Welcome to the 12th Workshop on Cyanobacteria!

The 12th Workshop on Cyanobacteria marks the continuation of a successful series of student- and postdoc-centered workshops that are held in North America typically every three years and that are covering all aspects of cyanobacteriology. The organization has been facilitated and registration fees have been kept low through the generous support of our Gold (Algenol, Arizona State University, International Society of Photosynthesis Research, and Reliance Industries Limited) and Silver (Walz) sponsors. Moreover, we gratefully acknowledge grant support from the National Science Foundation (BIO/MCB Molecular and Cellular Biosciences) and the US Department of Energy (Basic Energy Research in the Office of Science); these grants fund the registration fees of many students and postdocs at this Workshop. We also thank the committee of graduate students who have played a major role in the selection of participants who received a registration fellowship and of oral presenters.

We wish you all a pleasant and productive meeting!

The Organizing Committee:

Rob Burnap
Ferran Garcia-Pichel
Kathleen Tucker
Wim Vermaas
Michelle Watson
Locations:

- Oral presentations are in the Marston Theater in ISTB-4.
- Poster presentations are in the Biodesign Institute (BDA on the map), first floor and basement; enter through the South entrance. Refreshments and snacks are served at poster sessions.
- Note that the Biodesign Institute is a secure facility. Posters are accessible only during the times of poster hanging and of the poster sessions, and 7:30 am – 6:00 pm on Friday.
- Meals and dorms are in the Barrett Honors Hall.
- Saturday dinner is at the Desert Botanical Garden (bus transportation is provided).

*Note that badges must be worn at all times; there is no access to the poster area without a badge.*
Oral presentations

All oral presentations, except the invited lectures, will be 12 minutes plus three minutes of discussion. **Timing will be strictly enforced.** As timing is tight, all speakers must upload their presentation (as a PowerPoint file) on the computer at the desk outside the Marston Theater in ISTB-4 at least two hours prior to the start of the session, and check that all figures show correctly. Speakers must name their file with their last name and the session number. To minimize the time between presentations, **speakers are not allowed to use their own computer for their presentation.**

Posters

**Your poster size should not exceed 4 ft x 4 ft (1.2 m x 1.2 m).** There are 8-ft (2.40 m) boards in the poster room, so please share your side of the board with a neighbor.

There are four poster sessions:

1. Thursday, 8-10 pm
2. Friday, 3:30-6 pm
3. Friday, 8-10 pm
4. Saturday, 1:15-4:15 pm

Please be present near your poster for one of the four sessions according to the following schedule: Divide the number of your poster in your session by 4 (e.g., for poster 3.10, this would be 2.50), and:

- If your number after division ends in .25, then please present in poster session 1.
- If your number after division ends in .50, then please present in poster session 2.
- If your number after division ends in .75, then please present in poster session 3.
- If your number after division ends in .00, then please present in poster session 4.

There is ample time for poster presentation and discussion. For those of you who are not so experienced in conferences and workshops, this is your opportunity to introduce yourself to others, tell about your work, network, make new friends, etc. Don’t be shy. Sometimes the best ideas for new experiments come from these informal interactions in the poster room.

In the program, only the presenting author of the oral or poster presentation is indicated. For a complete list of authors, please see the abstracts.

**Note:** You will have the option to give a five-minute oral presentation on your poster. We will have a projector and screen set up adjacent to the poster space, and will project a poster there for exactly five minutes. During this time, the poster presenter may explain her/his poster in **five minutes** to whoever is listening. Questions and discussion will follow at the poster. If you are interested in orally presenting your poster, please send a note to this effect to Kathleen Tucker (ktucker@asu.edu) so we expect your poster PDF, and send a one-page PDF of your entire poster to Kathleen by **Monday, May 16**. There are less oral poster presentation slots than that there are posters, so it will be first-come, first-serve.
Talks: Marston Theater in ISTB-4. Posters: BioDesign (badge required; South entrance). Meals: Barrett Dining Hall.

Program

Thursday, May 19:

3:00-7:00 pm  **Registration and dorm check-in**, Barrett Honors College; please let Kathleen Tucker (ktucker@asu.edu) know beforehand if you are going to be late.

3:00-7:00 pm  **Poster hanging**, Biodesign Institute (please check in first; no access to Biodesign without a badge; enter through the South entrance across from ISTB-4).

7:00-7:15 pm  **Welcome and practical announcements**, Marston Theater in ISTB-4

7:15-8:00 pm  K.1. **Jack Meeks, UC-Davis**, Nitrogen-fixing symbiotic interactions between *Nostoc punctiforme* and the hornwort *Anthoceros* spp. as a paradigm to enable *Nostoc*-crop plant associations: pipeline or pipedream? (Keynote Lecture 1)

8:00-10:00 pm  **Poster session and welcome reception** (Biodesign)

Friday, May 20:

6:45-8:15 am  **Breakfast**, Barrett Honors College Dining Hall

Session 1, Biotechnology:  **Tasios Melis and Kostas Stamatakis, Co-Chairs**

8:30-9:15 am  K.2. **Shota Atsumi, UC-Davis**, Global metabolic rewiring of an obligate photoautotrophic cyanobacterium for production of chemicals under diurnal light conditions (Keynote Lecture 2)

9:15-9:25 am  Introduction by the session co-chairs

9:25-9:40 am  1.A. **Ping Xu, Shanghai Jiao Tong University**, Engineering cyanobacteria as photosynthetic platform for sustainably producing valuable chemicals directly from CO₂

9:40-9:55 am  1.B. **Fiona Davies, Colorado School of Mines**, Engineering photosynthetic limonene and bisabolene production in the cyanobacterium *Synechococcus* sp. PCC 7002

9:55-10:10 am  1.C. **Elias Englund, Uppsala University**, Production of manoyl oxide, a precursor to the medically active compound forskolin, in *Synechocystis* PCC 6803

10:10 am  **Group photo**, outside the entrance to ISTB-4

Until 10:40 am  **Coffee break**, ISTB-4 lobby
Talks: Marston Theater in ISTB-4. Posters: BioDesign (badge required; South entrance). Meals: Barrett Dining Hall.

10:40-10:55 am 1.D. Wei Xiong, National Renewable Energy Laboratory, The plasticity of cyanobacterial metabolism supports direct CO₂ conversion to ethylene

10:55-11:10 am 1.E. Tylor Johnson, South Dakota State University, Increasing the tolerance of filamentous cyanobacteria to next-generation biofuels via directed evolution

11:10-11:25 am 1.F. Yi Ern Cheah, Vanderbilt University, Isotopically nonstationary $^{13}$C metabolic flux analysis guided strain engineering of isobutyraldehyde producing *Synechococcus elongatus* PCC 7942

11:25-11:40 am 1.G. Pramod Wangikar, Indian Institute of Technology Bombay, $^{13}$C-Metabolic flux analysis of *Synechococcus* sp. PCC 7002 and its glycogen mutant under photoautotrophic conditions

11:40-11:55 am 1.H. Que Chen, University of Amsterdam, Expression of holo-proteorhodopsin in *Synechocystis* sp. PCC 6803

11:55-1:15 pm Lunch, Barrett Honors College Dining Hall

**Session 2, Molecular Physiology:** Christiane Funk and Lou Sherman, Co-Chairs

1:15-1:25 pm Introduction by the session co-chairs

1:25-1:40 pm 2.A. Meghan Barnhart-Dailey, Sandia National Laboratories, Pigment localization and dynamics in individual cyanobacterial cells

1:40-1:55 pm 2.B. Melissa Cano, National Renewable Energy Laboratory, Carbon partitioning and management of energy in a glycogen-deficient *Synechocystis* strain

1:55-2:10 pm 2.C. Kirstin Gutekunst, Christian-Albrechts-University, Kiel, The Entner-Doudoroff pathway is an overlooked glycolytic route in cyanobacteria and plants

2:10-2:25 pm 2.D. Gina Gordon, University of Wisconsin-Madison, Tunable gene repression in *Synechococcus* sp. strain PCC 7002 using CRISPRi

2:25-2:40 pm 2.E. Susan Cohen, UC-San Diego, Dynamic localization of the cyanobacterial circadian clock

**Session 3, Interactions and Environment:** Brian Palenik, Chair

2:40-2:45 pm Introduction by the session chair

2:45-3:00 pm 3.A. Stephanie Hays, Harvard University and Wyss Institute, Seen and unforeseen interactions in engineered photosynthetic consortia
Talks: Marston Theater in ISTB-4. Posters: BioDesign (badge required; South entrance). Meals: Barrett Dining Hall.

3:00-3:15 pm  3.B. **Maria Agustina Dominguez-Martín**, *Universidad de Córdoba*, Effect of low concentration of nitrate on the nitrogen metabolism of *Synechococcus* WH7803

3:15-3:30 pm  3.C. **Ana Giraldo Silva**, *Arizona State University*, Nursing the biological soil crust restoration: cyanobacteria isolation, lab cultivation, scaling up and inoculum conditioning

3:30-6:00 pm  **Poster session**

6:00-7:15 pm  **Dinner**, Barrett Honors College Dining Hall

7:15-8:00 pm  7.K. **Cheryl Kerfeld**, *Michigan State University/LBNL/UC-Berkeley*, Modularity in cyanobacterial photoprotection and CO$_2$ fixation (Keynote Lecture 3)

8:00-10:00 pm  **Poster session**

**Saturday, May 21:**

6:45-8:15 am  **Breakfast**, Barrett Honors College Dining Hall

**Session 4, Nitrogen Fixation: Terry Thiel, Chair**

8:30-8:35 am  Introduction by the session chair

8:35-8:50 am  4.A. **Andrea Balassy**, *Washington University*, Transcriptional regulation of nitrogen fixing genes

8:50-9:05 am  4.B. **Loralyn Cozy**, *Illinois Wesleyan University*, Regulation of heterocyst commitment in *Anabaena* sp. strain PCC 7120

9:05-9:20 am  4.C. **Romain Darnajoux**, *Université de Sherbrooke*, In vivo characterization of nitrogenase kinetics in *Anabaena variabilis* ATCC 29413 using cavity ring-down spectroscopy

**Session 5, Photosynthesis and CO$_2$: Bob Blankenship and Jindong Zhao, Co-Chairs**

9:20-9:30 am  Introduction by the session co-chairs

9:30-9:45 am  5.A. **Min Chen**, *University of Sydney*, Structure and function of red-shifted phycobilisomes isolated from Chl f-containing cyanobacterium *Halomicronema hongdechloris*
Talks: Marston Theater in ISTB-4. Posters: BioDesign (badge required; South entrance). Meals: Barrett Dining Hall.

9:45-10:00 am  5.B. Veerle Luimstra, University of Amsterdam and Wetsus, Light color matters in cyanobacterial photosynthesis

10:00-10:05 am  In memoriam for Hans Matthijs, University of Amsterdam

10:05-10:35 am  Coffee break, ISTB-4 lobby

10:35-10:50 am  5.C. Meng Li, University of Tennessee, A novel tetrameric form of photosystem I widespread in cyanobacteria: Structure, occurrence, function and evolution

10:50-11:05 am  5.D. Adam Pérez, Penn State University, A [3Fe-4S]^{1+/0} cluster in the F_{b} site of a C14G PsaC mutant of Synechococcus sp. PCC 7002 can sustain photoautotrophic growth

11:05-11:20 am  5.E. Hualing Mi, Shanghai Institutes for Biological Sciences, Localization and functions of several subunits of NDH-1 complexes in the cyanobacterium Synechocystis sp. PCC 6803

11:20-11:35 am  5.F. Juliana Artier, Oklahoma State University, Structure-function studies of CupA, a protein involved in the carbon uptake system NDH-1_{3} of Synechocystis sp. PCC6803

11:35-11:50 am  5.G. Raul Gonzalez-Esquer, Michigan State University, Streamlined construction of the carboxysome core via protein domain fusion

11:50-1:15 pm  Lunch, Barrett Honors College Dining Hall

1:15-4:15 pm  Poster session. Please take down your poster at the end of the poster session and make your way over to the Marston Theater

4:30-5:15 pm  K.4. Devaki Bhaya, Carnegie Institution for Science, Diversity and dynamics of phototrophic communities (Keynote Lecture 4)

5:15-5:30 pm  Bus loading to go to the Desert Botanical Garden (dbg.org), 1201 N. Galvin Parkway, Phoenix, AZ 85008. All registered participants are invited. Turn right when exiting the ISTB-4 building. Buses depart at 5:30 pm.

6:00-9:30 pm  Workshop dinner at the Desert Botanical Garden (Dorrance Center)

9:30 pm  Bus returns to the Barrett Honors College
Talks: Marston Theater in ISTB-4. Posters: BioDesign (badge required; South entrance). Meals: Barrett Dining Hall.

**Sunday, May 22:**

6:45-8:15 am  *Breakfast*, Barrett Honors College Dining Hall

**Session 6, Physiology and Metabolism: Ruanbao Zhou and Brian Pfleger, Co-Chairs**

8:30-8:40 am  Introduction by the session co-chairs

8:40-8:55 am  6.A. Benjamin Rubin, UC-San Diego, The essential gene set for cyanobacteria

8:55-9:10 am  6.B. Maria del Carmen Muñoz-Marín, UC-Santa Cruz, Transcriptome of the N₂-fixing cyanobacterium UCYN-A over diel cycles

9:10-9:25 am  6.C. Ralf Steuer, Humboldt University of Berlin, Towards multiscale models of cyanobacterial growth: a modular approach

9:25-9:40 am  6.D. Thomas Mueller, Penn State University, Modeling regulation and metabolism in cyanobacteria

9:40-9:55 am  6.E. Sara Pereira, Universidade de Porto, Genes and proteins involved in the assembly and export of cyanobacterial extracellular polymeric substances (EPS)

9:55-10:10 am  6.F. Carlos Quiroz Arita, Colorado State University, Scalability of flat photobioreactors: Incorporating Lagrangian fluid mechanics in growth models

10:10-10:40 am  *Coffee break*, ISTB-4 lobby

10:40-11:25 am  K.5. Himadri Pakrasi, Washington University, Engineering nitrogen fixation ability in Synechocystis 6803 (Keynote Lecture 5)

**Final session:**  Wim Vermaas, Rob Burnap and Ferran Garcia-Pichel, Co-Chairs

11:25-11:50 am  Awards

11:50-12:00 n  Next meeting

12:00 n  *Adjournment*. A box lunch and a bottle of water will be provided for on the go.

12:00-2:00 pm  *Check out from dorms*. Barrett Honors Dorm front desk
Poster sessions are in Biodesign, 1st Floor and Basement. A badge is required for entry (South entrance).

1. Posters, Session 1, Biotechnology and Genomics

1.1. Qingfang He, University of Arkansas at Little Rock, Cyanobacterial platform for production of phenylpropanoids

1.2. Shahrah Alqahtani, University of Arkansas at Little Rock, Genetic engineering of Synechocystis sp. PCC 6803 for sustainable production of cinnamic acid

1.3. Han Min Woo, Korea Institute of Science and Technology and Korea University, Pathway engineering for production of photosynthetic acetone and isoprenoids production from CO₂ using engineered cyanobacteria

1.4. Jacob Sebesta, Colorado State University, Metabolic engineering of Synechocystis sp. PCC 6803 for improved terpenoid production

1.5. Christie Peebles, Colorado State University, Engineering astaxanthin production in Synechocystis sp. PCC 6803: Challenges and successes

1.6. Anastasios Melis, UC-Berkeley, Cyanobacterial Biosynthetics: fuel and chemicals production

1.7. Julie Chaves, UC-Berkeley, Controlling isoprene synthase expression in the cyanobacterium Synechocystis: a molecular-biological approach

1.8. Rhiannon Carr, University of Wisconsin-Oshkosh, Metabolic engineering for β-pinene production in Synechococcus sp. PCC 7002

1.9. Valerie Wagner, University of Wisconsin-Oshkosh, The impacts of β-pinene on the growth and metabolism of native and isoprene-producing Synechococcus sp. PCC 7002 cyanobacteria

1.10. Megan Raebel, University of Wisconsin-Oshkosh, Regulated promoters to control toxic genes in the methylyerythritol phosphate (MEP) pathway of Synechococcus sp. PCC 7002

1.11. Ankita Kothari, Lawrence Berkeley Natl Lab, Alkane production in Synechococcus sp. PCC 7002

1.12. Bo Wang, National Renewable Energy Lab, Mitigating rate-limiting steps in cyanobacterial production of ethylene

1.13. Masahiro Kanno, UC-Davis, Continuous 2,3-butanediol production in an obligate photoheterotrophic cyanobacterium in diurnal light conditions

1.14. Fei Tao, Shanghai Jiao Tong University, Engineering cyanobacterial xylitol production from CO₂

1.15. Emmanuel Reyna-Gonzalez, University of Potsdam, One-pot synthesis of leader peptide-free microviridins

1.16. Patrick Videau, Oregon State University, Assessment of Anabaena sp. strain PCC 7120 as a heterologous expression host for cyanobacterial natural products: production of lyngbyatoxin A

1.17. Christopher Jones, University of Wisconsin-Madison, Translational fusions facilitate high-level heterologous gene expression in Synechococcus sp. PCC 7002

1.18. Haojie Jin, Carnegie Institution, Orthogonal T7 RNA polymerase/promoter system controls gene expression in Synechocystis sp. PCC 6803
Poster sessions are in Biodesign, 1st Floor and Basement. A badge is required for entry (South entrance).

1.19. **Kostas Stamatakis**, *NCSR Demokritos*, An in situ antimicrobial susceptibility testing method based on cyanobacterial chlorophyll a fluorescence

1.20. **Aiko Turmo**, *Michigan State University*, Carboxysomes redesigned as nanoreactors for renewable chemical production in cyanobacteria

1.21. **Isaac Plant**, *Harvard University*, Developing genetic tools for polyploid prokaryotes

1.22. **Kristen Wendt**, *Washington University*, A transient CRISPR/Cas editing system to introduce markerless genome modifications in the cyanobacterium *Synechococcus elongatus* UTEX 2973

1.23. **Kui Wang**, *Algenol Biotech LLC*, Novel shuttle vector capable of transforming multiple genera of cyanobacteria

1.24. **James Golden**, *UC-San Diego*, Broad-host-range genetic tools for cyanobacteria


2. **Posters, Session 2, Molecular Physiology**

2.1. **Anika Wiegard**, *Heinrich-Heine-University Duesseldorf*, Diversity of putative clock systems

2.2. **David Welkie**, *UC-San Diego*, Deletion of the circadian clock protein KaiA in *Synechococcus elongatus* PCC 7942 results in impaired diurnal growth

2.3. **Yingying Wang**, *University of Kiel*, The physiological functions of different ferredoxins in *Synechocystis* sp. PCC 6803

2.4. **Louis Sherman**, *Purdue University*, Altering the structure of carbohydrate storage granules in the cyanobacterium *Synechocystis* sp. PCC 6803

2.5. **Claudia Durall**, *Uppsala University*, Analysis of engineered *Synechocystis* PCC 6803 cells containing additional copies of phosphoenolpyruvate carboxylase

2.6. **Friedrich Kirsch**, *University of Rostock*, Modulating sucrose metabolism in cyanobacteria: Knocking out competing pathways and overexpressing selected enzymes in *Synechocystis* sp. PCC 6803

2.7. **Jianping Yu**, *National Renewable Energy Lab*, Phosphoketolase pathway contributes to cyanobacterial carbon metabolism

2.8. **Steven Holland**, *Oklahoma State University*, Changes in electron flow observed in carbon sink mutants of the cyanobacterium *Synechocystis* sp. PCC 6803

2.9. **Charles Halfmann**, *South Dakota State University*, The effects of glycogen deficiency on the nitrogen-stress response in *Anabaena* sp. PCC 7120

2.10. **Rachel Hood**, *UC-Berkeley*, The stringent response regulates adaptation to darkness in the cyanobacterium *Synechococcus elongatus*

2.11. **Christiane Funk**, *Umeå University*, The Deg proteases of the cyanobacterium *Synechocystis* sp. PCC 6803
Poster sessions are in Biodesign, 1st Floor and Basement. A badge is required for entry (South entrance).


2.14. Deng Liu, *Washington University*, Improved O₂ tolerance of nitrogenase via uptake hydrogenase in engineered *Synechocystis* 6803 containing the minimal *nif* cluster

2.15. Carol Baker, *Penn State University*, Expression of a carotenoid oxygenase gene by constitutive and inducible promoters in *Synechococcus* sp. PCC 7002

2.16. Alicia Muro-Pastor, *CSIC and Universidad de Sevilla*, Regulatory RNAs involved in nitrogen assimilation

2.17. Manuel Brenes-Álvarez, *CSIC and Universidad de Sevilla*, Identification of small RNAs in heterocystous cyanobacteria

2.18. Jeffrey Cameron, *University of Colorado-Boulder*, Probing mechanisms of RNA turnover in cyanobacteria using next-generation sequencing

2.19. Andrea Busch, *Michigan State University*, The tryptophan-rich sensory protein (TSPO) in the cyanobacterium *Fremyella diplosiphon*

2.20. Priscila Herrera, *Centro de Investigación y Estudios Avanzados del IPN*, Complementary chromatic acclimation in the cyanobacterium *Synechococcus* PCC 7335

3. Posters, Session 3, Interactions and Environment

3.1. Brian Palenik, *Scripps Institution of Oceanography*, Marine *Synechococcus* aggregate formation

3.2. Mizuho Ota, *UC-San Diego*, A barcoded transposon mutant library reveals genes involved in amoebal grazing

3.3. Kevin Becker, *Woods Hole Oceanographic Institution*, The microbial lipidome of subtropical Pacific surface waters dominated by *Prochlorococcus*

3.4. Ángeles Muñoz, *University Autonoma of Madrid*, Analysis of molecular diversity within single *Rivularia* colonies by Illumina sequencing

3.5. Ariana Eily, *Duke University*, Understanding nutrient exchange in the Azolla-Nostoc symbiosis

3.6. Nathali Maria Machado de Lima, *Sao Paolo State University*, Diversity of cyanobacteria of biological soil crusts in Brazilian savannah


4. Posters, Session 4, Nitrogen Fixation and Heterocyst Differentiation

4.1. Ruanbao Zhou, *South Dakota State University*, Transformation of *Anabaena cylindrica* implicates a role of AcaK in akinete development
Poster sessions are in Biodesign, 1st Floor and Basement. A badge is required for entry (South entrance).

4.2 Teresa Thiel, University of Missouri-St Louis, Regulation of the nif1 and nif2 gene clusters in Anabaena variabilis

4.3 Megan Smeets, Illinois Wesleyan University, Restoration of heterocyst production to a ΔhetP strain of Anabaena

4.4 Xudong Xu, Institute of Hydrobiology, Modulation of the expression of patS by HetZ, an ssDNA-binding protein, in Anabaena sp. PCC 7120

4.5 Emiko Sano, University of Montana, Local adaptation by an ancient global polymorphism for heterocyst function

4.6 Douglas Risser, University of the Pacific, Defining the gene regulatory network promoting hormogonium development in Nostoc punctiforme

4.7 Amin Omairi-Nasser, University of Chicago, How do Anabaena cells communicate?

4.8 Jeff Elhai, Virginia Commonwealth University, Inviability of Anabaena lacking three RG(S/T)GR pentapeptide-containing negative regulators of differentiation

5. Posters, Session 5, Photosynthesis and CO₂

5.1 Qiang Wang, Institute of Hydrobiology, Thf1 helps to stabilize PSI under high light in Synechococcus sp. PCC 7942

5.2 Marcia Ortega Ramos, Penn State University, Discovering the roles of PSI variants in heterocysts

5.3 Visily Kurashov, Penn State University, Investigation of Photosystem I embedded in a trehalose glass matrix

5.4 Vicki Moore, Arizona State University, Examining the role of photosystem stoichiometry in Synechocystis

5.5 Gaozhong Shen, Penn State University, Acclimatory responses and biogenesis of IsiA-family proteins in cyanobacteria

5.6 Hui-Yuan Steven Chen, Washington University, The role of carotenoids in energy transfer within the IsiA pigment protein

5.7 Travis Korosh, University of Wisconsin-Madison, Examining the effects of alternative electron transport in cyanobacteria

5.8 Wim Vermaas, Arizona State University, The electron transfer pathway upon H₂ oxidation by the NiFe bidirectional hydrogenase of Synechocystis sp. PCC 6803 in the light shares components with the photosynthetic electron transfer chain in thylakoid membranes

5.9 Neil Miller, Oklahoma State University, Subunits of NDH-1 in Synechocystis sp. PCC 6803 are regulated by light and inorganic carbon

5.10 Feiyan Liang, Uppsala University, Overexpressing photosynthetic carbon flux control enzymes in Synechocystis PCC 6803
5.11 Julian Eaton-Rye, University of Otago, Bicarbonate-reversible inhibition of the iron-quinone acceptor complex of Photosystem II lacking low-molecular-weight proteins or with targeted mutations to the D1 protein

5.12 Tina Summerfield, University of Otago, D1´-containing PS II reaction center complexes under different environmental conditions in Synechocystis sp. PCC 6803

5.13 Rob Burnap, Oklahoma State University, Structural rearrangements preceding dioxygen formation by the water oxidation complex of photosystem II

5.14 Anton Avramov, Oklahoma State University, Membrane fluidity as a limiting factor for PS II repair mechanism

5.15 Paul Janssen, Belgian Nuclear Research Center, Unique features of Arthrospira D1 proteins

5.16 Erin Bonisteel, Mount Allison University, Quantitative characterization of the FtsH protease in relation to PsbA turnover in Prochlorococcus and marine Synechococcus

5.17 Matthew Melnicki, Lawrence Berkeley National Laboratory, Structure, diversity and evolution of a new family of soluble carotenoid-binding proteins

5.18 Robert Blankenship, Washington University, Native mass spectrometry characterization of fluorescence recovery protein and its interaction with orange carotenoid protein

5.19 Haijun Liu, Washington University, Photoactivation and relaxation studies on the cyanobacterial OCP in the presence of different metal ions

5.20 Sigal Lechno-Youssef, Michigan State University, Towards understanding of function of the evolutionary related homologs of the orange carotenoid protein in Fremyella diplosiphon

5.21 Ming-Yang Ho, Penn State University, Far-red light photoacclimation (FaRLiP) in Synechococcus sp. PCC 7335: Central control and a novel combination of antenna structures

5.22 Nathan Soulier, Penn State University, Heterologous expression and characterization of far-red absorbing phycobiliproteins from two photoacclimative responses

5.23 Jesús Barrera-Rojas, CINVESTAV-IPN, Characterization of photosynthetic membrane complexes from Prochlorococcus marinus MIT 9313

5.24 Henning Kirst, UC-Berkeley, Antenna engineering in cyanobacteria to improve solar-to-biomass energy conversion efficiency

5.25 Christina Kronfel, University of New Orleans, Characterizing the function of CpeF in phycoerythrin biosynthesis

5.26 Lyndsay Carrigee, University of New Orleans, Characterizing the function of bilin lyases CpeY and CpeU in marine Synechococcus RS9916

5.27 Adam Nguyen, University of New Orleans, Characterization of the putative bilin lyase MpeY from Synechococcus RS9916

5.28 Fei Cai, Lawrence Berkeley National Laboratory, Understanding β-carboxysomes through production of synthetic carboxysome shells
Poster sessions are in Biodesign, 1st Floor and Basement. A badge is required for entry (South entrance).

