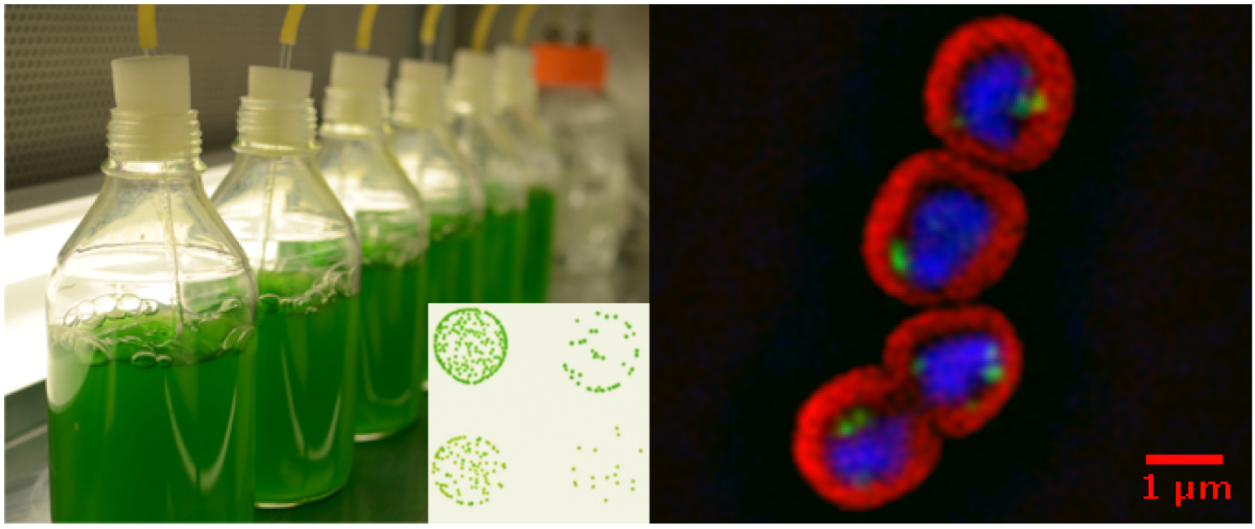
Program and Abstracts

13th Workshop on Cyanobacteria



University of Colorado-Boulder

Renewable and Sustainable Energy Institute

National Renewable Energy Laboratory

June 6-9, 2019
Boulder, CO

Foreword and Acknowledgements

Welcome to the 13th Workshop on Cyanobacteria!

This student and postdoc centered meeting is held in North America every three years and covers all aspects of cyanobacteriology.

The Renewable and Sustainable Energy Institute (RASEI) is a joint institute between the University of Colorado-Boulder and the National Renewable Energy Laboratory (NREL). We thank RASEI and NREL for their generous administrative and financial support. In particular, we thank Carlyla Dawson for her major role in organization of the conference.

We gratefully acknowledge the financial support provided by the International Society of Photosynthesis Research and the US Department of Energy Bioenergy Technologies Office (BETO). These funds will be used to cover the registration fees of several students and postdocs at this workshop.

We also thank the committee of students and postdocs involved in selection of oral presentations and registration fellowships.

Organizers:

Jianping Yu, NREL
Jeffrey Cameron, CU/RASEI/NREL

Carlyla Dawson, RASEI

Cover Image:
Jeffrey Cameron

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Locations

All oral presentations and poster sessions are located in the Sustainability, Energy, and Environment Complex (SEEC).

SEEC
4001 Discovery Drive
Boulder, CO 80309

- Oral Presentations are in the central conference room: SEEC C120
- Poster presentations are in the SEEC Atrium by the Café.
- The banquet dinner is located at Chautauqua Dining Hall. A shuttle bus has been arranged for transportation between venues.
  https://www.chautauqua.com/contact-us/map-directions/

Campus Accommodations

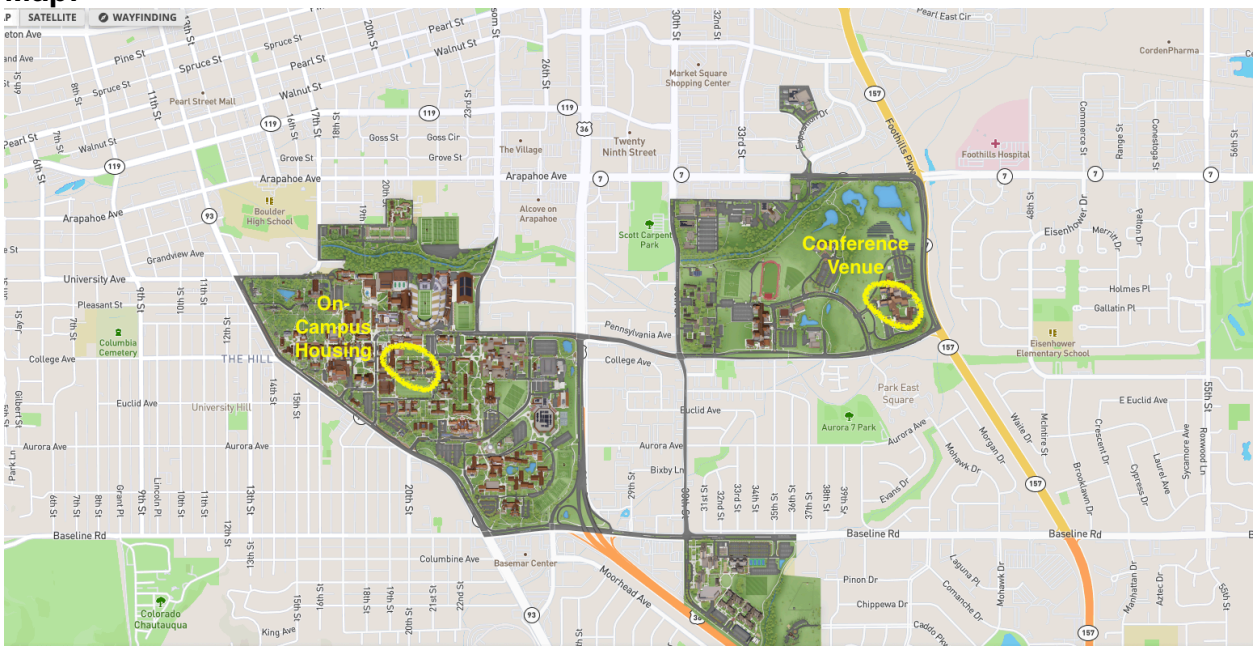
You will be staying in Baker Hall during your time on campus. Please reference the campus map https://www.colorado.edu/map/ for location.

Baker Hall:
2005 Baker Street
Boulder, CO 80310
Phone: 303-492-6896
Check-in Time: 3:00 PM (Standard) Check-in Location: Baker Hall
Check-out Time: 12:00 PM – 1:00PM (Standard) Check-out Location: Baker Hall

If check-in or check-out service is needed outside of building hours (before 6:00 AM or after Midnight), or for 24-hour front desk assistance, visit the below halls. Use the following link to find their locations: http://www.colorado.edu/campusmap/

- Willard Hall
  2200 Willard Loop Drive, Boulder, CO 80310; phone: 303-492-5378

Map:



Linen Service:
Linen service in residence halls includes a linen package (includes one set of sheets, two towels, one washcloth), pillow, pillowcase. blanket and/or bedspread. There is no housekeeping service in individual guest rooms. Housekeeping service is provided in restrooms and public areas. Rooms are furnished with extra-long twin beds, dresser, bookcase, desk and closet. Telephones are not provided in rooms and phone jacks are not active. A microfridge (microwave, refrigerator, and freezer) is provided in each room. Telephone wake-up service is not provided. TVs are located in building lounges. All rooms are non-smoking. Alternating bed height is prohibited.

Rooms are furnished with extra-long twin beds at a height of 35”.

Laundry:
Coin-operated (or campus cash) laundry facilities are located in each residence hall. Participants need to provide their own laundry detergent. $1.25 per load to wash; $1.25 per load to dry (quarters only).

Maintenance:

If maintenance is required to fix something in a room, inform the staff at the front desk of your residence hall. If the front desk is not staffed when the report needs to occur, visit one of the 24-hour desk locations listed above.

Mail:
To mail a letter or package to someone visiting campus, please mail it to the following address:

CU Conference Services
Attn: Conference Name/Recipient Name 2480 Kittredge Loop Dr. #1953
Boulder, CO 80310

Requirements & Guidelines for Event Sponsor and Participant Conduct

1. Event Sponsor is required to inform participants of all conference Residence Hall and Dining Center rules and policies and to enforce compliance by the Participants.
2. Event Sponsor and participants must treat all University of Colorado staff courteously.
3. Event Sponsor may not sell food or beverages on Residence Hall property. Selling food and/or beverages of any kind in the Residence Halls is a violation of the University of Colorado vending contract.
4. Room or roommate switches must be authorized by Residence Hall staff.
5. Rooms are to be left in the same condition as at check-in, tidy and with furniture returned to its original location. The use of glitter, balloons, confetti, etc. is prohibited. Cost of damages and/or the cost of extra cleaning beyond normal wear-and-tear will be charged to the Event Sponsor.
6. Cleats, skates, roller blades and golf spikes may not be worn inside the Residence Hall and/or Dining Center.
7. Sports equipment: balls, pucks, sticks, racquets, etc. may not be used in the Residence Hall and/or Dining Center. All damages to University of Colorado property will be charged to the Event Sponsor.
8. The following Owner buildings do not have potable water in the outside faucets: Aden, Baker, Brackett, Cheyenne Arapaho, Crosman, Farrand, Hallett, Kittredge (Andrews, Arnett, Bucking ham, Smith, and Kittredge West), Libby, Sewall, and Willard. Event Sponsor shall advise Participants not to fill water bottles from the outside faucets.

CU Residence Hall and Campus Policies:

- CU reserves the right to enter any room/apartment for the purpose(s) of inspection, repairs, extermination services, or to control the room/apartment in the event of an epidemic, emergency or any other reason in accordance with university policies.
- CU reserves the right to remove any person(s) causing a disruption from the premises.

- CU is not responsible for any damage or loss of personal property. Belongings left behind will be deemed abandoned and disposed of per university policy.
- Participants are prohibited from altering, damaging or defacing buildings, floors, furniture, fixtures or equipment on campus.
- Pets are not allowed in campus facilities with the exception of documented ADA service animals requested and given written approval more than 30 days prior to the start of your event.

Meal Information:
Use your lodging & dining or guest keycard for meals. Meals are available for purchase on-site at open dining centers by credit card if they are not already included in your lodging & dining package, commuter, or guest meal package. Summer dining hours are as follows:

Farrand Dining (June 7 Breakfast):

Weekdays (Monday – Friday) *Times are subject to change*.

Breakfast	7:00 a.m. – 9:30 a.m.
Lunch	11:00 a.m. – 2:00 p.m.
Dinner	4:30 p.m. – 6:30 p.m.

Weekends (Saturday & Sunday) *Times are subject to change*.

Breakfast	7:00 a.m. – 9:30 a.m.
Brunch/Lunch	11:00 a.m. – 2:00 p.m.
Dinner	4:30 p.m – 6:30 p.m.

Center for Community (C4C) (June 8 and 9 Breakfast):

Weekdays (Monday – Friday) Times are subject to change.

Breakfast	7:00 a.m. – 9:00 a.m.
Lunch	11:00 a.m. – 4:30 p.m.
Dinner	4:30 p.m. – 6:30 p.m.

Weekends (Saturday & Sunday) Times are subject to change.

Breakfast	7:00 a.m. – 10:30 a.m.
Brunch/Lunch	9:00 a.m. – 4:30 p.m.
Dinner	4:30 p.m – 6:30 p.m.

Please reference the campus map https://www.colorado.edu/map/ for locations.

Special Dietary Needs:

For participants with any allergies or dietary restrictions please contact our Registered Dietician Lisa Whalen (Lisa.Whalen-1@Colorado.EDU, 303-735-8241) in order to guide and support participants to prepare a plan prior to arriving to campus.

Campus Dining Hall Policies

- For "All You Care To Eat" operations: While in the dining hall, guests are welcome to eat as much as they like from any of the venues within the dining center, and carryout items are limited to a piece of fruit or hand-held dessert. Bringing containers into the dining hall for use in removing food or containing outside food is not permitted. Removing any items from the dining hall is prohibited and may involve Campus Police.
- Shoes and shirt are required for entrance into the dining hall.
- Please eat at the dining center designated on your meal cards associated with your conference and in the timeframe provided by your conference.
- CDS does not reserve space or sections of tables in the dining rooms. Dining space is first come first serve.

Campus Cash:

Campus guests may load funds onto their lodging & dining or guest keycard to pay for laundry machines and retail store items within Housing & Dining Services (including Starbucks at the UMC) or printing services at applicable places on campus. Campus cash is available on a 'use it or lose it' basis and expires upon your group's departure. No refunds will be given for campus cash loaded onto guest access card or campus cash card.

1. A campus cash card may be purchased and loaded with funds using a credit card at the BuffOne Card Office at the Center for Community (C4C). Please check with their office regarding card expiration dates and applicable rules.
2. A campus cash card may be purchased and loaded using cash at a campus cash card machine
   (located in the entryway area of the C4C outside of the BuffOne Card Office and at the UMC).
3. Funds using a credit card can be loaded onto your lodging & dining or guest keycard at the following website (insert the card # when asked for the Patron ID #): https://services.jsatech.com/paymentcc.php?tab=credit&cid=59

Parking:

For information on pay own parking (credit card machines) see the below "Short Term Parking" link: https://www.colorado.edu/pts/short-term-parking. **If you plan to park on-campus, please contact Carlyla Dawson at Carlyla.dawson@colorado.edu**

Parking Citations:

Parking in incorrect lots or without a valid permit or proper payment can result in parking citations or possible towing. Associated fees are the responsibility of the person operating the vehicle. Parking citations must be paid or appealed within 14 calendar

days of the issue date. Appeals must be filed before the closing of the PTS office on the 14th day after the date of the issuance of the citation.
Pay your citation online: www.colorado.edu/pts/citations

ADA Parking Needs:
Campus parking permits or paying at a meter are required for those with disability placards or license plates. Visit the following website for more information: www.colorado.edu/pts/permits/ada-accessible-parking.

Transportation:
Airport shuttle service to/from DIA (Denver International Airport) & Boulder:
https://www.flydenver.com/parking_transit/transportation_den

- Green Ride Boulder: www.greenrideboulder.com 303-997-0238
- SuperShuttle: www.supershuttle.com 800-258-3826
  RTD Buses (regional service to/from DIA & Boulder): http://www.rtd-denver.com/Schedules.shtml#regional
- View Skyride schedules for AB/AB1/AB2 airport runs & regional routes
- View Flatiron Flyer for Denver/Boulder routes
  Boulder Local Buses: https://bouldercolorado.gov/goboulder/bus The Skip, Bound, and Hop pass campus daily.

Car Rentals:

- eGo CarShare: www.carshare.org
- Zipcar: www.zipcar.com
  Boulder B-Cycle (a community non-profit bike sharing system): https://boulder.bcycle.com/ Daily and month passes available

City walking paths and maps: https://bouldercolorado.gov/goboulder/walk

Internet Access:

- Access wireless service on campus by selecting UCB Guest wireless from your available Wi-Fi network options and accepting the terms and conditions upon opening your web browser. You will be prompted to re-accept these terms and conditions every 24 hours.
- If you encounter difficulty accessing the Internet, call 303-735-HELP (4357) or email help@colorado.edu for assistance during their business hours (7:30 am – 7:00 pm, Monday-Friday).
- Check firewalls or security settings on your computer that could possibly complicate accessing the campus Wi-Fi system before you arrive.

Eduroam:
The eduroam service is an international secure federated access service, allowing for members of participating institutions to access a secure wireless network when on the CU Boulder campus. Campus visitors from institutions participating in eduroam can

simply select eduroam from their operating system Wi-Fi options and connect to eduroam using their institution's credentials. If you experience any issues with this while visiting CU Boulder, please contact your home institution's service desk. Campus visitors not from a participating institution should use the UCB Guest wireless service.

Recreation Center:
The central campus and Bear Creek recreation centers are accessible to campus guests during your stay. Show your lodging & dining or guest access card as ID and pay upon entering unless the entrance fees are covered by your conference. Youth under the age of 15 must be accompanied by an adult.
For more information visit: http://www.colorado.edu/recreation/membership-lockers/membership-rates.

Printing, Copying, Faxing and Shipping Services:
For printing, copying or shipping, please visit one of the below:

- The UPS Store
  2480 Kittredge Loop Drive (north side of Kittredge Central building) 303-442-2601, Ext. #102
- The Ink Spot Copy Center
  1st Floor, University Memorial Center, UMC 130C 303-492-7878
- FedEx Office Print & Ship Center
  2616 Baseline Rd, Boulder, CO 80305 (sound end of campus) 303-494-2622

Campus Bookstore:
University Memorial Center
Euclid Avenue, Boulder, CO 80309 (303) 492-6411; (800) 255-9168
www.cubookstore.com

Health & Safety Information:

• Campus and Off-Campus Emergencies	911
• CU Police Department (On-Campus, Non-Emergency)	303-492-6666
• City of Boulder Police Department (Non-Emergency)	303-441-3333
• Boulder Community Hospital 4747 Arapahoe Ave, Boulder, CO 80303	303-415-7000

Campus Closures, Extreme Weather, and Other Emergencies: Be in the know. Know what to do.

- CU Conference Services is committed to keeping our guests notified with up-to-date and real-time information in the infrequent event of campus closures, extreme weather and other emergencies. CUCS accomplishes this goal by automatically signing up on-site clients and adult attendees to our automated RAVE Alert System. Adults' (18+) e-mail and/or cell phone number(s) provided at registration/check-in are sent to the CU Boulder Alert administrator to be included in the alert database. Individuals are active in the database only for the duration

of the event or program on campus. Depending on the contact information provided, alerts are sent straight to either e-mail accounts and/or mobile devices via our text messaging service.

- The University of Colorado Boulder is committed to providing timely warnings and/or emergency notifications for situations that represent a serious or continuing threat to the campus community and visiting guests. If warranted, warnings may be followed by a clarification and/or instructional statement from CU Conference Services administration.

How to find additional information in an emergency
- On your mobile device—Watch for text or e-mail alerts in the case of a campus closure or if there is a threat to personal safety.
- On the web—Visit CU home page www.colorado.edu for detailed campus closure and emergency information and updates.
- On the phone—Call the campus info line at 303-492-INFO (4636) for recorded information and updates relating to campus alerts.
- By e-mail—Check your e-mail after an emergency for support and resource information.
- On social media—Like CU Boulder on Facebook and follow @cuboulder and @cuboulderalerts on Twitter.

Sustainability:
CU Boulder is a leader in climate and energy research, interdisciplinary environmental studies and in engaging in sustainability and "green" practices on campus. Bring a reusable water bottle and refill free, use the recycling bins across campus, and compost where available. We encourage visitors to consider the purchase of carbon offsets for travel. For more information about CU Boulder's sustainability initiatives and carbon-offset purchases, visit the following websites:

Research, Degrees, Outreach & Operations: http://www.colorado.edu/sustainability The Environmental Center:http://www.colorado.edu/ecenter Colorado Carbon Fund (carbon offsets): http://www.coloradocarbonfund.org Native Energy (carbon offsets): http://www.nativeenergy.com

Weather:
Boulder is 5,430 feet in elevation and has a sunny, semi-arid climate. Hydrating during your stay is very important, as altitude sickness (headache, nausea, shortness of breath, dizziness, and tiredness) can occur. Weather conditions can change rapidly throughout each day and from day to day. Bring sunscreen, lipstick/lip moisturizer, a hat, walking shoes, and layers for cooler evenings.

Smoking:
The University is a smoke-free environment; smoking of any type of substance is not permitted anywhere on campus. Electronic cigarettes are included in the smoking ban.

Alcohol & Drug Policy:
The University of Colorado Boulder is committed to excellence in all aspects of personal and academic life. We recognize that alcohol abuse and misuse is a significant impediment to achieving this excellence. Therefore, CU-Boulder permits only the responsible, legal consumption of alcohol. The university complies with all federal, state, and local laws concerning alcohol and illegal drugs.

Laws Related to Alcohol & Drug Use:
Persons under 21 years of age cannot legally possess or consume alcoholic beverages. The furnishing of alcoholic beverages to underage persons is prohibited. Individuals who are of legal drinking age may possess and consume alcohol only in the privacy of their room with the door closed in their assigned residence hall room or at official conference catered events. Alcohol cannot be consumed or carried in open containers on any street, sidewalk, alley, automobile, or public area on campus. Any participant who consumes or possesses alcohol contrary to the above is subject to request for departing the premises and, upon request, shall leave the premises immediately.

The possession, use, sale, manufacturing, or distribution of Illegal ("Illegal" means unlawful under Colorado state law or federal law) drugs in the residence halls, including marijuana and drug paraphernalia including but not limited to pipes, hookahs, bongs, water pipes, etc. is not permitted. Marijuana remains a controlled substance under the federal Controlled Substance Act and, accordingly, is Illegal. Any participant who involves themselves in the use of possession of Illegal drugs is subject to the campus's request to depart the premises and, upon request, shall leave the premises. A participant may also be subject to legal action.

Possession of firearms, explosives, fireworks, incendiary devices, ammunition, other weapons, or instruments designed to look like any of the above will result in the possible immediate removal from campus.

For additional general information, visit https://www.colorado.edu/conferenceservices/

Oral Presentations

All oral presentations, except the invited keynote lectures, will be 12 minutes plus three minutes of discussion. Timing will be strictly enforced by the session chairs. All speakers must upload their presentations (as a PDF or Powerpoint file) on the computer prior to the beginning of their session. Both standard (4:3) and wide screen (16:9) format are acceptable.

Posters

For the poster presentations, posters should be of a size that fits a presentation board 48” in width and 36” in vertical height where push pins can be used for mounting.

Session 1: Thursday, June 6 (Odd Number Posters):

5:30-7:00 pm Poster hanging
8:00-9:30 pm Poster session 1

Please take down posters after poster session and transfer poster to wall on LEFT side of SEEC C120 using provided push pins.

Session 2: Friday, June 7, (Even Number Posters):

5:30 pm Poster hanging
8:00-9:30 pm Poster Session 2

Please take down posters after poster session and transfer poster to wall on RIGHT side of SEEC C120 using provided push pins.

