2025
PROJECT CATALOG
SciLifeLab Stockholm Summer Intern program

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    Erdinc Sezgin, Karolinska Institutet (SciLifeLab), erdinc.sezgin@scilifelab.se

1.

Exploring dynamic perturbations at the pMHC interface

PI: Adnane Achour (adnane.achour@scilifelab.se)
Supervisor: Tom Resink (tom.resink@scilifelab.se)

Project aim
Investigate the structural and dynamic bases for the differential immunogenicity of related peptide-MHC complexes

Project description
The immunogenic responses of cytotoxic $CD8^+$ T lymphocytes are guided by the interaction between their T cell receptors (TCRs) and cognate major histocompatibility complexes (MHCs) presenting peptide epitopes. While various structural and biophysical parameters governing the TCR-dependent recognition of peptide-MHCs (pMHCs) have been uncovered, the role of residue dynamics is often understudied or overlooked. Our lab focuses on these molecular bases of immunogenicity and is seeking to expand our understanding of dynamic patterns at the pMHC interface and how these may explain the differential responses elicited by closely related peptides.

During the project, the student will prepare several initial models to perform molecular dynamics (MD) simulations. They should become familiar with the steps involved in running MD experiments, from energy minimization to the final isothermal-isobaric production runs. This project will also provide hands-on experience with running and managing computational jobs on high performance computing (HPC) systems. While the simulations are running, the student will be expected to analyze previous MD datasets. The first dataset will be generated prior to them joining the lab; after this, all following datasets will come from their own simulations. By the end of the project, the student will be comfortable in analyzing and interpreting structural and dynamic data. This project will be purely computational. Thus, an understanding of the Linux command line and some experience with Python or R would be beneficial, although not required. A strong understanding of protein structure and intermolecular interactions is desirable. If this project is promising, it could provide an excellent foundation for a future master's thesis.

Techniques student will use/learn
- Running atomistic MD simulations (GROMACS)
- Biomolecular structure analysis (PyMOL/Coot)
- Data analysis and plotting (Python/R)
- Working on HPC systems (Linux)
- Interpreting structural and dynamic data

Supervision
Day-to-day supervision, training, and guidance will be handled in person by the PhD student (Tom Resink), who has experience with all the techniques.

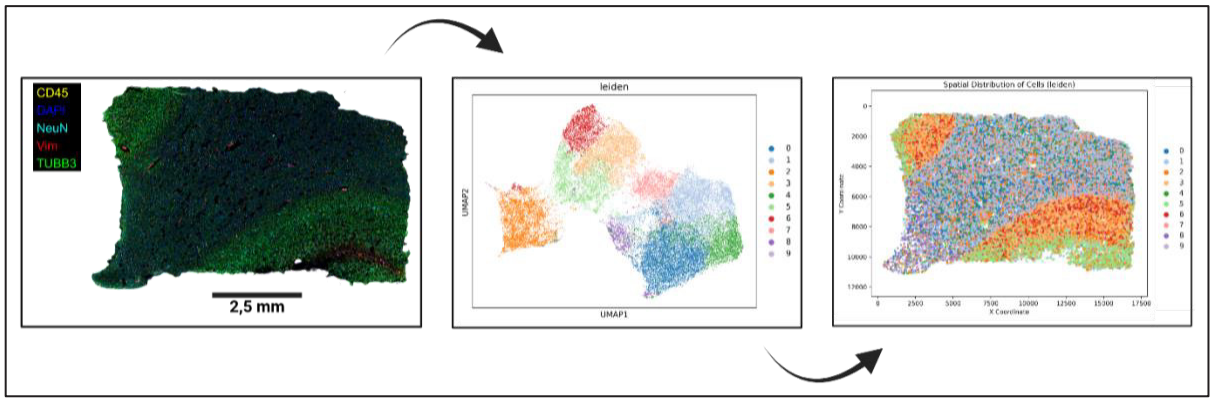
2.

Untangling the complexity of neurodegenerative diseases using spatial proteomics

PI: Burcu Ayoglu, Dept. of Protein Science, KTH burcu.ayoglu@scilifelab.se
On-site co-supervisor: Iva Sutevski, Dept. of Protein Science, KTH iva.sutevski@scilifelab.se

Project background and aims:

The human brain is composed of billions of neuronal and glial cells, present in hundreds to thousands of unique cell subtypes, distributed across nearly 200 distinct brain regions. Given this immense complexity, it is not surprising that neurodegenerative diseases (NDDs) disrupt multiple molecular, cellular, and tissue-level processes.
This project focuses on a group of NDDs known as Lewy Body Diseases (LBDs), including Parkinson's disease, the most common form. Like other NDDs, LBDs are characterized by the accumulation of misfolded proteins, synaptic dysfunction, impaired energy metabolism, cytoskeletal disorganization, proteostasis defects, neuroinflammation, and progressive neuronal cell death. However, the precise relationships between these pathological processes remain poorly understood, and as a result, no disease-modifying treatments are currently available.
Within a multi-omics research initiative, we leverage cutting-edge **spatial proteomics** assays to analyse post-mortem brain tissue from LBD patients. Our aim is to uncover the cellular and molecular features underlying LBDs across multiple brain regions with the goal of identifying clinically relevant biomarkers for early diagnostic and prognosis.



