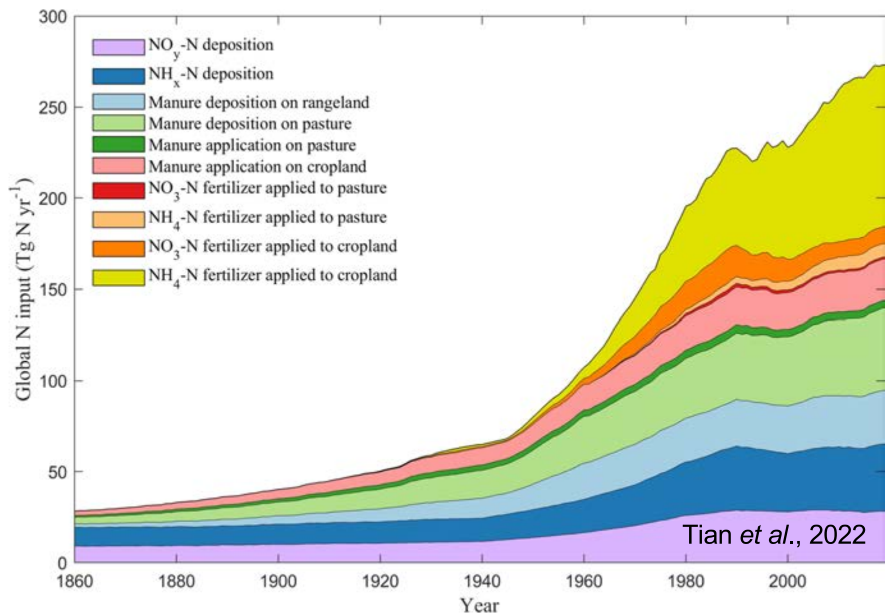
A Day and a Night in the Life of a Photosynthetic Diazotroph

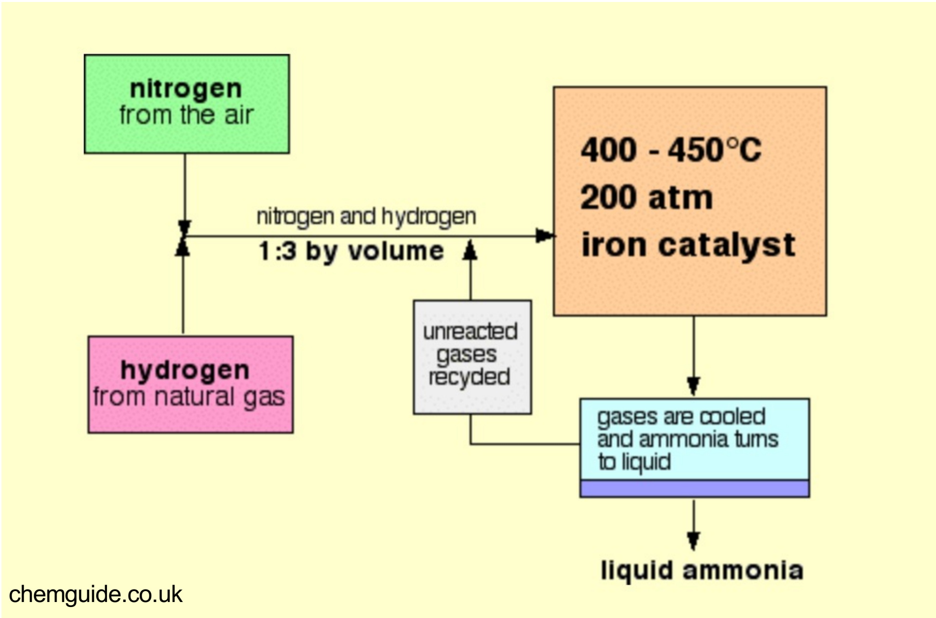
Himadri Pakrasi

Nitrogen is essential for life



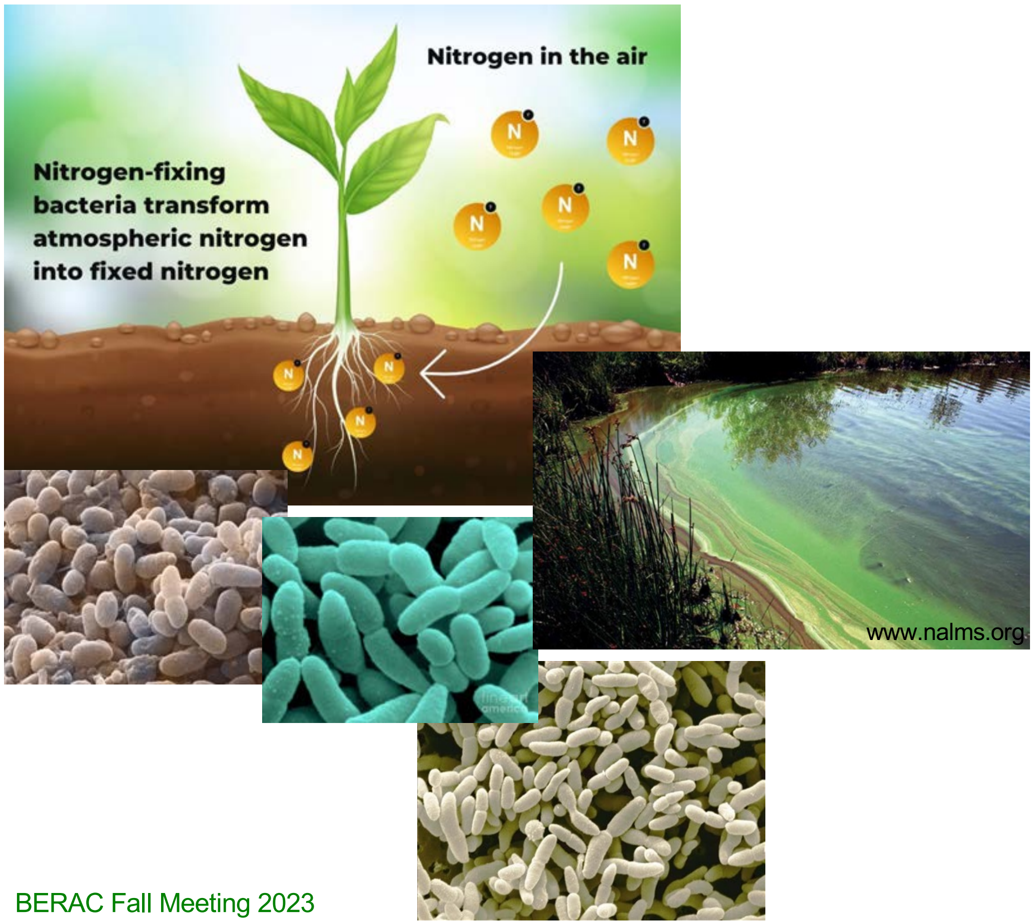
- Global nitrogen demand continues to increase
- Atmospheric nitrogen needs to be reduced (fixed) to be bioavailable

Industrial nitrogen fixation is energy intensive and environmentally unfriendly



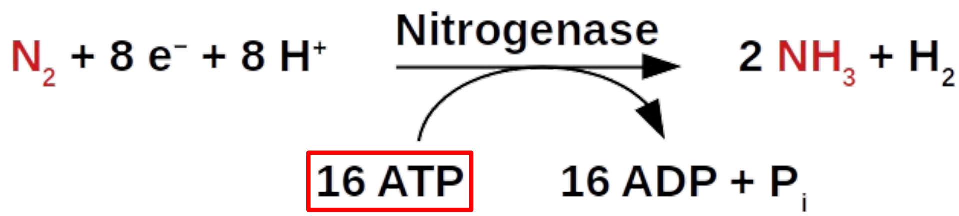
- Currently, the major source of reduced nitrogen is the Haber-Bosch process
- The reaction has a massive carbon footprint and accounts for 1.2% of global annual CO_2 emission (2.8 tons of CO_2 for every ton of NH_3)

Biological nitrogen fixation-a greener alternative



- Only prokaryotes can fix N_2
- Catalyzed by the nitrogenase enzyme

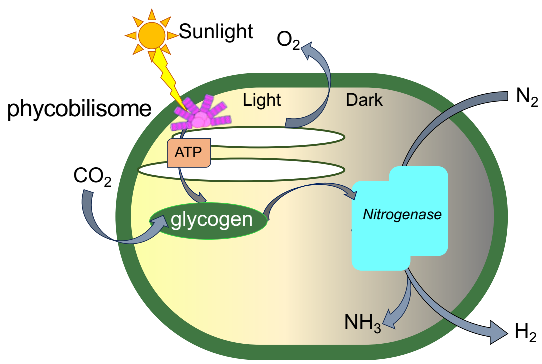
Biological nitrogen fixation

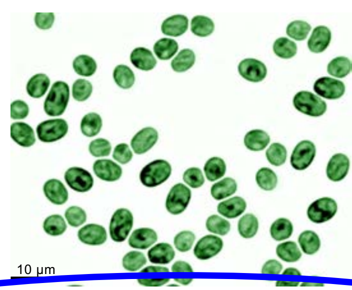


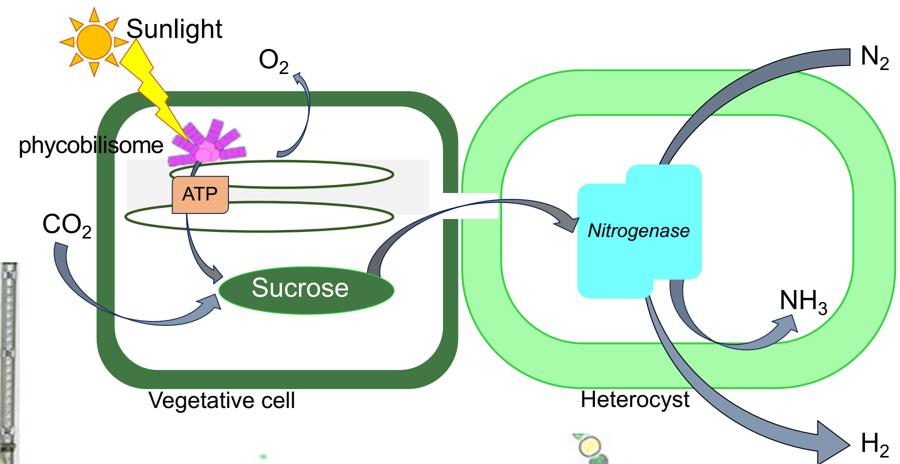
- Energy intensive process
- Therefore, fueling N_2 fixation with solar energy is an attractive paradigm
- Diazotrophic cyanobacteria can utilize solar energy to fix nitrogen

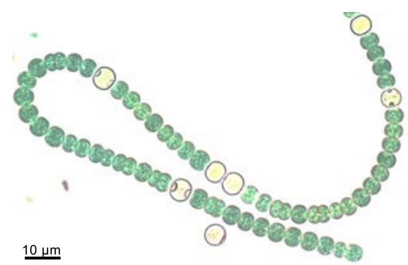
Two Strategies to protect nitrogenase in cyanobacteria

Nitrogenase is highly sensitive to O_2





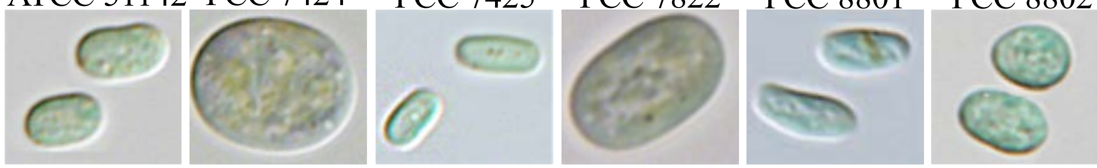




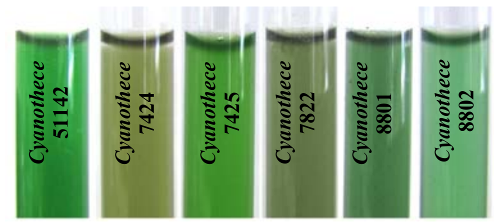
Unicellular - temporal separation

Filamentous - spatial separation

Cyanothece is a diverse group of unicellular diazotrophic cyanobacteria



Cyanothece Strain	ATCC 51142	PCC 7424	PCC 7425	PCC 7822	PCC 8801	PCC 8802
Cell size	4-5μm	7-8μm	3-4μm	8-10μm	4-5μm	4-5μm
Isolated from	Port Aransas, Texas	Rice field, Senegal	Rice field, Senegal	Rice field, India	Rice field, Taiwan	Rice field, Taiwan
Linear chromosomes	1	0	0	3	0	0

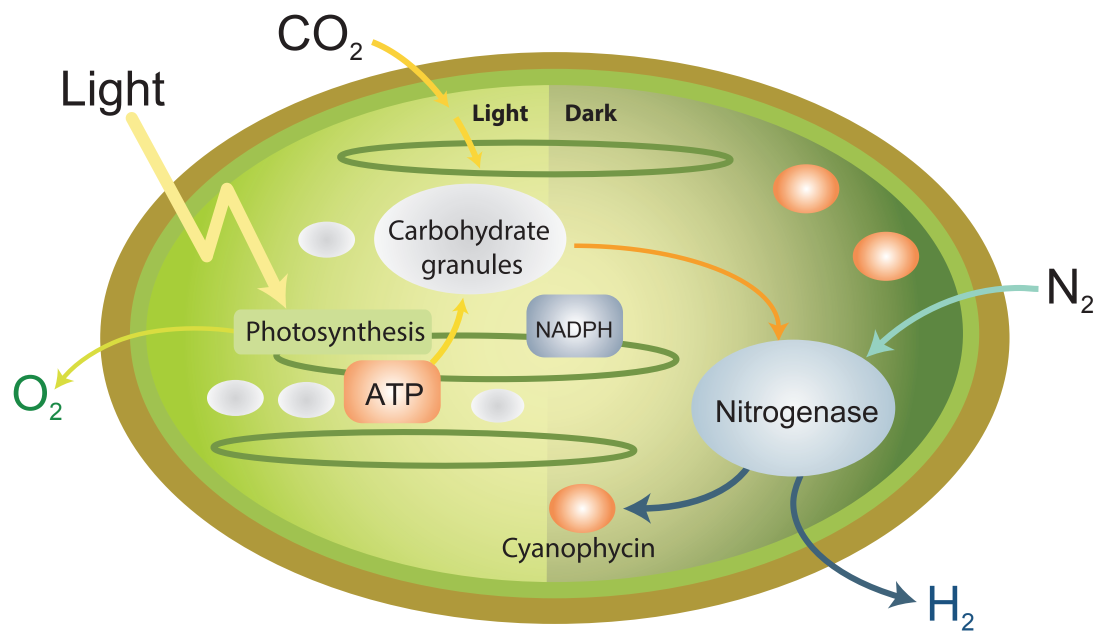


- Present in diverse environments (freshwater, intertidal habitat, open ocean).
- Close relatives are recognized for their contributions to nitrogen fixation in the oceans (Zehr et al., *Nature* 2001)
- Robust circadian rhythm

