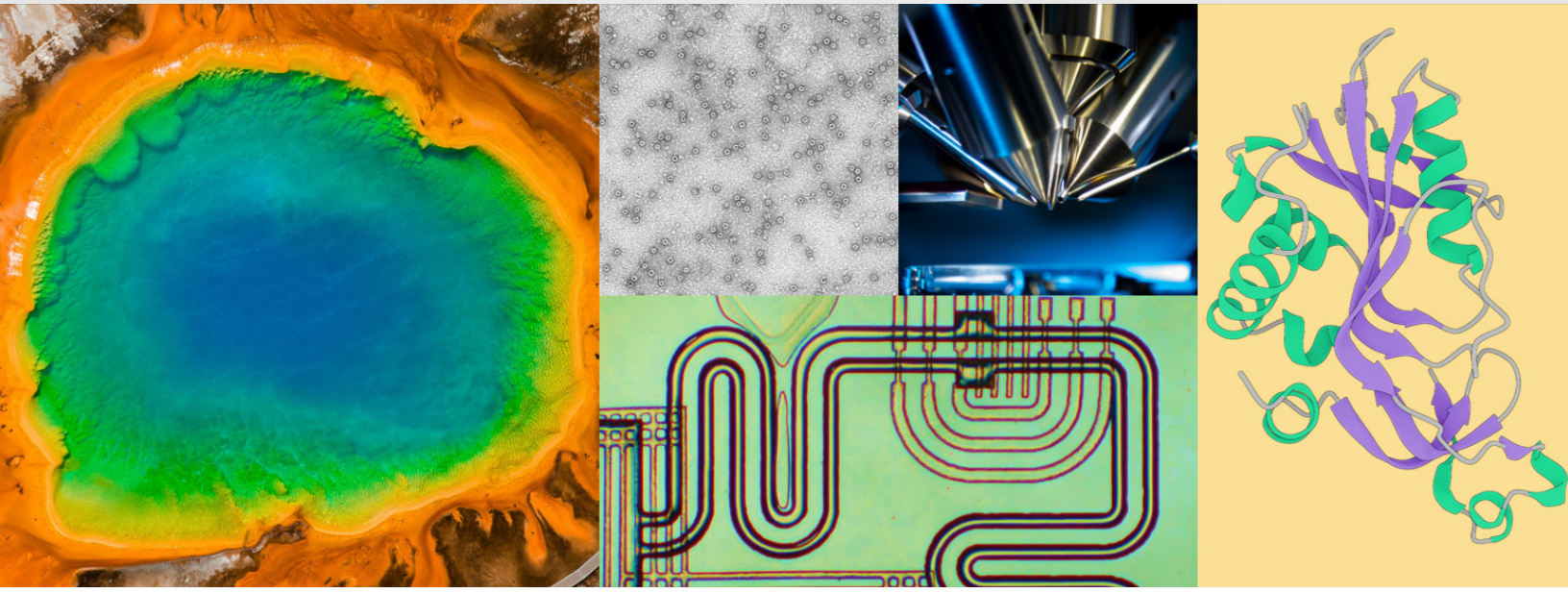
PROHITS

Prokaryote proteomics at high temperature for single cells



Issue 3
August 2026

This issue of the newsletter has been edited by Kenneth Valerio Aguilar (DC4) and Mansi Jain (DC10).

ISSUE 3 **AUGUST 2026**

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Co-funded by the European Union

The University of Debrecen has received the funding 2020-2.1.1-ED-2023-00269 from the Hungarian National Research, Development and Innovation Fund to cover the participation of DC10 in PROHITS.

PROHITS at a glance

The project in a nutshell

PROHITS is a Marie Skłodowska-Curie Doctoral Network (MSCA-DN) project with the overall aim of resolving the proteome of prokaryotes at single-cell level and at a range of temperatures. This will be achieved by integrating experimental and computational approaches, and will improve our understanding of thermophile biology to optimally design cell factories and the related industrial processes.

