diseases including cancer, metabolic and neurodegenerative diseases. The group is currently involved in research projects investigating these diseases using its high-resolution molecular approaches.

To develop the field of Microbial Systems Ecology, the group has pioneered a number of key methodologies and technologies that allow the systematic and high-resolution study of microbial community-driven processes. These range from wet lab techniques and microfluidics-based in vitro co-culture systems to downstream bioinformatic analysis methods for the integration of high-resolution molecular data (“integrated multi-omics”). The overall vision of the group is to use an integrated omics approach to identify key processes governing microbial community structure, function as well as products, which may inform control strategies for microbial communities in the future, especially those within the gut.

Key projects
Integrated omics
High-resolution molecular biology approaches are vital for discovering and characterising microbial consortia, and understanding the interaction of microbial communities with biotic and abiotic environmental factors. Integrated omics, comprising community genomics, transcriptomics, proteomics and metabolomics, are able to reveal the links between genetic potential and functionality in a truly systematic fashion. Our recently developed methods range from a patented comprehensive biomolecular extraction method called BioMolXtract, yielding high-quality DNA, RNA, protein and metabolite extracts from single, unique samples, to new bioinformatic analysis tools. This enables meaningful integration of the generated multi-omics data for downstream analysis and modelling of mixed microbial communities, including those of human-health interest. For example, we have obtained new insights into functional changes within the gut microbiota in the context of type 1 diabetes. Furthermore, we have recently found that caesarean section delivery leads to the disruption of the transfer of key functional strains from mother to infant very early on which results in differences in immune system stimulation when compared to vaginally delivered infants. Financial support is provided by the Luxembourg National Research Fund (FNR) and the European Commission (EC).

The human microbiome and its role in Parkinson’s disease
Parkinson’s disease (PD) is hypothesised to start in the enteric nervous system and the olfactory bulb, potentially triggered by a so far unknown pathogenic agent. Aberrations within the gastrointestinal and nasal microbiomes may play a role in the initiation of this process. Using our high-resolution molecular methods, we are investigating whether changes in microbial community structure and function in the gastrointestinal tract, as well as the nasal cavity accompany PD onset, its progression and its prodromal REM sleep behaviour disorder (RBD). Recent results highlight observable differences in the gut microbiome of PD patients, in bacteria associated with mucus foraging, most notably Akkermansia spp. and Bacteroides spp. Overall, the project has the potential for the discovery of microbiome signatures as early progression and risk biomarkers for PD and for contributing to our understanding of disease development. Our collaborators on this project are Prof. Wolfgang Oertel from the University of Marburg (Germany) and Prof. Brit Mollenhauer from the University of Göttingen (Germany). Financial support is provided by the FNR and the Michael J. Fox Foundation.
Microfluidics-based human-microbial interaction models

Recent scientific evidence suggests that a range of human diseases may result from microbial dysbiosis. In order to ascertain causal links, we have developed a representative microfluidics-based in vitro model, named HuMiX, which allows the prolonged co-culture of differentiated human epithelial cell lines and human microbiota under biomimetic conditions. The device is particularly well-suited for investigating the nature and impact of molecular crosstalk between human and microbial contingents, how imbalances therein may be involved in disease pathogenesis, and how the diet-related food components as well as pro-, pre- and anti-biotics may affect human-microbial molecular interactions. Furthermore, we have included representative immune cell populations in the model and are working on the integration of representative neuronal cell populations. Finally, we are constructing an in vitro model of the entire human gastrointestinal tract, termed GUT, which, among other applications, will be suited to study the pharmacokinetics of drugs. Our collaborators on these projects are Prof. Frederic Zenhausern from the University of Arizona (USA) and Prof. Kenya Honda from the RIKEN Center for Integrative Medical Sciences (Japan). Financial support is provided by the FNR and the EC.

A role for extracellular bacterial RNA in host-microbe interactions

Extracellular RNAs (exRNAs) are involved in within-host intercellular communication, e.g., in tumor microenvironments. We were first to report that enteric bacteria also export RNAs. To better understand the potential of host-microbe interactions via exRNAs, we studied the outer membrane vesicle (OMV)-associated complement of the enteric pathogen *Salmonella enterica serovar Typhimurium*. We found growth-condition-specific extracellular enrichment of OMV-associated RNAs as well conservation of full-length transcripts. Motivated by these findings, we are currently investigating the OMV-associated RNA complement of gut bacteria related to colorectal cancer (e.g. *Fusobacterium nucleatum*) to test whether these RNAs could have a functional role in disease and/or could be used as biomarkers. Our collaborators on these projects are Prof. David Galas from the Pacific Northwest Research Institute (USA) and Prof. Peer Bork from the European Molecular Biology Laboratory (Germany). Financial support is provided by the FNR.

Systems biology of natural microbial assemblage

We are investigating specific lipid accumulating microbial (LAM) communities, which are found at the air-water interface of certain wastewater treatment plants and whose phenotypes may be harnessed for the concomitant treatment of wastewater and production of biofuel. By using a time-resolved integrated omics approach, we aim to link biotic and abiotic factors to population abundance, to reconstruct the community-wide gene regulatory as well as metabolic networks, and to understand the key processes involved in lipid processing, assimilation and storage by LAM communities. We are specifically investigating factors related to the microbial ecology of these communities, e.g. population-level genetic heterogeneity, overall community metabolism as well as the evolutionary arms race between virus infection, mobile genetic elements and host defense. Financial support is provided by the FNR.

Key publications

Environmental Cheminformatics group

Overview
The Environmental Cheminformatics group focuses on the comprehensive identification of known and unknown chemicals in our environment to investigate their effects on health and disease. The environment and the chemicals to which we are exposed is incredibly complex, with over 125 million chemicals registered in the largest chemical registry and over 70,000 in household use alone. All detectable molecules in complex samples can now be captured using high resolution mass spectrometry (HRMS). Non-target HRMS provides a “snapshot” of all chemicals present in a sample and allows for retrospective data analysis through digital archiving. However, scientists cannot yet identify the vast majority of the tens of thousands of features in each sample, leading to critical bottlenecks in identification and data interpretation. Identifying the chemical unknowns in living organisms and our environment is essential for unravelling the causes of disease and toxicity, improving our understanding of biological processes and developing new strategies to counteract diseases. For instance, the causes of Parkinson’s disease (PD) are largely unidentified and hypothesised to be due to a complex combination of environmental and genetic factors. While non-target HRMS methods now provide a basis to identify unknowns, this remains extremely time consuming and, in many cases, a matter of luck. Prioritising efforts to find significant metabolites or potentially toxic substances responsible for observed effects is the key, which involves reconciling highly complex samples with expert knowledge and careful validation. This group will pursue a fundamental shift away from single-substance assessments and develop generic approaches scalable to tens of thousands of chemicals, features and samples.

Key projects

Environmental Cheminformatics to Identify Unknown Chemicals and their Effects (ECHIDNA)
ECHIDNA is a five year ATTRACT fellowship from the Luxembourg National Research Fund (FNR) starting late 2018 to build computational methods suitable for investigating and elucidating unknowns and causes of effects using HRMS of small molecules. Computational and experimental developments will improve structure elucidation, including cheminformatics approaches as well as stable and dynamic labelling of samples. New cheminformatics methods will improve our understanding of the fundamentals of HRMS and work towards the “holy grail” of a full Computer-Assisted Structure Elucidation (CASE) system for HRMS. A microbiome-PD cohort study will yield complex samples with patient/early stage/control information but scientists cannot yet identify the vast majority of the tens of thousands of features in each sample, leading to critical bottlenecks in identification and data interpretation. Identifying the chemical unknowns in living organisms and our environment is essential for unravelling the causes of disease and toxicity, improving our understanding of biological processes and developing new strategies to counteract diseases. For instance, the causes of Parkinson’s disease (PD) are largely unidentified and hypothesised to be due to a complex combination of environmental and genetic factors. While non-target HRMS methods now provide a basis to identify unknowns, this remains extremely time consuming and, in many cases, a matter of luck. Prioritising efforts to find significant metabolites or potentially toxic substances responsible for observed effects is the key, which involves reconciling highly complex samples with expert knowledge and careful validation. This group will pursue a fundamental shift away from single-substance assessments and develop generic approaches scalable to tens of thousands of chemicals, features and samples.

