Francesca Aguilo Payeras

Wallenberg Centre for Molecular Medicine, Umeå University

Associate Senior Lecturer at the Department of Medical Biosciences

francesca.aguilo@umu.se

https://www.umu.se/wallenberg-centrum-for-molekylar-medicin/

forskning/fellows/francesca-aguilo/

Area of interest

Epigenetic and RNA-based gene regulation of cell fate and breast cancer

Our main interest is to decipher novel epigenetic and epitranscriptomic mechanisms affecting global gene expression and their implication in cell fate and cancer initiation and progression with a focus on breast cancer.

RNA is not only an essential intermediate in the flux of genetic information from DNA to proteins, but rather is a molecule that plays crucial roles in the regulation of fundamental cellular processes, being the dysregulation of certain RNAs implicated in a number of important pathological processes, including cancer. The coding and non-coding transcriptome is extensively and dynamically regulated by chemical modification adding a new layer of complexity and functionality to the emerging roles of RNAs in health and disease. The impact of these modifications has recently begun to be explored within a new field of study: ‘Epitranscriptomics’. We believe that, by understanding the role of RNA modifications in physiology and pathology, novel and powerful disease biomarkers and drug targets could be identified.

The Aguilo’s lab is part of different networks, including *COST Epitran* at the European level, and EpiCoN at UmU, to enhance local and international collaborations. We also participate in the *RNA Society of Sweden* in order to increase the communication between scientist and students in the field of RNA biology in Sweden.

Strengths in lab

We combine classical biochemical methods with state-of-the-art genome-wide sequencing and proteomic techniques to interrogate the role of RNAs modifications and the interplay with other epigenetic marks in stem and cancer cells. Different types of cell culture, including stem cells, iPSC, and 3D cell culture models, patient samples, as well as mouse orthotropic transplantation are used in our studies.

Abstract

Chemical modifications of RNA provide a direct and rapid way to modulate the existing transcriptome, allowing the cells to adapt rapidly to the changing environment. Among these modifications, *N6*-methyladenosine (m6A) has recently emerged as a widely prevalent mark of messenger RNA in eukaryotes, linking external stimuli to an intricate network of transcriptional, post-transcriptional and translational processes. m6A modification virtually affects all aspects of RNA metabolism, including mRNA decay, translation and splicing. Both m6A modification and the enzymes that control m6A metabolism are essential for normal development. Recently, aberrant m6A deposition has been associated with multiple types of cancer, including glioblastoma, lung and breast cancer. Depending on the type of cancer, the different regulators of the m6A mark can serve as either oncogenic or tumor-supressor. For instance, in breast cancer the expression of the methyltransferase METTL3 is significantly upregulated, suggesting that high level of m6A modification associate with the activation of prooncogenic signals that result in cancer progression.

Marta Bally

Wallenberg Centre for Molecular Medicine, Umeå University

Associate Senior Lecturer at the Department of Clinical Microbiology

marta.bally@umu.se

https://www.umu.se/en/staff/marta-bally/?expandaccordion=b

Area of interest

Virus-membrane interactions

Marta Bally’s interdisciplinary approach bridges medical sciences, engineering, and physics to study the mechanisms governing the interactions between cell surfaces and biological nanoparticles (e.g., viruses, drug delivery vehicles, and extracellular vesicles).

With a background in engineering and a fascination for fundamental biology, I strive to take advantage of my expertise in the development of bioanalytical assays as well as enthusiasm for translational research to investigate the mechanisms by which biological nanoparticles interact with the cell surface. To address such questions, my group adopts a multidisciplinary approach based on the use of in vitro cell-surface mimics of various complexities and on live-cell microscopy. By combining platforms which span the spectrum, in terms of control and complexity, our aim is to elucidate interaction mechanisms occurring at the cell membrane that have failed to be understood previously.

My current main research focus is centered on elucidating the interactions between viruses and the cell surface; in particular, I study the mechanisms modulating binding and release of Herpes Simplex Viruses from cell-surface carbohydrates. Other activities include the design of new liposome-based vaccine vectors, the development of bioanalytical assays to detect, sort, and characterize biological nanoparticles, as well as the development of platforms for testing anti-viral drugs.

Strengths in lab

As a complement to traditional cell studies used in the field of virology, our group develops surface-based assays in combination with advanced microscopy techniques. We work with minimal models of the cell membrane (cell-membrane mimics), to study processes occurring at the cell surface in a highly controlled manner. Cell-surface mimics are model systems whose composition can be fine-tuned to study specific interactions occurring at the cell surface with great precision and accessibility by many surface-sensitive analytical techniques. Using total internal reflection fluorescence (TIRF) microscopy, we analyze the binding kinetics and diffusion behavior of virus particles at the single particle level.

As a complement to cell-membrane mimics, we plan to add single particle tracking in live-cell microscopy experiments to our assay portfolio. Live-cell microscopy allows for interactions to be investigated within the complex milieu of natural components and provide physiological feedback on interactions taking place at the cell surface.

We also have a track-record in studying biomolecular interactions with surface-based analytical methods such as the Quartz Crystal Microbalance and Surface Plasmon Resonance.

Finally, we develop and implement methods to characterize biological nanoparticles at the single particle level in order to study heterogeneities in virus or extracellular vesicle populations. In this context, we have recently developed a microfluidic tool working as a nano- flow cytometer which allows for the fluorescence based detection, quantification, and characterization of biological nanoparticles.

Abstract

A number of enveloped viruses, including herpes simplex viruses attach to susceptible host cells via interaction between their glycoproteins and cell-surface glycosaminoglycans (GAGs). This initial recognition needs to be tightly regulated to ensure virus diffusion through the GAG-rich glycocalyx, attachment to the cell membrane for virus internalization and release of the virus upon egress.

In our work, we study the molecular and physical mechanisms modulating HSV binding and release from cell surface GAGs. Our approach relies in the first place on minimal models of the cell surface consisting of substrate immobilization of GAG chains. These in vitro cell-surface models make it possible to study the details of virus-GAG interactions. TIRF microscopy allows for the quantitative analysis of affinities and diffusion coefficients of GAG -bound viruses on a single virus level. AFM-based force spectroscopy is used to quantify virus-GAG interaction forces. These biophysical experiments are further complemented with single particle tracking on live cell microscopy to study viral entry.

With our research, we gain insight into the modulatory function of protein glycosylation and interrogate the role of GAG sulfation in the process: We show that mucin-like regions found on the glycoproteins of HSV play an important role in modulating the interaction, an observation further supported by cell experiments. We further show that the diffusion of virions on the surface depends on the type of GAGs and their degree of sulfation. Taken together, our research contributes to a better understanding of the mechanisms underlying the interaction between a virus and the surface of its host. Such insights will without doubt facilitate the design of more efficient antiviral drugs or vaccines.

Staffan Berglund

Wallenberg Centre for Molecular Medicine, Umeå University

Senior Consultant in Pediatrics, University Hospital of Umeå

Researcher at the Department of Clinical Sciences

staffan.berglund@umu.se

https://www.umu.se/institutionen-for-klinisk-vetenskap/forskning/

pediatrik/

Area of interest

A newborn child will double its weight in less than six months and the key to a successful development is a well-balanced nutrition. Exploring this early nutrition and how it impacts on child health is our area of interest. The challenge is to identify relevant nutrients and their best biomarkers, but also to identify clinically relevant measures of health and development.

In our group, we focus on two important micronutrients; iron and lactoferrin. Iron is an essential element for most tissues and it plays an important role in a newborn child. Not only is iron necessary for the oxygen transport molecule hemoglobin, it is also essential for brain development where it is important in cell migration, neurotransmitter synthesis and myelination. We assess the need of iron in different stages of infancy and in different settings using clinical studies. To optimize this research, we also need to know more about the physiological mechanisms that are involved in iron homeostasis. Therefore, we are currently investigating several important biomarkers such as ferritin, hepcidin and erythroferron and their role in the newborn infant.

Lactoferrin is one of the most common proteins in breast milk and believed to play an important immunological role in infancy through different bioactive mechanisms. This protein may be one important reason for the reduced risk of infections that is associated with breast feeding, and we aim to learn more of its role in vivo. In a large randomized trial LIME, we are currently exploring the effect of adding bovine lactoferrin to infant formula.

A novel clinical trial for improved infant formula

**Background:**

Breast milk is the gold standard of early infant nutrition and breastfed infants have advantages in several developmental outcomes compared to those formula-fed. We hypothesized that two reasons for these difference are that infant formula have lower content of the bioactive protein lactoferrin and higher levels of iron.

**Method:**

In the randomized controlled trial LIME, we included 180 healthy formula fed infants with normal birthweight (2500-4500g). The infants were randomized to three different infant formulas given between 6 weeks and 6 months of age: Experimental formula 1 (n=72) containing 2 mg/L of iron and 1 g/L of bovine lactoferrin, experimental formula 2 (n=72) containing 2 mg/L of iron and no added bovine lactoferrin, and a controls formula (n=36) containing 8 mg/L of iron and no lactoferrin. Another 72 breast fed infants were included as a reference group. At 4, 6, and 12 months of age, we plan to assess anthropometrics, infections, gastrointestinal symptoms, iron status, C-reactive protein, cytokines, (interleukin 2, TNF alpha, TGF beta), vaccine antibodies, microbiota, metabolomics and Bayley test of neurodevelopment (only 12 months).

**Results:**

176 (98%) formula fed and 70 (97%) breast fed infants completed the intervention phase and were assessed at 6 months of age. Blood was drawn from 165 (92%) and 67 (93%) infants respectively.

The LIME study successfully fulfilled the intervention phase with uniquely low dropout rate. The un-blinded analyses will start in May 2019.

Ronnie Berntsson

Wallenberg Centre for Molecular Medicine, Umeå University

Associate Senior Lecturer at the Department of Medical Biochemistry

and Biophysics

ronnie.berntsson@umu.se

https://www.biostruct.umu.se/principal-investigators/ronnieberntsson/

Area of interest

My lab is focused on understanding the underlying molecular structure and function of Type 4 Secretion Systems (T4SS). This will lead to a deeper insight into one of the main processes responsible for horizontal gene transfer events, including the spread of antibiotic resistance genes, in bacteria.

The proteins involved in forming the T4SS are be studied biochemically, structurally and biophysically. While the T4SSs of gram-negative bacteria are fairly well understood, gram-positive T4SSs are not. Since gram-positive T4SSs are very dissimilar from their gram-negative counterparts, little can be deduced from the few gram-negative systems so far studied. Furthermore, they occur in a number of pathogens, such as enterococci, streptococci and staphylococci. Another aspect that makes gram-positive T4SSs interesting is that they are used to efficiently transfer not only antibiotic resistance, but also virulence factors. This makes them attractive targets for the development of novel anti-virulence drugs; deactivation of T4SSs would lead to attenuation of the pathogen followed by easier clearance of an infection by the host immune system.

We are specifically working with a couple of model conjugative plasmids and their T4SSs, trying to determine the structures of the proteins involved and understanding how the initial cell adhesion and DNA processing works.

**Strengths in lab**

Main methods used in the lab are recombinant (membrane) protein overproduction and purification followed by biochemical and biophysical assays like EMSAs, pull-down experiments and ITC. The proteins are then structurally determined using mainly protein crystallography but also cryo electron microscopy.

PrgB promotes Aggregation, Biofilm Formation and Conjugation through DNA binding and Compaction

Andreas Schmitt1, Kai Jiang2, Martha I. Camacho3, Venkateswara Rao Jonna1, Anders Hofer1, Fredrik Westerlund2, Peter J Christie3 and Ronnie P-A Berntsson1

1Department for Medical Biochemistry and Biophysics, Umeå University, Sweden   
2Department of Biology and Biological Engeneering, Chalmers University of Technology, Sweden   
3Department of Microbiology and Molecular Genetics, McGovern Medical School, USA

Gram-positive bacteria deploy type IV secretion systems to facilitate horizontal gene transfer. The T4SSs of Gram-positive bacteria rely on surface adhesins as opposed to conjugative pili to facilitate mating. *Enterococcus faecalis PrgB* is a surface adhesin that promotes mating pair formation and robust biofilm development in an extracellular DNA (eDNA) dependent manner. Here we report the structure of the adhesin domain of PrgB. The adhesin domain binds and strikingly also compacts DNA in vitro. In vivo, PrgB deleted of its adhesin domain does not support cellular aggregation, biofilm development and conjugative DNA transfer. PrgB also binds lipoteichoic acid (LTA), which competes with DNA. Our findings support a hypothetical mechanism whereby: i) PrgB forms long-range intercellular contacts through binding of eDNA filaments and through DNA condensation establishes shorter range contacts; ii) PrgB then alternatively binds LTA exposed on target cell surface to stabilize mating junctions enabling highly efficient T4SS-mediated gene transfer.

Anders Björkman

Wallenberg Centre for Molecular Medicine, Lund University

Associate Professor and Senior Consultant, Department of Translational Medicine – Hand Surgery, Lund University, and Skåne University Hospital, Malmö, Sweden.

anders.bjorkman@med.lu.se

www.med.lu.se/wcmm/researchers

Area of interest

Nerve injury, neuropathy and stroke – from the peripheral nerve to brain plasticity

All manual daily activities such as turning a key or gripping a glass are based on interactions between the peripheral and central nervous system. The complexity of this interaction becomes obvious when an individual suffers a nerve injury, neuropathy, stroke or in amputees who use a hand prosthesis. Even if state of the art treatment, including microsurgery, is used the outcome following nerve injury, neuropathy and stroke is often a severe impairment in hand function as well as decreased ability to perform activities of daily living and possibility to work. Hand amputees most often use a hand prosthesis however, modern day hand prosthesis lack sensory feedback and they are difficult to control and only allow very simple grips.

This project has three aims: 1/ mapping cerebral plasticity, 2/ development of treatment strategies using guided plasticity for patients with traumatic nerve injury, neuropathy and stroke, and 3/ development of systems for sensory feedback and motor control of hand prosthesis.

The effects of a peripheral nerve injury, neuropathy, stroke and hand amputation are examined both in the peripheral nerve and in the brain using advanced high (3 tesla) and ultra-high (7 tesla) MRI. Results from MRI are compared with results from neurophysiological- and clinical tests. Based on results from imaging and clinical tests different treatment strategies are developed, where the dynamic capacity of the brain i.e plasticity is guided to improve hand functions.

Systems for sensory feedback in hand prosthesis are studied with focusing on creating a modality-matched feedback applicable in existing hand prosthesis. Furthermore, a new approach to the control of prosthetic hands is suggested. Control algorithms based on intramuscular EMG or high-density surface EMG will be used in order to control modern, multi-degree of freedom, prosthetic hands.

**Strengths in lab**

• Techniques for advanced clinical assessment of peripheral nerve injuries in humans

• Techniques for functional MR imaging in humans at high (3T) and ultra-high (7T) field-strength

• Techniques for advanced neurophysiological assessment of peripheral nerve function in humans

• A lab at the technical university in Lund (LTH) focused on signal and measuring techniques

Loss of inhibition in ipsilateral somatosensory areas following altered afferent nerve signaling from the hand

Anders Björkmana and Andreas Weibullb

aDepartment of Translational Medicine - Hand Surgery and  
bDepartment of Medical Radiation Physics, Lund University, Skåne University Hospital, Malmö, Sweden

**Background**

Cutaneous stimulation of the hand results in increased neural activity in the contralateral primary somatosensory cortex (S1) in humans, whereas an inhibition of neurons is seen in the ipsilateral S1. The aim of this study was to assess changes in neural activity in the S1 bilaterally, with a focus on the ipsilateral hemisphere, following altered afferent nerve signaling from the hand.

**Patients and methods**

Three cohorts, all with altered afferent nerve signaling from the hand, participated in the study. There were: 18 patients with traumatic median nerve injury, 10 patients with vibration induced neuropathy and 11 healthy subjects who had their dominant hand and wrist immobilized for 72 hours. In addition, 36 healthy subjects were included as controls. Each subject was examined using functional magnetic resonance imaging at 3 Tesla.

**Results**

All three study cohorts showed enlarged activation in the contralateral S1 during tactile stimulation compared to healthy controls. Moreover, inhibition of the ipsilateral S1 was significantly decreased or completely lost. Thus, somatosensory areas of both hemispheres respond to changed afferent nerve signaling from the hand.

**Conclusion**

The loss of inhibition of neurons in the ipsilateral S1 suggests an important role of the ipsilateral hemisphere in the cerebral adaptation following a change in afferent nerve signaling

Jeremie Boucher

Wallenberg Centre for Molecular and Translational Medicine,

University of Gothenburg

Co-opted Senior Lecturer, Institute of Medicine

Principal Scientist, Associate Director, AstraZeneca R&D

jeremie.boucher@gu.se

jeremie.boucher@astrazeneca.com

wcmtm.gu.se/research-groups/boucher

Area of interest

Area of interest: Type 2 diabetes, Obesity and NASH

Being a Principal Scientist at AstraZeneca with research time within the Wallenberg Center, my goal is to identify novel targets and pathways for the treatment of type 2 diabetes, obesity, and nonalcoholic steatohepatitis. In particular, my group is working on modulating adipose tissue phenotype for the treatment of metabolic diseases:

1) Increasing brown adipose tissue abundance or activity or converting white into brown adipocytes holds promise for the treatment of metabolic diseases. We are characterizing the molecular pathways controlling the development, differentiation and function of white, beige and brown adipose cells, and the role that BMPs and the transcription factors PPARα and γ play in those processes

2) Senescence (aging of cells) of adipose tissue is closely associated with common cardiometabolic complications including type 2 diabetes and cardio-vascular disease. We are investigating the key drivers of senescence in the different adipose tissue cells (endothelial cells, mesenchymal stem cells, adipocytes) in metabolic diseases, how senescent cells communicate with other cells and tissues to impact metabolism, and how to inhibit senescence in adipose

We have an extensive national and international collaboration network with researchers from Harvard Medical School, University of Pennsylvania, University of Virginia, University of Campinas, INSERM, the Novo Nordisk Foundation Center for Basic Metabolic Research, and the Integrated Cardio Metabolic Centre/Karolinska Institute.

Strengths in lab

My group adopts a multidisciplinary approach and has both in vitro and in vivo capabilities: the lab has developed expertise in mouse and human preadipocyte and adipocyte culture methods. It uses a combination of molecular biology techniques and functional assays such as lipolysis, glucose uptake, lipogenesis and glucose and fatty acid oxidation. Obese, insulin resistant, diabetic and NASH mouse and rat models are routinely used for in vivo studies. The metabolic status of animals is assessed by performing glucose and insulin tolerance tests, Comprehensive Lab Animal Monitoring System (CLAMS) measurements, and metabolic tracer and clamp studies.

The dual PPARα/γ-agonist tesaglitazar robustly induces browning of white fat *in vitro* and *in vivo*

Tobias Kroon1, Matthew Harms1, Daniel Nilsson5, 6, Anna Lindblom1, Peter Gennemark2, Gavin O’Mahony3, Victoria Osinski4, Coleen McNamara4 and Jeremie Boucher1, 5, 6

1Bioscience,

2DMPK,

3Medicinal Chemistry, Cardiovascular, Renal and Metabolism, IMED Biotech Unit, AstraZeneca, Gothenburg, Sweden

4Department of Medicine, Cardiovascular Research Center, University of Virginia, Charlottesville, VA, USA

5The Lundberg Laboratory for Diabetes Research, University of Gothenburg, Sweden

6Wallenberg Centre for Molecular and Translational Medicine, University of Gothenburg, Sweden

Increasing brown adipose tissue abundance or activity or converting white into brown adipocytes holds promise for the treatment of type 2 diabetes and obesity. Several PPARγ agonists, including rosiglitazone, have been shown to robustly induce browning of white adipose tissue (WAT) *in vitro* but their effects *in vivo* are less pronounced, while PPARα agonists show modest browning effects both in vitro or *in vivo*. In the present study, we investigated whether PPARα and PPARγ dual agonism would have synergistic effects in inducing browning of WAT. We found that all tested dual PPARα/γ agonists robustly increased uncoupling protein 1 (*Ucp1*) expression in both mouse and human preadipocytes, with tesaglitazar giving the largest *Ucp1* induction. Notably, tesaglitazar also strongly induced browning of WAT *in vivo* in both lean and obese mice at thermoneutrality, largely exceeding the increase in *Ucp1* observed with rosiglitazone. Mechanistically, we found that selective PPARγ agonism was sufficient for the conversion of white into brown-like adipocytes in vitro, but dual PPARα/γ agonism was superior to selective PPARγ agonism at inducing white fat browning *in vivo*, at least in part via a PPARα-mediated increase in fibroblast growth factor 21 (Fgf21). In human preadipocytes *in vitro*, combined treatment with rosiglitazone and Fgf21 resulted in a robust and synergistic increase in *UCP1* mRNA levels, superior to rosiglitazone- or FGF21-monotreatments. Tesaglitazar-induced browning in obese mice was associated with increased energy expenditure, enhanced insulin sensitivity, reduced liver steatosis, and an overall improved metabolic profile compared to rosiglitazone and vehicle control groups. In conclusion, we found that dual PPARα/γ agonism robustly promotes browning of white fat in *vivo*, via synergistic action of FGF21 and PPARγ agonism. Our findings identify a novel opportunity to develop compounds with robust browning of WAT, via dual modulation of PPARα and PPARγ.

Paul Bourgine

Wallenberg Centre for Molecular Medicine, Lund University

Associate Senior Lecturer at Department of Clinical Sciences

in Lund, Lund University

paul.bourgine@med.lu.se

www.bourginelab.com

Area of interest

Human skeletal and hematopoietic regeneration

**Bone** is a complex **Organ** offering structural and mechanical support of our body, but also consisting in our principal hematopoietic center. During **development** and **repair**, bones pre-dominantly form through the **endochondral ossification** route. This involves the condensation of **mesenchymal cells**, forming a **cartilage** tissue progressively vascularized and remodeled into a mature **Bone Organ**, hosting functional hematopoiesis.

The timely cellular and molecular mechanisms occurring throughout Bone Organ formation remain elusive. These include the distinct and successive stages of **cartilage**, **vasculature**, **bone** and **hematopoiesis** establishment. Compiling human-specific knowledge on these processes may have tremendous applications in **regenerative medicine**, toward the development of innovative therapies for skeletal and hematopoiesis tissues repair.

My lab will aim at deciphering the mechanisms driving human **bone** and **bone marrow formation** to establish repair strategies. Toward this objective, we developed robust **3D in vitro** and **in vivo** systems capable of recapitulating the tissue stages of human **Bone Organ** formation. These biotechnological platforms were primarily designed for skeletal repair but can also be exploited for the study/regeneration of the hematopoietic tissue. Together with the manipulation of dedicated human mesenchymal lines, these models will allow gaining considerable fundamental knowledge on human bone and bone marrow biology, for translational applications. Ultimately, we target the design of **cell-free** biological matrices **molecularly customized** in composition, as **grafts** capable to instruct tissue regeneration.

Keywords: cartilage, bone, human hematopoiesis, 3D culture systems, mesenchymal cells, biomaterial, tissue engineering, extracellular matrices.

**Strengths in lab**

• 3D culture systems

• Death-inducible cell lines

• Engineering of biological extracellular matrices

• Cartilage and bone tissue generation

• In vitro and In vivo engineering of human hematopoietic niches

Björn Burmann

Wallenberg Centre for Molecular and Translational Medicine,

University of Gothenburg

Associate Senior Lecturer at Department of Chemistry and

Molecular Biology

bjorn.marcus.burmann@gu.se

wcmtm.gu.se/research-groups/burmann

Area of interest

Dr. Björn Burmann, Associate Senior Lecturer (Assistant Professor) in Chemistry oriented towards life science, investigates macromolecular protein machines by high-resolution Nuclear Magnetic Resonance (NMR) underlying essential cellular functions, e.g. protein quality control and DNA repair processes.

His group aims to elucidate the respective function of the different protein complexes at the atomic level in order to understand their dysfunction underlying several neurodegenerative diseases and cancer-types. The group studies these large molecular protein complexes (~500–800 kDa) by sophisticated NMR-methods, to be able to derive structural and dynamical adaptions of these complexes at the atomic level in solution. These NMR studies are complemented and combined with additional information from other structural biology and biophysical methods. Lately, the group also started to investigate the feasibility of different native vesicles for their usage in functional and structural studies.

These integrated structural biology approaches are used to understand the possible allosteric mechanism of these proteins and their respective complexes underlying their functionality. This knowledge will subsequently be used to understand the effect of disease-related mutations and for the future design of either novel antibiotics or new drugs.

Strengths in lab

The Burmann lab has extensive experience in expressing, specific isotope-labeling, and purification of a wide range of different proteins and nucleic acids, which are subsequently studied by a variety of biophysical methods. Further, we are establishing currently bacterial outer membrane vesicles and related systems as a novel platform for functional and structural studies. Besides our main technique, high-resolution advanced NMR-spectroscopy, we also study our proteins and their complexes with additional biophysical methods like SEC-MALS, and Bio-Layer Interferometry, which we also combine with bioinformatical methods.

Abstract

Studying proteins and their respective complexes atomic resolution in their native environment is since long ago the aim of almost every structural biologist. Current efforts in the group towards the aforementioned aim are mainly focused on two emerging techniques: *in-cell* and *in-situ* NMR spectroscopy.

Studies on the interaction of Parkinson´s related a-synuclein and its regulation by molecular chaperones by *in-cell* NMR within mammalian cells will be presented. Our results establish a novel regulatory role of molecular chaperones for a-synuclein under physiological conditions. This fundamental new role molecular chaperones might open new mechanistic perspectives for therapeutic interventions for Parkinson´s disease.

To date, no methods exist to experimentally mimic the complex architecture of bacterial outer membranes in order to study biological processes at the native bilayer. To address this issue, we recently developed a method to manipulate the protein content of bacterial outer membrane vesicles (OMVs). These vesicles circumvent the limitations of established methods relying on membrane-mimetic systems or artificially reconstituted membranes and for the first time allow detailed biophysical studies of outer membranes under native conditions. Initial data exploring the potential of engineered OMVs to study the structure/function relationship of outer membrane components at the molecular level *in situ* using a variety of structural and biophysical methods will be presented.

Claudio Cantù

Wallenberg Centre for Molecular Medicine, Linköping University

Senior Lecturer at the Department of Clinical and Experimental Medicine (IKE)

Faculty of Medicine and Health Sciences

claudio.cantu@liu.se

liu.se/en/employee/claca29

Area of interest

Gene Regulation Downstream of Signaling Pathways

Cells communicate by exchanging signalling molecules to instruct each other about their position, function and fate. When a signal is received from a cell, it culminates - after complicated biochemical cascades - in the activation of specific combinations of genes, inducing cells to acquire distinct identities and functions.