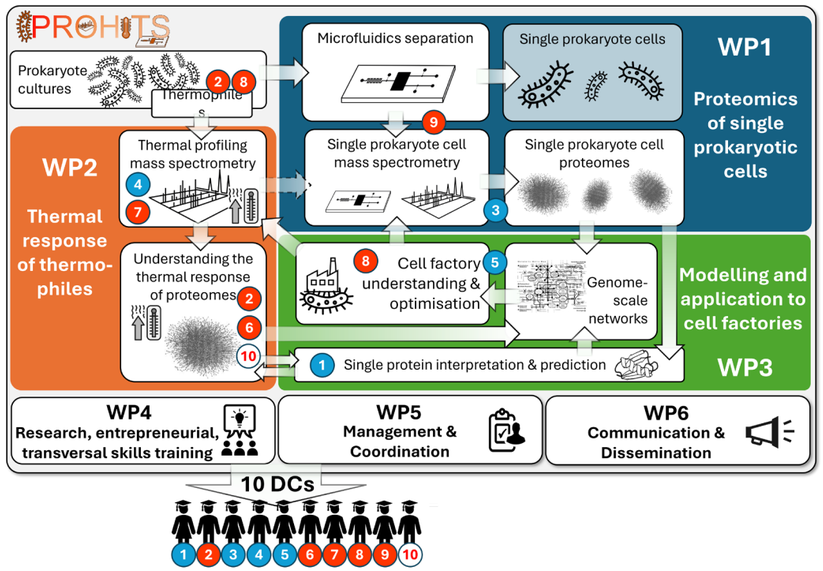
The PROHITS DN will advance 1) microfluidics to separate single prokaryotic cells, 2) mass spectrometry (MS) and its associated bioinformatics to handle single prokaryotic cells, and 3) MS-based thermal proteome profiling to study the thermal response of the proteome of thermophiles. We will use the information generated to 4) understand the in vivo stability of proteins, and 5) create genome-scale models of thermophiles to 6) optimize them as cell factories. Two main technological developments are thus expected:

- Instrumentation to determine the proteome of single prokaryotic cells and its response to temperature changes.
- Improved cell factory modelling by including proteome and thermal response information, so guiding metabolic engineering approaches to optimize environmentally sustainable industrial processes.

PROHITS represents a platform to train ten doctoral candidates (DCs) with translational and interdisciplinary, as well as transversal and entrepreneurial skills , resulting in:

- Excellent understanding of both experiment and computation, and of the challenges and opportunities at the interface between these.
- The ability to solve challenges in the determination, (biological) interpretation, and (industrial) application of thermophilic proteomes, along with broader method applicability to all prokaryotes.
- Engagement with the societal impact of their research, and awareness of innovation opportunities.

The Network is organised into six Work Packages as follows:



For more information, visit https://prohits.eu.

Consortium

PROHITS brings together 6+1 beneficiary institutions from six different European countries. Four academic organisations (VUB, VIB, UiB, UniVie, CNRS and UD) and one company (NovoArc GmbH) join forces with the support of two partner universities (Universiteit Gent and Université de Strasbourg) and a partner company (Bruker Daltonics GmbH& Co. KG) to build up a highly interdisciplinary network to tackle the ambitious goals of the project.

Partner institutions

Vrije Universiteit Brussel (VUB)
Prof. Dr. Wim Vranken
Prof. Dr. Eveline Peeters

Vlaams Instituut voor Biotechnologie (VIB) and Universiteit Gent (UGent)
Prof. Dr. Lennart Martens

Universitetet i Bergen (UiB)
Prof. Dr. Harald Barsnes

Universität Wien (UniVie)
Prof. Dr. Jürgen Zanghellini

Centre National de la Recherche Scientifique (CNRS) and Université de Strasbourg (UNISTRA)
Dr. Christine Carapito

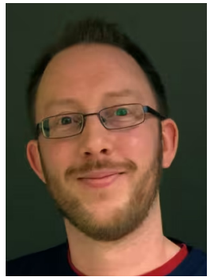
NovoArc GmbH
Dr. Julian Quehenberger

Debreceni Egyetem (UD)
Prof. Dr. Éva Csősz

External co-supervisor
Dr. Anjali Seth

Bruker Daltonics GmbH& Co. KG
Mr. Pierre-Olivier Schmit

Meet the supervisors



Prof. Harald Barsnes

Prof. Dr. Harald Barsnes holds a PhD in bioinformatics and currently works as a professor at the Department of Biomedicine at the Faculty of Medicine at the University of Bergen (UiB) in Norway. After finishing his PhD in 2010, he worked briefly as a senior engineer before securing his own postdoc funding. After 3.5 years as a postdoc and 2.5 years as a researcher, he again obtained his own research funding and has since 2016 led a research group focused on the development of user-friendly and open-source bioinformatics tools that enable and empower researchers to analyze and share their own data. His research group is connected to both the Proteomics Unit at the University of Bergen (PROBE) — a national core facility for large-scale protein analysis using mass spectrometry — and the Computational Biology Unit (CBU) — an interdisciplinary center focusing on the intersection of computer and life science. When not working, you will most likely find him hiking in the mountains.