Non-target Screening with High Resolution Mass Spectrometry
Analytical and computational methods for high throughput non-target high resolution mass spectrometry methods (NTHRMS) will be developed at the LCSB in conjunction with the Metabolomics Platform, the Enzymology & Metabolism Group and the Bioinformatics Core. A large focus will be on developing open computational methods, primarily using the programming language R. This will incorporate the workflows developed during the SOLUTIONS project (www.solutionsproject.eu) and the collaborative trial run by the US EPA (ENTACT). Open packages such as enviMass, enviPat, nontarget, RMassBank and others will be integrated with other packages under development (ReSOLUTION, RChemMass) and connected with initiatives from the NORMAN Network (see below). MetFrag (http://c-ruttkies.github.io/MetFrag/), developed in collaboration with the IPB within SOLUTIONS, will form the basis for non-target identification.
Open Data Exchange and the NORMAN Network

Ensuring the open availability of high quality data is critical to improving computational methods and this group will continue several initiatives within the NORMAN Network (www.norman-network.com) and beyond. We will continue to coordinate the NORMAN Suspect Exchange (http://www.norman-network.com/?q=node/236) and curating/integrating this into the United States Environmental Protection Agency (US EPA) CompTox Chemistry Dashboard (https://comptox.epa.gov/dashboard/). This is tightly connected with unique European initiatives launched within several NORMAN working groups (Prioritization, EDA and Non-target screening) such as NormaNEWS, the NORMAN Digital Sample Freezing Platform and MassBank.EU (see below).


This group will continue collaborative activities in the European MassBank server (www.massbank.eu), initiated within the NORMAN Network in 2012. This includes the contribution of high quality mass spectral data as well as uploading of external contributions and continuing input into server and functional developments (https://github.com/MassBank/). Collaborative development on the spectral processing software “RMassBank” (https://github.com/MassBank/RMassBank/) will continue for automated processing, calibration and annotation of mass spectra using online services such as CACTUS, CTS, PubChem, ChemSpider and the CompTox Dashboard for chemical annotation. These methods are applicable for MassBank and computational mass spectrometry workflows in general. Our group will focus on pushing the boundaries of the possible with tentative libraries annotated with appropriate confidence levels and structural information to develop methods to deal with complex mixtures in real samples in a robust manner. Initiatives such as the SPectraL hASH (SPLASH, https://github.com/berlinguyinca/spectra-hash) and the collaboration with the Dashboard (https://comptox.epa.gov/dashboard/) will enable cross-resource retrieval and searching, ensuring improved access to data, independent of the data upload point.

Key publications


Principal Investigator
Emma Schymanski
Start: September 2017

STAFF
Postdocs
Corey Griffith (jointly with Linster lab)
Todor Kondic
Randolph Singh
PhD candidates
Lorenzo Favilli
Adelene Lai Shen Lyn
German Preciat Gonzalez (jointly with Leiden University, the Netherlands)
Flagship projects

LCSB’s flagship projects bring together several of our research groups and are highly interdisciplinary by nature. The following pages highlight important infrastructure such as the national ELIXIR Node in Luxembourg and the NCER-PD Parkinson cohort as well as research and education endeavors such as the Parkinson’s disease (PD) map or the three Doctoral Training Units that are coordinated by the LCSB in the areas of PD, the microbiome and critical transitions.

National Centre of Excellence in Research on Parkinson’s Disease

Programme coordinators: Rudi Balling, Rejko Krüger

NCER-PD (www.parkinson.lu) unites clinical, biomedical, and computational research in Luxembourg. The aim is to pave the way for emerging precision medicine concepts for PD, including earlier diagnosis, patient stratification, and personalised treatments. The first phase of the project was accomplished in 2019, and the Luxembourg National Research Fund (FNR) recently approved the second funding period until 2023.

During its first four years, NCER-PD has established a nation-wide and deep-phenotyped cohort comprising around 1,500 participants from Luxembourg and the Greater Region, called LuxPARK. These include patients diagnosed with PD and other forms of parkinsonism and matching control subjects. This cohort ranks already amongst the 7% largest Parkinson’s cohorts worldwide. Research physicians, psychologists, and study nurses trained at the Parkinson’s Research Clinic – situated at the Centre Hospitalier de Luxembourg (CHL) and with local recruitment hubs in hospitals, nursing homes, and laboratories throughout Luxembourg – are routinely collecting detailed clinical and neuropsychological data, as well as biosamples such as blood, urine, stool, and saliva. These data and samples are then stored, curated, and integrated into state-of-the-art data and biobank facilities for further processing within the NCER-PD programme and beyond.

Aiming to determine PD biomarker signatures, LuxPARK and other high-quality cohorts’ biological samples undergo multi-omic analyses, focusing on metabolomics and genetics. Data is then processed using sophisticated computational modelling, machine learning, and network analyses to develop a mechanistic understanding of the disease.

Building on Luxembourg’s capabilities in data management of integrated clinical and molecular data, NCER-PD also develops a data hub to host and analyse PD data. The platform aims to provide a single entry point for access, curation, and analysis of PD data, including clinical, neuropsychological, and biomedical data. It serves both as the national PD research database and as an international repository for genome data.

The multi-faceted nature of NCER-PD is also reflected by the combined expertise of major stakeholders in the Luxembourgish research landscape: the LCSB, the CHL, the Luxembourg Institute of Health, the Integrated Biobank of Luxembourg, and the Laboratoire National de Santé. Also, NCER-PD is privileged to collaborate with excellent partners in the area of PD based at the Universities of Marburg (Germany), Oxford (The United Kingdom), Tübingen (Germany), and the Parkinson-Clinic in Kassel (Germany). NCER-PD is providing momentum to attract complementary PD research initiatives, such as the Michael J. Fox Foundation (MJFF), European Institute of Innovation & Technology (EIT-Health), and Genetic Epidemiology of Parkinson’s Disease Consortium (GEoPD), making Luxembourg an attractive hub for PD research. The NCER-PD consortium is looking into a fruitful second funding period for the next four years, harvesting on the data collected and exploring new avenues of biomedical research, translating findings into advanced therapies for PD.

Figure: NCER-PD is an international cross-disciplinary research initiative on PD centred in Luxembourg, combining clinical, biomedical, and computational efforts for personalised medicine.
ELIXIR Luxembourg

Head of Node: Reinhard Schneider

In research projects all over Europe, valuable data are generated but usually remain local and lack a broader use. ELIXIR, the European infrastructure for life science information, aims to provide access to this wealth of biological data as well as bioinformatics tools. ELIXIR offers the possibility to archive, integrate, analyse and exploit the large and heterogeneous data sets of modern life science research beyond the life-time of individual research projects. It has been acknowledged as one of the most strategic research infrastructures in Europe. Luxembourg-based ELIXIR implementation is supported by the Ministry of Higher Education and Research.

Luxembourg – a data hub for Translational Medicine

ELIXIR-LU, the Luxembourgish ELIXIR node, is based at the LCSB. The node focuses on long-term sustainability of tools and data for translational medicine. Translational medicine data integrate clinical information with molecular and cellular data for a better understanding of diseases. They bridge the gap between the molecular level, findings from the laboratory, and the clinical observations and applications. ELIXIR-LU facilitates long-term access to those research data. This allows the reuse of previously generated translational medicine data to address new research questions and dramatically save time and costs.

With our ELIXIR-LU node we want to overcome the major hurdles in the availability of biomedical data for a wider research community: fragmentation of data types, different terminologies and the legal challenges of sharing personal data. Through suitable data management systems that collect and integrate all types of data, support in the curation and harmonisation of clinical data and secure data hosting and analysis platforms we will open up existing translational medicine data to the research community.

ELIXIR-LU is offering the following services, available for life-science researchers as well as biomedical stakeholders in the EU and worldwide:

Secure storage and access management: GDPR compliant data sharing
Integration of well-curated clinical and molecular data from cohorts and large consortia and giving efficient access in line with security and data protection requirements of biomedical research.

Data catalogue: choose your most interesting data set!
An indexation of metadata that allows to find and select data for research projects.

Tool registry: mining the data
Platforms and tools to facilitate the analysis of translational medicine data that can be applied directly on data in the ELIXIR-LU repository or used for the analysis of own data sets.

User training: proficiency in translational medicine data
Workshops and courses on translational medicine data management, curation, analytics, and visualisation as well as reproducibility in research.

1 General Data Protection Regulation
Parkinson’s disease map

Project team: Piotr Gawron, Marek Ostaszewski, David Hoksza, Ewa Smula

Although many genetic and environmental factors contributing to the risk of PD have been identified, no unique causal mechanism is defined. Information on various aspects of PD pathogenesis is rapidly increasing and needs to be efficiently organised, so that the resulting data is available for exploration and analysis. To that end, the LCSB, in collaboration with the Systems Biology Institute in Tokyo, has developed the Parkinson’s disease map (PD map), the first freely accessible and high quality knowledge repository of molecular mechanisms contributing to the complex aetiology of PD (pdmap.uni.lu).

The PD map is a manually curated knowledge repository established to describe molecular mechanisms of PD. Cellular processes implicated in PD pathogenesis such as synaptic and mitochondrial dysfunction, impaired protein degradation, and neuroinflammation have been thoroughly integrated. The map compiles information from more than one thousand research articles into an easy to explore molecular interaction map that can be used on PC, tablets and smartphones. In addition, it offers research-facilitating tools such as the overlay of experimental data and identification of drug targets.

We envision the PD map as a hub for the PD community, constantly organising the increasing information on PD. We encourage researchers to support us to continuously refine and expand the knowledge within the PD map. Therefore, we organise regular hands-on workshops with experts. Moreover, a built-in commenting function allows any user to provide feedback to PD map moderators.