A relatively small number of signalling cascades is known, and their components have been historically discovered by their epistatic effects. Therefore, it is widely accepted that signaling pathways are conserved linear series of molecular events, irrespective of the cell-type in which they are triggered. How can a small number of conserved signaling cascades drive the wide-range of differentiation fates occurring during animal development? Understanding this is important, as several human pathological conditions arise when these mechanisms are perturbed. The discovery of novel molecular details of embryonic development bears the potential of generating new diagnostic markers and, perhaps in the future, novel therapeutic avenues for several pathological conditions, including cancer. We make use of sophisticated state-of-the-art technologies, from mouse genetics to high-throughput biochemical approaches (e.g. protein-protein interaction screens, ChIP-seq), to discover the composition of the protein “arsenal” that, in different cell types, allows the activation of the correct genes, while leaving silent many others. Our experimental efforts are focused on the so-called ‘Wnt signalling pathway’, a molecular cascade important for virtually all aspects of embryonic development, and whose deregulation causes human malformations and several forms of aggressive cancer.

Wnt Signalling at the Interface between Development and Cancer

Colorectal cancer (CRC) is prominently caused by uncontrolled activation of the Wnt signalling pathway. However, a complete inhibition of this pathway would have devastating effects on normal tissue homeostasis. Using mouse model of CRC, we previously discovered that inhibiting the interaction between the Wnt signaling co-factors BCL9 and β-catenin does not perturb intestinal homeostasis, but strongly impairs metastatic traits (Moor et al., 2015). This suggested that therapeutic strategies aimed at targeting BCL9 (by interfering with its interaction with β-catenin) will be well tolerated and can be used without perturbing intestinal renewal, but yet prove sufficient to dramatically reduce CRC progression and improve disease outcome. It remains unknown, however, what are the protein partners that allow the β-catenin/BCL9 complex to drive a metastatic program. High-throughput protein-protein interaction studies allowed us to uncover new candidate proteins that specifically bind to select domains of BCL9L. Among them, a T-box containing transcription factor - never previously implicated in CRC, referred to as TboxTF – appeared to co-operate with the β-catenin/BCL9 complex in CRC and other specific developmental contexts. In intestinal epithelial cells, its expression correlates with Wnt activity: it is higher in Lgr5+ stem cells and becomes overexpressed in CRC. In vivo genome-wide DNA-protein interaction assays reveal that a large fraction of TboxTF-bound regions is co-occupied by β-catenin/BCL9. Notably, TboxTF precisely occupies previously described TCF-dependent WRE. This raised a model in which tissue-specific transcription factors can modulate the Wnt signalling-dependent transcriptional response via the action of the adaptor proteins BCL9.

Changchun Chen

Wallenberg Centre for Molecular Medicine, Umeå University

Associate Senior Lecturer at Umeå Center for Molecular Medicine

changchun.chen@umu.se

https://www.umu.se/forskning/grupper/changchun-chen/

Area of interest

Animals are experiencing frequent changes in O2 availability in their living environments, and they have evolved sophisticated mechanisms to cope with both acute and chronic alterations in O2 supply. Importantly, interruption of O2 supply for more than a few minutes could lead to irreversible pathogenesis of many major causes of mortality in humans. Despite intensive research, the molecular and neural circuit bases of O2 sensing remain unclear. We are investigating acute and chronic O2 sensation in the nematode *C. elegans*. Studying O2 sensing in *C. elegans* provides many unique advantages over other systems. *C. elegans* robustly respond to the changes in O2 levels, and is amenable for high-throughput behavioural screens to identify functionally relevant molecules without prior knowledge. Its fully-constructed nervous system allows us to trace flow of information from sensory inputs to motor outputs. We will combine large-scale genetic screen, biochemistry, calcium imaging, optogenetics, and single neuron transcriptional profiling to delineate O2 sensing mechanisms at both molecular and neural circuit levels. Our research has the potential to gain important new insights into the neuronal basis of behavioral and physiological adaptations that are important for an organism to survive better under extreme conditions.

Strengths in lab

Behavioral genetics, high-throughput genetic screen, calcium imaging, optogenetics.

Abstract

Although powerful, forward genetic dissection of complex traits is limited by the laborious mapping required to link phenotypes to specific genes. Here we describe a method that allows molecular characterization of hundreds of mutants simultaneously. The method is based on a ‘multiple hits strategy’ – that is, on searching for multiple alleles of the same gene in collections of mutants selected for specific phenotypes. It omits preliminary experimental steps, and relies on *post hoc* computational analyses to identify phenotype-causing mutations in sequenced strains. The analysis pipeline filters background variants and sequencing noise, selects mutations likely to be induced by the chemical mutagen employed, highlights genes with multiple mutant “hits”, and describes how each lesion alters the open reading frame. The pipeline is general, and can be applied to both model and non-model organisms accessible to forward genetics. We demonstrate its utility by sequencing 449 *C. elegans* mutants defective in aggregation behavior and identifying associated defective genes.

Erika Comasco

SciLifeLab/Uppsala University

Associate Senior Lecturer, Department of Neuroscience,

Uppsala University

erika.comasco@neuro.uu.se

https://www.neuro.uu.se/research/research-groups/erika-comasco/

Area of interest

Biological Psychiatry

By using state-of-the-art techniques, we aim to further understand the biological substrates of psychiatric disorders, such as addiction and premenstrual dysphoric disorder.

In collaboration with Prof. Nylander, we research on the effects of exposure to early-life stress and addictive drugs (e.g. alcohol and nicotine) on brain and behaviour. Stressors during critical periods of brain development, such as childhood maltreatment, have the potential to leave signatures on endocrine and neural systems, thus malprogramming emotional and cognitive brain circuits. Focusing on adolescence, (epi)gene-environment interactions are investigated in rodents as well as human population-based samples to identify biomarkers of vulnerability to addiction.

Together with Prof. Sundström-Poromaa, we investigate interactive effects of sex hormones and drugs (e.g. nicotine and antidepressants) on brain and behavior of healthy subjects as well as psychiatric patients. The psychoneuroendocrine underpinnings of sex differences and gonadal hormone effects on mental health indeed remain largely unknown, thus impeding the development of sex-specific treatments. Ongoing studies of the group aim to make a major headway in understanding the psychobiology of women’s behavior and mental health. By integrating genetic, endocrine, pharmacological, neurophysiological and neuroimaging measures, we aim to characterize diagnosis- and treatment-related biomarkers of sex-specific disorders such as premenstrual dysphoric disorder.

Strengths in lab

We aim to contribute to a nosology of psychiatric disorders informed by disease neurobiology, therefore we make use of state-of-the-art techniques available at Uppsala University, such as the MR and PET centers. We use validated psychological assessment tools, gold standard preclinical and clinical procedures, as well as perform ad hoc genotyping and gene expression analyses and neuroimaging mapping.

Brain structure correlates of PMDD

Premenstrual dysphoric disorder (PMDD) is the prototypical sex-specific psychiatric disorder in which symptoms onset and offset require a particular hormonal milieu. The present study investigated diagnosis-related markers of brain structure in the late luteal phase, i.e. the symptomatic phase of the menstrual cycle. Patients with PMDD and healthy women filled in a diary to monitor PMDD symptoms throughout the menstrual cycle and underwent a magnetic resonance scan during the late luteal phase. The Daily Record of Severity of Problems total score and subscales scores were computed considering the final five days of the luteal phase of the menstrual cycle when the brain scan took place. The subscales were Depressive Symptoms, Physical Symptoms, and Anger/Irritability. Acquisition of high-resolution Magnetic Resonance (MR) imaging data was conducted with a 3.0 Tesla whole-body scanner. All images were preprocessed and then segmented into grey matter, white matter, cerebrospinal fluid, bone, soft tissue and background probability maps. MR preprocessed data was used to conduct Voxel-Based Morphometry analyses, using the Statistical Parametric Mapping software implemented in MATLAB. Preliminary analyses showed a correlation, adjusting for age and brain size, between irritability during the luteal phase and the anterior cingulate cortex and the insula, key nodes of the salience network of relevance to anger and irritability. Cytoarchitecture brain analyses are ongoing. Information on neural correlates will lead to a nosology of PMDD informed by disease neurobiology beyond the DSM classification, to impact the theoretical framework of PMDD, as well as to characterize the psychobiology of women’s mental health during their reproductive age.

Martin Dalin

Wallenberg Centre for Molecular and Translational Medicine,

University of Gothenburg

Researcher, Department of Pediatrics, Institute of Clinical Sciences, University of Gothenburg

Resident physician, Department of Pediatric Oncology,

Sahlgrenska University Hospital;

martin.dalin@gu.se

https://wcmtm.gu.se/research-groups/dalin

Area of interest

Genetic disease markers in childhood cancer

Despite significantly improved treatment outcomes of most pediatric cancers during the last decades, around 20% of children diagnosed with cancer in Sweden and most other industrialized countries today die from their disease. At the same time, severe side effects and long-term complications of chemotherapy and other cancer treatments are frequently reported. Finding the balance between effective treatment and acceptable side effects for each patient is one of the greatest challenges of pediatric cancer management.

The goal of this project is to investigate the potential of using circulating cell-free tumor DNA (ctDNA) as a marker for disease burden, treatment response, and relapse in childhood cancer. To date, most ctDNA studies have investigated the potential of well-known hotspot oncogenic mutations (such as KRASG12D or BRAFV600E) as plasma markers of disease in adult cancer patients. However, pediatric cancers are characterized by few somatic point mutations, and a large number of structural chromosomal rearrangements leading to copy number alterations and fusion genes. They also show a striking genetic diversity, indicating that personalized design of ctDNA assays are most likely required to investigate the potential of this method as a biomarker in pediatric cancers.

Martin Dalin is a resident physician in pediatric oncology at the Sahlgrenska University Hospital. After a PhD in experimental cancer biology at the University of Gothenburg and a postdoc in cancer genetics at Memorial Sloan Kettering Cancer Center, New York, he has started this translational project applying his research background in molecular cancer biology to his clinical interest in pediatric oncology. He will start his position at the Wallenberg Centre for Molecular and Translational Medicine, University of Gothenburg in May 2019.

Strengths in lab

Our areas of interest include pediatric cancer genetics, whole exome and whole genome sequencing, bioinformatics, and circulating tumor DNA analyses. For example, we are using Simple multiplexed PCR-based barcoding of DNA for ultrasensitive mutation detection by next-generation sequencing (SimSenSeq), developed by Dr. Anders Ståhlberg and coworkers.

We have a biobank of blood samples drawn sequentially before, during and after therapy in children with all types of cancer. Blood plasma and cells are stored separately. This collection of patient material may be of interest for collaborators in the field.

Circulating tumor DNA as a biomarker in pediatric cancer

In any cancer of the human body, tumor cells are continuously dying, leading to a leakage of fragmented tumor DNA that circulates freely in the blood stream. This circulating cell-free tumor DNA (ctDNA) opens up new possibilities to detect and monitor cancer using mutation analysis of plasma samples. Due to the recent breakthrough in large-scale DNA sequencing techniques, analysis of ctDNA has emerged as a potential biomarker in cancer. Studies in adult patients suggest the method to be highly specific and sensitive, although more research is needed before it can be implemented in clinical use. The aim of this study is to investigate the correlation between ctDNA levels and clinical parameters in pediatric cancer.

All children diagnosed with cancer at the Sahlgrenska University Hospital are eligible for the study. We first analyze the genetic alterations causing the disease using whole exome and whole genome sequencing. Based on the point mutations, fusion genes and copy number alterations detected in each specific case, we design individual sequencing assays in order to monitor the level of ctDNA in plasma samples sequentially acquired during and following treatment. If proven successful, this novel marker of disease burden may contribute with prognostic information that will help guiding treatment decisions. It may also serve as a sensitive marker for relapse of the disease. Lastly, it may reduce the need for more invasive or potentially harmful methods of treatment evaluation, such as bone marrow analysis in children with leukemia and repeated CT scans in children with solid tumors.

João Duarte

Wallenberg Centre for Molecular Medicine, Lund University

Associate Senior Lecturer, Department of Experimental

Medical Science, Lund University

joao.duarte@med.lu.se

www.lunduniversity.lu.se/lucat/group/v1001219

Area of interest

Diabetes and brain function

The increasing prevalence of type 2 diabetes (T2D) in western societies is closely associated with obesity, sedentary life-styles and the excessive consumption of food products rich in fat and sugar. The whole-body metabolic imbalance in diabetes has a detrimental impact on brain function, leading to increased risk of dementia.

Our previous work in rodent models of T2D has identified diabetes-induced alterations of brain energy metabolism and metabolic profiles, neuromodulation systems, astrogliosis and synaptic dysfunction underlying memory dysfunction. We further found that pharmacological interventions that target the adenosinergic neuromodulation system partly restore T2D-induced brain dysfunction.

Our lab mainly focuses on understanding early metabolic dysfunction that might precede and be involved in the diabetes-induced neurodegenerative process. In particular, we aim at understanding T2D-induced alterations of brain insulin-dependent metabolic regulation in neurons and astrocytes. Counteracting insulin signalling defects in brain areas regulating memory processes might provide a means of correcting energy fuelling to synaptic activity and plasticity, and prevent diabetes-induced synaptic dysfunction, neurodegeneration and memory dysfunction.

Abstract

Diabetes affects the morphology and plasticity of the hippocampus, and leads to learning and memory deficits. Caffeine has been proposed to prevent memory impairment upon multiple chronic disorders with neurological involvement. We tested whether long-term caffeine consumption prevents type 2 diabetes (T2D)-induced spatial memory impairment and hippocampal alterations, including synaptic degeneration, astrogliosis and metabolic modifications. Control Wistar rats and Goto-Kakizaki (GK) rats that develop T2D were treated with caffeine (1 g/L in drinking water) for four months. Spatial memory was evaluated in a Y-maze. Hippocampal metabolic profile and glucose homeostasis were investigated by 1H magnetic resonance spectroscopy. The density of neuronal, synaptic and glial-specific markers was evaluated by Western blot analysis. GK rats displayed reduced Y-maze spontaneous alternation and a lower amplitude of hippocampal long-term potentiation when compared to controls, suggesting impaired hippocampal-dependent spatial memory. Diabetes did not impact the relation of hippocampal to plasma glucose concentrations, but altered the neurochemical profile of the hippocampus, such as increased in levels of the osmolites taurine (P<0.001) and myo-inositol (P<0.05). The diabetic hippocampus showed decreased density of the pre-synaptic proteins synaptophysin (P<0.05) and SNAP25 (P<0.05), suggesting synaptic degeneration, and increased GFAP (P<0.001) and vimentin (P<0.05) immunoreactivities that are indicative of astrogliosis. The effects of caffeine intake on hippocampal metabolism added to those of T2D, namely reducing myo-inositol levels (P<0.001) and further increasing taurine levels (P<0.05). Caffeine prevented T2D-induced alterations of GFAP, vimentin and SNAP25, and improved memory deficits. We conclude that caffeine consumption has beneficial effects counteracting alterations in the hippocampus of GK rats, leading to the improvement of T2D-associated memory impairment.

Olof Eriksson

SciLifeLab/Uppsala University

Associate Senior Lecturer, Department of Medicinal Chemistry, Uppsala University

olof.eriksson@ilk.uu.se

https://www.ilk.uu.se/research-groups/theranostics\_en/

Area of interest

Molecular Imaging in metabolic disease

During the last decades there have been significant progress in the molecular understanding of type 1 and type 2 diabetes (T1D/ T2D). This is partly due to the use of several important animal models of diabetes including but not limited the NOD mouse, the Zucker-Fatty rat, many transgenic strains etc. We can now reverse both induced and spontaneously developed diabetes in these models. Despite this remarkable progress, we are far from a cure for human diabetes, which presumably is due to discrepancies between the models and the clinical situation. Most importantly, it is due to a failure in identifying the areas in which the model and the human physiology are actually congruent, and in which translating promising preclinical treatments could lead us closer to a cure of human diabetes.

My proposal to solve this problem is to develop new tools for non-invasive in vivo molecular studies of the human beta cell and metabolic and inflammatory processes involved in the development of diabetes. The field of molecular imaging has been revolutionized during the last 20 years with progress in hybrid Positron Emission Tomography (PET)/ Magnetic Resonance (MR) hardware, image analysis software and radiochemistry, but so far this has not been fully utilized in the study of diabetes.

Strengths in lab

• Molecular Imaging

• Positron Emission Tomography (PET)

• Single Photon Emission Tomography (SPECT)

• Magnetic Resonance Imaging (MRI)

• Radiopharmaceutical development

• Disease models (diabetes, xenografts)

• Large animal studies

• Clinical studies

Quantification of beta cell mass in humans by PET

The natural history of both T1D and T2D with respect to changes in total beta cell-mass (BCM) remains unknown. Currently, there exists no validated method for direct measurement of beta cell mass. Changes in biomarkers in blood, e.g. glucose, c-peptide, insulin, HbA1c etc., are all composite parameters critically dependent on a complex interaction between the functional activity of the beta cells and the total beta cell mass. Non-invasive quantification of BCM would allow assessment of the effect of new treatments aiming for beta-cell regeneration, prevention of autoimmune beta-cell destruction, engraftment and survival of transplanted islets as well as more basic biological questions such as the etiology and disease progression of both T1D and T2D.

Positron Emission Tomography (PET) is a molecular imaging technique, which is minimally non-invasive, and has high sensitivity and resolution in comparison to similar imaging techniques. Importantly, it is quantifiable, a requirement for accurate measurement of molecular processes and tissue protein expression levels in vivo in human and large animals. Hybrid PET/MRI scanners combine functional and structural imaging techniques allowing simultaneous assessment of multiple endpoints.

Novel beta cell enriched imaging targets - not present in the exocrine or ductal pancreas - are identified by proteomic and transcriptomic screening. These novel targets constitute not only imaging biomarkers, but also largely unexplored therapeutic targets. As an example, we recently reported the GPR44 receptor as a potential target and developed GPR44 PET radioligands [11C]AZ12204657 and [11C]MK7246, now in clinical and large animal phase imaging studies.

Daniel Globisch

SciLifeLab/Uppsala University

Associate Professor, Department of Medicinal Chemistry,

Uppsala University

daniel.globisch@scilifelab.uu.se

www.ilk.uu.se/research/afk\_en/biomarker-discovery/

Area of interest

Discovery of Unknown Metabolic Interactions of Microbiota and Human Host: Combining Novel Metabolomics and Chemical Biology Methodologies

The laboratory of Associate Professor Daniel Globisch, PhD combines metabolomics with chemical biology methodologies, chemical synthesis and systems biology for the selective investigation of microbiota metabolism. Our comprehensive and interdisciplinary projects represent an advanced strategy for metabolite biomarker discovery for pancreatic cancer.

I started my independent laboratory in September 2015 as a SciLifeLab Fellow at Uppsala University with the goal to discover unknown metabolic biomarkers for pancreatic cancer. The multidisciplinary nature of my research projects includes chemical synthesis, mass spectrometry, bioassays, biochemical pathway analysis, and systems biology.

Biomarker discovery is a challenging task in any type of human specimen as these are comprised of a complex mixture of biomolecules. The analysis of metabolites is termed metabolomics, the newest ‘omics’-research field. One of the most exciting scientific developments in the past decade has been the understanding that gut microbiota profoundly impact human physiology. This complex consortium of trillions of microbes possesses a diverse range of biochemical and metabolic activities and plays a crucial role in multiple physiological processes. This metabolic interspecies communication represents a tremendous and new opportunity for biomarker discovery. However, tools for the selective analysis are lacking. We have developed unique tools at the interface of chemistry and biology for analysis of specific metabolite classes with focus on microbiota human-host co-metabolism. These methods will allow for the discovery of unknown metabolites in medical relevant samples to evaluate their potential as biomarkers.

Strengths in lab

My laboratory has developed new state-of-the-art tools combining Chemical Biology and metabolomics analysis. We have synthesized a unique chemoselective probe and enzymatic assays to achieve an advanced metabolites analysis using ultra-performance liquid chromatography-coupled with tandem mass spectrometry (UHPLC-MS/MS). We quantitatively and qualitatively analyze metabolites in any human and other mammalian sample type such as urine, plasma, feces, saliva, and tissue. Our strength lies in the analysis of biosynthetic pathways, metabolite structure elucidation and chemical synthesis of isotope labeled internal standards for precise quantification.

Discovery of Unknown Metabolic Interactions of Microbiota and Human Host: Combining Novel Metabolomics and Chemical Biology Methodologies

The detailed investigation of metabolites in human samples (serum, plasma, urine, saliva, feces or tissues), termed metabolomics, carries a great potential for the discovery of unknown biomarkers.[1] The analysis of metabolites is termed metabolomics, the newest ‘omics’-research field, which is mostly lacking of advanced chemical tools compared to other ‘omics research fields. One of the most exciting scientific developments in the past decade has been the understanding that gut microbiota profoundly impact human physiology. The complex consortium of trillions of microbes possesses a wide range of metabolic activity. This metabolic interspecies communication represents a tremendous opportunity for biomarker discovery as only limited information on this co-metabolism has been elucidated on a molecular level.[2]

We have developed new state-of-the-art Chemical Biology techniques for an enhanced metabolomics analysis using liquid chromatography-coupled with tandem mass spectrometry (UPLC-MS/MS).[3,4] We have recently developed a unique chemoselective probe for analysis of fecal samples. Immobilized to magnetic beads, this complex probe allows for facile extraction of metabolites from human fecal samples and led to increased mass spectrometric sensitivity by a factor of 2000.[3] In a second new method, we utilized selective enzymatic treatment of metabolites in human samples, UPLC-MS/MS and bioinformatics analysis (XC-MS) to easily identify converted metabolites and to elucidate the chemical formula.[4,5] We subsequently chemically synthesized each identified molecule to unequivocally validate the molecular structure. Using this specific workflow, we have successfully identified three times as many sulfated metabolites than reported in the Human Metabolome Database (HMDB).

Our unique metabolite-analyzing methodologies at the interface of Chemistry and Biology are aimed at overcoming limitations in mass spectrometry-based metabolomics research. We are applying these methods for the discovery of unknown metabolites in medical relevant samples to evaluate their potential as biomarkers for pancreatic cancer. We anticipate the discovery of unknown early-stage biomarkers, which are crucial for the development of new diagnostics.[6]

Literature: [1] D. Globisch, A.Y. Moreno, M.S. Hixon, A.A.K. Nunes, J.R. Denery, S. Specht, A. Hoerauf, K.D. Janda ***Proc. Natl. Acad. Sci. U S A.*** 2013, 110, 4218–4223. [2] D. Globisch, C.A. Lowery, K.C. McCague, K.D. Janda ***Angew. Chem. Int. Ed.*** 2012, 51, 4204–4208. [3] N. Garg, L.P. Conway, C. Ballet, M.S.P. Correia, F.K.S. Olsson, M. Vujasinovic, J.M. Löhr, D. Globisch ***Angew. Chem. Int. Ed.*** 2018, 57, 13805 –13809. [4] C. Ballet, M.S.P. Correia, L.P. Conway, T.L. Locher, L.C. Lehmann, N. Garg, M. Vujasinovic, S. Deindl, J.M. Löhr, D. Globisch ***Chem. Sci.*** 2018, 9, 6233–6239. [5] M.S.P. Correia, C. Ballet, H. Meistermann, L.P. Conway, D. Globisch ***Bioorg. Med. Chem.***, 2019 *accepted*. [6] R. J. Shirey, D. Globisch, L. M. Eubanks, M. S. Hixon, K. D. Janda ***ACS Infect. Dis.*** 2018, 4, 1423-1431.

Eric Daniel Głowacki

Wallenberg Centre for Molecular Medicine, Linköping University

Group Leader at the Laboratory of Organic Electronics (LOE), Norrköping

Faculty of Science and Engineering

eric.glowacki@liu.se

Area of interest

Optoelectronic biointerfaces – stimulation and optopharmacology

Our team works on developing novel electronic devices for biomedical implants which can stimulate and record biophysical processes. Our flagship technology is the photocapacitor – a nanoscale platform which transduces an optical signal into directly-evoked action potentials in neurons.

We focus on processes driven with light. A large part of our research focuses on fabricating optoelectronic devices which can artificially stimulate neurons. The devices operate in the tissue transparency window of the near-infrared, where light can penetrate deeply through skin and bone. The motivation is to provide a wireless and minimalistic implant which can perform the duty of standard implantable electrodes, but without the wiring. The devices we fabricate are not only wireless, but also 100-1000 times thinner than most existing technologies. Making implants have as small as possible mechanical footprint improves the efficacy of bioelectronic medical treatments by minimizing the risk for inflammation and making surgical implantation less invasive. On the other hand, the other block of our research efforts is directed at devices which, when stimulated with light, deliver controlled amounts of reactive oxygen species (mostly peroxide). We study the effects of photoelectrochemically-generated peroxides on physiological processes, with the hope of developing novel therapeutic approaches to neurodegenerative diseases.

Strengths in lab

The research that we carry out is at the interface of semiconductor physics and technology and applied electrophysiology and neuroscience. The diverse background of our team reflects this, as does the laboratory space. Part of our team consists of materials scientists and physicists, who work in a clean room semiconductor device fabrication facility located in Norrköping, Sweden. There we have full access to typical methods of nano- and microfabrication of metals, semiconductors, and biomedical-grade plastics. We have constructed custom setups for studying the fundamental photophysical and electrochemical behavior of our devices. On the other hand, other members of the team are electrophysiologists or biochemists, who work on deploying our devices at the level of single-cell experiments to *in vivo* studies and interactions with preclinical researchers. We have access to an electrophysiological and cell culture lab for our experiments, while in vivo work is carried out with collaborators.

Light-induced extracellular stimulation using organic electrolytic photocapacitors

Marie Jakešováa,b, Malin Silverå Ejnebyb,c, Magnus Berggrena, Daniel Simona, Fredrik Elinderc, and Eric Daniel Głowackia,b

a Laboratory of Organic Electronics, ITN Campus Norrköping, Linköping University, Bregatan 33, 602 21 Norrköping, Sweden

b Wallenberg Centre for Molecular Medicine, Linköping University, SE-58185, Linköping, Sweden E-mail: eric.glowacki@liu.se

c Division of Cell Biology, Department of Clinical and Experimental Medicine, Linköping University, SE-581 85 Linköping, Sweden

We report on a nanoscale semiconducting optoelectronic system optimized for neuronal stimulation: the organic electrolytic photocapacitor. The devices comprise a trilayer of metal and p and n semiconductors. When illuminated in physiological solution, these metal-semiconductor devices charge up, transducing light pulses into localized displacement currents that are strong enough to stimulate cells. The devices are freestanding, requiring no wiring or external bias, and are stable in physiological conditions. We have systematically evaluated the ability of photocapacitor devices to alter the cell membrane potential of single nonexcitable cells, generate action potentials in neuronal cell cultures, and stimulate explanted light-insensitive embryonic retinas.

Markus Hansson

Wallenberg Centre for Molecular Medicine, Lund University

Associate Professor and Senior Consultant, Department of Hematology, Skåne University Hospital and Lund University

markus.hansson@med.lu.se

www.med.lu.se/wcmm/researchers

Area of interest

Focus on Multiple Myeloma

Markus Hansson combines clinical work as a senior consultant in hematology at Skåne University Hospital with clinical trials and basic science regarding multiple myeloma (MM). He heads the clinical myeloma team and the myeloma research group including three PhD students (MDs), three research nurses, one assistant nurse and two laboratory technicians.