5.29 **Cecilia Blikstad, UC-Berkeley/LBNL**, CcmM and its interaction with Rubisco and CcmN in cyanobacterial β-carboxysomes

5.30 **Manuel Sommer, UC-Berkeley and LBNL**, Characterization of the selective permeability of carboxysome shell proteins and implications for CO₂ fixation efficiency

6. **Posters, Session 6, Physiology and Metabolism**

6.1 **Julia Walter, University of Turku**, A novel calcium-binding protein in *Anabaena* sp. PCC 7120 is crucial for growth in calcium-depleted conditions

6.2 **Claudia Hackenberg, European Molecular Biology Laboratory**, Understanding the role of microcystin and CP12-CBS in *Microcystis aeruginosa*

6.3 **Alexander Makowka, University of Kiel**, Physiology of the Entner-Doudoroff pathway in cyanobacteria

6.4 **Bethany Hazen, California State University-Fresno**, Glutathione and glutathione-dependent enzymes protect *Synechococcus* PCC 7942 against stress

6.5 **Sean Geiger, California State University-Northridge**, Npun_F0288 plays a role in lipid droplet production and filament integrity in *Nostoc punctiforme*

6.6 **Nicole Fuentes, California State University-Northridge**, Carotenoid biosynthesis associated with lipid droplets in *Nostoc punctiforme*

6.7 **Ryan Clark, University of Wisconsin-Madison**, Kinetic modelling of light-limited cyanobacterial growth and chemical production

6.8 **Saratram Gopalakrishnan, Penn State University**, Cyanobacterial genome-scale mapping models for $^{13}$C-metabolic flux analysis

6.9 **Nanette Boyle, Colorado School of Mines**, Metabolic modeling of a nitrogen fixing bacterium

6.10 **Rajib Saha, Washington University**, Diurnal regulation of cellular processes in the cyanobacterium *Synechocystis* sp. PCC 6803: Insights from transcriptomic, fluxomic and physiological analyses

6.11 **Rey Allen, Arizona State University**, Axenic biofilms of *Synechocystis* PCC 6803 require cell surface structures and occur under nutrient limitation

6.12 **Ryan Simkovsky, UC-San Diego**, Prophage control of cyanobacterial growth behaviors

6.13 **Jacob Lamb, Norwegian University of Science and Technology**, The importance of pili-mediated metal acquisition in primary productivity

6.14 **Anne Vogel, Norwegian University of Science and Technology**, Nanowire-mediated iron acquisition in *Synechococcus* sp. PCC 7002

6.15 **Lisa Dirks, Arizona State University**, Enhancing phosphate uptake in cyanobacteria

6.16 **Jindong Zhao, Peking University**, An amidase that is required for proper nanopore formation on cell wall septa in cell-cell communication in *Anabaena* sp. PCC 7120

6.17 **Bianca Brahamsha, Scripps Institution of Oceanography**, Cell-cell signaling in marine *Synechococcus*: Dual transcriptome analysis of antagonistic strains
Poster sessions are in Biodesign, 1st Floor and Basement. A badge is required for entry (South entrance).

6.18 Derek Fedeson, Michigan State University, Development of surface-display in Synechococcus elongatus PCC 7942: Engineering a cyanobacterial attachment system toward multispecies photosynthetic consortia

6.19 Tanya Soule, Indiana University-Purdue University Fort Wayne, Timing of the Precambrian rise in atmospheric oxygen through molecular evolutionary reconstruction of the cyanobacterial sunscreen scytonemin

6.20 Anne Ruffing, Sandia National Laboratories, Ionizing radiation resistance in cyanobacteria

6.21 Hideaki Shiraishi, Kyoto University, Cryopreservation of the edible filamentous cyanobacterium Arthrospira platensis

6.22 Shashi Kiran Nivas, St Aloysius College, Environmentally sustainable microalgae as a high value supplement to fodder industry
Abstracts

Abstracts are arranged in the following order:

1. **Keynote lectures**, in chronological order
2. **Oral presentations**, ordered by session and in chronological order of the talks
3. **Poster presentations**, ordered by session and loosely arranged according to subtopic

*Editor’s note:* In some cases abstracts for poster or regular oral presentations exceeded 250 words for the complete abstract (title, affiliation, abstract body, and references). If so, the abstract may have been edited to come closer to the word limit without changing the main message of the abstract.

**Keynote Lectures**

**K.1. Nitrogen-fixing symbiotic interactions between *Nostoc punctiforme* and the hornwort *Anthoceros* spp. as a paradigm to enable *Nostoc*-crop plant associations: pipeline or pipedream?**

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The filamentous cyanobacterium *Nostoc punctiforme* is an exceptional experimental system. Amongst many other phenotypic traits, its vegetative cells have three conditionally dependent developmental fates: nitrogen-fixing-heterocysts that are present in a non-randomly spaced pattern; cold and desiccation resistant spore-like akinetes, that may initially be spaced relative to heterocysts; and transiently differentiated, non-growing, motile hormogonium filaments. Enabled by hormogonium and heterocyst differentiation, *N. punctiforme* can also grow in symbiotic association with a fungus and terrestrial plants that represent three of the major phylogenetic divisions: Bryophyte hornworts and liverworts, Gymnosperm Cycads and Angiosperms of the family Gunneraceae. We developed the hornwort *Anthoceros punctatus*-*N. punctiforme* association as an experimental system to identify the genetic and physiological traits that are essential for establishment of plant symbiotic nitrogen fixation. The results focusing on the *N. punctiforme* partner have allowed us to define distinct (i) hormogonium-dependent infection and (ii) heterocyst-dependent functional stages of symbiotic interaction that appear largely to be under control of the plant partner. The symbiotic growth, developmental, morphological and physiological characteristics of *N. punctiforme* will be discussed (1). We are transitioning to a new plant partner, *Anthoceros agrestis*, which is amenable to genetic manipulation and displays the gametophyte-sporophyte life cycle in axenic culture (2). Our ultimate goal is to enable engineering of similar associations with crop plants, such as rice and/or maize. The reasonable possibility of engineering a *N. punctiforme* association with crop plants has been enhanced by the recognition of a common symbiotic signaling pathway between essentially only leguminous plants and nitrogen-fixing rhizobia bacteria and nearly all plants and arbuscular mycorrhizal fungi (3), the pathway of which will be presented. Genetic markers of the common pathway are present in hornworts and our initial assays are to determine their expression in response to the presence of *N. punctiforme*.

**References**


K.2. Global metabolic rewiring of an obligate photoautotrophic cyanobacterium for production of chemicals under diurnal light conditions

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Chemical production in photosynthetic organisms is a nascent technology with great promise for renewable chemical production. Cyanobacteria are under investigation as a means to utilize light energy to directly recycle CO$_2$ into renewable chemical compounds currently derived from petroleum. However, while genetic engineering tools are readily available for model organisms such as *Escherichia coli* and *Saccharomyces cerevisiae*, this is not the case for cyanobacteria. We have previously engineered production of the chemical feedstock 2,3-butanediol (23BD) from an obligate photoautotrophic cyanobacterium, *Synechococcus elongatus* PCC 7942$^1$. We subsequently explored the optimization of 23BD production by varying ribosomal binding site and promoter strength, operon organization, and gene expression at the transcriptional and translational level$^{2,3,4}$. The resulting engineered strains exhibited enhanced total carbon fixation and 23BD production under continuous light conditions$^4$. We concurrently observed an increase in oxygen evolution correlating to high carbon redirection away from metabolism, indicating the possibility of an increase in photosynthetic efficiency overall. Any large-scale cyanobacterial production scheme must rely on natural sunlight for energy, thereby limiting production time to only lighted hours during the day. To overcome this limitation we engineered *S. elongatus* for production of 23BD in continuous light, diurnal light, and continuous dark conditions via supplementation with glucose or xylose$^5$. This study achieved efficient 23BD production under diurnal conditions, which was comparable with that under continuous light conditions. These advances in engineering cyanobacteria provide additional tools to understand cyanobacterial metabolism as well as increase the amount of CO$_2$ converted to valuable chemicals by these microorganisms.

References:

K.3. Modularity in Cyanobacterial Photoprotection and CO$_2$ fixation

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Broadly speaking, biological “modules” are cellular components or subsystems that are semi-autonomous functional units (1). They span the scales of biological organization; in cyanobacteria, for
example, modules include protein domains, operons, metabolic pathways and subcellular compartments such as the carboxysome. A modular perspective not only provides a framework for approaching structure--function studies, but it also foregrounds how changes in intramodule connectivity lead to new functions. Essentially, modules are powerful elements for “plug and play” into new contexts through evolution or engineering. Recent studies on the Orange Carotenoid Protein (2-4) and the carboxysome (5-7) will be used to illustrate how a modular perspective is leading to new insights into the evolution of photoprotection and the carboxysome, and provides a foundation for their applications in synthetic biology (8).

References:


K.4. Diversity and dynamics of phototrophic communities

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Cyanobacteria are important and ubiquitous in many diverse and extreme environments. An understanding of how cyanobacteria adapt and thrive is crucial from several perspectives. We focused on microbial communities that form stratified biofilms or mats in hot-springs where 16S rRNA diversity has previously been correlated with environmental gradients of temperature and light. The genomes of two Synechococcus isolates that dominate at different temperatures in the mats, has provided many insights into genomic and metabolic diversity within these populations. I will describe some of our key findings. The metagenomic data in combination with deep amplicon sequencing from these communities, reveal an unexpectedly high degree of genomic micro-diversity. I will discuss the advantages and drawbacks of these approaches and our future plans. I will present our recent attempts to explore the co-evolution of host and cyanophage populations in the microbial mats, by creating viromes and exploiting CRISPR spacers. Finally, we are also interested in probing the role and importance of phototaxis in these structured communities.
K.5. Engineering nitrogen fixation ability in *Synechocystis* 6803

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Certain cyanobacterial strains are capable of atmospheric nitrogen fixation, a counter-intuitive task, since nitrogenase, the enzyme that fixes nitrogen, is rapidly inactivated in the presence of oxygen. To achieve this goal, unicellular cyanobacteria such as *Cyanothece* 51142 separate these two activities temporally - they photosynthesize during the day and fix nitrogen during nighttime. Phylogenetically, *Cyanothece* 51142 is closely related to *Synechocystis* 6803. However, the latter strain cannot fix nitrogen. Our objective is to use a systems biology knowledgebase, exemplified by detailed models of metabolism and regulation, along with advanced synthetic biology tools to endow *Synechocystis* 6803 cells with the ability to fix nitrogen. *Cyanothece* 51142 has one of the largest intact contiguous *nif* gene cluster found in nitrogen-fixing cyanobacteria. The entire 28 kb cluster has been successfully transferred to *Synechocystis* 6803 and has remained stable for over two years. Under appropriate environmental conditions, the transformants can fix nitrogen. Genome-scale metabolic models have been developed for both *Cyanothece* 51142 and *Synechocystis* 6803, and are being implemented to aid in the fine-tuning of nitrogen fixation ability in the engineered *Synechocystis* cells. Supported by funding from the NITROGEN program of the National Science Foundation.
Oral presentations

Oral presentations are numbered by session. The speaker sequence is indicated by a letter, in alphabetical order.

1.A. Engineering cyanobacteria as photosynthetic platform for sustainably producing valuable chemicals directly from CO₂

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Many useful chemicals such as plant natural products are mainly produced by extraction from higher plants, which cannot keep up with the surging global demand. Furthermore, the over-felling of many medicinal plants has undesirable effects on the ecological balance. In our research, we tried to engineer cyanobacterial and construct autotrophic photosynthetic platforms to directly convert the greenhouse gas CO₂ into a range of the valuable chemicals, including resveratrol, naringenin, bisdemethoxycurcumin, p-coumaric acid, caffeeic acid, and ferulic acid. These compounds can be easily branched to many other precious and useful natural products. Various strategies have been systematically investigated to increase the production, including introducing a feedback-inhibition-resistant enzyme, creating functional fusion proteins, and increasing malonyl-CoA supply. The highest titer of these natural products reached 128.2 mg/L from the autotrophic system, which even exceeded those obtained by many other heterotrophic microorganisms using carbohydrates. Several advantages such as independence from carbohydrate feedstocks, assembly of functional P450s, and availability of plentiful NADPH and ATP demonstrate that this photosynthetic platform is uniquely suited for producing the plant natural products.

1.B. Engineering photosynthetic limonene and bisabolene production in the cyanobacterium Synechococcus sp. PCC 7002

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The plant terpenoids limonene and α-bisabolene are hydrocarbon precursors to various industrially-relevant chemicals. We have engineered *Synechococcus* sp. PCC 7002 to photosynthetically produce limonene or α-bisabolene through heterologous expression of the *Mentha spicata* L-limonene synthase or the *Abies grandis* (E)-α-bisabolene synthase genes, respectively. To increase partitioning of photoassimilated carbon and reductant toward terpenoid metabolism, we blocked the competing glycogen biosynthetic pathway through deletion of the ADP glucose pyrophosphorylase-encoding gene. Without glycogen biosynthesis the mutant excreted a suite of organic acids as an energy-spilling mechanism. However, these metabolites were not effectively utilized for terpenoid metabolism. We have conducted a thorough investigation of the dynamics of photosynthesis in the glycogen-deficient mutant to understand how cyanobacteria respond to major carbon-sink manipulation. Unexpectedly, the mutant maintained a higher photosynthetic capacity than wild type over prolonged nitrogen
deprivation, supporting continuous organic acid secretion in the absence of biomass accumulation, and retained a nutrient-replete-type ultrastructure including an extensive thylakoid membrane network. These findings suggest that multiple global signals for nitrogen deprivation are not activated in the mutant, thus allowing maintaining an active photosynthetic machinery under conditions where photosynthesis would normally be abolished. This unusual metabolism has important biotechnological implications toward engineering a photocatalytic cell factory that directly converts carbon dioxide to terpenoid hydrocarbons without cell growth and division.

References

1.C. Production of manoyl oxide, a precursor to the medically active compound forskolin, in
_Synechocystis_ PCC 6803

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Terpenoids (isoprenoids) are a large, diverse family of natural compounds including sterols, carotenoids and hormones, many of which are commercially interesting. Forskolin is a complex C_{20} diterpenoid that is produced in the root cork cells of the plant _Coleus forskohlii_, and that has pharmacological activities. Because of its complex molecular structure, chemical synthesis of forskolin is not economically attractive, and extraction and purification from _C. forskohlii_ plants is laborious and resource intensive. We have engineered the unicellular cyanobacterium _Synechocystis_ PCC 6803 to produce the forskolin precursor 13R-manoyl oxide (13R-MO) by expressing the two _C. forskohlii_ enzymes CfTPS2 and CfTPS3. These two nonnative genes were integrated into the genome at three different locations with two different promoters, with the top producing strains yielding production titers of 0.25 mg/L/g DCW 13R-MO. To further increase the yield, 13R-MO producing strains were engineered by introduction of selected enzymes from the _C. forskohlii_ non-mevalonate pathway, which resulted in improving the titer to 0.46 mg/L/g DCW in the highest producing strain. By cultivating the strains at different light intensities, large differences in product accumulation were observed, attributed to changes in promoter activities. Finally, we quantified carotenoid and chlorophyll content in our strains as those pigments are made or partially made from the same terpenoid precursor as 13R-MO and the results suggests that heterologous expression of 13R-MO influences pigment composition. This work is of interest for future engineering of terpenoid products in cyanobacteria and provides insight into promoter selection.

1.D. The plasticity of cyanobacterial metabolism supports direct CO$_2$ conversion to ethylene

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The cyanobacterial tricarboxylic acid (TCA) cycle functions in both biosynthesis and energy generation. However, it has until recently been generally considered to be incomplete with limited flux, and few attempts have been made to draw carbon from the cycle for biotechnological purposes. We demonstrated that ethylene can be sustainably and efficiently produced from the TCA cycle of the recombinant cyanobacterium _Synechocystis_ 6803 expressing the Pseudomonas ethylene forming
enzyme (Efe). A new strain with a modified ribosome binding site upstream of the efe gene diverts 10% of fixed carbon to ethylene and shows increased photosynthetic activities. The highest specific ethylene production rate reached 718 ± 19 μl l⁻¹ h⁻¹ per A_{730} nm. Experimental and computational analyses based on kinetic ¹³C-isotope tracer and liquid chromatography coupled with mass spectrometry (LC–MS) demonstrated that the TCA metabolism is activated by the ethylene forming reaction, resulting in a predominantly cyclic architecture. The outcome significantly enhanced flux through the remodelled TCA cycle (37% of total fixed carbon) compared with a complete, but bifurcated and low-flux (13% of total fixed carbon) TCA cycle in the wild type. Global carbon flux is redirected towards the engineered ethylene pathway. The remarkable metabolic network plasticity of this cyanobacterium is manifested by the enhancement of photosynthetic activity and redistribution of carbon flux, enabling efficient ethylene production from the TCA cycle.

References:

1.E. Increasing the tolerance of filamentous cyanobacteria to next-generation biofuels via directed evolution

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Renewable biofuels can lessen our reliance on fossil fuels. Cyanobacteria are being investigated for the production of biofuels directly from carbon dioxide, thus eliminating the steps of biomass production, harvest, logistics, and conversion required for 1st and 2nd generation biofuels. This study determined the initial tolerance of _Anabaena_ sp. PCC 7120, _Anabaena variabilis_ ATCC 29413, and _Nostoc punctiforme_ ATCC 29133 to four potential biofuels: farnesene, myrcene, linalool, and limonene. These cyanobacteria were then subjected to three rounds of directed evolution where the strains were exposed to increasing titers of each chemical. This led to a library of 12 putative mutants with higher tolerance to individual chemicals. These mutants were assessed for growth performance at chemical titers higher than the wildtype strains could tolerate. An _Anabaena_ 7120 mutant was developed that could tolerate 0.32 g/L farnesene, compared to 0.1 g/L in the wildtype. Similar, statistically significant improvements in linalool tolerance were obtained with _A. variabilis_ (0.72 g/L compared to 0.45 g/L for the wildtype) and _N. punctiforme_ (0.54 g/L compared to 0.45 g/L for the wildtype). This work serves as proof-of-concept that directed evolution is a valid methodology to increase the tolerance of filamentous cyanobacteria to biofuels. These strains can now be genetically engineered to produce the biofuel they have developed an increased tolerance to. Potentially, this will lead to increased biofuel production compared to wildtype strains that have also been genetically engineered to produce the biofuel.

1.F. Isotopically nonstationary ¹³C metabolic flux analysis guided strain engineering of isobutyaldehyde producing _Synechococcus elongatus_ PCC 7942

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Cyanobacterial biotechnologies are paving the way for environmentally conscious and renewable biosynthesis of industrially relevant commodities. These nascent systems, though promising, need to be
further optimized in order to achieve yields suitable for industrial applications. Understanding cellular metabolism in industrial conditions is important for strain improvement. We previously developed isotopically nonstationary metabolic flux analysis (INST-MFA) to characterize the photoautotrophic metabolism of *Synechocystis* PCC 6803 (Young et al. 2011). On-going research in our lab focuses on applying INST-MFA to characterize and predict pathway bottlenecks in engineered cyanobacterial production systems. This presentation will focus on our efforts in applying INST-MFA to an isobutyraldehyde (IBA) producing strain of *Synechococcus elongatus* PCC 7942 (SA590 (Atsumi et al. 2009)). We also discuss the effect of overexpressing the predicted pathway bottlenecks on cellular metabolism and IBA production. Our goal is to develop INST-MFA as an effective tool for guiding strain engineering of cyanobacterial systems.

References:

1.G. 13C-Metabolic flux analysis of *Synechococcus* sp. PCC 7002 and its glycogen mutant under photoautotrophic conditions

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Isotopically non-stationary 13C labeling studies were performed on *Synechococcus* sp. PCC 7002 using the wild type (WT) and a mutant lacking the genes encoding glycogen synthase (*A1532/glgA1* and *A2125/glgA2*) and impaired in glycogen synthesis. Removal of this native carbon sink could increase carbon flux towards other value-added products. Comparative analysis of the WT and glycogen deficient mutant provided insight into the re-routing of carbon through primary intermediates of metabolism and the flux ratios at critical branch points. Slow labeling of TCA Cycle relative to glycolysis and Calvin Cycle was observed in both strains. Labeling of 2-phosphoglycolate (2PG), the first committed intermediate in photorespiratory metabolism, is slow, thus the carboxysome-based carbon concentrating mechanism may be fully active in these strains. Also, in the WT strain 13C-enrichment of intermediates at steady state reveals the existence of inactive pools of metabolites that get labelled at reduced rates. Currently, transient labeling data for the mutant are being used to estimate the intracellular fluxes with an isotopically nonstationary metabolic flux analysis approach (INST-MFA). Transient labeling of the glycogen precursor ADP-glucose and soluble sugar UDP-glucose was slower in the mutant than in WT. Comparison of flux maps for WT and mutant will provide further insights that will be of value in metabolic engineering efforts in cyanobacteria. Authors acknowledge financial assistance from Reliance Industries Ltd, India.
1.H. Expression of holo-proteorhodopsin in *Synechocystis* sp. PCC 6803

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Retinal-based photosynthesis may contribute to the free energy conversion needed for growth of an organism carrying out oxygenic photosynthesis, like a cyanobacterium. After optimization, this may even enhance the overall efficiency of phototrophic growth of such organisms in sustainability applications. As a first step towards this, we here report on functional expression of the archetype proteorhodopsin in *Synechocystis* sp. PCC 6803. Upon use of the moderate-strength psbA₂ promoter, holo-proteorhodopsin is expressed in this cyanobacterium, at a level of up to $10^5$ molecules per cell, presumably in a hexameric quaternary structure, and with approximately equal distribution (on a protein-content basis) over the thylakoid and the cytoplasmic membrane fraction. These results also demonstrate that *Synechocystis* sp. PCC 6803 has the capacity to synthesize all-trans-retinal. Expressing a substantial amount of a heterologous opsin membrane protein causes a substantial growth retardation *Synechocystis*, as is clear from a strain expressing PROPS, a non-pumping mutant derivative of proteorhodopsin. Relative to this latter strain, proteorhodopsin expression, however, measurably stimulates its growth.

2.A. Pigment Localization and Dynamics in Individual Cyanobacterial Cells

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The identity, abundance, and localization of photosynthetic pigments within cyanobacterial cells are critical to understanding the cell’s response to its environment. Unfortunately, photosynthetic pigments are collocated in pigment-protein complexes and have an inherently high degree of spectral overlap, making them difficult to differentiate using traditional fluorescence microscopy methods. We have previously shown that hyperspectral confocal fluorescence microscopy coupled with multivariate analysis is uniquely suited to untangle the highly overlapped spectral signatures from photosynthetic pigments and reveal pigment localization in intact, living cells [1,2]. Here, we utilize this technique to identify and map multiple photosynthetic pigments in individual cyanobacterial cells in response to changing environmental conditions (nitrogen starvation). This method provides increased understanding of global pigment dynamics and function, specifically highlighting heterogeneity of cellular response unavailable from bulk analytical techniques. The results have important implications for synthetic biology and development of biohybrid or bio-inspired devices.

References

2.B. Carbon partitioning and management of energy in a glycogen-deficient Synechocystis strain

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The regulation of photosynthetic carbon partitioning remains to be understood. We previously reported that removal of a major carbon sink can redirect the carbon flow towards the production of specific metabolites under stress conditions: when deprived of nitrogen, the cyanobacterial Synechocystis sp. PCC6803 mutant strain incapable of glycogen synthesis (∆glgC) excretes the organic acids 2-oxoglutarate (alpha-ketoglutarate) and pyruvate for several days, without concomitant increase in cell biomass, in a process described as “overflow metabolism”¹. Our recent observations that the ratio between those two products can be differentially altered by environmental conditions have led us to suggest that the regulation of carbon partitioning might be tightly linked to photosynthate (ATP and NADPH) availability. Following these new insights, we studied the energy and redox state of wild-type Synechocystis (WT) and ∆glgC strains under various growth conditions. We show that ∆glgC exhibits a higher energy charge than WT even under non-saturating light conditions and uses overflow metabolism as a strategy to maintain energy homeostasis.


2.C. The Entner-Doudoroff pathway is an overlooked glycolytic route in cyanobacteria and plants

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Glucose degradation pathways are central for energy and carbon metabolism and provide ATP, NAD(P)H, and precursors for amino acids, nucleotides and fatty acids. Cyanobacteria and plants oxidize carbohydrates via glycolysis and the oxidative pentose phosphate (OPP) pathway. However, both possess a third, previously overlooked pathway of glucose breakdown, the Entner-Doudoroff (ED) pathway². Its key enzyme 2-keto-3-deoxygluconate-6-phosphate (KDPG)-aldolase is widespread in cyanobacteria, moss, ferns, algae and plants. Active KDPG-aldolases from Synechocystis and barley were biochemically characterized. KDPG, a metabolite unique to the ED-pathway was detected in both in vivo indicating an active ED-pathway. Phylogenetic analyses revealed that photosynthetic eukaryotes acquired KDPG-aldolase from the cyanobacterial ancestors of plastids via endosymbiotic gene transfer. Studies on Synechocystis mutants lacking key enzymes of all three glucose degradation pathways revealed that the ED pathway is physiologically significant especially under mixotrophic conditions (light and glucose) and under autotrophic conditions in a day/night cycle, which is commonly encountered in nature. The ED-pathway has lower protein costs and ATP yields than glycolysis², in line with the observation that oxygenic photosynthesizers are nutrient- rather than ATP-limited. Furthermore, the ED-pathway does not generate futile cycles in organisms that fix CO₂ via the Calvin-Benson-cycle.

References
2.D. Tunable Gene Repression in *Synechococcus* sp. strain PCC 7002 using CRISPRI

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Tunable gene repression presents novel opportunities as a means to disrupt essential proteins and metabolic pathways. We have adapted the CRISPR-Cas9 system from *Streptococcus pyogenes* to silence genes in cyanobacterium *Synechococcus* sp. strain PCC 7002. The CRISPR (clustered regularly interspersed palindromic repeats) system has been adapted to repress genes and coined as CRISPR interference (CRISPRi). A specific target sequence can be repressed by the action of a single guide RNA (sgRNA) that is complementary to the target sequence. The catalytically inactive Cas9 protein recognizes the sgRNA and binds to that site, preventing RNA polymerase from transcribing messenger RNA.\(^1\) We have adapted this system for PCC 7002 and achieved inducible repression of heterologous yellow fluorescent protein, the phycobilisome, and essential genes encoding the carboxysome. This is the first example of titratable repression in cyanobacteria using CRISPRi, allowing strains to be grown and manipulated before silencing a specific target. This tool facilitates the study of essential genes of unknown function and enables groundbreaking metabolic engineering capability, by providing a straightforward approach to redirect metabolism and carbon flux in the production of high-value chemicals.