With the continuous development of dedicated tools and the curation of content with the help of the scientific community, the PD map opens new avenues in research on neurodegenerative diseases. In addition, the technology will be used as a blueprint for the generation of other disease maps.

Figure: (A) Biological overview of the cellular environment of a neuron. (B) The PD map organizes the knowledge of relevant mechanism into a hierarchical structure, from tissue to molecular details. (C) Thanks to representing the literature findings in a standardized notation, the PD map can be efficiently explored, analysed and interpreted.

Key publication


Doctoral Training Unit: Critical Transitions in Complex Systems (DTU - CriTiCS)

**Project coordinators:** Jorge Gonçalves, Alexander Skupin, **Start:** Autumn 2016

Catastrophic events occur in various fields and at various levels. Examples include earthquakes, stock market crashes and, for individuals, the onset of diseases such as cancer. If we could understand the critical transitions (CTs) that induce catastrophes, we would be better equipped to prevent them arising or at least to mitigate their effects. Yet, despite much multidisciplinary endeavour, current tools often lack rigorous theoretical foundation and sometimes exhibit poor predictive power.

The research within the Doctoral Training Unit (DTU) CriTiCS confronts this problem within a range of disciplines in the areas of clinical science, immunology, biology, and finance. Diverse approaches are employed, including data collection (from new experiments and literature), statistical analysis, mathematical modelling and theory development (critics.uni.lu).

Through synthesizing the work undertaken within each discipline, the project as a whole is designed to enable the development of a more robust, generalized, interdisciplinary theory of CTs. Such theory may be used, first, to classify CTs according to their dynamics and then to provide the foundations for:

- Identifying early warning signals to enable timely and reliable predictions of catastrophes
- Developing tools to model, analyse, and detect CTs in diverse areas of application

Ultimately, the goal of the project overall is two-fold: to support more advanced research of CTs within scientific disciplines and, in multiple fields, to improve society’s ability to anticipate CTs to undesirable states.

The CriTiCS consortium is led by the LCSB, which hosts its two coordinators and is comprised of eleven doctoral students, ten supervisors, one postdoctoral researcher, and three international external researchers. The interdisciplinarity of the project is increased by the presence of supervisors from other research units of the University of Luxembourg from the Physics and Materials Science Research Unit, the Luxembourg School of Finance and the Life Sciences Research Unit, as well as from another national partner, the Luxembourg Institute of Health. The collaboration and interaction among these partners is of great importance for the development of the project. This comes for instance from the mutual benefit from the interaction between computational/analytical partners with experimental partners having complementary approaches, but also from the interaction of partners with similar backgrounds to which the project provides interlocutors on the same ground. Regular meetings and workshops are held to enhance the interactions between the students and to allow them to present their research and to identify potential collaborations across projects.

For the students, the ambition of this project is not only to enable them to achieve more than discipline-specific expertise, but also to experience first-hand the development of integrated research that:

- Produces cross-fertilisation between disciplines,
- Synthesises empirical investigation and theoretical development, and
- Combines basic and applied scientific approaches.

This project is funded by the Luxembourg National Research Fund (FNR).
In a changing and interconnected world, the functions of microbiomes have to be better understood in view of sustaining the health of the human population. Two important healthcare challenges of our time are linked to microbiomes in different ecosystems, notably the spread of antimicrobial resistance genes and the increasing prevalence of chronic diseases. The field of microbiology is currently undergoing a revolution driven largely by a shift away from classical reductionist approaches towards the study of microorganisms directly in their native environments through advanced analytical methods. These approaches are allowing us to unravel the interdependencies between microbiomes associated with animals, the environment and humans.

The concept of “One Health” as well as our Doctoral Training Unit, MICROH, recognise that human health is connected to the health of animals and the environment. MICROH is a truly interdisciplinary doctoral training programme aimed at studying the interactions within and between microbiomes in relation to human health by using systems-level approaches. We focus on two main research areas which represent frontier research topics of immediate public health relevance: (i) the spread of antimicrobial resistance genes and their acquisition by clinically relevant microorganisms, and (ii) the role of the human microbiome in chronic diseases.

The Doctoral Training Unit started in September 2018 and will leverage the existing world-leading expertise in microbiology and systems biology in Luxembourg. The DTU links all major national research institutions active in the field of microbiology, namely the LCSB, the Life Sciences Research Unit and Physics and Materials Science Research Unit of the University of Luxembourg, the Luxembourg Institute of Health, the Luxembourg Institute of Science and Technology, the Laboratoire National de Santé as well as the private company Laboratories réunis. By regrouping individual doctoral research projects focused around the two themes, MICROH will generate important new knowledge on how to leverage system-wide big data for the management of human health in the future. We provide an excellent, interdisciplinary research and training environment hosting 17 doctoral students and 2 postdocs/project managers, all connected via 21 supervisors, from 7 national as well as 3 international partner institutions. The learning and networking opportunities of the MICROH DTU are extended through the FNR-funded Lecture Series “From Single Organisms to Systems Ecology and Evolution” in which word-leading experts in the microbiome field present their cutting-edge research to the MICROH students, postdocs, supervisors, and interested listeners. This will facilitate the education and training of the next generation of microbiome scientists with excellent skills and knowledge essential to pursuing successful career paths in the future.

This project is funded by the Luxembourg National Research Fund (FNR).
Doctoral Training Unit: Molecular, Organellar and Cellular Quality Control in Parkinson’s disease and other Neurodegenerative Diseases (DTU - PARK-QC)

Project coordinator: Jens Schwamborn Start: July 2018

Ageing is the most important risk factor for neurodegenerative diseases, such as Parkinson’s disease (PD), and linked to a progressive decline of molecular, organellar and cellular homeostasis quality control mechanisms. As for other incurable neurodegenerative diseases, mutations in genes responsible for monogenic forms of PD have been identified, that directly interfere with quality control mechanisms that affect (i) molecular quality control, e.g., via protein misfolding and aggregation or (ii) key instances of organellar quality control, e.g., via impaired mitochondrial clearance, and (iii) cellular quality control for selective elimination of damaged cells.

To define the missing link between these functional networks, in PARK-QC we will dissect mechanisms of quality control surveillance using a unique panel of advanced cellular and animal models of PD and applying novel computational modelling strategies for the identification of complex regulatory networks underlying dysfunctional quality control mechanisms.

Based on these novel experimental approaches and advanced screening platforms for in vitro and in vivo models, we will use pharmacological and genetic interventions to develop novel therapeutic approaches. Our interdisciplinary approach directly synergises with current national research programmes on PD and is envisaged to translate into novel diagnostic and treatment options for neurodegenerative diseases. The PARK-QC Doctoral Training Unit (DTU) will host 18 PhD students, all connected via 9 supervisors, from 3 national research institutions.

Since cellular transitions play an important role in this new DTU, synergies are foreseen in the research approaches with already ongoing DTUs CriTiCS and NextImmune.

This project is funded by the Luxembourg National Research Fund (FNR).
Enzymology & Metabolism group

Overview
Several observations clearly indicate that our understanding of cellular metabolic networks, and the regulation thereof, is far from being complete. A main objective of our group is to exploit genomic and post-genomic data to discover new enzyme functions relevant to human disease and ageing. Our research is currently focusing on (1) better understanding the physiological role of recently identified protein and metabolite repair mechanisms, (2) characterising poorly studied enzymes involved in rare diseases, and (3) developing strategies for accelerating enzyme function discovery. Comparative genomics-based bioinformatics tools are used for in silico predictions of gene functions. On the experimental level, we use gas or liquid chromatography and mass spectrometry-based (GC- or LC-MS) metabolomics methodologies, as well as the model organisms Saccharomyces cerevisiae and zebrafish, and human cell lines as central tools. In addition, recombinant protein expression and purification and development of enzymatic assays are important for biochemical validation and characterisation of predicted gene functions. Reverse genetic strategies are employed to study the role of enzymes of interest in the complex setting of whole cells or organisms. Yeast, zebrafish and human cell lines are also used for modelling rare metabolic diseases and performing small molecule screens to find potential drug candidates. Our research is intended to impact fields ranging from fundamental biochemistry to metabolic modelling, metabolic engineering and medicine.

Figure: Principles of enzymatic metabolite repair mechanisms.

