International Pre-doctoral program 2025

Microcosm Earth Center: Max Planck Institute for Terrestrial Microbiology (MPI-TM) and Philipps-Universität Marburg (UMR)

Project booklet

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Microcosm Earth Center: Max Planck Institute for Terrestrial Microbiology (MPI-TM) and Philipps-Universität Marburg (UMR)

Name of Institution	MPI for Terrestrial Microbiology
Name of host laboratory / group	Biophysics of environment sensing by motile microorganisms
Website of host laboratory / group	https://www.mpi-marburg.mpg.de/colin
Project title	Effect of motility and chemotaxis on the self-organization of cooperating bacteria
Project duration (3-6 months)	5-6 months
Prerequisites (educational)	Background in microbiology or physics

Project proposal: description and expected outcomes of project (500 words max.):

In the lab, we typically study homogeneous populations of bacteria growing in well-mixed batch cultures. In contrast, natural bacterial populations present a wide diversity of phenotypes that interact with each other and live in highly structured environments, both physically and chemically (e.g. the soil). There, they are exposed to and they shape gradients of various environmental conditions, notably of nutrients, which they can navigate thanks to flagellated motility, coupled to the chemotaxis sensory pathway, to migrate towards more favorable conditions. In most microbial community, a fraction of the species is motile, the other not. Previous works from our lab have shown that the physical interactions between the two phenotypes lead to spatiotemporal organization patterns that are specific to such mixed populations. Natural microbial communities also often show metabolic entanglement, where a species produces metabolites needed by the other, while receiving some it needs in return. Chemotactic motility is hypothesized to facilitate the metabolic interaction, thanks to the navigation up the metabolite gradients, but evidences are scarce.

The aim of this project is to understand which roles motility plays in a phenotypically mixed and metabolically entangled community. Our lab develops microfluidics devices, with which we submit bacterial population to well-controlled structured environments, which we combine with various fluorescence microscopy and image analysis methods to study various aspects of their behavior. During the project, we will first construct *Escherichia coli* mutant strains that are either motile or non-motile, fluorescently tagged and carry amino-acid-auxotrophy mutations, to make them dependent on each other for growth in the appropriate minimal media. We will then monitor with fluorescence

microscopy the population growth and self-organization, first in an unstructured environment (a simple liquid culture) and then in a microfluidic maze which we will build up. With this project, we aim to understand to which extent is motility and chemotaxis facilitating metabolic interactions and improving bacterial yield, in homogeneous and in structured environments, and how the metabolic interaction affects the specific motility-based patterning.

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Microcosm Earth Center: Max Planck Institute for Terrestrial Microbiology (MPI-TM) and Philipps-Universität Marburg (UMR)

Name of Institution	Max Planck Institute for Terrestrial Microbiology
Name of host laboratory / group	Dept. of Biochemistry and Synthetic Metabolism/AG Erb
Website of host laboratory / group	https://www.mpi-marburg.mpg.de/erb
Project title	Self-regulated Metabolic and Genetic linked in vitro Network
Project duration (3-6 months)	~ 6 Months
Prerequisites (educational)	Biotechnology, Biology,Biochemistry, Molecular Biology

Project proposal: description and expected outcomes of project (500 words max.):

Cellular resource allocation and efficient utilization are paramount for survival and functionality of living organisms. Central to this process is the regulation of gene expression in response to key metabolites, a fundamental strategy employed by the living cells for efficient utilization of the resources. However, establishing such a complex system *in vitro* from bottom up has been challengeing. Recently in our lab we estasblished the metabolic and genetic linked *in vitro* network (MGLN) by integration of metabolic layer (i.e., CETCH Cycle) with genetic layer (i.e., PURE TXTL).

The goal of the project is to bottom-up assemble an *in vitro* self-regulated metabolic and genetic linked network (MGLN) able to perform "decision-making" *i.e.*, it autonomously activates its anaplerotic module upon producing a target metabolite using gene regulation. Engineering such a complex *in vitro* system will paves the way to the development of innovative applications in biotechnology, biomanufacturing, and bioengineering.