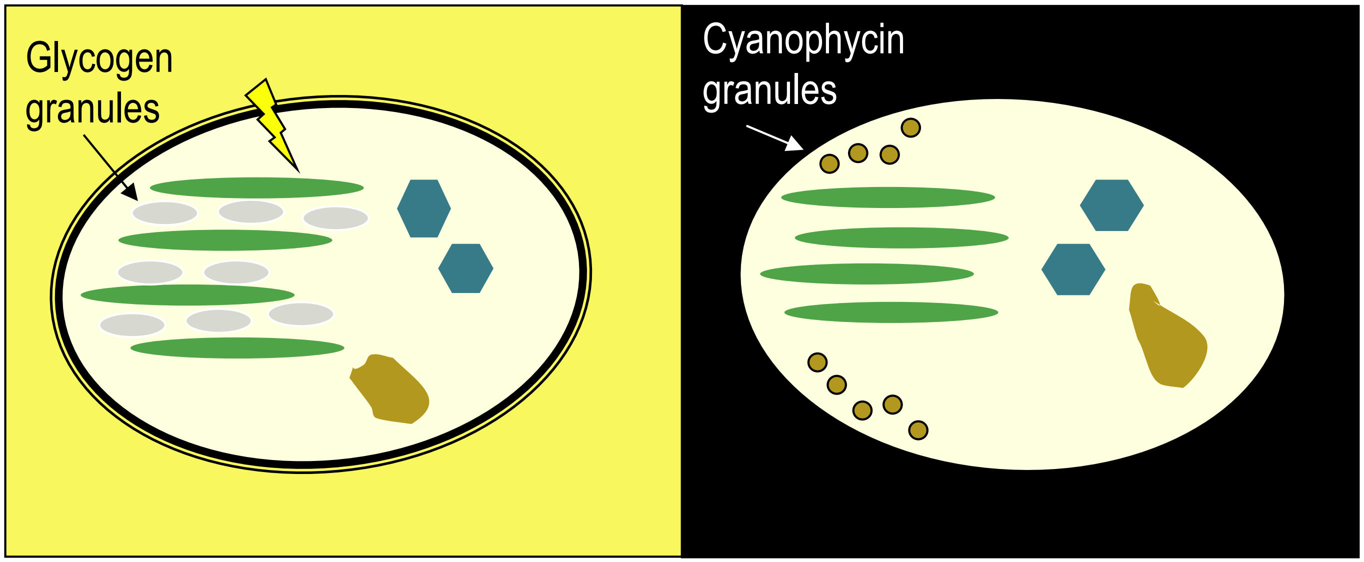
Bandyopadhyay et al., mBio, 2011

Cyanothece sp. ATCC 51142 is a unicellular diazotrophic cyanobacterium

- Extensively studied, exhibits robust growth and high light tolerance, genetically transformable (Liberton et al., 2019)
- Performs oxygenic photosynthesis and nitrogen fixation in the same cell
- Assimilates and stores metabolic products in inclusion bodies



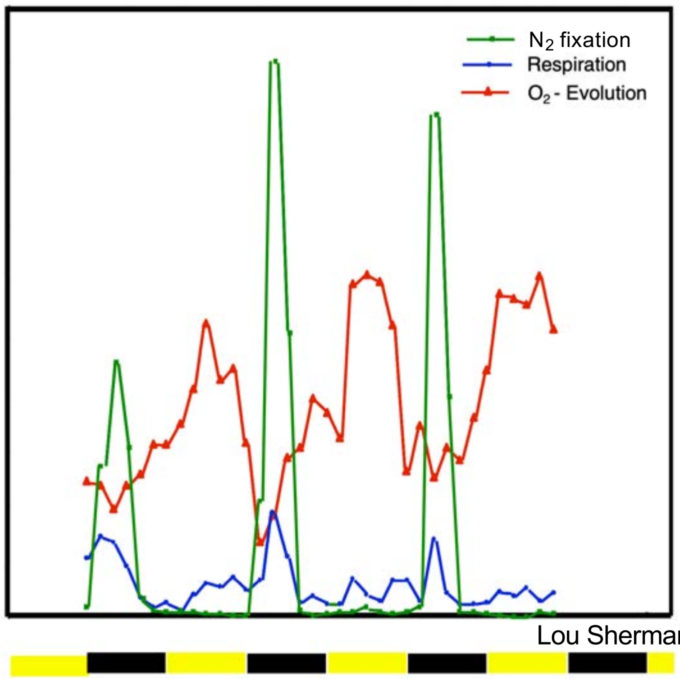
Cyanothece 51142 diurnal cycle



Photosynthesis | Nitrogen Fixation

Photosynthesis and nitrogen fixation are separated *temporally* in the same cell

Day and night in *Cyanothece*



Lou Sherman

- Photosynthesis results in O_2 production during the day.
- High rates of respiration generate low intracellular oxygen tension during early night hours.
- Oxygen-sensitive enzymes such as nitrogenase can then function in the same cell.

Systems–level analysis of *Cyanothece* 51142

- An omics-based knowledgebase is essential to develop diazotrophic cyanobacteria as synthetic biology production chassis
- Over the past years, we have used systems approaches to understand the metabolic blueprint of *Cyanothece* 51142 under diverse environmental conditions

Systems–level analysis of *Cyanothece* 51142

EMSL Grand Challenge in Membrane Biology

Washington University in St. Louis

DONALD DANFORTH PLANT SCIENCE CENTER

NCMIR NATIONAL CENTER FOR MICROSCOPY AND IMAGING RESEARCH

Pacific Northwest National Laboratory
Operated by Battelle for the U.S. Department of Energy

Richard Smith

- Ultrastructure
- Genomics
- Transcriptomics
- Proteomics
- Metabolic profiling
- Structural biology
- Physiology

Dave Koppenaal

Lou Sherman

William R. Wiley EMSL Environmental Molecular Sciences Laboratory

PURDUE UNIVERSITY

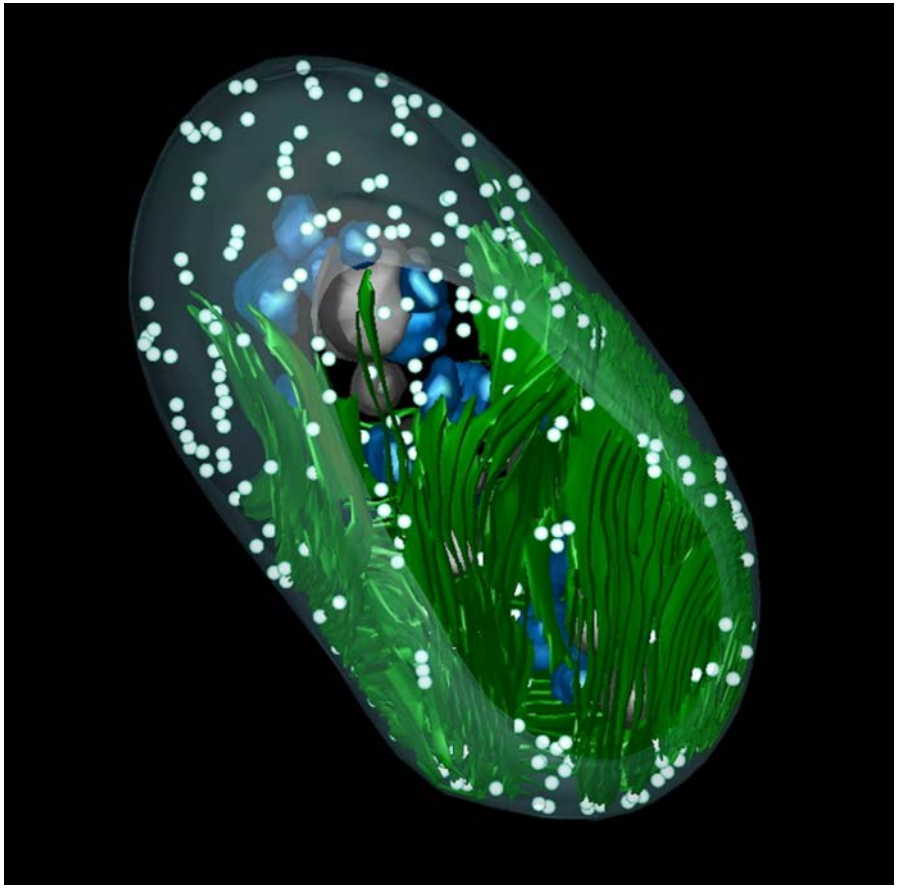
SAINT LOUIS UNIVERSITY

Office of Science U.S. DEPARTMENT OF ENERGY

SIPPE Institute of Plant Physiology and Ecology, SIBS, CAS

Modeling cellular architecture of *Cyanothece*

- Quantitate components on whole cell level
  - 21 carboxysomes, 250-600 nm
  - ~60 polyphosphate bodies, 50-700 nm
  - ~250 lipid bodies, 60-100 nm
- New details of thylakoid membrane system
  - occupies ~15% of cellular volume
  - Single system with unique morphology
- Tomograms from light/dark timepoints showed that some components appear stable (thylakoids, carboxysomes, lipid bodies, polyphosphate), while others oscillate (carbohydrate, cyanophycin)

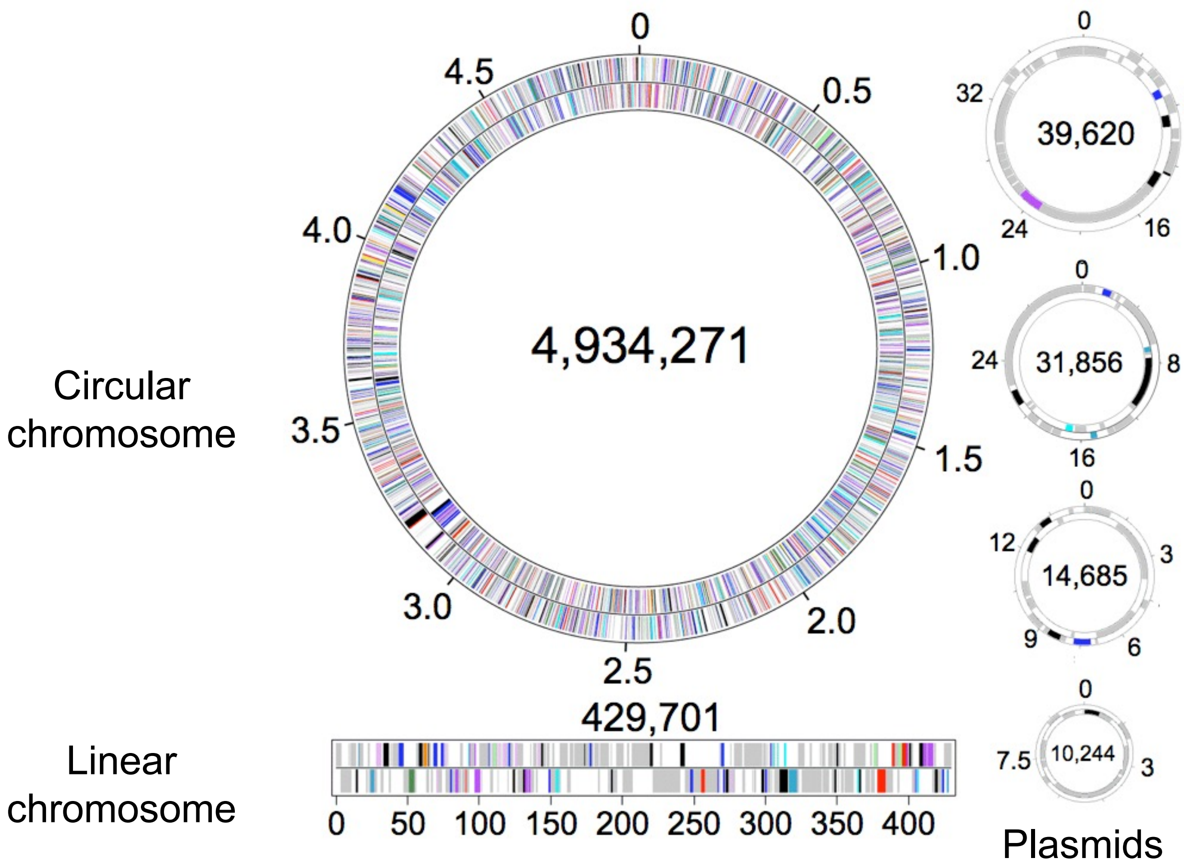


Michelle Liberton

Green = Thylakoid membranes; White = Lipid bodies; Blue = Carboxysomes; Gray =Polyphosphate

Liberton et al., Plant Physiol 2011

Cyanothece 51142 genome

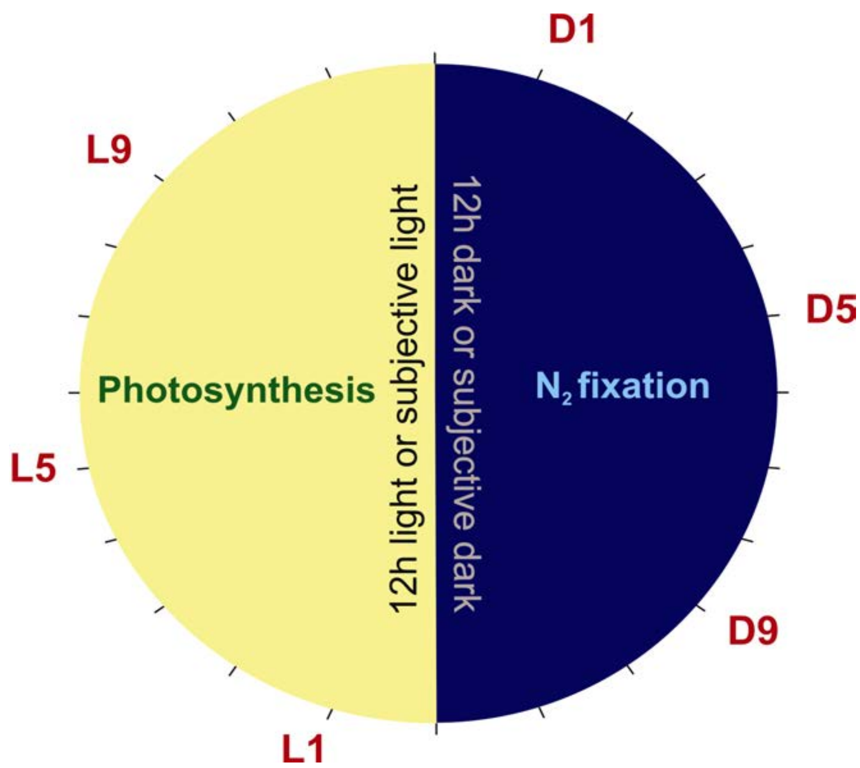


- 5.46 Mb
- 5304 predicted ORFs
- 2735 genes assigned to functional categories
- 0.43 Mb linear chromosome