Key projects
Role of damaged forms of NADH and NADPH in neurodegeneration
We have discovered a widely conserved enzymatic repair mechanism for hydrated, redox-inactive forms of NADH and NADPH (designated NAD(P)HX), two central cofactors of cell metabolism. With our collaborators, we have recently discovered that genetic defects in this repair system result in febrile-induced and eventually lethal forms of infantile neurodegeneration. We now aim to better understand how and under which circumstances NAD(P)HX is formed in the cell and what cellular functions are affected by the accumulation of NAD(P)HX. These studies include transcriptomics and metabolomics analyses, growth rate and cell viability determination, and mitochondrial function studies in multiple environments. In yeast, we could already demonstrate that NAD(P)HX repair deficiency leads to a blockage of the main serine synthesis pathway and we elucidated the underlying molecular mechanism. Establishment of zebrafish models and drug screens for this new infantile neurodegenerative disease are ongoing. The central roles of NAD(P)H in living cells, the conservation of the NAD(P)HX repair system across all kingdoms of life and its causal association with neurodegeneration in humans suggest that a better understanding of the NAD(P)HX repair system will reveal new fundamental aspects of cell metabolism as well as the preservation of neuronal health. This research is supported by the Luxembourg National Research Fund (FNR), the Juniclair foundation, and the Lions Club Luxembourg.

2-Hydroxyglutarate metabolism and its role in disease
D-2-hydroxyglutarate (D-2HG) and L-2-hydroxyglutarate (L-2HG) are metabolites that accumulate in several types of inherited neurometabolic diseases and certain forms of cancer, such as glioblastomas and acute myeloid leukaemia. In each of these diseases, mutations in specific dehydrogenases cause 2HG accumulation. The pathways leading to 2HG formation and the roles of 2HG in disease and aging are still unclear. We recently filled a prominent gap in yeast metabolism by discovering the enzymes responsible for the formation and degradation of D-2HG in this organism. Our results show that in yeast, D-2HG metabolism links the main serine synthesis pathway to the mitochondrial respiratory chain. Ongoing experiments are designed to find additional genes involved in D-2HG metabolism and to better understand the interplay between D-2HG and mitochondrial function as well as epigenetic regulation. This work may lead to a better understanding of the pathophysiology of D-2HG associated diseases and to the identification of molecular drug target candidates that could be tested for the treatment of certain forms of cancer and severe neurometabolic diseases. This research is supported by the FNR and the University of Luxembourg.
Comparative genomics- and metabolomics-based strategies for enzyme function discovery

In collaboration with the Bioinformatics Core, we identified more than 600 and 2000 putative enzyme genes among the about 2000 and 6500 genes of unknown function in yeast and human, respectively. Prioritising putative enzyme genes with predicted roles in metabolism and/or disease, we develop, in collaboration with the Metabolomics platform hosted by our group and with the Environmental Cheminformatics Group, targeted and non-targeted LC-MS-based approaches to compare metabolite profiles of control cells and cells with specific gene deletions. Metabolites whose levels differ significantly between these cells help to predict endogenous reactions catalysed by the enzymes of interest. Those predictions can then be validated on recombinant protein level through appropriate enzymatic assays. This approach has already allowed to identify a new eukaryotic D-ribulokinase. With this project, we aim to address a major post-genomic challenge that can only be tackled by a community-wide effort: progress from knowing the code to understanding the message by decreasing the number of genes of unknown function. This project is supported by the FNR.

Crosstalk between a protein repair methyltransferase, cell signalling, and brain function

PCMT1 is a highly conserved repair enzyme that recognises spontaneously formed isoaspartyl residues in proteins and, through methylation, facilitates their reconversion back into the normal aspartyl precursors. PCMT1 deficiency leads to accumulation of high levels of damaged proteins, especially in the brain, and massive seizures in a knockout mouse model. In addition, PCMT1 overexpression leads to a longer life span in worms and flies. Other studies suggest roles for PCMT1 also in neurodegenerative disease and cancer. In this project, we have established and phenotyped zebrafish and mouse hippocampal cell models with PCMT1 deficiency. Our studies indicate that PCMT1 plays an important role in supporting normal calcium fluxes in the brain and our models can now be used to investigate the underlying molecular mechanisms. This project was funded by the FNR.

Using the ‘awesome’ power of yeast to investigate complex traits and rare neurodegenerative diseases

Connected to the yeast platform developed in our group, we pursue several projects involving Quantitative Trait Loci (QTL) analyses. In one project, we used our experimental pipeline for high-throughput ageing phenotyping in two distinct segregating populations, obtained by crossing short- and long-lived natural Saccharomyces cerevisiae isolates. QTL analyses led to the identification of two major genes causally involved in life span regulation. In addition, we use yeast models, as well as human cell and zebrafish models, for Batten disease and Zellweger syndrome to screen for small molecules with potential disease-modifying properties. Juvenile Batten disease is the most common inherited neurodegenerative disease found in children and Zellweger syndrome is a peroxisome biogenesis disorder. Efficient treatment options for these fatal diseases are currently not available. We recently identified two drugs that were able to rescue defects observed in yeast and zebrafish models of a form of juvenile Batten disease. This work is supported by various funding sources including the ATOZ foundation, the LOSCH foundation, the Rotary Club, and private donors.

Key publications


Principal Investigator
Carole Linster
Start: January 2013

STAFF
Postdocs
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Adhish Walvekar

Scientific Staff
Zlatan Hodzic
Christian Jäger

PhD candidates
Marat Kasakin
Myrto Patraskaki

Technicians
Dean Cheung
Jean-François Conrotte
Xiangyi Dong
Floriane Vanhalle
**Integrative Cell Signalling group**

**Overview**

The general approach of the group is to combine state-of-the-art imaging and single cell techniques with mechanistic modelling and bioinformatics analyses to investigate how the emergent behaviour of cells, organs and organisms originates from molecular entities. To understand the underlying cell signalling mechanisms, we focus on live cell imaging and single cell transcriptomics analyses complemented by multi-scale simulations. Our major interests are signalling pathways related to neurodegeneration and epileptogenesis with a particular focus on mitochondrial dysfunction and dynamic coupling between intercellular energy metabolism and cell signalling such as Ca$^{2+}$ dynamics. Therefore, we use induced pluripotent stem cell (iPSC) lines, human *post mortem* brain samples, and zebrafish for phenotyping assays including sophisticated image analysis, bioinformatics and information theory-based methods to reveal the dynamic consequences of disease-related perturbations at single cell resolution.

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**Key projects**

**Dissecting cell population heterogeneity dynamics**

Cell fate commitment is the key process for understanding how a multicellular organism’s genotype gives rise to phenotypic traits including disease development and progression. To understand the role of epigenetically-induced cell heterogeneity and its importance for development and pathogenesis, we characterise population dynamics of diverse models including iPSC differentiation, human breast cancer cells and development of zebrafish brains at single cell resolution. We quantify cellular heterogeneity dynamics by combining single cell RNA sequencing approaches, microscopy and flux cytometry with advanced bioinformatics analyses. The resulting multi-scale data enables to get a comprehensive picture of cell fate dynamics and is integrated into a developmental modelling framework. The methods developed to characterise critical transitions of cell states in this project are also applied to changes of mitochondrial dynamics.

**Cellular heterogeneity in neurodegeneration**

The human brain is probably the most complex system in the universe and perturbations of its homeostasis are linked to severe diseases such as neurodegeneration and epilepsy. Besides neurons, glial cells like microglia and astrocytes play a critical role in the pathogenesis of neurodegenerative diseases. For example, a subfraction of microglia seems to lose their protective functions and become harmful to the brain. These subgroups of microglia engage maladaptive inflammatory or phagocytic responses that promote neuroinflammation and neuronal cell death and may contribute to the progression of Alzheimer’s disease (AD). To characterise this intercellular phenomenon, we image *post mortem* human brain samples from AD subjects and controls, and develop an unbiased automatic image analysis pipeline. This enables to extract a wide range of cellular features from individual cells including standard morphological characteristics to more innovative graph-based features accounting different patterns of arborisation and complexity. Comparing brain tissues from patients and controls for different brain regions, we identify alterations of the potentially harmful microglia, characterise their key phenotypic features and impact on the disease. Additionally, this methodology provides new tools for the understanding of microglia, and its involvement in neurodegenerative processes and other brain alterations.
Brain energy metabolism in neurodegeneration

Brain energy metabolism is based on the fine-tuned interplay of glycolysis and mitochondrial activity, as the energy providing pathways, and intercellular energy transfer. It represents a promising unifying perspective for neurodegenerative diseases because mitochondrial dysfunction, as a common hallmark, interrelates several known pathological processes including dopamine synthesis, oxidative stress and proteostasis. To account for the complex interplay of different cell types in brain energy metabolism, we combine high-resolution imaging and mechanistic spatial modelling of intra- and intercellular energy homeostasis. In collaboration with Mark Ellisman at the National Center of Microscopy and Imaging Research (NCMIR) at La Jolla, we use super-resolution 3D electron microscopy (EM) to image astrocytic morphologies and mitochondria to develop spatiotemporal in silico models of intracellular energy metabolism that allow us to investigate the dynamic consequences of potentially disease related morphological and molecular impairments. To elucidate the underlying dynamic mechanisms of impaired brain energy metabolism, we study mitochondrial dynamics at different levels. First, we analyse the crosstalk between Ca$^{2+}$ signalling and mitochondrial activity by live cell fluorescent imaging and dynamic modelling, and show how the coupling can increase robustness of energy homeostasis within cells. Second, we use PD-related iPSC cell lines and pharmacological perturbations together with multiscale modelling to study mitochondrial transport and its impact on mitochondrial turnover. This fission- and fusion-based processes ultimately determine the energy profiles along neurites and their functionality. Finally, we develop a microscopic in silico model of mitochondria to investigate how mitochondrial morphology is affecting energy production. This project is partially funded by the FNR.

