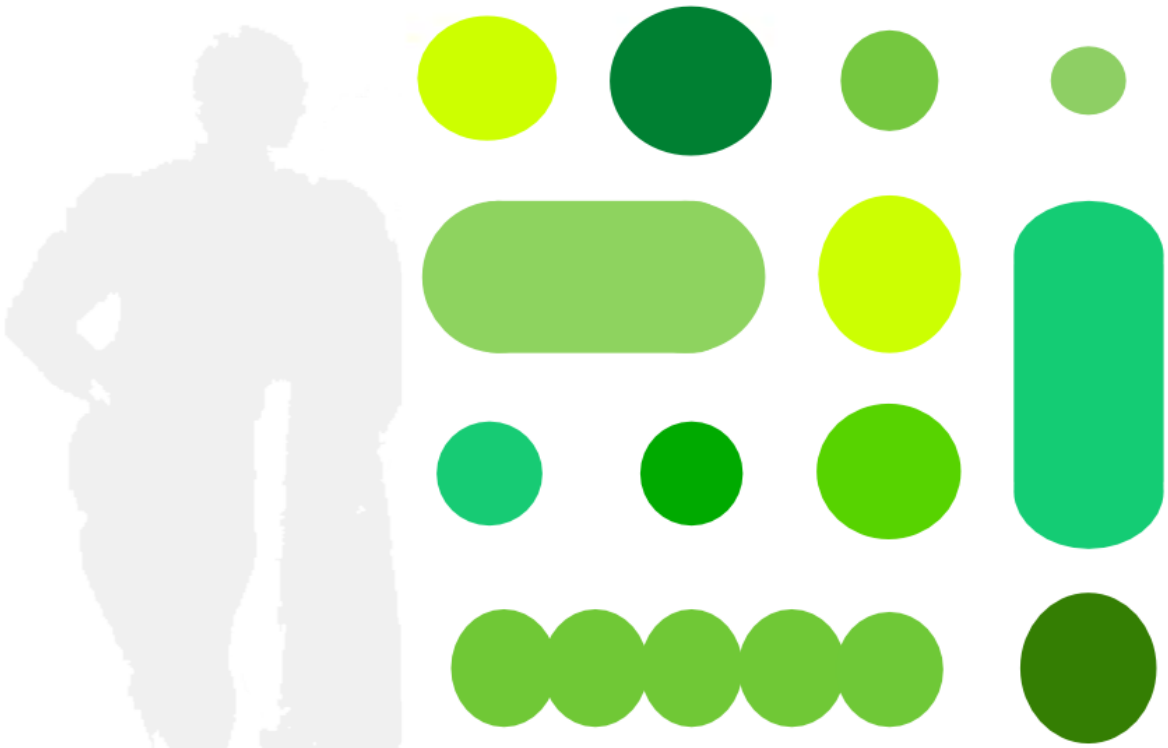


Cyano Kassel 2023

Conference program

Welcome to the Cyano2023

8th Early Career Researcher Symposium on Cyanobacteria VAAM Special Group Cyanobacteria Meeting

27th-29th September 2023 in Kassel, Germany

Dear Participants,

We would like to welcome you to the Cyano2023 which is hosted by the Gutekunst Lab from the University of Kassel. Our exciting schedule includes contributions from young scientists in the form of presentations and posters alongside top of the research presentations by five keynote speakers. We encourage plenty of scientific exchange in discussion panels, while also getting to know the people behind the research with a social program. Please be aware that we won´t print out this booklet, so if you want a printed version, please bring your own.

The organizers:

Frauke Caliebe

Johanna Caliebe

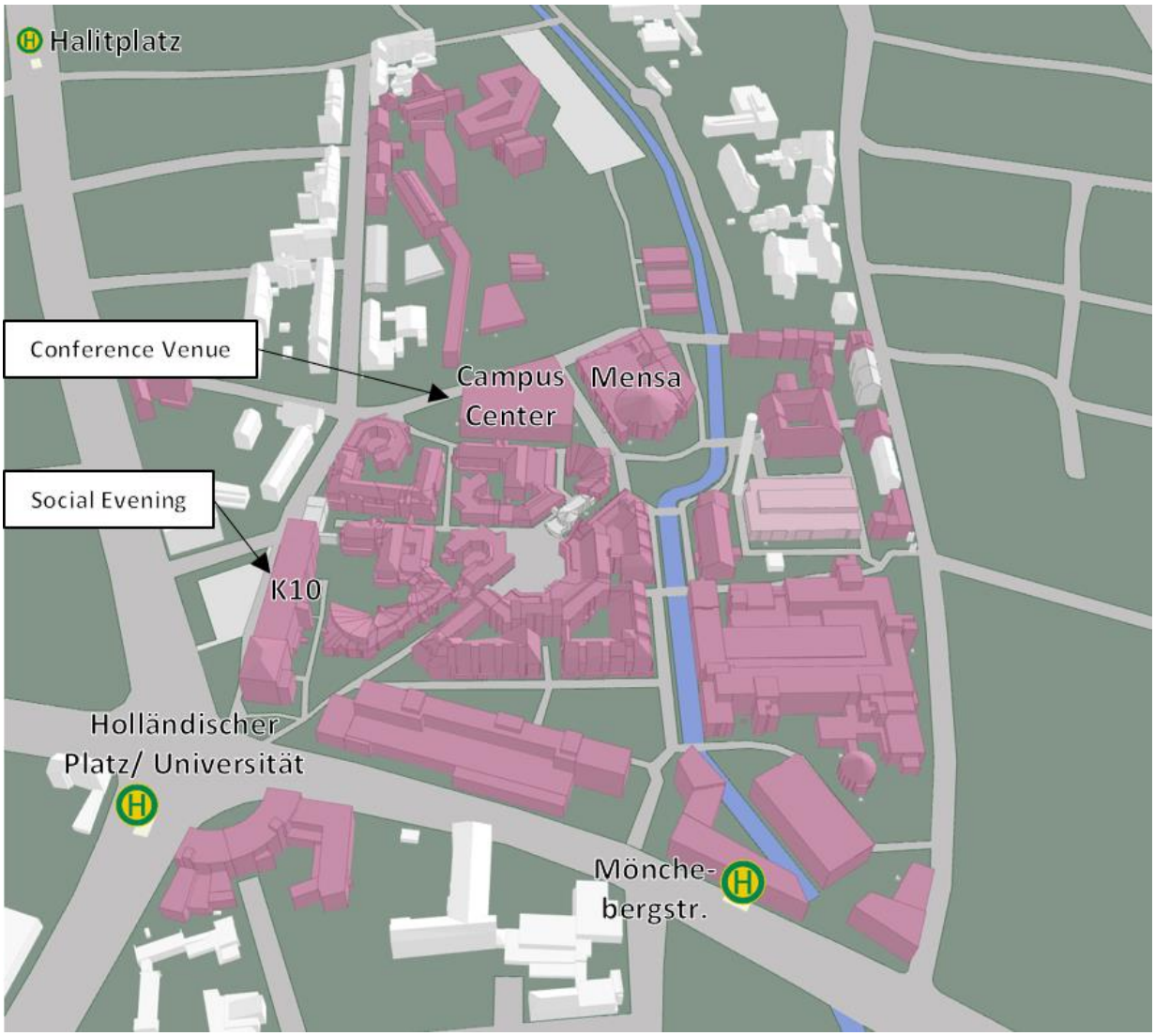
Florian Paul

Nadine Strabel

Marius Theune

We thank our sponsors and contributors:

The Venue and Getting Around in Kassel



The Cyano2023 takes place inside the Campus Center at the University of Kassel:

Moritzstraße 18, 34127 Kassel.

The Campus Center is located on the Campus ‘Holländischer Platz’ and is completely barrier-free. Registration, coffee breaks and poster sessions will take place on the ground floor, while talks are being held in a lecture hall on the first floor. Sanitary facilities can be found on the first floor as well.

To get to the venue via public transportation, it takes a 5-minute foot walk from the station “Holländischer Platz/ Universität” which in turn can be reached, for instance from downtown, with the tram lines 1 “Vellmar”, Bus 52 "Salzmannshausen" or Bus 100 "Calden Flughafen" as well as several other lines from other directions.

Schedule Cyano2023

Wednesday, 27.09.2023

12:00 pm - 1:45 pm Registration

1:45 pm - 2:00 pm Welcome remarks

2:00 PM – 3:00 PM	**Keynote Talk 1: Sofia Doello, University of Tübingen** Regulatory mechanisms controlling the metabolism of glycogen
3:00 PM – 3:20 PM	Adrian Tüllinghoff *Establishing an in vivo cascade in cyanobacteria for light-driven redox bio catalysis on gram scale*
3:20 PM – 3:40 PM	Luisa Hemm *Identification of RNA-based interactions and regulation in the cyanobacterial model Synechocystis sp. PCC 6803*
3:40 PM - 4:00 PM	**Coffee break**
4:00 PM - 4:20 PM	Nils Schmidt *DNA methylation, a regulator of keystone enzyme of chlorophyll biosynthesis in Synechocystis sp. PCC 6803*
4:20 PM - 4:40 PM	Jorge Guio *Deciphering novel transcriptional regulatory networks in the cyanobacterium Anabaena sp. PCC 7120 and their potential involvement in nitrogen metabolism*
4:40 PM - 5:00 PM	Laura Antonaru *Common loss of far-red light photo acclimation in cyanobacteria from hot and cold deserts*
5:00 PM - 5:20 PM	Mirka Kutzner *Conserved residues in the linker connecting helices 1 and 2 control the activity of the Synechocystis IM30 protein*
5:20 PM - 7:00 PM	**Poster session 1**

Schedule Cyano2023

Thursday, 28.09.2023

Session II, Chair: Florian Paul

9:00 AM - 10:00 AM	**Keynote Talk 2: Kevin Redding, Arizona State University** Light-driven electron transport in Heliobacteria
10:00 AM - 10:20 AM	Ute Hoffmann *Enzyme engineering to accelerate the Calvin-Benson-Bassham cycle in cyanobacteria*
10:20 AM - 10:40 AM	Alexandra Schirmacher *Formation of synthetic nanofilaments in cyanobacteria for protein co-localization*
10:40 AM - 11:00 AM	**Coffee break**
11:00 AM - 11:20 AM	Elisabeth Lichtenberg: *Translation-independent RNA-localization in Synechocystis*
11:20 AM - 11:40 AM	Shujie Wu *Diversity and distribution of far-red photoacclimation in desert-dwelling cyanobacteria from sabkha oum dba (Morocco)*
11:40 AM - 12:00 PM	Aleksandar Stanojkovic *The global continuum of Microcoleus species revealed through population genomics*
12:00 PM - 12:20 PM	Vendula Krynická *The cyanobacterial ftsh4 protease controls accumulation of protein factors involved in the biogenesis of photosystem I*
12:20 PM - 1:30 PM	**Lunch break**
Session III, Chair: Nadine Strabel	
1:30 PM - 2:30 PM	**Keynote Talk 3: Alistair McCormick, University of Edinburgh** New tools and fast-growing strains for engineering biology in cyanobacteria
2:30 PM - 2:50 PM	Alexander Kraus *Small but powerful! The small protein NirP1 controls the nitrogen assimilation in cyanobacteria*
3:00 PM - 5:30 PM	Social activity (4 options)
5:30 PM - 7:00 PM	**Poster session 2**
7:00 PM - open end	Social evening

Schedule Cyano2023

Friday, 29.09.2023

Session IV, Chair: Johanna Caliebe

9:00 AM – 10:00 AM	**Keynote Talk 4: Anna Matuszynska, RWTH Aachen** Computational models of *Synechocystis sp.* PCC 6803
10:00 AM – 10:20 AM	Luisa Wittemeier *Positional isotopologue analysis of aspartate to determine PEPC activity in vivo*
10:20 AM – 10:40 AM	Rune Höper *Understanding cyanobacterial phototrophic growth through genome-scale modelling*
10:40 AM - 11:10 AM	**Group picture, Coffee break**
Session V, Chair: Frauke Caliebe	
11:10 AM - 11:30 AM	Mara Reis *Studying the impact of high manganese stress on the cyanobacterium Synechocystis sp. PCC 6803 by transcriptomics*
11:30 AM – 12:30 PM	**Keynote Talk 5: Stephan Klähn, UFZ Leipzig** Balancing & Re-routing: Distinctive control principles and synthetic biology applications of cyanobacterial metabolism
12:30 PM - 1:00 PM	**Concluding remarks, poster prices**

Invited Speakers

It is our great pleasure to have keynote lectures given by and be able to discuss with the following invited speakers:

Sofia Doello	University of Tübingen
Kevin Redding	Arizona State University
Alistair McCormick	University of Edinburgh
Anna Matuszynska	RWTH Aachen
Stephan Klähn	UFZ Leipzig

Lunch

On Thursday, lunch in the canteen (Mensa) on campus will come at a reduced price of 2,50-4,50€ if you bring your student ID (applies to internationals as well), regular pricing will be 7,70-9,70€, to be paid by EC-card. There are also several cafés and restaurants in walking distance.
During coffee breaks, vegetarian and vegan snacks will be provided.

Social Program

To get in touch with each other beyond the science, there are some fun social activities planned. You can go on a guided city tour about the Grimm brothers, learning more about the history of the famous fairytale authors and their opus in this region of Germany. Kassel is famous for the contemporary art exhibition “documenta”, which takes place every 5 years and has left its mark all around the city. In the guided tour you will learn about this international art event. To get to the starting point of the guided tours you can either walk from the venue to the Fridericianum (20 minutes’ walk) or take the tram from “Holländischer Platz/ Universität” to “Friedrichsplatz” (around 15 minutes total with walking to/ from the tram stop, 1,90€ for short distance, one way).

If you feel a bit sportier, you can explore the Fulda River via canoe. Hopefully, you don´t get wet! To get there, you will meet at the conference venue and walk there (about 10 minutes). The visit to the Botanical Garden is perfect for all the botanists amongst you Cyanobacteria enthusiasts. To get to the botanical garden you have to take the tram from “Holländischer Platz/ Universität” to “Park Schönfeld” (15 minutes, 3€ for one way) and walk about 10-15 minutes through the park. There are also group tickets for the tram, which are cheaper. If you intend to use the tram, it might be a good idea to bring some cash to facilitate organizing a group ticket. Please make sure to be ready for the activities and back to the venue after them on time since the schedule is pretty tight. The cost for the guided tours “Documenta” and “Grimm brothers” is 12,00 €, the cost for canoeing is 10,00 €. The visit to the botanical garden is free. You can either pay in cash at the registration (please bring the exact amount, as we might not be able to give change!) or by PayPal to marius-theune@web.de. If you use PayPal, please give “Cyano 2023, name (first, last)” as reference.

Poster Session and Get-Together

On Wednesday and Thursday evening there will each be a poster session. You can get together and talk about your current research project in a relaxed atmosphere. Snacks and drinks will be provided. You can hang up your poster as soon as you arrive, latest during the coffee break before the poster session. The sessions are arranged so that posters with odd numbers (01, 03, ...) will be presented on Wednesday and even numbers (02, 04, ...) on Thursday. This way, everyone should have enough time to participate. You can look up your poster number in the list given below. From Thursday 6 PM until Friday 11 AM, you will have one vote to choose your favorite poster. This will be done via a tool called LamaPoll; there are two links since only 50 participants are allowed per poll. On Friday, three winners will be announced and receive a small surprise that we prepared.

1. https://survey.lamapoll.de/Poster-Voting-Cyano2023_1
2. https://survey.lamapoll.de/Poster-Voting-Cyano2023_2

Statement

The University of Kassel sees itself as a gender-equitable, family-friendly, open, inclusive, and intercultural university. It considers the diversity of its members to be a productive resource and aims to eliminate discrimination on the grounds of individual or social characteristics. We intend to host the Cyano 2023 in this spirit and want everyone to feel welcomed and encouraged to participate in the program openly.

Contributed Talks Overview

FIRST AUTHOR	TITLE
Adrian Tüllinghoff	Establishing an in vivo cascade in cyanobacteria for light-driven redox biocatalysis on gram scale
Luisa Hemm	Identification of RNA-based interactions and regulation in the cyanobacterial model *Synechocystis* sp. PCC 6803
Nils Schmidt	DNA methylation, a regulator of keystone enzyme of chlorophyll biosynthesis in *Synechocystis* sp. PCC 6803
Jorge Guio	Deciphering novel transcriptional regulatory networks in the cyanobacterium *Anabaena* sp. PCC 7120 and their potential involvement in nitrogen metabolism
Laura Antonaru	Common loss of far-red light photo acclimation in cyanobacteria from hot and cold deserts
Mirka Kutzner	Conserved residues in the linker connecting helices 1 and 2 control the activity of the *Synechocystis* IM30 protein
Ute Hoffmann	Enzyme engineering to accelerate the Calvin-Benson-Bassham cycle in cyanobacteria
Alexandra Schirmacher	Formation of synthetic nanofilaments in cyanobacteria for protein co-localization
Elisabeth Lichtenberg	Translation-independent RNA-localization in *Synechocystis*
Shujie Wu	Diversity and distribution of far-red photoacclimation in desert-dwelling cyanobacteria from sabkha oum dba (Morocco)
Aleksandar Stanojkovic	The global continuum of Microcoleus species revealed through population genomics
Vendula Krynická	The cyanobacterial ftsh4 protease controls accumulation of protein factors involved in the biogenesis of photosystem I
Alexander Kraus	Small but powerful! The small protein NirP1 controls the nitrogen assimilation in cyanobacteria
Luisa Wittemeier	Positional isotopologue analysis of aspartate to determine PEPC activity in vivo
Rune Höper	Understanding cyanobacterial phototrophic growth through genome-scale modelling
Mara Reis	Studying the impact of high manganese stress on the cyanobacterium *Synechocystis* sp. PCC 6803 by transcriptomics

Poster Presentations overview

The odd numbers (marked in grey) will present in poster session 1 (Wednesday), the even numbers (marked in white) will present in poster session 2 (Thursday). Please dismount your poster only after the second poster session, but by Friday 1 pm at the latest.

Poster Overview

#	Titel	First Author
01	Characterization of the individual PsaA and PsaB assembly intermediates of photosystem I in *Synechocystis* 6803	Sadanand Gupta
02	A novel cyanobacterial membrane protein involved in the biogenesis of photosystem I	Anna Wysocka
03	Exploring the role of *Synechocystis* CurT protein in the biogenesis of photosynthetic apparatus	Petra Skotnicova
04	Rational design of biophotoelectrodes for in vitro biocatalysis	Anna Frank
05	New mechanisms regulating inorganic carbon uptake in Cyanobacteria	Peter Walke
06	Characterisation of the molecular mechanism of CO2 capture and sequestration by formation of intracellular amorphous carbonates (iACC) inclusions	Monis Athar Khan
07	Cyanobacterial toxins as modulators of carbon fixation	Arthur Guljarnow
08	Unravelling the physiological function of the Entner-Doudoroff pathway in *Synechocystis* sp. PCC 6803 by enzyme characterization	Ravi Ojha
09	SbtB: A substrate of FtsH4	Surbhi Sharma
10	Structure and activation of SynDLP, the fusogenic dynamin-like protein of the cyanobacterium *Synechocystis* sp. PCC 6803	Lara Mernberger
11	Exploring the Role of the Auxiliary Metabolic Gene petE from the Cyanophage Syn9 during infection	Nicola Sekulara
12	Formation of IM30 punctae requires a structured core domain	Ndjali Quarta
13	The regulation of the activity of ferredoxin-nitrite reductase by the ferredoxin-nitrite reductase regulator protein 1 and thioredoxin a in *Synechocystis* sp. PCC 6803	Vincent Reinmuth
14	Nitrite secretion by cyanobacteria is controlled by the small protein NirP1	Alexander Kraus
15	Membrane remodeling and curvature induction by a small transmembrane protein	Pia Briger
16	Thylakoid targeting improves expression and stability of a plant cytochrome P450 in the cyanobacterium *Synechocystis* sp. PCC 6803	Sayali Hanamghar
17	The role of the MCE (mammalian cell entry) protein in biogenesis of the thylakoid membrane of *Synechococcus elongatus* PCC 7942	Xiaowei Dong
18	Iterative analysis of metabolic modulation in the cyanobacterium *Aphanizomenon flos-aquae* 2012 KM1/D3 upon nitric oxide synthase derived NO induction	Neha Gupta
19	Liberation of mineral-bound iron by marine cyanobacteria *Synechococcus* sp. PCC 7002 using siderophores	Sean Hegan
20	Temperature-induced dynamics of secondary metabolites, biocondensates and growth of microcystis strains	Souvik Roy
21	Utilization of the key regulatory protein CP12 to re-direct energy flux towards products in cyanobacteria	Amadeus Itzenhäuser
22	A chimeric KaiA-like regulator extends the KaiB3-KaiC3 clock system in bacteria	Kim Nadine Sebastian
23	Is Sll0518 required for RNA translocation?	Wongsik Seo

Poster Presentations overview

24	Computational screen for novel RNA structures in cyanobacteria	Adrian Geissler
25	A minimal coarse-grained model of cyanobacterial growth	Mona Nehse
26	*Aphanizomenon flos-aquae* 2012 KM1/D3 upon nitric oxide synthase derived NO induction	Tobias Pfennig
27	Spatio-temporal regulation of type IV pilus assembly in *Synechocystis* sp. PCC 6803	Jonas Hammerl
28	Chlorophyll-binding domain of the cyanobacterial ferrochelatase is indispensable under cold stress	Divya Aggarwal
29	Toxic cyanobacteria *Nodularia spumingena*, a winner in future baltic sea climate? Long term adaptation of *N.spumigena* to temperature and salinity change	Carolin Peter
30	Sucrose utilization in recombinant *E.coli* strains for efficient cofactor regeneration	Lucija Sovic
31	Blue-green travelers: cultivation of cyanobacteria associated with sea turtles	Lucija Kanjer
32	Direct positive effect of stoniness on the activity of photoautotrophs in the dryland biological soil crusts	Naw Than Than Aye
33	Light dependent morphogenesis of cyanobacterium *Synechococcus elongatus* PCC 7942	Soumila Mondal
34	Clarification of chromatic acclimation-4 in *Synechococcus* WH8109	Jeegna Solanki
35	Ca + signaling in filamentous heterocyst forming cyanobacteria	Teresa Müller
36	A promising approach for long-term cryopreservation of *Anabaena* sp. PCC 7120	Inés Federio
37	Membrane inlet mass spectrometry	Friederike Hörsch
38	Enhancing cyanobacterial growth and culture stability through co- cultivation with heterotrophic bacteria	Miriam Verucchi
39	Quantitative analysis of structured, mixed-species microbial consortia in microfluidic flow-cell platform	Selina Hanisch
40	Engineering cyanobacteria as the basis for a synthetic microbial community	Dennis Hasenklever
41	Responses and adaptations to high CO_2 in cyanobacteria	Elena Carrasquer-Alvarez
42	Regulation of the carbon flux in *Synechocystis* using the PGAM-PirC switch	Nathalie Sofie Becker
43	Engineering the cyanobacterium S*ynechocystis* into a synthetic organelle	Jeannine Volke
44	Photosynthesis-driven whole-cell biocatalysis using *Synechocystis* sp.PCC 6803 for the conversion of cyclohexane to cyclohexanone.	Nina Siebert
45	Cyanobacterial biofilms for photoelectrochemistry	Nathan Wright
46	Design and implementation of a $NADP^+$ /ferredoxin-reducing O_2 -tolerant hydrogenase in *Synechocystis*	Diletta Sacco
47	Anoxic culture conditions promote higher growth rates for *Pseudanabaena* and potentially other biotechnological strains.	Kimberly Kaiser

Computational models of *Synechocystis sp. PCC* 6803

Jun.Prof. Dr. Anna Matuszyńska
Computational Life Science
RWTH Aachen University
Germany

anna.matuszynska@rwth-aachen.de

Mathematical and computational models have emerged as indispensable tools in modern biological research, particularly in the context of understanding and exploring complex systems. In this talk, we highlight the significance of these models in deciphering the primary energy production capacity of cyanobacteria and their biotechnological relevance.