Aim :-

1. Purify the relevant proteins of CETCH cycle and anaplerotic module.
2. Test the compatability of anaplerotic module proteins with MGLN using mass spectrometry and fluoroscence based plate reader assay
3. Integration and optimization of anaplerotic module with self regulated MGLN.

Reference:-

1. Giaveri S., Bohra N., Diehl C., *et al.* , Integrated translation and metabolism in a partially self-synthesizing biochemical network. Science 385,174-178(2024). DOI:10.1126/science.adn3856
2. Shimizu, Y., Inoue, A., Tomari, Y. *et al.* Cell-free translation reconstituted with purified components. *Nat Biotechnol* **19**, 751–755 (2001). https://doi.org/10.1038/90802
3. Diehl, C., Gerlinger, P.D., Paczia, N. *et al.* Synthetic anaplerotic modules for the direct synthesis of complex molecules from CO_2. *Nat Chem Biol* **19**, 168–175 (2023). https://doi.org/10.1038/s41589-022-01179-0

International Pre-doctoral program 2025

Microcosm Earth Center: Max Planck Institute for Terrestrial Microbiology (MPI-TM) and Philipps-Universität Marburg (UMR)

Name of Institution	MPI-TM
Name of host laboratory / group	Department of Biochemistry and Synthetic Metabolism/ Dr. Maren Nattermann
Website of host laboratory / group	https://www.mpi-marburg.mpg.de/biochemistry-and-synthetic-metabolism
Project title	Enzyme engineering for orthogonal metabolic pathways
Project duration (3-6 months)	6 months
Prerequisites (educational)	For this project, we are looking for a highly motivated student with a background in natural sciences. Previous laboratory experience is a bonus, but not required. The main language in our lab is English, therefore good English proficiency (B2-C1) is important.

Project proposal: description and expected outcomes of project (500 words max.):

The reduction of atmospheric CO_2 is one of the most pressing challenges of the 21st century. In recent years, many synthetic pathways for the efficient use of CO_2 by engineered organisms have been developed. These routes are more efficient than those evolved by nature and can be coupled to biological production pathways for bulk and fine chemicals, providing a large toolbox for biotechnological CO_2 re-capture and upcycling.

However, the implementation of synthetic pathways is challenging due to the delicate balance required between growth and product generation. This matter is particularly pressing in organisms relying on low-energy feedstocks such as CO_2. Here, any energy spent on off-pathway products or futile cycles can impede the implementation of a pathway: key cellular resources like ATP or NADPH may be drained or important metabolite pools such as acetyl-CoA disrupted, rendering the organism incapable of growth. In pathway engineering, a lot of effort is placed on minimizing these interactions. This concept is referred to as pathway orthogonality, where synthetic pathways interact with core metabolism as little as possible, allowing for a better allocation of cellular resources and a higher likelihood of successful implementation in vivo.

This project will focus on generating short orthogonal cascades for the production of chemicals. Mainly, the synthetic enzymes required for efficient catalysis will be developed. Here, you will purify

enzymatic candidates, compare their activities, develop high-throughput screens and screen mutant libraries to find improved variants. Towards the end of the project, the variants generated will be characterized biochemically via kinetic assays based on UV-Vis, fluorescence or HPLC-MS analytical methods.