MM is the second most common malignancy of the blood, with an incidence of 600 patients per year in Sweden. The disease is characterized by an uncontrolled growth of an abnormal malignant plasma cell clone in the bone marrow, producing monoclonal antibodies that can be detected as a paraprotein in serum (”M component”). MM arise from a premalignant disease, monoclonal gammopathy of undetermined significance (MGUS), which is a common condition with a prevalence of 1% in the Swedish population35. In contrast to MGUS, which is without symptoms, MM is clinically characterized by bone marrow failure (leading to anemia and compromised immunity), lytic bone destructions (leading to pain, pathological fractures and hypercalcemia) and renal failure. Current therapy includes corticosteroids, chemotherapy, immunomodulators, proteasome inhibitors, and autologous stem cell transplant. Even with ASCT most patients get relapses and MM remains incurable and fatal, with a survival of 3 to 8 years, depending on age at diagnosis.

Markus Hansson has since 2013 been working in the board of the Nordic Myeloma Study Group (NMSG) that coordinates many clinical trials in the Nordic region. He is also in the board of the Swedish myeloma biobank and chairman of the Swedish myeloma group and the Swedish national treatment guidelines group.

Strengths in lab

The Swedish myeloma biobank (located in Lund) and a Swedish myeloma registry. Locally we have excellent experience in clinical trial design, in advanced multicolor flow cytometry and cell sorting, protein purification, cell culture and immuno-fluorescence microscopy. Furthermore, we share laboratory with Björn Nilssons group creating a strong multi-disciplinary environment with computational, experimental or clinical expertise.

Abstract

Our main goal is to improve outcome for the painful and fatal tumor disease, multiple myeloma (MM). To do this we will pursue three lines of research; i) use investigator initiated clinical trials to test prevention strategies and ii) to test new drug combinations and iii) investigate phagocyte subsets and functions during treatment and progression of MM. If successful, this project will i) show that elimination of common subclinical infections could abrogate the small plasma cell clones and possibly prevent or decrease the risk of progression into MM ii) improve MM treatment and iii) gain knowledge of how phagocytes contribute to progression of MM, this could also lead to completely new treatment strategies targeting the MM supporting bone marrow milieu.

Einar Heiberg

Wallenberg Centre for Molecular Medicine, Lund University

Associate Professor and Biomedical Engineer at Department of

Clinical Sciences Lund, Clinical Physiology, Skåne University Hospital and Lund University

einar.heiberg@med.lu.se

https://www.med.lu.se/klinvetlund/klinisk\_fysiologi/forskning/cardiac\_mr\_group

Area of interest

Image processing is fundamental issue in medical imaging, as without image processing no quantitative data can be derived. Image segmentation is the process of discriminating an image into an object of interest from background. Currently the two main areas of interest are:

Large scale image analytics

Large-scale cross-sectional research has an important role for pre-clinical research through hypothesis generation and identification of future treatment targets. Existing clinical analysis software packages are not adequate for analyzing large patient cohorts of 1,000-100,000 patients. The main reasons are 1) analysis time per patient is too long and comes with significant need for manual interaction, and 2) existing commercial tools do not save the results in an open format that allows re-processing to extract new data. Ongoing work is to process SCAPIS cardiac CT data sets, initially at two sites and 12 000 subjects.

Image segmentation for 3D printing and virtual reality applications

Software have been developed to perform segmentation and convert segmented objects to a 3D printable format. Applications are anatomical models, cutting and drilling guides, and 3D printed implants.

Strengths in lab

Einar Heiberg is part of the steering committee of Lund cardiac MR group, which is a large research group of approximate 35 members. The group is active in a wide range or research including; cardiac pump physiology, ischemic heart disease, cardioprotection, image processing, MR physics, congenital heart disease, computational flow modelling, cardiac perfusion, pulmonary artery hypertension, and exercise physiology. The group also maintains a large animal line for research around acute cardiac syndrome and cardioprotection.

Main competence of Einar Heiberg is development of medical image processing algorithms and software. Einar Heiberg has developed the software Segment (http://segment.heiberg.se) that is freely available for research and widely used for medical image processing. Map shows locations where the software was used the last 12 months.

3D printing of supportive implants

The central idea is to be able to directly 3D print supportive implants. This will be performed using a newly purchased 3D printer (M220, Apiumtec, Germany) that is able to print in the biocompatible thermoplastic PEEK (polyether ether ketone). This material has been used for implants during the last 30 years, but it is only recently it is possible to use the material for 3D printing. The material has several advantageous properties for implants, such as it can be autoclaved, biological inertness, and has a comparable elasticity modulus as native bone. First applications that first will be explored are implants that are not load bearing, and specifically replacement of complete carpal bones and cranioplasty. Another research direction is on the how the 3D printing process can be adjusted to produce porous prints, and whether this can be used to produce osseointegration at desired parts of the implant.

The project is performed together with researchers at Skåne University Hospital and Anders Björkman (WCMM, Lund).

Oskar Hemmingsson

Wallenberg Centre for Molecular Medicine, Umeå University

Associate Senior Lecturer at the Department of Surgical and Perioperative Sciences

Senior Consultant in Surgery, University Hospital of Umeå

oskar.hemmingsson@umu.se

https://www.umu.se/personal/oskar-hemmingsson/

Area of interest

The *RAS* genes are the most commonly mutated protooncogenes in human cancer. The RAS proteins mediate signals emanating from cell surface receptors, including receptor tyrosine kinases, into the cell interior. Other components in the RAS signaling pathway are also frequently activated in human cancer. For example, upstream receptors such as EGFR or ERBB2 or downstream effector kinases such as RAF or MAPK. Because of its central role in this oncogenic signal transduction web, mutated RAS may seem an ideal therapeutic drug target. However, RAS has so far proven to be an intractable drug target and no RAS-targeted drug is approved for cancer treatment. An alternative approach may be to target other components of the RAS pathway, or to target interacting proteins that are necessary for the oncogenic function of RAS. The nematode *C. elegans* has been extensively used to delineate genetic pathways that are common among animals. Thus, research using *C. elegans* has provided fundamental insights regarding the RAS/RAF/MAPK pathway. Oncogenic mutations in the RAS signaling pathway results in tissue specific phenotypes in *C. elegans* that can be studied *in vivo*. We apply the nematode model on cancer research focusing on *B-RAF* activated melanoma and *K-RAS* activated colon cancer. The aim is to identify novel drug targets and substances to inhibit RAS pathway-driven human cancer.

Strengths in lab

Expertise and equipment for *C. elegans* research. Cell cultures and gene editing including CRISPR/Cas9 technology. Cancer tissue studies. Proximity to our clinical institution with access to registers, biobanks and clinical studies, enabling a translational research environment.

Abstract

Metastatic melanomas frequently harbor an activating mutation in *B-RAF* and can be treated by B-RAF inhibitors. This results in a prolonged progression-free survival but eventually drug resistance results in treatment failure. Activation of receptor tyrosine kinases (RTK) is a known resistance mechanism and LRIG1 is a tumor suppressor downregulating RTKs. Our results indicate that LRIG1 is a suppressor of the RAS signaling pathway in melanoma through suppression of EGFR. This LRIG1-dependent tumor suppression is lost during development of drug resistance against BRAF inhibitors in melanoma cell cultures. This shows that LRIG1 is a clinically relevant target for further studies to improve cancer treatment. Our results also suggest that LRIG/*sma-10* genetically cooperates with RAS/*let-60* in *C. elegans*. The RAS/*let-60(G13D)* gain of function mutation in worms causes a constitutive activation of the protein, resulting in inappropriate vulval cell specification events during larval development. The resulting multivulva (Muv) phenotype is easily scored and represents a good readout for inappropriate RAS signaling. The Muv phenotype in RAS/*let-60(G13D)* mutants is enhanced by the LRIG/*sma-10(wk89)* mutation, showing that LRIG/*sma-10* is a conserved suppressor of RAS signaling.

Frank J. Hernandez

Wallenberg Centre for Molecular Medicine, Linköping University

Group Leader at the Department of Physics, Chemistry and Biology (IFM)

Faculty of Science and Engineering

frank.hernandez@liu.se

https://liu.se/en/research/nat-lab

Area of interest

The Nucleic Acids Technologies Lab (NAT-Lab) explores the use of nucleic acids as biorecognition molecules to develop highly specific and sensitive systems, with various detection modalities. Our ultimate goal is to generate diagnostic and therapeutic approaches with properties outside the scope of the existing technologies for their use in a broad range of applications. We have developed a platform for the identification of activatable probes for targeting human diseases with high incidence and mortality. We exploit the tremendous diversity and widespread expression of nucleases for targeting several bacterial species (e.g. Sthapylococcus aureus) and pathological conditions such as cancer (e.g. breast cancer).

Our lab is working on three main research lines:

• Screening of nuclease activity as biomarker for targeting diseases.

• Adaptation of nucleic acid probes to several detection modalities.

• Therapeutics based on nucleic acid probes.

Strengths in lab

Screening of nucleic acid probes, *in vitro* and *in vivo* models of infectious diseases and cancer.

Biological GPS for detecting infectious diseases and cancer

Over expression of nucleases has been reported as promising biomarker for infectious diseases, and other human conditions such as cancer and autoimmune diseases. In our studies, we have demonstrated the capability of nucleases for detecting specific bacteria in animal models of disease. With this strategy we developed nucleic acid probes with high sensitivity and specificity that allows *in vivo* detection of bacteria in 45 min, clearly demonstrating the great potential of this technology for targeting applications where nuclease activity is present. Therefore any method based on this property (nuclease degradation activity) represents a novel alternative for diagnostic and therapeutic intervention.

As a proof-of- concept, we have previously developed a novel molecular imaging approach that rapidly and specifically detects *S. aureus* infections via fluorescent probe activation by a specific nuclease (micrococcal nuclease)secreted by *S. aureus* (Hernandez *et. al.* Nat Med. 2014, 20, 301-306). To overcome the limitations with the fluorescence-based probes regarding tissue penetration and background signal, we are now pursuing a novel approach based on activatable contrast agents that can be measured with magnetic resonance imaging (MRI).

Anetta S. Härtlova

Wallenberg Centre for Molecular and Translational Medicine, University of Gothenburg

Associate Senior Lecturer, Institute of Biomedicine, Department of Microbiology and Immunology

anetta.hartlova@gu.se

https://wcmtm.gu.se/research-groups/hartlova-group

Area of interest

Molecular mechanisms of innate immunity and inflammation

Research in our laboratory focuses on cellular and molecular mechanisms that control inflammation in the context of ageing and age-related pathologies.

Age-related diseases such as neurodegeneration and metabolic disorders, that were not traditionally considered to be cause by inflammation, have been recently associated with low grade chronic inflammation. Although chronic inflammation has been recognized as one of the hallmarks associated with the ageing process, little is known about the causes and molecular mechanisms that mediate systemic chronic inflammation. There is an increasing appreciation that changes in the tissue microenvironment, such as the accumulation of cell debris and systemic changes in metabolic and hormonal signals, contribute to macrophage polarization and the development of chronic inflammation. Despite its importance, there is little understanding of cellular and molecular mechanisms underlying macrophages plasticity required for maintenance of tissue homeostasis. The main research interest of the laboratory is to examine the cell type-specific roles of phagocytosis and innate immune sensing pathways in the regulation of inflammatory response. Our long term goal is to develop therapeutic strategies to modulate these pathways and prevent or treat age-related human diseases.

My laboratory has extensive collaborations with researchers at the University of Gothenburg and Umeå University. Internationally, the group collaborates actively with researchers at Newcastle University (U.K.), Francis Crick Institute London (U.K.), Imperial College London (U.K.), Weizmann Institute of Science (Israel), VIB Ghent (Belgium), Institute Pasteur de Lille (France) and Medical Faculty, University of Chille (Chille).

Strengths in lab

My laboratory is using a wide variety of approaches to address key questions in in the role of innate immunity in the regulation of inflammation *in vitro* and *in vivo*. We combined biochemistry, state-of-art high-resolution mass spectrometry, image-based approaches, *in vivo* conditional knockout mouse models and human patient samples.

MSR1 triggers JNK-mediated lipid-induced inflammation and promotes obesity-related non-alcoholic liver disease

Sine Kragh Petersen, Anetta Härtlova

Low-grade chronic inflammation is associated with metabolic dysregulation in obesity and associated with non-alcoholic fatty liver disease (NAFLD). The transition from simple steatosis towards non-alcoholic steatohepatitis (NASH) represents a key step in NAFLD pathogenesis, as it is associated with cirrhosis, portal hypertension and hepatocellular carcinoma. However, the regulatory networks controlling the obesity-induced inflammation and their impact on the onset and progression of NAFLD remain unclear. Here we show that the expression of macrophage scavenger receptor MSR1—an innate immune receptor and lipid sensor—strongly correlated with lipid accumulation along with inflammation in the liver tissue from patients with NAFLD; implicating the role of MSR1 in the early stage of NAFLD pathogenesis. High-fed diet mice lacking MSR1 were protected against glucose intolerance, hepatic triglycerides accumulation, liver inflammation and sinusoidal fibrosis compared to wild-type. We further showed that MSR1 triggers JNK-mediated lipid-induced inflammation independent of lipopolysaccharide stimulation. This inflammatory response was reversed by MSR1 ablation, the receptor inhibition by monoclonal antibody or JNK inhibition. We also identified two polymorphisms in the *MSR1* gene that are significantly associated with SAF activity score and fibrosis in a cohort of 399 NAFLD patients. This study provides evidence for the role of MSR1 in the early onset inflammatory response in obesity-associated NAFLD and a potential explanation of chronic liver inflammation during over-nutrition.

Walker S. Jackson

Wallenberg Centre for Molecular Medicine, Linköping University

Senior Lecturer at the Department of Clinical and Experimental Medicine

Faculty of Health Sciences and Medicine

walker.jackson@liu.se

https://liu.se/en/research/selective-vulnerability-to-neurodegeneration

Area of interest

Neurodegeneration

My lab is interested in why neurodegenerative diseases tend to target specific brain regions, a feature known as selective vulnerability. For example, why are brain regions involved in memory targeted in Alzheimer’s disease whereas brain regions important for motor control are targeted in Huntington’s disease? On the other hand, how do other brain regions resist these diseases? If we can learn the secrets from the resistant regions maybe we can transfer those traits to the vulnerable regions and slow disease progression. Since these diseases typically affect people later in life, even a modest deceleration could make a large impact.

We study several neurodegenerative diseases in genetically modified mice. Although mice and humans have obvious differences, in good mouse models the brain regions affected in humans are also affected in mice, with the same types of neuropathological lesions. Rather than using scalpels, we use molecular approaches to dissect the brain into component parts. We also study the models in vivo with a variety of techniques including automated video based behavioral analyses, magnetic resonance imaging and telemetric electroencephalography.

Once a human is clinically affected by a neurodegenerative disease the brain is drastically altered, causing current treatments to generally be insufficient. Therefore, we study models before there are clinical or neuropathological changes to identify very early disease mechanisms and therapeutic targets.

Strengths in lab

Our key technology is based on a tool we created to study, specifically in any cell type of interest, gene regulation at 4 levels: 1) epigenetics/chromatin regulation, 2) translating mRNAs, 3)Argonaute 2 bound miRNAs, and 4) pulse labeled RNA (useful to label mitochondrial RNA and lncRNAs, among others). We also have extensive experience with genome manipulation, especially for knock-in mouse lines, using conventional and CRISPR/Cas9 based methods.

Abstract

Neurodegenerative diseases progressively transform healthy adult brains into dysfunctional states, generally leading to premature death. Since initial stages of any given neurodegenerative disease targets only a small subset of cells, the large abundance of resistant cells masks affected cells, hampering progress in understanding the earliest changes. To reduce this complexity we have employed the RiboTag technique to capture and study mRNAs from specific cell types to study how they respond to neurodegenerative disease at a very early stage. Surprisingly, although GABAergic neurons are unfazed, glutamatergic neurons have an expression profile indicative of reshaping of neuronal architecture. Moreover, astrocytes are very sick early in the disease process. These results are the exact opposite of what was predicted from existing literature, emphasizing the usefulness of this approach.

Inspired by these results we developed a new mouse line to obtain even more information about changes in gene regulation. This new mouse line simultaneously expresses four separate protein components in a specific cell type to enable the capture of multiple types of nucleic acids. The nucleic acids captured include 1) the nucleus for epigenetics and RNA splicing 2) translating mRNAs, 3) miRNAs attached to Argonaute2 and 4) total RNAs (especially useful for lncRNAs and mitochondrial transcription). Although single cell transcriptomic techniques are gaining in popularity, the advantages of a “population” approach is that the detected transcripts are not limited to those that are highly expressed and fewer samples need to be collected, sequenced and analyzed to identify the most vulnerable cell types and their pathogenic processes. Moreover, this model can be used for marking specific cell types for single cell experiments, providing a nice bridge between the two methods.

Gauti Jóhannesson

Wallenberg Centre for Molecular Medicine, Umeå University

Associate Senior Lecturer at the Department of Clinical Sciences

Senior consultant in Ophtalmology, University Hospital of Umeå

gauti.johannesson@umu.se

Area of interest

Pathophysiology of Glaucoma

Gauti Jóhannesson, Consultant in Ophthalmology, Associate Professor and Associate Senior Lecturer (Assistant Professor) incorporates clinical investigations and neuroimaging techniques to improve the understanding of the pathophysiology of glaucoma.

The main thread of my research has been glaucoma. Through my career I have studied glaucoma with respect to its prevalence, follow-up and enhanced drug delivery with nanoparticles. Taking the next step in glaucoma research, I have gotten increasingly more interested in the pathophysiology of glaucoma. After my postdoctoral experience abroad, I am leading a project focused on intracranial blood flow measurements with advanced magnetic resonance imaging (MRI) in glaucoma patients using phase-contrast MRI as well as angiographic imaging and quantitative flow determination with optimized 4D flow MRI. This project studies the interaction between the intraocular pressure and the blood flow of intracranial arteries, specifically the ophthalmic artery, in different types of glaucoma and healthy controls. As a natural continuation of the blood flow project, we have also used the unique possibilities of simultaneous positron emission tomography – magnetic resonance imaging (PET-MRI) with the aim of getting a deeper understanding of the pathophysiology of glaucoma by studying the metabolism and blood flow of the visual pathways in the brain. Furthermore, we are investigating a possible link between glaucoma and dementia through functional MRI (fMRI) and cognitive testing.

I have national and international collaborations and have received several external grants for my research as main applicant. In particular the 4-year grant from the Swedish Society of Medical Research (SSMF) has enabled me to focus on my glaucoma pathophysiology research.

Strengths in lab

The methods we use include various magnetic resonance imaging techniques including combined PET-MRI, PC-MRI, 4D flow MRI and fMRI. For ocular imaging we use swept-source ocular coherence tomography, perimetry to determine visual field damage as well as tonometry for measurement of intraocular pressure.

Lowering of intraocular pressure does not affect volumetric flow rate of ipsilateral ophthalmic artery in ocular hypertension

**Purpose**

To investigate if lowering of intraocular pressure (IOP) affects the volume flow rate of the ophthalmic artery (OA) in patients with previously untreated ocular hypertension (OH).

**Material and methods**

47 patients with untreated ocular hypertension (OH) were included and underwent ophthalmological examination and a 3-Tesla scanner MRI investigation. MRI included a 3D time of flight (3DTOF) to localize the ophthalmic artery (OA) and a phase contrast magnetic resonance imaging (PCMRI), with the plane placed perpendicular to the vessel, to measure the OA flow rate. The spatial resolution was 0.35 x 35mm. The patients received latanoprost once daily in the eye with higher pressure, the untreated eye served as control. The same measurements were repeated approximately 1 week later. Eleven patients were excluded due to absence of OA (n=2), unable to perform MRI (n=1), poor MRI data (n=3), terminated participation (n=4) and treatment to both eyes (n=1). Thus, 36 patients were analyzed (15 female, mean age 67±8 years, range 47-81 years). Comparisons were performed using paired t-tests.

**Results**

The average OA volumetric flow rate before and after treatment in the treatment eye was 9.9±4.4 and 10.2±4.4ml/min respectively (mean±-SD;p=0.51). In the control eye, the OA volumetric flow rate was 11.0±5.0 and 10.5±3.6ml/min respectively (p=0.83). There was no significant difference between the average OA volumetric flow rate in the treatment eye and the control eye, before (p=0.19) or after treatment (p=0.36). The change in volumetric flow rate after treatment in the treatment eye (0.4±3.3 ml/min) was not significantly different from the change in the control eye (-0.2±4.0 ml/min, n=29) (p=0.87). There was no significant difference in blood pressure before and after treatment (systolic: p=0.21, diastolic: p=0.47). Latanoprost lowered IOP by 7.2±2.7mmHg in the treatment eye (p<0.01).

**Conclusions**

The results indicate that a significant lowering of IOP does not affect the volumetric flow rate of the ipsilateral OA. The ability to maintain blood supply to the eye independently of IOP could be a protective mechanism in preserving vision in ocular hypertension patients.

Andreas Josefsson

Wallenberg Centre for Molecular Medicine, Umeå University

Associate Senior Lecturer at the Department of Surgery and Perioperative Sciences

Senior consultant in Urology, University Hospital of Umeå

andreas.josefsson@umu.se

Area of interest

Biomarkers for prostate cancer - from screening to metastatic disease

Sweden has the highest rate of prostate cancer (PC) death per capita in the world (2,500 yearly). PSA-based screening decreases PC deaths but with both overdiagnosis and underdiagnosis (of significant tumors with low PSA-production) as major problems. To decrease overtreatment of indolent tumors, active surveillance is implemented, but with the risk of missing the window of curability for some men. There is a huge clinical need of blood- based biomarkers that can identify men with aggressive disease at an early stage when cure is possible without overdetection of indolent (present in more than 50% of elderly men) cases.

Treatment with curative intention for prostate cancer includes radiotherapy or surgery, but still 30% relapse. For those patients, antiandrogens are standard of care and the addition of docetaxel are currently under investigation in an international randomized phase III clinical trial (Urology PI: Andreas Josefsson).

In metastatic prostate cancer castration therapy has been the standard since 1940 but recently three additional therapies have shown to be beneficial and there is a need to identify which patients that are in need of which therapy.

It have previous shown that the phenotype of circulating tumor cells (CTC) in the blood mirror that in the bone metastases, and that CTC-characteristics are prognostic . Phenotypically different CTC can be isolated with a novel by me patented method (PCT-application).

Recent finding from the prostate cancer group in Umeå show the existence of phenotypically different subgroups of bone metastases with different molecular drivers. Subgroups that probably respond differently to different treatments. In collaboration we are now developing a subgroup specific panel for CTC characterization that allows the detection of PC molecular subgroup. In this way we can test if we can predict treatment responses in a newly started randomized adaptive multi-arm multi-center clinical trial.

In summary my goal is to improve the outcome of patients with prostate cancer by investigating the phenotypes prostate cancers in both tissues and liquid biopsies and to investigate therapeutic options, metastatic potential and tumor biology in both clinical samples and in-vivo and in vitro models.

Strengths in lab

I have a broad experience of both clinical trials, biomarker discovery and translational research with focus on isolation of Circulating tumor cells (CTC). My engagement in steering groups in clinical trials, both international (MSKCC, Hamburg and Homburg) and national (SPCG xxxx) in combination with unique biobanks and coupled clinical information this opportunity to work in WCMM in Umeå gives a great opportunity to further strengthen the prostate cancer research in Umeå.

Within my new position I have now initiated a project that will be able to “harvest” CTCs from patients, not only from prostate but also other cancers, with a more than 100-fold increase in number of cells retrieved for further characterization, culturing and implantation into nude mice.

Through my clinical work at the Urology clinic in Umeå and also twice a month in Gothenburg, the number of patients available for the clinical trials can be increased. The large biobank of prostate blood and tissue samples from prostate cancer patients in all phases of the disease collected in Umeå as part of the UCAN project will be an important asset. I aim at speeding up both the implementation of promising markers and further explore and understand the tumor biology behind lethal prostate cancers and how they should be treated.

Development of castration resistant prostate cancer is reflected in circulating tumor cells

Andreas Josefsson, Jan-Erik Damber, Karin Welén

**Background**

The development of castration resistant prostate cancer (CRPC) is associated with several alterations in gene expression. Specifically, several genes involved in steroidogenesis and androgen signaling are increased in CRPC. The amount of circulating tumor cells (CTCs) is a biomarker for CRPC progression, and CTC expression of the constitutively active androgen receptor (AR) splice variant 7 (ARV7) predicts poor response to drugs targeting the androgen signaling axis in CRPC. In the present study we investigate whether gene expression changes can be detected in CTCs during CRPC development.

**Methods**

CTCs were isolated from blood samples from 29 patients with metastatic prostate cancer before androgen deprivation therapy (ADT) and at development of CRPC. A panel of genes related to prostate cancer progression and metastasis was analysed with RT-qPCR after gene specific pre-amplification. Signals were normalized to the average signal strength of samples. The paired samples before ADT start and at relapse of CRPC were compared to identify induced gene expression changes in CTCs.

**Results**

Of the analyzed genes 14 displayed a significantly changed gene expression at CRPC relapse compared to before start of ADT. The genes with an increased expression at CRPC relapse were related to steroidogenesis and AR-signaling (AKR1C3, SRD5A1, AR, ARV7) and anti-apoptosis (BCL2). Genes downregulated at CRPC were related to epithelial characteristics (EPCAM, KRT19, TACSTD2, and HER2), prostate markers (FOLH1, KLK3 and PSMA) together with a gene related to neuroendocrine differentiation (AGR2) and epithelial-to-mesenchymal transition (TWIST1).

**Conclusion**

The need for clinically relevant liquid biomarker are urgently needed in metastatic prostate cancer and also tools for methods for focused and explorative approached to find mechanism of resistance and metastatic spread. This study show that a predefined and gene panel could validate and also follow the relative change in circulating tumor cells from sensitive to resistance to ADT, indicating that CTC is a reliable and promising tool for both precision medicine and also for exploring mechanism of resitance and metastatic spread.

Robin Kahn

Wallenberg Centre for Molecular Medicine, Lund University

Associate Professor and Consultant at Department of Clinical Sciences in Lund, Div. Pediatrics, Skåne University Hospital and

Lund University

robin.kahn@med.lu.se

http://portal.research.lu.se/portal/en/organisations- researchgroups/center-of-pediatric-rheumatology(0d51d02f- b2b7-47fe-a176-33f3c31089d1).html

Area of interest

– Can children get rheumatic joint disease? Is it a life-long disease? Does my child really need all these drugs!!!

These are the questions we hear every week when meeting families to children with newly diagnosed inflammatory joint disease.

To get a chronic disease will have a major impact on your day-to-day life and suddenly you are different… you need medications, repeated doctors’ visits… and you have pain.

– Why did I get this?!

In our research group, we try to answer these questions that patients and parents ask every day.

Although we nowadays have an extensive array of treatment options blocking inflammation, none of these will restore the immunological homeostasis of the joint; and even though juvenile arthritis has been described for several decades as a specific form of arthritis, we still don’t know the long-term prognosis in these children.

So…. the most important questions for the patient are still unanswered.