Program

Thursday, June 6:

3:00-7:00 pm	Registration and check-in to on-campus housing
5:30-7:00 pm	Poster hanging (Session 1; odd numbers)
6:30 pm	Shuttle bus transportation to SEEC
7:00-7:15 pm	Welcome and announcements
7:15-8:00 pm	**K.1. Keynote Lecture 1:** Don Bryant; *Life on the (far-red) edge: far-red light photoacclimation in terrestrial cyanobacteria*
8:00-9:30 pm	**Poster session 1 and welcome reception**
9:30 pm	Take down session 1 posters and transfer to lecture hall wall
9:45 pm	Shuttle bus transportation to on-campus housing

Friday, June 7:

7:00-8:15 am	Breakfast (On-campus housing: Farrand Dining Hall)
8:15 am	Shuttle bus transportation to SEEC
8:30-9:15 am	**K.2. Keynote Lecture 2:** Paul Hudson; *New metabolic engineering and systems biology possibilities in Synechocystis with the CRISPRi gene repression tool*

Session 1, Biotechnology: Chair, Carlos Quiroz-Arita

9:15-9:25 am	**Introduction by the session chair**
9:25-9:40 am	**O.1.** Carlos Quiroz-Arita; *Integrating Physical and Lifecycle Modeling for Design and Optimization of Cyanobacteria Cultivation Systems*

9:40-9:55 am **O.2.** Annesha Sengupta; *Promoter engineering for applications in pathway engineering in cyanobacteria*

9:55-10:10 am **O.3.** Bryan Bishe; *Modification of the RSF1010 Broad Host-Range Plasmid for Improved Conjugation and Cyanobacterial Bioprospecting*

10:10-10:25 am **O.4.** Alejandra A. Schiavon; *CyanoGate: A Golden Gate modular cloning suite for engineering cyanobacteria based on the plant MoClo syntax*

10:25-10:55 am **Group photo, then coffee break**

10:55-11:10 am **O.5.** Yeyan Qiu; *Developmentally Regulated Genome Editing in Terminally Differentiated N_2-Fixing Heterocysts of Anabaena cylindrica ATCC 29414*

11:10-11:25 am **O.6**. Daniel Norena Caro; *Mathematical modeling of cyanobacterial metabolism for production of valuable nitrogen-compounds in Anabaena sp. PCC 7120*

11:25-11:40 am **O.7.** Sean Craig; *Towards Photosynthetic Hydrogen Production*

11:40-11:55 am **O.8.** Wim Vermaas; *Production of fatty-acid-derived biofuel and green chemicals in cyanobacteria*

11:55-1:15pm **Lunch Provided in SEEC**

Session 2, Photosynthesis-Light Reactions: Chair, Xiying Li

1:15-1:25 pm **Introduction by the session chair**

1:25-1:40 pm **O.9.** Xiying Li; *Structural and functional insights into the tetrameric photosystem I from heterocyst-forming cyanobacteria*

1:40-1:55 pm **O.10.** Amanda Cockshutt; *Different Picocyanobacterial Strains Have Different PhotoSystem II Repair Strategies Mediated by FtsH Expression Capacity*

1:55-2:10 pm **O.11.** Nathan Soulier; *Characterization of Cyanobacterial Phycobiliproteins Absorbing Far-Red Light*

2:10-2:25 pm **O.12.** Pablo Ignacio Calzadilla; *Revisiting cyanobacterial state transitions: balancing photosystem activities*

2:25-2:40 pm	**O.13.** Maria Agustina Dominguez Martin; *Cyanobacterial photoprotection by the OCP-related proteins*
2:40-2:55	**Break**

Session 3, Interactions with the environment: Chair, Christie Peebles

2:55-3:05 pm	**Introduction by the session chair**
3:05-3:20 pm	**O.14.** Christie Peebles; *The dynamic metabolome of cyanobacteria in sinusoidal light dark cycles*
3:20-3:35 pm	**O.15.** Teresa Thiel; *Cobalt-induced expression of two nitrogenase activators, CnfR1 and CnfR2, in Anabaena variabilis*
3:35-3:50 pm	**O.16.** Ryan Simkovsky; *Sniffing out a bad pond: Mass Spectrometry-based detection of contamination*
3:50-4:05 pm	**O.17.** Nanette Boyle; *MultIscale MultiObjective Systems Analysis (MIMOSA): an advanced metabolic modeling framework for complex systems*
4:05-7:15 pm	**Free time and dinner on own (map and list of restaurants available in program)**
5:30-7:00 pm	Hang posters (Session 2; even numbers)
7:15-8:00	**K.3. Keynote Lecture 3:** Chen Yang; *Tracing dynamic metabolism in cyanobacteria with stable isotopes and metabolomics*
8:00-9:30 pm	**Poster Session 2**
9:30 pm	Take down session 2 posters and transfer to lecture hall
9:45	Shuttle bus transportation to on-campus housing

Saturday, June 8:

7:00-8:15 am	Breakfast (On-Campus housing: Center for Community C4C)
8:15am	Shuttle bus transportation to SEEC

Session 4, Photosynthesis-Carbon metabolism and regulation: Chair, Nicholas Hill

8:30-8:40 am	**Introduction by the session chair**
8:40-8:55 am	**O.18.** Nicholas Hill, *Carboxysome degradation in cyanobacteria*
8:55-9:10 am	**O.19.** Daniel Solymosi; *Cytochrome cM assists in the regulation of PQ-pool redox state in photomixotrophic Synechocystis sp. PCC 6803*
9:10-9:25 am	**O.20.** Bo Wang; *Towards a sustainable nitrogen economy via rewiring cyanobacterial nitrogen metabolism*
9:35-9:50 am	**O.21.** Lyndsay Carrigee; *MpeV is the PEB lyase-isomerase for the doubly linked bilin on the β-subunit of phycoerythrin I & II in Synechococcus RS9916*
9:50-10:20	**Coffee Break**
10:20-10:35 am	**O.22.** José Ángel Moreno-Cabezuelo, *Integrated proteomic and metabolomic analysis of glucose utilization in Prochlorococcus and Synechococcus*
10:35-10:50 am	**O.23.** Neil T Miller; *Cyanobacterial NDH-1 complexes contribute to the formation of ΔpH across the thylakoid membrane*
10:50-11:05 am	**O.24.** Brandon Rohnke; *Regulation of the Carbon Concentrating Mechanism in Dynamic Environments in the Cyanobacterium Fremyella diplosiphon*

Session 5, Physiology I-Omics: Chair, Marie Adomako

11:05-11:15 am	**Introduction by the session chair**
11:15-11:30 am	**O.25.** Marie Adomako; A walk on the wild side: *Using comparative genomics to study complex phenotypes in cyanobacteria*
11:30-11:45 am	**O.26.** Pramod P. Wangikar; *Proteomic investigations of fast growth phenotype of Synechococcus elongatus PCC 11801 under elevated CO_2 levels*
11:45-12:00 pm	**O.27.** Lauren Mills; *Spatial mapping of a cyanobacterial proteome reveals distinct subcellular compartment organisation and dynamic metabolic pathway*

12:00-12:15 pm	**O.28.** John I Hendry; *Genome-scale flux distribution of the fast growing cyanobacerium Synechococcus elongatus UTEX 2973*
12:15-4:30 pm	**Lunch provided in SEEC and free time**
4:30-5:15 pm	**K.4. Keynote Lecture 4:** Carl Johnson; *As Time Glows By: Circadian Rhythms in Cyanobacteria from Molecules to Populations*
5:30 pm	Shuttle bus loading to Chautauqua Dining Hall
6:00-9:30 pm	Workshop dinner at Chautauqua Dining Hall
9:30 pm	Shuttle bus return to on-campus housing.

Sunday, June 9:

7:00-8:15 am	Breakfast (On-Campus housing: Center for Community C4C)
8:15am	Shuttle bus transportation to SEEC

Session 6, Physiology II-regulation and characterization; Chair, Susan Golden

8:30-8:40 am	**Introduction by session chair**
8:40-8:55 am	**O.29.** Susan Golden; *How Cyanobacteria Tell Time*
8:55-9:10 am	**O.30.** Vinson Lam; *Freezing Time: visualizing cyanobacterial circadian clock complexes with cryo-electron tomography.*
9:10-9:25 am	**O.31.** Damini Jaiswal; *A Fast Growing, Naturally Transformable Cyanobacterium, Synechococcus elongatus PCC 11801 isolated from an urban lake in Mumbai*
9:25-9:40 am	**O.32.** Steeve Lima; *Classical and non-classical secretion mechanisms in cyanobacteria*
9:40-10:10 am	**Coffee Break**
10:10-10:25 am	**O.33.** Muzzopappa Fernando; *Molecular evolution of the Orange Carotenoid Protein: inter-domain interaction and the role of the linker in photoactivity*
10:25-10:40 am	**O.34.** Robert Nichols; *Discovery and characterization of a novel bacterial nanocompartment in Synechococcus elongatus PCC 7942*

10:40-10:55	**O.35.** Stephan Klähn; *The distinctive regulation of cyanobacterial glutamine synthetase*
10:55-11:25	**Break**

Final Session:

11:25-11:50 am	Poster Awards
11:50-12:00 am	Announcement of next meeting
12:00 pm	Adjournment. Remove all posters.
12:15 pm	Shuttle bus transportation to on-campus housing
12:00-1:00 pm	**Check out from on-campus housing in Baker Hall**

Posters

Session 1: Odd Numbers
Session 2: Even Numbers

P.1. Mathematical Modeling of Cyanobacteria: Biomechanics and Structured Population Dynamics
Sabina Altus

P.2. Isolation and Characterization of Novel Freshwater Cyanophages
Richard Alvey

P.3. Generation of a high tolerant platform strain for metabolic engineering applications using adaptive laboratory evolution
Ruth Amanna

P.4. Switching the carbon flux towards TCA cycle intermediates in Synechococcus elongatus PCC 7942 for enhanced succinate production
Neha Arora

P.5. Improving desiccation tolerance in Synechococcus sp PCC 7002 towards regeneration of biomaterials
Juliana Artier

P.6. Photoassembly of Photosystem II: The role of Ca2+ in Photoactivation and Dark Rearrangement of Mn4CaO5 cluster of PSII
Anton Avramov

P.7. Application of 13C flux analysis for debottlenecking isobutyraldehyde production in cyanobacteria
Piyoosh Babele

P.8. Quantifying potential human exposure to cyanotoxins from lettuce grown with contaminated water
Austin Bartos

P.9. Photosynthetic generation of heterologous terpenoids in cyanobacteria
Nico Betterle

P.10. Cooperativity in the NAD(P)H-dependent Oxygen Reduction Reaction Catalyzed by Synechocystis 6803 Flavodiiron 3 Homodimers
Kate Brown

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Keynote Lectures

K.1. Life on the (far-red) edge: far-red light photoacclimation in terrestrial cyanobacteria

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When grown in wavelengths longer than ~700 nm, some terrestrial cyanobacteria undergo an extensive photoacclimation response, known as far-red light photoacclimation (FaRLiP). Compared to cells grown in WL, cells acclimated to far-red light exhibit greatly enhanced oxygen evolution when assayed in FRL and can grow in FRL. A knotless red/far-red phytochrome/histidine kinase, RfpA, controls the expression of the highly conserved, 20-gene FaRLiP cluster. RfpC is small CheY-like phosphate shuttle, and RfpB is a response regulator/transcriptional activator with two CheY-like phospho-receiver domains and a DNA-binding domain. Expression of the FaRLiP gene cluster causes extensive remodeling of Photosystem I (PSI), PSII, and phycobilisomes. Gene products of the FaRLiP cluster replace seventeen core subunits of these three complexes produced by cells grown in white light. Cells additionally synthesize chlorophyll (Chl) f, a small amount of Chl d, and special phycobiliproteins, all of which contribute to enhanced absorption between 700 and 800 nm. The PSI complexes produced in FRL still employ a heterodimer of Chl a/Chl a´ as the special pair. The PSII complexes produced in FRL contain Chl d, Chl f, Chl a, pheophytin a and b-carotene. One of the genes in the FaRLiP gene cluster, a super-rogue paralog of psbA of PSII, known as chlF, encodes Chl f synthase, a photo-oxidoreductase that acts on Chl a or Chlide a. Recent transcription profiling studies indicate that the gene(s) responsible for Chl d synthesis also are encoded in the FaRLiP gene cluster. Progress towards the identification of these genes will be described.

K.2. New metabolic engineering and systems biology possibilities in Synechocystis with the CRISPRi gene repression tool

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Our group is using the inducible gene-repression tool CRISPRi in Synechocystis for multiple projects in metabolic engineering and systems biology. We have exploited the multiplex capability (6 sgRNAs) of CRISPRi to rapidly screen the effect of eliminating competing pathways on fatty alcohol production. In this case, we observed regulatory compensation when repressing genes around the acyl-ACP node. We also saw that repression efficiency of CRISPRi decreases with the number of guide sgRNAs. An inducible CRISPRi allows controlled repression of essential genes, and we have used repression of citrate synthase to arrest growth and direct flux to lactate, where specific productivities are increased several fold. However, repression of essential genes imposes a strong selection pressure for dCas9 or sgRNA mutants. I will discuss how sgRNA libraries, combined with droplet-based cell sorting for lactate secretion, can be used to find better strategies for arresting growth while keeping CO2 fixation high. Finally, I will give an example of how we use CRISPRi repression of enzymes to force accumulation of a metabolite, and perform subsequent transcriptomics to uncover metabolite-responsive gene regulation.

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K3. Tracing dynamic metabolism in cyanobacteria with stable isotopes and metabolomics

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Recently, isotope tracer technique and mass spectrometry (MS)-based metabolomics have made a rapid progress. Here we present examples demonstrating that combination of dynamic isotope tracers, metabolomics, and mathematical modeling approaches can be used to provide valuable insight into cyanobacterial metabolism. We studied how cyanobacteria adjust their metabolism to enable adaptation to changes in nutrient supply. Quantitative tracking of the dynamic responses of intracellular metabolome and metabolic fluxes to the perturbation allowed us to discover an active ornithine-ammonia cycle (OAC) in the non-diazotrophic Synechocystis sp. PCC 6803 and diazotrophic Cyanothece sp. ATCC 51142. The OAC involves a novel biochemical reaction catalyzed by arginine dihydrolase. We demonstrated that the OAC allows for rapid remobilization of nitrogen reserves under starvation and a high rate of nitrogen assimilation and storage after the nutrient becomes available. Disruption of OAC severely impaired the cellular adaptability to environmental nitrogen fluctuations. Serving as nitrogen storage and remobilization machinery, the OAC is widely distributed in N2-fixing cyanobacteria and may be crucial for their contribution to marine nitrogen fixation. On the other hand, with guidance provided by dynamic flux analysis and metabolite profiling, we engineered the cyanobacterium Synechococcus elongates to produce isoprene that is a key building block of synthetic rubber. The engineered strain directed about 40% of photosynthetically fixed carbon toward the isoprene biosynthetic pathway, resulting in an efficient production of isoprene from CO2. The constructed strains can be used to construct a photoautotrophic cell factory for the production of diverse terpenoids from CO2.

K.4. As Time Glows By: Circadian Rhythms in Cyanobacteria from Molecules to Populations

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Chronobiologists study biological oscillators, the most prominent being circadian rhythms that are circa-24 h clocks that act as biological timekeepers to help organisms adapt optimally to the daily light/dark (and temperature) cycles that result from the earth's rotation. Twenty-five years ago, chronobiologists did not believe that prokaryotic organisms (aka bacteria) had circadian oscillators. This idée fixe was overturned by discoveries from our laboratory and others that demonstrated a bona fide circadian clock system in prokaryotic cyanobacteria (aka blue-green algae). Since that time, tremendous strides have been accomplished in our understanding of this bacterial clock system, which has remained at the forefront of circadian rhythm research. For example, the cyanobacterial system provided the first rigorous tests of the adaptive significance of circadian clocks. Recent studies highlight the fitness advantage of these daily timekeepers in both intra- and inter-specific competitions. Moreover, the cyanobacterial clock proteins KaiA, KaiB, and KaiC were the first to have their crystal structures solved. Most remarkable was the first demonstration of a biochemical oscillator reconstituted from purified KaiA, KaiB, and KaiC proteins in vitro. In a fundamental sense eukaryotic clock systems may be organized very similarly to the cyanobacterial system, including multiple oscillators that are coupled to promote resilience. Finally, the cyanobacterial circadian program regulates gene activity and metabolic pathways, and it can be manipulated to improve the expression of practically useful bioproducts (e.g., biofuels, biopharmaceuticals) using cyanobacteria as bioreactors.

Oral presentations

Session 1, Biotechnology; Chair, Carlos Quiroz-Arita

O.1. Integrating Physical and Lifecycle Modeling for Design and Optimization of Cyanobacteria Cultivation Systems

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As part of a broad collaboration between industry, academia, and the national laboratories, we have developed models and experiments to quantify tradeoffs among the scalability, sustainability, and technical feasibility of cyanobacteria cultivation systems. A central hypothesis to this research is that the lifecycle energy costs and benefits, the cultivation productivity, and the scalability of any given organism or technology is governed by the fluid mechanics of the photobioreactor systems. The fluid characteristics of both open raceway ponds and flat photobioreactors are characterized through industrial-scale experiment and modeling. Turbulent mixing is studied by applying Acoustic Doppler Velocimetry (ADV), Particle Image Velocimetry (PIV), and computational fluid dynamics (CFD) characterization tools. The implications of these fluid conditions on photoautotrophic organisms are studied through cultivation and modeling of the cyanobacteria, Synechocystis sp. PCC6803. Growth-stage models of this cyanobacteria include functions dependent on incident radiation, temperature, nutrient availability, dark and photo-respiration. By developing an integrated approach to laboratory experimentation and industrial-scale growth experiments, we have validated models to quantify the scalability and sustainability of these novel biosystems. These capabilities are utilized to perform long-term and industrially-relevant assessments of the costs and benefits of these promising technologies and will serve to inform the biological engineering research and development of new organisms.

O.2. Promoter engineering for applications in pathway engineering in cyanobacteria

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Commercialization of cyanobacteria-based platform chemicals necessitates expansion of synthetic biology toolbox of cyanobacteria1 to improve productivity. Promoters are biological parts that control gene expression and understanding its regulatory elements responsive to abiotic factors will benefit towards optimizing product yield. In this study, two native promoters, cpcB (phycocyanin) and rbc (RuBisCo) of Synechococcus elongatus PCC 7942 were truncated and characterized with respect to their sequence-length, effect of light, CO2 and diurnal rhythm. The promoters exhibited diurnal and circadian rhythm with cpcB showing 2-fold higher strength than rbc under ambient CO2 levels. The cpcB promoter of length 300bp showed highest activity and 200 bp was identified as the minimum region for gene expression. The presence of a negative regulatory region made the promoter sensitive to high light however, the repression was alleviated under high CO2 levels. Therefore, suitable for expressing genes under high light and CO2 conditions. On the other hand, rbc was repressed by high CO2 concentrations except for the minimum active stretch (225bp), demarcating the regulatory region between 225 and 300bp from the start codon of rbcLS. The complete removal of this probable CO2 responsive region made this promoter active under high CO2 without having circadian control. Signifying the importance of this regulatory region for rbc promoter activity under carbon-limited state. This study will help expand the repertoire of synthetic biology tools for pathway engineering in cyanobacteria.

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O.3. Modification of the RSF1010 Broad Host-Range Plasmid for Improved Conjugation and Cyanobacterial Bioprospecting

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In order to facilitate the genetic engineering of diverse cyanobacterial strains, we have modified the broad host-range plasmid RSF1010 to improve its transmissibility, increase its copy number, and facilitate cloning manipulations. The RSF1010 plasmid replicates in a large number of diverse bacterial strains but produces low amounts of useable DNA for cloning experiments. We previously showed that a mutant of the RSF1010 plasmid, mobAY25F, yields greater amounts of useable plasmid DNA, but lowers the plasmid's conjugation efficiency. In this study, we modified the RSF1010 mobAY25F (pCVD047) plasmid's conjugation and replication systems to facilitate its use in cloning experiments and restore its conjugation efficiency. These modifications were shown to increase

conjugation efficiency in three diverse laboratory strains of cyanobacteria, Synechocystis sp. PCC 6803, Leptolyngbya sp. BL0902, and Anabaena sp. PCC 7120. We then demonstrated the use of an improved RSF1010 plasmid, pAM5404, in mating experiments with cultured samples of wild cyanobacteria and other microorganisms sampled from locations in Southern California. We showed that pAM5404, which confers spectinomycin/streptomycin resistance, allowed selection of exconjugant cyanobacteria carrying the plasmid and facilitated the isolation of genetically tractable strains. These experiments led to the isolation and description of several new cyanobacterial strains from wild cultures. The improved RSF1010 vector can be used for bioprospecting for genetically tractable strains and is compatible with the CYANO-VECTOR cloning system, which is a versatile tool for constructing plasmids for genetic engineering of diverse cyanobacterial strains.

O4: CyanoGate: A Golden Gate modular cloning suite for engineering cyanobacteria based on the plant MoClo syntax

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Cyanobacteria are attractive hosts for producing renewable fuels and high-value chemicals due to their genetic tractability, relatively fast growth, and their ability to utilize sunlight and CO2. Despite exciting progress, cyanobacteria still lag behind in the field of synthetic biology compared to bacterial, yeast and mammalian systems. Several new tools and parts are currently being developed for cyanobacteria to advance metabolic engineering to levels commensurate with other model cell factories. We have developed an easy-to-use cloning system called CyanoGate that is fully compatible with the syntax of the Golden Gate Modular Cloning (MoClo) Toolbox for plants. Here, we present a suite of parts and acceptor vectors for making i) marked/unmarked knock-outs or integrations using an integrative acceptor vector, and ii) transient multigene expression and repression systems (e.g. CRISPRi) using a known and a previously undescribed replicative vector. We have characterised these parts in two separate cyanobacterial species, Synechocystis sp. PCC 6803 and the more recently described fast-growing strain Synechococcus elongatus UTEX 2973. The system is publicly available on Addgene and can be readily expanded to accommodate other standardised MoClo parts to accelerate the development of reliable synthetic biology tools for the cyanobacterial community.

O.5. Developmentally Regulated Genome Editing in Terminally Differentiated N2-Fixing Heterocysts of Anabaena cylindrica ATCC 29414

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Some vegetative cells of Anabaena cylindrica are programed to differentiate semi-regularly spaced, single heterocysts along filaments. Since heterocysts are non-dividing cells, with the sole known function for solar-powered N2-fixation, is it necessary for heterocyst to retain entire genome (7.1 Mb) from its progenitor vegetative cell? By sequencing the genome of isolated heterocyst, we discovered and confirmed that at least six DNA elements (0.12 Mbp) are deleted from the heterocyst genome during heterocyst development. There were another 33 bigger DNA fragments (the length varies from 500 bp to 6,350 bp) were missing in our 286 heterocyst contigs after mapping to the chromosomal sequence of A. cylindrica PCC 7122. The six-element deletions led to restore five genes (nifH1, nifD, hupL, primase P4 and a hypothetical gene of jointed anacy-RS29550 and anacy_RS29775) that were interrupted in vegetative cells, and lose 172 genes that were presented in in the genome of vegetative cells. By aligning the nif genes (nifH1, nifD and hupL) of N2-fixing cyanobacterial species (multicellular and unicellular cyanobacteria) as well as non-photosynthetic N2-fixing bacteria (non-cyanobacteria), we found that interrupted nif genes (nifH1, nifD and hupL) uniquely occurred in heterocyst-forming N2-fixing cyanobacteria. These cyanobacteria all have the conserved core sequences that may be required for phage DNA insertion. This research demonstrated that (1) different genomes may occur in distinct cell types in a multicellular bacterium; and (2) genome editing is coupled to cellular differentiation and/or cellular function in a multicellular cyanobacterium.

O.6. Mathematical modeling of cyanobacterial metabolism for production of valuable nitrogen-compounds in Anabaena sp. PCC 7120

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Anabaena sp. PCC. 7120 (abbr., Anabaena) are filamentous cyanobacteria with potential applications for CO2 capture and sustainable production of high-value chemicals (e.g., poly aspartic acid, secondary metabolites, and pigments) because they can consume multiple sources of nitrogen to drive the production of cellular metabolites. One of those metabolites is phycocyanobilin (PCB), a blue chromophore attached to C-phycocyanin, a nitrogen reserve involved in the light harvesting complex of photosystem II. Genomic scale metabolic reconstruction (GSMR) coupled with Flux balance analysis (FBA) have been used to gain deep quantitative understanding of metabolic networks with

biotechnological potential. In this project, the GSMR of Anabaena is presented and FBA is used to predict cellular growth rates and bio-production fluxes of phycocyanobilin (PCB) in diazotrophic and non-diazotrophic cultures, using molecular nitrogen (N2) and sodium nitrate (NaNO3), respectively. To provide with a high-quality metabolic model, the Anabaena GSMR relies on updated information on the global chemical composition of these cyanobacteria. Likewise, the model predictions are compared with experimentally determined fluxes of N2 and NaNO3 demands, which constitute modeling constraints. FBA analysis is also used to estimate CO2 capture potential and photosynthetic efficiency. The current version of the GSMR gives an accurate biochemical and qualitative description of the metabolism of Anabaena, and model improvements are being implemented to increase the quantitative accuracy. Modeling and experimental results suggest that Anabaena's CO2 capture potential, photosynthetic efficiency, and PCB biosynthesis are higher for cultures growing with NaNO3. In addition, these capabilities may improve in liquid media with urea (CH4N2O).