Representative workflow in spatial proteomics data analysis. We start with immunofluorescent images targeting ca. 30 different protein targets. After image preprocessing and computational identification and segmentation of individual cells, clusters of similar cells are analyzed, and their spatial distribution within brain tissue is explored for cell type annotation.

Techniques and learning outcomes:
During this summer internship, we will supervise you to gain hands-on experience with:

- **Immunofluorescence microscopy** for validating antibody specificity
- **Antibody conjugation** with oligonucleotide barcodes to build high-plex antibody panels for spatial proteomics experiments
- **PhenoCycler Fusion** spatial proteomics platform for analyzing human brain samples
- **Computational image processing and analysis** using in-house developed tools and custom Python scripts

By the end of this internship, you will have acquired a comprehensive understanding of the full workflow-from sample preparation to data acquisition and analysis-involved in spatial proteomics, which was recently recognized as Method of The Year by journal Nature. You will also apply these state-of-the-art techniques to advance our understanding of tissue biology in LBDs.

3.

Investigating Preferential Na^+/K^+-ATPase Protein Clustering Using Multi-Colour MINFLUX Super-Resolution Microscopy

Supervisor
Hjalmar Brismar (hjalmar.brismar@scilifelab.se), PI

On-site supervisor
Bruno Stojcic (bruno.stojcic@scilifelab.se), PhD student

Project Description

Super-resolution microscopy techniques have revolutionized our ability to visualize molecular structures beyond the diffraction limit of conventional light microscopy. Among these, MINFLUX (Minimal Photon Fluxes) represents the current pinnacle, achieving nanometer-scale resolution by combining elements of STED and single-molecule localization microscopy (SMLM). Unlike conventional fluorescence microscopy, which is limited to a resolution of approximately 200–250 nm due to diffraction, MINFLUX enables localization precision in the single-digit nanometer range. This makes it an attractive tool for studying nanoscale protein organization.

The Na^+/K^+-ATPase (NKA) is a critical membrane protein complex responsible for maintaining cellular ion homeostasis by actively transporting sodium and potassium ions across the plasma membrane. Beyond its canonical ion transport function, NKA is increasingly recognized for its role in cellular signaling and membrane organization. Evidence suggests that NKA may form dynamic clusters, but their structural organization and functional implications remain unclear.

In this project, the student will help generate and develop the analysis pipeline for MINFLUX super-resolution datasets to investigate NKA clustering. The NKA will be labeled using established constructs in the lab, with its protein subunits tagged using ALFA tag and GFP. The student will utilize the DNA-PAINT approach to generate multi-color super-resolution images, enabling precise localization of individual NKA proteins. An advanced image analysis pipeline will be developed to quantify clustering behavior with high precision. By leveraging MINFLUX's single-digit nanometer resolution, this approach will provide new insights into the nanoscale organization of NKA and its potential regulatory mechanisms in the cell membrane. Through hands-on experience with cutting-edge microscopy and computational analysis, the student will gain valuable skills in both experimental and data-driven biophysics research.

3.