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Microcosm Earth Center: Max Planck Institute for Terrestrial Microbiology (MPI-TM) and Philipps-Universität Marburg (UMR)

Name of Institution	MPI-TM
Name of host laboratory / group	Höfer Laboratory / Bacterial Epitranscriptomics
Website of host laboratory / group	https://www.mpi-marburg.mpg.de/mprg/katharina-hoefer
Project title	Live cell NAD and NAD-RNA imaging using synthetic biology tools
Project duration (3-6 months)	6 months
Prerequisites (educational)	Biochemistry, Biology

Project proposal: description and expected outcomes of project (500 words max.):

RNA's simple chemical structure, composed of four nucleotides, contrasts with its highly complex functionality. Over 170 RNA modifications have been identified, impacting its function and stability. The Höfer lab primarily focuses on characterizing RNAs modified at their 5' terminus with the redox factor nicotinamide adenine dinucleotide (NAD). Since the discovery of NAD-modified RNAs in 2015, they have been found in all kingdoms of life.
Building on these findings, the Höfer lab aims to understand the functional role of NAD-RNA in model organisms such as *Escherichia coli* and bacteriophage T4. The lab's research integrates synthetic biology, biochemistry, chemistry, and bioinformatics to uncover the connections between redox biology, gene expression, and regulation. Recent discoveries, including enzymes responsible for NAD-RNA biosynthesis and removal, offer insights into NAD-RNA's biological significance e.g. regulating RNA stability. In 2023, the Höfer lab introduced the concept of RNAylation—how RNAs and proteins interact—which provides a new perspective on RNA functionality (Wolfram-Schauerte, Nature 2023). However, the broader biological roles of NAD-RNAs remain poorly understood.

The proposed project aims to investigate the presence of NAD-RNAs in detail and how intracellular NAD concentrations regulate their abundance in various bacterial species. To achieve this, we will measure NAD and NAD-RNA levels using novel synthetic biology tools, including NAD sensors and ribozymes—engineered, catalytically active RNAs—*in vivo*. As a proof of concept, we will detect known NAD-RNAs in *E. coli* and extend this system to the green alga *Chlamydomonas reinhardtii* to explore

the functional role of NAD and NAD-RNA regulation and their implications for CO_2 fixation for the first time.

The project unfolds in two objectives:

Objective 1: ***Live-Cell NAD Imaging Using a Genetically Encoded Fluorescent Sensor***
In the project's first phase, the student will establish a genetically encoded NAD sensor, FiNad, in *E. coli*. FiNad enables real-time monitoring of NAD dynamics. During the initial two months, the student will clone and express the FiNad sensor in *E. coli*, recording NAD fluctuations under different (stress) conditions using fluorescence measurements and Fluorescence-Activated Cell Sorting (FACS).

Objective 2: ***Ribozymes as a novel tool to detect and quantify NAD-RNAs in vivo***
After measuring NAD concentrations, the student will employ the ribozyme Mtr1 to introduce fluorescent labels into NAD-modified RNAs for identification and quantification. Mtr1 will be generated through *in vitro* transcription and purified via polyacrylamide gel electrophoresis (PAGE). Total RNA from *E. coli* and *C. reinhardtii*, isolated by the student, will be mixed with the ribozyme and a fluorescent dye to label NAD-modified RNAs. The student will then analyze NAD-RNA levels using PAGE, investigating how intracellular NAD concentrations affect NAD-RNA abundance for the first time.

Expected Outcomes and Training: This project offers the student a comprehensive research experience, building expertise in RNA handling in both *in vitro* and *in vivo* systems. The student will master essential techniques such as in vitro transcription, bacterial RNA isolation, FACS, and protein cloning. By expanding our understanding of NAD and NAD-RNA, this project contributes to scientific knowledge while training future researchers in biochemistry, synthetic biology, and molecular biotechnology.