This is the starting points of our research are:

• To study the long-term prognosis and comorbidities in children with juvenile idiopathic arthritis,

and how this has been affected by modern treatments

• To study the pathogenesis of juvenile arthritis with special focus on the immunological

profile of the joint

Strengths in lab

We are a truly translational laboratory where we take our clinical hypothesis to the lab-bench and back. As we are well integrated in the clinical practise at the children’s hospital in Lund, we have access to their biobank containing thousands of blood and joint fluid samples. We also collect synovial biopsies from children with arthritis. In our lab we investigate leukocyte polarization patterns using flow cytometry, qPCR and immunofluorescence. The most important strength of our lab is the translational connections.

Abstract

**Introduction**

Monocytes are heterogenous cells implicated in the pathogenesis of several autoimmune diseases. These cells are highly plastic, able to undergo polarization in response to the environment, acquiring pro-inflammatory (M1) or anti-inflammatory (M2) features. The polarization state influences their effector functions and the interaction with the microenvironment. Here, we aimed to map the polarization state and functional properties of synovial monocytes in Juvenile idiopathic arthritis (JIA).

**Methods**

Subtype distribution and polarization of synovial monocytes in patients with JIA were compared to paired circulating monocytes by flow cytometry and qPCR. Phagocytosis and ROS production were assessed by PhagoTest and PhagoBurst. Synovial fluid (SF) was analyzed for cytokines and polarization ability by stimulation of monocyte-derived macrophages.

**Results**

‘Intermediate’ CD14+CD16+ monocytes were more frequent in SF compared to blood (median (IQR): 59.8% (53.5-79.8) vs 5.9% (4.3-7.5), p<0.001). These monocytes were

polarized to M1(CD40+) (58.8% (38.5-79.4))- or chimeric M1M2(CD40+CD206+) (21.3% (12.4-40.8) phenotypes. Accordingly, synovial CD14+ monocytes had elevated mRNA expression of both M1 and M2 polarization markers and displayed an impaired phagocytosis of opsonized FITC-labeled *E.Coli*, measured by percentage of positive cells (25% (16.2-28.9) vs 70.7% (63.2-73.6, p<0.063)).

**Conclusion**

Synovial monocytes are mainly M1 and chimerically M1/M2 polarized. Their influence on synoviocytes and their impaired phagocytosis indicate an important role of monocytes in the pathogenesis of oligoarticular JIA.

Cecilia Koskinen Holm

Wallenberg Centre for Molecular Medicine, Umeå University

Associate Senior Lecturer at the Department of Odontology

Resident dental surgeon, Oral and Maxillofacial Surgery, University

Hospital of Umeå

cecilia.koskinen@umu.se

Area of interest

Inflammatory diseases affecting the tooth supporting tissues

The periodontium is highly specialised connective tissue, with the function to support the tooth and enable attachment of the tooth to the jawbone. It consists of the gingivae, alveolar bone, periodontal ligaments/fibers and root cementum. Chronic inflammatory conditions in the periodontium is triggered by commensal micro-organisms that colonise the tooth surface and induce an immune response, both of the innate and adaptive immune system. The early phase of disease is known as gingivitis. In some but not all individuals, untreated gingivitis proceeds to periodontitis, and as a result of a hypo- or hyperactive immune system an irreversible bone and connective tissue degradation is noticed. Ultimately the result of the disease is loss of teeth. The severity of the disease is not dependent on the amount commensal bacteria on the tooth surface, but rather on the host-susceptibility and immune response. In our group we study inflammatory driven bone resorption and connective tissue alterations in relation to periodontitis.

My main research project aims to study periodontal Ehlers Danlos syndrome (pEDS), a subtype of the connective tissue disease Ehlers Danlos syndrome. pEDS main feature is severe periodontitis debuting in early age with loss of teeth in young adulthood. It has been shown that individuals with pEDS exhibit heterozygous missense or in-frame insertion/deletion mutations in *C1R* or *C1S* encoding the complement factors C1r and C1s in the classical complement pathway. Therefore, the goal is to unravel by which mechanisms the mutated *C1R* and *C1S* contribute to pEDS in order to find possible treatment strategies.

Strengths in lab

In our lab we use techniques ranging from mouse and human primary cell culture to in vivo mouse models. We have the expertise to perform a wide range of cell and molecular biology technics in combination with histochemical and histomorphometric analyses of calcified and de-calcified tissue. Moreover, we have access to oral tissue and blood samples from well characterized individuals (periodontally healthy and diseased).

By which Mechanisms can Mutations in Complement factor genes contribute to Periodontal Ehlers-Danlos syndrome?

By which Mechanisms can Mutations in Complement factor genes contribute to Periodontal Ehlers-Danlos syndrome?

**The purpose** of this project is to study epithelial and connective tissue defects in relation to C1r and C1s, which are components of the immune complement system. With our research we hope to **find new treatment strategies** for periodontal Ehlers-Danlos syndrome (pEDS), which is a disorder that render in fragile, dysfunctional oral soft tissue and severe periodontitis in young age. Interestingly, it has recently been shown that pEDS is linked to mutations in *C1R* and *C1S*. Although, the mechanism between the complement factor mutations and the clinical symptoms of pEDS is still unrevealed.

The **specific aim** is therefore to investigate the importance of *C1R* and *C1S* mutations for fibroblast and keratinocyte function and behaviour. Moreover, the aim is to study the effects of the mutations in relation to the immune system. For those purposes, we will:

• Develop a **3D-gingiva** (oral tissue) model and introduce the ***C1R* and *C1S* mutations** in the

cells contained in the model

• Use the mutated and wild-type model to histologically and morphologically compare the cells

and the structure of the tissue

• Use the mutated and wild-type models to analyze and compare gene and protein expression of

importance for keratinocyte and fibroblast growth and function

• Develop a ***C1R*-mutant mouse** to be able to study the mutations impact on the immune system

• Use ***in vivo* inflammation mouse models** to investigate differences between wild-type and

mutated mice in relation to the innate immune response

Francisca Lottersberger

Wallenberg Centre for Molecular Medicine, Linköping University

Senior Lecturer at the Department of Medical and Health Sciences (IMH), Division of Drug Research (LÄFO)

Faculty of Health Sciences and Medicine

francisca.lottersberger@liu.se

liu.se/en/employee/fralo26

Area of interest

Chromatin Mobility and Genome Integrity

Francisca Lottersberger, PhD, Senior Lecturer, studies the molecular pathways that encompass DNA Damage Response (DDR) signaling, with the aim to develop tools for the diagnosis and tailored treatment of cancer.

The integrity of our DNA is continuously threatened by endogenous and exogenous sources of damage, which can potentially give rise to mutations and chromosome rearrangements. Eukaryotic cells have evolved complex DNA Damage Response pathways to recognize and repair such damages. Defects in these pathways promote genome instability, tumorigenesis, and cancer progression. At the same time, the linear chromosomes are at risk of being mistaken as sites of DNA damage and failure in protecting telomeres from the DNA Damage Response pathways has also been associated with genome instability and tumor progression.

The lab uses a variety of approaches in order to dissect the molecular pathways that encompass DNA Damage Response, chromatin dynamics, telomere maintenance, tumorigenesis and ageing. In particular, we are dissecting the mechanisms that regulate chromatin mobility, by identifying new molecular factors involved in it and by studying the consequences of altered mobility on genome integrity in both normal and cancer cells. As an ultimate goal, we aim to develop new tools for the diagnosis and tailored treatment of specific life-threatening cancers.

I graduated at the University of Milano-Bicocca in 2006, with a thesis investigating the functions of the highly conserved protein 14-3-3 in cell cycle regulation and DNA damage response in the yeast *S. cerevisiae*. In 2008, I joined the laboratory of Professor Titia de Lange at the Rockefeller University to elucidate the role of DNA resection and chromatin mobility in promoting DNA repair in mammalian cells. In 2017, I was appointed WCMM Fellow at the Linköping University.

Strengths in lab

The experimental techniques applied in the lab are: live-cell imaging and semi-automatic quantification of chromatin mobility, induction of DNA damage and analysis of cellular response, detection of chromatin binding proteins by immunofluorescence/FISH/ChIP, telomere length and single strand overhang detection and chromosomal rearrangements analysis, as well as mouse and human genetic approaches and standard biochemical and cell-biological methods.

Abstract

DNA Damage Response (DDR) is fundamental to prevent mutagenesis and cancer development. One of the most dangerous DNA lesions is a DNA Double Strand Break (DSB) and eukaryotic cells have evolved highly regulated pathways to repair it. Failures in these processes lead to genome instability and tumorigenesis. We previously defined dynamic cytoplasmic microtubules and the nuclear transmembrane LInker of Nucleoskeleton and Cytoskeleton (LINC) complex as key regulators of chromatin mobility at the sites of DNA damage, together with the chromatin binding factor 53BP1. We also demonstrated that deficiencies in promoting the roaming of the chromatin in the nucleus delay repair of DSBs that are distal, highlighting the importance of chromatin mobility in the context of the DDR. In a continuation of this published work, we are focusing on the influence of nuclear architecture and chromatin state over DNA mobility and damage repair. Recent findings on this issue will be presented.

Iben Lundgaard

Wallenberg Centre for Molecular Medicine, Lund University

Associate Senior Lecturer at Department of Experimental Medical Science, Lund University

iben.lundgaard@med.lu.se

lundgaardlab.com

Area of interest

The Glymphatic System: Glia-Immune Interactions

Despite the brain’s high level of metabolic activity the central nervous system (CNS) does not contain any lymphatic vessels. The cerebrospinal fluid (CSF) is driven into peri-vascular spaces where exchange of solutes takes place and this mediates brain-wide clearance. The peri-vascular bulk flow system was named the glia-lymphatic (glymphatic) system due to the crucial role of astrocytes’ aquaporin 4 (AQP4) water channels. The glymphatic system is akin to the lymphatic system and also connects with the conventional lymphatic system upon drainage from the CNS. Due to this clearance function, we believe that astrocytes and CSF are crucial for removing metabolites and thus maintaining a healthy milieu and preventing diseases.

Our lab is interested in the glymphatic system due to its function as a macroscopic clearance system. Among specific research topics at the Lundgaard laboratory is the role of the glymphatic system in normal physiology aging, neurodegenerative diseases and in CNS immune function.

Strengths in lab

Fluorescence microsopy, in vivo microscopy, animal models of neurodegenerative diseases, 3D reconstructions of lipid-cleared tissues (CLARITY/vDISCO), immunostainings.

CNS-to-lymph node solute transport perpetuates autoimmune disease

Multiple Sclerosis (MS) is an autoimmune demyelinating disease of the central nervous system. Lesions in MS patients are characterized by demyelination and immune cell infiltration often forming peri-vascular cuffing around blood vessels. In the healthy brain, perivascular spaces are highways of transport for the glymphatic system, which mediates brain-wide clearance of solutes. Here we used the experimental autoimmune encephalomyelitis (EAE) mouse model of MS to map glymphatic transport of cerebrospinal fluid (CSF). Perivascular CSF influx and clearance was reduced in the brain. Inhibition of glymphatic function or cervical lymphatic vessel occlusion significantly reduced the disease burden when begun in the pre-symptomatic, but not the symptomatic phase. Treatment with the immune-modulatory drug interferon beta 1 (IFN-b) rescued glymphatic function, whereas adaptive T-cell transfer inhibited glymphatic function similarly to regular EAE. These observations suggest that the glymphatic-to-lymphatic axis plays a role in the effector phase of EAE and targeting CSF transport might be a novel mechanism to curb disease burden in MS patients.

Pernille Lærkegaard Hansen

Wallenberg Centre for Molecular and Translational Medicine,

University of Gothenburg

Co-opted Professor, Institute of Neuroscience and Physiology

Senior Director, Head of Bioscience, CKD. CVRM, IMED, AstraZeneca

pernille.laerkegaardhansen@astrazeneca.com

Area of interest

Prof. Hansen has over 20 years of experience in renal physiology and pathophysiology. She holds a PhD in physiology from the University of Southern Denmark. After a postdoc from the National Institutes of Health, National inst. of Diabetes, Digestive and Kidney Diseases, USA she later became Professor and Deputy Department Head of Cardiovascular and Renal Research at the University of Southern Denmark.

Prof. Hansen has significant experience from the field of renal hemodynamics and thorough experience in *in vitro* and *in vivo* systems as well as translational approaches. Her main research interests are vascular function both in normal physiological settings and in cardiovascular and renal diseases and her research group use techniques from the molecular level, to *in vitro* techniques to the integrated in vivo level measuring continuously GFR, RBF and MAP in awake mice. The major aim of Prof Hansen’s research activities, in the academic setting, has fallen into three main areas; 1) calcium channels, 2) adenosine and 3) aldosterone and their involvement in cardiovascular and renal physiology and pathophysiology. She has many international collaborations and previously prioritized collaborations to Odense University Hospital and perform functional studies on freshly isolated human blood vessels.

Prof Hansen is working at AstraZeneca, Gothenburg, Sweden as Head of CKD, Early CVRM. The role as Senior Director at AstraZeneca includes scientific and management tasks being responsible for the early phase of the therapeutic area, Chronic kidney disease. Prof Hansen leads the strategy and the early pipeline, and she is responsible for new CKD relevant scientific projects and collaborations. Her interest in the renal hemodynamics has led to two recent collaboration projects on vasculature rarefaction in CKD and a newly established 3D bioprinted model together with colleagues at AstraZeneca and external experts. With Christer Betsholtz at Karolinska, they aim to understand the molecular drivers of vascular rarefaction in CKD by using single cell sequencing. With Jennifer Lewis, Harvard Medical School, their vision is to build a high through-put humanized vascularized tubular model that can be used for understanding cross talk between peritubular capillaries and the tubules. The outcome of the two projects will increase the understanding of drivers of loss of capillaries in CKD and inform upcoming projects on new medicine for treating Chronic Kidney Disease.

Cristina Maglio

Wallenberg Centre for Molecular and Translational Medicine, University of Gothenburg

Researcher at Institute of Medicine

Resident Physician at Department of Rheumatology and Inflammation

cristina.maglio@gu.se

https://wcmtm.gu.se/research-groups/maglio

Area of interest

Adiponectin at the crossroad between metabolism and inflammation

Adipokines are cytokines mainly produced by the adipose tissue and they play a role in metabolism, inflammation and immunity. In subjects with obesity, the release of adipokines is impaired, either increased or decreased.

Adiponectin is probably the most well studied adipokine. This adipokine is highly abundant in serum and is mostly produced by the white adipose tissue; nevertheless, circulating adiponectin levels are low in subjects with obesity and metabolic syndrome. However, recent studies have suggested that adiponectin might simply be a marker of glucose homeostasis rather than a key player.

Adiponectin is increased in both serum and joint fluids of patients affected by rheumatoid arthritis (RA), an inflammatory joint disease affecting about 1% of the Swedish population. Moreover, adiponectin serum levels correlate with RA disease activity. *In vitro* studies have also shown that adiponectin has both pro- and anti-inflammatory properties. In *in vitro* studies performed in joint fibroblasts from subjects with RA, adiponectin is able to stimulate the production of the pro-inflammatory cytokines interleukin 6 (IL-6), IL-8 as well as matrix metalloproteinases, suggesting a possible role of this molecule in the pathogenesis of RA.

A genome-wide association study published in 2013 (*Negi S et al, Arthritis and Rheumatism 2013*) has described a genetic variant in the ADP ribosylation factor like GTPase 15 (ARL15) gene to be associated with increased risk of developing RA. The same variant associates with increased circulating levels of adiponectin, thus suggesting a causal effect of this adipokine on the development of RA.

**Strengths in lab (technologies, methods):**

• Lab: cell isolation/collection from human material, cell culture, flow cytometry, ELISA, western

blot, confocal microscope etc.

• Biostatistics: including univariate/multivariate methods, power analysis, effect size, survival

analysis etc.

• Genetics: DNA extraction, DNA amplification, genotyping, sequencing, interpretation of

genome- and exome-wide association studies as well as Mendelian randomization studies.

• Epidemiology: collecting, analysing and interpreting data from big patient cohorts.

Role of adiponectin in the development of rheumatoid arthritis (RA)

In a cohort of about 4000 subjects with obesity followed-up for up to 29 years, serum adiponectin predicted the development of rheumatoid arthritis (RA). Obese subjects having both baseline adiponectin and C-reactive proteins above the median had an almost 3-times increased risk of developing RA compared with those with both adiponectin and C-reactive protein below the median.

To determine if other cytokines known to be elevated in subjects with RA were able to predict the development of RA as adiponectin, we performed a nested case-control study in the same cohort of obese subjects. We included 82 subjects who developed RA and 410 controls matched for age, sex, body-mass index and smoking and we measured serum levels of resistin, leptin and visfatin. Adiponectin showed to be predictive for the development of RA independently of resistin, leptin and visfatin.

To determine if adiponectin plays a causal role in the pathogenesis of RA and is not merely a marker of disease, we are currently performing *in vitro* studies in cells involved in RA, and a Mendelian randomization study. Both the *in vitro* studies and the Mendelian randomization project are ongoing. We have some preliminary data on cytokine production from healthy peripheral blood mononuclear cells (PBMCs) and fibroblast-like synoviocytes (FLS) stimulated using adiponectin. Adiponectin is able to induce IL-6 and TNF-α in healthy PBMCs, IL-6 and IL-8 in healthy FLS. These results suggest that adiponectin plays a role in the development of RA. Experiments using PBMCs and FLS from RA patients will be performed in short future to further confirm the effect of adiponectin on these RA-related cells.

Martin Magnusson

Wallenberg Centre for Molecular Medicine, Lund University

Associate Professor and Senior Consultant, Department of Clinical Sciences Malmö, and Div. of Cardiology Skåne University Hospital and Lund University.

martin.magnusson@med.lu.se

www.med.lu.se/wcmm/researchers/fellows#magnusson

Area of interest

The core my research is to bridge the surprisingly under-explored gap between the “omics” of epidemiology (e.g. genomics, metabolomics and proteomics) and biological and clinical function. Thus, a major component of my research aims to enhance the understanding of causes to progressing diabetes and cardiovascular disease where I together with my co-workers invest large efforts in metabolomics and proteomics. However, a central issue is that we do not stop at finding metabolites/proteins and metabolomics/proteomic patterns associated with risk of progressing disease, but also examine the importance of genetic predisposition behind such relationship to find causal association and we also aim to explore the underlying mechanisms (by *in vivo/vitro* experiments and even human trials if applicable). Here we have already discovered two novel candidates in the amino acids; isoleucine, phenylalanine, and tyrosine but also in dimethylglycine, which will be further tested to shed light on the biochemical underlying mechanisms. It is conceivable to assume that our causality assessment of biomarkers of disease will provide guidance on whether or not drug development targeted at the biomarker in question is worthy to pursue.

I have my clinical position at the heart failure clinic at Skåne University Hospital where I work as a senior cardiology consultant at the Department of Heart Failure. There, I have initiated a major translational heart failure research project (the **HeAR**t and failure in**VEST**igation study - HARVEST), as principal investigator, aiming to create a unique infrastructure for advanced heart failure research. The purpose is to identify optimal conditions for prevention and treatment of heart failure and associated comorbidities (e.g. cognitive dysfunction and dementia). A large cohort (1500 patients) will be established and followed prospectively through 6-moths re-examination and established Swedish national and local registers. This prospective study, with to date approximately 500 patients enrolled, has the potential to lead to the discovery of new mechanisms and potential therapeutic targets for the treatment of heart failure. Further, it will provide knowledge on how to treat heart failure to avoid concomitant dementia. This initiative has already generated several publications and submitted manuscripts. One of the submitted manuscripts originated from the HARVEST study shows that selenium deficiency is independently associated with of poor outcome in heart failure patients and encourages future studies examining if supplementation of selenium might improve prognosis (patented with Magnusson listed as co-inventor). The results from this particular study are presented as an abstract on this year (2019) NMMP-congress.

Adil Mardinoglu

SciLifeLab/KTH Royal Institute of Technology

Associate Professor of Systems Biology, KTH Royal

Institute of Technology

adilm@scilifelab.se

www.sysmedicine.com

Area of interest

Systems biology, or systems medicine that is the application of systems biology approaches to medical research and medical practice, provides powerful tools to elucidate pathophysiological responses as well as the underlying molecular mechanisms involved in the progression or occurrence of disease. The principal tools of systems biology are biological networks, including genome-scale metabolic models (GSMMs), gene regulatory networks (GRNs), protein–protein interaction networks (PPINs), signalling networks and gene co-expression networks (GCNs). These biological networks provide excellent functional contexts for integrating and making sense of large –omics datasets. This network knowledge can be converted into computational models and then used in network dependent analysis or simulations of biological systems.

**Strengths in lab (technologies, methods):**

My lab focusses on the generation of cell/tissue specific biological networks and analysis of the clinical data using such networks. The ultimate goal is the use of multi-omics data to reveal the underlying molecular mechanisms involved in the progression of the metabolic diseases and discover novel drug target and biomarkers that can be used in the development of efficient treatment strategies.

Abstract

To develop novel strategies for prevention and treatment as well as to gain detailed insights about the underlying molecular mechanisms of liver associated diseases – fatty liver disease, cirrhosis, type 2 diabetes and hepatocellular carcinoma, it is vital to study the biological functions of liver and how liver interacts with other human tissues as well as with the gut microbiota. Biological networks including metabolic, transcriptional regulatory, protein-protein interaction, signalling and co-expression networks can provide a scaffold for studying biological pathways operating in the liver in connection with disease development in a systematic manner. I will present our recent work where biological networks have been employed to identify the reprogramming in liver physiology in response to complex diseases including NASH/NAFLD and liver cancer. I will further discuss how this mechanistic modelling approach can contribute to identification of drug targets which may lead to design of targeted and effective treatment strategies. Finally, I will present a roadmap for the successful development of small molecules that can be used in the treatment of NASH/NAFLD and liver cancer.

Anja Meissner

Wallenberg Centre for Molecular Medicine, Lund University

Associate Senior Lecturer, Department of Experimental

Medical Science, Lund University

anja.meissner@med.lu.se

portal.research.lu.se/portal/en/persons/anja-meissner(6a464116-a3b7-4cd2-b418-2206a3f2d1b0).html

Area of interest

Cardiovascular disease such as heart attack, stroke, and peripheral vascular disease, is the number-one health problem in the world. Despite remarkable progress in diagnosis and prevention, cardiovascular diseases cause disability and death at an astounding rate. The best opportunities to develop and implement new strategies for preventing and treating cardiovascular disease lies in the understanding of its underlying mechanisms. Our research aims to isolate novel therapeutic targets that effectively prevent and most importantly, also reverse complications mediated by cardiovascular risk factors such as hypertension.

Specifically, we are interested in sphingosine-1-phosphate (S1P) signaling and its role in the regulation of the vascular and the immune system. We recently described a novel role for S1P and its generating enzyme SphK2 in the pathogenesis of experimental hypertension, whereby hematopoietic Sphk2 activity crucially regulates the hypertension-induced elevation of plasma S1P. Remarkably, elevated plasma S1P levels have also been reported in human hypertension. Thus far, our earlier work provides ample evidence that S1P plays a key role in immune cell recruitment, cytokine production and vascular tone regulation not only during experimental hypertension but also associated target organ damage. Inhibition of S1P-guided T-cell chemotaxis for instance, protects from a cerebral accumulation of CD3+ T-cells and the development of memory deficits in hypertensive mice.

Besides hypertension, our lab also investigates the role of S1P signaling as a novel molecular link in cardiopulmonary distress. In a mouse model of heart failure, we found strong indications that pro-inflammatory cytokines provoke an accumulation of S1P in the lung with implications for airway hyperresponsiveness, adverse remodeling and tissue inflammation. In a cross-disciplinary endeavor, we aim to explore a novel molecular mechanism, linking the heart and the lung in disease.

Strengths in lab

Animal models of hypertension, myocardial infarction and heart failure, cerebral small vessel disease and stroke

S1P mass spectrometry

Small vessel dissection, pressure myography

Flow cytometry analyses of different tissue compartments including brain, lung and larger blood vessels

Inhibition of SphK2 activity mitigates Experimental Hypertension by reducing Systemic and Vascular Inflammation

Nicholas Don-Doncowa, Yun Zhanga & Anja Meissnera,b

a Department of Experimental Medical Sciences, Lund University, Sweden

b Wallenberg Centre for Molecular Medicine, Lund University, Sweden

**Background:**

The immune system and particularly T-cells play a considerable role in the pathogenesis of hypertension. Despite substantial experimental efforts, the molecular mechanisms underlying the nature of T-cell activation contributing to the disease onset and perpetuation are still elusive. We recently reported a critical role of the bioactive phospholipid sphingosine-1-phosphate (S1P) in blood pressure (BP) responses to Angiotensin II (AngII) in a mouse model. By regulating S1P plasma levels and hence, T-cell mobilization from secondary lymphoid organs, the activity of the S1P generating enzyme SphK2 is crucially involved in the regulation of BP levels. Based on these findings, we presently aimed to investigate the therapeutic potential of pharmacological SPHK2 inhibition in experimental hypertension, focusing on T-cell responses.

**Materials and Methods:**

In a murine model of AngII-induced hypertension, we assessed the potential therapeutic effect of specific SPHK2 inhibition on *(1)* BP levels using tail cuff plethysmography, *(2)* immune cell populations using flow cytometry, and *(3)* S1P levels using mass spectrometry.

**Results:**

Therapeutic treatment with the SPHK2 inhibitor significantly lowered BP levels, which was accompanied by a decrease in the number of circulating T-cells. Remarkably, our data reveal a treatment-associated change in CD4+ T-cell phenotype with reduction of both T-helper (Th) 17 and Th1 cells, which might be causative to the attenuated tissue inflammation we observe in mice treated with SPHK2 inhibitor.

**Conclusion:**

Our results point to a critical contribution of SPHK2-S1P signaling in immune-cell responses and tissue inflammation during AngII-induced hypertension and reveal potential therapeutic properties of SPHK2 inhibition. Thus, the inhibition of S1P production by antagonizing SPHK2 activity might evolve as new therapeutic strategy to efficiently controlling BP, hypertension-related inflammation and associated target organ damage.

Ignacio Mir Sanchis

Wallenberg Centre for Molecular Medicine, Umeå University

Associate Senior Lecturer at the Department of Medical Biochemistry

and Biophysics

ignacio.mir-sanchis@umu.se

www.imsresearchlab.com

Area of interest

Staphylococcal Mobile Genetic Elements – Phage Therapy

Our main goal is to understand the fundamental biology of Mobile Genetic Elements (MGEs) and direct this knowledge towards phage therapy *-employment of bacteriophages to treat bacterial infections-* and other biotechnology applications.

Antimicrobial resistance is one of the most challenging threads we are facing worldwide. Bacteria become resistant to antibiotics by acquiring Mobile Genetic Elements (MGEs) by horizontal gene transfer mechanism. Using *Staphylococcus aureus* as model system, we study several MGEs involved in pathogenesis: i) staphylococcal cassette chromosome elements, which is responsible for the highly problematic phenotype methicillin resistant S. aureus or MRSA, ii) *Staphylococcus aureus* Pathogenicity Islands (SaPIS) and iii) staphylococcal bacteriophages. Both SaPIs and phages are involved in deadly toxinosis.

