ISPP 2022

17th International Symposium on Phototrophic Prokaryotes

21-25 August 2022, Liverpool, UK

Abstract Book

5

Increased photosynthetic production of sucrose by engineered Synechocystis sp. PCC6803 cells immobilized in polymer matrices

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Cyanobacteria are photosynthetic microbes with low nutrient requirements and a versatile metabolism, which makes them a desirable chassis for sustainable biomanufacturing of different solar chemicals. Genetically engineered cyanobacterium *Synechocystis* sp. PCC 6803, strain S02 [1] is capable of synthesizing sucrose via photosynthetic CO_2 fixation and excreting it into the medium. This provides a sustainable carbon source for different applications, including biosynthesis of high-value products by heterotrophic bacteria and yeasts in artificial photoautotroph-heterotroph consortia. For further improvement of sucrose production by *Synechocystis*, we entrapped cells in polymer matrices. Entrapment offers a protective 3D scaffold to the cells prolongs the lifespan of the cells and conserves energy, as cell growth is restricted. Furthermore, it alters the metabolism of the confined cells towards efficient sucrose production. We demonstrated that entrapment of S02 cells in Ca^{2+}-cross-linked alginate beads improves their specific productivity (normalized to chlorophyll *a* content) by 86% as compared to suspension cultures within seven days of cultivation under osmotic stress. The sucrose production process stops after seven days but it can be prolonged up to 27 days by applying a semi-continuous production strategy. Under the semi-continuous condition, ~3000 mg l^{-1} of sucrose is produced by the beads after 17 days. Photosynthetic activity of the cells and gas fluxes were studied in the suspension cultures and entrapped cells using fluorescence and membrane inlet mass spectrometry. We concluded that cell entrapment in Ca^{2+}-alginate beads is an effective method for prolonged sucrose production in cyanobacteria and allows the separation of cells from the medium, thus facilitating downstream process optimization. This property opens up novel possibilities for creating advanced artificial photoautotroph-heterotroph consortia for the sustainable production of chemicals using CO_2 and light energy.

[1] Thiel, K. et al. (2019) Redirecting photosynthetic electron flux in the cyanobacterium Synechocystis sp. PCC 6803 by the deletion of flavodiiron protein Flv3. Microb Cell Fact 18, 189.

6

Differential translation and novel small proteins and their functions in cyanobacteria

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The number of small proteins (s-proteins) <70 amino acids and their importance for life are underestimated. During genome annotation, the corresponding small open reading frames (sORFs) are often neglected and also the direct s-protein detection by mass spectrometry is not straightforward. However, acting as regulators, subunits, linkers or tags, s-proteins play important roles for cells and organisms. In Cyanobacteria, such proteins are known to function as protease tags like NblA [1] or subunits of photosystem complexes I and II [2,3]. We have been establishing alternative approaches for s-protein identification, based on the bioinformatic analysis of transcriptomic data [4] and adapting protocols for ribosome profiling (RiboSeq [5]) in *Synechocystis* sp. PCC 6803. In combination with Retapamulin treatment, an antibiotic that stalls ribosomes at translation initiation sites [6], novel sORFs can unambiguously be identified, even in gene-internal locations and antisense orientations. This has led to finding more than 250 novel s-protein candidates, several of which have been confirmed by epitope tagging, including intergenic, intragenic and antisense examples.
For the more detailed functional characterization we have been focusing on s-proteins that are induced by changing environmental conditions assuming that their functions might be related to stress responses and acclimation to unfavorable settings. Following a strategy including coimmunoprecipitation, the characterization of deletion, overexpression and complementation strains, we identified NblD as a new player in the programmed phycobilisome dismantling during nitrogen starvation [7], AtpΘ as a regulator of the ATP synthase reverse reaction [8] and several more that highlight intriguing molecular mechanisms and solve long-standing questions in the physiology and molecular biology of phototrophic prokaryotes.

[1] Collier & Grossman (1994) *EMBO J.* 13, 1039–1047.
[2] Kashino *et al.* (2002) *Biochemistry* 41, 8004–8012.
[3] Shi & Schröder (2004) *Biochim. Biophys. Acta* 1608, 75–96.
[4] Baumgartner *et al.* (2016) *BMC Microbiol.* 16, 285.
[5] Ingolia *et al.* (2009) *Science* 324, 218–223.
[6] Weaver *et al.* (2019) *mBio* 10, e02819-18.
[7] Krauspe *et al.* (2021) *Proc. Natl. Acad. Sci. USA* 118, e2012277118.
[8] Song *et al.* (2022) *Curr. Biol.* 32, 136-148.e5.

7

Identification of RNA-based interactions and regulation in the cyanobacterial model Synechocystis sp. PCC 6803

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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). This kind of regulation impacts disproportionately often photosynthetic processes. In *Synechocystis* sp. PCC 6803, genes encoding photosynthesis-relevant proteins are repressed by the sRNA PsrR1 after shifts to high light (2) and by IsaR1 during phases of iron starvation (3), while ApcZ antagonizes expression of the Orange Carotenoid Protein if conditions favor light harvesting via the phycobilisomes (4). 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 (5). 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 (6). 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 (7). This suggests that RNA binding proteins play an important role in the correct function of cellular mechanisms in phototrophic organisms. Therefore, the in-depth characterization of these photosynthesis-relevant RBPs, the identification of new RBPs and the verification and characterization of novel sRNAs 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. Georg, et al. Plant Cell. 2014 (9):3661–79
3. Georg et al. (2017) Current Biology 27, 1425–36.
4. Zhan et al. (2021) Plant Cell 33, 358–80.
5. Irastortza-Olaziregi M, Amster-Choder O. WIREs RNA. 2020;12(2):e1615.
6. Holmqvist E, Vogel J. Nat Rev Microbiol. 2018 (10):601–15.
7. Mahbub M, et al. Nat Plants. 2020 (9):1179–91.

8

CyanoTag: High-throughput protein localisation in cyanobacteria

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Photosynthetic prokaryotes are of fundamental importance to aquatic ecosystems and global carbon cycling and have exciting potential for use in sustainable bioindustries. However, much of their basic biology is not yet understood and their genomes still contain large numbers of uncharacterised protein-coding genes. To accelerate our understanding of these essential microorganisms we have developed a high-throughput pipeline for the scarless integration of tags that facilitate protein localisation and interactome profiling in the model cyanobacterium *Synechococcus elongatus* PCC7942. We have used this CyanoTag platform to fluorescently label over 320 *S. elongatus* proteins – more than 10% of the proteome. Localisation of these proteins via super-resolution imaging has provided novel biological insights into a diverse range of processes including the regulation of photosynthesis and cell division. In this presentation we will share some of these insights, our progress towards expanding the CyanoTag library to the whole *S. elongatus* proteome, and the opportunities this resource could provide for the cyanobacterial research community.

9

Diurnal regulation of inorganic carbon uptake in cyanobacteria

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Phytochromes are light receptors that regulate many processes in plants, algae, fungi and prokaryotes. *Bona fide* phytochromes can absorb red light (in nature day light) resulting in physiologically active P_{fr} conformation, whereas far-red light (in nature darkness) converts it back into physiologically non-active P_r. In the cyanobacterium *Synechocystis* sp. PCC 6803 two phytochromes are annotated. Cph2 is known for its role in cell motility, while the physiological function of Cph1 is not clear yet.
Recently, it has been shown that red/far-red light signals regulate the activity of the inorganic carbon-concentrating mechanism (CCM) in *Synechocystis* involving the phytochrome Cph1 and the carbon-regulatory protein SbtB [1]. However, the mechanism how phytochromes are activating the CCM during the day and inactivating it during the night is unknown. We are aiming to identify a signalling pathway connecting light sensing via Cph1 to regulation of the carbon concentrating mechanism via SbtB. Because the light-dependent histidine kinase *cph1* is found in an operon with the response regulator *rcp1*, we assume that Rcp1 is transducing the light signal. The Rcp1 belongs to the CheY family of response regulators with no annotated DNA-binding domain. Hence, a direct interaction with target proteins via phosphorelay is necessary. Mutants in the proposed signal transduction will be generated and compared in their ability to regulate CCM activity in response to light signals.

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[1] Oren N *et al* (2021) Red/far-red light signals regulate the activity of the carbon-concentrating mechanism in cyanobacteria. Sci. Adv. 7:eabg0435

10

Photobiocatalytic Oxyfunctionalization with High Rate using Baeyer-Villiger Monooxygenase from Burkholderia xenovorans in Metabolically Engineered Cyanobacteria

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The synthesis of ε-caprolactone, an important polymer precursor is currently performed using hazardous peracids from the oxygenation of cyclohexanone[1]. Baeyer-Villiger Monooxygenases (BVMO's), on the other hand could catalyse the same reaction under environmentally-benign conditions[2]. However, the requirement for auxiliary substrates needed for co-factor recycling as well as supplying the necessary oxygen for the reaction hinder this enzymatic route[3]. The recombinant production of BVMO in cyanobacteria allows substitution of auxiliary compounds with water and utilizes oxygen directly from water splitting. Herein, we report the identification of a BVMO from *Burkholderia xenovorans* ($BVMO_{Xeno}$) exhibiting higher reaction rates than previously tested BVMOs in the synthesis of ε-caprolactone. We report a 10-fold increase in specific activity (25 vs 2.3 U g_{DCW}^{-1}) as compared to cyclohexanone monooxygenase from *Acinetobacter* sp. ($CHMO_{Acineto}$). The faster reaction rate and minimal ketoreduction could be attributed to a 10-fold lower K_M value of $BVMO_{Xeno}$ as compared to $CHMO_{Acineto}$. Furthermore, the specific activity was enhanced by further manipulation of the photosynthetic electron transport chain (PETC). The photosynthetic oxygen evolution can contribute to alleviate the highly problematic oxygen mass-transfer limitation of oxygen-dependent enzymatic processes.

[1] Schmidt S *et al* (2015) An enzyme cascade synthesis of e-caprolactone and its oligomers. Angew. Chem., Int. Ed. 54:2784-7; [2] M.J.L.J. Fürst MJLJ *et al* (2019) Baeyer-Villiger Monooxygenases: Tunable Oxidative Biocatalyts. ACS Catal. 9:11207-11241 [3] Böhmer S *et a*l (2017) Enzymatic Oxyfunctionalization driven by Photosynthetic Water-Splitting in the Cyanobacterium *Synechocystis* sp. PCC 6803. Catalysts 7:240.

12

Longevity of photosynthesis under desiccated state

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The terrestrial cyanobacterium *Nostoc commune* is an anhydrobiotic organism with extreme longevity. Lipman (1941) reported that the specimen of *N. commune* from the Cryptogamic Herbarium of the Field Museum of Natural History (Chicago, IL) was revived and grown in culture solution eighty-seven years after collection and placement in the herbarium [1]. The laboratory culture of *N. commune* strain DRH1 was isolated from the desiccated materials of *N. commune* CHEN, which was collected in 1981 and stored for 10 to 12 years until being used as an inoculum source. Because *N. commune* does not differentiate into akinetes, vegetative cells are thought to sustain the ability to grow in a desiccated state. Recovery of photosynthesis in a short time after rehydration was examined using our laboratory stocks of dry *N. commune* thalli after long-term storage in a desiccated state [2]. In the samples stored at room temperature for over 8 years, photosynthetic oxygen evolution was barely detectable, whereas oxygen consumption was recovered. There was an exceptional case in which photosynthetic oxygen evolution recovered after 8 years of storage at room temperature. Carotenoids, scytonemin and chlorophyll *a* appeared to be intact but β-carotene and α-tocopherol characteristically disappeared in the dry thalli stored at room temperature. The ability to recover photosynthesis after rehydration in the dry thalli of *N. commune* is gradually damaged at room temperature and can only be maintained for several years. Two important questions remain to be answered: (1) how *N. commune* cells become dormant in response to dehydration and (2) how they safely recover biological activities by rehydration. A biochemical approach is a requisite to solve these questions with respect to the extreme desiccation tolerance of the photosynthetic machinery in *N. commune*.

[1] Lipman CB (1941) The successful revival of *Nostoc commune* from a herbarium specimen eighty-seven years old. Bull Torr Bot Club 68:664-666. DOI: 10.2307/2481755
[2] Sakamoto T *et al* (2022) Recovery of photosynthesis after long-term storage in the terrestrial cyanobacterium *Nostoc commune.* J Gen Appl Microbiol (in press)

13

Molecular mechanisms involved in the symbiotic interaction between Oryza sativa and Nostoc punctiforme

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The symbiotic cyanobacterium *Nostoc punctiforme* has been extensively used as a model to study symbiosis between cyanobacteria and plants. Here, we have used this cyanobacterium to promote a long-term, stable endophytic association with *Oryza sativa*, which has been studied by means of confocal microscopy [1]. In order to know the signalling mechanisms involved in recognition between the plant and the cyanobacterium, we conducted a quantitative proteomic analysis in both organisms at early stages of the pre-symbiotic process [2]. We found proteins differentially accumulated linked to several biological functions, including signal transduction, adhesion, defence-related proteins, and cell wall modification. In *Nostoc punctiforme* we found increased expression in 62 proteins that have been previously described in other *Nostoc*-plant symbioses, reinforcing the robustness of our study. Our findings reveal new proteins activated in the early stages of the *Nostoc-Oryza* symbiosis that might be important for the recognition between the plant and the host. *Oryza* mutants in genes in the common symbiosis signalling pathway (CSSP) show a reduced colonization efficiency providing first insights on the involvement of the CSSP for the accommodation of *N. punctiforme* inside the plant cells. This information may have long-term implications for a greater understanding of the symbiotic interaction between *Nostoc* and land plants.

[1] Álvarez C, Navarro JA, Molina-Heredia FP, Mariscal V (2020) Endophytic colonization of rice (*Oryza sativa* L.) by the symbiotic strain *Nostoc punctiforme* PCC 73102. Molecular Plant-Microbe Interactions 33: 1040–1045

[2] Álvarez C, Brenes-Álvarez M, Molina-Heredia FP, Mariscal V (2022) Quantitative Proteomics at Early Stages of the Symbiotic Interaction between *Oryza sativa* and *Nostoc punctiforme* reveals novel proteins involved in the symbiotic crosstalk. Plant and Cell Physiology, accepted.

14

Structure of a fusogenic cyanobacterial dynamin-like protein

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Dynamin-like proteins (DLPs) are large GTPases that utilize the energy of GTP hydrolysis to remodel membranes. Unlike many eukaryotic DLPs, only few bacterial DLPs (BDLPs) have been identified and characterized thus far. In fact, a defined *in vivo* activity of a BDLP has not been discovered yet. While cyanobacteria contain an extended and highly dynamic thylakoid membrane (TM) system, biogenesis and dynamics of this internal membrane structure is still poorly understood. An involvement of BDLPs in membrane remodeling in cyanobacteria is well conceivable.
We have identified *Syn*DLP, a novel DLP encoded in the cyanobacterium *Synechocystis* sp. PCC 6803. Isolated *Syn*DLP forms higher-ordered oligomeric structures and interacts with negatively charged TM lipids, and this interaction is enhanced by nucleotide binding. *Syn*DLP forms filamentous oligomers in solution in absence of lipids and/or nucleotides, features not observed with other BDLPs. *Syn*DLP is capable of liposome fusion *in vitro*, albeit this membrane fusion activity is nucleotide-independent. Consequently, membrane remodeling by *Syn*DLP appears not to directly require the energy released by GTP hydrolysis.
To better understand the structure and functions of *Syn*DLP, we also determined a cryo-TEM structure of *Syn*DLP filaments up to a resolution of 3.6 Å. Besides a typical dynamin-like modular domain arrangement, the structure revealed new DLP features, such as an intramolecular disulfide bond in the bundle signaling element (BSE) and an expanded interface between the BSE and the GTPase domain (GD). *Syn*DLP shows a relatively high basal GTPase activity, albeit established GD-activating contacts cannot be observed in the structure. Removal of the BSE stabilizing disulfide bond or BSE-GD contacts affects the *Syn*DLP GTPase activity and stability of *Syn*DLP oligomers, indicating a hitherto unique concept of DLP trans-activation.
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. *Syn*DLP interacts with TM lipids and is able to fuse TM-mimicking membranes. Thus, *Syn*DLP is a novel member of the DLP group of *fusion DLPs*.

15

Regulation of carbon acclimation in cyanobacteria

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Cyanobacteria fix inorganic carbon (Ci) via the Calvin-Benson cycle, which is supported by an inorganic carbon-concentrating mechanism (CCM). The activity of the CCM needs to be regulated according to fluctuating Ci conditions. The carbon acclimation on transcriptional level is quite well understood, however, changes in carbon flux and transport activities seem to be mainly regulated on the posttranscriptional level. During the last years, we provided evidence that the light-sensing phytochrome system is involved in the inhibition of Ci uptake in the darkness (1). Furthermore, phosphorylation of metabolic enzymes and transporters is possibly responsible for the fine-tuning of carbon fluxes into different metabolic branches (2). In addition, regulatory proteins such as PirC have been shown to modulate the flux of carbon into lower glycolysis (3). Our current knowledge of carbon regulation and its possible application in cyanobacterial biotechnology will be presented and discussed.

1. Oren N, Timm S, Frank M, Mantovani O, Murik O, Hagemann M (2021) Red/far-red light signals regulate the activity of the carbon-concentrating mechanism in cyanobacteria. *Science Advances* 7, eabg0435.

2. Spät P, Barske T, Maček B, Hagemann M (2021) Alterations in the CO_2 availability induce alterations in the phospho-proteome of the cyanobacterium *Synechocystis* sp. PCC 6803. *New Phytologist* 231, 1123-1137.

3. Orthwein T, Scholl J, Spät P, Lucius S, Koch M, Macek B, Hagemann M, Forchhammer K (2021) The novel PII-interactor PirC identifies phosphoglycerate mutase as key control point of carbon storage metabolism in cyanobacteria. *Proceedings of the National Academy of Sciences USA (PNAS)* 118, e2019988118.

16

Sustaining Rice Production through Biofertilization with N2-Fixing Cyanobacteria

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Current agricultural productivity depends on an exogenous nutrient supply to crops. This is of special relevance in cereal production, a fundamental part of the trophic chain that plays a trial role in the human diet. However, most common agricultural practices entail highly detrimental side-effects from an environmental point of view. Long-term nitrogen fertilization in croplands results in degradation of soil, water, and air quality, producing eutrophication and subsequently contributing to global warming. By this, there is a biotechnological interest in using nitrogen-fixing microorganisms to enhance crop growth without adding chemically synthesized nitrogen fertilizers. This is particularly beneficial in paddy fields, where about 60% of the synthetic fertilizer that has been applied is dissolved in the water and washed away. In these agricultural systems, N_2-fixing cyanobacteria show promising biotechnological potential as biofertilizers, improving soil fertility while reducing the environmental impact of the agricultural practice. In the current study, Andalusian paddy fields have been explored to isolate N_2-fixing cyanobacteria. These endogenous microorganisms have been subsequently re-introduced in a field trial to enhance rice production. Our results provide valuable insights regarding the use of an alternative natural source of nitrogen for rice production.
Iniesta-Pallarés M *et al* (2021) Sustaining Rice Production through Biofertilization with N_2-Fixing Cyanobacteria. Appl Sci 11, 4628:1-12

17

Investigating the role of substrate binding proteins in nutrient assimilation in marine picocyanobacteria

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Marine picocyanobacteria play an important role in ecosystem functioning, as primary producers. Picocyanobacteria are a phylogenetically diverse group that are highly abundant across the world's oceans, including oligotrophic regions. Phosphorus (P) is an essential macronutrient for picocyanobacteria and hence its availability in the oceans is one of the key limiting factors for their growth and primary productivity. They have adopted several strategies to acquire P, including high-affinity transporters such as PstSCAB. Some *Synechococcus* strains inhabiting oligotrophic oceans have multiple copies of these high-affinity transporters. For example, strain WH8102 has three homologs of the binding component (PstS) of the PstABC transporter as well as a more distant homolog SphX. Conversely, strain CC9311 that inhabits nutrient-replete coastal regions has only a single copy of PstS. We hypothesize that multiple copies of putative phosphate binding proteins (i.e., PstS) in WH8102 may be functionally distinct in terms of substrate specificity or affinity.

To characterise the substrate specificity and ecological role of PstS homologs in strain WH8102, histidine-tagged substrate binding proteins (SBPs) were heterologously expressed in *E. coli* and purified using affinity and size-exclusion chromatography methods. Substrate specificity for each of these proteins were tested for various organic and inorganic phosphorus (Pi) sources using differential scanning fluorimetry (DSF). Using a novel nanoDSF-based isothermal approach we determined the binding affinity of all three PstS proteins to Pi and polyphosphate. Our results show that all copies of PstS from WH8102, except for SphX, have a strong preference for P_i compared to other P sources tested. Furthermore, a copy of PstS1 mostly conserved across all picocyanobacterial strains has high affinity (Kd 0.44 □M) for P_i, while PstS2 exhibit comparatively lower affinity (Kd 4.3□M) to Pi. This suggests that the presence of accessory transporter components with different phosphate affinities in WH8102 may allow them a significant advantage for P uptake and hence survival in nutrient-deplete oceans.

18

Building the charge-separating D1/D2 assembly complexes of Photosystem II in cyanobacteria

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Photosystem II (PSII) is the pigment-binding light-driven oxidoreductase that feeds photosynthetic electron transport chain by electrons extracted from water. To investigate the earliest steps of PSII assembly, we used strains of the cyanobacterium Synechocystis sp. PCC 6803 arrested at initial stages of PSII biogenesis and expressing affinity-tagged PSII subunits to isolate PSII reaction center assembly (RCII) complexes and their precursor D1 and D2 modules ($D1_{mod}$ and $D2_{mod}$). RCII preparations isolated using either a His-tagged D2 or a FLAG-tagged PsbI subunit contained the previously described RCIIa and RCII* complexes that differ with respect to the presence of the Ycf39 assembly factor and high-light-inducible proteins (Hlips). The preparations also contained a larger complex consisting of RCIIa bound to monomeric photosystem I (PSI). All RCII complexes contained the PSII subunits D1, D2, PsbI, PsbE and PsbF and the assembly factors rubredoxin A (RubA) and Ycf48 but we also detected PsbN, Slr1470 and the Slr0575 proteins, which all have plant homologues. The RCII preparations also contained FtsH protease complexes and prohibitins/stomatins (Phb) of unknown function. Phb occurrence and requirement of high detergent concentration for solubilisation of the D1/D2 complexes suggest location of these complexes in raft-like membrane microdomains nearby the plasma membrane. RCII complexes were active in light-induced primary charge separation and bound chlorophylls, pheophytins, beta-carotenes and heme. The isolated $D1_{mod}$ consisted of D1/PsbI/Ycf48 with some Ycf39 and Phb3 while $D2_{mod}$ contained D2/cytochrome b559 with co-purifying PsbY, Phb1, Phb3, FtsH2/FtsH3, CyanoP and Slr1470. The results indicate that neighbouring small subunits and most accessory factors bind to D1 and D2 soon after their synthesis. As stably bound chlorophyll was detected in $D1_{mod}$ but not $D2_{mod}$, formation of RCII appears important for stable binding of most of the chlorophylls and both pheophytins. We suggest that chlorophyll can be delivered to RCII from either monomeric PSI or Ycf39/Hlip complexes and therefore we propose an existence of two different pathways for RCII formation in cyanobacteria.

[1] Knoppová et al. (2022) Assembly of D1/D2 complexes of photosystem II: binding of pigments and a network of auxiliary proteins. Plant Physiology, doi.org/10.1093/plphys/kiac045

21

Polymorphisms in extracellular matrix proteins in the terrestrial cyanobacterium Nostoc commune

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The terrestrial cyanobacterium *Nostoc commune* is tolerant to multiple stresses such as desiccation, UV irradiation, oxidation and heat. *N. commune* forms colonies in which cellular filaments are surrounded by extracellular matrices (ECM). The ECM consists of polysaccharides and protein components including WspA (water stress protein), SodF (Fe-superoxide dismutase), catalase and Dps (DNA-binding proteins from starved cells), which are thought to be involved in the multi-stress tolerance of *N. commune* [1]. Four *N. commune* chemo-genotypes are identified thus far [2], and the genes encoding the ECM proteins were characterized in these *N. commune* genotypes. The SodF and NpDps4 homologs were found to be highly similar among the *N. commune* genotypes, whereas the catalase and NpDps1 proteins were different with each other. Moreover, WspA proteins were highly diverse and thus *wspA* genes did not seem to be xenologs [1]. Two *wspA* genes (AB518000 and LC195125) from the laboratory culture strain KU002 of *N. commune* (genotype A) were heterologously expressed in *E. coli* cells to produce WspA proteins and the polyclonal antibodies against each WspA protein were obtained. The WspA proteins in the water-soluble extracellular proteins from the different genotypes of *N. commune* colonies were examined by immunoblot analysis using both antisera. A single band with a size of 36 kDa was detected in genotypes A and C, a band with a size of 33 kDa was detected in genotype B, and two bands with sizes of 38- and 32 kDa were detected in genotype D. This result indicates that the WspA proteins in different *N. commune* strains are immunologically related with each other. Despite the proposed WspA polymorphism in *N. commune*, only a single *wspA* copy is likely to be expressed in genotypes A, B and C of *N. commune*, although there are WspA proteins with different apparent sizes in genotype D.

[1] Inoue-Sakamoto K *et al* (2018) Characterization of extracellular matrix components from the desiccation-tolerant cyanobacterium *Nostoc commune*. J Gen Appl Microbiol 64:15-25

[2] Sakamoto T *et al* (2019) Four chemotypes of the terrestrial cyanobacterium *Nostoc commune* characterized by differences in the mycosporine-like amino acids. Phycol Res 67:3-11

(347 words)

22

Exploring cyanobacterial protein secretion for ecology and biotechnology

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Protein secretion is a universal process which mediates interactions with the environment and plays a key role in various aspects of cell physiology. Protein secretion systems are ubiquitous in the cyanobacterial phylum, however their function and regulation remain largely unknown [1]. Here I will show how our recent work sheds light on these enigmatic cellular complexes.

Previously, we utilised the fast-growing cyanobacterium *Synechococcus elongatus* UTEX 2973 to express and secrete the industrially relevant enzyme *Tf*AA10A, a lytic polysaccharide monooxygenase from the gram-positive bacterium *Thermobifida fusca.* *Tf*AA10A was recognized by the cyanobacterial secretion machinery and virtually all the protein was directed to the culture medium [2]. Using the *Tf*AA10A signal peptide, we then developed a nanoluciferase (NLuc)-based secretion reporter to further explore cyanobacterial protein secretion. Reporter benchmarking showed that secretion levels could be detected using small volumes (< 1 µL) and with a sensitivity at least 10 times higher than traditional western blotting [3]. The development of this reporter has now allowed us to start exploring protein secretion in cyanobacteria from several different directions. First, we demonstrated that the two-step secretion of NLuc is independent of T4aP using a collection of *Synechocystis* type IV pili mutants [3]. More recently, we have been targeting different *S. elongatus* PCC 7942 translocation systems using the NLuc reporter fused to a collection of native and heterologous signal peptides (e.g. Sec/Tat, type I secretion system, microcin pathway) to shed light on the efficiency and function of different secretion routes. Finally, NLuc secretion has also been measured in several closely related *Synechocystis* and *S. elongatus* strains and the results suggest that a small number of mutations can lead to significant differences in secretion yields.

The results of these experiments will be discussed and contextualized considering current knowledge. Advances in cyanobacterial protein secretion will enable a better understanding of cyanobacterial cell physiology and unlock new applications in photosynthetic biotechnology.

[1] Russo, DA & Zedler, JAZ (2021) Genomic insights into cyanobacterial protein translocation systems. Biol Chem 402:39-54

[2] Russo DA *et al* (2019). Expression and secretion of a lytic polysaccharide monooxygenase by a fast-growing cyanobacteria. Biotechnol Biofuels 12:1-13

[3] Russo DA *et al* (2022). Development of a highly sensitive luciferase-based reporter system to study two-step protein secretion in cyanobacteria. J Bacteriol 204: e00504-21

23

Adjusting intracellular NADPH concentration in photosynthetic cells targeting biotechnology applications

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In oxygen-evolving photosynthetic organisms such as cyanobacteria, light energy is converted to chemical energy to reduce CO_2 generating organic compounds. However, cyanobacteria can also use external carbohydrates under specific conditions, and they possess several intertwined glycolytic pathways to modulate NADPH and ATP levels to respond to changing environmental conditions (Makowka et al., 2020). Furthermore, glycolytic routes form shunts to replenish the CBB cycle under photoautotrophy. A prerequisite for all these reactions is controlled electron transfer between reducing and oxidising agents.
In cyanobacteria two common soluble electron carriers involved in photosynthesis and glycolysis are NADPH and NADH. Pyridine nucleotide transhydrogenase, PntAB, is an integral membrane protein complex coupling the oxidation of NADH to the reduction of $NADP^+$, hence regulating the intracellular redox balance. In *Synechocystis* sp. PCC 6803 (*Synechocystis*) PntAB resides in thylakoids and under low-light photomixotrophy it balances NADH/NADPH equilibrium specifically in the direction of NADPH[2].
To investigate how modulations in intracellular $NADP^+$/NADPH ratio affect the complex metabolic acclimation of *Synechocystis* during changing trophic strategies we constructed the PntAB overexpression strain and characterised it under different trophy conditions together with the Δ*pntA* mutant [2]. Our results demonstrate that *Synechocystis* can integrate a large surplus of PntAB into thylakoids where the translocation of protons along the membrane proton gradient generates energy for catalysis. Higher PntAB content in cells improves growth in the presence of additional glucose. Additionally, we investigated the effect of PntAB expression level to intracellular NADP(H) concentration and to proton motive force with fluorescence- and spectroscopy-based methods. Suitability of PntAB overexpression strain as a chassis for whole cell photobiotransformation is discussed; several enzymes utilised as photo-biocatalysts, like flavin oxidoreductases and Baeyer–Villiger monooxygenases, use NADPH as a substrate.

[1]. Makowka A et al (2020) Glycolytic Shunts Replenish the Calvin-Benson-Bassham Cycle as Anaplerotic Reactions in Cyanobacteria. Mol Plant. 13(3):471-482.
[2]. Kämäräinen J, Huokko T *et al* (2017) Pyridine nucleotide transhydrogenase PntAB is essential for optimal growth and photosynthetic integrity under low-light mixotrophic conditions in Synechocystis sp. PCC 6803. New Phytol, 214:194–204.

24

Photosynthesis-driven biocatalytic redox reactions for the synthesis of high-value chemicals

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With their excellent selectivity and often very mild reaction conditions, oxidoreductases play an important role in the chemical and pharmaceutical industries. Widely-used enzymatic redox transformations such as C-H oxyfunctionalization or C=C double bond reduction require a stoichiometric supply of electrons, which is usually provided from petrol-based or agricultural cosubstrates such as isopropanol or glucose. The use of an equivalent amount of organic cosubstrates for a process is highly problematic for its atom economy, an important metric for sustainability. Whole-cell redox biocatalysis in cyanobacteria uses electrons from photosynthetic water-splitting and saves organic cosubstrates [1].

Above a certain reaction rate, heterologous biotransformation reactions are limited by the intracellular electron supply. We have shown that the deletion of flavodiiron proteins as competing electron outlets can significantly increase the reaction rate of whole-cell photobiotransformations such as ene-reductases or Baeyer-Villiger monooxygenaseses. The physiological consequences of a very strong heterologous electron sinks on the cyanobacterial metabolism and strategies for an increase of the productivity of the cells will be discussed.

[1] K. Köninger, A. Gomez-Baraibar, C. Mügge, C. Paul., F. Hollmann, M. Nowaczyk and R. Kourist, Ang. Chem., Int. Ed., 2016, 55, 5582-5585, DOI: 10.1002/anie.201601200.
[2] L. Assil-Companioni, H. C. Büchsenschütz, D. Solymosi, N. G. Dyczmons, K. K. F. Bauer, S. Wallner, P. Macheroux, Y. Allahverdiyeva, M. M. Nowaczyk* and R. Kourist* (2020) ACS Catalysis, DOI: 10.1021/acscatal.0c02601.

25

Light-dependent phosphorylation of response regulators controls the orientation of phototaxis in Synechocystis sp. PCC 6803

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The unicellular cyanobacterium *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis*) can move towards or away from a unidirectional light source using type IV pili, a process called phototaxis. The Tax1 system, a homolog of the typical bacterial chemosensory system, regulates positive phototaxis of *Synechocystis* [1]. Interestingly, compared with the canonical chemosensory system, the methylesterase (CheB), the methyltransferase (CheR), and the phosphatase (CheZ) are not encoded in *Synechocystis*. Two CheY-like response regulators, PixG and PixH, were found in the Tax1 system, suggesting a noncanonical regulatory mechanism for this system in *Synechocystis*. Using autophosphorylation and phosphotransfer assays, we confirmed that the histidine kinase PixL can be autophosphorylated *in vitro* and transfer the phosphate to PixG and PixH. A mutagenesis study showed that D326 or D52 in PixG or PixH, respectively are the phosphorylation sites. Furthermore, mutation of the respective aspartic acid in PixG or PixH to alanine at the native locus in *Synechocystis* resulted in the loss of the positive phototaxis. Yeast two-hybrid assays showed that the D326A variant significantly decreased the interaction of PixG with PilB1 and PilC, two components of the motility system. In addition, overexpressing PixG in a *pixD* knock-out mutant (which exhibits negative phototaxis) can switch the movement direction from backward to forward, suggesting that PixG regulates positive phototaxis. However, upon overexpression of PixH in *Synechocystis*, the cells showed negative phototaxis, the same phenotype of *pixH* knock-out mutants [1], indicating that a proper concentration of PixH in the cell is crucial for phototaxis regulation. Further, pull-down of PixG or PixH harbouring phosphomimicking (D to E) or non-phosphorylation (D to A) mutations, followed by mass spectrometry, indicated that only phosphomimicking PixG can interact with PilT2, while both mutant versions of PixG may interact with PilM. In summary, our results suggest that the signal in the Tax1 system is transduced to the two CheY-like response regulators via phosphorylation, and phosphorylated PixG can interact with the motility system.

[1] Yoshihara S *et al.* (2000) Novel putative photoreceptor and regulatory genes Required for the positive phototactic movement of the unicellular motile cyanobacterium *Synechocystis* sp. PCC 6803. Plant Cell Physiol 41:1299–304

26

Light-dependent induction of cell polarity and switching of moving direction in a rod-shaped cyanobacterium Thermosynechococcus

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Light responses considerably impact the cellular physiology of photosynthetic organisms, as light is their crucial energy source. Some cyanobacterial strains exhibit phototactic motility to move toward or away from the light source depending on the quality and quantity of the light. However, how environmental light information is integrated to govern directional phototaxis is elusive. Furthermore, the cell biological mechanisms of how the type IV pilus motility machinery drives the directional phototactic movement in relation to the cell polarity were unknown.
We found that the rod-shaped cells of *Thermosynechococcus vulcanus* move toward green light under a microscope setup [1]. Additional blue light irradiation induced a reversal of the movement, leading to negative phototaxis away from the green light source. Our systematic analyses of photoreceptor gene disruption mutants uncovered that SesA was essential for this blue light-dependent directional switching of phototaxis. SesC was crucial for re-switching again to positive phototaxis after removal of the additional blue light illumination. SesA produces the bacterial nucleotide second messenger, c-di-GMP, in a blue light-dependent manner, whereas SesC has green light-activated c-di-GMP degrading activity [2]. These results suggest that under a high blue/green light ratio, cells trigger c-di-GMP signaling to induce the escape from the light.
Furthermore, we found that the cells can show directional movement when oriented perpendicular to the light vector and the moving direction. Using a fluorescent beads assay and maleimide-based click labeling of PilA1 cysteine knock-in mutant, we revealed that the active type IV pili are localized at both cell poles and guide the movement by their biased extension to the moving direction. Such a within-a-pole polarity regulation contrasts with many other bacteria, which show a pole-to-pole regulation to move directionally only when the cells are parallel to the moving direction.
[1] Nakane D and Enomoto G *et al* (2022) *Thermosynechococcus* switches the direction of phototaxis by a c-di-GMP dependent process with high spatial resolution. eLife in press.
[2] Enomoto G *et al* (2015) Three cyanobacteriochromes work together to form a light color-sensitive input system for c-di-GMP signaling of cell aggregation. PNAS 112(26), 8082-8087.

27

Distinct organization of biosynthetic genes influences the production of UV-absorbent mycosporine-like amino acids in cyanobacteria

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Text: Mycosporine-like amino acid UV-absorbing compounds (MAAs) synthesized by cyanobacteria are recognized as a potential source of ecologically friendly sunscreens. External stress-factors like the spectral composition of light, nitrogen concentration, salinity and heat stress can impact the MAAs production. However, the effect of distinct MAAs biossynthetic genes arrangement found in cyanobacteria genomes has not yet been evaluated. In this study we investigated the gene arrangement and the synthesis of shinorine (SHI) and porphyra-334 (P-334) in two bloom-forming cyanobacterial strains, *Arthrospira platensis* CENA597 and *Nodularia spumigena* CENA596, exposed to fluorescent white light versus UVA plus UVB, which stimulate MAA production. Genome annotations showed that in *A. platensis* CENA597 genes linked to SHI and P-334 production were spatially scattered while in *N. spumigena* CENA596 these genes were organized in a cluster. The CENA597 strain, with unclustered genes, did not showed any detectable SHI nor P-334 levels measured by LC/MS-MS in both the control (45 days) and the UV exposed conditions (after 72h of UVA plus UVB exposure). Conversely, CENA596 strain had $0.065 \pm 0.06 .10^{-2}$ μg_{SHI} /$mg_{Biomass}$ and 2.354 ± 0.05 $\mu g_{P\text{-}334}$ /$mg_{Biomass}$ in normal light conditions and $0.067 \pm 0.01 .10^{-4}$ μg_{SHI} /$mg_{Biomass}$ and 7.664 ± 0.45 $\mu g_{P\text{-}334}$ /$mg_{Biomass}$ after UV treatment, at the same experimental conditions. This results indicated that only clustered genes produces MAAs and can be successfully stimulated by UV radiation, therefore, evaluation of gene organization can provide information on the production or not of these compounds.

28

Dual phototrophy in Sphingomonas glacialis AAP5 isolated from an alpine lake

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Bacterium *Sphingomonas glacialis* AAP5 isolated from the alpine lake Gossenköllesee contains genes for anoxygenic phototrophy as well as proton-pumping xanthorhodopsin. *Sphingomonas* bacteria with the potential for dual phototrophy were identified in metagenomes collected from lake Gossenköllesee during winter and spring season, which indicates that these organisms are active at low temperatures. Therefore, we investigated the influence of temperature on AAP5 gene expression. Xanthorhodopsin is expressed when illuminated at temperatures below 16°C. In contrast bacteriochlorophyll-containing reaction centers are expressed between 4 and 22°C in the dark. Thus, cells grown at lower temperature under natural light-dark cycle produce both photosystems and use them simultaneously for energy generation. Xanthorhodopsin contains carotenoid nostoxanthin serving as an auxiliary antenna, which extends its absorption properties in a blue part of the spectrum. Upon illumination, xanthorhodopsin-containing cells reduced respiration, increased ATP synthesis and showed enhanced growth. This suggests, that dual phototrophy may represent a metabolic advantage in alpine lakes where photoheterotrophic organisms face limited organic substrates, low temperature, and extreme changes in irradiance.

29

Mg2+ deficiency affects thylakoid membrane bioenergetics in the cyanobacterium Synechocystis sp. PCC 6803

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Mg^{2+} is the most abundant divalent cation in living cells. It serves as a cofactor in many enzymatic reactions and is required for various intracellular functions. In photoautotrophic organisms, Mg^{2+} serves as the central ion of the light-harvesting pigment chlorophyll. Therefore, it is of particular importance in chloroplasts and cyanobacteria. In addition to being part of chlorophylls, Mg^{2+} serves as a counterion to balance the pH gradient that is built up across the thylakoid membrane due to the photosynthetic light reaction. Yet, as Mg^{2+} is so crucial, how does a Mg^{2+} limitation affect the physiology of a photoautotrophic cyanobacterium?
When the cyanobacterium *Synechocystis* sp. PCC 6803 was grown in BG11 medium with reduced Mg^{2+} content, the cellular abundance and activity of PS II was essentially not altered, whereas the PS I content was largely decreased as compared to the control. In agreement with a reduced donor-side limitation, the effective quantum yield of PS I, Y(I) was slightly higher and P_{700} re-reduction was faster when cells were grown under Mg^{2+} limitation.
Using the fluorescent dye acridine orange, we also monitored the light-induced generation of ΔpH across the *Synechocystis* thylakoid membranes (TM). Following a fast decrease of the fluorescence signal upon switching the light on, due to acidification of the thylakoid lumen, we observed a fluorescence increase, due to alkalization of the cytoplasm. We observed altered fluorescence signals when *Synechocystis* cells were grown in media with low Mg^{2+} concentrations. Here, the thylakoid lumen became slightly less acidified upon switching on the actinic light, and the simultaneous alkalization of the cytoplasm was also reduced. These differences observed under Mg^{2+}-limitation suggest a significant role of Mg^{2+} in the formation of a ΔpH across the cyanobacterial TM.

30

The role of Cyanobacteria in ameliorating stress conditions for bacterial community in Brazilian soda lakes

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The Brazilian's Pantanal biome, which is the largest tropical wetland in the world, host a variety of lakes and lagoons, including several soda lakes. Soda lakes are natural environments with high pH level and frequently associated high salinity. Even though their proximity, these soda lakes could be clustered in three different groups based on its limnological features: ET (eutrophic turbid), OT (oligotrophic turbid) and CVO (crystalline vegetated oligotrophic) lakes. ET lake has a constant natural occurrence of cyanobacterial blooms and so eutrophic conditions while OT lake is marked by a high dissolved organic matter and suspended ions in resulting in a dark water-color. CVO lake has a less stressful environment being colonized by macrophytes. Some studies described the microbiome and its adopted strategies to thrive on stressful conditions observed in these lakes, but how this microbiome interact remains unclear. Here, we aimed to understand the bacterial interactions on soda lakes and how these interactions could impact the soda lakes functioning. Applying the network analysis in metagenomic sequencing from these areas (one lake representative from each lakes' category sampled in triplicate), the ET lake had a lower complexity of interactions (reduced nodes and edges and average degree) with high modularity and connectivity. OT lake had an intermediated profile of complexity and modularity combined with an enrichment of negative correlations (competition). CVO lake had an intermediated complexity and low modularity with an enrichment of positive interactions (cooperation). The occurrence of the cyanobacterial bloom seems impacts the establishment of bacterial interactions. The bloom inputs carbon on this environment improving the nutritional status, that could stimulate the acquisition of nutrients by heterotrophic bacteria resulting in a competitive interaction. However, the cyanobacteria detoxify the high concentration of natural arsenic on this lake ameliorating the stress conditions. This scenario could be explained by the stress-gradient hypothesis where high toxicity and high nutritional status in a stressful environment result in a balanced interactions between the organisms. For OT and CVO lakes, other eco-evolutionary processes could be more relevant to drive the bacterial interactions.

32

Modes of peptidoglycan growth in the multicellular cyanobacterium Anabaena

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Anabaena represents a group of cyanobacteria that form filaments of communicated cells exhibiting features of multicellular organisms such as signaling by morphogens and coupled circadian oscillations. In bacteria, the murein sacculus is a giant peptidoglycan molecule deposited outside the cytoplasmic membrane and is essential for maintenance of the specific cell morphology, osmotic balance and viability. The *Anabaena* filament presents a continuous outer membrane that delimits a continuous periplasm including a single peptidoglycan molecule that is engrossed in the intercellular septa between contiguous cells. In *Anabaena,* lateral peptidoglycan incorporation, which in model rod-shaped bacteria elongate the cell during growth, is weak, whereas midcell incorporation during cell division, which forms the poles of the daughter cells, takes place at substantial levels. Additionally, in contrast to other bacteria, in *Anabaena* peptidoglycan incorporation is maintained in the mature intercellular septa after daughter cell separation. In *Anabaena*, the proteins MreB, MreC and MreD, normally involved in lateral peptidoglycan growth, localize throughout the cell periphery including the cell poles but, remarkably, also to the divisome, where they stay all through the process of septum construction. *Anabaena* derivatives with inactivated *mreB*, *mreC*, or *mreD* genes present conspicuous alterations in the filament morphology, including the formation of rounded cells with irregular size and distortions of the filament linearity [1]. These mutants also show altered septal peptidoglycan growth bands, which are wider and thicker than in the wild type, and frequently appear irregularly spaced and tilted with regard to each other. Moreover, the division plane, visualized by FtsZ localization, also appears aberrantly placed. Finally, both lateral and septal peptidoglycan incorporation are impaired in mutants that down-express cell division proteins. We propose that, as many other septal proteins of *Anabaena*, MreB, MreC and MreD localize to the cell septa by interactions with the divisome during cell division. In the septa, MreB, MreC and MreD may regulate peptidoglycan remodeling involved in localization and maintenance of the septal junction-protein arrays mediating intercellular communication.

[1] Velázquez-Suárez C *et al* (2020) The inorganic nutrient regime and the *mre* genes regulate cell and filament size and morphology in the phototrophic multicellular bacterium *Anabaena*. mSphere 5:e00747-20.

33

Directing cyanobacterial photosynthesis

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Photosynthesis holds the key to the sustainable generation of useful products from light. To realise this potential, photosynthesis must be engineered to redirect energy and reductant derived from photons to desired products. Cytochrome P450s, which catalyse a broad array of reactions, have been engineered into a variety of photosynthetic organisms. They have been shown to be photosynthesis-dependent and furthermore, the addition of P450s can increase the photosynthetic capacity of the host organism. We have developed this technology further by expressing a P450 (CYP1A1) in the cyanobacterium *Synechococcus* sp. PCC 7002. By rationally engineering photosynthesis via the removal of a competing electron sink (the respiratory terminal oxidase COX), we significantly increased the activity of CYP1A1. This enhanced CYP1A1 activity was facilitated via an increase in the flux of electrons through photosystem I (PSI). A transcriptomic analysis was carried out on the designed strains and we describe a complex response including genes involved in photosynthesis and electron transfer. Specifically, the expression of CYP1A1 resulted in the reduction in expression of other natural electron dissipation pathways. These results highlight the potential of engineering photosynthetic organisms, but also identifies the need to consider the broader impacts on cellular metabolism of any rationally induced changes.