O.7. Towards Photosynthetic Hydrogen Production

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The need to develop a sustainable energy is crucial and one of the foremost global challenges facing humanity, driven by a continuing global population expansion and anthropogenic climate change. Hydrogen (H2) is a clean burning fuel which, however production relies on fossil fuels that is both unsustainable and environmentally unfriendly. The current global market is currently estimated at $115.25 Billion and expected to rise to $154.74 Billion by 2022.

Microbes such as cyanobacteria offer a sustainable method of H2 production platform with minimal environmental impact, making them an extremely attractive proposition. Cyanobacteria can possess two functionally distinct [NiFe] hydrogenases: an uptake enzyme, only capable of hydrogen oxidation and bidirectional enzyme capable of reducing protons to evolve hydrogen. Synechococcus sp. PCC 7002 encodes one bidirectional [NiFe] which is a heteropentameric enzyme composed of a hydrogenase module (HoxH & HoxY), forming the catalytic core and the diaphorase module (HoxE, HoxF & HoxU).

We have previously demonstrated that the hydrogenase of Synechocystis sp. PCC 6803 is thylakoid associated forming two distinct hydrogenase populations, one dispersed throughout the thylakoid and the other forming distinct puncta which correlate with hydrogen evolution (Burroughs et al., 2014). We show that the hydrogenase of Synechococcus sp. PCC 7002 is also thylakoid localised, forming similar populations as described in Synechocystis sp. PCC 6803. We demonstrate that the hydrogenase puncta of Synechococcus sp. PCC 7002 directly interact with the NDH-1 complex which increase upon the addition of glycerol.

O.8. Production of fatty-acid-derived biofuel and green chemicals in cyanobacteria

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Cyanobacteria are an excellent group of organisms for production of excreted biofuels and green chemicals because they are photosynthetic (producing compounds from CO2, water and light) and typically excrete produced compounds much more easily than other phototrophs such as algae do. Excretion of product helps to alleviate feedback inhibition of product formation and enhances the economic feasibility of the process. However, a drawback of an excreted product is that other organisms may be able to consume it. We have generated a laurate-producing and -excreting strain of the cyanobacterium Synechocystis sp. PCC 6803 that contains a thioesterase from the plant Umbellularia californica, releasing the fatty acid laurate when native fatty acid biosynthesis reaches the C12 stage. However, laurate is readily consumed by many heterotrophic prokaryotes. Therefore, we added a methylation step to convert laurate to the more stable and water-insoluble methyl laurate. This conversion of laurate to methyl laurate is done by a S-adenosyl methionine (SAM)-dependent enzyme. Main advantages over current biofuel products are methyl laurate's immediate application as biodiesel and its limited solubility in water, thus reducing the availability to heterotrophs in the culture and increasing the ease of harvesting. Morever, lauroyl esters have many additional applications. This approach provides a 'one-stop-shop' cyanobacterial platform that generates liquid transportation fuel from CO2 and water with sunlight as the energy input.

Session 2, Photosynthesis-Light Reactions: Chair, Xiying Li

O.9. Structural and functional insights into the tetrameric photosystem I from heterocyst-forming cyanobacteria

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Cyanobacteria have two photosystems (PSII and PSI) and carry out oxygenic photosynthesis. Several oligomer states of PSI are found, including trimeric and tetrameric forms. The function of PSI oligomerization in cyanobacteria is not well understood. The light energy for electron transfer of PSI comes from PSI chlorophylls themselves and phycobilisomes (PBS), which are the major light harvesting antennae in these organisems. Most cyanobacteria contain two types of PBS, hemidiscoidal PBS (refer as PBS) and CpcL-PBS. PBS and CpcL-PBS are both attached to the thylakoid membranes through a hydrophobic domain of the linkers ApcE and CpcL, respectively. PBS are more closely associated with PSII while CpcL-PBS are associated with PSI. However, the energy absorbed by PBS could be transferred to PSI through the terminal emitter ApcD and it is regulated by a process called state transitions. To understand the function of PSI oligomerization, we purified PSI from heterocyst-forming Anabaena 7120 and studied it with cryo-EM. Here, we report the 2.37-Å structure of a tetrameric PSI complex from Anabaena 7120. Four PSI monomers, organized in a dimer of dimer, form two distinct interfaces that are largely mediated by specifically orientated polar lipids, such as sulfoquinovosyl diacylglycerol. The structure depicts a more closely connected network of chlorophylls across monomer interfaces than those seen in thermophilic cyanobacterial trimeric PSI, potentially allowing a more efficient energy transfer between monomers. Our physiological data also revealed a functional link of PSI oligomerization to electron transport and thylakoid membrane organization.

O.10. Different Picocyanobacterial Strains Have Different PhotoSystem II Repair Strategies Mediated by FtsH Expression Capacity

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Picocyanobacteria are numerically dominant photoautotrophs of the oligotrophic regions of Earth's oceans. These organisms are characterized by their small size and highly reduced genomes. In this study, we grew three strains of picocyanobacteria: the low light, high nutrient strain Prochlorococcus marinus MIT 9313; the high light, low nutrient Prochlorococcus marinus MED 4; and the high light, high nutrient marine Synechococcus strain WH 8102; under low and high growth light levels. We then performed matched photophysiology, protein and transcript analyses. The strains differ significantly in their rates of Photosystem II repair under high light and in their capacity to remove the PsbA protein as the first step in the Photosystem II repair process. When grown under low light, MIT 9313 loses active Photosystem II quickly when shifted to high light, but has no measurable capacity to remove PsbA. MED 4 and WH 8102 show less rapid loss of Photosystem II and considerable capacity to remove PsbA. MIT 9313 has less FtsH protease thought to be responsible for the removal of PsbA in other cyanobacteria. Furthermore, the predominant FtsH isoform expressed in MIT 9313 is homologous to the

FtsH 4 isoform characterized in Synechocystis PCC 6803, rather than the FtsH 2 and 3 isoforms thought to be responsible for PsbA degradation. MED 4 on the other hand shows high light inducible expression of the isoforms homologous to FtsH 2 and 3, consistent with its faster rate of PsbA removal. MIT 9313 has adapted to its low light environment by diverting resources away from Photosystem II repair.

O.11. Characterization of Cyanobacterial Phycobiliproteins Absorbing Far-Red Light

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Some cyanobacteria are able to synthesize far-red light-absorbing paralogs of allophycocyanin, the main core protein of the primary cyanobacterial light-harvesting complex, the phycobilisome. These proteins play a part in two photoacclimative responses in cyanobacteria: far-red light photoacclimation (1), and low-light photoacclimation (2). In this study, the entire series of far-red allophycocyanins involved in these photoacclimative responses have been characterized through optical spectroscopy, mass spectrometry, and mutagenesis. This characterization has enabled us to define their role in each type of photoacclimation, as well as their post-translational modifications both in native and heterologous systems. Through comparisons with the well-characterized, red-light absorbing allophycocyanin-B from Synechocystis sp. PCC 6803, several residues have been identified as being important for far-red absorbance in these paralogous allophycocyanins. ApcD1 from Synechocystis sp. PCC 6803 was successfully used as a platform for the conversion of a red-light phycobiliprotein to a far-red light variant. This study has defined the contributions of these individual proteins to their respective photoacclimative response, and demonstrated diversity within the far-red light photoacclimative responses of two cyanobacterial strains, Leptolyngbya sp. JSC-1 and Synechococcus sp. PCC 7335. It has also clarified the mechanism by which these proteins achieve far-red absorbance, which can be applied in the creation of far-red fluorophores.

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O.12. Revisiting cyanobacterial state transitions: balancing photosystem activities

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Photosynthetic organisms need to sense and respond to fluctuating environmental conditions to avoid the formation of dangerous reactive oxygen species. The excitation energy arriving at each photosystem permanently changes due to variations of intensity and spectral properties of the absorbed light. Cyanobacteria, like plants and algae, have developed a mechanism, named state transitions, that sense and respond to these fluctuating conditions. In this work, we characterize the role of the cytochrome b6f and phosphorylation reactions in cyanobacterial state transitions using Synechococcus elongatus PCC 7942 and Synechocystis PCC 6803. A large Photosystem II fluorescence quenching was observed in State II which seems not to be related to spillover. This membrane-associated process was inhibited by betaine, sucrose and high concentrations of phosphate. Then, using different chemicals affecting the PQ pool redox state and the activity of the cytochrome b6f, we demonstrated that this complex is not involved in S. elongatus and Synechocystis PCC6803 state transitions. Finally, by constructing and characterizing 21 kinase and phosphatase mutants and using chemical inhibitors, it was clearly shown that phosphorylation reactions are not essential in cyanobacterial state transitions. Thus, signal transduction is completely different in cyanobacteria and plant (green alga) state transitions.

O.13. Cyanobacterial photoprotection by the OCP-related proteins

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In the majority of cyanobacteria, the Orange Carotenoid Protein (OCP) is a photoreceptor responsible for thermal dissipation of excess light energy captured by the phycobilisome. Recently, new homologous families of the constituent domains of OCP have been identified (Melnicki et al. 2016). Nine different clades of N-terminal domain homologs have been described across diverse cyanobacteria species and are named Helical Carotenoid Proteins (HCPs). Homologs to the C-terminal domain (CTDHs) have also been found in nearly every genome encoding an HCP. Most likely, OCP was derived from a combination of HCP with CTDH forming a single polypeptide. Our investigation focuses on the structure, spectroscopic properties, and function of HCPs and CTDH, particularly whether they constitute a modular photoprotective system. Tolypothrix PCC 7601 is an ideal model system for dissecting out the function of these proteins since it contains two OCPs, one CTDH, and three HCPs (HCP1, HCP2, and HCP3). We overexpressed them in

Tolypothrix allowing us to identify their natively bound carotenoid. We have recently successfully characterized the structure and spectroscopic properties of these HCPs and CTDHs (Melnicki et al., 2016. Lechno-Yossef et al., 2017 and Dominguez-Martin et al., 2019). Further, we probed the interaction between the HCPs and CTDH. Our results contribute to the efforts to understand the functional roles of this large, newly discovered family of pigment proteins, which at present remain enigmatic.

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2. Lechno-Yossef et al. (2017). Plant Journal.
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Session 3, Interactions with the environment: Chair, Christie Peebles

O.14. The dynamic metabolome of cyanobacteria in sinusoidal light dark cycles

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Cyanobacteria and algae have been touted as a promising platform for the production of biofuels and chemicals. So far strain engineering efforts in cyanobacteria and algae have fallen short of expectations. This is likely due to the fact that strain engineering efforts have occurred under low- and continuous-light conditions with minimal understanding of how natural sunlight availability affects the physiology of the cell. Our lab previously demonstrated that engineered free fatty acid production is decreased in daily light-dark cycles as opposed to continuous light relative to wild-type. Toward this goal, we have improved systems biology understanding in day/night cycles. Specifically, we engineered a photobioreactor system which enables diurnal sinusoidal light cycles with peak-light intensities reaching over 1,500 µmol photons m^-2 s^-1. We developed and implemented a multi-platform metabolomics study to investigate the dynamic behavior of metabolism of Synechocystis sp. PCC 6803 in sinusoidal day/night cycles and have found dramatic changes across metabolism. We observed widespread oscillations across the light cycle with 90%, 94%, and 40% of the identified polar/semi-polar, non-polar, and polymeric metabolites displaying statistically significant oscillations, respectively. This presentation will discuss the observations we have made from this high-resolution temporal data. (https://doi.org/10.1111/tpj.14320)

O.15. Cobalt-induced expression of two nitrogenase activators, CnfR1 and CnfR2, in Anabaena variabilis

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Regulation of cyanobacterial nitrogen fixation is still poorly understood. NtcA is known to be important for all aspects of nitrogen metabolism, but its specific role as a direct regulator of nitrogen fixation genes has yet to be clearly defined. The activator CnfR (PatB) has been shown to be important for nitrogenase genes expressed in heterocysts and in anaerobic vegetative cells (Tsujimoto, et al., 2014, Pratte & Thiel, 2016). Anabaena variabilis is unusual because it has two Mo-nitrogenases. Nif1 functions only in heterocysts while Nif2 functions only in anaerobic vegetative cells. Each of these nif gene clusters has its own CnfR activator protein. In order to better define the roles of CnfR1 and CnfR2 in cell-type specific activation of nifB1 (heterocysts) and nifB2 (anaerobic vegetative cells), respectively, we have expressed cnfR1 and cnfR2 under the control of the cobalt-inducible coaT promoter (Gonzalez, et al., 2016). Cobalt induced expression of cnfR1 or cnfR2 50-100 fold, producing the activator proteins in cell types and under conditions in which each protein is not normally made. The two proteins behaved differently. CnfR1 activated expression of both nifB1 and nifB2 in vegetative cells with or without oxygen; however, CnfR2 could only activate nifB2 and only in anaerobic vegetative cells, not in heterocysts.

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Tsujimoto R, Kamiya N & Fujita Y (2014) ProcNatl Acad Sci USA 111: 6762-6767.

O.16. Sniffing out a bad pond: Mass Spectrometry-based detection of contamination

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Contamination of industrial-scale growth systems by pathogens, predators, and non-productive contaminating species continues to be a major obstacle to the robust and economically sustainable production of cyanobacterial and algal biomass and bioproducts. Appropriate management of these pests requires a sensitive and continuous monitoring system that can detect and identify contaminants and competitors as early as possible. In comparison to current detection methodologies, such as quantitative PCR or FlowCam monitoring systems, a mass spectrometry (MS)-based detection system is orders of magnitude more sensitive and can be readily automated for continuous monitoring of multiple production ponds.

We have developed a chemical ionization mass spectrometry-based detection system capable of real time monitoring of volatile compound abundances in the air over an algal culture. Using this system, we have examined the headspace over healthy algal cultures throughout multiple growth phases, under abiotic stresses, and through culture crashes resulting from infecting the cultures with predators. The resulting data has allowed us to confirm previously characterized molecular signatures derived from breakdown pathways occurring in the culture liquid. These experiments have allowed us to expand our catalog of molecules that indicate the health of the cyanobacteria or contamination, as well as to determine thresholds for detection of contaminants and the kinetics of culture crashes post-infection. Altogether, this research is rapidly advancing the development of a field-deployable instrument for monitoring contamination in algal cultures.

O.17. MultIscale MultiObjective Systems Analysis (MIMOSA): an advanced metabolic modeling framework for complex systems

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Photosynthetic microorganisms have the potential to be a renewable source of a wide variety of fuels and chemicals but their potential has not yet been realized due to the complexity of these organisms and a general lack of tools when compared to the premiere model organisms such as E. coli and yeast. We have developed a new modeling tool, MIMOSA; which is a multi-scale, multi-paradigm modeling approach that can track individual cells in a population, account for differences in the environment (such as nutrient or light availability) and allows for multi-objective optimization. We have used MIMOSA to investigate nutrient cycling in the marine nitrogen fixing cyanobacterium, Trichodesmium erythraeum. Integration of diffusion and rules of cellular physiology help to explain the role of metabolic oxygen sequestration methods, like Mehler's reactions, to reduce intracellular oxygen concentration and halt inhibition of nitrogenase through poisoning. It is effective in predicting when nitrogenase was limited when more energetically favorable nitrogen compounds like nitrate were available, yielding 0% to 55% inhibition of nitrogenase between 0 and 10 μM NO3-, consistent with values reported through experimentation. Overall, the model is effective in predicting the confluence of metabolism and physiology in governing whole-population behaviors.

Session 4, Photosynthesis-Carbon metabolism and regulation: Chair, Nicholas Hill

O.18. Carboxysome Degradation in Cyanobacteria

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Carboxysomes are large (~1 GDa) protein complexes found in cyanobacteria that play a vital role in sequestering inorganic carbon for photosynthesis. Carboxysomes consist of a selectively permeable protein shell that encapsulates the enzymes carbonic anhydrase and ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco). As part of the cyanobacterial CO2 concentrating mechanism (CCM), high levels of CO2 are concentrated inside the carboxysome around Rubisco, increasing the rate of Rubisco carboxylation, while minimizing the rate of its competitive oxygenation reaction. While it is known how the essential components assemble into a functional carboxysome, degradation of these protein complexes has never been identified. Here, we use time-lapse microscopy to track both the position and activity of individual carboxysomes within single cells over time, showing evidence of carboxysome degradation events. Carboxysome degradation likely occurs due to breakage of its protein shell, rendering the carboxysome inactive and exposing its cargo Rubisco to the cytoplasm. The exposed Rubisco is then recruited to a cell pole. To our knowledge, this is the first time the catalytic activity of a single protein complex has been tracked over time in vivo. By identifying previously unknown degradation events, we anticipate that this work will influence the design of downstream biotechnological applications, such as engineering more stable carboxysome shells and incorporating carboxysomes into plant chloroplasts for improved crop photosynthesis.

O.19. Cytochrome cM assists in the regulation of PQ-pool redox state in photomixotrophic Synechocystis sp. PCC 6803

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Photomixotrophy is an intriguing ability to simultaneously utilize different carbon sources, which requires the fine-tuning of carbon fixation, glycolysis, photosynthetic and respiratory electron transport. A small thylakoid-localized heme protein, cytochrome cM, assists in the regulation of the photomixotrophic bioenergetics. When cytM is deleted, Synechocystis sp. PCC 6803 gains a marked growth advantage both under photomixotrophic and under heterotrophic conditions [1]. However, the molecular mechanisms behind the phenotype had to be clarified. In order to understand the role of CytM in photomixotrophic bioenergetics, we constructed various transgenic mutants and tested different growth regimes. We applied (i) high

throughput, quantitative proteomics, to study changes on the proteomic landscape; (ii) membrane inlet mass spectrometry (MIMS), to monitor real time gas exchanges; (iii) chlorophyll fluorescence and P700 oxido-reduction, to characterize the photosynthetic apparatus.

Our results show, that the deletion of cytM significantly delays the over-reduction of PQ-pool, enabling a high net photosynthesis and respiration. Although the growth advantage is glucose-dependent, thylakoid respiratory terminal oxidase complexes are dispensable for the phenotype. Proteomics analysis revealed slight rearrangements in the intermediary carbon metabolism, accumulated phosphate and metal uptake proteins and elevated levels of cofactor biosynthetic enzymes.

We propose, that cytM is widely associated with the thylakoid electron transport chain and by assisting in the regulation of the PQ-pool redox state, it affects glucose utilization and photosynthesis, independently of thylakoid respiratory terminal oxidases.

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O.20. Towards a sustainable nitrogen economy via rewiring cyanobacterial nitrogen metabolism

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Development of sustainable economy calls for alternative energy storage and utilization technologies. Although battery- and carbon-based routes have gained tremendous attention, nitrogen-based routes have rarely been exploited so far. Guanidine (CH_5N_3) which contains 71.1% nitrogen by mass is an exemplary chemical to explore the nitrogen-based routes of energy storage and utilization. Guanidine has a variety of applications including its use as propellant, slow-release fertilizer, or precursor to pharmaceuticals and antimicrobial polymers. Conventional chemical synthesis of guanidine exclusively relies on fossil fuels, is energy intensive, and is detrimental to the environment. Herein, guanidine is photosynthetically produced via a *de novo* guanidine biosynthesis cycle in the engineered *Synechocystis* sp. PCC 6803 and *Anabaena* sp. PCC 7120 strains, which represents the first biological conversion of renewable solar energy into the chemical energy stored in the nitrogen-rich compound guanidine. Furthermore, a guanidine degradation pathway is, for the first time, discovered in cyanobacteria. Our findings could shed light on harnessing the biological nitrogen metabolism for alternative strategies for energy storage and utilization.

O.21. MpeV is the PEB lyase-isomerase for the doubly linked bilin on the β-subunit of phycoerythrin I & II in Synechococcus RS9916

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Marine cyanobacteria in the genus Synechococcus are found ubiquitously around the world due to their ability to utilize various wavelengths of light for photosynthesis. The phycobilisome (PBS) in Synechococcus sp. RS9916 (hereafter, RS9916) contains two types of phycoerythrin (PEI and PEII) as the phycobiliproteins most distal on the rods. In RS9916, specialized lyases called lyase-isomerases, attach phycoerythrobilin (PEB) and simultaneously isomerize it to phycourobilin (PUB). MpeV is a putative lyase-isomerase in RS9916 which isomerizes PEB to PUB at Cys-50 and Cys 61 on the beta subunit of PEI (CpeB). MpeV is similar to CpeF from Fremyella diplosiphon (Fd) which attaches PEB to CpeB [1]. Using a protein expression system in E. coli, purified recombinant CpeB expressed with MpeV and bilin synthesis genes was analyzed by absorbance and fluorescence spectroscopy followed by SDS-PAGE and LC-MS-MS. Results show MpeV covalently attaches PUB to Cys-50, 61 of CpeB. This activity increases when CpeB is partially chromophorylated by CpeS, a lyase that adds PEB to Cys-82, with a further increase in efficiency when coexpressed with CpeZ, a homolog of a chaperone-like protein in Fd [2]. Activity of MpeV on the beta subunit of PEII (MpeB) was similarly analyzed, and PUB ligation is also detected at the Cys-50, 61 residues. MpeV showed no detectable lyase activity on the alpha subunits of PEI or PEII.

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O.22. Integrated proteomic and metabolomic analysis of glucose utilization in Prochlorococcus and Synechococcus

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Prochlorococcus and Synechococcus are the most abundant oxygenic phototrophs on Earth and responsible for an important part of the global primary production. Previous results from our group showed that Prochlorococcus can transport glucose, increasing the expression of genes involved in its metabolization[1]. In addition, glucose transport was detected in natural populations of Prochlorococcus[2]. Our working hypothesis is that mixotrophy confers an evolutionary advantage to Prochlorococcus against other microorganisms sharing the same ecological niche.

Using metabolomic and proteomic approaches, we have initiated studies to compare the effect of 100 nM and 5 mM glucose on the metabolism of Prochlorococcus and Synechococcus, under light and dark conditions. The results show that 5 mM glucose addition led to a strong metabolic shift toward overall anabolic patterns in all the studied strains. 469 metabolites increased their concentration after 5 mM glucose addition. We will focus on the study of carbohydrates, especially those more related to glucose metabolism in cyanobacteria; glycolysis, TCA cycle, Calvin cycle and pentose phosphate pathway. Proteomic results show that the relative abundance of proteins related with photosynthetic apparatus, photosynthetic pigments, ribosomes, and ATPase decreased under darkness and in presence of glucose. Our metabolomics and proteomics results confirm that Prochlorococcus and Synechococcus use glucose.

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O.23. Cyanobacterial NDH-1 complexes contribute to the formation of ΔpH across the thylakoid membrane

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Most organisms possess an NAD(P)H Dehydrogenase Complex (NDH-1) (commonly known as Complex I) in the respiratory electron transport chain. Cyanobacteria are unique in possessing multiple NDH-1 complexes per cell, with some able to hydrate CO2 to HCO3-, thereby trapping inorganic carbon in the cytoplasm to supply Rubisco. These complexes have been seen to participate in cyclic electron flow around PSI and locate to the thylakoid membrane [1]. They have been well studied in E. coli, with structures known and point mutants constructed to explore the mechanism of coupling electron transport to proton pumping [2]. By comparing the amino acid sequences, it is seen that many of the residues important for proton pumping activity are conserved in cyanobacteria. Based on this, it may be inferred that these complexes perform proton pumping, though this has not yet been shown experimentally. To test proton pumping in cyanobacterial NDH-1 complexes, Wild Type Synechocystis sp. PCC 6803 and the mutant M55, lacking functional NDH-1 complexes, were assayed for their ability to form ΔpH across the thylakoid membrane by measuring the quenching of acridine orange fluorescence upon actinic illumination in the presence of inhibitors and ionophores.

