Carbon Fixation in Cyanobacteria

Joint Graduate Student Seminar

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(Year 2 PhD)

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Date: 10 December 2024

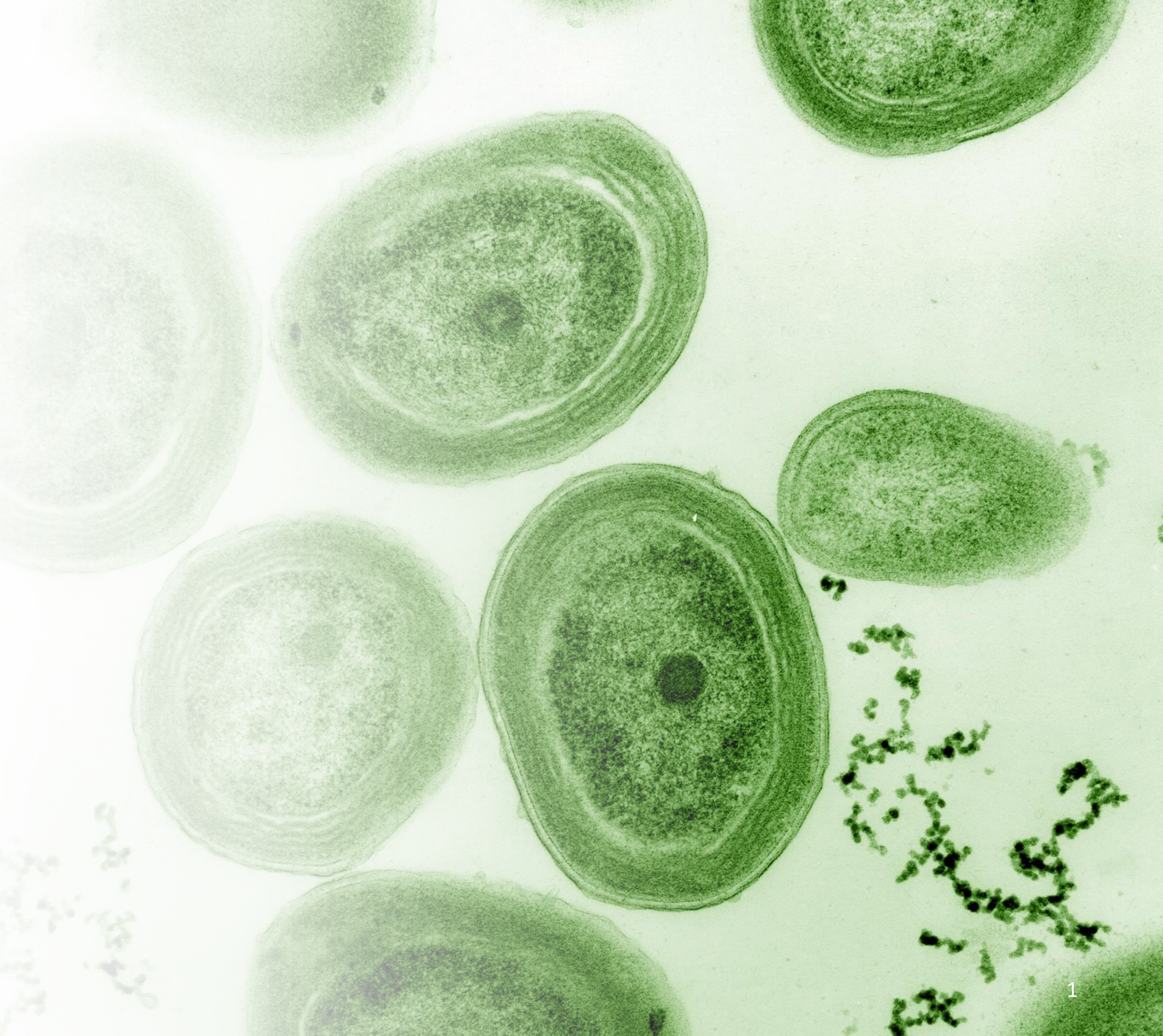


Image credit: [Thompson & Watson (CC0 1.0)]