Our computational endeavors focus on modeling cyanobacteria, with a specific emphasis on the cyanobacteria strain *Synechocystis* sp. PCC 6803. We present our most recent mechanistic model of photosynthesis, which provides insights into the dynamic interplay of light quality and quantity on its efficiency. By investigating the role of these parameters, we aim to enhance our understanding of the underlying mechanisms governing photosynthetic activity in cyanobacteria.

Furthermore, we delve into the importance of comprehending photosynthetic activity to explore the theoretical boundaries of terpenoid production in cyanobacteria. Through our modeling efforts, we shed light on the intricate secondary metabolism in cyanobacteria, laying the groundwork for potential advancements in the field.

This talk serves as an opportunity to present our latest findings and preliminary work in computational modeling, providing valuable insights into the fundamental aspects of cyanobacterial biology. Ultimately, our research contributes to the broader goal of harnessing the untapped potential of cyanobacteria for biotechnological applications.

New tools and fast-growing strains for engineering biology in cyanobacteria

Alistair J. McCormick
Institute of Molecular Plant Sciences and Centre for Engineering Biology, School of Biological Sciences, University of Edinburgh, EH9 3BF, UK

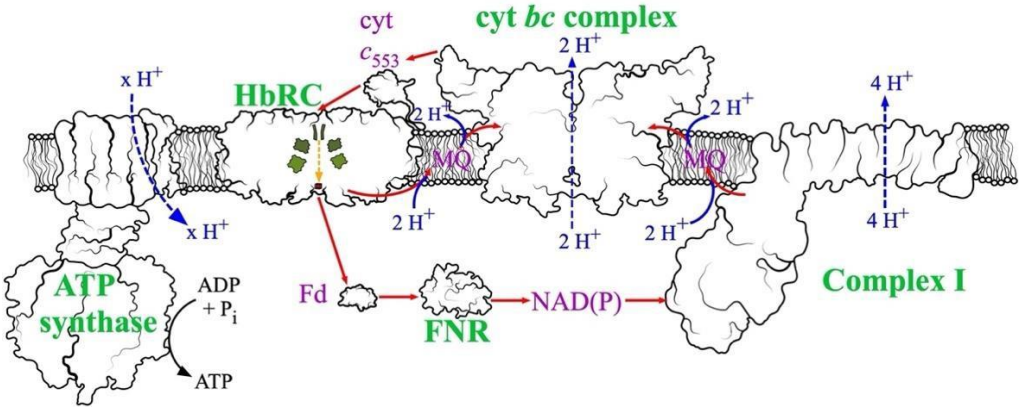
Recent advances in engineering/synthetic biology-based tools have spurred a prolific increase in fundamental cyanobacterial research and biotechnological exploitation. Nevertheless, even in the most widely studied species, *Synechocystis* sp. PCC 6803 (Synechocystis), only ~30% of the predicted gene coding sequences have an assigned function. I will firstly overview our work to develop the CyanoGate modular cloning kit for rapid plasmid assembly and transformation/transconjugation in cyanobacteria. I will then discuss our ongoing collaborative efforts to develop CyanoSource (https://cyanosource.ac.uk), a publicly available barcoded Synechocystis mutant library and plasmid resource for the research and biotechnology communities. Lastly, I will outline a suite of new CyanoGate-compatible molecular tools that we have developed to help unlock the engineering potential of the fast-growing marine cyanobacterial strain *Synechococcus* sp. PCC 11901. We hope that these tools will lay the foundations for further adoption of PCC 11901 as a robust model strain for engineering biology and green biotechnology.

Light-driven electron transport in Heliobacteria

Gregory Orf[1], Shreya Shaw[1], Sabrina Leung[1], Karim Walters[2], Patricia Baker[1], John H. Golbeck[2] and Kevin E. Redding[1]*

1School of Molecular Sciences, Arizona State University
2Dept. Biochemistry & Molecular Biology, Pennsylvania State University
*Kevin.Redding@asu.edu

The heliobacteria, a family of anoxygenic phototrophs, possess the simplest known phototrophic system, utilizing a homodimeric Type I reaction center (RC) within only 2 subunits (PshA and PshX) and lacking any peripheral antenna complexes. Although they are photoheterotrophs in the light, the heliobacteria can also grow fermentatively on pyruvate in the dark like their clostridial cousins. They are likely the result of a lateral gene transfer of genes conferring phototrophy (e.g., RC subunits, bacteriochlorophyll synthesis). I will discuss how we created a transformation system, and how we have leveraged the endogenous CRISPR/Cas system to create the means to cleanly delete genes from the chromosome of *Heliomicrobium modesticaldum*. Using this technology, we have launched a genetic study of the light-driven cyclic electron transport pathways within this organism. Mutants lacking the genes for the RC (*pshA*) or cytochrome *bc* complex (*petABCD*) are nonphototrophic and exhibit a >100-fold slower re-reduction of cyt *c* after a laser flash. Mutants lacking the minor RC subunit PshX are phototrophic but show a slight drop in RC levels and the loss of a low-energy pigment. Deletion mutants of each of the 3 genes encoding a small di-cluster ferredoxin shown to bind and be reduced by the acceptor side of the heliobacterial RC resulted in no effect, indicated functional redundancy within the ferredoxin pool.



Regulatory mechanisms controlling the metabolism of glycogen

Sofía Doello
Interfaculty Institute for Microbiology and Infection Medicine, University of Tübingen, Germany

Glycogen is a wide-spread reserve polymer important for the survival to environmental stresses of all kinds of organisms. In the cyanobacterium *Synechocystis* sp. PCC 6803, glycogen is necessary to overcome dark and starvation periods. During nitrogen limitation, glycogen synthesis and degradation are required for survival. Cells that are unable to produce glycogen fail to properly enter the state of dormancy that allows them to cope with nitrogen-starved conditions, and cells uncapable to degrade glycogen are impaired in the awakening process. Given the important role of this polysaccharide, its metabolism is subjected to a complex regulation. The activity of the enzymes involved in glycogen synthesis and degradation must be tightly controlled to ensure rapid access to the sugar stores when required. Such a responsive and fine control is achieved via post-translational modifications, including phosphorylation, redox-induced modifications, and allosteric interactions. The known regulatory mechanisms of the glycogen synthesis and degradation machinery will be summarized and discussed. Interestingly, some of these mechanisms are not specific of cyanobacteria, but evolutionary conserved from bacteria to humans.

Balancing & Re-routing: Distinctive control principles and synthetic biology applications of cyanobacterial metabolism

Stephan Klähn, Helmholtz Centre for Environmental Research – UFZ, Leipzig

Within a cell, all metabolic routes are closely connected. Therefore, cells have to manage the distribution of resources, i.e. the overall metabolism is coordinated and balanced according to the availability of energy equivalents as well as nutrients. As bacteria have a high surface to volume ratio they are even more challenged by constant changes of environmental as well as biochemical parameters. To maintain homeostasis and to adjust metabolism bacterial cells therefore require a plethora of sophisticated regulatory mechanisms that continuously process external or internal signals to determine in a situation-dependent way whether metabolic fluxes are finally directed into one or another route. However, it is scarcely understood how all these processes are regulated and coordinated in cyanobacteria. It turned out that data from typical model bacteria like *E. coli* or *Bacillus subtilis* can regularly not be applied because even for ubiquitous pathways cyanobacteria evolved unique regulatory mechanisms (as exemplified by the regulation of glutamine synthetase, BIOspektrum 25, p.610–613). However, to rationally engineer cyanobacteria for biotechnological or pharmaceutical applications, e.g. by channeling metabolic fluxes to obtain the maximum yield of a desired chemical product, it is of paramount importance to fully comprehend underlying molecular processes that control primary metabolism. Our research tackles these problems and aims at the basic understanding of key regulatory switches as well as their utilization to engineer synthetic cyanobacterial cell factories. Our investigation is targeting all regulatory levels: sensing and signaling (via sensory proteins or RNAs), transcriptional regulation of genes (via transcriptional regulators), RNA-based mechanisms targeting the posttranscriptional level (small regulatory RNAs and riboswitches) as well as the modulation of enzyme activities by molecular effectors (small regulatory proteins). In addition, we also make use of these intrinsic regulators to enable inducible expression of heterologous genes (for enzymes or entire pathways) or to direct metabolic fluxes towards specific products. The obtained knowledge will provide a scientific fundament for the rational design of photosynthesis-driven applications.

ESTABLISHING AN *IN VIVO* CASCADE IN CYANOBACTERIA FOR LIGHT-DRIVEN REDOX BIOCATALYSIS ON GRAM SCALE

Adrian Tüllinghoff *[,a], Harcel Djaya-Mbissam[a], Jörg Toepel [a], and Bruno Bühler[a]
[a] *Helmholtz-Centre for Environmental Research (UFZ) Leipzig, Environmental Microbiology, Permoserstr .15, 04318 Leipzig, Germany*
* *e-mail: adrian.tuellinghoff@ufz.de*

Cyanobacteria are ideal host organisms for truly sustainable whole-cell biocatalysis, as both reduction equivalents and O_2 are provided *in vivo* via water oxidation with light as sole energy source.[1] This attracted the coupling of redox reactions, especially oxygenases, to the photosynthetic light reaction via NADPH and O_2 in recombinant cyanobacteria. Introducing heterologous reactions into microbial hosts often suffers from reactant toxicity. Based on a recombinant *Synechocystis* sp. PCC 6803 strain harboring a Baeyer-Villiger monooxygenase (BVMO),[2] we could implement the first artificial light-driven redox cascade for the conversion of cyclohexanone to the polymer building block 6-hydroxyhexanoic acid. BVMO and lactonase co-expression, both from *Acidovorax* sp. CHX100, enabled this two-step conversion with an activity of up to 63.1 ±1.0 U g_{CDW}^{-1} without accumulating inhibitory ε-caprolactone.[3] Thereby, one of the key limitations of biocatalytic reactions, i.e., reactant toxicity, was overcome. Besides design and development of the cascade, scale-up to Lab scale reactors and process optimization will be presented. With our strategy, titers up to 23.50 ± 0.84 mM (3.11 ± 0.12 g L^{-1}) 6-HA with product yields of 0.96 ± 0.01 mol mol^{-1} were achieved, illustrating the potential of producing this non-toxic product in a synthetic cascade. Product balancing revealed that the onset of the biotransformation improves light conversion, as the product formation does not hamper growth, but increases photosynthetic efficiency. This study shows the feasibility of light-driven biocatalytic cascade operation in cyanobacteria and highlights respective metabolic limitations and engineering targets.

_____

1. J. Toepel, R. Karande, S. Klähn, B. Bühler. *Curr Opin Biotechnol*, **2023**, 80, 102892.
2. A. Tüllinghoff, M.B. Uhl, F.E.H. Nintzel, A. Schmid, B. Bühler, J. Toepel. *Front Catal*, **2022**, 1, 780474
3. A. Tüllinghoff, H. Djaya-Mbissam, J. Toepel, B. Bühler. *Plant Biotechnol J*, **2023**

IDENTIFICATION OF RNA-BASED INTERACTIONS AND REGULATION IN THE CYANOBACTERIAL MODEL SYNECHOCYSTIS SP. PCC 6803

Luisa Hemm*[,a], Matthias Riediger[a], Satoru Watanabe[b] and Wolfgang R. Hess[a]

[a] Institute of Biology 3, University of Freiburg, Freiburg, Germany
[b] Department of Bioscience, Tokyo University of Agriculture, Tokyo, Japan
** e-mail: luisa.hemm@biologie.uni-freiburg.de*

Cyanobacteria are exposed to various changing environmental conditions, such as nutrient availability, temperature shifts and varying light intensities. In response to these changes, cyanobacteria have evolved different regulation mechanisms.
Various types of regulatory RNA molecules contribute to the regulation of gene expression in bacteria. One part of this RNA-based regulation is carried out by small noncoding RNAs (sRNAs) which frequently modulate the translation of their target mRNAs via complementary base pairing[1]. Furthermore, it was shown that the localization of the mRNA in the cell also plays an important role for the protein expression and function of these proteins[2]. Both of these mechanisms require assistance by RNA-binding proteins (RBPs). RBPs regulate a variety of processes in the bacterial cell, ranging from transcription termination and translation initiation to RNA decay[3]. We contributed to work which showed that Rbp2 and Rbp3, belonging to a family of conserved cyanobacterial RBPs, are important for the correct localization of photosynthetic mRNAs, such as psbA and psaA, at the thylakoid membrane[4]. This suggests that RNA binding proteins play an important role in the correct function of cellular mechanisms. Therefore, the in-depth characterization of these photosynthesis-relevant RBPs and the identification of new in the cyanobacterial model Synechocystis sp. PCC 6803 is being addressed in this study.

_____

1 Georg J, Hess WR. Microbiol Spectr. 2018 Jul 13;6(4):6.4.12.
2 Irastortza-Olaziregi M, Amster-Choder O. WIREs RNA. 2020;12(2):e1615.
3 Holmqvist E, Vogel J. Nat Rev Microbiol. 2018 (10):601–15.
4 Mahbub M, et al. Nat Plants. 2020 (9):1179–91.

DNA METHYLATION, A REGULATOR OF KEYSTONE ENZYME OF CHLOROPHYLL BIOSYNTHESIS IN *SYNECHOCYSTIS* SP. PCC6803

Nils Schmidt*[,a], Satoru Watanabe[b], Roman Sobotka[c], Wolfgang R. Hess[d] and Martin Hagemann[a]

[a] *Department of Plant Physiology, University Rostock, Albert-Einstein-Straße 3, 18059 Rostock, Germany*

[b] *Department of Bioscience, Tokyo University of Agriculture, Tokyo, Japan*

[c] *Institute of Microbiology, Centre Algatech, Novohradská 237 - Opatovický mlýn, 379 01 Třebon, Czech Republic*

[d] *Genetics and Experimental Bioinformatics, University of Freiburg, Schänzlestr. 1, 79104 Freiburg, Germany*

** e-mail: nils.schmidt@uni-rostock.de*

Epigenetics acts as an important regulator of gene expression in eukaryotes as well as prokaryotes. In bacteria, it regulates DNA repair, cell replication and gene expression by methylation of specific nucleotides in the DNA (1). The modifications were provided by DNA methyltransferases (MTases). The model organism *Synechocystis* sp. PCC 6803 harbors at least five functional MTases (2).

The aim of this project is to reveal the purpose of the genomic DNA methylation in cyanobacteria and analyze the impact of the different MTases. Mutants lacking the MTase M.Ssp6803II (*sll0729*) possess an altered phenotype. Cells of these strains are decreased in size, contain less chlorophyll a and are sensitive for UV exposure. However, this phenotype is unstable, because after long-term cultivation of the Δ*sll0729* strain single clones appeared that are displaying wild-type-like phenotype (suppressor clones) (3). Whole genome sequencing of suppressor clones revealed a single nucleotide exchange in the promoter of *slr1790*. This gene encodes the protoporphyrinogen IX oxidase (HemJ), a keystone enzyme of the chlorophyll biosynthesis. Partial knockout of HemJ displays a reduced chlorophyll content and is complemented by overexpression of homolog enzyme (4). Transcriptome data of the original Δ*sll0729* clones showed significantly reduced amounts of *slr1790* transcripts. HPLC measurements revealed accumulation of phototoxic chlorophyll precursors in Δ*sll0729* cells. To verify the hypothesis that the mutated *slr1790* promoter is responsible for the wild-type like phenotype of the Δ*sll0729* suppressor clones, *slr1790* promoter::Δ*sll0729* double mutants with and without a specific *slr1790* promoter mutation in the methylation side were analyzed. Phenotype, long-term cultivation behavior and chlorophyll precursor accumulation of these strains support the *slr1790* promoter hypothesis. The native promoter double mutant shows similarly high levels of chlorophyll precursors like the Δ*sll0729* single mutant. The chlorophyll precursors in the mutated promoter double mutant are on a wild-type-like level. To analyze differences in HemJ levels in all these strains, a specific antibody will be used for immunoblotting. An induced expression system shall enable expression of *slr1790* temporally to complement the Δ*sll0729* phenotype. In summary, we propose altered expression of *slr1790* as a reason for Δ*sll0729* phenotype and suppressor clones, suspecting indirect regulation by promoter methylation due to M.Ssp6803II.

1. M. Sánchez-Romero and J. Casadesús. Nat Rev Microbiol, **2020**, 18, 7-20

2. M. Hagemann, K. Gärtner, M. Scharnagl, P. Bolay, S. C. Lott, J Fuss, B. Huettel, R. Reinhardt, S. Klähn, W. R. Hess. DNA Res, **2018**, 25, 343-352

3. K. Gärtner, S. Klähn, S. Watanabe, S. Mikkat, I. Scholz, W. R. Hess, M. Hagemann. Front Microbiol, **2018**, 10, 1233

4. K. Kato, R. Tanaka, S. Sano, A. Tanaka, H. Hosaka. PNAS, **2010**, 107, 16649-16654

DECIPHERING NOVEL TRANSCRIPTIONAL REGULATORY NETWORKS IN THE CYANOBACTERIUM *ANABAENA* SP. PCC 7120 AND THEIR POTENTIAL INVOLVEMENT IN NITROGEN METABOLISM

Jorge Guío*[a], Marta Acero [a], M. Teresa Bes [a], M. Luisa Peleato [a], María F. Fillat [a] and Emma Sevilla [a]

[a] *Department of Biochemistry and Molecular and Cell Biology. University of Zaragoza, Zaragoza 50009, Spain.*
** e-mail: jguio@unizar.es*

Cyanobacteria are able to adapt to diverse environmental conditions thanks to the existence of differential gene expression mechanisms. In the cyanobacterium *Anabaena* sp. PCC 7120, FUR (Ferric Uptake Regulator) proteins are a family of global transcriptional regulators which control a wide set of cellular processes, ranging from photosynthesis to nitrogen metabolism. Apart from directly regulating their target genes, previous studies have suggested that FUR proteins may also indirectly regulate many cellular processes by means of other regulatory proteins. Consequently, FUR proteins could be cornerstones of novel regulatory networks in cyanobacteria.

In this work we have identified nearly 30 genes with regulatory functions directly regulated by FUR proteins, namely transcriptional regulators, two-component systems and sigma factors. Besides, we have found that some of them are also regulated by the global nitrogen regulator NtcA, suggesting that these transcriptional regulatory networks could be involved in orchestrating responses to nitrogen deficiency.

In order to elucidate the function of these newly discovered targets of FUR proteins, some of these uncharacterized transcriptional regulators have been obtained as recombinant proteins and their potential role has been studied. Our results show that two of these regulators, Alr1976 and All0345, control several genes involved in nitrogen metabolism and heterocyst differentiation, revealing that the components of these networks could play key roles in the physiology of cyanobacteria.

COMMON LOSS OF FAR-RED LIGHT PHOTOACCLIMATION IN CYANOBACTERIA FROM HOT AND COLD DESERTS

Laura A. Antonaru[1,2]*, Vera M. Selinger[1,3], Patrick Jung[4], Giorgia Di Stefano[5], Nicholas D. Sanderson[6,7], Leanne Barker[6], Daniel J. Wilson[8], Burkhard Büdel[9], Daniel P. Canniffe[10], Daniela Billi[5], Dennis J. Nürnberg[1,3]

[1]*Institute for Experimental Physics, Freie Universität Berlin, Berlin, Germany.*

[2]*Department of Life Sciences, Imperial College, London, UK.*

[3]*Dahlem Centre of Plant Sciences, Freie Universität Berlin, Berlin, Germany.*

[4]*Department of Integrative Biotechnology, University of Applied Sciences Kaiserslautern, Pirmasens, Germany.*

[5]*Department of Biology, University of Rome Tor Vergata, Rome, Italy.*

[6]*Nuffield Department of Medicine, University of Oxford, Oxford, UK.*

[7]*NIHR Oxford Biomedical Research Centre, Oxford, UK.*

[8]*Big Data Institute, Nuffield Department of Population Health, University of Oxford, Oxford, UK.*

[9]*Department of Biology, University of Kaiserslautern, Kaiserslautern, Germany.*

[10]*Institute of Systems, Molecular and Integrative Biology, University of Liverpool, Liverpool, UK.*

** e-mail: laura.antonaru@fu-berlin.de*

Deserts represent an extreme challenge for photosynthetic life. Despite their aridity, they are often inhabited by diverse microscopic communities of cyanobacteria. These organisms are commonly found in lithic habitats, where they are partially sheltered from extremes of temperature and UV radiation. However, living under the rock surface imposes additional constraints, such as limited light availability, and enrichment of longer wavelengths than are typically usable for oxygenic photosynthesis. Some cyanobacteria from the genus *Chroococcidiopsis* can use this light to photosynthesize, in a process known as far-red light photoacclimation, or FaRLiP[1,2]. This genus has commonly been reported from both hot and cold deserts[3]. However, not all *Chroococcidiopsis* strains carry FaRLiP genes, thus motivating our study into the interplay between FaRLiP and extreme lithic environments. The abundance of sequence data and strains provided the necessary material for an in-depth phylogenetic study, involving spectroscopy, microscopy, and determination of pigment composition, as well as gene and genome analyses. Pigment analyses revealed the presence of red-shifted chlorophylls *d* and *f* in all FaRLiP strains tested. In addition, eight genus-level taxa were defined within the encompassing *Chroococcidiopsidales*, clarifying the phylogeny of this long-standing polyphyletic order. FaRLiP is near universally present in a generalist genus identified in a wide variety of environments, *Chroococcidiopsis sensu stricto*, while it is rare or absent in closely related, extremophile taxa, including those preferentially inhabiting deserts. This likely reflects the evolutionary process of gene loss in specialist lineages.

1. Antonaru, L. A., Cardona, T., Larkum, A. W., & Nürnberg, D. J. *ISMEJ*, **2020**, *14(9)*, 2275-2287.

2. Zhang, Z. C., Li, Z. K., Yin, Y. C., Li, Y., Jia, Y., Chen, M., & Qiu, B. S. *Environ. Microbiol.*, **2019**, *21(4)*, 1497-1510.

3. Bahl, J., Lau, M. C., Smith, G. J., Vijaykrishna, D., Cary, S. C., Lacap, D. C., Lee, C. K., Papke, R. T., Warren-Rhodes K. A., Wong F. K. Y., McKay, C. P. & Pointing, S. B. *Nat. Commun.*, **2011**, *2(1)*, 1-6.

CONSERVED RESIDUES IN THE LINKER CONNECTING HELICES 1 AND 2 CONTROL THE ACTIVITY OF THE *SYNECHOCYSTIS* IM30 PROTEIN

Mirka Kutzner*[,a], Carmen Siebenaller[a,] Nadja Hellmann[a] and Dirk Schneider[a,b]

[a] *Department of Chemistry, Biochemistry and*
[b] *Institute of Molecular Physiology, Johannes Gutenberg University Mainz*
** e-mail: mikutzne@uni-mainz.de*

The inner membrane associated protein of 30 kDa (IM30) is essential for the formation and maintenance of thylakoid membranes in cyanobacteria and chloroplasts[1]. The exact physiological function of IM30 is still unclear, but membrane stabilization and membrane fusion are assumed activities[3]. IM30 monomers, which consist of seven α-helices, assemble in solution to form large homooligomeric ring structures[2]. Yet, the precise role of these ring structures is enigmatic. It is assumed that the ring is necessary for the fusion activity of the protein[3].