References:

2.E. Dynamic Localization of the Cyanobacterial Circadian Clock

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Circadian rhythms, regulated by a 24-h biological clock, are vitally important for controlling temporal programs of cellular physiology. Cyanobacteria are the only prokaryotes known to have a circadian clock. The *Synechococcus elongatus* PCC7942 oscillator, encoded by the kaiA, kaiB and kaiC genes, regulates global patterns of gene expression and the timing of cell division. Through the use of functional fusions to fluorescent proteins, we observe that KaiA and KaiC localize as discrete foci at the poles of cells. Clock protein localization is itself a circadian regulated process where enhanced polar localization is observed at night in a clock-dependent fashion. The molecular mechanism that localizes KaiC to the poles is conserved in *Escherichia coli* suggesting that KaiC localization is not dependent on other clock- or cyanobacterial-specific factors. In contrast, KaiA localization is entirely dependent on kaiC; consistent with this notion, KaiA and KaiC co-localize with each other as well as with CikA, a key input factor previously reported to display unipolar localization. Expression of a cikA mutant variant that displays diffuse localization throughout the cell results in de-localization of KaiC. We propose a model in which the observed spatio-temporal pattern of localization facilitates interactions with metabolites of photosynthesis, including the redox-active quinone pool in the membranes, and protein complex assembly, which together contribute to the synchronization and robustness of the circadian clock.
3.A. Seen and unforeseen interactions in engineered photosynthetic consortia

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Microbes constantly communicate; within consortia of limited complexity or communities containing diverse species, cellular conversations are being had. Properties that emerge from interaction include the ability to divvy up tasks, what we call division of labor, and the ability to survive perturbation, or robustness. Photosynthetic consortia demonstrate these characteristics with stunning clarity: division of labor is inherent in the relationship between phototrophs and heterotrophs while well-known photosynthetic consortia like lichens and cyanobacterial mats display robustness to extreme environmental perturbations. In an effort to better engineer and observe interactions, division of labor, and robustness in photosynthetic consortia, we constructed flexible photosynthetic communities containing Synechococcus elongatus and the model heterotrophs Escherichia coli, Bacillus subtilis, and Saccharomyces cerevisiae. We take advantage of S. elongatus to provide the fixed carbon source for our microbial communities. Within these consortia we witness foreseen and unforeseen microbial interactions that result in division of labor as well as stability over day-to-month time scales and robustness to certain environmental perturbations. Not only have we observed these populations, but we have also added heterotrophs that produce valuable compounds to demonstrate photoproduction from microbial communities. Altogether, this work demonstrates simplified photosynthetic consortia that recapitulate characteristics of natural systems while also demonstrating photoproduction from microbial communities.

3.B. Effect of low concentration of nitrate on the nitrogen metabolism of Synechococcus WH7803

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The versatility of marine Synechococcus spp. has been related to its ability to grow over a wide range of light intensities and to utilize a wide variety of nitrogen sources. On the other hand, it has been described that in stratified Sargasso seawater nanomolar changes in nitrate concentrations occurred. This change was stoichiometrically consistent with the subsequent cellular production of a Synechococcus bloom (Glover et al, 1988). With that background, the question to answer is how are Synechococcus spp. able to successfully coexist with Prochlorococcus spp.? For that, we analyzed the effect of very low concentrations of nitrate on key enzymes and key genes related to nitrogen metabolism. We also determined the uptake under this condition. The results were interesting and showed the different regulation system respect to other model cyanobacteria of the level of nitrogen. To test this hypothesis a study using RNAseq is performed in order to give us new insights.

References:
3.C. Nursing the biological soil crust restoration: cyanobacteria isolation, lab cultivation, scaling up and inoculum conditioning
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Biological soil crust (biocrusts) communities provide important ecosystems services to arid lands regarding soil fertility and stability. Filamentous cyanobacteria, such as Microcoleus vaginatus and Microcoleus steenstrupii, are considered biocrust pioneers and assist in soil surface stabilization. Soil stabilization promotes the successional establishment of other biocrusts organisms, including other cyanobacteria. Human activities, ranging from cattle grazing to military training, have resulted in significant deterioration of biocrusts surface cover of soils. Aiming at developing an effective restoration strategy, we established a “biocrusts-nursery” that serves as an inoculum supply for restoration. We isolated and grew large quantities of the main biocrusts cyanobacteria from a variety of soil types (sandy and silty) in cold and warm deserts of military lands in the southwestern US. Traditional scale-up methods from the algae industry gave good yields when growing some of the target cyanobacteria (Nostoc sp., Tolypothrix sp. and Scytonema sp.). Yet, when growing Microcoleus spp., biomass yields were very low. We developed a method that gives excellent biomass yield. By implementing this new approach, we obtained exponential and fast growth of the biocrust pioneers Microcoleus vaginatus and Microcoleus steenstrupii. Our inoculum formulation is based on pedigreed laboratory cultures that match the cyanobacterial relative abundance of the original sites, and additionally, have been conditioned to dry-wet cycles and increasing light exposure, with the goal of increasing field adaptation and survival rates. Ultimately, this nursery approach should help us to increase field recovery rates.

4.A. Transcriptional regulation of nitrogen fixing genes
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Nitrogen is essential to plant growth. However, N2 gas cannot be directly assimilated for biosynthesis. The goal of our project is to develop biological design principles that enable engineering nitrogen-fixing (diazotrophic) plants. The major challenge of fixing nitrogen by oxygenic organisms is to separate the oxygenic photosynthesis from the oxygen-sensitive nitrogen fixation process. Cyanothece 51142, a diazotrophic cyanobacterium accommodates both processes in a single cell by temporal separation. The specific aim for our research is apply synthetic biology tools that enable a non-diazotrophic unicellular cyanobacterium, Synechocystis 6803 that mimics the chloroplast of plant cells, to fix nitrogen. It has been demonstrated in a collaborative project that an engineered Synechocystis with the nif cluster of Cyanothece showed nitrogenase activity. However, the highest level of activity achieved only 2.5% compare to Cyanothece. To overcome this issue, the main approach incorporates the engineering of PatB, a Cyanothece transcription factor that may serve as a transcriptional regulator of nif genes in Cyanothece. PatB regulation1,2 has been revealed in cyanobacteria that use different strategies to fix atmospheric nitrogen. We aim to explore the native transcriptional regulation of nif genes and characterize the specific binding of PatB to key promoter regions in the nif cluster. Furthermore, we will evaluate the PatB regulatory role on nitrogenase activity in vivo in the engineered Synechocystis.

References:
4.B. Regulation of Heterocyst Commitment in *Anabaena* sp. strain PCC 7120
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Multicellular organisms must coordinate the differentiation of physiologically distinct cell types. This change from a pluripotent to a specialized cell is often irreversible and therefore termed terminal differentiation. The study of molecular mechanisms that underlie the commitment to terminally differentiate, however, is challenging because commitment takes place before any obvious morphological changes have occurred and can be occluded by the three-dimensional complexity of tissues. The multicellular cyanobacterium, *Anabaena*, serves as a model for the study of commitment as it consists of only two cell types: the pluripotent vegetative cell and the terminally differentiated heterocyst. The timing of commitment to a heterocyst fate in *Anabaena* has been described but the regulatory mechanisms that govern this timing are not yet fully understood (1). To clarify the regulation of cell fate commitment in this model system, a series of mutations were created and their epistatic and biochemical relationships were assessed. We show that the heterocyst regulator, HetP, functions in concert with three homologous proteins, Alr3234, Alr2902, and Asl1930, to modulate the timing and efficiency with which differentiating cells achieve a heterocyst fate commitment. We suggest that these four proteins may regulate a downstream switch that terminally restricts cell fate.

References:

4.C. *In vivo* characterization of nitrogenase kinetics in *Anabaena variabilis* ATCC 29413 using cavity ring-down spectroscopy
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Cyanobacteria are important contributors of the nitrogen cycle, particularly in the climate change sensitive boreal biome. The study of biological nitrogen fixation regulation and metabolism is made particularly difficult due to the lack of temporal resolution of classical methods used to measure N fixation. In this work, we used acetylene reduction by cavity ring down spectroscopy (ARACAS, Cassar et al. 2012) to study, for the first time *in vivo*, kinetic parameters of the molybdenum and vanadium nitrogenase in pure culture of *Anabaena variabilis* ATCC 29413. Such study was only achieved using purified enzymes so far (Miller and Eady 1988). We confirmed that the molybdenum and the vanadium nitrogenases achieved similar activity at low temperature (<10°C), mainly driven by Michaelis’s constant dependency to temperature. These results illustrate that the ARACAS method is a powerful tool to investigate the physiology of cyanobacteria and other nitrogen fixers. This also raises questions regarding the role of the vanadium nitrogenase in boreal biomes and elsewhere.

References
5.A. Structure and function of red-shifted phycobilisomes isolated from Chl f-containing cyanobacterium *Halomicronema hongdechloris*

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Phycobilisomes are the main light-harvesting protein complexes in cyanobacteria and some algae. These complexes only absorb green and orange light, complementing chlorophyll absorbance. We present a new phycobilisome derived complex that consists only of allophycocyanin core subunits, having red-shifted absorption peaks of 653, 712 nm and a shoulder at ~730 nm. These red-shifted phycobiliprotein complexes were isolated from the chlorophyll *f*-containing cyanobacterium, *Halomicronema hongdechloris*, grown under monochromatic 730 nm-wavelength (far-red) light. The 3D model obtained from single particle analysis reveals a double disk assembly of 120–145 Å with two α/β allophycocyanin trimers fitting into the two separated disks. They are significantly smaller than typical phycobilisomes formed from allophycocyanin subunits and core-membrane linker proteins, which fit well with a reduced distance between thylakoid membranes observed from cells grown under far-red light. The distribution of those red-shifted phycobiliproteins *in situ* was investigated using confocal fluorescence microscopy. Our findings show that red-shifted phycobilisomes are required for assisting efficient far-red light harvesting and transfer the energy to Chl *f*-containing photosynthetic protein complexes. These red-shifted absorption features may be part of a reduced model of phycobilisome, with altered pigment binding states within the protein complexes. These new red-shifted phycobilisomes have implications for flexible strategies in chromatic acclimation, which would improve light-harvesting efficiency.

5.B. Light colour matters in cyanobacterial photosynthesis

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Microalgal growth is often limited by the amount of usable light; in nature the conversion efficiency of light energy into biomass ranges from less than 1% to maximally 6.4% in plants and up to 10% for microalgae¹. This low energy efficiency in nature implies many opportunities for efficiency improvement for algal growth under artificial light conditions. Light emitting diodes (LEDs) produce quite narrow-banded emission peaks at low energy costs, hence seem promising for artificial lighting. We have used LED light at 440 nm, 625 nm and 660 nm, absorbed by chlorophyll *a*, phycocyanin and chlorophyll *b* (and chlorophyll *a*) respectively, to contrast the growth efficiency of *Synechocystis* sp. PCC6803 in light of different colours. The results demonstrate that light harvesting capacity and distribution of light to the reaction centres of photosystems 2 and 1 and the ratio of both photosystems are strongly regulated. Furthermore, the light-to-biomass conversion showed a much lower efficiency at blue light than at orange and red light.

References:

5.C. A Novel Tetrameric Form of Photosystem I Widespread in Cyanobacteria: Structure, Occurrence, Function, and Evolution

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Photosystem I (PSI) forms trimeric complexes in most characterized cyanobacteria. We reported the tetrameric form of PSI, possibly in equilibrium with monomeric and dimeric species, in the unicellular cyanobacterium, Chroococcidiopsis sp. TS-821 (TS-821). Using CryoEM, a 3D model of PSI tetramer structure at ~11.5 Å resolution was obtained and a 2D map within the membrane plane of ~6.1 Å. These data were used to build a model that was compared with the high-resolution structure of the PSI of T. elongatus at 2.5 Å. This comparison reveals key differences in the two different interfaces. Phylogenetic analysis based on Psal sequences shows that TS-821 is closely related to heterocyst-forming cyanobacteria. We speculated that PSI in all heterocyst-forming cyanobacteria are tetrameric and here we test this hypothesis. Our results show that heterocyst-forming cyanobacteria and close relatives have tetrameric or dimeric PSI. This tetrameric form of PSI was closely correlated with a different genomic organization of the psa genes. These cyanobacteria showed different stability and tendency to form tetrameric PSI. Some potential physiological and environmental factors that potentially affect tetrameric PSI quantity have been investigated. Nitrogen source and salinity have little effect on the presence of tetrameric PSI. Further investigation will determine the physiological significance of the tetrameric PSI vs the trimeric counterpart. The evolutionary implication of non-trimeric PSI is discussed as well.


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In vitro solar biohydrogen systems using cyanobacterial Photosystem I (PSI) as the light-driven photochemical module have been developed where cysteine 14 of the PsaC stromal subunit is replaced by glycine (C14G), eliminating a ligand to the terminal [4Fe-4S]²⁺/¹⁺ F₈ cluster of the PSI electron transfer chain (ETC). C14G PsaC has an exchangeable coordination site capable of chemical rescue by sulfhydryl-containing compounds allowing tethering of exogenous components to PSI enabling electron tunneling from F₈ to redox-active catalysts. In Synechococcus sp. PCC 7002, replacement with C14G PsaC is possible and, unexpectedly, capable of photoautotrophy. This strain produces PSI complexes capable of forward electron transfer to soluble redox acceptors and charge recombination kinetics with diminished efficiency, but comparable to wild-type PSI. Electron paramagnetic resonance (EPR) spectroscopy shows characteristic signals from F₈ are absent while those from F₆₇, also a [4Fe-4S]²⁺/¹⁺ cluster in PsaC, are present. The dark spectrum of C14G PsaC-containing PSI complexes shows an axial paramagnetic spectrum indicative of a [3Fe-4S]¹⁺ cluster. This signal is sensitive to increasing illumination providing evidence the cluster is part of the ETC. In vitro reconstitution of the iron-sulfur cluster in these PSI complexes yields EPR resonances characteristic of a [4Fe-4S] cluster. This study suggests a [3Fe-4S]¹⁺/⁰ cluster participates in photosynthetic ETC of the C14G PsaC strain and establishes the groundwork for a light-driven PSI module.
5.E. Localization and functions of several subunits of NDH-1 complexes in the cyanobacterium *Synechocystis* sp. PCC 6803

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The cyanobacterial NAD(P)H dehydrogenase (NDH-1) complexes play crucial roles in variety of bioenergetic reactions. However, the localization and the physiological roles of several subunits of NDH-1 are not fully understood. Here, by constructing fully segregated *ndhM, ndhN, ndhO, ndhH, ndhJ* null mutants in *Synechocystis* sp. PCC 6803, we found that deletion of these genes but not *ndhO* severely impaired the accumulation of the hydrophilic subunits of the NDH-1 in the thylakoid membrane, causing the disassembly of NDH-1MS, NDH-1MS’ as well as NDH-1L, resulting in lethal phenotype in air or in the presence of glucose. In contrast, deletion of NdhO results in the suppression of the growth in the presence of glucose or at pH 6.5 in air. In the cytoplasm, either NdhH, NdhJ, or NdhM deleted mutant, but neither NdhN nor NdhO deleted mutant, failed to accumulate the NDH-1 assembly intermediate consisting of NdhH, NdhJ, NdhK and NdhM. Based on these results, we suggest that NdhM, NdhN, NdhH and NdhJ are essential for the stability and the function of NDH-1 complexes, while NdhO for the NDH-1 functions under the condition of inorganic carbon limitation. We discuss the roles of these subunits and propose a new NDH-1 model.

5.F. Structure-function studies of CupA, a protein involved in the carbon uptake system NDH-1, of *Synechocystis* sp. PCC6803

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Cyanobacteria are photosynthetic microorganisms that have evolved mechanisms to thrive in diverse situations. In aquatic environments, inorganic carbon (C\textsubscript{i}) is present as dissolved CO\textsubscript{2} and bicarbonate (HCO\textsubscript{3}\textsuperscript{-}), both used by cyanobacteria on its energy production. *Synechocystis* 6803 has five C\textsubscript{i} concentrating systems responsible for increasing C\textsubscript{i} concentration inside of the cell: three HCO\textsubscript{3}\textsuperscript{-} transporters and two CO\textsubscript{2} uptake systems. The latter catalyzes the (non)reversible conversion of CO\textsubscript{2} to HCO\textsubscript{3}\textsuperscript{-}, possibly with release of a proton across the membrane. They also avoid loss of internal CO\textsubscript{2} due to leakage, since CO\textsubscript{2} is diffusible. These systems are specialized NDH-1 complexes, NDH-1\textsubscript{3} (NdhF3/NdhD3/CupA/CupS) and NDH-1\textsubscript{4} (NdhF4/NdhD4/CupB), which have very little known about their mechanism. Mutants lacking one of these proteins present impaired CO\textsubscript{2} uptake. We constructed knockout mutants of the whole operon and later complemented with an ectopic overexpressing system under RuBisCO promoter control. Physiological analysis of these mutants, by chlorophyll fluorescence and O\textsubscript{2} evolution driven by bicarbonate, show a high C\textsubscript{i} requirement feature, but the complementation strain seems to have a phenotype more like wild type. Further studies will be directed to analyze mutants with point mutations on His/Cys of CupA protein and evaluate its possible role on carbonic anhydrase activity.
5.G. Streamlined Construction of the Carboxysome Core via Protein Domain Fusion

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In cyanobacteria, carbon fixation occurs in a bacterial microcompartment (BMC) called carboxysome, that encloses ribulose-1,5-bisphosphate carboxylase/oxygenase and carbonic anhydrase within a protein shell. BMCs hold great potential for industrial applications, as they inherently serve the purpose of increasing enzymatic reaction efficiency, sequestering volatile intermediates, separating toxic intermediates from the cell lumen. We have successfully re-engineered the carboxysome core of Synechococcus elongatus PCC 7942 by consolidating domains from four different gene products into a single chimeric protein. The resulting carboxysome resembles the native carboxysome in form and function. Our efforts are directed now in the deployment of the minimal carboxysome in heterologous hosts. Our results are a proof-of-concept of the feasibility of BMC engineering using a domain-based approach based on protein architectures. This lays the foundation for generating carboxysome-based cyanobacterial nanoreactors for the production of renewable chemicals, by demonstrating novel core construction via protein domain fusions.

References:

6.A. The Essential Gene Set for Cyanobacteria


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To identify the comprehensive set of genes and intergenic regions that impact fitness of the model cyanobacteria, S. elongatus, we created a pooled library of approximately 250,000 transposon mutants and used sequencing to identify the insertion locations. By analyzing the distribution and survival of these mutants we identified 718 of the organism’s 2,723 genes as essential for survival. The validity of the essential gene set is supported by its tight overlap with well-conserved genes and its enrichment for core biological processes. The differences noted between our dataset and these predictors of essentiality, however, have led to surprising biological conclusions. One such finding is that genes in the second half of the tricarboxylic acid (TCA) cycle are dispensable, suggesting that S. elongatus does not require a cyclic TCA process. Furthermore, the density of the transposon mutant library enabled statements on the essentiality of non-coding RNAs, regulatory elements, and other intergenic regions. Our survey of essentiality for every locus in the S. elongatus genome serves as a powerful resource for understanding the organism’s physiology and defines the essential gene set required for the growth of a photosynthetic organism.
6.B. Transcriptome of the N₂-fixing cyanobacterium UCYN-A over diel cycles

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An unusual symbiosis has been recently discovered between two uncultured picoplankters, a small (≤3 µm) prymnesiophyte and the unicellular diazotrophic cyanobacteria UCYN-A (Thompson et al 2012). UCYN-A lacks key metabolic pathways such as oxygenic photosynthesis and the Calvin cycle, however still retains the photosystem I (PSI) (Zehr et al 2008, Tripp et al 2010). In order to determine if UCYN-A expresses PSI genes and to identify genes with diel expression patterns, we developed a whole genome array over two diel cycle. The transcription patterns showed a high transcription during the day in the N₂-fixation and a high transcription during the night in the PSI genes. These results are completely opposite from those of most cyanobacteria in this phylogenetic group. In addition, we used a double-CARD-FISH (Catalyzed Reporter Deposition-Fluorescence in situ Hybridization) assay to study cell division of the host and symbiont during diel cycles. Preliminary observations with two UCYN-A cells per host before the sunset suggest that the cyanobacterial growth and division occur prior to the prymnesiophyte cell division.

References:

6.C. Towards multiscale models of cyanobacterial growth: a modular approach

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While many aspects of phototrophic growth are well understood, it remains a considerable challenge to elucidate the dependencies and interconnections between the diverse cellular processes that together constitute cellular growth. Computational modeling allows us to quantitatively describe the individual cellular processes relevant for growth. As yet, however, computational models are mostly confined to the inner workings of individual processes, rather than describing the manifold interactions between them in the context of a living cell. This contribution seeks to summarize existing computational models that are relevant to describe phototrophic growth and outlines how these models can be integrated into a coherent whole. Our ultimate aim is to understand cellular functioning and growth as the outcome of the coordinated operation of diverse yet interconnected cellular processes. In particular, we are interested in the temporal resource allocation problem of phototrophic growth. Based on a comprehensive account of intracellular interconversions and constraints, we seek to describe the processes and fluxes required to synthesize new cellular components. We demonstrate that appropriately constructed “whole-cell models” allow us to derive emergent properties of cyanobacterial functioning, such as the temporal dynamics of glycogen synthesis, based only on a narrow set of well-defined parameters and assumptions.

References:
6.D. Modeling Regulation and Metabolism in Cyanobacteria

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Computational modeling of cellular processes (regulation, metabolism) provides insights into an organism and guides future work. As nitrogen fixation requires an anaerobic environment, diazotrophic organisms such as Cyanothece 51142 temporally separate photosynthesis and nitrogen fixation. To incorporate this process into the non-diazotrophic Synechocystis 6803, regulatory networks over a diurnal cycle were developed for both Cyanothece and Synechocystis and used to identify two transcription factors native to Synechocystis, LexA and Rcp1, that are promising candidates to control the nif gene cluster and other pertinent metabolic processes, respectively. While regulatory networks can identify broad intervention strategies, modeling metabolism provides a more granular perspective, and enables both the identification of the metabolic repertoire of an organism and future strain design. Genome-scale metabolic (GSM) models were developed for two closely related strains, Synechococcus 7942 and the Synechococcus 2973. Despite the 99.8% genome sequence identity between the two strains, under similar conditions Synechococcus 2973 grows 3.7 times faster. Incorporation of experimental data as constraints on the model gave a fold change in growth rates on the same order of magnitude as the experimental observations, identifying carbon fixation rate as a contributing factor. The construction of metabolism and expression (ME) models for these two organisms has increased the scope of modeled cellular processes and allowed for the testing of additional causes of the differing growth rates.

6.E. Genes and proteins involved in the assembly and export of cyanobacterial extracellular polymeric substances (EPS)

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Many cyanobacteria produce extracellular polymeric substances (EPS), mainly composed of polysaccharides that can remain attached to the cell surface or be released to the environment (RPS-released polysaccharides). The mechanisms of EPS biosynthesis and export remain poorly understood. A phylum-wide analysis of genes/proteins putatively involved in the assembly and export of EPS revealed that cyanobacterial EPS production may not strictly follow one of the pathways previously characterized in other bacteria [1]. Several Synechocystis knockout mutants on EPS-related genes are being generated to unveil the role of the encoded proteins. In other organisms, EPS chain length is determined by the autophosphorylation of the Wzc C-terminal tyrosine residues and its dephosphorylation by the Wzb phosphatase. In Synechocystis, a Wzc homolog (Sll0923) is involved in EPS production [2]. Now, deletion mutants of wzc (construct kindly provided by [2]) and wzb (slr0328) were generated. For both, a decrease in the amount of RPS was observed, and a decrease in CPS (capsular polysaccharides) in the Δwzc. To elucidate whether Wzc is a substrate of Wzb, these proteins were overexpressed in E. coli, purified and used in in vitro assays. The results showed that the purified Wzb has phosphatase activity and dephosphorylates Wzc. The results obtained for other mutants will also be discussed.

References:

6.F. Scalability of Flat Photobioreactors: Incorporating Langrangian Fluid Mechanics in Growth Models

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Langrangian fluid mechanics was applied on a flat photobioreactor to model the tracking of cyanobacteria particles for scalability purposes. Particle Image Velocimetry (PIV) was applied to obtain the velocity field as an Eulerian representation of the flow of a bench scale flat photobioreactor. The PIV of the flat photobioreactor was utilized to validate a Computational Fluid Dynamics (CFD) model, where Langrangian mechanics was applied obtaining particles tracking. Our research suggests that unlike previous models where fluid dynamics is ignored, the rapid changes of dark: light cycles influenced by mixing impact the growth of photoautotrophic microorganisms. This significant information obtained from PIV and CFD models will be utilized to scale down the rapid light changes, observed in scaled-up photo bioreactors, at the lab to resemble more realistic conditions and predict reliable growth rates of photoautotrophic microorganisms for scalability purposes.

Posters

1.1. Cyanobacterial Platform for Production of Phenylpropanoids

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Because of their photosynthetic ability and biosynthetic versatility, cyanobacteria are promising candidates for the production of certain plant metabolites, including phenylpropanoids. These compounds are difficult to produce in nonphotosynthetic bacteria (such as \textit{E. coli}) because they lack the ability to produce functional plant cytochrome P450 enzymes. Tyrosine ammonia lyase catalyzes the production of p-coumaric acid from tyrosine. We produced p-coumaric acid in a strain of transgenic \textit{Synechocystis} 6803. Whereas a strain of \textit{Synechocystis} 6803 genetically engineered to express \textit{sam8}, a tyrosine ammonia lyase gene from the actinomycete \textit{Saccharothrix espanaensis}, accumulated little or no p-coumaric acid, a strain that both expressed \textit{sam8} and lacked \textit{slr1573}, a native hypothetical gene shown here to encode a laccase that oxidizes polyphenols, produced <82.6 mg/L p-coumaric acid, which was readily purified from the growth medium. \textit{Synechocystis} 6803 is also able to produce caffeic acid from p-coumaric acid. A strain expressing the \textit{Arabidopsis thaliana ref8} gene, which encodes a P450 enzyme coumarate-3-hydroxylase, was able to produce caffeic acid at a concentration of 7.2 mg/L from p-coumaric acid under oxygenic photosynthetic growth conditions. These studies demonstrated that cyanobacteria are well suited for the bioproduction of plant secondary metabolites that are difficult to produce in other bacterial systems.
1.2. Genetic Engineering of *Synechocystis* sp. PCC 6803 for Sustainable Production of Cinnamic Acid

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Phenylpropanoids belong to the largest group of the secondary metabolites produced by plants. They play important roles in plant growth and development, in addition to protecting plants against diseases, wounding, UV light and other stresses. All plant phenylpropanoids are derived from cinnamic acid, which is formed from phenylalanine by the phenylalanine ammonia-lyase (PAL). The cinnamic acid is a promising compound that is highly beneficial to human health for their anticancer, antitoxic and anti-inflammatory functions. Currently, the major industrial production methods of cinnamic acid are chemical synthesis or extraction from plants. However, the pitfalls associated with these methods are low production yield and high cost. Cyanobacterial platforms could be highly suitable for phenylpropanoid production due to potential high yield and eco-friendly, cost effective production processes. The primary purpose of this research is to explore the possibility of using the cyanobacterium *Synechocystis* PCC 6803 for biosynthesis of cinnamic acid. In this research, we genetically engineer a strain of *Synechocystis* 6803 to express a gene encoding a phenylalanine ammonia lyase (PAL), which converts phenylalanine into cinnamic acid.