Outline

- Part I: Roles of Autotrophs in Carbon Cycle
- Part II: Carboxysomes: A Group of Bacterial Microcompartments (BMCs)
- Part III: Applications of Cyanobacteria in Biotechnology
- Conclusion
- References

Part I:
Roles of Autotrophs in Carbon Cycle

Imbalanced Global Carbon Cycle

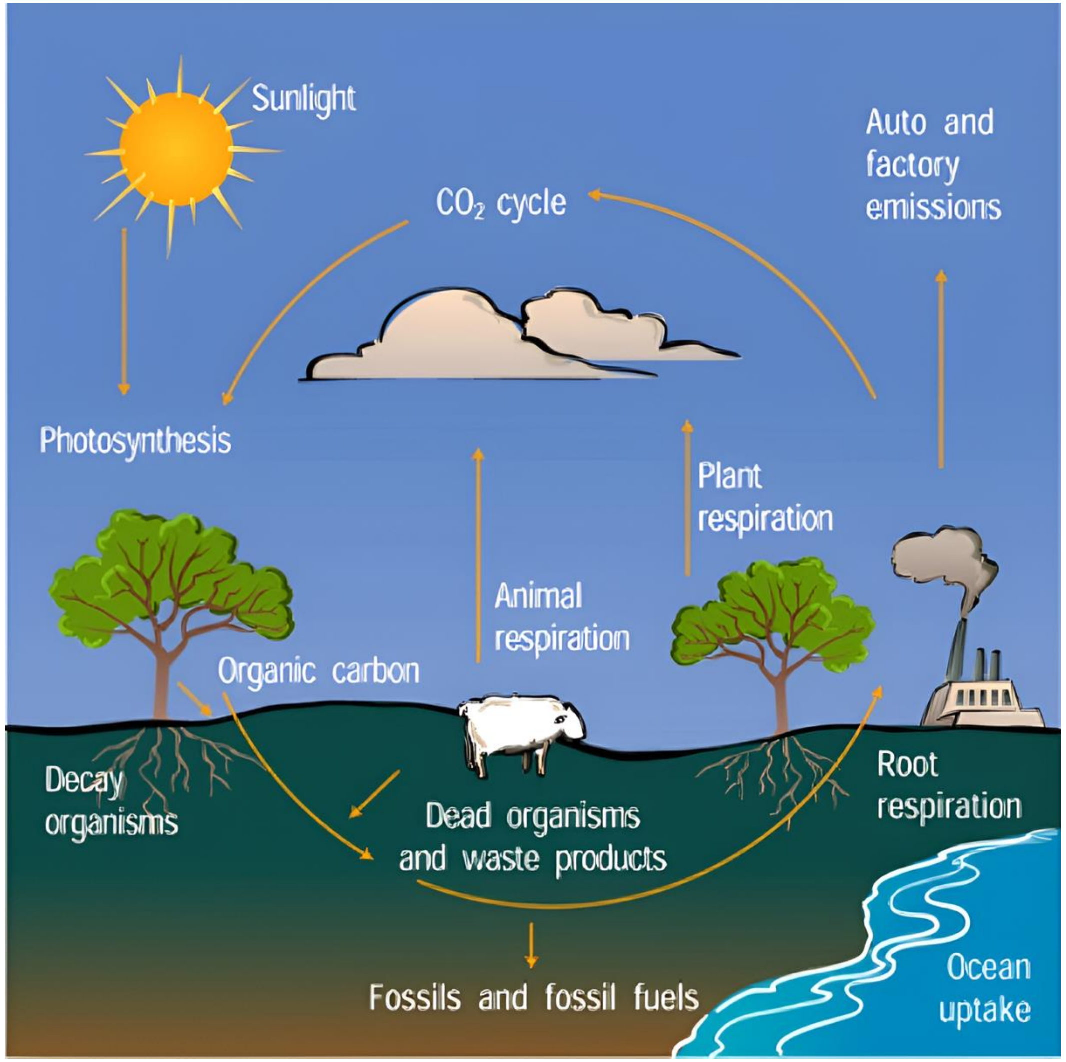


Image credit: [University Corporation for Atmospheric Research (2024)]