An IM30 mutant in which three conserved residues in the linker connecting helices 1 and 2 are replaced still forms typical ring structures, yet the mutated protein is not able to induce membrane fusion[4]. Therefore, this mutant appears to be well-suited to study the protein properties that are important for fusion in detail. Overall, our results do not indicate a particular importance of any of the individual amino acids, yet an additive effect of the mutations. Contrary to our expectations, the mutations resulted in impaired oligomerization of the protein with blocked ring formation, yet a fusion activity was observed, albeit drastically reduced. These results suggest that the ring structure may not be necessary for IM30-triggered membrane fusion.

1. J. Heidrich, A. Thurotte, D. Schneider. *BBA - Biomembranes*, **2017**, *1859*, 537-549.
2. T. K. Gupta, S. Klumpe, K. Gries, S. Heinz, W. Wietrzynski, N. Ohnishi, et al. *Cell*, **2021**, *184*, 3643-3659.
3. C. Siebenaller, B. Junglas, D. Schneider, *Front. Plant Sci.*, **2019**, *10*, 1500.
4. R. Hennig, J. Heidrich, M. Saur et al. *Nat. Commun.*, **2015**, *6*, 7018.

ENZYME ENGINEERING TO ACCELARATE THE CALVIN-BENSON-BASSHAM CYCLE IN CYANOBACTERIA

Ute A. Hoffmann*[,a], Anna Z. Schuppe[a], Emil Sporre[a], Axel Knave[a], Rui Miao [a] and Elton P. Hudson[a]

[a] School of Engineering Sciences in Chemistry, Biotechnology and Health, Science for Life Laboratory, KTH – Royal Institute of Technology, Tomtebodavägen 23B, SE-17165 Solna, Sweden
** e-mail: ute.hoffmann@scilifelab.se*

Flux control in the Calvin-Benson-Bessham (CBB) cycle is distributed over several reactions operating far from equilbrium.[1] In particular Rubisco and F/SBPase have significant control over carbon fixation for growth or biosynthesis.[2] We have developed an enzyme engineering platform with the ultimate goal of increasing carbon fixation and conversion by the model cyanobacterium *Synechocystis* sp. PCC 6803. Starting with targeted mutagenesis libraries, we used competitive growth coupled to deep sequencing to compare the properties of Rubisco and F/SBPase mutants under different cultivation conditions. As the type I Rubisco of cyanobacteria, green algae and land plants may be phylogenetically constrained regarding mutations and catalytic parameters,[3] we used a type II Rubisco, as these show more sequence diversity and lower folding requirements.[4] We show that the platform identifies enzyme variants of both the type II Rubisco and F/SBPase enzymes that affect cell growth in different conditions. The establishment of this platform is a first step toward creating optimized enzyme variants to accelerate the CBB cycle in cyanobacteria and possibly chloroplasts.

1. Hudson EP. *Sem. Cell Dev. Biol.*, **2023**, in press.
2. F. Liang, E. Englund, P. Lindberg, P. Lindblad. *Metab. Eng.*, **2018**, *46*, 51–59.
3. J. W. Bouvier, et al. *Mol. Biol. Evol.*, **2021**, *38(7)*, 2880–2896.
4. D. Davidi, et al. *EMBO J.*, **2020**, *39*, e104081.

FORMATION OF SYNTHETIC NANOFILAMENTS IN CYANOBACTERIA FOR PROTEIN CO-LOCALIZATION

Alexandra M. Schirmacher*, Julie A. Z. Zedler

Matthias Schleiden Institute, Friedrich Schiller University, Dornburger Str. 159, 07743 Jena, Germany

** e-mail: alexandra.schirmacher@uni-jena.de*

Cyanobacteria hold great potential as production chassis for industrial applications in a future bioeconomy. However, product titres are often too low and not competitive compared to heterotrophic hosts.[1] To address this challenge, we aim to develop intracellular co-localization strategies for heterologous biosynthetic pathways. Bringing enzymes in close proximity increases local substrate concentrations and reduces cross-talk with native pathways.[2] This is expected to increase pathway efficiency and ultimately product yields.

Here, we used two different strains in a proof of concept study: the model organism *Synechocystis* sp. PCC 6803 and the fast-growing cyanobacterium *Synechococcus elongatus* UTEX 2973. We have successfully established protein-based nanofilaments that can span the entire cell in both strains based on a self-assembling small hexameric protein.

To investigate if the nanofilaments can serve as scaffolds to co-localise heterologous proteins, we targeted two enzymes for the production of ethanol to the nanofilaments. The enzymes were fused with short peptides to mediate the interaction to the nanofilament. We discovered that the fusion of the enzymes with these peptides resulted in increased protein levels and an inversion of enzyme stoichiometry. This led to up to 30x increase in ethanol production, even in absence of the scaffold. By applying this system, we directly compared the fast-growing *S. elongatus* UTEX 2973 with the closely related, but slower growing strain *Synechococcus* sp. PCC 7942 for their production capacities. The strains displayed differences regarding total ethanol production, productivity rates and protein levels. These results and the tools developed here will benefit future applications in the green bioeconomy.

1. G. Luan, S. Zhang, X. Lu. *Curr Opin Biotechnol*, **2020**, *62*, 1–6.

2. M.H. Abernathy,L. He, Y.J. Tang. *Biotechnol Adv,* **2017**, *35*, 805–814.

TRANSLATION-INDEPENDENT RNA LOCALISATION IN *SYNECHOCYSTIS*

Elisabeth Lichtenberg*[,a], Dominik Rabsch[b], Ute A. Hoffmann[c] and Annegret Wilde[a]

[a] *University of Freiburg, Institut of Biology III, Schänzlestr. 1, D-79104 Freiburg, Germany*

[b] *University of Freiburg, Institut of Informatics, Georges-Koehler-Allee, building 106, D-79110 Freiburg, Germany*

[c] *School of Engineering Sciences in Chemistry, Biotechnology and Health, Science for Life Laboratory, KTH – Royal Institute of Technology, Tomtebodavägen 23B, SE-17165 Solna, Sweden*

** e-mail: Elisabeth.Lichtenberg@biologie.uni-freiburg.de*

RNAs are known to localize heterogeneously throughout eukaryotic cells. Here, different mechanisms involved in RNA-trafficking were discovered over the past few years. Special cis-acting sequential motifs determine the final RNA localization and further provide binding sites for RNA-binding proteins, necessary for RNA-transport. Although those processes were thought to solely occur in eukaryotes, recent evidence suggest that translation-independent, directed RNA-trafficking could be important for local regulation of gene expression in prokaryotes as well[1]. We aim to get a better understanding of translation-independent RNA organization using two independent experimental approaches. The RNA-sequencing technique "RNA localisation-sequencing (Rloc-seq)" combining cell fractionation and RNA-sequencing and the visualization technique "fluorescence in situ hybridization (FISH)" combined with high-resolution microscopy[2,3]. Using Rloc-seq we further intent to identify different sequential or structural motifs responsible for directed RNA-transport. Current results show, that different transcripts encoding proteins involved in the photosynthesis accumulate at the thylakoid membrane in a translation-independent manner. However, not only the process of RNA-transportation plays an important role determining the fate of RNAs. Other players like RNA polymerase, RNA degradasomes and ribosomes could be involved in spatio-temporal RNA organization. Therefore, we try to unravel the fate of mRNAs in Synechocystis sp. PCC 6803 by investigating not only the subcellular RNA organization, but also RNaseE mediated RNA degradation occurring in a 5'- phosphorylation dependent manner[4].

1. Irastortza-Olaziregi, M. & Amster-Choder, O. RNA localization in prokaryotes: Where, when, how, and why. Wiley Interdiscip. Rev. RNA 12, 1–27 (2021).

2. Mahbub, M. et al. mRNA localization, reaction centre biogenesis and thylakoid membrane targeting in cyanobacteria. Nat. Plants 6, 1179–1191 (2020).

3. Kannaiah, S., Livny, J. & Amster-Choder, O. Spatiotemporal Organization of the E. coli Transcriptome: Translation Independence and Engagement in Regulation. Mol. Cell 76, 574-589.e7 (2019).

4. Hoffmann, U. A. et al. Transcriptome-wide in vivo mapping of cleavage sites for the compact cyanobacterial ribonuclease E reveals insights into its function and substrate recognition. Nucleic Acids Res. 49, 13075–13091 (2021).

DIVERSITY AND DISTRIBUTION OF FAR-RED PHOTOACCLIMATION IN DESERT-DWELLING CYANOBACTERIA FROM SABKHA OUM DBA (MOROCCO)

Shujie Wu*[1,2], Laura A. Antonaru[1], Niels-Ulrik Frigaard[3], Michael Kühl[3], Daniel P. Canniffe[4] and Dennis J. Nürnberg[1,2]

[1]*Institute of Experimental Physics, Free University of Berlin, Germany.*

[2]*Dahlem Centre of Plant Sciences, Free University of Berlin, Germany.*

[3]*Department of Biology, University of Copenhagen, Denmark.*

[4]*Department of Biochemistry and Systems Biology, University of Liverpool, UK.*

** Shujie.wu@fu-berlin.de*

Oxygenic photosynthesis has been considered to be restricted to visible light. However, some cyanobacteria can extend their photosynthetic range into far-red light (FRL) by undergoing a complex and extensive acclimation process known as far-red light photoacclimation (FaRLiP) which leads to the formation of the red-shifted chlorophylls (chl) *d* and *f*, as well as modified photosystems and phycobilisomes. In shaded areas, FRL is usually enriched, and it has been shown that for some habitats such as stromatolites, beach rocks, or caves, FaRLiP cyanobacteria are commonly found within them. Nevertheless, our understanding of the impact of FRL photosynthesis on a global scale is largely unknown. Here, we focus on Sabkha Oum Dba (Morocco), an arid habitat inhabited by photosynthetic soil crusts. We prove that it contains large quantities of chl *f*, suggesting that FRL is a key driver of photosynthesis.

By isolating several new FaRLiP cyanobacteria from this habitat, we were able to characterize their photophysiology and define their position in the phylogenetic tree. Two characteristic spectral features were observed for all FRL-grown isolates: (i) the fluorescence emission spectra were shifted to 730-750 nm and (ii) the reflection spectra showed an additional peak at 708 nm. High-performance liquid chromatography (HPLC) experiments confirmed that all FRL samples contain chl *d* and *f*. The 16S-23S rRNA phylogenetic tree revealed that the majority of the isolates with the capability to perform FaRLiP belong to phylogenetically early-branching cyanobacteria of the order *Phormidesmiales*. These are typically characterized by growing in thin filaments with a diameter of 0.8-1.1 µm. When replicating the original sand environment in the laboratory, the isolates formed 1 mm-thick cyanobacterial crusts, growing about 2 mm below the sand surface. This was similar to the original site. HPLC results confirmed that these crusts contained chl *f*, and the first photosynthetic parameters could be determined by microsensor measurements to estimate the importance of FRL photosynthesis.

THE GLOBAL CONTINUUM OF *MICROCOLEUS* SPECIES REVEALED THROUGH POPULATION GENOMICS

A. Stanojković*, S. Skoupý and P. Dvořák

Faculty of Science, Palacký University Olomouc, Šlechtitelů 27, 78371 Olomouc, Czech Republic

** e-mail: aleksandar.stanojkovicdbe@gmail.com*

Speciation is a continuous and complex process signifying any stage of ecological and genetic divergence within populations[1]. An integral facet of understanding speciation involves quantifying gene flow and identifying barriers that hinder it between populations at various stages of speciation[2]. Here, we explored diversification patterns, species boundaries, and the genetic underpinnings driving the differentiation of cosmopolitan cyanobacterium *Microcoleus vaginatus*. Our dataset included 291 genomes, of which 202 strains and eight herbarium specimens were sequenced specifically for this study. Our findings unveil a global continuum encompassing at least twelve distinct *Microcoleus* species, each characterized by varying levels of gene flow as well as genetic and ecological divergence. The diversification of *Microcoleus* began 29.6 million years ago, and it has been shaped by a complex interplay between selection and genetic, ecological, and geographic factors. Genome scans uncovered that genes associated with stress response and biosynthetic processes might have governed cyanobacterial adaptations, enabling them to thrive in heterogeneous soil systems. This study provides novel insights into the notion of microbial species and argues that the speciation of *Microcoleus* involves divergence over the whole genome through periodic allopatric speciation, ecological speciation, and extensive gene flow.

1. B. J. Shapiro, M. F. Polz, Ordering microbial diversity into ecologically and genetically cohesive units. *Trends in Microbiology*, **2014**, *22*, 235–247.

2. L. M. Bobay, H. Ochman, Biological Species Are Universal across Life's Domains. *Genome Biology and Evolution*, **2017**, 9, 491–501.

THE CYANOBACTERIAL FTSH4 PROTEASE CONTROLS ACCUMULATION OF PROTEIN FACTORS INVOLVED IN THE BIOGENESIS OF PHOTOSYSTEM I

V. Krynická*[,a], P.Koník[a,b], P. Skotnicová[a], S. Gupta[a,b], M. Tichý[a], S. Sharma[a,b], J. Komenda[a], R. Sobotka[a]

[a]Institute of Microbiology of the Czech Academy of Sciences, Centre Algatech, Novohradská 237, Třeboň, 379 01, Czech Republic
[b]Faculty of Science, University of South Bohemia, Branišovská 1645/31a, České Budějovice, 370 05, Czech Republic
** e-mail: krynicka@alga.cz*

Membrane-bound FtsH proteases are universally present in prokaryotes and in the mitochondria and chloroplasts of eukaryotic cells. These metalloproteases are often critical for viability and play both protease and chaperone roles to maintain cellular homeostasis. In contrast to most bacteria bearing a single *ftsH* gene, cyanobacteria typically possess four FtsH proteases (FtsH1-4) forming heteromeric (FtsH1/3 and FtsH2/3) and homomeric (FtsH4) complexes. The functions and substrate repertoire of each complex are however poorly understood.

To identify substrates of the FtsH4 protease complex we established a trapping assay in the cyanobacterium *Synechocystis* PCC 6803 (*Synechocystis*) utilizing a proteolytically inactivated ^{trap}FtsH4-His. Around 40 proteins were specifically enriched in ^{trap}FtsH4 pulldown when compared with the active FtsH4. The list of putative FtsH4 substrates contained Ycf4 and Ycf37 assembly factors of Photosystem I (PSI), its core PsaB subunit and the IsiA chlorophyll-binding protein that associates with PSI during iron stress. We focused on the PSI-related proteins and analysed their degradation by FtsH4 *in vivo* in *Synechocystis* mutants and *in vitro* using purified substrates. The data confirmed that FtsH4 degrades Ycf4, Ycf37, IsiA and also the individual PsaA and PsaB subunits in the unassembled state but not when assembled within the PSI complexes.

SMALL BUT POWERFUL!

THE SMALL PROTEIN NIRP1 CONTROLS THE NITROGEN ASSIMILATION IN CYANOBACTERIA

Alexander Kraus[a*], Vincent L. H. Reinmuth[a], Philipp Spät[b], Stefan Timm[c], Martin Hagenmann[c], Boris Maček[b]. Marko Böhm[d], Kirstin Gutekunst[d], Wolfgang R. Hess[a]

[a] *Genetics and Experimental Bioinformatics, Institute of Biology 3, University of Freiburg, D-79104 Freiburg, Germany*

[b] *Department of Quantitative Proteomics, Interfaculty Institute for Cell Biology, University of Tübingen, D-72076 Tübingen, Germany*

[c] *Plant Physiology Department, Institute of Biosciences, University of Rostock, D-18059 Rostock, Germany*

[d] *Molecular Plant Physiology and Bioenergetics in Photoautotrophs, Institute of Biology, University of Kassel, D-34132 Kassel, Germany*

** e-mail: alexander.kraus@biologie.uni-freiburg.de*

In shifts from high CO_2 (HC) to low CO_2 (LC) conditions, cyanobacteria secrete nitrite, a toxic intermediate of nitrate assimilation, but the underlying molecular mechanism remains unknown.[1,2] We identified an uncharacterized gene in the cyanobacterium *Synechocystis* sp. PCC 6803, which was previously reported to produce the noncoding RNA ncr1071, to encode the small protein NirP1. Ectopic overexpression of *nirP1* led to a chlorotic phenotype and delayed growth, which was correlated by the excretion of nitrite and severe changes in amino acid pools. Coimmunoprecipitation experiments revealed the nitrite reductase (NiR), a central enzyme in the assimilation of ammonia from nitrate, as the direct regulatory target of NirP1.[3] In natural environments, the excreted nitrite will be utilized by other microorganisms[2]. Because NirP1 is widely conserved in cyanobacteria, it impacts the activities and composition of the surrounding microbiome and plays a crucial role in the coordination of C and N primary metabolism by targeting the activity of one central enzyme.[3] Here we report thioredoxin A as a further candidate interacting with NirP1, likely as a regulator of the NirP1-NiR binding, transmitting the cellular redox status.

1. I. Suzuki, T. Sugiyama, T. Omata, Regulation of nitrite reductase activity under CO2 limitation in the cyanobacterium Synechococcus sp. PCC7942. Plant Physiol. 107, 791–796 (1995).

2. Berube PM, O'Keefe TJ, Rasmussen A, LeMaster T, Chisholm SW. Production and cross-feeding of nitrite within Prochlorococcus populations. mBio (2023); e01236-23

3. A. Kraus, P. Spät, S. Timm, A. Wilson, R. Schumann, M. Hagemann, B. Macek, W. R. Hess, Nitrite secretion by cyanobacteria is controlled by the small protein NirP1. bioRxiv preprint (2023), doi: https://doi.org/10.1101/2023.08.04.552085

POSITIONAL ISOTOPOLOGUE ANALYSIS OF ASPARTATE TO DETERMINE PEPC ACTIVITY IN VIVO

Luisa Wittemeier*[,a], Martin Hagemann[b] and Joachim Kopka[a]

[a] *Max Planck Institute of Molecular Plant Physiology, Am Mühlenberg 1, D-14476 Potsdam-Golm, Germany*

[b] *Department of Plant Physiology, University of Rostock, Albert-Einstein-Straße 3, D-18059 Rostock, Germany*

** e-mail: wittemeier@mpimp-golm.mpg.de*

Photoautotrophic organisms use light energy and inorganic carbon (Ci) to produce organic material. Cyanobacteria fix Ci (CO_2 and HCO_3-) mainly by two important enzymes, ribulose-1,5-bisphosphate carboxylase/oxygenase (RUBISCO), which generates two molecules 3 phosphoglycerate, and phosphoenolpyruvate carboxylase (PEPC), which forms oxaloacetate. While 1,2,3-C of oxaloacetate carbon backbone and respective downstream metabolites (i.e. malate and aspartate) is derived from RUBISCO assimilation, 4-C is specific to PEPC assimilation. Positional isotopologue analysis of aspartate, the major downstream metabolite in *Synechocystis sp*. PCC 6803, allows differentiation between RUBISCO and PEPC assimilation of Ci. Exploring *in source* fragmentation of gas chromatography-electron impact ionization-mass spectrometry (GC-EI-MS) at nominal mass resolution and GC-atmospheric pressure chemical ionization-MS (GC-APCI-MS) at high mass resolution enabled determination of ^{13}C fractional enrichment ($E^{13}C$) in each carbon position of aspartate. Validation was performed by measurements of positional labelled aspartic acid standard mixtures. Combined with dynamic $^{13}CO_2$ labelling of *Synechocystis sp.* PCC 6803 cultures, it was possible to determine PEPC activity *in vivo*. Accurate quantification of aspartate concentration and positional $E^{13}C$ provided molar Ci assimilation rates. In addition, it was possible to reveal the impact of PEPC on total carbon assimilation, i.e. the ratio of Ci fixation by PEPC and RUBISCO.

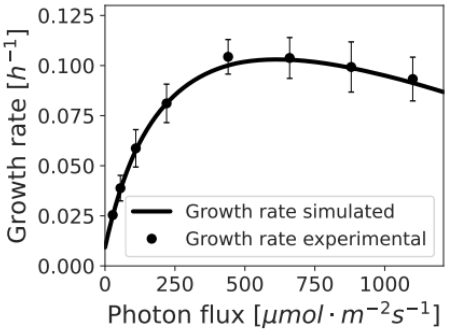
UNDERSTANDING CYANOBACTERIAL PHOTOTROPHIC GROWTH THROUGH GENOME-SCALE MODELING

Rune Höper*[,a], Tomáš Zavřel[b] and Ralf Steuer[a]

[a] *Institute for Theoretical Biology, Humboldt-University of Berlin, Unter den Linden 6, 10099 Berlin, Germany*

[b] *Department of Adaptive Biotechnologies, Global Change Research Institute of the Czech Academy of Sciences, Brno, Czech Republic*

Flux balance analysis (FBA) is a powerful tool for decoding metabolic networks and predicting phenotypic outcomes. Models of cyanobacterial growth, however, remain scarce due to the difficulty of modeling light-harvesting and electron transport chain processes. We present an updated genome-scale metabolic network reconstruction of *Synechocystis* sp. PCC 6803[1] that introduces a novel approach for describing light absorption and utilization. The model effectively predicts quantitative properties of phototrophic growth when fitted to experimental data,[2] including light-dependent O_2 evolution and the ratio of ATP versus NADPH production. The Model also enables calculating metabolic costs of cellular components, aiding simulation of biotechnological production pathways and engineered strains.[3] This work empowers researchers and engineers in cyanobacterial biotechnology, providing insights into the intricate metabolic dynamics of these photosynthetic microorganisms.



1. H. Knoop, & R. Steuer. *Front. Bioeng. Biotechnol.*, 2015, *3*, 47.

2. T. Zavřel, M. Faizi, C. Loureiro, G. Poschmann, K. Stühler, M. Sinetova, A. Zorina, R. Steuer, J. Červený. *eLife*, 2019 *8*:e42508.

3. JS. Rodrigues, L. Kovács, M. Lukeš, R. Höper, R. Steuer, J. Červený, P. Lindberg, T. Zavřel. *Bioresour. Technol.*, 2023, *380,* 129068.

Studying the impact of high manganese stress on the cyanobacterium *Synechocystis sp.* PCC 6803 by transcriptomics

Mara Reis[1], *Prisca Viehöver*[2], *Andrea Bräutigam*[1], *Marion Eisenhut*[1]
[1] *Computational Biology, CeBiTec and Faculty of Biology, Bielefeld University, Germany*
[2] *Genetics and Genomics of Plants, CeBiTec and Faculty of Biology, Bielefeld University, Germany*
mara.reis@uni-bielefeld.de

Photosynthetic organisms rely on sufficient supply of manganese (Mn) to form the oxygen evolving complex (OEC), which is essential for oxygenic photosynthesis. The OEC drives the oxidation of H_2O and thus serves as electron donor for photosystem II. Nevertheless, Mn has detrimental effects if excessively accumulating inside the cell, eventually leading to cell death. Hence, proper homeostasis is crucial to avoid an excess on the one hand, but also ensure sufficient Mn supply on the other hand [1]. To enable tightly controlled homeostasis, several Mn transporters serve proper subcellular Mn allocation in the model cyanobacterium *Synechocystis sp*. PCC 6803. Among them, the thylakoid membrane Mn exporter (Mnx) facilitates Mn export from the cytoplasm into the thylakoid lumen. Accordingly, the Δ*mnx* knock-out mutant is sensitive towards elevated Mn levels in the medium [2]. The reason for Mn toxicity in cyanobacteria is unknown.

In this study, we aimed at unraveling the cause of Mn sensitivity in Δ*mnx.* To this end, we investigated the impact of excess Mn on global gene expression in the WT and Δ*mnx*.