References:


1.3. Pathway engineering for production of photosynthetic acetone and isoprenoids production from CO\textsubscript{2} using engineered cyanobacteria

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Cyanobacteria have been gathering global interests regarding CO\textsubscript{2} bio-mitigation due to the ability to direct utilization of CO\textsubscript{2}. Engineered cyanobacteria have been enabled to produce industry-relevant chemicals from CO\textsubscript{2}. Our engineering of *Synechococcus elongatus* PCC 7942 enabled continuous conversion of CO\textsubscript{2} to acetone as sole product via both ATP-driven malonyl-CoA synthesis pathway and heterologous phosphoketolase-phosphotransacetylase pathway [1]. Because of strong correlations between the metabolic pathways of acetate and acetone, supplying the acetyl-coA directly from CO\textsubscript{2} in the engineered strain, led to sole production of acetone without changing nutritional constraints, and without an anaerobic shift. Subsequently, 5% (v/v) CO\textsubscript{2} gas-stripping-based recovery systems allowed collection of the sole production of acetone from CO\textsubscript{2}. In addition, further modular engineering of cyanobacterial strains was applied to produce plant-derived secondary chemicals including isoprenoids from CO\textsubscript{2}. Our engineered strains could be modified to create bio-solar cell factories for sustainable photosynthetic production of acetyl-CoA-derived biochemicals. This work was supported Korea CCS R&D Center (KCRC) (no. 2014M1A8A1049277) and the National Research Foundation of Korea grant-funded by the Korean Government (Ministry of Science, ICT and Future Planning) (2016, University-Institute Cooperation program).

References:

1.4. Metabolic engineering of *Synechocystis* sp. PCC6803 for improved terpenoid production

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Plants produce a stunning diversity of terpenoids that have potential applications in industries from fuels to pharmaceuticals. The expression of the enzymes necessary to produce these molecules in bacterial cultures promises to dramatically reduce the cost of their production. Researchers have made significant progress in improving the titer, yield, and productivity in the production of terpenoids in *E. coli* and *S. cerevisiae* cultures. Production in these organisms, however, relies on providing carbon feedstocks which may be costly and may exacerbate food insecurity. Production of valuable molecules in cyanobacteria combines the advantages of the fast growth rates and easy genetic modifications of bacteria and yeast, with photosynthetic capability that obviates the requirement for a carbon feedstock. Researchers have had limited success producing terpenoids in cyanobacteria, with yields typically orders of magnitude less than what has been achieved in *E. coli* and *S. cerevisiae*. In this work, we apply metabolic engineering principles used to improve the productivity of non-photosynthetic model organisms to the production of the diesel replacement sesquiterpenoid, α-bisabolene, in *Synechocystis* sp. PCC6803.

1.5. Engineering Astaxanthin Production in *Synechocystis* sp. PCC 6803: Challenges and Successes

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*Synechocystis* sp. PCC 6803 is an attractive production platform for a variety of fuels and chemicals due to rapid doubling times, photosynthetic production from CO$_2$, a sequenced genome, and established molecular biology techniques. Unfortunately, the full potential of this technology has yet to be realized due to low product titers and productivity. Advances in pathway engineering strategies for cyanobacteria are necessary to develop industrial production strains. This presentation will focus on our efforts to develop additional molecular biology tools to control gene expression, to develop genome scale modeling to better understand metabolism, and the application of some of these tools to engineer astaxanthin production in *Synechocystis* sp. PCC 6803.
1.6. **Cyanobacterial Biosynthetics: fuel and chemicals production**

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The concept of a direct Photosynthesis-to-Fuels and Photosynthesis-to-Chemicals approach [1,2] entails application of a single organism acting both as photocatalyst and processor, absorbing sunlight, photosynthesizing, and generating useful bio-products. Reduction to practice of this concept is offered by the photosynthetic production of hemiterpene isoprene \( \text{C}_5\text{H}_8 \) and monoterpene \( \beta \)-phellandrene \( \text{C}_{10}\text{H}_{16} \) hydrocarbons by transformant cyanobacteria, generated from sunlight, carbon dioxide, and water. The work describes metabolic engineering approaches, whereby carbon flux in cyanobacteria is accelerated and diverted toward terpene hydrocarbon synthesis and release [3-5]. An important feature of this approach is the spontaneous product separation from the biomass, and from the liquid culture, simplifying product sequestration, harvesting, and quantification, thus enabling scale-up for process commercialization. The work will further discuss challenges encountered [5,6], including the regulation of endogenous cellular carbon partitioning between different biosynthetic pathways, over which the living cell exerts stringent control. Experimental approaches to up-regulate carbon flux through the terpenoid biosynthetic pathway, enhancing product-to-biomass carbon partitioning ratios in the cell and correspondingly enhancing the yield of hydrocarbons production will be discussed.

References:


1.7. **Controlling isoprene synthase expression in the cyanobacterium *Synechocystis*: a molecular biological approach**

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The development of cyanobacteria as photo-catalysts for bio-based fuel and chemical production has recently emerged in a growing effort to reduce global reliance on petrochemical products. Engineering the cyanobacterial genome for this purpose requires specific molecular biology tools for controlling the expression of heterologous genes. This work identifies a specific stem loop mRNA sequence that controls the genes that are transcribed before and after it in a heterologous construct. This sequence was shown to be a useful tool to successfully overexpress isoprene synthase to enhance isoprene production yields and carbon partitioning towards isoprene in the cyanobacterium *Synechocystis*. 
1.8. Metabolic Engineering for β-pinene Production in *Synechococcus* sp. PCC 7002

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Divestment from petroleum-derived products is of utmost environmental, economic, and political importance, as continuing to rely upon a rapidly-dwindling non-renewable resource for everyday functions ranging from food storage to transport is a losing endeavor. Microbes, and in particular cyanobacteria, present a promising avenue to replace not only conventional gasoline and diesel but also energy-dense aviation fuels (e.g. JP-5, JP-10) by commandeering native biosynthesis pathways. The β enantiomer of pinene, a dual-ringed aromatic terpenoid produced primarily by conifers, has comparable energetic properties to JP-5, and can be dimerized into a compound that is energetically equivalent to JP-10. β-pinene may be synthesized in *Synechococcus* PCC 7002 by supplementing the methyl-erythritol-phosphate (MEP) pathway with two enzymes, a geranyl diphosphate synthase (GPPS) and a pinene synthase (bPinS), adapted from *Abies grandis* and *Artemisia annua*, respectively. Transgenic cyanobacteria carrying the optimized genes for these enzymes have been isolated and verified. Initial assessments of these transgenic strains suggest relatively low production, as growth seems unperturbed despite pinene having been observed to have negative effects on PCC 7002 culture stability at concentrations above 40 mg/L; investigation of this toxic effect and the possibility of mitigating it by sequestering pinene in a dodecane overlay is ongoing. Characterization of pinene production by gas chromatography-mass spectroscopy is also underway and will be reported.

1.9. The impacts of β-pinene on the growth and metabolism of native and isoprene-producing *Synechococcus* sp. PCC 7002 cyanobacteria

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Cyanobacteria efficiently use solar energy and can be genetically modified to convert captured carbon into renewable bioproducts. Our group has engineered *Synechococcus* sp. PCC 7002 cyanobacteria to produce β-pinene, a precursor for numerous terpene products including high-density jet fuels. However, at high concentrations, pinene may accumulate in cell membranes, alter membrane integrity, and cause cell death. We are investigating the impacts of β-pinene on the growth, viability, and metabolism of *Synechococcus* to understand pinene toxicity and develop strategies to reduce its toxic effects. In sealed-bottle experiments, β-pinene began to inhibit growth at approximately 40 mg/L, and at 100 mg/L, β-pinene caused complete culture bleaching. Some colony-forming units could be recovered from bleached cultures, which suggests that cells may be selected for increased β-pinene tolerance. The effects of β-pinene on photosynthetic electron transport reactions in living cyanobacteria are being investigated with a BioLogic JTS-10 kinetics spectrophotometer. β-pinene did not have an immediate dramatic impact on cytochrome f/c\(_6\) and photosystem I P700 kinetics after 9 ms excitation but did slow re-reduction half-times by ~2-fold immediately following exposure to β-pinene at 100 mg/L. The impacts on electron transfer of longer term exposures are being investigated. Pinene sensitivity of enzymes in the methylerythritol phosphate (MEP) pathway, leading to isoprene, pinene and terpenes, is being investigated by assessing the impact of β-pinene on production of isoprene in engineered, isoprene-producing *Synechococcus* sp. PCC 7002 cyanobacteria. The results of these studies will be presented.
1.10. Regulated Promoters to Control Toxic Genes in the Methyerythritol Phosphate (MEP) Pathway of Synechococcus sp. PCC 7002

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We have engineered *Synechococcus* sp. PCC 7002 to produce isoprene, a volatile, gas-phase precursor of terpene bioproducts and biofuels. To further increase isoprene yield, genetic modifications can be made to enhance the MEP pathway. However, when expressed at high levels, some of the gene products, such as 1-deoxy-D-xylulose 5-phosphate synthase (DXS) may be toxic to the cyanobacteria. One strategy to alleviate this is to use regulated promoters. We are testing a temperature-sensitive repressor-promoter (cl857-pR) from bacteriophage lambda. At temperatures below ~32°C, the repressor binds to the promoter and prevents gene expression, but at higher temperatures, the repressor denatures, allowing gene expression. A cl857-pR regulator has been used in cyanobacteria, but not optimized for *Synechococcus* sp. PCC 7002. We first linked this regulator to a yellow fluorescent protein (yfp) gene to create a reporter construct that controls YFP expression. This construct has been introduced into *Escherichia coli* and tested in a turbidostat culture where it shows ~10-fold induction of gene expression as a function of temperature. The same cl857-pR-yfp reporter construct has been introduced into PCC 7002 where preliminary turbidostat data show temperature-dependent induction of ~4-fold. The cl857-pR regulator is being further refined for *Synechococcus* sp. PCC 7002 and has also been linked to potentially toxic MEP pathway genes to test temperature-controlled gene regulation as a strategy for increased isoprene and terpene production in *Synechococcus*.

1.11. Alkane production in Synechococcus sp. PCC 7002

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Cyanobacteria can utilize solar energy to convert CO2 into biofuels such as alkanes, the major constituents of gasoline, jet fuels and diesel. Certain cyanobacteria are known to have an alkane biosynthesis pathway consisting of an acyl–acyl carrier protein reductase (AAR) and an aldehyde decarboxylase (ADO), which convert the intermediates of fatty acid metabolism to alkanes. We attempt to heterologously express the AAR and the ADO enzymes from *Nostoc punctiforme* ATCC 29133 and *Lyngbya aestuarii* BL J in *Synechococcus* sp. PCC 7002, a fast growing euryhaline bacterium that can be modified using acrylic acid-based counter selection. Since this strain has no native AAR and ADO enzymes it forms an excellent chassis for alkane production. Heterologous expression of AAR and ADO genes from the strain ATCC 29133 in PCC 7002 resulted in a strain with high alkane production capacity (1). *Lyngbya aestuarii* BL J is a marine cyanobacterium isolated from a marine intertidal mat in Baja California. Since microbial mats are known to contain alkanes (2, 3), it is likely that the cyanobacterial strains from the mats harbor AAR and ADO that can result in high alkane production. We study the alkane production capacity of the resulting strains along with the possibility of developing an alkane sensor to monitor the alkane produced.

References

1.12. Mitigating Rate-Limiting Steps in Cyanobacterial Production of Ethylene

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As environmental and sustainability concerns on utilizing fossil fuels continue to mount, there is strong motivation to seek alternative pathways to produce biofuels and commodity chemicals from renewable resources. Cyanobacteria, which are able to utilize sunlight and fix CO₂, become attractive targets in the field of metabolic engineering and synthetic biology. Despite success in initial demonstration of production of a variety of biofuels and chemicals in engineered cyanobacteria, the intrinsic regulation of carbon and nitrogen (C/N) metabolism in cyanobacteria is still one of the hurdles that obstruct turning cyanobacteria to efficient cell factories. Cyanobacterial production of ethylene is rendered by expression of a single protein, ethylene-forming-enzyme (Efe). The reaction catalyzed by Efe has been proposed to utilize α-ketoglutarate, arginine and oxygen as the substrate, and to produce ethylene, succinate, pyrroline-5-carboxylate, guanidium and CO₂ as the products. α-Ketoglutarate is a pivotal signaling molecule that regulates the intracellular C/N metabolism, while arginine as a nitrogen-rich compound may affect photosynthesis in cyanobacteria. Herein, efforts to further improve ethylene productivity in cyanobacteria focus on exploring and mitigating the rate-limiting steps, such as increasing expression of the Efe, investigating the substrate supply, and tracking the byproduct accumulation.

References:

1.13. Continuous 2,3-Butanediol Production in an Obligate Photoautotrophic Cyanobacterium in Diurnal Light Conditions

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Cyanobacteria are under investigation as a means to utilize light energy to directly recycle CO₂ into chemical compounds currently derived from petroleum¹². Any large-scale photosynthetic production scheme must rely on natural sunlight for energy, thereby limiting production time to only lighted hours during the day. Here the obligate photoautotrophic cyanobacterium Synechococcus elongatus PCC 7942 was engineered for enhanced production of 2,3-butanediol (23BD) in continuous light, 12h:12h light-dark diurnal, and continuous dark conditions via supplementation with glucose or xylose. Glucose or xylose consuming pathways³ were installed into 23BD production strains to allow for heterotrophic growth and production⁴. This study achieved 23BD production under diurnal conditions comparable to production under continuous light conditions, with a maximum 23BD titer of 3.0 g·L⁻¹ in 10 days. By demonstrating chemical production under dark conditions, this work enhances the feasibility of using cyanobacteria as industrial chemical-producing hosts.

References:
1.14. Engineering cyanobacteria xylitol production from CO₂

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Xylitol is a good sweetener, and it has been widely used in food, medicine and chemical industry. However, organisms tend to accumulate only low quantities of xylitol, so that extraction and purification are often complicated. Chemical synthesis is also technically challenging and uneconomical. Recent advances in synthetic biology and metabolic engineering have enabled the production of xylitol via microbial metabolism of carbohydrates. However, economical and sustainable biosynthesis of natural products remains a tremendous challenge. In our research, we tried to engineer cyanobacteria for xylitol production. By extending a natural pathway of PPP to construct a new approach to produce xylitol in S. elongatus PCC7942, the engineered strain PX can produce 25.5 mg/l xylitol from CO₂ and solar energy in sixteen days. To optimize the synthesis pathway of xylitol, xpdh and the gene (phoA) encoding phosphatase were introduced into S. elongatus PCC7942 to construct recombinant bacteria PXP. Engineered strain PXP can produce 16.94 mg/l xylitol under the condition of continuous ventilation.

1.15. One-Pot Synthesis of Leader Peptide-Free Microviridins

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Microviridins are a type of ribosomally synthesized and post-translationally modified peptides (RiPPs) produced by different strains of cyanobacteria. During the biosynthesis of microviridin the linear precursor peptide is thrice cyclized, cleaved off of its leader peptide and N-terminally acetylated. The presence of the leader peptide is crucial for the cyclizations to occur[1]. Since microviridins are important from the pharmaceutical point of view due to their ability to inhibit proteases, large efforts have been made aiming to improve them and reduce their size[2]. An in vitro approach was desired but difficult to accomplish mainly because of the need of the presence of the leader peptide and the lack of an appropriate protease for maturation of the microviridin. We tried a new methodology attaching the leader peptide to the amino terminus of the ATP-grasp ligases making them constitutively active. Using these modified enzymes we were able to synthesize mature microviridin from short synthetic core peptides in a one-pot reaction. This approach allows us to screen designed peptide libraries for novel and more potent microviridins, including variants containing non-canonical amino acids.

References:
1.16. Assessment of *Anabaena* sp. strain PCC 7120 as a heterologous expression host for cyanobacterial natural products: production of lyngbyatoxin A

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Cyanobacteria are well known producers of natural products of highly varied structure and biological properties. However, the long doubling times, difficulty in establishing genetic methods for marine cyanobacteria, and low compound titers have hindered research into the biosynthesis of their secondary metabolites. While a few attempts to heterologously express cyanobacterial natural products have occurred, the results have met with varied success. Here we report the first steps in developing the model freshwater cyanobacterium *Anabaena* sp. strain PCC 7120 (*Anabaena* 7120) as a general heterologous expression host for cyanobacterial secondary metabolites. We show that *Anabaena* 7120 can heterologously synthesize lyngbyatoxin A in yields comparable to the native producer, *Moorea producens*, and detail the design and use of replicative plasmids for compound production. We also demonstrate that *Anabaena* 7120 recognizes promoters from various biosynthetic gene clusters from both free-living and obligate symbiotic marine cyanobacteria. Through simple genetic manipulations, the titer of lyngbyatoxin A can be improved up to 13-fold. The development of *Anabaena* 7120 as a general heterologous expression host enables investigations of interesting cyanobacterial biosynthetic reactions and genetic engineering of their biosynthetic pathways.

1.17. Translational fusions facilitate high-level heterologous gene expression in *Synechococcus* sp. PCC 7002

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*Synechococcus* sp. PCC 7002 is an ideal organism for the photosynthetic production of chemicals and fuels [1]. Due to low separation costs; monoterpenes are attractive targets for chemical production in this organism [2]. However, monoterpene synthases display low turnover numbers and catalytic efficiencies [3] necessitating high expression levels in host organisms. We therefore generated a suite of expression constructs in *Synechococcus* sp. PCC 7002 using Pinene Synthase from *Abies grandis* which indicate that expression of Pinene Synthase can be vastly improved by the use of translational fusions. These results hint at a universal mechanism to facilitate high levels of heterologous gene expression in *Synechococcus* sp. PCC 7002.

References:


**1.18. Orthogonal T7 RNA polymerase/promoter system controls gene expression in Synechocystis sp. PCC 6803**

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To potentially reduce the dependence on industrial fertilizer in agriculture, one strategy would be to transfer nitrogen fixing capacity into non-leguminous crop plants. This is a major challenge at several levels. Our plan is to use the unicellular cyanobacterium *Synechocystis* sp. PCC6803 as a chassis for developing and testing tools of synthetic biology with the purpose of transferring nitrogen fixation capacity to this organism. It was chosen not only for its energy self-sufficiency, but also because it is a progenitor to the modern chloroplast, which can provide energy and reduction for the nitrogen fixation process. Seminal studies using synthetic biology studies in *E. coli* have shown that the nitrogenase gene cluster needs to be separated into at least 4 operons to keep a stoichiometric balance for functional nitrogenase activity (Temme, K., 2012). In our study, we try to introduce the T7 RNA polymerase (T7RNAP) regulator system which is extremely promoter-specific, into *Synechocystis* sp. PCC6803. We are currently testing this system. The T7RNAP regulator system in *Synechocystis* sp. PCC6803 will not be restricted for the expression of the nitrogenase gene cluster. Thus, we envisage that once this system has been built, tested and optimized it can be used for the expression of complex operons.

**1.19. An in situ antimicrobial susceptibility testing method based on cyanobacterial chlorophyll a fluorescence**

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Up to now antimicrobial susceptibility testing (AST) methods are indirect and generally involve the manual counting of bacterial colonies following the extraction of microorganisms from the surface under study and their inoculation in a separate procedure. In this work, an in situ, direct method for the evaluation and assessment of antibacterial properties of materials and surfaces is proposed. Instead of indirectly manual colony count or inhibition zone measurement, the proposed procedure, employs the measurement of Chl a fluorescence of the exclusively phototrophic *Synechococcus* sp. PCC 7942, and of the *Synechocystis* sp. PCC 6714, capable of both phototrophic and heterotrophic growth in order to add versatility and better reflect the antibacterial effects of surfaces under study towards nonphotosynthetic bacteria. In contrast with existing methods of determination of antibacterial properties, it produces high resolution and quantitative results and is so versatile that it could be used to evaluate the antibacterial properties of any compound (organic, inorganic, natural or plasma nanotextured superhydrophobic surfaces) under any experimental conditions, depending on the targeted application. Acknowledgement: this work is supported by the LoveFood2Market Horizon Project.
1.20. Carboxysomes Redesigned as Nanoreactors for Renewable Chemical Production in Cyanobacteria
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Bacterial microcompartments (BMCs) are proteinaceous organelles widespread in bacterial phyla. BMCs sequester metabolic reactions to increase their efficiency, segregate toxic metabolites from the cytoplasm or retain volatile intermediates1. Carboxysomes, the cyanobacterial BMCs for CO2 fixation, are the best characterized. Using methods in synthetic biology, the carboxysome architecture can be redesigned for the development of novel protein nanoreactors1. Previously, we successfully re-engineered the carboxysome core by the use of protein domain fusions2. We are expanding the possibilities of carboxysome function by testing the compartmentalization of heterologous enzymes for the production of isoprene in the cyanobacterium Synechocystis sp. PCC 6803. Carboxysomes (and other BMCs) have tremendous potential to impact the “green” chemical industry, as customizable protein scaffolds that optimize the biological production of renewable resources.

References:

1.21. Developing Genetic Tools for Polyploid Prokaryotes
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Polyploidy, in prokaryotes such as cyanobacteria, poses problems for the use of classic genetic tools that were originally developed in monoploid bacteria.1 In this work, we describe a set of genetic tools that enable classic monoploid genetic tools in the model organism Synechococcus elongatus PCC 7942. First, we demonstrate that CRISPR/Cas9 behaves as expected in PCC 7942. Next, we show that this system can be used to force segregation of a desired chromosomal insertion, drastically reducing the time needed to make targeted knockouts. We then focus on tuning the DNA repair mechanisms to promote errors upon dsDNA breaks. Finally, we look at using Cas9 gRNA libraries against the entire PCC 7942 genome in combination with error prone strains to make completely segregated random knockouts.

References:
1.22. A transient CRISPR/Cas editing system to introduce markerless genome modifications in the cyanobacterium *Synechococcus elongatus* UTEX 2973

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As photoautotrophic prokaryotes, cyanobacteria are ideal organisms for industrial production of a wide array of valuable biosynthetic products. The newly characterized strain *Synechococcus elongatus* UTEX 2973 is a particularly promising candidate for serving as a biological factory because of its rapid growth rate (1). We have implemented a CRISPR/Cas markerless editing system that will allow for extensive genomic engineering of *Synechococcus* 2973 (2). We targeted the *nblA* gene in this organism for deletion because of its well-known role in phycobilisome degradation under nitrogen starvation conditions. In developing this system, we discovered that Cas9 from *Streptococcus pyogenes* is toxic in cyanobacteria, and conjugation with stable, replicating constructs containing *cas9* resulted in lethality. However, implementing a system that allowed for transient expression of *cas9* facilitated markerless editing in 100% of exconjugants. This platform for introducing markerless mutations will allow for increased efficiency in cyanobacterial genome editing.

References:

1.23. Novel Shuttle Vector Capable of Transforming Multiple Genera of Cyanobacteria

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A novel shuttle vector system based on an endogenous plasmid isolated from a proprietary *Cyanobacterium* sp. strain was constructed and successfully used to stably transform three different cyanobacterial genera: *Synechocystis* PCC 6803, *Synechococcus* PCC 7002 and *Cyanobacterium* sp. The shuttle vector system contains a codon-optimized antibiotic resistance gene for selection. Transformation was confirmed by PCR and plasmid rescue for the three cell lines tested. In addition, the utility of the vector for expressing transgenes in cyanobacteria was exhibited by successful expression of a GFP gene in the transformed cell lines, which was confirmed by epifluorescence microscopy. The broad host range and easy manipulation of this new shuttle vector make it an efficient and versatile gene delivery vehicle for genetic engineering of many different species of cyanobacteria.

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Improved genetic tools for cyanobacteria will provide new opportunities for scientific research and biotechnology. We have developed a set of broad host range genetic tools for diverse cyanobacterial strains. These tools allow the creation of modular vectors containing standardized genetic parts. The resulting modular vectors are designed for the component parts to be easily replaced or additional parts to be easily inserted. Different types of vectors can be assembled, including autonomously replicating vectors and integrating vectors for gene knockout and gene expression from the chromosome. A library of donor plasmids carrying standardized parts has been made and the parts have been tested in several cyanobacterial strains. The library of vector parts includes origins of replication for \textit{E. coli}, origins of transfer for conjugation, origins of replication and neutral sites for various cyanobacteria, antibiotic markers, expression cassettes with different promoters, and reporter cassettes. Assembled modular vectors allowed a thorough characterization of different genetic parts and devices in a number of cyanobacterial strains. These tools have been used for the characterization of mutant versions of the broad-host-range plasmid RSF1010, a theophylline-inducible riboswitch, a set of constitutive promoters with different levels of activity, vectors based on the \textit{S. elongatus} plasmid pANS, and for expression of a large gene cluster required for the biosynthesis of the polyunsaturated fatty acid EPA.

1.25. CyanoBase Status 2016

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In 1996, we reported the first-ever complete genome of the cyanobacterial genome of \textit{Synechocystis} sp. strain PCC 6803. CyanoBase (http://genome.microbedb.jp/cyanobase) was then released in 1998 and has since been maintained to offer researchers access to information on the genome of this and additional 38 cyanobacterial and related species\cite{1}. The database also contains repository facilities, TogoAnnotation\cite{2,3}. With use this system, we carry out a manual annotation that extracts gene and/or protein entities from full papers. We have processed over 5,000 full papers for cyanobacteria. Consequently, each gene page in CyanoBase has a list of exhaustive collection of the papers, in which the gene was mentioned. We are on the way to incorporating the new features into the database to store and share massive omics research data from NGS’s for bacterial species. Our plan and current status of such extensions of the database will be presented.

References:
2.1. Diversity of putative clock systems

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The circadian clock of 	extit{Synechococcus elongatus} PCC 7942 is composed of KaiA, KaiB and KaiC. Input factors synchronize the core oscillator with environmental signals and metabolism. Output factors read out KaiC’s phosphorylation state and control global cyclic gene expression. Interestingly, cyanobacterial timing systems display diversity (1). For instance, 	extit{Prochlorococcus} might use a KaiBC-based timing system, whereas 	extit{Synechocystis} sp. PCC 6803 contains additional KaiB and KaiC homologs next to KaiA. The diverged KaiC proteins exhibit autophosphorylation, but only 	extit{Synechocystis} KaiC1 is affected by KaiA (2). Recent in vitro studies with an extended set of proteins indicate that kinase activity of cyanobacterial and archaeal KaiC homologs is well conserved. To decode putatively reduced or more complex timing systems we performed a reciprocal BLAST analysis revealing the distribution of Kai proteins as well as input and output factors in bacteria and archaea. Co-occurrence analysis with more than 100 unique cyanobacterial timing systems hint to a core module for circadian timing that will be discussed.