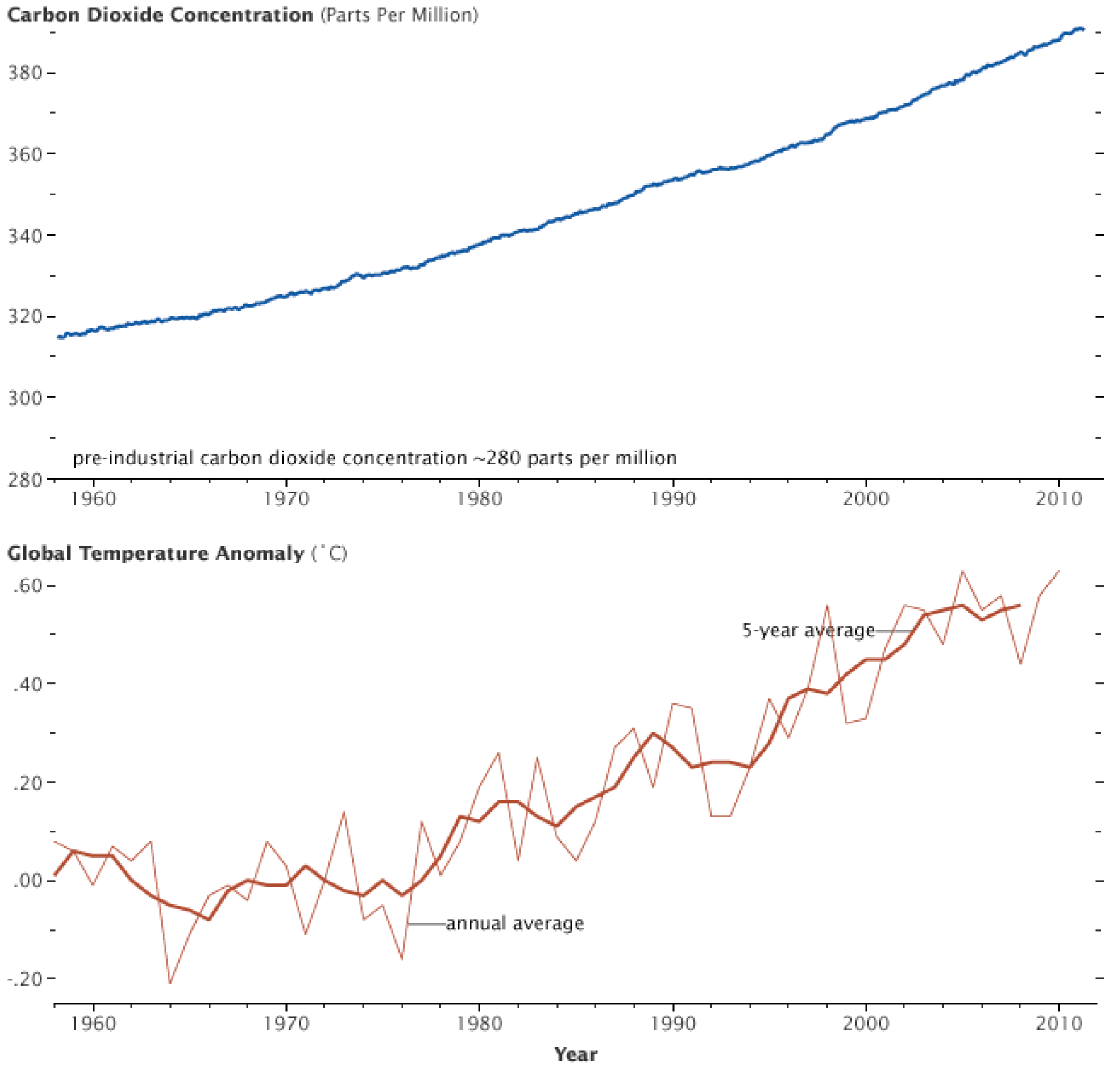
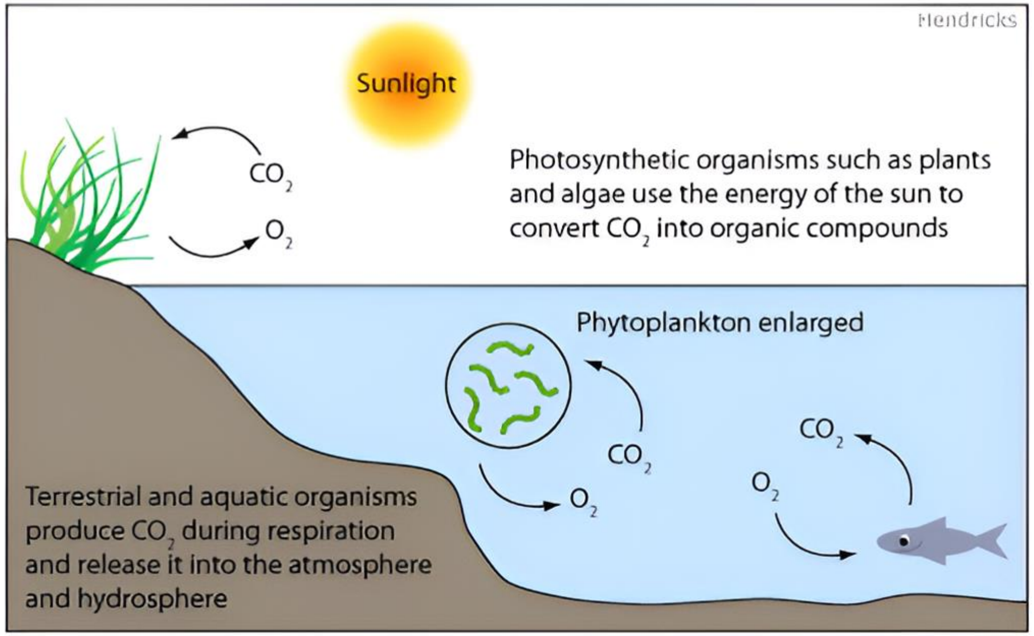