34

Whole genome-based phylogenomics of phototrophic bacteria: an overview of accessible tools for any microbiologist

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With whole genome sequencing being more mainstream and available, it has become a more valuable tool for phylogenetic and evolutionary studies, particularly for prokaryotes. The number of complete and draft whole genomes available in public databases has increased exponentially these past decades, and new genome sequences can nowadays be more readily obtained, by in-house sequencing or through paid services, than ever before. The support value of whole genome-based comparisons in addition to traditional single gene-based analyses and physiological characterization has been shown in many examples. On the other hand, many microbiologists may not have access to the bioinformatic tools to performed detailed whole genome-based comparisons. However there are many open access, sever-based tools available that require limited bioinformatics skills to perform such analysis. This presentation will provide an overview for microbiologists and biochemists of whole genome-based comparative tools that can be used without prerequisite programming skills. We will discuss how whole genome-based phylogenetic trees (using PATRIC services [1]) and average nucleotide comparisons (using JSpecies [2]) can be used to support taxonomic and evolutionary discussions related to phototrophic prokaryotes. We will highlight examples of recently described new taxa of *Heliomicrobium*, *Rhodocyclus*, and the new bacterial family of *Halorhodospiraceae,* that were discovered using these techniques [3,4,5].

[1] Wattam AR *et al* (2017) Improvements to PATRIC, the all-bacterial Bioinformatics Database and Analysis Resource Center. Nucleic Acids Res 45, D535–D542

[2] Richter M *et al* (2016) JSpeciesWS: A web server for prokaryotic species circumscription based on pairwise genome comparison. Bioinformatics 32, 929–931

[3] Kyndt JA *et al* (2021) Phylogenetic relationship of phototrophic heliobacteria and systematic reconsideration of species and genus assignments based on genome sequences of eight species. Int J Syst Evol Microbiol 71(4), 4729-4739

[4] Kyndt JA *et al* (2022) Comparative genome analysis of the photosynthetic beta-proteobacteria of the genus *Rhodocyclus*: heterogeneity within strains assigned to *Rhodocyclus tenuis* and description of *Rhodocyclus gracilis sp. nov*. as a new species. Microorganisms 10, 649

[5] Imhoff JF *et al* (2022) Genomic comparison, phylogeny and taxonomic reevaluation of the *Ectothiorhodospiraceae* and description of *Halorhodospiraceae fam. nov.* and *Halochlorospira gen. nov.* Microorganisms 10, 295

35

Another surprise from the aerobic anoxygenic phototrophs: Wide range of metallophores

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Aerobic anoxygenic phototrophs (AAP) do not stop surprising us from the time of discovery. Firstly a few decades ago, it was totally unexpected to uncover the obligate dependence on oxygen among bacteria producing bacteriochlorophyll a and performing anoxygenic photosynthesis. Additional significant news from AAP came after locating them near Black Smokers of the Juan de Fuca Ridge in the Pacific Ocean and announcing 2000 m deep hydrothermal vents as their new home. Finally, they revealed a phenomenal physiological feature: High resistance to oxyanions tellurite, tellurate, selenite, selenate and vanadate. Some AAP could tolerate 2300 to 2700 µg/ml of K_2TeO_3, usually toxic to microbes at as low as 1 µg/ml. Phylogenetically they belong to - *α*, *b*- and γ-*Proteobacteria*, and can only synthesize and use photosynthetic light harvesting systems in the presence of oxygen. They are incapable of anaerobic photosynthesis and photoautotrophy, distinguishing them from all other anoxygenic phototrophic species, and have been isolated from a vast variety of environments including marine ecosystems, freshwater and meromictic lakes, hypersaline springs, and biological soil crusts.

The most recent AAP surprise was uncovered in the study of over 100 strains representing 44 species from 27 genera that were chosen to be investigated for their interactions with iron and other metal cations such as Mg, V, Mn, Co, Ni, Cu, Zn, Se and Te using a chromeazurol S assay to detect sidero-(metallo-)phore production. Representatives from 12 species within 8 genera of *α-Proteobacteria*, or 21.8% of tested strains, produced highly diffusible siderophores that could bind one or more metals. In addition, γ-*proteobacterial Chromocurvus halotolerans*, strain EG19, excreted a brown compound into growth medium, which was purified and confirmed to act as a siderophore. It had an approximate size of 341 Da and drew similarities to the siderophore rhodotorulic acid, a member of the hydroxamate group, previously found only among yeasts. This study is the first to discover sidero-(metallo-)phore production to be wide spread among AAP, which may be another key method of metal chelation and potentially detoxification within their habitats.

36

Disjointed biosynthetic gene clusters for the shared production of two structural distinct microbial sunscreens

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Mycosporine-like Amino Acids (MAAs) are small, colourless and water-soluble secondary metabolites, with high molar extinction coefficients that makes them efficient UV sunscreens. They possess a unique mechanism where the absorbed UV radiation is dissipated to surroundings as heat without the creation of free radicals and some MAAs were also shown to have free radical scavenging activity. Although over 40 different MAA variants have been reported, only shinorine (λmax=334 nm) biosynthetic pathway has been fully established involving four enzymes encoded by the biosynthetic gene cluster *mysABCD (*or *NRPS* instead of *mysD)*. Here we report two structurally distinct MAAs from *Nostoc* sp*.* UHCC 0926. This lichen symbiont produces the previously described 756 Da tri-core MAA (C34H53N4O15) and a novel 582 Da diglycosylated aplysiapalythine C - like variant (C23H39N2O15). The chemical structures were determined using a combination of mass spectrometry and analysis of one- and two-dimensional NMR spectra. We have also obtained an 8.3 Mb complete genome sequence for *Nostoc* sp*.* UHCC 0926 in order to identify the MAA biosynthetic gene cluster. Surprisingly, we have found three separate mycosporine biosynthetic gene clusters encoded at distant locations in the complete genome. These results provide a new perspective into the evolution of secondary metabolite biosynthesis and broadens the chemical diversity of MAAs to include a new 582 Da diglycosylated aplysiapalythine C - like MAA variant.

38

RNA chaperones in cyanobacteria

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Since the earliest times of evolution, RNA has played a crucial role as catalytic tool, as guardian of all genetic information, as well as a key molecule in almost all kinds of regulatory mechanisms. To accomplish all these tasks, proteins have evolved that were able to interact with RNA. One subclass of these are the so called ‘RNA chaperones’. They support a variety of RNAs in their folding, interactions and stability. There are RNA chaperones well-studied in several bacteria, like CsrA, ProQ or Hfq. However, in cyanobacteria homologs either do not exist or have taken over other tasks in the course of evolution. For this reason, we have screened through a Grad-Seq experiment for potential RNA-binding proteins in *Synechocystis* sp. PCC 6803 (1). Hereby a structural homologue to KhpA was found, which functions in certain non-photosynthetic bacteria as a global RNA-binding protein. Further, it was described to be associated with another protein, KhpB, which could also be found in *Synechocystis*. First phenotyping experiments with the knock-out strains indicated an essential function under glucose or high light stress. Through cell fractionation, we found that KhpA could be (partially) membrane-associated. Moreover, *khpA* (*slr0287*) is located in a transcriptional unit with *rps16*, a ribosomal protein. In addition, transcriptome data show significant coregulation with many other ribosomal proteins. Therefore, we are now addressing a possible ribosomal association through ribosomal profiling. Our data suggest a relevant function for the KhpA-KhpB system in cyanobacteria.

Riediger M *et al* (2021). “Analysis of a photosynthetic cyanobacterium rich in internal membrane systems via gradient profiling by sequencing (Grad-seq). The Plant Cell. Vol. 33, No. 2: pp. 248–269.

39

Novel prosthecate aerobic anoxygenic phototrophs discovered in alpine meromictic lakes of British Columbia, Canada

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Aerobic anoxygenic phototrophs (AAP) are a group of bacteria that use bacteriochlorophyll containing reaction centres and light harvesting complexes for photosynthesis in the presence of oxygen. All known members lack autotrophic pathways to generate organic compounds from CO_2, yet they are found in most illuminated aquatic ecosystems, composing a substantial part of microbial communities in euphotic zones. Stratified alpine lakes offered an atypical sampling location due to the illuminated nature of each steep chemocline and thermocline. Seven Gram-negative bacteria isolated from meromictic Mahoney and Blue Lakes were found to be flagellated prior to developing prosthecae. All became pink–red after 1-2 weeks of incubation, and contained bacteriochlorophyll *a*. The inability to grow under anaerobic conditions supported their identification as obligate AAP. Physiological tests revealed a preference for high salinity, where BL14^T tolerated up to 6.5% NaCl or 16.0% Na_2SO_4. In addition to recognizable phenotypic differences, analysis of 16S rRNA gene sequences found both strains BL14^T and ML37^T related to *Alkalicaulis satelles,* G-192^T with 98.41 and 98.84% respective similarity, and distantly associated to members of the non-phototrophic genus *Glycocaulis profundi,* ZYF765^T (95.59 and 95.36%, respectively) within the newly established *Maricaulales* order of *α-Proteobacteria*. Average nucleotide identity and digital DNA:DNA hybridization of their circularized genomes differentiated the new isolates from types species. Both BL14^T and ML37^T contained photosynthetic operons of 46,143 and 46,315 bp, where genes of BL14^T were uniquely split into two distal operons. Furthermore, *A. satelles* was not originally published as an AAP, however we found that it also contained a similar 45,131 bp fragment. Photosynthesis in combination with the distinct morphological feature of prosthecae formation is a significant finding, as it likely represents the first instance of AAP potentially capable of regulating buoyancy. These isolates bring further excitement as they relate to a family and order of α-*Proteobacteria* that previously contained no phototrophic members, thereby increasing the known diversity, physiology, and ecology of AAP.

40

Regulation of the PSI-specific phycobilisome linker protein CpcL

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Controlling the energy distribution between photosystem I (PSI) and photosystem II (PSII) is essential for proper photosynthesis under fluctuating environmental conditions. Phycobilisomes (PBSs) are the major light-harvesting antennae in cyanobacteria. Generally, PBSs are composed of core cylinders and several rods connected by the rod-core linker protein CpcG and transfer the light energy mainly to PSII. In contrast, there has been described a rod-type PBS, named CpcL-PBS, which is a PSI-specific antenna complex. The rod-membrane linker protein CpcL directly connects the PBS rod with PSI to form PBS-CpcL-PSI supercomplexes [1, 2]. How and for what cyanobacterial cells exploit two different PBSs remain elusive.
In the heterocyst-forming cyanobacterium *Anabaena* sp. PCC 7120, the amount of the PBS-CpcL-PSI supercomplex was increased under nitrogen starvation conditions. In addition, even larger supercomplexes containing PSI together with the CpcL-PBS were detected. The ratio between the CpcL to CpcG protein amount was higher in heterocysts than in vegetative cells. After CpcL overexpression and in the Δ*cpcG* mutant, we could observe the increased level of the PBS-CpcL-PSI supercomplex and the larger PBS-CpcL-PSI supercomplexes also in vegetative cells. These results suggest that not the CpcL amount but an increased ratio of CpcL/CpcG controls the formation of the PBS-CpcL-PSI supercomplexes. Northern blot analysis showed an increased ratio of *cpcL*/*cpcG* transcript under nitrogen starvation conditions, suggesting additional transcriptional regulation of the tandemly arranged *cpcL* and *cpcG* genes within the whole *cpc* operon. An antisense RNA encoded in the *cpcG* gene locus could be crucial for operon decoupling of *cpcL*-*cpcG* transcript upon nitrogen deprivation.

[1] Watanabe M *et al* (2014) Attachment of phycobilisomes in an antenna–photosystem I supercomplex of cyanobacteria. PNAS 111(7), 2512-2517.
[2] Noji T, Watanabe M *et al* (2021). Direct Energy Transfer from Allophycocyanin-Free Rod-Type CpcL-Phycobilisome to Photosystem I. The Journal of Physical Chemistry Letters, 12(28), 6692-6697.

41

Characterization of the individual PsaA and PsaB assembly intermediates of the cyanobacterial Photosystem I

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Photosystem I (PSI) is one of the largest multiprotein complexes in photosynthetic membranes of oxygenic phototrophs. The major protein components of PSI 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 the foundation for building this very complex protein assembly. Although the high-resolution structure and function of the PSI have 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 an 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. FLAG-PsaA contained 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 bound carotenoids. The complex showed a typical red-shifted 77K Chl fluorescence peak like the isolated PSI. In contrast, the isolated PsaB did not form a homodimer, did not associate with any PSI small subunits, and showed only a small number of associated Chls mostly fluorescing at 670 nm. Apart from previously identified PSI assembly factors, both preparations contained a few novel, most probably PSI-specific proteins. In conclusion, PsaA and PsaB markedly differ in their ability to homodimerize and bind pigments. As they are considered to be 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.

42

Regulatory RNAs involved in heterocyst differentiation and function

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Upon nitrogen deprivation, some filamentous cyanobacteria differentiate heterocysts, specialized nitrogen-fixing cells regularly spaced along the filaments. Differentiation of heterocysts is under transcriptional control of the master regulators NtcA and HetR and involves structural and metabolic changes that result in the microaerobic environment required for nitrogenase function. We have recently characterized several non-coding RNAs that regulate, at the post-transcriptional level, different aspects of this process. NsiR1 is an sRNA expressed specifically in heterocysts, that indirectly regulates the amount of HetR, resulting in higher concentration of HetR in developing heterocysts than in vegetative cells of *Nostoc* sp. PCC 7120 [1]. Also, NsiR1 represses the expression of a protein that acts as a brake of the commitment to differentiate [2]. The metabolic adaptations of heterocysts include the inhibition of CO_2 fixation. Both sRNAs and antisense RNAs are involved in this process. For instance, NsiR4, an NtcA-dependent sRNA that is expressed more strongly in heterocysts than in vegetative cells represses the expression of two enzymes involved in CO_2 fixation: the bifunctional sedoheptulose-1,7-bisphosphatase/fructose-1,6-bisphosphatase, additionally regulated by a heterocyst-specific antisense RNA [3], and phosphoglycerate kinase [4].
We have carried out a global approach for the identification of nitrogen-regulated antisense transcription in *Nostoc* sp. PCC7120 and we are currently evaluating the physiological relevance of some of these transcripts in heterocyst differentiation. Ultimately, the ability to manipulate heterocyst metabolism using regulatory RNAs could become a useful tool towards the use of heterocysts as cell factories for the production of oxygen-sensitive compounds of interest.

Funded by BFU2016-74943-C2-1-P and PID2019-105526GB-I00 from MCIN/AEI/10.13039/501100011033, cofinanced by the European Regional Development Fund.

[1] Brenes-Álvarez M *et al* (2020) NsiR1, a small RNA with multiple copies, modulates heterocyst differentiation in the cyanobacterium *Nostoc* sp. PCC 7120. Environ Microbiol 22:3325-3338
[2] Brenes-Álvarez M *et al* (2022) The heterocyst-specific small RNA NsiR1 regulates the commitment to differentiation in *Nostoc*. Microbiol Spectrum e0227421

[3] Olmedo-Verd E *et al* (2019) A heterocyst-specific antisense RNA contributes to metabolic reprogramming in *Nostoc* sp. PCC 7120. Plant Cell Physiol 60:1646-1655
[4] Brenes-Álvarez M *et al* (2021) A nitrogen stress-inducible small RNA regulates CO_2 fixation in *Nostoc*. Plant Physiol 187:787-798

43

Controlling distribution of photosynthetic electron flux between flavodiiron proteins, NDH-1, CO2 fixation, and exogenous sinks

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Upon changes in environmental conditions, cyanobacteria must avoid overreduction of the photosynthetic electron transport chain (PETC) and subsequent damage to the photosynthetic apparatus. Cyanobacteria strive to achieve this by precisely orchestrating the distribution of reducing power from the soluble electron carrier protein Ferredoxin to various sinks and regulatory electron transport pathways. The main electron acceptor processes include reduction of $NADP^+$ by Ferredoxin-$NADP^+$-oxidoreductase (FNR) and the subsequent use of NADPH in CO_2 fixation, the light-dependent reduction of O_2 in the Mehler-like reaction by flavodiiron proteins (FDPs), cyclic electron transport (CET) mediated mainly by NAD(P)H-deyhdrogenase-like complex 1 (NDH-1), as well as the regulatory thioredoxin system. However, it remains largely unknown how the coordination of the activities of these processes in achieved. To this end, we explored the distribution of photosynthetic reductants between the aforementioned electron acceptors in *Synechocystis* sp. PCC 6803.
Firstly, we employed a novel cyanobacterial bimolecular fluorescence complementation (BiFC) system to study protein–protein interactions as well as membrane-inlet mass spectrometry, near-infrared absorbance spectrophotometry, and NADPH fluorescence measurements to characterise photosynthesis in *Synechocystis* mutants deficient in Ferredoxins, FDPs, or FNR. We demonstrated that, like NDH-1, FDPs very likely use Ferredoxin 1 as their main electron donor, enabling intricate coordination of the activities of FDPs and CET, possibly via the thioredoxin system. Furthermore, by expressing a strong exogenous electron sink that uses NADPH as a cofactor in a biotransformation reaction, we achieved highly efficient oxidation of the PETC due to the heterologous enzyme outcompeting FDPs, CET, and CO_2 fixation. This suggests that the availability of $NADP^+$ as substrate for FNR –- or the activation state of FNR – is a deciding factor determining the fate of electrons from Ferredoxin. Our results also suggest interdependency between generation and regulation of the proton motive force, FDP activity and the thiol redox state of the cyanobacterial cell. In conclusion, these findings have strong implications for optimising the use of cyanobacteria as sustainable whole-cell biotransformation platforms by targeting the direction of photosynthetic reducing power towards desired reactions.

44

Formation of synthetic nanofilaments in cyanobacteria for protein co-localisation

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Cyanobacteria hold great potential as production chassis for industrial applications in a future bioeconomy. However, product titres are often still too low and not competitive compared to heterotrophic hosts. To address this challenge, we aim to develop intracellular co-localisation strategies for heterologous biosynthetic pathways. Bringing enzymes in close proximity increases local substrate concentrations and reduces cross-talk with native pathways. 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. Despite the metabolic burden of filament formation, growth of the transformants was not significantly impaired.
To investigate if this strategy can be used to co-localise heterologous proteins, we targeted a fluorescent protein to the nanofilaments. We verified co-localisation using confocal microscopy as well as cell lysate fractionation using sucrose gradients. The fluorescent protein and the nanofilament proteins were found in the same fraction suggesting the presence of an interaction. We now aim to expand this co-localisation concept to enzymes of a heterologous pathway to increase product titres. The tools developed here will benefit future applications in green biotechnology.

46

HetL provides immunity to HetR against PatS inhibition, and promotes patterning in Anabaena PCC 7120

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Anabaena is a multicellular bacterium able of cell differentiation. When combined nitrogen is abundant, *Anabaena* grows in long filaments formed by a same cell type named vegetative cells. When combined nitrogen becomes limiting, 5-10% of the vegetative cells are induced to differentiate into a specific cell type which provides a microoxic environment suitable for N_2-fixation. Heterocysts are semi-regularly distributed along the filaments, making from *Anabaena* the simplest model to study pattern formation. The establishment of this pattern follows a local activation and a long-range inhibition model where HetR activates the synthesis of its proper inhibitor PatS. Being diffusible PatS inhibits the activity of HetR in the vegetative cells neighbouring the heterocyst. HetL, a pentapeptide repeat protein**,** has been identified through a genetic screen based on the over-production of PatS[1]. HetL is therefore involved in PatS signalling but the molecular mechanism ongoing was unknown. We used a multidisciplinary approach, combining genetics, molecular modelling and biophysics, to investigate the possible function of HetL. This allowed to demonstrate that HetL is the immunity protein providing HetR protection against PatS inhibition[2]. Furthermore, Bacterial Two Hybrid assays and genomic analysis revealed that the interaction between HetL and HetR is one among numerous protein-protein interactions that build a network linking several factors involved in initiating the differentiation program, dictating the commitment state and establishing the final pattern[4]. Our results open up interesting perspectives for the elucidation of the mechanism of action of all these proteins and the importance of their interactions in establishing the pattern.

[1] Liu D *et al* (2002) *hetL* overexpression stimulates heterocyst formation in *Anabaena* sp. strain PCC 7120. *J Bacteriol*. 184(24):6873-81
[2] Xu X *et al* (2020) HetL, HetR and PatS form a reaction-diffusion system to control pattern formation in the cyanobacterium *Nostoc* PCC 7120*. eLife 7;9:e59190*
[3] Xu X *et al* (2022) Interaction network among factors involved in heterocyst-patterning in cyanobacteria. *Molecular Gentics and Genomics* under revision.

47

What We Are Learning From Explosion of Reaction Centre-Light Harvesting 1 Core Complex Structures

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A growing number of high-resolution RC-LH1 core complex structures have recently appeared, owing mainly to major advancements in single particle cryo-EM analysis. Among those with closed LH1 rings surrounding the RC, the thermal stability *Thermochromatium tepidum* core complex was attributed to strong interactions between Ca^{2+} ions and the 16 □□-heterodimeric subunits, which also drive the extended redshift of the LH1 Q_Y-absorption band [1]. Besides the menaquinone occupying the RC Q_A site, five ubiquinones were revealed, one in the Q_B site and four free Q molecules which participate in the Q redox species exchange pathway via passage through narrow pores between the LH1 subunits. This was verified for the *Rhodospirillum rubrum* complex [2], in which a previously unrecognized RC BChl is liganded to a phospholipid. For the BChl *b*-containing complex from *Blastochloris viridis* [1], a closed LH1 ring was revealed consisting of 16 □□□-heterotrimers and a single □□-heterodimer, with the missing □-subunit leaving a ring opening for Q exchange. Among the complexes containing open LH1 rings, two 14-membered rings are fused together to form a dimer in *Rhodobacter sphaeroides* [3]. The structure revealed two new proteins adjacent to ring openings in addition to PufX, that participate in the formation of channels that promote free Q-redox species diffusion. Likely participating Q exchange species were modeled for structures of the open ring complexes from *Rba. veldkampii* [4] and *Rhodopseudomonas palustris* [5], and an open RC-LH complex was also found in the filamentous anoxygenic phototroph *Roseiflexus castenhlozii* [1].

[1] Gardiner AT *et al*. (2020) A comparative look at structural variation among RC-LH1 'core' complexes present in anoxygenic phototrophic bacteria. Photosynth. Res. 145:83-86 [2] Qian P *et al.* (2021) Cryo-EM structure of the *Rhodospirillum rubrum* RC-LH1 complex at 2.5 Å. Biochem. J. 478:3253-3263 [3] Qian P *et al*. (2021) Cryo-EM structure of the dimeric *Rhodobacter sphaeroides* RC-LH1 core complex at 2.9 Å. Biochem J 478:3923-393 [4] Bracun L *et al*. (2021) Cryo-EM structure of the photosynthetic RC-LH1-PufX supercomplex at 2.8-Å resolution. Science Adv 7:eabf8864 [5] Swainsbury D.J.K *et al.* (2021) Structures of *Rhodopseudomonas palustris* RC-LH1 complexes with open or closed quinone channels. Science Adv. 7;eabe2631

48

Distinct metabolic states of individual filaments in Anabaena revealed by SIMS analysis

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Under combined-nitrogen deprivation, heterocyst-forming cyanobacteria trigger the segregation of CO_2 and N_2 fixation in two different cell types: vegetative cells and heterocysts, respectively. This metabolic strategy of separating physically the processes of oxygenic photosynthesis and N_2 fixation requires an intercellular exchange of carbon and nitrogen metabolites along the filament to support growth. To quantify and visualize the assimilation and distribution of recently fixed C and N in *Anabaena* sp. PCC 7120 at the single-cell level, we incubated the filaments with stable isotope-labelled substrates (^{13}C-bicarbonate and $^{15}N_2$) in a time-course experiment coupled to secondary ion mass spectrometry imaging (LG-SIMS and NanoSIMS) [1]. $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios showed higher enrichment of both isotopes with time in vegetative cells than in heterocysts, which is consistent with the transfer of fixed N from the heterocyst to the vegetative cells and the consumption of reduced C received from vegetative cells in the heterocyst. In addition, high ^{15}N enrichment was observed at the heterocyst necks, which suggests accumulation of recently fixed N in the cyanophycin granules. Whereas ^{13}C- and ^{15}N-enrichment was relatively homogeneous along stretches of vegetative cells in a filament, label was significantly different between filaments unraveling the presence of filaments with high variability in metabolic activity. Additionally, some filaments contained differently-labelled stretches of cells, which could be related to the presence of non-communicating cells [2], including heterocysts [3]. In summary, our results show the presence, in an *Anabaena* culture under controlled conditions, of filaments with different metabolic states and a distinct behavior of some cells that adds heterogeneity to a filament. These findings illustrate the importance of performing studies at the cellular level to understand metabolic aspects in multicellular organisms.

[1] Nieves-Morión M *et al* (2021) Single-Cell Measurements of Fixation and Intercellular Exchange of C and N in the Filaments of the Heterocyst-Forming Cyanobacterium *Anabaena* sp. Strain PCC 7120. mBio 12:e01314-21.

[2] Arévalo S *et al.* (2021) Coexistence of Communicating and Noncommunicating Cells in the Filamentous Cyanobacterium *Anabaena*. mSphere 6:e01091-20.

[3] Nürnberg DJ *et al* (2015) Intercellular diffusion of a fluorescent sucrose analog via the septal junctions in a filamentous cyanobacterium. mBio 6:e02109.

49

Regulatory crosstalk between tetrapyrrole and arginine biosynthetic pathways in cyanobacteria

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Cyanobacteria produce large quantities of tetrapyrroles (chlorophyll, heme and bilins) as cofactors for photosynthesis by a long branched pathway. Due to the phototoxicity of porphyrins the synthesis of tetrapyrroles must be carefully balanced with environmental conditions. Availability of nitrogen seems to be particularly crucial because bilin-rich phycobilisomes (PBS) serve also as a nitrogen reserve. Under fluctuating nitrogen availability, a large fraction of PBS and photosystems can be degraded and re-synthesized in a few hours, which creates a large temporal sink for new chlorophyll and bilins. How the cell regulates fluxes in the tetrapyrrole pathway remains enigmatic [1]. To clarify this question, we mapped the interactome of enzymes and protein factors that participate in tetrapyrrole biosynthesis in the cyanobacterium *Synechocystis* PCC 6803. Interestingly, we found that glutamyl-tRNA reductase (GluTR) - the first enzyme of the tetrapyrrole pathway, forms a stable complex with ArgJ, the first enzyme of arginine biosynthesis. This complex further includes BtpA protein linked previously with the stability of Photosystem I [2]. Another enzyme of the arginine pathway (ArgD) binds, together with cyanophycinase, to Gun4 – an essential protein for the magnesium chelation step of the chlorophyll formation [3]. Notably, ArgD interacts with Gun4 armadillo-repeat domain that is common in cyanobacteria and red algae but was lost in green lineage. Arg biosynthesis and catabolism plays a pivotal role in nitrogen homeostasis and we demonstrated a stimulatory effect of ornithine, a key metabolite of the Arg pathway, on the formation of Gun4-ArgD complex. A working model of how the Arg pathway modules the synthesis of chlorophyll and bilins will be presented.

[1] Chen GE *et al* (2021) Evolution of Ycf54-independent chlorophyll biosynthesis in cyanobacteria. PNAS 118:e2024633118
[2] Zak E and Pakrasi HB (2000) The BtpA protein stabilizes the reaction center proteins of photosystem I in the cyanobacterium Synechocystis sp. PCC 6803 at low temperature. Plant Physiol 123:215-22
[3] Sobotka R *et al* (2008) Improtance of the cyanobacterial Gun4 protein for chlorophyll metabolism and assembly of photosynthetic complexes. J Biol Chem 283:25794-25802.

52

Flv3A facilitates O2-photoreduction and affects H2-photoproduction independently of Flv1A in diazotrophic Anabaena filaments

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The model heterocyst-forming filamentous cyanobacterium *Anabaena* sp. PCC 7120 (*Anabaena*) is capable of simultaneously performing oxygenic photosynthesis in vegetative cells and O_2-sensitive N_2-fixation inside the heterocysts. In *Anabaena*, the flavodiiron proteins (FDPs) have already been shown to participate in photoprotection of photosynthesis by driving excess electrons to O_2 (Mehler-like reaction)[1,2]. Here, we addressed the physiological relevance of the vegetative cell-specific Flv1A and Flv3A on the bioenergetic processes occurring in diazotrophic *Anabaena* under variable CO_2-levels. We demonstrate that both Flv1A and Flv3A are required for proper induction of the Mehler-like reaction upon a sudden increase in light intensity, which is likely important for the activation of CO_2-concentrating mechanisms (CCM) and CO_2-fixation. Under ambient CO_2-levels and diazotrophic conditions, Flv3A is capable of mediating moderate O_2-photoreduction, independently of Flv1A, but in coordination with Flv2 and Flv4. Strikingly, the lack of Flv3A resulted in strong downregulation of the heterocyst-specific uptake hydrogenase, which led to enhanced H_2-photoproduction under both oxic and micro-oxic conditions. These results reveal a novel regulatory network between the Mehler-like reaction and the diazotrophic metabolism, which is of great interest for future biotechnological applications.

References

[1] Allahverdiyeva Y, Mustila H, Ermakova M, Bersanini L, Richaud P, Ajlani G. et al. 2013. Flavodiiron proteins Flv1 and Flv3 enable cyanobacterial growth and photosynthesis under fluctuating light. Proc Natl Acad Sci 110: 4111-4116.

[2] Ermakova M, Battchikova N, Allahverdiyeva Y, Aro EM. 2013. Novel heterocyst specific flavodiiron proteins in Anabaena sp. PCC 7120. FEBS Lett, 587: 82-87.

53

Extracellular vesicle-mediated secretion of tetrapyrrole pigments in the cyanobacterium Leptolyngbya boryana

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Protochlorophyllide (Pchlide) reduction in the late stage of chlorophyll *a* (Chl) biosynthesis is catalyzed by two enzymes: light-dependent Pchlide oxidoreductase (LPOR) and dark-operative Pchlide oxidoreductase (DPOR). The differential operation of LPOR and DPOR enables a stable supply of Chl in response to changes in light conditions and environmental oxygen levels in most oxygenic photosynthetic organisms including cyanobacteria. When a mutant (YFC2) lacking the *chlL* gene encoding one subunit of DPOR of the cyanobacterium *Leptolyngbya boryana* is grown in the dark, the Chl biosynthesis is arrested at the Pchlide reduction resulting in accumulation of Pchlide in the cells and a significant portion of accumulated Pchlide is secreted into the culture medium. The mechanism by which Pchlide is secreted into the culture medium remains unknown. To understand the secretion mechanisms, we analyzed Pchlide in the culture medium using various techniques. YFC2 was grown under dark heterotrophic conditions, and the culture supernatant was collected. Pchlide in the supernatant was recovered in a precipitate by ultracentrifugation, and the Pchlide precipitate was separated into two fractions by sucrose density gradient centrifugation. This behavior was in good agreement with that of extracellular vesicles (EVs) of other microorganisms. Analyses of the fractions using SDS-PAGE and mass spectrometry revealed that proteins that are localized in the outer membrane are main components together with Pchlide. Furthermore, EV-like structures with various sizes were observed by transmission electron microscopy. These characteristics of the Pchlide fractions suggested that Pchlide is secreted via EV in YFC2. Considering that Pchlide is a strong potent photosensitizer, EV secretion of Pchlide could be a protection mechanism to avoid photosensitization. The same analyses were applied to the culture supernatant of the wild-type culture grown in the light conditions, revealing that EVs are also produced in wild type, but the content of EVs is mainly carotenoids rather than Pchlide. Based on these results, we will discuss new roles of EV in *L. boryana*.

[1] Usui K *et al* (2022) Extracellular vesicle-mediated secretion of protochlorophyllide in the cyanobacterium *Leptolyngbya boryana* Plants, in press

54

Functional analysis of an essential gene in cyanobacteria that is conserved among oxygen-evolving photosynthetic organisms

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In oxygenic phototrophs, photosynthetic electron transport occurs in the special inner membranes called thylakoid membranes (TM). Cyanobacteria are the only prokaryotes that perform oxygenic photosynthesis on TM, and are thought to be share the common ancestor with chloroplasts of algae and plants. TM harbor various protein complexes (e.g., Photosystem I, Photosystem II, Cytochrome *b6f*, ATP synthase) inside a lipid bilayer. Although each membrane lipid and protein has been well studied, molecular mechanisms of biogenesis of entire membranes are still enigmatic. So far, only a few genes that are involved in TM formation, such as vipp1 and curT, have been reported.

Here, we analyzed a cyanobacterial gene that is highly conserved in oxygenic phototrophs (hereafter gene A). Gene A in higher plants is encoded in their nuclear genomes, and its product is predicted to be transported into chloroplasts. Moreover, gene A is absent from the genome of cyanobacteria belonging to the genus *Gloeobacter*, which do not have TM. These genomic data suggest that gene A takes part in TM formation. Because we were unable to create knockout mutants of gene A in some model cyanobacterial strains, a knockdown mutant (KD) of the gene was produced in *Synechococcus elongatus* PCC 7942. The growth of KD was retarded along with reduced pigment content and photosynthetic activity. In KD, the amount and composition of TM lipids were not significantly different from the WT, but ambiguous structure of lipid bilayer of TM was observed. Moreover, a layered structure of TM was partly disrupted. These results suggest, this essential gene is related to the construction of TM, at least in cyanobacteria.

55

Development of gene overexpression system based on the broad host range vector in cyanobacteria

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Cyanobacteria is expected to the ecology-friendly host for producing biomaterials, such as biofuels. Genetic engineering is one approach to promote utilization cyanobacteria, however, cyanobacteria still lag behind the more advanced *E. coli* toolkit. Especially, there is limited information on expression vectors available for gene overexpression in cyanobacteria. Currently, RSF1010-based vector systems are used for bioproduction using cyanobacteria, although expression levels of exogenous genes is limited due to the low copy number [1,2]. Thus, high-copy vector systems that can be used with a variety of cyanobacteria are required.
Here, we have developed a new expression vector pYS that can be used by multiple cyanobacterial species, *Synechococcus elongatus* PCC 7942, *Synechocystis* sp. PCC 6803, and *Synechococcus* sp. PCC 7002. Our comprehensive library screening revealed that a fragment containing ORF630 encoded in the endogenous plasmid pcc5.2 of *S.*6803 was shown to be maintained at a high copy number in a heterologous cyanobacteria host, *S.*7942. We further used GFP reporter system to evaluate the availability of pYS. In *S.*7942, compared to the expression system in chromosome, the strain carrying pYS1-GFP expressed 10-fold of GFP in an IPTF-dependent manner. Similarly, pYS1-GFP was found to be maintained at high copy number in *S.*6803 and *S.*7002 but was no longer regulated by IPTG. To improve the regulation by IPTG, we have constructed the pYS4-GFP, which carrying the double *lacO* operators around the *trc* promoter [3]. pYS4-GFP can be used for IPTG-fully dependent control in *S.*7002. These findings suggest that pYS is useful as a broad host range vector and that the strictness of promoter control depends on the host.

[1] Mermet-Bouvier *et al* (1993) Transfer and replication of RSF1010-derived plasmids in several cyanobacteria of the genera *Synechocystis* and *Synechococcus*. *Current Microbiology* 27:323–327
[2] Huang *et al* (2010) Design and characterization of molecular tools for a synthetic biology approach towards developing cyanobacterial biotechnology. *Nucleic Acids Research* 38:2577–2593
[3] Markley *et al* (2015) Synthetic biology toolbox for controlling gene expression in the cyanobacterium *Synechococcus* sp. strain PCC 7002. *ACS Synthetic biology* 4:595-603

56

Regulation of photosynthesis-dependent nitrogen fixation in the nonheterocystous cyanobacterium Leptolyngbya boryana

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Leptolyngbya boryana is a filamentous and nonheterocystous cyanobacterium that has the nitrogen-fixing growth ability under microoxic conditions. Nitrogenase catalyzing the nitrogen fixation reaction is extremely vulnerable to oxygen. Thus, nitrogen fixing organisms should have some mechanisms to protect nitrogenase from oxygen. While *L. boryana* requires anaerobic environment for nitrogen fixing growth, nitrogenase activity is dependent on light, indicating that nitrogenase is driven by ATP and reductant generated by photosynthesis. In addition, photosynthetic oxygen evolution is maintained well when expression of the *nif* genes is induced by transfer to anaerobic and nitrate-depleted conditions. These characteristics suggest that *L. boryana* has some molecular mechanisms that protects nitrogenase from photosynthetically produced oxygen. However, the mechanisms remain largely unknown [1]. To elucidate the mechanisms, we conducted transposon-tagging mutagenesis to isolate mutants that showed poor nitrogen-fixing growth [2]. From more than 4,000 mutants conferred streptomycin resistance by transposon tagging, 34 mutants showing abnormal nitrogen-fixing growth were isolated. Many of these mutants were accompanied by poor heterotrophic growth in the dark. Genome resequencing was performed for all mutants to identify the site where the transposon was inserted. Based on phenotype and the annotations of responsible genes, we present an updated sketch of the molecular mechanisms that allow nitrogen fixation to coexist with photosynthesis.

[1] Tsujimoto R et al (2014) Transcriptional regulators ChlR and CnfR are essential for diazotrophic growth in nonheterocystous cyanobacteria. Proc Natl Acad Sci USA 111: 6762-6767
[2] Tomatsu C et al (2018) *In vivo* transposon tagging in the nonheterocystous nitrogen-fixing cyanobacterium *Leptolyngbya boryana*. FEBS Lett 592: 1634-1642

57

A novel signaling cascade regulates growth of cyanobacteria according to carbon dioxide

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High CO_2 enhances photosynthetic activity and growth of cyanobacteria. How cells sense the amount of inorganic carbon and balance cellular functions accordingly remains poorly understood. Our recent results reveal a novel signaling cascade that connects growth to the amount of inorganic carbon in the cyanobacterium *Synechocystis* sp. PCC 6803. Transfer of the *Synechocystis* control strain (CS) cells from ambient air to 3% CO_2 was found to activate the expression of photosynthesis and growth related genes by reducing the formation of RNA polymerase (RNAP) holoenzymes containing the growth-restricting SigC sigma factor. Acclimation of *Synechocystis* cells to high CO_2 is prevented if the small ω subunit of the RNA polymerase (RNAP) core is deleted [1, 2]. Without the ω subunit, the formation of the RNAP-SigC holoenzyme was enhanced in high CO_2, and transcriptome analysis showed decreased expression of numerous photosynthetic and cell wall genes in the ω-less ΔrpoZ cells compared to the CS cells in high CO_2. Due to these transcriptional changes, high-CO_2-induced enhancement of photosynthesis was largely missing in ΔrpoZ cells and inefficient synthesis of the peptidoglycan layer of the cell wall induced lysis of dividing ΔrpoZ cells in high CO_2. Spontaneous secondary mutations in the *ssr1600* gene rescued the high-CO_2-sensitive phenotype of the ΔrpoZ strain. Our results show that the Ssr1600 protein functions as an anti-SigC antagonist regulating the formation of the RNAP-SigC holoenzyme. The amount of the Ssr1600 protein responds to CO_2 concentration, regulating the formation of the growth restricting RNAP-SigC holoenzyme according to available inorganic carbon. We suggest a partner switch mode of action for the Ssr1600 protein. Formation of the anti-SigC antagonist Ssr1600/anti-SigC complex releases the SigC sigma factor from the SigC/anti-SigC complex and growth restricting RNAP-SigC holoenzymes are formed.

[1] Gunnelius L *et al* (2014) The omega subunit of RNA polymerase core directs transcription efficiency in cyanobacteria. Nucleic Acids Res 42:4606-4614.
[2] Kurkela J *et al* (2017) Acclimation to high CO_2 requires the ω subunit of the RNA polymerase in *Synechocystis.* Plant Physiol 174:172-184.

58

Generation of synthetic shuttle vectors enabling modular genetic engineering of cyanobacteria

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Cyanobacteria have raised great interest in biotechnology due to their potential for a sustainable, photosynthesis-driven production of fuels and value-added chemicals. This has led to a concomitant development of molecular tools to engineer the metabolism of those organisms. In this regard, however, even cyanobacterial model strains lag behind compared to their heterotrophic counterparts. For instance, replicative shuttle vectors that allow gene transfer independent of recombination into host DNA are still scarce. Here, we introduce the pSOMA shuttle vector series comprising ten synthetic plasmids for comprehensive genetic engineering of *Synechocystis* sp. PCC 6803. The series is based on the small endogenous plasmids pCA2.4 and pCB2.4 each combined with a replicon from *E. coli*, different selection markers as well as features facilitating molecular cloning and the insulated introduction of gene expression cassettes. We made use of genes encoding green fluorescent protein (GFP) and a Baeyer-Villiger monooxygenase (BVMO) to demonstrate functional gene expression from the pSOMA plasmids *in vivo*. Moreover, we demonstrate the expression of distinct heterologous genes from individual plasmids maintained in the same strain and thereby confirmed compatibility between the two pSOMA sub-series as well as with derivatives of the broad-host-range plasmid RSF1010. We also show that gene transfer into the filamentous model strain *Anabaena* sp. PCC 7120 is generally possible, which is encouraging to further explore the range of cyanobacterial host species that could be engineered via pSOMA plasmids. Altogether, the pSOMA shuttle vector series displays an attractive alternative to existing plasmid series and thus meets the current demand for the introduction of complex genetic setups and to perform extensive metabolic engineering of cyanobacteria.

59

Effects of DNA methylation on gene expression and environmental stressors in the cyanobacterium Synechocystis PCC6803

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The field of epigenetics has raised increasing interest over the last decades. Epigenetic factors, such as DNA methylation are of overwhelming relevance in eukaryotes. In bacteria they play important roles in the regulation of cell cycle control [1] or in restriction-modification systems [2]. However, the role of DNA methylation has been little explored in the regulation of gene expression and physiological functions in bacteria, especially in cyanobacteria. Quick adaptations to changing environments are of crucial importance for these global important prokaryotes, where epigenetic factors are considered to play essential roles. Six different DNA methylases have been described for the cyanobacterium *Synechocystis* sp. PCC 6803 [3]. Based on a genome-wide dataset of ^{m4}C and ^{m5}C methylation sites, we investigated variations in the degree of epigenetic modifications associated with selected stress conditions and their relation to changes in transcript levels. The DNA methylase M.Ssp6803II (Sll0729) modifies the first cytosine in GGCC to N4-methylcytosine (GG^{m4}CC). Knockout mutants of *sll0729* showed strongly diminished growth and a reduced amount of chlorophyll a. Recurring suppressor mutants of Δ*sll0729* recovered to a WT-similar phenotype [4]. In these cells a mutation was found directly downstream of the -10 element within the discriminator region of the gene *slr1790* promoter. The mutation led to a GGCC to GGTC single nucleotide substitution. The Gene *slr1790* encodes protoporphyrinogen IX oxidase, a key enzyme of tetrapyrrole biosynthesis and essential for survival. In an heterologous reporter system, co-expression of M.Ssp6803II enhanced the expression of a reporter driven by the *slr1790* promoter. This indicates that the methylation in the discriminator sequence of this promoter leads to a higher affinity towards the RNA polymerase. The data show that the multiple effects of DNA modification in bacteria deserve further attention.

[1] Fioravanti *et al* (2013), PLoS Genet., 9, e1003541.
[2] Tock, M. R., and Dryden, D. T. (2005). Curr. Opin. Microbiol. 8, 466–472.
[3] Hagemann, M *et al.* (2018). DNA Res. 25, 343–352.
[4] Gärtner K *et al.* (2019). *Front. Microbiol.* 10:1233.

60

Dissecting the enzymatic machinery of peptidoglycan synthesis in the filamentous heterocyst-forming cyanobacterium Anabaena PCC 7120

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Heterocyst-forming cyanobacteria grow as chain of cells (known as trichomes or filaments) that can be hundred of cells long. The filament consists of individual cells surrounded by a cytoplasmatic membrane and peptidoglycan (PG) layer. The cyanobacterial PG differs from the best-investigated PG in that it is thicker and possess a higher degree of cross-linking between PG chains than those in other Gram-negative bacteria [1]. However, the essential enzymatic machinery of PG biogenesis and metabolism, including glycosyltransferases, transpeptidases and hydrolases, may be conserved in cyanobacteria [2]. The spatial and temporal growth dynamics of the PG determine the maintenance of the cell shape both during growth and division. Two multiprotein complexes operate in PG growth that carry out localized PG synthesis: the elongasome, which in rod-shaped bacteria directs lateral and peripheral synthesis in the central region of cells during growth, and the divisome, which directs septal PG synthesis during cell division. Both the elongasome and the divisome incorporate a battery of PG-synthesizing enzymes that include bifunctional enzymes with transpeptidase and glycosyltransferase activities, which are known as class A penicillin-binding proteins (PBPs), and monofunctional transpeptidases, known as class B PBPs [3]. The growth of PG has been studied in detail in model rod-shaped bacteria with a heterotrophic metabolism, but much less is known in bacteria with other morphologies or metabolic alternatives. The purpose of this research is to dissect the complexes for lateral and septal PG synthesis in the photosynthetic filamentous heterocyst-forming cyanobacterium *Anabaena* sp. PCC 7120. For this, we intend to generate derivative strains with mutations in each of the predicted PBPs, and to study their localization by means of fusions to fluorescent proteins.