Eric Welsh

Welsh et al., PNAS 2008

Transcriptomics Experimental Design



Growth conditions

12h dark/12h light
minus-nitrate

Time course

sampling over 48h

Time points

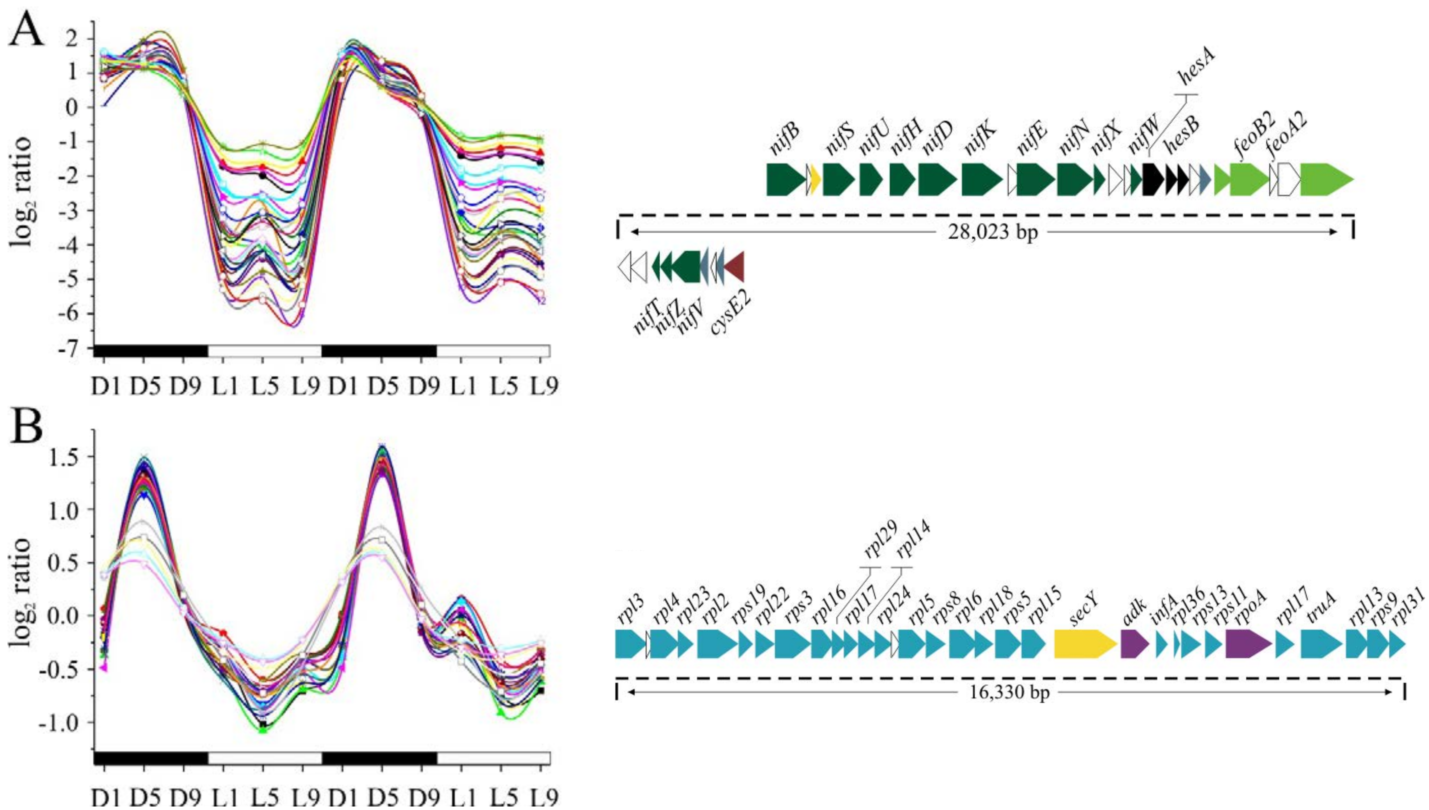
12 samples

Control

mixture of RNA

Jana Stockel

Expression profiles of genes involved in nitrogen fixation and genes encoding ribosomal proteins

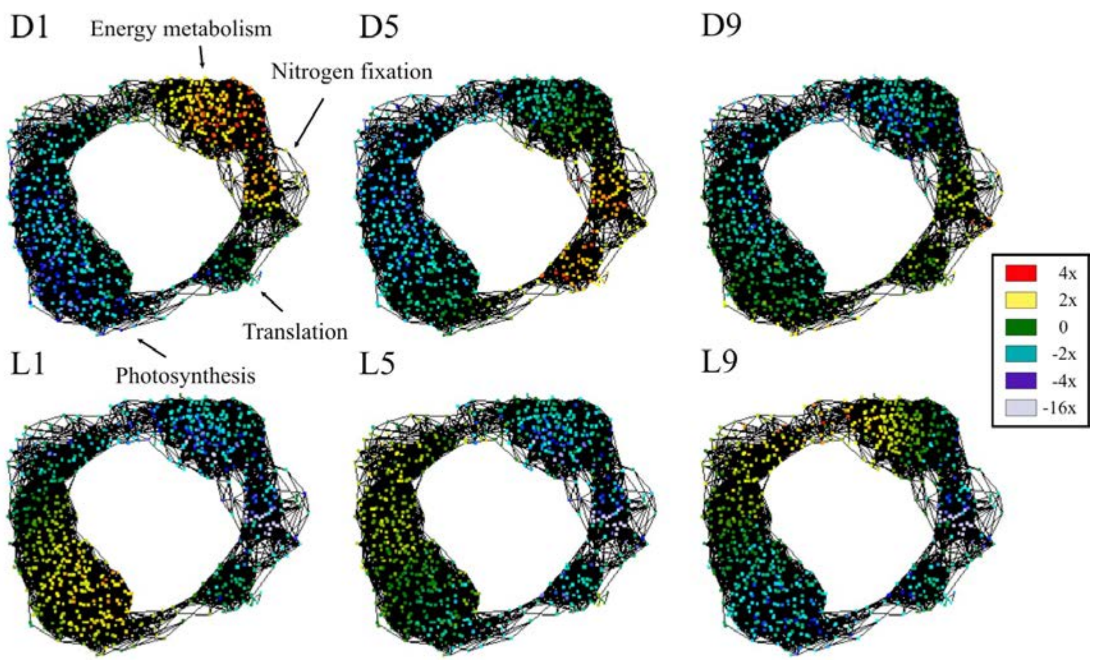


nif genes are expressed only in darkness

Stöckel et al., PNAS 2008

Global Transcriptomics

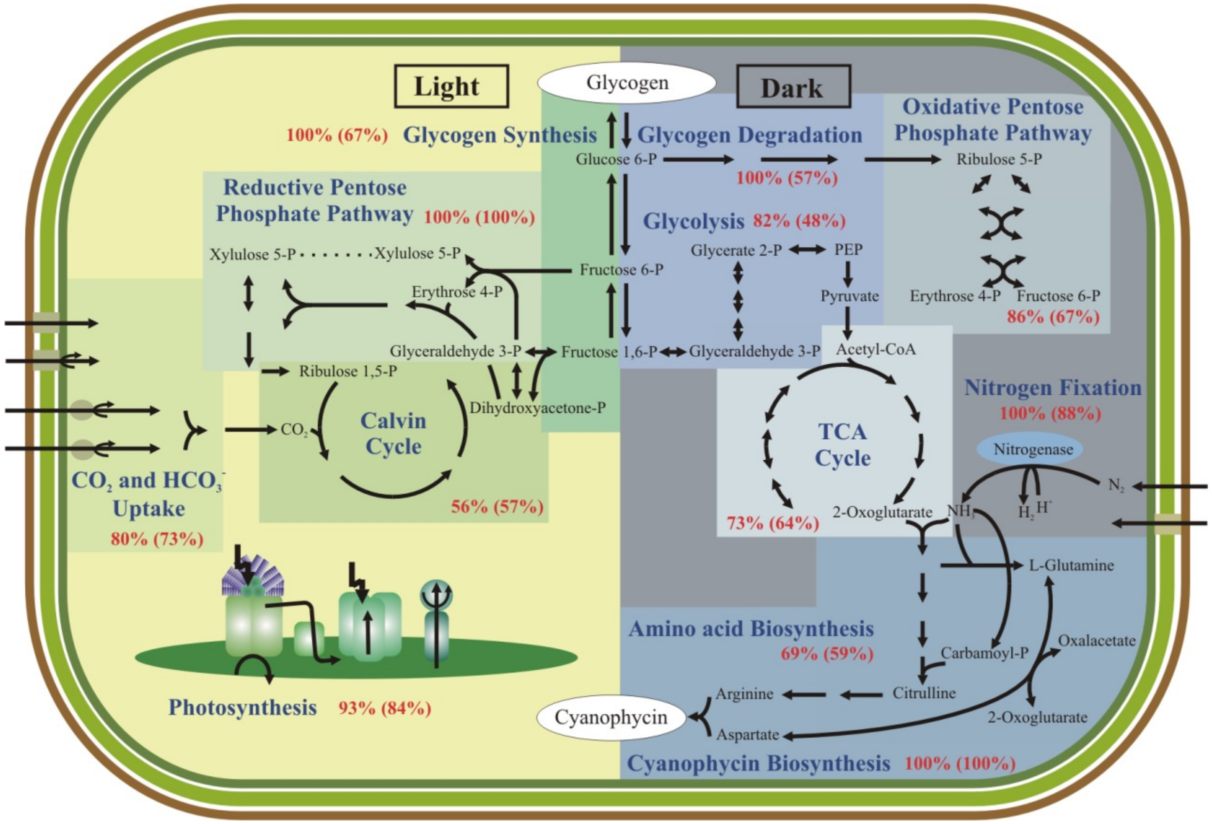
Diurnal cycling at the transcriptional level



Central metabolic pathway genes are co-regulated & maximally expressed at specific and distinct times

Stöckel et al., PNAS 2008

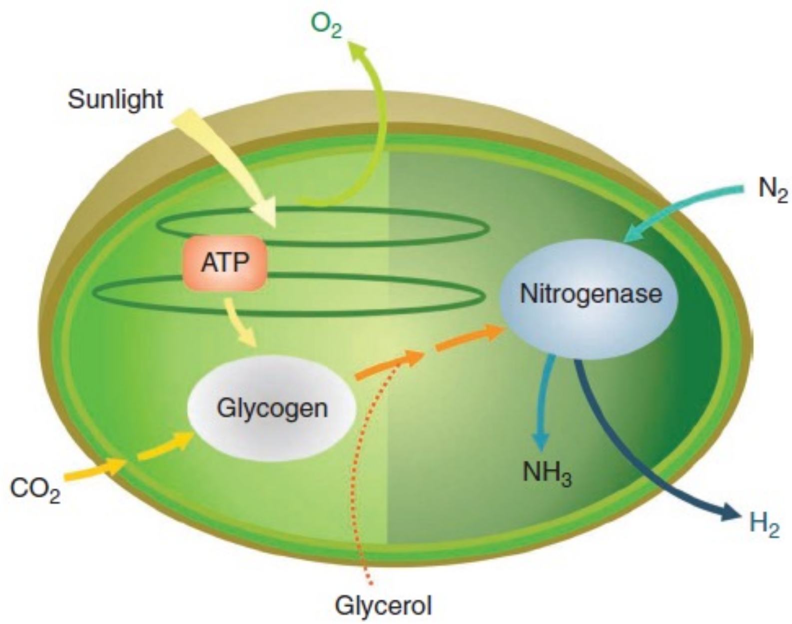
Robust oscillations of central metabolic processes at the transcriptional level in *Cyanothece* 51142



- Diurnal oscillation of many different biochemical processes in *Cyanothece* cells
- *Cyanothece* is metabolically highly active during both day and night

Stöckel et al., PNAS 2008

Photobiological H_2 production in *Cyanothece* 51142

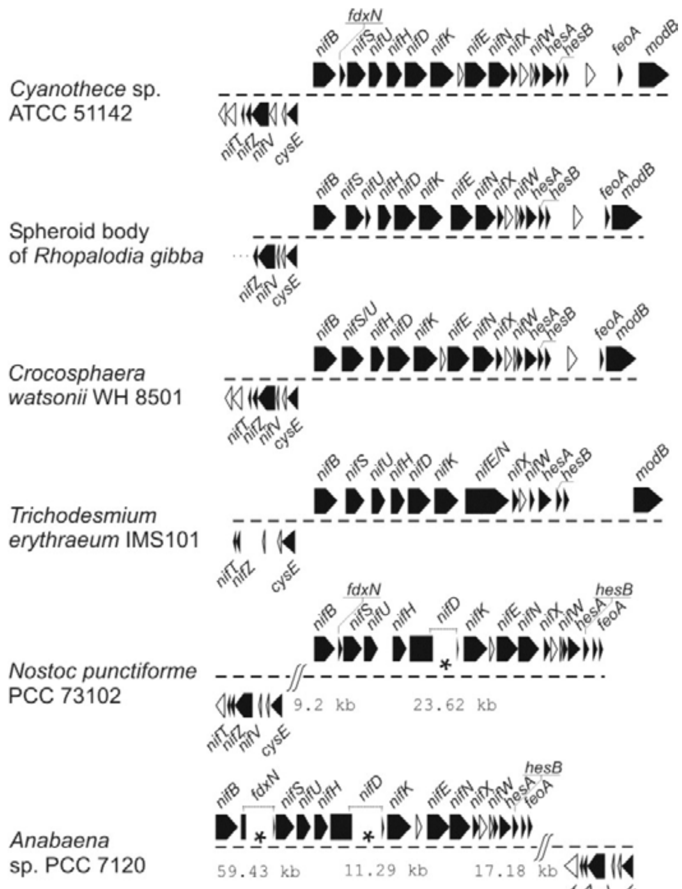


$$N_2 + 8H^+ + 8e^- + 16ATP \longrightarrow 2NH_3 + H_2 + 16ADP + P_i$$

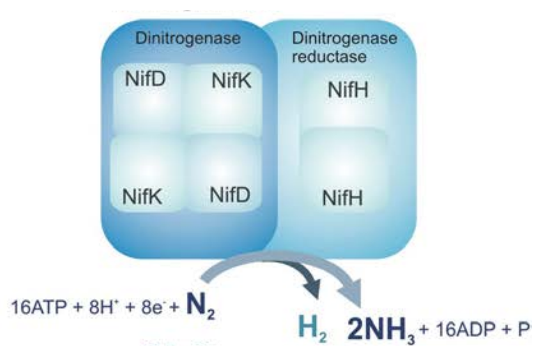
Temporal separation of nitrogen fixation from photosynthesis allows nitrogenase to function under suboxic intracellular conditions created during the dark period

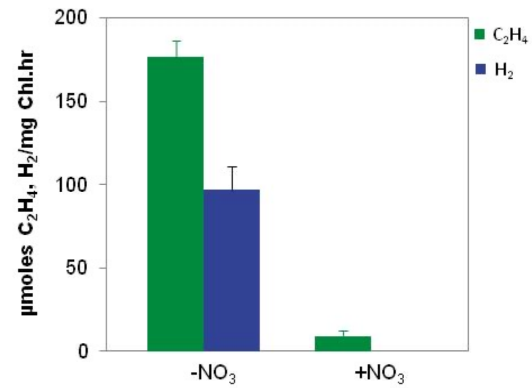
Bandyopadhyay et al., Nature Comm 2010

A large intact nitrogenase gene cluster in *Cyanothece* leads to high rates of N_2 fixation and H_2 production



Welsh et al., PNAS 2008





- *nif* genes expressed only under $–NO_3$
- Hydrogen production is nitrogenase-mediated

Bandyopadhyay et al., Nature Comm 2010

High rates of H_2 production

$$N_2 + 8\,e^- + 8\,H^+ \xrightarrow{\text{Nitrogenase}} 2\,NH_3 + H_2$$

$$16\ \text{ATP} \rightarrow 16\ \text{ADP} + P_i$$

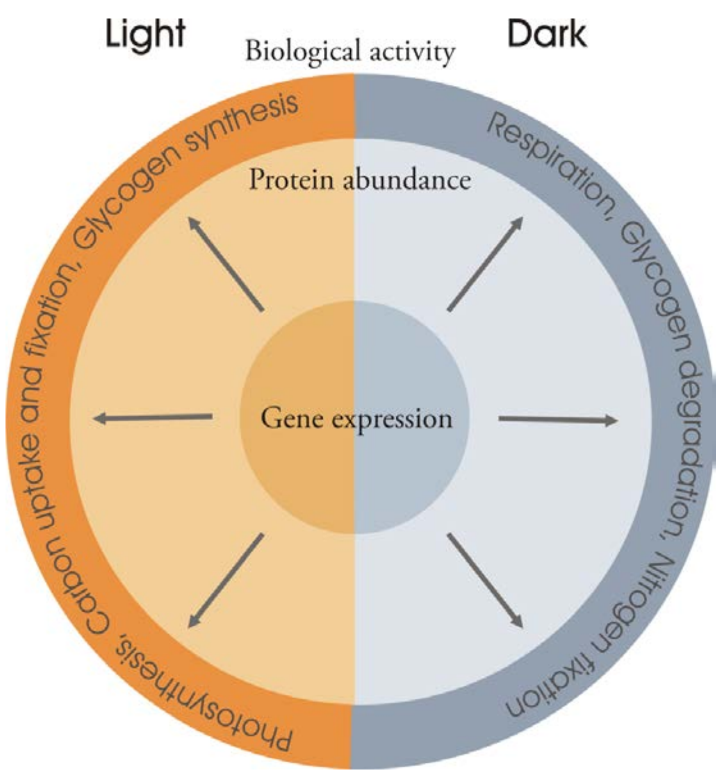
Strain	Enzyme	Specific rates (aerobic incubation)	Specific rates (anaerobic incubation)	
		(µmoles/mg Chl.h)	(µmoles/mg Chl.h)	(µmoles/mg Prot.h)
C. reinhardtii	Hydrogenase	-	**6.6**[1]	
Synechocystis 6803	Hydrogenase	-	**1.2**[2]	
Anabaena 29413	Nitrogenase	-	**45.17**[3]	
R. palustris	Nitrogenase			**0.92**[4]
Cyanothece 51142	Nitrogenase	**152**	**373**	**3.5**