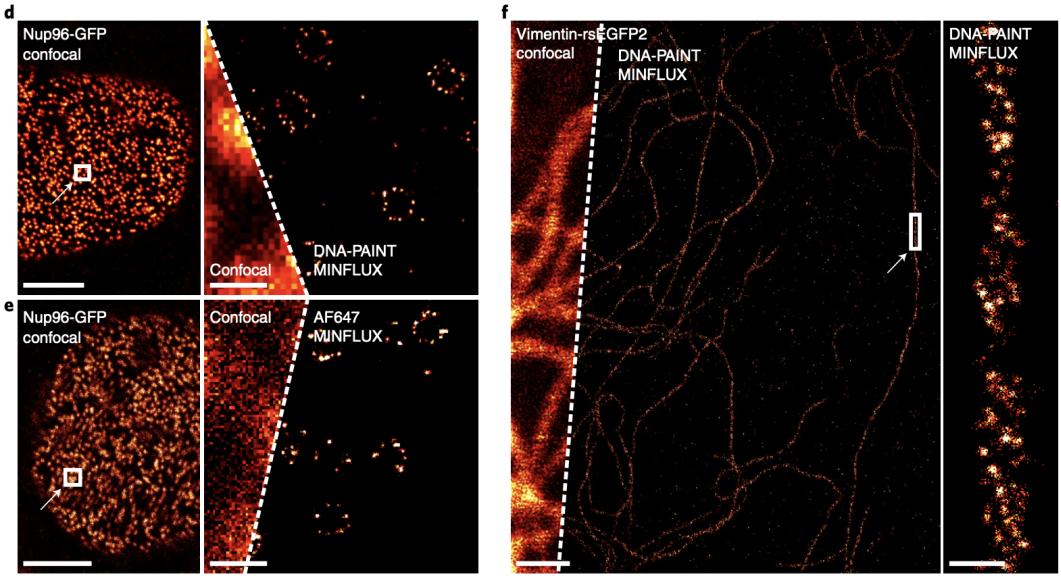


Figure 1: Excerpt from Ostersehlt et al., showcasing the comparison between the resolution of DNA-PAINT MINFLUX and conventional confocal microscopy on several subcellular protein targets. *Ostersehlt, L. M., Jans, D. C., Wittek, A., Keller-Findeisen, J., Inamdar, K., Sahl, S. J., Hell, S. W., & Jakobs, S. (2022). DNA-PAINT MINFLUX nanoscopy. Nature Methods, 19(9), 1072–1075.* https://doi.org/10.1038/s41592-022-01577-1

This summer you will gain valuable hands-on experience in:

- Cell culture and cell transfection transfection (if interested)
- MINFLUX and super-resolution microscopy sample preparation
- DNA-PAINT image acquisition (spectral unmixing and exchange PAINT)
- Advanced Image Analysis and Interpretation (using Python and/or Matlab)
- Implementation of clustering analysis algorithms (i.e. DBSCAN)

Project supervision plan

The intern will be supervised by Bruno Stojcic (PhD student), with support from the PI, other lab members, as well as the Advanced Light Microscopy (ALM) facility. All data acquisition components such as sample preparation and microscopy will be done with hands-on supervision, with more flexibility offered to the intern when designing an image processing pipeline. If there is interest, the intern is welcome to learn several fundamental wet lab techniques used in the lab such as cell culture, cell transfection, bacterial transformation, etc, as well as other microscopy modalities available in the lab, when appropriate for the project.

Unveiling Food Fraud: RNA-Based Species Identification

Project PI: Marc Friedländer; ERC-funded wet-lab postdoc to be recruited
Supervisor email: marc.friedlander@scilifelab.se

Project description:
Over recent years, our group has discovered that RNA can surprisingly persist in fragmented form in environmental samples, including food and air, and in extinct or even ancient animals (Figure 1) (1,2). In one research line funded by ERC, we apply RNA profiling to ensure food safety and detect food fraud. We are collaborating with the Food Fraud department of Livsmedelsverket to discover new RNA biomarkers. Most types of food substitutions (e.g., horse instead of beef) can be easily detected using DNA gene markers, however this is not possible when the animals are closely related. For instance, gourmet wild boar meat is in high demand but can easily be substituted with meat from domestic pigs that are virtually indistinguishable at the DNA level. We have preliminary evidence that these species can be distinguished at the RNA level - as they express different genes related to fat metabolism and muscle development. The summer student will profile RNA from food samples using next-generation sequencing and analyze the resulting RNA-seq data to detect putative biomarkers and distinguish the related species.

Techniques used:
The student will apply RNA extraction to food products, next-generation sequencing library prep, sequencing using our Illumina NextSeq2000 instrument, and computational analyses using HiSat2, featureCounts, DESeq2, Kraken2, and our in-house software, including miRTrace2.

Supervision plan:
For the wet-lab work, the student will be supervised hands-on by a postdoc we are currently recruiting for our ERC proof-of-concept project. For the dry-lab work, most supervision will be conducted by PI Marc Friedländer, mainly on-site but also via Zoom for some weeks over the summer. The student will be included in any publications and patents resulting from their work.

Figure 1: Tasmanian tiger specimen from which we recently extracted, sequenced, and profiled RNA (left). Air sampler that we have used to collect and profile airborne RNA from Skansen (middle). Example boar meat cut (right).

1. Friedländer MR; Gilbert MTP. *'How ancient RNA survives and what we can learn from it'*. **Nature Reviews Molecular Cell Biology**, 2024.

2. Mármol-Sánchez E, Fromm B, Oskolkov N, Pochon Z, Kalogeropoulos P, Eriksson E, Biryukova I, Sekar V, Ersmark E, Andersson B, Dalén L, Friedländer MR, *'Historical RNA expression profiles from the extinct Tasmanian tiger'*, **Genome Research**, 2023.