References:


2.2. Deletion of the circadian clock protein KaiA in 	extit{Synechococcus elongatus} PCC 7942 results in impaired diurnal growth

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The circadian clock in 	extit{Synechococcus elongatus} PCC 7942 is based on a central oscillator consisting of proteins KaiA, KaiB, and KaiC. Cyclic phosphorylation of KaiC over a 24-h period sets the timing of the clock and output signals are relayed to gene expression through the SasA–RpaA two-component system in which RpaA is a transcription factor targeting many genes, including those involved in nighttime metabolic processes. While removing the whole clock complex (KaiABC) does not impair light-dark (LD) growth, surprisingly, we have found that deletion of kaiA does. To investigate why KaiA is essential for LD growth, we sampled the ΔkaiA mutant over two diurnal cycles. We observed significantly reduced levels of RpaA and KaiC phosphorylation, as well as reduced class I gene expression measured via a P\textit{kaiBC} luciferase reporter. ROS was also higher than wild type cells. Moreover, disrupting cikA relieved LD sensitivity in the KaiA mutant. Considering that CikA and KaiA compete for binding to KaiB, the KaiA mutant would have hyperactive CikA phosphatase activity on RpaA. This supports the hypothesis that KaiA plays a key role in regulating the day/night transition and agrees with the model that P\textasciitilde RPaA is crucial for oxidative pentose phosphate pathway expression required for LD growth in obligate phototrophs.
2.3. The physiological functions of different ferredoxins in *Synechocystis* sp. PCC 6803

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Ferredoxins are the first soluble acceptors of electrons on the stromal side of chloroplast electron transport chain and use iron-sulfur cluster to distribute electrons to various metabolic pathways, such as carbon fixation and nitrate reduction\(^1\). There are totally 6 plant-like and 3 bacterial-like ferredoxins in *Synechocystis* sp. PCC 6803. Different ferredoxins may be involved in different metabolic pathways. Latest research found that ferredoxin and flavodoxin IsiB directly reduce the bidirectional NiFe-hydrogenase in cyanobacterial\(^2\). In addition to the linear electron flow of photosynthesis, ferredoxins are also involved in the cyclic electron transfer (CET), which balances the amount of NADPH and ATP production in photosynthesis. Furthermore, CET functions as a safety valve for excess electrons under adverse environmental conditions\(^3\). However, which of the ferredoxins are involved in these processes is still unknown. We deleted most of the 9 ferredoxins in *Synechocystis*. The respective mutants were examined under different conditions. The involvement of distinct ferredoxins in hydrogen production and CET around PSI will be discussed.

References:


2.4. Altering the structure of carbohydrate storage granules in the cyanobacterium *Synechocystis* sp. PCC 6803

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Carbohydrate storage is important in cyanobacterial and chloroplast metabolism. Understanding how to manipulate this carbohydrate metabolism can aid in harnessing cyanobacteria for biotechnology. While most cyanobacteria produce glycogen, some accumulate water-insoluble amyllopectin-like granules. This alternative form, termed “semi-amyllopectin”, is formed by cyanobacterial species harboring three branching enzyme (BE) homologs. In this study, we mutagenized the native BE gene in *Synechocystis* sp. PCC 6803 through N-terminal truncations and replaced one of the two native debranching enzymes (DBE) with a heterologous DBE from a semi-amyllopectin forming strain. Resulting polysaccharide granule morphology, growth, and glycogen content did not significantly differ in the mutant strains compared to wild type, and ultrastructure analysis revealed modest changes to granule morphology. However, glucan chain length distribution analysis revealed that the resulting glycogen shared structural characteristics similar to granules found in semi-amyllopectin producing strains. This is the first investigation of the impact of branching enzyme truncations on the structure of storage carbohydrates in cyanobacteria and is an important contribution towards understanding the relationship between the enzymatic repertoire of a cyanobacterial species and the morphology of its storage carbohydrates.
2.5. Analysis of engineered *Synechocystis* PCC 6803 cells containing additional copies of phosphoenolpyruvate carboxylase

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It is possible to genetically engineer cyanobacteria to produce a variety of biofuels [1-3] but productivity is typically low. Increased carbon fixation can increase the production of carbon based biofuels and other products of interest in photosynthetic organisms. In addition to RuBisCO, phosphoenolpyruvate carboxylase (PEPc) may also fix carbon in cyanobacteria. I have designed three engineered strains by overexpressing *pepc* in the cyanobacterium *Synechocystis* PCC 6803 and created cells with one (WT+PEPc) or two (WT+ 2xPEPc) additional copies of *pepc*, as well as cells with additional copies of *pepc*, *ppsa* and *mdh* (WT+PPSA+ PEPc+MDH). The additional copies were designed to replace the *psbA2* gene in the genome. However, since the additional copy of *pepc* is identical to the native, single recombination with the native *pepc* has occurred in all engineered cells. SDS-PAGE/Immunoblot demonstrated that more PEPc protein is present in the engineered cells compared to in wild-type cells with an increased level of PEPc with increasing *pepc* copy number. Interestingly, the WT+2xPEPc engineered cells grow faster than the control strain in low light and it has shown a higher *in vitro* PEPc activity [4]. Presently, the *in vivo* carbon fixation activity is being examined in the engineered strains.

References:


2.6. Modulating sucrose metabolism in cyanobacteria – Knocking out competing pathways and overexpressing selected enzymes in *Synechocystis* sp. PCC 6803

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Due to their photoautotrophic lifestyle, cyanobacteria are increasingly attractive as cellular factories for the production of organic compounds. One example is sucrose, which is synthesized upon salt acclimation of many cyanobacterial strains and plays a role as carbon source for N₂-fixing heterocysts. In contrast to the well documented role in plant metabolism, the function of sucrose in cyanobacteria remains fragmentary. The aim of this study is to analyze the physiological role of sucrose in cyanobacterial carbon metabolism with regard to the potential of cyanobacterial hosts for sucrose production. *Synechocystis* sp. PCC 6803 strains were generated that express specific genes for sucrose synthesis or in which competing reactions were deleted, to enable enhanced carbon fluxes into sucrose. Here we will report on two target genes for manipulation of sucrose metabolism. Knockouts of *ggsS* (*sll1566*), encoding for the key enzyme of salt-induced glucosylglycerol biosynthesis, and *inv* (*sll0626*), encoding for a putative sucrose-degrading invertase, were generated. Compared to the wild-type, the combined knockout of *ggsS* and *inv* alters the sucrose accumulation pattern in response to salt stress. The double mutant shows enhanced sucrose contents even under salt-free conditions, although sucrose accumulation remains clearly stimulated by salt addition. The redirection of carbon fluxes offers possibilities to enhance the yield of cyanobacterial produced sucrose. Moreover, the salt-dependence of sucrose accumulation makes it a promising compound in seawater-based production.
2.7. Phosphoketolase pathway contributes to cyanobacterial carbon metabolism

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Central carbon metabolism in cyanobacteria comprises the Calvin-Benson-Bassham cycle, glycolysis, the pentose phosphate pathway and the TCA cycle. Redundancy in this complex metabolic network renders the rational engineering of cyanobacterial metabolism for the generation of biomass, biofuels and chemicals a challenge. Here we report the presence of a functional phosphoketolase pathway - which splits xylulose-5-phosphate (or fructose-6-phosphate) to acetate precursor acetyl phosphate - in an engineered strain of the model cyanobacterium *Synechocystis (ΔglgC/xylAB)*, in which glycogen synthesis is blocked, and xylose catabolism enabled through the introduction of xylose isomerase and xylulokinase. We show that this mutant strain is able to metabolise xylose to acetate on nitrogen starvation. To see whether acetate production in the mutant is linked to the activity of phosphoketolase, we disrupted a putative phosphoketolase gene (*slr0453*) in the *ΔglgC/xylAB* strain, and monitored metabolic flux using $^{13}$C labelling; acetate and 2-oxoglutarate production was reduced in the light. A metabolic flux analysis, based on isotopic data, suggests that the phosphoketolase pathway metabolises over 30% of the carbon consumed by *ΔglgC/xylAB* during photomixotrophic growth with both xylose and CO$_2$ as the carbon sources. Disruption of the putative phosphoketolase gene in wild type *Synechocystis* also led to a deficiency in acetate production in the dark, indicative of a contribution of the phosphoketolase pathway to heterotrophic metabolism. We suggest that the phosphoketolase pathway, previously uncharacterised in photosynthetic organisms, confers flexibility in energy and carbon metabolism in cyanobacteria, and could be exploited to increase cyanobacterial carbon efficiency and photosynthetic productivity.

Reference:

2.8. Changes in electron flow observed in carbon sink mutants of the cyanobacterium *Synechocystis* sp. PCC 6803

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Two strains of *Synechocystis* sp. PCC 6803 with alterations in their carbon metabolism were analyzed for their photosynthetic efficiency under high and low inorganic carbon (C$_i$) availability. The *ΔglgC* mutant is unable to form glycogen, while the JU547 mutant has been engineered to produce ethylene. In each mutant, a sink of electrons and carbon has been either closed or added, respectively. The aim was to examine changes in electron flow and carbon acquisition resulting from these alterations. Under high C$_i$, the *ΔglgC* mutant displayed highly reduced PQ and NADPH pools, delayed initiation of the Calvin-Benson-Bassham cycle, and a large impairment of linear electron flow and cyclic electron flow around PSI. These were partly reversed under low C$_i$. In JU547, enhanced expression of low C$_i$ proteins, slightly more oxidized PQ and NADPH pools and increased rates of cyclic electron flow were observed. These results point to unexpected interplay between regulatory circuitry and promise for future integration of the ethylene forming enzyme into the *ΔglgC* mutant background.
2.9. The effects of glycogen deficiency on the nitrogen-stress response in *Anabaena* sp. PCC 7120

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The nitrogen stress response (NSR) is an adaptive mechanism that prepares the cell for nitrogen starvation. In the filamentous cyanobacterium *Anabaena* sp. PCC 7120 (*Anabaena*), the NSR activates a major morphological transformation, converting a proportion of vegetative cells into specialized N$_2$-fixing cells called heterocysts. The internal mechanisms that control the NSR in *Anabaena* are deeply rooted to the carbon/nitrogen status of the cell. Glycogen, a multi-branched glucose polymer, serves as a major carbon sink in *Anabaena*. Upon nitrogen starvation, wild-type cells accumulate glycogen to counteract the absence of nitrogen assimilation, while activating heterocyst development and N$_2$-fixation. To understand the interplay between glycogen metabolism and the NSR in N$_2$-fixing cyanobacteria, we have created a glycogen-deficient mutant of *Anabaena* through an inactivation of glucose-1-phosphate adenylyltransferase (*agp*), the enzyme responsible for the first committed step in glycogen synthesis. Our analysis reveals that during nitrogen deprivation, the Δ*agp* mutant displays several physiological changes compared to the wild-type, including decreased growth rate and photosynthetic activity, inability to degrade light-harvesting phycobilisomes, and reduced nitrogenase activity. These findings reveal that disrupting glycogen synthesis alters many characteristics associated with nitrogen stress, and suggests a relationship between glycogen metabolism and the NSR in *Anabaena*.

2.10. The stringent response regulates adaptation to darkness in the cyanobacterium *Synechococcus elongatus*

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The cyanobacterium *Synechococcus elongatus* relies upon photosynthesis for growth and reproduction. During darkness, *Synechococcus* stops growing, derives energy from its glycogen stores, and greatly decreases rates of macromolecular synthesis via unknown mechanisms. We have shown that the stringent response, a stress response pathway whose enzymes are conserved across bacteria and in plant plastids, contributes to this dark adaptation. Levels of the stringent response alarmone ppGpp rise after a shift from light to dark, indicating that darkness is analogous to starvation for cyanobacteria. High levels of ppGpp are sufficient to stop growth and dramatically alter many aspects of cellular physiology, including levels of photosynthetic pigments, polyphosphate, and DNA content. Cells lacking the stringent response display pronounced growth defects in light/dark cycles. The stringent response regulates expression of a number of genes in *Synechococcus*, including components and regulators of carbon and nitrogen metabolism. One of the most strongly (p)ppGpp-regulated genes encodes ribosomal hibernation promoting factor (*hpf*), and we demonstrate that *hpf* controls translation in response to light status. Although the metabolism of *Synechococcus* differentiates it from typically studied bacterial model systems, the logic of the stringent response remains remarkably conserved, while at the same time having adapted to the unique stresses of the photosynthetic lifestyle.
2.11. The Deg proteases of the cyanobacterium *Synechocystis* sp. PCC 6803

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Plants and cyanobacteria contain a variety of chaperones and proteases to monitor proper folding and function of proteins. Particularly interesting candidates are enzymes of the multifunctional Deg family of ATP-independent serine endopeptidases. The three Deg proteases of the cyanobacterium *Synechocystis* sp. PCC 6803, called HtrA (high temperature requirement A), HhoA (HtrA homologue A) and HhoB (HtrA homologue B), are important for survival under high light and temperature stresses. Using difference gel electrophoresis (DIGE) and N-terminal combined fractional diagonal chromatography (COFRADIC) the proteome of wild type *Synechocystis* cells was compared with the ones of single insertion mutants (ΔhhoA, ΔhhoB or ΔhtrA) or a mutant lacking all three Deg proteases (Δdeg). Deletion of all three Deg proteases lead to the down-regulation of proteins related to the biosynthesis of outer cell layers and affected protein secretion. During the late growth phase of the culture Deg proteases were found to be secreted to the extracellular medium of the wild type strain. While cyanobacterial Deg proteases seem to act mainly in the periplasmic space, deletion of the three proteases influences the proteome and metabolome of the whole cell. Impairments in the outer cell layers of the triple mutant might explain the higher sensitivity towards light and oxidative stress.

References:

2.12. Occurrence and function of DNA methylation in the cyanobacterium *Synechocystis* sp. PCC 6803

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Methylation of the prokaryotic genome is a crucial epigenetic signal for various mechanisms, including DNA repair, recognition of foreign DNA or chromosome partitioning. DNA methylation is catalysed by DNA-methyltransferases, utilizing S-adenosyl methionine as methyl group donor. Recent studies suggested a regulative role of DNA methylation within gene expression (Lluch-Senar et al., 2013). However, the underlying mechanism of epigenetic control is entirely unknown. We aim to unravel the links between DNA methylation, control of gene expression and physiological effects in *Synechocystis* sp. strain PCC 6803. Single molecule real-time DNA sequencing (SMRT-Sequencing) was performed to analyse base specific DNA modification in *Synechocystis* wild type. Interposon mutagenesis was used to knockout genes for putative DNA methyltransferases, and microarrays were applied to search genome-wide transcriptional changes. The genome of *Synechocystis* harbors genes for three methyltransferases, but no restrictase. The gene slr1803 codes for MSsp68033II methylating 5'-'GA"TC-3'. This dam-like activity is essential for the viability of *Synechocystis*. The genes slr0214 (Scharnagl et al., 1998) and sll0729 code for non-essential, orphan methyltransferases MSsp6803I and MSsp6083II. Mutants lacking slr0214 or sll0729 differ in gene expression and phenotypes compared to wild type. Moreover, SMRT-Sequencing identified an additional DNA modification within the palindromic sequence 5'-'GA"AGGC-3'. In silico analysis detected three genes potentially coding for the missing methyltransferase. These genes code for non-essential proteins as segregated knockout mutants were identified. Thus, we identified up to five methyltransferases and started analysing their physiological functions.

References:
2.13. Oxygen-Responsive Genetic Circuits Constructed in *Synechocystis* sp. PCC 6803

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Photoautotrophic organisms, particularly cyanobacteria, are promising sources for the sustainable generation of value-added bioproducts using carbon dioxide and sunlight. However, few transcriptional regulators have been developed for cyanobacteria that provide specific and orthogonal control of gene expression. In addition, the synthetic regulatory circuits that have been employed in cyanobacteria respond to only a single chemical input. Genetic devices that respond to environmental signals could be used to connect heterologous gene expression to the conditions important for that expression, as opposed to irrelevant chemicals used in research labs. We developed an inducible promoter that responds to oxygen and utilized the synthetic regulator in a two-input AND gate in *Synechocystis* sp. PCC 6803 to address these issues. We tested the performance of the oxygen-responsive promoter with either an oxygen-independent fluorescent reporter or a heterologous *nifHDK* which encodes the structural proteins of the oxygen-sensitive enzyme nitrogenase. Transcripts of both genes were only produced in low oxygen conditions. To enable more specific transcriptional control, we expanded this simple oxygen-responsive circuit into a two-input AND gate. The more complex circuit allowed expression of the fluorescent reporter only when both signals, low oxygen and high anhydrotetracycline, were present. The AND gate is, to our knowledge, the first complex logic gate created in cyanobacterial species. The transcriptional regulators created in this work expand the synthetic biology tools available for complex gene expression in cyanobacteria.

2.14. Improved O$_2$ Tolerance of Nitrogenase via Uptake Hydrogenase in Engineered *Synechocystis* 6803 Containing the Minimal nif-cluster

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In order to engineer nitrogen fixation activity in *Synechocystis* 6803, we have transferred to it an intact cluster of 35 *nif*-genes from *Cyanothece* 51142, integrated into a replicating plasmid. We successfully detected nitrogenase activity in this engineered strain under special conditions. Next, we examined the requirement of minimal number of *nif*-genes for nitrogenase activity in *Synechocystis* 6803. By deleting certain genes in the cluster, we have demonstrated that the cluster between *nifT* and *hesB* is minimally required for nitrogenase activity. We also determined that *nifX* and *nifW* are critical for the activity since knocking out either one leads to a dramatic reduction of the activity. Meanwhile, we found that the nitrogenase enzyme in the engineered strains is very sensitive to O$_2$, a tiny amount of which repressed the activity by almost 50-fold. In order to improve the activity under microoxic conditions, genes coding for an uptake hydrogenase in *Cyanothece* 51142 were introduced into the engineered strain containing the minimal *nif*-cluster. Interestingly, nitrogenase activity under microoxic conditions was enhanced through the expression of uptake hydrogenase. We are currently pursuing various approaches to enhance oxygen tolerance of nitrogen fixation in engineered *Synechocystis* 6803 strains. Supported by funding from the NITROGEN program of the National Science Foundation.
2.15. Expression of a Carotenoid Oxygenase Gene by Constitutive and Inducible Promoters in *Synechococcus* sp. PCC 7002

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β-carotene is essential for photoprotection in cyanobacteria and plants. In most animals β-carotene is a substrate for cleavage by carotenoid oxygenase (CO) enzymes to produce retinoids such as all-trans-retinal. We introduced a CO enzyme from *Drosophila melanogaster*, BDiox, into *Synechococcus* sp. PCC 7002 (Syn7002) to symmetrically cleave β-carotene into two all-trans-retinal. The β-diox cDNA was cloned and expressed by a modified constitutive cpcBA promoter (Pcpc 9.4) in a Syn7002 expression plasmid. This construct produced viable transformants. A zinc-inducible promoter system was also employed for CO gene expression. In the presence of Zn2+, the response regulator SmtB is released from the smtA promoter upstream of β-diox. Viable Syn7002 transformants were obtained when grown in ‘zinc-free’ medium. β-diox was induced following ZnCl2 addition (<100 μM). In both constitutive and inducible transformants, BDiox presence was confirmed by western blot and its activity assayed by HPLC of total cellular β-carotene content. Sufficient uncleaved β-carotene was retained for incorporation into the reaction centers for photoprotection when β-diox is regulated by either promoter. The all-trans-retinal produced by BDiox is being used to explore the functional co-expression of light-driven opsin proteins such as halorhodopsin that require one covalently bound molecule of all-trans-retinal.

Reference:

2.16. Regulatory RNAs involved in nitrogen assimilation

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Non-coding regulatory RNAs are currently recognized as essential post-transcriptional regulators involved in every aspect of bacterial physiology. RNA-Seq analysis of the *Nostoc* sp. PCC 7120 transcriptome shows abundant transcription of both antisense RNAs and small non-coding RNAs (sRNAs). We are undertaking several approaches to the identification of regulatory RNAs in cyanobacteria, including a predictive algorithm based on transcriptomic information and phylogenetic conservation (1). Whereas some of the identified sRNAs are encoded in every cyanobacterial genome analyzed, several sRNAs appear exclusively in heterocystous strains. We are further analyzing some sRNAs that participate in regulatory circuits under control of NtcA or HetR. This is for instance the case with NsiR4 (nitrogen stress induced RNA 4), an element of an NtcA-controlled coherent negative feed-forward loop that modulates post-transcriptional regulation of glutamine synthetase (2).

References:
2.17. Identification of small RNAs in heterocystous cyanobacteria
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Bacterial small non-coding RNAs (sRNAs) are involved in the regulation of a wide range of responses to nutritional stresses. We are interested in the possible role of sRNAs in the regulation of adaptation to nitrogen deficiency and, in particular, in the differentiation of heterocysts. A dRNAseq transcriptomic analysis in *Nostoc* sp. PCC 7120 (1) identified many possible transcription start sites that could correspond to sRNAs. A pipeline (2) that combines the transcriptomic data with predicted Rho-independent terminators and additionally takes into account phylogenetic conservation identified most of previously described sRNAs and many novel sRNAs. Some of them are only conserved in heterocystous cyanobacteria and/or their transcription is heterocyst-specific. We hypothesize that they may play a role in differentiation and/or function of the heterocyst. We have validated transcription of several of them and are currently undertaking their characterization.

References:

2.18. Probing mechanisms of RNA turnover in cyanobacteria using next-generation sequencing
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Multiple enzymes participate in RNA turnover, a critical process for maintaining cellular protein homeostasis. While this process is conserved across all domains of life, the mechanisms of RNA turnover are not well understood in cyanobacteria. To address this knowledge gap, we compared the diversity of mRNA degrading systems in 66 diverse cyanobacterial strains. We found that unlike many other bacteria including as *Escherichia coli* and *Bacillus subtilis*, the genomes of many cyanobacterial species encode multiple homologues of RNase III, an endonuclease that targets double-stranded RNA. Since RNase III is conserved from bacteria to animals and has many important functions including ribosomal RNA maturation, viral RNA processing, and RNA processing in native CRISPR systems, we aimed to investigate the function of the three RNase III homologues in *Synechococcus* sp. PCC 7002 using genetics and next-generation sequencing. Our results suggest that RNase III activity is crucial for optimal cellular fitness and that the three RNase III homologues in this strain are not functionally redundant.

References
2.19. The Tryptophan-Rich Sensory Protein (TSPO) in the Cyanobacterium *Fremyella diplosiphon*

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The tryptophan-rich sensory protein (TSPO) is a nearly ubiquitous integral membrane protein. TSPO has been associated with stress- and disease-related processes as well as pigment biosynthesis in organisms from bacteria to mammals, but full elucidation of its functional role is lacking for most TSPO-containing organisms, including cyanobacteria. We describe the regulation and function of a TSPO homolog in the chromatically acclimating cyanobacterium *Fremyella diplosiphon* (FdTSPO). Chromatically acclimating cyanobacteria are capable of tuning cellular photosynthetic phycobiliprotein content and morphology to external changes in available light. Accumulation of the FdTSPO transcript is upregulated by green light and in response to stress. A *F. diplosiphon* TSPO deletion mutant and overexpression strain showed altered responses compared to the wild type under various stress conditions, including salt treatment, osmotic stress, and induced oxidative stress. FdTSPO ligand binding activity for tetrapyrroles is in the low micromolar range while cholesterol, a known ligand of mammalian and proteobacterial TSPOs, binds poorly. Taken together, our results provide support for involvement of membrane-localized, bilin-binding FdTSPO in mediating cellular responses to stress in *F. diplosiphon* and suggest an involvement of FdTSPO in tetrapyrrole metabolism and regulation in vivo.

2.20. Complementary Chromatic Acclimation in the Cyanobacterium *Synechococcus* PCC 7335

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The phycobilisome (PBS) is a supercomplex composed of chromophore-binding proteins (phycobiliproteins) and linker proteins. PBS collects light energy, funnels it to the core and then transfers it to photosystems. Some cyanobacteria have capacity to remodel its phycobilisome in response to light color by complementary chromatic acclimation (CCA). Recent studies show CCA in far-red, which include phycobiliproteins, linkers, photosystems and chlorophyll f¹². *Synechococcus* PCC 7335 is a unicellular cyanobacterium isolated from intertidal zone of Puerto Peñasco, Sonora Mexico by J.B. Waterbury. Its genome has been sequenced and is in the process of being annotated. This cyanobacterium has gene clusters for CCA in green, red and far-red, a gene for CpcL and a complete cluster for N₂ fixation. We will present the characterization of cells and PBS from cells grown in white light, red light and far red light by absorbance and fluorescence spectra. The components of the three type of PBSs identified by SDS-PAGE, followed by mass spectrometry; and the liposoluble pigments resolved by HPLC.

References:


3.1. Marine Synechococcus aggregate formation

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Marine Synechococcus is a typically unicellular cyanobacterium found in diverse marine environments. Recent reports have documented the likely presence of clumps of these cells. We similarly report here that clumps and aggregates are frequently found at a Pacific coastal monitoring site. In lab experiments large clumps of cells are found during the co-culturing of Synechococcus sp. strain CC9311 and nanoflagellate grazers suggesting that Synechococcus may evolve to form clumps to avoid grazing. A knockout mutant in a predicted atypical polyketide synthesis gene in Synechococcus sp. strain CC9311 causes clumping in this strain, suggesting a potential mechanism for clump formation.

3.2. A barcoded transposon mutant library reveals genes involved in amoebal grazing

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Grazing by protists exerts a significant selective pressure on cyanobacterial populations. Analyses of genetic factors that influence susceptibility to grazing provide insight into the molecular details of these predator-prey interactions. A random barcode transposon mutant library in the freshwater cyanobacterium Synechococcus elongatus PCC 7942 was subjected to grazing by two free-living amoebae, HGG1 and LPG1, on solid and liquid media. The fitness of mutants in the resulting population was quantified by barcode sequencing to identify genes that influence grazing survival. The results of the experiments on solid media with either amoeba strongly corroborate previous studies demonstrating that defects in lipopolysaccharide biogenesis confer grazing resistance. A number of mutants were resistant to grazing in one amoeba but not the other, suggesting these gene products are involved in specific interactions. Additionally, it appears that the factors influencing grazing efficiency are substantially disparate between different environments, as mutants resistant to grazing on plates often did not have a fitness advantage in liquid media.