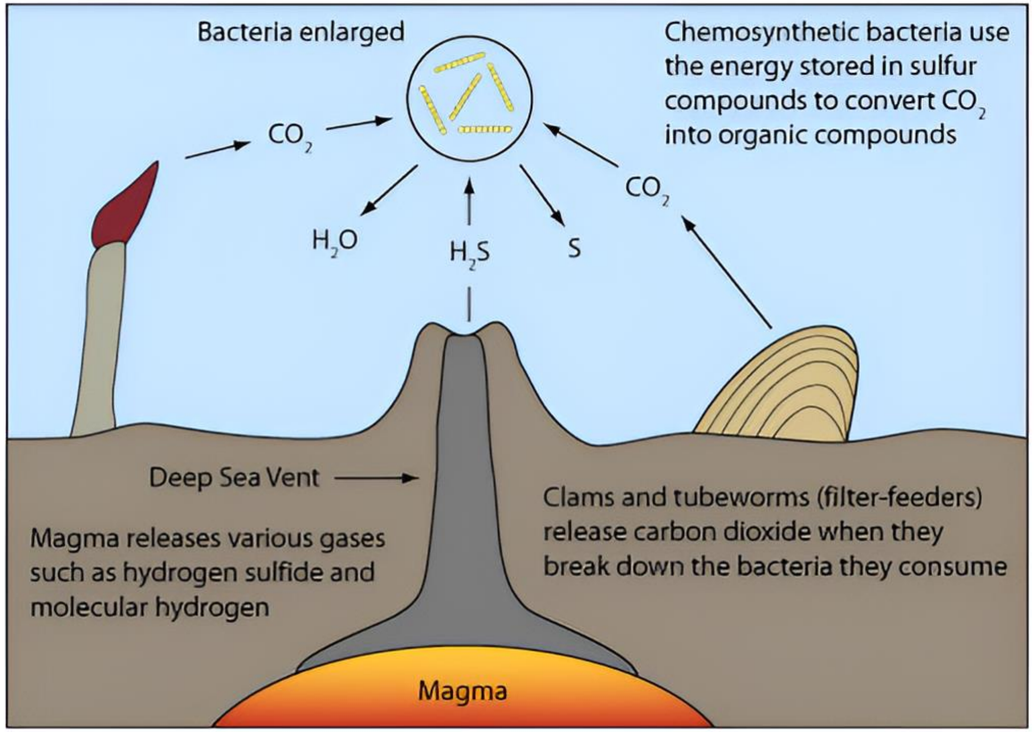
Image credit: [NASA Earth Observatory (2011)]