5.

Project title: Subcellular proteomics of acute myeloid leukemia (AML)

Principal Investigator (PI): Prof. Janne Lehtiö, janne.lehtio@scilifelab.se, KI
Supervisor: Yanbo Pan (PhD, Senior Research Specialist), yanbo.pan@scilifelab.se, KI

Objectives

- Generate subcellular proteomic profiles of AML cell lines.
- Identify proteins with significant re-localization linked to drug response.
- Provide hands-on experience in mass spectrometry-based proteomics and bioinformatics.

Project description

Protein localization plays a vital role in cellular function, and its misregulation can contribute to disease progression and drug resistance in AML. **Subcellular proteomics** has proven to be a powerful tool in identifying disease mechanisms by tracking protein distribution changes[1].

We have developed **SubCellBarCode** [2-4], which offers a straightforward method for robust protein localization and re-localization analysis for more than 15000 proteins. This technique has been successfully applied to study protein re-localization in response to various cellular perturbations, such as EGFR inhibition and protein farnesylation inhibition. By expanding its application to AML cells treated with anti-leukemia drugs, we aim to gain insights into drug mechanisms of action, resistance, and off-target toxicity.

Here, we are looking for a motivated student with an interest in subcellular proteomics. The student will learn to apply the SubCellBarCode method on AML cells with/without drug treatment, to explore the protein locations and translocations at a proteome-wide level. The SubCellBarCode method is a combination of laboratory work and bioinformatic analysis, we expect the student to work in a well-documented and reproducible way and the focus of the student will be the adaption of well-developed SubCellBarCode method on AML cells to generate the new knowledge to understanding leukemia. The project will provide the student with many new insights into MS-based proteomics, spatial proteomics, bioinformatic pipelines, as well as the involved leukemia biology.

Key techniques the student will use and learn

- Protein subcellular fractionation strategies for subcellular proteomics.
- Sample preparation for MS analysis, such as cell culturing, protein extraction/digestion.
- MS-based quantitative proteomics including both Data Independent Acquisition (DIA) and Tandem Mass Tagging (TMT) labeling methods.
- Reproducible computational subcellular proteomics analysis by **SubCellBarCode** R package.

Supervision

- Daily support from Yanbo Pan and senior lab member.
- Weekly meetings with Yanbo Pan to track progress, troubleshoot challenges, and refine methods.
- Mid-internship evaluation to assess progress and adjust goals if needed.
- Final review session with Yanbo Pan and Prof. Janne Lehtiö to discuss findings.

Deliverables

- A processed dataset with annotated subcellular localization and re-localization.
- A short scientific report/presentation summarizing methods, findings, and interpretations.

Reference

1. Josie A Christopher et al. ***Nat Rev Methods Primers***. 2021, 1:32.
2. Lukas Orre[#], Mattias Vesterlund[#], Yanbo Pan[#], et al. ***Mol Cell***. 2019, 3;73(1):166-182.e7.
3. Taner Arslan[#], Yanbo Pan[#], et al. ***Nat Protoc***. 2022, 17(8):1832-1867.
4. Yanbo Pan[#], et al. ***iScience*** 2025, doi: https:// doi.org/10.1016/j.isci.2025.111864.

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6.

Plasma proteomic signatures of patient-reported symptoms: linking molecular measurements to patient experiences

PI: Janne Lehtiö, janne.lehtio@ki.se
Supervisor: Noora Sissala, noora.sissala@ki.se
Affiliation: Department of Oncology-Pathology, Karolinska Institutet

Project description

Proteomics and other omics technologies have been widely used to understand disease mechanisms and discover biomarkers predicting clinical outcomes, such as treatment response and prognosis. However, far less attention has been given to subjective patient experiences, such as symptoms and sensations of illness, despite their importance to patients' quality of life. This project aims to address this knowledge gap by investigating how the plasma proteome—the proteins in the blood—correlates with patients' symptoms. Our goal is to gain new insights into how molecular changes influence which symptoms develop and how they are perceived by patients (e.g. the severity). Ultimately, understanding the molecular basis of symptoms could enable the discovery of biomarkers to inform symptom management and thereby improve patients' quality of life.

This project offers a valuable opportunity for a master student to gain hands-on experience analyzing data from cutting-edge proteomics technologies while contributing to interdisciplinary research at the intersection of molecular biology and patient care. The student will apply computational methods, preferably in R, to analyze and integrate proteomics data with detailed self-reported symptom data from patients with lung cancer and other lung conditions. Through this work, the student will develop skills in proteomics, bioinformatics, and biological interpretation. The student's objectives will be to:

1. Explore correlations between plasma protein levels and individual symptoms
2. Identify clusters of co-occurring symptoms and investigate their correlations with plasma protein levels
3. Compare the relationships between plasma proteins and symptoms across different lung conditions

The student will be jointly supervised by a PhD student (main supervisor) and PostDoc (co-supervisor) at Lehtiö lab (https://lehtiolab.github.io/).