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Microcosm Earth Center: Max Planck Institute for Terrestrial Microbiology (MPI-TM) and Philipps-Universität Marburg (UMR)

Name of Institution	Microcosm Earth Center
Name of host laboratory / group	Working Group Klatt
Website of host laboratory / group	https://www.uni-marburg.de/en/microcosm-earth/wg-klatt
Project title	Molecular response of oxygenic phototrophs to high-arsenic exposure
Project duration (3-6 months)	
Prerequisites (educational)	Microbiology; Biochemistry; Molecular Biology

Project proposal: description and expected outcomes of project (500 words max.):

Arsenic is known for its detrimental toxic effects in all domains of life. Since reaching appreciable levels already billions of years ago, almost every organism encodes detoxification strategies. However, the notion of arsenic as a potentially beneficial semi-metal has arisen in the past decades, whether in fueling anoxygenic photosynthesis or serving as a terminal electron acceptor in microbial respiration. Recently, mechanisms for the production of complex lipid-soluble organic arsenic species have been described, appearing to be unique to phototrophic microbes. Despite the elusive mechanisms for organoarsenic production, phototrophic bacteria exhibit strong viability but display distinct morphology when exposed to this potentially toxic element, suggesting profound molecular-level adaptations that remain unknown.

In this Pre-doctoral project, the candidate will explore physiological variations in cyanobacteria caused by arsenic exposure, which we have recently observed to alter phenotypes under environmental stress. This will involve the description of the phenotype through hyperspectral imaging and/or electron microscopy, alongside e.g. transcriptomics/proteomics to characterize the molecular underpinnings of adaptation to or utilization of arsenic. The candidate may also perform in-vitro characterization of potential catalytic mechanisms, test functional predictions in knock-out strains or dive into structural biological predictions based on native cryo-EM. The projects' specific definition will be developed in close collaboration with the candidate, tailored to their background and interests.

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Microcosm Earth Center: Max Planck Institute for Terrestrial Microbiology (MPI-TM) and Philipps-Universität Marburg (UMR)

Name of Institution	Microcosm Earth Center
Name of host laboratory / group	WG Klatt
Website of host laboratory / group	https://www.uni-marburg.de/en/microcosm-earth/wg-klatt
Project title	Echoes of the early Earth: Exploring microbial primary production and respiration in shallow hydrothermal ecosystems.
Project duration (3-6 months)	3 or 6 months
Prerequisites (educational)	End of M.Sc. or already graduated

Project proposal: description and expected outcomes of project (500 words max.):

The oxygenation of Earth's atmosphere was a transformative event that enabled the evolution of complex life. Despite its significance, the processes driving early oxygenation, particularly during the Precambrian era, remain poorly understood. Critical to this process are the interactions between microbial life, biogeochemical cycles, and geological factors that governed carbon transformations within the oxygen cycle. In ancient oceans, primary production in microbial mats played a pivotal role in sequestering carbon, while the subsequent burial of this biomass influenced atmospheric oxygen levels. Modern analogs, such as microbial mats in hydrothermal environments, provide a unique opportunity to study conditions resembling early Earth. These mats, driven by complex interactions of photosynthesis, chemosynthesis, and various respiration pathways, offer a window into how early microbial ecosystems might have functioned. Understanding the balance of primary production and respiration in such systems is essential for unraveling the biogeochemical processes that contributed to Earth's initial oxygenation.

This research will investigate primary production, respiration pathways, and microbial interactions within microbial mats and hydrothermal plumes in a fluctuating sunlit hydrothermal environment, analogous to early Earth. The project may involve fieldwork in Milos, Greece. Alternatively or additionally, the hydrothermal vent environment will be

simulated in the lab in Marburg, using mesocosms for the microbial mats and/or roller tanks to study microbial aggregation in the plumes above the vents. The specific focus of the project will depend on the candidate's background and interests, within the broader framework of an interdisciplinary study by international collaborators. Under the guidance of the Klatt group, which specializes in real-time assessment of microbial processes, the candidate will have the opportunity to learn in-situ microsensor techniques to monitor light, oxygen, temperature, and sulfide concentrations in microbial mats over diel cycles. Additionally, stable isotope (^{13}C) probing can be employed to explore aspects of carbon cycling. Concentrations of oxygen, iron, arsenic, and sulfide can also be analyzed in subsamples or visualized in 2D using 'diffusive equilibrium in thin-films' (DET) techniques and planar optodes to assess their role in microbial metabolism and carbon cycling. Meta-omics analyses may supplement biogeochemical analyses to assess the molecular underpinnings of observed processes.