Cells were grown under standard growth conditions (9 µM $MnCl_2$) to an OD_{750} of 0.8 and then split into two cultures. For 24 h, the cells were either further cultivated under standard growth conditions or supplemented with 90 µM $MnCl_2$. Afterwards, RNA was extracted, rRNA depleted, and RNAseq performed using Illumina® NextSeq500 platform with 76 BP SR (single read) at a HighOutput Flowcell. Transcriptomics data were analyzed using EdgeR and the Benjamini Hochberg procedure in RStudio. RNAseq data analysis revealed that the Mn stress transcriptome was very similar to a Fe limitation transcriptome. However, signature genes, such as *isiA/isiB* were not affected by the Mn treatment. Especially genes encoding proteins participating in uptake of metals via the outer membrane were reduced in transcript levels. Furthermore, the Mn stress conditions affected global transcript abundances in the Δ*mnx* mutant much stronger than in the wild type. The Δ*mnx* mutant displayed a reduction of transcripts coding for proteins involved in carbon fixation, ATP generation, and photosynthesis in general.

Our transcriptomics data do not provide clear evidence for the causality of Mn toxicity in cyanobacteria. We hypothesize that the cells downregulate the entrance for Mn into the cell to return to and maintain Mn homeostasis. However, since the outer membrane passage is also used by Fe molecules, this strategy initially induces a transcriptional Fe limitation syndrome. In the long term, this eventually leads to inhibited photosynthesis and energy metabolism and death if the high intracellular Mn load cannot be adjusted, as in the case of the Δ*mnx* mutant.

1. Eisenhut, M. Manganese homeostasis in cyanobacteria. *Plants* **9**, (2020).
2. Brandenburg, F. *et al.* The Synechocystis manganese exporter mnx is essential for manganese homeostasis in cyanobacteria. *Plant Physiol* **173**, 1798–1810 (2017).

CHARACTERIZATION OF THE INDIVIDUAL PSAA AND PSAB ASSEMBLY INTERMEDIATES OF PHOTOSYSTEM I IN SYNECHOCYSTIS 6803

S. Gupta*[a,b], M. Tichy[a], L. Bučinská[a], J. Pilny[a] , and J. Komenda[a]

[a]*Institute of Microbiology, Centre Algatech, Opatovický mlýn, 37901 Třeboň, Czech Republic*
[b]*University of South Bohemia, Branišovská 31, 37005 České Budějovice, Czech Republic*

** e-mail: gupta@alga.cz*

Photosystem I (PSI) is one of the largest multiprotein complexes in photosynthetic membranes of oxygenic phototrophs. The major protein components are two homologous protein subunits PsaA and PsaB. Together these subunits form a heterodimeric type I reaction center (RCI), which is believed to act as a foundation for building very complex protein assembly. Although the high-resolution structure and function of the PSI has been largely elucidated, the molecular details of its earliest assembly phase preceding heterodimerization remain unknown due to the rapid nature of the process. Hence, our study focused on the earliest assembly steps involving the synthesis of individual major subunits PsaA and PsaB, incorporation of pigments into them and association of auxiliary assembly factors, before they form the heterodimer. For this purpose, we individually deleted psaA and psaB genes and the remaining gene for the second large subunit was provided with a N-terminal sequence encoding a FLAG-tag. The isolated tagged PsaA and PsaB subcomplexes were characterized with respect to their spectroscopic properties and pigment-protein composition. We found the FLAG-PsaA predominantly exists as a homodimer which is associated with PsaK protein as the only detectable small PSI subunit. It contains a large number of chlorophyll (Chl) molecules including epimer of Chl a (Chl a'), which is a part of PSI special pair (Chl a'/Chl a heterodimer), and also binds carotenoids. The complex showed a typical red-shifted 77K Chl fluorescence peak like isolated PSI. In contrast, the isolated PsaB does not form a homodimer, does not associate with any PSI small subunits and shows only a small number of associated Chl mostly fluorescing at 670 nm. Apart from previously identified PSI assembly factors, both preparations contained few novel PSI-associated proteins. In conclusion, PsaA and PsaB markedly differ in their ability to homodimerize and bind pigments. As they considered as a result of gene duplication, data suggest that despite their large similarity, PsaA kept properties of primordial homodimeric RCI protein while PsaB significantly diverged during evolution.

A NOVEL CYANOBACTERIAL MEMBRANE PROTEIN INVOLVED IN THE BIOGENESIS OF PHOTOSYSTEM I

Anna Wysocka*[,a,b], Josef Komenda[b] and Roman Sobotka[b]

[a] *Faculty of Science, University of South Bohemia, Branišovská 1760, 370 05 České Budějovice, Czech Republic*
[b] *Centre Algatech, Institute of Microbiology, Czech Academy of Sciences, Novohradská 237, 379 01 Třeboň, Czech Republic*
** e-mail: wysocka@alga.cz*

The core subunits of Photosystem II and Photosystem I (PSII and PSI) are large transmembrane proteins that bind a number of hydrophobic cofactors such as chlorophylls and carotenoids. It is expected that all photosystems core proteins are inserted into the thylakoid membranes by Sec translocon (SecYEG trimer) subjoined with YidC insertase and chlorophyll synthase enzyme[1]. However, which other proteins participate in this process is not known. Using *Synechocystis* sp. PCC 6803 we have recently identified a small transmembrane protein Ssr0332 potentially involved in the biogenesis of PSI. The D*ssr0332* mutant grows slower photoautotrophically in comparison to wild type and possesses significantly lowered level of PSI while the PSII level remains unchanged. To clarify the protein's function we produced a *Synechocystis* strain expressing the FLAG-tagged Ssr0332 and performed a pulldown assay. When compared to the control, the elution was enriched by Sec translocase proteins, several proteins involved in PSI biogenesis and Ndh complex subunits. To determine how the PSI biogenesis is affected in the KO strain we analyzed the transcription of genes encoding PSI core subunits and the synthesis/assembly of PSI complexes by [35]S pulse radiolabeling followed by 2D clear-native/SDS PAGE. Our data show that the biogenesis of PSI subunits is affected at translation and/or early assembly steps and that the Ssr0332 protein may be also involved in chlorophyll delivery to nascent polypeptides of PSI subunits.

1. J. Komenda, R. Sobotka. *Adv Botanical Res*, **2019**, *91*, 195-223.

EXPLORING THE ROLE OF *SYNECHOCYSTIS* CURT PROTEIN IN THE BIOGENESIS OF PHOTOSYNTHETIC APPARATUS

Petra Skotnicová*[,a], Martin Tichý[a], Peter Koník[a,b], Lenka Bučinská[a] and Roman Sobotka[a]

[a]*Institute of Microbiology of the Czech Academy of Sciences, Centre Algatech, Novohradská 237, Třeboň, 379 01, Czech Republic*

[b]*Faculty of Science, University of South Bohemia, Branišovská 1645/31a, České Budějovice, 370 05, Czech Republic*

** e-mail: skotnicova@alga.cz*

Curvature Thylakoids (CurT) proteins have been described in *Arabidopsis thaliana* as proteins essential for the thylakoid architecture by inducing the curvature of the membrane.[1] CurT is an abundant protein in the cyanobacterium *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis*) as its abundance is comparable with the level of Photosystem II (PSII).[2] This important protein was localized to the thylakoid convergence zones with low Chl autofluorescence,[3] and was also detected in transversal membrane sheets.[4] The crucial role of CurT for the *Synechocystis* viability is obvious from the fact that the *curT* deletion mutants were very difficult to fully segregate[4] or did not segregate at all.[1] According to published data,[4] the inactivation of *curT* gene in *Synechocystis* results in a disorganization of the thylakoid membrane system, a 50% reduction of PSII compared to wild-type, and the loss of competence for DNA uptake. In this work we segregated our own Δ*curT* strain, however, even partially segregated cells generate very frequently suppressor mutations. We obtained a collection of Δ*curT* suppressor strains including photoautotrophic variants. We sequenced these strains and identified a number of suppressor mutations. We have also constructed a strain producing Flag-tagged version of CurT. This protein was purified and the obtained pull-downs were analysed by protein mass spectrometry. Altogether our new data indicate that the CurT is specifically located in membrane zones active in the biogenesis of photosynthetic apparatus.

---

1. U. Armbruster, M. Labs, M. Pribil, S. Viola, W. Xu, M. Scharfenberg, A. P. Hertle, U. Rojahn, P.E. Jensen, F. Rappaport, P. Joliot, P. Dörmann, G. Wanner, D. Leister. *Plant Cell*, **2013**, *25*, 2661-2678.

2. P. J. Jackson, A. Hitchcock, A. A. Brindley, M. J. Dickman, C. N. Hunter. *Photosynth. Res.*, **2023**, *155*, 219-245.

3. A. Rast, M. Schaffer, S. Albert, W. Wan, S. Pfeffer, F. Beck, J. M. Plitzko, J. Nickelsen, B. D. Engel. *Nat Plants*, **2019**, *5*, 436-446.

4. S. Heinz, A. Rast, L. Shao, A. Gutu, I. L. Gügel, E. Heyno, M. Labs, B. Rengstl, S. Viola, M. M. Nowaczyk, D. Leister, J. Nickelsen. *Plant Cell*, **2016**, *28(9)*, 2238-2260.

RATIONAL DESIGN OF BIOPHOTOELECTRODES FOR IN VITRO BIOCATALYSIS

Anna Frank*[,a], Panpan Wang[b], Felipe Conzuelo[c], Nadine Strabel[d], Jens Appel[d], Kirstin Gutekunst[d], Wolfgang Schuhmann[b], and Marc M. Nowaczyk[a]

[a] *University of Rostock, Albert-Einstein-Straße 3, 18059 Rostock, Germany*
[b] *Ruhr University Bochum, 44801 Bochum, Germany*
[c] *Institute of Chemical and Biological Technology, 2780-157 Oeiras, Portugal*
[d] *University of Kassel, Heinrich-Plett-Straße 40, 34132 Kassel, Germany*

** e-mail: anna.frank@uni-rostock.de*

The use of the photosynthetic protein complex Photosystem I (PSI) for the fabrication of solar energy conversion devices is a promising strategy due to its natural abundance, stability and high quantum efficiency. A challenge in such devices is to overcome internal short circuiting processes. One approach is oriented immobilization of PSI complexes in so-called Langmuir monolayers. Charge recombination occurring at the gaps between disc-shaped PSI trimers could be successfully minimized by additionally employing smaller PSI monomers, filling the gaps and resulting in increased surface coverage and overall performance[1]. To promote efficient wiring between PSI complexes and the electrode surface, rationally designed redox polymers can be used as an effective tool: They enhance electron transfer and film stability and furthermore enable the deposition of additional enzymes such as oxidoreductases to the electrode, in order to make use of the high energy electrons generated by PSI, as could be shown for a hydrogenase[2] as well as for a PSI-hydrogenase fusion complex[3], resulting in light-driven H_2 production.

1. P. Wang, A. Frank, F. Zhao, J. Szczesny, J.R.C. Junqueira, S. Zacarias, A. Ruff, M. M. Nowaczyk, I. A. C. Pereira, M. Rögner, F. Conzuelo, W. Schuhmann, *Angew Chem Int Ed*, **2020**, *60*, 2000 – 2006.

2. F. Zhao, P. Wang, A. Ruff, V. Hartmann, S. Zacarias, I. A. C. Pereira, M. M. Nowaczyk, M. Rögner, F. Conzuelo, W. Schuhmann, *Energy Environ. Sci.*, **2019**, *12*, 3133 – 3143.

3. P. Wang, A. Frank, J. Appel, M. Boehm, N. Strabel, M. M. Nowaczyk, W. Schuhmann, F. Conzuelo, K. Gutekunst, *Adv. Energy Mater.*, **2023**, *13*, 2203232 (1-9)

NEW MEACHANISMS REGULATING INORGANIC CARBON UPTAKE IN CYANOBACTERIA

Peter R. Walke*[,a], Stephan Klähn[b] and Martin Hagemann[a]

[a] *Pflanzenphysiolgie, Universität Rostock, A.-Einstein-Str. 3, D-18059 Rostock, Germany*
[b] *UFZ, Permoserstr. 15, D-04318 Leipzig, Germany*
** e-mail: peter.walke@uni-rostock.de*

Cyanobacteria concentrate inorganic Carbon (C_i), i.e. CO_2 and HCO_3^-, using different uptake systems. The Δ5 mutant in *Synechocystis* sp. PCC 6803 does not possess any of the transporters[1]. Under ambient air conditions this mutant is not viable. Reintroducing the native sodium-dependent bicarbonate transporter SbtA into the mutant, rescues the lethal phenotype and allows specific examination of SbtA-related regulatory effects.
sbtA is located in an operon with *sbtB*. SbtB is regulating the activity of SbtA dependent on adenylnucleotides and redox[2]. In its regulatory T-loop interacting with SbtA, there are two phosphorylation sites described[3]. Creating site specific mutations, we aim to identify its physiological function.
Upstream of *sbtA*, a novel small protein could be identified: SbtC. Partial co-regulation with *sbtAB* and other C_i-related phenotypes support hypotheses that SbtC is interacting with SbtA, its regulator SbtB and/or transcription factors related to the central carbon metabolism.

1. Xu M, Bernát G, Singh A, et al. Properties of Mutants of Synechocystis sp. Strain PCC 6803 Lacking Inorganic Carbon Sequestration Systems. *Plant Cell Physiol*. 2008;49(11):1672-1677. doi:10.1093/pcp/pcn139
2. Mantovani O, Reimann V, Haffner M, et al. The impact of the cyanobacterial carbon-regulator protein SbtB and of the second messengers cAMP and c-di-AMP on CO_2-dependent gene expression. *New Phytol*. 2022;234(5):1801-1816. doi:10.1111/nph.18094
3. Spät P, Maček B, Forchhammer K. Phosphoproteome of the cyanobacterium Synechocystis sp. PCC 6803 and its dynamics during nitrogen starvation. *Front Microbiol*. 2015;6. doi:10.3389/fmicb.2015.00248

Characterisation of the molecular mechanism of CO_2 capture and sequestration by formation of intracellular amorphous carbonates (iACC) inclusions

Monis Athar. Khan *[a], Soufian. Ouchane [a], Karim. Benzerara [b], Franck. Chauvat [a] and Corinne-Cassier. Chauvat [a]

[a] Université Paris-Saclay, CEA, CNRS, Institute for Integrative Biology of the Cell (I2BC), Gif-sur-Yvette, France,
[b] Sorbonne Université, Muséum National d'Histoire Naturelle, UMR CNRS 7590, Institut de Minéralogie, de Physique des Matériaux et de Cosmochimie (IMPMC), Paris, France
**email: monis-athar.khan@universite-paris-saclay.fr*

Cyanobacteria are photosynthetic organisms that play an important role in the geochemical cycle of our planet (O2 evolution and CO2 capture). They are a phylogenetically diverse phylum that is also involved in biomineralization with the formation of carbonate sedimentary deposits and impact on biogeochemical cycles of calcium (Ca) and carbon (C). This biomineralization was thought to occur only by an extracellular process that is not genetically controlled. However, recently several cyanobacterial species that form intracellular amorphous calcium carbonate (iACC) inclusions in thermodynamically unfavourable intracellular environment were isolated in various environment. (1) The known peculiarities of the iACC forming species are- i) increased Ca sequestration, ii) presence of microcompartment surrounding the inclusions, iii) different pattern of inclusions localization and iv) capacity to also form strontium carbonate. The molecular mechanism(s) involved in the formation of iACC, and their genetic control remain unknown. Using a bioinformatic approach, our collaborators identified a candidate gene named *ccyA*, present in all iACC-forming cyanobacteria and absent in the genome of other cyanobacteria as a marker of the iACC formation. (2) Furthermore, *ccyA* is also interesting in encoding an orphan protein (named calcyanin) harbouring new structural protein domains. The proteins of the calcyanin family consists of a conserved C-terminal domain and a variable N-terminal domain that can be distinguished into four types. The objective of this study is to identify the *in vivo* function of the *ccyA* gene by using a genetic approach. For that purpose, deletion and overexpression of *ccyA* is to be carried out in 2-4 iACC- forming unicellular cyanobacteria, selected on the basis of i) the ability to be manipulated in the laboratory; ii) the scattered position or the localization of the iACC granules (at the middle and/or the poles of the cells); and iii) the amino-acids sequences of the N-variable domain. There has been no report of gene deletion in three of the selected strains: *Synechococcus* sp. PCC 6312 (S6312), *Cyanothece* sp. PCC 7425 (C7425) and *Synechococcus calcipolaris* PCC 11701 (S11701), therefore, different strategies: natural transformation, suicide conjugative plasmid (single and double crossover) and CRISPR-Cas12 are being explored to obtain a deletion mutant. The other strategy is to study the function of *ccyA* by overexpressing it in iACC forming and non-iACC forming cyanobacteria. As caclyanin harbours variable N-ter domain, conjugative expression vector (3)(4) allowing the constitutive expression of *ccyA* from S6312, C7425, S11701 and *Gloeomargarita lithophora* (C7) were constructed and transferred successfully to C7425 and non-iACC forming cyanobacteria, *Synechococcus elongatus* PCC 7942 (S7942). We showed that overexpression of *ccyA* from S6312 and C7 in S7942 resulted in observation of Ca hotspots in these mutants hinting towards the involvement of *ccyA* with Ca and thus iACC formation. (2) We now have successfully constructed overexpression mutants of *ccyA* in C7425 (iACC forming). Preliminary growth experiments in varying Ca concentrations of C7425 mutants showed that the fitness of C7425 mutants is different when compared to the wild-type. Whether these differences are correlated to changes in Ca concentration and formation of iACC is being explored by various techniques such as SEM, STXM, XRF and ICP-MS.

---

1) K. Benzerara, F. Skouri-Panet, J. Li, C. Férard, M.Gugger, T. Laurent, E. Couradeau, M. Ragon, J. Cosmidis, N. Menguy, I. Margaret-Oliver, R. Tavera, P. López-García, D. Moreira. *Proc Natl Acad Sci USA*, **2014**, 29;111(30):10933-2) K. Benzerara, E.Duprat, T. Bitard-Feildel, G. Caumes, C. Cassier-Chauvat, F. Chauvat, M. Dezi, SI. Diop, G. Gaschignard, S. Görgen, M. Gugger, P. López-García, M. Millet, F. Skouri-Panet, D. Moreira, I. Callebaut. *Genome Biol Evol*, **2022**, 2;14(3).
3) T. Veaudor, M. Ortega-Ramos, T. Jittawuttipoka, H. Bottin, C. Cassier-Chauvat, F. Chauvat. *PLoS One,* **2018**, 7;13(6).
4) C. Chenebault, E. Diaz-Santos, X. Kammerscheit, S. Görgen, C. Ilioaia, S. Streckaite, A. Gall, B. Robert, E. Marcon, DA. Buisson, K. Benzerara, JF. Sassi, C. Cassier-Chauvat, F. Chauvat. *Front Microbiolgy,* **2020**, 18;11:586601.

CYANOBACTERIAL TOXINS AS MODULATORS OF CARBON FIXATION

Arthur Guljamow[1], Tino Barchewitz[1], Rebecca Große[1], Stefan Timm[2], Martin Hagemann[2], Elke Dittmann[1]

[1]Department of Microbiology, Institute for Biochemistry and Biology, University of Potsdam, 14476 Potsdam-Golm, Germany.

[2]Department of Plant Physiology, Institute of Biological Sciences, University of Rostock, 18059 Rostock, Germany.

email: guljamow@uni-potsdam.de

The freshwater cyanobacterium *Microcystis* frequently forms dense “blooms” which can accumulate the hepatotoxic peptide microcystin. This compound plays an extracellular role as an infochemical but also acts intracellularly by binding to proteins of the carbon metabolism, most notably to RubisCO. Diel fluctuations of extracellular microcystin levels directly correlate with the binding of the peptide to proteins inside the cell. Concomitantly, RubisCO reversibly relocates from the cytoplasm to the cell's periphery. In connection with this we found indications that a substantial fraction of RubisCO can be found outside of carboxysomes, at least temporarily. We replicated these effects by adding microcystin externally to cultures grown under continuous light. Interestingly, the activity of RubisCO isolated from the microcystin-free mutant depends on the cultivation light regime, whereas wild type Rubisco activity remains constant. We propose that microcystin acts as a modulator of carbon fixation and may be part of a fast response to conditions of high light and low carbon that contributes to the metabolic flexibility and the success of Microcystis in the field.

Unravelling the physiological function of the Entner-Doudoroff pathway in *Synechocystis* sp. PCC 6803 by enzyme characterization

Ravi Ojha[1], Christopher Bräsen[1], Kirstin Gutekunst[2], Bettina Siebers[1]

[1]Molecular Enzyme Technology and Biochemistry, University of Duisburg-Essen, 45141 Essen, Germany, [2]Department of Molecular Plant Physiology, Bioenergetics in Photoautotrophs, University of Kassel, Germany

Email: ravi.ojha@uni-due.de

Introduction

Although the Entner-Doudoroff (ED) pathway has been demonstrated as one of four glycolytic routes in the cyanobacterial model organism *Synechocystis* sp. PCC 6803 its physiological function remains to be elucidated[1]. Deletion of the *eda* gene, encoding ED aldolase (EDA), revealed significance of the ED pathway for glycogen utilization in the light, resuscitation from nitrogen starvation and high to low CO_2 shift[2,3,4]. However, ^{13}C flux analysis of photomixotrophically grown cells revealed no flux via the ED pathway and an additional unknown function of EDA was suggested[5].

Objective

This study aims to further decipher the role of the ED pathway and its key enzyme EDA.

Materials and Methods

The codon optimized gene encoding a His-tagged version of the *Synechocystis* EDA (*sll0107*) was heterologously expressed in *E. coli*. The enzyme was purified and biochemically characterized with respect to substrate specificity, kinetic and regulatory properties.

Results

EDA catalyzes the cleavage of 2-keto 3-deoxy 6-phosphogluconate with a high catalytic efficiency of 0.026 $sec^{-1}mM^{-1}$ (V_{max} of 9 U/mg, K_m of 0.15 mM). In contrast, 4-hydroxy 2-oxoglutarate was only converted with a low catalytic efficiency of 0.0000120 $sec^{-1}mM^{-1}$ (V_{max} of 0.95 U/mg, K_m of 2.8 mM). Moreover, effector studies revealed that 6-phosphogluconate (6PG) inhibits EDA activity with a K_i of 1.6 mM.

Conclusion

Our findings confirm that *sll0107* possesses EDA activity. The regulation by 6PG points towards a more sophisticated regulation of the ED pathway in interaction with the complex carbon network that enables adaptation to metabolic and energetic needs in a changing environment.