References:
3.3. The microbial lipidome of subtropical Pacific surface waters dominated by *Prochlorococcus*

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In the oligotrophic ocean, mortality and cell production of *Prochlorococcus* have been shown to be strongly linked to day/night cycles (1). Yet, it remains unknown whether the cell loss of *Prochlorococcus* results from viral lysis or consumption by grazers. We investigated the microbial lipidome at high temporal resolution over 8 day/night cycles in the North Pacific subtropical gyre to examine the microbial community composition and its responses to mortality. Preliminary results show that diagnostic microbial membrane lipids and respiratory quinones reflect diel cycles of both photosynthetic and heterotrophic bacterial communities. While the photosynthetic signals (e.g., sulfoquinovosyl diacylglycerol and plastoquinone) follow production rates of *Prochlorococcus*, aerobic heterotrophic biomarker abundances (e.g., ubiquinones) occur where *Prochlorococcus* mortality rates are highest. This suggests that grazers are predominantly responsible for *Prochlorococcus* mortality in the subtropical Pacific gyre.

References:

3.4. Analysis of molecular diversity within single *Rivularia* colonies by Illumina sequencing

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Macroscopic hemispherical colonies, of about 0.5-3 cm of diameter, identified as belonging to genus *Rivularia*, can be typically found as dominant near the source of highly calcareous streams (1). Previous analysis indicated that *Rivularia* colonies might include more than one genotype (1, 2). In order to study the variability and the proportion of genotypes within single colonies, several *Rivularia* colonies from five Spanish rivers were analyzed by Illumina sequencing and parallel optical microscopy. Specific cyanobacterial primers, CYA359F, and CYA781Ra and Rb, were used to amplify the V3 and V4 hypervariable region of 16S rRNA gene. Results showed clear genetic differences depending on the colony, although they showed overlapping morphology. Some colonies were dominated (93-97%) by a genotype previously identified as corresponding to *R. biasolettiana* (2), while in others the percentage of this genotype was around 60-70%. A novel genotype with no matches in the database dominated (97-98%) other colonies.

References:

3.5. Understanding nutrient exchange in the *Azolla-Nostoc* symbiosis

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Azolla, a small genus of aquatic ferns, has immense green potential to positively impact the globe. *Azolla* owes this distinction to the nitrogen-fixing cyanobacterium, *Nostoc azollae*, which it harbors within specialized cavities in each of its leaves1. The *Azolla-Nostoc* symbiosis is unique among known plant-bacteria endosymbioses, in that the cyanobiont is intimately associated with the fern perpetually throughout its life cycle. *Nostoc azollae* filaments are enclosed within a mucilaginous network in the leaf cavity, and are closely associated with specialized hairs that arise from the adaxial epidermal cells of *Azolla* that form the boundary of the leaf cavity2. Earlier studies2,3, unveiled two important nutrients (ammonia and sucrose) exchanged between these symbiotic partners. Here, we use a combination of phylogenetics, transcriptomics, and confocal microscopy to understand the molecular mechanisms of how these nutrients are exchanged in this intimate association.

References:


3.6. Diversity of cyanobacteria of biological soil crusts in Brazilian savannah

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Biological soil crusts (biocrusts) are phototroph-built microbial communities that form on soil surfaces in habitats where plant cover is restricted. They have extreme ecological relevance protecting soil particles against erosive forces, local hydrology and fertility. The last decade has seen an effort to define the identify of the main cyanobacterial players in soil cruts. We have investigated biocrusts from six sites in subtropical savannah in Brazil. Some parks presented *Leptolyngbya* as the most abundant genus, other, besides this, also presented *Microcoleus* in similarity to reports of South African drylands (BÜDEL et al., 2009). Three were dominated by *Porphyrosiphon*, similar to data found in Australia (BÜDEL et al., 2012). These results highlight the diversity of BSC’s in Brazil, emphasizing the importance of this type of work to deepen in the study of biocrusts.

References


3.7. Evidence for substrate specialization among marine euendolithic cyanobacteria from Mona Island, Puerto Rico

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Endolithic cyanobacteria that bore their way into solid carbonates are responsible for eroding coastal limestones, damaging mollusk shells, coral skeletons and historical landmarks. They have been extensively described in the traditional literature, and shown to be active in a variety of mineralogically distinct carbonates and phosphates. Whether there exists a specialization among boring cyanobacteria towards the type of substrate has not been addressed. Yet, the model strain Mastigocoleus testarum (1) clearly prefers calcium carbonate in the laboratory. To answer this question, we interrogated endolithic microbial communities from marine intertidal limestones (CaCO₃), dolostones (MgCaCO₃) and apatites (Ca₅(PO₄)₃(OH,Cl,F)) collected on Mona Island, Puerto Rico. The mineral and the microbial community compositions were determined using X-ray diffraction and next-generation 16S rDNA libraries sequencing respectively. The analysis detected no correlation between the mineral and microbial compositions at low taxonomic resolution, but one arose at the species(OTU) level among likely euendolithic Cyanobacteria. We found that close phylogenetic relatives can exhibit totally opposite substrate preference. Our results also suggest that there must exist alternate boring mechanisms to the one described in the model strain Mastigocoleus testarum.

References:

4.1. Transformation of Anabaena cylindrica implicates a role of AcaK in akinete development

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Vegetative cells of Anabaena cylindrica form heterocysts in the presence of combined nitrogen and when nitrogen-deprived. An A. cylindrica vegetative cell can also differentiate into an akinete (a kind of spore) and a terminally positioned, tapered cell. An akinete normally develops adjacent to a heterocyst or another akinete, presenting a rare embryogenetic-type induction in a bacterium (1). P_aack, a presumptive promoter of an akinete marker gene (2), acaK, together with its coding region, were fused to a promoter-less green fluorescent protein gene (gfp) in a pDU1-based plasmid. The resulting construct (P_aacK-acaK-gfp) was transferred into A. cylindrica by conjugation, aided by plasmid pRL2602 that contains four methylase genes to protect sequences recognized by corresponding restriction endonucleases. GFP fluorescence from P_aacK-acaK-gfp originates principally in akinetes. Fluorescence was also detected in some heterocysts and in what appear to be meristematic cellular zones that contain a terminally positioned, tapered cell and its neighboring cells, most of which divide actively. Overexpression of acaK led to earlier, more abundant, akinete formation, sometimes distant from heterocysts. These observations suggest that over-expressed AcaK may show a relationship between akinetes, tapering cells, and heterocysts.
References:

4.2. Regulation of the \textit{nif1} and \textit{nif2} gene clusters in \textit{Anabaena variabilis}

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The heterocystous cyanobacterium \textit{Anabaena variabilis} has two Mo-nitrogenases - Nif1, expressed in heterocysts under N-limiting conditions, and Nif2, in vegetative cells only in N-limiting conditions under anoxic conditions. For both large \textit{nif} operons the primary promoter is upstream of \textit{nifB1/nifB2} with multiple motifs conserved between the two \textit{nifB} genes. Hybrid promoters, fusing the conserved motifs from the \textit{nifB2} promoter to the downstream \textit{nifB1} promoter region were able to drive normal, heterocyst-specific expression of \textit{lacZ}, indicating that the conserved motifs upstream of the \textit{nifB1} promoter are not responsible for heterocyst-specific expression. The \textit{cnfR (patB)} gene was previously identified as a possible regulatory protein in \textit{Anabaena} 7120 (1) and in \textit{Leptolyngbya boryana} (2). A close homologue, \textit{cnfR1}, is present in \textit{A. variabilis} and another, \textit{cnfR2}, is downstream of the \textit{nif2} cluster. Mutations in \textit{cnfR1} or \textit{cnfR2} decreased expression of \textit{nifB1} and \textit{nifB2}, respectively, and abolished nitrogenase activity. Expression of \textit{P}_{\textit{nifB2}:lacZ} \textit{from A. variabilis} in anoxic vegetative cells of \textit{Anabaena} sp. PCC 7120 required CnfR2, suggesting that CnfR2 acts on the \textit{nifB2} promoter.


4.3. Restoration of heterocyst production to a \textit{ΔhetP} strain of \textit{Anabaena}

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\textit{Anabaena} sp. Strain PCC 7120 is a filamentous cyanobacterium capable of differentiating a nitrogen-fixating cell type called a heterocyst. The \textit{hetP} gene has been identified as being required for the normal magnitude and timing of heterocyst formation. Where as a wild type strain produces 10\% heterocysts in 23 hours, a \textit{ΔhetP} strains produces only 2-3\% heterocysts in 48 hours. (1) How the loss of \textit{hetP} leads to this phenotype is currently unknown. To identify genes downstream of \textit{hetP} in the differentiation pathway that could be aiding in its function during heterocyst formation we performed a forward genetic screen. A Tn5 transposon was introduced via conjugation into a \textit{ΔhetP} strain and plated on nitrogen deficient media with selection. Surviving colonies were assayed for restoration of wild-type heterocyst accumulation and timing of development. The DNA of these strains was isolated and the transposon location was identified. We demonstrated a class of genes whose inactivation is capable of restoring wild-type heterocyst formation in the absence of \textit{hetP}. Future work will examine the function of these genes in relation to \textit{hetP}.

References:
4.4. Modulation of the expression of *patS* by HetZ, an ssDNA-binding protein, in *Anabaena* sp. PCC 7120

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*patS* is a gene involved in heterocyst patterning in *Anabaena*. It encodes a 17-aa peptide (PatS17) that is processed into inhibitors of heterocyst differentiation. *hetZ* and *hetP* are two genes involved in control of heterocyst differentiation and directly regulated by the master regulator HetR. At 6 hours after N-stepdown, the up-regulation of *patS* was completely blocked in a *hetR*-null mutant, greatly reduced in a *hetZ*-null mutant, but not in a *hetP*-null mutant. In EMSA, purified recombinant HetZ showed no binding to dsDNA upstream of *patS*, but strong binding to a 50-base ssDNA in the same region. Substitutions at the predicted DNA-binding site of HetZ abolished the ssDNA-binding activity. On the ssDNA fragment, the HetZ-recognition site was delimited to a 10-base region, and 6 of the 10 bases were found to be required for the specific binding. As shown with *gfp*, mutations at the HetZ-binding site led to significantly reduced transcription from the *patS* promoter in differentiating cells. We also showed that HetZ interacts with PatU3 and HetR and that PatS17 is processed into PatS7 (DERGSGR), then PatS6 (ERGSGR) and PatS5 (RGSGR). The ssDNA-binding activity of HetZ was inhibited by PatU3 but not HetR or PatS derivatives. Consistently, inactivation of *patU3* increased the expression of *patS*.

4.5. Local Adaptation by an Ancient Global Polymorphism for Heterocyst Function

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A gene content polymorphism involving two genes on the heterocyst envelope polysaccharide (HEP) expression island is found in a population of heterocyst-forming *Mastigocladus laminosus* at White Creek—a N-limited, geothermally heated stream in Yellowstone NP. At White Creek, the deletion allele predominates in *M. laminosus* from downstream sites where temperature is cooler (~39-43°C mean annual temperature) but is absent from upstream sites (47-54°C). This spatial distribution suggests local adaptation at alternative sites. Based on the characterized function of the HEP expression island, we predicted that the deletion increases gas flux across the heterocyst envelope, and our investigations of a deletion mutant of *Anabaena* sp. PCC 7120 support this. We propose that spatial variation in temperature at White Creek favors alternative alleles at this locus due to thermodynamic effects on heterocyst physiology. Remarkably, the deletion has an ancient origin and has spread globally. We conclude that functional variation maintained by long-term selection over extraordinary time scales can not only drive local adaptation and population divergence but also shape the distribution of microbial diversity on a global scale.
4.6. Defining the gene regulatory network promoting hormogonium development in *Nostoc punctiforme*

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Filamentous cyanobacteria commonly exhibit gliding motility, which facilitates a number of biological processes including phototaxis, the formation of colonial aggregates, and the establishment of plant-cyanobacterial symbioses. In a subset of these organisms, gliding is restricted to hormogonia, differentiated motile filaments. Currently, the gene regulatory network that governs hormogonium development is not well defined. To identify components of this network we employed a forward genetic screen to isolate non-motile mutants of the filamentous cyanobacterium *Nostoc punctiforme*. This screen has identified several genes as essential for normal hormogonium development and motility, including *ogtA*, which encodes a putative O-linked β-N-acetyl glucosamine transferase (OGT). In many eukaryotes, OGTs modulate protein activity via transient O-GlcNAcylation of target proteins, resulting in subsequent effects on a number of cellular and developmental processes. In *Nostoc punctiforme*, transcription of *ogtA* is upregulated early in hormogonium differentiation and deletion of *ogtA* completely abolished motility. The *ogtA*-deletion strain could be induced to differentiate filaments with hormogonium-like morphologies, but unlike wild-type hormogonia, these filaments fail to accumulate PilA. These results implicate O-GlcNAc protein modification as an essential early event in hormogonium development.

4.7. How do *Anabaena* cells communicate?

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The filamentous nitrogen-fixing cyanobacterium *Anabaena* sp. PCC 7120 differentiates specialized cells, heterocysts, that fix atmospheric nitrogen and transfer the fixed nitrogen to adjacent vegetative cells. Reciprocally, vegetative cells transfer fixed carbon to heterocysts. Several routes have been described for metabolite exchange within the filament, one of which involves communicating channels that penetrate the septum between adjacent cells. Several *fra* gene mutants were isolated 25 years ago on the basis of their phenotypes: inability to fix nitrogen and fragmentation of filaments upon transfer from N+ to N- media. Cryopreservation combined with electron tomography and immunoelectron tomography, were used to characterize channels between cells as well as investigate the role of three *fra* gene products in channel formation. We were able to visualize and measure the dimensions of channels that breach the peptidoglycan between vegetative cells and between heterocysts and vegetative cells. The channels appear to be straight tubes, 21 nm long and 12 nm in diameter for the latter and 12 nm long and 12 nm in diameter for the former¹. We also show that, FraC and FraG are clearly involved in channel formation while FraD has a minor part. Additionally, FraG was located close to the cytoplasmic membrane and in the heterocyst neck, using immunogold labeling with antibody raised to the N-terminal domain of the FraG protein².

References
4.8. Inviability of *Anabaena* lacking three RG(S/T)GR pentapeptide-containing negative regulators of differentiation

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HetR protein is called the master regulator of patterned heterocyst differentiation, but it arose in filamentous cyanobacteria long before the appearance of heterocysts, terminally differentiated cells specialized for aerobic nitrogen fixation. Its function in nonheterocyst-forming cyanobacteria remains obscure. In *Anabaena* PCC 7120, two negative regulators of HetR, a short peptide PatS and the protein HetN, both contain an RGSGR pentapeptide essential for their activity and are known to guide heterocyst pattern formation by controlling the binding of HetR to DNA and the protein's turnover. However, this control mechanism cannot be universal since PatS and RGSGR-containing HetN orthologs are absent from most heterocyst-forming cyanobacteria. We will present evidence that all HetR-containing cyanobacteria encode orthologs of another small RG(S/T)GR-containing protein, PatX, and a variable set of additional RG(S/T)GR-containing peptides and proteins. In *Anabaena* PCC 7120, PatX has partially overlapping functions with PatS in controlling heterocyst differentiation. Deprivation of all three negative regulators – PatS, PatX and HetN – results in the differentiation of all vegetative cells into heterocysts in combined nitrogen-free medium and extensive fragmentation and cell lysis and death in nitrogen-replete conditions. Thus HetR activity unrestrained by RG(S/T)GR-containing negative regulators is lethal under different growth conditions in *Anabaena* PCC 7120. The possible roles of HetR in the evolution of multicellularity and determination of cell fate in cyanobacteria will be discussed.

5.1. Thf1 helps to stabilize PSI under high light in *Synechococcus* sp. PCC7942

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The thylakoid formation protein (Thf1) is conserved in all photosynthetic organism. The level of Thf1 is regulated by various stress conditions in *Synechococcus* sp. PCC7942 (hereafter *Synechococcus*), up (N and S starvation, high light (HL) and chilling (LT)) or down (P depletion and HS). To further explore the localization and function of Thf1, the deletion strain, \(\Delta Thf1\) was constructed. It was found that the thylakoid membrane system in the mutant was not affected under normal growth condition. Comparing with the WT, the \(\Delta Thf1\) showed reduced PSII activity, but increased fluorescence emission, D1 protein level and chlorophyll content under HL condition, suggested blocked damaging D1 degradation and thus inhibited PSII reparation, which could be further confirmed by the down-regulated FtsH protease. Besides PSII, PSI was even more seriously affected in \(\Delta Thf1\) indicated by reduced fluorescence emission, electron transport activity as well as re-reduction rate, even under low light (LL) condition, suggested PSI damage could be the primary effect of thf1 deletion in *Synechococcus*. The mutant cells had lower PSI subunit content under higher light condition, and exhibited similar pattern as the PSI assembly factor BtpA mutant under LT stress, shown as reduced PSI activity and PSI subunit accumulation but not PSII. Thf1 tends to be more soluble under LL but more membrane-bound as a peripheral protein under HL, sucrose gradient fractionation of the membrane protein complexes and further crosslinking and western-blot analysis indicated that Thf1 was correlated with PSI.
5.2. Discovering the roles of PSI variants in heterocysts

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Cyanobacterial photosystem I (PSI) is a heterodimer of PsaA and PsaB that binds cofactors involved in electron transport. Despite the pseudo-C₂ symmetry, kinetic investigations showed biphasic forward electron transfer (ET) from A₁A and A₁B to Fₓ. In 2003, Ishikita and Knapp proposed that the biphasic kinetics could be explained by different redox potentials of the A₁A and A₁B quinones, and that Asp-575PsaB/Gln-588PsaA were crucial for the asymmetry in their energetics. In 2011, Magnuson et al. noted that the divergent PsaB subunit of PSI, PsaB2, is only present in filamentous nitrogen fixing bacteria and contains the residue Gln-575, making it symmetrical to the B-side. This mutation would be expected to alter the redox potential of the A-side quinone. PsaB2 contains additional changes relative to PsaB, including an inserted Gln-Q581 in the helix close to Fₓ that could affect its orientation, and hence, the kinetics of electron transfer. We tested the possible effect of these alterations in the PsaB1 protein of Synechocystis sp. 6803 by generating three variants: (i) Asp-575 replaced by Gln-575, (ii) insertion of Gln-581, (iii) the combination of both. The variants of PSI are being characterized by various spectroscopic techniques. Additionally, phenotypic analysis should provide insight into the physiological effect of these mutations.

References:

5.3. Investigation of Photosystem I Embedded in a Trehalose Glass Matrix

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The disaccharide trehalose protects against desiccation and protein denaturation. Its high glass transition temperature, which is a function of residual water concentration, makes it suitable for studying immobilized proteins at room temperature. This work focuses on investigating the effect of a room temperature trehalose glassy matrix on electron transfer in photosystem I (PS I) reaction centers from Synechocystis sp. PCC 6803. Flash-induced time-resolved spectroscopy at 830 nm, which measures P₇₀₀⁺, showed that wild type PS I embedded in a trehalose glass has broadly distributed recombination kinetics. In solution, the dominant recombination phase has characteristic time of ~50 ms, which is ascribed to the back reaction between [F₆/F₈]⁻ and P₇₀₀⁺. Trehalose glass samples of wild-type PS I demonstrate multiple kinetic phases. These new kinetic phases have life times of 150 ms, 6 ms, 285 µs and 16 µs and most probably correspond to recombination from [F₆/F₈]⁺, Fₓ⁻, A₁A⁻ and A₁B⁻ respectively, with P₇₀₀⁺. This hypothesis is supported by the data obtained from reaction centers lacking [F₆/F₈] and both [F₆/F₈] and Fₓ. Removal of [F₆/F₈] causes disappearance of the 150 ms phase and removal of [F₆/F₈] and Fₓ causes a loss of both the 150 ms and 6 ms kinetic phases. Therefore, different microstates of PS I appear to be present in the trehalose glass such that forward electron transfer is inhibited depending on the precise microstate ‘frozen in’. Future studies will include measurements of electron transfer from the quinone using time-resolved optical spectroscopy at 480 nm and transient EPR spectroscopy along with incorporating different variants of PS I in the trehalose glass.
5.4. Examining the Role of Photosystem Stoichiometry in *Synechocystis*

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Oxygenic photosynthesis uses energy from photons to drive electron transport through two photosystems (PSII and PSI), with the goal of producing both ATP and NADPH, some of which is used for carbon fixation. However, PSII and PSI do not always exist at parity, with the cyanobacterium *Synechocystis* sp. PCC 6803 maintaining 2- to-5-fold more PSI than PSII, depending on conditions. The physiological significance of this proportional disparity is a long-standing question. A possible explanation is that this is an accommodation that allows cyclic electron flow around PSI to generate sufficient ATP for cellular needs, since these cells lack much respiratory activity. We set out to test the hypothesis that a high PSI/PSII stoichiometry in this organism is of functional importance. We generated photoautotrophic mutants with a 2- to-4-fold reduction in PSI levels. At 50 μmol photons m\textsuperscript{-2} s\textsuperscript{-1} the mutant strains grow more slowly than wild type does, with about a 40% reduction in growth rate. However, at higher light intensity there is no significant difference between most strains and wild type for photosynthetic oxygen evolution. PSI content affects the light saturation point, with low-PSI strains requiring higher light intensity to become fully saturated. Low-PSI mutants show greater productivity on a per-chlorophyll basis, suggesting that reduction in PSI content may allow for more productive light utilization within cultures.

5.5. Acclimatory Responses and Biogenesis of IsiA-Family Proteins in Cyanobacteria

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Cyanobacteria exhibit significant diversification in their light-harvesting complexes and have evolved diverse regulatory mechanisms to acclimate to changes in their growth environments. In the siderophilic, filamentous cyanobacterium, *Leptolyngbya* sp. strain JSC-1, five different isiA-family genes occur in two gene clusters. The relative transcript levels for the genes in these clusters are significantly up-regulated during acclimation to Fe limitation, although to somewhat different extents. Chl-protein complexes were isolated and further purified from cells grown under Fe-replete and Fe-depleted conditions. TEM image classification and LC-MS analysis were employed to elucidate the complex acclimative mechanism that modifies light harvesting in response to iron starvation in *Leptolyngbya* sp. strain JSC-1. The acquisition and expression of isiA-like genes during evolution should also be beneficial to cells growing under very low light intensity, as has been observed in some low-light-adapted ecotypes of *Synechococcus* ecotypes isolated from microbial mats. Low-light-ecotypes A14-63 and A6-63 have a gene cluster, *apcD4-apcB3-isiX*, that is evolved acclimation to low light growth. These genes do not occur in the high-light adapted A1-LI strain. Pigment-protein complexes were purified and characterized by spectroscopic comparison and LC-MS analysis. The IsiX, ApcD4 and ApcB3 proteins were identified in the fraction together with some of PS II subunits. The evolution and regulation of specialized photoacclimation mechanisms, which allow low-light-adapted cyanobacteria to grow under low irradiance or specialized nutrient conditions, will be discussed.
5.6. The role of carotenoids in energy transfer within the IsiA pigment protein
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IsiA is a chlorophyll-binding membrane protein produced by cyanobacteria when grown in iron-deficient environments. IsiA forms various ring-shaped supercomplexes consisting of different numbers of IsiA and photosystem I subunits (1). Earlier spectroscopic studies suggested that carotenoids in IsiA may be involved in energy dissipation (2). However, direct spectroscopic evidence of this role of carotenoids in IsiA is lacking. Energy transfer within isolated IsiA particles was probed at cryogenic temperature using time-resolved fluorescence and absorption spectroscopy, and the interactions between different spectral forms of chlorophyll $a$ and carotenoids were monitored. When chlorophyll $a$ was excited, an excited state absorption band originating from carotenoids was not observed, indicating that the chlorophyll excited state was not quenched by a carotenoid quencher. We conclude that carotenoids do not quench the energy absorbed by chlorophyll $a$, but rather may have a structural role within IsiA-containing supercomplexes.

References:

5.7. Examining the Effects of Alternative Electron Transport in Cyanobacteria
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The photosynthetic apparatus utilizes light-dependent electron flow to generate a tightly coupled ratio of ATP/NADPH. Under environments that would negatively affect photosynthetic productivity, changes in the demand and production of ATP and NADPH for metabolic pathways may arise, and the photosynthetic machinery must balance the generation and consumption of both ATP and NADPH through alternative electron transport (AET) processes. In an effort to determine the effects of modulating the ATP/NADPH levels through these mechanisms, we have characterized genetically-engineered strains of the cyanobacteria Synechococcus sp. strain PCC 7002 (PCC 7002) overexpressing heterologous flavodiiron proteins to stimulate the Mehler pathway (Helman et al. 2003), or NADPH dehydrogenase subunits enhancing cyclic electron flow (Shikanai 2007). We have demonstrated that enhancement of AET through these processes improves growth rates, increases tolerance to abiotic stresses, as well as alters carbohydrate metabolism. We believe our study would be invaluable to a deeper understanding of the regulation (Wilde and Hihara 2015) that govern the much of metabolism, while also validating, and leading to more predictive genome-scale metabolic models for cyanobacteria (Nogales et al. 2012).

References:
5.8. The electron transfer pathway upon H₂ oxidation by the NiFe bidirectional hydrogenase of *Synechocystis* sp. PCC 6803 in the light shares components with the photosynthetic electron transfer chain in thylakoid membranes

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In anaerobic conditions the NiFe hydrogenase in the cyanobacterium *Synechocystis* sp. PCC 6803 catalyzes transient H₂ production upon a darkness-to-light transition, followed by a rapid H₂ uptake. We measured H₂ uptake in *Synechocystis* mutants lacking photosystem I, photosystem II or terminal oxidases and in the wild-type strain with and without active cytochrome *b*₆₆. Rapid light-induced H₂ uptake was dependent on cytochrome *b*₆₆ and the presence of photosystem I. We propose light-dependent electron transport from H₂ to plastoquinone, probably via NAD(P)H dehydrogenase, and on to cytochrome *b*₆₆ and photosystem I. In darkness H₂ uptake is ~10-fold slower than in the light and is independent of thylakoid redox components. The plastoquinone redox state may be key in determining the ultimate H₂ redox partner. H₂ uptake and production in darkness likely use the same redox partners. NADH and NADPH, but not reduced ferredoxin, were confirmed as hydrogenase redox donors in vitro.

5.9. Subunits of NDH-1 in *Synechocystis* sp. PCC 6803 are regulated by light and inorganic carbon

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*Synechocystis* sp. PCC 6803 possesses variations of NADPH Dehydrogenase complex 1 which hydrate CO₂ to HCO₃⁻ as part of the CO₂ concentrating mechanism (NDH-1₃,₄), and perform Cyclic Electron Flow (CEF, NDH-1¹,²). The NDH-1 complexes all share a core module that receives electrons from ferredoxin, establishing a proton motive force for ATP production with no net change in [NADPH] [1]. Called the NDH-1M complex, the core is composed of 14 subunits whose regulation is poorly understood, although it is known that the expression of some subunits are upregulated upon carbon limitation [1,2]. The functions of NDH-1 complexes arise from specialized modules comprised of paralogs of NdhF and NdhD which experience differing regulation from the core [1,2]. The regulation behind the modules will allow for cyanobacterial physiology to be understood for their contribution to carbon fixation and to CEF. Carbon limitation and/or light excess will cause transcriptional upregulation of the core complex alongside the specialized modules, resulting in changes to the plastoquinone reduction state and CEF activity. This will be shown by luminescence based promoter probe analysis alongside P700 absorbance and PQ reduction based on chlorophyll fluorescence.