Learning outcomes

- An understanding of proteomics technologies and plasma proteomics
- Quality control and pre-processing of different types of proteomics data
- Statistical analysis of proteomics and categorical (symptoms) data, including correlation, regression, and clustering analyses
- Data visualization in R
- Interpreting and presenting scientific data

7.

The use of sodium- or proton-motive force among bacteria: salinity-dependent prevalence and genetic context

Group leader: Anders Andersson (anders.andersson@scilifelab.se)
Supervisor: Krzysztof Jurdzinski (krzysztof.jurdzins@scilifelab.se)

Background:

Generating and utilizing electrochemical gradients is the basis of bioenergetic processes, especially ATP synthesis, for almost all lifeforms. Most organisms utilize proton-motive force (PMF). However, some bacteria use a sodium gradient instead, despite the utilization of sodium-motive force (SMF) being energetically less efficient than the use of PMF (Mulkidjanian et al., 2008)maintaining lower concentration of Na+ in the cytoplasm than in the surrounding milieu. In the vast majority of bacteria, as well as in mitochondria and chloroplasts, export of Na+ occurs at the expense of the proton-motive force. Some bacteria, however, possess primary generators of the transmembrane electrochemical gradient of Na+ (sodium-motive force. Still, around 72% of marine bacteria have been estimated to use NAD(P)H to produce SMF, compared to around 4% of freshwater bacteria (Walsh et al., 2013). Whether SMF generation using NAD(P)H always leads to SMF utilization for ATP generation, and whether it excludes PMF generation, remains unknown. Moreover, we have recently shown that gains of genes related to SMF-dependant ATP generation rarely accompany evolutionary transitions to a higher salinity level (Jurdzinski et al., 2023). We speculate that utilization of SMF requires a broad genetic repertoire, the acquisition of which is rare. Still, the genetic context in which SMF is used as the main energy-generating mechanism remains unmapped.

Project description:

The first task in the project will be to refine the methods used to identify SMF- and PMF-utilizing bacteria based on their genomes. Here, we will use orthology-based functional annotation of genes within metagenome-assembled genomes (MAGs) as obtained with EggNOG-Mapper (Huerta-Cepas et al., 2017)because predicting orthology is computationally intensive at large scale, and most pipelines are relatively inaccessible (e.g., new assignments only available through database updates. We have already run the annotation on thousands of MAGs from freshwater, brackish, and marine waters (Jurdzinski et al., 2023), and the student will analyze the results. More specifically, a proper set of genes indicating that a bacterium uses PMF or SMF needs to be identified. This step will most likely based on the identification of H^+ or Na^+ translocating NADH:ubiquinone oxidoreductases. Additionally, we will develop a proper statistical correction for the incompleteness of MAGs, the variability in the components comprising protein complexes, and the uncertainty of gene annotation. As a double-check, we also plan to deploy AlphaFold3 (Abramson et al., 2024) to assess whether the F_1F_o-ATPase genes found in the MAGs encode translocases having Na^+ or H^+ as a ligand. Ultimately, the refined identification of SMF and PMF utilization will be used to analyze the prevalence of these strategies among freshwater, brackish, and marine bacteria.

The second part of the project will focus on the genetic context in which SMF rather than PMF is used to generate ATP. Here, we will use the functional annotation of genes in the predicted SMF-utilizing MAGs from the first part of the project, and compare the gene sets to the ones found for the bacteria using PMF instead. Here, we can use classical (or Bayesian) statistics, network analysis, or machine learning-based methods (e.g., the Boruta algorithm), depending on the initial literature review and discussions with the student.

Some techniques and approaches used in the project

1. Orthology-based gene functional annotation, gene ontologies;
2. AlphaFold3;
3. Classical (or Bayesian) statistics, network analysis, or machine learning-based methods (e.g., the Boruta algorithm) for contextual gene-content analysis;
4. Data analysis with R and/or Python.

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7.

Brief plan of the project

Week 1	Weeks 2-3	Weeks 4-5	Week 6
Literature review, planning	Analyzing the functional gene annotation and running AlphaFold3 to identify SMF- and PMF-utlizing bacteria	Analyzing the genetic context of SMF-utilization	Combining and summarizing the results

Supervision plan

Krzysztof will hold regular meetings with the student 1-2 times a week and will be available to answer questions and help with troubleshooting throughout the internship. Anders will facilitate the planning and the synthesis of the project results and participate in regular meetings according to his availability during the summer.