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O.24. Regulation of the Carbon Concentrating Mechanism in Dynamic Environments in the Cyanobacterium Fremyella diplosiphon

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In order to optimize photosynthesis under dynamic environmental conditions, cyanobacteria must tune their carbon concentration mechanism (CCM) based on external stimuli. The CCM has several major components, including multiple inorganic carbon (Ci) transporters and proteinaceous bacterial microcompartments called carboxysomes, which together serve to import Ci and concentrate CO2 around carboxysome-localized Rubisco. Our previous work has shown that both Ci and light availability impact the stoichiometry of CCM components in F. diplosiphon, resulting in changes to carboxysome morphology and organismal fitness (Rohnke et. al 2018). Here, we present novel methods in cyanobacteria for assaying carbon assimilation as a dynamic function of carbon availability (i.e., Carbon Response Curves, CRCs) based on similar methods used for plants. Our analyses demonstrate that CRCs are straightforward and provide rich detail about carbon fixation in cyanobacteria. CRCs, carboxysome morphology, and CCM gene expression analyses under variations in Ci availability, light quality, and light intensity provide insight on the functional impact and mechanisms of CCM regulation. Our results support the hypothesis that alterations in CCM component stoichiometry can fine tune carboxysome morphology and that a cyanobacterium's ability to do so has a functional effect on carbon fixation and organismal fitness.

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Session 5, Physiology I-Omics: Chair, Marie Adomako

O.25. A walk on the wild side: Using comparative genomics to study complex phenotypes in cyanobacteria

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Cyanobacteria are important photosynthetic members of biofilm communities in diverse environments, and their biofilms have potential applications in wastewater purification, bioremediation, and suppression of biofouling. The cyanobacterium Synechococcus elongatus PCC 7942 is a tractable model organism used to research photosynthesis, circadian rhythms in prokaryotes, and photosynthetic production of valuable chemicals. Under normal laboratory growth conditions PCC 7942 is exclusively planktonic, but will form biofilms upon mutation of specific genes. Here we introduce a wild isolate of S. elongatus, strain UTEX 3055, recently retrieved from an environmental sample, which forms robust biofilms and exhibits phototaxis, as a model for studying these complex phenotypes. We have sequenced the genome of UTEX 3055, and the average nucleotide identity with PCC 7942 is 98.46%, indicating that they are the same species. We are using comparative genomics to gain insight into complex phenotypes in S. elongatus by harnessing the genetic similarity of these two strains and their distinct phenotypic differences. A randomly-barcoded transposon mutant (RB-TnSeq) library in PCC 7942 generated in our lab is a robust tool for unbiased genetic screens; a complementary RB-TnSeq library is under construction in UTEX 3055. A comparative genetics approach using the results from screens in both libraries will be used to study the genetic basis of phototaxis and biofilm behaviors in S. elongatus. The comparison of a lab-adapted strain and a wild isolate of the same species will also aid the understanding of the role of cyanobacteria in environmental communities and the processes of microbial domestication.

O. 26. Proteomic investigations of fast growth phenotype of Synechococcus elongatus PCC 11801 under elevated CO2 levels

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The growing interest in the use of cyanobacteria as industrial hosts for photosynthetic production of biofuel(s) coupled to CO2 sequestration has led to a critical need of investigating the regulation of metabolism in response to changes in growth conditions or introduction of heterologous pathways. The heterologous expression of any pathway in cyanobacteria leads to significant redirecting of carbon towards desired product generating more demand of carbon that can be fulfilled by cultivating engineered strain in

the presence of high CO2. In this study, we investigated the metabolic responses to increasing CO2 concentrations for fast-growing, novel freshwater cyanobacteria, Synechococcus elongatus PCC 11801, using quantitative proteomics (iTRAQ). The protein expression data revealed that this organism responded to elevated CO2 level by upregulation of proteins involved in photosynthesis, glycolysis, carbon fixation, nitrogen absorption, and transport. There was a concomitant decline in proteins involved in inorganic carbon uptake, oxidative pentose phosphate pathway, TCA cycle, photo protection, and redox maintenance. This is the first comprehensive study that resulted in a detailed assessment of molecular changes in this novel strain while shifting from carbon-limited to carbon-sufficient conditions. The insights gained from here may help in rationalizing strategies of pathway engineering for production of sustainable fuels through efficient CO2 capture in PCC 11801 and related neighbor strains.

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O.27. Spatial mapping of a cyanobacterial proteome reveals distinct subcellular compartment organisation and dynamic metabolic pathway

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Cyanobacteria are complex prokaryotes, incorporating a Gram-negative cell wall and internal thylakoid membranes. However, localisation of proteins within cyanobacterial cells is poorly understood. Using subcellular fractionation and quantitative proteomics we report the most extensive subcellular map of the proteome of a cyanobacterial cell, identifying ~67% of Synechocystis sp. PCC 6803 proteins, ~1000 more than previous studies. 1,712 proteins were assigned to six specific subcellular regions. Proteins involved in energy generation localised to thylakoid membranes whereas transporters and regulatory proteins predominantly reside in the plasma membrane. The majority of metabolic enzymes are soluble although numerous biosynthetic pathways terminate in membranes. Ribosomal proteins and enzymes synthesising the storage compound polyhydroxybuyrate localise to distinct regions of the cell. Moreover, heterogeneity within membrane and cytoplasmic regions is observed, indicating further cellular complexity. Cyanobacteria thylakoid membrane protein localisation is conserved in Arabidopsis thaliana chloroplasts, suggesting similar proteome organisation in higher photosynthetic organisms. The dynamic organisation of a cyanobacterial cell we reveal will aid our understanding of these environmentally and biotechnological important organisms.

O.28. Genome-scale flux distribution of the fast growing cyanobacerium Synechococcus elongatus UTEX 2973

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With a short doubling time of 1.5 hr, Synechococcus elongatus UTEX 2973, has emerged as a potential platform for the solar based production of biochemicals. Estimation of intracellular fluxes in this organism will inform metabolic engineering strategies to maximize the production rate of target chemicals. In this meta-analysis, we elucidated the genome-scale flux distribution of this organism using Isotopic Non-stationary 13C-Metabolic Flux Analysis by fitting the labeling pattern of intracellular metabolites obtained from an earlier study (Abernathy et al., 2017). To achieve this, a genome-scale carbon mapping model, imSyu593, was constructed starting from the previously available mapping model for Synechocystis sp PCC 6803 (Gopalakrishnan et al., 2018). imSyu593 traces the flow of carbons through 593 reactions spreading across the central carbon metabolism, amino acid metabolism and peripheral pathways. Flux estimation revealed a near complete assimilation (>96%) of the fixed carbons into biomass. This high carbon conversion is the result of reincorporation of carbons oxidized during photorespiration and anabolic pathways and preferential usage of non-decarboxylating reactions such as phosphoketolase. The reincorporation of oxidized carbons compensated for the carbon loss associated with the usage of photorespiratory C2 cycle for synthesizing glycine and serine. Interestingly, malic enzyme was found to be dispensable with pyruvate being synthesized via Pyruvate kinase. And instead of using pyruvate dehydrogenase, Acetyl CoA was synthesized using the carbon efficient phosphoketolase pathway. These findings suggest the existence of a carbon efficient metabolism in UTEX 2973 in conjunction with fast growth rate and supports the development of this organism into an ideal photoautotrophic production platform.

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Session 6, Physiology II-regulation and characterization; Chair, Susan Golden

O.29. How Cyanobacteria Tell Time

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Cells of diverse organisms, from cyanobacteria to humans, execute temporal programs that are driven by circadian oscillators. The circadian clock of the cyanobacterium Synechococcus elongatus is a discrete nanomachine comprising three proteins – KaiA, KaiB, and KaiC – which interact progressively to set up the timekeeping mechanism, and two kinases whose activities are altered by engaging the Kai oscillator. The key events that enable the clock to tell time, become set to local time, and regulate global patterns of gene expression and metabolism, rely on these five proteins plus the target of the kinases: a transcription factor, RpaA. The clock is permissive late in the day for processes that prepare the cell for night, when photosynthetic metabolism will be inoperative. Transcripts of genes peak at dusk that encode enzymes of a night-time metabolic program. During the night, glycogen stored during the day is broken down to fuel the synthesis of a crucial reductant, NADPH. Prior to dawn, the night-time program is turned off by the clock, enabling the cell to switch from utilizing stored carbon to synthesizing cellular components when light becomes available to power photosynthesis.

O.30. Freezing Time: visualizing cyanobacterial circadian clock complexes with cryo-electron tomography.

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Circadian clocks are nearly ubiquitous throughout life, appearing in eukaryotes and prokaryotes, and modulate metabolism and behavior in anticipation of regular, predictable environmental changes. One of the simplest prokaryotic clocks is based on the tri-molecular KaiA-KaiB-KaiC oscillator found in the cyanobacterium Synechococcus elongatus, which signals time by the phosphorylation state of KaiC over a 24-hour period. Additionally, KaiC, KaiA, and other key clock network proteins are recruited to the cell pole during nighttime to form a distinct focus as visualized by fluorescent labeling. These foci are critical for circadian rhythmicity, as strains defective in foci formation also exhibit irregular circadian phenotypes.

To study the molecular organization of these clock complexes, we are using cryo-electron tomography (CET) to reconstruct 3D cellular architecture in situ. With rapid freezing to

prevent ice crystallization, we can take a snapshot of what the cell looks like at any point in time. With focused ion-beam (FIB) milling, we can reduce sample thickness and open windows to the interior of a bacterium by creating ~180 nm thick sections that are accessible to high-resolution CET. With correlative light- and electron-microscopy (CLEM), we can compare fluorescent localization signals to high-resolution structures obtained from CET for the same sample.

Our current results with cryo-CLEM suggest that KaiC may form filamentous structures near the thylakoid membranes. Through computational image processing, we can assign positions and orientations to each KaiC molecule within the focus. Ongoing structural refinement will allow us to elaborate on the role of KaiC in maintaining robust circadian rhythms.

O.31. A Fast Growing, Naturally Transformable Cyanobacterium, Synechococcus elongatus PCC 11801 isolated from an urban lake in Mumbai

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Although a number of cyanobacterial strains have become popular in laboratory research, the quest for a suitable industrial strain continues. The desired characteristics include fast growth, genetic amenability, and tolerance to high light, CO2 and temperature. In this context, Synechococcus elongatus UTEX 2973 has attracted attention as a potential industrial chassis. Notably, UTEX 2973 is a close cousin of the hugely popular S.elongatus PCC 7942 with 99.8% sequence identity and differences only at 55 genetic loci. Here, we report a fast growing strain Synechococcus elongatus PCC 11801, isolated from an urban lake in India, that is tolerant to high temperature, light, CO2 and salt. Genome sequence of PCC 11801 shows only 83% identity with its closest neighbor PCC 7942 and thus adds significant diversity. In this study, we describe the unique attributes of PCC 11801 that shows a doubling time of 2.3 h, fastest growth for any cyanobacteria reported so far under ambient CO2 conditions. The genome of PCC 11801 shows several genes that do not have homologs in neighbor strains PCC 7942 and UTEX 2973, some of which may be responsible for adaptation to various abiotic stresses. The remarkably fast growth rate of PCC 11801 coupled with its robustness and ease of genetic transformation makes it an ideal candidate for the photosynthetic production of fuels and chemicals.

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O.32. Classical and non-classical secretion mechanisms in cyanobacteria

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TolC is an outer membrane channel-tunnel protein involved in the secretion of structurally different components. Coupling with different inner membrane complexes, TolC is part of at least two different secretion systems: the type I secretion system (together with ABC-type transporters), and the multidrug efflux system (together with resistance-nodulation-division efflux pumps). TolC-dependent secretion mechanisms have been extensively studied in Proteobacteria mainly due to their impact on antimicrobial resistance. However, knowledge about these secretion mechanisms in cyanobacteria remains elusive. In our group, we have recently identified TolC in Synechocystis sp. PCC 6803, along with 11 putative inner membrane translocase components of TolC-mediated secretion. Gene-inactivation of each of the candidate genes followed by a comprehensive phenotypic characterization allowed to link specific protein components to the processes of protein export (e.g. S-layer protein) and drug efflux (antibiotics, detergents and dyes). Furthermore, an increased capacity of outer membrane vesicles (OMVs) formation and release was also found in the tolC-deletion mutant. The release of OMVs by bacterial cells represents an alternative, but largely overlooked mechanism of secretion, in which biologically active molecules (including toxins, enzymes and nucleic acids) can be trafficked to distant targets in a concentrated, protected and targeted manner. By studying the release of OMVs on mutants with impaired secretory functions we suggest that the hyper-vesiculating phenotype of the TolC-deficient mutant is related to cell envelope stress management. Altogether, these findings highlight how both classical (TolC-mediated) and non-classical (OMVs-mediated) secretion systems are crucial for cyanobacterial cell homeostasis.

O.33. Molecular evolution of the Orange Carotenoid Protein: inter-domain interaction and the role of the linker in photoactivity

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The cyanobacterial Orange Carotenoid Protein (OCP) is a photoactive protein that plays a major role in dissipating the excess energy arriving at the photosynthetic apparatus. The OCP is composed by two domains connected by a flexible loop. The three paralogous of the OCP (OCP1, OCP2 and OCPX) were originated by gene fusion of ancestral domain genes. We report here the first characterization of an OCPX. Using phylogenetic and biochemical approaches, we characterized one OCP from each subfamily focusing on the inter-domain interaction and the role of the linker. Specific amino acids in the linker provided additional regulation by allowing protein deactivation, enhancing antenna binding and regulating the photoactivation. Most of these features are kept by the relatively ancestral OCPX, including the dimer-to-monomer transition already described in OCP1. Our results suggested, that OCP2 had accumulated mutations in specific residues that increased the inter-domain interaction and preserve the fast deactivation. On the other hand, oligomeric regulation was lost in OCP2. During evolution OCP1 deactivation became slower allowing further regulation by interaction with the FRP, a protein which accelerate the deactivation. Both OCP1 and OCPX have conserved the negative regulation of the photoactivation provided by the linker that is important for these OCP which are constitutively expressed. By contrast, OCP2 developed a positive regulation of the photoactivation by the linker, which counteract the strong domain affinity. This allow the OCP2 to be effective in stress conditions.

O.34. Discovery and characterization of a novel bacterial nanocompartment in Synechococcus elongatus PCC 7942

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A defining feature of cells is their ability to spatially organize their cytosolic milieu into specialized compartments. Many bacteria possess protein-based organelles that compartmentalize enzymes with metabolic activities that are incompatible with the rest of the cell. One example is the carboxysome, a bacterial microcompartment, which compartmentalizes RuBisCO and carbonic anhydrase to optimize CO2 fixation. Although bacterial microcompartments are well-studied, another class of proteinaceous organelle, known as bacterial nanocompartments, remains poorly understood. Recent bioinformatic evidence has revealed that there may be many distinct families of these nanocompartments widespread throughout prokaryotic phyla with novel cargo proteins and physiological functions. Here, we have identified a novel family of bacterial nanocompartment found in the freshwater cyanobacteria Synechococcus elongatus PCC 7942. We have shown that this nanocompartment encapsulates a cysteine desulfurase cargo which is directed to the interior of the compartment via an N-terminal targeting

sequence and have implicated its potential role in sulfur starvation response. Using Cryo-EM, we have determined the structure of this nanocompartment to 2.6Å resolution.

O.35. The distinctive regulation of cyanobacterial glutamine synthetase

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The increasing importance of cyanobacteria as microbial cell factories is contradicted by the current state of knowledge about metabolic regulation. In many aspects cyanobacteria differ from other bacterial groups and hence, data that were obtained from genetic models like E. coli can often not be applied. This will be exemplified by the regulation of glutamine synthetase (GS), the key enzyme of bacterial nitrogen assimilation. The classic example, the comprehensively characterized GS of enterobacteria is subject to exquisite regulation at multiple levels, among them gene expression regulation to control GS abundance as well as feedback inhibition and covalent modifications to control enzyme activity. The cyanobacterial GS, however, features a fundamentally different regulation which includes the interaction with small proteins, the so-called inactivating factors (IFs) that inhibit GS linearly with their abundance. The model strain Synechocystis sp. PCC 6803 harbours two of these factors called IF7 and IF17 whose abundance is mainly controlled at the transcriptional level. In addition, cyanobacteria evolved unique RNA-based regulatory mechanisms to tightly tune IF abundance. For instance, the nitrogen stress induced RNA 4 (NsiR4) was found to interact with and to affect translation of the mRNA encoding IF7 (Klähn et al., 2015). Moreover, recent analyses revealed a glutamine riboswitch as another key element that controls IF17 synthesis in a glutamine-dependent manner (Klähn et al. 2018). These intriguing differences in cyanobacterial GS regulation suggest that further metabolic reactions might be also controlled differently compared to other bacteria.

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Poster presentations

P.1. Mathematical Modeling of Cyanobacteria: Biomechanics and Structured Population Dynamics

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Mathematical modeling is a powerful tool for furthering our understanding of cyanobacteria and photosynthesis in support of and beyond insight gained through experimentation alone. This talk will demonstrate this valuable relationship in two main areas; the first being specific colony morphology as driven by biomechanical force. For example, time-lapse fluorescence microscopy images suggest that cyanobacteria regulate photosynthetic activity in response to mechanical stress. The resulting growth dynamics lead to a repeatedly observed, highly organized colony morphology that persists through the early 16- to 32-cell stages [1]. A biomechanical model derived from the principles of Newtonian mechanics will be presented along with simulations replicating the biological and physical features, specifically the force of cell-to-substrate friction and cell-to-cell interactions, required to observe this morphology. This result opens the door to the exciting possibility of using cyanobacteria to metabolize light energy into kinetic energy. The latter portion of this talk will focus on the influence of population structure on growth dynamics. The ability of cyanobacteria to perform photosynthesis, and therefore grow, is controlled by carboxysome productivity. Partial differential equation models will be presented for this population which include key features such as the age- and size-distribution of cells as well as the age (or productivity) of carboxysomes. Comparing model predictions with experimental data will help evaluate the claim that carboxysome productivity declines over time, and point to the appropriate models for carboxysome degradation.

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P.2. Isolation and Characterization of Novel Freshwater Cyanophages

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While recent advancements in DNA sequencing technologies have resulted in an ongoing revolution in understanding the universe of bacteriophages, the cyanophages that infect freshwater model cyanoabacterial species have remained largely unexplored. Here we report on the discovery of new cyanophage isolates with varied plaque morphologies that infect Anabaena sp. PCC 7120. Phenotypic characterization of these new isolates includes an examination of the infection process using time-lapse light microscopy and phage particle morphology using transmission electron microscopy. Genomic sequencing of one isolate, a Myoviridae, has further revealed several interesting commonalities with a pair of recently sequenced cyanophages, A-1 and N-1, isolated in the early 1980s. As these viral entities are believed to be abundant and diverse, this work lays a foundation for the development of a collection such phages that promises to provide not only a more complete understanding of the pressures these model cyanobacterial species face in their natural habitats but also a suite of potential new tools for working with them.

P.3. Generation of a high tolerant platform strain for metabolic engineering applications using adaptive laboratory evolution

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There is a necessity to replace petroleum-derived products with sustainable and replenishable forms. Even though cyanobacteria are emerging as promising hosts for creation biofuels such as n-butanol, ethanol etc they suffer from product inhibition at low concentrations or are prone to product inhibition of their proteobacteria counterparts' titers. This affects their growth and metabolism, thereby, limiting their economic viability. Here, we employ adaptive laboratory evolution (ALE) approach for increasing tolerance of a fast-growing local isolate Synechococcus elongatus PCC 11801 (Jaiswal et al., 2018) towards n-butanol and 23BD. We achieved a 150% increase of n-butanol tolerance from 2 g/L to 5 g/L after 100 passages (333 days) and a 100% increase of 23BD tolerance from 15 g/L to 30 g/L after 91 passages (352 days). Apart from increased solvent specific tolerance, cells showed increased tolerance towards other alcohols of similar and varying lengths. Along with increased tolerances, these strains should also be photosynthetically healthy. Chlorophyll a content and health (via phytoPAM) analysis reveals minimal stress being experienced by the adapted strains. Evolved strains accumulate both transient and permanent mutations, our results show that the changes occurred are permanent, allowing the cell to retain its ability to grow at high concentrations even after 15 subcultures without any stress. This study provides the first time generation of a highly tolerant, metabolically stable adapted strain and provides description of physiological changes related to the increase of n-butanol and 23BD tolerance in the adapted cyanobacteria.

P.4. Switching the carbon flux towards TCA cycle intermediates in Synechococcus elongatus PCC 7942 for enhanced succinate production

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Succinate is one of the top building blocks for an array of valuable products such as polymers, bioplastics, solvents and nylon precursors. Photosynthetic cyanobacteria are naturally endowed with the capacity to synthesize and secrete succinate via the tricarboxylic acid (TCA) cycle variant pathways. In the present study, a variant of TCA cycle consisting of isocitrate lyase (ICL) and malate synthase (MS) were overexpressed in Synechococcus elongatus PCC 7942. Native strong constitutive promoters of PCC 7942 were utilized for expressing these genes. The engineered strain was able to produce 3-4 fold higher succinate in 36 hours as compared to the wild type without any retardation in growth rate. The results suggested, rerouting of the carbon flux towards the production of succinate. The enzymatic activity of the expressed genes was validated via respective enzymatic assays and quantitative gene expression. Further, the succinate titers were estimated using succinate estimation kit and Gas-chromatography (GC-FID). However, in order to further increase the succinate titers, more carbon flux needs to be diverted towards the TCA cycle along with the reduction in the synthesis of storage molecules.

P.5. Improving desiccation tolerance in Synechococcus sp PCC 7002 towards regeneration of biomaterials

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Cement is traditionally used in general construction, but its use comes at an environmental cost. There is an increasing interest in the use of biocement, which takes advantage of microbially induced calcium carbonate precipitation. This biomaterial has several benefits, such as potential for self-healing, a property that depends on the microorganism survival under ambient conditions to be able to regenerate and produce new biomaterial. Cyanobacteria has been shown to induce precipitation of calcite, dependent on photosynthesis, with the advantage of capturing and storing CO2. Our group has demonstrated that the fast-growing cyanobacterium Synechococcus sp. PCC

7002 is able to induce calcite precipitation, which we are currently using in a hydrogel-based living cement-free mortar, with good mechanical properties. Yet, this organism has to survive the considerable stress of desiccation under nonaquatic ambient conditions. We were able to highly increase viability, after complete cell dehydration for over 7 days, by exogenously suppling Trehalose. This glucose dimer is widely used by organisms as a desiccation defense mechanism, including several cyanobacteria. Hence, we reengineered a strain to produce Trehalose, where treZ and treY genes from Anabaena sp. PCC 7120, a moderate desiccation tolerant aquatic organism, were introduced in Synechococcus sp. PCC 7002, under an inducible or a constitutive promoter control. The results of cell survivability under intense desiccation stress will be presented.

Funding for this work from DARPA Engineering Living Materials Program.

P.6. Photoassembly of Photosystem II: The role of Ca2+ in Photoactivation and Dark Rearrangement of Mn4CaO5 cluster of PSII

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Photosystem II (PSII) utilize solar energy to catalyze one of the important and most thermodynamically demanding reactions: the oxidation of water into protons and molecular oxygen. The PSII continuously experiences damage due to high light intensity resulting in the loss of photosynthetic activity. To perform a highly efficient photosynthetic activity, damaged D1 protein should be replaced, with consequent reassembly of PSII [1]. The key step in obtaining functional PSII de novo is the assembly of Mn4CaO5 core, driven by series of photo-oxidative reactions with incorporation of Mn and Ca ions into the coordination environment of PSII with the poorly studied rate limiting dark rearrangement step after first photooxidation event [2]. We have developed technique that combines site directed mutagenesis and in vitro PSII photoactivation as function of inorganic ions accessibility to better understand the role of coordination environment in PSII photoassembly.