Anindita Bandyopadhyay

Systems level understanding of *Cyanothece* led to the highest cyanobacterial H_2 production rates

Bandyopadhyay et al., Nature Comm 2010

Global Proteome Analysis



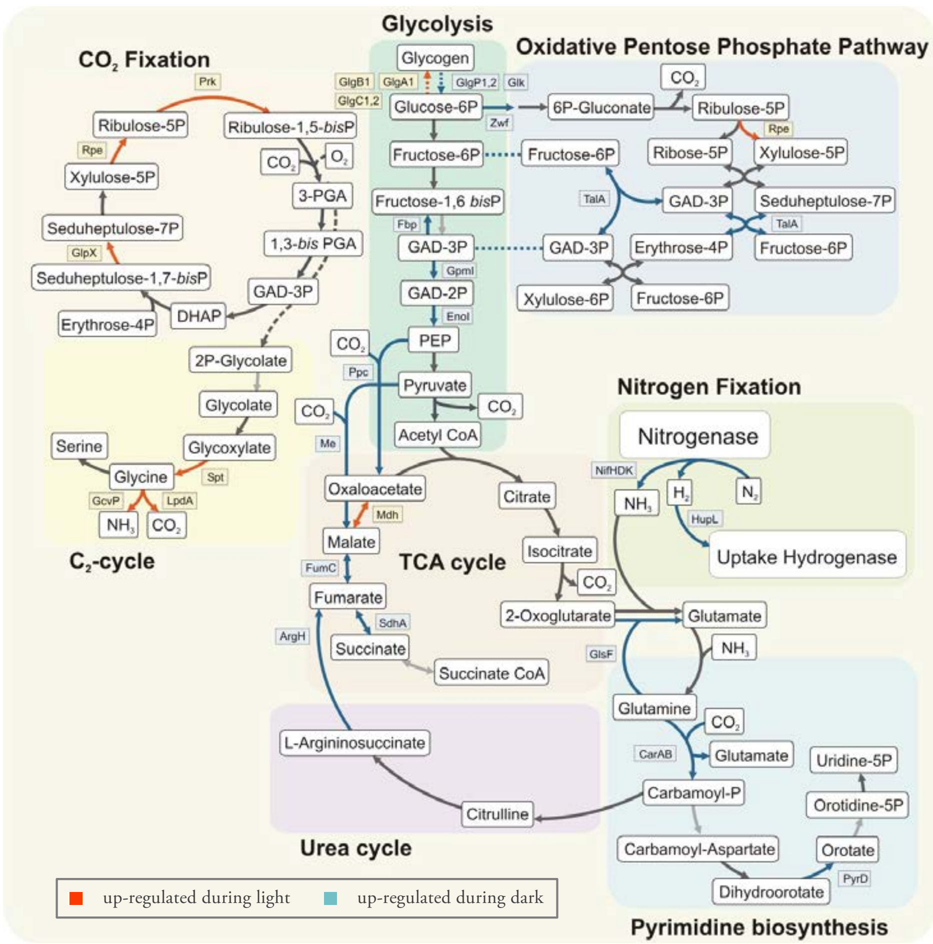
- What is the impact of diurnal rhythms on the protein levels in *Cyanothece* 51142?
- How well are the observed diurnal changes at the transcriptional level reflected at protein level?
- Is there a correlation between temporally regulated physiological phenomena and protein abundance?

Jana Stockel

Jon Jacobs

Stöckel et al., PLoS ONE 2011

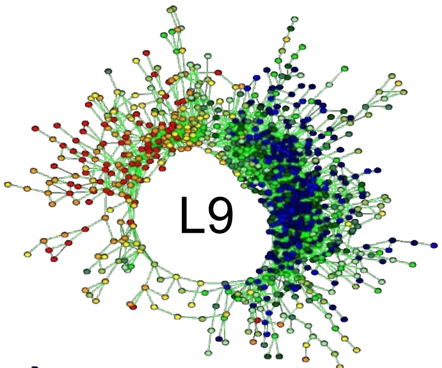
Cyclic expression of proteins related to central metabolic processes in *Cyanothece* 51142

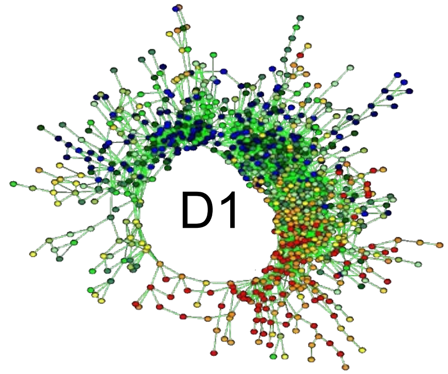


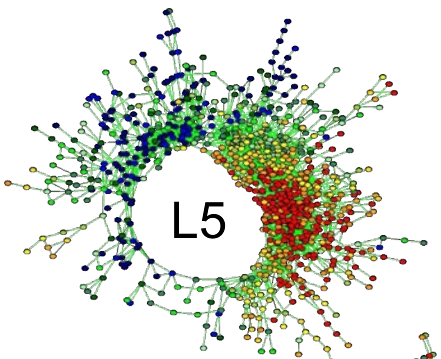
Stöckel et al., PLoS ONE 2011

Several enzymes related to different central metabolic pathways such as glycogen degradation, oxidative pentose phosphate pathway, glycolysis, TCA-cycle, nitrogen fixation, CO_2 fixation and glyoxylate cycle **reveal cyclic changes in their protein abundances** during a diurnal cycle.

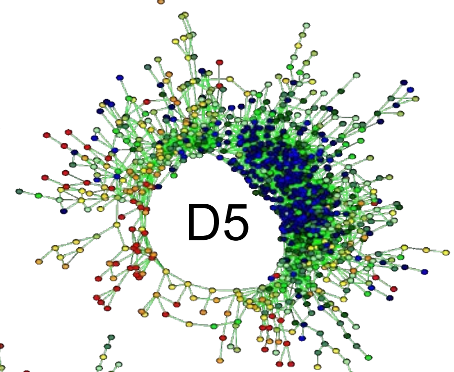
Proteome Cycling



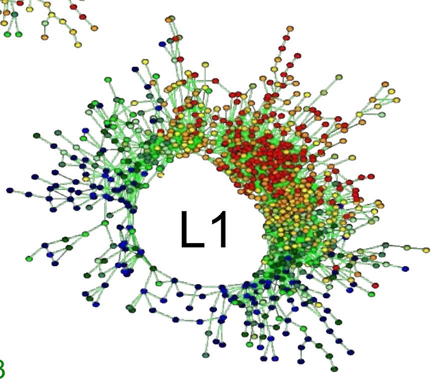


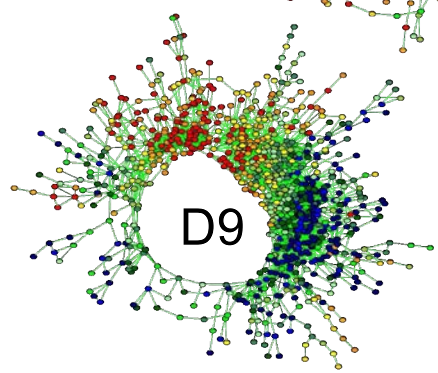


Cycling of proteins across different time points during a diurnal period



Core metabolic pathway proteins are co-regulated and co-expressed during the diurnal cycle



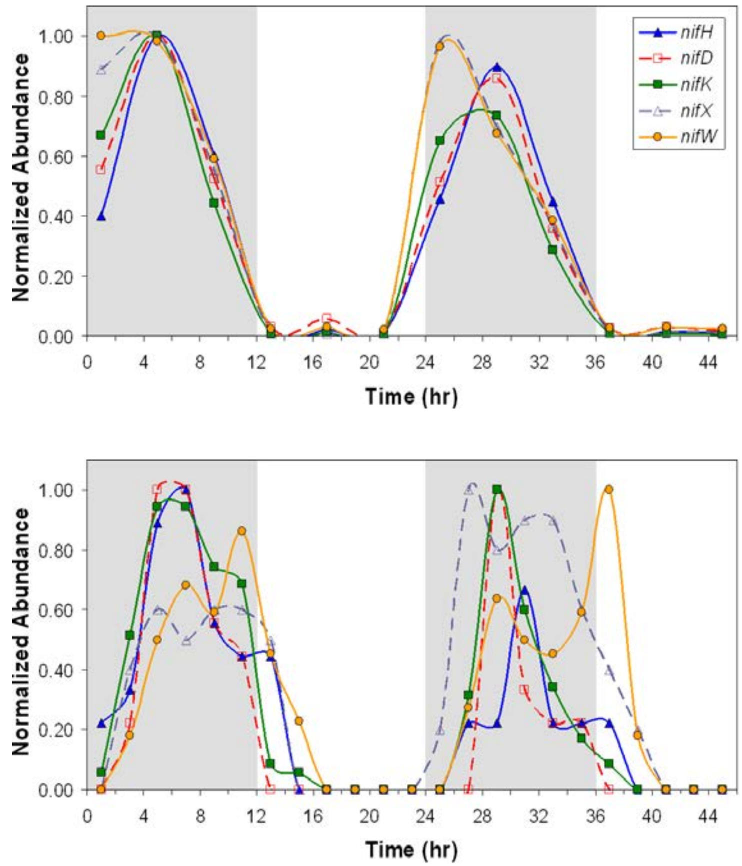


High expression
Low expression

Nitrogenase-related genes cycle in both transcript and protein abundances

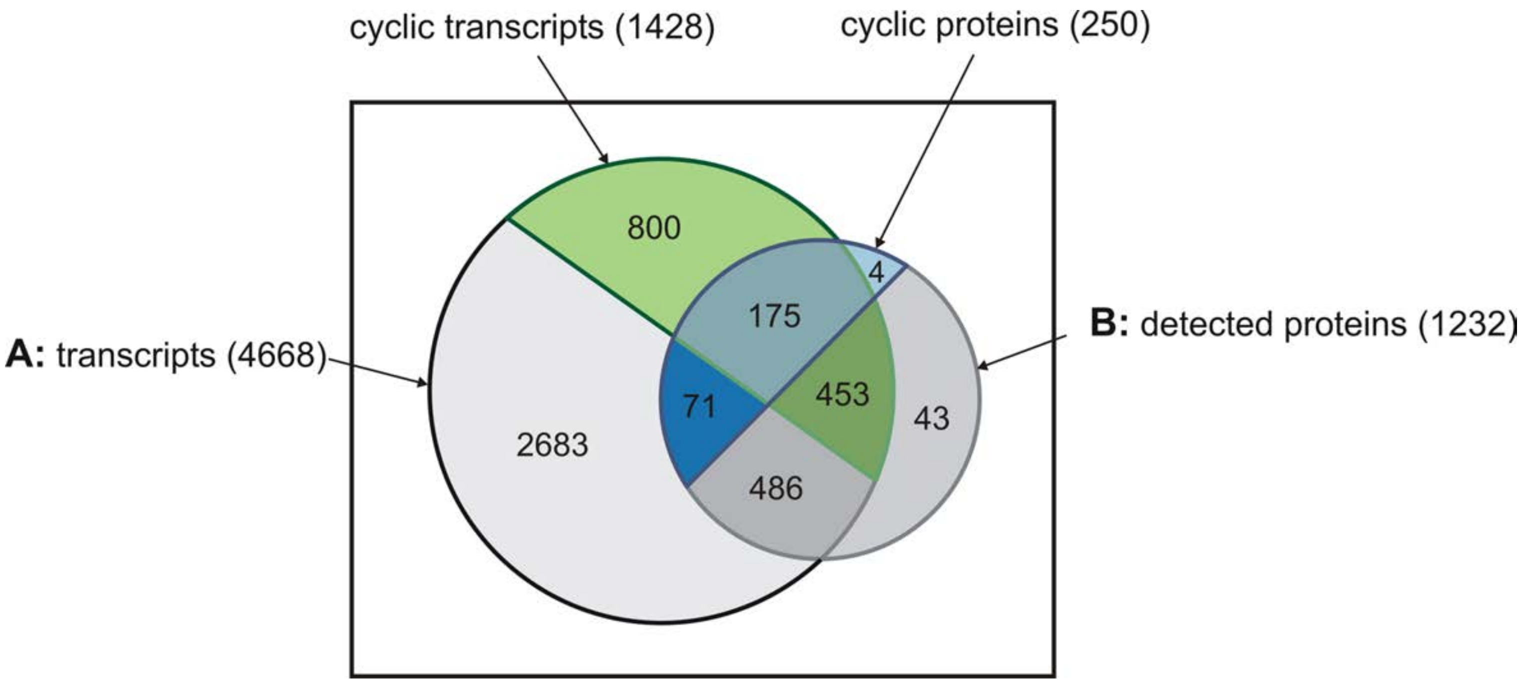
Transcript

Protein



Transcript and protein levels are high during the dark period and decrease during light

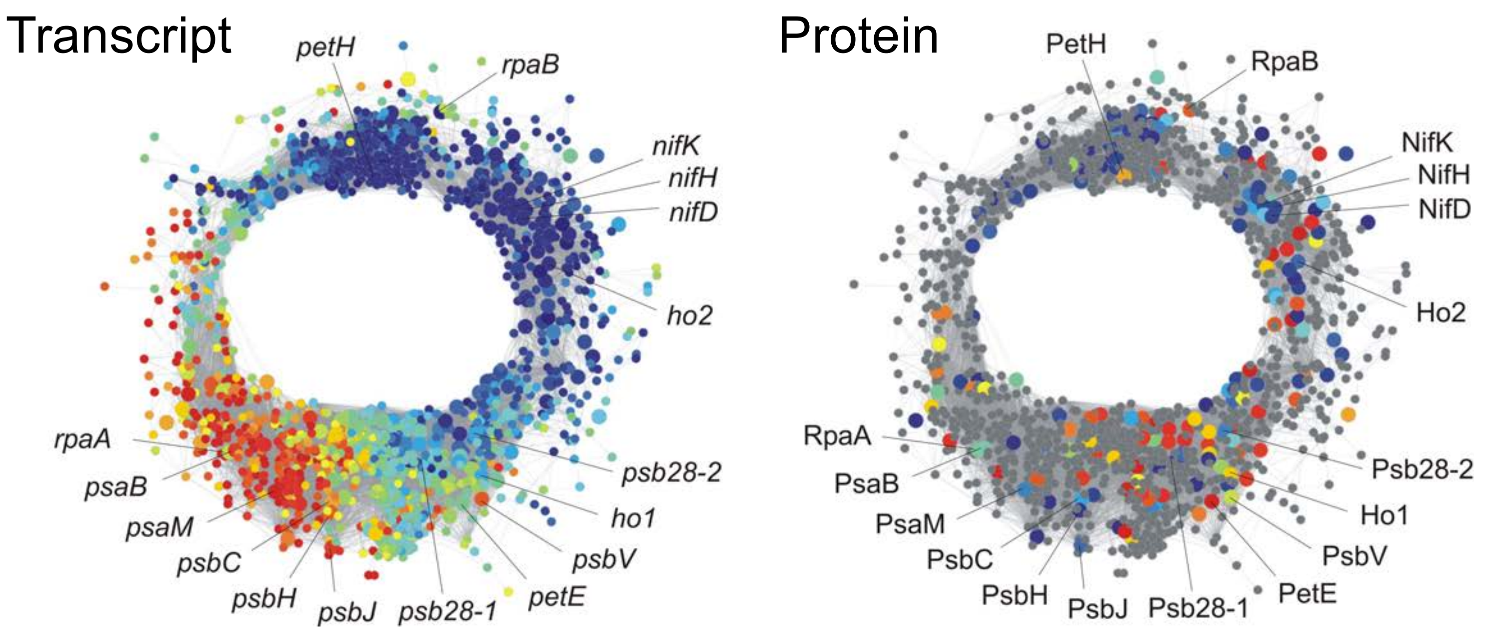
Integration of transcriptomic and proteomic data