Mechanisms of epilepsy establishment

Epilepsy is characterised by a hyperexcitability of the brain that can lead to seizures. To investigate how this hyperexcitability is established within the brain and how seizures build up, we monitor epileptogenesis in zebrafish models at single cell resolution. For this purpose, we combine EEG measurements, Ca$^{2+}$ dynamics imaging and single cell RNA sequencing experiments with machine learning algorithms and mechanistic modelling to characterise perturbation specific mechanisms of seizure dynamics and disease development. Our integrative approach identifies distinct brain cell populations for different disease causes and our advanced analysis methods allow for reliable seizure predictions. These insights may also support future translational intervention in patients.

Key publications

Interventional Neuroscience group

Overview
The Interventional Neuroscience group is a highly translational research unit that transfers innovative neurosurgical methods from bench to beside and back. Their main focus is applied computational science for clinical practice in Neurosurgery. Researchers and clinicians develop methods that use artificial intelligence and machine learning algorithms as well as tools used in image-guided procedures and biomedical modelling in Neurosurgery and Systems Biomedicine. Besides scientists and clinicians, PhD, MD and Master students are working hand in hand in highly interdisciplinary research projects. Collaboration partners are the Centre Hospitalier de Luxembourg (CHL), the Luxembourg Institute of Health (LIH) and the Laboratoire National de Santé (LNS). This allows to analyse results from clinical research on a highly scientific level and transfer those efficiently from the lab setting into the clinic and back. Innovations from research directly benefit patient care, and clinical observations can lead to new insights. The developed tools in the Interventional Neuroscience group offer highly innovative potential and are interesting for industrial partners.

Key projects

Integrated neurosurgical perioperative imaging (INSITU® study)
This study evaluates the diagnostic accuracy of state-of-the-art laser technologies, such as Raman spectroscopy (RS) and optical coherence tomography (OCT), in the evident intraoperative tumour diagnosis. Tissue specimens taken during the surgery of tumours of the nervous system (brain, spinal cord, peripheral nerves) are examined with a novel robotised device (SOLAIS System®, Synaptive, Canada). Recorded data are classified using methods of artificial intelligence/machine learning and correlated with conventional tumour diagnostic methods (histopathology, computed tomography, etc.). The aim of this project is to support a clear intraoperative tumour diagnosis, which in the future will help to perform resection control during surgery, as well as to clarify perioperatively if a tumour has been sufficiently removed or if there are still remaining parts. These new techniques may be able to replace or supplement intraoperative MRI and will help the surgeon in direct analysis of the data under the surgical microscope. Further findings will reveal new information on the tumour composition at the protein level. This could improve personalised adjunctive postsurgical treatment and could potentially close the gap between epigenetics, histopathology, imaging and biochemistry. This innovative research project, within a collaborative partnership between the National Service for Neurosurgery at the CHL, the LIH and the LNS, is funded by the Luxembourgish Cancer Foundation.

Deep brain stimulation planning and postoperative analysis with deep learning methods
Deep Brain Stimulation (DBS) is a clinically proven surgical treatment of the symptoms of Parkinson’s disease and other neurological and psychiatric diseases. Patients receiving DBS have electrodes implanted into specific brain structures containing different functional areas. The trajectory for such electrode insertion has to be a straight line through the brain, without injuring brain structures or blood vessels. The post-operative reconstruction of the DBS electrodes is important for an efficient stimulation parameter tuning. A major limitation of existing DBS approaches is that they are manual or semi-automatic, and thus both time-consuming and subjective. High quality 3D images are needed to define the best path in each patient for inserting the electrodes and to decide on a precise electrode positioning. Sophisticated device programming to reach an optimal electrical stimulation for each patient after surgery is currently lacking as well. The PaCER ® algorithm developed in our team most accurately detects the electrode position, based on postoperative CT scans. Furthermore, we work...
on algorithms for automatic trajectory planning for DBS. The aim is to develop an automatic-image processing pipeline offering access to high quality 3D images and guidance for planning and execution of this procedure. To define optimal electrical stimulation for each patient after surgery, we build real-time feedback loops to deliver best electrical stimulation, thus to design personalised feedback systems optimised for each patient.

**Wearable sensors to capture movement disorders for a feedback-controlled deep brain stimulation**

The project focus on novel evaluation, classification and treatment strategies for movement disorders. Given the dynamic nature of motor impairments and disease progression, for example in the case of Parkinson’s disease, DBS parameters are frequently adapted by the treating physician to suppress motor symptoms and to reduce stimulation-induced side effects. This procedure, however, leads inevitably to a time-consuming effort to achieve the best treatment reflecting in clinic and at home settings. Moreover, the process of stimulation programming is becoming increasingly complex with directional leads. Hence, physicians prefer objective tools to improve their clinical work. Therefore, the project aims to analyse and classify motor symptoms in movement disorders and to derive an appropriate control strategy in the clinical setting. The work is in collaboration with the Systems Control group, National Department of Neurosurgery at CHL, and Trier University of Applied Sciences in Germany. This project received funding from the FNR.

**Deep learning tools for microscopical images**

Automation of biological image analysis is essential to boost biomedical research. The recent advances in computer vision and deep learning outperformed other technologies becoming state-of-the-art. The project’s aim is to build precise and intuitive tools for pattern recognition tasks using deep learning techniques to analyse microscopy images from quality control to disease diagnosis.

**Projects in neuromodulation for pain: Pathophysiology and clinical analysis of high-frequency and pulsed radiofrequency spinal cord stimulation**

Together with the teams of Prof. Fernand Anton and Dr Marian van der Meulen from the Integrative Research Unit on Social and Individual Development at the University of Luxembourg and our partners from CHL, as well as an industrial partner, we examine the pathophysiology of high frequency spinal cord stimulation (SCS) in chronic pain patients (HF-10 SCS). Today, the exact pathophysiological mechanisms of the HF-10 therapy are not fully understood. Within our project, the HF-10 stimulation effects of patients are examined and pathophysiological conclusions will be drawn, to better understand the treatment modalities.

**EPIPULSE-projects**

Together with the Clinical and Epidemiological Investigation Center at LIH and an industrial research partner, our team at the CHL has been working out the clinical scientific base for pulsed radiofrequency stimulation of the spinal cord for the treatment of chronic pain syndromes within a multicentric retrospective (EPIPULSE-Retro®) and a prospective monocentric trial (EPIPULSE-Pro®).

**Key publications**

Neuropathology group

Overview
The Neuropathology group is a translational group linking more clinically orientated neuropathological diagnostics and research at the Laboratoire national de Santé (LNS) with basic neurodegenerative research at the LCSB, as well as neurooncological research at the Luxembourg Institute of Health (LIH) and is supported by the PEARL Excellence Programme for Research in Luxembourg of the Luxembourg National Research Fund (FNR). The overarching goal is to translate clinico-pathological findings into cell culture- and animal-based experimental laboratory models and to validate basic research findings in patient cohorts. With this approach, we aim at deciphering the pathogenesis of neurological disorders as well as translate basic laboratory findings into diagnostic and therapeutic applications.

Key projects
Neurodegeneration (Luxembourg Centre for Systems Biomedicine)
Our aim is to understand the pathological causes and processes of Parkinson’s disease (PD) with the help of mouse models. One of the advantages of mouse models is the possibility to study neurological disease in a mammalian brain that has the basic architecture and cellular composition at least partially similar to those found in the human brain.

In a first approach, PD-like disease is induced in experimental mouse models by either genetic engineering (transgenic, knockout, knockin for familial PD genes), or toxin administration (neurotoxin such as 6-Hydroxydopamine, or α-synuclein fibrils). Disease induction is followed up by longitudinal characterisation of pathological changes by behavioral, neuropathological, biochemical, and systems biological measurements.

Using these methods, two novel observations have been made:

1. The earliest pathological change detected in all models is a distinctive shift in the gene expression signature of midbrain dopaminergic neurons, notably in regulatory transcription factors unique to these neurons. This change is detected months before any visible neurodegeneration is observed. Investigations are underway to determine what causes this gene signature change, and whether it can also be found in the peripheral nervous system (PNS) and could be a biomarker candidate for early PD detection.

2. In a PD induced model that incorporates prion-like spreading of α-synuclein pathology, an exceptionally strong α-synuclein aggregation independent microgliosis was found. Current investigations are aimed at determining what causes this microgliosis and if it is a driver of neurodegeneration.