[1] Hoiczyk E, Hansel A (2000) Cyanobacterial cell walls: news from an unusual prokaryotic envelope. J Bacteriol 182:1191-1199. [2] Leganés F *et al*. (2005) Wide variation in the cyanobacterial complement of presumptive penicillin-binding proteins. Arch Microbiol 184:234-248. [3] Typas A *et al*. (2012) From the regulation of peptidoglycan synthesis to bacterial growth and morphology. Nat Rev Microbiol 10:123-136

61

Artificial phycoerythrobilin biosynthesis in Synechocystis sp. PCC 6803

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In cyanobacteria, red algae and cryptophytes, enhanced utilization of photon energy for oxygenic photosynthesis is facilitated by light-harvesting complexes consisting of phycobiliproteins (PBP). These PBPs include open-chain tetrapyrrole chromophores, called phycobilins, which bind to the apo-proteins. Formation of holo-proteins is ensured by covalent linkage via thioether bonds to conserved cysteine residues inside the polypeptide backbone. Proper stereochemical attachment of pigments is provided by so-called PBP lyases. Cryptomonad light-harvesting relies on a highly degenerated, single type of PBP, which is localized in the thylakoid lumen [1]. The cryptophytic light-harvesting apparatus employs the hetero-tetrameric phycoerythrin PE545 consisting of CpeA and CpeB, however, the preceding formation to a fully mature PBP remains mostly elusive [2].
Here we present approaches for the functional characterization of the *Guillardia theta* PE545 post-translational modification machinery. Hereby, *E. coli* and *Synechocystis* sp. PCC 6803 serve as bacterial hosts for heterologous expression of the PE545 subunits and its related lyases. Prior to pigment attachment to CpeA, a PBP lyase (CpeX), specific for the attachment of the chromophore DHBV was produced in *E. coli*. The biochemical activity of the heterologously synthesized CpeX was analyzed spectroscopically by chromophore-binding assays to evaluate its phycobilin-associating activity. Subsequent transfer of DHBV to CpeA requires further characterization. In an alternative approach, assembly of the complementary CpeB is realized via synthetic operons in *Synechocystis* sp. PCC 6803. Consequently, PBP lyase mediated pigment transfer to recombinantly produced PE545 will be investigated by spectroscopic-, HPLC- and MS-analyses.
Being the first completely sequenced cryptomonad, *G. theta* provides a promising opportunity to identify novel PBP lyases and characterize their biochemical activity during maturation. This knowledge will offer an in-depth understanding of how a dramatically degenerated light-harvesting complex is still able to efficiently capture and transfer solar energy to the photosystems' reaction center.

[1] Spear-Bernstein L & Miller K (1989) Unique location of the phycobiliprotein light-harvesting pigment in the cryptophyceae. J. Phycol. **25**, 412–419.
[2] Kieselbach *et al* (2017) Proteomic analysis of the phycobiliprotein antenna of the cryptophyte alga *Guillardia theta* cultured under different light intensities. *Photosynth. Res.* **135**, 149–163.

62

Role of cytochrome cM in Synechocystis sp. PCC 6803 to restart photoautotrophy after prolonged darkness

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In their natural environments photoautotrophic cyanobacteria have to endure both diurnal as well as prolonged periods of darkness, such as, in the extreme, during a Nordic winter. However, it remains largely unknown how cyanobacteria adjust their bioenergetics and metabolism to survive prolonged darkness and, as importantly, to allow them to safely recommence photoautotrophy upon re-illumination.

Cytochrome c_M (CytM) is an enigmatic *c*-type heme protein found in nearly every sequenced cyanobacterial species. We recently identified CytM as a major player in decreasing photosynthesis under photomixotrophic growth conditions in the model cyanobacterium *Synechocystis* sp. PCC 6803.[1] However, a role of CytM in photoautorophy, and its molecular mechanism, remained elusive. This prompted us to ask whether CytM could also have a role in the bioenergetic acclimation of the cyanobacterial cell under dark to light transitions. Here, we investigated the role of CytM in dynamic photoautotrophy by probing the physiology of the ΔCytM mutant under different dark/light regimes. We observed that while ΔCytM and wild-type cells grew similarly under continuous illumination, when a dark period was introduced of 20 h per day, the ΔCytM mutant grew slower than wild-type cells. Further, we discovered that the ΔCytM mutant was unable to resume photoautotrophic growth when kept in darkness for at least 5 days and then shifted to light. We investigated the cause of the phenotype by probing the photosynthetic electron transport chain using a suite of biophysics techniques. We identified the bottleneck in the dark-treated ΔCytM mutant was at PSI, even after the cells were shifted to illumination for 7 days. Further, 77K fluorescence and immunoblotting showed a significant deficiency of PSI centers in comparison to PSII in ΔCytM. Therefore, we concluded that CytM has an essential role in restarting photoautotrophy after prolonged darkness by adjusting bioenergetics in a way that allows reconstitution of the photosynthetic electron transport chain. Combined with our previous knowledge about CytM hindering photosynthesis during photomixotrophic growth, we concluded that CytM is a key player in the regulatory network allowing cyanobacterial cells' bioenergetics response to changing trophic modes.

[1] Solymosi D *et al* (2020) Cytochrome *c* M Decreases Photosynthesis under Photomixotrophy in *Synechocystis* sp. PCC 6803. *Plant Physiol.* 183: 700–71

63

Characterising the role of cyanophage plastocyanin

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Cyanophage infecting *Prochlorococcus* and *Synechococcus* are ubiquitous in marine systems. How cyanophage infections play out, particularly in terms of subverting host metabolism, is however poorly understood. Genome sequencing and infection studies have demonstrated a great diversity in cyanophage genome size and infection strategies. Moreover, within these genomes are a number of host-like metabolic genes, named Auxiliary Metabolic Genes (AMGs). Cyanophage AMGs are diverse, encoding genes related to photosynthesis, nutrient depletion and central carbon metabolism. Intriguingly, some cyanophages are capable of inhibiting CO2 fixation while maintaining photosynthetic electron transport, supposedly to ensure consistent ATP production for phage replication. However, there is relatively little work focused upon characterising the function of specific cyanophage AMGs during host infection. Here, we set out to characterise the role of the cyanophage-encoded plastocyanin gene, *petE*. Plastocyanin is a key electron transport protein in cyanobacterial respiration and photosynthetic electron transport chains and the high abundance of the *petE* gene in cyanophage genomes suggests an important role during infection. Using the freshwater strain *Synechococcus elongatus* spp. PCC7942 as a heterologous host, we generated knock-in mutants where the PCC7942 gene was replaced with the *petE* gene from the marine *Synechococcus* sp. WH7803 or cyanophage S-RSM4. We compared growth and photophysiology measurements under different light intensities to assess if the cyanophage plastocyanin facilitates greater tolerance to light. Alongside this we are using proteomics to investigate where the cyanophage petE protein, as well as other cyanophage AMGs, localise during infection of a marine *Synechococcus* host, to give further clues of the role of such AMGs in the infection process.

64

NirR1 is a small protein that controls nitrite-to-ammonium reduction in cyanobacteria

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Different from all other bacteria, the physiology of cyanobacteria is based on the assimilation of organic compounds from inorganic carbon powered by oxygenic photosynthesis. This setting poses particular regulatory challenges. One of these challenges is the integration of carbon and nitrogen metabolism. Several regulatory factors control the primary nitrogen metabolism, such as the PII protein and multiple different proteins interacting with it (PirA, PirC or PipX) [1], [2], [3] or the glutamine synthetase with its inactivating factors IF7 and IF17 [4]. However, regulatory proteins of other enzymes in the nitrogen assimilation pathway have not been described thus far. Here we reveal the 81 amino acid regulatory protein NirR1 in the cyanobacterium *Synechocystis* sp. PCC 6803 to function as inhibitor of nitrite reductase, the key enzyme in the assimilation of ammonia from nitrate/nitrite. Ectopic overexpression of the *nirR1* gene in *Synechocystis* under standard growth conditions led to phenotypic changes, which included the excretion of nitrite. Nitrite excretion has previously been associated with the shift from high to low CO_2 conditions [5]. Indeed, the expression of *nirR1* goes up under low CO_2, whereas it is repressed by shifts to nitrogen starvation [6]. These data demonstrate that NirR1 plays a crucial role in the coordination of C and N primary metabolism in cyanobacteria by targeting the activity of one of the central enzymes. Our findings suggest that many of relatively small proteins exist that could control the integration of carbon and nitrogen metabolism in cyanobacteria by modulating the activity of relevant enzymes.

[1] Espinosa *et al*., 2006, Mol. Microbiology, DOI: 10.1111/j.1365-2958.2006.05231.x

[2] Bolay *et al*., 2021, mBio, DOI: 10.1128/mBio.00229-21

[3] Orthwein *et al*., 2021, PNAS, DOI: 10.1073/pnas.2019988118

[4] García-Domínguez *et al*., 1999, PNAS, DOI: 10.1073/pnas.96.13.7161

[5] Suzuki *et al*., 1995, Plant Physiology, DOI: 10.1104/pp.107.3.791

[6] Kopf *et al*., 2014, DNA Res., DOI: 10.1093/dnares/dsu018

65

How to implement an oxygen tolerant hydrogenase from Ralstonia eutropha into Synechocystis sp. PCC 6803?

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Cyanobacteria are potential candidates to couple oxygenic photosynthesis and H_2 production with the help of hydrogenases. One of the major challenges in using native cyanobacterial hydrogenases is their high oxygen sensitivity. We addressed this challenge, by the introduction of an O_2-tolerant hydrogenase from *Ralstonia eutropha* into a phototrophic bacterium, namely the cyanobacterial model strain *Synechocystis* sp. PCC 6803 (*Syn*6803) (Lupacchini et al. 2021). We characterized the strain *Syn_Re*SH$^+$ in detail and found that activity is limited by a low enzyme production and maturation. Therefore, we designed an advanced *Re*SH expression system in *Syn*6803, adapting the CyanoGate cloning system, whereby we achieved a higher level of synthesized protein and elevated activity *in vivo*. Interestingly, we found that maturation of the protein complex limits activity and we develop strains to improve this step in *Syn6803*. Furthermore, we determined limitations and improved enzyme activity by optimizing physiological conditions, in particular the light availability, substrate (H_2) supply and a sufficient electrons sink (CO_2). Finally, we follow several strategies to improve the H_2 production in the designed *Syn*6803 strains and we will present challenges that need to be addressed in future to develop a sustained H_2 production with phototrophic microorganisms.

Lupacchini, S., J. Appel, R. Stauder, P. Bolay, S. Klähn, E. Lettau, L. Adrian, L. Lauterbach, B. Bühler, A. Schmid, and J. Toepel. 2021. 'Rewiring cyanobacterial photosynthesis by the implementation of an oxygen-tolerant hydrogenase', *Metab Eng*, 68: 199-209.

66

Effects of light and oxygen on chlorophyll d biosynthesis in a marine cyanobacterium Acaryochloris marina

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A marine cyanobacterium *Acaryochloris marina* synthesizes chlorophyll (Chl) *d* as a major Chl in addition to a small amount of Chl *a*. Chl *d* has a formyl group at its C3 position instead of a vinyl group in Chl *a.* The modification allows Chl *d* to absorb far-red light in addition to visible light. Chl *d* has been assumed to share the biosynthetic pathway with Chl *a* with the unidentified enzyme catalyzing the formation of the C3-formyl group. Experiments using oxygen isotopes suggested that the Chl *d* biosynthesis is catalyzed by an oxygen-dependent oxygenase [1] though detailed reaction mechanism remains unknown. The situation is different from that of the biosynthetic enzyme of Chl *f* that has a formyl group at its C2. The enzyme was recently found to be a paralogue of the PSII core subunit that catalyzes the formylation reaction in a light-dependent manner [2]. In this study, we focused on light and oxygen, the most important external factors in Chl biosynthesis, and investigated their effects on Chl *d* biosynthesis in *A. marina*. The amount of Chl *d* in heterotrophic dark-grown cells was comparable to that in light-grown cells, indicating that *A. marina* has a light-independent pathway in Chl *d* biosynthesis. Interestingly, the Chl *a/d* ratio increased significantly in the dark. Under anoxic conditions, the amount of Chl *d* increased with growth in light, however no growth was observed in dark, indicating that *A. marina* synthesizes Chl *d* normally even under such “micro-oxic” conditions caused by endogenous oxygen evolution. Interestingly, accumulation of pheophorbide *d* was observed in anoxic dark conditions, suggesting that Chl *d* degradation is induced by anaerobicity and darkness although the mechanism for the oxygen requirement for Chl *d* biosynthesis has not been evaluated yet.

[1] Schliep, M., et al. (2010) ^{18}O labeling of chlorophyll *d* in *Acaryochloris marina* reveals that chlorophyll *a* and molecular oxygen are precursors. J Biol Chem, **285**(37): p. 28450-6

[2] Ho, M.Y., et al. (2016) Light-dependent chlorophyll *f* synthase is a highly divergent paralog of PsbA of photosystem II. Science, **353**(6302)

67

How do multicellular cyanobacteria escape the viral trap?

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Viruses are pervasive and critical for the structure and diversity of bacterial communities in aquatic ecosystems. Much like viral infections in our own population, reducing transmission of the virus is key to preventing and halting large-scale viral infections throughout the population. Spatial isolation can achieve this, where increasing cell-to-cell distance can reduce the encounter rate of virus to cell. However, independent lineages of bacteria, and particularly within the cyanobacteria have evolved multicellularity. Any benefit to multicellular cooperation may be outweighed by viral pressure. How multicellular cyanobacteria reduce viral transmission between neighbouring cells and thus escape the viral trap is currently unknown.

This project aims to reveal whether multicellular cyanobacteria can resist viral infection, and if so, through which mechanisms. We will isolate phage against two strains of cyanobacteria that each possess different multicellular phenotypes. We will seek to understand how transmission occurs between neighbouring cells and how the colony as a whole can protect itself.

68

Membrane destabilization and fusion induced by the Synechocystis IM30 protein

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The inner membrane-associated protein of 30 kDa (IM30, also known as “the vesicle-inducing protein in plastids 1, Vipp1”) is essential in chloroplasts and cyanobacteria. IM30 appears to be crucially involved in thylakoid membrane biogenesis and/or maintenance, where it either stabilizes membranes or triggers membrane fusion processes. These apparently contradicting functions have to be tightly controlled and separated spatiotemporally in chloroplasts and cyanobacteria. IM30’s fusogenic activity depends on Mg^{2+} [1], which directly binds to IM30, resulting in a rearrangement of the IM30 structure, involving exposure of extended hydrophobic surface regions on IM30 rings [2]. Interaction of Mg^{2+} with IM30 results in increased binding of IM30 to native as well as model membranes. Via Atomic Force Microscopy (AFM) in liquid, IM30-induced bilayer defects were observed in solid-supported bilayers in presence of Mg^{2+}. These defects start as small holes within individual IM30 *puncta* and expand to larger defect structures. The observed defect structures differ dramatically from the membrane-stabilizing carpet structures, which we previously observed in the absence of Mg^{2+} [3]. Thus, Mg^{2+}-induced alterations of the IM30 structure switches the IM30 activity from a membrane-stabilizing to a membrane-destabilizing function, a crucial step in membrane fusion. Likely, Mg^{2+}-loaded IM30 initially binds to and stabilizes spontaneously occurring small and transient bilayer defects. Sequential local accumulation of more IM30 protomers potentially promotes growth of the bilayer defects and might finally result in a bilayer-spanning toroidal or barrel-stave pore. The bilayer defects observed on solid-supported lipid bilayers likely trigger *in vitro* fusion of liposomes and thylakoid membrane fusion *in vivo*, in living cyanobacterial cells.

[1] Hennig, R *et al.* (2015) IM30 triggers membrane fusion in cyanobacteria and chloroplasts, Nature Comm 6, 7018
[2] Heidrich J *et al*. (2018) Mg^{2+}-binding triggers rearrangement of the IM30 ring structure, resulting in augmented exposure of hydrophobic surfaces competent for membrane binding, J Biol Chem 293, 8230-8241
[3] Junglas B, *et al*. (2020) IM30 IDPs form a membrane protective carpet upon supercomplex disassembly, Comm Biol 3, 595

70

Nitrogen exchange in marine diatom-heterocystous cyanobacteria symbioses

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In open ocean environments the concentration of nitrogen is extremely low and some diatoms establish symbioses with N_2-fixing cyanobacteria. Some of these symbioses involve heterocystous cyanobacteria of the *Richelia* genus, whose location in diatoms varies from cytoplasmic to periplasmic to fully external [1]. While the role of the symbiotic cyanobacterium is to provide nitrogen to the diatom host, the draft genomes of the periplasmic (*R. intracellularis* RC01) and cytoplasmic (*R. euintracellularis* HH01) symbionts show lack of some nitrogen metabolism enzymes and pathways suggesting metabolite exchange between host and symbiont [1, 2]. Thus, HH01 lacks glutamine-2-oxoglutarate aminotransferase (GOGAT), the RC01 lacks the proline biosynthesis pathway, and both symbionts lack a complete polyamine biosynthesis pathway. The comparison between the genomes of the symbionts and those of model free-living heterocystous cyanobacteria has allowed the identification of membrane transporters potentially involved in metabolite exchange [2]. The symbionts have ABC transporters homologous to the *Anabaena* systems for non-polar amino acids (N-I), for polar and acidic amino acids (N-II), and for polyamines (Pot) [2]. We have expressed in *E. coli* and purified the solute binding proteins (SBPs) of these transporters, and their binding specificity for different solutes was tested by means of intrinsic tryptophan fluorescence quenching. We have shown that the N-II SBP from HH01 (RintHH_12770, NatF) can bind glutamate *in vitro* with high affinity, suggesting a possible glutamate/glutamine exchange between the diatom and the symbiont; we have also shown that the Pot SBP from HH01 (RintHH_7180, PotD) can bind specifically spermidine *in vitro*, suggesting a possible exchange of arginine or agmatine for the polyamine spermidine in this symbiosis; and we are currently studying solute binding to the N-I SBP from HH01 (RintHH_11820, NatB). These results show the feasibility of studying the function of proteins from non-cultivable symbionts by heterologous gene expression and have unraveled previously unknown aspects of the physiology of these symbioses.

[1] Hilton JA, *et al* (2013) Genomic deletions disrupt nitrogen metabolism pathways of a cyanobacterial diatom symbiont. Nat Commun 4:1767.
[2] Nieves-Morión M, *et al* (2020) Predicting substrate exchange in marine diatom-heterocystous cyanobacteria symbioses. Environ Microbiol 22:2027-2052.

72

Identification of the biosynthetic pathway for the production of the potent trypsin inhibitor radiosumin

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Cyanobacteria are a prolific source of natural products with potent biological activities. Radiousmin is a complex dipeptide first isolated from *Plectonema radiosum* NIES-515, comprised of Aayp (2-amino-3-(4-amino-2-cyclohexene-1-ylidene) propionic acid), Aacp (2-amino-3-(4-amino-2cyclohexylidene) propionic acid) and two acetyl groups. Radiousmin is a strong trypsin inhibitor active in submicromolar conentrations. Here we identified 16 putative radiousmin biosynthetic gene clusters from the genomes of cyanobacteria. Heterologous expression of the 16.8 kb radiosumin biosynthetic pathway from *Dolichospermum planctonicum* UHCC 0167 in *Escherichia coli* BAP1 resulted the production of radiosumin B. These results provided insights into the biosynthetic origins of the potent trypsin inhibitor radiosumin in cyanobacteria and experimentally confirmed the involvement of the radiosumin gene cluster in the biosynthesis of radiosumins.

73

Genomic and phenotypic characterization of Chloracidobacterium isolates provides evidence for multiple species

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Chloracidobacterium is the first and until now the sole genus in the phylum *Acidobacteriota* (formerly *Acidobacteria*) whose members perform chlorophyll-dependent phototrophy (i.e., chlorophototrophy). An axenic isolate of *Chloracidobacterium thermophilum* (strain B^T) was previously obtained by using the inferred genome sequence from an enrichment culture and *in situ* diel metatranscriptomic profiling analyses to direct adjustments to the growth medium and incubation conditions. Based on these studies, a defined growth medium for *Chloracidobacterium thermophilum* was developed which consequently was used for the isolation of eight additional strains of *Chloracidobacterium* spp. from microbial mat samples collected from Mushroom Spring, Yellowstone National Park, USA, at temperatures of 41, 52, and 60 °C; and an axenic strain from Rupite hot spring in Bulgaria. All isolates are obligately photoheterotrophic, microaerophilic, non-motile, thermophilic, rod-shaped bacteria. *Chloracidobacterium* spp. synthesize multiple types of (bacterio-)chlorophylls and have type-1 reaction centers similar to those of green sulfur bacteria. Light harvesting is accomplished by the bacteriochlorophyll *a*-binding Fenna-Matthews-Olson protein, and chlorosomes containing bacteriochlorophyll *c*. The genomes of these isolates are approximately 3.7 Mbp in size and comprise two circular chromosomes with sizes of approximately 2.7 Mbp and 1.0 Mbp. Comparative genomic studies and phenotypic properties suggest that the nine isolates represent three species within the genus *Chloracidobacterium*. In addition to *C. thermophilum*, the microbial mats at Mushroom Spring contain a second species, tentatively named *Chloracidobacterium aggregatum*, that grows as aggregates in liquid cultures. The Bulgarian isolate, tentatively named *Chloracidobacterium validum*, will be proposed as the type species of the genus, *Chloracidobacterium,* the type genus of a new family, *Chloracidobacteriaceae,* within new order *Chloracidobacteriales,* the class *Blastocatellia,* and the phylum *Acidobacteriota.*

74

Sigma factors in cyanobacteria: a novel proposal of functional classification and evolutionary distribution

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Cyanobacteria are gram-negative prokaryotes that perform oxygenic photosynthesis, and found almost all over the earth. They are diverse, including unicellular, filamentous, freshwater, marine, thermostable, and drought-resistant species [1]. Transcription in bacteria is carried out by a multisubunit core RNA polymerase (RNAP) that associates with an interchangeable sigma (σ) subunit and directs the transcription machinery to specific promoter regions [2,3]. The sigma factors can be divided into two classes: σ70 and σ54. Unlike other bacteria, cyanobacteria have a great diversity of alternative σ70 factors with highly modular composition in functional domains, which contributes to the high ability of cyanobacteria to adapt to a wide range of environmental and physiological changes [4]. Several studies have explored the diversity and distribution of σ70 among in cyanobacteria, leading to 3 or 4 family groups of sigma proteins [1,4]. However, these studies used traditional full-length protein comparison methods which in our opinion might give misleading results on σ70 protein classification. In this work, we take advantage of methods that compare proteins based on functional domain patterns, to identify sigma factors in the 360 cyanobacterial genomes. The modular functional domain composition of σ70, combined to the comparative genomics analysis lead to a broaden classification of σ70 proteins into 13 clans with distinct functional families, for which each of them was functionally explored and described. Finally, the diversity and distribution of sigma factors were analyzed in relation with cyanobacterial cell types, morphological and physiological traits. Our results provide insights into the functional relationship among the vast diversity of σ70 protein groups, with their evolutionary distribution among cyanobacterial lineages.

References

[1] Imamura & Asayama (2009) Sigma factors for cyanobacterial transcription. Gene Regulation and Systems Biology:3 65–87.
[2] Saecker et al. (2011) Mechanism of bacterial transcription initiation: RNA polymerase-promoter binding, isomerization to initiation-competent open complexes, and initiation of RNA synthesis. J Mol Biol 412:754 –771.
[3] Helmann (2019). Where to begin? Sigma factors and the selectivity of transcription initiation in bacteria. Mol Microbiol 112, 335-347.
[4] Srivastava et al. (2020) Cyanobacterial Sigma Factors: Current and Future Applications for Biotechnological Advances. Biotechnology Advances 40: 107517.

75

FurC engagement in the regulatory network of heterocyst development and nitrogen fixation in Anabaena

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FurC is one of the three paralogs of FUR (Ferric Uptake Regulator) proteins present in *Anabaena* sp. PCC 7120. Initially, FurC was described as the PerR ortholog in this cyanobacterium since it was involved in the modulation of the oxidative stress response. However, afterwards experimental data obtained in our laboratory revealed that a *furC*-overexpressing strain (EB2770FurC) was unable to develop heterocysts which pointed to a potential participation of FurC in the regulation of this process.

In this work the transcriptome of a *furC*-overexpressing strain was compared with those of *Anabaena* sp. PCC7120 (wild type strain) under standard and nitrogen-deficiency conditions. Under standard conditions, the *furC* overexpression modified the mRNA levels of 197 genes. Interestingly, genes potentially regulated by FurC were engaged in relevant functional categories such as photosynthesis, iron metabolism and nitrogen and energy metabolisms among others. Subsequently, EMSA assays revealed that *psbAIV*, the *fec* system, a succinyl-CoA synthetase and some regulatory genes such as *all7016* and *alr9013* were direct targets of FurC.

Under nitrogen starvation conditions, RNA seq comparative analyses indicated that 504 genes showed altered expression in the EB2770FurC strain related to *Anabaena* sp. PCC7120. This differential expression under nitrogen starvation conditions suggested that FurC could display a relevant role in the adaptation of *Anabaena* to this situation. Among the heterocyst differentiation and nitrogen fixation category, 20 genes were found to be deregulated in the EB2770FurC strain. Finally, EMSA assays revealed novel FurC-direct targets including some regulatory elements that control heterocyst development (*hetZ* and *asr1734*), genes directly involved in the heterocyst envelope formation (*devBCA* and *hepC*) and genes which participate in the nitrogen fixation process (*nifHDK* and *nifH2*, *rbrA* rubrerythrin and *xisHI* excisionase). In summary, this work shows a new player in the complex regulatory network of heterocyst formation and nitrogen fixation.

76

A PROTEASE- MEDIATED MECHANISM REGULATES THE CYTOCHROME C6/ PLASTOCYANIN SWITCH IN CYANOBACTERIA

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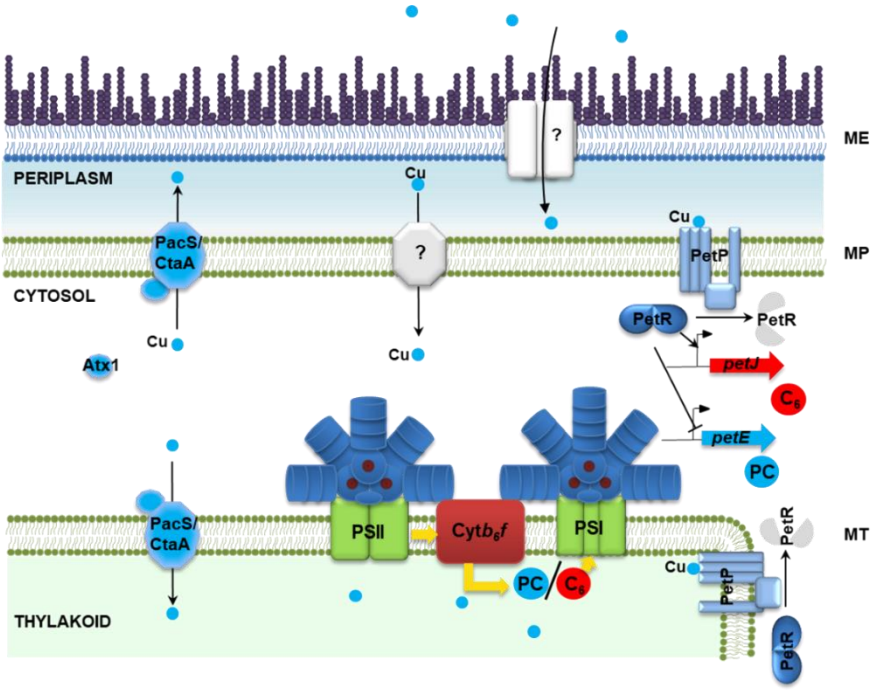
After the Great Oxidation Event (GOE) iron availability was greatly reduced and photosynthetic organisms evolved several alternative proteins. One of these proteins is plastocyanin, a type I blue-copper protein able to replace cytochrome c6 as soluble electron carrier between cytochromeb_6f and PSI. In most cyanobacteria, expression of these two alternative proteins is regulated by copper availability but the regulatory system remained unknown. Here we provide evidence that the regulatory system is composed by a BlaI/CopY family transcription factor (called PetR) and a BlaR membrane protease (called PetP). PetR represses *petE* (plastocyanin) expression and activates *petJ* (cytochrome c_6) while PetP controls PetR levels *in vivo*. Using whole cell extracts we prove that PetR degradation requires the presence of both PetP and copper. Transcriptomic analysis has revealed that PetRP system regulates only 4 genes (*petE*, *petJ*, *slr0601* and *slr0602*) highlighting its specificity. Furthermore, the presence of *petE* and *petRP* in early branching cyanobacteria indicate that acquisition of these genes could have been an early adaptation to reduced iron bioavailability after GOE. New data on how copper is detected and how the signal is transduced by the PetRP system will presented. In addition, we will also present evidence of the function of this system in *Anabaena* sp. PCC 7120.



Copper metabolism and transport in Cyanobacteria: Scheme showing the regulatory system for *petE*/*petJ* switch and the putative copper transporters in cyanobacteria.

Funding: Grant PID2020-112645GB-I00 funded by MCIN/AEI/ 10.13039/501100011033

77

Novel regulatory networks performed by Ferric Uptake Regulator (FUR) proteins in Anabaena sp. PCC 7120

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In cyanobacteria FUR (Ferric Uptake Regulator) proteins are a family of key transcriptional regulators which control a wide set of cellular processes, ranging from photosynthesis to nitrogen metabolism [1,2]. The cyanobacterium *Anabaena* sp. PCC 7120 contains three FUR paralogues, which have important roles in the response to abiotic stresses: FurA, which is involved in responses to iron deficiency, nitrogen starvation and oxidative stress; FurB, which controls responses to zinc deficiency and oxidative stress; and FurC, which has been found to be implicated in responses to oxidative stress and nitrogen deficiency [1,2].
Apart from directly regulating their target genes, previous studies have suggested that FUR proteins could also control the expression of many other transcriptional regulators and two component systems, whose targets and activities remain unknown. Interestingly, some of these regulators do not show orthologs in heterotrophic bacteria, indicating that at least some of them could be specific of cyanobacteria. This indicates that FUR proteins could be cornerstones of novel regulatory networks in cyanobacteria, which would expand their regulons and would allow them to indirectly regulate many other cellular processes.
In this work we have identified around 30 genes with regulatory functions directly regulated by FUR proteins, including adenylate cyclases, transcriptional regulators, sigma factors, sensor kinases and response regulators. Besides, as FUR paralogues are involved in the responses of this cyanobacterium to nitrogen deficiency, the coregulation of these regulatory networks by the global regulator of nitrogen metabolism NtcA has also been analysed, obtaining that some of these genes are coregulated by FUR proteins and NtcA. This has allowed us to define new transcriptional networks in cyanobacteria, which could be involved in orchestrating their cellular responses to abiotic stresses.
[1] Sevilla E *et al* (2021) Fur-like proteins: Beyond the ferric uptake regulator (Fur) paralog. Arch Biochem Biophys 701:108770.
[2] Fillat MF (2014) The FUR (ferric uptake regulator) superfamily: Diversity and versatility of key transcriptional regulators. Arch Biochem Biophys 546:41-52.

78

Consequences of Zur (FurB) deregulation unveil novel metabolic networks in Anabaena sp. PCC7120.

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The genome of *Anabaena* sp. PCC7120 encodes three FUR paralogs called FurA (ferric uptake regulator), FurB/Zur (zinc uptake regulator) and PerR (Peroxide stress regulator). Functional characterization of these proteins evidenced that their activity goes beyond the control of metal homeostasis, since they are also engaged in the modulation of photosynthesis, nitrogen metabolism and different stress responses. Previous work showed that the regulon of FurB consists of genes mainly involved in the adaptation of the cyanobacteria to zinc deficiency and the prevention of oxidative damage in the cell [1,2]. Differential expression analysis of RNA-seq data provided us a deeper knowledge of the consequences of deregulation of zinc homeostasis in *Anabaena*. This study also unveiled potential metabolic networks linked to the activity of FurB. Deletion of the *zur* gene caused significative changes (fold-change >$\pm$2 and p-value >0.05) in the transcription of 406 genes, of which 166 were annotated and belonged to different functional categories. In addition to the set of genes previously identified as FurB targets [1], the deletion of *zur* affected the transcription of genes involved in photosynthesis, regulatory functions, nitrogen metabolism, transposition, cell wall, transporters and carbon metabolism. The presence of FurB boxes and the binding ability of FurB to these potential targets were analyzed. One of the most outstanding groups of genes with altered transcription were involved in carbohydrate metabolism and included a set of glycosyltransferases and other enzymes potentially involved in biofilm formation. Therefore, we set a protocol for the establishment of the appropriate culture conditions and the monitorization of biofilm formation in different variants of *Anabaena* sp. PCC7120. Interestingly, the amount of FurB in the cell was related to the ability of these *Anabaena* variants to form biofilms. Furthermore, we also observed that the level of FurB expression slightly affects the frequency of heterocyst formation.

[1] Napolitano M *et al* (2012) Characterization of the response to zinc deficiency in the cyanobacterium *Anabaena* sp. PCC 7120. J Bacteriol 194:2426-36.
[2] Sein-Echaluce VC *et al* (2015) Zur (FurB) is a key factor in the control of the oxidative stress response in *Anabaena* sp. PCC 7120. Environ Microbiol 17:2006-17.

79

Thioredoxin mediated redox modulation of the Ferric Uptake Regulator FurA in Anabaena sp. PCC 7120

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The Ferric Uptake Regulator FurA from *Anabaena* sp. PCC 7120 is a multifunctional regulator that contains five cysteines, four of them arranged into two CXXC motifs. Lack of structural zinc ion enables FurA to develop disulfide reductase activity and allows the occurrence of intra and intermolecular thiol-disulfide exchanges. Indeed, previous studies showed that four of these five cysteins are involved in the formation of two intramolecular disulphide bridges and that FurA could coexist in different redox states in the cytosol [1]. Interestingly, FurA oxidation state determines its activity as regulator and its ability to bind to different metabolites. However the precise mechanism underlying the reduction of FurA, as well as its functional electron donor, remain still unknown. Because of the relationship between FurA and the control of genes involved in oxidative stress defence and photosynthetic metabolism, we sought to investigate the role of type *m* thioredoxin TrxA as a potential redox partner mediating dithiol-disulfide exchange reactions necessary to facilitate the interaction of FurA with its different ligands.
Our results demonstrate that TrxA is able to interact both *in vitro* and *in vivo* with FurA from *Anabaena* sp. PCC 7120, as it was seen in cross-linking and bacterial two-hybrid assays. Light to dark transitions resulted in reversible oxidation of a fraction of the regulator present in *Anabaena* sp. PCC 7120. Reconstitution of an electron transport chain using *E. coli* NADPH-thioredoxin-reductase followed by alkylation of FurA reduced cysteines evidenced the ability of TrxA to reduce FurA. Furthermore, the use of site-directed mutants allowed us to propose a plausible mechanism for FurA reduction. Taken together, these results point to TrxA as one of the redox partners that modulates FurA performance [2].
[1] Botello-Morte L *et al* (2013) Unraveling the Redox Properties of the Global Regulator FurA from *Anabaena* sp. PCC 7120: Disulfide Reductase Activity Based on Its CXXC Motifs. Antioxid Redox Signal 20:1396-1406.
[2] Guío J *et al* (2021) Thioredoxin Dependent Changes in the Redox States of FurA from *Anabaena* sp. PCC 7120. Antioxidants 10(6):913.

80

Evolution of novel mycosporine-like amino acids (MAAs) in Cyanobacteria and characterisation of their photochemical properties

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Ultraviolet radiation emitted by the sun −particularly UVA radiation, with wavelengths between 315 and 400 nm− can be harmful to humans, causing sunburn and DNA damage resulting in skin cancer. UVA radiation can also impair other organisms, which develop strategies to avoid this damage. Some of these organisms, including photosynthetic cyanobacteria, produce a range of small UV-absorbing molecules called mycosporine-like amino acids (MAAs) as a protection mechanism against UV radiation. These molecules absorb UV radiation between 310 nm and 362 nm and could be potentially used as sunscreens for humans, substituting some of the currently used chemical sunscreens that can be toxic for the environment.
The main goal of this project is to use cyanobacterial strains *Chlorogloeopsis fritschii* PCC 6912 and PCC 9212 to produce and characterise novel MAAs that could be used in sunscreen formulations. To date, two known MAAs (mycosporine-glycine and shinorine) and two unknown UV-absorbing molecules have been extracted from *C. fritschii*, and some stability assays have been carried out before scaling up and purifying the novel MAAs for structural elucidation.

81

Thioredoxin TrxA regulates carbon and nitrogen flow in Synechocystis sp PCC 6803

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Thioredoxins (Trxs) are thiol-disulfide oxidoreductases. In photosynthetic organisms, Trxs regulate the activity of target enzymes in a light-dependent manner. Three Trxs are conserved among cyanobacteria and eukaryotic chloroplasts: *m*-type (also known as TrxA), *x*-type (TrxB) and *y*-type (TrxQ) Trxs. The fourth Trx, named TrxC, is unique to cyanobacteria. In *Synechocystis* sp PCC 6803 only TrxA is essential for growth. To investigate the role of this Trx, our group has recently developed a strategy based on low-level expression of TrxA (1). The mutant strain, named STXA2, in the presence of an inducer, showed TrxA levels of 10% compared to the wild type but only small phenotypic differences. TrxA levels decreased 3-fold after removal of the inducer, causing strong phenotypic changes. We performed a transcriptomic analysis to explore the changes in gene expression associated with these phenotypic changes in the STXA2 strain. The analysis shows that the response of many genes for the major cellular processes are highly coordinated. We observed a strong down-regulation of genes related to photosynthesis, ATP synthesis or CBB cycle, while genes related to photoprotection, alternative electron flows or amino acid metabolism were up-regulated in response to low TrxA levels. Physiological and metabolic analysis also show that low TrxA levels lead to accumulation of nitrogen and carbon storage polymers, as well as changes in amino acid pools. Interestingly, STXA2 cells rapidly respond to re-addition of the inducer and return to the initial phenotype. Taken together, our results suggest a role for TrxA in the regulation of carbon and nitrogen balance.

(1) Mallén-Ponce, Manuel J., et al (2021) Depletion of M-Type Thioredoxin Impairs Photosynthesis, Carbon Fixation, and Oxidative Stress in Cyanobacteria. *Plant Physio*187: 10.1093/plphys/kiab321.

82

Electron flow measurements enable determination of cyclic electron flow and redox state in cyanobacteria

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The exact contributions of linear and cyclic electron transport are difficult to measure accurately and have been a matter of debate since long. Here, we describe the development of a method that allows quantification of electron flow in absolute terms through photosystem I in a photosynthetic organism for the first time. Specific newly established protocols allowed to discern the redox states of plastocyanin, P700 and the FeS-clusters including ferredoxin at the acceptor site of PSI in the cyanobacterium *Synechocystis* sp. PCC 6803 with the near-infrared spectrometer Dual-KLAS/NIR. P700 absorbance changes determined with the Dual-KLAS/NIR correlated linearly with direct determinations of PSI concentrations using EPR. We used dark-interval relaxation kinetics measurements ($DIRK_{PSI}$) to determine electron flow through PSI under different conditions [1].
Combination of $DIRK_{PSI}$ with oxygen evolution measurements yielded a proportion of 35 % of surplus electrons passing PSI compared to PSII. We attribute these electrons to cyclic electron transport, which is twice as high as assumed for plants. Counting electrons flowing through the photosystems allowed determination of the number of quanta required for photosynthesis to 11 per oxygen produced, which is close to previously published values [1].
On the basis of these investigations we could show using the Dual-KLAS/NIR instrument that photomixotrophic cells more strongly reduce their NAD(P)H pool and in parallel their ferredoxin. This results in a shift from using NAD(P)H dependent enzymes to ferredoxin dependent ones including the ferredoxin dependent GOGAT and the pyruvate:ferredoxin oxidoreductase. Especially the latter is surprising since this enzyme is known to be oxygen sensitive in many organisms [2].
[1] Theune ML, Hildebrandt S, Steffen-Heins A, Bilger W, Gutekunst K, Appel J (2021) *In-vivo* quantification of electron flow through photosystem I – cyclic electron transport makes up about 35 % in a cyanobacterium. BBA-Bioenerg 1862:148353

[2] Wang Y, Chen X, Spengler K, Terberger K, Boehm M, Appel J, Barske T, Timm S, Battchikova N, Hagemann M, Gutekunst K (2022) Pyruvate:ferredoxin oxidoreductase and low abundant ferredoxins support aerobic photomixotrophic growth in cyanobacteria. Elife 11:e71339

83

The fate of mRNAs: Sub-cellular localization and degradation of RNAs in bacterial cells

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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 “Rloc-sequencing” combining cell fractionation and RNA-sequencing and the visualization technique fluorescence in situ hybridization (FISH) combined with high-resolution microscopy[2,3]. Using Rloc-sequencing we further intent to identify different sequential motifs and corresponding RNA-binding proteins 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].

Key literature:

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).

85

Efflux transporter capability in Synechocystis: roles and application

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The multidrug and toxin efflux (MATE) protein family comprises multipass membrane transporters that are ubiquitous across evolution. Their common occurrence is accompanied by important functions. They operate in antibiotic resistance and anticancer drug toxicity, or regulate plant fertility via seed and guard-cell chemistry. In Man, the pair of MATEs is most strongly expressed in the kidney, removing cation drugs from the body, but with interesting differential expression of MATE1 and MATE2. In *Arabidopsis*, a large group of approx. 57 duplicated family members is typical of the angiosperms [1]. These representatives have distinct specificities for compounds that range from ions to a hormone, secondary metabolites and xenobiotics. In *Synechocystis,* two MATEs were previously reported to be responsible for efflux of toxic cations, but not a range of other compounds, and one of the pair was reported to be involved in paraquat resistance. One of the pair is a eubacterial NorM-type MATE, and the other is similar to the common bacterial and archaeal DinF-type. Transcription assays revealed a complex expression profile which was regulated by other transporters or xenobiotic application [2]. Meanwhile, our previous findings on flavonoid transport by the *A. thaliana* FFT MATE [3], which is similar in structure to both NorM and eukaroytic MATEs, led us to hypothesise that the cyanobacterial pair might be capable of effluxing cyanobacterial natural products. As well as studying function *in vivo*, a synthetic biology assembly-pipeline has been set up to test these transporters' ability to efflux high-value products from the cell.

[1] Subramanian NM et al. (2022) A systematic phylogenomic classification of the multidrug and toxic compound extrusion transporter gene family in plants. Front Plant Sci 13 doi 10.3389/fpls.2022.774885.
[2] Ongley SE et al. (2016) A multidrug efflux response to methyl viologen and acriflavine toxicity in the cyanobacterium Synechocystis sp. PCC6803. J Appl Phycol doi 10.1007/s10811-016-0816-5.
[3] Thompson EP et al. (2010) An Arabidopsis flavonoid transporter is required for anther dehiscence and pollen development. J Exp Bot 61 doi10.1093/jxb/erp312.

86

Uncovering the role of FurC/PerR in the regulation of processes dependent of C/N balance.

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FurC from *Anabaena* sp. PCC7120 is transcriptional regulator previously described as the Peroxide Stress Regulator (PerR) [1]. However, recent studies revealed that FurC regulation goes beyond the response to hydrogen peroxide, modulating the expression of genes involved in photosynthesis [2], iron homeostasis and nitrogen metabolism [3]. Previous differential gene expression analysis of a *furC*-overexpressing strain (EB2770FurC) allowed the inference of a putative FurC-box [3]. In the present work, a more restrictive version of the FurC box was searched in the promoter regions of the whole *Anabaena* sp. PCC7120 genome with the aim to explore the scope of FurC regulon. The prediction was validated by gel retardation assays revealing 19 new direct FurC targets that belonged to different functional categories. Some of them display relevant roles in nitrogen fixation (*hetR* and *hgdC*) and central carbon metabolism (*cmpR*, *glgp1* and *opcA*) suggesting a key role of FurC in the control of processes dependent of the C/N balance. Moreover, the gene expression levels of the selected newly identified targets were measured by Real Time RT-PCR in the *furC*-overexpressing strain compared to those in *Anabaena* sp. PCC7120. Interestingly, most of the tested FurC targets were upregulated in the *furC*-overexpressing strain, supporting the role of FurC as a transcriptional activator of some genes. Additionally, isothermal titration calorimetric experiments revealed that in presence of manganese and DTT FurC was able to bind to 2-oxoglutarate (2-OG), a molecule that inform about the C/N balance. These results suggest that FurC could tune the regulation of genes involved in the C and N metabolisms by sensing 2-OG levels.

[1] Yingping, F *et al* (2014) Unravelling the cross-talk between iron starvation and oxidative stress responses highlights the key role of PerR (*alr0957*) in peroxide signalling in the cyanobacterium *Nostoc* PCC 7120. Environ Microbiol Rep 6, 468-475.
[2] Sevilla, E *et al* (2019) Regulation by FurC in *Anabaena* Links the Oxidative Stress Response to Photosynthetic Metabolism. Plant Cell Physiol 60, 1778-1789.
[3] Sarasa-Buisan, C *et al* (2022) FurC (PerR) from *Anabaena* sp. PCC7120: a versatile transcriptional regulator engaged in the regulatory network of heterocyst development and nitrogen fixation. Environ Microbiol 24, 566-582.