We hypothesize that dark rearrangement is a translocation of the first oxidized Mn3+ from the high affinity oxidation site to the specific Mn1 binding site guided by Ca2+ through the formation of Mn3+(-OH)-Ca2+ scaffold intermediate. Presented work will show the progress in in vitro photoactivation of Mn depleted PSII in thylakoid membranes isolated from several site directed Synechocystis sp. PCC6803 mutants. Our work aims to present the role of Mn2+ and Ca2+ ions availability and accessibility during the assembly of the Mn4CaO5 cluster of PSII.

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P.7. Application of 13C flux analysis for debottlenecking isobutyraldehyde production in cyanobacteria

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This project aims to develop a systematic fluxomics approach to identify the bottleneck metabolic reactions that limit carbon flux towards enhanced isobutyraldehyde (IBA) production in genetically engineered cyanobacteria. Our lab has previously developed and successfully utilized isotopically nonstationary 13C-MFA (INST-MFA) (1-4) to delineate the photoautotrophic metabolism of cyanobacteria (4) and plant leaves (5). Recently, we combined INST-MFA with rational metabolic engineering to improve the productivity of an IBA producing mutant of the cyanobacterium Synechococcus elongatus PCC7942 (6). This presentation describes our current effort at refactoring previously engineered cyanobacteria strains (in (6)) to identify the metabolic phenotypes that contribute to enhanced strain performance. The results indicate that the bottleneck to IBA lies around the pyruvate node. By perturbing the fluxes around pyruvate, we were successful at further increasing IBA productivity. Our studies demonstrate the importance of INST-MFA which might be crucial for the engineering of metabolically efficient cyanobacterial and algal strains for biotechnological/industrial applications.

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P.8. Quantifying potential human exposure to cyanotoxins from lettuce grown with contaminated water

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The prevalence of harmful algae blooms and their associated cyanotoxins in lakes near urban centers is an increasing global issue that poses an environmental and human health hazard. Exposure to cyanotoxins via consumption can result in acute and chronic neuro- and hepatoxic effects. Microcystin, Nodularin, and B-methylamino-L-alanine are common cyanotoxins that pose a threat to humans who use contaminated water for crop and garden irrigation. Experimental data is lacking on the fate of these toxins in the environment, standard methods for cyanotoxin extraction from soil and plants, and on the capacity for bioaccumulation in food crops. To fill these knowledge gaps, we will first create an efficient extraction procedure from soil and vegetation and quantify uptake in lettuce crops irrigated with contaminated water. Second, a range of agricultural soils will be tested for their ability to affect cyanotoxin mobility and availability to plants. Third, three environmentally relevant concentrations of cyanotoxins will be applied to lettuce grown in a random block design in a greenhouse. Cyanotoxin concentrations in soil and roots and shoots of lettuce will be quantified using an enzyme-linked immunosorbent analysis kit and confirmed with high performance liquid chromatography. Results will provide data to improve the ability to model the fate of cyanotoxins in the environment. We will develop guidelines regarding the use of cyanotoxin contaminated soil and irrigation water and on the consumption of affected crops.

P.9. Photosynthetic generation of heterologous terpenoids in cyanobacteria

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The work aims to convert the secondary slow metabolism of the terpenoid biosynthetic pathway into a primary activity in cyanobacteria, and to generate heterologous products using these photosynthetic microorganisms as cell factories. Case study is the production of the 10-carbon monoterpene β-phellandrene (PHL) in Synechocystis sp. PCC 6803 (Synechocystis). Barriers to this objective include the slow catalytic activity of the terpenoid metabolism enzymes that limits rates and yield of product synthesis and accumulation. Fusion constructs as protein overexpression vectors were applied in the overexpression of the geranyl diphosphate synthase (GPPS) and β-phellandrene synthase (PHLS) genes, causing accumulation of GPPS up to 4% and PHLS up to 10% of the total cellular protein. Such GPPS and PHLS protein overexpression compensated for their slow catalytic activity and enabled transformant Synechocystis to constitutively generate 24 mg of PHL per g biomass (2.4% PHL:biomass, w:w), a substantial

improvement over earlier yields. The work showed that a systematic overexpression, at the protein level, of the terpenoid biosynthetic pathway genes is a promising approach to achieving high yields of prenyl product biosynthesis, on the way to exploiting the cellular terpenoid metabolism for commodity product generation.

P.10. Cooperativity in the NAD(P)H-dependent Oxygen Reduction Reaction Catalyzed by Synechocystis 6803 Flavodiiron 3 Homodimers

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Photosynthetic flavodiiron (Flv) proteins are a unique class of flavodiiron proteins that catalyze the oxygen reduction directly from reduced pyridine pools. In cyanobacteria the soluble Flvs are known to be essential for managing photosynthetic electron flow and providing protection for PSI during periods of high and fluctuating light. The biochemical and kinetic properties of Flvs that enable this are currently only partially understood. Here we address this knowledge gap through analysis of purified recombinant Synechocystis 6803 flavodiiron 3 (Flv3). Structural models of Flv3 generated from mass spectrometry showed Flv3 forms homodimers with preferential binding of FMN. The cofactor arrangements appear to support direct oxidation of pyridines and inter-monomer electron transfer processes. Reduction of the flavin cofactors show formation two electron reduced states with no semiquinone observed at steady state conditions, suggesting that Flv3 may be tuned to minimize one electron radical formation. Reduction of Flv3 with NAD(P)H shows a Fe(II)Fe(III) redox state signal observed in other non-heme iron and flavodiiron proteins, that was lost under reduction by dithionite. Flv3 catalyzed ORR using either NADH or NADPH as the electron donor with kinetics consistent with cooperativity. Analysis of the Flv3 binding isotherm of NAD(P)H by fluorescence showed sigmoidal dependence on NAD(P)H concentration, indicative of cooperative binding between two sites within a dimer. These properties finely tune Flv3 reactivity to function under the dynamic levels of oxygen and reduced pyridine nucleotides that are produced during photosynthetic growth, and to enable the modulation of electron flow under changing conditions.

P.11. The Fluctuating Cell-Specific Light Environment and its Impacts on the Physiology of *Synechocystis*

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Individual cells of cyanobacteria or algae are supplied with light in a highly irregular fashion when grown in industrial scale photobioreactors (PBRs). These conditions coincide with significant reductions in growth rate compared to static light environments commonly used in laboratory experiments. We grew a dense culture of the model cyanobacterium *Synechocystis* sp. PCC 6803 under a sinusoidal light regime in a bench-top PBR (the Phenometrics ePBR). We developed a computational fluid dynamics model of the ePBR which predicted that individual cells experienced rapid fluctuations (~6 s) between 2000 and <1 μmol photons m^{-2} s^{-1}, due to vertical mixing and self-shading. The daily average light exposure of a single cell was 180 μmol photons m^{-2} s^{-1}. Physiological measurements across the day showed no *in situ* occurrence of non-photochemical quenching nor was there significant photoinhibition. An *ex situ* experiment showed that up to 50% of electrons derived from PSII were diverted to alternative electron transport in a rapidly changing light environment modeled after the ePBR. Collectively, our results suggest that modification of non-photochemical quenching may not increase productivity of cyanobacteria in PBRs with rapidly changing light. Instead, our results suggest that tuning the rate of alternative electron transport and increasing the processing rates of electrons downstream of photosystem I are potential avenues to enhance productivity. The approach presented here could be used as a template to investigate the photophysiology of any aquatic photoautotroph in a natural or industrially relevant mixing regime.

P.12. Direct conversion of CO2 to squalene by metabolically-engineered in cyanobacteria

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Metabolic engineering of cyanobacteria has enabled photosynthetic conversion of CO2 to value added chemicals as bio-solar cell factories [1]. The using cyanobacterial for direct production of squalene is advantage, since they can just grow carbon dioxide from air and sunlight without energy sources based on glucose. However, the production levels of squalene among several isoprenoids in engineered cyanobacteria were quite low, compared to other microbial hosts. Here, we engineered S. elongatus PCC 7942 with modular metabolic pathways consisting of the methylerythritol phosphate pathway enzymes and the squalene synthase for production of squalene, originated from 4 type microorganisms [2]. Sequentially, to increase production of squalene from CO2 by application of the push-and-pull strategy. Squalene synthase (SQS) was fused to either a key enzyme (farnesyl diphosphate synthase) of the methylerythritol phosphate pathway or the β-subunit of phycocyanin (CpcB1). Moreover, the best squalene producer was cultivated in a scalable photobioreactor (6 L) with light optimization [3]. Further development of the photo-bioprocessing conditions with strain improvement will promote establishment of an engineered bio-solar cell factory for industrial-scale CO2 conversion. This work was financially supported by Korea CCS 2020 Program and Basic Research Program granted by National Research Foundation of Korea.

Keywords: Metabolic engineering, Cyanobacteria, Synthetic biology, Isoprenoids, squalene

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P.13. Roles for ClpXP in regulating the circadian clock in Synechococcus elongatus

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Circadian rhythms, regulated by a 24-h biological clock, are vitally important for controlling temporal programs of cellular physiology. The Synechococcus elongatus circadian oscillator, encoded by the kaiA, kaiB and kaiC genes, regulates global patterns of gene expression and the timing of cell division. Regulated proteolysis is an important quality control mechanism necessary for a broad range of cellular processes. The ClpXP protease consists of ClpX, an AAA+ ATPase that recognizes substrates targeted for degradation and translocates them into the ClpP peptidase chamber. The S. elongatus genome encodes one homolog of ClpX and three ClpP peptidase paralogs. The clpXP gene products play roles in both cell division and circadian rhythms, as clpX mutants display elongated cells, and disruption of clpX, clpP1 or clpP2 results in long-period circadian phenotypes. Remarkably, deletion of clpX and clpP2 resulted in cells capable of a 12-h phase shift of the circadian peak of gene expression in response to a phase resetting dark pulse, compared to a wild-type 5-h shift. Moreover, the cell morphology defect of clpX mutant cells was suppressed by two-fold expression of the bacterial tubulin homolog FtsZ and by modulation of the clock. Expression of active-site mutant variants in the ClpP paralogs lengthens the circadian period but supports normal cell morphology. We propose that chaperone activities of ClpX are required to coordinate clock control of cell division, whereas the protease activities of the ClpXP1P2 complex are required to maintain appropriate periodicity of the clock and its synchronization with the external environment.

P.14. Development of a Proximity-based Proteomics Technique in Cyanobacteria

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Cyanobacteria possess unique intracellular organization, including thylakoid membranes enclosing a thylakoid lumen, an inner and outer cytoplasmic membrane enclosing the periplasmic space, and proteinaceous organelles called carboxysomes. Characterizing subcellular regions of cyanobacteria with mass spectroscopy-based proteomics can lead to a better understanding of cellular organization and physiology. False positives and negatives are often obtained when analyzing isolated subcellular regions purified using imperfect purification techniques. Furthermore, certain subcellular regions cannot be isolated with existing purification methods. Proximity-based proteomics offers a unique opportunity to interrogate proteomes of organelles and sub-cellular regions that avoids some problems associated with classical purification techniques. In this strategy, proteomes of interest are covalently labeled in live cells with biotin by specifically targeted enzymes, purified using the biotin tag, and analyzed via mass spectroscopy. I have optimized a proximity-based proteomics protocol utilizing APEX2, an engineered ascorbate peroxidase that biotinylates protein in the presence of biotin-phenol and H2O2, in Synechococcus sp. PCC 7002. This is the first use of APEX2 in a photosynthetic organism or in a prokaryote. As a proof of concept, proximity-based proteomics of the cytoplasm identified >30% of proteins encoded by the genome. I will use proximity-based proteomics to determine the proteome of the cytoplasm, periplasm, and thylakoid lumen in PCC 7002 with specifically targeted APEX2. In the future, this technique can be used to identify protein interaction candidates or to interrogate specific cellular processes in non-membrane bound regions of the cell.

P.15. Metabolic engineering of a fast-growing novel strain Synechococcus elongatus PCC 11801 for the production of succinic acid

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Succinic acid is used extensively as a platform chemical in agriculture, pharmaceutical, food industry as well as also forms a precursor for many chemical compounds. Therefore, augmenting its production for its use as a base chemical has always been the objective of the extensive research. Photoautotrophic organisms like cyanobacteria can harvest light and CO2 and therefore can be an ideal platform to directly convert CO2 feedstock into value-added compounds. Some pioneer study has been done

by engineering Synechococcus elongatus PCC 7942 for the production of succinic acid, however, the titers reported were low (1).

Here, we report an improvement of succinate titers by engineering a novel fast growing and naturally transformable local isolate Synechococcus elongatus PCC 11801 (2). We expressed the genes encoding for α-ketoglutarate decarboxylase and succinate semialdehyde dehydrogenase for engineering PCC 11801 under the effect of constitutively expressing strong truncated native promoter, which resulted in a strain capable of producing higher titers of succinate. The expression of an additional gene PEP carboxylase resulted in directing the carbon flux towards succinate and thereby further boosting the succinate titers than reported previously. We, therefore have engineered a cyanobacterial strain to produce succinate and this engineered strain represent an excellent platform cell factory for future development of bioprocesses.

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P.16. A Phage-Type Tyrosine Integrase Is Responsible for Excision of a nifH1 element of Anabaena cylindrica ATCC 29414

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Many heterocyst-forming cyanobacteria have instances of large DNA elements interrupting nitrogenase genes in vegetative cells. These DNA elements are excised from the genome in specialized nitrogen fixing cells termed heterocysts, which allows these interrupted genes to be reconstructed and expressed in heterocysts. One such instance is present within the nifH1 gene of Anabaena cylindrica. This DNA element (5736 bp) contains anacy_2116, a phage tyrosine-integrase gene. The data presented here shows Anacy_2116, XisA, is sufficient for excision of the nifH1 DNA element in the E. coli system. A site-directed mutation of this tyrosine-integrase protein was preformed to altering conserved Y414 to F414. This mutant protein is unable to excise out this DNA element. The importance of the Core Recognition Sequence, CRS, was also evaluated. The CRS in nifH1 element is the conserved 8 bp nucleotide CCGTGAAG. This CRS was mutated to match the CRS of Alr1442, XisA, gene from Anabaena PCC 7120 responsible for the nifD element excision, which is the 11 bp nucleotide sequence GGATTACTCCG. CRS sequence swapping did not allow Alr1442 to excise nifH1 element, although the

positive control Alr1442 can excise nifD element in E. coli system. Results here suggests corresponding flanking region surrounding the CRS are involved in this specific excision event. All data presented here suggests excision of the nifH1 element is not solely dependent on the integrase for excision. This phenomenon shares many similarities with tyrosine-recombinase systems from bacteriophage and suggests an evolutionary assimilation between cyanobacteria and bacteriophage resulting in a host-controlled prophage.

P.17. Single amino acid substitution in RpaA leads to moderate arrhythmic clock output

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Circadian regulation of cellular processes in Synechococcus elongatus PCC 7942 is controlled by the post-translational oscillator, KaiABC. The oscillator serves as the core amid a complex network of clock proteins, integrating environmental 'input' signals to coordinate temporal regulation of 'output' gene expression. Genetic effectors of circadian rhythms in S. elongatus have been discovered through rigorous chemical and transposon mutagenesis screens. Previous work described an arrhythmic gene-expression phenotype caused by insertion of a transposon in the putative crm open reading frame (crm1). Initial evidence suggested that crm may encode a peptide that influences clock-mediated gene expression through undefined interactions with the response regulator, RpaA. The present study shows that phenotypes previously ascribed to the disruption of crm are instead caused by a mutant allele of rpaA linked to the crm locus; crm is positioned 201 base pairs upstream of rpaA. Resequencing of the mutagenesis cosmid that harbors the crm1 insertion flanked by chromosomal DNA revealed a missense SNP in rpaA, producing RpaAR121Q. Reconstruction of the crm1 mutant using the above mutagenesis cosmid produced two subpopulations: arrhythmic crm1 mutants encoding RpaAQ121, and rhythmic crm1 mutants encoding RpaAR121. Markerless editing with CRISPR/Cpf1 was used to generate rpaA SNPs in a wild-type background, confirming that RpaAQ121 is necessary and sufficient to cause arrhythmic output from clock-controlled promoters. The R121Q substitution is predicted to disrupt interaction between RpaA and either of the competing histidine kinases/phosphatases, SasA and CikA. Ongoing work seeks to clarify the altered biochemical properties of RpaAQ121 that lead to arrhythmic clock behavior.

P.18. Chemotaxonomy as a new and rapid tool to identify marine cyanobacteria

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Tropical marine cyanobacteria produce an array of biologically active secondary metabolites. Despite, the taxonomic classification of cyanobacteria is not yet resolved and it is constantly under revision. Modern taxonomic approach composed of morphological and phylogenetic analysis. However, there are limitations in terms of the morphological approach. The current study focused on whether the secondary metabolites produced by cyanobacteria is species specific and whether these chemicals can be used to foster the taxonomic relationship between the phylogenetically distinct various marine cyanobacterial species. We also aimed to develop a rapid chemotaxonomic tool for the identification of cyanobacteria on the basis of its major secondary metabolites. We are hypothesizing that each species would always produce the major chemotype irrespective of the geographical locations and these will be the major chemical markers of those species. To examine this, we compared the secondary metabolite compositions and 16S rRNA sequences among several collections of cyanobacteria from South Florida, the Caribbean, and Hawaii region. The chemical profiling was performed by using ESI-LC-MS to screen for secondary metabolites. MALDI-TOF was used for rapid identification of the metabolites. We found out that the several collections of cyanobacterial species always produce the major chemotype irrespective of geographical locations. We also showed that the MALDI-TOF can contribute to rapid and accurate identification of the core chemotype and hence more accurate taxonomies.

P.19. Engineering the methylerythritol phosphate pathway in cyanobacteria for photosynthetic isoprene production from CO2

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Isoprene, a key building block of synthetic rubber, is currently produced entirely from petrochemical sources. Growing concerns over global warming and fossil-energy shortage have stimulated efforts to develop microbial isoprene production from renewable feedstock or directly from CO2. To produce isoprene directly from CO2, we engineered the isoprene biosynthetic pathway in the cyanobacterium Synechococcus elongatus, with guidance provided by dynamic flux analysis and metabolite profiling1. We identified the pathway bottlenecks and increased the isoprene biosynthetic flux by overexpressing bottleneck enzymes, optimizing precursor levels, and constructing enzyme fusions. A highly efficient conversion of the photosynthate to isoprene was achieved, resulting in the production of 1.26 g L−1 isoprene from CO2. This titer is among the highest records

reported for photosynthetic manufacturing. The strains developed in this study can serve as platform hosts for photoautotrophic production of a broad range of terpenoids.

Reference: Gao, X. et al. Engineering the methylerythritol phosphate pathway in cyanobacteria for photosynthetic isoprene production from CO2. Energy & Environmental Science 9, 1400-1411 (2016).

P.20. Interactions of Phototropism and Gravitropism in Cyanobacteria

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Gene expression in bacteria has previously been shown to be affected by near-zero or extremely high gravity. However, no mechanistic basis for gravitropic behavior has been established in bacteria previously. Taking advantage of the expression of several categories of pigment in the cyanobacterium Synechococcus sp. PCC 7002 (hereafter Synechococcus) and mutant strains thereof, we demonstrate that the distribution of pigmentation within the cell, and within cell colonies, is regulated by combined directional sensing of incoming light, adhesion to a surface via extracellular matrix, and applied external force, including the normal force of gravity applied to the cell. Cells grown on a substrate orient their thylakoids on the faces of the cell proximal and distal to the substrate and express both chlorophyll and phycobilins in both of these membrane regions. Phycobilins are primarily expressed in the membrane region nearest to the light source, while chlorophyll is preferentially expressed in the region opposite the overall external force applied to the cell. Cells also respond to the presence of nearby cells by producing an overall pigment distribution which follows the same rules as within the cell, but applied to the scale of the overall group. The mechanism for distribution of pigments appears to be regulated by presence of polyphosphate bodies within the cell. Lack of polyphosphate near a given thylakoid region produces a distinct spotted pigment distribution wherein pigment is expressed in small patches of membrane. Additionally, removal of polyphosphate completely negates the cell's ability to sense external forces.

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P.21. Hydrocarbon Production and Biological Nitrogen Fixation in Anabaena sp. PCC 7120

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Cyanobacteria photosynthetically produce long-chain hydrocarbons, which are considered as infrastructure-compatible biofuels. However, native cyanobacteria do not produce these hydrocarbons at sufficient rates or yields to warrant commercial deployment. This research sought to identify specific genes required for photosynthetic production of alkanes to enable future metabolic engineering for commercially viable production of alkanes. The two putative genes (alr5283 and alr5284) required for long-chain hydrocarbon production in Anabaena sp. PCC7120 were knocked out through a double crossover approach. The knockout mutant abolished the production of heptadecane (C17H36). The mutant is able to be complemented by a plasmid bearing the two genes along with their native promoters only. The complemented mutant restored photosynthetic production of heptadecane. This combined genetic and metabolite (alkanes) profiling approach may be broadly applicable to characterization of knockout mutants, using N2-fixing cyanobacteria as a cellular factory to photosynthetically produce a wide range of commodity chemicals and drop-in-fuels from atmospheric gases (CO2 and N2 gas) and mineralized water.

Many cyanobacteria produce heptadecane, but its physiological role is unknown. The alkane knockout mutant showed reduced ability to adapt to nitrogen fixing conditions. Mutant cultures adapted slowly to nitrate-free medium, displaying a fragmented phenotype while the wildtype cultures grew with long filaments. However, after four weeks' adaptation to nitrate-free medium, the mutant culture was comparable to the wildtype both in filament length and heterocyst counts. Nonetheless, nitrogenase levels were lower in the mutant compared to the wild-type, suggesting a connection between alkane production and nitrogen fixation in Anabaena sp. PCC7120.

P.22. Characterizing unusual enzymes from the cylindrocyclophane biosynthesis pathway in cyanobacteria

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Cylindrocyclophanes are a family of macrocyclic hydrocarbons produced by some cyanobacteria. We are investigating two enzymes in cylindrocyclophane biosynthesis which catalyze unusual reactions in biology. The first enzyme of interest, CylC, acts early in biosynthesis to halogenate an unactivated carbon atom in a fatty acyl chain. CylC requires none of the cofactors or cosubstrates that are known for other halogenases, suggesting CylC represents a new type of halogenase which we hypothesize uses a diiron cofactor. The second enzyme of interest, CylK, is the final essential enzyme in cylindrocyclophane biosynthesis. In a reaction analogous to the Friedel-Crafts alkylation, CylK uses the halogen atom installed by CylC to perform a dimerizing macrocyclization. Together, CylC and CylK enable an unusual biosynthetic strategy to form a carbon-

carbon bond at a previously unactivated position. We are exploring the substrate and product specificities of these enzymes to gauge their potential as biocatalysts for synthetically useful reactions.

P.23. BROAD-HOST-RANGE GENETIC TOOLS FOR CYANOBACTERIA AND HETEROLOGOUS EXPRESSION OF NATURAL PRODUCTS

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Improved genetic tools for cyanobacteria provide new opportunities for scientific research and biotechnology. We have developed a set of broad host range standardized genetic parts and devices for genetic modification and engineering of diverse cyanobacterial strains. The parts and devices are carried in a library of donor vectors and include origins of replication for E. coli, origins of replication and neutral sites for cyanobacteria, origins of transfer for conjugation, antibiotic-resistance markers, expression cassettes with different promoters, and reporter cassettes. These genetic parts and devices were characterized in several diverse cyanobacterial strains. The system has been used to develop and validate improved versions of the broad-host-range plasmid RSF1010, theophylline-inducible riboswitches, a set of constitutive promoters with a wide range of activity, and vectors based on the S. elongatus plasmid pANS. The genetic tool kit has been used for the heterologous expression of several genes, including a 20-kb gene cluster required for the biosynthesis of the polyunsaturated fatty acid EPA, a hs_bmp7 gene and hs_bmp7 to hs_bmp12 gene cluster involved in synthesis of polybrominated diphenyl ethers, and the dehydroascorbate reductase (DHAR) gene from Brassica juncea (BrDHAR) to increase resistance to oxidative stress.