Out of 250 cyclic proteins, 175 overlapped with genes previously identified as cyclic at the transcript level

Stöckel et al., PLoS ONE 2011

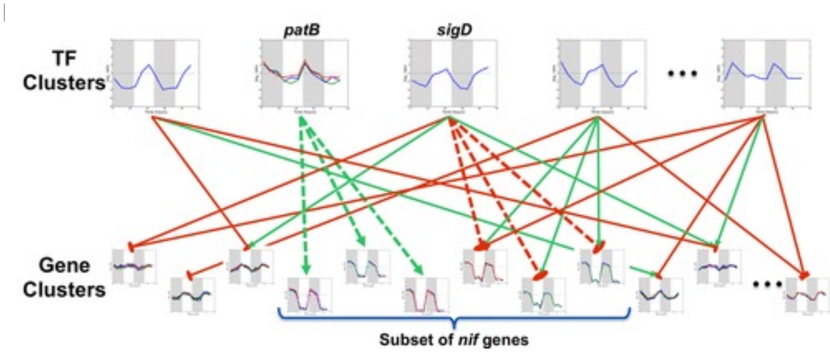
Integration of transcriptomic and proteomic data



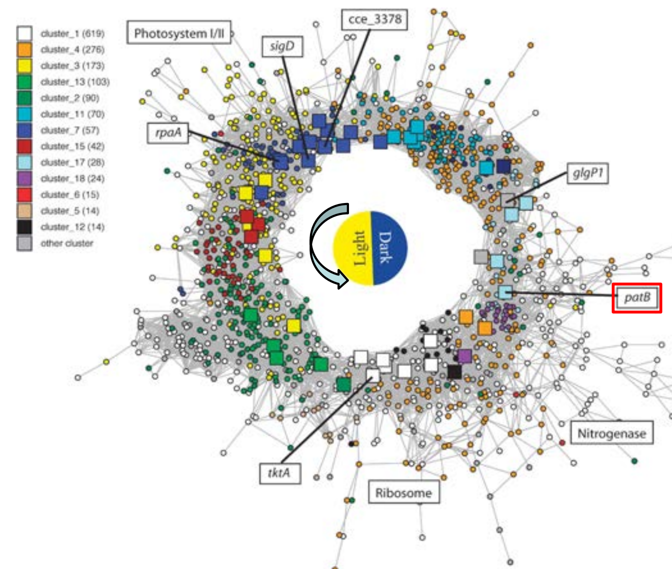
Transcript and protein abundances at various time points showed that post-transcriptional events are important for temporal regulation of key processes

Stöckel et al., PLoS ONE 2011

Modeling regulatory networks for nitrogen fixation in *Cyanothece*



Predicted interactions in part of the *Cyanothece* regulatory network: activating (green); inhibiting (red)



Topology of cyclic wreath network of *Cyanothece* transcription

Mueller et al., ACS Synth Biol 2016

Costas Maranas

McDermott et al., Mol. Biosystems 2011

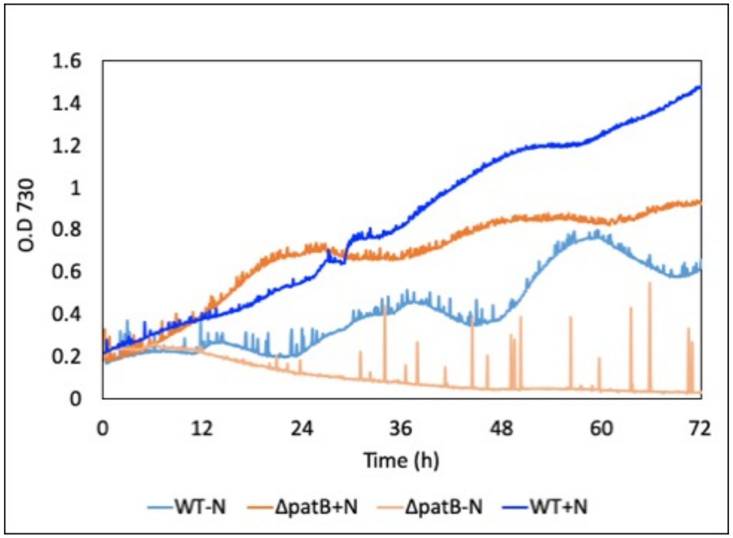
Predicted bottlenecks in cellular processes

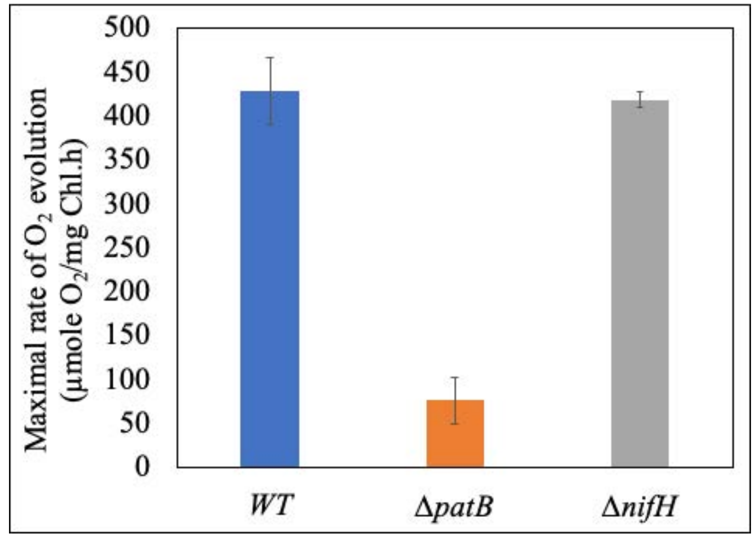
Table 1 Top 25 topological bottlenecks

ID	Rank	Name	Cyclicity	Description	Cluster	Transition
cce_4095	1		circadian	unknown; contains UPF0004	7	L to D
cce_3378	2		diurnal	**Two-component response regulator**	7	L to D
cce_1898	3	***patB***	circadian	**Transcriptional regulator (nitrogen fixation)**	17	D
cce_3594	4	***sigD***	circadian	**RNA polymerase sigma factor 2**	7	L to D
cce_0579	5	*fdxB*	circadian	Ferredoxin III	18	D
cce_4627	6	***tktA***	diurnal	**Transketolase**	1	D to L
cce_3149	7		circadian	unknown	1	D to L
cce_4205	8		circadian	hypothetical protein; contains a GCN5-related N-acetyltransferase domain	4	D
cce_3446	9		circadian	unknown	10	*
cce_3607	10		circadian	putative D-xylulose 5-phosphate/D-fructose 6-phosphate phosphoketolase	7	L to D
cce_3564	11		diurnal	unknown	3	L
cce_1844	12		circadian	unknown; contains an EF-Hand type domain	17	D
cce_1629	13	*glgP1*	circadian	Glycogen phosphorylase	10	*
cce_0043	14	*gmhA*	diurnal	Phosphoheptose isomerase	7	L to D
cce_2449	15		diurnal	unknown; contains a glycoside hydrolase, family 57 domain	1	D to L
cce_3617	16	*leuB*		3-isopropylmalate dehydrogenase	1	D to L
cce_0298	17	***rpaA***	diurnal	**Two-component response regulator (circadian rhythm)**	7	L to D
cce_1749	18		circadian	hypothetical protein; contains a conserved TM helix domain	7	L to D
cce_0072	19		diurnal	UPF YGGT-containing protein	3	L
cce_4510	20	*shc*	diurnal	Squalene-hopene-cyclase	7	L to D
cce_2625	21	*psbU*	diurnal	photosystem II 12 kD extrinsic protein	3	L
cce_2535	22	*opcA*	circadian	OxPPCycle protein	11	D
cce_2552	23		diurnal	unknown; contains amidinotransferase and CHP300 domains	1	D to L
cce_2500	24		circadian	hypothetical protein; contains a radical SAM domain	7	L to D
cce_1482	25		circadian	conserved hypothetical protein	11	D

McDermott et al., Mol. Biosystems 2011

Role of PatB in *Cyanothece* 51142



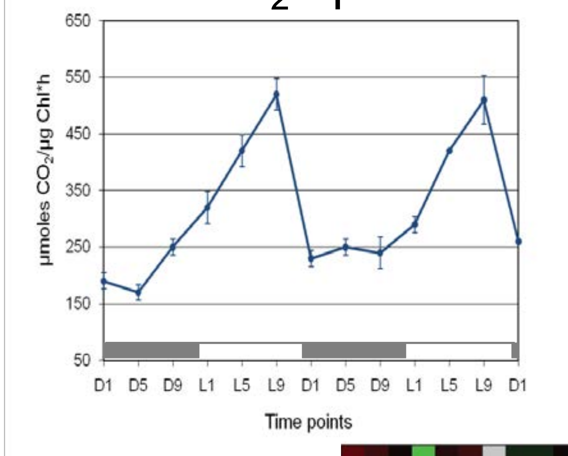


- PatB is essential for growth under nitrogen fixing conditions
- Δ*patB* strain degrades phycobilisomes and shows reduced rates of oxygen evolution
- Comparison to Δ*nifH*, a strain also unable to fix nitrogen, suggests a role for PatB in regulating photosynthesis as well

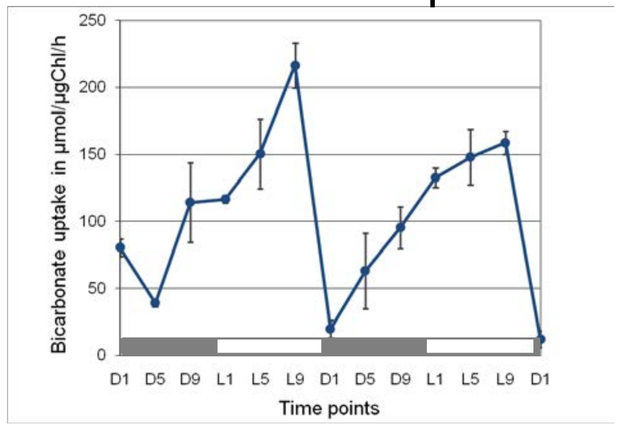
Bandyopadhyay et al., in preparation

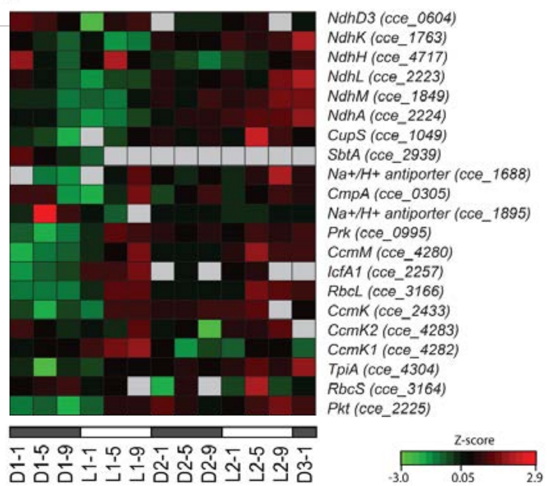
Diurnal cycles in carbon uptake capacity

CO_2 uptake



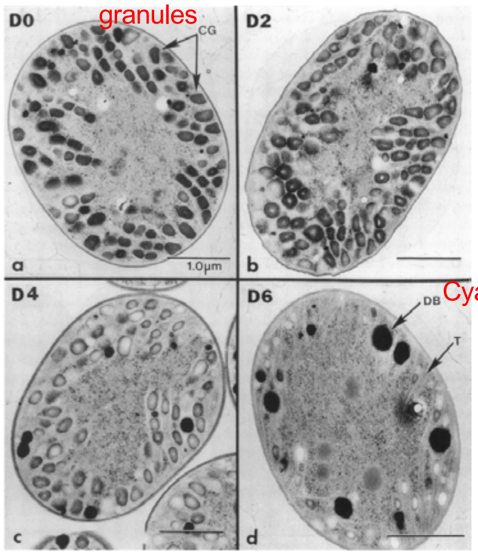
Bicarbonate uptake

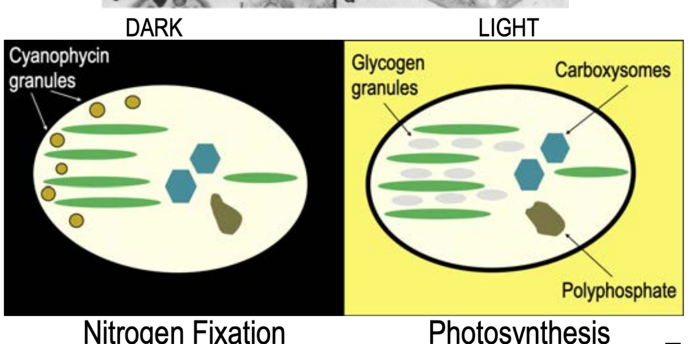


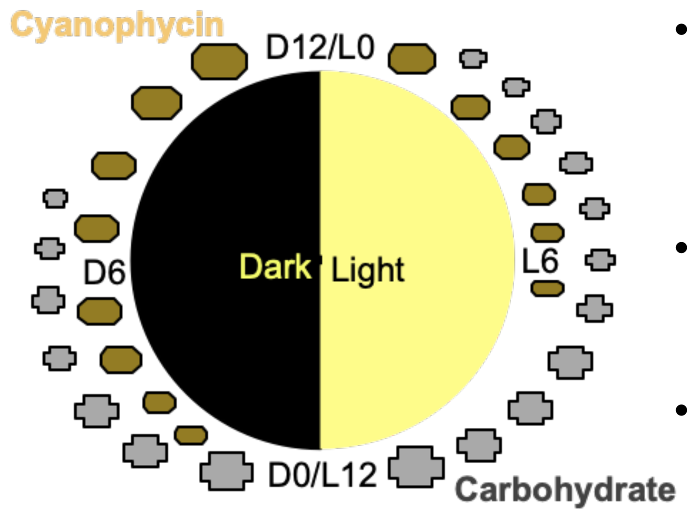


The capacities for CO_2 and bicarbonate uptake oscillate during the diurnal cycle and correlate with temporal changes in the expression of proteins involved in CO_2 fixation, such as RbcL and the carbonic anhydrase IcfA.