In a second approach, a mouse genetic reference population, the Collaborative Cross (CC), is used to identify novel genetic regulators of dopaminergic neuron integrity. The demise of these neurons is responsible of the PD-typical decline in motor function. A number of mutations associated with familial PD or sporadic PD risk have been identified, yet few are known that modulate specifically PD-linked decline in motor function. The Neurodegeneration project uses the CC mouse genetic reference population, composed of mouse strains that differ in their dopaminergic function and architecture, to identify novel genetic modulators of these neurons. Neuropathology, neurochemistry, molecular genetics, cell biology, and bioinformatics approaches are used to pursue these goals. Toward the end of this project, it is projected to link the findings, by working with human geneticists, to new gene candidates that govern dopaminergic neuron function in humans.

Figure: Bridging from clinical to fundamental research and back.
Neurooncology (Luxembourg Institute of Health)

Glioma cells display a high degree of genetic and epigenetic alterations that frequently lead to altered metabolism. A second important feature of glioma cells is their diffuse infiltration into residual CNS tissue. Our hypothesis is that certain metabolic reprogramming in tumor cells impacts their invasive properties. We therefore aim at deciphering the metabolic signature of diffusely infiltrating cells, compared to the tumor core and normal brain tissue by utilizing non-targeted metabolic analysis of different tumor- and brain regions of the established PDX models. Additionally, we aim at integrating metabolomics with proteomic- and epigenetic data for a better understanding of how tumor cell migration and metabolism are interconnected in glioblastoma (GBM). The outcome of this project will be the identification of novel metabolic targets to attenuate or possibly prevent the diffuse infiltration of GBM cells.

Key publications

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Start: January 2017

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Alessia Sciortino
Rédouane Slimani (LIH)
Oihane Uriarte Huarte

Technicians
Camille Cialini (LIH)
Jean-Jacques Gérardy (LNS)
Medical Translational Research group

Overview
The motto of the Medical Translational Research group is to combine research with clinical care and clinical research. Translational research is carried out in close collaboration with the Bioinformatics Core of the LCSB. The team, moreover, provides education on disease-related and general medical knowledge within the interdisciplinary environment of the LCSB. It emphasises research activities among physicians in Luxembourg and the Greater Region consisting of Luxembourg, France and Germany. While actively providing clinical care to patients, the Medical Translational Research group forms the ideal link between bench and bedside.

Many chronic diseases often share common underlying mechanisms such as inflammation, disturbed signalling or polyclonal proliferation. The interplay between chronic disease mechanisms are currently underexplored. The Medical Translational Research group is interested in exploring the nature of these common mechanistic features by employing NGS or omics methods to sample material from cell culture, mouse models and human clinical trials. Major foci of research are metabolism disorders, microbiome, cancer, inflammation and comorbidities, in addition to sequencing both familial and nonfamilial.

Key projects

Metabolism disorders
Metabolic disorders include obesity, insulin resistance, diabetes and fatty liver diseases. Several clinical and experimental projects are ongoing in collaboration with several clinical partners such as the University of Magdeburg, the Saarland University Medical Center in Homburg/Saar and the University of Lorraine in Nancy. Together with Charité Berlin, we will be investigating the effects of calorie restriction/fasting on inflammation and especially Rheumatoid arthritis.

Microbiome
We are interested in studying dynamic changes in the microbiome of healthy individuals and patients. Understanding microbiome-related consequences in health and disease opens a huge opportunity for designing personalised diagnostics and treatments. Together with collaborators at the LCSB and the hospitals we focus on microbiome-dependent changes in conditions such as malignant diseases or inflammation.

In the SMART (saliva microbiome and radiation therapy) clinical study, we are investigating the changes in the saliva microbiome of head and neck cancer patients undergoing radiation therapy. The microbiome signatures are expected to facilitate understanding the severity of side effects as well as to discover novel and individualised treatment options. In the MICROH-Liver project, using 3D liver models, we will be investigating the role of microbial metabolites in glucose and lipid homeostasis. Our microbiome research is supported by the University of Luxembourg and the Luxembourg National Research Fund (FNR).

Endocrinology
An ongoing effort relies in the detection of candidate genes for endocrine disorders, both in familial or sporadic settings. Together with the University of Wuerzburg we sequence a series of adrenal tumors and the corresponding leukocyte DNA to find the causing mutations for the onset of sporadic Cushing’s disease. Conn syndrome is another adrenal disease. It is an aldosterone-producing adenoma which may result in hypertension, muscle cramps and weaknesses as well as chronic headaches. With our partners from Alicante and Munich, we have identified a family with a suspected inherited genetic cause of Conn syndrome. We have sequenced the family to investigate the genetic causes. We are validating the results with a CRISPR/Cas gene-edited in vitro model. The results of the project will help identify modifiers of the disease and discover new drug targets in aldosterone regulation.
Inflammation and signalling

Inflammatory vascular disease such as atherosclerosis or vascular inflammation affect many subjects of the world population. Since vascular diseases represents a lipid-driven inflammation the pathophysiology requires a wiring of metabolism and immune system. IRG1 is a macrophage specific gene characteristic for immune system activation and is induced by bacterial stimuli as well as oxLDL. The IRG-1 dependent production of the substrate itaconic acid (ITA) directly interferes with cellular tricarboxylic cycle (TCA) metabolism. IRG-1 also mediates the production of reactive oxygen species (ROS) in macrophages. These data suggest that IRG-1 links innate immune activation with metabolism. We aim to characterise the role if IRG-1 in inflammatory vascular disease in vivo and in vitro. This project aims at defining a novel player as potential target mechanism for inflammatory processes in the vasculature. Another molecule of interest is β3 integrin, which is shown to be involved in various pathophysiologic processes such as cancer/neoangiogenesis, platelet aggregation, inflammation, bone resorption, and vascular disease. We further elucidate the role of β3 integrin in a cell-specific fashion using Cre/Lox technology in vivo, in conditional β3 deletion in vitro models and in silico utilising high-throughput data acquisition followed by modelling of the signalling pathway based on the experimental data. This research has received funding from the European Union’s Seventh Framework Programme.

Comorbidities

In collaboration with Charité Berlin, providing a huge cohort of tinnitus and comorbid disorders, we are investigating the epigenome and metabolome of these patients to discover mechanistic causative factors to stratify the study population. As preparatory work, we will be collaborating with the Life Sciences Research Unit, the Luxembourg Institute of Health and the Integrated Biobank of Luxembourg for the development of analytic methods. In another study, PERMENTI, we are investigating the psychological effects, especially depression, caused by inflammatory bowel disease (IBD). Together with the Centre Hospitalier Emile Mayrich in Esch-sur-Alzette, we will investigate the effects of immunological factors in IBD patients on the mental well-being of the patients.

Shaping the biomedical landscape in Luxembourg and beyond

In addition to the research projects described above, Prof. Jochen Schneider also holds various functions impacting the biomedical landscape in Luxembourg and beyond by:

- Serving as deputy director for the first year of medical education of students at the University of Luxembourg
- Being a member of the Comité National d’Ethique de Recherche (CNER), Luxembourg
- Being a member of the Animal Experimentation Ethics Committee (AEEC)

Key publications

The Molecular & Functional Neurobiology Group employs molecular, ‘omics’ and single-cell approaches to decipher the genetic and non-genetic origins of Parkinson’s disease (PD). We explore the role of the mitochondrial genome in the pathogenesis of idiopathic PD (IPD) using postmortem and iPSC-derived tissues. We aim to identify novel cellular pathways which may be the targets for therapeutic intervention in the future. With regards to genetic PD, we are targeting cellular factors that are associated with penetrance i.e. that determine the likelihood that an individual carrying a certain gene variant also develops the disease. These biomarkers may either serve to indicate advanced disease progression or they may, ideally, also direct us to cellular processes that can be modulated by drugs to delay the onset of PD.

Key projects

The role of the mitochondrial genome in idiopathic Parkinson’s disease (IPD)

One of the cellular hallmarks of PD is mitochondrial dysfunction due to respiratory chain complex deficiencies and, hence, disturbance of the neuronal energy metabolism in the dopaminergic neurons of the substantia nigra (SN). However, the molecular pathways involved in respiratory failure and subsequent neurodegeneration remain unknown. In IPD, neurohistological studies have linked respiratory chain dysfunction to somatic alterations in the mitochondrial DNA (mtDNA).

In collaboration with researchers from the Newcastle University, we identified impaired mito-nuclear signalling as the cause of mtDNA disintegration in IPD. To study this phenomenon in a longitudinal fashion, we performed an in-depth mitochondrial characterisation of seven IPD cases and four age-matched controls investigating parameters such as (i) mtDNA integrity, (ii) mtDNA maintenance, (iii) respiratory chain function, (iv) mitochondrial morphology, and (v) mitophagy in 2D neuronal cultures. In collaboration with Prof Jens Schwamborn, we additionally assessed (vi) cell viability, (vii) neuronal development, (viii) neuronal function, and (ix) astrocyte function in midbrain organoids. Applying machine-learning approaches, we are currently analysing our manifold data to determine, which parameters have the highest capacity to discriminate between IPD patients and aged controls. Cluster analyses of our preliminary results from 2D and 3D cell cultures highlight phenotypic similarities between a subgroup of IPD patients in both data sets. Moreover, an initial principal component analysis of the mitochondrial phenotyping data identified PGC-1α as key driver of mitochondrial dysfunction in IPD. As the master regulator of mitochondrial biogenesis, PGC-1α can enter the mitochondria to trigger mtDNA transcription. This finding supports our key hypothesis and strengthens the relevance of mitochondria-nuclear crosstalk in the pathogenesis of IPD. This project is funded through the ATTRACT programme of the Luxembourg National Research Fund (FNR).