1. Describe your background, your company, your goals, and contributions to the field.
My master's degree is in the field of bioinformatics with a focus on proteomics data, in particular on how the development of user-friendly software can help when interpreting and understanding such relatively complex data sets. This has been a continuous goal throughout my carrier ever since, although the specific objectives may have changed along the way, from being able to share proteomics data in standard formats to how to interactively explore the same data in a user-friendly manner that does not require advanced programming skills. This has resulted in a long list of open-source proteomics software, including lots of training material which has hopefully led many people to a better understanding of proteomics data analysis.

2. Present your reseach group.
My research group is connected to both the Proteomics Unit at the University of Bergen (PROBE) — a national core facility for large-scale protein analysis using mass spectrometry — and the Computational Biology Unit (CBU) — an interdisciplinary center focusing on the intersection of computer and life science. We therefore have access to both the wet lab expertise at PROBE and the dry lab experience at CBU. At this crossroads we are trying to build the bridge between high-throughput omics analyses and novel biomedical knowledge. During the last four years I have also taken on an increasing amount of administrative responsibilities, such as leading the Bergen Biomedical Research School to improve the local PhD education, functioning as the acting head of the Proteomics Unit, and recently becoming the deputy head of the Department of Biomedicine, thus expanding my role beyond that of mainly leading a research group.

3. What inspired you to pursue a career based on research?
I am not sure that I ever made a conscious choice to pursue a career based on research. I just made the best of the opportunities that came my way and never really had a grand plan for where I would end up. My motivation rather came more from the individual challenges presented along the way and how they could best be solved. The fact that there is always a new challenge around the next corner, and that you never know what it will be until you get there, has also been quite inspiring.

4. What has been a significant moment in your research path?
Halfway through my PhD an opportunity to develop a system for making mass spectrometry data publicly available came along, and something that was originally meant to be a short three-month assignment ended up as an important part of my thesis, resulted in a high impact publication in Nature Biotechnology, and started a still-ongoing collaboration with Lennart Martens (which just happens to be one of the other PROTHITS supervisors). Thus, proving my point above that you have to make the best of the opportunities that come your way.

5. What outlines a good supervising and/or management strategy in your eyes, and how do you implement it in your research group?
The most important element is that the group members feel that they are part of a team and that they can rely on each other. In our research group we therefore spend a fair amount of time together also outside of the direct research activities. It can be something as basic as making sure that we all eat lunch together, to the occasional off-campus activity such as hiking or shuffleboard. This helps minimize the distance between the members and makes it easier to communicate during the more stressful times. Additionally, it is very important that the individual group members take ownership of their part of the project. I therefore give them a lot of freedom to come up with ideas and expect them to have their own internal motivation rather than requiring, for example, fixed deadlines in order to make progress.

6. What does PROHITS mean to you?
So far, PROHITS has been a great example of how research is supposed to be carried out, i.e., a group of individuals with diverse backgrounds come together with a common research goal in mind. Seeing how the different pieces of the larger puzzle are now starting to fit has been a very rewarding experience and shows that we are on the right track!

7. What is your and your team's contribution to PROHITS?
My main role is to supervise Kenneth (the doctoral candidate located in Bergen) and guide his work on the analysis framework for interpreting the generated proteomics data and make sure that the developed software is both user-friendly and accessible for the rest of the PROHITS members. I am also co-supervising Dinu (one of the doctoral candidates located in Strasbourg) and leading the Data management subcommittee.

8. Which milestones do you think are the most important?
To me, the most important milestone will be when we are able to put all of the different pieces together and the doctoral candidates start understanding how their individual parts all contribute to the big picture required to improve our understanding of thermophile biology. and models for data analysis and interpretation.

9. What challenges might we expect in PROHITS?
The challenges so far have mainly been related to getting an overview of the field and trying to learn how to build on the previous knowledge. Specifically, when it comes to software development, the challenge is always to figure out where the current limitations are with regards to analyzing our particular type of data, and how to best fill those gaps without reimplementing everything from scratch, while at the same time also making sure that the results are novel enough to be published.

