Impact Report 2022 on the Professorship of Cryo-Electron Microscopy of

Professor Martin Pilhofer

ETH Foundation

Thank you!

The Professorship of Cryo-Electron Microscopy led by Martin Pilhofer was made possible thanks to donations from the NOMIS Foundation, among others. The Baugarten Foundation and the von Finck family have also contributed substantially to the upgrade of existing equipment and the purchase of a new cryo-EM device at ETH Zurich.

We would like to express our heartfelt thanks for this generous support on behalf of all those involved.

In 2019, Martin Pilhofer was appointed to the Professorship of Cryo-Electron Microscopy at ETH Zurich.

Labor Martin Pilhofer

Current composition of the research group

Postdocs: 5
Doctoral students: 11
Technical staff: 1
Lab manager: 1
Project leader: 1
Administrative staff: 1

New to the group

Since last autumn, many new researchers have joined the group.

Anastasiia Kokhanovska

Technical staff member

BSc in Biotechnology, Kiev Polytechnic Institute
Nationality: Ukrainian

Marlen Petersen

Doctoral student (Weiss subgroup)

MSc University of British Columbia Vancouver
Nationality: German

Jessie Malit

Doctoral student

MSc University of Hong Kong
Nationality: Filipino

Highlights

In September 2022, the SNSF (Swiss National Science Foundation) approved a proposal for a project to investigate the structure and mechanism of contractile injection systems (CIS), the research group's core interest.

Drawing on the findings of the last few years, the team will investigate the contraction mechanism and any conformational changes in the baseplate of these systems. The baseplate binds specifically to target cells, enabling the pricking of target cells and the transfer of effector molecules (see below). In addition, a contractile injection system will be studied in primordial microbes called archaea, where no similar systems have been observed so far.

Research activities and outlook

The aim of Martin Pilhofer and his team is to investigate the interactions of bacteria with other cells. They are particularly interested in investigating the macromolecular structures that mediate these cell-cell interactions. By combining the key technology of cryo-electron tomography (cryoET) with other imaging techniques and functional assays, the group integrates information from the molecular to the cellular and intercellular scale.

The research is primarily concentrated on three areas:

1. Research into different types of cell-cell interactions.
2. Further development of cryo-electron microscopy and other imaging techniques.
3. Investigation of urinary tract infections by the Weiss sub-group.

Bacterial cell-cell interactions

The interaction between bacteria and other cells is based on the transfer of effector molecules from the bacteria to other cells. For this to happen, macromolecular machines are needed – that is, cell organelles that ensure that the effector molecules are channelled out of the bacterium and into the target cell. The various types of bacteria exhibit entirely different strategies for these secretion mechanisms. One of these is the contractile injection system, in which the bacterium pierces the target cell and thus transfers its effector molecule. Revealing these underlying mechanisms with the help of cryoET creates the necessary conditions for understanding the progression of disease in infections, and in a second step helps in the development of suitable prevention methods and therapies. These interactions are also important in the environment; for example, in the formation of biofilms and in the interaction of cyanobacteria with a large number of other organisms.

Summary of current research work

Investigation of

- contractile injection systems in three types of marine bacteria, multicellular cyanobacteria, streptomycetes and archaea;
- interactions between bacterial episymbionts (epixenosomes) and their host (ciliates);
- the interaction between marine bacteria and choanoflagellates;
- the secretion mechanism of Tc toxins;
- filaments from metabolic enzymes and meiosis in yeast;
- cell-cell communication in multicellular cyanobacteria;
- cell-cell interactions in hot springs and a desert oasis;
- evolutionarily interesting ciliate symbiosis in Lake Zug.

The newly discovered injection nanomachine of cyanobacteria is located in an unusual place, namely in the thylakoid membrane (green). (Graphic: from Weiss G., et al, Nature Microbiology 2022)

Highlights 2021–2022

Over the past year, Martin Pilhofer and his team have made significant progress in the identification and characterisation of new bacterial contractile injection systems. These results will substantially improve understanding of the structure, mechanisms and evolution of bacterial contractile injection systems. Two pioneering studies were published in Nature Microbiology in January 2022 (Weiss et al. and Xu et al., see publication list). These describe two novel injection systems: one found in cyanobacteria (blue-green algae), and one in the marine bacterium Algoriphagus machipongonensis.