Carbon and nitrogen storage occurs as part of the diurnal cycle of photosynthesis and nitrogen fixation



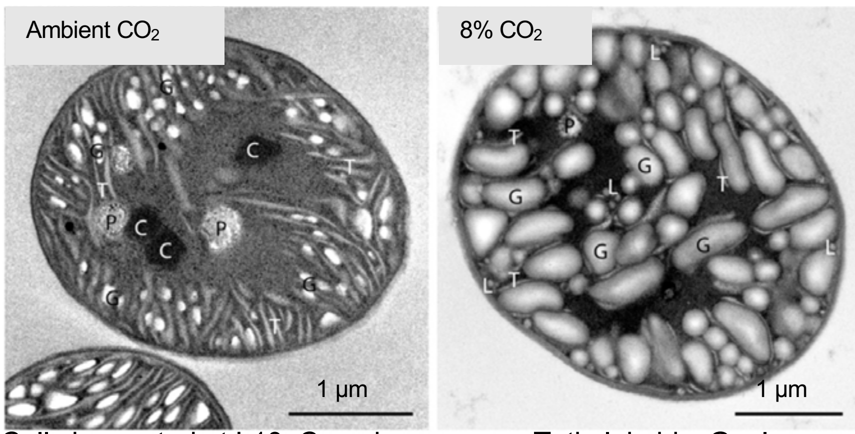




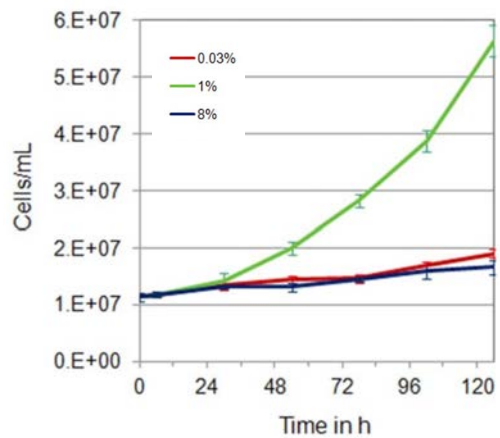
From Schneegurt et al., J Bacteriol 1994

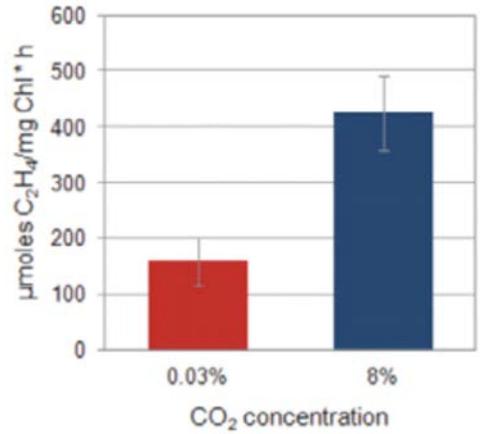
- Carbon is stored in carbohydrate granules
- Nitrogen is stored in cyanophycin bodies
- Both accumulation and degradation pathways were probed

High carbon impacts cell morphology and nitrogen fixation



Cells harvested at L10. C, carboxysomes; T, thylakoids; G, glycogen



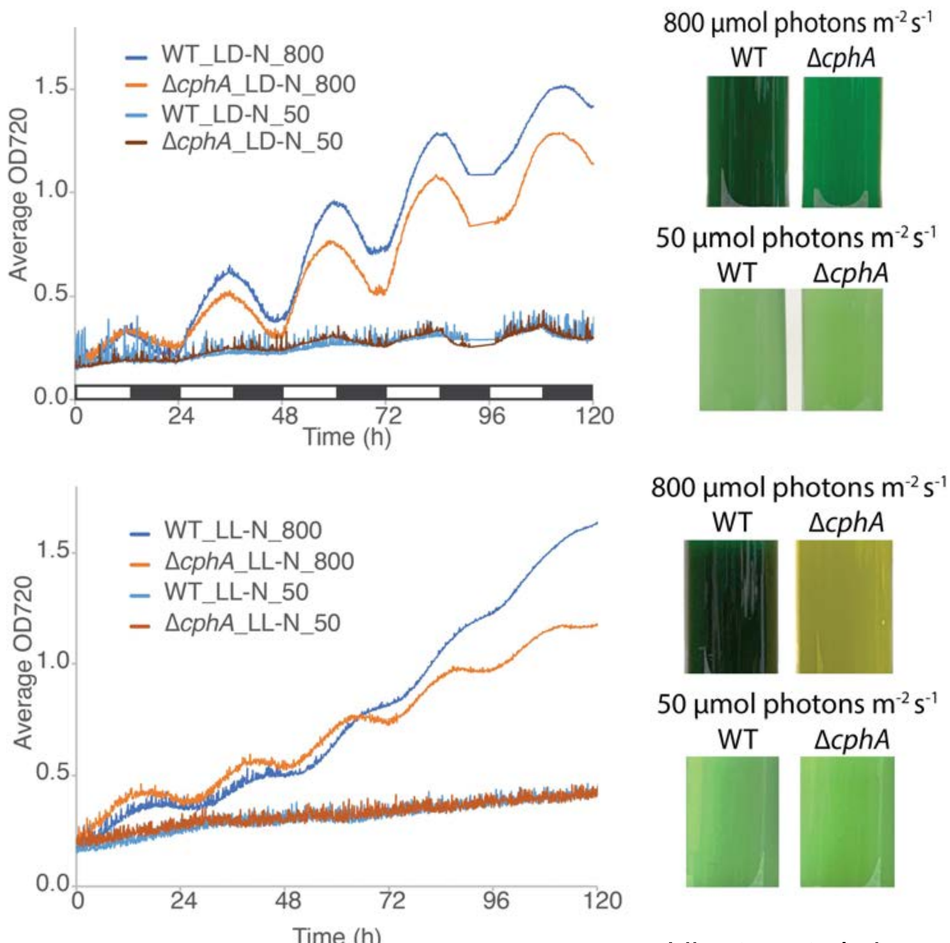


- High CO_2 triggers the production of carbon-rich compounds and increases nitrogen fixation

33

Stöckel et al., PLoS ONE 2013

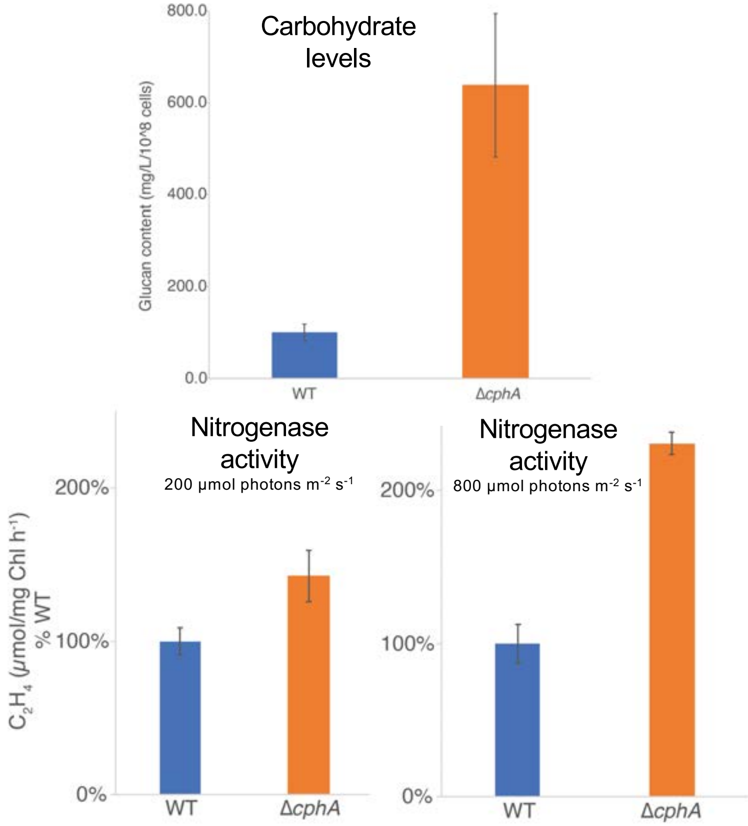
Disruption of cyanophycin formation



Liberton et al., in preparation

- Deletion of the cyanophycin synthetase *cphA* eliminates cyanophycin production
- Δ*cphA* strain exhibits slower growth in high light
- Under continuous high light (LL), phycobilisomes are degraded as a nitrogen source

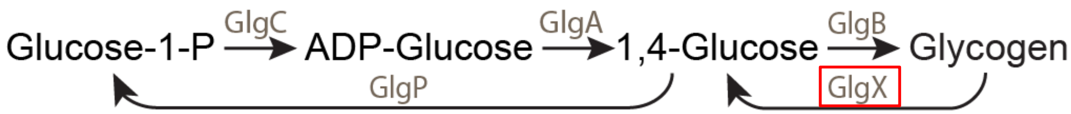
Carbohydrate storage and nitrogen fixation in the Δ*cphA* strain



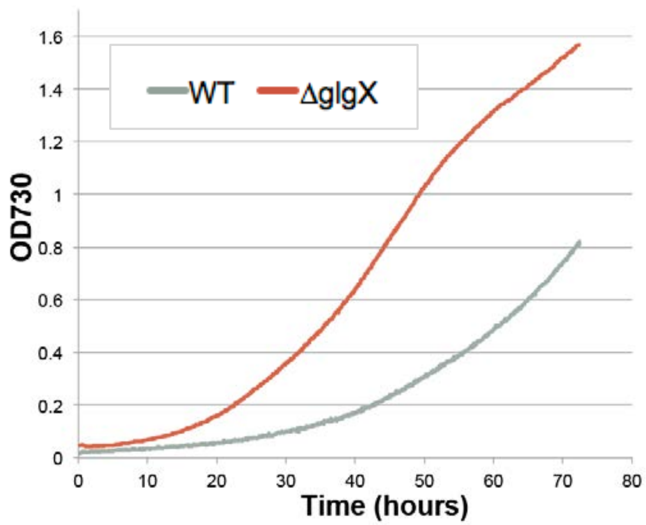
- Excess carbon is stored when cellular nitrogen levels are low
- N_2-fixation is increased to compensate for the lack of stored nitrogen

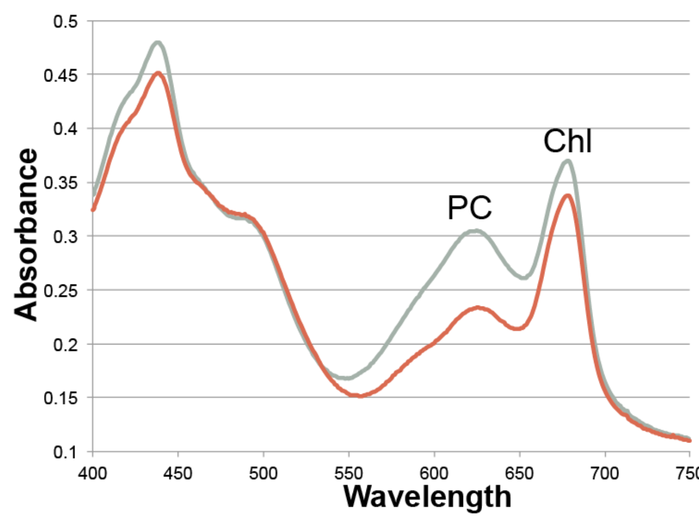
Liberton et al., in preparation

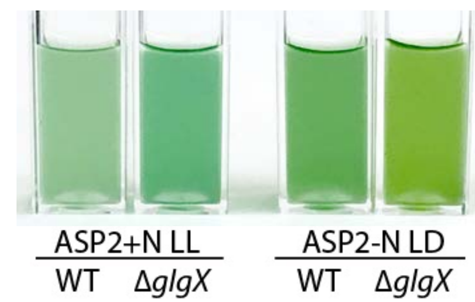
Carbohydrate storage in *Cyanothece* 51142



GlgX is the glycogen debranching enzyme



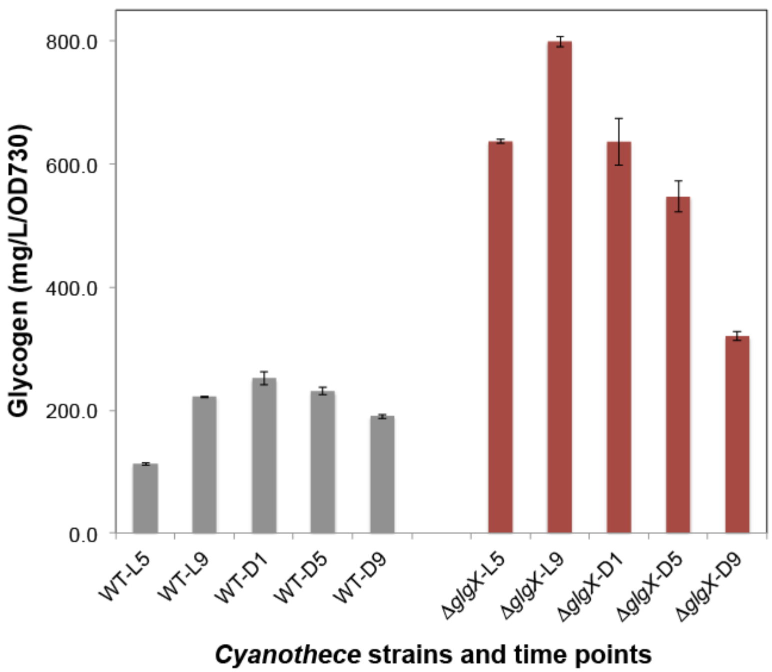




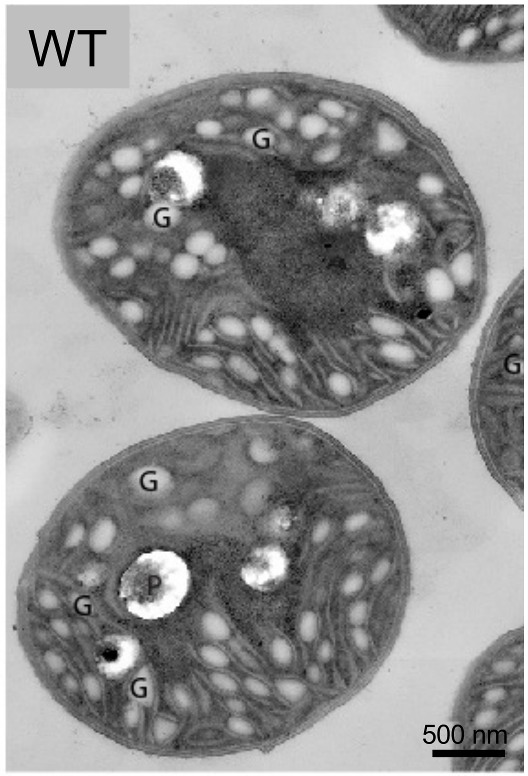
The Δ*glgX* strain has a higher growth rate and decreased phycobilisome (PC) content