P.24. Biomanufacturing with Intelligent Adaptive Control

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Cyanobacteria are considered a promising platform for the production of biofuels and renewable chemicals. Cyanobacteria may also be utilized as a resource for metabolites with biotechnological potential. For example, cyanobacteria can produce a wide variety of molecules, such as siderophores, herbicidal molecules, plastic precursors, etc. To

exploit such diversity, we are developing machine learning (ML) tools that optimize the discovery and design of metabolic modules that comprise or influence a given biosynthetic pathway. As a proof-of-concept, we are evaluating polyhydroxyalkanoate (PHA) production across the cyanobacterial phylum. Pfam domains derived from the CyanoGEBA genome sequence dataset, combined with growth data of ten select cyanobacterial strains and the corresponding PHA yields, will be analyzed through ML, to discover sets of genes that influence (whether positively or negatively) PHA production. Predicted genetic modifications and optimized culture conditions will then be validated in the model organism Synechocystis sp. PCC6803. We expect that this approach will allow us to discern and predict cooperative and opposing biochemical effects of the expression of combined sets of genes. In addition, by leveraging the metabolic diversity of cyanobacteria to accelerate the production of renewable polymers, we will advance the goal of a circular bioeconomy.

P.25. Engineering Improved Ethylene Production: Leveraging Systems Biology and Metabolic Engineering.

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Ethylene is a small hydrocarbon gas, widely used in the chemical industry. Its annual worldwide production currently exceeds 150 million tonnes, surpassing any other organic compound. Ethylene is currently produced from steam cracking of ethane, which produces vast quantities of CO2, contributing to global warming. Ethylene is the monomer for the most common plastic, polyethylene, and annual global production is approximately 80 million tons. Therefore, unlocking a sustainable or carbon neutral alternative to ethylene production is imperative. Ethylene has been produced in a wide variety of different microorganisms utilising the ethylene forming enzyme (EFE) from P. syringae pv phaseolicola. However, despite recent advances, substantial developments still need to be made to fully realise the potential of biological ethylene production in a self-sustaining chassis. Substantial improvements in ethylene production will need to address key bottlenecks such as enzyme solubility and biochemical precursor availability. We have utilised a combination of systems biology, transcriptional engineering, mutagenesis, continuous competitive fermentation and designer protein evolution, coupled to high-throughput screening to enable the accelerated development of a high efficiency ethylene producing strain. Yielding a 15-fold increase in ethylene productivity, the most significant improvement reported in the literature to date.

P.26. MpeU is a lyase-isomerase for MpeA in marine cyanobacterial species Synechococcus RS 9916

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Marine Synechococcus sp RS9916 employs Type 4 chromatic acclimation (CA4) to optimize the ratio of two chromophores, green-light absorbing phycoerythrobilin (PEB) and blue-light absorbing phycourobilin (PUB), within their light harvesting complexes. Synechococcus sp RS9916's ability to perform CA4 occurs via a horizontally acquired genomic island [1-2]. The gene mpeU is found in all Synechococcus strains that adapt to blue light [3]. The mpeU gene is located in a genomic region which includes genes involved in the biosynthesis of PEII. In order to study the role of MpeU, an mpeU interruption mutant was generated in RS9916 and grown under green light and blue light conditions. Analysis of PBS from mpeU deletion mutants in RS9916 revealed that MpeU was required for the addition of PUB to either PEI or PEII [3]. The MpeA protein (alpha PEII subunit) from mutant cells grown under blue light conditions was observed to have a lower PUB:PEB ratio than WT MpeA that was isolated from blue light-grown PBS. The MpeA protein from mutant samples grown in blue light was digested with trypsin and analyzed by LC-MS. In BL, normally MpeA contains phycourobilin (PUB) at all three Cys sites. However, in the mpeU mutant, PEB was found at C-140 on MpeA, suggesting that MpeU is the lyase-isomerase which is responsible for the attachment of PUB to Cys-140 of MpeA under blue light conditions. Results from recombinant co-expressions with MpeA, MpeU and the enzymes necessary for phycoerythrin synthesis in E. coli will be presented. Nickel affinity chromatography will be used to purify His6x tagged MpeA followed by fluorescence and absorbance spectroscopy to assess the α subunit's chromophorylation state.

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P.27. A Novel Antenna Protein Complex in the Life Cycle of Photosystem II

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Photosystem II (PSII) is a unique membrane-protein complex that catalyzes light-driven oxidation of water. PSII, which undergoes frequent damage due to its demanding photochemistry, is repaired and reassembled in an intricate process, many aspects of which remain unknown. We have isolated a novel PSII subcomplex from the cyanobacterium Synechocystis sp. PCC 6803 that lacks the core reaction center components of PSII. This pigment-protein complex does contain the antenna proteins CP47 and CP43 as well as most of their associated low-molecular-mass subunits and the assembly factor Psb27. This finding challenges the current model of the PSII repair cycle in which, following damage, PSII is disassembled into separate CP43 and CP47 sub-complexes. We call this complex No Reaction Center (NRC) and propose that it is involved in PSII repair following photodamage. Furthermore, we propose that formation of this pigment protein complex maximizes efficiency of repair and minimizes collateral damage by maintaining the unharmed PSII components in a structured complex to await re-insertion of nascent reaction center proteins.

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P.28. An Efficient, Markerless Strain Construction Method for Synechococcus sp. PCC 7002

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Metabolic engineering efforts typically require the ability to make multiple and successive genomic modifications in a particular host. While this might be a trivial task for microbes such as Escherichia coli, such is still not the case for cyanobacteria. Thus, as cyanobacteria continue to emerge as a popular chassis for carbon-neutral chemical production, it is imperative that more efficient methods for genome engineering in this host be developed. Here, we present a two-step method for the markerless strain construction in Synechococcus sp. PCC 7002 using site-specific recombinases and a novel counterselection method. First, an antibiotic resistance cassette, flanked by two loxP sequences, is delivered into the genome at the loci of interest via homologous recombination. Second, CRE recombinase is transiently expressed by integrating into an essential gene where segregation cannot occur and the strain instead remains as a merodiploid. CRE recombinase-dependent excision of the antibiotic resistance cassette under these conditions is 100% efficient and results in fully segregated, markerless strains immediately post-transformation. In the end, removal of CRE recombinase is

facilitated by simply growing the cells in the absence of antibiotic selection. The versatility of this approach, conditions favoring the use of this natural counter-selection system, and a comparison of this method to current strain construction methods will all be discussed.

P.29. Identification of the amino acids that confer bilin isomerase activity in a marine cyanobacterial lyase-isomerase enzyme family

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Many marine Synechococcus strains use a process known as Type 4 chromatic acclimation to optimize the ratio of two chromophores, green-light absorbing phycoerythrobilin (PEB) and blue-light absorbing phycourobilin (PUB), within their light harvesting complexes. These strains encode a family of closely related bilin lyase enzymes which attach PEB, and in some cases isomerize it to PUB. The MpeQWYZ [1-4] family of enzymes were compared through sequence alignments to understand how these proteins have evolved and to identify potential sites responsible for the isomerase function. Previous studies of the PecEF bilin lyase-isomerase in Mastigocladus laminosus suggested residues that may contribute to the isomerase function [5]. The identified domain, NHCQGN, was not found in the lyase-isomerases of the MpeQWYZ family [5]. These sites in MpeQ (lyase-isomerase) were mutated to the equivalent residue in MpeW (lyase) using combined overlapping PCR. Wild type and mutant MpeQ proteins were coexpressed with the alpha subunit of phycoerythrin II and the enzymes necessary for phycoerythrin synthesis in E. coli. The purified samples were analyzed by absorbance and fluorescence spectroscopy and on SDS polyacrylamide gels. Our initial results suggest that the sites V319, T320, and Y323 contribute to isomerase activity.

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P.30. Industrial carbon capture and conversion to biomass by fast-growing cyanobacteria cultivated on wastewater

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Fast-growing cyanobacteria that are amenable to synthetic bioengineering hold great potential for sustainable carbon capture and bioproduction. Synechococcus sp. PCC 7002 and UTEX 2973 are among the fastest-growing microalgae on earth with doubling times of less than 3.5 and 2 hours, respectively. To develop an inexpensive, industrial growth medium, we have analyzed waters from the Waupaca Wisconsin Wastewater Treatment Plant (WWTP) and developed media formulated from stages of the treatment process. The best of these media support growth of both PCC 7002 and UTEX 2973 at rates and yields comparable to those on standard growth media. To demonstrate viable industrial carbon capture by algal biomass production and conversion to fuels and coproducts, we are working with Waupaca Foundry Inc. (WFI) to test PCC 7002 and UTEX 2973 growth on foundry flue gas emissions and WWTP media. We find that both PCC 7002 and UTEX 2973 grow vigorously on WFI flue gas and WWTP media in LED-illuminated photobioreactors at the foundry, with no evidence of growth inhibition from components of the flue gas. These findings set the stage for process scale-up with techno-economic analysis to develop economically-viable, industrial carbon capture with sustainable production of fuels that may feed back into industrial operations. Moreover, we have engineered both Synechococcus PCC 7002 and UTEX 2973 to produce terpene bioproducts that can contribute to process economics and sustainability. Finally, if algal carbon capture and recycling can be established in the heart of the Wisconsin winter, then it should be viable anywhere in the world.

P.31. Metabolic engineering of cyanobacteria for cinnamic acid production

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Cinnamic acid (CA) and p-hydroxycinnamic acid (pHCA) are phenylpropanoids that have wide application as precursors for flavoring agents, health and cosmetic products. Traditionally, these compounds are obtained by extraction from plant tissues or chemical synthesis. An alternative production method for these aromatic acids relies on the engineered heterotrophic microorganisms. However, usage of phototrophic organisms such as cyanobacteria and green algae would allow making this process truly sustainable.

In our research, we currently focused on the metabolic engineering of Synechocystis for CA production. This compound can be obtained by a single enzymatic step from phenylalanine by the action of enzyme phenylalanine ammonia-lyase (PAL). Although this enzyme has been found in a few cyanobacteria [1], it is not present in Synechocystis. We selected five PAL candidates for heterologous expression based on their kinetic properties according to literature data. Expression cassettes were

successfully introduced into Synechocystis. Obtained strains were tested based on their growth and productivity. The usage of Synechocystis as the host cyanobacterial strain allowed us to avoid native regulation of the PAL enzyme required for CA formation as well as to reduce the likelihood that CA is further metabolized in the cell. Additionally, a central metabolic role of phenylalanine, the precursor for the formation of CA by PAL, favors obtaining high titers of the product.

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P.32. Characterizing the phycobilisome abundance regulator psoR in the cyanobacterium Fremyella diplosiphon

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Certain species of cyanobacteria use a process called complementary chromatic acclimation (CCA) to tune their light-harvesting pigments to optimize utilization of the light energy they absorb and to protect themselves against excess excitation that can cause cellular damage. A spontaneous mutant with an interrupted psoR gene was shown to have disrupted CCA under red and green light conditions in the freshwater cyanobacterium Fremyella diplosiphon. The psoR mutant was shown to have decreased growth under green light, but increased growth under red light compared to wild-type. Spectral scans revealed an increased absorbance at 620 nm consistent with elevated phycocyanin content under all light conditions tested. Additionally, a decreased absorbance was observed at 565nm indicative of decreased levels of phycoerythrin. The Phyre2 protein structure prediction web portal was used to model the structure of PsoR, and structural similarity to metallo-β-lactamase fold proteins containing a β-CASP domain was predicted. β-CASP proteins are nucleic acid-processing enzymes that include proteins that function as DNA-repair and RNA-processing proteins. Future research on the psoR gene can aid in our understanding of how CCA works and give us a clearer understanding of the complex light-dependent sensory network in F. diplosiphon.

P.33. Characterising cyanobacterial electron export pathways from the thyalkoid membranes to the external environment

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Cyanobacteria have evolved elaborate electron transport pathways to carry out photosynthesis and respiration, and to dissipate excess energy in order to limit cellular damage. In Synechocystis sp. PCC 6803, we have shown that deletion of thylakoid membrane localized terminal oxidases, cytochrome c oxidase (COX) and quinol oxidase (Cyd), and a third complex, the alternative respiratory terminal oxidase, located in the plasma membrane electron transport chain, results in increased electron export from the cell1. Using a novel microscale flow-based BPV device and a mutant deficient in all three terminal oxidases and two other electron sinks, flavodiiron 1/3 and 2/4, we generated power densities of over 0.5 W m-2, double that obtained with wild-type cells2. Overall, this suggests that an uncharacterised pathway is present which is able to efficiently transport a large flow of electrons generated in the thylakoid membrane to first the plasma membrane, then the cellular exterior, which may constitute an important electron sink3. Characterisation of this pathway is key to developing optimised strains for BPVs, facilitating their development as a renewable energy source, and for understanding the physiological role of electron export in cyanobacteria. In order to do this we will take advantage of a Synechocystis sp. PCC 6803 mutant library and high-throughput BPVs to identify and characterise unknown proteins involved in electron transport.

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P.34. Characterization of RubisCO activase in the cyanobacterium Fremyella diplosiphon

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RubisCO, the enzyme that catalyzes the first step of carbon assimilation in photosynthesis in most photosynthetic organisms, is prone to inhibition by sugar phosphate small molecules, including its substrate ribulose 1,5 bisphosphate (RuBP) and intermediate products of its reaction. In plants and green algae, the enzyme RubisCO activase (Rca) is involved in regulating RubisCO activity, by catalyzing the removal of bound inhibitors. Cyanobacterial RubisCO was shown to be less sensitive to inhibition compared to its plant counterpart. Nonetheless, many diverse species of cyanobacteria contain a gene for a protein that has a AAA+-ATPase domain, similar to that found in

Rca. Additionally, the great majority of these cyanobacterial proteins also contain a C-terminal sequence extensions with high similarity to the small subunit of RubisCO (Small Subunit Like Domain, SSLD). We term this group of RubisCO Activase-Like Cyanobacterial proteins – ALC. Because cyanobacterial RubisCO is less sensitive to inhibition, it is not clear if the ALC functions in a similar manner to that of the Rca. We are studying the expression, cellular localization and biochemical function of the ALC in the cyanobacterium Fremyella diplosiphon.

P.35. Pathway engineering for high-level photosynthetic acetone biosynthesis from CO2 in cyanobacteria

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To replace petroleum-based chemicals, tremendous efforts have been successfully enabled to produce photosynthetic short-chain-alcohols, biochemicals, and isoprenoids by manipulating metabolic pathways and introducing synthetic pathways in engineered cyanobacteria. Here, we introduce a synthetic acetate-acetyl-CoA (AA) bypass to redirect carbon flux toward acetyl-CoA by secreting and assimilating acetate. To demonstrate the AA-bypass, two different synthetic acetone production pathways (AAD and NAD) were applied to produce photosynthetic acetone from CO2. SeHL120 (AA-bypass with the AAD pathway) was constructed and cultivated for acetone production, resulting in 378.1 mg L-1 acetone and 19.9 mg L-1 acetate. Thus, the successful development of the cyanobacterial strains with the novel AA-bypass, combined with bioprocessing, will accelerate the development of bio-solar cell factories for the direct conversion of CO2 to acetyl-CoA-derived bio-chemicals. This work was supported by Korea CCS R&D Center (KCRC), and the Basic Science Research Program through the National Research Foundation of Korea.

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P.36. Manganese oxidation by Cyanobacteria and the evolution of photosystem II

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Oxygenic photosynthesis is arguably the most important bioenergetic innovation in Earth history. Manganese plays a special role in this history because of its role in the water-oxidizing complex (WOC) of photosystem II (PSII). Both comparative biology and the sedimentary rock record provide evidence that Mn(II)-oxidizing phototrophy may have been a direct evolutionary precursor to oxygenic photosynthesis. If this was the case, it is possible that extant Cyanobacteria are still capable of phototrophic Mn(II) oxidation.

Here we present data with Synechocystis sp. PCC 6803 wild-type cells, PSII D1 mutants, and isolated membranes that show Mn(II) oxidation occurring by a light-dependent, PSII-dependent mechanism. This effect demonstrates at least some phylogenetic breadth (examined in Synechocystis, Nostoc, and Gloeobacter). To evaluate the ecological scope of this process, we have also begun investigating Mn-oxidation by Cyanobacterial communities in the environment via two different settings: 1) modern fluvial environments with particularly high Mn abundance in the Northern Cape Province of South Africa, and 2) ancient biosignatures of this process preserved in unique Mn-rich stromatolites in the sedimentary rock record, deposited close in time to the rise of oxygen. Taken together, these data suggest that Cyanobacteria play a role in Mn cycling in both modern and ancient environments—and that Mn-oxidation appears to have been an important gateway process to the evolution of biological water-splitting. Ongoing work is focused on understanding the physiological value and molecular mechanics of this process, and its evolutionary implications for the origin of the WOC.

P.37. Multi-Generational Analysis of Polyploidy in Cyanobacteria

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Polyploidy, the presence of multiple identical chromosomes in a single-cell, is rarely associated with prokaryotes. However, it is becoming increasingly apparent that many ecologically, medically, and biotechnologically important prokaryotes are polyploid. Currently, there is little experimental evidence to support theories regarding the fitness benefits associated with differences in chromosome copy number in prokaryotes, and it is unclear whether the well-defined systems of DNA replication and inheritance in monoploid cells are essential for polyploid strains. To determine how chromosome dynamics are regulated in a polyploid cyanobacteria, we analyzed long-term, time-lapse fluorescence microscopy images of individual cells with fluorescently labeled chromosomes. Quantitative image processing with custom algorithms enabled us to track

individual cells and monitor chromosome number and segregation over multiple generations for single-cell derived lineages. Using this method, we were able to define the effect of growth rate on chromosome dynamics and gene expression. We also measured the impact of depleting genes essential for cell functions, such as cell division and DNA replication, on chromosome number and segregation. Lastly, we determined the effects of chromosome loss on cyanobacterial physiology. Our data indicate that while the principles governing DNA dynamics are conserved from monoploid cells to polyploid cells, the mechanisms regulating those dynamics have diverged.

P.38. Effects of glucose addition to natural Prochlorococcus populations at Aloha Station, Hawaii

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The marine cyanobacterium Prochlorococcus can utilize glucose as energy source (1-3). However, the relative importance of inorganic and organic carbon assimilation in these cyanobacteria are still poorly understood.

We carried out diel cycles studies after glucose addition at ALOHA Station, Hawaii. We have studied the shift in the microbial community structure in response to glucose, observing minor changes driven by the growth of three major taxa, including Prochlorococcus.

We have also analyzed the transcription of Prochlorococcus genes involved in carbon metabolism and photosynthesis. Preliminary results have shown an increase in the transcription levels of genes involved in glycolysis and in genes encoding a glucose transporter in response to glucose addition. Photosynthetic genes showed no differences after the treatment, with high transcription levels in all samples.

Moreover, primary production and glucose uptake studies combined with flow cytometry cell sorting were performed. Our results showed a temporal separation of glucose uptake, with maximum uptake during the day and minimum at night. Moreover, less than 1% of total C uptake was assimilated via mixotrophic pathways relative to primary production in natural populations of Prochlorococcus.

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P.39. Precise Flux Map of Synechococcus sp. PCC 7002 with 13C isotopic labeling of metabolites and fragment ions quantified via SWATH

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13C Metabolic Flux Analysis (MFA) uses 13C-labeled substrate as a tracer followed by the measurement of mass isotopologue distribution (MID) of metabolites to estimate the carbon fluxes. The success of MFA depends on the precise measurement of MIDs. The positional labeling information contained in the fragment MIDs obtained via collision-induced dissociation has been demonstrated to improve the precision of flux estimates theoretically and experimentally. The use of specifically labeled tracers offers an advantage to obtain precise flux estimates in heterotrophic organisms. In the case of a photoautotrophic organism that utilizes CO2 as a sole carbon source, specifically labeled tracers could not be used. In such a scenario, isotopic non-stationary MFA involving a dynamic 13C labeling experiment is crucial to get fluxes with good confidence. Here, we demonstrate that the use of precise fragment MIDs of metabolites obtained via SWATH-MS/MS helps to achieve precise flux estimates in photoautotrophic metabolism of a model cyanobacterium Synechococcus sp. PCC 7002. We compared the flux estimates with and without fragment MIDs. We observed a significant decrease in 95% confidence interval for fluxes of reactions in the Calvin cycle when product MIDs were used. The differential labeling information contained in fragment MIDs as a result of formation of a particular metabolite by a reaction between two intermediate metabolites drawn from different pathways can be more informative than the intact precursor MID. This is the first report where precise flux estimates is obtained in cyanobacteria using fragment MIDs.

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P.40. An improved natural transformation protocol for Synechocystis sp. 6803.

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Cyanobacteria are photoautotrophic prokaryotes capable of converting sunlight into chemical energy via oxygenic photosynthesis. Fast growth rates, ease of maintenance and the speed with which mutants can be generated, in comparison to terrestrial plants, has resulted in certain cyanobacterial species becoming model organisms for study in the field of photosynthesis and cyanobacterial biotechnology. Synechocystis sp. 6803 is one such species; unicellular and naturally transformable with a fully sequenced and annotated genome. Synechocystis mutants have been used to reveal the molecular mechanisms of photosynthesis from light harvesting to water splitting and carbon fixation. In recent years, Synechocystis mutants have yielded chemical commodities from sunlight and atmospheric carbon. However, Synechocystis is a polyploid organism, possessing multiple copies of its genome per cell. The time required to generate mutants is far greater than for its heterotrophic monoploid counterpart, Escherichia coli; heterologous DNA integrated into the genome must first be segregated across all copies before mutant phenotypes are revealed. We have developed a novel natural transformation protocol that reduces that time required to obtain fully segregated mutants by temporarily inducing monoploidy in Synechocystis. We envisage our simple protocol will aid our colleagues in labs around the world working with Synechocystis.

P.41. A Cyanobacterial Sidestream Nutrient Removal Process and Its Life Cycle Implications

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This study proposes a novel integration of a municipal wastewater treatment facility (WWTF) with a cyanobacterial nutrient removal process for sidestream wastewater treatment. A life cycle assessment (LCA) approach was used to determine the effectiveness and environmental performances of the integrated system. The LCA is populated by models of wastewater process engineering, material balance, cyanobacterial growth, and kinetics of anaerobic digestion. The cyanobacteria growth model incorporates chlorophyll synthesis, nitrogen uptake, photosynthesis, centrate inhibition, and competition for nitrogen between cyanobacteria and nitrifiers. Modeling results are validated against experiments with Synechocystis sp. PCC6803 grown in sludge centrate. With a maximum specific growth rate of 1.09 day−1, the nitrogen removal rate of the proposed WWTF would be increased by 15% when compared to the baseline wastewater treatment facility with a biological nutrient removal process. Incorporating the cyanobacterial nutrient removal process as the sidestream wastewater treatment of a conventional activated sludge process reduces the total nitrogen concentrations discharged from the WWTF from 25.9 to 15.2 mg.l−1. Methane yield was found to be

increased by 4% of the baseline value when cyanobacterial biomass was co-digested with the activated sludge. Life cycle energy use and greenhouse gas emissions were found to be reduced by 8% and 17%, respectively, relative to a baseline wastewater treatment facility. Overall, a cyanobacteria-based sidestream municipal wastewater treatment process could be an effective and environmentally sustainable biological nutrient removal process in the future addressing the water-energy-food nexus.