References:

Abramson, J., Adler, J., Dunger, J., Evans, R., Green, T., Pritzel, A., Ronneberger, O., Willmore, L., Ballard, A.J., Bambrick, J., Bodenstein, S.W., Evans, D.A., Hung, C.-C., O'Neill, M., Reiman, D., Tunyasuvunakool, K., Wu, Z., Žemgulytė, A., Arvaniti, E., Beattie, C., Bertolli, O., Bridgland, A., Cherepanov, A., Congreve, M., Cowen-Rivers, A.I., Cowie, A., Figurnov, M., Fuchs, F.B., Gladman, H., Jain, R., Khan, Y.A., Low, C.M.R., Perlin, K., Potapenko, A., Savy, P., Singh, S., Stecula, A., Thillaisundaram, A., Tong, C., Yakneen, S., Zhong, E.D., Zielinski, M., Žídek, A., Bapst, V., Kohli, P., Jaderberg, M., Hassabis, D., Jumper, J.M., 2024. Accurate structure prediction of biomolecular interactions with AlphaFold 3. Nature 630, 493–500. https://doi.org/10.1038/s41586-024-07487-w

Huerta-Cepas, J., Forslund, K., Coelho, L.P., Szklarczyk, D., Jensen, L.J., von Mering, C., Bork, P., 2017. Fast Genome-Wide Functional Annotation through Orthology Assignment by eggNOG-Mapper. Mol. Biol. Evol. 34, 2115–2122. https://doi.org/10.1093/molbev/msx148

Jurdzinski, K.T., Mehrshad, M., Delgado, L.F., Deng, Z., Bertilsson, S., Andersson, A.F., 2023. Large-scale phylogenomics of aquatic bacteria reveal molecular mechanisms for adaptation to salinity. Sci. Adv. 9, eadg2059. https://doi.org/10.1126/sciadv.adg2059

Mulkidjanian, A.Y., Dibrov, P., Galperin, M.Y., 2008. The past and present of sodium energetics: May the sodium-motive force be with you. Biochim. Biophys. Acta BBA - Bioenerg., 15th European Bioenergetics Conference 2008 1777, 985–992. https://doi.org/10.1016/j.bbabio.2008.04.028

Walsh, D.A., Lafontaine, J., Grossart, H.-P., 2013. On the Eco-Evolutionary Relationships of Fresh and Salt Water Bacteria and the Role of Gene Transfer in Their Adaptation, in: Gophna, U. (Ed.), Lateral Gene Transfer in Evolution. Springer, New York, NY, pp. 55–77. https://doi.org/10.1007/978-1-4614-7780-8_3

8.

SciLifeLab Summer Fellow 2025 - Lappalainen lab - KTH - SciLifeLab

Analysing single cell drug response experiments via gene expression recalibration

Project PI: Tuuli Lappalainen

Project-Supervisor: Philipp Rentzsch

Project description

In recent years, there has been significant progress in leveraging single cell experiments as a massively parallel platform to perform molecular differential expression experiments. Depending on the experimental design, this enables the generation of large datasets that contain hundreds to thousands of tests of genomic elements, molecules, or drug targets. One very big such data set is Tahoe-100M, a drug response screen that measured the effect of 1,200 drug treatments on gene expression in 50 different cell lines.

In the project described here, we are seeking a student to analyze the Tahoe-100M dataset in conjunction with gene expression recalibration, a method we developed recently. Recalibration adjusts measured expression changes relative to the genetic variability between individuals, offering a novel view on where observed changes are not only on an absolute scale, but relative to the natural dosage range. For example, our method does less look at whether a change doubles the expression of a gene, but how that change compares to the difference observed between two healthy individuals, and whether it possibly is beyond the range observed in the human population. We have found that recalibration alters the focus of experiments by de-ranking highly variable genes and moving focus from, often well-known, response genes towards metabolic and regulatory genes.

We are interested in this project for two main reasons: Firstly, we think that recalibration can help in the analysis of this and other large drug screening experiments by providing a better view for identifying the genes directly affected by a drug. Secondly, we want to investigate the relationship between current genetic variability estimates that are measured at steady state and the genetic variability observed in this dataset, which is measured after the treatment in an excited, perturbed state. As such, we consider this an ongoing project and may be continued as a student-assistant or master's thesis project.

Technologies & techniques

- Working in an HPC environment
- Differential expression testing
- Data analysis in R or Python
- Data visualization

The student will be supervised by post-doc Philipp Rentzsch as an extension of his work on differential expression recalibration. We are able to provide a desk, access to computational resources and obviously provide in-person (or online, if preferred) help with the project.