1. Chen X, Schreiber K, Apple J *et al*., PNAS 2016; 113:5441-5446
2. Makowka A, Nichelmann L, Schulze D *et al*., Mol. Plant 2020; 13(3):471-482
3. Doello S, Klotz A, Makowka A. *et al*., Plant Physiol. 2018; 177(2):594-603
4. Lucius S, Makowka A, Michl K *et al*., Frontier Plant Sci. 2021; 12:787943
5. Schulze D, Kohlstedt M, Becher J *et al*., Microb Cell Fact. 2022; 21:69

SbtB: A substrate of FtsH4

S. Sharma, V. Krynicka

Centre Algatech, Institute of Microbiology, Czech Academy of Sciences, 37901 Třeboň,

Czech Republic

email:sharma@alga.cz

Membrane-bound FtsH proteases are universally present in prokaryotes and in the mitochondria and chloroplasts of eukaryotic cells. These metalloproteases are often critical for the viability of the cell and play both protease and chaperone roles to maintain cellular homeostasis. In contrast to most bacteria having a single *ftsH* gene, cyanobacteria typically possess four FtsH proteases (FtsH1-4) that form heteromeric (FtsH1/3 and FtsH2/3) and homomeric (FtsH4) complexes. The function and substrate repertoire of each complex is poorly understood. Recent data showed that FtsH4 plays a role in stress acclimation by regulating high light inducible proteins. To identify new substrates of the FtsH4 protease complex, we established a trapping assay in the cyanobacterium *Synechocystis* PCC 6803 utilizing a proteolytically inactivated ^{trap}FtsH4-His. Around 40 proteins were specifically enriched in ^{trap}FtsH4 pulldown when compared with the active FtsH4. The list of putative FtsH4 substrates contained seven proteins involved in carbon concentrating mechanism (CCM) in *Synechosystis* such as SbtA, SbtB, and CupA. Interestingly, SbtB and Fvl proteins were, unlike the other CCM putative substrates, copurified exclusively with the ^{trap}FtsH4-His but not with the active FtsH4 form. SbtB is a noncanonical member of the P_{II} signal transduction superfamily, which is involved in C_i acclimation by signalling the cellular carbon status via cAMP. Our work demonstrates that overexpression of FtsH4 reduces the levels of SbtB which results in sensitivity of the mutant to carbon depletion. On the contrary, deletion of FtsH4 leads to accumulation of SbtB and its sensitivity elevates the levels of inorganic carbon in *Synechosystis*. *In vivo*, proteolytic assay proved the role of FtsH4 in SbtB degradation. Therefore, we suggest the SbtB as a new substrate of FtsH4. These results, together with the fact that the FtsH4-His is copurified with Slr0374, another protein important for CO_2 uptake, suggests that the FtsH4 plays an important role in the acquisition of inorganic carbon.

- V. Krynická, M. Tichý, J. Krafl, J. Yu, R. Kaňa, M. Boehm, P. Nixon, J. Komenda. *J. Mol. Biol.* **2014**, *94(3)*, 609-24.
- V. Krynická, J. Georg, P. Jackson, M. Dickman, C. Hunter, M. Futschik, W. Hess, J. Komenda. *The Plant Cell*. **2019**, *31(12)*, 2912-28.
- V. Krynická, P. Skotnicová, P. Jackson, S. Barnett, J. Yu, A. Wysocka, R. Kaňa, M. Dickman, P. Nixon, C. Hunter, J. Komenda. *Plant Commun*. **2023,** *4(1).*
- K. Selim, F. Haase, M. Hartmann, M. Hagemann, K. Forchhammer. *PNAS*. **2018**, *115(21)*, E4861-9.

Structure and activation of *Syn*DLP, the fusogenic dynamin-like protein of the cyanobacterium *Synechocystis* sp. PCC 6803

L. Gewehr[a], L. Mernberger[a,*], R. Jilly[a], B. Junglas[c,d], C. Sachse[c,d], R. Heermann[b] and D. Schneider[a,b]

[a] *Department of Chemistry, Biochemistry*

[b] *Institute of Molecular Physiology, Johannes Gutenberg University Mainz*

[c] *Ernst-Ruska Centre for Microscopy and Spectroscopy with Electrons, ER-C-3/Structural Biology*

[d] *JuStruct: Jülich Center for Structural Biology, Forschungszentrum Jülich, Germany*

*E-Mail: mernberger@uni-mainz.de

The dynamin-like proteins (DLPs) are a superfamily of large GTPases (>60 kDa) that utilize the energy of GTP hydrolysis to remodel membranes1. Their functions include remodeling events, such as membrane fusion, membrane fission and cell division. Unlike many eukaryotic DLPs, only few bacterial DLPs (BDLPs) have been identified and characterized thus far2. Regarding the poorly understood but highly dynamic thylakoid membrane (TM) system in cyanobacteria, biogenesis and dynamics of this internal membrane structure presumably involve proteins such as BDLPs.

SynDLP is a dynamin like protein encoded in the cyanobacterium Synechocystis sp. PCC 6803. Isolated SynDLP forms oligomeric structures in solution in absence of lipids and/or nucleotides, features not observed with other BDLPs. SynDLP interacts with negatively charged TM lipids, albeit the binding affinity toward DOPG containing liposomes is not affected upon addition of nucleotide. SynDLP is capable of liposome fusion in vitro, while this membrane fusion activity is also nucleotide independent. Consequently, membrane remodeling by SynDLP appears not to directly require the energy released by GTP hydrolysis.

The structural elucidation by cryo-EM revealed a typical dynamin-like fold of the protein as well as the formation of higher oligomers via defined interfaces in the stalk domain (SD). In addition to that, the existence of an intramolecular disulfide bond in the bundle signaling element (BSE) domain and an expanded intermolecular GTPase domain (GD)-BSE interface could be shown. SynDLP shows a relatively high basal GTPase activity, albeit established GD-activating contacts cannot be observed in the structure. Interestingly, the longitudinal GD-BSE interface as well as the intramolecular disulfide bond in the BSE affect the GTPase activity of the protein, thus indicating a novel concept of GD activation in a DLP3.

In summary, the cyanobacterium Synechocystis sp. PCC 6803 expresses a previously uncharacterized, novel BDLP. The structure of the oligomeric protein revealed new regulative DLP features, such as an intramolecular disulfide bond in the BSE and an expanded BSE-GD interface. SynDLP interacts with TM lipids and fuses TM-mimicking membranes, although the function of GTP hydrolysis remains unclear. Thus, SynDLP is a novel member of the DLP group of fusion DLPs.

1. Daumke, O. & Praefcke, G. J. K. Invited review: Mechanisms of GTP hydrolysis and conformational transitions in the dynamin superfamily. *Biopolymers* vol. 105 580–593 Preprint at https://doi.org/10.1002/bip.22855 (2016).

2. Guo, L., Satler, L., Shafqat, S., Graumann, P. L. & Bramkamp, M. A Bacterial Dynamin-Like Protein Confers a Novel Phage Resistance Strategy on the Population Level in Bacillus subtilis. *mBio* **13**, (2022).

3. Gewehr, L. *et al.* SynDLP is a dynamin-like protein of Synechocystis sp. PCC 6803 with eukaryotic features. *Nature Communications 2023 14:1* **14**, 1–17 (2023).

Exploring the Role of the Auxiliary Metabolic Gene *petE* from the Cyanophage Syn9 During Infection

Nicola Sekularac*[,a], Miguel Fernández[a], Ran Tahan[b], Debbie Lindell[b], Michelle M. Gehringer[a], Eugenio Pérez Patallo[a], Susanne Zehner[a], Nicole Frankenberg-Dinkel[a]

[a] *Faculty of Biology, University of Kaiserslautern-Landau, Germany;*
[b] *Faculty of Biology, Technion-Israel Institute of Technology, Haifa, Israel.*

** e-mail: sekulara@rptu.de*

During phage infection, cyanobacteria undergo significant changes in host metabolic pathways such as photosynthesis, carbon-, and fatty acid- metabolism. These changes are supposed to be induced by so called auxiliary metabolic genes (AMGs) encoded in the phage genome. These host-like genes are thought to improve the host metabolism during the infection cycle. AMGs are frequently found in cyanophage genomes.[1,2] We are investigating the role of a particular AMG, the plastocyanin-like gene *petE* from the cyanophage Syn9. Plastocyanins are small blue copper proteins, that transfer electrons during photosynthesis from the b_6f complex to photosystem I and are common to most cyanobacteria, algae, and plants.[3] To verify the functionality of the phage gene, *Syn9petE* was heterologously expressed in *E. coli*. The purified recombinant protein displayed the blue color typical for plastocyanins and an absorption maximum at ~600 nm. The protein can shift between different redox states. Furthermore, EPR spectroscopy confirmed its identity as a type I copper-binding protein. In parallel, a recombinant Syn9 phage lacking a functional *petE* was generated. Recombinant phage particles were first enriched and subsequently purified. Recombinant phage lysates will be assessed for their efficiency in infection and viral particle production. In addition, the photosynthetic efficiency of infected host cells will be analyzed. In summary, our objective is to elucidate the role of the cyanophage-encoded plastocyanin and understand its impact on the interaction with the host.

_____

1. L.R. Thompson, Q. Zeng, L. Kelly, K.H. Huang, A.U. Singer, J. Stubbe, S.W. Chisholm. *Proc Natl Acad Sci U S A*., **2011**, 108(39), E757-64.

2. D. Lindell, J.D. Jaffe, Z.I. Johnson, G.M. Church, S.W. Chisholm. *Nature*, **2005**, 438(7064), 86-89.

3. E.L. Gross. *Photosynthesis Research*, **1993**, 37(2), 103-116.

FORMATION OF IM30 PUNCTAE REQUIRES A STRUCTURED CORE DOMAIN

Ndjali Quarta*[,a,b] and Dirk Schneider[b,c]

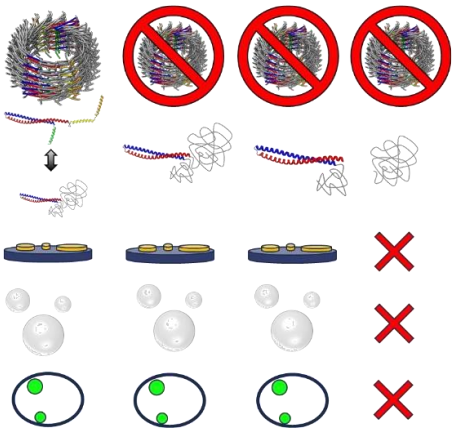
[a] *Johannes Gutenberg University, Mainz, Germany*

[b] *Max Planck Graduate Center, Mainz, Germany*

[c] *Institute of Molecular Physiology, Mainz, Germany*

** e-mail: ndquarta@uni-mainz.de*

Cyanobacteria and chloroplasts require the inner membrane-associated protein of 30 kDa (IM30), also known as Vipp1, for the biogenesis and maintenance of their thylakoid membranes.[1] IM30 forms large ring structures in solution,[2] which disassemble on membranes where they form carpet structures.[3] Ring disassembly involves partial unfolding of the IM30 monomers, and about half of the protein becomes intrinsically disordered. Partially unfolded IM30 forms condensates in solution under physiologically relevant conditions. *In vivo* IM30 is known to form *punctae*, yet little is known about the driving forces.[4] We used atomic force, DIC and fluorescence microscopy to show that formation of IM30 assemblies, both *in vitro* (carpet structures and condensates) and *in vivo* (*punctae*), requires a structured core. This suggests that the *in vivo* formation of IM30 *punctae* is related to the formation of IM30 condensates.



PDB: 7O3Y

1. D. Kroll, K. Meierhoff, N. Bechtold, M. Kinoshita, S. Westphal, U. C. Vothknecht, J. Soll, P. Westhoff. *Proceedings of the National Academy of Sciences*, **2001**, *98*, 4238-4242.

2. T. K. Gupta, S. Klumpe, K. Gries, S. Heinz, W. Wietrzynski, N. Ohnishi, J. Niemeyer, B. Spaniol, M. Schaffer, A. Rast, M. Ostermeier, M. Strauss, J. M. Plitzko, W. Baumeister, T. Rudack, W. Sakamoto, J. Nickelsen, J. M. Schuller, M. Schroda, B. D. Engel. *Cell*, **2021**, *184*, 3643-3659.

3. B. Junglas, R. Orru, A. Axt, C. Siebenaller, W. Steinchen, J. Heidrich, U. A. Hellmich, N. Hellmann, E. Wolf, S. A. L. Weber, D. Schneider. *Communications Biology*, **2020**, *3*, 595.

4. A. Gutu, F. Chang, E. K. O'Shea. *Molecular Microbiology*, **2018**, *108*, 16-31.

NITRITE SECRETION BY CYANOBACTERIA IS CONTROLLED BY THE SMALL PROTEIN NIRP1

Alexander Kraus[a*], Vincent L. H. Reinmuth[a], Philipp Spät[b], Stefan Timm[c], Martin Hagenmann[c], Boris Maček[b]. Marko Böhm[d], Kirstin Gutekunst[d], Wolfgang R. Hess[a]

[a] *Genetics and Experimental Bioinformatics, Institute of Biology 3, University of Freiburg, D-79104 Freiburg, Germany*

[b] *Department of Quantitative Proteomics, Interfaculty Institute for Cell Biology, University of Tübingen, D-72076 Tübingen, Germany*

[c] *Plant Physiology Department, Institute of Biosciences, University of Rostock, D-18059 Rostock, Germany*

[d] *Molecular Plant Physiology and Bioenergetics in Photoautotrophs, Institute of Biology, University of Kassel, D-34132 Kassel, Germany*

** e-mail: alexander.kraus@biologie.uni-freiburg.de*

In shifts from high CO_2 (HC) to low CO_2 (LC) conditions, cyanobacteria secrete nitrite, a toxic intermediate of nitrate assimilation, but the underlying molecular mechanism remains unknown.[1,2] We identified an uncharacterized gene in the cyanobacterium *Synechocystis* sp. PCC 6803, which was previously reported to produce the noncoding RNA ncr1071, to encode the small protein NirP1. Ectopic overexpression of *nirP1* led to a chlorotic phenotype and delayed growth, which was correlated by the excretion of nitrite and severe changes in amino acid pools. Coimmunoprecipitation experiments revealed the nitrite reductase (NiR), a central enzyme in the assimilation of ammonia from nitrate, as the direct regulatory target of NirP1.[3] In natural environments, the excreted nitrite will be utilized by other microorganisms[2]. Because NirP1 is widely conserved in cyanobacteria, it impacts the activities and composition of the surrounding microbiome and plays a crucial role in the coordination of C and N primary metabolism by targeting the activity of one central enzyme.[3] Here we report thioredoxin A as a further candidate interacting with NirP1, likely as a regulator of the NirP1-NiR binding, transmitting the cellular redox status.

1. I. Suzuki, T. Sugiyama, T. Omata, Regulation of nitrite reductase activity under CO2 limitation in the cyanobacterium Synechococcus sp. PCC7942. Plant Physiol. 107, 791–796 (1995).

2. Berube PM, O'Keefe TJ, Rasmussen A, LeMaster T, Chisholm SW. Production and cross-feeding of nitrite within Prochlorococcus populations. mBio (2023); e01236-23

3. A. Kraus, P. Spät, S. Timm, A. Wilson, R. Schumann, M. Hagemann, B. Macek, W. R. Hess, Nitrite secretion by cyanobacteria is controlled by the small protein NirP1. bioRxiv preprint (2023), doi: https://doi.org/10.1101/2023.08.04.552085

THE REGULATION OF THE ACTIVITY OF FERREDOXIN-NITRITE REDUCTASE BY THE FERREDOXIN-NITRITE REDUCTASE REGULATOR PROTEIN 1 AND THIOREDOXIN A IN *SYNECHOCYSTIS* SP. PCC 6803

V. Reinmuth*[,a], A. Kraus[a], P. Spät[b], M. Böhm[c], K. Gutekunst[c], B. Macek[b] and W. R. Hess[a]

[a] *Institut für Biologie III, Albert-Ludwigs-Universität Freiburg, D-79104 Freiburg, Germany*

[b] *Department of Quantitative Proteomics, Interfaculty Institute for Cell Biology, University of Tübingen, D-72076 Tübingen, Germany*

[c] *Molekulare Pflanzenphysiologie, Bioenergetik in Photoautotrophen, University of Kassel, D-34132 Kassel*

** e-mail: v.reinmuth@students.uni-freiburg.de*

Cyanobacteria assimilate nitrate (NO_3^-) via reduction to nitrite (NO_2^-) and subsequently to ammonium (NH_4^+). In the cyanobacterium *Synechocystis* sp. PCC 6803 (*Synechocystis*) the second reaction is catalyzed by the ferredoxin-nitrite reductase (NiR).[1] The previously discovered small protein ferredoxin-nitrite reductase regulator protein 1 (NirP1) has been shown to regulate the nitrogen assimilation in *Synechocystis*. Ectopic overexpression of NirP1 led to a slower growth with a chlorotic phenotype and excretion of NO_2^-. In pull-down experiments using NirP1-3xFlag as bait protein, NiR and the electron-donor Thioredoxin A (TrxA) were the most enriched proteins.[2] With data from protein structure models it was hypothesized that NirP1 acts as an inhibitor of NiR, while TrxA regulates the activity of NirP1.[2] The exact mode of this regulation as well as how TrxA affects the NirP1-NiR-complex was unknown. The results of this work show that TrxA binds to NirP1, possibly releasing and reactivating inhibited NiR in a mechanism seemingly controlled by the redox state of the cell.

---

1. E. Flores, A. Herrero. *Biochem. Soc. Trans.*, **2005**, *33*, 164-167

2. A. Kraus et al., **2023** (preprint: https://www.biorxiv.org/content/10.1101/2023.08.04.552085v1)

MEMBRANE REMODELING AND CURVATURE INDUCTION BY A SMALL TRANSMEMBRANE PROTEIN

Pia Briger*[,a] and Dirk Schneider[a]

[a]*Department of Chemistry, Biochemistry, Johannes Gutenberg University Mainz*

**e-mail: pbriger@uni-mainz.de*

The curvature thylakoid protein (*Syn*CurT), a homolog of the *Arabidopsis thaliana* Curt1 protein, significantly affects the structure and organization of thylakoid membranes in the cyanobacterium *Synechocystis*[1]. The protein is predominantly localized in thylakoid membrane margin regions, where it induces/stabilizes membrane curvature. Consequently, deletion of *Syn*CurT results in a severely altered thylakoid membrane organization.

The predicted *Syn*CurT structure includes two transmembrane helices connected by a short loop, an amphipathic helix at the N-terminus and an additional helix at the C-terminus. Membrane adhesion of amphipathic helices as well as wedge shaped transmembrane regions are known to induce curvature. The primary objective is to identify specific protein constituents responsible for inducing curvature in thylakoid membranes and to determine the significance of protein oligomerization for *Syn*CurT-induced membrane bending. We successfully expressed and purified truncated *Syn*CurT variants, as well as the full-length protein in absence as well as presence of fluorescent protein tags. We use the isolated proteins to investigate the impact of different protein regions on induction of membrane curvature and protein oligomerization via measuring Förster resonance energy transfer (FRET) as well as electron microscopy of proteoliposomes and engineered *Synechocystis* cells.

_____

1. U. Armbruster, M. Labs, M. Pribil et al. *Plant Cell*, **2013**, 25, 2661-2678.

THYLAKOID TARGETING IMPROVES EXPRESSION AND STABILITY OF A PLANT CYTOCHROME P450 IN THE CYANOBACTERIUM *SYNECHOCYSTIS* SP. PCC 6803

Sayali S. Hanamghar[a], Lisbeth Mikkelsen[b], Silas B. Mellor[b], Christoph Crocoll[b], David A. Russo[d], Poul Erik Jensen[c], and Julie A. Z. Zedler[a]

[a] *Synthetic Biology of Photosynthetic Organisms, Matthias Schleiden Institute, Friedrich Schiller University Jena, Germany*

[b] *Section for Molecular Plant Biology, Department of Plant and Environmental Sciences, University of Copenhagen, Frederiksberg, Denmark*

[c] *Section for Food Analytics and Biotechnology, Department of Food Science, University of Copenhagen, Frederiksberg, Denmark*

[d] *Bioorganic Analytics, Institute for Inorganic and Analytical Chemistry, Friedrich Schiller University Jena,Germany*

** e-mail: sayali.hanamghar@uni-jena.de*

Plants produce a large array of natural products of interest for pharmaceuticals, nutraceuticals, food colorants and flavoring agents. Usually, these compounds are natively produced at low titres and involve complex biosynthetic pathways which often include cytochrome P450s (P450s). P450s are known to be difficult to express in traditional heterotrophic chassis. However, cyanobacteria have shown promise as a sustainable alternative for heterologous expression of plant P450s and light-driven product biosynthesis[1]. In this study, we explore strategies for improving plant P450 stability and membrane insertion in cyanobacteria. We have chosen as our model system the cyanobacterial model organism *Synechocystis* sp. PCC 6803 and the well-studied P450 CYP79A1 from the dhurrin pathway of *Sorghum bicolor*. Combinations of the P450 fused with different elements of PetC1 were tested. PetC1 is the major Rieske protein in the cytochrome b_6f complex and localizes to the thylakoid membrane. All tested CYP79A1 variants led to oxime production. Interestingly, the relative oxime levels of variants using elements of PetC1 resulted in up to 20 to 25 times higher oxime levels compared to the unmodified CYP79A1.These findings are promising to improve heterologous P450 expression in cyanobacteria and can ultimately contribute to advancing recombinant plant natural product biosynthesis in cyanobacterial chassis.

———————

[1]Wlodarczyk et al., *Metab Eng, 2016, 33*, 1–11.

THE ROLE OF THE MCE (MAMMALIAN CELL ENTRY) PROTEIN IN BIOGENESIS OF THE THYLAKOID MEMBRANE OF *SYNECHOCOCCUS ELONGATUS PCC 7942*

Xiaowei Dong*[,a], Daniel Walker[a], Vidya Darbari[a] and Conrad Mullineaux[a]

[a] *School of Biological and Behavioural Sciences, Queen Mary University of London, E1 4LS London, UK*

** e-mail: x.dong@qmul.ac.uk*

Cyanobacteria have a specialized internal membrane called thylakoids to host the photosynthetic machinery. Photosynthesis in thylakoid membranes is affected by the type and integrity of lipids on the membrane. MCE proteins are conserved lipid-binding proteins in double-membrane bacteria and eukaryotic chloroplasts. In *E. coli*, an MCE transport system called Mla has been implicated in phospholipid trafficking and outer membrane integrity. A similar complex TGD has been found in the *Arabidopsis thaliana* chloroplast, which was suggested to transport phosphatidic acid from the endoplasmic reticulum to the chloroplasts to synthesize galactolipids. MCE domain-containing proteins are also found in cyanobacteria, but their functions are unknown.

In *Synechococcus elongatus PCC 7942*, a single protein Syf0351 has a conserved MCE domain. An MCE mutant was generated, but the fully segregated knockout cells could not be achieved, which indicates its essential role in *Synechococcus*. As the MCE protein could not be knocked out, a conditional expression mutant was generated. The phenotype differences will be studied in high and low expression levels of the MCE protein. The lipidomic analysis of the thylakoid membrane will be carried on in different MCE expression levels to explore if it engages in lipid transport. In addition, an unknown but essential question is where the MCE protein is located, thylakoid or plasma membrane? Since the native MCE has a low expression level and the high expression level might change the actual localisation, the expression level of the MCE protein will be adjusted to the phenotype that is the same as the wild type. Then the localisation will be determined by membrane fractionation and western blot.

Iterative analysis of metabolic modulation in the cyanobacterium *Aphanizomenon flos-aquae* 2012 KM1/D3 upon nitric oxide synthase derived NO induction

Neha Gupta, Arun Kumar Mishra*
Laboratory of Microbial Genetics, Department of Botany, Institute of Science,
Banaras Hindu University, Varanasi- 221005
Email: neha.bhu65@gmail.com
akmishraau@hotmail.com

Nitric oxide synthase (NOS) in mammals is recognized for its essentialities in several metabolism including blood vascular relaxation and nerves transmission etc. yet their functionalities in prokaryotes is lesser appreciated. Pioneering studies unveiled the existence of NOS homologs not only in bacteria but also in photosynthetic organism, exhibiting the essentiality of NOS in these life forms. Besides, NOS seems to have global functions that differ from their mammalian counterparts. Therefore, in our study, the distribution, diversity, abundance and phylogeny of NOSs among cyanobacteria were evaluated. Further, physiological and biochemical importance of NOS derived NO production was investigated in *Aphanizomenon flos-aquae* 2012/KM1/D3 and influence of NOS inhibition on cellular physiology, metabolic activities and DNA integrity were examined in cyanobacterium. The accumulation of NO was dramatically reduced upon NOS inhibitor L-N^G-Nitro arginine methyl ester (L-NAME) supplementation, exhibiting significant NO synthesis by NOS, whereas addition of L-arginine increase NOS derived NO in a dose dependent manner. Moreover, the reduction in the growth and metabolic activities of the cyanobacterium were evident upon L-NAME treatment that possibly pertained to decline in photopigments, PSII efficiency, impaired membrane fluidity, cellular integrity, protein oxidation and DNA damage due to oxidative burst which culminated into cell death. Further, L-arginine supplementation maintained Asc/DHAsc and GSH/GSSG ratio, conferred redox homeostasis. The present study provided considerable evidences to support the vitality of NOS derived NO production in neutralizing the oxidative burst by influencing antioxidative system, thereby maintain stable cyanobacterial growth. These finding strengthens the foundation to access biological significance of this enzyme in relation to stress tolerance and physiology of cyanobacteria.