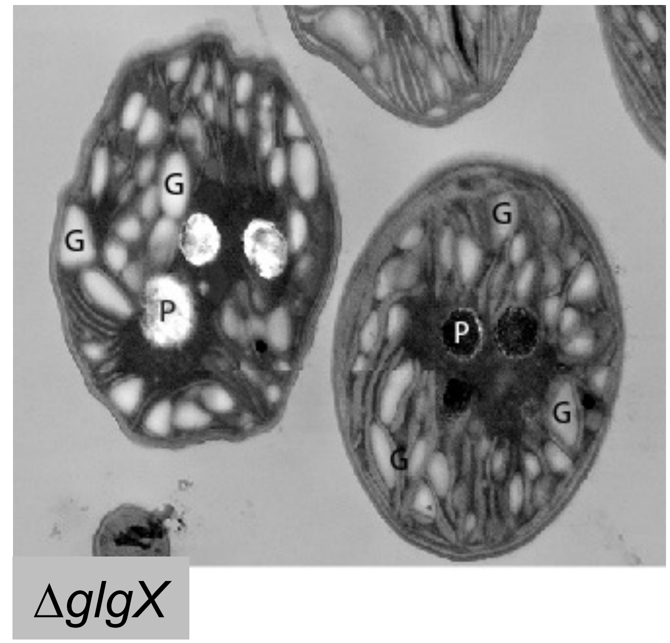
Liberton et al., AEM 2019

Carbohydrate accumulation is higher in the Δ*glgX* strain compared to WT



Liberton et al., 2019

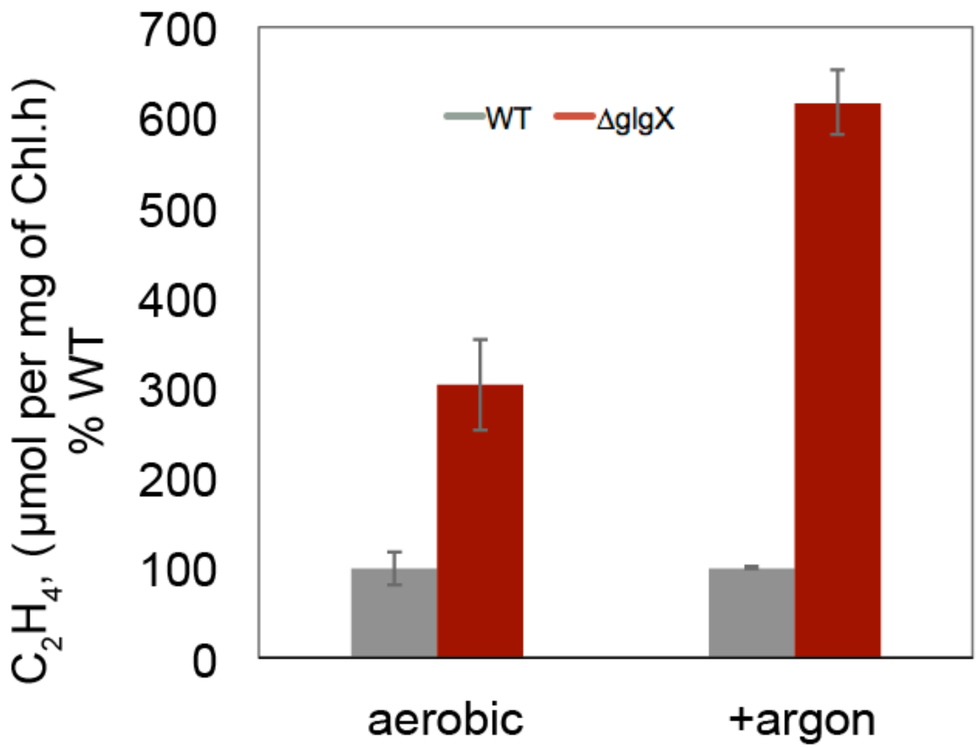




Carbohydrate granules are larger and more irregularly shaped in the Δ*glgX* mutant

Liberton et al., AEM 2019

N_2-Fixation is significantly enhanced in the Δ*glgX* strain

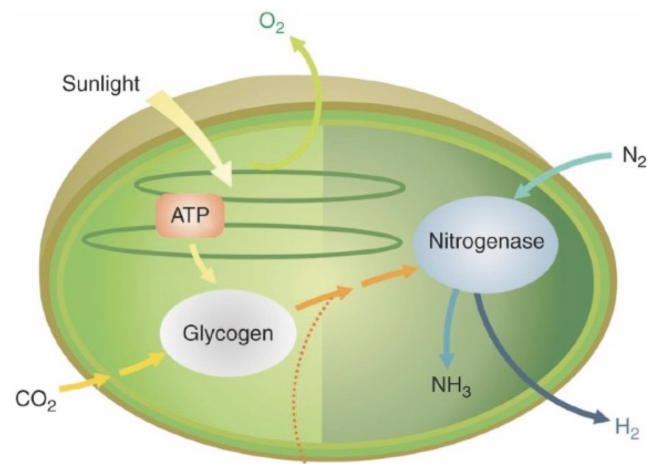


Rates of N_2-fixation increased 3- to 6-fold in the mutant compared to WT

Liberton et al., AEM 2019

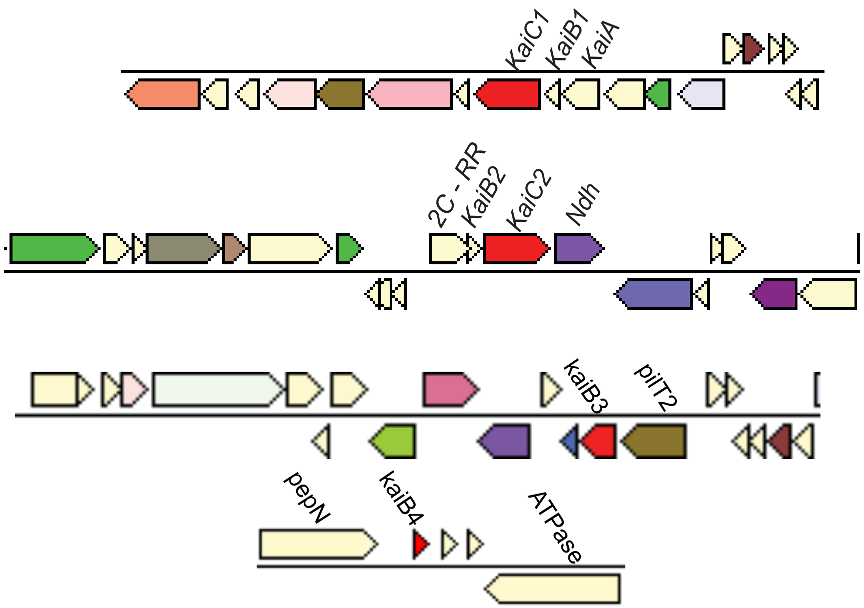
The circadian clock function is critical for unicellular diazotrophic cyanobacteria

- N_2 fixation by unicellular diazotrophic cyanobacteria led to the discovery of the circadian rhythm in prokaryotes.
- Unicellular diazotrophic cyanobacteria require temporal separation of photosynthesis and nitrogen fixation.
- Under diurnal growth conditions, the separation is automatically imposed.
- Under continuous light (CL) and altered day length conditions, the internal clock function is critical.
- Best studied clock system is in the unicellular non-diazotrophic cyanobacterium *S. elongatus.* Three genes – KaiA, KaiB and KaiC.
- Clock mechanism not yet studied in unicellular diazotrophic cyanobacteria.

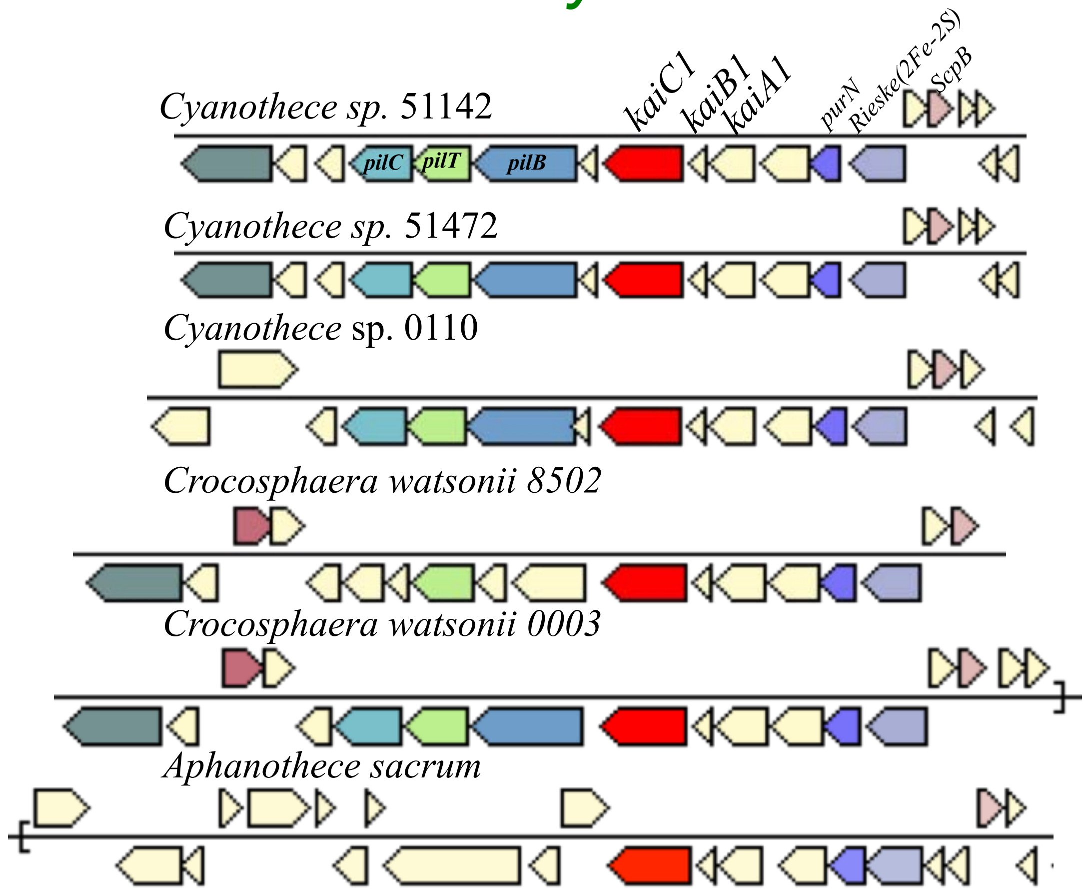


Bandyopadhyay et al. (2023) doi.org/10.21203/rs.3.rs-2625388/v1

Investigating the role of the circadian clock in *Cyanothece* 51142



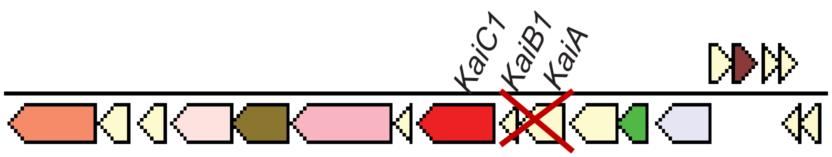
Cyanothece 51142 genome harbors multiple *kai* genes

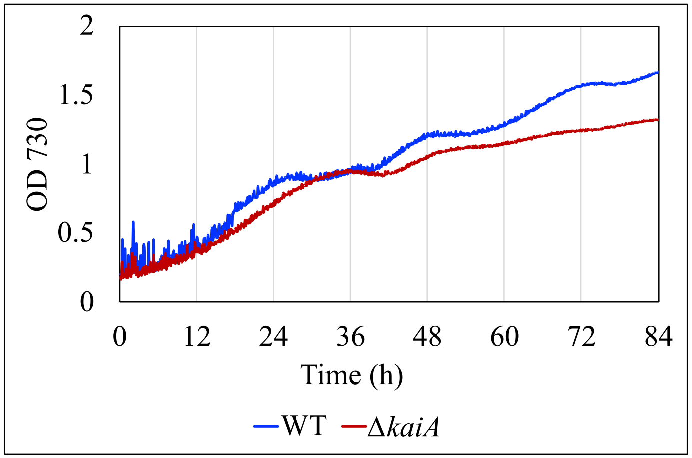


The *kaiABC* cluster and its neighborhood are conserved among unicellular diazotrophic cyanobacteria

Bandyopadhyay et al., doi.org/10.21203/rs.3.rs-2625388/v1

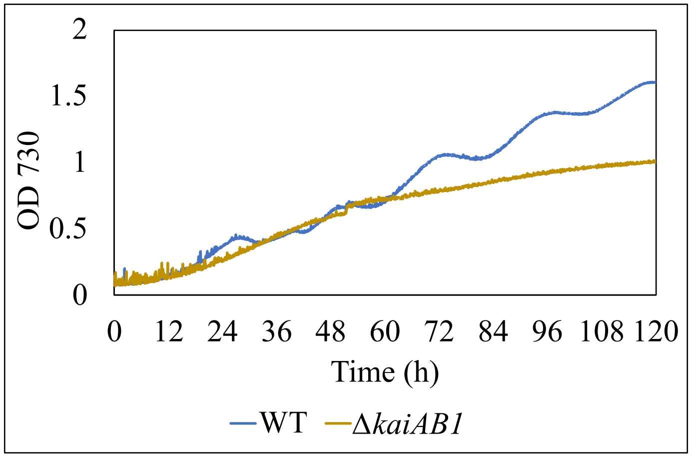
The *kaiABC* gene cluster is indispensable in *Cyanothece* 51142





WT – robust rhythm in growth under CL

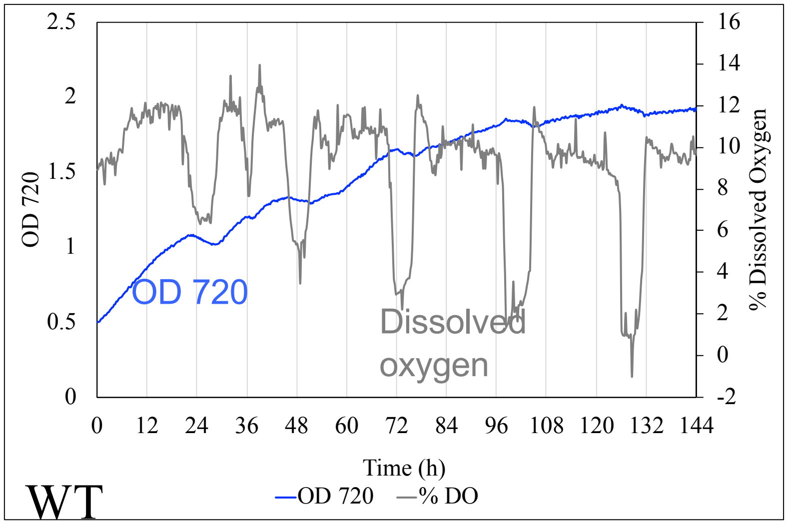
ΔkaiA, *ΔkaiAB* - loss of rhythm

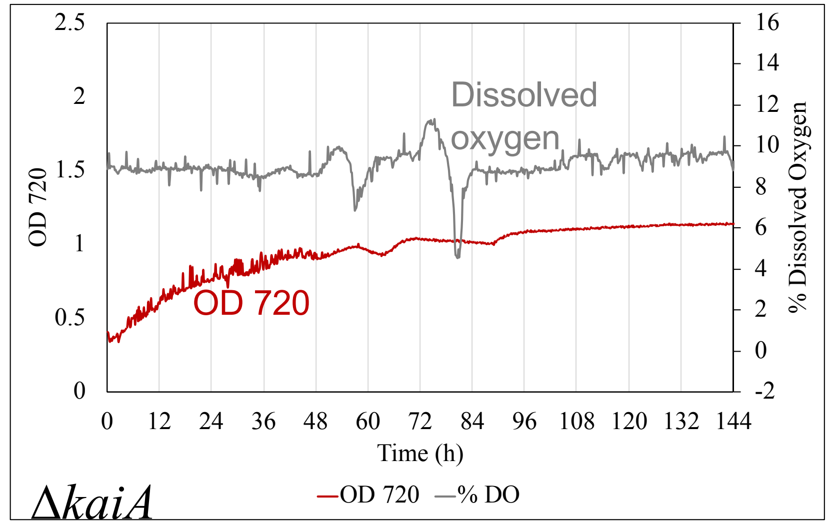


Bandyopadhyay et al., doi.org/10.21203/rs.3.rs-2625388/v1

The Δ*kaiA* mutant exhibits disruption in cellular oxygen dynamics

Continuous Light culture



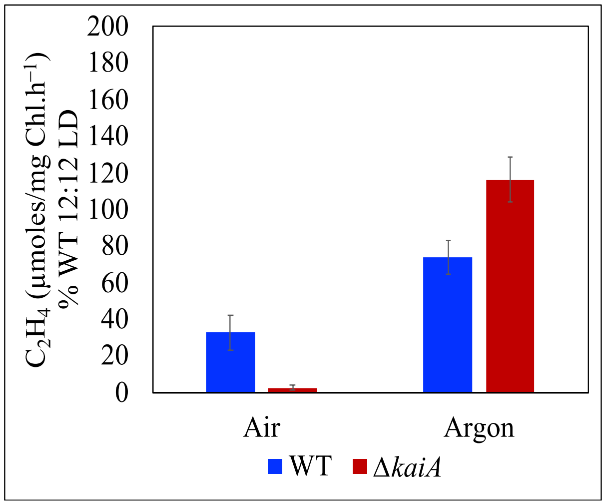


- Robust oscillations in DO levels correspond with drop in OD 730 and increase in nitrogenase activity in the WT

- Oscillations are greatly dampened and only a few random dips in DO levels in the mutant