87

7dSh, a bioactive sugar from Synechococcus Elongatus with herbicidal properties

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The simple, unicellular cyanobacterium *Synechococcus elongatus* (*S. elongatus*) excretes a bioactive compound in culture supernatant that inhibits the growth of various prototrophic organisms including species of cyanobacteria. The compound was isolated and identified to be the unusual sugar 7-deoxy-sedoheptolose (7dSh). Usually, bioactive compounds are produced from enzymes that are encoded within specific secondary metabolite gene clusters. In contrast, 7dSh is the product of promiscuous enzyme activity derived from unusual salvage of 5-deoxyadenosine (5dAdo), the by-product of SAM-radical enzyme activity. We showed that the 5dAdo recycling route in *S. elongatus* uses promiscuous enzymes that lead to the excretion of 5-deoxy-D-ribose (5dR), which is further converted to 7dSh using the general transketolase reaction. We established a chemoenzymatic synthesis of 7dSh using *S. elongatus* transketolase as catalyst and 5dR as substrate. The synthesis of 7dSh in sufficient quantities allowed for thorough antimicrobial and herbicidal bio-profiling. 7dSh showed strong growth inhibition on *Arabidopsis thaliana* at low micromolar concentrations. Untargeted metabolome analysis of 7dSh-treated organisms showed a strong accumulation of 7-deoxy-D-arabino-heptulosonic acid 7-phosphate, indicating that 7dSh targets 3-dehydroquinate synthase (DHQS), a key enzyme of the shikimate pathway. Inhibition of purified DHQS by 7dSh confirmed this assumption. This explains the herbicidal activity of 7dSh, since the shikimate pathway is essential in bacteria, fungi, and plants. As this pathway is absent in animals and humans, it is a top target for the development of herbicides. In fact, glyphosate, by far the most used herbicide worldwide, targets the shikimate pathway. Further work is required to develop a biological herbicide derived from 7dSh for future glyphosate substitution.

[1] **Brilisauer, K et al.** (2019). Cyanobacterial antimetabolite 7-deoxy-sedoheptulose blocks the shikimate pathway to inhibit the growth of prototrophic organisms. *Nature Communications*, 10 (545), https://doi.org/10.1038/s41467-019-08476-8.

[2] **Rapp, Johanna et al.** (2021). “A Bioactive Molecule Made by Unusual Salvage of Radical SAM Enzyme Byproduct 5-Deoxyadenosine Blurs the Boundary of Primary and Secondary Metabolism.” *Journal of Biological Chemistry* 296. https://doi.org/10.1016/j.jbc.2021.100621

[3] **Rapp, Johanna et al.** (2021). “In Vivo Inhibition of the 3-Dehydroquinat Synthase by 7_Deoxysedoheptulose Depends on Promiscuous Uptake by Sugar Transporters in Cyanobacteria.” *Frontiers in Microbiology* 12: 1720

88

New players in the control of arginine synthesis in cyanobacteria

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Due to their photoautotrophic lifestyle cyanobacteria arouse interest as potential hosts for the sustainable production of fuel components and high-value chemicals. However, their broad application as microbial cell factories in biotechnology is hampered by the limited knowledge about the regulation of metabolic fluxes in these organisms. Even for common metabolic routes cyanobacteria evolved quite different mechanisms, exemplified by the well-investigated regulation of glutamine synthetase (GS) via small proteins termed inactivating factors (IFs) and specific regulatory RNAs [1, 2]. With PirA we revealed another small protein composed of 51 amino acids to control a key enzymatic reaction in *Synechocystis* sp. PCC 6803 [3]. This regulation is achieved by the interaction of PirA with the central carbon/nitrogen control protein P_{II}. P_{II} controls the entry into the cyanobacterial ornithine-ammonia cycle (OAC) by activating the key enzyme N-acetyl-L-glutamate kinase (NAGK). We propose that PirA competes with NAGK for P_{II} binding, thereby prevents NAGK activation and diminishes the synthesis of downstream metabolites such as arginine. Similar to the GS IFs, PirA accumulates in response to ammonium upshift due to de-repression of the *pirA* gene from negative control by NtcA, the global transcriptional regulator of nitrogen assimilation. As the interaction of PirA with P_{II} also requires ADP, we propose that PirA determines the flux into nitrogen storage compounds by integrating signals for nitrogen availability but also the energy state of the cell. Moreover, at the post-transcriptional level *pirA* is regulated by the nitrogen stress induced RNA 4 (NsiR4) [4], which targets the corresponding mRNA close to the ribosome binding site and thereby interferes with its translation. An inverse regulation of NsiR4 and PirA by NtcA results in a coherent feed-forward loop that enables fine tuning of arginine synthesis and conveys additional metabolic flexibility under highly fluctuating nitrogen regimes. Altogether, pairs of small protein inhibitors and of sRNAs that control the abundance of these enzyme effectors at the post-transcriptional level appear as fundamental building blocks in the regulation of primary metabolism in cyanobacteria.

[1] Bolay *et al* (2018), *Life* 8: 52. [2] Klähn *et al* (2018), *Nucleic Acids Research* 46: 10082-10094. [3] Bolay *et al* (2021), *mBio* 12:e00229-21. [4] Klähn *et al* (2015), *PNAS* 112: E6243-E6252.

89

Elucidating the role of SepI in peptidoglycan biosynthesis in Anabaena

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Bacterial cells are surrounded by a thick layer made of peptidoglycan (PG) that confers rigidity to the cell, providing a line of defense against external threats as well as determining the cell shape. Despite its rigidity, the PG layer must be continuously remodeled to allow for cell growth and, eventually, cell division. These processes require the coordinated action of amidases and PG synthases. In the cyanobacterium *Anabaena* sp. PCC 7120, cells form long filaments of up to hundreds of cells, and neighboring cells are joined by proteinaceous structures termed septal junctions (SJ) that are involved in intercellular metabolite exchange as well as cell-cell adhesion [1]. Cell division in filamentous cyanobacteria, rendering joined cells connected through septal junctions instead of separated cells, calls for a specific cell division mechanism, and several unique proteins, specific to cyanobacteria, have been identified in the division machinery of *Anabaena*. One of such proteins is SepI (Alr3364), a cytoplasmatic protein that has been recently described to localize at the cell division ring as well as in the intercellular septal regions. A *sepI* inactivation mutant shows filament fragmentation and inability for diazotrophic growth, and it also bears unsealed septal PG disks. BACTH experiments have revealed that SepI interacts with ZipN, SepF, FtsI and SepJ, key proteins of the divisome and SJ formation [2]. These findings suggest SepI to be a new septal protein, whose function has remained uncharacterized so far. In this work, we have analyzed the role of SepI in PG synthesis, focusing in the interaction between SepI, FtsI and FtsW (putative transpeptidase and PG synthase in *Anabaena*, respectively). We are using an *in vitro* approach with purified proteins to investigate a possible effect of SepI on PG synthesis, including the PG polymerization rate as well as PG crosslinking pattern.

[1] Nieves-Morrión M *et al* (2017) Molecular diffusion through cyanobacterial septal junctions. mBio 8(1):e01756-16. [2] Springstein BL *et al* (2020) A novel septal protein of multicellular heterocystous cyanobacteria is associated with the divisome. Mol Microbiol.113 (6): 1140-1154.

90

BCCM/ULC: A PUBLIC CULTURE COLLECTION TO SAFEGUARD CYANOBACTERIAL DIVERSITY AND TAXONOMIC REFERENCE STRAINS

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BCCM/ULC: A public culture collection to safeguard cyanobacterial diversity and taxonomic reference strains

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The BCCM/ULC public collection (https://bccm.belspo.be/about-us/bccm-ulc) aims to gather representative cyanobacterial strains from a variety of ecosystems, with a particular focus on the polar environments. So far, the genetic diversity of the 243 strains for which the 16S rRNA gene sequence was determined, corresponded to 93 OTU's (99% 16S rRNA similarity). The collection's aim is to preserve the deposited biological material, to valorize it by performing research on it, to provide it to interested parties for fundamental and applied research, and to provide services linked to the identification of the Cyanobacteria for the scientific community. An ISO 9001 certificate was obtained for the public deposition and distribution of strains, as part of the multi-site certification for the BCCM consortium.

The collection includes several reference (or 'type') strains for newly described taxa. They include *Plectolyngbya hodgsonii* (ULC009), *Shackletoniella antarctica* (ULC037), *Timaviella circinata* (ULC401) and *T. karstica* (ULC402), *Cephalothrix komarekiana* (ULC718), *Parakomarekiella sesnandensis* (ULC591) and the reference for the new family Petrachlorosaceae, *Petrachloros mirabilis* (ULC683). Recently, the BLCC (Berthold-Laughinghouse Culture Collection) deposited 196 strains with several new taxa from different ecosystems in Florida. These deposits include *Johannesbaptistia floridana* (ULC590) isolated from benthic coastal substrata, *Iningainema tapete* (ULC575), which is able to produce nodularin and was isolated from a greenhouse, *Brasilonema fioreae* (ULC548), *B. santannae* (ULC544) and *B. wernerae* (ULC573) from terrestrial environments, *Leptochromothrix* (ULC597), *Ophiophycus* (ULC599) and *Vermifilum* (ULC454) from benthic mats in mangrove forests, *Neolyngbya biscaynensis* (ULC530) and *Affixifilum floridanum* (ULC525) from marine benthic cyanobacterial mats, and *Tigrinifilum guerandense* (ULC525) and *Capilliphycus guerandensis* (ULC593) from saline benthic mats. These ecosystems offer a huge and still largely unexplored diversity of cyanobacteria. Moreover, they are a potential source of novel secondary compounds. For

example, species of *Neolyngbya* and *Brasilonema* have been shown to produce compounds with antibiotic and antifungal properties.

References:

Barbosa et al. (2021). Diversity of the genus *Brasilonema* (*Nostocales*, *Cyanobacteria*) in plant nurseries of central Florida (USA) with the description of three new species: *B. fioreae* sp. nov., *B. santannae* sp. nov. and *B. wernerae* sp. nov. *Fottea* 21, 82-99.

Berthold et al. (2021). *Iningainema tapete* sp. nov. (*Scytonemataceae*, *Cyanobacteria*) from greenhouses in central Florida (USA) produces two types of nodularin with biosynthetic potential for microcystin-LR and anabaenopeptins. *Harmful Algae* 101: 101969.

Berthold et al. (2021). Untangling filamentous marine cyanobacterial diversity from the coast of South Florida with the description of *Vermifilaceae* fam. nov. and three new genera: *Leptochromothrix* gen. nov., *Ophiophycus* gen. nov., and *Vermifilum* gen. nov. *Mol. Phylogenet. Evol*. In press.

Berthold (2020). *Johannesbaptistia floridana* sp. nov. (*Chroococcales*, *Cyanobacteria*), a novel marine cyanobacterium from coastal South Florida (USA). *Fottea* 20:152-159.

Caires et al. (2018). Biotechnological potential of *Neolyngbya* (Cyanobacteria), a new marine benthic filamentous genus from Brazil. *Algal Res*. 36. 1-9.

Da Silva Malone et al. (2015). *Cephalothrix* gen. nov. (*Cyanobacteria*): towards an intraspecific phylogenetic evaluation by multilocus analyses. *Int. J. Syst. Evol. Microbiol*. 65, 2993-3007.

Lefler et al. (2021). The occurrence of *Affixifilum* gen. nov. and *Neolyngbya* (*Oscillatoriaceae*) in South Florida (USA), with the description of *A. floridanum* sp. nov. and *N. biscaynensis* sp. nov. *J. Phyco.* 57, 92-110.

Sciuto et al. (2017). Cryptic cyanobacterial diversity in the giant cave (Trieste, Italy): The new genus *Timaviella* (*Leptolyngbyaceae*). *Cryptogamie Algol* 38, 285-323.

Soares et al. (2020). *Parakomarekiella sesnandensis* gen. et sp. nov. (*Nostocales*, *Cyanobacteria*) isolated from the Old Cathedral of Coimbra, Portugal (UNESCO World Heritage Site). *Eur. J. Phycol.*

Soares et al. (2022)…….

Strunecky et al. (2020). Diversity of cyanobacteria at the Alaska North Slope with description of two new genera: *Gibliniella* and *Shackletoniella. FEMS Microbiol. Ecol*. 96:fiz189.

Taton et al. (2011). *Plectolyngbya hodgsonii*: a novel filamentous cyanobacterium from Antarctic lakes. *Polar Biol*. 34, 181-191.

91

Chlorophyll-binding domain of the cyanobacterial ferrochelatase is indispensable under cold stress

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Oxygenic phototrophs produce all tetrapyrroles by a long and branched biosynthetic pathway. At the branch point between heme and chlorophyll (Chl) pathways, ferrochelatase (FeCh) and Mg-chelatase compete for the same substrate – protoporphyrin IX. Insertion of iron into protoporphyrin IX by FeCh leads to heme. A unique feature of FeCh in oxygenic phototrophs is the presence of the Chl-binding (Hlip) domain resembling the cyanobacterial single-helix highlight-inducible proteins (Hlips). As shown recently, the binding of Chl to *Synechocystis* FeCh stabilizes the dimeric form of this enzyme [1] but the role of Hlip domain remains unknown. We constructed a *Synechocystis* strain (FΔChl) with mutated FeCh lacking Chl-binding ligands in its Hlip domain. Interestingly, shifting this mutant to cold stress (18°C) impaired its growth and the phenotype got enhanced with increasing light intensity; under 18°C/300 µE of photons (HL) the growth of the mutant was arrested. Interestingly, if a short (1h) pre-treatment with HL was included before the cold stress, FΔChl strain grew essentially like wild type. It implies that HL-induced factor(s) can substitute for the photoprotective function of the FeCh Hlip domain but, during sudden cold, the binding of Chl to FeCh is essential. Analysis of FΔChl cells after 6h of cold/HL stress revealed a sharply reduced level of PSII. Consistently, $^{[35]}$S pulse-labelling of membrane proteins depicted inefficient synthesis of the D1 subunit of PSII in the mutant; this defect was accompanied by the accumulation of unbound Chl molecules in membranes and by accelerated dissociation of PSI trimers. We co-purified FeCh as well as its separately expressed Hlip domain from cold/HL stress with an atypical form of PSI (PSI[1]*) which we identified previously in a complex with Chl synthase [2]. We speculate that the FeCh Hlip domain is involved in the transfer of the Chl molecule to D1 protein synthesized for the repair of PSII.

[1] Pazderník M *et al* (2019) The antenna-like domain of the cyanobacterial ferrochelatase can bind chlorophyll and carotenoids in an energy-dissipative configuration. J Biol Chem. 294:11131-11143
[2] Chidgey JW *et al* (2014) Cyanobacterial chlorophyll synthase-HliD complex associates with the Ycf39 protein and the YidC/Alb3 Insertase. Plant Cell 26:1267-1279

92

Anoxygenic photosynthesis and its regulation in Gemmatimonas phototrophica

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Photoheterotrophic bacteria represent an important part of aquatic microbial communities. *Gemmatimonas phototrophica* is the first photoheterotrophic organism representing the phylum Gemmatimonadota. It was isolated from the freshwater Swan lake in the Gobi desert in 2014 [1]. *G. phototrophica* is the first known example of acquisition of the entire photosynthetic gene cluster via horizontal gene transfer between phototrophic and non-phototrophic representatives of distant bacterial phyla [1]. Here we report the time-resolved transcriptome analysis following the transition from dark to light conditions in *G. phototrophica*. The change from heterotrophic growth in the dark to photoheterotrophic growth in the light was implemented to identify gene-regulatory patterns following this transition. The results of RNA sequencing showed consistent but very weak repression of the photosynthesis gene cluster and upregulation of the ferrochelatase gene. This suggests a rerouting of the porphyrin-anabolism away from bacteriochlorophyll *a* to heme biosynthesis. A specific response to singlet oxygen evolution and strong repression of photosynthetic genes as in other aerobic anoxygenic phototrophic bacteria was not observed. Our findings suggest a weak adaptation of *G. phototrophica* to the usage of its acquired photosynthesis apparatus through horizontal gene transfer.
[1] Zeng Y *et al* (2014) Functional type 2 photosynthetic reaction centers found in the rare bacterial phylum Gemmatimonadetes. Proc Natl Acad Sci USA 111:7795-7800

93

Rational Design of Biophotoelectrodes for In Vitro Biocatalysis

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The use of photosynthetic protein complexes for the fabrication of solar energy conversion devices is a promising strategy due to their natural abundancy and high quantum efficiency. Particularly, one of the main photosynthesis-driving enzymes, photosystem I (PSI), is a stable protein complex able to convert visible light into high-energy electrons – making it an attractive candidate for the fabrication of biohybrid devices. One of the challenges in such devices is to overcome short-circuiting processes between the light-generated electrons and the electrode. One approach is oriented immobilization of PSI complexes in so-called Langmuir monolayers by taking advantage of their amphiphilic character, thus enabling anisotropic electron flow. Residual charge recombination occurring at the gaps between disc-shaped PSI trimers could be successfully closed 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-active 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 photoelectrode, in order to make use of the high-energy electrons generated by PSI, as could be shown for a hydro-genase, resulting in light-driven H_2 production (2). Further optimization of PSI-based biohybrid devices can be achieved via the use of transparent, 3D-nanostructured electrodes that enable a significantly increased protein loading density (3) and via decorating PSI complexes with additional light-harvesting antennae, closing the “green gap” and thus increasing the system’s overall quantum efficiency (4).

(1) Wang P *et al* (2020) Closing the Gap for Electronic Short-Circuiting: Photosystem I Mixed
Monolayers Enable Improved Anisotropic Electron Flow in Biophotovoltaic Devices**.** *Angew Chem Int Ed*,

(1) Zhao F *et al* (2019), A photosystem I monolayer with anisotropic electron flow enables Z-scheme like photosynthetic water splitting, *Energy Environ. Sci.*

(2) Bobrowski T, et al (2020) Scalable Fabrication of Biophotoelectrodes by Means of Automated Airbrush Spray-Coating, *ChemPlusChem*

(3) Hartmann V and Harris D *et al* (2020) Improved quantum efficiency in an engineered light harvesting/ photosystem II super-complex for high current density biophotoanodes. *Journal of Materials Chemistry*

94

Effect of isoprene production on cyanobacterial cell economy when grown under different light regimes

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In order to achieve a carbon-neutral economy, photosynthetic microorganisms such as cyanobacteria have been extensively studied as solar-powered cell factories for the production of organic compounds with industrial interest, using the atmospheric CO_2 as carbon source.
Among other metabolites, cyanobacteria produce terpenoids, a diverse group of natural products that play pivotal roles in the cell. These metabolites have industrial relevance as fragrances, colorants and biofuel precursors[1]. Our research focuses on the metabolic engineering of cyanobacteria for terpenoid production, specifically isoprene. Isoprene production has been shown to be possible in cyanobacteria and previous studies demonstrated some bottlenecks in the terpenoid metabolism, namely the first and last enzymes of the MEP pathway[2,3]. In this study, we addressed how isoprene production in *Synechocystis* PCC6803 can affect the cell metabolism and how its production can be improved by optimizing cultivation conditions such as light quality, light intensity and temperature. Three strains were used: an isoprene-producing strain, which expresses the isoprene synthase from *Eucalyptus globulus* and overexpresses the two abovementioned MEP bottlenecks[3]; a control strain, only overexpressing the two MEP bottlenecks; and another control strain, expressing only the antibiotic resistance cassette. All strains were generated by genome integration. We then grew all strains in turbidostat mode and compared growth, carbohydrate, protein and lipid pools, pigments, CO_2 fixation, photosynthetic efficiency and isoprene production when grown under red, green, blue or violet light, as well as at different light intensities and temperatures. To monitor isoprene production, we made significant modifications to membrane-inlet mass spectrometer to make it compatible with isoprene detection. Preliminary results show changes in carbon fixation and pigments between strains and changes in isoprene production between the different wavelengths and temperatures.

1 – Rodrigues, J.S., Lindberg, P. (2021) Engineering Cyanobacteria as Host Organisms for Production of Terpenes and Terpenoids, Cyanobacteria Biotechnology, chapter 9, Wiley,267-300.
2 – Gao, X. *et al.* (2016) Engineering the methylerythritol phosphate pathway in cyanobacteria for photosynthetic isoprene production from CO2, *Energy Environ. Sci.*, 9,1400.
3 – Englund, E. *et al.* (2018) Systematic overexpression study to find target enzymes enhancing production of terpenes in Synechocystis PCC6803, using isoprene as a model compound, *Metab. Eng.*,49:164-177.

96

Photosystem II biogenesis in Synechocystis 6803 occurs on SecYEG-YidC-SecDF holotranslocons associated with FtsH proteases

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The core subunits of Photosystem II (PSII) are large transmembrane proteins that bind a number of hydrophobic cofactors such are chlorophylls and carotenoids. It is expected that all PSII core proteins are inserted into the thylakoid membrane by Sec translocon (SecYEG trimer) subjoined with YidC and chlorophyll synthase enzyme [1]. However, what other proteins participate on this process is not known. Using *Synechocystis* 6803 we have recently identified SecD and SecF proteins among proteins specifically co-eluted with the CP47 assembly module, a building block of PSII [2]. In heterotrophic prokaryotes SecDF heterodimer associates with SecY and participates in export of proteins into periplasm. We found that the *Synechocystis secDF* genes cannot be deleted and, to clarify their function in cyanobacteria, we FLAG-tagged SecF and purify this protein under native conditions together with a control. The obtained pulldown contained nearly stoichiometric levels of FLAG-SecF and SecD and relatively high content of FtsH4 and FtsH2 proteases. Other FLAG-SecF interactors consist SecYE and YidC, several PSII assembly factors, and a number of uncharacterized proteins. To confirm a close association between FtsH4 and SecDF, we isolated tagged FtsH4 and, indeed, SecDF belong to specific interactors. We propose that in cyanobacteria PSII complexes are produced on SecYEG-YidC-SecDF holotranslocons that are localized in a specialized domain of thylakoid membrane. The SecDF heterodimer, powered by proton gradient, might facilitate the insertion of multi-span membrane protein such as CP47. Associated FtsH proteases provide quality control of the synthesized PSII subunits and degrade assembly factors that are either damaged or need to be cleaned as a response to environmental conditions.

[1] Komenda J and Sobotka R (2019) Chlorophyll-binding subunits of photosystem I and II: Biosynthesis, chlorophyll incorporation and assembly. Adv Botanical Res, 91:195-223
[2] Konert MM *et al* (2022) High-light-inducible proteins HliA and HliB: pigment binding and protein-protein interactions. Photosyn Res, accepted

97

Charge separation induced by a lower-energy bacteriochlorophyll in the reaction center complex of Heliobacterium modesticaldum

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A major pigment in the photosynthetic reaction center complex of heliobacteria (HbRC) is bacterochlorophyll (BChl) *g*. The X-ray structure of HbRC (PDB ID: 5V8K) was solved for a complex the thermophilic heliobacterium *Heliobacterium modesticaldum*. It has revealed that over ~60 molecules of BChl *g* are densely packed in the core-protein, while an apparent dimeric configuration is observed only for the primary electron donor P800, which is a special pair of BChl *g*'. We have recently reported that a ground state bleaching (GSB) of BChl *g*, whose absorption peak is longer than P800, is observed prior to the primary charge separation [1]. This suggests that some of BChl *g* in HbRC is a "red-BChl *g*" with a lower excitation energy than P800; however, the lack of dimer-like antenna BChls *g* in the X-ray structure has made it difficult to confirm and specify which is the red-BChl *g*. Strong excitonic coupling between some chlorophylls of electron transfer chains could also explain such a lower excitation energy.
To identify the responsible site for the red-BChl *g*, we further tracked excitation energy transfer and the primary charge separation reactions by ultrafast transient absorption spectroscopy with a femto-second resolution. The GSB signal of the red-BChl *g* was reproducibly observed at both low and room temperatures. Its rising was completed within 1 ps and always associated with energy equilibrations between bulk antenna BChls *g*. Global analysis by multiple exponential decay functions showed that decay of the red-BChl *g* GSB signal always associates the primary charge separation. Its lifetime was relatively long and varied to 10-50 ps depending on measurement conditions. Any GSB signal attributable to formation of an excited state of P800 was never observed before the primary charge separation, the 10-50-ps lifetime suggests limitation of energy diffusion from the red-BChl *g* to the primary electron donor. A recent theoretical estimation has proposed a similar energy transfer profile [2]; we will discuss the responsible BChl *g* for the red-BChl *g* in HbRC.
[1] R. Kojima *et al.* (2020) *J. Photochem. Photobiol. A* 401:112758.
[2] A. Kimura *et al.* (2021) *J. Phys. Chem. B* 125:3727-3738. .

98

Cryo-EM structure of the photosynthetic complex from Gemmatimonas phototrophica at 2.4 Å

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Phototrophic Gemmatimonadetes evolved the ability to use solar energy following horizontal transfer of photosynthesis-related genes from an ancient phototrophic proteobacterium [1]. The electron cryomicroscopy structure of the *Gemmatimonas phototrophica* photosystem at 2.4 Å reveals a unique, double-ring complex. Two unique membrane-extrinsic polypeptides, RC-S and RC-U, hold the central type-2 reaction center within an inner 16-subunit light-harvesting 1 (LH1) ring, which is encircled by an outer 24-subunit antenna ring (LHh) that adds light-gathering capacity. Each LH subunit is populated by one novel ketocarotenoid called gemmatoxanthin [2]. The outer LHh antenna contains 24 bacteriochlorophyll molecules absorbing at 800 nm and 24 bacteriochlorophyll pairs absorbing at 816 nm. The inner antenna is formed by 16 bacteriochlorophyll pairs absorbing at 868 nm. Since the pigments in the outer ring have higher energy than the pigments in the inner antenna the whole complex serves as a funnel. The energy absorbed by the pigments in the LHh antenna is transferred within several picoseconds down the energy gradient to the reaction center. This structural and functional study shows that *Gem. phototrophica* has independently evolved its own compact, robust and highly effective architecture for harvesting and trapping solar energy [3].

[1] Zeng Y *et al* (2014) Functional type 2 photosynthetic reaction centers found in the rare bacterial phylum Gemmatimonadetes. P. Natl. Acad. Sci. USA 111: 7795-7800.
[2] Nupur *et al*. (2021) Structure elucidation of the novel carotenoid gemmatoxanthin from the photosynthetic complex of *Gemmatimonas phototrophica* AP64. Sci. Rep. 11: 15964.
[3] Qian P *et al* (2022) 2.4-Å structure of the double-ring *Gemmatimonas phototrophica* photosystem. Sci. Adv. 8: eabk3139. https://doi.org/10.1126/sciadv.abk3139

99

Inhibition of cyanobacteria under high CO2

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Photosynthetic microorganisms have adapted to sequester CO2 from the atmosphere at very low CO2 concentrations, which are often limiting for growth (around 400 ppm). However, these organisms are typically not naturally adapted to high CO2 concentrations and are often inhibited at concentrations corresponding to 1–5% CO2 in the gas phase. Our goal is to understand why cyanobacteria are inhibited under high CO2 and to bioengineer the organisms such that they function under high CO2. We will characterize the physiological behaviour under conditions of controlled CO2 concentrations and use measurements of growth, photosynthesis, and transcriptome responses. A better understanding of the physiology under high CO2 may enable more flexible uses of photosynthetic microbes in biotechnology, for example, if the microbes are cultivated in exhaust gases containing high CO2.

100

Photomorphogenesis in Synechocystis sp. PCC 6803

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The intensity of Sunlight, the most important environmental factor for life on Earth, is constantly changing: seasonally, on a daily basis and on a minute/second time scale. In aquatic habitats, the light spectrum also changes according to location and depth of water column. Photoautotrophic microorganisms have developed several survival strategies to effectively respond to these changes and to maintain effective light energy utilization. Here, we report acclimation of the model cyanobacterium *Synechocystis* sp. PCC 6803 to nine monochromatic light regimes covering the whole range of photosynthetically active radiation (PAR) spectrum. Using a combination of biochemical and biophysical methods, we describe a great plasticity in *Synechocystis* pigmentation, cellular composition, and efficiency of electron transport in dependence of cultivation wavelength. PAR wavelengths determine the redox state of the plastoquinol (PQ) pool, which further controls processes such as PSII/PSI ratio or cyclic electron flow around PSI. Orange/red wavelengths warranted the highest electron flow through PSII, allowing *Synechocystis* to reach optimal growth rates, and to accumulate more carbohydrates and lipids during the growth phase. Cells acclimated to bluish light demonstrated a reduced necessity of non-photochemical quenching mediated by orange carotenoid protein (OCP-NPQ), possibly as a consequence of a reduced PQ pool compared to red-light acclimated cells. By following state transitions, we show that efficiency of State 1 → State 2 transition optimizes donor-side limitation of PSI and efficiency of State 2 → State 1 transition helps to reduce the OCP-NPQ necessity. Our results show plasticity in wavelength-dependency of *Synechocystis* metabolism and a central role of the redox state of PQ pool in wavelength sensing. However, the results also suggest that specific photoreceptors are involved in light quality sensing and signalling, and that further studies are required to elucidate a key role of these photoreceptors in photomorphogenesis of *Synechocystis*.

101

From single cell growth to productive ecosystems: Insights from mathematical modelling

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Abstract:
The evolution of oxygenic photosynthesis in the ancestors of modern-day cyanobacteria gave rise to perhaps the most important biological process within our biosphere. While many properties of growth in axenic cultures are reasonably well understood, cyanobacteria and other microorganisms have evolved as parts of interconnected and dynamic ecosystems. Phototrophic microorganisms are primary engines of biogeochemical cycles and dominate primary production in marine ecosystems.
Mathematical modelling can help us to understand how marine microbes interact and collaborate: what are the prerequisites and energetic trade-offs for cooperation and division of labor? How do metabolic diversity and mutualistic relationships emerge? To tackle these challenging questions, we can build upon high quality quantitative models of microbial (in particular cyanobacterial) growth and resource allocation developed over the past decade. Our premise is that the perspective of cellular resource allocation offers a unique opportunity to understand the constraints and energetic trade-offs that govern the emergence of dependencies between photo- and heterotrophic microorganisms in marine environments.
The contribution will present a novel computational model that describes interactions between photo- and heterotrophic microorganisms. Our starting point are coarse-grained models of cellular metabolism and growth. The model allows us to evaluate the costs and benefits of interactions, and allows us to outline a plausible evolutionary pathway for the emergence of metabolic dependencies.

102

Overexpression of PMM/PGM compensates the lack of PGM in glycogen metabolism in Synechocystis sp.PCC6803

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Cyanobacteria are widespread microorganisms that harvest light energy through oxygenic photosynthesis to fuel the assimilation of CO_2 into organic compounds. As a light-dependent microorganism, part of the carbohydrates are stored as glycogen, a dynamic branched glycan that allows cells to cope with light-dark cycles and a changing environment. Glycogen is permanently recycled, and its pathway converges to glucose 1-P, being the first and last metabolite of its cycle. Finally, the interconversion of glucose 1-P and glucose 6-P connects glycogen to central carbon metabolism. This bidirectional reaction is performed by enzymes from the phosphohexomutase superfamily with phosphoglucomutase (PGM) activity[1]. In the cyanobacterium *Synechocystis* sp. PCC6803, two enzymes, PGM (encoded by *sll0726*) and PMM/PGM (a bifunctional phosphomannomutase/phosphoglucomutase enzyme, encoded by *slr1334*), are capable of this interconversion[2].

Since phosphoglucomutase activity is crucial in glycogen metabolism, we addressed the implications of loss of this activity. Since complete segregation of *slr1334* null-mutants is not possible, a conditional mutant was constructed. This mutant showed a drastic lethal phenotype upon inductor removal, confirming its essential role in cyanobacteria. On the contrary, deletion of the *sll0726* gene is viable, leading to a strain with almost undetectable PGM activity, which ultimately affects the amounts of glycogen. In this mutant, the reduced interconversion of phosphoglucoses by PMM/PGM causes an impaired phenotype similar to that of glycogen-less mutants such as the ADP-glucose pyrophosphorylase (AGP) mutant that includes an improper response to high light or nitrogen starvation. However, in contrast to AGP, the flux to glucose 1-P in the PGM mutant is enough for adaptation to a saline medium or dark-night cycles. Furthermore, overexpression of the PMM/PGM enzyme in a PGM mutant suppresses fitness drawbacks, restoring the phenotype without any apparent detriments in growth performance, indicating that an increase in PMM/PGM is enough to overcome the lack of PGM.

1. Stiers, K. M., Muenks, A. G. & Beamer, L. J. Biology, Mechanism, and Structure of Enzymes in the α-D-Phosphohexomutase Superfamily. in *Advances in Protein Chemistry and Structural Biology* vol. 109 265–304 (Academic Press Inc., 2017).
2. Liu, L., Hu, H. H., Gao, H. & Xu, X. D. Role of two phosphohexomutase genes in glycogen synthesis in Synechocystis sp. PCC6803. *Chinese Science Bulletin* **58**, 4616–4621 (2013).

This research was funded by grant PID2019-104513GB-I00 from the Ministerio de Ciencia e Innovación. Pablo Ortega is recipient of a predoctoral contract from Ministerio de Ciencia e Innovación y Universidades (contract number FPU18/06580).

103

Resuscitation from nitrogen chlorosis: Deciphering a program for maintenance of viability

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Cyanobacteria that do not fix nitrogen survive prolonged periods of nitrogen starvation in a dormant state where cell growth and metabolism are arrested. This quiescent state is also known as chlorosis, since it involves degradation of the photosynthetic pigments. Upon nutrient availability, these pale and dormant cells return to vegetative growth within 2–3 days. This resuscitation process, as exemplified with the model *Synechocystis* PCC 6803, is highly orchestrated and relies on the stepwise re-installation and activation of essential cellular structures and functions. We have been investigating the transition to chlorosis and the return to vegetative growth as a simple model of a cellular developmental process and a fundamental survival strategy. We employed studies at all levels of cellular activity, from subcellular morphology, transcriptomics, and quantitative proteomics/phosphoproteomics to the level of metabolic control. Thereby we could describe the transcriptomic and proteomic landscape of a dormant cyanobacterium and its dynamics during awakening, as well as identify different layers of regulatory processes that are essential for survival.
One aspect of prime importance in the transitions into and out of the dormant state, is the regulation of energy homeostasis. By investigating the regulation of ATP homeostasis during dormancy, we unraveled a critical role for sodium bioenergetics in dormant cells. During nitrogen starvation, cells reduce their ATP levels and engage sodium bioenergetics to maintain the minimum ATP content required for viability. When nitrogen becomes available, energy requirements rise and cells immediately increase ATP levels using a sodium motive force. Rapid activation of glycogen catabolism provides the cells with carbon skeletons and reductant. The phosphoglucomutase reaction plays a key role for the tight control of glycogen turn-over. The emergence of highly orchestrated mechanisms that support the resuscitation program suggests that this process is deeply rooted in the evolution of cyanobacteria and plays pivotal roles in natural selection.

References:

Klotz A, Georg J, Bucinska L, Watanabe S, Reimann,V, Januszewski W, Sobotka R, Jendrossek D, Hess WR, Forchhammer K. 2016. *Curr Biol.* 26, 1-11
Doello, S., Klotz, A., Makowka, A., Gutekunst, K. & Forchhammer, K. 2018. *Plant Physiol.* 177, 594-603.
Spat, P., Klotz, A., Rexroth, S., Macek, B. & Forchhammer, K. 2018. *Mol Cell Proteomics* 17, 1650-1669.
Doello, S., Burkhardt, M. & Forchhammer, K. 2021. *Current Biology,* 31:1-10
Neumann, N., Doello, S. and Forchhammer. K. 2021. *Microb Physiol* 31:78–87
Doello, S., Neumann, N. and Forchhammer, k. 2021. *BioRxiv*. https://doi.org/10.1101/2021.04.15.439997

104

Enabling Synthetic Biology for the Chlorobiaceae

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The Chlorobiaceae have physiological properties that make them attractive candidates for biotechnological applications including native synthesis of intriguing molecules (e.g. wax esters, glycolipids), mixotrophic growth with CO_2 and organic acids, use of reduced sulphur compounds as electron donors, and low absolute light requirements for phototrophic growth. Therefore, the Chlorobiaceae use resource streams distinct from those for crop plants, cyanobacterial, and algal platforms. Some genetic tools and global data sets also exist, primarily for *Chlorobaculum tepidum*, but are rare for other members. Therefore, a better underlying basic knowledgebase for multiple organisms and additional tools are required to make the Chlorobiaceae feasible and attractive synthetic biology platforms. Data will be presented on three separate projects laying the necessary ground work for synthetic biology and metabolic engineering:

1) Improved systems for heterologous gene expression and genome manipulation
2) Comparative metabolomics of diverse Chlorobiaceae
3) Light regulated transcription and cell composition changes

Together, these basic science data and tolls will provide a pathway for the rational engineering of multiple Chlorobiaceae based on shared and unique metabolic pathways, a common set of tools, and an improved understanding of transcriptional regulation and cellular composition under different growth regimes. Furthermore, they will provide tools for more precise and subtle manipulative experiments to probe basic physiological responses and regulatory systems apart from engineering and applications driven research.

105

Photophysiological consequences of lipid substitution in a marine Synechococcus at high light intensities

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Lipid membranes are essential for cellular life forming a barrier towards the environment and, in phototrophs, acting as a key component of thylakoid membranes. Under phosphorus (P) limitation, membrane composition changes as non-P-containing lipids substitute P-containing lipids to provide P for other cellular functions.

To understand the consequences of membrane lipid changes during P-depletion on carbon dioxide (CO_2) fixation capacity and photophysiology in marine cyanobacteria, we characterised a *sqdB* knockout mutant (□*sqdB*), a gene encoding UDP-sulfoquinovose synthase, in *Synechococcus* sp. PCC7002 [1]. This mutant is unable to synthesize the non-P-containing lipid sulfoquinovosyl diacylglycerol (SQDG), which substitutes phosphatidylglycerol (PG) under low P condition. Using liquid chromatography - mass spectrometry we found that while PG concentration decreased under P-depletion in WT cells resulting in an increased SQDG:PG ratio, the PG content remained constant in □*sqdB* cells. Additionally, mutant cells showed a decreased growth rate compared to the WT, reaching stationary phase after a few days with a distinctly reduced cell yield. Interestingly, P depletion had a marked effect on CO_2 fixation capacity at high light intensities in WT cells (40% reduction), a feature which was much more pronounced in the □*sqdB* mutant (80% reduction). Concomitantly, rapid light curves of mutant cells showed a lower effective quantum yield compared to the WT and significantly lower photosynthetic rate and maximum electron transport rate values in the mutant. Gene expression analysis between WT and □*sqdB* cells is currently in progress to provide a mechanistic basis for the observed phenotype.

In summary, our data indicates that membrane lipid changes following P stress affect CO_2 fixation capacity and the photophysiological performance of a marine *Synechococcus*, particularly under high light intensities, environmental parameters that feature widely in oligotrophic open ocean waters.

[1] Sato N, et al. (2016) Dispensability of a sulfolipid for photoautotrophic cell growth and photosynthesis in a marine cyanobacterium, *Synechococcus* sp. PCC7002. Biochem Biophys Res Comm 477: 854-860.

106

Using Label Free Proteomics to map protein enrichment through the photosynthetic transition in purple bacteria

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The facultative phototroph *Cereibacter sphaeroides* (formerly *Rhodobacter sphaeroides*) belongs to the class of α-proteobacteria, a morphologically diverse taxon thought to include the progenitor of the mitochondria. *C. sphaeroides* has been studied as a model organism for anoxygenic photosynthesis for over 60 years, and more recently also for the assembly of photosynthetic membranes [1] [2]. This is in part due to the tractability of its photosynthetic regulatory system, which is activated in micro-oxic conditions via the PrrABC redox sensing pathway and the AppA/PpsR repressor pair. Advances in the resolution and high throughput ability of Label Free Quantification (LFQ) proteomics have made it a powerful system to study the proteomes of photosynthetic fractions of purple bacteria, in order to probe patterns of enrichment in mature and precursor membrane [2]. Here we present relative quantification data of purified precursor (upper pigmented band, UPB) and mature (intra-cytoplasmic membrane, ICM) photosynthetic membrane fractions, detailing how key spectral complex peptides, putative assembly factors, and biosynthesis enzymes are respectively enriched. Furthermore, preliminary LFQ data of both fractions sampled over a time-course from a respirative to photosynthetic mode of growth is presented, that could provide direct evidence for the order of spectral complex enrichment in each fraction when combined with absolute quantification techniques such as QconCAT [3]. With the aid of molecular genetics techniques to knock out candidate genes, these data may also prove to be an effective tool for the identification of new players in the photosynthetic assembly process.

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[1] PucC and LhaA direct efficient assembly of the light-harvesting complexes in *Rhodobacter sphaeroides*, *Molecular Microbiology*, 2016, V 99, I 2, 307-327
[2] Quantitative proteomic analysis of intracytoplasmic membrane development in *Rhodobacter sphaeroides*, *Molecular Microbiology*, 2012, V 84, I 6, 1062-1078
[3] QconCAT: Internal Standard for Protein Quantification, *Methods in Enzymology*, 2016, V 556, 289-303

107

Period of cyanobacterial circadian rhythm correlates with solution pH in vitro

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The circadian clock of cyanobacteria can be reconstituted in vitro. By mixing three clock proteins, KaiA, KaiB, KaiC with ATP, the phosphorylation state of KaiC exhibits a circadian rhythm. KaiC, a duplicate P-loop ATPase belonging to the RecA superfamily, consists of N-terminal CI and C-terminal CII domains and forms an ATP-dependent hexametric ring. Whereas it has been shown that solution pH also changes the properties of many P-loop ATPase, effect of pH on KaiC and Kai-protein-based circadian oscillator have been unclear so far. In this study, effect on the solution pH on Kai-protein-based circadian oscillator was examined. Buffers in the pH range of 6-9 were used to investigate the effects of pH on in vitro KaiC phosphorylation rhythms, Kai protein interactions, and KaiC ATPase activity. The results showed that the period of the in vitro KaiC rhythm correlated with solution pH, varying from 15 hours at pH 6.5 to 36 hours at pH 8.5. Solution pH slightly affected the ATPase activity of KaiC and altered the autophosphorylation and autodephosphorylation activity of KaiC and the function of KaiB on KaiC. Solution pH appears to alter the surface charge of the interface between the two ATPase domains of KaiC, thereby affecting the autophosphorylation and autophosphorylation activities of KaiC via interdomain interactions.

108

Ecological niche and importance of chlorophyll f-containing cyanobacteria in extreme beachrock biofilms

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Cyanobacteria with far-red light photoacclimation (FaRLiP) can modify their photopigmentation by synthesizing red-shifted phycobiliproteins and special chlorophylls, i.e., chlorophyll (Chl) *d* and *f*. This enables use of near-infrared radiation (NIR) for oxygenic photosynthesis in habitats depleted of visible light (VIS). Cyanobacteria with FaRLiP are widespread but their ecological niche and especially their importance for primary production in natural habitats remains largely unexplored.
Here we report on the occurrence and photosynthetic activity of Chl *f*-containing cyanobacteria in biofilms covering beachrock, i.e., a widespread, intertidal rock formation along (sub)tropical coastlines exhibiting extreme daily variation in solar irradiance, chemical dynamics and desiccation. We used a combination of gas exchange measurements, hyperspectral and luminescence lifetime-based O_2 imaging to quantify how endolithic cyanobacteria with far-red chlorophylls contribute to the primary production of an intertidal beachrock habitat exposed to visible and near-infrared radiation. While VIS-driven photosynthesis predominantly took place in the dense cyanobacterial surface biofilm of beachrock, NIR-driven oxygenic photosynthesis was mainly confined to a subsurface layer in the beachrock containing endolithic cyanobacteria with Chl *f*.
Our study shows that NIR-driven photosynthesis by cyanobacteria with FaRLiP reaches high rates (up to ~30% of areal photosynthesis rates driven by visible light at comparable photon irradiance) and thus can contribute significantly to the primary production of beachrock biofilms. The ecophysiological plasticity of cyanobacteria expressing FaRLiP can thus have important implications for the primary production of their natural habitats.

109

Metabolic engineering of Synechocystis PCC 6803 for photosynthetic isobutanol production

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As an effective approach to alleviate the increased demand of energy and the concerns of dramatically increased CO_2 emission, it is urgent to develop carbon-neutral biofuels to replace traditional fossil fuels. Cyanobacteria, photosynthetic microorganisms, are promising novel cell factories for chemicals production, including biofuels. Isobutanol, a four-carbon alcohol, is considered as a superior candidate as a biofuel for its high energy density with suitable chemical and physical characteristics. The unicellular cyanobacterium *Synechocystis* PCC 6803 (hereafter *Synechocystis*) has been successfully engineered for photosynthetic isobutanol production from CO_2 and solar energy in a direct process [1]. Heterologous expression of α-ketoisovalerate decarboxylase ($Kivd^{S286T}$) is sufficient for isobutanol synthesis via the 2-keto acid pathway in *Synechocystis*. With additional expression of acetolactate synthase (AlsS), acetohydroxy-acid isomeroreductase (IlvC), dihydroxy-acid dehydratase (IlvD), and alcohol dehydrogenase ($Slr1192^{OP}$), the resulting *Synechocystis* strain, with a functional 2-keto acid pathway, showed enhanced isobutanol production reaching 98 mg L^{-1} in short-term screening experiments [2]. On the other hand, through modulating $kivd^{S286T}$, encoding a previously identified bottleneck enzyme, copy numbers as well as the composition of the 5'-region, a final *Synechocystis* strain with three copies of $kivd^{S286T}$ showed a significantly improved isobutanol production of 144 mg L^{-1}, an 177% increase compared to the previously reported best producing strain under identical conditions [2]. Systematic analysis in proteomics level will be performed on isobutanol-producing *Synechocystis* strains, to reveal the intrinsic regulation of the cell and provide metabolic engineering guidelines for further enhancement of isobutanol production. Additionally, developing a photobioreactor device with an automatic isobutanol harvesting and upgrade system will make an essential step towards industrialization of photosynthetic isobutanol produced from photoautotrophic microorganisms.

[1] Liu X *et al* (2021) Current advances in engineering cyanobacteria and their applications for photosynthetic butanol production. Curr Opin Biotechnol 73:143-50
[2] Xie H *et al* (2022) Expressing 2-keto acid pathway enzymes significantly increases photosynthetic isobutanol production. Microb Cell Fact 21, 17

110

A cyanophage lacking PSII reaction centre genes is sensitive to high light

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Viruses of bacteria frequently possess genes that may augment host metabolism during infection, beyond that required for viral morphogenesis. In viruses of cyanobacteria, these auxiliary metabolic genes (AMGs), often participate in photosynthesis. The first identified AMGs encode the core photosystem II (PSII) reaction centre proteins D1 and D2 that together bind all proteins and cofactors required for PSII function. Yet, to date, an exact function for the viral D1 has been established. D1 was thought to be found in all genomes of the cyanomyoviral group but recently, a Myovirus was isolated that lacked the *psbA* gene encoding D1. Here we show that the development of this virus is inhibited by high light and has impaired PSII function during infection. Further, we examined available metagenomes to show that this sequence type is enriched in low-light environments. Together, these data enforce the role of a viral D1 in maintained and active PSII repair cycle to supply energy for phage morphogenesis.