We are primarily interested in understanding how these MGEs express their genes and replicate their DNA. DNA replication of MGEs is particularly attractive, in the sense that not all components for DNA replication are encoded by the mobile element but some are hijacked from the host. This feature invariably will lead us to identify new complexes as drug targets.

Although some staphylococcal phages encode toxins and virulence genes, there are some phages that do not encode known virulence genes, are lytic and infect a broad spectrum of bacterial strains, being good candidates for phage therapy. For those phages proposed as candidates for phage therapy, more than 70% of their gene content has *unknown function*. We aim to unveil those unknowns as well as to engineer staphylococcal bacteriophages with enhanced features to be used as treatment.

Strengths in lab

We use genetics and molecular biology approaches combined with biochemistry and structural biology techniques.

Abstract

Methicillin Resistant *Staphylococcus aureus* (MRSA) is a worldwide public threat, killing hundreds of thousands of people annually. The methicillin resistant phenotype is caused by a mobile element called Staphylococcal Cassette Chromosome (SCC). In nature these elements move horizontally from donor to recipient cells, but this horizontal gene transfer between bacteria has not been detected in laboratory conditions. Although we know that SCC elements encode their own site-specific recombinases responsible for the integration/excision of the element, nothing else is known about the biology of these elements. Despite these elements were classified as non-replicative mobile elements we propose that they become replicative at some point during their horizontal transfer. Here I will show that in addition to the site-specific recombinases, SCC elements encode other conserved genes whose products are related to DNA replication. I will show the X-ray crystal structure of two conserved proteins: Cch, an ATPase with helicase activity (2.9 Å resolution) and LP1413, a single stranded DNA binding protein (2.7 Å resolution). Cch has three domains, forms a three layered ring shaped hexamer and its ATPase domain is structurally related to the MCM replicative helicases from the archaea and eukaryotic cells. LP1413, the single stranded DNA binding protein, adopts a winged helix turn helix motif and has a hydrophobic pocket that might be used presumably to interact to other replication related proteins. I will speculate about the role of these proteins *in vivo* during SCC’s biology and transfer.

Johan Normark

Wallenberg Centre for Molecular Medicine, Umeå University

Senior Consultant in Infection Medicine, University Hospital of Umeå

Researcher at the Department of Clinical Microbiology

johan.normark@umu.se

Area of interest

Many important pathogens in man have their normal ecological niche in humans where healthy carriage dominates over disease. The ability of these commensal pathogens to cause disease depends on several microbial factors as well as on genetic and environmental factors in the human host. The interplay between infectious agents affects in carriage and disease impacts on the clearing capacity mediated by the innate and adaptive immune system. This delicate relationship between the microbe and host modulates not only the likelihood for a commensal pathogen to cause disease, but also disease type and disease severity. We have focused upon pediatric malaria infection, relapsing fever and invasive pneumococcal disease.

We aim to understand how multiple infections affect carriage and disease progression.

Strengths in lab

We have developed several murine co-infection models in order to emulate the relationships in a controllable environment. The ulterior goal is to improve the management of the respective diseases and understand the immunological drivers of the acute phase response.

Abstract

Several studies have observed serum lipid changes during malaria infection in humans. All of them were focused at analysis of lipoproteins, not specific lipid molecules. The aim of our study was to identify novel patterns of lipid species in malaria infected patients using lipidomics profiling, to enhance diagnosis of malaria and to evaluate biochemical pathways activated during parasite infection. Using a multivariate characterization approach, 60 samples were representatively selected, 20 from each category (mild, severe and controls) of 690 study participants between age of 0.5-6 years. Lipids from patient's plasma were extracted with chloroform/methanol mixture and subjected to lipid profiling with application of the LCMS-QTOF method. We observed a structured plasma lipid response among the malaria-infected patients as compared to healthy controls, demonstrated by higher levels of a majority of plasma lipids with the exception of even-chain length lysophosphatidylcholines and triglycerides with lower mass and higher saturation of the fatty acid chains. An inverse lipid profile relationship was observed when plasma lipids were correlated to parasitaemia. This study demonstrates how mapping the full physiological lipid response in plasma from malaria-infected individuals can be used to understand biochemical processes during infection. It also gives insights to how the levels of these molecules relate to acute immune responses.

Hanna Nyström

Wallenberg Centre for Molecular Medicine, Umeå University

Resident Physician in Surgery, University Hospital of Umeå

Researcher at the Department of Surgical and Perioperative Sciences

hanna.nystrom@umu.se

https://www.umu.se/personal/hanna-nystrom/

https://www.umu.se/wallenberg-centrum-for-molekylar-medicin/

forskning/wcmm-associerade-forskare/hanna-nystrom/

Area of interest

Colorectal cancer (CRC) is globally the third most common cancer and over 7000 people receive the diagnosis yearly in Sweden. 30-50% of patients with colorectal cancer will develop colorectal liver metastases (CLM) and for those patients, the only hope of a lasting cure is that the metastases can be surgically removed. For those than can undergo liver surgery the 5-year survival is over 50% which is unique for a metastatic disease.

CLM can grow with three different growth patterns (GP) in the liver and these patterns have been linked to prognosis after liver surgery and they also might determine how well the tumour responds to oncological treatment. For example, anti-angiogenic drugs seem to work well in patients with one of the GPs whereas another GP does not require angiogenesis, but instead uses the mechanism of vascular co-option in the existing liver parenchyma. There also seems to be a difference in how the tumour cells are detected (or not detected) by the immune system as well on how the metastatic tumour cells reorganize their microenvironment in the liver.

These findings imply that patients with CLM may benefit from a personalized treatment, but would then mean that we need a way to classify them prior surgery. Currently the GPs are classified based on the tissue after surgery. The differences found in the GPs also means that there might me new targets for drug-development to detect. It is also not known whether these properties can be found already in the primary tumour.

**Strengths in lab**

Areas of expertise: Tumor stroma, extracellular matrix biology, circulating tumor markers, surgery, colorectal cancer, metastatic colorectal cancer, imaging (MRI and PET-CT).

Technologies/methods: Circulating tumor marker analyses focusing on the stroma (ELISA, Multiplex assays), organotypic models for cell invasion studies and studies focusing on stromal-tumor cell interactions, in situ hybridization, RNA sequencing, human Imaging (MRI and PET-CT).

Abstract

Colorectal cancer (CRC) is globally the third most common cancer. 30-50% of patients with colorectal cancer will develop colorectal liver metastases (CLM) and for those patients, the only hope of a lasting cure is that the metastases can be surgically removed. For those than can undergo liver surgery the 5-year survival is over 50% which is unique for a metastatic disease.

CLM can grow with three different growth patterns (GP) in the liver and these patterns have been linked to prognosis after liver surgery and they also might determine how well the tumour responds to oncological treatment. For example, anti-angiogenic drugs seem to work well in patients with one of the GPs whereas another GP does not require angiogenesis, but instead uses the mechanism of vascular co-option in the liver parenchyma. There also seems to be a difference in how the tumour cells are detected (or not detected) by the immune system as well on how the metastatic tumour cells reorganize their microenvironment in the liver.

One of our goals is to find a way to classify the growth pattern in CLM in patient’s prior surgery using modern imaging as well as circulating biomarkers to enable a personalized medicine concept. A second goal is to investigate how the transcriptome differs between the GP and if this hopefully new molecular insights can be used for the development of targeted oncological and surgical therapy. Lastly, we want to understand if these molecular differences can be found in the primary tumour.

Roger Olofsson Bagge

Wallenberg Centre for Molecular and Translational Medicine,

University of Gothenburg

Associate Professor at Department of Surgery, Institute of Clinical Sciences, Sahlgrenska Academy at the University of Gothenburg

Senior Consultant, Department of Surgery, Sahlgrenska

University Hospital

roger.olofsson.bagge@gu.se

wcmtm.gu.se/research-groups/olofssonbagge

Area of interest

Translational Cancer Research

Roger Olofsson Bagge is an Associate Professor and Senior Consultant Surgeon focusing his research interest on translational cancer research. The main clinical interest is breast cancer and malignant melanoma and the overall aim of the research program is to increase the basic understanding of cancer biology and treatment by including patients into clinical trials with a strong translational back-bone. He is Director of the Sahlgrenska Breast Cancer Center and also Head of the Department for Breast and Melanoma Surgery.

A special interest is isolated limb perfusion, which is a unique surgical cancer treatment where a part of the body is isolated surgically, connected to a heart-lung machine and perfused with a deadly dose of a chemotherapeutic agent. Roger is the only surgeon in Sweden performing this treatment, and he also treats international patients via Sahlgrenska International Care. The unique feature of this treatment is that the part of the body having metastases receives a very high dose of chemotherapy while the rest of the body is untreated, this also includes leaving an intact immune system in the patient. His research was the first to show that part of this very effective treatment is mediated by an immunological activation. This original finding has been further studied and refined in the laboratory and have now been translated back to the clinic with a new clinical trial combining isolated limb perfusion with modern immunotherapy using a PD-1 inhibitor.

Another interest concerns the role of exosomes in both cancer progression and as potential treatments. Already in his thesis he explored tumour specific exosomes and their contents derived directly from the liver circulation of patients with uveal melanoma metastases. Based on these very early experiments he is now focusing his pre-clinical research into the basic understanding of the intricate cell to cell communication by exosomes in mediating effects of immunotherapy in melanoma.

All work is heavily based on an extensive translational collaboration and includes several research partners, both in Gothenburg and internationally, and also together with partners in the pharmaceutical industry.

Minimally-invasive isolated limb perfusion (MI-ILP) - a first report of a new technique

Roger Olofsson Bagge1, Per Carlson2, Roya Razzazian2, Christoffer Hansson3, Anders Hjärpe3, Dimitrios Katsarelias1 and Jan Mattsson1

1Department of Surgery, Institute of Clinical Sciences, Sahlgrenska Academy at the University of Gothenburg, Sahlgrenska University Hospital, Gothenburg, Sweden

2Department of Radiology, Institute of Clinical Sciences, Sahlgrenska Academy at the University of Gothenburg, Sahlgrenska University Hospital, Gothenburg, Sweden

3Department of Thoracic Surgery, Institute of Clinical Sciences, Sahlgrenska Academy at the University of Gothenburg, Sahlgrenska University Hospital, Gothenburg, Sweden

**Background**

Isolated limb perfusion (ILP) and isolated limb infusion (ILI) are treatments for patients with locally advanced melanomas and sarcomas of the extremities. The techniques allows for the administration of very high doses of a chemotherapeutic agent regionally with low systemic toxicity. ILP have higher response rates, but requires open surgery for vascular access, whereas ILI is minimally invasive with percutaneous placement of the catheters. A technique that combines the benefits of each method is newly developed, and the aim is to present the feasibility of this new minimally-invasive ILP (MI-ILP) procedure.

**Method**

Six patients, five with melanoma in-transit metastases and one patient with squamous cell carcinoma, were included in a phase I feasibility trial. Percutaneous vascular access of the ipsilateral extremity vessels was performed using the insertion of 8-10 Ch arterial and 12-14 Ch venous Bio-Medicus NextGen® catheters under ultra-sound guidance. The catheters were then connected to an oxygenated perfusion system and the limb was perfused during 60-90 minutes using melphalan alone (n=5) or melphalan and TNF-alpha (n=1). Response (according to WHO criteria) and the worst local toxicity (according to Wieberdink) was evaluated at 3 months.

**Results**

All six treated patients underwent the procedure without the need for conversion to open surgery. The median number of tumors were three, with the largest size of the metastasis measuring in median 15 mm. There were five femoral and one brachial perfusions and the median operating time was 164 minutes. The median perfusion flow was 555 ml/min (range 110-711) and the leakage during perfusion was 0.1% (range 0.0%-3.2%). The complete response (CR) rate was 67%, two patients had a stable disease (SD). Four patients (67%) had a Wieberdink grade II reaction and two patients (33%) had a grade III reaction.

**Discussion**

MI-ILP is a new minimally-invasive technique for tumor treatment in extremities. All the six procedures were successful, without any need for conversion to open surgery, and with similar perfusion characteristics as for standard open-surgery ILP. The major limitation is the limited number of patients treated and further studies are needed to establish the role of MI-ILP in future cancer care.

Antonios Pantazis

Wallenberg Centre for Molecular Medicine, Linköping University

Senior Lecturer, Division of Neurobiology, Dept. of Clinical and Experimental Medicine, Linköping University

Faculty of Health Sciences and Medicine

antonios.pantazis@liu.se

https://liu.se/en/employee/antpa45

Area of interest

The Molecular Determinants of Cellular Excitability

Ion channels are fascinating macromolecular complexes that endow our cells with the ability to sense and generate electrical signals. The Pantazis laboratory combines cutting-edge experimental and computational approaches to understand how the intricate molecular architecture of ion channels relates to their function and regulation; and how ion channel function or dysfunction governs cellular excitability in health and disease.

My main area of research is on ion channels, which are membrane proteins governing cellular excitability: that is, the ability of cells to generate, sense, and respond to, the electrical signals used in nerves, muscles and the heart. I am particularly interested in neuronal voltage-gated calcium (CaV) channels, which couple electrical messages to the potent Ca2+-mediated cytosolic signaling system, responsible for neurotransmitter release, synaptic tuning and even gene expression. CaV channels possess a highly intricate molecular structure, uniquely suited to their varied biophysical properties: my laboratory will study how the CaV channel dynamic structure responds to electrical signals, to ensure the precise amplitude and timing of the Ca2+ signal. Importantly, neuronal CaV channels are critical, and largely underused, drug targets, for many familial and acquired neurological disorders including epilepsy, ataxia and chronic pain. Therefore, our research is not only important for understanding how our bodies work at the molecular level, but also for the development of next-generation drugs with superior potency and selectivity.

Strengths in lab

The Pantazis laboratory will combine cutting-edge and innovative experimental and computational approaches to (i) unravel the complex activation and regulation mechanisms of ion channels; (ii) discover how their intricate structure and conformational rearrangements relate to their functional properties; and (iii) understand how ion channel function (and dysfunction) regulates physiological electrical signaling or causes aberrant excitability and disease. The structural and functional properties of normal, and disease-causing, ion channel macromolecular complexes will be interrogated in heterologous expression systems using voltage clamp fluorometry, distance-encoding photoinduced electron transfer (DEPET) and functional and structural modeling. Ion channel role in neuronal excitability and diseases such as epilepsy will be evaluated in cultured cells and excised tissues using hybrid electrophysiological-computational approaches (dynamic clamp) and Ca2+ imaging.

The molecular transitions underlying infantile epilepsy: a case for translational biophysics

An exome-based panel in an infant suffering from epilepsy revealed a de novo c.906 T>G change in KCNA2, which encodes the pore-forming subunit of KV1.2 channels. The missense mutation results in substitution F302L, at the S4 helix of the KV1.2 voltage-sensing domain (VSD). Our previous electrophysiological investigations revealed that KV1.2-F302L channels activate up to 2-fold faster, and exhibit a more hyperpolarized voltage dependence (~13 mV) compared to KV1.2-WT. Voltage clamp fluorometry revealed that augmented channel opening is accompanied by enhanced VSD activation.

Moreover, KV1.2-F302L channels exhibit accelerated slow inactivation by up to ~3-fold and slower inactivation recovery (~2-fold at –80mV) than wild-type. Their inactivation voltage dependence is shifted to more hyperpolarized potentials (ΔV0.5=–13mV). Investigations at more physiological temperature (33°C) recapitulated the results of room-temperature experiments: F302L enhances both channel activation (gain of function) and inactivation (loss of function).

Computational simulations of a neuronal excitability model revealed that the F302L gain-of-function effect is dominant: a classical Hodgkin-Huxley neuron (1μF/cm2) was complemented with KV1.2 conductance models (36mS/cm2) fit to electrophysiological data from either WT or F302L channels. HH neurons with KV1.2-F302L were less excitable than those with KV1.2-WT, having a higher stimulus threshold for tonic firing (WT: 4.8μA; F302L: 14.2μA).

Based on our experimental and computational results, it is likely that the seizures arise due to inhibitory neuron suppression, increasing overall circuit excitability. This is consistent with the patient’s current anti-epileptic therapy (topiramate), which enhances inhibitory neurotransmission.

Gesine Paul-Visse

Wallenberg Centre for Molecular Medicine, Lund University

Associate Professor and Senior Consultant, Department of Clinical Sciences Lund, Neurology, Skåne University Hospital and

Lund University

gesine.paul-visse@med.lu.se

http://portal.research.lu.se/portal/en/organisations-researchgroups/translational-neurology-tny(c6575435-f465-4477-99f4-36fad083aaeb).html

Area of interest

Neuroprotection & neuroregeneration: From target

identification to clinical trials

Being a neurologist, my vision is to contribute to discovery, development and clinical implementation of novel therapies for neurological disorders. I work with patients with all neurological disorders, but have a specialized profile in Parkinson’s disease. As such, I am head of the clinical movement disorder team and elected board member of the Swedish Movement Disorder Society (Swemodis), the Swedish Parkinson Academy and the Network of European CNS Transplanation and Restoration (NECTAR). As a GCP- trained clinician, I have considerable experience in clinical studies, with a focus on not just therapeutic improvement but structural regeneration.

Having also a background in basic science, I lead a preclinical research group “Translational Neurology” at Lund University, which allows me to work with research questions using a translational approach.

We examine disease mechanisms of neurodegenerative disorders, in particular how neurovascular changes intitiate or sustain neurodegeneration. We have a special focus on pericytes, perivascular cells at the interface between blood and brain and investigate the hypothesis that pericytes are key players in inflammation and neurodegeneration. We study neurovascular changes in disease models of Parkinson’s disease, Huntingtons disease, stroke and glioblastoma multiforme and respective human samples. Our focus is to elucidate pathological changes in blood-brain barrier properties and cell signaling between cells forming the blood-brain barrier with the aim to identify new cellular and molecular therapeutic targets.

Strengths in lab

We work with cell culture models, including human cell lines, life cell imaging. We also utilize several disease models in mice in vivo that we combine using transgenic mouse strains to test specific questions. In addition, we corroborate rodent findings with or primarily investigate different patient samples (post mortem tissue, CSF, blood). We apply confocal analysis, functional behavioral tests, transcriptomics, proteomics, and secretome analysis as well as analysis of clinical data.

I am clinical investigator in a clinical multicenter trial using fetal-cell derived dopaminergic neurons for cell replacement in Parkinson’s disease (www.Transeuro.org.uk) and in two clinical trials investigating the neurorestorative effect of the intracerebral administration of growth factors (PDGF-BB; CDNF: Treater.eu) in Parkinson’s disease. We are currently preparing the first clinical trial using embryonic stem-cell derived dopaminergic neurons for patients with Parkinsons disease.

Abstract

**Background and Purpose**

In ischemic stroke, breakdown of the blood brain barrier (BBB) aggravates brain damage. Pericyte detachment contributes to BBB disruption and neurovascular dysfunction, but little is known about its regulation in stroke. Here we investigated how loss of Regulator of G protein signaling-5 (RGS5) in pericytes affects BBB breakdown in stroke and its consequences.

**Method**

We used RGS5 knockout (KO) and control mice and applied a permanent middle cerebral occlusion (pMCAO) model. We analyzed pericyte numbers, phenotype and vessel morphology using immunohistochemistry and confocal microscopy. We investigated BBB breakdown by measuring endothelial coverage, tight junctions (TJs) and aquaporin 4 (AQP4) in addition to BBB permeability (fluorescent conjugated dextran extravasation). Tissue hypoxia was assessed with pimonidazole hydrochloride and neuronal death quantified with the TUNEL assay.

**Results**

We demonstrate that loss of RGS5 increases pericyte numbers and their endothelial coverage, which is associated with higher capillary density and length, and significantly less BBB damage after stroke. Loss of RGS5 in pericytes results in reduced vascular leakage and preserved TJs and AQP4, decreased cerebral hypoxia and partial neuronal protection in the infarct area.

**Conclusions**

Our findings show that loss of RGS5 affects pericyte-related BBB preservation in stroke and identifies RGS5 as an important target for neurovascular protection.

Felipe Pereira

Wallenberg Centre for Molecular Medicine, Lund University

Associate Senior Lecturer at Department of Laboratory Medicine, Div. of Molecular Medicine and Gene Therapy, Lund University

filipe.pereira@med.lu.se

https://www.med.lu.se/lund\_stem\_cell\_center/molecular\_medicine\_ and\_gene\_therapy/research\_groups/filipe\_pereira

Area of interest

Cell Reprogramming in Hematopoiesis and Immunity

The focus of my laboratory is to understand the molecular determinants underlying cell reprogramming and hematopoietic specification. In humans, the 200 differentiated cell states are normally stable and inherited through cell division. Under certain conditions, cell fate can, however, be modified or reversed. Cell reprogramming can be achieved experimentally in different ways, including nuclear transfer, cell fusion or expression of transcription factors. The emergent ability to directly reprogram any human cell into desired hematopoietic cell-types is opening avenues to the discovery of new therapies for immune and blood diseases.

The main goals of our research are:

1. To understand at the molecular level how hematopoietic cellular identities are specified

during development employing cellular reprogramming.

2. To use this knowledge to manipulate genes and pathways that ultimately may allow

the generation of patient-specific hematopoietic and immune cells for regenerative

medicine and immunotherapy.

Our research will increase the understanding of the minimal intrinsic determinants underlying hematopoietic cell diversity, allowing us to delve into the mechanistic regulation of progenitor and effector cell developmental specification. This knowledge may allow the re-creation of these unique cell identities from any human cell. In the long-term we believe that our research will contribute to personalized hematopoietic regeneration as well as to develop novel cancer immunotherapies for leukemia, melanoma and other aggressive cancers.

Strengths in lab

We use a variety of experimental approaches to understand hematopoietic reprogramming, including: lentiviral gene transduction, flow cytometry, single cell gene expression and chromatin profiling, directed differentiation of mouse embryonic stem cells, Crispr/Cas9-based gene editing, cellular transplantation, high-content automated image acquisition and analysis and mouse genetics.

Alongside with the understanding of the basic biology of hematopoietic specification, we aim to apply our findings to the treatment of human diseases. Our translation efforts include: a) development of new viral vectors and reprogrammed cells for gene as cell therapy, b) use these new sources of patient-specific cells for the identification of small molecules using chemical screens, c) analysis of patient cohorts with haematological and solid cancers that have received immunotherapies.

Cooperative Transcription Factor Induction Mediates Hemogenic Reprogramming

During development, hematopoietic stem and progenitor cells (HSPCs) arise from specialized endothelial cells by a process termed endothelial-to-hematopoietic transition (EHT). However, despite extensive studies in various animal models, the genetic program driving human hematopoietic stem cell (HSC) emergence remains largely unknown. We have previously reported the generation of hemogenic precursor cells from mouse fibroblasts with the expression of the transcription factors (TFs) Gata2, cFos, Gfi1b and Etv6. These TFs induce a dynamic, multi-stage hemogenic process that progresses through an endothelial-like intermediate, recapitulating developmental hematopoiesis *in vitro*. Here, we demonstrate that human fibroblasts can be reprogrammed into hemogenic cells by the same transcription factors. Induced cells display dynamic EHT transcriptional programs, generate hematopoietic progeny, possess HSPC cell surface phenotype, and repopulate immunodeficient mice for 3 months. Mechanistically, GATA2 and GFI1B interact and co-occupy a cohort of targets. This cooperative binding is reflected by engagement of open enhancers and promoters, initiating silencing of fibroblast genes and activating the hemogenic program. However, GATA2 displays dominant and independent targeting activity during the early phases of reprogramming. These findings shed light on the processes controlling human HSC specification and support generation of reprogrammed HSCs for clinical applications.

Silvia Remeseiro

Wallenberg Centre for Molecular Medicine, Umeå University

Associate Senior Lecturer at Umeå Center for Molecular Medicine

silvia.remeseiro@umu.se

www.umu.se/en/research/groups/silvia-remeseiro/

Area of Intrest

Long-range gene regulation and 3D organization of the Glioblastoma Genome

Most genetic variants that predispose to cancer reside outside the coding genome and are located in regions enriched in putative enhancer elements. In cancer, somatic copy number alterations with a very high impact on gene expression are rather explained by systematic occurrence of rearrangements in non-coding regulatory regions or changes in the 3D chromatin organization (e.g. enhancer hijacking, disruption of insulated neighborhoods). In this context, the contribution of the non-coding regulatory genome to glioblastoma (GBM) is largely unexplored. Despite the number of specific susceptibility loci identified in GBM vs non-GBM tumors, a mechanistic and functional understanding of glioblastoma initiation and progression is missing. This is due to the complexity of genetic, epigenetic and microenvironment initiating events, together with high inter- and intra-tumor heterogeneity.

My group aims to study how reprogramming of gene regulatory mechanisms and changes in 3D genome organization contribute to gene dysregulation underlying glioblastoma initiation and progression, processes that I have investigated earlier in my career in other tumorigenic contexts. I intend to build an interdisciplinary group joining expertise from different biological and technical backgrounds to incorporate ideas not only from cancer but also from neuroscience and development, and with a strong basis of molecular biology and bioinformatics. We will study how alterations in the regulatory and topological architecture of the genome might contribute to the high tumor heterogeneity of glioblastoma and its local invasive capacity, and how this can modulate the crosstalk with elements of the tumor microenvironment providing such aggressiveness and invasiveness to migrating glioma cells.

**Strengths in lab**

My research background ranges from mouse models of cancer, mouse genetics, CRISPR-Cas9-based genome editing to a broad knowledge of “omics”-based approaches (e.g 4C-seq, ChIP-seq, RNA-seq), which I have mainly applied to the study of 3D chromatin organization and long-range gene regulation in the field of cancer. I have extensively used Chromosome Conformation Capture methods (i.e. 4C, HiC) to interrogate the 3D organization of chromatin, and combined this with other genome-wide approaches such as ChIP-seq and RNA-seq to study the interplay with the epigenetic and transcriptional landscape, as well as with imaging and FISH methods to reach the single-cell resolution. In the lab we will combine state-of-the-art approaches in the fields of next generation sequencing and imaging, together with mouse models, 3D cultures and human samples from glioblastoma.

Nelly Romani Vestman

Wallenberg Centre for Molecular Medicine, Umeå University

Senior Consultant in Endodontics, University Hospital of Umeå

Researcher at the Department of Odontology

nelly.romani.vestman@umu.se

https://www.umu.se/en/wallenberg-centre-for-molecular-medicine/research/wcmm-associerade-forskare/nelly-romani-vestman/

**Area of interest**

Future directions in regenerative endodontics

Our translational research team investigates the treatment of immature necrotic teeth in young individuals due to dental trauma. Dental trauma injuries during childhood and adolescence can have an adverse impact on oral health throughout life. If immature teeth get infected and lose their vitality, the root formation stops. These teeth with thin and fragile dentinal walls have consequently questionable long-term survival.