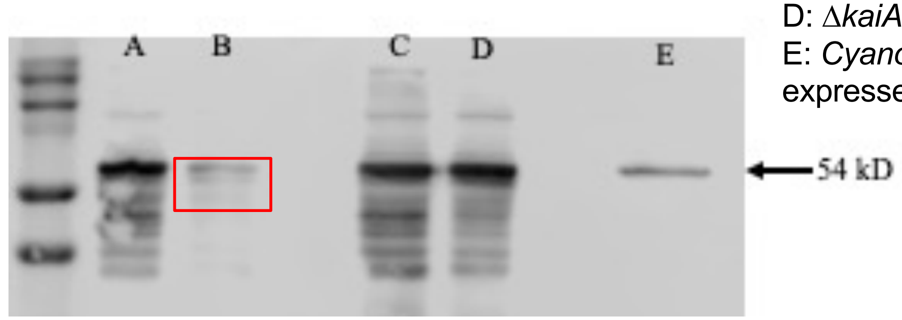
Bandyopadhyay et al., doi.org/10.21203/rs.3.rs-2625388/v1

KaiA is essential for maintaining the integrity of the nitrogenase enzyme under high oxygen conditions



Nitrogenase activity under CL

NifD in cells under CL

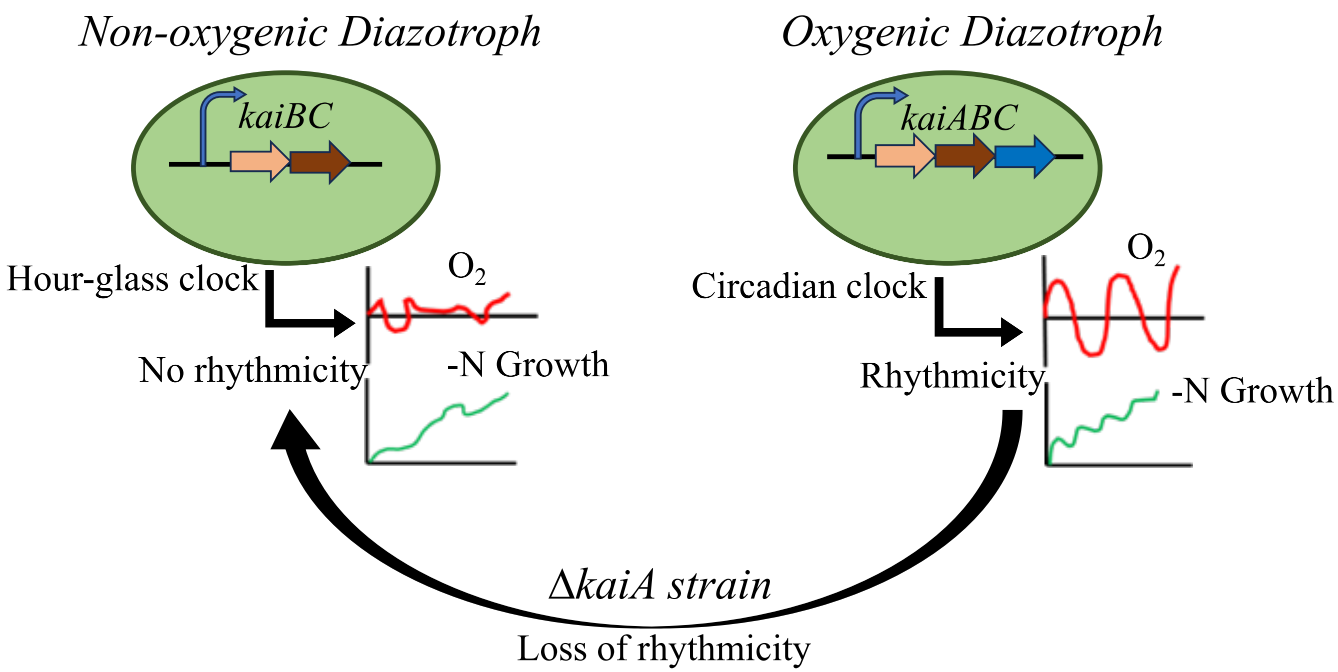


A: WT – aerobic incubation
B: Δ*kaiA* - aerobic incubation
C: WT - anaerobic incubation
D: Δ*kaiA* - aerobic incubation
E: *Cyanothece* NifD protein expressed in *E.coli*

- KaiA does not directly affect nitrogenase activity
- KaiA regulates intracellular oxygen tension, providing an environment conducive for nitrogenase function

Bandyopadhyay et al., doi.org/10.21203/rs.3.rs-2625388/v1

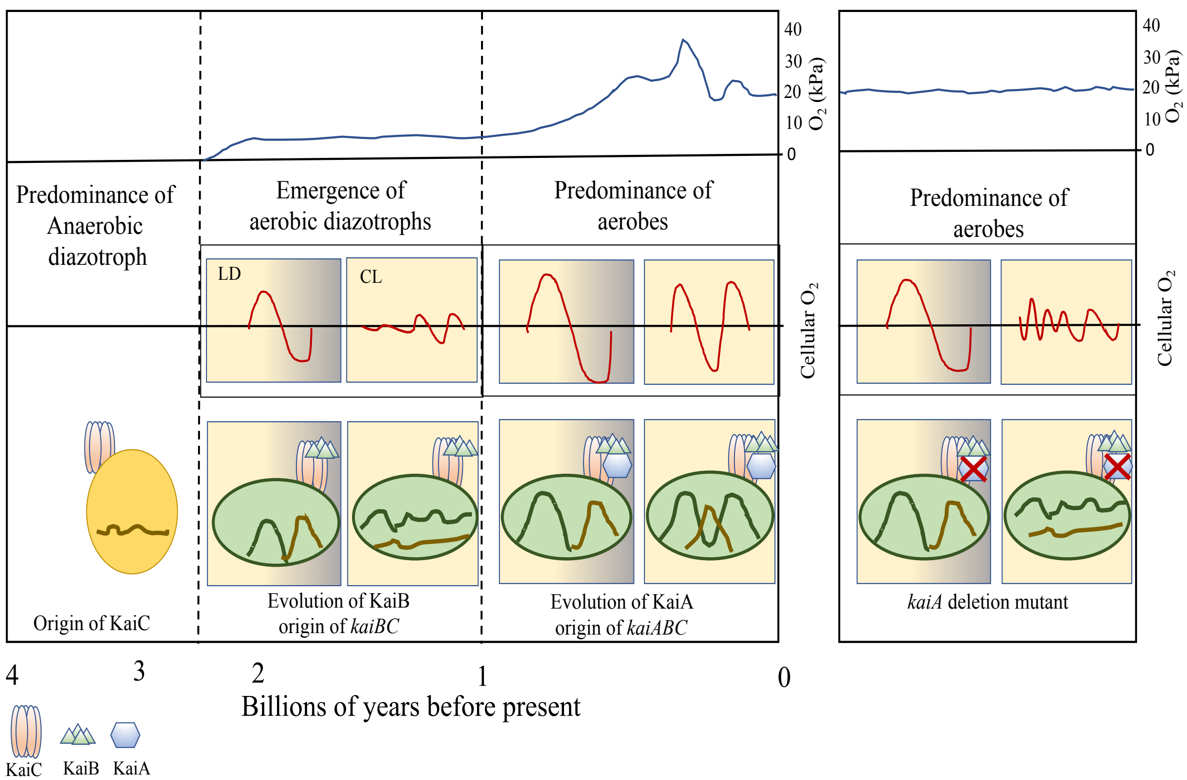
KaiA - a cyanobacterial invention?



KaiA is likely an addition to the prokaryotic hourglass clock comprised of KaiBC

Bandyopadhyay et al., doi.org/10.21203/rs.3.rs-2625388/v1

Importance of KaiA in regulating cellular oxygen dynamics in unicellular diazotrophic cyanobacteria



- Under LD cycles, temporal separation of photosynthesis (green) and N_2 fixation (brown) are enforced by external LD cues and KaiA is dispensable.
- Under CL, the separation is controlled by the internal clock that enforces the rhythms of photosynthesis and N_2 fixation and ensures optimal cellular oxygen levels (red) conducive for nitrogen fixation.
- KaiA is essential for the maintenance of robust self-sustained rhythms. With increasing oxygen levels in the atmosphere over the course of evolution, addition of KaiA to the KaiBC clock was an adaptive strategy that unicellular diazotrophic cyanobacteria resorted to.

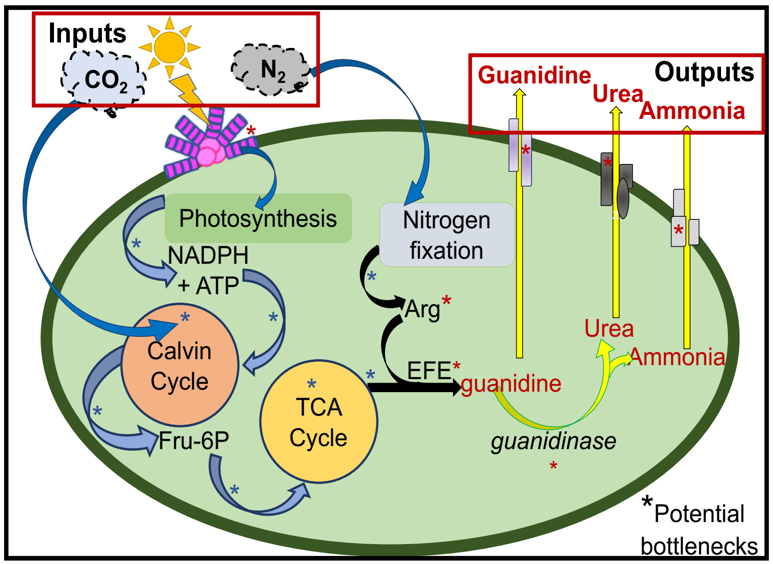
Bandyopadhyay et al., doi.org/10.21203/rs.3.rs-2625388/v1

Future Directions

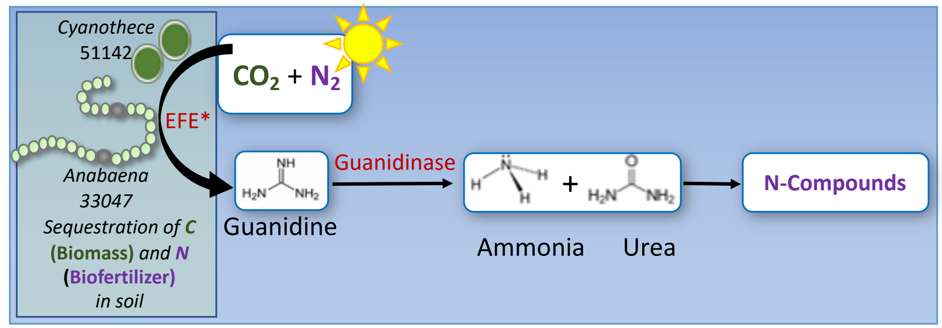
- *Cyanothece* 51142 has emerged as a model microbe to study processes related to CO_2 and N_2 fixation in a photosynthetic organism
- Plethora of genetic tools are now available to address long-standing questions related to photosynthesis and nitrogen fixation in such a unicellular diazotroph
- Stage is set to develop these versatile organisms as production chassis strains

Converting N_2 and CO_2 into N-rich products

DOE Science Foundations for Energy Earthshots



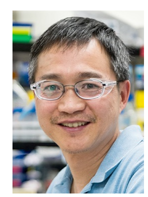
Leveraging cyanobacterial metabolism for the solar-powered conversion of N_2 and CO_2 into N-rich fertilizer compounds



Overview of the proposed technology for bio-fertilizer production and accelerated carbon sequestration using engineered N-fixing cyanobacteria to produce guanidine, ammonia, and urea.


Yinjie Tang


Yixin Chen


Jianping Yu


Wei Xiong


Harvey Hou

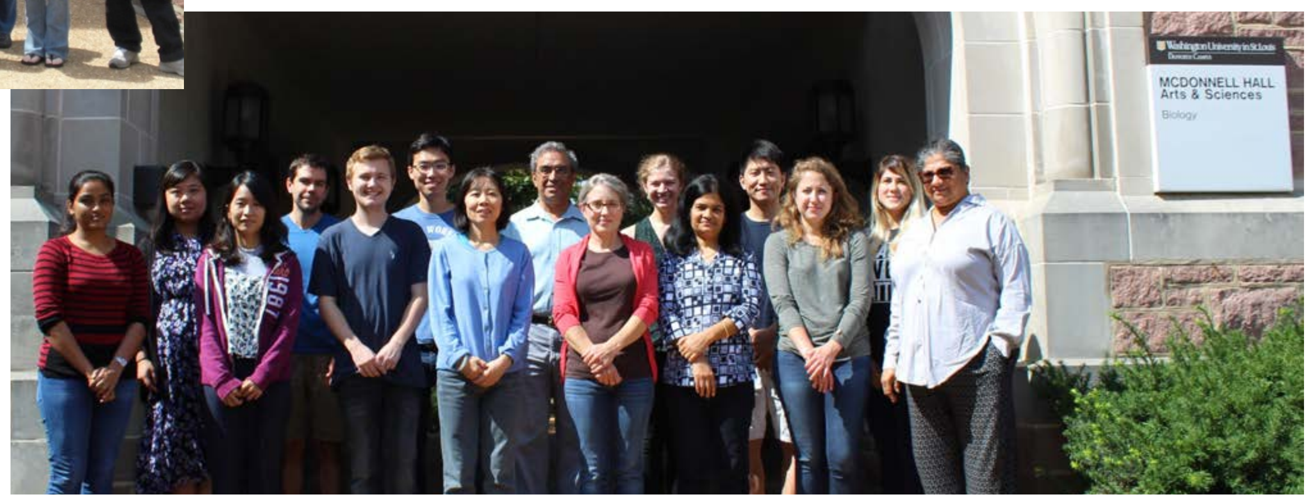

Vida Dennis

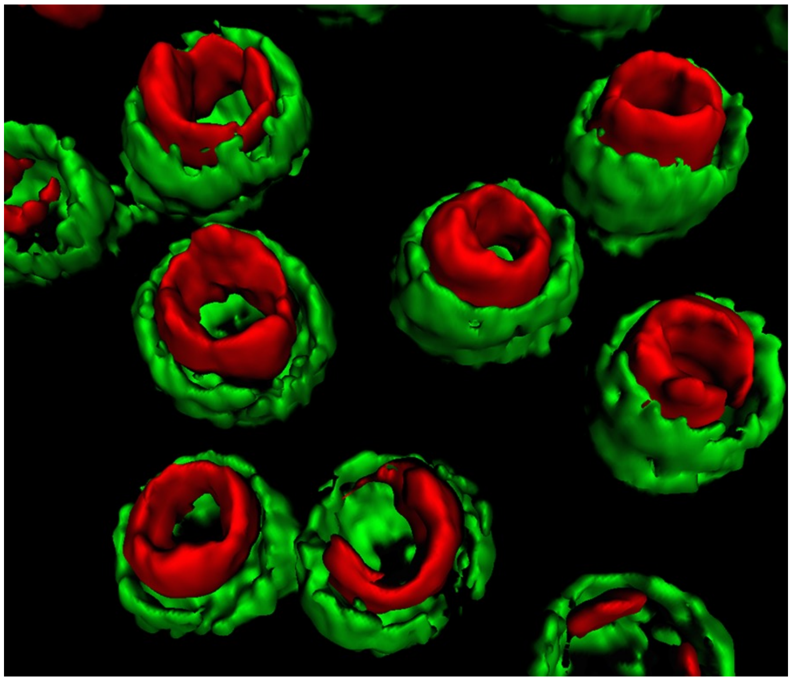
NREL Transforming ENERGY

Alabama State University 1867

Pakrasi Lab

Washington University in St. Louis





https://sites.wustl.edu/photosynthbio/

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