111

Metabolic engineering of cyanobacteria for phenylpropanoids production

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Cinnamic acid (CA) and *p*-hydroxycinnamic acid (*p*HCA) are phenylpropanoids that have wide application as precursors for flavoring agents, health and cosmetic products. Traditionally, these compounds are obtained by extraction from plant tissues or chemical synthesis. An alternative production method for these aromatic acids relies on the engineered heterotrophic microorganisms. However, usage of phototrophic organisms such as cyanobacteria and green algae would allow making this process truly sustainable. In our research, we currently focused on the metabolic engineering of *Synechocystis* PCC 6803 for CA production. This compound can be obtained by a single enzymatic step from phenylalanine by the action of enzyme phenylalanine ammonia-lyase (PAL). Although this enzyme has been found in a few cyanobacteria [1], it is not present in *Synechocystis*. We selected five PAL candidates for heterologous expression based on their kinetic properties according to literature data. Expression cassettes were successfully introduced into *Synechocystis*. Obtained strains were tested based on their growth and productivity. The usage of *Synechocystis* as the host cyanobacterial strain allowed us to avoid native regulation of the PAL enzyme required for CA formation as well as to reduce the likelihood that CA is further metabolized in the cell. Additionally, a central metabolic role of phenylalanine, the precursor for the formation of CA by PAL, favors obtaining high titers of the product.
Our work contributes to the establishing cyanobacteria as a promising platform for production of valuable plant metabolites and commodities such as phenylpropanoids [2].

[1] Moffitt MC *et al* (2007) Discovery of two cyanobacterial phenylalanine ammonia lyases: kinetic and structural characterization. Biochemistry 46:1004-1012.
[2] Kukil, K., Lindberg, P. (2022) Expression of phenylalanine ammonia lyases in *Synechocystis* sp. PCC 6803 and subsequent improvements of sustainable production of phenylpropanoids. Microb Cell Fact 21, 8. https://doi.org/10.1186/s12934-021-01735-8

112

Elucidating environmental selection of cyanophage auxiliary metabolic gene carriage

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The marine *Synechococcus* genus is one of the most abundant groups of phototrophs on planet Earth and are thus key primary producers. Cyanophage infecting these organisms possess distinct copies of host genes in their genome so-called auxiliary metabolic genes (AMGs) whose expression during the infection process is thought to facilitate manipulation of host metabolic pathways. AMGs include components of the photosynthetic machinery e.g. *psbA*, *petE*, *ptoX* as well as genes involved in nutrient acquisition e.g. *pstS*. However, little is known about how environmental conditions select for AMG carriage. Here, we set out to address this question via the isolation of cyanophages infecting *Synechococcus* sp. WH7803, using samples from an Atlantic Meridional Transect (AMT24). This AMT transect traverses both temperate mesotrophic waters and oligotrophic gyre systems in the North and South Atlantic, as well as equatorial upwelling regions and hence includes a range of temperature, light and nutrient conditions. Cyanophages are currently being isolated from samples along the transect and will be subjected to genome sequencing to elucidate specific AMG carriage. Subsequently, we will combine traditional assays of infection dynamics with cyanophage genetic manipulation to elucidate the functional role of specific AMGs.

113

Cryo-EM structure of the Synechocystis cytochrome b6f complex with the regulatory PetP subunit

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In oxygenic photosynthesis, the cytochrome b_6f (cytb_6f) complex links the linear electron transfer (LET) reactions occurring at photosystems I and II and conserves part of the redox potential energy as a transmembrane proton gradient via the Q-cycle. In addition to this central role in LET, cytb_6f also participates in a range of processes including cyclic electron transfer (CET), state transitions and photosynthetic control. Many of the regulatory roles of cytb_6f are facilitated by auxiliary proteins that differ depending upon the species, yet because of their weak and transient nature the structural details of these interactions remain unknown. In cyanobacteria, an apparent key player in the regulatory balance between LET and CET is PetP, a ~10 kDa protein which is absent in plants and green algae. We used cryogenic electron microscopy to determine the structure of the *Synechocystis* sp. PCC 6803 cytb_6f complex in the presence and absence of PetP. Our structures show that PetP interacts with the cytoplasmic side of cytb_6f, displacing the C-terminus of the PetG subunit and shielding the C-terminus of cytochrome b_6, which binds the heme c_n molecule that is suggested to mediate CET. The structures also highlight key differences in the mode of plastoquinone binding between cyanobacterial and plant cytb_6f complexes, which we suggest reflects the unique combination of photosynthetic and respiratory electron transfer in cyanobacterial thylakoid membranes.

114

Highly productive batch cultivation using membrane-mediated CO2 supply and nutrient-rich media in CellDEG screening platforms

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The economic viability of phototrophic microorganisms is often limited by low biomass concentrations and growth rates due to insufficient light utilization and low rates of CO_2 transfer into the medium. A prerequisite for highly productive batch cultures is a medium containing all essential minerals in sufficient concentration to prevent nutrient deficiency at high cell density.
In the present study, we evaluate the growth of two common cyanobacterial lab strains, *Synechocystis sp.* PCC 6803 and *Synechococcus sp.* PCC 7002 with the aim to maximize productivity (biomass per time) and final cell density in batch cultures carried out in parallel CellDEG membrane cultivators. While media with high NaCl concentration are commonly used for culturing marine cyanobacteria, it is not known whether these strains tolerate high initial nitrate concentrations.
To investigate this problem, we used CellDEG screening platforms combined with an exponential increase in photon flux density (PFD) and balanced, bicarbonate-buffered mineral media with 64 mM and 121 mM nitrate, respectively. The CellDEG high-throughput screening platforms and HD10 (10 ml) membrane cultivators were used to ensure high levels of dissolved CO_2. To prevent photoinhibition at low initial cell density, the PFD was increased from an initial 100 to a constant 1000 µmol photons m^{-2} s^{-1} with a doubling time of 8 h.
After the exponential growth phase, we observed a phase with almost constant productivity of 8 g CDM L^{-1} d^{-1} for both organisms, and final cell densities above 20 g CDM L^{-1} using the 121 mM nitrate medium. The results were further evaluated using a computational model of phototrophic growth, with good agreement between model-derived and experimentally observed behavior. The model will be instrumental to identify further factors that influence phototrophic productivity.
Our experiments show that up to 45 parallel 10 ml batch cultures of *Synechocystis sp.* and *Synechococcus sp.* can be cultured with high productivity to high cell densities using the CellDEG screening platforms, balanced mineral media with a nitrate concentration of 121 mM, membrane-mediated CO_2-supply and controlled increase in light intensity.

115

Exopolysaccharide features under desiccation stress from Phormidium ambiguum and Scytonema javanicum biofilm and biocrust

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Cyanobacteria are capable of forming soil biocrust and biofilms under harsh environmental conditions, excreting exopolysaccharides (EPS) that are supposed to protect cells against several stresses. The present work was aimed at evaluating the role of the EPS in protecting cyanobacteria from the desiccation in two different structures, biocrusts (i.e. structures composed by soil particles and a network of cyanobacterial filaments and EPS) and biofilms. *Phormidium ambiguum* and *Scytonema javanicum* were inoculated to produce biofilms in flasks and biocrusts in sand microcosms. The microcosms were daily watered (35 ml). After 28 days of biocrust and biofilm formation, three biocrust samples were subjected to desiccation stress by stopping the watering for 5 days, while in controls the watering continued. In three biofilm samples the culture medium was removed, only maintaining the biofilm sticked on the bottom of the flasks. In biocrusts, the Loosely Bound (LB)-EPS were recovered with distilled water and the Tightly Bound (TB)-EPS with 0.1 M Na_2-EDTA at 2 sampling times (after 28 days and after the desiccation period). The EPS released by the biofilm in the culture medium (RPS) and the sheaths of the cyanobacteria were recovered, respectively, by precipitation with isopropyl alcohol, and by treating the biofilm sticked on the glass with distilled water (80°C, 1h). The period of desiccation induced the production of a significantly higher (P< 0,05) amount of TB-EPS for both cyanobacteria microcosms in comparison with the biocrusts formed after 28 days of watering and in controls, indicating a possible protection role of the TB-EPS against desiccation. Similarly, in biofilms it was observed a significantly higher amount of sheath EPS than RPS for all treatments. Desiccation also induced a different monosaccharidic composition between the sheath and the RPS. Indeed, after the desiccation period, rhamnose, galactose and xylose were found in the sheath and not in the RPS. On the other hand, glucose was the main monosaccharide present in the EPS extracted from biocrust and from biofilms under all the conditions tested. The two cyanobacterial species showed a plasticity in the response to the desiccation stress in terms of amount and quality of EPS produced.

117

Architecture and components of the gated cell-cell communication system of filamentous cyanobacteria

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The cyanobacterium *Nostoc* PCC 7120 has multicellular traits: the filaments consist of hundreds of cells, which are communicating by cell-cell connections. These multi-protein complexes form channels thru the septa and are called septal junctions (SJ). The activity and mechanism of the cell-cell communication system can be investigated using fluorescent marker molecules in FRAP measurements [1]. The *in situ* architecture of SJs can be studied by imaging focused ion beam-thinned filaments with cryo-electron tomography [2]. The SJs exhibit a five-fold symmetric cytoplasmic cap module, a membrane-embedded plug domain and a tube traversing the septum thru peptidoglycan nanopores. Depending on the cellular condition, the SJs close by conformational changes, and switch back to the open state, rendering SJs gated cell-cell connections analogous to gap junctions [2,3]. The impact of the cap and plug module on SJ closure was demonstrated by investigating a *fraD* mutant, which lacked both modules, leading to a non-gating phenotype [2]. The FraD-GFP fusion protein localizes to the plug domain [2,3].
Via co-immunoprecipitation another putative SJ protein, SepN, was identified. The protein localizes to the septa where it is essential for proper SJ assembly. A mutant in *sepN* exhibited reduced rates of intercellular communication, and lost the ability of SJ gating like the *fraD* mutant [4]. Cryo-electron tomograms of the *sepN*-mutant revealed SJs lacking the plug module [4]. The combination of missing plug but present cap allowed to deduce the importance of the plug module for proper SJ assembly and in sealing the diffusion area in the closed SJ state.
Further proteins, identified by co-IP, will be investigated in future studies, in order to identify all components of the oligomer SJ complex and their functions in gated intercellular communication.

[1] Mullineaux CW *et al* (2008) Mechanism of intercellular molecular exchange in heterocyst-forming cyanobacteria. EMBO Journal 27:1299-308
[2] Weiss GL *et al* (2019) Structure and function of a bacterial gap junction analog. Cell 178: 374-384
[3] Kieninger A-K & Maldener I (2021) Cell–cell communication through septal junctions in filamentous cyanobacteria. Curr Opin Microbiol 61:35–41
[4] Kieninger A-K *et al (*2022) SepN is essential for assembly and gating of septal junctions in *Nostoc* sp. PCC7120 bioRxiv 2022.01.26.477872

118

In vivo kinetics of the CP12 dependent Calvin–Benson–Bassham cycle regulation under non-continuous conditions

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Cyanobacteria live in a dynamic aquatic environment and must adapt to many changing conditions. One of them is the sudden illumination change due to wave-induced light flashes and shadowing of the cell. Therefore the Calvin–Benson–Bassham (CBB) cycle must be tightly regulated to keep photosynthesis as effective as possible while avoiding oxidative stress. One known way of controlling the CBB cycle is the redox-dependent complex formation of its key enzymes Phosphoribulokinase (PRK), Glyceraldehyde-3-phosphate dehydrogenase 2 (GAP2), and the small unstructured protein CP12. This interaction is regulated by the thioredoxin system and is therefore tightly connected to the cell's redox status. We developed a setup to investigate the PRK-CP12-GAP2 complex formation *in vivo* on a single cell level in the model cyanobacterium *Synechocystis* sp. PCC 6803. The investigation of a ∆cp12 mutant confirmed the observations as the CP12 dependent regulation of the CBB cycle. With this setup in place, we were able to measure the kinetics of this critical regulatory step of the CBB cycle upon light-dark transition and under various environmental conditions.

119

Light-driven hydroxylation of testosterone by Synechocystis sp. PCC 6803 expressing the heterologous CYP450 monooxygenase CYP110D1

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The development of chemical and pharmaceutical industries greatly improved the quality of human life but also led to high amounts of chemical wastes and CO2 threatening the environment and the climate. Compared to chemical synthesis, biocatalysis has several advantages since enzymes generally exhibit high selectivity and the reactions are typically performed in non-hazardous solvents and mild conditions. The use of heterotrophic organisms as whole-cell biocatalysts requires exogenous supply of sacrificial electron donors for cofactor regeneration. In contrast, photobiocatalysis using cyanobacteria offers the possibility of harnessing the reducing power generated by photosynthetic water oxidation to regenerate cofactors. Cytochrome P450 monooxygenases are particularly suitable to be expressed in cyanobacteria, as they catalyse reactions consuming O_2, a by-product of photosynthesis, and they can accept electrons from endogenous ferredoxins naturally present in these microorganisms [1]. In this work, the heterologous cytochrome P450, CYP110D1 from *Nostoc* sp. PCC 7120 (*alr4766*) [2] was expressed in the model cyanobacterium *Synechocystis* sp. PCC 6803 under the control of different regulatory parts [3] to catalyse the light-driven selective hydroxylation of testosterone into 15β-hydroxytestosterone. In addition, the reaction conditions including cell density, aeration, and substrate concentration were optimized, leading to a maximum specific activity of 1 U g_{CDW}^{-1}. Altogether, the results obtained demonstrated the efficiency and sustainability of testosterone hydroxylation using our engineered *Synechocystis* chassis compared to biocatalysis with heterotrophic microorganisms or chemical synthesis. However, there is still room for improvement, namely by using tailored regulatory elements, chassis with streamlined electron flow and enhancing enzymatic activity by protein engineering.

[1] Wlodarczyk A *et al* (2016) Metabolic engineering of light-driven cytochrome P450 dependent pathways into *Synechocystis* sp. PCC 6803. Metab Eng 33:1–11
[2] Agematu H *et al* (2006) Hydroxylation of testosterone by bacterial cytochromes P450 using the *Escherichia coli* expression system. Biosci Biotechnol Biochem 70:307–311
[3] Ferreira EA *et al* (2018) Expanding the toolbox for *Synechocystis* sp. PCC 6803: validation of replicative vectors and characterization of a novel set of promoters. Synth Bio 3:1–15

120

Heterologous production of compatible solutes using Synechocystis sp. PCC 6803-based chassis

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Microorganisms can cope with environmental stresses like temperature or salinity through the production of compatible solutes (that can accumulate intracellularly in high concentrations, without interfering with metabolism) [1]. Among compatible solutes, glycine betaine has various applications in nutrition, pharmaceuticals, and cosmetics. Currently, this compound can be extracted from sugar beet plants or be obtained by chemical synthesis resulting in low-yields or high-carbon footprint [2], and thus an eco-friendly and effective production process is required. Therefore, we aimed at exploring the heterologous production of this compatible solute using *Synechocystis* sp. PCC 6803 and, for this purpose, mutants lacking the native solutes sucrose or/and glucosylglycerol (Δ*sps*, Δ*ggpS*, and Δ*sps*Δ*ggpS*), were generated and characterized. In parallel, a synthetic device for the production of glycine betaine was designed based on the metabolic pathway described in *Aphanothece halophytica* [3]. This device was assembled with a synthetic constitutive promoter [4], and then introduced into the generated mutants. Transcription of the ORFs comprising the device was stable and insulated from *Synechocystis'* native regulatory network. Production of glycine betaine was achieved in all chassis tested, and shown to increase with salinity. The maximum glycine betaine production 64.29 µmol/gDW was reached in the Δ*ggpS* chassis grown under 3% NaCl (seawater-like salinity). The main key players involved in carbon fluxes under salinity conditions were also identified, and will enable the optimization of the production of this, or other compatible solutes, using *Synechocystis*-based chassis [5].

[1] Klähn S & Hagemann M (2011) Compatible solute biosynthesis in cyanobacteria. Environ Microbiol 13:551–562
[2] DuPont (2015) Betafin® natural betaine sustainable and substantiated. http://animalnutrition.dupont.com/fileadmin/user_upload/live/animal_nutrition/documents/open/Betafin__Natural_Betaine_Lifecycle_Analysis.pdf
[3] Nyyssölä A *et al* (2000) Extreme Halophiles Synthesize Betaine from glycine by Methylation. J Biol Chem 275:22196–22201
[4] Ferreira EA *et al* (2018) Expanding the toolbox for *Synechocystis* sp. PCC 6803: validation of replicative vectors and characterization of a novel set of promoters. Synth Bio 3:1–15

[5] Ferreira EA et al (2022) Heterologous production of glycine betaine using *Synechocystis* sp. PCC 6803-based chassis lacking native compatible solutes. Front Bioeng Biotech 9:821075

121

Green rehabilitation system for burned soils based on the inoculation of native cyanobacteria and microalgae

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Mediterranean-type ecosystems are prone to intense seasonal wildfires, severely affecting the countries within these regions on a wide range of ecological and socio-economic aspects. The **GreenRehab project** aims at developing a low-cost, eco-friendly and easy-to-implement system for the rehabilitation of burned soils based on the inoculation of native cyanobacteria and microalgae. It also proposes to establish a protocol to evaluate the post-fire recovery. The photosynthetic microorganisms are particularly important in biocrust formation and soil ecosystem functions, allowing the emergence of more complex above-soil communities. Within GreenRehab, several cyanobacteria/microalgae were isolated from soil biocrusts collected at Mortágua (Viseu, Portugal), a municipality devastated by fires in 2017. The photosynthetic microorganisms were characterized using a polyphasic approach, including morphological and ultrastructural characteristics, sequencing of molecular makers, and phylogenetic analyses. In addition, a metabarcoding approach to assess the soil/soil biocrust cyanobacterial community was developed, revealing a higher degree of complexity with several sequences corresponding to not yet described genera/species. The isolated microorganisms were tested in microcosm experiments to select the ones that were able to improve the soil properties/act as growth enhancers using methodologies previously developed [1]. Other applications for these soil microorganisms such as standard ecotoxicological assays were also developed [2]. In parallel, a protocol to assess the fire impact and post-fire vegetation recovery based on a remote sensing approach [3] using an unmanned aerial vehicle equipped with a multispectral camera was established. Currently, the best performing microorganisms are being tested as a consortium in further microcosm experiments and cultivated in large scale for field experiments. The consortium will be inoculated in restricted burned areas, and the soil properties, microbial community, edaphic fauna, and vegetation will be assessed before and after inoculation.

[1] Brito Â *et al* (2022) A new cyanobacterial species with a protective effect on lettuce grown under salinity stress: Envisaging sustainable agriculture practices. J Appl Phycol 34:915-928

[2] Carvalhido V *et al* (2021) Development of an ecotoxicological test procedure for soil microalgae. Sci Total Environ 781:147006
[3] Marcos *et al* (2021) A framework for multi-dimensional assessment of wildfire disturbance severity from remotely sensed ecosystem functioning attributes. Remote Sens 13:780

122

Microbial community structure in subtropical beachrock with phototrophic biofilms containing chlorophyll f

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Beachrock is formed by calcification of sand in the intertidal zones of certain subtropical and tropical coastlines. Microbial biofilms form on the beachrock surface and subsurface cavities that are photosynthetically highly active. We have investigated the structure and environment of such microbial communities in the beachrock on the subtropical Heron Island, Australia. 16S rRNA and pigment analyses showed that the microbial diversity was low and varied across the environmental gradients from pink (wettest) to brown to black (driest) beachrock and vertically within the beachrock biofilm. The phototrophic communities in all beachrock types were dominated by cyanobacteria. Algae were essentially absent from the driest portions of the beachrock. The nonphotosynthetic populations were dominated by Anaerolineae (phylum Chloroflexi). Dense *Neolyngya* populations dominated the wettest beachrock (pink), whereas *Rivularia*-like and *Chroococcidiopsis*-like populations were highly abundant in the driest beachrock (brown and black). Chlorophyll (Chl) *f*, which enables far-red light harvesting, was abundant in all beachrock biofilm in amounts corresponding to about 5% of that of Chl *a*. Endolithic cyanobacterial populations related to *Halomicronema* and *Chroococcidiopsis* were found inside the beachrock cavities and appeared to account for most of the Chl *f*. The beachrock also contained high amounts of cyanobacterial-type phycoerythrin and phycocyanin. A relatively low content of these phycobiliproteins compared to the Chl *a* content suggested that the cyanobacteria was nitrogen-limited even though evidence suggests that the cyanobacteria are fixing nitrogen. Overall, the microbial populations of beachrock constituted low-diversity and highly specialized communities in an oligotrophic environment constrained by the diel and tidal cycles and extreme conditions of light and desiccation.

123

Growth of Synechocystis sp. strain PCC 6803 on nitrate is dependent on sll1454 (narB) gene

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The nitrate reductase of unicellular cyanobacterium *Synechococcus elongatus* strain PCC 7942 is a molybdoprotein [1] and has been shown to be repressed by ammonium [2] and to be sensitive towards tungsten, which poisons the enzyme by replacing molybdenum [1, 3], whereas addition of the latter to a medium containing tungsten can rescue the activity of the enzyme. In *Synechocystis* sp. strain PCC 6803 the gene *sll1454* (*narB*) encoding a functional nitrate reductase has already been deleted by Baebprasert *et al.* to check whether the hydrogenase and the nitrate reductase compete for the same electrons in this strain [4], however, the phenotype of the *sll1454* knock out mutant strain concerning the assimilation of nitrate as the only available nitrogen source as well as the effect of tungsten to the molybdoenzyme in the wild type has not been analyzed so far. In this work we deleted *sll1454* by a neomycin phosphate transferase in direct and complementary orientation and both mutant strains lost the capacity to grow in a medium with nitrate as sole nitrogen source but grew well in medium (without molybdenum or tungsten) containing ammonium instead. Replacing molybdenum by tungsten in this medium impaired but did not completely inhibit the growth of the wild type of *Synechocystis* sp. strain PCC 6803, indicating the cells kept molybdenum within their nitrate reductase even though the cells had been cultivated in the absence of both nitrate and molybdenum for several generations.

[1] Guerrero MG *et al* (1981) The assimilatory nitrate-reducing system and its regulation. Ann Rev Plant Phys 32:169-204
[2] Herrero A *et al* (1981) Regulation of nitrate reductase levels in the cyanobacteria *Anacystis nidulans*, *Anabaena* sp. strain 7119, and *Nostoc* sp. strain 6719. J Bacteriol 145:175-180
[3] Herrero A and Guerrero MG (1986) Regulation of nitrite reductase of the cyanobacterium *Anacystis nidulans*. J Gen Microbiol 132:2463-2468
[4] Baebprasert W *et al* (2011) Increased H_2 production in the cyanobacterium *Synechocystis* sp. strain PCC 6803 by redirecting the electron supply via genetic engineering of the nitrate assimilation pathway. Metab Eng 13:610-616

125

Cyanobacterial communities in Antarctic and sub-Antarctic lacustrine microbial mats

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During the BelSPO funded CCAMBIO project, based on an extensive sampling of about 80 samples from 8 Antarctic Conservation Biological Regions (ACBRs) on the Antarctic continent and two sub-Antarctic islands, two goals were pursued. First, the diversity of cyanobacteria growing in the microbial mats of freshwater lakes was described, including a comparison of rare and abundant taxa and the determination of the proportion of endemic taxa. Second, the influence of geographic isolation and environmental parameters (pH and conductivity) were studied at three different spatial scales: circum-polar, continental, and bioregional. In total, 310 cyanobacterial OTUs were obtained and covered a large taxonomic diversity, with 6 orders containing 64 genera. The most frequent genera were *Leptolyngbya* (31.4 % of the OTU abundance), *Phormidesmis* (29.2 %), *Phormidium* (10.5 %), *Oscillatoria* (5.2 %), and *Cyanobium* (3 %). The cyanobacterial communities appeared dominated by filamentous cyanobacteria on the Antarctic continent, whereas the sub-Antarctic islands present a more balanced diversity, with a higher presence of unicellular morphotypes. A MetaMDS analysis on the Bray-Curtis dissimilarity matrix of OTU shows that the cyanobacterial community structures differ between Antarctica and the sub-Antarctic islands. Within Antarctica, the PERMANOVA analysis reveals that the differences in community structures between maritime and continental samples, even if significant, are smaller than with the sub-Antarctic islands. Based on our GenBank annotation, the geographic distribution of OTUs found in Antarctica and in the sub-Antarctic islands shows different patterns. Indeed, even if cosmopolitan taxa are important in both places, Antarctica shows a higher proportion of “cold” taxa (Alpine and Polar = 48.3 %), compared to the sub-Antarctic (Alpine and Polar = 15.2 %). The data suggests that the cyanobacterial communities are not structured according to the ACBRs. The factors explaining the cyanobacterial community differences appear to depend on the spatial scales we focus on. Indeed, distance effect is an important factor at the circumpolar scale, and then decreases in favor of the environmental parameters tested (pH and conductivity) within the Antarctic continent or in the sub-Antarctic islands.

126

Cyanobacterial extracellular vesicles: from biology to biotechnology

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The active transport of material from the interior to the exterior of the cell, or secretion, represents a very important mechanism of adaptation to the surrounding environment. In bacteria, secretion is mediated by a series of multiprotein complexes that cross the membrane(s). In addition, biological material may also be released from the bacterial cell in the form of vesicles, a secretion strategy that has been highly overlooked in cyanobacteria [1]. Extracellular vesicles (EVs) are discrete and non-replicable proteoliposomal nanoparticles, ranging in size between 20 and 400 nm in diameter [2]. Here, we show that vesiculation is a common bacterial response mechanism to high-copper concentrations, including in cyanobacteria. Our results suggest that release of EVs represent a novel copper-secretion mechanism, shedding light into alternative mechanisms of bacterial metal resistance [3].
In a more biotechnological approach, we tested cyanobacterial EVs as nanocarriers of heterologous proteins for applications in fish. We started by incubating zebrafish larvae with *Synechocystis* sp. PCC6803 EVs, isolated from mutant strains with different cell envelope characteristics. Results show that *Synechocystis* EVs are biocompatible with fish larvae, regardless of their structural composition. We established also that cyanobacteria are amenable to engineering heterologous protein expression and loading into EVs, for which we used the reporter sfGFP. Moreover, upon immersion treatment, we demonstrate that sfGFP loaded *Synechocystis* EVs accumulate in the gastrointestinal tract of zebrafish larvae, opening the possibility of using cyanobacterial EVs as a novel biotechnological tool in fish, e.g. for modulating their nutritional status or stimulating specific adaptive immune responses.
Altogether, this highlights the importance of studying EVs in cyanobacteria for better understanding their cell biology and physiology, but also for exploring novel biotechnological applications.

[1] Lima S *et al.* (2020) Extracellular vesicles: an overlooked secretion system in cyanobacteria. Life 10:129
[2] Biller SJ *et al.* (2022) Isolation and characterization of cyanobacterial extracellular vesicles. J Vis Exp 180:e63481

[3] Lima S *et al.* (2022) Extracellular vesicles as an alternative copper-secretion mechanism in bacteria. J Hazard Mat 431:128594
[4] Matinha-Cardoso J *et al.* (2022) Novel protein carrier system based on cyanobacterial nano-sized extracellular vesicles for application in fish. Microb Biotechnol. Accepted

127

Proteomic analysis of a filamentous anoxygenic phototrophic bacterium during the transition from respiration to phototrophy

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Chloroflexus aurantiacus is a thermophilic filamentous anoxygenic phototroph in the phylum *Chloroflexota*. This bacterium grows chemotrophically under oxic conditions and phototrophically under anoxic conditions by using functional enzymes encoded in the genome [1, 2]. However, the proteomic manner of *C. aurantiacus* along with the changing conditions is not well elucidated. In this study, the high-resolution time-course proteomic analysis was performed to understand the dynamics of proteins expressed during the transition from respiration to phototrophy.

The aerobic culture of *C. aurantiacus* J-10-fl was cultivated in 5 L of the medium containing organic carbons in 10 L fermenter for 48 days in the light with the gas-phase of N_2:O_2 (98:2). Cells collected from the fermenter were subjected to spectroscopic and LC-MS/MS analysis.

The bacterial growth was not observed immediately after the cultivation started. The phototrophic growth was first detected on 12th day of cultivation. The growth curve showed two exponential growth phases. The doubling time in the 1st phase was 3.2 ± 0.5 days until 17th day. After 20th day, the growth rate became slower, 11.8 ± 1.6 days (2nd phase). The relative bacteriochlorophyll (BChl) *c* content per cell was increased remarkably in the 1st phase. The time-course proteomic analysis detected 2677 proteins out of 3934 CDSs contained in *C. aurantiacus* genome. The majority of enzymes for BChl biosynthesis were abundant at 1st phase, consistent with the relative BChl *c* content per cell. Structural proteins for photosynthetic apparatus increased progressively in abundance during the cultivation. Simultaneously, enzymes for the carbon fixation pathway were highly expressed at the phototrophic growth phase. From these results, co-expression of not only photosynthesis-related proteins but carbon fixation enzymes are suggested to be important for the anoxygenic phototrophy of *C. aurantiacus*.

1. **Klatt CG, Liu Z, Ludwig M, Kühl M, Jensen SI, *et al.*** Temporal metatranscriptomic patterning in phototrophic *Chloroflexi* inhabiting a microbial mat in a geothermal spring. *ISME J* 2013;7:1775–1789.
2. **Cao L, Bryant DA, Schepmoes AA, Vogl K, Smith RD, *et al.*** Comparison of Chloroflexus aurantiacus strain J-10-fl proteomes of cells grown chemoheterotrophically and photoheterotrophically. *Photosynth Res* 2012;110:153–168.

129

Differential phototactic behavior of closely related cyanobacterial isolates from Yellowstone hot spring biofilms

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Phototrophic biofilms in most environments experience major changes in light levels throughout a diel cycle. Phototaxis can be a useful strategy for optimizing light exposure under these conditions, but little is known about its role in cyanobacteria from thermal springs. We examined two closely related *Synechococcus* isolates (*Synechococcus* OS-A dominates at 60-65℃ and OS-B' at 50-55℃), from outflows of Octopus Spring in Yellowstone National Park. Both isolates exhibited phototaxis and photokinesis in white light, but with differences in speed and motility bias. OS-B' phototaxed towards UVA, blue, green and red wavelengths while OS-A primarily phototaxed towards red and green. OS-A also exhibited negative phototaxis under certain conditions. The repertoire of photoreceptors and signal transduction elements in both isolates were quite different to those characterized in other unicellular cyanobacteria. These differences in the photoresponses between OS-A and OS-B' in conjunction with *in situ* observations, indicate that phototactic strategies may be quite versatile and finely tuned to the light and local environment.

130

Metabolic engineering of Synechocystis sp. PCC 6803 for the photoproduction of the sesquiterpene valencene

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Abstract
Cyanobacteria are extremely adaptable, fast-growing, solar-powered cell factories that, like plants, are able to convert carbon dioxide into sugar and oxygen and thereby produce a large number of important compounds. Due to their unique phototrophy-associated physiological properties, i.e. naturally occurring isoprenoid metabolic pathway, they represent a highly promising platform for terpenoid biosynthesis. Here, we implemented a carefully devised engineering strategy to boost the biosynthesis of commercially attractive plant sequiterpenes, in particular valencene. Sesquiterpenes are a diverse group of bioactive metabolites, mainly produced in higher plants, but with often low concentrations and expensive downstream extraction. In this work we successfully demonstrate a multi-component engineering approach towards the photosynthetic production of valencene in the cyanobacterium *Synechocystis* sp. PCC 6803. First, we improved the flux towards valencene by markerless genomic deletions of *shc* and *sqs*. Secondly, we downregulated the formation of carotenoids, which are essential for viability of the cell, using CRISPRi on *crtE*. Finally, we intended to increase the spatial proximity of the two enzymes, *ispA* and *CnVS*, involved in valencene formation by creating an operon construct, as well as a fusion protein. Combining the most successful strategies resulted in a valencene production of 19 mg/g DCW in *Synechocystis*. In this work, we have devised a useful platform for future engineering steps.

131

Heterologous expression of far-red light photosystem II subunits in Synechocystis sp. PCC 6803

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Certain terrestrial cyanobacteria acclimate to far-red light (FRL) by remodelling their photosynthetic apparatus to bind pigments such as chlorophyll *d* (Chl *d*) and Chl *f* that are synthesised in response to FRL. This acclimation response is termed far-red light photoacclimation (FaRLiP) and is under the control of a ~20 gene cluster (FaRLiP cluster) encoding the Chl *f* synthase (ChlF) and FRL paralogues of core subunits of photosystem I (PSI), photosystem II (PSII) and the phycobilisome (PBS). Heterologous expression of PsbA4/ChlF encoded in the FaRLiP cluster drives Chl *f* production in non-FaRLiP cyanobacteria but at relatively low levels compared to native systems [1]. This might be due to the lack of the FRL-PSI and FRL-PSII subunits encoded in the FaRLiP cluster that naturally bind Chl *f*. A recent cryo-EM structure of a native FRL-PSII core complex has revealed the presence of 30 Chl *a*, 1 Chl *d* and 4 Chl *f* per PSII monomer [2]. The Chl *d* occupies the Chl_{D1} position in the PsbA3/PsbD3 reaction centre complex and the four Chl *f* are found in the antenna (three in PsbB2 and one in PsbC2) [2]. Here, we replaced individual endogenous PSII subunits in *Synechocystis* sp. PCC 6803 (Syn6803) with their FRL paralogues (PsbA3 (FRL-D1), PsbB2 (FRL-CP47), PsbC2 (FRL-CP43) and PsbD3 (FRL-D2)) encoded by *Chroococcidiopsis thermalis* PCC 7203 to test whether these FRL-PSII subunits accumulate in the absence of Chl *f* and Chl *d* and whether they are incorporated into hybrid PSII complexes. Growth tests showed that none of these FRL-PSII mutants grew photoautotrophically. However, a combination of His-tag pull-downs and immunoblotting revealed that PsbA3, PsbC2 and PsbD3 could accumulate within the membrane and were able to form complexes with the endogenous Syn6803 PSII subunits. In contrast, levels of PsbB2 were drastically reduced.

[1] Ho M-Y *et al* (2016) Light-dependent chlorophyll f synthase is a highly divergent paralog of PsbA of photosystem II. Science, 353:aaf9178.

[2] Gisriel CJ *et al* (2022) Structure of a monomeric photosystem II core complex from a cyanobacterium acclimated to far-red light reveals the functions of chlorophylls d and f. J Biol Chem, 298:101424.

132

The role of the Ycf48 accessory factor in cyanobacterial photosystem II assembly

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Oxygenic photosynthesis describes the light-driven conversion of carbon dioxide to biomass in plants, algae and cyanobacteria. During this process, photosystem II (PSII), a multi-subunit thylakoid membrane protein complex, catalyses the light-driven oxidation of water at a Mn_4CaO_5 cluster. The structure and function of PSII have been intensively studied. However, less is known is about the PSII assembly process, which occurs in a stepwise process and involves a variety of accessory proteins [1].
The Ycf48 accessory factor plays a physiologically important role at an early stage of cyanobacterial PSII assembly [2]. Ycf48 binds to unassembled precursor D1 and promotes the formation of the PSII reaction centre (RCII) assembly complex [2, 3] from the smaller D1 module ($D1_{mod}$) containing D1 and PsbI and D2 module ($D2_{mod}$) containing D2 and cytochrome b-559 [1].
We have used cryo-EM to determine the structure of a RCII/PSI complex isolated from the cyanobacterium *Synechocystis* sp. PCC 6803. Surprisingly, the PSI monomer was oriented in the opposite orientation to the RCII complex. Our results reveal that Ycf48, which is a 7-bladed beta propeller protein, interacts with D1 through a conserved ‘Arg patch’ and that binding of Ycf48 distorts the normal binding site of the Mn_4CaO_5 cluster found in the mature PSII complex. Consequently, Ycf48 prevents the premature binding and oxidation of Mn ions and its detachment is an obligatory step for light-driven assembly of the Mn_4CaO_5 cluster.

[1] Komenda J *et al* (2012). Assembling and maintaining the photosystem II complex in chloroplasts and cyanobacteria. Current opinion in plant biology, 15:245-251
[2] Komenda J *et al* (2008). The cyanobacterial homologue of HCF136/YCF48 is a component of an early photosystem II assembly complex and is important for both the efficient assembly and repair of photosystem II in Synechocystis sp. PCC 6803. Journal of Biological Chemistry, 283:22390-22399
[3] Yu J *et al* (2018). Ycf48 involved in the biogenesis of the oxygen-evolving photosystem II complex is a seven-bladed beta-propeller protein. Proceedings of the National Academy of Sciences, 115:E7824-E7833

133

A proteolytic pathway coordinates cell division and heterocyst differentiation

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The cyanobacterium *Anabaena* PCC 7120 is able to form a particular cell type, heterocyst, for nitrogen-fixation upon deprivation of combined nitrogen in the growth medium [1]. We have shown previously that a functional cell division is required for heterocyst development since the inhibition of certain component or the inactivation of some of the genes involved in cell division, blocked heterocyst development [2-4]. The underlying mechanism remained unknown. HetF is a putative protease required for heterocyst differentiation (5). We demonstrated that HetF was a component of the divisome complex for cell division (3). Therefore, HetF is a candidate for the coordination of cell division and heterocyst differentiation. We'll present evidence on the identification of a HetF substrate, cleaved specifically by HetF at the division site. This proteolytic pathway times the commitment step of heterocyst development to the success of cell division, through a licence-and-brake mechanism [6].

[1] Zeng X and Zhang C-C (2022) The making of a heterocyst in cyanobacteria. Ann Rev Microbiol 76: doi.org/10.1146/annurev-micro-041320-093442. In press.
[2] Sakr S *et al* (2006) Inhibition of cell division suppresses heterocyst development in *Anabaena* sp. strain PCC 7120. J. Bacteriol. 188:1396-1404.
[3] Xing WY *et al* (2021) HetF protein is a new divisome component in a filamentous and developmental cyanobacterium. mBio. 12:e01382-21.
[4]. Wang L *et al* (2021) The developmental regulator PatD modulates assembly of the cell-division protein FtsZ in the cyanobacterium *Anabaena* sp. PCC 7120. *Env. Microbiol.* 23:4823-4837.
[5] Wong FCY and Meeks JC (2001) The HetF gene product is essential to heterocyst differentiation and affects HetR function in the cyanobacterium *Nostoc punctiforme*. J Bacteriol 183: 2654-2661.
[6] Xing WY et al. A proteolytic pathway coordinates cell division and heterocyst differentiation through a license-brake mechanism. In preparation.

134

PII-PirC-PGAM - The regulatory triad of cyanobacterial carbon metabolism

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The balancing of the cyanobacterial C/N-metabolism is a crucial mechanism for the maintenance of its homeostasis. During vegetative growth, newly fixed carbon is mainly used for the formation of essential building blocks. When non-diazotrophic cyanobacteria, like *Synechocystis* sp. PCC 6803, are starved by combined nitrogen, newly fixed carbon is stored as glycogen. Carbon partitioning occurs at the cofactor-independent phosphoglycerate mutase (PGAM) reaction, which is controlled by the regulatory axis of PII-PirC-PGAM. PII acts as a sensor of the energy- and nitrogen status using ADP, ATP, and 2-oxoglutarate (2-OG) as sensory molecules. Nitrogen limitation leads to increased 2-OG accumulation, causing dissociation of the PII-PirC complex. Released PirC now acts as an inhibitor of cofactor-independent phosphoglycerate mutase (PGAM), which finally leads to glycogen formation (1). In a PirC-deficient mutant, carbon flow towards Acetyl-CoA is not turned down, leading to increased polyhydroxybutyrate (PHB) accumulation during nitrogen starvation. Combining the PirC mutation with increased expression of phaA-phaB genes (encoding the enzymes for the first steps towards PHB) leads to a *Synechocystis* strain that reaches PHB levels up to 80% of their cell dry mass (2). To further elucidate the interaction between PII and PirC, we used Biolayer interferometry with PirC and various PII variants, confirming that the T-loop of PII is critically involved in binding PirC. Bioinformatic analysis of available PGAM sequences (Uniprot) revealed a highly conserved additional sub-domain in cyanobacterial PGAM sequences, predicted to form a loop structure. A *Synechocystis* mutant expressing the PGAM with deleted loop subdomain has a growth disadvantage when cultivated at low CO_2. conditions. The elucidation of the structure of PGAM and PirC is a prospective goal for a better understanding of the regulatory axis of PII, PirC, and PGAM.

References

1. T. Orthwein et al. (2021) The novel PII-interactor PirC identifies phosphoglycerate mutase as key control point of carbon storage metabolism in cyanobacteria. Proceedings of the National Academy of Sciences of the United States of America
2. M. Koch et al. (2020) On the Role and Production of Polyhydroxybutyrate in the Cyanobacterium Synechocystis sp. PCC 6803. Life (Basel, Switzerland)

135

Metabolite interactions in the bacterial Calvin cycle and implications for flux regulation

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Metabolite-level regulation of enzyme activity is important for coping with environmental shifts. Improved understanding of such regulation could guide attempts to engineer more efficient strains for biochemical production. Recently developed proteomics methods allow for mapping of post-translational interactions, including metabolite-protein interactions, that may be relevant for quickly regulating pathway activity. While feed-back and feed-forward regulation in glycolysis has been investigated, there is relatively little study of metabolite-level regulation in the Calvin cycle, particularly in bacteria. Here, we applied limited proteolysis small molecule mapping (LiP-SMap) to identify and compare metabolite-protein interactions in four bacteria that fix CO_2 using the Calvin cycle, including two cyanobacteria and two chemoautotrophs. Species-specific interactions were observed, such as interactions with glucose-6-phosphate in the chemoautotroph *Cupriavidus necator* and interactions with glyoxylate in the cyanobacteria *Synechocystis sp.* PCC 6803, which suggests that metabolite-level regulation can be specific for a certain metabolic network or bacteria lifestyle. Metabolites interacting with Calvin cycle enzymes fructose-1,6/sedoheptulose-1,7-bisphosphatase (F/SBPase) and transketolase were tested for effects on catalytic activity using kinetic assays. GAP increased the activity of both *Synechocystis* and *Cupriavidus* F/SBPase, which may act as a feed-forward activation mechanism in the Calvin cycle. A kinetic model incorporating regulations on F/SBPase generally enhanced flux control of ATP and NADPH supply over the cycle. We show that LiP-SMap is a promising technique to explore and uncover novel post-translational metabolic regulation, although the method could benefit from improved sensitivity and specificity.

136

α-cyanobacteria possessing form IA RuBisCO globally dominate aquatic habitats

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RuBisCO (ribulose 1,5-bisphosphate carboxylase/oxygenase) is one the most abundant enzymes on Earth and virtually all food webs depend on its activity to supply fixed carbon. To compensate RuBisCO's frailties, organisms have evolved carbon concentrating mechanisms (CCMs) to concentrate CO_2 around the active site. In bacteria, the carboxysome represents one such CCM, of which two independent forms exist: α and β. Cyanobacteria are key players in the planet's carbon cycle with the vast majority of the phylum possessing a β carboxysome and a form IB RuBisCO, including most cyanobacteria used as models in laboratories. The exception are the exclusively marine *Prochlorococcus* and *Synechococcus* genera that numerically dominate open ocean systems and possess α-carboxysomes and a form IA RuBisCO. To date, the reason why marine systems favour an α-form has remained elusive. Here, we report the genomes of 58 cluster 5 picocyanobacteria, closely related to marine *Synechococcus* that were isolated from freshwater lakes across the globe. Remarkably, we find all these isolates possess α-carboxysomes accompanied by a form 1A RuBisCO. Moreover, we demonstrate α-cyanobacteria dominate freshwater lakes worldwide. Hence, the paradigm of a separation in carboxysome type across the salinity divide does not hold true, and instead the α-form dominates all aquatic systems. We thus question the relevance of β-cyanobacteria as models for aquatic systems at large and pose several hypotheses for the reasons for the success of the α-form in nature.

Keywords: RuBisCO, α/β-cyanobacteria, marine, freshwater, cluster 5 picocyanobacteria

137

Transcriptome-wide cleavage sites of cyanobacterial RNase E reveal insights into its function and substrate recognition

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Ribonucleases are crucial enzymes for RNA metabolism and the rapid acclimation to environmental changes. Not much is known about the roles and substrate affinity of the two essential ribonucleases RNase E and RNase J in cyanobacteria. To analyse the targetome of endoribonuclease RNase E, we engineered a mutant strain of *Synechocystis* sp. PCC 6803 in which RNase E is inactive at an elevated temperature of 39°C. We used this strain to map 1,472 RNase-E-dependent cleavage sites on a transcriptome-wide level by RNA-seq before and after shifting the cells from 30°C to 39°C [1]. Furthermore, we engineered a strain harbouring RNase E with inactivated 5' sensing, which is one of its several substrate affinity mechanisms. Performing RNA-seq on this strain in comparison to wild-type *Synechocystis* enabled us to map further RNase-E-dependent cleavage sites.
The resulting data sets provide a transcriptome-wide overview of RNase E cleavage sites. It has been long known that RNase E plays an important role in the acclimation to changing light conditions. Our data sets substantiate this function by mapping RNase-E-dependent cleavage sites in important photosynthetic transcripts, e.g. the *cpcBA* transcript, which encodes phycocyanin subunits. An RNase-E-dependent cleavage site downstream of *petD*, which encodes subunit 4 of the cytochrome b6f complex, might liberate an sRNA with regulatory function. Further targets include the sRNA PmgR1, which is essential for photomixotrophic growth and *gifB*, encoding glutamine synthetase inactivating factor 17. Besides these most likely regulatory functions, we found that RNase E matures several RNA species such as 5S rRNA and tRNAs. Furthermore, our data hints towards RNase E regulating the copy numbers of plasmids pSYSA and pSYSM. Our results are an important first step to understand this multifaceted ribonuclease and provide an important starting point to characterise specific cleavage events and their impact on regulation, acclimation events and cell physiology.