This research will shed light on the metabolic pathways and biogeochemical processes within microbial ecosystems under early Earth-like conditions, advancing our understanding of the environments that fostered life’s evolution.

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Name of Institution	Microcosm Earth Center
Name of host laboratory / group	WG Klatt
Website of host laboratory / group	https://www.uni-marburg.de/en/microcosm-earth/wg-klatt
Project title	**Role of reactive oxygen species in environmental Bacteria-Phage interaction**
Project duration (3-6 months)	3 or 6 months
Prerequisites (educational)	End of M.Sc. or already graduated

Project proposal: description and expected outcomes of project (500 words max.):

Phages, or bacteriophages, are viruses that infect bacteria and play a vital role in biogeochemical cycling. By lysing bacterial cells, phages release organic matter and nutrients, contributing to nutrient turnover in ecosystems. In addition to causing bacterial lysis, phages regulate bacterial metabolism throughout infection, potentially altering key biochemical processes. These interactions are well understood in stable environments, such as the open ocean, where phage-induced bacterial mortality influences carbon, nitrogen, and phosphorus cycles through the "viral shunt," which enhances primary production by recycling nutrients.

While phages significantly impact large-scale nutrient cycles, their infection dynamics are heavily influenced by changing environmental factors. However, we lack a comprehensive understanding of how these factors affect phage-host interactions. Most studies have focused on isolated organisms in constant environments, rarely considering the dynamic, fluctuating conditions found in nature. For instance, light has been shown to modulate cyanobacterial infection, suggesting that phage-induced mortality may follow a diel cycle. Temperature also plays a critical role, as rising global temperatures generally increase viral production relative to bacterial growth. Environmental fluctuations in light, redox conditions, and temperature are also closely linked to the production of reactive oxygen species (ROS), such as hydrogen peroxide (H2O2). ROS can be generated by microorganisms as a stress response or through

abiotic processes in fluctuating environments. Notably, H2O2 has been associated with phage induction, but the relationship between ROS dynamics and phage infections in real-world ecosystems remains poorly understood. This project aims to fill this knowledge gap through a combination of in-situ measurements in dynamic aquatic microbial ecosystems and controlled laboratory experiments. The specific focus of the project will be shaped by the candidate's background and interests.

Possible methods to be used: biogeochemical analyses, microbial cultivation, viral counts, qPCR, meta-omics

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Name of Institution	MPI for terrestrial Microbiology
Name of host laboratory / group	Geochemical Protoenzymes/WG Preiner
Website of host laboratory / group	https://www.mpi-marburg.mpg.de/martina-preiner https://www.uni-marburg.de/en/microcosm-earth/wg-preiner
Project title	Synthesis of mineral nanoparticles as prebiotic catalysts
Project duration (3-6 months)	4 to 6 months
Prerequisites (educational)	MSc in (Inorganic) Chemistry

Project proposal: description and expected outcomes of project (500 words max.):

We are interested in mineral-based catalysis in the context of prebiotic chemistry and have shown that nanoparticular minerals such as magnetite (Fe_3O_4), awaruite (Ni_3Fe) and greigite (Fe_3S_4) can catalyze the reduction of CO_2 with H_2 to organic acids found in the first steps of autotrophic CO_2 fixation in prokaryotes (Preiner *et al.*, 2020; Beyazay *et al.*, 2023). Since then, we have tested several different synthetic mineral catalysts for cofactor-based prebiotic reactions (Henriques Pereira *et al.*, 2022; Henriques Pereira et al., *in prep.*) and are currently establishing a mineral nanoparticle production pipeline in our lab. Via this pipeline, the internship's main focus will be based on synthesis pathways established in heterogeneous catalysis research groups, using organic templates and tube furnaces to produce transition metal oxides, and intermetallic compounds (Yu, Chan and Tüysüz, 2018; Yu *et al.*, 2019). Furthermore, we will look at processes to coat porous material with the desired catalysts. The catalyst synthesis will be monitored via in-house XRD and µ-RFA measurements and on-campus electron microscopy. After successful synthesis, the catalysts will be tested for their abilities to promote several prebiotic reactions in our lab (CO_2 fixation, reduction of redox cofactors).