LIBERATION OF MINERAL-BOUND IRON BY MARINE CYANOBACTERIA *SYNECHOCOCCUS* SP. PCC 7002 USING SIDEROPHORES

Sean P. Hegan*, Sofia Conrad, Nicole Frankenberg-Dinkel and Michelle Gehringer

[a] *Department of Microbiology, University of Kaiserslautern-Landau, Paul-Ehrlich-Straße Bld. 23, 67663 Kaiserslautern, Germany*

** e-mail: hegan@rptu.de*

Marine cyanobacteria are an important primary producer for marine environments and require iron (Fe) as a cofactor in the production of photosynthetically essential chromophores such as Chlorophyll α and phycobilins, limiting their synthesis under iron limiting conditions[4,5]. One method cyanobacteria use to make insoluble Fe(III) bioavailable is using siderophores which chelate iron species, allowing uptake through TonB-dependent transporters[1,2,4]. Here we investigate the ability of wild type *Synechococcus* sp. PCC 7002 siderophores, synechobactins, to reduce Fe(III) from basalt under iron depleted conditions. Levels of total iron in the medium were tracked using Ferrozine assay, while the Chrome Azurol S (CAS) assay was used to monitor levels of siderophores[1,3]. Experiments were buffered to maintain the culture medium at a pH of 8 to prevent the chemically induced weathering of basalt to clay and eliminate shading effects. Addition of basalt to iron depleted cultures restored chlorophyll α levels to that observed in iron replete cultures. Further analyses will reveal whether other metals were reduced from the basalt in the presence of the siderophore. Future investigations will repeat these experiments on mutants of *Synechococcus* sp. PCC 7002 that do not synthesize synechobactin (G0023-25) or have no TonB transport system[2].

1. A. J. Hermann, J. Sorwat, J. M. Byrne, N. Frankenberg-Dinkel, M. M. Gehringer. *Nat. Comm.*, **2021**, *12.1*, 2069

2. C. Yong, B. Deng, L. Liu, X. Wang, H. Jiang. *AEM. Bio.,* **2022**, *89.1*, e01732–22

3. D. B. Alexander and D. A. Zuberer. *Biol. Fertil. Soils*., **1990**, *12*, 39–45

4.L. S. Fresenborg, J. Graf, H. Schätzle, E. Schleiff: Advances in Cyanobacterial Biology. (P. K. Singh), Academic Press 2020, pp. 85–117.

5. T. C. Enzigmüller-Bleyl, J. S. Boden, A. J. Hermann, K. W. Ebel, P. Sánchez-Baracaldo, N. Frankenberg-Dinkel, M. M. Gehringer. *Geo. Bio.,* **2022**, *20.6*, 776–789

"TEMPERATURE-INDUCED DYNAMICS OF SECONDARY METABOLITES, BIOCONDENSATES AND GROWTH OF *MICROCYSTIS* STRAINS"

Souvik. Roy*[,a], Dr. Arthur Guljamow[a] and Prof. Dr. Elke Dittmann[a]

[a] *Department of Microbiology, Institute of Biochemistry and Biology, University of Potsdam, Germany*

* *e-mail: roy@uni-potsdam.de*

The growth of cyanobacteria poses a risk to water quality and human health due to the production of the toxic hepatotoxin microcystin. The Microcystis genus is known to produce this toxin, which can vary between the cellular and extracellular pool as well as between free and protein-bound forms. Our investigation focused on how different temperatures affect the growth dynamics and production of secondary metabolites in the *M. aeruginosa* strains. We recently observed that the wild-type strain grew better at 20, 30, and 35°C, while the Δ*mcyB* mutant performed better at 25°C[1]. A similar reversal of the competitive dominance has been previously reported for different CO_2 levels[2]. Additionally, we found that soluble microcystin quotas were relatively higher at 20°C than at higher temperatures, and that the two microcystin congeners were affected differently by temperature changes[1]. Temperature also influences the biocondensation of microcystin, with a critical threshold between 20 and 25°C. This threshold also applied to the biocondensates and localization of the carbon-fixing enzyme RubisCO, which likely contributed to the growth advantages of the wild type and Δ*mcyB* mutant at 20°C and 25°C[1]. All in all, our study reveals how microcystin production in M. aeruginosa responds to temperature, influencing its adaptability and phenotypic plasticity. These insights emphasize the role of microcystins and other metabolites in the organism's rapid adaptation to temperature changes, underscoring the need to account for temperature-dependent processes in MC quota interpretation. Our latest findings, which provide insights into the success of *Microcystis* in rising global water temperature, will be showcased in our poster.

---

1. Roy. S., Guljamow. A., & Dittmann. E. (2023). Impact of temperature on the temporal dynamics of microcystin in M. aeruginosa PCC7806. Frontiers in Microbiology - Aquatic Microbiology. Manuscript ID: 1200816. (In review)

2. Van de Waal, D. B., Verspagen, J. M., Finke, J. F., Vournazou, V., Immers, A. K., Kardinaal, W. E., Tonk, L., Becker, S., Van Donk, E., Visser, P. M., & Huisman, J. (2011). Reversal in competitive dominance of a toxic versus non-toxic cyanobacterium in response to rising CO2. ISME J, 5(9), 1438–1450.

Utilization of the key regulatory protein CP12 to re-direct energy flux towards products in cyanobacteria

M.A. Itzenhäuser[1*], F. Brandenburg[1], S. Grimm[1], S. Klähn[1]

1 - Department of Solar Materials, Helmholtz Centre for Environmental Research – UFZ, Leipzig, Germany

Cyanobacteria as phototrophic microorganisms bear great potential to produce chemicals and fuels, including biotechnological hydrogen from sustainable resources such as light, H_2O, and CO_2. The recently gained knowledge on metabolic electron and carbon fluxes and their regulation brings about opportunities to channel the resources towards product formation. For instance, intrinsic regulators might pose interesting engineering targets to direct metabolic fluxes towards products. With recent research opening the window towards a more comprehensive view on fundamental principles of metabolic regulation, small regulatory proteins that are often unique to cyanobacteria got into focus. For instance, the CALVIN-BENSON-BASSHAM (CBB) cycle and thereby the assimilation of inorganic carbon is mainly controlled by a redox-controlled small protein named CP12 that interacts with and inhibits glyceraldehyde-3-phosphate dehydrogenase and phosphoribulokinase, when light availability is not sufficient. [1,2]

Some cyanobacteria, including model strain *Synechocystis* sp. PCC 6803, are capable of hydrogen formation via intrinsic, bidirectional [NiFe] hydrogenases that function as a valve for surplus electrons. However, hydrogen formation is in direct competition with the CBB cycle as it is the major electron sink. Here we made use of phage-based CP12 variants to downregulate the CBB cycle minimizing the electron drain in *Synechocystis*. Unlike the native CP12 of *Synechocystis*, which only inhibits the CBB cycle in the dark and gets deactivated by a reduced cell status, CP12 homologs from cyanophages appear to be active when the infected host cell is exposed to light. This, together with other metabolic interventions, lead to an enhanced electron availability for phage reproduction. [3] To simulate this circumstance, we constructed *Synechocystis* strains that encode those CP12 homologs under the control of the Ni^{2+}-inducible *nrsB* promoter. Indeed, an inhibitory effect on growth upon induction of heterologous gene expression was observed. Moreover, the generated strains showed enhanced production and a reduced re-uptake of hydrogen. With this, we demonstrate that CP12 homologs from phages can be used to engineer the metabolism of *Synechocystis* to boost processes that are directly fueled by electrons from the photosynthetic machinery.

---

[1] C. McFarlane, N. Shah, B. Kabasakal, J. Murray. PNAS, **2019,** 116(42): 20984-20990.

[2] S. Lucius, M. Theune, S. Arrivault, S. Hildebrandt, C. Mullineaux, K. Gutekunst, M. Hagemann. Front. Plant Sci., **2022**, 13: 1028794.

[3] L. Thompson, Q. Zeng, L. Kelly, S. Chisholm. PNAS, **2011**, 108: E757-E764.

A chimeric KaiA-like regulator extends the KaiB3-KaiC3 clock system in bacteria

Kim N. Sebastian*[a], Christin Köbler[a,1], Nicolas M. Schmelling[b,1], Alice Pawlowski[b,1], Philipp Spät[c], Nina M. Scheurer[a], Lutz Berwanger[b], Boris Maček[c], Anika Wiegard[b], Ilka M. Axmann[b,*], Annegret Wilde[a,*]

[a]Institute of Biology III, Faculty of Biology, University of Freiburg, 79104 Freiburg, Germany; [b]Institute for Synthetic Microbiology, Biology Department, Heinrich Heine University Düsseldorf, 40225 Düsseldorf, Germany; [c]Department of Quantitative Proteomics, Interfaculty Institute for Cell Biology, Eberhard Karls University Tübingen, 72076 Tübingen, Germany

e-mail: kim.sebastian@biologie.uni-freiburg.de

Due to earth's rotation, organisms are subjected to certain environmental changes. To anticipate these daily rhythms, organisms of all kingdoms of life have evolved diverse timing mechanisms. In cyanobacteria, the interplay of the proteins KaiA, KaiB and KaiC forms a chemical oscillator and the phosphorylation state of KaiC drives cyclic gene expression [1]. In addition to this standard protein oscillator, *Synechocystis* sp. PCC 6803, a model organism for cyanobacterial research, harbors several, diverged clock homologs. One such non-standard system, KaiB3-KaiC3, appears to influence metabolic changes in response to darkness [2], [3]. This study provides compelling evidence for a direct interaction of KaiC3 with Sll0485, a potential new chimeric KaiA homolog, named KaiA3. The N-terminus of KaiA3 shares similarities to a NarL-type response regulator receiver domain. However, the resemblance drastically diminishes in the C-terminal domain, which resembles the standard clock protein KaiA. We detect the formation of a high molecular weight complex of KaiA3 together with KaiB3 and KaiC3. Furthermore, phosphorylation of KaiC3 exhibits a rhythmic pattern of 48 hours in the presence of KaiA3 and KaiB3 *in vitro* as well as *in-vivo*. The deletion of *kaiA3* leads to KaiC3 dephosphorylation and results in growth defects during mixotrophic growth and in darkness. In conclusion, we propose that KaiA3 represents a novel nonstandard homolog of KaiA, thereby expanding the understanding of the KaiB3-KaiC3 system in cyanobacteria. We suggest that the KaiA3B3C3 system is an autonomous oscillator and, in conjunction with the canonical KaiA1B1C1 system, plays a role in regulating the transition between auto-/heterotrophic growth in *Synechocystis*.

[1] N. Takai *et al.*, "A KaiC-associating SasA-RpaA two-component regulatory system as a major circadian timing mediator in cyanobacteria," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 103, no. 32, pp. 12109–12114, 2006, doi: 10.1073/pnas.0602955103.

[2] A. Wiegard *et al.*, "Synechocystis KaiC3 Displays Temperature- and KaiB-Dependent ATPase Activity and Is Important for Growth in Darkness.," *J. Bacteriol.*, vol. 202, no. 4, Jan. 2020, doi: 10.1128/JB.00478-19.

[3] A. Wiegard *et al.*, "Biochemical analysis of three putative KaiC clock proteins from Synechocystis sp. PCC 6803 suggests their functional divergence," *Microbiology*, vol. 159, no. Pt_5, pp. 948–958, May 2013, doi: 10.1099/MIC.0.065425-0.

COMPUTATIONAL SCREEN FOR NOVEL RNA STRUCTURES IN CYANOBACTERIA

Adrian Sven Geissler[a], Elena Carrasquer-Alvarez[b], Niels-Ulrik Frigaard[b], Jan Gorodkin[a], and Stefan Ernst Seemann[a,*]

*[a] Center for non-coding RNA in Technology and Health, Department of Veterinary and Animal Sciences, University of Copenhagen, Denmark; [b] Marine Biological Section, Department of Biology, University of Copenhagen, Denmark; * e-mail: seemann@rth.dk*

RNA structures in cyanobacteria are known to regulate key molecular mechanisms, such as the photosynthesis-regulating small RNA structure PsrR1.[1] However, the number of currently known RNA structures in cyanobacteria is limited, because the search space for RNA structure predictions has so far been limited to nucleotide sequence similarity (*e.g.* via BLAST search).[2,3] In this study, we conduct a phylum-wide screen for novel RNA structures with the CMfinder tool, which predicts RNA structures based on conservation of both nucleotide sequence and RNA secondary structure.[4] We screened the intergenic regions adjacent to 931 ortholog coding genes in 202 cyanobacterial genomes. We scored each prediction with respect to its RNA structure conservation (CMfinder's metric) relative to the local phylogeny of the ortholog genes (inferred with IQ-TREE2).[4,5] Furthermore, we estimated the false discovery rate (FDR) of each predicted RNA relative to additional CMfinder runs on the shuffled, but both phylogeny and di-nucleotide content preserved, intergenic search regions.[6] In total, our screen predicts 1815 RNA structure motifs of which 825 motifs have an FDR ≤ 10%. For the evaluation of the known structure recall, we conducted a sequence and structure homology search for known structures (relative to the Rfam database) and bacterial transcription termination structures (RNIE model).[7,8] The structure homology search had low expected FDRs for the reference structures (< 0.1%), and with this search as reference, we observe a recall of small RNA (3 of 14 families) and cis-regulatory (*e.g.,* riboswitch) RNA structure families (7 of 16 families recalled) overlapping the search regions. Given that CMfinder uses a much smaller phylogeny than the model drawn from Rfam this is in line with the expectations. Interestingly, we found 423 of the 825 predicted motifs to have no overlap with known structures, such that we predict that these are novel RNA structures. For downstream functional analysis, we manually selected candidate RNA structures adjacent to gene orthologs that are part of important metabolic pathways, including photosynthesis, nitrogen assimilation, biosynthesis of nucleotides, lipopolysaccharides, vitamin B_{12}, and NAD(P)H, and molecular processes of RNA degradation and DNA topology arrangement.

1. Georg, J. *et al.* The Small Regulatory RNA SyR1/PsrR1 Controls Photosynthetic Functions in Cyanobacteria. *Plant Cell* **26**, 3661–3679 (2014).
2. Axmann, I. M. *et al.* Identification of cyanobacterial non-coding RNAs by comparative genome analysis. *Genome Biol.* **6**, R73 (2005).
3. Brenes-Álvarez, M., Olmedo-Verd, E., Vioque, A. & Muro-Pastor, A. M. Identification of Conserved and Potentially Regulatory Small RNAs in Heterocystous Cyanobacteria. *Front. Microbiol.* **7**, (2016).
4. Yao, Z., Weinberg, Z. & Ruzzo, W. L. CMfinder-a covariance model based RNA motif finding algorithm. *Bioinformatics* **22**, 445–452 (2006).
5. Minh, B. Q. *et al.* IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era. *Mol. Biol. Evol.* **37**, 1530–1534 (2020).
6. Gesell, T. & Washietl, S. Dinucleotide controlled null models for comparative RNA gene prediction. *BMC Bioinformatics* **9**, 248 (2008).
7. Gardner, P. P., Barquist, L., Bateman, A., Nawrocki, E. P. & Weinberg, Z. RNIE: genome-wide prediction of bacterial intrinsic terminators. *Nucleic Acids Res.* **39**, 5845–5852 (2011).
8. Kalvari, I. *et al.* Non-Coding RNA Analysis Using the Rfam Database. *Curr. Protoc. Bioinforma.* **62**, e51 (2018).

Is Sll0518 required for RNA translocation?

Wongsik Seo , Luisa Hemm and Wolfgang R. Hess

Genetics & Experimental Bioinformatics,

Institute of Biology, University Freiburg, D-79104 Freiburg Germany

The localization of mRNA in eukaryotes is well studied, like the targeting of mRNA to the membrane of the endoplasmic reticulum. The proteins are translated there to be transferred into the membrane (transmembrane proteins) or to be secreted. However, the topic was underrepresented in prokaryotes as they lack cellular compartmentalization. But recent studies showed, that in many cases, transcripts in prokaryotes are distributed throughout the cell highlighting the importance of RNA localization in prokaryotes [1] . Due to the highly dense membrane structures in cyanobacteria, the mechanism of mRNA localization of membrane proteins is of great interest to the community. We contributed to a study showing that the RRM containing RNA binding proteins Rbp2 and Rbp3 play a major role in translocation of photosynthetic mRNAs to the thylakoid membrane. In a double deletion mutant of Rbp2 and Rbp3 mRNA encoding for membrane proteins of the photosynthesis apparatus are more concentrated in the cytoplasm as in the wild type where the mRNA

is distributed along the thylakoid membrane[2]. In a further unpublished study, the unknown protein Sll0518 was identified as an interaction partner of Rbp3. Since the function of the protein is yet unknown, we performed structural analysis using the

ColabFold[3] and Foldseek[4] webserver. This revealed that Sll0518 contains two OB−fold barrels[5] and two intrinsically disordered region (IDR) in the N−terminal as well as in the C−terminal region. The OB−fold motif is frequently found in single−stranded DNA binding proteins (SSB) and is of great interest for this study as it may be involved in nucleotide binding together with Rbp3. Interestingly, these two OB−folds are connected in a different way from the known structure of two tandem OB−folds. This specific arrangement of two OB−folds is very rare in bacteria in general, but is found in some hypothetical proteins of cyanobacteria. To further analyze this protein and its involvement in mRNA localization, several mutants were constructed. With these strains we aim to perform phenotypical analysis, pull down assays and protein tracking to characterize Sll0518 and its cellular functions in more detail.

[1] Irastortza-Olaziregi M., et al., (2021), WIREs RNA Vol. 12(2)

[2] Mahbub M., et al., (2020), Nature Plants Vol. 6: 1179-1191

[3] Mirdita M., et al., (2022), Nature Methods

[4] van Kempen, M., et al., (2023), Nat Biotechnol

[5] Youkharibache P., at al., (2019), Structure Vol. 27(1): 6-26

A MINIMAL COARSE-GRAINED MODEL OF CYANOBACTERIAL GROWTH

Ralf Steuer[a], Mona Nehse*[,a]

[a] *Institute for Theoretical Biology, Humboldt-Universität zu Berlin, Berlin, Germany*
** e-mail: nehsemon@hu-berlin.de*

Because of their high growth rates and their amenability to genetic modification, cyanobacteria are promising organisms for green biotechnology. A key requirement to further advance cyanobacterial biotechnology, however, is a better understanding of the factors that determine the limits of phototrophic growth. In particular, the maximal phototrophic growth rate is limited by a finite proteome, and understanding how cyanobacteria allocate their finite resources is crucial for maximizing growth and phototrophic productivity. To investigate optimal proteome allocation, we present a coarse-grained model of cyanobacterial phototrophic growth based on ordinary differential equations. The model consists of three molecular species: precursor molecules, photosynthetic units and ribosomes. The finite proteome results in a trade-off between photosynthesis and the translation of proteins. We are particularly interested in how the cyanobacterial cell regulates the allocation of proteins to these two processes. To this end, we develop a "self-optimizing" model that is capable of attaining maximal growth rates by adjusting its proteome allocation based on the reduction of the photosynthetic electron transport chain as an internal light sensor. The model reproduces several characteristic properties of cyanobacterial growth and can be readily extended to further study protein allocation strategies in phototrophic growth.

SHEDDING LIGHT ON BLUE-GREEN PHOTOSYNTHESIS: A WAVELENGTH-DEPENDENT MATHEMATICAL MODEL OF PHOTOSYNTHESIS IN *SYNECHOCYSTIS* SP. PCC 6803

Tobias Pfennig*[,a,b], Elena Kullmann[a], Tomáš Zavřel[c], Andreas Nakielski[a,d], Oliver Ebenhöh[b,e], Jan Červený[c], Gábor Bernát[f], and Anna Matuszyńska[a,b]

[a] *Computational Life Science, RWTH Aachen University, Germany*
[b] *Cluster of Excellence on Plant Science, HHU Düsseldorf, Germany*
[c] *Department of Adaptive Biotechnologies, Czech Academy of Sciences, Czechia*
[d] *Institute for Synthetic Microbiology, HHU Düsseldorf, Germany*
[e] *Institute of Theoretical and Quantitative Biology, HHU Düsseldorf, Germany*
[f] *Balaton Limnological Research Institute, Eötvös Loránd Research Network (ELKH), Hungary*
** e-mail: tobias.pfennig@rwth-aachen.de*

Cyanobacteria hold great potential to revolutionize industries and farming practices with their light-driven chemical production. However, to fully exploit their photosynthetic capacity, intricate knowledge about their photosynthetic machinery is necessary which can be provided by mathematical models.

Therefore, we have developed an ordinary differential equation-based model for *Synechocystis* sp. PCC 6803 to assess its performance under various light sources. Our model accurately predicts electron partitioning through four main pathways, and the rates of O_2 evolution, and carbon fixation. Additionally, it captures behaviour in Pulse Amplitude Modulation measurements. We explore proposed state transition mechanisms, favouring PSII quenching based on theoretical evidence. Moreover, we evaluate metabolic control for biotechnological production under diverse light conditions.

Our model can, therefore, provide valuable knowledge integration and support for experimental advances.

Spatio-temporal regulation of type IV pilus assembly in *Synechocystis* sp. PCC 6803

Jonas Hammerl*[,a,b], Yu Han[a], Nils Schürgers[a], Annegret Wilde[a]

[a] *Institute of Biology III, University of Freiburg, Schänzlestraße 1, Freiburg, Germany*
[b] *Spemann Graduate School of Biology and Medicine (SGBM), University of Freiburg, Freiburg, Germany*
** e-mail: jonas.hammerl@sgbm.uni-freiburg.de*

During cyanobacterial phototaxis polar activity of type IV, pilus machinery mediates directional movement towards or away from a light source. In *Synechocystis* sp. PCC 6803 phototaxis is governed by multiple signal transduction pathways that employ CheY and PatA-like response regulators. The latter have been shown to interact with the pilus extension ATPase PilB1 which is proposed as a central regulatory hub during phototactic decision making[1]. We hypothesize that polar interactions of pilus motor components and response regulators orchestrate phototactic motility. Employing CLSM and radial fluorescence intensity profiling, we aim to elucidate subcellular localization of PilB1 and response regulators during phototactic motility. Preliminary results show localization of PixG at the leading cell edge during positive phototaxis similar to reported PilB1 localization[2]. Furthermore, to study interactions of PilB1 with phototactic response regulators on a subcellular scale *in vivo* we are establishing a FLIM-FRET system in *Synechocystis*. We visualized membrane tethered PilB1-mTurquoise2 and used the known PilB1 interactor PixE as a mVenus fusion to optimize the FLIM-FRET system. In the future, we will investigate the impact of response regulator phosphorylation on subcellular localization and the ability to modulate polar pilus activity. Moreover, to identify novel interaction partners of pilus components, we have established a bacterial two-hybrid library screening system. Taken together, response regulators and pilus components show differential localization correlating with movement direction.