Dental management of necrotic teeth with aberrant root formation represents a challenging clinical situation. The classical approaches for treating these teeth provide little or no benefits on root development. Our research group is interested in studying a biological treatment referred as “revascularization”, or “regeneration”. This treatment aims to regenerate pulp-like tissue within the root canal space after inducing an influx of stem cells from the apical papilla that results in reestablishment of pulp protective functions. It is actually a promising treatment to maintain immature necrotic teeth in young individuals with otherwise poor long-term prognosis.

Furthermore, we aim to analyse the microbial profiles of immature necrotic teeth using a Next Generation Sequencing Protocol and elucidate how human stem cells from the dental apical papilla interact with endodontic bacteria pathogens. Because of the translational nature of this project including clinical and laboratory studies, the results are attempted to provide strong scientific evidence for the field of regenerative endodontics while allowing for clinical treatment optimization.

Nelly Romani Vestman combines research and clinical work as senior consult in Endodontics in the region of Västerbotten. She obtained her PhD from the Swedish National School of Odontological Science/Umeå University and performed an internship in Microbiology and Molecular genetics at The Forsyth Institute, Boston. Dr. Romani Vestman has recently joined The Wallenberg Centre for Molecular Medicine at Umeå University as clinical research associate.

**Strengths in lab**

My research team combines experimental investigation with clinical research. We have experienced conducting randomized controlled trials in which the experimental intervention is added to a standard treatment. We have long clinical experience in treating immature necrotic teeth and analysing microbiological samples from root canals. We use cultural and molecular methods to explore the complex oral microflora. Furthermore, we have international collaborators and are particularly skilled in evaluating the overall microbiota in oral cavity using high-throughput sequencing techniques.

Microbiota composition profile of immature necrotic teeth

Considering the significance of infection removal for regeneration procedures, it is important to get knowledge of the microbiota isolated from these teeth. To the best of our knowledge, this will be the first study using a next generation sequencing technique to explore the microbial composition of immature necrotic teeth.

**Aim**

The aim of this study is to analyse the microbial profiles of necrotic immature teeth due to dental trauma using viable culture techniques and a Next Generation Sequencing protocol.

**Hypothesis**

The microbial profile of immature necrotic teeth is similar to that of primarily permanent teeth

**Material and methods**

Young individuals aged 6-18 treated at the specialist clinics of endodontics (County of Västerbotten, North Sweden) were included. The inclusion criteria included patients with immature teeth and confirmed pulp necrosis caused by dental trauma. Exclusion criteria were teeth with extensive loss of coronal tissue and patients who had received antibiotic treatment during the previous 3 months. Ethical approval from the Ethics Committee at Umea University was obtained.

Root canal biofilms were sampled after rubber dam application and cleaned. A sterile saline solution was introduced into the canal and instrumentation was performed so that material could be taken from the root canal by soaking up the fluid with paper points. Samples were used for cultivation and DNA extraction for further molecular identification analysis.

Samples was cultured on selective and non-selective media at different dilutions. The plates were incubated at 37°C in anaerobic conditions for up to 7 days and the colony-forming units (CFUs) was counted transformed into actual counts based on known dilution factors. In addition, isolates were selected from each plate and identify by comparing 16S rRNA gene sequences to databases.

DNA was extracted and analysed by The Human Oral Microbe Identification using Next Generation Sequencing (HOMINGS) at the facilities of the Forsyth Institute, Boston. In addition, detection of selected endodontic pathogens was performed by PCR of the 16S rRNA genes using specific primers and cycling parameters and comparing sequences to databases.

Preliminary results from the culture and molecular study would be presented in this poster.

Anders Rosengren

Wallenberg Centre for Molecular and Translational Medicine,

University of Gothenburg

Senior Lecturer at Institute of Neuroscience and Physiology

anders.rosengren@gu.se

wcmtm.gu.se/research-groups/rosengren

Area of interest

Type 2 diabetes - from basic science to clinical trials

Anders Rosengren, MD, Senior Lecturer (Assistant Professor), integrates clinical investigations, bioinformatics and experimental studies and aims to better understand the pathophysiology of type 2 diabetes and to identify more specific treatment targeted at the underlying disease mechanisms.

He is PI for the “Detailed Mapping of Type 2 Diabetes” (DIACT) study, which is a longitudinal patient study that investigates how the major pathophysiological components in type 2 diabetes are interlinked and develop over time. This is combined with global genetic, gene expression and metabolite data to identify biomarkers associated with pathophysiological components.

Network analysis and other bioinformatics approaches are used to integrate genetic and gene expression data from the patients and to identify disease genes. Candidate genes identified from these analyses are studied experimentally to investigate underlying disease mechanisms. Moreover, a method for drug repositioning is used that compares the gene networks that are perturbed in T2D with a large library of gene expression signatures from drugs to identify potential anti-diabetic compounds.

Anders Rosengren has extensive international collaborations, which includes e.g. Sage Bionetworks in Seattle and University of Oxford. Anders Rosengren was appointed a Ragnar Söderberg researcher in Medicine 2013. He has also recently been awarded a 5-year Future Research Leader grant by the Swedish Foundation for Strategic Research.

Anders Rosengren is also PI for PriusHealth, a study aiming to develop new research-based web tool for patients with type 2 diabetes. This tool will integrate biological and lifestyle aspects of type 2 diabetes and aims to improve patient self-management and is developed in close collaboration with patients in a research study.

Strengths in lab

We have an experimental and a translational arm in the lab. The experimental techniques we work with are: electrophysiology, Ca2+ imaging, in vivo metabolic challenges of mice and rats as well as standard biochemical and cell-biological methods.

The translational arm integrates bioinformatics methods, especially network analysis of gene expression data and statistical analysis of genetic, gene expression and patient phenotypes, with patient studies using various metabolic challenges.

Abstract

The poster will present an overview of our research on combining bioinformatics with cell-physiological techniques and clinical studies to identify new disease mechanisms and therapies for type 2 diabetes (T2D). We have recently identified Sox5 as a regulator of beta-cell phenotype. Sox5 knockdown induced gene expression changes similar to those observed in T2D and diabetic animals and had profound effects on insulin secretion, including reduced depolarization-evoked Ca2+-influx and beta-cell exocytosis. SOX5 overexpression reversed the expression perturbations observed in a mouse model of T2D, increased the expression of key beta-cell genes and improved glucose-stimulated insulin secretion in human islets from donors with T2D. The findings suggest that human islets in T2D display changes reminiscent of dedifferentiation and highlight SOX5 as a regulator of beta-cell phenotype and function. The poster will also present our findings on sulforaphane as a new potential anti-diabetic compound that reduces hepatic gluconeogenesis, including a translation to T2D patients.

Rolf B. Saager

Wallenberg Centre for Molecular Medicine, Linköping University

Senior Lecturer at the Department of Biomedical Engineering (IMT)

Faculty of Science and Engineering

rolf.saager@liu.se

https://liu.se/en/employee/rolsa28

Area of interest

Quantitative Optical Imaging of Skin

My primary focus is to develop methods and instrumentation that advance spectroscopy, light transport modeling, and imaging of tissue; creating non-invasive tools for the clinical detection, monitoring or treatment of skin diseases and injuries.

Specifically, our group advances spectroscopic methods in a way that exploits the molecular (absorption and fluorescence) and structural (light scattering) sensitivity, quantitation and non-invasiveness through a technique called Spatial Frequency Domain Imaging/Spectroscopy (SFDI/S). This is a relatively new technique that has several appealing attributes as a general measurement platform: low cost, quantitative in optical contrast, depth selective and spatially scalable. This technique measures the differentiated response of remitted light from tissue, when patterns (sinusoidal intensity projections of varying spatial frequency) are projected on to it. This approach quantifies the effective Modulation Transfer Function (MTF) of a diffuse optical system (in this case: tissue) and relates this function in terms of contributions from absorption and scattering.

The main thrust of our research is focused deploying these imaging platforms in applications and collaborations that seek to address unmet needs in either primarily dermatological or models of disease in pre-clinical (small animal) settings.

Strengths in lab

Optical Instrumentation Development: Our group designs and develops custom spectral imaging systems for use in a variety of translational settings, (i.e. benchtop to beside). This includes a variety of optical testing setups, standards, and validation resources. We also design and fabricate custom tissue simulating phantoms to emulate the optical properties from a variety tissue types, structures and potential pathologies. These are used to determine instrument performance as well as the accuracy and robustness of data processing/analysis methods and models. Additionally, we have also developed methodologies for virtual instrument design, based on an investigational *in-vivo* spectral measurement platform.

Advanced Optical Modeling and Simulation Tools: We offer several model based methods to interpret responses of multiply scattered light from tissue. These include simple analytical methods for layered tissue structures to full tomographic reconstruction. Simulation tools for light transport modeling are also available, based on either simplified analytical approaches or stochastic methods (e.g. Monte-Carlo)

Abstract

Spatial Frequency Domain Imaging/spectroscopy (SFDI/S) is a relatively new technique that has several appealing attributes as a general measurement platform: low cost, quantitative in optical contrast, depth selective and spatially scalable. This technique measures the differentiated response of remitted light from tissue, when patterns (sinusoidal intensity projections of varying spatial frequency) are projected on to it. This approach quantifies the effective Modulation Transfer Function (MTF) of a diffuse optical system (in this case: tissue) and relates this function in terms of contributions from absorption and scattering. Our lab advances this method in a way that exploits the molecular sensitivity, quantitation and non-invasiveness of SFDI/S, but extends the spatial resolution and depth selectivity through the use of recent advances in imaging technology (e.g. snap-shot hyperspectral imagers) and compressive sensing (e.g. single pixel imagers), as well as novel computational methods and models of light transport in order to translate these technologies into clinical settings.

The primary thrust of our research is focused on applications and collaborations that seek to address unmet needs in either primarily dermatological or models of disease in pre-clinical (small animal) settings. Current projects include: (1) refining the sensitivity to tissue scattering properties to non-invasively extract greater structural detail and specificity from *in-vivo* tissue, (2) incorporate mechanisms of fluorescence in existing quantitative, depth-sensitive models for light transport in tissue, enabling full quantitative tomography in small animal imaging platforms, and (3) development of custom, handheld imaging systems for dedicated clinical use and for deployment in low resource settings.

Kristoffer Salholm

Wallenberg Centre for Molecular Medicine, Umeå University

Associate Senior Lecturer at the Department of Integrative

Medical Biology

kristoffer.sahlholm@umu.se

https://www.umu.se/forskning/grupper/forskargrupp-kristoffersahlholm/

Area of interest

Psychotic disorders (e.g., schizophrenia, bipolar disorder with mania, and schizoaffective disorder) are believed to affect about 3% of the global population and are characterized by delusions and hallucinations. Dopamine receptors form the main targets for antipsychotic therapy. However, current medication does not adequately address all aspects of schizophrenia symptomatology and, in addition, is fraught with troublesome side effects. It has been postulated that the time course of drug-receptor interactions, as well as the differential engagement of dopamine receptor subtypes, may influence both side effect profiles and clinical efficacies of antipsychotics.

Our group investigates the modes of action of current and experimental antipsychotic drugs, with the aim to inform the development of novel, improved therapeutics. We are also interested in the interplay of different dopamine receptor subtypes, at the signaling- as well as the whole-animal level.

We use live-cell signaling assays to examine drug-receptor interactions, as well as behavioral readouts in rodents to characterize the in vivo actions of antipsychotics. We will also incorporate in vivo imaging modalities to investigate drug effects in different regions of the living brain.

Strengths in lab

Time-resolved GPCR signaling assays (in particular using electrophysiology), mouse behavior.

STRUCTURAL DETERMINANTS OF RECOVERY FROM INHIBITION AT THE DOPAMINE D2 RECEPTOR

Kristoffer Sahlholm1,2, Richard Ågren2,3, Hugo Zeberg2, Sean W. Reilly4, Robert H. Mach4, Peter Århem2, Johanna Nilsson2,3

1Department of Integrative Medical Biology, Umeå University, Sweden,

2Department of Neuroscience, Karolinska Institutet, Sweden,

3Department of Clinical Neuroscience, Karolinska Institutet, Sweden,

4Department of Radiology, University of Pennsylvania, PA, United States

We previously investigated antipsychotic interactions with the dopamine D2 receptor (D2R) using activation of G protein-activated inward rectifier potassium (GIRK) channels as readout of receptor occupancy by dopamine [1]. Recovery of D2R responsiveness to dopamine following antipsychotic washout was often submaximal compared to control. We previously assumed that sub-maximal recovery was caused by lipophilicity-driven accumulation of antipsychotics in the cell membrane and/or interior.

In the present study, we investigated aripiprazole analogues comprising an orthosteric N-2-methoxyphenyl piperazine moiety linked to a tetrahydroquinolinone (presumably contacting the D2R “secondary binding pocket” [2]), by a saturated linker containing 3, 4, or 5 carbon atoms. Surprisingly, the 3- and 5-carbon spacer compounds allowed for a similar extent of recovery (about 80% of control), whereas recovery was about 20% upon washout of the 4-carbon compound. Curve shift experiments performed with the 4-carbon compound were consistent with a competitive mechanism. Mutation of residues presumably contacting the linker and/or secondary fragment revealed a strong decrease of Ki, and both rate and extent of recovery of V91A and E95A, whereas L94A produced no decrease in Ki, a less produced increased in rate of recovery of about 2-fold, and a an increase in extent of recovery to about 75%.

Our results suggest that interaction with the secondary binding pocket, rather than lipophilicity, determines the extent of recovery for the phenylpiperazines studied here. A heterobivalent competitive mode of action [3] might explain the low extent of recovery from the 4-carbon compound, by enabling a high rate of rebinding of the ligand.

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Alireza Salami

Wallenberg Centre for Molecular Medicine, Umeå University

Associate Senior Lecturer at the Department of Integrative

Medical Biology

alireza.salami@umu.se

https://www.umu.se/wallenberg-centrum-for-molekylar-medicin/forskning/fellows/alireza-salami/

Area of interest

Cognitive impairments impede the functioning of older people and create major individual and societal costs. Developing ways to preserve functioning in old age is thus of great importance. However, current knowledge about brain mechanisms that underlie cognitive decline is insufficient to enable effective intervention programs. It is critical to find measurement tools that can predict future severe cognitive decline, such as the one typically observed in demented elderly people, as early as possible, before substantial irreversible damage has been caused to the brain. Our group uses advanced functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) in order to explore the functional and molecular bases of cognitive decline in aging. We hope that this kind of research pave the road for successful design and implementation of intervention programs that may improve cognitive abilities in old age.

Strengths in lab

We have access to both MRI and PET techniques to explore functional and molecular basis of aging human brain.

Striatal iron modulates neural signatures of working memory

Intracellular non-hem iron in the brain is involved in numerous biological processes, such as neurotransmitter synthesis, synaptic plasticity, and myelination, which are crucial for early life development. However, elevated iron content in old age has found to be deleterious for brain cells because of triggering oxidative stress. Given previous evidence for the association between iron and cognitive measures, it is plausible that such an association varies across different age groups. Moreover, iron content could directly impact brain activity via astrocytic dysfunction: the astrocytes, cells where iron concentration increases with aging, are involved in the neurovascular coupling on which the blood oxygen level-dependent (BOLD) signal measured with functional MRI (fMRI) relies. Here we investigated age-related differences in brain iron content and their associations with differences in working memory and concomitant alterations in brain activation across the adult lifespan. We found that striatal iron was positively associated with working memory performance in the younger group, whereas a negative association was found in the older group. Critically, a significant pattern revealing positive and negative associations of striatal iron to BOLD response in the striatal-cortical circuit was observed in younger and older adults, respectively. Taken together, striatal iron was related to working memory ability and associated BOLD response in an age-dependent manner, and future research needs to shed light on a mechanism behind the age-varying relationship.

Volkan Sayin

Wallenberg Centre for Molecular and Translational Medicine, University of Gothenburg

Associate Senior Lecturer at the Department of Surgery at Institute of Clinical Sciences

volkan.sayin@gu.se

http://wcmtm.gu.se/research-groups/sayin

Area of interest

My lab is well positioned in the intersection between lung cancer therapeutics, cancer metabolism and redox biology. Utilizing a lot of expertise, we are now building a precision medicine platform combined with the latest technologies in lung cancer research and metabolomics to establish an extensive translational lung cancer group at Sahlgrenska.

Initially, we plan to identify druggable targets in RAS and RAF driven cancers by combining a high-throughput screening platform using lentiviral-CRISPR/Cas9 strategies that was established during my postdoctoral studies with metabolomics and state-of the art genetically engineered mouse models of lung adenocarcinoma (Sayin VI et al 2017, *ELife, & Nat. Med.*). We will use lentiviral-CRISPR/Cas9 strategies to inactivate any gene-of-interest at tumor onset or later during tumor progression and metastasis. We will also develop patient-derived xenograft (PDX) models by transplanting fresh human lung tumor pieces subcutaneously in humanized-immunocompromised mice, and then serially transplant them to cohorts of mice for experiments and pre-clinical trials. We will use this platform for a range of experiments in my own group and in collaborative efforts at GU. As an example, we have recently shown that activation of the endogenous antioxidant pathway in lung cancers through genetic or small molecule inactivation of Keap1, rewires cancer cell metabolism and increase cancer progression at the cost of a druggable metabolic dependence on glutaminase. These findings are already fueling ongoing clinical trials and development of novel inhibitors.

The overarching goal of our research is to improve the quality of life and survival rates of cancer patients, guide personalized clinical trials, repurpose pre-existing drugs and to find novel combination therapies that can synergize with current standard of care therapies including chemo- and immune therapies.

*Keap1* loss promotes Kras-driven lung cancer through metabolic rewiring resulting in a druggable dependence on glutaminolysis

Targeting KRAS-mutant lung cancer remains a major clinical challenge. Genetic and metabolic diversity among KRAS-mutant cancers might underlie the limited success of synthetic lethal targets in translational oncology. Here we combined a mouse model of KRAS-driven lung cancer with the CRISPR/Cas9 system to functionally characterize the Keap1/Nrf2 antioxidant pathway, which is mutated in ~30% of lung cancers. We show that loss of *Keap1* accelerates lung cancer progression and metastasis through hyper-activation of the Nrf2–antioxidant pathway.

Combining metabolomics and focused genetic screening, we uncover that *Keap1/Nrf2* mutant cancers are metabolically rewired to depend on glutaminolysis. We demonstrate that this dependence can be therapeutically exploited in both patient derived xenografts and mouse models of lung cancer through glutaminase inhibition. Furthermore, we show that cancers beyond *Keap1/Nrf2* mutants can become metabolically rewired to depend on glutaminolysis. Finally, through both screening and targeted approaches we identify resistance mechanisms to glutaminase inhibition. Our study establishes a precision-medicine platform through which we provide a mechanism and a rationale for genotype based sub-stratification of *Keap1/Nrf2* mutant lung cancer patients as responders to glutaminase inhibitors.

Mikael Sellin

SciLifeLab/Uppsala University

Associate Senior Lecturer, IMBIM, Uppsala University

mikael.sellin@imbim.uu.se

https://www.imbim.uu.se/research-groups/infection-and-defence/sellin-mikael/

Twitter: @SellinLab

Area of interest

Gut infections constitute a leading cause of morbidity worldwide, with estimates of up to two billion disease cases annually. Enterobacteria, such as *Salmonella*, *Escherichia*, and *Shigella* species account for more than half a billion of these infections. Pathogenic enterobacteria are characterized by the ability to bind and/or invade the epithelium of the intestinal mucosa, thereby triggering an inflammatory tissue response. Antibiotic treatment has proven remarkably inefficient at clearing these infections and may in some cases even increase bacterial shedding from the infected individual. Moreover, the heavy use of antibiotics in healthcare and agriculture has led to a fast spread of resistance mechanisms among enterobacterial isolates. Hence, we have both curiosity-driven and clinical incentives to better understand the relevant microbe – host interactions that drive progression of intestinal inflammatory disease.

The mechanisms of pathogen - host cell interplay have traditionally been studied in simplified cell culture settings, where pure bacteria and tumour-derived cell lines are mixed in a culture medium. Such experiments continue to uncover a wealth of potential biochemical interactions between microbe and host cell. However, to understand the relevant molecular and physiological underpinnings of a “real” gut infection, additional approaches are needed. Recent developments in high-resolution microscopy techniques and experimental models now allow us to tackle how enterobacterial disease progresses on the cellular and molecular level also under more physiological conditions. The Sellin lab employs comparative cell biology, organotypic tissue culture, analysis of intact infected tissues, state-or-the-art microscopy, and clinically relevant pathogens (i.e. *Salmonella* and *Shigella* species), to explore the mechanisms sparking enterobacterial gut disease. The ambition is that our fundamental work will form the basis for future therapeutic approaches against these challenging infectious agents.

Strengths in lab

Organoid tissue culture, Intestinal pathology analysis, Bacterial infection biology, Bacterial genetics, Mammalian cell line culture, Murine models of gut infection, Live 3D microscopy.

The Sellin Lab @Uppsala University – Bacterial gut infections

It has likely not escaped anyone’s notice that bacterial infections constitute a re-emerging global health threat. Invasive gut bacteria comprise a prevalent and challenging group of infectious agents that can spark intestinal inflammatory disease and are often surprisingly resilient to antibiotic therapy. The Sellin laboratory is embedded in the department IMBIM at Uppsala University and affiliated with SciLifeLab and Uppsala Antibiotics Center. We study the molecular warfare between invading gut bacteria, for example *Salmonella* and *Shigella* species, and the epithelium of the hosts intestinal mucosa. We conduct bacterial infection experiments both under simplistic cell line culture settings, in cultured organoid 3D-mimics of the gut mucosa, and in murine in vivo models of gut disease. Our overarching aim is to understand the physiologically relevant mechanisms that explain each step of bacterium – host cell interplay during development of inflammatory gut disease. Here, we give an overview of ongoing activities in our laboratory.

Karolina Skibicka

Wallenberg Centre for Molecular and Translational Medicine,

University of Gothenburg

Senior Lecturer at Department of Neuroscience and Physiology

karolina.skibicka@neuro.gu.se

wcmtm.gu.se/research-groups/skibicka

Area of interest

Obesity - Neural Substrates of Energy Balance; From Molecule to Circuit to Behavior

Karolina Skibicka, PhD, Senior Lecturer, Associate Professor (Docent) in Physiology, investigates the behavioral and neuroendocrine processes that govern fundamental homeostatic and reward controls of food intake, and ultimately how these systems fail in obesity. She aims to identify a more effective obesity treatment targeting the neural circuits underlying overeating.

By integrating careful experimental decomposition of behavior with neuropharmacology, genetic manipulations, and molecular methods we aim to gain insight into how food and feeding behavior affects the brain, and in turn how the brain regulates feeding and food choices.

Recent discoveries by my group include findings that satiety or hunger hormones, for example glucagon-like peptide 1 or ghrelin, which are altered by nutritional status, affect far more than feeding behavior and body weight. They profoundly affect reward derived from food but also alcohol, emotionality and decision-making. This impact on behavior is paralleled by neurochemical and molecular changes in brain circuits regulating them. These findings are now pursued in clinical trials.

I have extensive international collaborations, which include researchers from University of Pennsylvania, University of Southern California, Cambridge University, University of Freiburg, and Karolinska Institute, in addition to multiple local collaborative projects. I was appointed a Ragnar Söderberg Fellow in Medicine 2015. I have also been awarded the Fernström Prize in Medicine 2016 for young investigators. Recently (2019) I have also received the Swedish Research Council Consolidator Award.

**Strengths in lab**

CNS microinjections and microinfusions; optic stimulation and DREADD manipulation of genetically selected neuronal population; brain, BAT, & WAT histology and immunohistochemistry; virally-mediated neural tract tracing and si/shRNA neuroanatomically selective gene knockdown; energy expenditure measurements (telemetric core and BAT temperature measurement, infrared thermography, spontaneous activity); ingestive behavior measures (meal size, frequency, macronutrient preference); motivated behavior (operant conditioning paradigms, place preference); impulsive behavior (delay discounting, go-no go, DRL); emotionality and sociability tests (depression and anxiety-like behaviors, novel cage mate interaction-based tests).

Additionally we are also collaborating with Chalmers (an engineering university; group of Ann-Sofie Cans), to develop novel implantable microfabricated sensors for in vivo real time analysis of neurotransmitter release.

GLP-1 modulates the supramammillary nucleus-lateral hypothalamic neurocircuit to control ingestive and motivated behavior in a sex divergent manner.

Lorena López-Ferreras L1,3, Kim Eerola1, Devesh Mishra1,3, Olesya T Shevchouk1, Jennifer E Richard 1, Fredrik H Nilsson 1, Matthew R Hayes2, Karolina P Skibicka1,3

1 Department of Physiology/Metabolic Physiology, Institute of Neuroscience and Physiology, The Sahlgrenska Academy at the University of Gothenburg, Sweden.

2 Translational Neuroscience Program, Department of Psychiatry, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania

3 Wallenberg Centre for molecular and translational medicine, University of Gothenburg, Sweden

The supramammillary nucleus (SuM) is nestled between the lateral hypothalamus (LH) and the ventral tegmental area (VTA). This neuroanatomical position is consistent with a potential role of this nucleus to regulate ingestive and motivated behavior. Through the application of anterograde and retrograde neural tract tracing with novel designer viral vectors, the current findings show that SuM neurons densely innervate the LH in a sex dimorphic fashion. Glucagon-like peptide-1 (GLP-1) is a clinically targeted neuro-intestinal hormone with a well-established role in regulating energy balance and reward behaviors. Here we determine that GLP-1 receptors (GLP-1R) are expressed throughout the SuM of both sexes, and also directly on SuM LH-projecting neurons, and investigate the role of SuM GLP-1R in the regulation of ingestive and motivated behavior in male and female rats. SuM microinjections of the GLP-1 analogue, exendin-4, reduced ad libitum intake of chow, fat, or sugar solution in both male and female rats, while food-motivated behaviors, measured using the sucrose motivated operant conditioning test, was only reduced in male rats. These data contrasted with the results obtained from a neighboring structure well known for its role in motivation and reward, the VTA, where females displayed a more potent response to GLP-1R activation by exendin-4. In order to determine the physiological role of SuM GLP-1R signaling regulation of energy balance, we utilized an adeno-associated viral vector to site-specifically deliver shRNA for the GLP-1R to the SuM. Surprisingly, and in contrast to previous results for the two SuM neighboring sites, LH and VTA, SuM GLP-1R knockdown increased food seeking and adiposity in obese male rats without altering food intake, body weight or food motivation in lean or obese, female or male rats. Taken together, these results indicate the potential for SuM to contribute to ingestive and regulation of motivated behaviors; an effect contingent on sex, diet/homeostatic energy balance state and behavior of interest. These data also extend the map of brain sites directly responsive to GLP-1 agonists, and highlight key differences in the role that GLP-1R play in three interconnected closely neighboring nuclei.