[1] Hoffmann UAH *et al* (2021) 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

138

Expanded solar energy conversion by engineered biohybrid photosystems employing components from anoxygenic and oxygenic photosynthesis

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Expanded solar energy conversion by engineered biohybrid photosystems employing components from anoxygenic and oxygenic photosynthesis.
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Reaction centre (RC) and RC-light harvesting 1 complexes from anoxygenic photosynthetic prokaryotes such as *Rhodobacter* (*Rba.*) *sphaeroides* have been incorporated into a variety of devices for *in vitro* solar energy conversion (e.g. [1,2]). Recent years have seen impressive increases in the photocurrents and photovoltages achievable by these proteins, and the development of device architectures for stable operation [1]. However, a drawback of these photoproteins is their limited harvesting of visible sunlight, their bacteriochlorophyll pigmentation absorbing primarily blue/near-ultraviolet and near-infrared light. To address this, we have explored mechanisms for expanding the range of solar energy harvested by *Rba. sphaeroides* RCs by conjugation to synthetic and/or natural materials with complementary absorbance. Non-covalent conjugation of RCs to synthetic quantum dot (QD) nanocrystals [3] enhances the harvesting of visible light by RCs through high-efficiency Förster resonance energy transfer (FRET) with a lifetime of a few tens of nanoseconds [4]. Conjugation to QDs of a mixture of *Rba. sphaeroides* RCs and chlorophyll-containing LHCII antenna complexes from oxygenic phototrophs further enhances light harvesting through both direct LHCII□RC and indirect LHCII□QD□RC FRET [5]. Both LHCII and dimeric LHCI complexes can also be covalently conjugated to RCs through a genetically-encodable, all-protein linking system to produce self-assembling polychromatic complexes capable of enhanced solar energy conversion in solution and on surfaces [6]. Key findings on these biohybrid photosystems will be described.

[1] Friebe, V.M. *et al.* (2022) Sustaining electron transfer pathways extends biohybrid photoelectrode stability to years. *Angew. Chem. Int. Ed.* e202201148

[2] Paul, N. *et al.* (2022) Plasmonic protein electricity generator. *Nanoscale Horizons* **7**, 220-234.
[3] Liu, J. *et al.* (2019) Mechanisms of self-assembly and energy harvesting in tuneable conjugates of quantum dots and engineered photovoltaic proteins. *Small* 15:1804267
[4] Amoruso, G. *et al.* (2021) High efficiency excitation energy transfer in biohybrid quantum dot–bacterial reaction center nanoconjugates. *J. Phys. Chem.* 12:5448–5455.
[5] Liu, J. *et al.* (2020) Minding the gap between plant and bacterial photosynthesis within a self-assembling biohybrid photosystem. *ACS Nano* 14:4536-4549.
[6] Liu, J. *et al.* (2020) Polychromatic solar energy conversion in pigment-protein chimeras that unite the two kingdoms of (bacterio)chlorophyll-based photosynthesis. *Nat. Comms.* 11:1542.

140

Association of the multitask regulator PipX with the ribosome-assembly GTPase EngA

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The Cyanobacterial Linked Genome (CLG), a web tool that generates flexible gene networks based on synteny (https://dfgm.ua.es/genetica/investigacion/cyanobacterial_genetics/dCLG/) connects PipX, a small protein exclusive of cyanobacteria that binds to the nitrogen regulators NtcA and PII according to the C/N ratio and energy status, with non-anticipated proteins. One of them is the ribosome-assembly GTPase EngA, conserved in eubacteria and plants. In contrast to PipX, EngA is essential in all systems studied so far. It plays essential roles in ribosome biogenesis in both bacteria and chloroplasts and has been found membrane associated in *E. coli* and in *Arabidopsis thaliana* thylakoids, where it has been connected with the photosystem II repair cycle. EngA shows a unique domain structure in which two G domains are tandemly repeated (GD1_GD2_KH-like). We recently showed that there is physical interaction between the GD1 domain of EngA and PipX, providing the first proof of concept for the CLG and paving the way to additional discoveries in the field based on synteny networks.

For a long time, we have been speculating that “PipX toxicity”, an arrest of growth that was observed whenever the PipX/PII ratio is increased, could be caused by the binding to an unknown protein partner, and we now wondered whether EngA could be that enigmatic PipX target. So far, our genetic analyses support the idea that PipX binds to EngA to slow down growth under certain conditions, including the environmentally relevant temperature of 20ºC.

To integrate previous information on PipX with our latest results concerning PipX-EngA interactions we propose that PipX binds to EngA to slow down growth under conditions in which the levels of PII and EngA effectors (together signalling energy status and C/N ratios) allow formation of a significant number of PipX-EngA complexes that interfere with ribosome assembly. This scenario, that can be reached by genetic manipulations increasing the intracellular PipX/PII ratio, would occur naturally under non-optimal growth conditions. We will discuss recent results supporting the current model for PipX-EngA regulatory interactions as well as new approaches aimed to understand the peculiarities and idiosyncrasy of regulatory mechanisms that may be widespread in oxygenic photosynthetic organisms.

141

The Evolution of the Cytochrome c6 Family of Photosynthetic Electron Transfer Proteins

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During photosynthesis, electrons are transferred between the cytochrome b_6f complex and photosystem I. This is carried out by either plastocyanin or cytochrome c_6 in many cyanobacteria and eukaryotic algal species or plastocyanin in plant chloroplasts. However, three distinct and highly conserved cytochrome c_6 homologues have been found: cytochromes c_{6B} and c_{6C} in cyanobacteria, and cytochrome c_{6A} in plants and green algae. Despite high sequence conservation across their respective lineages, the function of these proteins remains subject to investigation.

As part of this investigation, the evolutionary relationship between the members of the cytochrome c_6 family in photosynthetic organisms was analysed comprehensively. Our phylogenetic analyses show that the cyanobacterial cytochromes c_{6B} and c_{6C} are likely to be orthologues that arose from a duplication of cytochrome c_6, but that there is no evidence for separate origins for cytochrome c_{6B} and c_{6C}. We therefore propose re-naming cytochrome c_{6C} as cytochrome c_{6B}. We show that the cyanobacterial cytochrome c_{6B} gave rise to the eukaryotic cytochrome c_{6A} and investigate cytochromes $c_{6(A/B)}$ distribution across photosynthetic microorganisms. Finally, we present evidence for an independent origin of proteins with some of the features of cytochrome c_{6A} in peridinin dinoflagellates.

We conclude with a new comprehensive model of the evolution of the cytochrome c_6 family which is an integral part of understanding the function of the enigmatic cytochrome c_6 homologues in cyanobacteria and chloroplasts. The improved understanding of the evolutionary connections within this protein family will aid experimental studies of their function in phototrophic organisms across phylogenetically distant lineages.

Slater, B., Kosmützky, D., Nisbet, R. E. R. and Howe, C. J. (2021). The Evolution of the Cytochrome c_6 Family of Photosynthetic Electron Transfer Proteins. Genome Biol. Evol. **13**, 1–12.

142

Photoelectrochemical Analysis of Thylakoid Membrane Electron Transport

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The wiring of photosynthetic systems to electrodes, from the scale of individual proteins to biofilms, is a new approach for analysing the electron transport processes underlying oxygenic photosynthesis. Furthermore, this technology forms the basis of bio-photoelectrochemical systems, where electrons generated from natural photosynthesis are utilised for power and fuel production [1]. My research focuses on the wiring of photosynthetic thylakoid membranes from the cyanobacterium *Synechocystis* sp. PCC 6803 to microporous and translucent inverse-opal indium tin oxide (IO-ITO) electrodes. Photocurrent profiles (the changes in current recorded upon illumination) recorded from these thylakoids have a characteristic and reproducible shape and magnitude. Through analysis of these photocurrent profiles the mechanism of electron transport between the thylakoids and the electrodes has been identified, with electrons being found to originate from both the photosynthetic and respiratory electron transport chains. The ability of this tool to probe cyanobacterial thylakoid membrane electron transport was demonstrated by using it to measure changes to plastoquinone redox pool state in different mutants and chemical conditions, which match results found in previous studies. The technique has also been extended to studying electron transport in the thylakoids of other photosynthetic organisms. These results establish photoelectrochemistry as a powerful technique for studying thylakoid membrane electron transport, particularly in cyanobacteria where fluorescence-based methods are complicated by the presence of phycobilisomes. To our knowledge, this is also the first demonstration of wiring an entire natural electron transport chain to an electrode, which has implications for the development of improved bio-photoelectrochemical systems.
[1] Wey LT *et al* (2019) The development of biophotovoltaic systems for power generation and biological analysis. ChemElectroChem 6:5375-5386

143

The role of the γ subunit in the photosystem of the lowest energy phototrophs

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Purple phototrophic bacteria use a core 'photosystem' consisting of light harvesting complex 1 (LH1) antenna surrounding the reaction centre (RC), which primarily absorbs far-red–near-infrared light and converts it to chemical energy. Species in the *Blastochloris* genus, which are able to use light >1000nm for photosynthesis, use bacteriochlorophyll (BChl) *b* rather than the more common BChl *a* as their major photopigment, and also uniquely assemble LH1 with an additional polypeptide subunit, LH1γ, encoded by multiple open reading frames in their genomes. In order to assign a role to this subunit, we deleted the four LH1γ-encoding genes in the model *Blastochloris viridis*. Interestingly, growth under the halogen bulbs we routinely use for cultivation of anoxygenic phototrophs yielded cells displaying an absorption maximum of 825 nm, similar to that of the RC complex without LH1, but growth under white light fluorescent bulbs yielded cells with an absorption maximum at 972 nm. HPLC analysis of pigment composition and sucrose density gradient fractionation demonstrate that the mutant grown in white light assembles RC–LH1, albeit with an absorption maximum blue-shifted by 46 nm relative to the WT complex. Wavelengths between 900–1000 nm transmit poorly through the atmosphere due to strong absorption by water, thus our results provide an evolutionary rationale for the incorporation of the γ subunit into the LH1 ring; this polypeptide red-shifts the absorption maximum of the complex to a range of the spectrum where the photons are of lower energy but are more abundant. Finally, we transformed the mutant with plasmids carrying genes encoding natural LH1γ variants, and demonstrate that the polypeptide found in the WT complex red-shifts absorption back to 1018 nm, but incorporation of a distantly-related variant results in only a moderate red-shift. This result suggests that tuning the absorption maximum of this organism is possible, and may permit light capture past the current low-energy limit of natural photosynthesis.

144

The ancient evolution of far-red light photoacclimation

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A diverse subset of cyanobacteria can photosynthesize using near-infrared light. This rare acclimation process, advantageous in shaded environments, is encoded by a cluster of ca. 20 genes, and involves the synthesis of substituted chlorophyll *f*. Much has been discovered recently about the structural, spectral and regulatory aspects of this expansion of the classical red limit of oxygenic photosynthesis. However, the origin and the evolutionary history of the cluster in deep time remain unclear (1). This project clarifies this by relying on the vast increase in genomic and metagenomics data in recent years. Metagenomics databases, together with metagenomes from non-unialgal cultures, represent treasure troves of data. Here, we use enriched cultures from extreme environments, such as salt lakes and deserts. Bioinformatics techniques, including sequencing, metagenome assembly and phylogenetic tree networks, were used for the purpose of recovering and synthesizing data. More than six hundred new far-red light-associated genes were obtained, and Metagenome Assembled Genomes (MAGs) were built. Contrary to the original hypothesis that genes for far-red light photoacclimation were spread via horizontal gene transfer, the new data shows their distribution by vertical descent and loss. With the additional resolution, it is clear that chlorophyll *f* photosynthesis is an ancient adaptation, potentially associated with microbial mat evolution and stromatolite formation in the early Paleoproterozoic.

(1) Antonaru, L.A., Cardona, T., Larkum, A.W. and Nürnberg, D.J., 2020. Global distribution of a chlorophyll *f* cyanobacterial marker. *The ISME journal*, *14*(9), pp.2275-2287.

145
Natural competence in filamentous cyanobacteria

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The ability to take up DNA from the environment via natural transformation, termed natural competence, relies in gram negative bacteria on the type IV pilus and additional natural competence specific proteins. Inside the cyanobacterial phylum the complete set of the respective genes for natural competence is present in the majority of species, except members of the picocyanobacteria. However, experimental evidence for natural competence, i.e. demonstration of DNA uptake via natural transformation in a cyanobacterial strain, has been only available for few species.
Here, we show DNA uptake via natural transformation in two filamentous species, *Spirulina major* PCC 6313 and *Chlorogloeopsis fritschii* PCC 6912, members of the subsection III and V, respectively. Transformation into *S. major* was established with a plasmid for chromosomal integration by homologous recombination. This is to our knowledge the first report for gene transfer into the genus of *Spirulina*. Transformation of *C. fritschii* is the first demonstration of natural competence in subsection V and was established with the replicating plasmid pRL25C. While transformation of *C. fritschii* can be achieved with 200 ng plasmid and more, the lowest tested amount of plasmid DNA, which resulted in the transformation of *S. major*, is 3 ng.
Presence of the complete set of natural competence genes in numerous cyanobacteria together with rising number of experimentally validated examples of natural competence underline the relevance of this trait in major parts of the phylum and raise the question to its potential evolutionary advantage, e.g. allele transfer inside a population or potential route for DNA repair. Beside research questions directly related to natural competence in filamentous cyanobacteria, the establishment of gene transfer into new species open avenues for recombinant work in new parts of the phylum, relevant for both basic and applied research in the field.

147

Construction of a synthetic microbial community of Synechococcus elongatus and Azotobacter vinelandii using proteomics approach

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While microbial communities can undertake more complex tasks than individual populations and can achieve continuous production of valuable products, an increasingly number of attempts have been made to cultivate synthetic or artificial microbial consortia composed of defined species. However, details regarding their interactions and stability are often inadequate. A detailed understanding of the composition and dynamics of the proteome is necessary to gain fundamental insights into protein interactions among species.

In this study, we established a synthetic microbial consortium based on the cyanobacterium *S. elongatus* cscB/SPS and nitrogen-fixing bacterium *A. vinelandii* ΔnifL, in which members can survive without carbon and nitrogen sources within their media. We hypothesise that this foundation can support a third member specialised in producing desired biomolecules, such as amino acid. Moreover, an approach for performing proteomics experiments and data analysis in a co-culture system has been established to investigate interactions among defined microbial consortia. The resulting high-quality dataset consists of 19510 unique peptides corresponding to 2854 proteins, of which 1201 proteins belong to *A. vinelandii* and 1663 proteins belong to *S. elongatus* with a coverage of 24% and 58% respectively. Quantitative determination of protein abundances has resulted in the identification of 1287 differentially regulated proteins. We also found some metabolic pathways that changed significantly in co-culture, including photosynthesis, starch and sucrose metabolism, TCA cycle, quorum sensing, glycolysis/ gluconeogenesis, cysteine and methionine metabolism and two-component system, indicating the adjustment of acquisition of carbon and nitrogen under nutrient stress.

149

The photosynthetic response to disrupted chlorophyll synthesis in Synechocystis sp PCC 6803

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Electrons are distributed from photosystem I to many electron sinks by a small, [2Fe-2S] cluster protein called ferredoxin. All photosynthetic organisms contain multiple genes for ferredoxin, and previous work has established that some of these electron carriers show specific affinity for down-stream enzymes, and therefore regulate the partitioning of electrons into different metabolic pathways (Kimata-Ariga et al., 2000; Yonekura-Sakakibara et al., 2000; Voss et al., 2011). There is a class of ferredoxin proteins with extended C-terminii, here called FdC2, that is highly conserved from cyanobacteria, through algae to higher plants. Until now the function of FdC2 has remained elusive, although it appears essential in cyanobacteria (Cassier-Chauvat and Chauvat, 2014) and even single point mutations in rice result in a severe yellow leaved phenotype (Li et al., 2015; Zhao et al., 2015). It has recently been reported that FdC2 is essential for the Mg-protoporphyrin IX monomethyl ester cyclase step of chlorophyll synthesis (Stuart et al., 2021). We have studied the function of FdC2 in Synechocystis sp. PCC 6803 by disrupting its function through mutagenesis (Schorsch et al., 2018), and will present data showing the impact of this decreased chlorophyll content on photosynthesis, with particular reference to low iron adaptation.

Cassier-Chauvat C & Chauvat F (2014) Recent Advances. Life (Basel) 4: 666-680
Kimata-Ariga Y et al. (2000) The EMBO journal 19: 5041-5050
Li C et al. (2015) Plant Sci 238: 127-134
Schorsch et al. (2018) PNAS 115: E12111-E12120
Stuart D et al. (2021) *Plant Cell* 33: 2834–2849,
Voss I et al. (2011) The Journal of biological chemistry 286: 50-59
Yonekura-Sakakibara K et al. T (2000) Plant physiology 122: 887-894

150

Photosynthetic Antenna Architecture and its Impact on Energy Transfer

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Photosynthesis is the biological process of converting light energy into chemical energy. Organisms such as plants, algae, and cyanobacteria, utilize this process to sustain life and carry out many cellular processes. Cyanobacterial cells contain unique membranes, housing the photosynthetic system. The system is composed of many protein complexes, where the two key components initiating the process are antenna complexes and reactions centers (RC). The antenna complexes are called light harvesting complexes (LHC), corresponding to their function. Upon light irradiation, light energy is absorbed by the LHCs and immediately transferred to a RC, where the chemical and electron transfer reactions occur. The energy transfer (EET) from the LHC to the RC is highly efficient and relies on an overlap between the energy emitted by the LHC to that absorbed by the RC. The current mechanisms do not fully explain the EET occurring inside and between the complexes. In my research, we wish to investigate this energy transfer in a unique cyanobacteria, *A. marina*, for which there is a large energetic and physical gap between LHC and RC, yet it remains efficient. In practice, we wish to utilize a unique phenomenon found only in the *A. marina* membranes, where there is heterogenous separation between domains that contain the main LHC (the Phycobilisome, PBS) and domains lacking this complex. These unique domains, that we call LR-islands, contain all the components of the photosynthetic system, which will enable us to isolate it in its entirety from the cells. We use structural, biochemical, spectroscopic, and analytical tools to analyze this unique system. The complete isolated system may present properties typically existing only in vivo, which will help us decipher the full EET mechanism. In addition, we investigate particularly the *A. marina* PBS (AmPBS) complex, which is known to contain only phycocyanin (PC), assembled from two isoforms of the α and β subunits, in varying ratios. Using structural tools such as crystallography and CryoEM we can shed more light on the role of each isoform within the phycobilisome.

153

Localisation and characterisation of AncM, a thylakoid membrane-shaping factor in Synechocystis sp. PCC 6803

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The major photosynthetic complexes of cyanobacteria and chloroplasts are embedded in thylakoid membranes (TMs), a specialized internal membrane system. In the cyanobaterium Synechocystis sp. PCC 6803, TMs are arranged in 3 to 4 peripheral sheets, interrupted by TM convergence zones (TCZs) where the thylakoids are curved towards the plasma membrane (PM). At TCZs several thylakoid sheets are interconnected, forming local contact sites named thylapses between the two membrane systems (TM and PM) (Rast et al., 2019). One of the main drivers of TCZ formation is the membrane-curving protein CurT (Heinz et al., 2016). By whole-genome sequencing of a curT- suppressor strain, the protein anchor of convergence membranes (AncM) was identified as a factor required for the attachment of thylakoids to the PM at thylapses (Ostermeier et al., 2022). An ancM- mutant is shown to have i.) a photosynthetic phenotype affecting photosystem II and I, ii.) an altered thylakoid ultrastructure with additional sheets and converged thylakoids detached from the PM and iii.) an antagonistic function to CurT in shaping TM ultrastructure. Furthermore, we provide evidence that AncM co-localizes with the pD1 precursor protein which serves as a marker of early PSII assembly intermediates and, thus, assignes a biogenic function to TCZs of Synechocystis 6803.

Heinz, S., Rast, A., Shao, L., Gutu, A., Gugel, I. L., Heyno, E., Labs, M., Rengstl, B., Viola, S., Nowaczyk, M. M., Leister, D., & Nickelsen, J. (2016). Thylakoid Membrane Architecture in Synechocystis Depends on CurT, a Homolog of the Granal CURVATURE THYLAKOID1 Proteins. *Plant Cell*, *28*(9), 2238-2260. https://doi.org/10.1105/tpc.16.00491

Ostermeier, M., Heinz, S., Hamm, J., Zabret, J., Rast, A., Klingl, A., Nowaczyk, M. M., & Nickelsen, J. (2022). Thylakoid attachment to the plasma membrane in Synechocystis sp. PCC 6803 requires the AncM protein. *The Plant Cell*, *34*(1), 655-678.

Rast, A., Schaffer, M., Albert, S., Wan, W., Pfeffer, S., Beck, F., Plitzko, J. M., Nickelsen, J., & Engel, B. D. (2019). Biogenic regions of cyanobacterial thylakoids form contact sites with the plasma membrane. *Nat Plants*, *5*(4), 436-446. https://doi.org/10.1038/s41477-019-0399-7

154

Expression and localization of thylakoid associated mRNAs during heterocyst development in Anabaena sp. PCC 7120

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Thylakoid membranes are re-organized when heterocysts (specializing in nitrogen fixation) are differentiated in the filamentous cyanobacterium *Anabaena* sp. strain PCC 7120. Honeycomb thylakoids generated at the sub-polar region of the heterocyst cells contain terminal oxidases (Cox2 and Cox3) to consume the oxygen mainly from neighbouring vegetative cells. Although we have gained a great knowledge about the structure and function of the thylakoid membranes, the biogenesis of thylakoid membranes and the restructuring of this internal membrane system in heterocysts remain to be elucidated. RNA-FISH was used to probe the expression and localization of four mRNAs encoding thylakoid components. The mRNA signals were found to be localized at the inner edge of thylakoids, suggesting that the inner surface of thylakoid membranes is the synthesis site of thylakoid membrane proteins. Further experiments with puromycin treatment showed that the proteins in question were targeted to thylakoid membranes in an mRNA-based translation-independent manner. This suggests that specific RNA-binding proteins are involved in membrane protein targeting as previously observed in the unicellular cyanobacterium *Synechocystis* sp. PCC 6803 [1]. The re-organization of thylakoids during heterocyst differentiation was studied by probing the localization of *cox2* mRNA at different timepoints after nitrogen step-down. After 8-16h, Cox2 was synthesized at the existing peripheral thylakoids, suggesting that it undergoes a later redistribution to the sub-polar region with thylakoid membrane migration as observed in Santamaría-Gómez *et al*. [2]. We will next study the effect of two RBPs, RbpF and All4377, on mRNA expression and localization.

[1] Mahbub M *et al* (2020) mRNA localization, reaction centre biogenesis and thylakoid membrane targeting in cyanobacteria. Nat Plants 6:1179-1191.
[2] Santamaría-Gómez J *et al* (2018) Mechanisms for protein redistribution in thylakoids of *Anabaena* during cell differentiation. Plant Cell Physiol 59:1860-1873.

155

Role of mRNA localisation in membrane protein targeting and assembly in cyanobacteria

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Cyanobacterial thylakoid membrane is the sole site of photosynthetic electron transport and the main site of respiration. The proteome of the thylakoid membrane is distinct from the plasma membrane, yet they use the same protein trafficking machinery. Intensive investigations have failed to detect any distinct feature in the leader sequences of the plasma membrane and thylakoid membrane-targeted proteins. Therefore, the protein sorting mechanisms between the two membrane systems are still a mystery. Nevertheless, there are controversies regarding the biogenic sites of photosynthetic proteins. Our recent study on the in vivo mRNA localisation in two strains of cyanobacteria suggested that the translation of major photosynthetic proteins is localised in tight spots at the inner edge of the thylakoid membrane system[1]. The photosynthetic mRNAs were found to locate near the thylakoid membrane irrespective of ribosome association. This translation independent localisation of mRNAs suggests that the protein targeting signal could reside in the mRNAs rather than in the proteins[1]. We also found two RNA Binding Proteins (RBPs) involved in localising the photosynthetic mRNAs near the thylakoid membrane[1]. We suggest that the photosynthetic core proteins might have an mRNA-mediated targeting mechanism toward the thylakoid membrane, where they anchor to the membrane system by the dedicated RBPs and then protein translation initiates. It is yet to find how proteins target the plasma membrane. Therefore, we plan to use Fluorescence in situ Hybridisation technique to probe mRNAs encoding various plasma membrane and thylakoid membrane integral proteins to understand the basis of protein sorting mechanisms in different membrane systems of cyanobacteria. To explore deep into the biogenic regions, we will study the structural organisation of translation zones by Atomic Force Microscopy and track the later stages of photosynthetic protein assembly along with the corresponding mRNA localisation. We will work on identifying the specific features of mRNA molecules that might control the destination of the proteins. A clear understanding of protein sorting mechanisms will enable methods to put heterologous proteins into the correct locations in the cells.

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

156

Epibiotic Cyanobacteria Associated with Sea Turtles – from Metabarcoding to Culturing

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An increasing number of studies have shown that sea turtle-associated biofilms are highly diverse contain epibionts different from the ones that are found in other marine benthic biofilms. A sea turtle's carapace and skin can be the home to a large variety of macro-epibionts (e.g., barnacles, green and red algae) and micro-epibionts (e.g., bacteria, diatoms, protozoa), and many of them are found exclusively on sea turtles. Cyanobacteria are known for establishing symbiotic relationships with different hosts, however, little is known about the cyanobacteria associated with sea turtles. Recently, cyanobacteria have gained more attention as an important part of the sea turtle microbiota, but little is known about their identity, ecological role, or their possible effect on a sea turtle host and vice versa. Our previous research on cyanobacteria within the epizoic biofilm of 26 turtles (via amplicon sequencing of the V4 region of the 16S rRNA gene) showed that cyanobacteria comprised 3% of all ASVs on average, ranging from 0.01 to 19.77% across individual samples. More than 20 cyanobacterial families were found living on Mediterranean loggerheads, with the four most abundant being *Phormidesmiaceae*, *Paraspirulinaceae*, *Xenococcaceae*, and *Limnotrichaceae*. However, a large proportion of the sequences remained unidentified. To reduce this knowledge gap, we aimed to culture epizoic cyanobacteria sampled from microbial biofilm growing on sea turtles. The biofilm samples were enriched in ASN-III culture medium under 12h light period at 20 °C and single cells or colonies are picked using micropipette under inverted light microscope. Two xenic *Phormidium*-like cyanobacterial strains were obtained from the loggerhead carapace biofilm, they were examined morphologically using scanning electron and light microscopy. Their DNA was isolated and 16S sequences obtained, contributing to polyphasic characterization of the strains. Marker gene sequences from those cultures will contribute to the reference database and more accurate DNA barcoding and metabarcoding hits of cyanobacterial taxa in the future. Hopefully, this research will help better understand understudied cyanobacterial communities associated with vertebrate hosts.

157

Fructose phosphate metabolism in cyanobacteria: an in-silico analysis

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Many cyanobacterial strains can utilize sugars such as glucose or fructose in addition to CO_2. However, the cyanobacterium *Synechocystis* sp. PCC 6803 can grow on glucose, whereas fructose is toxic. In contrast, other cyanobacteria such as *Nostoc* sp. can utilize fructose. The question arises here, “why different strains show different resistance to external sugars?”. To counteract toxicity when accumulating fructose and converting it into fructose phosphate, cyanobacterial fructose metabolism is equipped with different classes of enzyme that can effectively metabolise phosphates of fructose. Here we performed various bioinformatics analyses, focused on the occurrence of enzyme classes involved in fructose metabolism, such as kinases, aldolases, ketolases, isomerases and/or hydrolases, among cyanobacteria. For example, the phophoketolases of *Synechocystis* PCC 6803 are mostly specific to heterotrophic and autotrophic conditions, while phosphofructokinases (PFK) are most active under mixotrophic conditions, but silencing PFK isoenzymes have no impact. A comparison of the fructose phosphate metabolising genes among fructose tolerant and intolerant cyanobacteria known so far (Ungerer *et al*., 2008) revealed that the fructose tolerant cyanobacteria (*Nostoc* sp.) have a greater number of enzymes and isoenzymes in the above-mentioned classes compared to the fructose sensitive ones. Many of these isoenzymes are even acquired via horizontal gene transfer. Also, the numbers of isoenzymes per class varies among the species to species under different classes, though most of them are without visible impacts on the metabolism. Therefore, we hypothesize, 1) the combination of these enzymes are for protection against evolutionary stress and sudden changes in their natural habitat, 2) fructose toxicity of cyanobacteria could be mitigated by efficient metabolism of fructose and its phosphates in cyanobacteria if they are equipped with these additional enzymes (i.e., extra PFKs or transaldolase); which are mostly believed to be complimentary but might be for sugar or fructose stress tolerance among cyanobacteria.

Reference:

Ungerer, J. L., Pratte, B. S., & Thiel, T. (2008). Regulation of fructose transport and its effect on fructose toxicity in Anabaena spp. *Journal of bacteriology*, *190*(24), 8115–8125. https://doi.org/10.1128/JB.00886-08

158

A cyanophage encoded RuBisCO deactivase

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Photosynthesis and CO_2 fixation are key biological processes crucial for life on Earth. In marine ecosystems unicellular cyanobacteria of the genera *Synechococcus* and *Prochlorococcus* contribute around a quarter of this fixed carbon.
We have recently shown that viruses infecting marine *Synechococcus* inhibit CO_2 fixation during the infection process (Puxty et al, 2016). However, the light reactions of photosynthesis which generate ATP and reductant are maintained, presumably to facilitate phage development. Thus, viral infection is important not only in controlling the abundance of marine picocyanobacteria but also affects global primary production estimates.
However, the underlying mechanism by which cyanophage inhibit CO_2 fixation is currently unknown. What is known is that the key CO_2-fixing enzyme RuBisCO can be metabolically inhibited by several naturally occurring sugar phosphates which bind to the active site rendering the enzyme inactive. This is typically solved by RuBisCO activase, an ATP dependent chaperone that can 'open' the RuBisCO active site and release the inhibitor.
Via analysis of cyanophage genomes we identified a gene (which we have named *cinI*) which is present in all cyanomyoviruses but not other phage, which we suggest is responsible for this inhibition of host CO_2 fixation during infection. CinI contains a domain similar to that of a RuBisCO activase but rather than being an activase we hypothesize CinI is a deactivase forming a direct protein-protein interaction with the RuBisCO complex in a similar, but functionally opposite, fashion to RuBisCO activase. In support of this we show that CinI from cyanophage S-RSM4 recombinantly expressed in *E. coli* can inhibit the activity of purified RuBisCO from *Synechococcus* sp. WH7803 *in vitro*. Moreover, CinI fixed on an IMAC column co-elutes with RuBisCO indicating direct binding. Furthermore, similarly to RuBisCO activase, CinI shows ATPase activity. Finally, heterologous expression of CinI in the freshwater cyanobacterium *Synechocystis* sp. PCC6803 showed a growth cost under photoautotrophic but not heterotrophic growth as well as a significantly reduced CO_2 fixation rate compared to control strains.
Thus, CinI appears to be the first reported protein inactivator of RuBisCO. A mechanistic understanding of its action might facilitate future applications whereby photosynthesis can be directly manipulated.

159

A toolkit for engineering the highly productive cyanobacterium Synechococcus sp. PCC 11901

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Synechococcus sp. PCC 11901 (PCC 11901) is a recently discovered fast-growing marine cyanobacterial strain. It has a capacity for sustained biomass accumulation to very high cell densities, even outperforming the marine model strain *Synechococcus* sp. PCC 7002. Nevertheless, very few genetic tools have been characterised in this strain. Here, we outline our progress towards the development of a synthetic biology toolkit based on the CyanoGate MoClo system to unlock the biotechnological potential of PCC 11901. We have characterised several neutral sites suitable for stable genomic integration that do not affect growth even at high densities. Furthermore, we have found that PCC 11901 is amenable to transconjugation and have characterised a suite of known and new genetic parts, including constitutive promoters, terminators, inducible systems, and CRISPR interference tools. We envision that this toolkit will lay the foundations towards the adoption of *Synechococcus* sp. PCC 11901 as a robust model strain for engineering biology and green biotechnology.

160

Characterisation of amino acid biosynthesis in Synechocystis sp. PCC 6803 via analysis of auxotrophic mutants

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Amino acid biosynthesis is not well understood in cyanobacteria and several pathways remain uncharacterised. Greater knowledge of these pathways may aid some synthetic biology applications as many amino acids are precursors for industrial compounds. Moreover, most amino acid biosynthetic pathways are conserved between cyanobacteria and plants, with numerous enzymes being targets for herbicides. Therefore, cyanobacteria could be excellent organisms to screen novel herbicides. To characterise amino acid biosynthesis, we used multiple approaches to generate auxotrophic mutants using knockout plasmids generated via CyanoSource, the developing *Synechocystis* sp. PCC 6803 gene knockout library. In our initial approach, generation of mutants cultured on plates with the respective amino acids did not result in any segregated knockouts, suggesting the absence of importers or insufficient import to compensate for loss of the biosynthetic pathways. In our second approach, mutants were generated on plates with a broad range of peptides of varying length, which did result in segregated knockouts of genes in different amino acid pathways. Thus, we hypothesise that peptides are imported via the oligopeptide transporter involved in peptidoglycan recycling. In combination with expression of characterised amino acid importers from *E. coli*, we are generating mutants of every gene encoding enzymes putatively involved in amino acid biosynthesis to confirm their function. This approach will also be used to identify candidates in the methionine, tyrosine and phenylalanine pathways, which are not characterised in *Synechocystis* sp. PCC 6803. This is the first study in which cyanobacterial amino acid auxotrophic mutants have been generated and will be key to complete characterisation of these pathways in cyanobacteria.

161

Hydrocarbons induce cyanobacterial membrane curvature required for light-to-dark thylakoid remodelling

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All cyanobacteria contain genes for the synthesis of C15-19 alkanes and alkenes, implying that these compounds play a key cellular function. Previous data from our laboratory have shown that hydrocarbons accumulate in cyanobacterial membranes *in vivo*, and hydrocarbon deficient mutants have significant growth, cell size and division defects, in addition to reduced membrane curvature. Membrane curvature is essential for optimal cellular growth, movement, division and vesicle budding/fusion, and is known to be induced by either asymmetrical lipid packing or curvature generating proteins. However, our data suggests a novel mechanism of curvature induction in which long chain hydrocarbons integrate into the centre of the lipid bilayer. To further investigate the role of hydrocarbons in cyanobacterial membranes we used a variety of different techniques with intact cells and lipid vesicles, as well as computer modelling. Small angle neutron scattering (SANS) on wild-type *Synechocystis* PCC 6803 cells, compared to a hydrocarbon-deficient mutant, revealed that in the absence of hydrocarbons, the normal membrane rearrangement in response to light to dark transitions is not observed, suggesting reduced membrane flexibility. SANS and small angle X-ray scattering (SAXS) on liposomes both show that there is an increase in membrane bilayer thickness in response to increasing hydrocarbon addition, consistent with hydrocarbons being present between the leaflets of the bilayer. *In silico* molecular dynamics simulation modelling of membranes with differing amounts of hydrocarbons demonstrate increased membrane thickness and curvature and faster lateral diffusion of lipids as hydrocarbon content is increased. Therefore, we propose that hydrocarbons function to increase membrane flexibility and likely facilitate efficient cell division and organisation of the photosynthetic thylakoid membranes.

162

Tools for genome editing by RNA-guided transposition to use in filamentous cyanobacteria

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Floating ferns from the genus *Azolla* constitute a symbiosis with the N_2-fixing, heterocyst-forming filamentous cyanobacterium *Nostoc azollae.* The ferns receive fixed nitrogen from the cyanobacteria allowing the symbiosis to thrive in the complete absence of N-fertilizers. *Azolla* species have diverse applications including being used as a biofertilizer and protein feed, making them important organisms of study. *N. azollae* is an obligatory symbiont with an eroded genome whose life cycle is tightly coordinated with that of its host [1]. As a consequence, its culture under laboratory conditions has proved impossible, which precluded stable genetic editing. Here, we develop the tools for and report on efficient RNA-guided transposition with the CAST system [2] to catalyze a directed insertion of DNA using the model filamentous cyanobacterium *Anabaena* sp. PCC 7120. To facilitate sharing and modular cloning, the CAST-encoding genes, sgRNA, Tn7 borders and specific cyanobacterial promoters were domesticated in level 0 modules for use in GoldenGate cloning [3]. For ease of testing, single guide (sg) RNA that bind the cas12k were designed to target the widely used *gfp*, as well as specific chromosomal loci in *Anabaena*. Plasmids generated were tested in different *Anabaena* strains: CSVT15 (*amt1::gfp*), CSAM137 (*sepJ::gfp*) and wild type. The experiments showed the efficacy of the system in creating targeted genetic modifications providing the sgRNA encoded leading strand of the gene targeted. The general advantage, over homologous recombination that relies on the host cell machinery, is the high efficiency of the catalyzed insertion: the CAST expression cassettes are provided along with the transposon substrate. Future research will focus on optimizing RNA-guided transposition for efficient genome editing in other cyanobacteria, including the *N. azollae* symbiont.

[1] Ran L *et al* (2010) Genome erosion in a nitrogen-fixing vertically transmitted endosymbiotic multicellular cyanobacterium. PLos One 5:e11486.

[2] Strecker J *et al* (2019) RNA-guided DNA insertion with CRISPR-associated transposases. Science 365:48-59.

[3] Vasudevan R *et al* (2019) CyanoGate: a modular cloning suite for engineering cyanobacteria based on the plant MoClo syntax. Plant Physiol 180:39-55.

163

On The Evolution and Function of Cyanobacterial NADPH Oxidases

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NADPH oxidases (NOX) are transmembrane redox proteins which utilise electrons from NADPH to reduce molecular oxygen into superoxide (O_2^-). Genes encoding NOX are widespread throughout animals, fungi, and plants, and have recently been identified in some prokaryotes, including cyanobacteria[1,2]. Cyanobacteria have evolved multiple mechanisms for dealing with the toxic repercussions of oxygen evolution, and it is therefore surprising that they encode homologs of NOX, which generate reactive oxygen species (ROS). NOX activity has been associated with signalling, metal acquisition, multicellularity, and predation defence. However, to date they have been characterised at the biochemical level only in eukaryotic cells or heterotrophic bacteria. In this study, we sought to investigate the evolutionary origins of cyanobacterial NOXs and understand their function in oxygenic phototrophic prokaryotes.
Bioinformatic analysis revealed that *nox* genes are widespread but sporadic in cyanobacteria, with both a long and a short isoform present. Long isoforms possess an additional EF-hand domain at the N-terminus and are highly homologous to eukaryotic NOX5, while short isoforms are closely related to prokaryotic NOX1-4. A putative short *nox* gene was amplified from the genome of *Chroococcidiopsis thermalis* PCC 7203, with its transcription under photoautotrophic growth conditions confirmed. Quenching the ROS generation of this species impaired its ability to perform extracellular iron reduction. *C. thermalis* NOX was further investigated by heterologous expression in *Synechocystis sp.* PCC 6803, which lacks *nox* genes. NOX-expressing mutants were demonstrated to have significantly enhanced superoxide generation compared to negative controls.
Taken together, these results provide first insights into cyanobacterial NOX activity. We show that NOX proteins are functional in photosynthetic prokaryotes, propose a role in metal acquisition and discuss possible hypotheses for their evolution in cyanobacteria.

[1] Hajjar C *et al* (2017) The NOX Family of Proteins Is Also Present in Bacteria. mBio 8(6):e01487-17
[2] Miles J *et al* (2021) The evolutionary origins of peroxynitrite signalling. Biochemical and Biophysical Research Communications 580 107-112

164

Light activation of Orange Carotenoid Protein

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Orange Carotenoid protein (OCP) is the only known photoreceptor which uses carotenoid for its activation1,2. It is found exclusively in cyanobacteria, where it functions to control light-harvesting of the photosynthetic machinery. However, the photochemical reactions and structural dynamics of this unique photosensing process are not yet resolved. We present time-resolved crystal structures at second-to-minute delays under bright illumination, capturing the early photoproduct and structures of the subsequent reaction intermediates. The first stable photoproduct shows carotenoid *trans*/*cis* isomerization at the C7'-C8'double bond and structural changes in the N-terminal domain with minute timescale kinetics. These are followed by a thermally-driven *cis*/*trans* isomerization that recovers to the dark state carotenoid configuration. Structural changes propagate to the C-terminal domain, resulting, at later time, in the H-bond rupture of the carotenoid keto group with protein residues. The isomerization and its transient nature are confirmed in OCP crystals and solution by FTIR and UV/Vis spectroscopy. This study reveals the isomerization of the carotenoid and subsequent thermal structural reactions as the basis of OCP photoreception. Understanding and potentially controlling the OCP dynamics offers the prospect of novel applications in biomass engineering3 as well as in optogenetics and bioimaging.

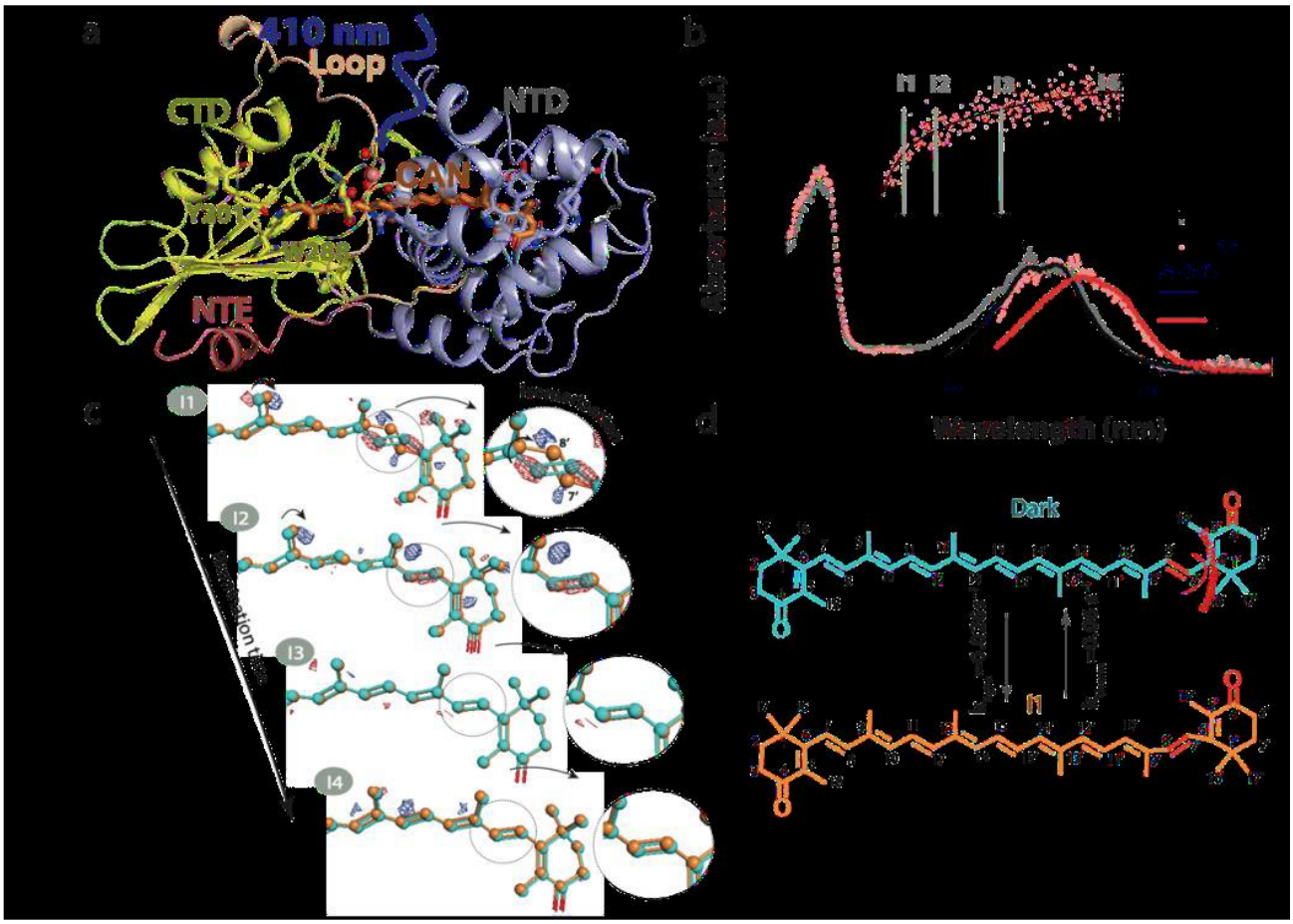

Figure a) Structure of OCPCAN in the 'dark' state. (b) Absorbance changes of OCP in solution (solid lines) and crystalline state (dotted lines) upon illumination. Inactive state OCPO and active state OCPR are shown in black/gray and red/pink respectively. Insert: Absorbance changes (pink dots) in the crystalline state detected at 550 nm upon 10 min of illumination with violet light. (c) Difference electron density (DED) maps (blue/red at ±3.0σ) were obtained for the four states: (I1) 1 min, (I2) 2 min,(I3) 5 min , (I4)10 min. Carotenoid coordinates of dark and light states are represented by cyan and orange lines respectively. (d) All-trans CAN is present in OCPDark while C8'-C7' cis conformation is observed in OCPI1. The latter thermally recovers back to all-trans state with prolonged illumination time (after 2-5 min).