10. What impact do you think PROHITS will have? Both within science but also societal?
The main impact of PROHITS will be the future research careers of the ten very promising doctoral candidates taking part in the project. Through PROHITS they will be exposed to a larger variety of challenges than most other doctoral candidates, learn how to work together as a large and coherent research team, and build a close-nit international network that the larger research community will benefit from for years to come.

About - PROBE

The Proteomics Unit at the University of Bergen (PROBE) is a national core facility for large-scale protein analysis using mass spectrometry, and a member of the Norwegian National Network of Advanced Proteomics Infrastructure. PROBE is equipped for a wide range of proteomics analysis, from the identification of proteins present in a sample to the comparison of protein expression profiles across samples. Expertise in experimental design, sample preparation, analysis and data interpretation is also available. PROBE offers service, support and collaborations to those interested in doing mass spectrometry-based protein analysis on any type of sample.
The Computational Biology Unit (CBU) works at the intersection of computer and life science. Its primary focus is on developing and implementing novel computational methods and applying these in the pursuit of fundamental biological questions. CBU is a collaboration between two faculties and five departments at the University of Bergen (UiB).
Both PROBE and CBU are based at the University of Bergen located in the city of Bergen on the west coast of Norway.

Meet the supervisors

Prof. Éva Csősz

Dr. Csősz is a biologist/biochemist and full professor at the University of Debrecen, serving as head of department of the Department of Biochemistry and Molecular Biology and head of the Proteomics and Metabolomics Core Facilities. Her research focuses on proteomics, metabolomics, mass spectrometry, and systems biology, with particular emphasis on biomarker discovery and the biomedical application of omics technologies. She earned the Doctor of the Hungarian Academy of Sciences (DSc) degree and has authored more than 80 peer-reviewed scientific publications. She is actively involved in several international scientific organizations, including leadership roles in the Hungarian Biochemical Society, European Proteomics Association (EuPA) and the Human Proteome Organization (HUPO). As an educator and mentor, she is dedicated to talent development and the creation of innovative biochemistry, proteomics and metabolomics courses for BSc, MSc, and PhD students.

DEBRECENI EGYETEM

1. Describe your background, your company, your goals, and contributions to the field.
I am a molecular biologist by training, and I came across with mass spectrometry only after finishing my PhD. During my early career, I was balancing between running a core facility, doing science, teaching students both in the classroom and in the lab and raising kids. That was quite challenging, but I learned a lot. My kids are now grown-ups, but balancing still continues. I am now a full professor at the Department of Biochemistry and Molecular Biology, teaching biochemistry, proteomics and metabolomics to medical, science and PhD students and I am the leader of the Proteomics and Metabolomics Core Facility at the Faculty of Medicine, University of Debrecen, Debrecen, Hungary. I am also teaching science master students in Cluj Napoca, Romania and I am doing various public service activities being executive committee member of the European Proteomics Association (EuPA), Council Member of Human Proteome Organization (HUPO), founder president of the Proteomics Division of the Hungarian Biochemical Society and vice-president of the Hungarian Biochemical Society. My major research goal is to better understand what leads to type 2 diabetes in obese patients and what are the factors protecting all those obese patients who do not develop diabetes. I love mass spectrometry and I am admiring all the possibilities that open up with this methodological arsenal, but I am also a big fun of the non mass spec-based proteomics. Outside of the lab, I like hiking and being outdoors. I like gardening, having a coffee in the shade, playing with my dog. I love all forms of water, I especially like the rapid mountain streams; one inspired me so much that I dedicated a book with children short stories to it. When I have a bit more time, I am writing; I have one storybook in Hungarian for children and currently I am working on the second one.

2. Present your reseach group.
I have an international and diverse research group and I am very proud of all of them. Colleagues from six different countries are working currently in the lab and it is so good to see when everyone brings its unique mosaic to make a colorful big picture. We are a relatively small research group with 5 PhD students, 2 technicians, 2 postdocs and 2 senior colleagues. We only have two mass specs, an Orbitrap Fusion for proteomics and a 5500 QTRAP for metabolite analysis but besides mass spectrometry, we perform Olink (Target and Reveal) analyses as well. The group is part of the Department of Biochemistry and Molecular Biology, Faculty of Medicine, taking considerable part in teaching of medical and science students. Being part of the Faculty of Medicine, having many contacts with physicians, shapes our research activity, having a multitude of health- and patient sample-related analyses. However, we have good relations with the biotechnologists, taking part in various projects in the field. In this sense, PROHITS perfectly fits to this biotech-related branch of research of our laboratory.