[1] Y. Han, A. Jakob, S. Engel, A. Wilde, N. Schürgers. Mol. Microbiol., 2022,117, 790-801

[2] N. Schürgers, D. J. Nürnberg, T. Wallner, C. W. Mullineaux, A. Wilde. Microbiology, 2015, 161, 960-966

CHLOROPHYLL-BINDING DOMAIN OF THE CYANOBACTERIAL FERROCHELATASE IS INDISPENSABLE UNDER COLD STRESS

Divya Aggarwal*[a], Jan Janouškovec[a], Josef Komenda[a], Roman Sobotka[a]

[a] *Centre Algatech, Institute of Microbiology, Czech Academy of Sciences, 37901 Třeboň, Czech Republic*

** aggarwal@alga.cz*

Oxygenic phototrophs produce all tetrapyrroles by a long and branched biosynthetic pathway. At the branch point, either magnesium chelatase inserts magnesium for chlorophyll (Chl) biosynthesis or ferrochelatase (FeCh) adds ferrous iron for heme biosynthesis. An intriguing feature of FeCh in oxygenic phototrophs is the C-terminal extension (Hlip domain) that origins in an ancient fusion between FeCh and the Chl-binding HliC protein[1]. HliC is a transmembrane, single helix protein, belonging to the family of high light inducible proteins (Hlips), which must dimerize to bind Chl and carotenoids[2]. Yet, the role of FeCh Hlip domain remains enigmatic. As shown recently, the FeCh in the cyanobacterium *Synechocystis* PCC 6803 can dimerize via its Hlip domain and binds Chl, β-carotene and zeaxanthin if there is an excess of free Chl in membranes[3]. To clarify the role of pigment binding to FeCh, we prepared *Synechocystis* strains possessing FeCh variants with point mutations in Hlip domain affecting the FeCh dimerization. Interestingly, these strains are cold sensitive and cannot survive combination of cold (18°C) and high light (300 µE of photons). By using protein $^{[35]}$S radiolabelling and 2D electrophoresis we found that, during stress conditions, the mutant strains are not able to synthesize enough of the core Photosystem II subunit D1 to maintain the pool of active PSII. This defect was accompanied by the accumulation of unbound Chl molecules in membranes and by accelerated dissociation of PSI trimers. Similar phenotype is observed for strains mutated in the FeCh catalytic site (low active FeCh). Our data indicate that the dimerization of FeCh, promoted by binding of free Chl molecules, is essential for the PSII biogenesis under cold stress.

1. J. Janouškovec, M. Milichovská, A. Pazuki, L. Kovářová, R. Sobotka. The origin of the extended protein superfamily. *In preparation.*
2. MK. Shukla, MJ. Llansola-Portoles, M. Tichy, AA. Pascual, B. Robert, R. Sobotka. *Photosyn Res* **2018**, 137, 29-39.
3. M. Pazdernik, J. Mares, J, Pilny, R. Sobotka. *J Biol Chem* **2019**, 294, 11131-11143.

TOXIC CYANOBACTERIA *NODULARIA SPUMINGENA*, A WINNER IN FUTURE BALTIC SEA CLIMATE? LONG TERM ADAPTATION OF *N. SPUMIGENA* TO TEMPERATURE AND SALINITY CHANGE

Carolin Peter*[,a], Elin Lindehoff[a] , Hanna Farnelid[a] and Catherine Legrand[a, b]

[a] *Linnaeus University, Universitetsplatsen 1, 39231 Kalmar, Sweden*
[b] *Halmstad University, Kristian IV:s väg 3, 30118 Halmstad, Sweden*
** e-mail: carolin.peter@lnu.se*

In southern and central basins of the Baltic Sea, the diazotroph *Nodularia spumigena* is a typical component of the summer bloom community. Due to its toxicity, the impact of *N. spumigena* blooms can be substantial. Predicted increases in temperature might lead to an increase in bloom frequency, intensity and duration as well as a northward expansion. However, the lower salinities of the northern basins might hamper growth and prevent bloom formation. While most efforts focus on short-term adaptations, this study examines the physiological adaptation of two *N. spumigena* strains (KAC11, KAC66) to decreased salinity (5, 3psu) compared to ambient bloom conditions (7 psu) for more than eight years in culture. After three years, the strains already exhibited distinctive strategies. While KAC66 maintained original growth rates, toxicity and phenology, KAC11 initially displayed a reduction in growth rate, nodularin:chla ratio and filament biovolume. Over an acclimation period of three years, the growth rate recovered and the nodularin:chla ratio even surpassed the original values, while the biovolume remained at only 10%. This could increase this strain's availability as food item which would have important implications for the food web especially in combination with increased toxicity. In the current experiment, the growth rate, toxicity and morphology of both strains after more than eight years of adaptation and their capability for recovery when re-introduced to original conditions were evaluated. This study presents invaluable insight to long-term adaptation in *N. spumigena* and thereby helps improve the predictions for toxic cyanobacterial blooms in the Baltic Sea.

Sucrose utilization in recombinant *E. coli* strains for efficient cofactor regeneration

Lucija Sovic[1], Lenny Malihan-Yap[1], Robert Kourist[*1]
[1] *Graz University of Technology, Institute of Molecular Biotechnology*
Petersgasse 14, 8010 Graz, Austria
sovic@tugraz.at

Many oxidoreductases such as Baeyer-Villiger monooxygenases (BVMO) depend on expensive cofactors such as NADPH, which impairs their industrial application and sustainability of the process. Efficient cofactor regeneration can be coupled to the cellular metabolism, however this might require additional carbon sources, lowering the atom economy. Alternatively, a cheap and abundant carbon source such as sucrose can be utilized. The photoautotrophic cyanobacterium *Synechocystis* sp. S02 produces sucrose from carbon dioxide, light and water and is able to secrete it into the medium. In a proof-of concept study shown in **Figure 1** the photosynthetically derived sucrose was utilized by co-cultivated *E. coli* W ΔcscR to regenerate NADPH for conversion of cyclohexanone to ε- caprolactone *via* recombinantly produced BVMO [1]. Herein, our goal was to expand the co-cultivation onto standard laboratory strains, which are natively unable to utilize sucrose and to use a faster BVMO variant isolated from *Bulkoderia xenovorans.* [2]. Additionally, we investigated essential genetic features needed for *E. coli* strains to successfully utilize sucrose in a whole-cell biotransformation.

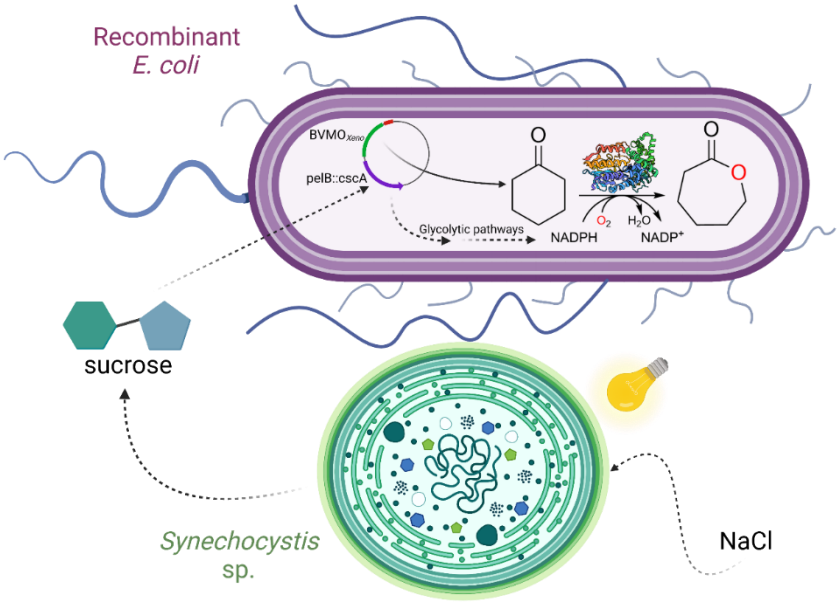


Figure 1. Graphical representation of the concept used in our study. Recombinant *E. coli* strains utilize sucrose, which is secreted into the medium by the *Syn* sp. S02 cells and in turn used for the NADPH cofactor regeneration needed in the target reaction.

[1] Tóth, G. S.; Siitonen, V.; Nikkanen, L.; Sovic, L.; Kallio, P.; Kourist, R.; Kosourov, S.; Allahverdiyeva, Y. Photosynthetically Produced Sucrose by Immobilized Synechocystis Sp. PCC 6803 Drives Biotransformation in E. Coli. *Biotechnol. Biofuels Bioprod.* **2022**, *15* (1), 146. https://doi.org/10.1186/s13068-022-02248-1.
[2] *Photobiocatalytic Oxyfunctionalization with High Reaction Rate using a Baeyer–Villiger Monooxygenase from Burkholderia xenovorans in Metabolically Engineered Cyanobacteria* | *ACS Catalysis*,***2021***. https://pubs.acs.org/doi/full/10.1021/acscatal.1c04555 (accessed 2023-03-06).

BLUE-GREEN TRAVELERS: CULTIVATION OF CYANOBACTERIA ASSOCIATED WITH SEA TURTLES

Lucija Kanjer*[,a], Marcelo Vaz[b] , Annick Wilmotte[b] and Sunčica Bosak[a]

[a] *Faculty of Science, University of Zagreb, Horvatovac 102a, 10 000 Zagreb, Croatia*
[b] *InBios – Molecular Diversity and Ecology of Cyanobacteria, BCCM/ULC Collection of Cyanobacteria, University of Liège, Allée du 6 Août 11, B-4000 Liège, Belgium*
** e-mail: lucija.kanjer@biol.pmf.hr*

The ecological role and diversity of cyanobacteria are important within diverse ecosystems, actively contributing to important functions like nutrient cycling, primary productivity and nitrogen fixation. Understanding their taxonomy and culturing cyanobacteria is of paramount importance for unraveling their ecological significance While cyanobacterial symbiotic relationships, such as those with marine invertebrates like corals and sponges, have garnered attention, the specific epibiotic cyanobacteria associated with vertebrates like sea turtles have remained notably unexplored in both study and cultivation.

This study addresses this gap by focusing on the cultivation and taxonomic exploration of cyanobacterial strains associated with sea turtles. The study aims to isolate and characterize cyanobacterial strains from the surfaces of sea turtles, shedding light on their taxonomic identity and potential ecological roles. A total of 11 cyanobacterial strains were successfully isolated from various sea turtle species. The genetic markers 16S rRNA and ITS were amplified and sequenced, revealing that all isolated strains belong to the homocytous filamentous cyanobacteria group.

By bridging the knowledge gap regarding these often overlooked microorganisms, this research paves the way for a deeper understanding of the intricate relationships between cyanobacteria and their host animals. The successful cultivation and taxonomic identification of these strains provide a foundation for future studies that could tell us more about the functional significance of these cyanobacteria within the sea turtle microbiome context.

Direct positive effect of stoniness on the activity of photoautotrophs in the dryland biological soil crusts

Naw Than Than Aye[a], Hezi Yizhaq[b], Eli Zaady[c], Nina Kamennaya[d,e]

[a]*Albert Katz International School for Desert Studies, Ben-Gurion University of the Negev, Israel*

[b]*Alexandre Yersin Department of Solar Energy and Environmental Physics, Ben-Gurion University of the Negev, Israel*

[c]*Department of Natural Resources, Agricultural Research Organization, Institute of Plant Sciences, Gilat Research Center,*

Israel

[d]*Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, Israel*

[e] *Goldman-Sonnenfeldt Schools of Sustainability and Climate Change, Ben-Gurion University of the Negev, Israel*

aye@post.bgu.ac.il, ninaka@bgu.ac.il

Approximately 70% of soil surface is covered with a thin metabolically active layer termed a biological soil crust (biocrust). Biocrusts harbor micro- and macro-scopic photoautotrophs, i.e., cyanobacteria, algae, lichens, and mosses, who play an important ecological role by enriching the underlaying soil with organic carbon and nitrogen. Because of prolonged dry periods, biocrusts in drylands are, however, dominated mostly by cyanobacteria and algae (Redfield et al., 2006). Soil stoniness-the presence of gravel, stones, and rocks in the soil profile-affects the biocrust properties and composition and increases accumulation of ammonium and organic carbon. It is assumed that in areas with high level of stoniness moisture availability in soil is higher compared to the areas with low stoniness (Zaady et al., 2021) . Here, we examined the effect of soil stoniness on the abundance and metabolic activity of the biocrust photoautotrophs. We collected crust samples from low-stoniness and high-stoniness areas in three dryland regions in the northern Negev Desert in Israel. In these samples we quantified (1) photosynthetic pigments, (2) microscopically counted and measured sizes of cyanobacteria and algae to estimate their total biovolumes, and (3) determined the rates of photosynthetic CO_2 and N_2 fixation. On average, the biocrust chlorophyll content was ~1.2 fold higher in the high-stoniness areas versus the low-stoniness areas, whereas the biovolumes of cyanobacteria and algae were ~2.8-fold and ~1.5-fold greater, respectively. The carbon fixation rates were ~2.4-fold higher in the high-stoniness areas versus the low-stoniness areas and N_2 fixation could only be detected in the high-stoniness areas. Therefore, we conclude that stoniness has a direct, positive effect on the photoautotrophic activity in the dryland biocrusts.

1. Redfield, E., Barns, S. M., Belnap, J., Daane, L. L., & Kuske, C. R. (2006). Comparative diversity and composition of cyanobacteria in three predominant soil crusts of the Colorado Plateau. *FEMS Microbiology Ecology*, *40*(1), 55–63. https://doi.org/10.1111/j.1574-6941.2002.tb00936.

2. Zaady, E., Stavi, I., & Yizhaq, H. (2021). Hillslope geodiversity effects on properties and composition of biological soil crusts in drylands. *European Journal of Soil Science*, *72*(5), 2308–2315. https://doi.org/10.1111/ejss.1309

LIGHT DEPENDENT MORPHOGENESIS OF CYANOBACTERIUM *SYNECHOCOCCUS ELONGATUS* PCC 7942

Soumila Mondal*[,a], Shailendra P. Singh[b] and Karl Forchhammer[a]

[a] *Interfaculty Institute of Microbiology and Infection Medicine, University of Tübingen, Tübingen 72076, Germany*

[b] *Centre of Advanced Study in Botany, Institute of Science, Banaras Hindu University, Varanasi 221005, India*

** e-mail: soumila.mondal@uni-tuebingen.de*

Cyanobacteria are photosynthetic, prokaryotic, Gram-negative organisms that are emerging model systems for the carbon dioxide (CO_2) sequestration of sustainable production of different secondary metabolites that have industrial and pharmaceutical values. However, the spectral composition of light in the fluctuating light environment can affect the growth, development and fitness of the cyanobacteria. Here in this study, we analysed the photobiology of *Synechococcus elongatus* PCC 7942 using different quality of lights such as white light (WL), red light (RL), green light (GL) and blue light (BL) to investigate the response of this organism to different wavelengths of photosynthetic active radiation (PAR). From the results of this study, it was observed that *S. elongatus* PCC 7942 cannot efficiently use GL and BL wavelengths of light, and thus growing under these light conditions compromised the fitness and growth of the organism by increasing the cellular level of reactive oxygen species (ROS). GL and BL, interestingly, increased the total amount of lipid inside the cell and caused decoupling of phycobilisomes (PBS) from the thylakoid membranes. It was also observed that different light quality also alters the morphology of the cyanobacterium. Furthermore, analysis of the expressions of different morphogenes suggested that GL and BL could regulate cell shape by altering the expression of cytoskeleton protein-encoding genes. Thus, it is evident that the growth and fitness of *S. elongatus* PCC 7942 can be compromised in dense culture or at higher depths in the water column where GL and/or BL-enriched environment prevails. However, decreased fitness is offset by increased lipid content and elongated cellular morphology.

CLARIFICATION OF CHROMATIC ACCLIMATION-4 IN *SYNECHOCOCCUS* WH8109

J. Solanki*, Michelle M. Gehringer, N. Frankenberg-Dinkel

Department of Microbiology, RPTU Kaiserslautern-Landau, Kaiserslautern, 67665,Germany
** e-mail: Solanki@rptu.de*

Marine phytoplankton are responsible for nearly half of the primary productivity on Earth, and the second most abundant picocyanobacteria *Synechococcus* contributes up to 16% of this total.[1] To optimize their fitness, various *Synechococcus* strains exhibit a finetune mechanism known as chromatic acclimation (CA). CA-4 makes use of different chromophores for photosynthetic light harvesting, depending on the available light color niche, particularly in the blue and green range (450-570 nm).[2] There have been conflicting reports about the chromatic acclimation ability of *Synechococcus* WH8109.[3,4] To address this uncertainty, our study aimed to investigate the chromatic acclimation process in WH8109. We grew WH8109 under blue and green light to check CA-4 by measuring changes in absorbance, as well as emission (570 nm) of the excitation of chromophore PUB (495 nm) and PEB (545 nm) and, compared the results to those generated using the positive CA-4 strain, *Synechococcus* RS9916. While the expected change in the PUB:PEB ratio (Exc 495:545) under blue (0.6) and green (1.6) range was observed for RS9916, a PUB:PEB ratio 0.7 was obtained for WH8109 grown under both light, even after transitioning from blue to green light, and *vice versa*. Our data, therefore, supports the observation of Humily et al,[4] that WH8109 does not undergo CA, despite possessing all the necessary genetic machinery for chromatic acclimation type4.

Refrences

1. J.E. Sanfilippo, A.A. Nguyen, L. Garczarek, J.A. Karty, S. Pokhrel, J.A. Strnat, F. Partensky, W.M. Schluchter, D.M. Kehoe. *PNAS*, **2019**, 116, 6457-6462.
2. J.E. Sanfilippo, L. Garczarek, F. Partensky, D.M. Kehoe. *Annu. Rev. microbiol*, **2019**, 73, 407-433.
3. R. Lovindeer, L. Abbott, H. Medina, K.R.M. Mackey. *Front. Mar. Sci*, **2021**, 8, 1-13
4. F. Humily, F. Partensky, C. Six, G.K. Farrant, M. Ratin, D. Marie, L. Garczarek. *PLOS*, **2013**, 8, 1-16.

Ca^{+} SIGNALING IN FILAMENTOUS HETEROCYST FORMING CYANOBACTERIA

Teresa A. Müller*[,a], Karl Forchhammer[a] and Khaled A. Selim[a,b]
[a] *Microbiology / Organismic Interactions, University of Tübingen, Auf der Morgenstelle 28, 72076 Tübingen, Germany*
[b] *Microbiology / Molecular Physiology of Prokaryotes, University of Freiburg, Germany*
** e-mail: te.mueller@student.uni-tuebingen.de*

Free calcium (Ca^{2+}) ions can be highly toxic as they can precipitate phosphate ions, which are important for various metabolic pathways. Therefore, cyanobacteria regulate their intracellular Ca^{2+}-concentration $[Ca^{2+}]_i$ via pumps, channels and calcium binding proteins.[1,2] In multicellular cyanobacteria, Ca^{2+} signaling also plays an important role in heterocyst differentiation.[3] Shortly after nitrogen depletion, a condition that triggers heterocyst differentiation, a transient increase of $[Ca^{2+}]_i$ [4] in pro-heterocysts have been reported.[3] In the filamentous heterocyst forming model organism *Nostoc* sp. PCC 7120 two calcium binding proteins have been identified: CcbP (cyanobacterial calcium binding protein)[3] and CSE (Ca^{2+} Sensor EF-hand).[5] CcbP is mainly known for its buffer property in capturing free Ca^{2+} ions.[3] It is negatively regulated via the heterocyst transcription regulators NtcA and HetR and appears to be absent in heterocysts.[3,6] CSE binds Ca^{2+} via two characteristic Ca^{2+} sensor EF hand domains.[5] The CSE appears unfolded in the absence of calcium and undergoes a strong conformational change upon Ca^{2+} binding. CSE is strongly downregulated during nitrogen depletion.[5,7] Although there is a strong connection between Ca^{2+} signaling and heterocyst differentiation, the specific functions of both calcium binding proteins, CcbP and CSE, remain elusive. Our aim is to further investigate the role of calcium signaling and calcium homeostasis in multicellular cyanobacteria with respect to heterocyst formation and photosynthesis. Therefore, we investigate the characteristics of single and double knockout or overexpressing mutants of those proteins in *Nostoc* sp. PCC 7120 regarding cell-cell-communication, growth under nitrogen limitation and heterocyst formation.

1. D.E. Clapham, *Cell*, **2007**,*131*, 1047–1058.
2. V. Norris *et al.*, *J. Bacteriol*., **1996**, *178 (13)*, 3677–3682.
3. Y. Zhao *et el., Proc. Natl. Acad. Sci. U. S. A.*, **2005**, *102 (16)*, 5744–5748.
4. I. Torrecilla *et al., Microbiol.,* **2004**, *150*, 3731-3739.
5. J. Walter *et al., BBA Bioenerg.,* **2019**, *1860 (6)*, 519-532.
6. Y. Shi *et al., PNAS*, **2006**, *103 (30)*, 11334-1339.
7. J. Walter *et al., BMC Microbiol.*, **2020**, *20:57*.

A PROMISING APPROACH FOR LONG-TERM CRYOPRESERVATION OF *ANABAENA* SP. PCC 7120

Inés Federío*[a], Jorge Guío [a], M. Teresa Bes [a], M. Luisa Peleato [a], María F. Fillat [a] and Emma Sevilla [a]

[a] *Department of Biochemistry and Molecular and Cell Biology. University of Zaragoza, Zaragoza 50009, Spain.*
** e-mail: ifederiozalaya@gmail.com*

Most cyanobacteria are preserved through subcultures in liquid medium or re-streaking on plates, which entails certain drawbacks, such as an increased risk of contamination and the possibility of considerable genetic diversity among the same strains. Thus, a simple and safe technique is required for the long-term preservation of cyanobacteria.

Cryopreservation in liquid nitrogen allows the preservation of genome stability over extended periods, reduces the risk of contamination, and saves labour and space. Although these storage techniques are widespread and optimized for many microorganisms, most of the described methods for cyanobacteria exhibit low viability after freezing and thawing. In the case of the cyanobacterium *Anabaena* sp. PCC 7120, optimization of a cryopreservation method is required, since this construction of mutants in this strain is time consuming and the viability achieved with current methods prevent its cryopreservation.

In this work, a cryopreservation method based on the use of a thermal ramp proved effective for cryopreservation of *Anabaena* sp. PCC 7120, maintaining viability after conservation for extended periods, and preserving their morphological, physiological, biochemical, and genetic properties.

MEMBRANE INLET MASS SPECTROMETRY

Friederike Hörsch*[,a], Jens Appel[a] and Kirstin Gutekunst[a]

[a] *Molecular Plant Physiology, Bioenergetics in Photoautotrophs, University Kassel, Heinrich-Plett-Straße 40, 34132 Kassel, Germany*
** e-mail: uk094802@uni-kassel.de*

Mass Spectrometry can be used to investigate the uptake and evolution of gases during photosynthesis in cyanobacteria. A membrane inlet spectrometer (MIMS) is particularly useful as it allows working in vivo with liquid cultures. Our setup can detect oxygen, argon and carbon dioxide isotopes as well as hydrogen isotopes. The use of different isotopes makes it possible to distinguish between different processes and enables us to monitor the gas exchange during respiration, photosynthetic oxygen production as well as hydrogen production in cyanobacteria.