Relevant publications/links

1. Rentzsch P, Kollotzek A, Mohammadi P and Lappalainen T 2024. Recalibrating differential gene expression by genetic dosage variance prioritizes functionally relevant genes. bioRxiv 2024.04.10.588830; doi: https://doi.org/10.1101/2024.04.10.588830
2. Vevo Therapeutics 2025. Press release: Vevo Therapeutics to Open Source Milestone Tahoe-100M Dataset. https://www.prnewswire.com/news-releases/vevo-therapeutics-to-open-source-milestone-tahoe-100m-dataset-302348631.html

9.

Mapping of Allosteric Regulation Sites in Metabolism and Gene Expression

PI: Vicent Pelechano (KI, MTC) - vicent.pelechano@scilifelab.se
Supervisor: Carlos Santolaria Bello (Postdoc) - carlos.santolaria@scilifelab.se

Background:

Metabolic enzymes have allosteric sites that interact with small molecules from metabolism, allowing for rapid and dynamic regulation of their activity based on cellular demands. These enzymes act as intracellular sensors, integrating signals from different metabolic pathways and responding to changes in the biochemical environment.

Allosteric regulation influences cancer development by affecting metabolism and other pathways crucial for cell survival. However, despite its importance, it has been less studied than other mechanisms, such as post-translational modifications or changes in enzyme expression. Investigating allosteric regulation is challenging due to its dynamic nature and the lack of experimental methods for proteome-wide analysis.

Project Description:

This project investigates how allosteric regulation influences cellular mechanisms like metabolism and transcription, and how these processes are coordinated. To achieve this, we identify putative allosteric sites across the proteome using computational predictions from AlphaFill.

Next, we perform a genome-wide perturbation of these predicted sites using MAGESTIC, a powerful multiplexed precision genome editing technique based on CRISPR-Cas9. This method allows us to introduce tens of thousands of mutations in a pooled format, without the need for colony isolation or resequencing. Additionally, it enables precise quantification of strain-specific abundance using Next-Generation Sequencing (NGS).

After genome editing with CRISPR-Cas9, we evaluate the fitness consequences of allosteric site perturbations by assessing the competitive growth of different strains in a pooled format and identifying them using NGS. Once we map the phenotypic landscape of allosteric sites, we focus on those with clear and reproducible phenotypes.

These sites will be further analyzed in the context of 3D protein structures to determine their functional relevance. Finally, we will validate key sites by generating and isolating individual mutant strains, confirming their phenotypes through genetic (RNA-seq, ribosomal profiling, ChIP-seq) and biochemical (enzyme activity assays, pull-down assays, LIP-MS) approaches.

Student Objectives:

- The student will be involved in performing competitive growth assays, preparing libraries for NGS, and identifying strains using NGS.
- The student will gain experience in wet lab techniques and in the analysis and integration of NGS data.

Relevant Techniques:

- Cell culture
- Molecular biology techniques
- Library preparation for NGS
- Data analysis of NGS results

10.

Project: Effects of diet on neuronal gene expression

PI: Iskra Pollak Dorocic (iskra.pollak@scilifelab.se)
Supervisor: Charlotta Henningson (charlotta.hennings@scilifelab.se)

Objective:
The objective of this project is to investigate the impact of diet on single-neuron mRNA expression patterns in specific brain regions of mice. The project aims to elucidate whether differences in diet, particularly between a regular diet and a high-fat diet, influences the expression of neuropeptidergic receptors in distinct neuronal populations. By analyzing fluorescent in situ hybridization images of mouse brain sections labeled with various mRNA probes, the student will explore how dietary factors shape neuronal diversity and receptor expression profiles at the single-cell level.

Key Responsibilities:

1. **Literature Review:**

Conduct a review of literature on the effects of diet on neuronal gene expression, neuropeptidergic systems, and relevant methodologies in neuroscience research.
Summarize key findings regarding the influence of diet on neuronal function, receptor expression, and associated behavioral outcomes. Identify gaps in knowledge and potential research directions for investigating diet-induced changes in mRNA expression.

2. **Data Exploration:**

Familiarize oneself with the fluorescent in situ hybridization images of mouse brain sections labeled with specific mRNA probes.
Utilize image analysis software such as Qupath with Cellpose and Fiji to explore spatial distribution, and mRNA expression patterns at single-neuron resolution.
Collaborate with team members to identify regions of interest and neuronal populations for further analysis.

3. **Data Analysis:**

Employ computational techniques to quantify and compare mRNA expression levels in neurons from mice on regular and high-fat diets.
Utilize statistical methods to identify diet-induced differences in neuropeptidergic receptor expression across distinct brain regions and neuronal subtypes.