P.42. Characterization of HmpF, a novel component of the Hmp chemotaxis-like system that controls cyanobacterial motility

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Many species of filamentous cyanobacteria exhibit gliding motility, likely via a conserved type IV pilus (T4P) system arrayed in rings at the cell poles. This motility facilitates a variety of biological processes including phototaxis and the establishment of nitrogen-fixing symbioses with plants. In the model filamentous cyanobacterium Nostoc punctiforme, the activity of the T4P system is thought to be regulated by the Hmp and Ptx chemotaxis-like systems, required for motility and positive phototaxis respectively. However, the precise nature by which these systems interact with the T4P motors to regulate motility is currently unknown. Recently, HmpF, a novel component of the Hmp system was identified and characterized (1). HmpF is an SMC-like protein that is essential for motility, and displays dynamic, coordinated, polar localization at the leading poles of cells in motile filaments. To further investigate the role of HmpF in motility, its localization is currently being investigated in various genetic backgrounds. Preliminary results do not support direct interaction between HmpF and cytoplasmic facing components of the T4P system, are consistent with a role for the Hmp system in establishing coordinated polarity of HmpF and motor activation, and indicate that the light sensing Ptx system, and perhaps other light sensing systems, may act cooperatively with, or upstream of the Hmp system to facilitate phototaxis.

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P.43. Metabolic Engineering of Cyanobacteria for Bisabolene Production

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The ever-increasing consumption of non-renewable resources in an unsustainable fashion has been a driving force for the expansion of the Biotechnology field over the last decades, especially in the genetic engineering of microorganisms to produce biofuels and other high-value compounds. Most approaches rely on heterotrophic microorganisms; however, their need for carbon supplementation is disadvantageous, as it competes with the food sector and increases substantially production costs. Unlike the bio-production in heterotrophs, the usage of photosynthetic microorganisms, such as cyanobacteria and green algae, allows the solar-powered fixation of carbon dioxide from the atmosphere and its conversion into desired organic chemicals. This second approach is more advantageous in the sense that carbon supplementation is not required, making the entire production process cheaper, and its application on large scales would allow the establishment of a carbon-neutral based economy.
Among other secondary metabolites, cyanobacteria produce terpenoids, a structurally diverse group of natural products that play different pivotal roles in the cell. These metabolites have high relevance to the industrial sectors as fragrances, colorants and precursors for drugs and biofuels [1,2]. Our research is currently focused on the metabolic engineering of cyanobacteria for terpenoid production, specifically bisabolene. This 15-carbon-atom terpenoid has been shown to be a suitable biofuel, when chemically hydrogenated into bisabolane, and its production in heterotrophic microorganisms in high titres was already reported [3].

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P.44. Development of Genetic and Computational Tools for CRISPRi/a Screening in Synechococcus sp. PCC 7002

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This effort seeks to develop CRISPR interference and activation (CRISPRi/a) tools in Synechococcus sp. PCC 7002 (hereafter 7002) to enable high-throughput screening of guide RNA (gRNA) libraries. A CRISPRi system based on dead Cas9 from Streptococcus pyogenes (dSpCas9) has already been demonstrated in 7002[1]. We modified this CRISPRi system to develop a 2-hybrid CRISPRa system, comprised of dSpCas9, a modified gRNA containing an MS2 hairpin loop, and the MS2 coat protein (MCP) fused to a transcriptional activator (SoxS). MCP-SoxS binds to the modified gRNA of the dSpCas9-gRNA complex to enable activation of downstream genes. We are also developing a computational pipeline for identifying gRNA sequences for CRISPRi/a in 7002. This pipeline uses Benchling tools to identify gRNA sequences in 7002 based on

the protospacer adjacent motif (PAM) for dSpCas9. The gRNA sequences are then aligned to the 7002 genome, and a penalty score is calculated based on the length and number of potential off-target sites in the genome. We will then apply an algorithm, currently under development, which incorporates experimental data for CRISPRi/a based on the distance from the transcription start site of the gene and positional nucleotide bias of the gRNA target sequence. The predicted experimental effectiveness of the gRNA will then be combined with the off-target penalty score to assign an overall score to each gRNA sequence. By combining these genetic and computational CRISPRi/a tools, we will develop interference and activation gRNA libraries for screening 7002.

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P.45. Depicting the role of alternative electron sinks in sucrose production in Synechococcus elongatus PCC 7942

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Photosynthetic organisms use energy dissipation mechanisms to protect themselves under high light conditions. However, these mechanisms inherently reduce their photosynthetic efficiency - an important property to consider when engineering photosynthetic microbes for bioproduction applications. Cyanobacteria use two primary mechanisms to dissipate a large proportion of their excess light energy: alternative terminal oxidases (ARTOs) (Lea-Smith et al., 2013) and flavodiiron proteins (FDPs) (Allahverdiyeva et al., 2013). Here, we consider if heterologous engineered pathways (e.g., sucrose production; Abramson et al., 2016 and/or cytochrome P450 enzymes; Berepiki et al., 2016) can be used to partially replace these endogenous protective mechanisms, and instead conserve captured energy in useful metabolic pathways. To explore this hypothesis, we have constructed mutants lacking FDPs (Δflv3/flv1) and/or other alternative oxidases in Synechococcus elongatus PCC 7942, and introduced heterologous pathways that can act as carbon or electron sinks (sucrose export and/or cytochrome P450s, respectively). We find that there are certain conditions under which engineered sinks compete for reducing equivalents and/or ATP with photoprotective elements in the cyanobacteria. Likewise, some engineered sinks (e.g., sucrose export) can partially compensate for the loss of photoprotection genes (e.g., Δflv3/flv1) with regard to some photosynthetic properties, such as feedback-induced backup of electrons on PSI. Taken together, our results suggest specific conditions under which engineered sinks may be used to redirect energy away from wasteful photoprotection pathways and instead utilize a higher proportion of captured light energy for useful metabolic work.

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P.46. Improving heterologous protein expression in Synechocystis sp. PCC 6803 for alpha-bisabolene production

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Plants use enzymes to generate a massively diverse array of terpenoids, many of which have present applications in industries including pharmaceuticals and food. Expressing such enzymes in bacteria would lead to lower costs of production of these chemicals due to faster growth rates and simpler harvesting. One challenge faced by microbial production of plant terpenoids is the difficulty of expressing enough of the heterologous enzymes that produce the terpenoid of interest to compete with the native pathways. Our objective is to improve cyanobacterial production of bisabolene, a precursor to a potential diesel replacement, bisabolane, to facilitate conversion of sunlight into liquid fuel. This work focused on modulating the important gene expression regulatory elements, ribosome binding sites and codon usage, to improve bisabolene production in the cyanobacteria, Synechocystis sp. PCC6803. A small set of 20 strains of this organism was generated, each with a different combination of ribosome binding site designed using the RBS Calculator and bisabolene synthase codon optimization. The strains were tested for bisabolene production with titers ranging from 0 (non-detect) to 7.9 ± 0.6 mg/L after five days of growth in continuous light. Similar titers were achieved for strains grown in 12hr:12hr light:dark cycles after 10 days. Bisabolene synthase abundance was found to correlate well with bisabolene titer.

P.47. Promoter engineering for applications in pathway engineering in cyanobacteria

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Commercialization of cyanobacteria-based platform chemicals necessitates expansion of synthetic biology toolbox of cyanobacteria1 to improve productivity. Promoters are biological parts that control gene expression and understanding its regulatory elements responsive to abiotic factors will benefit towards optimizing product yield. In this study, two native promoters, cpcB (phycocyanin) and rbc (RuBisCo) of Synechococcus elongatus PCC 7942 were truncated and characterized with respect to their sequence-length, effect of light, CO2 and diurnal rhythm. The promoters exhibited diurnal and circadian rhythm with cpcB showing 2-fold higher strength than rbc under ambient CO2 levels. The cpcB promoter of length 300bp showed highest activity and 200 bp was identified as the minimum region for gene expression. The presence of a negative regulatory region made the promoter sensitive to high light however, the repression was alleviated under high CO2 levels. Therefore, suitable for expressing genes under high light and CO2 conditions. On the other hand, rbc was repressed by high CO2 concentrations except for the minimum active stretch (225bp), demarcating the regulatory region between 225 and 300bp from the start codon of rbcLS. The complete removal of this probable CO2 responsive region made this promoter active under high CO2 without having circadian control. Signifying the importance of this regulatory region for rbc promoter activity under carbon-limited state. This study will help expand the repertoire of synthetic biology tools for pathway engineering in cyanobacteria.

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P.48. Deficiencies in FRL-Apc subunits lead to inability to assemble FRL-bicylindrical cores, reduced accumulation of Chl d, and impaired biogenesis of FRL-PSII

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To utilize far-red light (FRL), cyanobacteria capable of Far-Red Light Photoacclimation (FaRLiP) synthesize new pigments (Chl f and Chl d) and remodel three major photosynthetic complexes FRL-PBP complexes, FRL-PSII and FRL-PSI. In Synechococcus sp. PCC 7335, FaRLiP leads to assembly of bicylindrical core (BC) complexes that uniquely contain five subunits encoded by apcD5, apcB2, apcD2, apcE2, and apcD3 genes in the FaRLiP gene cluster. To explore the functions of the FRL-apc genes, deletion mutations were constructed for each of those five genes in Synechococcus sp. PCC 7335. Segregation of the mutant transformants was achieved with cells grown in white light (WL). As demonstrated by cell growth and absorption spectroscopic measurements and compared to the WT, no effect could be observed in the mutant cells grown under WL. However, the FRL absorption feature beyond 700 nm was significantly reduced in cells grown under FRL for all mutants. The results of low-temperature fluorescence emission spectroscopy demonstrated that deletion of the FRL-apc genes led to a defect in the synthesis of the FRL absorbing pigments and photosynthetic complexes, and mostly diminution in the accumulation of FRL-PBP complexes. Characterization of the isolated phycobiliproteins from mutant cells grown in FRL clearly demonstrated that no FRL-BC cores could be assembled in the mutants in which any FRL-apc gene was deleted. HPLC analyses of the pigment extracts showed that Chl d synthesis was significantly reduced in the mutant cells grown in FRL. The Chl d content of one apcD3 mutant strain was below detection limits. Relevant to the severe reduction in Chl d accumulation and as suggested by the low-temperature fluorescence emission spectroscopy, the assembly of FRL-PSII complexes was also dramatically reduced in all FRL-apc mutant cells grown in FRL.

P.49. BIOCHEMICAL STUDIES ON THE ROLES OF PERIPHERAL PATHWAYS IN PHOTOSYNTHETIC ELECTRON TRANSFER

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In photosynthetic microorganisms, complex biochemical and metabolic pathways balance energy generation and utilization. In order to understand and engineer energy flow within photosynthetic systems, we are investigating the coordination between peripheral redox pathways and photosynthetic electron transport (PET). We aim to resolve the roles of and interactions between pathway components, and determine the mechanisms underlying the coordination of these pathways. Of primary focus are the flavodiiron (Flv) proteins that are essential for catalyzing the oxygen reduction reaction (ORR) necessary for both the protection of photosynthetic reaction centers under changing conditions, and the acclimation of energy flow during transitions to photosynthetic growth. Using multiple Flv1 and Flv3 knockout strains in Synechocystis 6803, we have applied in vivo and in vitro strategies to determine the role of these proteins in regulating electron and photon

flux, with implications for the structure of photosynthetic reaction centers. In addition, recombinantly produced Synechocystis 6803 Flv3 served as a model for investigating the biochemical, kinetic and biophysical properties for catalyzing ORR. Flv3 purified as a homodimer and contained stoichiometric amounts of Fe and flavin following reconstitution. The recombinant Flv3 catalyzed ORR in the presence of either NADH or NADPH, where the reaction kinetics and structural models indicate cooperative kinetics and reactivity that is allosterically controlled. Our recent results provide greater insight into how and why peripheral redox and core photosynthetic pathways coordinate electron and photon flux.

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P.50. Direct conversion of CO2 to Fatty Acid Ethyl Esters (FAEEs) by Engineered Cyanobacteria

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Metabolic engineering of cyanobacteria has received attention as a sustainable strategy to convert carbon dioxide to fatty acid-derived chemicals that are widely used in the food and chemical industries. Herein, Synechococcus elongatus PCC 7942, a model cyanobacterium, was engineered for the first time to produce fatty acid ethyl esters (FAEEs) from CO2. Due to the lack of an endogenous ethanol production pathway and wax ester synthase (AtfA) activity in the wild-type cyanobacterium, we metabolically engineered S. elongatus PCC 7942 by expressing heterologous AtfA and introducing the ethanol pathway, resulting in detectable peaks of FAEEs. To enhance FAEE production, we used two different strategies. AtfA was replaced to the mutated variant wax ester synthase (AtfA-G355I) or a heterologous phosphoketolase pathway was introduced in the FAEE-producing strain to supply acetyl-CoA. Engineered S. elongatus PCC 7942 at first strategy produced FAEEs (2.2 ± 0.7 mg/L/OD730). Subsequent optimization of the cyanobacterial culture with a hexadecane overlay resulted in engineered S. elongatus PCC 7942 that produced photosynthetic FAEEs (10.0 ± 0.7 mg/L/OD730) at second strategy. This study is the first report of photosynthetic production of FAEEs from CO2 in cyanobacteria.

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P.51. CyAn: A cyanobacteria image analysis toolbox

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Many biological measurements, such as cell growth, are carried out at the population level using bulk cultures. However, cells can exhibit non-homogenous responses that are biologically relevant. Time-lapse microscopy has emerged as a powerful tool that enables biologists to observe single cell responses over time. Unfortunately, the task of analyzing individual cells from images is repetitive, time consuming, and can be influenced by individual bias. Here, we describe the development of an automated image analysis toolbox in MATLAB (tentatively called CyAn). The toolbox carries out: (1) cell identification (or segmentation), (2) tracking, and (3) analysis of the data. We show examples of each step, as well as analysis from our recent experiments in tracking single carboxysomes and counting chromosomes in cyanobacteria.

Funding was provided by the U.S. Department of Energy Biological and Environmental Science contract number DE-SC0019306 to J.C.C.

P.52. Synechococcus sp. PCC 7002 Zam is a redox regulated RNA binding protein

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Zam is an RNase II/R family member protein conserved across the cyanobacterial lineage. It was discovered in Synechocystis PCC 6803 as a gene whose deletion provided resistance to the carbonic anhydrase inhibitor acetazolamide. In Synechococcus PCC 7002, deletion of Zam alters pigmentation and causes a mild growth phenotype. How these complex phenotypes are caused in either strain by deletion of Zam is unknown; though, due to its homology to an RNA degradation enzyme, is is assumed Zam is regulating RNA metabolism.

Since cyanobacteria, especially PCC 7002, are a potential chassis for industrial relevant product production, detailing how they function at a molecular level is essential for enabling their successfully engineering. RNA metabolism is a particularly important process to understand because it governs so much of gene expression. By elucidating the mechanism by which Zam regulates RNA metabolism to produce phenotypic

changes, we will gain greater insight into how to control gene expression in cyanobacteria.

This poster shows bioinformatic evidence which allowed us to form hypotheses about the mechanism of function of Zam. It also details our purification strategy and provides preliminary results of in vitro experiments.

P.53. Characterization of salt stress on cyanobacterial photosynthesis

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The effects of NaCl on photosynthetic electron transport were studied in the cyanobacterium Synechocystis PCC 6803 by the combination of variable Chl fluorescence (including single cell level detection by using microfluidic devices) and P700+ absorption change measurements. Our data show that incubation of Synechocystis 6803 cells in the presence of 0.5- 1.0 M NaCl decreases the Fv/Fm value of variable Chl fluorescence and slows down the middle phase of Chl fluorescence relaxation after a short saturating flash, which reflect the limitation of electron transport at the water oxidizing complex, and at the QB plastoquinone binding site of PSII, respectively.

The NaCl effect on PSI electron transport was assessed by P700+ absorption change measurements. The steady-state level of P700+ when illuminated by weak far-red light is decreased in the presence of NaCl, while the relaxation rate of the P700+ in the dark following illumination is accelerated. Electron transport rates through PSII (ETR-II), and PSI (ETR-I) are both inhibited by NaCl. However, ETR-I is affected much less than ETR-II, which shows that PSI is more resistant to NaCl than PSII.

It is concluded that NaCl inhibits electron flow through PSII by acting at both the donor (water-oxidizing complex) and the acceptor side (QB binding site). NaCl also accelerates the delivery of electrons from stromal components to the thylakoid embedded PSI complex most likely via a cyclic electron transport pathway.

Acknowledgement: The work was supported by Hungarian Ministry for National Economy (GINOP-2.3.2-15-2016-00026).

P.54. Genes involved in high light tolerance in the fast-growing cyanobacterium Synechococcus elongatus UTEX 2973

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Cyanobacteria have adapted to grow in a wide range of environments including those with high light intensities found near the equator. There are diverse mechanisms for high light tolerance although many of them are not well understood and others remain to be discovered. The cyanobacterium Synechococcus elongatus UTEX 2973 is closely related to Synechococcus elongatus PCC 7942, yet they have a number of striking phenotypic differences. For instance, UTEX 2973 grows fastest under extreme high light conditions, around 1500 μE·m−2·s−1, whereas PCC 7942 grows fastest at a much lower light intensity of 400 μE·m−2·s−1 ¬[1]. The aim of this project is to identify and understand the genetic factors that are responsible for the extreme high light tolerance of UTEX 2973. To pin point the specific genetic changes that account for the light tolerance differences of the two strains, we screened a collection of mutants generated using CRISPR/Cpf1. To date, we have identified three separate genes that contribute to high light tolerance. Detailed analysis of these genes and their encoded proteins will be presented.

Supported by funding from DOE-BER. PW has been supported by a Bayer Graduate fellowship at Washington University.

1. Ungerer, J., et al., Adjustments to Photosystem Stoichiometry and Electron Transfer Proteins Are Key to the Remarkably Fast Growth of the Cyanobacterium Synechococcus elongatus UTEX 2973. mBio, 2018. 9(1).

P.55. PURIFICATION OF THE NDH-1S AND NDH-1S' COMPLEX USING AN AFFINITY TAG SYSTEM

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The NADPH Dehydrogenase Type 1 (NDH-1) complexes in cyanobacteria are involved in the CO2 concentrating mechanism (CCM), a process which allows cells to provide sufficient amounts of inorganic carbon (Ci) to Rubisco in low Ci conditions. These complexes possess subunits specially designed for high or low affinity CO2 uptake, CupA and B respectively. Deletion of either gene impairs cell growth and CO2 uptake with respect to Ci, but the mechanism by which these proteins function remains unknown. In a recent breakthrough, the 3D structure of the respiratory NDH-1 cyanobacterial complex has been determined by cryo-EM. However, the structure of Cup-containing NDH-1 complexes remains unresolved. In order to address this, a genetic system has been developed for the purification of CupA and B. Systematically tagging the D and F subunits of the NDH-1 complex using His6 or Step affinity tags could be the first step in isolating

complexes containing CupA and B without inhibiting function, allowing for further structural analysis by cryo-EM.

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2. Schuller, J. M., Birrell, J. A., Tanaka, H., Konuma, T., Wulfhorst, H., Cox, N., Nowaczyk, M. M. (2018). Structural adaptations of photosynthetic complex I enable ferredoxin-dependent electron transfer. Science, 363, 257-260.

P.56. Photosynthetic Production of (R)-3-Hydroxybutyrate

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Concerns about CO2 emission from fossil fuels utilization and the environmental pollution from fossil-derived plastic waste are escalating, which calls for the sustainable production and utilization of renewable biodegradable plastics. (R)-3-Hydroxybutyrate (3HB) is the most common building-block for polyhydroxyalkanoates, biodegradable and biocompatible thermoplastics with thermal and mechanical properties comparable to conventional plastics. It also serves as a precursor to a variety of value-added stereospecific chemicals. Although photoautotrohic production of (R)-3HB has been demonstrated using engineered cyanobacteria, higher titer and rate of cyanobacterial 3HB production are critical for commercial applications. Herein, a facile method was used to identify the bottleneck of the cyanobacterial 3HB biosynthesis pathway. Subsequent mitigation of the bottleneck led to dramatic increase of the 3HB productivity and titer, reaching 1.84 g L-1 within 10 days, using CO2 and light as the sole carbon and energy sources. Moreover, 13C-metabolic flux analysis delineated the sequential two-phase metabolism profile in the (R)-3HB-producing cells. During the early growth phase, carbon fluxes primarily contribute to biomass formation, whereas when cells enter the production phase, most of carbon fluxes go to the heterologous pathway, with 68.8% carbon input redirected to (R)-3HB (51.3%) and acetate (17.5%) formation under the investigated experimental conditions.

P.57. Glycogen metabolism jump-starts photosynthesis through the G6P shunt in cyanobacteria

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Cyanobacteria experience drastic changes in their carbon metabolism under daily light-dark cycles. In the light, the Calvin-Benson cycle fixes CO2 and divert excess carbon into glycogen storage. At night, glycogen is degraded to support cellular respiration. Dark-light transition represents a crucial environmental stress for cyanobacteria and other photosynthetic lifeforms. Recent studies in the field revealed the essential genetic background necessary for the fitness of cyanobacteria during diurnal growth. However, the mechanism behind the dark-light transition is not well understood. In this study, we discovered that glycogen metabolism can jump-start photosynthesis in the cyanobacterium Synechococcus elongatus PCC 7942 when photosynthesis reactions start upon light. Compared to the wild type, the glycogen mutant (ΔglgC) showed much lower photosystem II efficiency and slower photosystem I-mediated cyclic electron flow rate when photosynthesis starts. Proteomics analyses indicated that glycogen is degraded through the oxidative pentose phosphate pathway (G6P shunt) during dark-light transition. We also provide evidence indicating that the G6P shunt jump-starts photosynthesis by modulating NADPH levels during the transition period. This ingenious strategy helps jump-start photosynthesis in cyanobacteria following dark respiration, and stabilize the Calvin-Benson cycle under fluctuating environmental conditions. It has evolutionary advantages for the survival of photosynthetic organisms using the Calvin-Benson cycle for carbon fixation.

P.58. Understanding differential natural transformation between sister cyanobacteria Synechococcus 7942 and Synechococcus 2973

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Natural transformation is the process by which bacteria are able to take up and maintain naked DNA. This trait can serve as a valuable tool for genetic manipulation in the laboratory. Although many species of cyanobacteria have been deposited in culture collections, natural transformation has only been demonstrated in a handful of these diverse organisms (1). Two closely related cyanobacterial species provide a unique opportunity to understand natural transformation in cyanobacteria: Synechococcus elongatus PCC 7942 and Synechococcus elongatus UTEX 2973 (2). Although these organisms are 99.9% genetically identical, Synechococcus elongatus PCC 7942 is naturally transformable and Synechococcus elongatus UTEX 2973 cannot undergo natural transformation. Using a CRISPR/Cpf1 genome editing system, individual polymorphisms in Synechococcus elongatus UTEX 2973 were substituted with corresponding sequences in Synechococcus elongatus PCC 7942 (3). Mutant lines were

then assayed for restored natural transformability. Polymorphisms in three genetic loci were found to be key to differential natural transformation between these two species: the promoter for the pilMNOQ operon, which encodes components of the natural transformation pilus; the coding region of the transformation pilus sheath component pilN; and both the promoter and coding region of the global circadian transcriptional regulator RpaA.

(1) K.E. Wendt and H.B. Pakrasi, Frontiers in Microbiology, 2019. (2)J. Yu, et al., Scientific Reports, 2014. (3)J. Ungerer, et al., PNAS, 2018.

Acknowledgements: This study was funded by an NSF INSPIRE grant to HBP. KEW is supported by the William H. Danforth Plant Sciences Fellowship Program at Washington University.