Fredrik H. Sterky

Wallenberg Centre for Molecular and Translational Medicine,

University of Gothenburg

Researcher, Inst. Biomedicine, University of Gothenburg

Resident Physician, Dept. Clinical Chemistry, Sahlgrenska University Hospital

fredrik.sterky@gu.se

wcmtm.gu.se/research-groups/sterky

Area of interest

The human brain is a remarkably complex structure: Nearly 100 billion neurons wire together via some 1015 synapses to form the neural circuits that underlie its function. The formation, specification and maturation of synaptic connections are thought to depend on specific cell adhesion molecules that span the synaptic cleft to form physical interactions between pre- and postsynaptic neurons. The details of these processes are not well understood, but their impairment predispose to neurodevelopmental and psychiatric disorders including autism and schizophrenia.

We are interested in the molecular mechanisms that regulate the formation and specification synaptic connections. By using a combination of protein biochemistry and genetic models, we are addressing the role of two carbonic anhydrase-related proteins that may regulate synaptic cell adhesion via the well-known Neurexin family of adhesion receptors. We are also developing tools for rapid genetic engineering of human lineage-derived neurons to both study mechanisms of synapse formation and to address the functional impact of genetic variants identified in patients with neurodevelopmental delay and autism.

Strengths in lab

• Genetic modification of human embryonic stem cells (by CRISPR-Cas9 and/or AAV)

• Direct lineage-conversion of human ES/iPS-cells to neurons

• Mouse primary neurons

• Production and transduction of lentivirus and adeno-associated virus (AAV)

• Confocal microscopy

• Protein biochemistry and proteomics

A toolbox for rapid generation of conditionally mutant human neurons

The ability to precisely edit the genome of model organisms has been fundamental to the last decades of biomedical research, as carefully controlled genetic experiments are gold-standard to infer causality in mechanistic studies. Recent advances in embryonic or induced pluripotent stem (ES/IPS) cell culture in combination with the discovery and application of CRISPR methodologies have made routine genome editing of human cells for research purposes increasingly feasible, allowing generation of more disease-relevant cells and a means to assess the functional role of precise genetic variants identified in patients. Yet, conventional approaches inevitably necessitate clonal selection of the gene-edited cells, with the accompanied risk for amplifying stochastic mutations, epigenetic changes etc. that can never fully be controlled for, and require comparisons between multiple independent clones and/or rescue experiments in all subsequent experiments. The analysis of the cells thus easily becomes very laborious, despite generating them was fast. We have chosen to circumvent some of these problems by utilizing *Cre/loxP*-technology – commonly in mouse genetics – to generate cells for which the same clone can express alternative genetic variants depending on exposure to site-specific recombinases such as *Cre*. A drawback of this approach, however, is the time-consuming design and cloning of gene-specific targeting vectors. To speed up this process from months to ~2 weeks, we have developed a molecular toolbox and universal one-step cloning strategy. We are now utilizing this approach to generate human neurons with conditional mutations in genes linked to neurodevelopmental delay and autism.

Anders Ståhlberg

Wallenberg Centre for Molecular and Translational Medicine, University of Gothenburg

Associate Professor at Department of Pathology and Genetics and Head of Translational Genomics Platform within WCMTM at Clinical Pathology and Genetics, Sahlgrenska University Hospital

anders.stahlberg@gu.se

https://wcmtm.gu.se/research-groups/genomics-platform

https://cancercenter.gu.se/svenska/forskargrupper/anders-stahlberg

Area of interest

Anders Ståhlberg, Associate Professor, working as principal investigator at Sahlgrenska Cancer Center, University of Gothenburg. He is also Head of the Translational Genomics Platform at Clinical Pathology and Genetics, Sahlgrenska University Hospital.

Our research group’s goal is to increase the survival of patients suffering from breast cancer or sarcomas characterized by specific fusion oncogenes. To accomplish this we are identifying and targeting cancer stem cell (CSC) specific features associated with therapy resistance and monitoring treatment efficiency using blood plasma. To achieve these goals we have access to (i) advanced single-cell methodologies to study CSCs, (ii) experimental systems that allow us to study the role of the microenvironment, and (iii) ultrasensitive mutation detection techniques, enabling treatment monitoring using blood plasma.

The Translational Genomics Platform is a research initiative with the attempt to bring innovation into healthcare. Ultrasensitive techniques allow individual DNA molecules related to diseases like cancer to be detected. Tumor DNA enters the blood in cancer patients and by analyzing the amount of these disease-specific DNA sequences the tumor burden is assessed. Thereby the method not only opens up the possibility for early diagnostics, but also to customize the treatment for patients before tumors are observed through traditional imaging methods. Today, we apply our platform and techniques in several national and international research projects in cancer and beyond.

We have developed several strategies for gene expression profiling and rare molecule analysis, especially at the single-cell and single-molecule level. Anders is also a co-founder of TATAA Biocenter and iScaff Pharma.

Strengths in lab

Our translational research includes in vitro and in vivo systems as well as handling and using clinical samples. We are applying a wide range of methods related to cell and molecular biology. We have also developed several technologies related to DNA, RNA and protein analysis. Our expertise includes the whole workflow from sample collection to final data analysis with a focus on single-cell analysis and liquid biopsies. We have also expertise in experimental systems related to the microenvironment and 3D-bioprinting.

Analysis of cell-free tumor DNA using SiMSen-Seq

Analysis of circulating cell-free tumor DNA (ctDNA) in liquid biopsies offers new means for early cancer diagnostics, real-time monitoring of treatment efficiency and detection of relapse. Despite its potential use, ctDNA remains challenging to detect and to quantify as it represents only a small fraction of total plasma circulating cell-free DNA. We have developed an ultrasensitive sequencing technology, SiMSen-Seq, that allows allele frequencies < 0.1% to be detected, using several kilobases of DNA. SiMSen-Seq is simple to perform, flexible in multiplexing and requires minimal DNA input. SiMSen-Seq allows detection of variant alleles with easy customization of library content and a protocol that can be implemented in any molecular biology laboratory. Here, we present how SiMSen-Seq can be implemented in a liquid biopsy workflow, including sample collection, cell-free DNA extraction, sequencing and finally bioinformatics, to quantify extremely lowly prevalent disease-specific mutations.

Vinay Swaminathan

Wallenberg Centre for Molecular Medicine, Lund University

Associate Senior Lecturer, Department of Clinical Sciences Lund,

Div. Oncology and Pathology, Lund University

vinay.swaminathan@med.lu.se

http://portal.research.lu.se/portal/en/persons/vinay-swaminathan(81d8aa93-6072-4130-8c14-6be52a0b3e09).html

Area of interest

Cells in our body exist not only in a rich biochemical environment but also in a highly complex and dynamic mechanical environment. Whether it is blood flow, lung expansion, heart contraction or the matrix that surrounds cells everywhere, this environment is critical for most important functions in life. Starting from embryogenesis, tissue development and differentiation to immune, cardiovascular, musculoskeletal and brain function, the mechanical environment plays an important role in all these processes through regulatory interactions with cellular function. Not surprisingly, breakdown or mis-regulation of the interactions between cellular functions and the mechanical environment results in developmental defects, immune disorders, cardiomyopathies and cancer.

We are primarily but not exclusively interested in cell migration and role of integrin-based adhesions as sensors, transmitters and transducers of mechanical information. Our hypothesis is that the multi-molecular adhesion structure allows a cell to sense and respond to mechanical cues such as stiffness & fluid flow by encoding that information and response in its dynamic architecture, composition and regulation of the cell cytoskeleton. We test our hypothesis by utilizing an array of quantitative microscopy-based approaches and tools from engineering, physics and cell biology and investigate the relationship between forces/mechanics and molecular, sub-cellular and cellular organization, signaling and cellular dynamics.

Our ultimate aim is to extend our fundamental and mechanistic knowledge to 3D-cancer models where changes in the microenvironment leads to tumor growth and metastasis through breakdown of homeostasis between cells and their mechanical environment. By identifying pathways that go awry in cancer, we hope to contribute in developing novel therapeutics and targeting strategies.

Strengths in lab

Our lab primarily relies on quantitative light microscopy-based approaches to gain insight into the interactions of cells with its environment. We use TIRF microscopy, Confocal microscopy as well as have developed new approaches using fluorescence polarization microscopy. In addition, we have also developed biophysical tools for microscopy including magnetic tweezers and nano-indentation methods.

Our expertise is fundamental cell biology with focus on the cell cytoskeleton and adhesions. The lab falls under the specific area of cellular mechanotransduction, a rapidly growing and evolving field with broad applications in cancer biology, stem cell biology, development, immune biology and tissue regeneration.

Cell mechanics, mechanotransduction and focal adhesions: Introducing the Lab of Cell and Molecular Mechanobiology.

Every cell in our body has a complex and critical relationship with its extracellular environment. This relationship is required for all cellular functions, starting from embryogenesis and tissue specification in development to immune response, wound healing and cardiac function in adults. While the extracellular environment can be biochemical, advances over the past 25 years have highlighted the equal if not more critical importance of the mechanical environment. The mechanical environment includes the forces exerted by cells on each other, the strain from expanding and contracting organs like the lung, the flow of fluids such as blood in the vasculature and the diverse mechanical properties of the extracellular matrix that surrounds each and every cell. The Laboratory of Cell and Molecular Mechanobiology at Lund University explores the molecular basis and cellular consequence of these mechanical interactions between the cell and its environment. In the poster, we will present a snap-shot of the novel and state of the art microscopy tools used by the lab to understand how cells sense mechanical cues from the environment, primarily via a remarkable set of cellular organelles called focal adhesions. We will also present results where, using a combination of high-resolution microscopy and tools from structural biology, we discovered a novel mode of molecular organization in cells that enables it to sense and respond to complex cues and regulate the critical function of cell migration. Finally, we will highlight our plan on combining approaches from physics and engineering with fundamental cell and molecular biology and quantitative microscopy to understand cell-mechanical interactions from the molecular to tissue scale in normal physiology and in the context of regeneration and cancer metastasis.

Ka-Wei Tang

Wallenberg Centre for Molecular and Translational Medicine,

University of Gothenburg

Researcher at Institute of Biomedcine, University of Gothenburg

Resident Physician at Department of Clinical Microbiology

kawei.tang@gu.se

wcmtm.gu.se/research-groups/tang-group

https://cancercenter.gu.se/research/ka-wei-tang

Area of interest

Tumour virology - Virus genes essential for tumour maintenance and mutational landscapes during tumour development

Ka-Wei is a resident physician in Clinical Microbiology with research time within Wallenberg Centre. He aims to identify novel targets for the treatment of Epstein-Barr virus associated malignancies.

The majority of adults in the world are infected by Epstein-Barr virus (EBV). Following a primary infection during childhood or adolescence, EBV remains latent in our B-lymphocytes for the rest of our lives. For most of us this latent infection will go unnoticed. But for approximately 200,000 patients world-wide each year EBV-infection turns into a fatal disease in the form of hematological (Hodgkin’s lymphoma, Burkitt’s lymphoma and post-transplantation lymphoproliferative disease) and epithelial malignancies (gastric adenocarcinoma and nasopharyngeal carcinoma). However, no specific treatment is currently available for EBV-associated malignancies.

Our projects encompass molecular and clinical studies of the EBV-associated malignancies with particular focus on viral gene expression and mutational landscapes. We employ genetic manipulation techniques as well as standard clinical assays to affirm potential targets as clinically significant markers and important factors for proliferation.

Strengths in lab

Our translational team consists of members trained in biochemistry, bioinformatics, molecular biology/virology and clinical microbiology.

Characterization of viral long non-coding RNA in cancer harbouring Epstein-Barr virus

Epstein-Barr virus (EBV) is the causative agent for 200’000 cases of neoplasia worldwide every year. The virally encoded long non-coding RNA *RPMS1* is detected in all types of EBV-infected cells including latently infected non-malignant B-lymphocytes. Recently it has been shown that *RPMS1* is the main EBV-encoded gene expressed in EBV-associated gastric adenocarcinoma, indicating a significant role for *RPMS1* in tumor maintenance. Further bioinformatic characterization of EBV transcriptomes derived from Burkitt’s lymphoma and nasopharyngeal carcinoma reveal novel splice-junctions and through transcription start site and poly-A signal analysis determines strand specificity, which has been a controversy in the field. In our previous studies knock-out of *RPMS1* promoter severely impaired cell proliferation and survival. This extended complexity of *RPMS1* splice variants detected in different EBV-associated malignancies warrants further studies of differential interactomes and will influence *RPMS1*’s potential to act as a future drug target.

Alexandra Teleki

SciLifeLab/Uppsala University

Assistant Professor, SciLifeLab Fellow, Department of Pharmacy, Uppsala University

alexandra.teleki@farmaci.uu.se

http://farmaci.uu.se/research/drugdelivery/

**Area of interest**

Functional nano-enabled oral drug delivery systems for personalized medicine

The laboratory of Alexandra Teleki, PhD, Assistant Professor and SciLifeLab Fellow at Uppsala University, is in the area of pharmaceutical nanotechnology and uses nanomaterials to develop multifunctional and clinically translatable drug delivery systems. The aim is to bridge the gap of innovative and scalable nanotechnology research to clinically relevant, patient-compliant diagnosis and treatment.

The work capitalizes on the scalable and reproducible manufacture of functional nanoparticles by flame spray synthesis. We have advanced the flame synthesis of nanoparticles by developing a reactor that produces in a single step core-shell nanoparticles with fine and independent control over core particle properties and shell coating thickness. A wide spectrum of materials can be produced with this technology, such as magnetic (superparamagnetic iron oxide: SPION), plasmonic (Au, Ag), luminescent (nanophosphors), and pH sensitive nanoparticles. The coating layer (SiO2) enables the facile surface functionalization of such nanoparticles with targeting ligands to selected disease biomarkers. These nanomaterials are then used in the assembly of microcapsules that host specific drug molecules where the nanoparticles in the shell enable bioimaging, targeted delivery or controlled release. We employ rigorous solid state characterization of our nanomaterials to ensure reproducibility. The advanced multiscale drug delivery systems are tested in state-of-the-art in vitro cell- and tissue-based intestinal models. Currently, we are particularly interested in developing a novel MRI-based diagnostic technique for inflammatory bowel disease using SPION. Furthermore, we employ the SPION in the shell of colloidal capsules, that carry biological drugs with hyperthermia triggered local release at the disease site. Another focus area is the 3D printing of personalized oral dosage forms for pediatrics incorporating hybrid, lipid nanocarrier drug delivery systems solubilizing poorly water-soluble drug compounds. The development of such novel drug formulations at the interface of materials and pharmaceutical sciences will broaden their functionality to meet the future needs of the healthcare industry in establishing personalized treatment of patients.

**Strengths in lab**

Our key technologies focus on nanomaterial synthesis and characterization as well as their incorporation in drug formulations:

• Scalable and reproducible nanoparticle synthesis in flame reactors

• Enabling drug delivery formulations (i.e. lipid-based formulations, amorphous solid dispersions,

nanocarriers, mesoporous carriers, particle-stabilized emulsions)

• Solid state characterization techniques (i.e. DLS, FTIR, Raman, XRD, DSC)

• 3D printing of dosage forms (e.g. Cellink)

Furthermore, we share laboratories with the Drug Delivery group at Uppsala University, creating a strong multidisciplinary environment with world-leading *in-vitro* intestinal digestion and absorption assays, cell-based models and tissue studies in Ussing chambers for intestinal permeability as well as computational pharmacy (e.g. molecular dynamics simulations).

Abstract

A future vision of the healthcare industry is personalized medicine tailored either towards individual needs of patients or to dosage forms optimized for specific patient groups e.g. children and elderly. This requires drug formulation strategies that yield optimal therapy while maintaining high patient compliance; a need that cannot be matched by state-of-the-art drug delivery systems. Classical, poorly water-soluble small drug molecules are today often solubilized in lipid-based formulations (LBF) and administered orally as liquid-filled gelatin capsules. However, such formulations suffer from poor physical and chemical stability during storage and difficult handling during manufacturing. Creating a solid formulation at an early stage could minimize these stability concerns. Emerging biological therapies mostly rely on systemic administration that not always results in optimal, local therapeutic effects and can be associated with adverse effects. A recent development in pharmaceutical manufacturing is 3D printing that enables on demand production of advanced dosage forms with increased flexibility. In our research we aim to establish a drug delivery platform suitable for 3D printing of personalized dosage forms. The formulation strategy involves colloidal capsules with nanoparticles in their shell. We explore the use of functional nanoparticles in these multiscale structures that are synthesized by flame aerosol technology with proven scalability and reproducibility to ensure a rapid translation to the clinics. Here, we demonstrate the particle stabilization of hydrophobic LBFs using silica nanoparticles. The emulsions are prepared by high-pressure homogenization and the resulting droplet size is characterized by dynamic light scattering as well as cryo-scanning electron microscopy. Furthermore, the *in vitro* intestinal lipid digestion and subsequent drug release from Pickering LBF emulsions is characterized in a state-of-the-art lipolysis set up using simulated intestinal media. The particle-stabilized LBFs have submicron droplet sizes and display stability against coalescence for several months. We also assemble micronsized colloidal capsules with silica nanoparticles in their shell that can host biological drugs in their core. The encapsulation efficiency of such capsules can be further increased by applying outer biopolymer coating layers. Finally, we 3D print the particle-stabilized lipid emulsion into minitablets suitable as an oral dosage form for children.

Ilaria Testa

SciLifeLab/KTH Royal Institute of Technology

Assistant Professor, Department of Applied Physics at the School of Engineering Science, KTH Royal Institute of Technology

ilaria.testa@scilifelab.se

www.scilifelab.se/researchers/ilaria-testa/

Area of interest

Advanced light microscopy techniques

My research interest is focused on the establishment and development of advanced light microscopy techniques and its application to the life sciences.

Fluorescence microscopes, and especially their confocal and two-photon variants, are unique in their ability to directly observe morphological changes and molecular reactions in living cells. However, due to diffraction of light, the lateral resolution of conventional light microscopes is limited to about 200-300 nm. This limitation is overcome with great success by the field of super-resolution microscopy. Here, fluorescence molecules do not only act as probes to highlight features of interest, but their photophysical properties are used for overcoming the diffraction limit of light. By controlling those properties in space or time with light it is possible to improve the spatial resolution of an optical microscope down to the molecular scale (10-20nm).

My overarching scientific objective is to develop novel paradigms and concepts based on super-resolution microscopy to address contemporary challenges in biophysics and molecular biology. To achieve these goals I will push forward the quantitative aspect of live cell imaging by setting-up and applying different concepts of super-resolution microscopy based on single molecule detection (PALM/STORM/GSDIM) and targeted switching (STED/RESOLFT). These next generation microscopes will allow the precise identification of populations of biomolecules depending on their localization, abundance and dynamics inside their native environment. A special effort will be dedicated to investigate neuronal proteins, especially in synapses, where trafficking organelles and protein complexes are packed so tight in space that resolving them requires high resolution in space and time.

Live cell imaging at the nanoscale

Fluorescence microscopy was unable to discern fluorescently labeled structures closer than 200 nm for decades, until the recent breaking of the diffraction resolution barrier made nanoscale (20-50 nm) imaging routine. Reported nanoscopy variants switch fluorescently labeled structures either in a target manner with intense laser beams, or molecule by molecule followed by computation in a stochastic fashion. In my laboratory we showed that emergent MoNaLISA (Molecular Nanoscale Live Imaging with Sectioning Ability) fluorescence nanoscopy enables fast and continuous imaging of living cells and tissues in super resolved detail by producing raw data images using only ultralow levels of light. This advance has been facilitated by the generation of fluorescent proteins (rsFP) that can be reversibly photoswitched numerous times. Distributions of functional rsFP-fusion proteins in living mammalian cells are imaged at < 40 nm resolution. Using a fast-switching rsFP variant, we increased the imaging speed over our first reported methods, which in turn enabled us to record spontaneous and stimulated changes of dendritic actin filaments and spine morphology occurring on sub seconds time scales. Furthermore, 3D and adaptive scanning implementation of our concept enable precise localization of synaptic proteins located in the pre-synaptic side directly inside 3D tissues. Our powerful next generation super resolution technique represents a new paradigm for non-invasive induction and monitoring of ultrastructural dynamics of synaptic plasticity at the nanoscale.

Alesia Tietze

Wallenberg Centre for Molecular and Translational Medicine, University of Gothenburg

Associate Senior Lecturer, Department of Chemistry and

Molecular Biology

alesia.tietze@gu.se

https://wcmtm.gu.se/research-groups/-tietze-group

www.tietze-lab.com

Area of interest

A major focus of my research is a development of synthetic strategies for the synthesis of bioactive peptides, i.e. cysteine-rich peptides, which are derived from biologically active natural sources (i.e. cone snails, spiders, snakes), their targets (membrane proteins) and their structural analogues as well as engineering of bioinspired materials based on functional peptides/proteins and artificial carriers (i.e. nanopores, polymers).

We perform synthesis of bioactive peptides, derived from the venom of gastropods and study their mode of action with their targets, i.e. membrane proteins or/and transmembrane receptors, in order to develop more potent structures and proteolytically stable peptide analogs.1 Moreover, we develop new strategies for the chemical synthesis of membrane proteins, targets of bioactive peptides, since their synthesis is challenging due to their highly hydrophobic properties. Recently we developed method to improve their solubility and therefore facilitate the availability of this class of proteins/peptides.2

Additionally, we seek to craft these substances (pharmacologically interesting peptides and membrane proteins) onto a solid support or surface (e.g. polymers) with the goal to create new, bioinspired sensory or catalytic systems, such as bioinspired nanopores.3

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**Strengths in lab**

We use methodological approaches ranging from medicinal and peptide chemistry (organic chemistry, solution and solid phase techniques, chemical conjugation), to analytical chemistry (RP-HPLC, mass spectrometry (ESI and MALDI), UV-vis, fluorescence spectroscopy/microscopy and amino acid analysis) and structure determination (CD, NMR).

Selective and ultrasensitive sensor for early diagnostics of Alzheimer’s disease

During the last two decades studies have been performed in order to confirm the involvement of copper in β-amyloid plaques procession. The results of these studies show that determination of non-bound Cu2+ in urine and serum can be used as a basis for a screening method for the early diagnosis and/or monitoring of diseases linked to abnormal copper concentrations, for example AD or Wilson’s disease. Therefore, we designed functional peptide crafted onto a nanopore. Applying fluorescence microscopy observing the quenching of fluorescence signal upon Cu(II) binding, the concentration of bound Cu(II)-ions (LOD ~ 13 nM) can be precisely determined. Additionally, *I-V* measurements provide more sensitive detection of Cu-binding event up to femtomolar concentrations. The hybrid system is selective in the presence of Ni (II), Zn (II) ions upon pH shift and stable up to a year when the peptide is immobilized to the solid support.

Elin Trädgårdh

Wallenberg Centre for Molecular Medicine, Lund University

Associate Professor and Consultant, Department of Translational Medicine, Skåne University Hospital Malmö and Lund University

elin.tragardh@med.lu.se

www.petris.nu

http://portal.research.lu.se/portal/sv/organisations-researchgroups/nuclear-medicine-malmoe(1e478330-6da6-4c32-bf6a-fdafdf8e5a72). html#%C3%96versikt

Area of interest

New imaging biomarkers in PET-CT based on deep learning

Positron Emission Tomography with Computed Tomography (PET-CT) is the fastest growing imaging modality and an important part of the evaluation of patients with cancer for assessment of the primary tumour, for lymph node staging and for detection of metastases. A strength of PET-CT is that different radiotracers can be used to assess the underlying altered biology in cancer, including glucose metabolism, fatty acid metabolism, amino acid metabolism, and activity levels of a variety of receptors, enzymes and other cancer-specific and non-specific biomarkers.

Current imaging techniques, including PET-CT, are challenged by time consuming manual analysis, lack of quantification, reproducibility and most importantly, clinical validation. We believe that with automated quantification, analysis time will decrease, reproducibility will increase, and observer variability will decrease. Objectively measured imaging biomarkers that reliably quantify the PET-tracer activity would help realize the true clinical potential of PET-CT.

The aim is therefore to use new deep learning algorithms to develop novel PET-CT imaging biomarkers and to generate empirical evidence for analytical and clinical validation of the imaging biomarkers as indicators of prognosis and treatment efficacy in patients with cancer. In a first step, patients with prostate cancer are included. Work in progress includes also patients with lymphoma, lung cancer, cervical cancer, neuroendocrine tumours and breast cancer.

Validated imaging biomarkers could transform future clinical care, clinical trials, and drug discovery for cancer, and could transform future imaging biomarker development in cancer using other imaging modalities.

**Strengths in lab**

Nuclear medicine, PET-CT, image reconstruction

3D skeletal uptake of 18F sodium fluoride in PET-CT images is associated with overall survival in patients with prostate cancer

**Background**

Sodium fluoride (NaF) positron emission tomography combined with computer tomography (PET-CT) has shown to be more sensitive than the whole-body bone scan in the detection of skeletal uptake due to metastases in patients with prostate cancer. We aimed to calculate a 3D index for NaF PET-CT and investigate its correlation to overall survival (OS) in a group of patients with prostate cancer.

**Methods**

NaF PET-CTs were studied in 48 patients with prostate cancer. Automated segmentation of the thoracic and lumbar spines, sacrum, pelvis, ribs, scapulae, clavicles, and sternum were made in the CT images. Hotspots in the PET images were selected using both a manual and an automated method. The volume of each hotspot localized in the skeleton in the corresponding CT image was calculated. Two PET-CT indices, based on manual (manual PET index) and automatic segmenting using a threshold of SUV 15 (automated PET15 index), were calculated by dividing the sum of all hotspot volumes with the volume of all segmented bones.

**Results**

Manual PET index and automated PET15 index were significantly associated with OS and concordance indices were 0.69, and 0.70, respectively. The median manual PET index was 0.53 and patients with a manual PET index >0.53 had a significantly shorter median survival time than patients with a manual PET index <0.53 (2.5 years vs not reached after 5 years of follow-up (p<0.001)). The median automated PET15 index was 0.11 and patients with an automated PET15 index >0.11 had a significantly shorter median survival time than patients with an automated PET15 index <0.11 (2.3 years vs not reached after 5 years of follow-up (p<0.001)).

**Conclusions**

PET-CT indices based on NaF PET-CT are significantly associated with overall survival in patients with prostate cancer.

Eleonore von Castelmur

Wallenberg Centre for Molecular Medicine, Linköping University

Group Leader at Department of Physics, Chemistry

and Biology (IFM)

Faculty of Science and Engineering

eleonore.von.castelmur@liu.se

https://liu.se/en/employee/elevo74

Area of interest

Molecular mechanisms underlying protein function in health and disease

I am interested in examining at the molecular level how alterations in the sequence and structure of proteins affect their function. Combining techniques such as X-ray crystallography with NMR and solution scattering techniques allows us to explore the structure and dynamics of proteins and complexes of varying size and complexity. Integrating these techniques with biochemistry, biophysics and cell biology allows us to probe their function and regulation at the molecular as well as cellular level.

In particular, we are employing these integrated structural biology techniques to explore the evolutionary relationship and functional repurposing of human proteins acquired by picornaviruses.

Picornaviruses are a major cause of infections in humans and as such, it is of great interest to identify and characterise “host factors” (cellular proteins) necessary for viral infection, as well as their viral homologs. We want to understand whether the viruses have acquired these proteins to become independent of the cellular variant, and how these proteins have evolved in the viruses to fulfil new functions in short evolutionary time-frames. Such discoveries provide target candidates for the development of novel antiviral therapeutics and help us gain a better understanding of the lifecycle of these biomedically important viruses.