The newly discovered contractile injection systems (CIS) operate in a very different way from previously described systems and have a number of unique features. As a result, they shed light on the evolutionary differences between different types of injection systems. You can find more about the research and the contractile injection systems at https://ethz.ch/en/news-and-events/eth-news/news/2022/03/like-bacteria-firing-spearguns.html.

A further study, recently shared with the scientific community on a preprint server (Casu et al., see publication list), identified a new type of contractile injection system in Streptomyces bacteria, which could be a crucial tool for identifying and producing antibiotics. Contractile injection systems induce cell death in Streptomyces under stress conditions and play a key role in their complex life cycle.

Further development of cryo-electron microscopy

CryoET represents a key method of allowing insights into the structure of cell-cell interactions in the nanometre range. The method can be successfully combined with other imaging techniques in order to understand interactions on several "scales" or at different levels of magnification. In this way, light microscopy datasets provide information in the micrometre range, while structural biology methods offer insights at the atomic level in the nanometre range and below.

Cryo-electron tomography poses two challenges: combining and correlating the datasets, and applying it to complex samples, such as organoids, environmental samples or patient samples. These challenges are currently top priorities in the group's work.

Summary of current research work

Development of

— instruments and processes for cryo light microscopy, in collaboration with Carl Zeiss and Leica Microsystems.

(Further) development of processes and methods

— processes to "print" samples specifically on the slide (micropatterning);

— processes to freeze samples using high pressure;

— methods to concentrate bacteria in environmental samples and identify them by means of microscopy. In particular, a novel method is being investigated for isolating and examining/identifying cells individually after they have been imaged with cryo-EM;

— for automatically generating samples for cryoET using a focused ion beam (cryo-focused ion beam milling);

— for recording data using cryoET.

Highlights 2021–2022

Martin Pilhofer (main applicant) applied with ten other ETH professors to the SNSF and ETH for funding for a new type of microscope, known as Arctis. The Arctis microscope will enable scientists to prepare samples for cryo-EM using a plasma ion beam. This is particularly advantageous for patient and environmental samples which, due to their large volumes, can only be processed with great difficulty, if at all, using current methods. The applications were approved in autumn 2022.

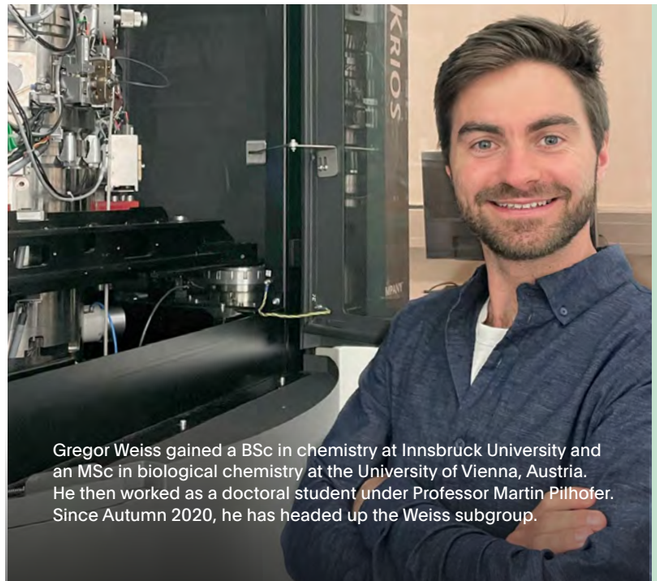
Investigating uropathogens (Weiss sub-group)

The Weiss sub-group comprises project leader Gregor Weiss, doctoral student Karolina Roganowicz and Master's student Simon Hauser, and focuses on the pathogens involved in urinary tract infections (UTIs). Another doctoral student, Marlen Dietrich, will join the team on 1 January 2022.