Exploring the involvement of PD-associated protein Parkin in mtDNA maintenance, replication, and transcription

Mutations in Parkin explain up to three quarters of early onset familial PD cases. Regarding the protein’s function, there is strong evidence that Parkin is involved in the clearance of depolarised mitochondria. Interestingly, recent work in neuronal cell lines and an mtDNA “mutator” mouse model suggested a direct interaction of the PD-associated protein Parkin with the mitochondrial transcription factor A (TFAM), which was shown to package the mitochondrial genome in nucleoids and which we found to be depleted in IPD SN neurons. Accordingly, we hypothesise that Parkin has the capacity to protect the mtDNA through its interaction with TFAM and that this function is impaired in PD patient neurons lacking Parkin. Assessing mtDNA integrity in iPSC-derived neurons from four Parkin-mutant patients and matched controls, we observed less mtDNA-associated 7S DNA, which is required during mtDNA transcription initiation, and a reduction in mtDNA gene expression. Sc-mtDNA analysis in post mortem SN tissue confirmed lower 7S DNA levels in dopaminergic neurons from a PD patient with Parkin mutations. Next, we will investigate the capacity of wildtype Parkin to rescue mtDNA-associated phenotypes in iPSC-derived neurons with an error-prone mtDNA polymerase γ. To further explore the cellular pathways involved in mtDNA...
disintegration in PD, we optimised a protocol for nuclei isolation from post mortem midbrain tissue to perform single-neuron RNAseq with 10X Genomics technology. First trials in control tissue have been successful, warranting the inclusion of rare brain tissue from Parkin- and IPD patients. The study will elucidate a novel and potentially more physiologically relevant role of Parkin in the pathogenesis of genetic PD.

Mitochondrial DNA as a trigger of neuroinflammation in PD
Recent studies suggest that mitochondria are key regulators of neuroinflammation in PD. Particularly, the release of mitochondrial DAMPs (damage-associated molecular patterns), which might include mitochondrial-derived ROS, proteins, lipids, and mtDNA, have been shown to elicit innate immune responses. In a PD context, the release of these mitochondrial DAMPs seems to be secondary to mtDNA instability or mitochondrial dysfunction. However, the signalling cascade involved in this inflammatory process and the extension to which it contributes to cell death and disease propagation in PD, is unclear. To answer these questions, we are studying mtDNA dynamics and stability in familial and idiopathic PD models, including dopaminergic neurons differentiated from patient-derived iPSCs and genetically modified neuronal cell lines, in the framework of the DTU - CriTiCS (page 23).

Markers and mechanisms of reduced penetrance in LRRK2 mutation carriers of PD
Although individuals may harbour an identical mutation for instance in the LRRK2 gene, their disease manifestation and age at onset may vary considerably. So far, only few factors have emerged, which impact on disease penetrance or constitute signs of advanced progression in LRRK2-PD. By contrast, in fibroblasts from manifesting and non-manifesting carriers of the G2019S mutation in LRRK2, we recently showed a correlation between mtDNA deletions and disease status. Interestingly, an increase in LRRK2 auto-phosphorylation and reduced levels of urate were previously identified as penetrance markers in LRRK2-associated PD. We now speculate that, in manifesting G2019S mutation carriers, increased LRRK2 kinase activity interferes with urate-sensitive NRF2 antioxidant signalling, which in turn (i) promotes mtDNA damage (by reducing the expression of TFAM), (ii) mediates ccf-mtDNA release, and eventually (iii) triggers pro-inflammatory signalling. We are currently exploring this hypothesis in patient iPSC-derived neurons and microglia. This project is part of the Research Unit “ProtectMove” (www.protect-move.de), which is co-funded by the FNR and the DFG.

mtDNA epigenetic analysis in PD
There is currently no consensus in the literature about the existence and disease relevance of mtDNA methylation. To conclusively clarify this research question for PD, together with Patrick May (LCSB) and Lasse Sinkkonen (LSRU), we are assessing the abundance of 5-methylcytosine and 5-hydroxymethylcytosine in peripheral blood monocytes from IPD patients and matched controls using latest NGS technology. Applying Nanopore sequencing to native (non-amplified) mtDNA, an unprecedented resolution of methylation patterns can be achieved. In mtDNA, extracted from control monocytes, we detected only five CpG islands with relevant levels of methylation. Moreover, using our novel approach, we could confirm an inverse correlation between methylation detection and mtDNA sequencing depth. To validate our initial findings, we will repeat the analysis in additional control monocytes before moving on to blood samples from IPD patients.

Key publications
Systems Control group

Overview
The Systems Control Group collaborates with biologists and clinicians to improve our understanding of diseases, with the goal to develop new therapies. The group produces theoretical and computational methods to achieve the goal of identifying how diseases arise and how they are mitigated. A key part is to build mathematical models that help pinpoint the locations and underlying mechanisms of diseases. The models capture an important feature of biological systems: the fact that they change over time in response to internal or external stimuli. Hence, the group focuses mostly on time-series data. Time-series data consist of snapshots of system properties at different time points, such as molecular concentrations (e.g. RNA, proteins or metabolites) or clinical data (e.g. ECGs or blood tests). While time-series data is more expensive to produce than steady-state data, tracing a system’s transient behaviour provides essential information on how different species interact with each other. With diverse backgrounds in mathematics, computational sciences, biology and medicine, members of the group engage with this overall problem from different perspectives. The group collaborates closely with biologists and clinicians to develop mathematical models with key predictive powers that are subsequently experimentally validated. This ensures that the group’s research has broad-ranging applications within biology and biomedicine, including Parkinson’s disease, deep brain stimulation, heart attacks and arrhythmias, mechanisms of circadian rhythms and the immune system.

Key projects

Real-time prediction of heart attacks and arrhythmias
The aim of this project is to detect and predict heart arrhythmias before they occur. Heart attacks and arrhythmias typically happen without noticeable warning. Predicting heart arrhythmias, such as atrial fibrillation, in real-time and with enough anticipation allows patients to seek immediate medical attention and prevent potential health complications. This project searches for subtle changes in heart dynamics, captured via ECG or PPG (via a smartwatch, for example), using state-of-the-art machine learning tools. The project is funded by the Luxembourg National Research Fund (FNR).

Network inference
The aim of this project is to understand how networks of genes and other molecules interact over time to produce disease-related phenotypic behaviours. Constructing reliable models of these regulatory networks allows us to simulate hypotheses that can be used to guide experimental testing, thus accelerating our understanding of how diseases function. Networks are inferred from time-series data. This also contributes to broader theoretical research into mathematical, algorithmic and software tools for understanding causal dynamic network structures. These tools have been applied to understand the mechanisms of action of circadian clocks and the immune system. The project aims to identify changes in biological network structures that occur when a system is perturbed in responses to stimulation, pharmacological intervention or genetic mutations. This project involves a collaboration with the Department of Plant Sciences at the University of Cambridge.

Critical transitions
Catastrophic events (i.e. sudden changes that affect systems stability) occur in various fields and at various levels. Examples include earthquakes, stock market crashes, and population extinction; for individuals, it is hypothesised that the onset of diseases such as cancer follows similar patterns. If we understood the critical transitions (CTs) that induce catastrophes, we would be better equipped to prevent them arising or at least to mitigate their effects. The proposed research confronts...
this problem within a range of disciplines in the areas of clinical science, immunology, biology, and finance. It aims to classify CTs according to their general dynamical features and then to provide the foundations for: a) identifying early warning signals to enable more timely, reliable predictions of catastrophes; b) developing tools to model, analyse, and detect CTs in diverse areas of application. Ultimately, the overall goal of the project is two-fold: to support more advanced research of CTs within scientific disciplines; and, in multiple fields, to improve society’s ability to anticipate CTs to undesirable states. The project entails eleven doctoral students and ten supervisors and it is funded by the FNR (see also DTU-CriTiCS, page 23).

Next generation of deep brain stimulation (DBS)
Deep Brain Stimulation (DBS) is a surgical therapy for several movement disorders (e.g. Parkinson’s disease (PD) and Essential tremor) and psychiatric diseases. During this procedure, an electrode is implanted into the brain, constantly delivering electrical pulses to specific regions of the brain. This project aims to considerably improve the efficiency of DBS while reducing side effects. In particular, it develops algorithms to deliver personalised stimulations that adapt to the patient’s needs, and computational tools to aid physicians initiate DBS parameters. This interdisciplinary project involves collaborations with the Centre Hospitalier de Luxembourg (CHL) and the University of Oxford. This project is funded by the FNR.