References
5.10. Overexpressing photosynthetic carbon flux control enzymes in *Synechocystis* PCC 6803

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It is environmentally sustainable to convert inorganic carbon directly into desired compounds with photoautotrophic organisms, like higher plants, algae, and cyanobacteria. The Calvin cycle is the primary carbon fixation pathway in photoautotrophic organisms. In higher plants, Ribulose-1,5-biphosphate carboxylation (RuBP) carboxylation and RuBP regeneration together control the carbon flux in this process based on experimental data and dynamic mimic data. Rubilose-1,5-carboxylase/oxygenase (RuBisCO) is the enzyme catalyzing RuBP carboxylation. For RuBP regeneration, Seduhetoluse-1,7-biphosphatase (SBPase), aldolase (FBA) and transketolase (TK) are the three main enzymes controlling photosynthetic rate (Zhu et al. 2007). Data from cyanobacteria is still missing. We succeeded overexpressing native RuBisCO, SBPase (fructose-1,6-/sedoheptoluse-1,7-biphosphatase, FBP/SBPase), FBA and TK separately in *Synechocystis* PCC 6803. The engineering strains showed different phenotypes compared to control strain under 100 μmol photons m⁻² s⁻¹ light conditions in shaking flasks. Growth rate, maximal *in vivo* oxygen evolution rate and dry weight increased in RuBisCO, FBP/SBPase, FBA overexpressed strains. TK overexpressed strain showed chlorotic phenotype and increased dry weight. These results indicate these four enzymes are key enzymes in Calvin cycle in *Synechocystis* PCC 6803 and subsequently are potential targets to increase photosynthetic rates and yields.

**References:**


5.11. Bicarbonate-reversible inhibition of the iron-quinone acceptor complex of Photosystem II lacking low-molecular-weight proteins or with targeted mutations to the D1 protein

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The chlorophyll-binding CP47 pre-assembly complex of Photosystem II (PSII) is associated with up to six low-molecular-weight proteins: PsbH, PsbL, PsbM, PsbT, PsbX and PsbY. We have used gene knockouts in *Synechocystis* sp. PCC 6803 to create a series of mutants to investigate the function of these proteins. In this presentation we will highlight novel findings surrounding the role of PsbT in stabilizing the bicarbonate ligand to the non-heme iron of PSII and we will present new data on the function of PsbH, PsbX and PsbY. These observations include the existence of a stable population of the reduced primary plastoquinone electron acceptor, Qₐ⁻, in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea in cells lacking PsbH indicating that the removal of this protein can substantially modify back reactions with the S2 oxidation state of the oxygen-evolving complex. Our data also suggest a role for these proteins in cyclic electron transfer around PSII which may be important in protecting against photodamage to nascent PSII complexes during assembly. We have also targeted residues on the D1 protein that stabilize bicarbonate suggesting this cofactor may play a role in the susceptibility of PSII to photodamage as well as contributing to the function of the iron-plastoquinone acceptor complex.
5.12. D1’-Containing PS II Reaction Center Complexes under Different Environmental Conditions in *Synechocystis* sp. PCC 6803

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The D1 protein of Photosystem II is the primary target of photodamage and environmental stress can accelerate this process. Cyanobacterial response to stress includes transcriptional regulation of genes encoding D1, including low-oxygen-induction of *psbA1* encoding the D1’ protein in *Synechocystis* sp. PCC 6803. The *psbA1* gene is also shown to be transiently up-regulated in high light and its deletion increased ammonium-induced photoinhibition. We investigated the role of D1’-containing PS II centers under different environmental conditions. A strain containing only D1’-PS II centers under aerobic conditions exhibited increased sensitivity to ammonium chloride and high light compared to a D1-containing strain. Additionally a D1’-PS II strain was outperformed by a D1-PS II strain under normal conditions; however, a strain containing low-oxygen-induced D1’-PS II centers was more resilient under high light than an equivalent D1 strain. These D1’-containing centers had chlorophyll a fluorescence characteristics indicative of altered forward electron transport and back charge recombination with the donor side of PS II. Our results indicate D1’-PS II centers may play a role in the reconfiguration of thylakoid electron transport in response to high light and low oxygen.

5.13. Structural rearrangements preceding dioxygen formation by the water oxidation complex of photosystem II

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Water oxidation is catalyzed by the Mn₄CaO₅ cluster of photosystem II. Recent studies implicate an oxo bridge atom, O5, of the Mn₄CaO₅ cluster, as the ‘slowly exchanging’ substrate water molecule. D1-Val185 is part of the broad aqueous channel as it passes the Mn₄CaO₅ cluster and in the close vicinity of O5. The D1-V185N mutant is shown to retard both the lag and O₂ release phases of the S₃⁺→S₀ transition. The pH dependence, D/H isotope effect, and temperature dependence on the O₂ release kinetics for this mutant were studied using time-resolved O₂ polarography and made comparison with wild-type and two mutants of the putative proton gate D1-D61 (1). Both two kinetic phases in V185N are independent of pH and buffer concentration and have weaker H/D kinetic isotope effects. Each phase is characterized by a parallel or even lower activation enthalpy, but a less favorable activation entropy than the wild-type. The results indicate new rate-determining steps for both phases. It is concluded that the lag does not represent inhibition of proton release, but rather, slowing a previously unrecognized kinetic phase involving a structural rearrangement or tautomerism of the S₃⁺ ground state as it approaches a configuration conducive to dioxygen formation. The parallel impacts on both the lag and O₂–formation phases suggest a common origin for the defects surmised to be perturbations of the H-bond network adjacent to O5. Possible mechanisms will be discussed.

**5.14. Membrane fluidity as a limiting factor for PSII repair mechanism**

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Photosystem II reaction centers (RCs) exposed to light could be photodamaged. RCs must be repaired to maintain highly efficient electron-transfer rate [1]. FtsH proteases, which are distributed both in the cytoplasmic and thylakoid membranes and factors involved in assembly of the Mn cluster of PSII supposed to be the main repair factors [2]. Repair mechanism depends on RCs and repair factors migration through thylakoid membrane. It is not clear what could affect the migration process and membrane fluidity and whether the migration of repair factors concentration could be the limiting factors for PSII repair machinery. The assembly of the Mn cluster is a light-driven process called photoactivation. It is hypothesized that the release of Mn enables the migration of damaged PSII RSs and the repair proteins within the thylakoid membranes. I will present the results of experiments examining the efficiency of assembly of the Mn cluster. We suppose that environment temperature could have a significant effect on the membrane fluidity and consequently affect the repair mechanism. To examine this statement, we perform series of photoactivation experiments in vivo with different treatment temperatures. The designed experiments show the correlation between the environmental temperature, membrane fluidity and rate of water oxidation by PSII RCs.

References:

**5.15. Unique features of *Arthrospira* D1 proteins**

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*Arthrospira* species possess two isoforms of PSII protein D1, tentatively called nD1 and aD1 (1). While nD1 is a D1:2 type encoded by one to three *psbA* gene copies, aD1 is a D1' type encoded by a single *psbA* gene. Both isoforms nD1 and aD1 differ in 44 residues from each other but both have a glutamate at position 130 (E130) indicative for adaptation to high light conditions. Although aD1 displays D1' typical features, including aa residues A80, L158, S162, L186, and A286, it also carries unique features as compared to the dataset holding D1 sequences of over 80 cyanobacteria: the pair R235/R238, the A270 residue, the unique motif AS-A-ACF starting at position 286, and a carboxy-terminal extension containing K349/N352/N357. Aminoacidic information of D1 isoforms in strain *Arthrospira* sp. PCC 8005, to be used in the MELiSSA bioreactor for long-haul expeditions in space (http://ecls.esa.int), is important in respect to its known tolerance to high light conditions and high levels of ionising radiation (> 5 kGy of gamma rays) (2).

References:
5.16. Quantitative Characterization of the FtsH Protease in relation to PsbA turnover in *Prochlorococcus* and marine *Synechococcus*

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Picocyanobacteria, despite their minute size, are the most abundant oxygenic photosynthesizing organisms in the majority of the oligotrophic ocean, contributing 32 to 80 percent of oceanic primary productivity. The PsbA protein (Photosystem II complex D1) becomes routinely photoinactivated at a rate driven by instantaneous light level. The membrane-bound protease FtsH is responsible for removing photoactivated Photosystem II complexes to allow for the synchronized insertion of a newly synthesized D1 subunit. Six *et al.*, (2007), showed that in response to increased irradiance, *Synechococcus* cell types all rapidly induced an increased rate of PSII repair [1]. Further, low-light ecotype *Prochlorococcus* SS120 showed negligible PSII repair while high-light ecotype *Prochlorococcus* PCC 9511 exhibited modest repair [1]. We investigated the relation between FtsH content and the capacity to limit photoinhibition in generalist *Synechococcus* WH8102, low light ecotype *Prochlorococcus* MIT9313 and high light ecotype *Prochlorococcus* MED4, after culture growth under low or high light. High-light grown cultures show significantly higher cellular FtsH to PSII ratios relative to low-light cultures. Furthermore the FtsH:PSII ratio closely predicted cellular capacities to remove D1 protein and counter photoinactivation across strains and growth conditions.

References:


5.17. Structure, Diversity and Evolution of a New Family of Soluble Carotenoid-Binding Proteins

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Using a phylogenomic approach, we have identified and subclassified a new family of all-helical carotenoid-binding proteins (HCPs) with sequence homology to the N-terminal domain (NTD) of the Orange Carotenoid Protein (OCP). These proteins comprise at least nine distinct clades and are found in all orders of cyanobacteria except the Prochlorales, frequently as multiple copies. These seem to be out-paralogs maintained from ancient duplications associated with subfunctionalization. All members share conservation of the carotenoid-binding residues and we confirm carotenoid binding as a fundamental property. We solved two crystal structures of one paralog (HCP1) from *Anabaena* PCC7120, binding different carotenoids, suggesting that the proteins flexibly bind a range of carotenoids without major structural alterations. We propose that one subtype (HCP4) represents the evolutionary ancestor of the OCP-NTD which arose by domain fusion. We suggest that the majority of these proteins have functions distinct from the OCP-NTD. HCP4 from *Anabaena* was shown to induce permanent thermal energy dissipation of phycobilisomes, HCP2 and HCP3 were shown to be very good singlet oxygen quenchers, and HCP1 did not show any apparent photoprotective function.
5.18. Native mass spectrometry characterization of fluorescence recovery protein and its interaction with orange carotenoid protein

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Cyanobacterial Orange Carotenoid Protein (OCP) protects reaction centers from damage by dissipating excess energy collected by phycobilisomes (PBS) as heat. Dissociation of the PBS-OCP complex is facilitated by another protein known as the Fluorescence Recovery Protein (FRP). Structural studies show that FRP can exist in different conformational and oligomeric states: two different conformations of dimer and one conformation of tetramer. We used native mass spectrometry (MS), a technique that uses volatilization and ionization of biological samples in their near-native states, to characterize FRP. Dimeric FRP is the predominant state while a smaller amount of higher order oligomers are present, especially tetramers. Dimeric FRP is also more resistant to collision-induced dissociation than other oligomers, suggesting that it is a more stable state. All the oligomers became less compact with the application of increasing trap collision energy as observed by a larger collision cross section, while the monomer didn’t exhibit structure expansion during activation. Native collision induced fragmentation of FRP mostly occurs at the peptide bonds where Aspartate (Asp) provides the amino group, indicating high flexibility or accessibility of Asp adjacent regions. To study OCP-FRP interactions, we generated the N-terminal domain (NTD) and C-terminal domain (CTD) of OCP by partial proteolysis. Using native mass spectrometry, we identified a complex of FRP-CTD as well as FRP-CTD-NTD, but not FRP-NTD. These results strongly suggest that dimeric FRP facilitates the conversion of red active OCP to its orange inactive form by bridging both NTD and CTD together rather than by associating with only the CTD.

5.19. Photoactivation and Relaxation Studies on the Cyanobacterial OCP in the Presence of Different Metal Ions

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Photosynthesis starts with light energy being absorbed by light-harvesting antenna complex and produces energy-rich organic compounds. All photosynthetic organisms face a challenge when light absorption by antennas exceeds photochemical conversion capacity. In cyanobacteria, non-photochemical quenching performed by the Orange Carotenoid Protein (OCP) is one of the most important mechanisms to regulate the light energy capture and thus photochemical conversion to be compatible with cellular energy requirements under fluctuating light conditions. In this communication, factors affecting the photoactivation and relaxation of OCP were examined. It was found that many heavy metals have no significant effects on the kinetics of either photoactivation or relaxation. It appears, however, that zinc ion inhibits photoactivation of OCP at sub-millimolar concentration. Copper ion has no effect on the kinetics of the photoactivation phase. Relaxation of red OCP to orange OCP, however, is completely blocked in the presence of copper, even in the presence of the fluorescence recovery protein. Native PAGE and SDS-PAGE analysis indicates the heterogeneous population of copper locked red OCP. Copper binding capacity to either N-terminal domain or C-terminal domain of OCP were also examined. Cu\textsuperscript{2+}-OCP binding constants were also estimated. Detailed structural analysis are in progress with the aim of elucidating the structural binding site of copper to the photoactivated red OCP. Functional roles of copper binding OCP \textit{in vivo} are also discussed.
5.20. Towards understanding of function of the evolutionary related homologs of the orange carotenoid protein in *Fremyella diplosiphon*

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The Orange Carotenoid Protein (OCP) is involved in photoprotection in cyanobacteria and is important for primary productivity of cyanobacteria and its potential use as a simple photoswitch. This is a modular protein consisting of an effector and a sensor domain, and in which a carotenoid molecule is involved in light sensing. In the dark, OCP is in an orange, inactive conformation. A conformational change and a shift of the carotenoid molecule cause a change of the color from orange to red, under high light conditions. This change of shape allows OCP to bind reversibly to the phycobilisome core, and mediate non-photochemical quenching. OCP is comprised of two main domains, an all-alpha helical N’ terminal domain (NTD), and an alpha-beta C-terminal domain (CTD), similar in shape to a nuclear transport factor 2 (NTF2). While the NTD is mostly involved in non-photochemical quenching, the CTD is involved in light sensing, and reverting to the non-active orange form. Interestingly, along with genes for full-length OCP, many cyanobacteria also contain genes homologous to the CTD or NTD. These domain homologs might have been the evolutionary ancestors of functional full length OCP, however, it is not clear what their function is. We are studying, in vitro, these domain homologs from *Fremyella diplosiphon* in attempt to learn their function(s) and for potential use as a synthetic photoswitch.

5.21. Far-red light photoacclimation (FaRLiP) in *Synechococcus* sp. PCC 7335: central control and a novel combination of antenna structures

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In response to far-red light (FRL), some cyanobacteria synthesize chlorophyll (Chl) d and Chl f and remodel photosystem (PS) I, PS II, and phycobilisomes (PBS), a process called Far-Red Light Photoacclimation (FaRLiP) to utilize FRL for oxygenic photosynthesis. The FaRLiP gene cluster, identified in 13 cyanobacterial strains, contains genes for Rfp regulators and paralogous PS I, PS II, and allophycocyanin subunits. In this study, three antibiotic resistance cassettes were tested and used to generate deletion mutants in *Synechococcus* sp. PCC 7335, and mutants of the *rfpA*, *rfpB*, and *rfpC* regulators were constructed. The three *rfp* mutants are unable to perform FaRLiP but can still undergo complementary chromatic acclimation (CCA) and produce additional phycobiliproteins (PBPs). The study of PBS and PBPs in *Synechococcus* sp. PCC 7335 shows that PBS are not remodeled as occurs in *Leptolyngbya* sp. JSC-1. Instead, bicylindrical cores containing paralogous allophycocyanin subunits encoded in FaRLiP cluster are produced and coexist with normal PBS in FRL-grown cells. These and other results show that variations in acclimation responses to FRL occur among cyanobacteria in spite of similarities in their FaRLiP gene clusters.
5.22. Heterologous Expression and Characterization of Far-Red Absorbing Phycobiliproteins from Two Photoacclimative Responses

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Far-Red Light Photoacclimation (FaRLiP) is possible in some species of cyanobacteria because of a large gene cluster encoding paralogs of most of the photosynthetic core components (1). Included in the cluster are genes encoding paralogous allophycocyanins, a class of phycobiliproteins found in the cores of phycobilisomes. These paralogous allophycocyanin genes were expressed heterologously in E. coli in order to assess their potential contributions to the far-red light-harvesting properties of the FaRLiP phycobilisome. A paralogous allophycocyanin from a low-light adapted thermophilic Synechococcus sp. Type A was also expressed to define its role in Low Light Photoacclimation (LoLiP; 2). Most but not all of the recombinant allophycocyanin paralogs are capable of absorbing far-red light and are potential contributors to the far-red absorbance observed in cyanobacterial cells that have undergone FaRLiP and LoLiP.

References:

5.23. Characterization of photosynthetic membrane complexes from Prochlorococcus marinus MIT 9313

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Most of cyanobacteria use the phycobilisome (PBS) as antenna to absorb and transfer light energy to the reaction centers of photosystems, also, it has been shown that ferredoxin:NADP+ reductase (FNR) is associated with PBS rods (1). The genus Prochlorococcus does not have PBS; it uses CP43 paralogs as antenna (PcBB); and from FNR amino acid sequence a transmembrane helix domain is predicted. We will present mass spectrometry data from sucrose gradient centrifugation and clear native electrophoresis after membrane solubilization with dodecyl maltoside of the cyanobacterium Prochlorococcus marinus MIT 9313. We found the PcbB-photosystem II supercomplex and the co-migration of FNR with the components of cytochrome bc1 complex.

References:
5.24. Antenna engineering in cyanobacteria to improve solar-to-biomass energy conversion efficiency

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Photosynthetic organisms evolved sizable arrays of light-harvesting antenna systems, composed of pigments like chlorophylls, phycobilins, and carotenoids, which absorb the energy of sunlight and funnel the excitation toward the photosystem-II or photosystem-I reaction centers. A large light-harvesting chlorophyll or phycobilisome antenna size affords the organism a competitive advantage in the wild, as it enables absorption of usually limited light in the natural ecotype. This in turn promotes growth, which, otherwise, would be severely stunted. Under bright sunlight, however, the large arrays of light-harvesting pigments absorb photons far in excess of what is needed to saturate photosynthesis. Over-absorbed energy is dissipated as heat, thus compromising the energy-conversion efficiency and potential productivity of photosynthesis. In a commercial production system, where high densities of cyanobacteria are grown under direct sunlight, this large light-harvesting antenna configuration may cause the unacceptable loss of up to 80% of the absorbed energy. Minimizing the antenna size of the photosystems will prevent the over-absorption of sunlight, alleviate unnecessary shading, and permit greater sunlight penetration deeper into the culture, leading to more efficient overall utilization and to greater productivity. We generated Δ-cpc mutants, lacking the genes coding for the peripheral phycocyanin rods. This truncated phycobilisome antenna size substantially limited the light-harvesting capacity of photosystem II. Nevertheless, Δ-cpc mutants under high light and mass culture conditions accumulated biomass faster by about 50% than the corresponding wild type. Application and approaches for further improvements to maximize sunlight-utilization efficiency will be discussed.

5.25. Characterizing the function of CpeF in phycoerythrin biosynthesis

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Cyanobacteria are important contributors to primary productivity in many ecosystems. Their success is in part due to their ability to harvest light that other photosynthetic organisms cannot, due to their phycobilisome complex. *Fremyella diplosiphon* can perform Type 3 chromatic acclimation in which it can change its phycobilisome protein content if its cells are experiencing more red or green light. Phycoerythrin (PE), the main protein that is synthesized to best absorb green light, contains five covalently attached phycoerythrobilin (PEB) chromophores for efficient photosynthetic light capture in *F. diplosiphon*. Chromophore ligation on phycobiliprotein α and β subunits occurs through bilin lyase catalyzed reactions. It is believed that each bilin attachment site is chromophorylated by specific bilin lyases. The cpeF gene in *F. diplosiphon* belongs to the CpcEF family of bilin lyases according to its similarity in sequence and predicted 3D structure. Recombinant protein studies were conducted and the results suggest that CpeF may be responsible for attaching PEB to the beta subunit of PE (CpeB). A cpeF deletion mutant in *F. diplosiphon* was generated and compared to wild type as well. The mutant results also suggest that CpeF plays a role in PE biosynthesis, possibly as a bilin lyase for CpeB at Cys-48/59.
5.26. Characterizing the Function of Bilin Lyases CpeY and CpeU in marine Synechococcus RS9916

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Many Synechococcus isolates are tuned to absorb specific light colors, but approximately one third are able to adjust the bilin chromophore composition of their phycobilisomes (PBS) to optimize light capture in changing light environments of the oceans through a process known as type IV chromatic acclimation (CA4) (1). Synechococcus contains two types of the phycobiliprotein phycoerythrin (PEI and PEII) increasing the complexity of the PBS light harvesting capabilities. Lyases are the enzymes which catalyze the addition of the linear tetrapyrrole bilins to specific cysteine residues on phycobiliproteins. Two potential lyases involved in CA4 in the marine Synechococcus RS9916 were identified as CpeY and CpeU. Initial analysis of recombinant CpeY revealed robust addition of phycoerythrobilin (PEB) to Cys-83 on the \( \alpha \) subunit of PEI (CpeA) in our \( E. coli \) expression system. HPLC analyses of \( cpeU \)-mutant PBS show defects in bilin addition to CpeA and MpeA in green light and to CpeA in blue light. We are awaiting results from LC-MS-MS analyses to determine which sites are affected on these subunits.

References:

5.27. Characterization of the Putative Bilin Lyase MpeY from Synechococcus RS9916

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Synechococcus strains are able to efficiently capture blue-green light and are able to maintain a high level of photosynthetic activity by continuously adjusting their photosynthetic machinery to changes in the ratio of blue to green light in their environment through a process called Type 4 chromatic acclimation (CA4). During CA4, there are three sites of differential chromophore attachment to phycoerythrin (PE) I and II subunits within the phycobilisomes (PBS) [1]. We are interested in identifying the bilin lyases/isomerases which are responsible for the chromophorylation changes seen during CA4. MpeY is similar to other known lyase/isomerases like MpeZ [1], [2]. The \( mpeY \) gene was inactivated in Synechococcus RS9916, and PBS were purified from WT and \( \Delta mpeY \) mutant strains grown under green light (GL) and blue light (BL) conditions. Spectral analyses of HPLC separated mutant PBS samples indicate that \( mpeY \) may be responsible for the addition of PEB to MpeA under GL conditions. LC MS/MS results indicate C-83 is the site affected in the \( \Delta mpeY \) mutant. Recombinant protein coexpression of MpeY with PEII proteins in \( E. coli \) confirms that this enzyme attaches PEB to MpeA.

References:
5.28. Understanding β-Carboxysomes through Production of Synthetic Carboxysome Shells

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Cyanobacteria evolved a CO₂ concentrating mechanism (CCM) to enhance the carbon fixation activity of the inefficient enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO). A central component of cyanobacterial CCM is a proteinaceous organelle named carboxysome. Two types of carboxysomes, α and β, encapsulate form IA and form IB RubisCOs, respectively. While the compositions and stoichiometry of α-carboxysomes have been well documented, equivalent information of β-carboxysomes is absent due to the technique difficulties in isolating and purifying intact β-carboxysomes. Here we reported a system for production of synthetic β-carboxysome shells and encapsulation of non-native cargo. Recombinant shells with or without encapsulated cargo can be purified, which lead to better understanding of protein-protein interactions in the shell and core-shell interaction of β-carboxysomes. This synthetic shell system will also be used to probe the permeability properties of intact shells; these data are critically needed for an understanding of how the carboxysome is integrated with the subcellular and cellular environment.

References:

5.29. CcmM and its Interaction with Rubisco and CcmN in Cyanobacterial β-Carboxysomes

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In cyanobacteria, a proteinaceous organelle called the carboxysome encapsulates the key enzymes for CO\textsubscript{2} fixation, carbonic anhydrase and Rubisco. This provides Rubisco with a high local concentration of CO\textsubscript{2} and protects it from O\textsubscript{2}. CcmM is a multidomain protein consisting of a potential γ-carbonic anhydrase domain and a repeat of Rubisco small unit like domains \cite{1}. It acts as a scaffolding protein both initiating the assembly of the carboxysome and organizing its interior \cite{2}. Its small subunit like domains interacts with Rubisco by displacing the small subunit and thereby cross-linking several Rubisco proteins together. The γ-carbonic anhydrase domain interacts with CcmN which contain a C-terminal encapsulation peptide that recruits the shell protein and thus forming the functional carboxysome. Here the progress in characterizing the structure and function of the complexes between CcmM and its interaction partners Rubisco and CcmN will be presented.

References:
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5.30. Characterization of the Selective Permeability of Carboxysome Shell Proteins and Implications for CO\textsubscript{2} Fixation Efficiency

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Carboxysomes are defined by selectively permeable proteinaceous shells that enhance the oxygen-sensitive CO\textsubscript{2} fixation reaction catalyzed by RubisCO. Structural models of shell protein suggest that the charged RuBisCO substrates and products, namely RuBP, bicarbonate and 3PGA can cross the carboxysome shell, while it provides a barrier to the uncharged molecules CO\textsubscript{2} and O\textsubscript{2} \cite{1}. However, the selective permeability was not functionally studied to date. To study the role of the shell proteins CCMK3 and CCMK4 for carboxysome permeability in \textit{Synechococcus} PCC7942, we grew cultures of wildtype and ccmK3 ccmK4 mutant in 3% CO\textsubscript{2} and analyzed the RuBP and 3PGA metabolite pools. We find a resulting metabolic imbalance, suggestive of a role of CcmK3 and CcmK4 in transport of RuBisCO substrates. Site directed mutants of CcmK3 and CcmK4 were created and are currently characterized to understand the role of a small, central pore for the transport function.

References:
\begin{enumerate}
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6.1. A novel calcium-binding protein in *Anabaena* sp. PCC 7120 is crucial for growth in calcium-depleted conditions

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Calcium plays a pivotal role as a well-known second messenger in eukaryotic organisms. However, studies in prokaryotes, especially in cyanobacteria, are still rather scarce. In plant cells, calcium transients are evoked in order to respond to environmental conditions with special regards to the communication between photosynthetic plastids and cellular mechanisms. Cyanobacteria are claimed to be the chloroplast ancestors. Therefore, the possibility that the function of intracellular cyanobacterial calcium “signatures” and regulation may be comparable to chloroplast calcium signalling, can’t be neglected and may differ between various species. In our studies, we focus on the investigation and characterisation of calcium regulators in two model cyanobacteria, *Anabaena* sp. PCC 7120 (filamentous, nitrogen-fixing) and *Synechocystis* sp. PCC 6803 (unicellular, non-nitrogen-fixing). A search for putative candidates with the help of bioinformatics tools revealed an unreviewed protein in *Anabaena* called Calcium Sensing EF-hand (CSE). This small (8.5 kDa) soluble protein is highly conserved in some filamentous cyanobacteria as it consists only of 2 EF-hand domains. The predicted protein crystal structure highly resembles the same calcium-binding motifs in calmodulin while its high affinity calcium-binding ability was demonstrated *in vitro*. Characterisation of a mutant strain lacking CSE unveiled the significance of this novel calcium-binding protein on the culture growth under particular stress conditions in *Anabaena*.