3. What inspired you to pursue a career based on research?
When I was a child, I was often afraid of unfamiliar noises. Over time, however, I realized that every sound has a clear and identifiable source. From that moment on, I began to see the world differently, instinctively trying to understand the underlying causes of the phenomena around me. Before this realization, I was frequently bored; afterward, life became endlessly fascinating, full of things to discover.

I was drawn to chemistry and astronomy early on, but when I first encountered cellular and molecular biology, it completely captivated me. I found it so fascinating and intellectually stimulating that I continue to admire the elegant simplicity and complexity of life to this day. I am particularly interested in processes at the molecular level within cells, while also appreciating the diverse forms and mechanisms of evolution.This is one reason why the study of thermophiles appeals to me so strongly: it is remarkable to see how these organisms have adapted to,and thrive in environments where few others can survive.

4. What has been a significant moment in your research path?
I have experienced many significant moments in my life. I often feel a deep sense of happiness after a rewarding lecture or an engaging scientific discussion. It is always a special joy to see my students or group members achieve something significant or take an important step forward in their careers. I tend to value these smaller yet continuous milestones. However, if I were to highlight one particularly transformative experience, it would be when I began working with EuPA and later with HUPO. This marked the beginning of an entirely new chapter in my life, opening the door to a vibrant international community. I had the opportunity to meet many inspiring colleagues dedicated to excellent science, and this network has remained a constant source of motivation and inspiration. I feel truly privileged to be part of such an engaging and dynamic community.

5. What outlines a good supervising and/or management strategy in your eyes, and how do you implement it in your research group?
I believe it is very important to give students and colleagues enough freedom to find their own paths. At the same time, effective supervision requires balance: there are situations that call for closer guidance, and others where greater independence is more appropriate. It is the supervisor's responsibility to recognize these moments and adjust accordingly. I generally try to avoid micromanagement, although there are times when more direct involvement becomes necessary.Within the team, we hold one-to-one meetings every Friday. These sessions provide an opportunity to discuss individual needs and ongoing tasks, and to review or simply listen to plans for the coming week. This regular exchange allows us to make timely adjustments, set priorities, and reflect on both short- and long-term goals. I have been inspired by a quotation attributed to Antoine de Saint-Exupéry that I try to use during supervision: "If you want to build a ship, don't drum up people to collect wood and don't assign them tasks and work, but rather teach them to long for the endless immensity of the sea."

6. What does PROHITS mean to you?
For me, PROHITS means both excellent science and strong networking. As a supervisor, it is very rewarding to see how the DCs interact and support one another. At the beginning, there was some uncertainty, which is totally natural, but over time, I have seen them grow more confident, actively seeking out resources and connecting with the right people within the network to address their questions. They have begun to collaborate effectively, and alongside their hard work, this spirit of collaboration is, in my view, a key factor in their success.

7. What is your and your team's contribution to PROHITS?
We are optimizing methods for proper digestion of thermophiles and addressing questions using metabolomics on the metabolic adaptation of these organisms to the various, often very harsh conditions.

About - UD

The Biomarker Research Group, led by Prof. Dr. Éva Csősz, belongs to the Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Debrecen, Hungary, where the department's Proteomics Core Facility is located. The laboratory is dedicated to carrying out targeted proteomics studies and examining post-translational modifications of proteins, enabling research principally within the Faculties of Science and Medicine of the University of Debrecen, and undertakes research, collaborations, and analytical services for other universities and biotech companies in the proteomics and metabolomics field. The international team with members from 4 continents and 8 countries, specializes in body-fluid proteomics and metabolomics, focusing on the discovery and validation of biomarkers in minimally invasive human samples such as tears, saliva, sweat, and blood. Its mission is to undertake research and to provide access to state-of-the-art mass spectrometry and proximity extension assay (Olink) techniques.

Meet the supervisors

Continued from the previous page

8. Which milestones do you think are the most important?
I believe the most important milestone is obtaining the thermal response profile of the first organism. Once we have sufficient experience with this process, generating thermal profiles for the remaining organisms should become significantly less challenging. Reaching this milestone will also demonstrate that the consortium has established the full range of necessary expertise: the ability to culture the organism, isolate and digest proteins, perform thermal protein profiling experiments, and apply the appropriate software tools and models for data analysis and interpretation.