Beyazay, T. et al. (2023) 'Ambient temperature CO2 fixation to pyruvate and subsequently to citramalate over iron and nickel nanoparticles', Nature Communications, 14, p. 570. https://doi.org/10.1038/s41467-023-36088-w.
Henriques Pereira, D.P. et al. (2022) 'Role of geochemical protoenzymes (geozymes) in primordial metabolism: Specific abiotic hydride transfer by metals to the biological redox cofactor NAD+', The FEBS Journal, p. 16329. https://doi.org/10.1111/febs.16329.
Preiner, M. et al. (2020) 'A hydrogen-dependent geochemical analogue of primordial carbon and energy metabolism', Nature Ecology & Evolution, 4, pp. 534–542. https://doi.org/10.1038/s41559-020-1125-6.

Yu, M. et al. (2019) ‘Optimizing Ni–Fe oxide electrocatalysts for oxygen evolution reaction by using hard templating as a toolbox’, Applied Energy Materials, 2, pp. 1199–1209. https://doi.org/10.1021/acsaem.8b01769.
Yu, M., Chan, C.K. and Tüysüz, H. (2018) ‘Coffee-waste templating of metal ion-substituted cobalt oxides for the oxygen evolution reaction’, Chemistry and Sustainability, 11(3), pp. 605–611. https://doi.org/10.1002/cssc.201701877.

International Pre-doctoral program 2025

Microcosm Earth Center: Max Planck Institute for Terrestrial Microbiology (MPI-TM) and Philipps-Universität Marburg (UMR)

Name of Institution	MPI for terrestrial Microbiology
Name of host laboratory / group	Geochemical Protoenzymes/WG Preiner
Website of host laboratory / group	https://www.mpi-marburg.mpg.de/martina-preiner https://www.uni-marburg.de/en/microcosm-earth/wg-preiner
Project title	Coenzymes under environmental selection pressure in a prebiotic context
Project duration (3-6 months)	4 to 6 months
Prerequisites (educational)	MSc or in the final months of it (Chemistry or Biochemistry)

Project proposal: description and expected outcomes of project (500 words max.):

Our lab tests the hypothesis whether coenzymes preceded enzymes at life's emergence. With this in mind, we are testing how various coenzymes react when exposed to a variety of geochemical environments and under what conditions they can fulfil the functions they fulfil today (electron-transfer, methyl transfer etc.) – the hypothesis: geochemical conditions preselected certain molecules as coenzymes before biochemistry did. During this internship, a variety of coenzymes (from flavins to pterins and corrins) will be tested for their compatibility with and functionality in different environmental conditions with a focus on their interaction different mineral catalysts. A focus will lie on unbuffered, ocean-like, aqueous solutions. Concretely, we are planning hydride-transfer reactions with the flavin cofactor FMN and methyl transfer reactions with the corrin-cofactor cobamide.