1. Kerfeld, C. A. *et al.* The crystal structure of a cyanobacterial water-soluble carotenoid binding protein. *Structure* **11**, 55–65 (2003).
2. Leverenz, R. L. *et al.* A 12 Å carotenoid translocation in a photoswitch associated with cyanobacterial photoprotection. *Science .* **348**, 1463–1466 (2015).

165

Cyanobacterial photosynthesis: Integrating current knowledge into a mathematical model

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Well-designed mathematical models complement experimental scientific work. The mathematical representation of reaction networks allows for a detailed and systematic investigation of the system. It includes the analysis of a whole system, as well as its individual components. Cyanobacteria are of high economic value becoming a promising tool in biotechnological production [1]. Because of that potential, tremendous effort was put into elucidating its metabolic mechanisms. Still, no full understanding is reached yet, making cyanobacteria an ideal modelling target. Importantly, despite their evolutionary bond to plants, the structure and components of the electron transport chain differ with a high impact on the overall dynamics, prohibiting the usage of established plant-based models.
Because models offer a high potential for knowledge gain, we have developed an ordinary differential equation-based model of photosynthesis in *Synechocystis* sp. PCC 6803 [2]. It dynamically tracks the major photosynthetic processes from light capture to electron transport and carbon fixation. Throughout the development process, simple kinetic representations and literature parameterisation were chosen to avoid overfitting. Still, important experimental findings on metabolic or fluorescence levels could be qualitatively reproduced. These include, for example, simulated redox state of electron carriers, flux through alternative electron pathways, and dynamic fluorescence signals. We also incorporated knowledge about the light colour dependency of photosynthetic electron transport. Notably, the model showed that besides fundamental parameters of the environment, like irradiance and aeration, the light colour is crucial in controlling the cells' productivity.
With this model, we integrate systems-level knowledge on photosynthesis in cyanobacteria and provide a theoretical framework for further complex investigation.

[1] D. J. Lea-Smith and G. T. Hanke, "Electron Transport in Cyanobacteria and Its Potential in Bioproduction," *Cyanobacteria Biotechnol.*, pp. 33–63, May 2021, doi: 10.1002/9783527824908.CH2.
[2] M. van Aalst, O. Ebenhöh, and A. Matuszyńska, "Constructing and analysing dynamic models with modelbase v1.2.3: a software update," *BMC Bioinformatics*, vol. 22, no. 1, pp. 1–15, 2021, doi: 10.1186/s12859-021-04122-7.

166

Disentangling terrestrial cyanobacterial community composition in the Sør Rondane Mountains, East Antarctica

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The sparse ice-free regions of Antarctica are the coldest deserts on Earth. Yet, ice-free soils harbor substantial and diverse microbial communities that can vary significantly between regions and micro-climatic conditions. The factors responsible for driving the microbial diversity and community structure in inland nunataks of East Antarctica are still poorly understood. Within the BELSPO MICROBIAN project, three sampling campaigns took place in the Sør Rondane Mountains during the austral summers of 2018, 2019 and 2020, resulting in more than 100 samples which ranged from different types of barren bedrock to substrates covered by biofilms and well-developed biological soil crusts including lichens, mosses, microalgae and/or cyanobacteria. Cyanobacterial diversity was assessed by amplicon sequencing targeting the V3-V4 variable region of the 16S rRNA gene with cyanobacteria-specific primers using the Illumina MiSeq platform (2x300 bp). Whilst favorable habitats, such as sheltered spots in rocky areas, enhance the development of different kinds of cyanobacterial crusts, cyanobacteria were present even in the most extreme ones. Granitic soils were dominated by very diverse cyanobacterial crusts, mostly composed by filamentous cyanobacteria of the Leptolynbyaceae and Phormidiaceae families, and by Nostocaceae. In contrast, marble soils were dominated almost exclusively by unicellular taxa of the Chroococcidiopsaceae family. Moraine samples from very dry areas were mainly characterized by members of the Chroococcidiopsaceae and Nostocaceae families whereas moraine samples taken close to a lake were rich in filamentous taxa as well, mostly belonging to the Leptolynbyaceae family. Next to bedrock type, other abiotic variables such as pH, NO_3 and TOC were especially important drivers of the cyanobacterial community composition in each sampled site. Metagenomics analyses are ongoing to characterize the gene repertoires of the phototrophic prokaryotic organisms that may be involved in their survival during the long drought and freezing periods that often occur in the Antarctic ice-free areas.

167

Explore the role of MCE proteins in thylakoid membrane biogenesis in Synechocystis sp. PCC 6803

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Cyanobacteria are prokaryotes capable of oxygenic photosynthesis. They have a specialised 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. In the Mla system, a single MCE protein MlaD assembles with a Permease and an ATPase to form an inner membrane ABC transporter complex (MlaFEDB) [1]. A similar pathway has been found in the Arabidopsis *thaliana* chloroplast. TGD2, with the MCE domain, combines TGD1- a permease and TGD3- an ATPase- to form an ABC-like transporter, which transports phosphatidic acid from the endoplasmic reticulum to the chloroplasts for the synthesis of galactolipids [2].

In a model cyanobacterium *Synechocystis* PCC 6803, two proteins (Sll0751, Sll1002) have conserved MCE domains. Our studies revealed that the two single knockouts had chlorophyll fluorescence phenotypes. Under strong illumination, as in the confocal microscope, there was significantly reduced chlorophyll fluorescence compared to the wild type, although the cellular content of pigments and photosystems appears unperturbed. The effects of DCMU (a PSII inhibitor) and FCCP (a protonophore) on chlorophyll fluorescence suggest that the low fluorescence phenotype of the MCE mutants arises from a leaky thylakoid membrane, resulting in faster electron transport away from PSII centres due to loss of photosynthetic control. One explanation is that the MCE knockouts perturbed the lipid composition of the thylakoid membrane, causing increased proton permeability. We now aim to explore the membrane localisation of Sll0751 and Sll1002 in *Synechocystis* by YFP-tagging and confocal microscopy. Furthermore, we aim to determine the structure of individual MCE protein and ABC lipid transporter complexes from *Synechocystis* with X-ray Crystallography and Cryo-EM.

[1] Ekiert DC et al (2017). Architectures of lipid transport systems for the bacterial outer membrane. *Cell*, *169*(2), 273-285.
[2] Fan J et al (2015). Arabidopsis TRIGALACTOSYLDIACYLGLYCEROL5 interacts with TGD1, TGD2, and TGD4 to facilitate lipid transfer from the endoplasmic reticulum to plastids. *The Plant Cell*, *27*(10), 2941-2955.

168

High-throughput single-cell platform for identification and recovery of photosynthetic microbes with enhanced electrogenic capabilities

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There is an increasing global demand for the development of sustainable alternative energy technologies. As a potential alternative, Biophotovoltaic devices (BPVs) present an environmentally-friendly, self-renewing, low-cost approach to harvest solar energy and convert it into electrical current [1,4]. Photosynthetic organisms, such as microalgal and cyanobacterial species, constitute the primary providers of reductive power in BPVs. However, the efficiency of such devices is low at present [2]. One effective strategy towards achieving higher power outputs is through a targeted optimisation of electron-generating microbes [1,3]. The average reducing behaviour of a cyanobacterial population often masks any high-performing individual cells. To overcome this and reveal rare cells, a single-cell platform for photosynthetic microbes was developed by integrating droplet microfluidics with commercial fluorescence-activated cell sorting (FACS) [5]. The Double Emulsion (DE) – FACS platform enables the identification and isolation of individual photosynthetic microbes with enhanced reducing abilities by leveraging a fluorescent redox marker as an indicator of electron output. DE-FACS provides an alternative and effective approach for screening cyanobacterial cells with enhanced electrogenesis, and was deployed to enrich for a sub-population of highly reducing cells from the cyanobacterium *Synechocystis* sp. PCC 6803. We also demonstrate the successful recovery and growth of single cells displaying enhanced electrogenesis. The DE-FACS enriched strains were further characterised by a multi-modal analysis of bulk reducing behaviour, genetic variations and BPV performance. Photosynthetic-organism directed DE- FACS (podDE-FACS) is a powerful tool to investigate and isolate microbes with high electron generating capacity and provides a simple, unprecedented platform to translate the phenotype of a single cyanobacterium with its genetic and electrochemical background.

References:

(1) Tschörtner, Jenny, Bin Lai, and Jens O. Krömer. "Biophotovoltaics: green power generation from sunlight and water." Frontiers in microbiology 10 (2019): 866.
1. Howe, Christopher J., and Paolo Bombelli. "Electricity production by photosynthetic microorganisms." Joule 4.10 (2020): 2065-2069.

2. Bradley, Robert W., et al. "Biological photovoltaics: intra-and extra-cellular electron transport by cyanobacteria." Biochemical Society Transactions 40.6 (2012): 1302-1307.
3. McCormick, Alistair J., et al. "Biophotovoltaics: oxygenic photosynthetic organisms in the world of bioelectrochemical systems." Energy & Environmental Science 8.4 (2015): 1092-1109.
4. Aharoni, Amir, et al. "High-throughput screening of enzyme libraries: thiolactonases evolved by fluorescence-activated sorting of single cells in emulsion compartments." Chemistry & biology 12.12 (2005): 1281-1289.

169

Diversity of far-red cyanobacteria in extreme environments

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Some cyanobacteria exhibit a complex and extensive photoacclimation response, known as far-red light photoacclimation (FaRLiP), which extends the range of oxygenic photosynthesis towards 800 nm by using red-shifted chlorophylls *d* and *f*. This long-wavelength, low-energy light is enriched in shaded environments. It has been shown that FaRLiP cyanobacteria are widely distributed; however, it is largely unknown to what extent chlorophyll *f*-based photosynthesis contributes to global primary production. We propose that FaRLiP is a key acclimation process in extreme environments such as deserts and salt lakes where abiotic stresses can restrict photosynthetic life to shaded niches.
Here, we investigate the microbial community of Sabkha Oum Dba (Morocco) and Clifton Lake (Australia). We enriched various new FaRLiP cyanobacteria and characterized their photophysiology and phylogenetic placement. The reflection spectra of the cells showed a peak shift to longer wavelengths (708 nm), and the fluorescence spectra of FRL cells had an emission peak at 730-750 nm. Interestingly, many of these cyanobacteria belong to phylogenetically early-branching cyanobacteria. They are characterized by thin filaments of 0.9-1.1 µm in width. They have a special arrangement of thylakoid membranes, with newly formed far-red membranes in the center of the cell, and phycobilisome and Chl *a*-membranes in the septal regions. Chlorophylls *f* and *d* were found in all far-red light-adapted samples by HPLC.

170

Role of select amino acid residues in binding affinity of Cytochrome c6 to Photosystem I

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In cyanobacteria, electron transfer to Photosystem I (PSI) can be performed by Plastocyanin or Cytochrome c_6 (Cyt c_6). However, the mechanism of Cyt c_6 binding to PSI is not yet fully understood and due to its transient binding, neither crystal nor cryo-EM structures of the complex have been successfully resolved. Here, we replaced three amino acids in the Cyt c_6 of the cyanobacterium *Thermosynechococcus elongatus*, in order to increase its binding affinity to PSI. The mutated residues include Val24, Glu34 and Ala62 and combinations thereof. Increased binding affinity may allow for better structural characterization of the bound complex. The binding affinities of the Cyt c_6 variants to PSI were determined by comparing oxygen reduction rates using polarography with a Clark-type electrode. The polarographic measurements show that the effects of amino acid replacements are consistent with predictions of, and support the current model proposed by Kölsch et al. (2018).
Reference:
Kölsch A, Hejazi M, Stieger KR, Feifel SC, Kern JF, Muh F, et al. Insights into the binding behavior of native and non-native cytochromes to photosystem I from Thermosynechococcus elongatus. J Biol Chem. 2018;293(23):9090-100.

171

The domain of CikA responsible for redox signaling in Synechocystis sp. PCC 6803

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Some cyanobacteria are capable of phototaxis, combining the ability to sense light direction with control of the Type IV pilus (T4P) apparatus to move towards or away from a light source. Electron transport inhibitor studies suggest that localized light-induced oxidation of the plastoquinone (PQ) pool is essential for directional light perception in *Synechocystis*. In cyanobacteria, only one direct response regulator for sensing the redox status of the PQ pool was identified – the sensor histidine kinase CikA. *Synechococcus* CikA contains an unstructured N-terminal domain linked to a GAF domain lacking the conserved cysteine bilin-binding site, a sensor histidine kinase (HIK) domain and a *pseudo*-receiver (PsR) domain at the C-terminus lacking the conserved phospho-accepting aspartic acid residue. *Synechococcus* CikA is a sensor histidine kinase that directly binds quinones via a C-terminal domain and relays information on plastoquinone redox state to reset the phase of the circadian clock. These results suggested that *Synechococcus* CikA might sense light indirectly through the cellular redox state and then transmit this information to the circadian oscillator. However, it is not clear whether the photoreceptor function of *Synechocystis* CikA could influence redox sensing and phototaxis, or which domain of CikA is active in signal transduction for phototaxis, as *Synechocystis* CikA has the conserved chromophore-binding cysteine in GAF and the aspartic acid in the receiver domain (R) which are not found in *Synechococcus*. We used mutagenesis to probe the domains of CikA required for redox sensing and directional light perception, with a *cikA* null mutant, *cikA*-*egfp* and a mutant lacking the C-terminal domain of CikA. The total photosynthetic content per cell of the *cikA* null mutant is less than wild type, but the mutant lacking the C-terminal of CikA seems similar to wild type. The *cikA* null mutant loses directionality, but the mutant lacking the C-terminal domain of CikA is still capable of phototaxis. This suggests that CikA influences phototaxis indirectly through its effect on the photosynthetic apparatus. This effect on photosynthesis does not require the C-terminal receiver domain. We are continuing to investigate the roles of the CikA domains in light perception and signal
transduction.

172

FTIR of the carbonyl region in far-red PSI variants

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Some cyanobacteria adapt to low-energy light by a process called far-red light photoacclimation (FaRLiP). During this process, standard protein subunits of Photosystem I (PSI) are replaced by their far-red variants, and 7-8 of the existing ~100 chlorophylls (chl) a are exchanged by red-shifted chl f. It is currently under debate, where these chlorophylls are located and whether they are part of the electron transfer chain (ETC) of the PSI reaction center, or fulfill a role in light harvesting only. In this study, light-induced FTIR difference spectroscopy was used to investigate the carbonyl region of isolated PSI variants of different cyanobacteria. According to a previous study (Hastings et al., 2019, BBA), the C=O bonds of the P700 chlorophylls after oxidation and possibly intermediate chlorophylls of the ETC were resolved in the difference spectrum. An observed shift in the difference spectra of far-red versus white light PSI of the same cyanobacterial strain could possibly be assigned to a chl f substituting for chl a in this intermediary chl pair, supporting the proposition that chl f is part of the ETC.

173

Single-cell Raman Spectroscopy of Synechococcus sp. PCC 7002 and Synechocystis sp PCC 6803

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There are more than 750 carotenoids with unique structures and absorbance properties known to date. All photosynthetic organisms produce carotenoids. The properties of carotenoids allow for a broad range of functions to the producing organism, such as light harvesting pigments, thylakoid membrane structure and most importantly photo protection through dissipation of excess energy and scavenging of reactive oxygen species. These compounds provide a broad range of uses outside of the producing organism, including pharmaceuticals for eye health and anti-cancer, as well as food additives to name a few. The bioprospecting of novel carotenoids is of high importance due to the variety of benefits. Traditionally carotenoids are identified and measured using biochemical methods such as high-performance liquid chromatography (HPLC). However, this process involves a high degree of sample preparation with a risk for compound degradation. It also requires a substantial amount of cell material and is not suitable for high throughput screening. Resonance Raman spectroscopy can potentially compensate for these shortfalls as it is non-invasive and can be applied to individual cells. Coupled with microfluidics it could be used to sort environmental samples based on carotenoid spectra from single cells. This study aimed to evaluate Raman spectroscopy for this purpose. Firstly, we measured the Raman spectra of single cells of assess the possibility to distinguish between two cyanobacterial model strains, *Synechococcus sp*. PCC 7002 and *Synechocystis sp.* PCC 6803 and cultures grown under different light conditions. Secondly, we investigated to what degree Raman spectroscopy can differentiate between carotenoids in a complex sample by recording the spectra of pure carotenoids and the spectra of mixed carotenoids samples. The results obtained show a good potential of single-cell Raman spectroscopy for distinguishing strains and culture conditions but a limited potential for compound identification. We conclude that single-cell Raman spectroscopy offers a good opportunity to pick interesting cells from mixed samples but requires further analysis to identify the carotenoids underpinning the spectral differences.

174

Spectral tuning of Photosystem II in Synechocystis sp. PCC 6803 mutants

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PSII is a large multi-protein membrane complex at the centre of photosynthesis, and PsbA, also called D1, is one of its core proteins. D1 binds several key cofactors such as chlorophylls, quinones and the Mn_4CaO_5 cluster. Some cyanobacteria use alternative versions of D1 under specific light conditions. For example, D1 orthologues, within modified versions of PSII, are involved in expanding photosynthesis from visible light into the far-red region of the solar spectrum. This process also relies on red-shifted chlorophylls (chl), chl *d* and/or chl *f*. It has been suggested that chl *d* or chl *f* might be the primary electron acceptors in these PSII complexes, but the model requires further validation as does the process of water oxidation. Therefore, the far-red-D1 variants from chl *d*-containing *Acaryochloris marina* and chl *f*-based *Chroococcidiopsis thermalis* PCC 7203 were introduced into *Synechocystis* sp. PCC 6803, a cyanobacterium only containing chl *a*. They were meant to replace the standard white light (WL)-D1 variants. In addition, we identified two amino acids that might play a role in coordinating far-red chlorophylls and introduced these point mutations into the WL-D1 of *Synechocystis* sp. PCC 6803. Our results indicate that the far-red D1 variants are expressed but only restore photosynthetic activity in case of the chl *d*-variant from *A. marina*. The chl *f*-D1 containing mutant was unable to grow photoautotrophically. However, none of the point mutations resulted in a loss of oxygenic photosynthesis.

175

Robust, Coherent and Synchronized Circadian Clock-Controlled Oscillations along Multicellular Filaments of Anabaena cyanobacteria

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In contrast with the large number of studies in unicellular phototrophic organisms, little is known about *arrays* of coupled circadian clocks in multicellular cyanobacteria, where collective effects can be studied in a well-defined and controlled configuration. The existence of circadian clocks in multicellular cyanobacteria raises a number of fundamental questions: Are clocks in individual cells coupled and synchronized through cell-cell communication, or are clocks entrained only by external cues? What is the spatial extent of synchronization? What is the role of stochastic cell-cell variations in copy numbers of molecules comprising the KaiABC core clock, demographic noise, in setting the temporal pattern and its robustness? To tackle quantitatively these and other questions, we studied the dynamics of a circadian clock-controlled gene at the individual cell level in *Anabaena* sp. PCC 7120, a multicellular filamentous cyanobacterium. We found significant synchronization and spatial coherence along filaments, clock coupling due to cell-cell communication, and gating of the cell division, in the presence of significant noise in expression. Furthermore, we observed low-amplitude circadian oscillatory transcription of *kai* genes encoding the post-transcriptional core oscillatory circuit and high-amplitude oscillations of *rpaA*, coding for the master regulator transducing the core clock output. Transcriptional oscillations of *rpaA* suggest an additional level of regulation. In agreement with our theoretical predictions, our findings show that far from being detrimental, demographic noise may seed oscillations that can be synchronized by clock coupling. This provides a robust description of circadian oscillations in a multicellular organism such as *Anabaena*.

178

Psip1 is a high-affinity alkaline phosphatase in picocyanobacterial occupying P-deplete oligotrophic oceans.

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'Omic technologies have impinged on many aspects of marine microbial ecology mainly through next generation sequencing and meta-transcriptomic studies. Such studies attempt to shed light on the functional potential and activity of microorganisms, and in so doing understand more clearly the role microbes play in planetary biogeochemical cycles. Arguably, such studies have just revealed a plethora of genes of no known function, so-called 'known' unknowns. In the marine picocyanobacteria marine *Prochlorococcus* and *Synechococcus* ~85% of genes in the total pan-genome for both species are vaguely annotated or encode hypothetical proteins despite the fact that we might consider these organisms to be extremely well studied phototrophs. Here, we sought to initiate functional characterisation of a few of these genes of unknown function targeting genes that are expressed in the real-world environment and using a combination of bioinformatics tools and molecular microbiology approaches.
Bioinformatics analysis of a phosphate starvation induced protein (Psip1) with no previously known function revealed high similarity in protein structure to the known alkaline phosphatase PhoX. However, there is little or no sequence identity between the two proteins. Environmental data from *Tara Oceans* and an Atlantic Meridional Transect showed that Psip1 is highly expressed in marine picocyanobacteria in low phosphate regions like the Mediterranean Sea and North Atlantic gyre. Psip1 from *Prochlorococcus* MED4 was over-expressed in *E. coli*, and showed strong phosphatase activity when tested with the artificial substrate *p*-nitrophenyl phosphate (*p*NPP). We demonstrate that both calcium and iron are required as metal co-factors essential for its activity, whilst enzyme activity is optimal at a pH between 9.5-10. Finally, enzyme kinetic data revealed that Psip1 has a much higher affinity for the *p*NPP substrate than its PhoX counterpart.
We hypothesize that expression of Psip1 represents one of the last ditch responses to phosphate starvation consistent with it being regulated by PtrA which is downstream of the PhoBR system, representing a key phosphatase for *Prochlorococcus* and *Synechococcus* to obtain inorganic phosphate from organic phosphorus (P) sources in oligotrophic areas low in P.

179

Assessing synthetic Rsp. rubrum with electron cryo-tomography and serial cryo-sectioning

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Rhodospirillum (Rsp.) rubrum is a purple phototrophic bacterium that lacks the typical bacteriochlorophyll a (BChl a) and the common light-harvesting antenna protein (LH2). LH2 can only assemble in the presence of BChl a and is known to cause tight curvature of the membranes that it resides in. *Rsp rubrum* was mutated to produce these foreign antenna complexes and probed with electron cryo-microscopy techniques to assess the structural impact of their addition within cells.
Electron cryo-microscopy (Cryo-EM) of frozen-hydrated samples can be used to probe cellular structures in their near-native state. Relatively recent developments in biological cryo-TEM have allowed for near-atomic resolution of structures within cells by collecting tomographic “tilt-series” of thinned frozen samples. The samples can be thinned by focused ion beam scanning electron microscopes (FIB-SEM) using the ion beam to mill away material until the desired thickness is achieved. These same instruments can also be used for high-quality imaging themselves, in a method that complements the high-resolution information gained from traditional TEM cryo-tomography with lower-resolution, high-contrast images of cells. Serial cryo-sectioning involves exposing a surface containing biological material with the ion beam, imaging that surface with a scanning electron beam, then repeatedly milling a layer away and imaging, gradually building up a 3D volume from a series of images.
Together these two techniques have been used to compare membrane architecture of *R. rubrum* with and without the genes for LH2, as well as for mutants with genetically modified biosynthetic pathways for various photosynthetic pigments. The images acquired could also be further datamined for structural information of the photosynthetic apparatus as well as other cellular features under near-native conditions.

180

In Operando Fluorescence Microscopy and Electrochemistry of Synechocystis PCC6803 Biofilms

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Exoelectrogenesis is the export of energetic electron carriers out of a cell, and is observed in many phototrophic microorganisms including the model cyanobacterium *Synechocystis* PCC6803. The biochemical mechanism of exoelectrogenesis is poorly understood, despite being the underlying phenomenon employed in biophotovoltaic devices[1], a burgeoning field of sustainable biotechnology. Typical approaches to studying this phenomenon rely predominantly on electrochemical methods[2], which serve well to characterise the bulk current output of a biofilm of cells, but lack dynamic spatial resolution and are limited to probing solely the electrochemical output of the biofilm. Here we employ *in operando* fluorescence microscopy and electrochemistry to study the broader biochemistry of the biofilm and variation across the biofilm during exoelectrogenesis.
The *in operando* system is comprised of an ITO-coated glass working electrode, a flat PT counter electrode and a Ag wire pseudo-reference electrode, and is designed to be used with any inverted microscope. Using this tool, we interrogate how *Synechocystis* cells behave in real time in response to applied potential and light, beyond just photocurrent output. We address questions of how cells reorganise themselves in a biofilm under applied potential, and how this changes over the course of a photoelectrochemical experiment. Through the use of fluorescence probes, we further interrogate how the intracellular and extracellular environments change in response to light, to aid in the understanding of the mechanism of exoelectrogenesis. This includes the use of Acridine Orange to track changes in pH, 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) as a probe for reactive oxygen species (ROS) and Thioflavin T (ThT) to study membrane potential.

[1] Wey, L. T. *et al* (2019) The Development of Biophotovoltaic Systems for Power Generation and Biological Analysis. ChemElectroChem, 6 (21), 5375–5386. https://doi.org/10.1002/celc.201900997
[2] Zhang, J. Z. *et al* (2018) E. Photoelectrochemistry of Photosystem II in Vitro vs in Vivo. J. Am. Chem. Soc. 140 (1), 6–9. https://doi.org/10.1021/jacs.7b08563

181

System-wide study of photosynthetic activity of cyanobacteria

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We investigate *in silico* the dynamics of photosynthsis in cyanobacteria, treated either as an individual cell or a community member. We are applying numerous computational methods to create mechanistic, deterministic models of 1) photosynthesis, 2) secondary metabolism and terpene production, and 3) interactions with other organisms within an inter-kingdom communities, such as lichen. With our models we aim to answer multiple questions, e.g., how are unicellular organisms, such as cyanobacteria **balancing energy** distribution between the pigment synthesis for energy production (chlorophylls) and synthesis of secondary metabolites? How can we increase a production of a particular metabolite by modulating photosynthetic electron transport chain? What is the theoretical maximum of such increase? Is the photosynthetic activity of a cyanobacteria living in a symbiosis in plant affected by the interaction with other organisms and if yes, can cyanobacteria change their production strategy? Does evolutionary game theory provide a tool to investigate the dynamics of community formation?

In our work we are using a modular approach in the creation of computational models [1], so eventually all models will be assembled together to provide a **system-wide perspective** on photosynthesis in cyanobacteria: how it is performed, does it play a role in inter-kingdom interactions and how can we modulate it to increase synthetic production of higly valuable compounds.

Note: It is an ongoing work of a newly established computational lab. We work in a close collaboration with several European wet-labs and currently models are calibrated for the data collected in *Synechocystis sp.* PCC 6803.

[1] van Aalst, M., Ebenhöh, O. and **Matuszyńska, A.** (2021) Constructing and analysing dynamic models with modelbase v1.2.3: a software update. *BMC Bioinformatics,* 22, 203. doi: 10.1186/s12859-021-04122-7

182

Life of aerobic anoxygenic phototrophs near the Arctic Circle

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Biogenesis of bacterial photosynthetic machinery involves coordinated gene expression and membrane remodelling regulated by levels of oxygen and light. Photosynthetic microbial communities in polar regions experience half a year of constant daylight concomitant with low temperature facilitating high oxygen tension. Typical phototrophic bacteria including the aerobic anoxygenic phototrophs would become devoid of any bacteriochlorophyll or photosynthetic reaction centres in just a few tens of hours under such conditions. A pure culture of a bacterium formerly identified as *Sediminicoccus rosea* [1] was isolated from a littoral of a creek in north-western Iceland near Raufarhöfn. Transmission electron microscopy (TEM) and tomography imaging showed oval shaped cells featuring up to 180 chromatophores. The negative stain TEM images of purified RC-LH1 showed circular structure of the complex with a diameter of 15 nm. Photosynthesis gene cluster at the opposite site of chromosome replication origin has the two regulators (ppaA and ppsR) sensitive to light intensity and oxygen concentration at the end of the operon, preceded by half of the bacteriochlorophyll synthesis genes (bchFNBHLM) located on complementary strand. The single 4.9 MB chromosome also contains two intact and two remnant phage genomes and a complete set of genes coding for enzymes required for utilization of phosphonate and aerobic methanogenesis. Cellular adaptation to transition between light and dark (and *vice versa*) was followed by measurements of photosynthetic activity and transcriptomics.

[1] Qu J-H *et al* (2013) *Sediminicoccus rosea* gen. nov. sp. Nov., isolated from the sediment of a eutrophic lake. J Gen Appl Microbiol 59(6):463-8. doi: 10.2323/jgam.59.463.

183

Decoding the stoichiometric composition and cargo organisation of carboxysomes

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Carboxysomes are proteinaceous organelles for CO_2 fixation in cyanobacteria and chemoautotrophs [1]. The carboxysome comprises hundreds of protein homologs, which self-assemble to form an polyhedron shell that encapsulates the key CO_2-fixing enzyme, Rubisco. There are two distinct linkages of carboxysomes, α- and β-carboxysomes, differing in the forms of Rubisco and protein composition. Uncovering the precise stoichiometric compositions of carboxysomes and Rubisco arrangement within the carboxysome is fundamental for understanding carboxysome self-assembly and physiology.

Using single-molecule fluorescence microscopy, we characterised the protein stoichiometry of β-carboxysomes in a β-cyanobacterium *Synechococcus elongatus* PCC 7942 using single-molecule fluorescence microscopy and revealed their stoichiometric changes in response to the levels of CO_2 and light intensity [2]. With on a QconCAT-based quantitative mass spectrometry, we also determined the stoichiometric composition of native a-carboxysomes from a chemoautotroph *Halothiobacillus neapolitanus*. We further performed an in-depth comparison of the protein stoichiometry of native a-carboxysomes and their recombinant counterparts heterologously generated in *E. coli* [3]. Furthermore, we used cryo-electron tomography techniques to investigate intact carboxysomes in situ and determined the architecture and interior Rubisco organization of two types of α-carboxysomes from *H. neapolitanus* and an α-cyanobacterium *Cyanobium sp.* PCC 7001 [4]. Our results revealed markedly different 3D organizations of Rubisco inside the two α-carboxysomes, and more importantly, uncovered that the authentic intrinsically disordered linker protein CsoS2 interacts with Rubiscos in situ but functions distinctively in the two α-

carboxysomes. These findings were made possible by achieving unprecedented, near-atomic resolution for the native Rubisco complexes and higher-order assemblies in the context of carboxysomes.

Our findings provide mechanistic insight into the principles that mediate carboxysome assembly as well as the structural variability and remodeling of carboxysomes. Advanced knowledge about carboxysome assembly may aid in rational design and reprogramming of carboxysomes in new contexts for biotechnological applications.

References

1. Liu, L.N. (2021) Advances in the bacterial organelles for CO_2 fixation. Trends in Microbiology.
2. Sun, Y. et al. (2019) Single-Organelle Quantification Reveals Stoichiometric and Structural Variability of Carboxysomes Dependent on the Environment. Plant Cell 31 (7), 1648-1664.
3. Sun, Y. et al. (2022) Decoding the Absolute Stoichiometric Composition and Structural Plasticity of alpha-Carboxysomes. mBio, e0362921.
4. Ni, T. et al. (2022) Tales of Two α-Carboxysomes: the Structure and Assembly of Cargo Rubisco. bioRxiv, 2022.03.15.484529.

184

Cryo-EM analysis of the macromolecular structures of photosynthetic RC-LH1 complexes in species of Rhodobacter

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Text: The reaction center (RC) and light-harvesting complex 1 (LH1) form a RC–LH1 core protein supercomplex that is vital for the primary reactions of photosynthesis in purple photosynthetic bacteria. The native RC–LH1 complexes exhibit diverse structural forms in different species of purple bacteria, ranging from single RCs surrounded by closed rings of LH1 antennae, through those surrounded by opened rings, to two RCs connected by a single S-shaped LH1 ring [1]. They may also incorporate additional polypeptide chains of varying sizes. Our research focuses on the architectures, assembly and electron transport pathways of RC–LH1 complexes in *Rhodobacter* species using cryo-electron microscopy, genetic mutagenesis and computational simulations, with the intent of advancing our understanding of the molecular details of RC–LH1 biogenesis and functions, as well as the roles of individual components in ensuring the structural and functional integrity of the RC–LH1 cores. Our findings revealed that the native RC–LH1 core complex of *Rhodobacter veldkampii* appears as a monomer containing a ring of 15 LH1 heterodimers with a 30 Å gap created by an additional polypeptide called PufX. PufX acts as a molecular “cross brace” to reinforce the association of LH1 αβ-subunits and the RC. The unusually large PufX-mediated opening in the LH1 ring and the defined arrangement of proteins and cofactors provide the molecular basis for the efficient excitation energy transfer and quinone transport across the LH1 barrier [2]. Additionally, we characterised the monomeric and dimeric RC–LH1 core complexes of *Rhodobacter sphaeroides* [3]. The RC–LH1 dimer has an S-shaped LH1 ring with a total of 28 LH1 heterodimers interconnecting two RCs. Two PufX polypeptides are positioned at the interface of the dimerization mediating the interaction between the two individual monomers. We also identified another additional transmembrane peptide, designated PufY, which is located between the RC and LH1 subunits near the LH1 opening. PufY prevents LH1 subunits from completely encircling the RC, creating a channel for quinone/quinol exchange. Our results provide insights into the structural variability of bacterial photosynthetic complexes, which enable efficient light harvesting, excitation energy transfer, and electron transport that underpin photosynthesis in phototrophic bacteria.

References:

1. Gardiner, A.T. *et al.* (2020) A comparative look at structural variation among RC-LH1 'Core' complexes present in anoxygenic phototrophic bacteria. *Photosynth Res* 145, 83-96. 10.1007/s11120-020-00758-3
2. Bracun, L. *et al.* (2021) Cryo-EM structure of the photosynthetic RC-LH1-PufX supercomplex at 2.8-Å resolution. *Sci Adv* 7, eabf8864. 10.1126/sciadv.abf8864
3. Cao, P. *et al.* (2022) Structural basis for the assembly and quinone transport mechanisms of the dimeric photosynthetic RC–LH1 supercomplex. *Nature Communications* 13, 1977. 10.1038/s41467-022-29563-3

185

Uncovering the structural basis of α-carboxysome shell assembly

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Carboxysomes are the central CO_2-fixing machinery in all known cyanobacteria and many chemoautotrophic proteobacteria [1]. They are megadalton-sized complexes self-assembled from thousands of protein subunits which encapsulate ribulose-1,5-*bis*phosphate carboxylase/oxygenase and carbonic anhydrase within a semi-permeable polyhedral shell [2]. Such characteristics provide these proteinaceous organelles with huge biotechnological potential, as demonstrated recently by the repurposing of carboxysomes into hydrogen-producing nanoreactors by exploiting the identified encapsulation peptide which sufficiently targets foreign cargos to the shell [3].
The production of synthetic shells provides a means for in-depth evaluation of the assembly mechanisms and pairwise interactions that drive shell formation [4,5]. It also holds promise for the generation of novel synthetic nanocages in non-native environments. Using a heterologous *Escherichia coli*-based system, we generated multiple α-carboxysome mini-shells derived from the model proteobacterium *Halothiobacillus neapolitanus,* with diameters ranging from 20 to 40 nm. We observed structural formation of the mini-shells into three core polyhedral symmetries at different ratios. Intriguingly, cryo-electron microscopy of these structures shows that the C-terminal domain of CsoS2, an intrinsically disordered scaffolding protein, forms multivalent contacts with the shell hexamer (CsoS1A) and pentamer (CsoS4A) proteins, threading through the assembly interface to stabilise the shell structure. We further demonstrated that these interactions are key to shell formation.
In summary, our work provides mechanistic insights into carboxysome shell assembly and lays the groundwork for future bioengineering work.

References: [1] Liu LN (2021) Advances in the bacterial organelles for CO_2 fixation. Trends Microbiol https://doi.org/10.1016/j.tim.2021.10.004; [2] Kerfeld CA *et al* (2018) Bacterial microcompartments. Nat Rev Microbiol 16:277-290; [3] Li T *et al* (2020) Reprogramming bacterial protein organelles as a nanoreactor for hydrogen production. Nat Commun 11:5448; [4] Tan YQ *et al* (2021) Structure of a Minimal α-Carboxysome-Derived Shell and Its Utility in Enzyme Stabilization. Biomacromolecules 22:4095-4109; [5] Sutter M *et al* (2017) Assembly principles and structure of a 6.5-MDa bacterial microcompartment shell. Science 356:1293-1297.

186

Exploration and Alteration of Carbon Metabolism in Heliomicrobium modesticaldum

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The Heliobacteria make up the only phototrophic members of the phylum Firmicutes. *Heliomicrobium modesticaldum*, a moderate thermophile of this group, has largely been used in studies regarding its photosynthetic reaction center and other electron transfer pathway related enzymes. However, *H. modesticaldum* has also displayed interesting aspects in terms of its general metabolism; it is an obligate heterotroph, despite containing an almost full set of genes for the reverse Tricarboxylic Acid cycle (rTCA) cycle, a cycle used in several species to grow autotrophically. However, the short set of oTCA cycle enzymes present in *H. modesticaldum* appears essential, with most of the flux moving through this partial cycle to reach α-KG. In this study, we deleted the citrate synthase gene of *H. modesticaldum*, eliminating access to the oTCA cycle from acetyl-CoA. This mutant displays a necessity for exogenous electrons (either from formate or ascorbate) when grown with acetate as its carbon source, confirming our suspicions that the oTCA cycle largely assists in harvesting electrons from the more electron deficient source of acetate. In addition, the mutant required supplemental glutamine when grown in minimal media, confirming that flux to α-KG is primarily through the oTCA portion.
Further, using a constructed xylose inducible promoter tested through this study, we introduced a new citrate metabolism enzyme, citrate lyase, from *Clostridium sporosphaeroides*. This citrate lyase will be able to fill the final, missing enzyme role in the rTCA cycle, granting *H. modesticaldum* with all of the machinery needed to complete the rTCA cycle. This enzyme was active in *H. modesticaldum*, although also at a detriment to growth. Overall, these studies have displayed a great dependence on flux through certain aspects of carbon metabolism, and a necessity to change these aspects if our goal of constructing an autotroph is feasible.

187

Encapsulating viable cyanobacteria in biocoatings

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Biocoatings embed defined bacterial species within colloidal polymer films, a type of synthetic latex paint. These films are formed after being deposited from a waterborne suspension and dried, a process called film formation. Biocoatings enable a myriad of interesting applications ranging from wastewater treatment processes to sustainable energy production [1]. This encapsulation of the bacteria can facilitate their transport to reactors and can help establish mature biofilm communities faster. However, maintaining viability within these coatings is a major challenge. To ensure the flow of gases and nutrients, we increased the porosity of the biocoatings by the addition of halloysite nanotubes, which are biocompatible clay nanotubes [2].
To develop biocoatings to capture carbon, we used three strains of cyanobacteria: a freshwater strain *Synechocystis* sp. PCC 6803, a marine strain *Synechococcus* sp. PCC 7002 and extremotolerant *Chroococcidiopsis cubana* PCC 7433, which were originally isolated from a dried pool in Cuba [3].
The viability of the biocoatings was assessed using multiple methods, including Confocal Laser Scanning Microscopy (CLSM), flow cytometry and resazurin-reduction and luminescence-based assays. We assessed the physiological function of the cyanobacteria by measuring the oxygen evolution with oxygen sensors.
From the two colloidal polymer formulations used, one was found to be toxic to *Synechococcus* sp. PCC 7002. In addition, poor survival was observed after the desiccation process by our assays.
Synechocystis sp. PCC 6803 was found, via CLSM and our assays, to be viable within the biocoatings, both with and without halloysite. However, no oxygen evolution could be observed from the biocoatings which was attributed to the desiccation process, as neither did dried unencapsulated bacteria. Further materials and process developments will be used to increase the rates of carbon uptake.

References:

[1] Cortez S *et al* (2017) Biocoatings: A new challenge for environmental biotechnology. Biochemical Engineering Journal 121:25–37

[2] Chen Y *et al* (2020) Introducing Porosity in Colloidal Biocoatings to Increase Bacterial Viability. Biomacromolecules 21, 11, 4545–4558

[3] De Wever A *et al* (2019) Evidence of high Ca uptake by cyanobacteria forming intracellular $CaCO_3$ and impact on their growth. Geobiology 17:676–690

188

Structural and spectroscopic investigations of energy and electron transfer in purple bacteria RC-LH1 complexes.

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The utilization of light energy in purple bacteria is a rapid and efficient process and starts when light-harvesting antenna complexes absorb light and transfer the captured energy to the reaction centre (RC), initiating a charge separation. The light harvesting 1 (LH1) encircle RC subunit forming the RC-LH1 core complex.
In *Rhodobacter sphaeroides* the RC-LH1 appear in monomeric and dimeric form. Our recent research has showed the atomic structures of the monomeric and dimeric RC-LH1 core complexes using cryo-electron microscopy and genetic approaches [1]. Our results reveal that two PufX polypeptides are positioned in the center of the S-shaped RC–LH1 dimer, interlocking association between the components and mediating RC–LH1 dimerization. Moreover, we identify another transmembrane peptide, designated PufY, which is located between the RC and LH1 subunits near the LH1 opening. PufY binds a quinone molecule and prevents LH1 subunits from completely encircling the RC, creating a channel for quinone/quinol exchange.
Based on the structural findings, we further carry out measurements on the energy transfer rates and kinetics of electron transfer inside the RC-LH1 wild-type and mutant structures using transient absorption spectroscopy and cytochrome c oxidation assays, to further investigate the structural variation and the roles of PufX and PufY in determining the energy and electron transfer of RC-LH1 complexes.
Advanced knowledge about the assembly principles of RC-LH1 dimers and the structural and functional modulation of photosynthetic RC-LH1 complexes will provide essential information required for design and engineering of artificial photosynthetic systems for sustainable bioenergy production.

References

1. Cao, P. *et al.* (2022) Structural basis for the assembly and quinone transport mechanisms of the dimeric photosynthetic RC–LH1 supercomplex. *Nature Communications* 13, 1977. 10.1038/s41467-022-29563-3

190

Enhancing the spectral range of Light-Harvesting pigment-protein complexes using synthetic chromophores incorporated into lipid membranes

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Light-harvesting (LH) protein complexes have the role in nature of absorbing solar energy with high efficiency and transferring it on to reaction centre proteins where the primary photochemical processes of photosynthesis take place. These LH complexes contain a suite of natural pigments that each absorb light at specific wavelengths; however, the natural combinations of pigment within any one protein do not cover the full range of visible light. In a proof-of-principle study,[1] we enhanced the effective absorption range of the plant protein Light-Harvesting Complex II (LHCII) by developing a biohybrid system where LHCII was assembled into lipid-membrane vesicles alongside synthetic lipid-linked Texas Red (TR) chromophores. Recently, we organised LHCII and TR into lipid nanodiscs to provide an idealised system where the energy transfer between TR and single LHCIIs could be probed using ultrafast spectroscopy.[2] These measurements, alongside structural modelling of the system and theoretical calculations, allowed us to quantify the photophysical and structural interactions that can occur between lipid-tagged chromophores and LH protein complexes. We also present work that demonstrates the modularity of the approach with other LH proteins and synthetic pigments. We show that a range of hydrophilic lipid-linked pigments and hydrophobic free pigments can be employed to enhance the absorption of both plant LH proteins and bacterial LH proteins, spanning the visible to the near-infrared spectral ranges. Finally, we demonstrate that lipid bilayer localised dyes can also be used to enhance the effective absorption range of bio/hybrid membranes comprised of plant thylakoids fused with supported lipid bilayers. The system we have developed not only acts as a useful tool to investigate the photophysical interactions between synthetic and natural chromophores but also demonstrates that augmenting natural photosynthetic components with complementary synthetic dyes could significantly enhanced the effectiveness of bio-hybrid systems.

[1] Hancock, A. M *et al* (2019). "Proteoliposomes as energy transferring nanomaterials: enhancing the spectral range of light-harvesting proteins using lipid-linked chromophores." Nanoscale 11(35): 16284-16292.
[2] Hancock, A. M., M. Son, M. *et al* (2021). "Ultrafast energy transfer between lipid-linked chromophores and plant light-harvesting complex II." Physical Chemistry Chemical Physics 23(35): 19511-19524.

193

Patterning of the autotrophic, mixotrophic and heterotrophic proteomes of oxygen evolving cyanobacterium Synechocystis sp.PCC 6803

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Proteomes of an oxygenic photosynthetic cyanobacterium *Synechocystis* sp. PCC 6803 were analyzed under photoautotrophic (low and high CO_2, assigned as ATLC and ATHC), photomixotrophic (MT) and light activated heterotrophic (LAH) conditions. Allocation of proteome mass fraction to seven sub-proteomes as well as differential expression of individual proteins were analyzed, paying particular attention to the photosynthesis and carbon metabolism centered sub-proteomes affected by the quality and quantity of the carbon source and the light regime upon growth. A distinct common feature of the ATHC, MT and LAH cultures was a low abundance of the inducible carbon concentrating mechanisms and photorespiration related enzymes, independently of the inorganic or organic carbon source. On the other hand, these cells accumulated respiratory NAD(P)H dehydrogenase I complex (NDH-1_1) in the thylakoid membrane (TM). Additionally, in glucose supplemented cultures a distinct NDH-2 protein, NdbA, accumulated in the TM whilst the plasma membrane localized NdbC and terminal oxidase decreased in abundance in comparison to both AT conditions. Photosynthetic complexes were uniquely depleted under the LAH condition while accumulated under ATHC. MT proteome displayed several heterotrophic features typical of the LAH proteome, particularly including the high abundance of ribosome as well as amino acid and protein biosynthesis machinery-related components. It is also noteworthy that the two equally light-exposed ATHC and MT cultures allocated similar mass fractions of the total proteome to the seven distinct sub-proteomes. Unique, trophic condition-specific expression patterns were likewise observed among individual proteins, including the accumulation of phosphate transporters and polyphosphate polymers storing energy surplus in the highly energetic bonds under MT condition and accumulation under LAH of enzyme catalyzing cyanophycin biosynthesis. It is concluded that the rigor of cell growth in MT condition results to a great extent from combining of photosynthetic activity with high intracellular inorganic carbon conditions created upon glucose breakdown and release of CO_2, beside the direct utilization of glucose-derived carbon skeletons for growth. This combination provides the MT cultures with excellent conditions for growth that often exceeds that of the mere ATHC.