In the future, I’d also like to explore whether we can evolve these proteins further, e.g. for applications in biotechnology.

Strengths in lab

In my lab we have extensive experience in all aspects of protein crystallography, from construct design and (high-throughput, LIC) cloning, protein expression and purification, crystallization, data collection and processing, phasing using both molecular replacement as well as experimental phasing techniques (for novel structures), model building and analysis.

We also use general biochemistry and biophysics techniques to characterize protein stability and function as well as protein–protein as well as protein-ligand interactions.

Understanding Viral Entry Mechanisms Through Multi-Level Structural Analysis

Picornaviruses are a major cause of infections in humans and animals, including common cold, poliomyelitis, hepatitis A, and foot-and-mouth disease. To identify and characterise novel viral host factors will help us gain a better understanding of the lifecycle of these biomedically important viruses, and hopefully yield candidates for the development of novel antiviral therapeutics. By employing insertional mutagenesis in haploid human cells, we identified a poorly-studied phospholipid-modifying enzyme, PLA2G16, as a common host factor for multiple picornaviruses. We could show that this enzyme is required in the early steps of viral entry, enabling virion-mediated genome delivery into the cytoplasm (ahead of a viral pore-formation triggered clearance pathway involving the autophagy machinery). Remarkably, there is a group of picornaviruses whose 2A proteins share conserved sequence motifs with the human protein host factor, which led us to ask whether these viruses had acquired and evolved the host protein so as to become independent from the host factor.

We are currently characterising representative examples of this protein from all branches of the phylogenetic tree of viral 2AH-box proteins, to try and trace the possible evolutionary steps of this protein. The crystal structures of H-box containing proteins we have elucidated to date reveal a remarkable structural plasticity. Addressing how this structural plasticity correlates with the functional repurposing of the protein in the different picornaviruses will help answer still open questions on the molecular details of the picornavirus life cycle, contribute to our basic understanding of protein folding and function, and help guide rational enzyme design efforts.

Darcy Wagner

Wallenberg Centre for Molecular Medicine, Lund University

Associate Senior Lecturer at the Department of Experimental Medical Sciences, Lund University

darcy.wagner@med.lu.se

tinyurl.com/lungbiogen

Area of interest

Over three million people die each year and over 60 million people suffer worldwide from chronic lung diseases (CLDs). At present, there is no cure for CLDs, including chronic obstructive pulmonary disease (COPD), pulmonary hypertension, and pulmonary fibrosis. Lung transplantation is the only option at end-stage disease and is further complicated by shortage in organs available for transplantation and low efficacy. Five-year survival rate has remained at 50% for the last decade. New options are desperately needed for these patients.

Our lab focuses on understanding the role of the extracellular environment for endogenous and exogenous lung tissue regeneration in healthy and diseased lung. In particular, our work focuses on the design and use of biologic and synthetic scaffolds to bioengineer new lung tissue for transplantation. We further aim to build new models of human lung tissue to reduce animal usage, better understand how regeneration processes are deranged in CLDs, and for use as drug discovery and therapeutic screening platforms.

Strengths in lab

My lab has a translational approach which uses techniques ranging from the cell level to in *vivo* animal models and *ex vivo* human models. In addition to standard cell and molecular biology techniques, we have established the following techniques:

The bioengineering arm of my lab has established techniques for whole organ perfusion decellularization, physiologic recellularization in 2 and 3-dimensions, and *ex vivo* bioreactor culture (including whole native organs). We have recently been working on developing bioinks from biologic and engineered materials for 3D printing of lung tissue. In this regard, we have also developed the accompanying expertise to characterize bioinks with regards to their rheological and 3D bioprinting properties.

The lab also studies repair and regeneration and has established expertise in precision cut tissue slices, organoid culture of primary stem and progenitor cells, and mechanotransduction (stretch and stiffness studies). We have experience in chronic lung disease murine models of fibrosis (bleomycin) and emphysema (elastase induced), as well as measuring lung mechanics using the Flexivent. The lab also utilizes bioinformatics approaches such as gene set enrichment analysis applied towards understanding chronic lung diseases.

Development of a hybrid alginate-ECM hydrogel as a potential bioink for 3D bioprinting

Background

Lung transplantation is the only curative approach for end stage lung disease, but tissue shortage remains a problem. Generating lung tissue ex vivo could overcome tissue shortages. Synthetic and biologic scaffolds have been explored, yet both have limitations. Biologic scaffolds have low reproducibility, while synthetic scaffolds lack biologically inductive properties. Hybrid materials, comprised of both, have been used for tissue engineering, but have been limited to simple shapes. Recent advances in 3D printing may permit for complex shapes, but potential bioinks are unknown. We hypothesized that a hybrid hydrogel generated by combining a synthetic polymer with lung derived extracellular matrix (ECM) could be a suitable bioink for 3D bioprinting lung tissue.

Methods

We generated hybrid hydrogels from a synthetic polymer, alginate, and ECM derived from decellularised mouse lungs to make hybrid hydrogels. Different combinations of ECM and alginate hybrid pre-hydrogel solutions and crosslinked hydrogels were characterised by rheology, spectrophotometry for turbidity, histological stainings and scanning electron microscopy (SEM). Viability and proliferation of distal lung epithelial cell lines (MLE12 and A549) encapsulated in the hydrogel were measured via immunofluorescence (Ki67+) and flow cytometry (EdU+) after 7 days.

Results

A hybrid ECM gel was successfully generated from alginate and decellularised mouse lung ECM. Percentages of ECM and alginate in pre-gel solutions impacted viscosity. Both MLE12 and A549 cells remained viable and were distributed homogenously within manufactured hydrogels of various shapes. MLE12s in ECM-alginate hybrid hydrogels were significantly more proliferative (30% increase) than MLE12 cells seeded in an alginate hydrogel as assessed by flow cytometry.

Discussion

A hybrid hydrogel generated from alginate and decellularised lung derived ECM supports proliferation of lung epithelial cell lines and can be manufactured in a variety of shapes. The hybrid alginate-ECM solution is thus a promising candidate as a bioink for 3D bioprinting but processing parameters need to be further optimized to generate constructs more closely resembling lung tissue.

Anders Wittrup

Wallenberg Center for Molecular Medicine, Lund University

Associate Professor and Consultant in Oncology, Department of Clinical Sciences Lund, Skåne University Hospital and

Lund University

anders.wittrup@med.lu.se

http://www.med.lu.se/wcmm/researchers/fellows#Wittrup

Area of interest

Despite recent advances, there is still a substantial unmet medical need for novel cancer therapeutics. A new class of pharmacological compounds, based on RNA, have recently opened the prospect to specifically target numerous cancer cell vulnerabilities. However, cytosolic delivery of macromolecular RNA molecules is a major hurdle to translate these molecules into clinically useful drugs. Still, a few RNA based drugs have entered clinical use and delivery to certain human tissues, notably the liver and CNS, is possible. However, to reach other tissues, and in particular tumors, the delivery process has to be improved significantly.

In our lab we have two main focus areas: First, we develop methods to study the process of cytosolic delivery of RNA. In particular, we have developed high resolution microscopy methods to study RNA delivery in living cells. Second, based on the insights from these studies we are developing novel strategies to enhance RNA delivery to tumors. These efforts have the potential to specifically turn off driving cancer genes and ultimately halt disease progression.

Strengths in lab

In our lab we use of various fluorescence microscopy techniques on living cells, 3D organoids and tumor tissues. To visualize fast and dynamic cell biological processes we make use of high-speed fluorescence imaging, both wide-field and confocal microscopy. For high resolution imaging of living cells, we use structured illumination microscopy (SIM). A particular focus in the lab is to translate microscopic images to reliable quantitative information and to calculate the number of molecules involved in the studied processes. We have also extensive experience of handling large amounts of microscopic data and how to process this data to address specific scientific questions in statistically solid ways.

Imaging Release of siRNAs from Vesicles Damaged by Membrane Disrupting Drugs

Hampus Du Rietz, Hampus Hedlund, Anders Wittrup

Small interfering RNA (siRNA) is a promising class of new therapeutics for targeting of disease-causing genes by RNA interference. For most siRNA-delivery approaches however, endosomal trapping of endocytosed siRNAs severely hampers the therapeutic effect. Only a small fraction is believed to exit the endosomes, which poses a significant barrier to the development of new siRNA therapeutics – especially with targets beyond the liver. Co-administration of membrane destabilizing small molecule drugs is one frequently explored strategy aiming to enhance escape of endosomal contents. Such compounds are believed to selectively disrupt lysosomes, however experimental data clearly demonstrating this is lacking.

We have investigated a collection of membrane destabilizing drugs and found that they disrupt intracellular vesicles and trigger escape of endosomal cargo with varying potency. To study these processes in detail, we have established a sensitive live cell imaging approach for visualization and quantification of vesicle damage and release of siRNAs from individual endosomes. This method allows analysis of cargo-vesicle disruption frequency, escape efficiency and release kinetics. Here, we used cholesterol-conjugated siRNAs as a model construct, although this approach could be extended for investigation of other oligonucleotides and targeted siRNA-conjugates. In addition, we use observer-independent classification strategies to characterize the compartment-identity of vesicles damaged by membrane disrupting drugs.

Our findings show that membrane destabilizing drugs are diverse and not lysosome specific. Drug-induced disruption of siRNA-containing vesicles permits efficient endosomal escape. However, mismatch between cargo-containing and drug-targeted compartments limits the improvement of siRNA-mediated knockdown. This has implications for future efforts to enhance the delivery of siRNAs and other nucleic acid-based drugs.

Aleksej Zelezniak

SciLifeLab/Chalmers University of Technology

Assistant Professor, Department of Chemical and Biological Engineering, Chalmers University of Technology

alezel@chalmers.se

https://www.chalmers.se/en/departments/bio/research/systems-biology/zelezniak-lab/Pages/default.aspx

Area of interest

Our research group interested in studying how genetic, environmental factors affect the operation and regulation of cellular metabolic networks. At the single species level, we want to understand how complex phenotypes emerge from the underlying molecular levels organized via central biological dogma. At the multicellular level, we want to understand what is the role of metabolism in the cell-to-cell interactions, in particular, its role in the co-existence of microbial species. Answering these questions will allow us not only to design organisms with desired metabolic properties for biotechnology purposes but also engineer synthetic microbial communities with specific health benefits. We are combining best practices of data science with artificial intelligence to learn quantitative understanding about genotype-phenotype relationships in metabolism organized via central molecular dogma.

Strengths in lab

We are experienced in metabolic modeling, flux analysis, bioinformatics, and overall biological data analysis. One of our research areas involves the development of tools for mass spectrometry signal analysis. Specifically, we work with proteomics (data independent acquisition) to enable fast and precise data acquisition at a large scale. We employ model big data technologies (e.g. Spark, GPU acceleration) to make sense of raw mass signal and translate it to biological findings. We collaborate with experimental labs and clinicians to enable proteomics for clinical applications. We are actively working with state-of-the-art machine learning approaches (Deep Learning) to use machine intelligence for getting insights into complex molecular data (actively working with gene expression). We love yeast as an experimental model.

Deep learning predicts gene expression from DNA sequence:

Gene expression is evolutionary shaped through tuning DNA regulatory grammar

Jan Zrimec, Aleksej Zelezniak

Transcription is a fundamental biological process acting as an interface between DNA and protein synthesis machinery. It is however unclear, to what extent a wide transcriptional repertoire, ranging from a just few copy numbers up to several thousands of transcripts per cell, encoded in the DNA and what are the key determinants such variation. Through analysis of over 50 000 RNA-Seq experiments using deep neural networks, here we show using that genes expression levels can be predicted (<25% error) using only nucleotide code for 70%-80% genes in organisms from all kingdoms of life including human. We have identified the presence of interactions between DNA motifs in all gene elements, including promoter, untranslated regions and termination sequences that are crucial for transcription regulation in *Saccharomyces cerevisiae*​. We showed that genes are evolved together with its flanking regulatory elements by demonstrating coevolving mutational mutation rates between genes regions in over 3500 orthologous genes from 14 fungi species. Overall, our results advance the understanding of the regulatory code and suggest that protein coding regions with adjacent flanking elements is in essence an evolved transcription regulatory unit yielding a mechanism by which whole gene sequences with prespecified expression patterns can be designed.

Daniel Öhlund

Wallenberg Centre for Molecular Medicine, Umeå University

Associate Senior Lecturer at the Department of Radiation Sciences

Resident physician in Oncology, University Hospital of Umeå

daniel.ohlund@umu.se

https://www.umu.se/forskning/grupper/ohlund-laboratory/

Area of interest

Novel therapies for Pancreatic Cancer

The research in Daniel Öhlund’s laboratory aims to identify and explore novel therapeutic strategies for pancreatic ductal adenocarcinoma (PDAC) by targeting the tumor-associated stroma of this highly treatment resistant disease.

PDAC is characterized by a pronounced fibrotic stroma that surrounds clusters of cancer cells. The cancer cells are known to trigger both the recruitment and activation of heterogeneous populations of cells, such as cancer-associated fibroblasts (CAFs), immune cells, and neurons, and to stimulate the production of extracellular matrix (ECM). Many studies have suggested that certain stromal cells, and the ECM they produce, provide the cancer cells with essential signals that regulate cancer cell growth and survival, modulate drug response and contribute to therapy resistance. But recent data have also shown that nonselective approaches to target the stroma can give undesirable and unpredicted results. This highlights the complexity of the tumor-associated stroma and underlines the need for a better understanding of the stromal heterogeneity to be able to develop more precise drugs targeting the stroma. **The hypothesis** developed in the lab predicts that the stroma contains subpopulations of stromal cells, and ECM proteins, with different pathophysiological roles. Some stromal components are induced by the cancer cells to serve pro-tumorigenic roles, and others are driven by host defense mechanisms to serve anti-tumorigenic purposes. By deciphering the stromal composition and by developing strategies that selectively target the pro-tumorigenic elements of the stroma, or that is enhancing the efficacy of the anti-tumorigenic stromal elements, we believe that tumor inhibitory effects can be achieved.

**Strengths in lab**

To reveal the full complexity of the stroma, we are applying mass spectrometry-based methods, single cell sequencing techniques, and different *in situ* RNA sequencing techniques, on cancer tissue from genetically engineered mouse models (GEMMs) of pancreatic cancer and human pancreatic cancer tissue from biobanks with detailed clinical data available. To further identify which of the components in the stroma that serve as potential drug targets, we have developed organoid based co-culture systems where both neoplastic cells and different stromal cells are represented. Promising findings are tested *in vivo* in pre-clinical drug trials at our animal hospital. The team of scientists in the laboratory have different background and expertise, and the scientific questions are approached with a multidisciplinary mindset.

Targeting Tumor-stromal Interactions in Pancreatic Cancer

Pancreatic cancer is resistant to available adjuvant therapies, indicating that novel strategies to tackle the disease are needed. The pancreatic tumor is characterized by a pronounced tumor stroma that surrounds the cancer cells, containing extracellular matrix and cancer-associated fibroblasts that provide the cancer cells with important signals that regulate cancer cell growth and survival, and contribute to therapy resistance. **The overall goal** of this project is to get a deeper understanding in the stromal heterogeneity, and to reveal and explore potential drugable targets hidden within the stroma. First, we will decipher the composition of the extracellular matrix by applying mass spectrometry-based methods, then resolving the phenotypic diversity found in cancer- associated fibroblasts embedded within the tumor by utilizing single cell sequencing and spatial transcriptomic techniques. Next, we aim to determine which stromal elements that are important in regulating cancer cell growth, survival, immune escape, and drug resistance by using genetically engineered mouse models of pancreatic cancer and organoid based co-culture systems. Finally, we will develop and test drugs that target the most pro-tumorigenic stromal interactions with the aim to discover and explore novel therapeutic strategies.

Gustav Andersson

Wallenberg Centre for Molecular Medicine, Umeå University

Associate Senior Lecturer at the Department of Integrative

Medical Biology

Resident physician in Hand Surgery, University Hospital of Umeå

gustav.andersson@umu.se

https://www.umu.se/en/research/groups/laboratory-of-neural-repairand-cellular-therapy/

Area of interest

Diffusion magnetic resonance imaging of nerve injury and repair

Our research group combine cells and biomaterials to create nerve repair conduits to treat injuries to the nervous system. Using in vitro and in vivo systems we are investigating the biological reactions which impede regeneration with the goal to develop novel therapies to increase neuronal survival and reduce target organ atrophy. Development of non-invasive MRI techniques to evaluate the degree of injury and to follow regenerating axons following repair has been the focus of my research in recent years. By use of diffusion tensor imaging and diffusion kurtosis imaging we have been able to visualize the injured and healing nerves, and to differentiate the healthy nerve from the degenerating distal stump. In the preclinical setting the technique will be used primarily to study the effects of novel therapies such as cell-based interventions, and biomaterials promoting peripheral nerve regeneration. As the clinical field of hand surgery deals with devastating nerve injuries in the upper extremity – the brachial plexus injury being the most severe – application of these imaging techniques is of great interest and would allow us to better detect which nerves are damaged and to what extent, as well as provide a valuable tool to follow the regenerating nerves and whether reinnervation of the end-organ will occur before it atrophies beyond rescue. In the cases where reinnervation does not occur, grafting or transferring of other functional nerves can be performed surgically to save the end-organ and preserve motor function of the limb.

Strengths in lab

Our group has long experience in studying injury and repair of peripheral nerves and spinal cord, and has established reproducible animal models of these conditions. The group consists of basic scientists and clinicians which provide insight both into the clinical field and the techniques required to perform the research of interest. Our infrastructure consists of modern animal facilities and cell culture labs, as well as a close proximity to the clinical setting where patients with peripheral nerve injuries appear. A clinical MRI scanner of 3 Tesla and a preclinical (animal) scanner of 9.4 Tesla serve as the basis for our imaging studies on nerve injuries. International collaborations, primarily with Leeds in the UK, is a long tradition with exchange both of research and clinical expertise.

Emma Börgeson

Wallenberg Centre for Molecular and Translational Medicine,

University of Gothenburg

Associate Senior Lecturer, Institute of Medicine

emma.borgeson@gu.se

wcmtm.gu.se/research-groups/borgeson

www.borgesonlab.org

Area of interest

Therapeutic potential of immunomodulation in cardiometabolic pathophysiology

My lab is a translational research team, investigating the underlying disease mechanisms of obesity and cardiometabolic disease, with a special interest in inflammatory resolution. We investigate the therapeutic potential of immunomodulation in obesity-related cardiometabolic pathophysiology, aiming to identify the underlying cause of metabolic disease and a more effective treatment targeting the inflammation that underlies pathophysiology.

Inflammation is a key driver of obesity-induced cardiometabolic pathophysiology and consists of two phases: an initial acute phase followed by a resolving phase. The latter is actively regulated by specialized pro-resolving lipid mediators (SPMs). Lipoxins are one group of SPMs that act through defined receptors to promote the resolution of inflammation.

The overall aim of our research is to investigate and harness the therapeutic potential of lipoxins. Our previous studies suggest that treatment with lipoxins attenuates obesity-induced adipose inflammation and subsequent development of systemic disease (Börgeson et al, Cell Metabolism, 2015). The lab is currently investigating the underlying molecular mechanisms that mediate this protection, and whether the results from preclinical models can be translated to human pathophysiology. We are also attempting to identify novel inflammatory and cardiometabolic fingerprints and biomarkers that characterize metabolically healthy obese (MHO) and metabolically unhealthy obese (MUO) patient phenotypes.

Strengths in lab

My research team combines experimental studies with clinical basic research to address hypothesis in a translational manner. We primarily utilize ex vivo cultures of patient tissue biopsies (e.g. adipose and intestine explants), which provides valuable “proof-of-principle” models which allows us to correlate experimental data with human physiology. As experimental “readouts” we use standard molecular biology techniques (digital droplet PCR, western blot, immunohistochemistry, immunofluorescence, ELISA etc.) and extensive flow cytometry characterization of leukocyte phenotype and number.

Elias Johansson

Wallenberg Centre for Molecular Medicine, Umeå University

Resident Physician in Neurology, University Hospital of Umeå

Researcher at the Department of Pharmacology and

Clinical Neuroscience

elias.johansson@umu.se

https://www.umu.se/wallenberg-centrum-for-molekylar-medicin/

forskning/wcmm-associerade-forskare/elias-johansson/

Area of interest

We conduct stroke research with a large clinical component, but also preclinical aspects.

Carotid stenosis (atherosclerosis in the carotid arteries) is one of several common causes of stroke. In routine health care, patients with stroke or TIA (transient stroke) caused by carotid stenoses with ≥50% lumen reduction undergo surgery or stenting to remove the stenosis. One exception is the rare form of most severe stenoses called “near-occlusion”, as these are believed to have a good prognosis. Near-occlusions are marked by a collapse in distal artery size due to flow reduction. We have recently discovered that near-occlusions are neither rare nor have good prognosis. Methodological errors of previous studies in terms of diagnostics and timing of inclusion explains the previous misunderstanding.

We are now learning more about near-occlusion to pave the way for future randomized trial(s), including diagnostics, prognosis and pathophysiology. Possibly, a new treatment method will be warranted.

Our current study is a prospective observational study, including 500 patients with carotid stenosis. These are examined with several advanced imaging modalities *in vivo*. To augment the pathophysiological aspect, plaque histology and blood biomarkers are also assessed. A planned spin-off is to create virtual histology for ultrasound and CT-angio, by comparing with histology.

Strengths in lab

For own research: Timing – having discovered the errors of the current paradigm of near-occlusion, we know what questions to ask before others do.

In general: Access to large biobank material with very detailed patient information (based on detailed knowledge of the patients and in vivo imaging). Access to new, not commercially available, in vivo imaging (especially 4D flow MRI).

Niklas Mattsson

Wallenberg Centre for Molecular Medicine, Lund University

Associate Professor and Resident Neurologist, Department of Clinical Sciences Malmö, Neurology, Skåne University Hospital

and Lund University

niklas.mattsson@med.lu.se

Area of interest

Alzheimer’s disease (AD) is a common neurodegenerative disease, which causes immense suffering for millions of patients and their relatives. The disease is characterized by accumulation of β-amyloid and tau in the brain. There is still no cure or disease-modifying treatment for AD. This is partly due to a lack of understanding of the earliest events in the disease, and how the different components of the disease cascade are linked together.

Key research questions that I am interested in revolve around the mechanisms of the early stages of AD. For example, the accumulation of β-amyloid and tau appears to follow largely predictable spatiotemporal patterns, but why do these processes start, and what mechanisms controls their spread? β-amyloid accumulation appears to be necessary for the detrimental spread of tau across the brain, but why? How closely correlated is the accumulation of β-amyloid and tau with synaptic and neuronal loss, and by which mechanism is the injury mediated? Are there especially toxic forms of β-amyloid or tau that are responsible for the injury? Are other components (inflammation?) also necessary? What underlies the selective vulnerability in different brain regions? How does the mechanisms of pathology differ between AD and other neurodegenerative diseases? What can be learnt from comparisons with neuronal injury in other conditions (such as acute injury after cardiac arrest), and from the neurochemistry of normal brain functions (including for processes that are critically altered in AD, such as sleep or neuropsychiatric behavior)?

**Strengths in lab (technologies, methods):**

To tackle questions such as the ones listed above, I use technologies that span from cell biology and genetic studies to advanced biochemical and neuroimaging markers in living humans with very early stages of disease.

Vicent Pelechano

SciLifeLab/Karolinska Institutet

Assistant Professor

vicent.pelechano@scilifelab.se

http://pelechanolab.com

https://ki.se/en/mtc/vicente-pelechano-group

Area of interest

Genomics of gene expression

One of the biggest challenges in biology is to understand how apparently identical cells respond differently to the same stimulus. We are especially interested in understanding how the intrinsic complexity of gene expression contributes to non-genetic cellular adaptation. To deliver an integrated view of the mechanisms driving the appearance of divergent cellular phenotypes, as well as to refine our knowledge of the basic process of gene expression, we study: the epigenetic status, transcript isoform usage and post-transcriptional mRNA regulation.

In addition to our interest in the fundamental dissection of gene expression, our lab actively develops novel sequencing technologies. We have developed a diversity of approaches to study gene expression, chromatin organization and to improve clinical analysis. We investigate the complexity of overlapping human transcript isoforms simultaneously sequencing both the 5’ and 3’ ends of each RNA molecule (TIF-Seq). Our lab has also shown how the existence of widespread co-translational mRNA degradation allows studying ribosome dynamics by sequencing mRNA degradation intermediates (5P-Seq). By combining experimental and computational biology, we aim to decrease the gap between research fields and contribute to a better mechanistically understanding of the gene expression process.

Strengths in lab

Our group, combining experimental and computational work, aims to develop and apply novel genome-wide techniques to study eukaryotic transcription to address fundamental biological questions with medical implications. We are experts in the development of genome-wide methods based on massive parallel sequencing. To develop those approaches we use budding yeast and human cell lines. We are interested in: Chromatin and RNA Inmunoprecipitation (ChIP and RIP), transcript isoform measurement, alternative transcription start and polyadenylation site, ribosome profiling, RNA stability measurement, single-cell approaches and the development of novel clinical genomic tools.

Michael Schöll

Wallenberg Centre for Molecular and Translational Medicine,

University of Gothenburg

Associate Senior Lecturer, Institute of Physiology and Neuroscience

michael.scholl@neuro.gu.se

wcmtm.gu.se/research-groups/scholl

Area of interest

Early detection of neurodegenerative diseases

Neurodegenerative diseases are notoriously difficult to diagnose early and there is still no cure available for disorders such as Alzheimer’s disease (AD). Biomarkers derived from imaging modalities such as positron emission tomography (PET) and magnetic resonance imaging (MRI), as well as biomarkers based on the analysis of cerebrospinal fluid (CSF) or blood, have become immensely important especially for the early identification of individuals who are likely to develop neurodegenerative disorders, since an established notion is that potentially successful treatments should be deployed as early as possible in the disease process.

This early identification of neuropathological processes using adequate biomarkers currently not only supports reliable clinical diagnoses but also serves the recruitment of suitable candidates for clinical treatment trials, and renders possible the application of these biomarkers as outcome measures in treatment trials.

In particular the recent development of methods to map the accumulation of conformationally faulty forms of proteins and the subsequent synaptic impairment in vivo using PET has profoundly changed the way these processes can be identified at an early, pre-symptomatic disease stage. The Schöll group is using the most recent developments in molecular imaging by means of PET in combination with other neuroimaging- and fluid-based biomarkers, as well as neuropsychological profiling to develop holistic, validated, and usable tools for such an early identification.

Strengths in lab

Our group uses a truly multidisciplinary approach to create prediction models for neurodegenerative diseases. We combine advanced imaging- and fluid-based biomarker analyses with neuropsychological assessment and statistical modelling to validate both the predictive and diagnostic properties of each modality and combinations of modalities as well as to establish and validate novel, more accessible tools for the early, pre-symptomatic detection of pathogenic processes.