Literature:

Henriques Pereira, D.P. et al. (2022) 'Role of geochemical protoenzymes (geozymes) in primordial metabolism: Specific abiotic hydride transfer by metals to the biological redox cofactor NAD+', The FEBS Journal, p. 16329. https://doi.org/10.1111/febs.16329.
Preiner, M. et al. (2020) 'A hydrogen-dependent geochemical analogue of primordial carbon and energy metabolism', Nature Ecology &

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Name of Institution	MPI for terrestrial Microbiology
Name of host laboratory / group	Geochemical Protoenzymes/WG Preiner
Website of host laboratory / group	https://www.mpi-marburg.mpg.de/martina-preiner https://www.uni-marburg.de/en/microcosm-earth/wg-preiner
Project title	CO_2 fixation over natural minerals
Project duration (3-6 months)	4 to 6 months
Prerequisites (educational)	MSc in Chemistry, Geochemistry or similar (or experience in these kind of research)

Project proposal: description and expected outcomes of project (500 words max.):

Our group is interested in the chemical reactions that could have led to the first cells on early Earth, the focus hereby lies on the origin of autotrophic CO_2 fixation from geochemistry. During this internship, the intern will investigate CO_2 fixation within serpentine rock samples utilising a pressurized reactor with a mixture of CO_2 and H_2 at pressures ranging from 10 to 35 bars to emulate conditions potentially akin to serpentinizing systems found in the Earth's crust. During this study, the abiotic synthesis of organic carbon from CO_2 will be monitored quantitatively highlighting the catalytic potential of natural serpentine in the production of prebiotic organic molecules, changes within the mineral material will be measured by XRD, µ-FTIR and Electron microscopy. Depending on the progress of the project, the ability to use natural minerals for further prebiotic experiments involving coenzymes will tested. Also the influence of porosity of the rock samples will be a line of research, again, depending on the overall progress.

Literature:

Beyazay, T. et al. (2023) 'Ambient temperature CO2 fixation to pyruvate and subsequently to citramalate over iron and nickel nanoparticles', Nature Communications, 14, p. 570. https://doi.org/10.1038/s41467-023-36088-w.

Henriques Pereira, D.P. et al. (2022) 'Role of geochemical protoenzymes (geozymes) in primordial metabolism: Specific abiotic hydride transfer by metals to the biological redox cofactor NAD+', The FEBS Journal, p. 16329. https://doi.org/10.1111/febs.16329.

Preiner, M. et al. (2020) 'A hydrogen-dependent geochemical analogue of primordial carbon and energy metabolism', Nature Ecology &

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Name of Institution	MPI-TM
Name of host laboratory / group	Sourjik
Website of host laboratory / group	https://www.mpi-marburg.mpg.de/sourjik
Project title	Multicolor labeling of bacterial rhizosphere communities
Project duration (3-6 months)	6 months
Prerequisites (educational)	Good knowledge of molecular biology and microbiology; at least basic knowledge of fluorescence microscopy

Project proposal: description and expected outcomes of project (500 words max.):

The aim of this project is to optimize and apply a recently developed tool for simultaneous labeling of more than 10 different bacterial strains or species in the same culture. This tool relies on combinations of different fluorescent proteins and enables monitoring spatial arrangement of bacteria in communities using microscopy and tracking their population dynamics using flow cytometry. Within this project, the multicolor labeling approach will be applied for the communities of soil/rhizosphere bacteria, growing either on agar plates or in liquid and with or without plant seedlings, or in a more natural environment of the soil.

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Name of Institution	**Max Planck Institute for Terrestrial Microbiology (MPI-TM)**
Name of host laboratory / group	Anke Treuner-Lange
Website of host laboratory / group	https://www.mpi-marburg.mpg.de/treuner-lange
Project title	Identification of potential protein interaction partners using proximity-labeling
Project duration (3-6 months)	4-6 month
Prerequisites (educational)	Basic knowledge of microbiology and molecular biology

Project proposal: description and expected outcomes of project (500 words max.):

Bacterial motility is important for adaptation and colonization of various habitats and overall fitness by directing cells towards nutrients and away from toxins and predators. The most widespread form of surface-associated motility is twitching motility, which is mediated by Type IVa pili (T4aP). Twitching motility depends on cycles of T4aP extension, surface adhesion, and retraction. Because the T4aP adheres strongly to the surfaces, cells are pulled forward during retractions.