195

Single-cell coordination of photosynthesis with N2 fixation in Trichodesmium: New insights from fluorescence lifetime analysis

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All diazotrophic cyanobacteria face the challenge of protecting the N_2-fixing enzyme nitrogenase from photosynthetically evolved O_2, which is usually achieved by separation of the two incompatible processes in time (day vs night) or in space (heterocysts). The globally important filamentous N_2 fixer *Trichodesmium* is unique in that it is able to fix N_2 during day-time although it does not have heterocysts, apparently through subtle coordination of photosynthesis and N_2 fixation at small scales of time and space. Here, we analyzed the lifetime of chlorophyll and phycobiliprotein fluorescence in two strains of N_2-fixing and NO_3-using *Trichodesmium* at bulk level and at single-cell resolution. Combination of Fluorescence Lifetime Imaging Microscopy (FLIM) with Lugol's stain allowed us to directly relate fluorescence lifetime to staining patterns of a commonly used marker for N_2-fixing cells. We observed distinct stretches of cells within filaments displaying either longer or shorter than average lifetime, which occurred at different frequency depending on the nitrogen treatment and the strain. Morphological patterns, responses to high light as well as comparison to Lugol staining patterns indicate that elevated fluorescence lifetime can reflect either a N_2-fixing state or a more general stress response. We discuss these findings in the context of the expression and localization patterns of key proteins in photosynthesis and N_2 fixation based on mRNA FISH and immunolocalization. The study highlights the intricate interplay of photosynthetic electron flow with N_2 fixation at single-cell level in *Trichodesmium* and calls for a re-evaluation of the current understanding of nitrogenase protection by regulation of photosynthesis.

196

Microbial composition recovered from metagenomic analyses of oxygenic and anoxygenic hot spring microbial mats

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Oxygenic microbial mats developed in a hot spring environment are assemblages of microbial populations containing oxygenic cyanobacteria, while the latter is absent in anoxygenic microbial mats. In our study, we report the metagenomic analyses of the microbial composition and structure of these two different mats collected from Nakabusa hot springs (NK), Japan (oxygenic mats) and New Pit hot springs (NP), USA (anoxygenic mats). Analyses of the 16S rRNA amplicon have shown the abundance of cyanobacterial *Thermosynechococcus* and aerobic bacteria in NK mats, but these are not found in NP mats. While members of *Chloroflexota* dominated both mats, these are more abundant in the NP mats together with sulfate-reducing groups.
Metagenome-assembled genomes (MAGs) were recovered from these two phototrophic mats. In NK, 20 MAGs were retrieved from the metagenome assembly. Most recovered MAGs were of high quality >90% sequence completeness with 0-6% contamination. Ten clusters represent the most dominating members of this mat; one *Thermosynechococcus* cluster and two *Chloroflexota* clusters belonging to *Ca.* Roseilinea and *Anaerolinea*, seven *Bacteroidota* clusters, including *Ignavibacteria* and *Chlorobia*, two clusters for sulfate-reducing bacteria comprising *Thermodesulfovibrio* and *Caldimicrobium*. In NP, 26 clusters were retrieved from the metagenome. Sequences derived from *Chloroflexota* sequences dominate the NP spring metagenome, with one *Chloroflexus* cluster, one *Roseiflexus* cluster and eight *Anaerolinea* clusters comprising approximately 4%, 0.24% and 22% of the metagenomic sequences, totaling 26.24%. Also, the other five clusters representing the dominant sulfate-reducing bacteria belonging to *Deltaproteobacteria* were recovered.
The metagenomic analyses of two microbial mats clearly indicate the different community composition and structure of oxygenic and anoxygenic hot spring mats. This work is part of the more extensive efforts to understand how the taxa inhabiting the mats relate to the metatranscriptomic, microsensor, and metabolomic processes occurring in the microbial mat system.

199

The transcriptome of Nostoc punctiforme in endosymbiosis with a hornwort reveals its symbiotic growth state

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What does the transcriptome of symbiotic *Nostoc punctiforme* in association with

When *Nostoc* spp. are in endosymbiotic association with plants, their morphology and physiology differ from the free-living growth state. Heterocyst frequency and nitrogenase activity are elevated 3- to 8-fold, respectively, but growth, photosynthetic CO_2 fixation, and ammonium assimilation are depressed to about 10 to 15% of free-living. We have applied RNA-Seq to define the transcriptomes of *A*. *punctatus* and *N*. *punctiforme* as an approach to describe the interactions between symbiotic partners and their symbiotic growth states. In these experiments, symbiotic colonies of *N*. *punctatus* could routinely be observed by 7 days of coculture; samples were therefore processed at days 7, 10, 14, and 28 of coculture, plus free-living steady state dinitrogen grown *N*. *punctiforme*.

A total of 7,568 *N*. *punctiforme* transcripts were recorded for each growth state. Relative to the free-living state, symbiotic *N*. *punctiforme* displayed 1,338 differentially expressed genes at a $P \leq 0.05$. These DEGs were organized into temporal clusters and at days 7-14 about 60% genes were initially expressed at a lower level, while 34% were expressed at a higher level. Not all the results were predicted by the morphological and physiological symbiotic characteristics. Transcripts identified as DEGs included only one out of sixteen core nitrogenase genes (*glb*N = globin, up and then downregulated) and one out of forty-four major heterocyst genes (*het*Z, downregulated). Glutamine synthetase, glutamate synthase and ammonium transporters were not differentially expressed. Thirty-two photosynthetic reaction center genes were identified and eighteen were differentially expressed: PSI accounted for ten downregulated DEGs and PSII for eight (five up- and then downregulated and three immediately downregulated). Both *psa*A and *psb*A were immediately downregulated. Ribulose bisphosphate carboxylase/oxygenase subunits were highly transcribed but not differentially expressed. Unexpected genes upregulated in the symbiotic state included thirty-one transposases (422 in the genome); fourteen of the transcripts encode pseudogenes. Five genes encoding taurine family dioxygenases were all immediately upregulated from low to high expression. A polyketide biosynthetic cluster was also immediately upregulated in the symbiotic state. This cluster (Pks4) is distinct from cluster Pks2, identified as an autogenic regulator of hormogonium differentiation, which was marginally expressed in both states.

We hypothesize that the differential regulation of physiological and biochemical activity in the symbiotic state is largely by post-translational mechanisms, except photosynthetic electron transport, organic sulfur catabolism and synthesis of a polyketide may be transcriptionally regulated. The role of the symbiotic specific polyketide is unknown. The provision of organic sulfur has been reported in epiphytic associations and could be a characteristic of cyanobacterial symbioses. The expression of seventeen presumably active transposases implies that the symbiotic state of *N*. *punctiforme* in *A*. *punctatus* could be genetically unstable.

201

Rapid, massive remodelling of Chroococcidiopsis thermalis photosystems upon transition from near-infrared growth to white light

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A diverse group of cyanobacteria harbour a cluster of genes which allow them to adapt to growth in near-infrared light and continue to perform oxygenic photosynthesis outside of the visible spectrum. Expression of this far-red gene cluster during far-red light growth allows alternative isoforms of photosystem and phycobilisome proteins, as well as the chlorophyll *f* synthase, to be expressed and used to maintain photosynthesis in this extreme environment, requiring a complete remodelling of photosynthetic systems which takes many weeks. However, upon reintroduction of far-red light acclimated *Chroococcidiopsis thermalis* to full spectrum (white) light, the reversal of far-red light photoacclimation occurs within a matter of days in a (white) light dependent manner. We have characterized this far-red light to white light transition in *C. thermalis* to understand how a photosynthetic organism simultaneously, specifically, and completely breaks down pigment protein complexes, the far-red photosystem variants, while synthesizing new photosystems within a matter of days. This process must safely shuttle all of the chlorophylls in the cell during the breakdown and synthesis processes to avoid excitation of free chlorophylls and reactive oxygen species generation, all while maintaining photoautotrophic growth. The spectral differences between the far-red light and white light photosystems provide an exploitable marker to use low temperature fluorescence emission, in combination with native protein gels, to dissect the time-course of far-red photosystem breakdown and white light photosystem synthesis. This has been performed in combination with transcriptomic analysis to understand the expression profile of fully acclimated far-red light *C. thermalis*, as well as the expression changes during the transition back to white light photosynthesis. The spectral isolation of the four photosystems, far-red light photosystems I and II and white light photosystems I and II, combined with the simultaneous breakdown of far-red light photosystems and synthesis of white light photosystems, makes the far-red light to white light transition an excellent model to dissect the biosynthesis and turnover of photosystems, especially photosystem I, as well as the chlorophyll trafficking systems required to safely orchestrate the processes.

202

Nitrogenase inhibition limited oxygenation of Earth's Proterozoic atmosphere

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Cyanobacteria invented oxygenic photosynthesis. Atmospheric O_2 began accumulating 2.4 billion years ago [1] at the transition between the Archean and Proterozoic eons. This Great Oxidation Event (GOE) [2] was a result of the unique and unprecedented ability of cyanobacteria to split water. Thus water became an electron donor for photoautotrophic growth. For the next two billion years, something limited cyanobacterial primary production; O_2 production was restricted, and atmospheric oxygen remained low [2-5]. Roughly 450 million years ago atmospheric O_2 rose with the advent of land plants [4-5]. Why did the O_2 content of the atmosphere remain low for more than a billion years after the appearance of oxygenic cyanobacteria with their virtually unlimited supply of electrons? The limiting factor may have been biological, and enzymatic. We propose that O_2 production was kept low by oxygen inhibition of nitrogenase activity [6]. Nitrogenase is the sole N_2-fixing enzyme on Earth, and is inactive in air containing 2% or more O_2 by volume [7]. We further propose that inhibition of nitrogenase by O_2 kept cell growth low, and atmospheric O_2,low, until upright terrestrial plants could physically separate photosynthesis in shoots and leaves from N_2 fixation in the ground. Multicellularity and compartmentalization liberated nitrogenase from inhibition by atmospheric O_2.

[1] Fischer, WW, et al. (2016) Evolution of oxygenic photosynthesis. Ann Rev Earth Planet Sci 44: 647–683
[2] Holland, HD (2006). The oxygenation of the atmosphere and oceans. Phil Trans Roy Soc Lond B 361: 903–915
[3] Lyons, TW. et al. (2014). The rise of oxygen in Earth's early ocean and atmosphere. Nature 506: 307–315
[4] Stolper, DA & Keller, B (2018) A record of deep-ocean dissolved O2 from the oxidation state of iron in submarine basalts. Nature 553: 323–327
[5] Lenton, TM et al. (2016) Earliest land plants created modern levels of atmospheric oxygen. Proc Natl Acad Sci USA 113: 9704–9709
[6] Allen JF, Thake B, Martin WF (2019) Nitrogenase inhibition limited oxygenation of Earth's Proterozoic atmosphere. Trends Plant Sci 24: 1022-1031
[7] Fay P (1992) Oxygen relations of nitrogen fixation in cyanobacteria. Microbiol Rev 56: 340–373

203

Diversity & evolution of Glutathione S-Transferases (GST) among the cyanobacterial system

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Glutathione S-Transferases (GSTs) comprise a diverse group of protein superfamily involved in cellular detoxification of various harmful xenobiotics and endobiotics. Cyanobacteria, being the primordial photosynthetic prokaryotes, served as an origin for the evolution of GSTs with diversity in their structures, substrate recognition, and catalytic functions. Detailed sequence alignment (126 genome) and phylogenetic tree analysis resulted in identification of 12 GST classes namely Chi, cGSTX4, cGSTX1, cGSTX5, cGSTX2, cGSTX3, cGSTX7, cGSTX10, cGSTX9, cGSTX6, cGSTX8 and cGSTX11 among cyanobacterial systems but distributed differentially in each order as follows- four in *Pleurocapsales*, eight in *Chroococcales*, seven in *Oscillatoriales*, five in *Stigonematales*, and nine in *Nostocales.* Detailed evolutionary analysis of cyanobacterial GSTs suggested that the order *Pleurocapsales* served as the ancestry for GST evolution. The analysis also identified a conserved motif with signature residues, cysteine, serine, and tyrosine at the N-terminal end that serves as the initiating residue for detoxification. Based on this activating residues, cyanobacterial GSTs are categorized mainly into three types S/C-type, S/Y-type and Y-type for the first time ever. Alternatively, the grouping of cyanobacterial GSTs and their unique signature residues were located, which serve as a possible discriminating factor. New data on cyanobacterial GST may improve further our understanding on GST evolution and other possible divergences in cyanobacteria.

210

Exploring the untapped potential of Aphanizomenon flos-aquae – a high-value cyanobacterium

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Aphanizomenon flos-aquae (AFA) is a filamentous, nitrogen-fixing cyanobacterium that is edible and is known for its high nutritional value. I am working with an AFA strain that blooms naturally in Klamath Lake, Oregon, since this strain already has worldwide approval as a nutritional supplement and could be used as a cell platform for synthesis of valuable biologics and metabolites. The project is divided into three main aims: 1) the phylogenetic and genomic characterisation of Klamath AFA; 2) the optimisation of its cultivation in controlled photobioreactors (PBR), both at small-scale in a laboratory setting and at pilot scale, with the goal of commercial exploitation; and 3) the development of genetic engineering technology for AFA and exploration of its biotechnological applications. Samples from the lake have been used in molecular taxonomy studies where amplified DNA from 16S rDNA, Internal Transcribed Spacer (ITS) and the gene for phycocyanin has been sequenced and compared to those from other AFA strains and related cyanobacteria. These same lake samples have also been used to obtain fragments of its whole genome sequence. Whilst an axenic AFA isolate is being recovered from the lake samples, a proxy AFA strain obtained from a culture collection is being used to optimise growth parameters. My findings indicate that a temperature of 25°C, the use of Jaworski Modified medium, a pH of 8.5 and relatively low light intensities (130 µmol/m^2/s) allow for the optimal laboratory cultivation of this cyanobacterium, allowing us to achieve a doubling time of 50 h. Currently, I am paving the way to successful transformation of the AFA proxy strain by investigating its optimal plating parameters, testing its sensitivity to several different antibiotics and obtaining its whole genome sequence. Next steps include further optimisation of the growth parameters via the addition of CO_2 testing of different light wavelengths, testing AFA's ability to grow at larger scale using a hanging bag PBR system and, finally, obtaining a whole genome assembly.

211

Engineering a rhodopsin-based photo-electrosynthetic system in bacteria for carbon dioxide fixation

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A key goal of synthetic biology is to engineer organisms that can use solar energy to convert CO_2 to biomass, chemicals and fuels. We engineered a light-dependent electron transfer chain by integrating rhodopsin and an electron donor to form a closed redox loop, which drives rhodopsin-dependent CO_2 fixation. A light-driven proton pump comprising *Gloeobacter* rhodopsin (GR) and its cofactor retinal have been assembled in *Ralstonia eutropha* (*Cupriavidus necator*) H16. In the presence of light, this strain fixed inorganic carbon (or bicarbonate) leading to 20% growth enhancement, when formate was used as electron donor. We found that an electrode from a solar panel can replace organic compounds to serve as the electron donor, mediated by the electron shuttle molecule riboflavin. In this new photo-electrosynthetic system, GR is augmented by an external photocell that serves as the electron donor for reductive CO_2 fixation. We demonstrated that this hybrid photo-electrosynthetic pathway can drive the engineered *R. eutropha* strain to grow using CO_2 as the sole carbon source. In this system, a bioreactor with only two inputs, light and CO_2, enables the *R. eutropha* strain to perform a rhodopsin dependent autotrophic growth. Light energy alone, supplied by a solar panel, can drive the conversion of CO_2 into biomass with a maximum efficiency of 4.1%, which is comparable with photosynthesis in plants.

212

A Bayesian approach using genomic data and biosynthetic pathways to study salt tolerance in Cyanobacteria

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Cyanobacteria often have to deal with environmental stressors such as salinity and drought; to do this, they synthesise compatible solutes, which are small molecules whose presence within the cell balances high environmental salt concentrations, particularly by sustaining water influx. The production of compatible solutes is an important mechanism for salt tolerance, i.e. the potential of a bacterium to survive in environments with high concentrations of dissolved salt, such as marine waters.
Cyanobacteria are known to produce five compatible solutes: sucrose, trehalose, glucosylglycerol, glucosylglycerate and glycine betaine. The ability to synthesise compatible solutes varies between individual cyanobacterial strains, and their distribution can be used to obtain information about the evolution of salt tolerance in the group.
Trait evolution analyses can be used to determine at which point in time and in which phylogenetic group a biological feature has first appeared. This can be achieved through multiple techniques, including parsimony-based, maximum-likelihood and Bayesian methods. We use here a novel approach to study the evolution of the biosynthetic pathways responsible for salt tolerance, by integrating genomic data and a Bayesian framework (Stochastic Mapping).
Our analyses predict that the last common ancestor of Cyanobacteria (living at around 3180 Mya) had a 91% probability of a high salt tolerance and was likely able to produce glucosylglycerol and glucosylglycerate. We also speculate that the ability to produce glycine betaine appeared much later, in the Proterozoic, at around 1300 Mya. Finally, we show how stochastic mapping can be used to study complex traits such as biosynthetic pathways in a Bayesian framework, performing inferences in continuous time.

213

Computational Redesign of LH1 Bacteriochlorophyll a Binding Sites to Chlorophyll b binding sites

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Photosynthetic organisms tend to occupy a spectral niche, which allows them to compete with other phototrophic organisms. If this photosynthetically productive spectrum were expanded to cover a greater range, the energy available to an individual organism could be increased. The fundamental reason for doing this would be to improve the productivity of photosynthetic organisms, such as plants, which has obvious benefits in the global food crisis. One step toward this enhanced spectral coverage involves redesigning the evolution-perfected bacterial proteins to bind chlorophylls (Chls) rather than bacteriochlorophylls (BChls). If BChl biosynthesis pathways are diverted to synthesise various Chls, they will need a protein to bind them. LH1 is the multimeric, core antenna of purple photosynthetic bacteria. It contains two proteins, referred to as α and β each of which binds a BChl *a* molecule via one coordination bond and one hydrogen bond. This work outlines the preliminary steps taken towards the redesign of this binding pocket away from BChl *a* specificity and towards Chl *b* using a computational protein design program – Rosetta – in combination with a ligand docking tool – Autodock Vina. These tools allow the large-scale redesign of proteins, prediction of stability, and assessment of the binding affinity of novel binding pockets, which has directed the selection of mutants. Mutants will then be subject to an *in vitro* reconstitution to assess their capacity to bind Chl *b* vs BChl *a*.

214

Evolution and diversity of marine aerobic anoxygenic phototrophs unveiled by large-scale genome analysis

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Aerobic anoxygenic phototrophic bacteria (AAPB) are one of ecologically important groups for carbon cycle in marine environments. The rapid increase in deposited metagenomic information provides the opportunity to reconstruct events in the evolution of phototrophy in Proteobacteria, especially in AAPB. Here, we demonstrate that the phylogenetic relationship of marine phototrophic Proteobacteria based on 570 *pufM* genes from reconstructed 8500 metagenome-assembled genomes (MAGs). The phylogenetic analysis demonstrated that horizontal gene transfer of photosynthetic genes from a-*Proteobacteria* to b- and g-*Proteobacteria* occurred in a limited branch relatively late in the course of evolution. It was also observed in the comparison between PufM and genome trees that several traces of horizontal transfer of photosynthetic gene clusters occurred within a-*Proteobacteria*. The genotype of having two *puf* operons known in *Citromicrobium* was also found in the *Rhodobacteraceae* clade. MAG construction in this study uncovered unexpected genomic diversity that extend our understanding of marine Proteobacterial phototrophs.

215

Investigating a D2 insertion in Prochlorococcus spp. strains lacking the extrinsic proteins PsbU and PsbV

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The D1 and D2 heterodimer forms the reaction centre of Photosystem II. This heterodimer binds to a number of cofactors with most are bound to D1, although the quinone, Q_A bound to D2. Many cyanobacteria have two copies of the *psbD* gene which typically encode identical D2 proteins. *Prochlorococcus* strains contain one *psbD* copy. Genome comparisons indicate the D2 protein of most *Prochlorococcus* strains contains a 7 amino acid insertion in the DE-loop of D2. The insert was noted to be present in strains lacking the extrinsic proteins PsbU and PsbV [1]. Our analysis shows the majority of sequenced *Prochlorococcus* strains (>40) have an insertion in D2 and lack PsbU, PsbV and CyanoQ. Analysis of phylogenetically diverse cyanobacteria shows this D2 insertion is only found in *Prochlorococcus* strains and only these strains lack both PsbU, PsbV and CyanoQ. We identify two insertions sequences: one found in high light *Prochlorococcus* ecotypes and one found in low light ecotypes. Using the strain *Synechocystis* sp. PCC 6803 we are investigating the role of the DE-loop of D2 and whether the requirement for the extrinsic proteins is altered by the addition of the insertion this loop.

[1] Ting et al (2009) Minimal genomes, maximal productivity: comparative genomics of the photosystem and light-harvesting complexes in the marine cyanobacterium, Prochlorococcus. Photosynthesis Research 101: 1-19 DOI: 10.1007/s11120-009-9455-x

217

Visualization of heterogeneous nitrogen fixation in marine unicellular cyanobacterium Crocosphaera

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Biological nitrogen fixation is the process that biochemically converts the inert, atmospherically abundant nitrogen gas (N_2) to biologically available ammonium and forms the initial step of both terrestrial and aquatic nitrogen cycles. Unicellular diazotrophic cyanobacteria (UCYN), the genus *Crocosphaera,* are abundant in vast oligotrophic areas of oceans [1]. Since nitrogenase, the enzyme responsible for nitrogen fixation, is sensitive and irreversibly damaged by oxygen, UCYN fix nitrogen at night utilizing the carbon reserves accumulated by photosynthesis during the day. Recently, using the NanoSIMS analysis we have reported that only 60 % of *Crocosphaera* cells showed nitrogen fixation activity despite homogeneous carbon fixation [2]. In addition, the model simulation revealed that this cell-to-cell heterogeneity of nitrogen fixation is beneficial for expanding their ecological niche [2]. To further investigate and quantify the heterogeneity of nitrogen metabolisms, we have visualized the nitrogen fixation in single cells on the levels of protein and gene expression. First, we have visualized the accumulation of NifH, the protein responsible for nitrogen fixation, in *C. watsonii* PS0609 by immunocytochemistry. The pattern of heterogeneity was similar to previous activity results observed by NanoSIMS. Secondly, we have visualized the expression pattern of *nif* genes using a reporter strain created in *C. subtropica* ATCC 51142. This reporter was constructed by fusing the promoter of the *nif* gene cluster with a fluorescent protein. Fluorescence-detected activity of the *nif* promoter indicates which cells are active in nitrogen fixation. The results showed that the heterogeneity in nitrogen fixation occurs at the transcriptional level as well. In addition, we have also observed heterogeneous patterns of distribution of phycobilin proteins among single cells by confocal microscopy [3]. Overall, the intercellular heterogeneity in nitrogen metabolisms seems to be an inherent feature of nitrogen fixing UCYNs.

[1] Zehr JP (2011) Nitrogen fixation by marine Cyanobacteria. Trends Microbiol 19(4):162-73.
[2] Masuda T *et al* (2020) Heterogeneous nitrogen fixation rates confer energetic advantage and expanded ecological niche of unicellular diazotroph populations. Commun Biol 3(1):172
[3] Masuda T *et al* (2022) Crocosphaera watsonii. Trends Microbiol S0966-842X(22)00044-0.

219

Characterization of novel Indian cyanobacterial isolates as potential biofactory

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Abstract

CO_2 capturing cyanobacteria are attractive biochassis candidates for sustainable production of wide range of chemicals. However, the titers are still far from the benchmarks set by heterotrophic bacterial systems. Therefore, search for an alternate, efficient industrial host is always ongoing. Few challenges like slower growth rate, lesser known synthetic biology tools and sensitivity to stress hinder deployment of any cyanobacterial strain into model strain. In this study, we present six newly discovered *S. elongatus* strains and their entire genome including chromosomal and native plasmid sequence along with the finished genome sequences for *S. elongatus* PCC 11801 and 11802 strains. We have compared the genome sequences of these strains with earlier characterised *S. elongatus* PCC 11801 and 11802 strains. Our study focuses on detailed characterization of all the strains and correlate their phenotypes with genomic differences. The Indian cyanobacterial isolates share significant similarity at genome and protein level, and fall under the same clade. However, the plasmids harbored by these strains exhibit substantial diversity. Novel plasmids with genes for CRISPR, heavy metal efflux, and DNA Restriction-Modification systems were found along with few other high copy no. cryptic plasmids. We unveiled presence of resistance gene against a novel class of phosphonic antibiotic and genes for asparagine synthesis which were not reported for this clade before. The strains' natural transformability was confirmed using eYFP reporter system. We also showed that representative promoters from the reported promoter library for PCC 11801 were portable for all these strains as well. To emphasize on the strains' ability undergo pathway modification, we transformed for mannitol expressing pathway and PDGF expressing gene in all of them followed by a comparative study on their productivity. Out of this, one of the strain, IITB6 gave the highest mannitol titer of 487mg/L. Similarly, we could confirm PDGF synthesis through mRNA levels in PCC11801. These studies reveal the differences in the expression of heterologous genes across various strains, despite of close genome similarity. It also signifies the requirement of multi-omics studies for characterizing any strain in detail instead of emphasizing only on genome organization. As a next step, we would do comparative Metabolomics analysis in order to understand the differences in the pathway expression.

204

The different functions of cellular appendages in cyanobacterial behaviour

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Cyanobacteria like many other bacteria assemble type IV pili on their surface. Type IV pili are multi-functional: they are known to play important roles in adhesion, biofilm formation, aggregation, DNA-uptake and twitching motility. Though, now there is increasing knowledge on the architecture of type IV pili and the chemotaxis systems controlling their activity in heterotrophic bacteria, little is known about the cyanobacterial pilus machineries and what controls its different functions. Second messenger molecules, such as c-di-GMP and cAMP, are important regulators of bacterial life-style decisions. The accumulation of these signalling nucleotides is related to surface recognition and light quality and controls the expression of type IV pilus related genes in the model cyanobacterium *Synechocystis*. The type IV pilus fiber consists of major pilin subunits and contains also so called minor pilins. The expression of these minor pilins is highly regulated and the presence or absence of them influence the different pilus functions. We found that specific sets of minor pilins are important for aggregation of cells, whereas others are essential for natural competence or biofilm formation or motility. Another regulatory system controls the direction of motility in response to different light signals. This system is composed of a network of photoreceptors and chemotaxis-like regulators. Here, we found that PATAN-domain response regulators can switch the direction of phototaxis by binding to the pilus motor PilB1 and to the pilus platform protein PilC. Interestingly, the PATAN domain alone is sufficient to induce reversal of cellular movement, suggesting that this domain is the principal output component of the PATAN-domain CheY-like response regulators. In summary, we will discuss the molecular components of the cyanobacterial type IV pilus machinery and the dynamics of pilus motor biogenesis and its communication with the phototaxis signaling complexes.

206

Phototrophs as models for bacterial longevity.

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Many bacteria can survive in a growth-arrested state for long periods of time, on the order of months, without forming dormant structures like spores or cysts, but the molecular basis for this is poorly understood. We have been studying bacterial longevity in growth arrest using the phototrophic alpha proteobacterium *Rhodopseudomonas palustris* as a model. Since it can continuously generate ATP when incubated in light; it, unlike heterotrophs, can meet the basic energy requirements to sustain its viability in growth arrest. We have found that active protein synthesis is required for *R. palustris* longevity and identified several highly conserved ribosome-associated proteins that are required for sustained viability in growth arrest, but not for growth. In addition to active translation and optimized ribosomes, guanosine polyphosphate [(p)ppGpp] is required for longevity. We are continuing to identify new longevity genes in *R. palustris* and to assign them functions. Some longevity genes are specific to alpha proteobacteria and others are conserved in all bacteria.

208

Structures of Rhodopseudomonas palustris RC-LH1 complexes with open or closed quinone channels

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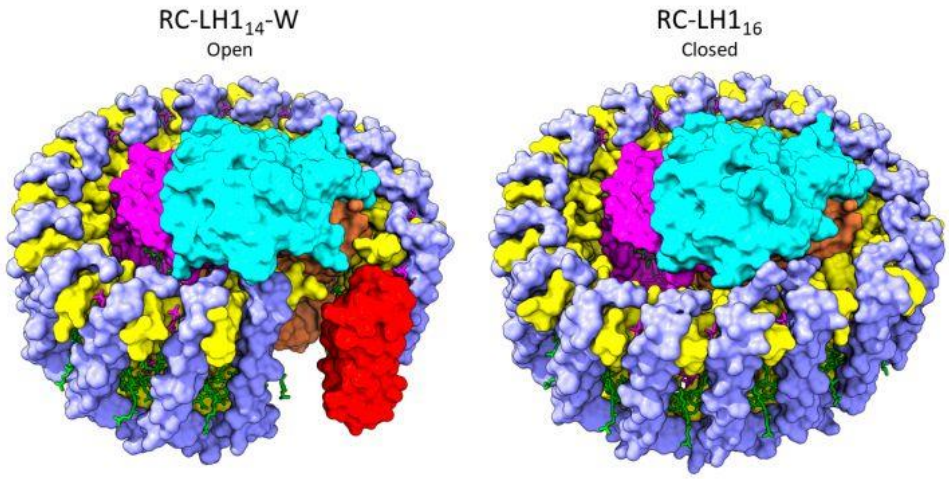
The reaction centre-light harvesting 1 (RC-LH1) complex is the core photosynthetic component in purple bacteria. We recently discovered that the model organism *Rhodopseudomonas palustris* produces two forms of its RC-LH1 complex; one with an additional subunit called protein-W and another that lacks protein-W. We resolved both of these structures by cryogenic electron microscopy. In the RC-LH1 complex without protein-W we show that 16 LH1 subunits completely encircle the RC, whereas with protein-W 14 LH1 subunits form an incomplete, open ring around the RC. Combining our structures with biochemical and spectroscopic analysis shows that protein-W creates a channel for enhanced quinone exchange, accelerating turnover at the RC at the expense of light-harvesting capacity. In addition to revealing the role of the structurally unique protein-W, this study provides important new insights into quinone dynamics in RC-LH1 core complexes, reveals a previously unidentified conformational change at the RC Q_B site, and shows the location of several accessory quinone binding sites that appear to aid quinone delivery to the RC.



209

Light-driven electron transport in Heliobacteria

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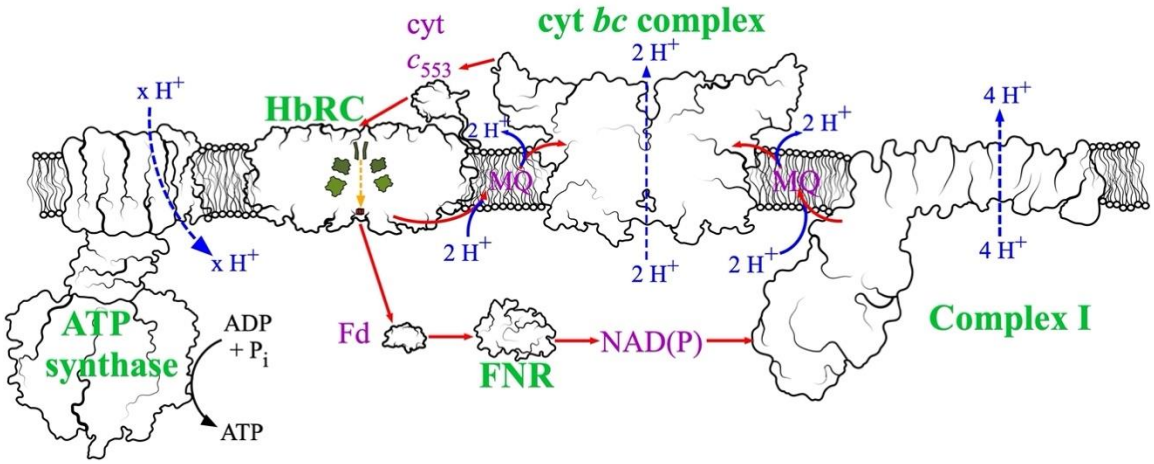
The heliobacteria, a family of anoxygenic phototrophs, possess the simplest known phototrophic system, utilizing a homodimeric Type I reaction center (RC) within only 2 subunits (PshA and PshX) and lacking any peripheral antenna complexes. Although they are photoheterotrophs in the light, the heliobacteria 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). Leveraging the endogenous CRISPR/Cas system, we have been able to delete several genes from the heliobacterial chromosome, including the ones encoding the PshX minor subunit of the RC and the cytochrome *bc* complex. Mutants lacking the cytochrome *bc* complex are nonphototrophic and exhibit a >100-fold slower re-reduction of cyt *c* after a laser flash. Mutants lacking PshX are phototrophic, but show a slight drop in RC levels and the loss of a low-energy pigment.



216

Harnessing the fundamental understanding of self-assembling protein organelles for bioengineering

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Self-assembly and compartmentalization provide the structural basis for confining metabolic reactions in space and time. A typical example of self-assembling and compartmentalizing systems in prokaryotes is the bacterial microcompartment, which can be functionally divided into anabolic carboxysomes and catabolic metabolosomes. The carboxysome is made of the key CO_2-fixing enzymes Rubisco and carbonic anhydrase, which are encapsulated by a protein shell that structurally resembles virus capsids. The nature assembly and architectural features of carboxysomes provide the foundation for their significant contribution to the global carbon cycle and have attracted increasing interest in repurposing carboxysome structures for new functions using synthetic biology. In this seminar, I will present our recent studies on (1) the protein stoichiometry, self-assembly, and architecture of carboxysomes, and (2) synthetic engineering of carboxysome structures in heterologous organisms for carbon fixation and bioenergy production, harnessing the knowledge about the molecular principles underlying carboxysome assembly and structure learned in nature.

References:

[1] Sun Y, et al. (2022) Decoding the absolute stoichiometric composition and structural plasticity of α-carboxysomes. *mBio* 13(2):e0362921.
[2] Liu LN (2022) Advances in the bacterial organelles for CO_2 fixation. *Trends Microbiol* 30(6):567-580.
[3] Chen T, et al. (2022) Incorporation of Functional Rubisco Activases into Engineered Carboxysomes to Enhance Carbon Fixation. *ACS Synth Biol* 11(1):154-161.
[4] Liu LN, Yang M, Sun Y, & Yang J (2021) Protein stoichiometry, structural plasticity and regulation of bacterial microcompartments. *Curr Opin Microbiol* 63:133-141.
[5] Li T, et al. (2020) Reprogramming bacterial protein organelles as a nanoreactor for hydrogen production. *Nat Commun* 11:5448.
[6] Huang F, et al. (2020) Rubisco accumulation factor 1 (Raf1) plays essential roles in mediating Rubisco assembly and carboxysome biogenesis. *Proc Natl Acad Sci USA* 117(29):17418-17428.
[7] Faulkner M, et al. (2020) Molecular simulations unravel the molecular principles that mediate selective permeability of carboxysome shell protein. *Sci Rep* 10(1):17501.

218

Lifting phylogeny by its bootstraps: Interpretations, overinterpretations and misinterpretations

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Phylogenetics has become a powerful tool in biology through methodological improvements, including the inclusion of sophisticated evolutionary algorithms, statistical models, and an ever-increasing capacity to encompass larger and larger sequence datasets. Because it now requires quite a bit of specialization from its practitioners, it brings about a real risk of losing sight of the tree by looking at the branches. Phylogenetics represents both a means to construct evolutionary hypotheses to be tested or a means to seek confirmation of evolutionary hypotheses arrived at by other means. As such, it is most useful when used in combination with theoretical frameworks or sets of data from other evolutionary pertinent disciplines. But current taxonomic work in microbiology, for example, is driven virtually exclusively by phylogenetic reconstructions, often neglecting other sources of biological information. Because of their ecological importance, today and in the distant past, few groups among microbes have been subject to so many and varied phylogenetic studies like the cyanobacteria. In this presentation, and using cyanobacteria as a model, I will review some instances where phylogenetic approaches alone fail us in our attempt to provide a realistic view of cyanobacterial evolutionary history, which include problems associated with the premises of ‘phylogenomics’, the role of convergent evolution and mutualisms, and the use of restricted datasets.

220

Diversity of far-red light photoacclimation responses in cyanobacteria

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Diversity of far-red light photoacclimation responses in cyanobacteria
The discovery of cyanobacteria capable of harvesting far-red light has changed the paradigm that oxygenic photosynthesis is only driven by visible light and that the associated photochemistry is initiated exclusively by chlorophyll *a*. There are two known types of far-red photosynthesis. Firstly, a constitutive adaptation that primarily uses chlorophyll *d* as the major photopigment, which is restricted to a single genus (*Acaryochloris*). More recently, an acclimation response known as Far-Red Light Photoacclimation (FaRLiP) was discovered, which uses chlorophyll *f* for photochemistry and is present in phylogenetically diverse cyanobacteria. FaRLiP involves the extensive remodelling of the photosynthetic machinery, via expression of a cluster of approximately 19 *genes coding for* paralogous subunits of Photosystem I, Photosystem II, phycobilisomes and regulatory elements. Here, I will highlight the similarities and differences in FaRLiP among cyanobacteria at genome, protein, membrane and cellular levels by using bioinformatics, biochemical and biophysical methods. Our study focusses on cyanobacteria of the genus “*Chroococcidiopsis*”, as well as the phylogenetically early-branching group of “*Halomicronema*/Synechococcales”. Few Synechococcales capable of FaRLiP have been described to date, but we have isolated numerous examples from the hypersaline environment of Sebkha Ooum Dba (Morocco), using stringent far-red cultivation methods. Furthermore, a strain was discovered that only contains a partial FaRLiP cluster. This strain displays normal growth behaviour under far-red light despite lacking genes for far-red PSI, indicating that the minimal requirements for FaRLiP are somewhat less than the ~19 generally conserved clustered genes. Based on genetic and structural comparisons, first attempts have been made to tune the absorption properties of the non-FaRLiP cyanobacterium *Synechocystis* sp. PCC 6803 towards far-red photosynthesis. In our approach, we targeted PSII and replaced the standard core PsbA subunit with several far-red variants. All mutants showed the assembly of a functional PSII complex with chlorophyll *a* only, suggesting the involvement of specific assembly factors for the introduction of long-wavelength chlorophylls at specific positions in far-red PSII.

222

Stealing from the photosynthetic electron transport chain

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The ‘light reaction’ in photosynthesis generates high energy carriers for the ‘dark reaction’ to carry out carbon dioxide fixation. The biohybrid research community can now electrochemically couple to the photosynthetic electron transport chain (PETC), both *in vitro* and *in vivo,* to either extract or insert electrons at various places for fundamental studies and for biotechnological applications.[1]
In this talk, I will show my group’s efforts to steal electrons from cyanobacterial PETC with greater efficiency and control using non-biological approaches. In particular, we utilise tailored 3D-electrodes to enhance the intrinsic activity of the cyanobacteria and to maximise electron exchange.[2] We also employ conductive extracellular matrices and exogenous molecular electron shuttles to accelerate extracellular electron transfer between the PETC and the electrode.[3] The ability to steal electrons from the PETC of phototrophic prokaryotes in a controlled manner will open up novel ways to probe and exploit their bioenergetics in biotechnologies.

References

1. Zhang, J. Z. & Reisner, E., (2020) Advancing photosystem II photoelectrochemistry for semi-artificial photosynthesis. *Nature Rev. Chem.*, *4* , 6-21.

2. Chen, X., Lawrence, J. M., Wey, L. T., Schertel, L., Jing, Q., Vignolini, S., Howe, C., Kar-Narayan, S., Zhang, J. Z., 3D-printed hierarchical pillar array electrodes for high performance semi-artificial photosynthesis. *Nat. Mater. in press (DOI: 10.1038/s41563-022-01205-5)*

3. Clifford, E. R., Bradley, R. W., Wey, L. T., Lawrence, J. M., Chen, X., Howe, C. J. & Zhang, J. Z., (2021) Phenazines as model low-midpoint potential electron shuttles for photosynthetic bioelectrochemical systems. *Chem. Sci.*, 12, 3328 - 3338.

223

Anoxygenic phototrophic Chloroflexota - uncovering metabolic flexibility by in-situ -omics analyses

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The phylum *Chloroflexota* (formerly *Chloroflexi*) is a deeply branching phylum of the *Bacteria*, which harbors extensive intraphylum diversity, and its members are found in a wide range of aquatic and terrestrial environments. Before the name *Chloroflexi* was first proposed in 2001, members of this phylum were referred to as ‘Green non-sulfur bacteria’. The phylum was named after the genus *Chloroflexus*, and its type species, *Chloroflexus aurantiacus*, a filamentous anoxygenic phototrophic bacterium that was first found in hot springs and described by Pierson and Castenholtz in 1974. As a ‘green’ phototrophic bacterium that lived in ‘non-sulphidic’ environments, it represented the group of ‘green non-sulfur bacteria’ (GNSB), which are now termed ‘filamentous anoxygenic phototrophs’ (FAP), a term used specifically for phototrophic members of the *Chloroflexota*. Phototrophic members are found in two classes within this phylum, the *Chloroflexia* and „*Candidatus* Thermofonsia“.

The class *Chloroflexia* contains all described FAP. The green FAP (e.g., genus *Chloroflexus*) contain the green bacteriochlorophyll (BChl) *c* as their major photosynthetic pigment, specifically found in light harvesting units called “chlorosomes”, as well as BChl *a* in their photosynthetic reaction centers. In the laboratory, they conduct anoxygenic phototrophy under anaerobic light conditions, as well as respiration in aerobic dark conditions. In their natural habitats, such as hot spring associated cyanobacterial mats, these organisms often encounter aerobic light conditions during the day, and an anaerobic dark environment at night. In contrast to lab cultivation studies, *in-situ* metatranscriptomic analyses confirm active phototrophy in aerobic conditions during midday in these mats, as well as a short period of aerobic respiration in the afternoon, when light decreases. Surprisingly, in the early morning hours, relative transcription peaks of genes encoding uptake hydrogenase, key enzymes for carbon fixation, respiratory complexes as well as enzymes for TCA cycle and acetate uptake suggest an aerobic chemomixotrophic lifestyle, which has been shown in laboratory studies of a *Cfl. aggregans* isolate, only very recently.

Red FAPs are found in the family *Roseiflexaceae*, with three described genera, which lack the characteristic green pigments of the green *Chloroflexia* members. These organisms are found in similar environments co-existing with green FAP. Similar to their green relatives, they show anoxygenic phototropic as well as respiratory metabolism. *In-situ* metatranscriptomic studies support the hypothesis that the expression of photosynthetic and respiratory components are controlled by oxygen, similar to other anoxygenic phototrophs, while active phototrophy is also carried out under aerobic conditions in their natural habitats.

224

Coexistence and Resistance among Cyanobacteria and their Phages in the Ocean

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Viruses are globally abundant and highly diverse. They influence the abundance, diversity and evolution of their hosts as well as biogeochemical cycles in the oceans. However, the lack of methods to measure virus populations and the extent of viral infection at taxonomically meaningful levels have precluded a quantitative understanding of their impact on these processes. Towards this end, we developed Polony and iPolony, single molecule solid-phase PCR techniques, to quantify discrete cyanophage families [1] and the extent to which they infect the unicellular marine cyanobacteria, *Synechococcus* and *Prochlorococcus* [2]. Using these methods, we found dramatic differences in cyanophage distribution and infection patterns across environmental gradients in the North Pacific Ocean [3]. A hotspot of cyanophage infection was observed in the transition zone between the subtropical and subpolar gyres, that at times, was high enough to limit the geographic range of *Prochlorococcus* [3]. However, in the vast North Pacific Subtropical Gyre cyanophage infection was low despite high cyanophage abundances, raising the possibility that resistance mitigates infection and allows for coexistence. Mechanisms of resistance differ against specialist and generalist cyanophages, with resistance being primarily at the stage of adsorption against specialist cyanophages, yet intracellular against generalist cyanophages [4]. Our results suggest that novel mechanisms of intracellular resistance are present in marine picocyanobacteria, one of which is the adaptive loss of cellular components essential for phage reproduction. Combined our findings highlight how changing environmental conditions and coevolutionary processes influence the effect of cyanophages on their cyanobacterial hosts.

References

[1] Baran, Goldin, Maidanik, Lindell. Quantification of diverse virus populations in the environment using the polony method. *Nature Microbiology*. 3:62-72 (2018).

[2] Mruwat, Carlson, Goldin, Ribalet, Kirzner, et al. A single-cell polony method reveals low levels of infected Prochlorococcus in oligotrophic waters despite high cyanophage abundances. *The ISME Journal* 15:41-54 (2021).

[3] Carlson, Ribalet, Maidanik, Durhman, Hulata, et al. Viruses affect picocyanobacterial abundance and biogeography in the North Pacific Ocean. *Nature Microbiology* 7:570-580 (2022).

[4] Zborowsky and Lindell. Resistance in marine cyanobacteria differs against specialist and generalist cyanophages. *Proc. Nat. Acad. Sci. USA*. 116:16899-16908 (2019).

225

The evolution of ancestral cyanobacteria

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Oxygenic phototrophs have played a fundamental role in Earth's history by enabling the rise of atmospheric oxygen paving the way for complex life. Their origin fundamentally transformed the biology and geochemistry of our planet. I will give an overview about key evolutionary events in the history of oxygenic phototrophs: 1) the Archean origin of PSII (the photochemical reaction centre that catalyses the light-driven oxidation of water to molecular oxygen), 2) the origin of the crown group of Cyanobacteria, 3) the emergence of filamentous forms around the Great Oxidation Event ~ 2.32 Ga, and 4) the late emergence of marine planktonic groups between 800-600 Mya. Molecular evolution analyses overall show that there is a huge lag between the Archean origin of oxygenic photosynthesis and planktonic forms at the end of the Precambrian. By studying the 'genomic record' we can now unravel how oxygenic phototrophs co-evolved with the Earth's biosphere during the Precambrian.