9. What challenges might we expect in PROHITS?
I think the greatest challenge at this stage is time, more specifically, ensuring that everything we have started can be successfully completed within the project's timeframe. Communication among the DCs, as well as between the DCs and supervisors, is good, and the DCs themselves are enthusiastic and hardworking. Given this, I see time management as the primary challenge we need to address.

10. What impact do you think PROHITS will have? Both within science but also societal?
I believe that PROHITS will provide highly valuable insights into how thermophiles adapt to heat stress. In particular, it has the potential to generate fundamental knowledge on protein stability, chaperone function, and the mechanisms that maintain protein integrity in the extreme environments where these organisms thrive.I also expect that many of these findings will be applicable in the biotechnology sector, helping to make the use of thermophiles more accessible and efficient, and enabling us to better harness the advantages they offer. In the long term, this could translate into meaningful societal benefits, such as the production of higher-quality, more affordable biologically derived compounds as alternatives to chemically synthesized ones.In addition, optimizing methods for studying prokaryotes at the single-cell level would provide a powerful tool for investigating evolution in unprecedented detail. Such approaches could reveal how frequent mutations contribute to adaptation in constantly changing environments, offering deeper insight into evolutionary processes at the most fundamental level.

Francis at the Chemistry Doctoral School Day


Dinu, Sunu, Mansi and Francis during a hiking

Project progress

Reporting (February - July 2026)

Deliverables

D1.1 Microfluidics instrumentation to separate single cells of at least 5 prokaryotes

Milestones

M2 Optimized MS sample prep. for single prokaryote cells
M12 Functional GEM for Saci
M8 Data analysis pipeline optimized for qualitative and quantitative analysis of TMT-labelled TPP data
M4 Identify unique peptides from analysis MS data single cells

Other

1st Periodic Report

Secondments (January 2026 - July 2026)

Completed:

DC1 Alexandre Bouillon at **UniVie, Austria**	Dec 2025 - Feb 2026	Duration: 3 month
DC10 Mansi Jain at **Cellenion, France**	January 2026	Duration: 1 months
DC4 Kenneth Valerio Aguilar at **UD, Hungary**	March 2026	Duration: 3 weeks
DC4 Kenneth Valerio Aguilar at **CNRS, France**	March 2026	Duration: 3 weeks

Project videos

- **Meet DC6, Dinu Ciobanu** https://youtu.be/MN1qhaPiPYM
- **Meet DC7, Sunu Lama** https://youtu.be/7EI3c5_Sfhs
- **Meet DC8, Ana Carvalho** https://youtu.be/7T4iave4YvI
- **Meet DC9, Onyeka Francis Offor** https://youtu.be/nIFNWVQqnjY
- **Meet DC10, Mansi Jain** https://youtu.be/ooYTWM1dzBg

Project progress

Workshop 3 - Project and DC consolidation. Cell factories and GEMs

Workshop 3 will take place on 4th - 10th September in Vienna (Austria), with UniVie acting as host. In addition to their progress presentations and a meeting of the Supervisory Board, the program includes a week full of training sessions:

- **Write a research proposal,** led by Prof. Wim Vranken (VUB).
- **IP rights,** led by Etz Hildegard and Gamauf Georg (Austrian Patent Office), and Inma Sánchez Romero (UniVie).
- **Metabolic modelling,** led by Prof. Jürgen Zanghellini (UniVie).
- **Digital lab management and the implementation of ISO9001 quality management system**, led by Christina Horn (NovoArc)

Recent contributions at scientific meetings

Carvalho, A.C., Ciobanu, D., Peeters, E., Zanghellini, J., Quehenberger, J. Towards a novel tetraether lipid producer Haloferax volcanii. Poster presented at the PhD retreat from the Doctoral School of the University of Vienna, 25th-27th February 2026 (Austria). https://doi.org/10.5281/zenodo.19004831

Rimón, M.J., **Carvalho, A.C.,** Zehetner, L., **Quehenberger, J., Zanghellini, J.** Pan genome-scale metabolic modeling reveals medium dependent metabolic diversity in Sulfolobus. Poster presented at the 10th Conference on Constraint-Based Reconstruction and Analysis (COBRA2026), 17th-19th March 2026 (Postdam). https://doi.org/10.5281/zenodo.19480749