P.59. Mechanical stress affects glycogen storage and pigment expression in *Synechococcus* sp. PCC 7002

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Understanding how mechanical forces impact carbon fixation and photosynthetic flux in carbon limited or light limited environments could allow more efficient engineering of cyanobacterial cells. *Synechococcus* sp. PCC 7002 (PCC 7002) accumulates glycogen as an energy storage molecule. PCC 7002 also contains a light-harvesting apparatus, the phycobilisome, which absorbs light from 500-650nm and can detach its rods as a photoprotective mechanism to prevent the absorption of too much light. Research on how mechanical forces influence glycogen storage and photoprotective mechanisms is relatively new. With single-celled microscopy indicating that mechanical forces play a role in regulating photosynthesis, it is possible that mechanical forces also regulate metabolism. PCC 7002 cultured in 2.5% agar, 3.0% CO2 initially decreases in absorbance at 634nm, normalized to OD730, from 0h to 24h, and then increases in absorbance from 24h to 96h, whereas PCC 7002 cultured in 0.5% agar, 3.0% CO2 decreases in absorbance at 634nm, normalized to OD730, from 0 to 96h. The increase in absorbance in the 2.5% agar condition could be due to a photoprotective mechanism with uncoupled phycobilisomes. Furthermore, PCC 7002 stores more glycogen, normalized to OD730, when cultured in 0.5% agar than in 2.5% agar, and more glycogen in 0.5% and 2.5% agar than in flask cultures.

P.60. PathParser, a computational toolbox for thermodynamics and kinetics analysis of metabolic pathways

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Metabolic engineering is essential in addressing global energy and environmental challenges via biotechnology. However most engineering efforts consist of trial-and-error in test tubes, and suffer from low productivity in part due to the lack of computational tools that help assess pathway properties based on thermodynamics and kinetics analysis. Therefore pathway design can benefit from computational tools that assess feasibility, cost and stability of the non-native metabolic pathways; such tools can also help us understand natural pathways and their regulation. Here we developed a computational toolbox, PathParser, which integrates the function of thermodynamics analysis, protein cost optimization and robustness analysis for both natural and synthetic pathways. Specifically, PathParser can minimize the free Gibbs energy of the least thermodynamically favorable reaction to optimize the driving force of a pathway, estimate the optimal enzyme protein cost for metabolic flux, and evaluate system response to enzyme expression perturbations. The toolbox was demonstrated with the Calvin–Benson–Bassham cycle and photorespiration pathway in the cyanobacterium *Synechocystis* PCC 6803 and validated with proteomics data. This toolbox may have broad applications in biotechnology.

P.61. Circadian rhythms in Synechococcus sp. strain PCC 7002

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Circadian clocks are self-sustained intracellular oscillators that underlie daily rhythms of sleep/waking, metabolic activity, gene expression, and many other biological processes. Circadian rhythms have three diagnostic characteristics, i.e. persistence in constant conditions, entrainment, and temperature compensation. Synechococcus elongatus PCC 7942 is the first prokaryotic organism known to exhibit circadian rhythms which have been well studied and documented. The kaiA, kaiB and kaiC genes encode the core components of the cyanobacterial circadian clock in S. elongatus PCC 7942. The unicellular, euryhaline cyanobacterium Synechococcus sp. strain PCC 7002 is a model organism for studies of cyanobacterial metabolism and biotechnological applications. Synechococcus sp. strain PCC 7002 also has a kaiABC gene cluster as in the strain PCC 7942, but it has one more copy of kaiB gene (named kaiB2). To test if Synechococcus sp. strain PCC 7002 shows circadian rhythmicity, we have generated two kinds of bacterial luxAB luminescence reporters. The first reporter uses the promoter from the

kaiB1 gene of the kaiABC cluster, i.e. kaiB1p::luxAB. The other reporter uses the promoter from the class I photosynthetic gene psbAI, i.e. psbAIp::luxAB. Both reporter strains exhibited robust circadian rhythms in PCC 7002 under constant light conditions. The kaiB1 promoter activities are much lower than those of the psbAI promoter. Moreover, the circadian rhythms of PCC 7002 showed longer circadian periods and different phase angles relative to those in PCC 7942 at 30°C. The responses of the clock reporters to different temperatures were also examined in Synechococcus sp. PCC 7002.

P.62. Genetic modification of the fast-growing nitrogen-fixing cyanobacterium Anabaena sp. 33047

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Anabaena sp. 33047 is the fastest-growing nitrogen-fixing cyanobacterium with a doubling time as short as 3.8 hours, making it a potential candidate for biological factory to produce biochemicals. However, genetic modifications of this strain was not previously possible, making it difficult to use it in synthetic biology studies. A potential barrier to gene transfer in this strain appeared to be the presence of an elaborate host restriction modification system. Filamentous cyanobacteria are known to harbor multiple sequence-specific type II restriction endonucleases. We anticipated that methylase or methyltransferase may protect exogenous DNA from degradation by the host. Therefore, we mined the genome sequence of Anabaena 33047 for genes for methylases in its restriction modification system. We targeted on several methylase or methyltransferase genes that are associated with a type II restriction system and selected five of them to be cloned into a helper plasmid. Use of this newly constructed helper plasmid in conjugation allowed facile targeted deletions of 4 genes in pathways involved in heterocyst differentiation, nitrogen assimilation, PBS degradation and other important processes, respectively. Initially, we used conventional homologous recombination plasmids to generate targeted mutant strains. Remarkably, even in the first generation, most, if not all of the colonies tested were double recombinant mutants, which is uncommon in other filamentous cyanobacteria. In the next step we plan to use CRISPR/Cpf1 system in this strain for scarless genome editing. These results suggest the feasibility of genetic modification and exploration of important processes in this amazing diazotrophic Anabaena strain.

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P.63. Constitutive Oxygen-Tolerant Nitrogenase Activity In Unicellular Cyanobacteria

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Cyanobacteria have played a profound role in shaping the biosphere, most notably through the Great Oxygenation Event (GOE) with the advent of photosynthesis. Cyanobacteria also contribute to global primary production through biological nitrogen fixation using nitrogenase, an oxygen-labile enzyme complex that evolutionarily predates the GOE. Current literature reports nitrogenase activity in unicellular cyanobacteria is protected from oxygen through diurnal separation from oxygenic photosynthesis. However, historic conditions of continuous-illumination and warm temperature at polar latitudes during the Triassic and Cretaceous may have created a selective advantage amongst unicellular cyanobacteria for non-temporal mechanisms of maintaining nitrogenase activity in the presence of oxygen. Here we report constitutive nitrogenase activity concurrent with a net-gain of oxygen through photosynthesis in a continuous-illumination adapted culture of the unicellular cyanobacteria, Cyanothece ATCC 51142. Nitrogenase activity in the adapted culture exhibited dependence on illumination and increased resilience to artificially raised oxygen-tension compared to a 12-hour light/12-hour dark cycle culture. We predict unicellular diazotrophic cyanobacteria closely related to Cyanothece ATCC 51142 also possess this physiology and we seek to understand how they do so by using genomic, transcriptomic and proteomic approaches. This work provides a model of oxygen-stable nitrogenase activity and suggests this physiology may be conserved in closely related unicellular diazotrophic cyanobacteria with implications for primary production in polar ecosystems and potential biotechnological application in sustainable agriculture.

P.64. Quantitative insights into the cyanobacterial cell economy

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Despite the importance of cyanobacteria as photosynthetic model organisms and as host organisms for green biotechnology, as yet only few studies have addressed quantitative growth properties and resource allocation even for well characterized model strains. Here,

we provide a quantitative analysis of light-limited, light-saturated, and light-inhibited growth of the cyanobacterium Synechocystis sp. PCC 6803 using a reproducible cultivation setup. We focused on light as the only variable environmental parameter – and identified trends in key physiological parameters, including growth rate, cell size, photosynthetic activity as well as proteome allocation as a function of growth rate. The interpretation of data was facilitated by a coarse-grained computational model of cyanobacterial resource allocation. Overall, the resulting growth laws (decrease of proteome fraction associated with light harvesting and increase of proteome fraction associated with translation with increasing light intensity and growth rate) are in good agreement with previous theoretical and experimental studies, whereas the observed invariance of the proteome fraction associated with metabolic processes differed from model predictions. Light, however, is not the only factor that affects photoautotrophic growth. Further studies are required to identify growth limitation under different environmental conditions, in particular limitations induced by other biotechnologically or environmentally relevant macro- or micronutrients. The proposed reproducible cultivation setup and the coarse-grained computational model used in this study provide a suitable framework and reference to facilitate and to contribute to such studies.

P.65. Inter-species competitions of cyanobacteria indicate fitness advantages conferred by circadian clocks

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Cyanobacteria are the only known bacteria that have been documented to have self-sustained circadian clocks. Previous intra-species competition research showed that for the cyanobacterium Synechococcus elongatus PCC 7942 (a model circadian clock organism), clock-disrupted strains can be out-competed in mixed cultures by strains with a functioning clock when cultured under conditions with 24 h cycles of light and dark. We are now testing inter-species competition systems among cyanobacteria using wild-type and several clock deficient strains of Synechococcus elongatus PCC 7942 versus Synechocystis sp. PCC 6803. Our goal is to study how circadian clock properties may be involved in conferring fitness advantages when competing against other species. We also employ metabolomic assays to discern the metabolic status of the cyanobacteria when they are competing with other species.

P.66. Construction of non-resistant screening gene expression platform in cyanobacterium Synechococcus sp. PCC 7002

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Marine cyanobacteria Synechococcus sp. PCC 7002 has the potential to become an important platform for producing chemical compounds through photosynthesis. In this work, we constructed an integrative vector, the pAQ3-Ex, for integration of foreign DNA cassettes with psbC gene rather than resistance genes as a selectable marker into neutral site located on the endogenous plasmids pAQ3 of this organism. By using green florescent protein (GFP) as the reporter for studying the parameters of this system, we screened positive strains in ordinary A-plus medium and found that the GFP was successfully expressed in positive strains. Then using this system, we expressed the ethylene-forming enzyme which is necessary for ethylene production by this single gene from common metabolites under several different promoters. The ethylene was successfully produced steadily. The production rate increased as cell cultures density increased. And the strain expressed under the chimeric psbA* promoter reached 4203 μl L-1 h-1 in the cell cultures density of OD730 2.4 while its grown rate with doubling times 4.9 h was almost the same as the wild type with doubling time 4.4 h. These results indicate that this non-resistant gene expression system was effective and suitable for foreign gene expression.

P.67. A Hybrid Histidine Kinase Initiates Hormogonium Development in the Filamentous Cyanobacterium Nostoc punctiforme

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Filamentous cyanobacteria display remarkable developmental complexity, particularly those in taxonomic subsections IV and V, which are capable of differentiating four distinct types of cells or filaments: vegetative cells, akinetes, heterocysts, and hormogonia. Hormogonia are motile filaments responsible for movement across solid surfaces and aid in establishing nitrogen-fixing symbioses with plants and fungi, but the genes regulating hormogonium development are largely unknown. A recent study, using the model filamentous cyanobacterium Nostoc punctiforme, which retains the full range of developmental complexity exhibited in wild isolates, found that a trio of sigma factors (sigJ, sigC, sigF) are essential for hormogonium development, with sigJ being the earliest acting of the three in the gene regulatory network (GRN). To further expand the GRN, additional components are being identified using transposon mutagenesis. One gene identified from this genetic screen was an orphan hybrid histidine kinase (Npun_R3825). This mutant was non-motile, did not differentiate into hormogonia, and lacked hormogonia specific proteins and polysaccharide. This phenotype implies that Npun_R3825 is an

early acting gene in the GRN, possibly upstream of sigJ. To further define its role in the network, RT-qPCR was used to determine how the expression of the hormogonium-specific sigma factors and their downstream targets were affected in the Npun_R3825-deletion strain. Deletion of Npun_R3825 significantly decreased transcript levels for all of these genes, indicating that Npun_R3825 acts upstream of the sigma factor cascade, and therefore at a very early point in the hormogonium GRN.

Abstract Addendum

*Added after the first “digital printing” of the abstract book

P.68. Identification of the siderophore biosynthesis operon of Synechococcus sp. PCC 7002

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Synechococcus sp. PCC 7002 produces siderophores, low-molecular weight molecules with high affinity for iron. Sequence information suggests a pathway for synechobactin biosynthesis, the siderophore produced by Synechococcus 7002. Two of the genes in the prospective siderophore operon, coding for a transporter (sidF) and an esterase/lipase (sidH) were genetically deleted. The resulting deletion strains were characterized by growth studies and light absorption characteristics. Compared to the wild type, deletion of ΔsidH strain results in increased spread of siderophore within agar, which was assessed by an in vivo assay. The results from ΔSidH studies support the hypothesized role of sidH in attaching fatty acid tails on synechobactin, anchoring the siderophores in the cell membrane. The ΔSidF strain has a phenotype similar to ΔSidH, most likely cause by disrupting the operon. Characterization of physiological response of wild type, SidF and SidH deletion strains to iron limitation indicate that the operon containing the genes for siderophore biosynthesis has been identified.

P.69. Creating a predictive metabolic model of photoautotrophic metabolism in cyanobacteria to improve bioengineering design

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The model cyanobacterium Synechococcus elongatus PCC 7942 is a proven photosynthetic organism for metabolic engineering and for investigating circadian regulation. A collaborative effort between the S. Golden and B. Palsson laboratories has produced a genome-scale metabolic model (GEM) of S. elongatus. This work a computational representation of obligate phototrophic metabolism that effectively models photon absorption and accounts for self-shading, improved gene annotations, and produced a powerful resource that allows researchers to better exploit cyanobacterial metabolism and test bioengineering principles.

A subsequent challenge is the need to simulate diurnal growth and the influence of the circadian clock on metabolite partitioning in order to use the model to realize higher bioproduct yields in outdoor cultivation strategies. Thus, we identified genes that are specifically necessary in a day-night environment by screening a dense bar-coded transposon library in both continuous light and diurnal growth conditions, comparing the fitness consequences of loss of each nonessential gene in the genome. In addition, we performed quantitative metabolomics time-course sampling spanning the day-night transitions to extend the modeling framework to the inherently non-steady state dynamics of diurnal growth. By combining time-course cell physiology data and metabolomics to characterize changes in resource allocation and metabolite pooling, respectively, such dynamics become in-scope for genome-scale modeling. By integrating both biomass and metabolite dynamics into a genome-scale model to simulate non-steady state processes, we hope to answer fundamental questions regarding regulation, photoperiod adaptation, and photoprotective mechanisms, while generating a distributable modeling platform that will enhance engineering of this important organism.

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P26 David M. Kehoe
O13 Cheryl A. Kerfeld
P34 Cheryl A Kerfeld
P23 Young-Saeng Kim
P10 Paul King
P49 Paul W. King
O12 Diana Kirilovsky
O33 Kirilovsky Diana.
O35 **Stephan Klähn**
P27 C. F. Klitzke
P33 Tuomas P.J. Knowles
P53 Sandeesha Kodru
P44 Raga Krishnakumar
P31 **Kateryna Kukil**
O34 Benjamin LaFrance
P68 Rahmi Lale
O30 **Vinson Lam**
P2 Zoephia Laughlin
P32 **Alicia Layer**
O19 David Lea-Smith
O4 David Lea-Smith
P33 **David Lea-Smith**
O13 Sigal Lechno-Yossef
P34 **Sigal Lechno-Yossef**
P24 Lee K
P35 **Hyun Jeong Lee**
P35 Mieun Lee
P50 Hyun Jeong Lee
O12 Claire Lemaire
O8 Shuqin Li
O9 Yanbing Li
O9 Xiying Li
P66 Xiying Li
O27 Kathryn Lilley
O32 **Steeve Lima**
P31 Pia Lindberg
P43 Pia Lindberg
P36 **Usha Lingappa**
P19 Deng Liu
P27 H. Liu

P27 T.M. Lohman
P30 Colin S. Long
P64 Cristina Loureiro
P10 Carolyn E. Lubner
P49 Carolyn E. Lubner
P23 Amy T. Ma
P3 Swati Madhu
P24 Maduwanthi N
P36 John Magyar
P60 PinChing Maness
O28 Costas D. Maranas
O13 **Maria Agustina Dominguez Martin**
O4 Alistair J. McCormick
P13 Briana M. McKnight
O26 Kanika Mehta
O10 Jenna Melanson
P9 Anastasios Melis
P34 Matthew R Melnicki
O23 **Neil T Miller**
P55 Neil Miller
O27 Lauren Mills
O7 Minton N
P25 Nigel Minton
P36 Danielle Monteverde
O24 Beronda Montgomery
P32 Beronda Montgomery
P34 Beronda L Montgomery
O13 Beronda L. Montgomery
P37 **Kristin A. Moore**
O22 **José Ángel Moreno-Cabezuelo**
P57 Rachael M. Morgan-Kiss
P10 David W. Mulder
P49 David W. Mulder
P33 Thomas Muller
P38 **María del Carmen Muñoz-Marín**
O10 Cole Murphy
O19 Dorota Mut-Pavlak
O33 **Muzzopappa Fernando**
O26 Monalisha Nayak
P30 Matthew E. Nelson
P26 Adam Nguyen
P29 Adam Nguyen
O34 **Robert Nichols**
P19 Xiaoqun Nie
P27 D. M. Niedzwiedzki
P28 David Nielsen
P30 Philip Nolan
O32 Paulo Oliveira
O28 Himadri B. Pakrasi
O31 Himadri B. Pakrasi
P54 Himadri B. Pakrasi
P58 Himadri B. Pakrasi
P62 Himadri B. Pakrasi
P69 Bernhard O. Palsson
P24 Parsons B
P29 Frédéric Partensky
P2 Ria Patel
P18 **Falguni Paul**
P25 Nicole Pearcy
O14 **Christie Peebles**
P46 Dr. Christie AM Peebles
P11 Graham Peers
P41 Graham Peers
P61 Brian F. Pfleger
O31 Ambarish Phadnavis
O34 Naiya Philips
P24 Pilania G
P29 Suman Pokhrel
O13 Tomas Polivka
O16 Robert Pomeroy
P40 **Matthew Pope**
P64 Gereon Poschmann
O14 Ashok Prasad
O26 Charulata Prasannan
P39 Charulata B. Prasannan
O16 Kim Prather
O5 **Yeyan Qiu**
P5 Jishen Qiu
O1 **Carlos Quiroz-Arita**

P41 **Carlos Quiroz-Arita**
P33 Clayton Rabideau
O1 Kenneth F. Reardon
P42 **Douglas D. Risser**
P67 Douglas Risser
P43 **João Rodrigues**
O24 **Brandon Rohnke**
P34 Brandon A Rohnke
P44 **Anne M. Ruffing**
P33 Kadi L. Saar
P7 Sarah Sacco
P26 Joseph Sanfilippo
P45 **María Santos-Merino**
O16 Jon Sauer
O34 David Savage
O4 **Alejandra A. Schiavon**
O21 Wendy Schluchter
P26 Wendy M. Schluchter
P29 Wendy M. Schluchter
P46 **Jacob Sebesta**
P30 Andrew Sellin
O2 **Annesha Sengupta**
O31 Annesha Sengupta
O31 Shinjinee Sengupta
P15 Annesha Sengupta
P4 Dr. Shinjinee Sengupta
P4 Ms. Annesha Sengupta
P47 **Annesha Sengupta**
O12 Pierre Sétif
K2 Kiyan Shabestary
P41 Sybil Sharvelle
P41 John J. Sheehan
O11 Gaozhong Shen
P11 Chen Shen
P48 **Gaozhong Shen**
P65 Stacy Sherrod
P57 Shrameeta Shinde
P27 M. Shinn
O5 Jagdeep Sidhu
P12 Sang Jun Sim
P50 Sang Jun Sim
O16 **Ryan Simkovsky**
P64 Maria Sinetova
P57 Sonali P. Singapuri
P44 Chuck R. Smallwood
P10 Sharon Smolinski
P49 **Sharon Smolinski**
O2 Sujata V. Sohoni
O31 Sujata Sohoni
P47 Sujata V. Sohoni
O12 Daniel Solymosi
O19 **Daniel Solymosi**
P35 Ji Gyeong Son
P50 **Jigyeong Son**
O11 **Nathan Soulier**
P48 Nathan Soulier
O26 Sanjeeva Srivastava
P3 Vaibhav Srivastava
P5 Wil Srubar
P64 Ralf Steuer
O27 Tim Stevens
P64 Kai Stühler
O2 Avinash Vellore Sunder
P47 Avinash Vellore Sunder
O13 Markus Sutter
O32 Paula Tamagnini
O28 Yinjie J. Tang
P23 Arnaud Taton
O18 Jian Wei Tay
P37 Jian Wei Tay
P51 **Jian Wei Tay**
O15 **Teresa Thiel**
P52 **Patrick Thomas**
O5 Shengni Tian
P23 Brian P. Tieu
P10 Monika Tokmina-Lukaszewska
O5 Trevor Van Den Top
P16 **Trevor Van Den Top**
P23 Kevin P. Trieu
O10 Brooke Turner

P48 Gavin Turner
O28 Justin Ungerer
P58 Justin Ungerer
P36 Joan Valentine
P53 **Imre Vass**
O4 Ravendran Vasudevan
P25 Sophie Vaud
O8 **Wim Vermaas**
O30 Elizabeth Villa
P68 Anne Vogel
P54 **Patricia Walker**
P55 **Ross Walker**
O12 Qiang Wang
O20 **Bo Wang**
O4 Baojun Wang
P56 **Bo Wang**
P57 **Xin Wang**
P60 Xin Wang
O2 Pramod P. Wangikar
O26 **Pramod P. Wangikar**
O31 Pramod P. Wangikar
P15 Pramod P. Wangikar
P3 Pramod P. Wangikar
P39 **Pramod P Wangikar**
P4 Pramod P. Wangikar
P47 Pramod P. Wangikar
O9 Peijun Wei
P27 D. A. Weisz
P69 **David G. Welkie**
P58 **Kristen E. Wendt**
O14 Allison Werner
P32 Melissa Whitaker
P5 Sarah L. Williams
O33 Wilson Adjele
P59 **Kevin Winkler**
P12 Han Min Woo
P35 Han Min Woo
P50 Han Min Woo
P60 **Chao Wu**
P60 Wei Xiong
O10 Kui Xu
P61 **Yao Xu**
P7 Yao Xu
K3 **Chen Yang**
P19 Chen Yang
K2 Lun Yao
P62 **Zi Ye**
P61 Jamey D. Young
P63 **James Young**
P7 Jamey D. Young
O20 Jianping Yu
P60 Jianping Yu
P64 **Tomáš Zavřel**
O12 Jiao Zhan
O9 Kun Zhang
P19 Hao Zhang
P57 Xiaohui Zhang
O9 Ning Gao Jingdong Zhao
P65 **Chi Zhao**
P66 Jindong Zhao
O9 Xiaoyu Zheng
P66 **Zhenggao Zheng**
O5 Ruanbao Zhou
P16 Ruanbao Zhou
P21 Dr. Ruanbao Zhou
P63 Ruanbao Zhou
P64 Anna Zorina
P67 **Esthefani Zuniga**

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Local Restaurant Guide

Links:
https://www.boulderdowntown.com/dining

Downtown Boulder:
Contemporary/Fine Dining

Amu Sake Bar and Restaurant
1221 Spruce St

Arcana Restaurant
909 Walnut St

Black Cat
1964 13th St

Boulder ChopHouse & Tavern
921 Walnut St

Boulder Dushanbe Teahouse
1770 13th St

Brasserie Ten Ten
1011 Walnut St

Corrida
1023 Walnut St

Frasca Food & Wine
1738 Pearl St

Jax Fish House
928 Pearl St

Jill's Restaurant & Bistro at St Julien Hotel and Spa
900 Walnut St

The Kitchen
1039 Pearl St

Leaf Vegetarian Restaurant
1710 Pearl St

Mateo
1837 Pearl St

The Mediterranean
1002 Walnut St

OAK at fourteenth
1400 Pearl St

PMG
2018 10th St

Riffs Urban Fare
1115 Pearl St

SALT
1047 Pearl St

Spruce Farm & Fish
2115 13th St

Steakhouse No. 316
1922 13th St

Upstairs
1039 Pearl St

Wild Standard
1043 Pearl St