Predicting the progression of PD
The clinical presentation and rate of progression of PD vary considerably among patients. Our understanding of this heterogeneity is still very limited, preventing clinicians from making accurate predictions. This project pools longitudinal time-series data from seven PD cohorts in Europe and the US. The large quantity of data is unprecedented in this context. It allows us to build and evaluate advanced models of disease progression using state-of-the-art techniques from statistics and machine learning. We are particularly interested in clustering as a mean to redefine PD subtypes, which will then allow a more personalised diagnosis and prognosis. Future work will apply this methodology to other diseases, such as Alzheimer’s. This work is funded by the University of Luxembourg and it is a collaborative effort between the University of Cambridge, Newcastle University, the Academic Medical Center in Amsterdam and the LCSB.

Predicting financial crashes
The project aims to identify hidden dynamics in financial systems and test potential early warning signals for critical transitions represented by mini-flash crashes in equity markets. Financial markets are known for their complexity due to the presence of many different types of agents whose actions belong to a very diverse set of strategies. Their collective behaviour in limit order markets results in time series of transaction prices for a particular security. The first part of the project studied causal effect of latency delays on occurrence of mini-flash crashes. This latency delay slows down the trading while reducing price impact, and results in decreased number of mini-flash crashes. The project aims at explicit descriptions of welfare gains due to usage of different order types, taking into account the microstructure of financial markets. The project is funded by the FNR.

Key publications
INFRASTRUCTURE

Cutting across disciplines in systems biomedicine, the LCSB integrates different technologies, models and expertise, from mathematical theory to practical medical requirements of the clinic. This allows the centre to adapt in the best possible way to the challenges of modern biomedical research. The goal of the LCSB is to establish and provide access to important infrastructure that supports the key research lines of the LCSB.

Larger infrastructure is established as ‘central platforms’ to make it easily accessible across all research groups, and to offer fee based services at national and international level. Besides offering customised services and method development, central platforms organise training workshops on new technologies, offer consultation for experimental design, data processing and troubleshooting. So far, the LCSB has established the following central platforms:

Aquatic Facility
The Aquatic Platform at the LCSB has been established as a breeder, supplier and user of zebrafish (*Danio rerio*). This teleost fish is a vertebrate organism widely used to study developmental biology and to model various human diseases. The Aquatic Platform consists of a modern, semi-automated facility that provides husbandry and embryo production services to support biomedical research and teaching activities. Currently, the facility can house up to 30,000 adult zebrafish, offering robotic feeding, maintenance of constant micro-environmental conditions (e.g. pH, temperature and conductivity), and semi-automated cleaning systems that ensure excellent conditions for research quality and for animal welfare. Among its services, the Aquatic Platform offers the generation of zebrafish disease models using morpholino antisense technology and CRISPR/Cas9 mutagenesis, as well as, pharmacological models and reporter lines using Tol2 transgenesis. In addition, the platform supports research activities by offering the possibility to perform diverse type of small-molecule screens, as well as toxicity screens for drug safety assessment and for eco-toxicological studies.

Rodent Facility
The rodent facility has been established to assist researchers in the development and analysis of various *in vivo* models including humanised and germ-free animals. The facility has the capacity to house more than 100 different mouse lines in 1,500 IVC cages in the Breeding barrier under SOPF status. The Experimental barrier with a capacity for 600 mouse and 300 rat cages, is dedicated to studies on transgenic rodents and offers as well the possibility to realise BSL-2 activities and housing xenograft models. Genetically modified rodent lines can be generated by our Transgenic Core Unit and kept either as live animals or as cryopreserved sperm. The facility offers the possibility of running a broad number of experimental procedures on the premises, including stereotoxic surgical procedures and motor behavior equipment as the Catwalk. Additionally, humanised mouse models can be provided within an SPF environment. The Germ-free Unit is established within the Breeding barrier to ensure a controlled health status. For colonization studies, germ-free mice can be inoculated with a suspension of a specific microbe (mono-colonisation), a defined finite group of microbes, or a polymicrobial mixture. This unit also employs a Sealed Positive Pressure IVC system to achieve the type of germ-controlled environment.

Sequencing Platform
The sequencing platform offers next-generation sequencing services to the University of Luxembourg researchers, as well as outside users using a combination of a NextSeq500 and a MiSeq sequencer from Illumina and a MinION nanopore sequencer from Oxford Nanopore Technologies, to address broad sequencing possibilities. The platform offers full service, starting with advice in experimental design up to sequencing data generation. Receiving RNA or DNA samples, sample quality check, library preparation, library quality control, sequencing run, raw data generation and preliminary data quality control report is performed. Last year, the platform performed 56 Illumina sequencing runs (processed 388 RNA and 215 DNA samples) and generated a combined ~3.13 Tbp of sequencing data, and supported 14 research groups. General applications of these projects included RNA sequencing, metatranscriptomics, single cell RNA sequencing, metagenomics, ChiP sequencing, DNasel sequencing, ATAC sequencing and targeted gene region sequencing. In 2018, the sequencing platform also acquired an automated library preparation robot, Biomek 4000, to further expedite the sequencing services turnaround time.
Metabolomics and Mass Spectrometry Platform
The Metabolomics Platform offers both GC-MS and LC-MS services, access to equipment for sample preparation and a comprehensive set of tools for reliable data analysis based on a statistical evaluation. Moreover, to address the needs in this fast changing field, the Metabolomics Platform continuously develops new analytical and tailormade methods to address upcoming biological and biomedical questions. This includes targeted and non-targeted GC-MS as well as LC-MS analyses, semi-quantification and absolute quantification of metabolites extracted from several sample types, including mammalian cells, bacteria, yeast, body fluids and soft tissues.

The platform hosts three cutting-edge LC-MS systems (Agilent 6560 Ion Mobility Q-TOF, Thermo Q Exactive, Sciex Q-Trap) and two Agilent GC-MS served by multifunctional sample preparation robots. Using these state-of-the-art technologies and standardised laboratory protocols, the Metabolomics Platform strives to offer high quality mass spectrometry-based measurements of a wide range of small molecules in biologically relevant samples.

Bioinformatics Platform
The LCSB Bioinformatics Core offers large data storage as well as bioinformatics expertise, which includes state-of-the-art workflows for data capture and data analysis using OpenStack based private cloud as well as high-performance computing (HPC) cluster of the University. In 2016, the core facility group has been made the Luxembourgish Node of the European bioinformatics infrastructure ELIXIR and serves as an international data hub for clinical and translational medicine data (see Flagship projects page 21). The bioinformatics platform further assists in the curation of existing clinical data for computational analysis, introducing international standards and performing quality control of entries. Support is given for the development of electronic data capture based on controlled vocabulary in CDISC standards and their storage in suitable tools for analysis such as tranSMART. A powerful genome analysis pipeline interprets data from exome and whole genome data while other bioinformatics tools have been developed to interpret differentially expressed genes within pathways and networks. The Bioinformatics Core further develops advanced dynamic visual analysis workflows and libraries (e.g. Fractalis) based on machine learning approaches and tools (e.g. ADA) for the integration of heterogeneous data across different data types and disciplines. Visualisation is supported by virtual and augmented reality, as well as disease map approaches, such as the PD map (see Flagship projects). Easy tools provided by the Bioinformatics team allow researchers to set up and fill their own disease maps.

Bioimaging Platform (bip)
The imaging platform offers a broad palette of microscopy systems: a laser scanning confocal microscope (Zeiss LSM 710) for high-resolution imaging of fixed samples, a spinning-disk confocal microscope (Zeiss Cell Observer) and an inverted epifluorescence microscope (Nikon Eclipse Ti-E) for prolonged live-cell imaging, a selective plane illumination microscope (SPIM) for low phototoxicity whole organ and organism imaging, as well as a high-content screening system (Perkin Elmer Opera). In addition, two super-resolution light microscopy approaches were recently established: a 3d STED microscope (Leica SP8 with FLIM capability) and the Rescan module (confocal.nl).

Technologies are used for the study of fixed and living cells and tissues with genetic or pharmacological perturbations, followed by sophisticated image analysis of cell morphology, topology and cellular dynamics. Furthermore, the facility comprises a flow cytometry branch with a cell sorter (BD FACS_AriaII) and high-speed analyser (BD LSRFortessa) allowing to quantify large cell populations at single cell resolution and to perform a variety of high-throughput assays and phenotypic selections. User trainings take place throughout the year through practical courses, seminars and workshops. Recently, a Scanning Electron Microscope (Zeiss Sigma 500) was built up in cooperation with the Laboratoire national de santé (LNS).

Further LCSB infrastructure is available through collaborative group platforms who also offer services. Details can be found on our webpage: https://www.uni.lu/lcsb/services


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