The T4aP extension/adhesion/retraction cycles are powered by the highly conserved T4aP machine (T4aPM), which in *M. xanthus*, is composed of at least 15 highly conserved proteins and spans from the outer membrane (OM) across the periplasm and inner membrane (IM) to the cytoplasm. In the cytoplasm the hexameric PilB and PilT ATPases associate with the cytoplasmic base of the core T4aPM in a mutually exclusive fashion to power T4aP extension and retraction.

In the rod-shaped bacterium *M. xanthus* T4aP are only extended at the leading cell pole, where upon extension and adhesion of T4aP, a force is created which pulls the cells in the direction of the adhered

T4aP. Consistent with this unipolar T4aP formation, the PilB extension ATPase almost exclusively localizes to the leading cell pole.

We recently discovered that the protein SopA is important for T4aP formation and involved in stimulating the polar localization of PilB. SopA interacts directly with SgmX, a protein composed of 14 TPR domains, which was suggested to stimulate T4aP formation by enabling PilB interaction with the base of the T4aPM. Our future goal is to understand how SgmX stimulates the interaction of PilB with the cytoplasmic base of the T4aPM and how SopA might further stimulate this interaction. So far no direct interaction between SgmX and PilB and SopA and PilB have been detected. To identify potential interaction partners of SopA, SgmX and/or PilB we aim to use mTurbo-based proximity-labeling experiments, which allows identification of low-affinity and/or transiently interacting protein partners. Depending on the outcome of these experiments direct protein interactions could be tested using the bacterial adenylate cyclase two-hybrid system (BACTH).

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Name of Institution	Max Planck Institute for Terrestrial Microbiology
Name of host laboratory / group	RG Yuan
Website of host laboratory / group	https://www.mpi-marburg.mpg.de/systems-and-synthetic-microbiology/jingyuan
Project title	Signaling from host to uropathogens via NAD precursors
Project duration (3-6 months)	6 months
Prerequisites (educational)	M.S.

Project proposal: description and expected outcomes of project (500 words max.):

Uropathogenic *Escherichia coli* (UPEC) and *Klebsiella pneumoniae* are the leading causes of urinary tract infections (UTIs), responsible for over 150 million cases globally each year. These uropathogens typically reside harmlessly in the human gut, forming a reservoir. Through fecal shedding, they spread to the periurethral area or vagina, where they enter the urethra, ascend the urinary tract, and trigger infections. Most UTIs are caused by the abundance of intestinal uropathogens, a major factor in recurrent UTIs. This highlights the critical role of gut colonization in the development of these infections. Gaining a deeper understanding of this colonization process may help in preventing UTIs and reducing the reliance on antibiotics for treatment.

The EvgS/EvgA two-component system plays a vital role in bacterial survival through the acidic environment of the human stomach. It also regulates virulence in both *E. coli* and *K. pneumoniae*. For uropathogens to establish a reservoir in the gut, it is crucial to prevent early activation of virulence genes, which requires deactivating the EvgS/EvgA system after passing through the gastric acid. Our research suggests that this system is involved in detecting NAD precursors generated from host-secreted nicotinamide, influencing bacterial infectivity. However, the exact pathways and mechanisms of this sensing process are not yet fully understood. It would be intriguing to explore whether the mammalian host could communicate with bacteria via NAD precursors, potentially affecting bacterial colonization in the gut.

During the six-month fellowship period, the student will characterize the detection of NAD precursors by *E. coli* via its EvgS/EvgA system using the GFP reporter and protocol established in my group. Specifically, the NAD precursor binding sites and signaling will be predicted and experimentally verified. In addition, the effect of NAD precursors on *E. coli* virulence will be studied using mammalian cell cultures in vitro. By the end of the fellowship, we expect to have identified the most important NAD precursor that has the greatest effect on EvgS activity, elucidated its binding site, and gained insight into its mechanism of action and its influence on *E. coli* virulence.