Valerio Aguilar, K.A., Bouwmeester, R.**, Martens, L., Barsnes, H.** Rethinking Thermal Proteome Profiling for Complex Perturbations Using Label-Free Proteomics. Poster presented at the Bioinformatics in Bergen 2025 Conference, 4th-5th May 2026 (Norway). https://doi.org/10.5281/zenodo.21625178

Valerio Aguilar, K.A., Bouillon, A., Juarez Garzon, M.A.**, Jachmann, C., Martens, L., Barsnes, H.** TPP-ANALYST: A comprehensive and user-friendly tool for the analysis of thermal proteome profiling data. Poster presented at the 3rd Norwegian Symposium on Proteomics and Biological Mass Spectrometry, 11th-12th May 2026 (Norway). https://doi.org/10.5281/zenodo.21625577

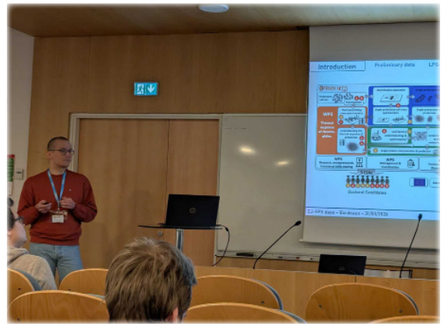
Andresen, J.B., Baes, R., Atmaca, H., Stroobants, A., Samanta, S., Majumder, S., **Ciobanu, D.Z.,** Heidig, S., Yasynska, O., **Vranken, Wim., Carapito, C.,** Efremov, R., Ghosh, A., **Peeters, E.** Elucidating the structure with an elusive subunit of the Hsp60 thermosome complex in S. acidocaldarius. Poster presented at the EMBO Workshop 'Molecular Biology of Archaea: Life Through the Prism of Archaea', 06th-10h July 2026 (UK). https://doi.org/10.5281/zenodo.21625798

Ciobanu, D.Z., Aguilar, K.V., Jachmann, C., Andresen, J.B., Baes, R., Peeters, E., Martens, L., Barsnes, H., Carapito, C. Thermal Proteome Profiling as a powerful approach to investigate the proteome stability of thermophiles and beyond. Poster presented at the SEMM Workshop: The Proteomics Revolution – Advancing Science and Society, 29th June-1st July 2026 (Italy). https://doi.org/10.5281/zenodo.21257520

Andresen, J.B., Baes, R., Peeters, E. Characterizing a thermophilic cyanobacterium with a proteomics-based investigation to enable biotechnological application. Oral prersentation at the 23rd Symposium of the International Association for Cyanophyte/Cyanobacteria Research, 3th-7th August 2026 (Belgium). **Prize to the best presentation.**

News

- Ana Carvalho (DC8) participated in the Long Night of Research at the University of Vienna, Austria, on 24 April 2026. During the event, she hosted an interactive booth titled "What's the Secret of Cells That Don't Fall Apart?".
- Francis Offor (DC9) participated in the Chemistry Doctoral School Day at the University of Strasbourg, France, on 24 March 2026.
- Dinu Ciobanu (DC6) participated in the Youth Club Days of the French Proteomics Society from 30 March to 2 April 2026.
- Ana Carvalho (DC8), Josephine Boel Andresen (DC2), and María José Rimón Martínez (DC5) participated in BIP EXPLIN course in Naples, Italy, from 15 June to 13 July 2026.



Dinu at Youth Club Day

Ana,Josie and María José at the BIP-EXPLIN course

Ana during the Long Night of Research

Journal club

The fellows meet online every 2-3 months to discuss about articles relevant to the project.

6st Journal club session:

Date: 20 April 2026

Article: Molecular crowding effects on protein stability in a bacterial proteome

DOI: https://doi.org/10.1038/s41598-026-359909

Chair: Dinu Ciobanu(DC6)

7nd Journal club session:

Date: 30 June 2026

Article: An automated workflow for label-free and multiplexed single cell proteomics sample preparation at unprecedented sensitivity

DOI:https://doirg/10.1101/2021.04.14.439828

Chair: Sunu Lama(DC7)

Stay tuned!

Linkedin
https://tinyurl.com/4cayu96f

PROHITS newsletter
https://tinyurl.com/3wesyhv3

Bluesky
https://tinyurl.com/535s4s9f

Instagram
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https://tinyurl.com/yc2538zz


PROHITS