Techniques:

RNAscope Hiplex Analysis: Utilize RNAscope Hiplex technology to detect and quantify mRNA expression in situ with single-cell resolution.
Qupath with Cellpose: Employ Qupath software with Cellpose plugin for automated cell segmentation and analysis of fluorescent images.
Fiji/ImageJ: Utilize Fiji/ImageJ for image processing and alignment of multiple rounds of imaging

Expected Outcomes:

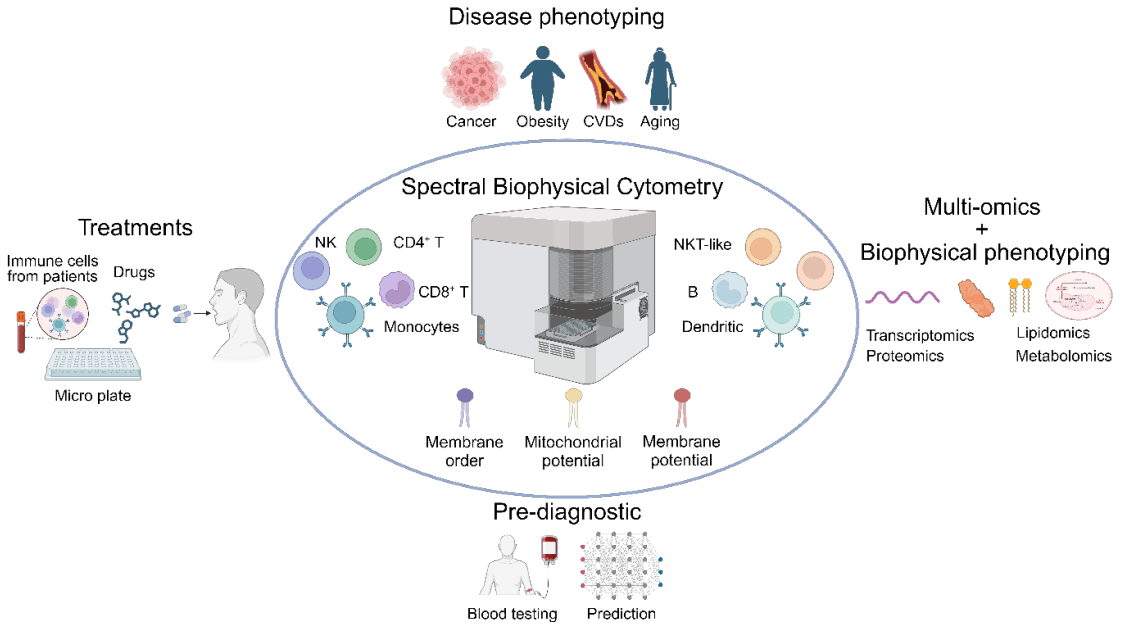
1. Gain hands-on experience in advanced molecular imaging techniques and computational analysis methodologies.
2. Contribute to uncovering novel insights into the effects of diet on neuronal gene expression and neuropeptidergic signaling pathways.

11.

Biophysical mapping in health and disease

PI: Erdinc Sezgin (KI/SciLifeLab) – erdinc.sezgin@ki.se
Supervisor: Cenk Gurdap (PhD student) – cenk.gurdap@ki.se
Description of the project: Cells undergo biophysical remodeling as a response to environmental changes, for survival and to execute their function. Changes in the biophysical properties of cells, such as membrane fluidity, tension, and membrane potential, are crucial to various diseases and have considerable potential as a diagnostic or prognostic marker. Over the years, numerous instruments, such as atomic force microscopy, micropipette aspiration, and optical traps have been developed to investigate the biophysical characteristics of cells. However, these technologies suffer from low throughput, which is a major obstacle to applying them to medical problems that require measuring thousands of cells. Therefore, there is still a major gap in our understanding of how collective biophysical properties of the cells alter during both physiological and pathological processes. We recently described a high-throughput and multi-parametric platform with single-cell resolution, which combines spectral flow cytometry and environment-sensitive dyes. Our platform enables fast and correlative measurement of the biophysical properties of model membranes, cell lines, and human blood samples.

The student will apply the method to 1) biophysically phenotype a disease or cellular states 2) integrate biophysical phenotyping with multi-omics technologies 3) predicting heath state of cells using machine learning based on biophysical remodeling 4) explore drug treatments on mentioned cells using the biophysical measurements as output.



List of main techniques: Spectral flow cytometry, environmental-sensitive dyes, cell culture, immune cell isolation, antibody and drug treatments, machine learning.
Supervision: The student will be mainly supervised by Cenk. Different parts of the project and techniques could be tailored to the student's interests. We provide training and daily supervision as needed. The student is more than welcome to engage in other lab activities, such as biophysics seminars and weekly lab meetings.

SciLifeLab