The general makeup of a MIMS consists of a mass spectrometer made up of ion-source, analyzer and detector and a special inlet, in which the liquid sample is separated from the vacuum by a thin membrane that only allows gases to pass through it while keeping the water out of the vacuum system.[1] To be able to use the MIMS for experiments it is necessary to calibrate it beforehand to be able to convert the MIMS signal into units commonly used for concentrations. This can be done by adding known amounts of a gas to the sample chamber and recording the corresponding MIMS signal.[2] It is also necessary to correct for the gas consumption of the instrument itself to be able to get correct rates of gas uptake and evolution of the cells in the sample.[3]

1. K. Beckmann,J. Messinger, M.R. Badger et al. *Photosynth Res*., **2009**, *102*, 511-522.
2. G. Hoch, B. Kok. *Arch. Biochem. Biophys*., **1963***, 101, 160-170.*
3. A. Burlacot, F. Burlacot, Y. Li-Beisson, G. Peltier. *Front. Plant Sci.,* **2020**, *11.*

ENHANCING CYANOBACTERIAL GROWTH AND CULTURE STABILITY THROUGH CO-CULTIVATION WITH HETEROTROPHIC BACTERIA

Miriam Verucchi*[,a], David A. Russo[b], Elke Dittmann[c] and Julie A.Z. Zedler[a]

[a] *Friedrich Schiller University Jena, Matthias Schleiden Institute, Synthetic Biology of Photosynthetic Organisms, Jena, Germany*
[b] *Friedrich Schiller University Jena, Institute for Inorganic and Analytical Chemistry, Bioorganic Analytics, Jena, Germany*
[c]*University of Potsdam, Department of Microbiology, Institute of Biochemistry and Biology, Potsdam-Golm, Germany*
** e-mail: miriam.verucchi@uni-jena.de*

Cyanobacteria have been proven to be promising sustainable production systems. However, their usage in industry remains limited. One reason for this is that monocultures are intrinsically fragile and limited in their metabolic capacity. Mixed production systems might offer several advantages in terms of culture robustness and metabolic capacity.
In this work we investigated the potential of a recently discovered, fast-growing cyanobacterium Synechococcus sp. PCC 11901 in co-cultivation with heterotrophic bacterial partners. The cobalamin auxotrophy of PCC 11901 served as a starting point to design stable co-cultures towards industrial applications.
We observed that co-cultures of this cyanobacterium and selected heterotrophic partners promoted growth and stability of the phototrophic strain. Axenic cultures died quickly, presumably due to a lack of vitamin B12. Co-cultures looked denser, more stable and maintained themselves in stationary phase until the end of the experiment. Growth promotion was also observed in co-cultures with a mutant of PCC 11901 which does not require vitamin B12, suggesting that there may be other, yet-undescribed, interactions that contribute to the observed effects.

QUANTITATIVE ANALYSIS OF STRUCTURED, MIXED-SPECIES MICROBIAL CONSORTIA IN MICROFLUIDIC FLOW-CELL PLATFORM

Hanisch, Selina*[,a], Karande, Rohan[b] and Bühler, Katja[a]

[a] *Helmholtz Centre for Environmental Research (UFZ), Permoserstr. 15, 04318 Leipzig*
[b] *Faculty of Life Sciences, University of Leipzig, Johannisallee 21-23, 04103 Leipzig*
** e-mail: Selina.Hanisch@ufz.de*

Nature-based solutions, such as the microbial mat cultivation platform, are resource-efficient concepts that are adapted to spatial areas facing environmental and economic challenges.[1] This project aims to design microbial mats, synthesizing the emission-free energy carrier biohydrogen and other commercially attractive products,[2] via a biological pathway using light as primary substrate. In contrast to single-species, these complex and robust consortia take optimal advantage of the solar spectrum,[3] thus, concerting the activity of the different locally-associated microorganisms for the design of energy-efficient photo-biocatalytic processes.
In static screening experiments, two photoautotrophic organisms, *Chlorella* spp. and *Synechocystis* sp. PCC 6803, were identified as promising consortia candidates for absorbing visible light and utilizing CO_2 for biomass production. They exhibited good compatibility with the chemoheterotrophic supporter strain *Pseudomonas taiwanensis* VLB120eGFP, whose role is to initiate biofilm formation and respire the produced O_2, and the photoheterotroph *Rhodopseudomonas palustris*, which absorbs infrared light. The different species combinations are to be assessed under continuous flow conditions in a developed microfluidic flow-cell set-up.
The goal is to obtain a quantitative understanding of the fundamental parameters needed to engineer such structured phototrophic microbial communities in biofilms.

1. G. Somarakis, S. Stagakis & N. Chrysoulakis. *ThinkNature-Nature-Based Solutions Handbook*, **2019**.
2. A. Eng & E. Borenstein. *Curr. Opin. Biotechnol.* **2019**, *58*, 117–128.
3. J. Seckbach & A. Oren: Microbial Mats: Modern and Ancient Microorg. in Stratified Systems. 14th Edition, Springer Science & Business Media: 2010.

ENGINEERING CYANOBACTERIA AS THE BASIS FOR A SYNTHETIC MICROBIAL COMMUNITY

D. Hasenklever*[,a], T. Berwanger[b], J. C. Pohlentz[b], K. Schipper[b], A. B. Matuszyńska[c] and I. M. Axmann[a]

[a] *Institute for Synthetic Microbiology, Department of Biology, Heinrich Heine University Düsseldorf, Universitätsstraße 1, 40225 Düsseldorf, Germany*
[b] *Institute of Microbiology, Department of Biology, Heinrich Heine University Düsseldorf, Universitätsstraße 1, 40225 Düsseldorf, Germany*
[c] *Computational Life Science, Department of Biology, RWTH Aachen University, Worringerweg 1, 52074 Aachen, Germany*
** e-mail: dennis.hasenklever@hhu.de*

In nature, different microorganisms form highly complex communities in which every species has a distinct role. One example are lichens, symbiotic associations of phototrophic cyanobacteria (or algae) with heterotrophic fungi. To increase our understanding of the complex structures and interactions within microbial communities and the role of each microbial partner, the design and analysis of simplified microbial model communities is necessary.

In this collaborative project, our goal is the *de novo* design of a synthetic microbial cross-kingdom community based on the well characterized and genetically amenable model organisms representing cyanobacteria (*Synechocystis* sp. PCC 6803, or *Synechococcus elongatus* PCC 7942), ascomycete (*Saccharomyces cerevisiae*) and basidiomycete fungi (*Ustilago maydis*).

Co-cultivation is based on the carbon source sucrose, which is produced by the phototrophic cyanobacterium using light and carbon dioxide. Sucrose secretion into the culture medium is achieved by inducible, heterologous expression of a sucrose permease gene in the cyanobacteria. This approach is often combined with salt stress and some other metabolic modifications such as overexpression of sucrose pathway genes, or the deletion of competing pathways or carbon sinks to increase sucrose production.

An important aspect for creating a synthetic community is to design and establish tools for the formation of stable co-cultures and the analysis/quantification of the microbial partners of the community. Thus, in this part of the project, we test different cultivation devices and photobioreactor setups for online monitoring of co-cultures as well as single-cell flow cytometry analysis for quantification of individual populations within the co-culture. Another important aspect for the formation of a synthetic microbial community is the characterization of the optimal cultivation conditions for cyanobacterial sucrose production and simultaneous growth of all co-culture partners. In order to track the carbon source sucrose and other important metabolites within the co-culture, we also intend to establish biosensors in cyanobacteria.

Exploiting these established tools, we will create a stable synthetic microbial community which will then be further used to characterize the nutrient exchange in microbial consortia with a special focus on carbon economics and logistics.

_____

RESPONSES AND ADAPTATIONS TO HIGH CO_2 IN CYANOBACTERIA

Elena Carrasquer-Alvarez* [a], Adrian S. Geissler [b], Jan Gorodkin [b], Stefan E. Seemann [b], Niels-Ulrik Frigaard [a]

[a] *Marine Biological Section, Department of Biology, University of Copenhagen, Denmark*

[b] *Center for non-coding RNA in Technology and Health, Department of Veterinary and Animal Sciences, University of Copenhagen, Denmark*

** e-mail: elena.carrasquer@bio.ku.dk*

Photosynthetic microorganisms have adapted to sequester CO_2 from the atmosphere at very low CO_2 concentrations, which are often limiting for growth (around 400 ppm). Therefore, these organisms are typically not naturally adapted to very high CO_2 concentrations and are often inhibited at concentrations corresponding to 5–15% CO_2 in the gas phase. Our goal is to understand why cyanobacteria are inhibited under high CO_2 and to bioengineer the organisms such that they function under these conditions. A better understanding of the physiology under high CO_2 may enable more flexible uses of photosynthetic microbes in biotechnology, for example, if the microbes are cultivated in anthropogenic exhaust gasses containing high CO_2.

We have characterized the physiological behavior of the cyanobacterium *Synechococcus* sp. PCC 7002 under conditions of controlled CO_2 concentrations (corresponding to 0.04 to 30% v/v in the gas phase) and different pH (6.5 to 8.5). We used measurements of growth, pigments, and transcriptome responses to evaluate the inhibitory effects of the different inorganic carbon species. Moreover, we studied the transcriptome and investigated which genes and pathways are differentially expressed in high CO_2 conditions with Ilumina sequencing of RNA samples. Mutants with genetically inactivated CO_2 and HCO_3^- uptake mechanisms will be tested to evaluate their significance at different CO_2 concentrations.

REGULATION OF THE CARBON FLUX IN *SYNECHOCYSTIS* USING THE PGAM-PIRC SWITCH

N. S. Becker*[,a], T. Orthwein[a], S. Klähn[b] and K. Forchhammer[a]

[a] *Microbiology/Organismic Interactions, University of Tübingen, Auf der Morgenstelle 28, 72076 Tübingen, Germany*

[b] *Helmholtz Centre for Environmental Research, Permoserstr. 15, 04318 Leipzig, Germany*

** e-mail: nathalie.becker@uni-tuebingen.de*

For a sustainable bioeconomy, CO_2-neutrality is pivotal. Therefore, *Synechocystis* sp. PCC 6803 is studied concerning the metabolic flux to broaden the bioengineering platform for biotechnological applications. An important control point of the carbon flux is the 2,3-bisphosphoglycerate-independent phosphoglycerate-mutase (PGAM), which converts the first CO_2 fixation product 3-phosphoglycerate to 2-phosphoglycerate. This reaction directs carbon flow towards lower glycolysis for the production of amino acids, fatty acids, biopolymers, such as polyhydroxybutyrate (PHB) and more.

Previous work in our lab has shown that the small protein PirC inhibits PGAM activity through binding.[1] PirC itself is repressed in presence of ATP or ADP by the PII protein, which acts as a signal integrator of the carbon and nitrogen.[1] Furthermore, on the basis of the PirC mutation, a strain was constructed that can produce up to 80 % PHB per cell dry mass under nitrogen starvation.[2] Additional, preliminary work indicates that PGAM production is downregulated in response to nitrogen starvation after 2 days. Our work aims to use this key hub to direct the metabolic flux towards lower glycolysis and the production of PHB and other feedstock chemicals. Therefore, inducible and tunable promoters, such as P_{petE} and P_{petJ}, will be tested in the pSEVA system to adjust both PGAM expression and repression by PirC and to deviate the carbon flux towards lower glycolysis.

Hence, by tuning the PGAM-PirC switch, a platform will be established for the redirection of carbon flow for enhanced valuable chemical production.

Engineering the cyanobacterium *Synechocystis* into a synthetic organelle

Jeannine Volke[*,1], Michael Feldbrügge[2,3], Marion Eisenhut[4], Andreas P.M. Weber[1,3]

[1] *Institute of Plant Biochemistry, Heinrich-Heine University Duesseldorf, Germany.*
[2] *Institute of Microbiology, Heinrich-Heine University Duesseldorf, Germany.*
[3] *Cluster of Excellence on Plant Sciences (CEPLAS), Heinrich-Heine University Duesseldorf, Germany.*
[4] *Institute of Computational Biology, University Bielefeld, Germany.*
**e-mail: jeannine.schlebusch@uni-duesseldorf.de*

Plastids in plants and algae evolved through the endosymbiotic incorporation of an ancient photosynthetic cyanobacterium. The reduction of the cyanobacterium to an organelle involved processes like endosymbiotic gene transfer, development of a protein targeting and import system, metabolic integration through the insertion of transporters, retrograde and anterograde signaling, and the synchronization of host and endosymbiont cell cycles [1]. It is suggested that the development of the plastid involved an additional partner, a Chlamydia-like intracellular pathogen. In this Ménage à trois hypothesis, the cyanobacterium and the bacterial pathogen coexisted within a parasitophorous vacuole and a hexose-phosphate and an adenine nucleotide transporter were acquired by the nascent plastid and cyanobacterial glycogen storage was lost early during this tripartite symbiosis [2].
We test the hypothesis that losing the glycogen storage capacity while being externally supplied with energy is a basic step towards metabolic cooperativity between cyanobacterial endosymbiont and host.
To this end, we use the model cyanobacterium *Synechocystis* sp. PCC6803 (*Synechocystis*) and generated a fully segregated mutant in ADP-glucose pyrophosphorylase (*glgC*). The Δ*glgC* mutant was impaired in glycogen accumulation and in growth under different light and dark rhythms. Mutant strains with additional deletions in the carbon partitioning pathway and expression constructs for different transport proteins (e.g., ATP transporter, hexose-phosphate carrier) were generated. The metabolic and physiological effects on the engineered strains are currently under work and will be discussed with respect to its relevance for metabolic cooperativity.

[1] S.B. Gould *et al.* (2008). *Annu. Rev. Plant Biol.* 59

[2] F. Facchinelli *et al.* (2013). *TiPS* 12

Photosynthesis-driven whole-cell biocatalysis using *Synechocystis* sp. PCC 6803 for the conversion of cyclohexane to cyclohexanone.

N. Siebert [*a], A. Tüllinghoff [b], B. Bühler [b] and R. Karande [a]

[a] *b-ACT matter - Interfaculty Centre for Bioactive Matter, Leipzig University, Germany*
[b] *Department Environmental microbiology, Helmholtz Centre for Environmental Research - UFZ, Leipzig, Germany*
** e-mail: nina_antonia.siebert@uni-leipzig.de*

Photosynthesis-driven whole-cell biocatalysis holds tremendous potential for developing sustainable and environmentally friendly processes [1]. Photoautotrophic organisms, such as cyanobacteria, convert inorganic carbon (CO_2) to organic carbon by using water, light energy, and various nutrients. The light energy absorbed by the cyanobacterial cell in the photosynthetic apparatus is used to split water molecules and produce activated reduction equivalents and O_2, which can serve as co-substrates for oxygenase-catalyzed reactions [2].
This study aims to design a recombinant photoautotrophic strain and gain insight into photosynthetically driven redox biocatalysis using a 2-step heterologous enzyme cascade, consisting of a cytochromeP450 monooxygenase and a cyclohexanol dehydrogenase. Both enzymes originate from the soil bacterium *Acidovorax* and are part of the initial cyclohexane degradation pathway [3]. In this poster, our cloning strategy to design the recombinant *Synechocystis* sp. PCC 6803 strain and the preliminary data for converting cyclohexane to cyclohexanone driven by photosynthetic water oxidation will be presented.

[1] J. Toepel, R. Karande, S. Klähn, & B. Bühler. Current Opinion in Biotechnology, **2023**, 80, art. 102892.
[2] A. Hoschek, B. Bühler, A. Schmid. Angew. Chem.-Int. Edit., **2017**, 56 (47), 15146 – 15149.
[3] R. Karande, D. Salamanca, A. Schmid, & K. Bühler. Biotechnol. Bioeng., **2018**, 115 (2), 312 – 320.

CYANOBACTERIAL BIOFILMS FOR PHOTOELECTROCHEMISTRY

Nathan V. Wright,[a] Conrad W. Mullineaux,[a] Ana J. Sobrido[b]

[a] *School of Biological and Behavioural Science, Queen Mary University of London, Mile End Road, London E1 4NS*
[b] *School of Engineering and Materials Science, Queen Mary University of London, Mile End Road, London E1 4NS*
** e-mail: n.v.wright@qmul.ac.uk*

Cyanobacteria can export high energy electrons to conductive substrates to generate electrical photocurrent in a process known as external electron transport (EET),[1] resulting in current generation that can be utilised directly as electricity to power electronic devices, or hypothetically, grid-level applications with a theoretical maximum efficiency up to 7.7 W m^{-2}.[2] Cyanobacterial photocurrent has also previously been demonstrated to power the reduction of protons into hydrogen at the cathode for energy storage in a three-electrode bio-photoelectrochemical device.[3] Type 4 Pili (T4P), cell surface appendages composed of major and minor pilin subunits are crucial for motility and conjugation and contribute to EET efficiency by controlling biofilm structure and organisation around the anode,[4] with current output correlating with chlorophyll loading density.[5]

Anode material is of equal importance. Electrospinning is a technique whereby carbon polymers are dissolved and extracted from a fine needle using a charge gradient and collected on a metal place to form a nanofibrous mesh. The resulting electrospun mat is a microporous semi-conductor with very high active surface area per unit mass.[6] Contemporary electrospun mats are generated from PAN (polyacrylonitrile), a petrochemical derivative, but sustainable sources of carbon such as lignin or cellulose derived from waste biomass also offer potential as semiconductors.

Compared to contemporary solar panels, bio-photovoltaics can offer cheap green energy from a fully carbon-based power source, with the potential for carbon capture in the form of biomass, energy storage in the form of hydrogen, low input costs from earth-abundant materials and low maintenance costs due to bacterial proliferation and self-repair. In this work, I investigate the importance of biofilm structure on photocurrent output by investigating the current profile of T4P mutant strains, focusing on minor pilin mutants. The aim is to engineer a bio-photoanode from sustainable electrospun fibres and cyanobacteria to produce photocurrent and hydrogen.

1. D. J. Lea-Smith, P. Bombelli, R.Vasudevan, C. J. Howe. *BBA*, **2015,** *1857,* 247-255.
2. A. J. McCormick, P. Bombelli, R. W. Bradley, R. Thorne, T. Wenzel, and C. J. Howe. *Energy Environ. Sci.*, **2015**, *8,* 1029-1109.
3. G. Saper *et al. Nat Commun,* **2018,** *9*, 2168.
4. L. T. Wey, J. M. Lawrence, X. Chen, R. Clark, D. J. Lea-Smith, J. Z. Zhang. C. J. Howe. *Electrochim. Acta,* **2021,** *395,* 139214.
5. J. Z. Zhang, P. Bombelli, K. P. Sokol, A. Fantuzzi, A. W. Rutherford, C. J. Howe, E. Reisner. *J. Am. Chem. Soc.,* **2017,** *140,* 6-9.
6. J. P. Tafoya, M. Thielke, G. Tian, R. Jervis, and A. B. Sobrido. *Curr. Opin. Chem. Eng.,* **2022,** *38***,** 100876.

DESIGN AND IMPLEMENTATION OF A NADP$^+$/FERREDOXIN-REDUCING O_2-TOLERANT HYDROGENASE IN *SYNECHOCYSTIS*

Diletta Sacco *[,a], Petra Till[b], Elisabeth Lettau[c], Lars Lauterbach[c], Jörg Toepel[a] and Bruno Bühler [a]

[a] *Dept. of Environmental Microbiology, Helmholtz Centre for Environmental Research –UFZ, 04318, Leipzig, Germany*

[b] *ICEBE, TU Wien, Wien, Austria*

[c] *iAMB Institute of Applied Microbiology (Bio IV) Aachen, Germany*

** e-mail: diletta.sacco@ufz.de*

Currently, the search for clean energy sources to mitigate climate change while preventing the shortage of available fuel is pushing towards H_2 as an energy carrier. In this context, biotechnological H_2 production driven by oxygenic photosynthesis is a desirable approach, as hydrogenase-catalyzed H_2 synthesis thereby mainly relies on light, water, and CO_2 fixation. Yet, the application of this biological process is hindered by the simultaneous release of O_2 being a strong inhibitor of most native NAD(P)H and ferredoxin (Fd) accepting hydrogenases. Previous studies showed the possibility to implement the O_2-tolerant NAD$^+$-dependent hydrogenase from *Cupriavidus necator* into *Synechocystis* sp. PCC 6803 [1]. The obtained results showed that efficient electron transfer to the hydrogenase by means of oxygenic photosynthesis requires a switch of cofactor dependence from NAD$^+$ to NADP$^+$/Fd. A viable option is the fusion of the hydrogenase module from C. *necator* with a NADPH/Fd binding subunit (diaphorase module) from a different strain. In this regard, we focus on the *Synechocystis* diaphorase module. Plasmids encoding chimeric hydrogenases were created and integrated into *Synechocystis*. At the moment, strains with chimeric enzymes are tested regarding hydrogenase assembly and activity. As an alternative strategy, we aim at changing the cofactor dependency of the NAD$^+$-reducing HoxF subunit from C. *necator* via targeted enzyme engineering. Variants are evaluated regarding their assembly and activity via *in vivo* assays. The latter includes cultivation under continuous illumination in yBG11 medium in sealed glass vials with a headspace gas mixture of 20% H_2, 10% CO_2, and 70% N_2. H_2 depletion is monitored via GC. Furthermore, we assess if H_2 oxidation by engineered hydrogenases can support growth of *Synechocystis*, by incubating cells in sealed vials flushed with the above-mentioned gas mix in the presence of DCMU. DCMU hinders water oxidation at PS II. Thus, growth only can occur via a lithoautotrophic lifestyle based on H_2. The cofactor dependency of variants is evaluated *in vitro* by assaying soluble protein fractions for H_2-dependent NAD$^+$ or NADP$^+$ reduction. Finally, the designed strains will be tested regarding sustained photo-H_2 production fueled by photosynthetic water splitting.

1. S. Lupacchini et al. *Metabolic Engineering*, **2021**, *68*, 199–209.

Anoxic culture conditions promote higher growth rates for *Pseudanabaena* and potentially other biotechnological strains.

Kimberly M. Kaiser[a]*, Sadia S. Tamanna[a], Sofia Conrad[a], Michelle M. Gehringer[a], Nicole Frankenberg-Dinkel[a]

[a]*Faculty of Biology, University of Kaiserslautern-Landau, Gottlieb-Daimler Straße, 67663 Kaiserslautern, Germany*
**schroedk@rptu.uni-kl.de*

Cyanobacteria evolved over 3.4 Ga, during a time period in Earth's history, in which the atmosphere did not contain O_2.[1] Investigations into photosynthetic efficiency using the deep branching, marine strain, *Pseudanabaena* sp. PCC7367, have demonstrated higher rates of O_2 release under anoxic conditions than under modern oxic conditions.[2] In this study, we measured the growth rate of *Pseudanabaena* sp. PCC7367 under three atmospheric conditions, (1) present atmospheric levels with low CO_2 (LC), (2) supplemented with CO_2 to 2000 ppm (HC) or (3) simulate an Archean atmosphere. The growth rate under Archean conditions was significantly higher ($p<0.001$) than under LC & HC conditions, while protein and glycogen levels were also significantly raised ($p<0.05$). Furthermore, medium O_2 levels were significantly higher under LC growth, when compared to both HC or simulated Archean atmospheres under stationary and stirred conditions. These observations indicate possible enhanced respiration rates for *Pseudanabaena* under present LC atmosphere.
The results of this investigation suggest that growing biotechnologically important strains such as *Synechocystis* sp. PCC6803 and *Synechococcus* sp. PCC7002, under anoxic conditions may increase growth rates, as well as protein and glycogen content.

1. Klatt J. M, Chennu A, Arbic B.K, Biddanda B.A, Dick G.J. (2021). Possible link between Earth's rotation rate and oxygenation. *Nat. Geosci*, 14 (8):564–570.
2. Herrmann, A., Sorwat, J., Byrne, J., Frankenberg-Dinkel, N., & Gehringer, M. (2021). Diurnal Fe (II)/Fe (III) cycling and enhanced O2 production in a simulated Archean marine oxygen oasis. *Nature Communications*, 12(1), 1-11.