The vast majority of UTIs are caused by uropathogenic E. coli (UPEC). UPEC are found not only in patient urine, but also in the cytoplasm of uroepithelial cells. Within this intracellular niche, UPEC are well protected against antibiotics or host defences, which can lead to recurrent and persistent infections. The main goal of the group is to understand the intracellular lifecycle of uropathogens by using an integrative approach based on infection biology and structural biology. A combination of light microscopy and cryoET makes it possible to analyse the infection cycle on several levels.

In 2021, the project "Towards the development of cryo-electron tomography as diagnostic tool for recurrent urinary tract infections" was supported by Personalized Health and Related Technologies (www.sfa-phrt.ch), a strategic research focus area of the institutions of the ETH Domain. This research project is being carried out in collaboration with University Children's Hospital Zurich and aims to further develop cryoET as a diagnostic instrument for direct analysis of intracellular bacterial communities (IBCs) in the uroepithelial cells of patients with acute UTIs. CryoET enables insights into the macromolecular structures of infected patient cells at unprecedented resolution. The first step is to establish workflows for processing uroepithelial cells from patient urine for subsequent cryoET imaging. This will help to discover new virulence factors that play a role in IBC formation. In the longer term, this will facilitate new approaches in the treatment of UTIs.

This project has now been approved by the Zurich Cantonal Ethics Committee, and the Weiss subgroup has started to systematically examine patients' urine for infected uroepithelial cells. For this purpose, a purification method using microfluidic chips has been implemented in the lab.



Gregor Weiss gained a BSc in chemistry at Innsbruck University and an MSc in biological chemistry at the University of Vienna, Austria. He then worked as a doctoral student under Professor Martin Pilhofer. Since Autumn 2020, he has headed up the Weiss subgroup.

Highlights 2021–2022

Gregor Weiss and his group have received a research grant of EUR 1 million from the Lopez Loreta Foundation. This funding will be used to advance the application of cryoET for clinical samples – in particular in using the high-resolution imaging technique for the direct analysis of tissue samples (biopsies) in order to complement current diagnostic methods.

Publications

Full list available at www.pilhoferlab.ethz.ch/publications

* = equal contribution/co-first author
bold = Pilhofer Lab members
✉ = corresponding author

2022

Casu B, Sallmen JW, ✉ Schlimpert S, ✉ **Pilhofer M**
Cytoplasmic contractile injection systems mediate cell death in *Streptomyces*
bioRxiv (www.biorxiv.org/content/10.1101/2022.08.09.503279v1)

Wohlfarth JC, **Feldmueller M**, Schneller A, Kilcher S, Burkolter M, **Pilhofer M**, Schuppler M, ✉ Loessner MJ
Gram-positive bacteria evade phage predation through endolysin-mediated L-form conversion
bioRxiv (www.biorxiv.org/content/10.1101/2022.05.24.493201v1)

*Kieninger AK, ***Tokarz P**, **Pilhofer M**, ✉ **Weiss GL,** ✉ Maldener I
SepN is essential for assembly and gating of septal junctions in *Nostoc* sp. PCC 7120
bioRxiv (www.biorxiv.org/content/10.1101/2022.01.26.477872v1)

***Weiss GL**, ***Eisenstein F**, Kieninger AK, **Xu J**, **Minas HA**, **Gerber M**, **Feldmüller M**, Maldener I, Forchhammer K, ✉ **Pilhofer M**
Structure of a thylakoid-anchored contractile injection system in multicellular cyanobacteria
Nature Microbiology (www.nature.com/articles/s41564-021-01055-y)
Read perspectives in ScienceNews (https://www.sciencenews.org/article/bacteria-molecular-syringe-cells-microscopy) and Nature Microbiology (www.nature.com/articles/s41564-022-01078-z)

***Xu J**, ***Ericson CF**, **Feldmüller M**, Rutaganira FUN, **Eisenstein F**, **Lien YW**, King N, ✉ **Pilhofer M**
Identification and structure of an extracellular contractile injection system from the marine bacterium *Algoriphagus machipongonensis*
Nature Microbiology (www.nature.com/articles/s41564-022-01059-2)
Read perspectives in ScienceNews (www.sciencenews.org/article/bacteria-molecular-syringe-cells-microscopy) and Nature Microbiology (www.nature.com/articles/s41564-022-01078-z)

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