6.2. Understanding the Role of Microcystin and CP12-CBS in *Microcystis aeruginosa*

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Microcystins (MC) are the most notorious toxins produced by harmful cyanobacterial blooms (CyanoHABs) in marine, brackish and freshwater habitats. It is assumed that MC increases the resistance of CyanoHABs towards various environmental stresses [1, 2]. Detailed knowledge about the actual molecular function of MC is, however, still fairly limited. Recent work has already revealed that MC is: a) covalently binding to certain proteins, such as CP12-CBS, protecting them against protease degradation [2], and b) possibly affecting the cellular ultrastructure of *Microcystis aeruginosa* under high light [3]. We aim to clarify the role of MC in *Microcystis aeruginosa*, one of the most common CyanoHABs in freshwater ecosystems, by investigating: (1) its interaction with CP12-CBS, a fusion protein of unknown function, consisting of the regulatory protein CP12 and the regulatory domain CBS [4], using protein crystallography and biochemical methods. CP12-CBS is uniquely found in cyanobacteria and its function is still unknown; and (2) the 3-dimensional cellular ultrastructure of *M. aeruginosa* wild type and its nontoxic mutant ΔmycB in response to high-light stress, using soft X-ray tomography.

References:

6.3. Physiology of the Entner-Doudoroff pathway in Cyanobacteria

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Our group has recently been able to show the presence of an operational Entner-Doudoroff pathway in the cyanobacterium *Synechocystis* sp. PCC 6803 (submitted1). This pathway embodies a third route of glucose oxidation next to classical glycolysis (Embden-Meyerhof-Parnas pathway) and the oxidative pentose phosphate pathway. Monitoring pathways of glucose oxidation in cyanobacteria has often been subject of scientific interest. While under heterotrophic conditions the oxidative pentose phosphate pathway seems to be the most abundant way of glucose oxidation, the situation under mixotrophic conditions remains unclear. We propose that the Entner-Doudoroff pathway might provide a missing link under mixotrophic conditions where glucose oxidation and carbon fixation (via the Calvin-Benson cycle) happen simultaneously. Previous experiments by our group have shown that a mutant where the key enzyme of the Entner-Doudoroff pathway, the 2-keto-3-deoxy-6-phosphogluconate aldolase (KDPG-aldolase), is deleted has impaired growth capability under mixotrophic conditions. Further studies to elucidate the physiology of the Entner-Doudoroff pathway and carbon metabolism in *Synechocystis sp.* PCC 6803 were done. I found differences in glycogen metabolism of the KDPG-aldolase mutant and wildtype. These data together with investigations on glucose uptake under autotrophic, mixotrophic and heterotrophic conditions will be discussed.

1Chen X et al (2016): The Entner-Doudoroff pathway is an overlooked glycolytic route in cyanobacteria and plants

6.4. Glutathione and glutathione-dependent enzymes protect *Synechococcus* PCC 7942 against stress

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Reactive oxygen species (ROS) are detrimental to cellular macromolecules.1 Low molecular weight (LMW) thiols detoxify ROS and maintain a reducing environment. Glutathione (GSH), a LMW thiol, maintains the cellular redox potential2 in *Synechococcus* PCC 7942. We characterized transposon mutants disrupted in *gshB*, required for GSH biosynthesis3, *gstC*, encoding a glutathione S-transferase (GST),4 *gscR*, encoding a dual activity nitrosothiol reductase/formaldehyde dehydrogenase5, and *grxC*, encoding a glutaredoxin.6 Growth of gshB::tn was inhibited by oxidants (H2O2, cumene hydroperoxide (CHP), and plumbagin), and metals, Cu2+ and Cr2O72-. Growth of gstC::tn was also inhibited by H2O2 and CHP, and the GST substrates 1-chloro-2,4-dinitrobenzene and 4-nitrobenzyl chloride. Both gscR::tn and gshB::tn were more susceptible to nitrosative stress and formaldehydes than wildtype. In addition, chlorophyll a was reduced in gshB::tn. GscR is able to act as a nitrosothiol reductase and formaldehyde dehydrogenase, and GrxC is a bona fide glutaredoxin that can be reduced by thioredoxin reductase, indicating cross-talk between the thioredoxin and glutaredoxin systems. Taken together, GSH and GSH-dependent enzymes protect *Synechococcus* PCC 7942 against different stresses.

References:
6.5. Npun_F0288 plays a role in lipid droplet production and filament integrity in *Nostoc punctiforme*

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*Nostoc punctiforme* lipid droplets (LDs) contain large amounts of un-charged lipids that stain with lipophylic dyes and contain enhanced levels of alkanes, alpha-tocopherol, and triacylglycerols [1]. To understand their physiological role, proteins associated with purified LDs were identified. Npun_F0288, a 12 kDa hypothetical protein containing two alpha-helical domains, was the highest expressed protein in lipid droplets of both wild type (WT) and LD overexpression strains. Structural prediction programs found similarities to cytoskeletal proteins, apolipoproteins, and vesicle fusion proteins. In order to determine protein localization and potential function, CFP translational-reporter and over-expression strains were created. The 0288CFP strain exhibited polar localization, reduced contacts between cells within filaments, and grew at a rate of 21% that of WT. Over-expression of *Npun_F0288* from a multi-copy plasmid resulted in increased LD production that were abnormally aggregated near the center of vegetative cells. The over-expression strain also exhibited a decreased growth rate, 56% that of WT. Together these results supported the hypothesis that Npun_F0288 plays a structural role in LD formation. *Npun_F0288* insertional and knockdown mutants are being created to further confirm the role of Npun_0288.


6.6. Carotenoid Biosynthesis Associated with Lipid Droplets in *Nostoc punctiforme*

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*Nostoc punctiforme* has been shown to produce intracellular lipid droplets (LDs) that increase in number as culture enters stationary phase (1). Five proteins putatively involved in carotenoid biosynthesis were found by a proteomic approach in LDs from cultures grown in both exponential and stationary phase. Three strains—CFP-protein fusion reporter, overexpressor, and mutant—were constructed for each gene encoding one of these lipid-associated carotenogenic proteins of interest. The effect of overproducing each CFP-protein fusion on growth or survival in high light and high heat was determined. Confocal and epifluorescence microscopy, along with BODIPY staining of LDs, allowed determination of the effect of these stresses on abundance or phenotypic characteristics of LDs. These techniques combined with Western blotting further supported co-localization of the proteins with LDs, which were isolated by ultracentrifugation from both exponentially grown and stationary cultures. Carotenoid composition of outer membrane, inner membrane, thylakoid membrane, and lipid droplets was profiled by HPLC and TLC after membrane fractionation. Comparison of these profiles hints at the site of origin of the LDs.

References:


6.7. Kinetic Modelling of Light-Limited Cyanobacterial Growth and Chemical Production

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A large variety of products have been produced from cyanobacterial photosynthesis, but higher productivities are required for implementation at industrial scale\(^1\). Kinetic modelling of bacterial growth\(^2\) and substrate utilization\(^3\) has long been an effective tool for understanding growth phases and energy utilization in bacteriology. In contrast to bacterial growth on organic substrates, light-limited cyanobacterial batch cultures spend a majority of their time in the growth deceleration region of the Monod plot due to the asymptotic decrease of cell-specific light exposure as a result of cell shading. In this work, a kinetic model was developed to study light-limited batch growth of cyanobacteria engineered for production of secreted or intracellular products. Parameters describing maintenance energy, photosynthetic efficiency, and target molecule productivity were determined by fitting the model to data from bioreactor growth experiments using strains of *Synechococcus* sp. PCC7002 engineered for the production of lysine as a secreted product or glycogen as an intracellular product. These parameters provided insight into strategies for strain improvement.

References:


6.8. Cyanobacterial genome-scale mapping models for \(^1\)C-Metabolic Flux Analysis

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The availability of a carbon mapping model remains the major bottleneck in the genome-scale \(^1\)C metabolic flux analysis of cyanobacteria. A 71% similarity of the *Synechocystis* genome-scale metabolic model with the fully mapped *E. coli* model results in only 221 reactions unique cyanobacterial reactions. Here, we have constructed *imSyn711* and *imSyf608*, the genome-scale metabolic mapping (GSMM) models describing reaction atom mapping for all carbon-balanced reactions contained within *Synechocystis* PCC 6803, *Synechococcus* PCC 7942, respectively, by appending to the already published *E. coli* mapping model, *imEco726*, reaction mapping information generated using the fast and accurate CLCA algorithm. We found that the 99.8% genomic sequence similarity of the *Synechococcus* PCC 7942 and the fast-growing *Synechococcus* UTEX 2973 gives rise to a 100% identity of carbon-balanced reactions contained within their respective genome-scale models, resulting in the applicability of *imSyf608* for \(^1\)C-MFA of *Synechococcus* UTEX 2973. However, *Synechococcus* and *Synechocystis* have 591 similar reactions, with 120 and 17 reactions unique to *Synechocystis* and *Synechococcus*, respectively. Furthermore, the presence of nine compounds possessing symmetry result in 49 reactions having multiple atom maps. Finally, the generation of an EMU model based on amino acid fragmentation obtained by GC-MS and glycolytic intermediates obtained by LC-MS reveals 67 novel carbon scrambling reactions unique to cyanobacteria arising from Calvin cycle, photorespiration, an expanded glyoxylate metabolism, and corrinoid biosynthetic pathways. The two mapping models *imSyn711* and *imSyf608* will be made available along with their corresponding EMU reactions.
6.9. Metabolic Modeling of a Nitrogen Fixing Cyanobacterium
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Trichodesmium erythraeum is a filamentous diazotrophic cyanobacteria which is responsible for approximately 42% of the global biological nitrogen fixation each year. Despite its importance standing in the global nitrogen cycle, little work has focused on quantifying intracellular and intercellular carbon and nitrogen fluxes. We have generated a genome-scale metabolic model of T. erythraeum based on the publically available genome sequence. A biomass formation equation has been developed based on laboratory data of trichomes grown on both N₂ and nitrate as N sources. Flux balance analysis (FBA) has been used to simulate fluxes for both distinct cell types (carbon fixing and nitrogen fixing) and in situ and previously reported laboratory data has also been used to help constrain the model and validate fluxes. We have also implemented dynamic FBA and cell-cell interactions to determine how a population determines the optimal composition of carbon fixing versus nitrogen fixing cells.

6.10. Diurnal Regulation of Cellular Processes in the Cyanobacterium Synechocystis sp. PCC 6803: Insights from Transcriptomic, Fluxomic and Physiological Analyses
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Certain cyanobacterial strains are amenable to facile genetic manipulation, thus enabling synthetic biology and metabolic engineering applications. Such strains are being developed as chassis for sustainable production of food, feed, and fuels. To this end, a holistic knowledge of cyanobacterial physiology and its correlation with gene expression patterns under the diurnal cycle is warranted. In this study, we measured and subsequently analyzed expression patterns of nearly 3,500 genes in Synechocystis 6803 over two consecutive diurnal periods. Based on this analysis, at least 39% of genes with many from major cellular processes including central carbon metabolism, transport, and regulation were found to exhibit oscillating expression profiles. The pattern of gene expression also led to the development of two distinct transcriptional networks of co-regulated oscillatory genes. These networks help describe how Synechocystis 6803 regulates its metabolism toward the end of dark period in anticipation of efficient photosynthesis during early light period. Furthermore, in silico flux prediction of important cellular processes and experimental measurements of cellular ATP, NADP(H), and glycogen levels showed how this diurnal behavior influences its metabolic characteristics. In particular, the NADPH/NADP⁺ showed strong correlation with the majority of the light peaking genes. We conclude that this ratio is a key endogenous determinant of the diurnal behavior of this cyanobacterium.
6.11. Axenic biofilms of Synechocystis PCC 6803 require cell surface structures and occur under nutrient limitation

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Phototrophic biofilms are key to nutrient cycling in natural and engineered environments. The literature on mixed-species phototrophic biofilms is extensive, but there are few studies describing biofilm formation by a single (axenic) species of phototrophic microbe. We hypothesize that phototrophic bacteria such as Synechocystis are able to form axenic biofilms, and use cell surface structures such as pili to attach and adhere to surfaces, similar to other biofilm-forming heterotrophic bacteria. Using microscopy and the crystal violet biofilm assay, we found that axenic wild-type (WT) Synechocystis forms biofilms of cells and extra-cellular material when shifted to nutrient-depleted medium. WT Synechocystis does not form biofilms in nutrient-replete medium (BG11). Unlike WT Synechocystis, mutants lacking genes required for synthesis of cell surface structures such as type IV pili and the S-layer do not form biofilms under nutrient limitation. We conclude that pili and the S-layer are necessary but not sufficient for biofilm formation by WT Synechocystis: additional factor(s) must be induced, such as through nutrient limitation, in order for pili and S-layer to facilitate biofilm formation. We will use mass spectrometry and RNA sequence of induced and uninduced WT cultures to further develop this model.

6.12. Prophage control of cyanobacterial growth behaviors

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We have discovered a prophage in the unicellular cyanobacterium Synechococcus elongatus PCC 7942 that represses both biofilm formation and expression of the photosynthetic apparatus. Previously identified as a small cryptic phage region, the 49 kb prophage contains over 51 open reading frames encoding a complete phage genome, including the genes necessary for a virion structure, switching between lysogeny and lysis, and genome replication and packaging. This prophage is present, with some modifications, in the closely related strains of S. elongatus PCC 6301 and S. elongatus UTEX 2973. Genetic deletion of the prophage from the PCC 7942 genome converts a planktonic, high-light bleaching strain into a biofilm-forming, non-bleaching strain. Further mutational analysis has identified a prophage region encoding two putative DNA-binding proteins as responsible for the photosynthetic bleaching phenotype. A recent isolate of S. elongatus from Waller Creek, Texas, named WC-1, lacks the prophage, readily forms biofilms, and does not bleach under high light. This new strain supports the hypothesis that the non-lysogenic strain of Synechococcus naturally grows as a biofilm at the bottom of the water column and is reprogrammed by prophage integration to disperse into the higher light conditions at the top of the water column.
6.13. The importance of pili-mediated metal acquisition in primary productivity
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Photosynthesis in *Synechocystis* sp. PCC 6803 requires a large quantity of iron and manganese [2,3] to maintain its electron transport chain and oxygen evolution, respectively. However, in most environments these elements are present in the form of insoluble oxides [1]. Whether cyanobacteria can utilize these sources of iron and manganese, and the potential molecular mechanisms involved remains to be defined. There is increasing evidence that pili can facilitate electron donation to extracellular electron acceptors, like iron oxides in non-photosynthetic bacteria [4-6]. In these organisms, the donation of electrons to iron oxides is thought to be crucial for maintaining respiration in the absence of oxygen. Our study investigates if any pilin proteins (including PilA1, the major pilin protein in *Synechocystis* sp. PCC 6803) may also provide a mechanism to convert insoluble forms of these elements into soluble forms for uptake by membrane transport systems. Growth experiments supported by spectroscopic data of a strain deficient in pilA1 indicate that the presence of the pilA1 gene enhances the ability to grow on various iron oxides. These observations suggest a novel function of PilA1 in *Synechocystis* sp. PCC 6803 iron acquisition.

References:

6.14. Nanowire-mediated iron acquisition in *Synechococcus* sp. PCC7002
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In marine environments, iron is often a limiting nutrient for the growth of phytoplankton. In oceans, iron exists in two redox states that are thought to have different bioavailability. Fe\(^{2+}\) is soluble and known to be readily bioavailable, whereas Fe\(^{3+}\) forms insoluble iron oxides. To what extent phytoplankton can access these iron oxides is unknown. Marine cyanobacteria such as *Synechococcus* sp. PCC7002 need efficient iron acquisition to maintain their large iron requirement. There is increasing evidence that nanowires (conductive type IV pili) facilitate donation of electrons to extracellular electron acceptors [1] such as iron oxides thereby reducing the complexed iron and increasing its bioavailability [2]. During this study, growth experiments and spectroscopic data of the *Synechococcus* sp. PCC7002 strain deficient in the pilA1 gene will be evaluated to assess whether PilA has a function in iron acquisition in marine cyanobacteria.

References:
6.15. Enhancing Phosphate Uptake in Cyanobacteria
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Phosphorus is a limited resource that is critical for life on Earth. However, current agricultural practices result in much phosphate waste in surface waters that in turn results in significant ecological damage. One way to enhance phosphate utilization and to reduce phosphate release into surface waters is to enhance phosphate uptake and storage in biological systems. We are exploring this approach using a cyanobacterium, *Synechocystis* sp. PCC 6803, that can be used for biotechnological applications. An additional advantage is that a phosphate-accumulating strain may have use as a phosphate-rich soil amendment, thus increasing phosphate recycling. We are testing phosphate uptake from BG11 medium and poly-phosphate accumulation in a mutant lacking the *phoU* gene, similar to the SPU101 mutant [1]. We measure the increased phosphorus uptake from the liquid medium via inductively coupled plasma mass spectrometry (ICP-MS) measuring total P (mg/L) in the medium. We follow intracellular poly-phosphate accumulation via DAPI staining and fluorescence microscopy. Additional mutations will be created to further increase phosphate uptake and accumulation via the overexpression of alkaline phosphatase (*phoA*), polyphosphate kinase (*ppk*), and the *pstSCAB* phosphate transporter genes (*pst1* and *pst2*).

Reference:

6.16. An amidase that is required for proper nanopore formation on cell wall septa in cell-cell communication in *Anabaena* sp. PCC 7120
Zhenggao Zheng, Xiying Li, Yan Zhang, Jindong Zhao*

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Intercellular communication is one of the most important features of multi-cellular organisms such as filamentous cyanobacteria. Here we report a gene encoding an amidase is required for cellular communication in *Anabaena* 7120. Although the gene product has no apparent signal peptide, its GFP fusion protein is located at the Z-ring in periplasmic space throughout cell cycle. A null mutant (M40) was constructed and studied with the method of FRAP. Movement of cytoplasm-loaded calcein between cells was aborted in M40. While the physical connection between the cells were similar in the wild type and M40 and arrays of the nanopores can be observed in the peptidoglycan septa of both strains, the average nanopore size on the wild type septa was significantly larger than that of M40. We also studied other filamentous cyanobacteria that do not form heterocysts. The cell-cell communication as revealed by FRAP varies among different filamentous cyanobacteria and it correlates well with the size and the number of nanopores on their septa. Thus, these filamentous cyanobacteria demonstrate various stages in evolution of multicellularity. The M40 was unable to grow diazotrophically and no mature heterocyst was observed on the filaments in the absence of combined nitrogen. Our results support the Turing model of heterocyst pattern formation in which cell-cell communication is essential.
6.17. Cell-cell signaling in marine Synechococcus: Dual transcriptome analysis of antagonistic strains

Javier Paz-Yepes¹,², Stephen M. Gross³, Gary P. Schroth¹, Brian Palenik¹, and Bianca Brahamsha*¹

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Synergistic or antagonistic microbial interactions in the environment ultimately affect the structures of microbial communities, with consequences for the ecosystem as a whole. Marine Synechococcus are a diverse group of coexisting species that contribute substantially to global primary productivity. Synechococcus sp. CC9605 inhibits the growth of other Synechococcus strains by producing a Microcin-C like compound (McC-like), a ribosome-synthesized peptide typical of Enterobacteriaceae that inhibits translation in susceptible strains. Some genes of the Microcin C-like (McC-like) biosynthetic gene cluster of Synechococcus sp. CC9605 are induced in response to co-culture with Synechococcus sp. CC9311. We have found that the induction appears to require cell to cell contact. No significant changes in gene expression occur when liquid co-cultures are separated by a 0.2 μm filter, allowing the diffusion of chemical cues, but not the passage of cells, nor does induction occur when strain CC9311 is pretreated sublethally with Proteinase K. To understand the molecular mechanisms underpinning this interspecies interaction, we examined the global transcriptional response of co-cultures of CC9311 and CC9605 using RNA-seq. In addition to those in the McC-like biosynthetic cluster of CC9605, we have detected a number of other differentially expressed genes in both strains, several of which are predicted to be involved in toxicity, defense and interspecific competition in general.


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Cyanobacteria are uniquely suited for development of sustainable bioproduction platforms, but are currently underutilized in scaled applications in part due to a lack of genetic tools. We have developed a surface display system in the cyanobacterial model Synechococcus elongatus PCC7942 via expression of modified versions of the outer membrane porin, SomA. Importantly, we have demonstrated the accessibility of heterologous functional groups on the recombinant porin to the external environment in living cells, but show that this requires the removal of occluding factors that include lipopolysaccharides and a putative surface layer protein. We show that displayed epitopes on SomA can be utilized to mediate physical adhesion between living cyanobacteria and abiotic surfaces or engineered partner Saccharomyces cerevisiae cells. Our work represents a significant step in the development of functional surface display system in cyanobacteria, with industrial and academic applications.

References:
6.19. Timing of the Precambrian Rise in Atmospheric Oxygen through Molecular Evolutionary Reconstructions of the Cyanobacterial Sunscreen Scytonemin

Ferran Garcia-Pichel\textsuperscript{1}, Martin Wojciechowski\textsuperscript{1}, Jonathan Lombard\textsuperscript{2}, Steven Wu\textsuperscript{1}, Sean Dunaj\textsuperscript{3}, and Tanya Soule\textsuperscript{3}*

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Most paleo-environmental models argue that Earth’s atmosphere underwent a change from mildly reducing and anoxic, to its present oxygen-rich character sometime after the evolutionary advent of oxygentic photosynthesis in cyanobacteria in the Great Oxidation Event (GOE), the timing of which remains a matter of contention. Presently favored estimates from the geochemical rock record place the GOE between 2.45-2.22 billion years ago (Ga), while fossil or molecular evidence for oxygenation is of a much later and uncertain date (2.1-1.65 Ga). We estimated that the minimal evolutionary age of scytonemin, an indole-alkaloid used by cyanobacteria as an ultraviolet-A (UVA) sunscreen, is approximately 2.3 ± 0.8 Ga through phylogenetic reconstruction of several genes that encode for proteins involved in the biosynthesis of scytonemin, as calibrated against the cyanobacterial fossil record. Since the biologically useful form of scytonemin requires an oxidizing environment, and UVA radiation is only damaging in the presence of free oxygen, the minimal age of the scytonemin biosynthetic operon places a direct constraint on the minimal age of the GOE. Our findings significantly push back in time the biological evidence, providing genomics-derived support for geochemical constraints of the timing of the GOE.

6.20. Ionizing Radiation Resistance in Cyanobacteria

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Cyanobacteria have been found to survive under high levels of ionizing radiation in contaminated environments like Chernobyl and Fukushima. While resistance to ionizing radiation has been observed in several cyanobacteria, very little is known regarding the fundamental mechanisms of ionizing radiation resistance in these organisms. As a preliminary investigation, we characterized three ‘model’ cyanobacteria: \textit{Synechococcus elongatus} PCC 7942, \textit{Synechocystis} sp. PCC 6803, and \textit{Synechococcus} sp. PCC 7002. All three species had moderate levels of radiation resistance, with 10% survival rates at ionizing radiation doses ranging from 550 – 750 Gy. This dose is nearly an order of magnitude higher than the 10% survival rate of other Gram-negative bacteria, like \textit{Escherichia coli}. To gain insight into the mechanisms contributing to this enhanced tolerance, we looked toward radiation resistance mechanisms known to be present in the highly radiation resistant bacterium, \textit{Deinococcus radiodurans}. To date, no single mechanism has been shown to be key for high radiation resistance in \textit{D. radiodurans}; instead, many mechanisms contribute, including multiple genome copies, condensed nucleoid structure, and manganese-mediated protection of proteins from reactive oxygen species. These three potential mechanisms of radiation resistance were investigated in the three cyanobacteria. Results suggest some overlap with the mechanisms found in \textit{D. radiodurans}, yet unique and unexplored mechanisms may exist in cyanobacteria, particularly given their phylogenetic distance.
6.21. Cryopreservation of the edible filamentous cyanobacterium *Arthrospira platensis*

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The edible filamentous cyanobacterium *Arthrospira (Spirulina) platensis* is industrially cultured in many tropical and subtropical regions and is consumed worldwide as a nutrient source and food additives. Despite their industrial usefulness, many *Arthrospira* strains have been maintained by regular sub-culturing rather than by cryopreservation, because efficient cryopreservation methods for them do not exist. To determine efficient cryopreservation conditions, cell survival rates were examined after freezing *A. platensis* NIES-39 under various conditions. As a result, it was found that more than 60% of cells were viable upon thawing when they had been frozen at a cooling rate of approximately \(-1°C \text{ min}^{-1}\) in the presence of 10% dimethyl sulfoxide. Decrease in the survival rate was not detectable after 12 months of storage, when they were kept in vapor phase above liquid nitrogen. Further examination with other *Arthrospira* strains showed that the cryopreservation conditions determined with *A. platensis* NIES-39 were unsuitable for many of them. Many *A. platensis* strains had strain-dependent optimal conditions for cryopreservation. By examining the cell survival rates after freezing various strains under various conditions, three sets of conditions that allow cryopreservation of *A. platensis* strains were found. The variety of successful cryopreservation conditions is useful when attempting to cryopreserve various *Arthrospira* strains.

6.22. Environmentally sustainable micro algae as a high value supplement to fodder industry

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The coastal region of Karnataka, India, at the foothills of Western Ghats with abundance of sunshine and heavy monsoon, is a rich source of algal diversity many of which could be utilized for their high value bio-products. Our Institute is situated along the coast and has easy access to local water bodies for easy collection of such algae. The work is focused on assessing isolates of micro algae for their high value bio-products. The microalgae were collected from the local water bodies and temporary ponds during the post monsoon months and were screened for lipid production after identification and isolation. Microalgae also offer multiple bio-products in addition to biofuel that can be used in variety of industry. Some of the algae even contain omega-3 fatty acids and can be used as feed supplement in cattle fodder for high value and environmental sustainability. The work is being carried out in phase wise manner like: (1) Identification and isolation (pure cultures from mixed cultures), (2) harvesting and collection, (3) extraction and analysis of lipids and high-value products, (4) laboratory scale up of algae biomass and (5) large-scale cultivation. After isolating pure algal cultures, growth and lipid production were compared to establish which strains are most stable and productive and most likely to be effective as a feed supplement for fodder industry. For large scale cultivation, the land used to grow algae can be non-arable land as long as it has access to a source of water maybe saline, brackish or even wastewater. Low-cost operational design for scaling up the mass is also in progress.
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