Autotrophs



Photosynthesis

E.g., terrestrial and aquatic organisms, including cyanobacteria



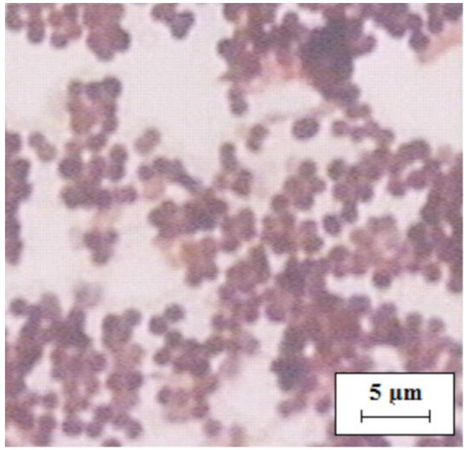
Chemosynthesis

E.g., sulphur bacteria, nitrifying bacteria, and methanogens

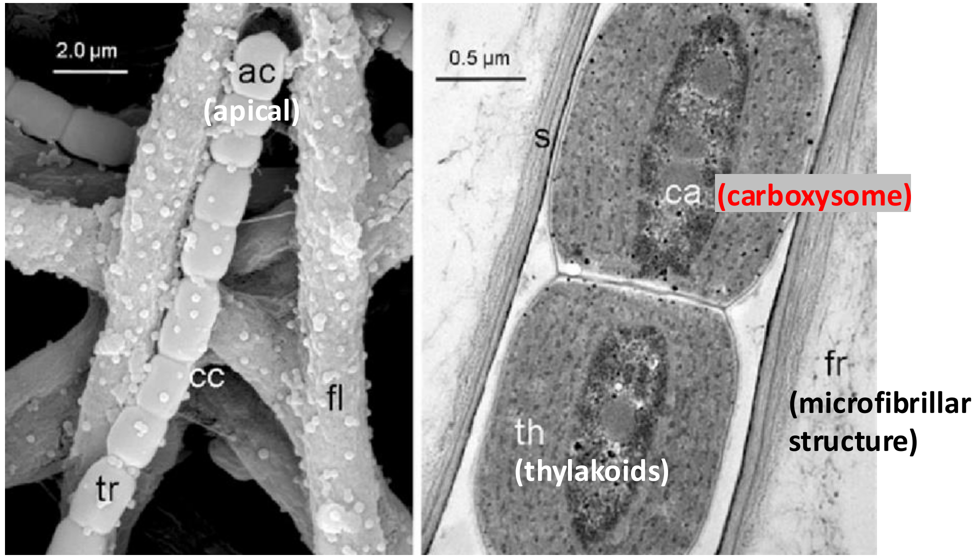
Cyanobacteria (aka. Blue-Green Algae)

- Gram-negative bacteria
- Share traits from both algae and bacteria
- Responsible for 20~30% of global carbon fixation [1]
- Convert up to 10% of sunlight energy into biomass [1]
    - 10× higher than terrestrial plant
    - 2× higher than algae

Oscillatoria and *Gleocapsa* orient towards light [2]



Gram-staining of *Synechocystis* spp. [3]



SEM (left) and TEM (right) of *Haloleptolyngbya alcalis* [4]

[1] Noreña-Caro & Benton, J. CO2 Util. **2018**, *28*, 335

[2] Berkshire Community College Bioscience Image Library (CC0 1.0)

[3] Kalaitzidou et al., HAICTA (conference). **2015**, 832

[4] Dadheech et al. Hydrobiologia. **2012**, *691*, 269

Cyanobacteria (aka. Blue-Green Algae)

Share traits from both algae and bacteria

Algae	Cyanobacteria	Bacteria
Eukaryote	Prokaryote	Prokaryote
Photosynthetic	Photosynthetic	Non-photosynthetic
Unicellular & multicellular	Unicellular & multicellular	Unicellular
Can be filamentous	Can be filamentous	Found in many diverse habitats
Found only in aquatic	Found in many diverse habitats	Capable of producing toxins
Does not produce toxins	Capable of producing toxins	Can cause increase of turbidity, not visible colonies
Can form visible colonies in water	Can form visible colonies in water	

Six CO_2-Fixation Pathways Used in Nature

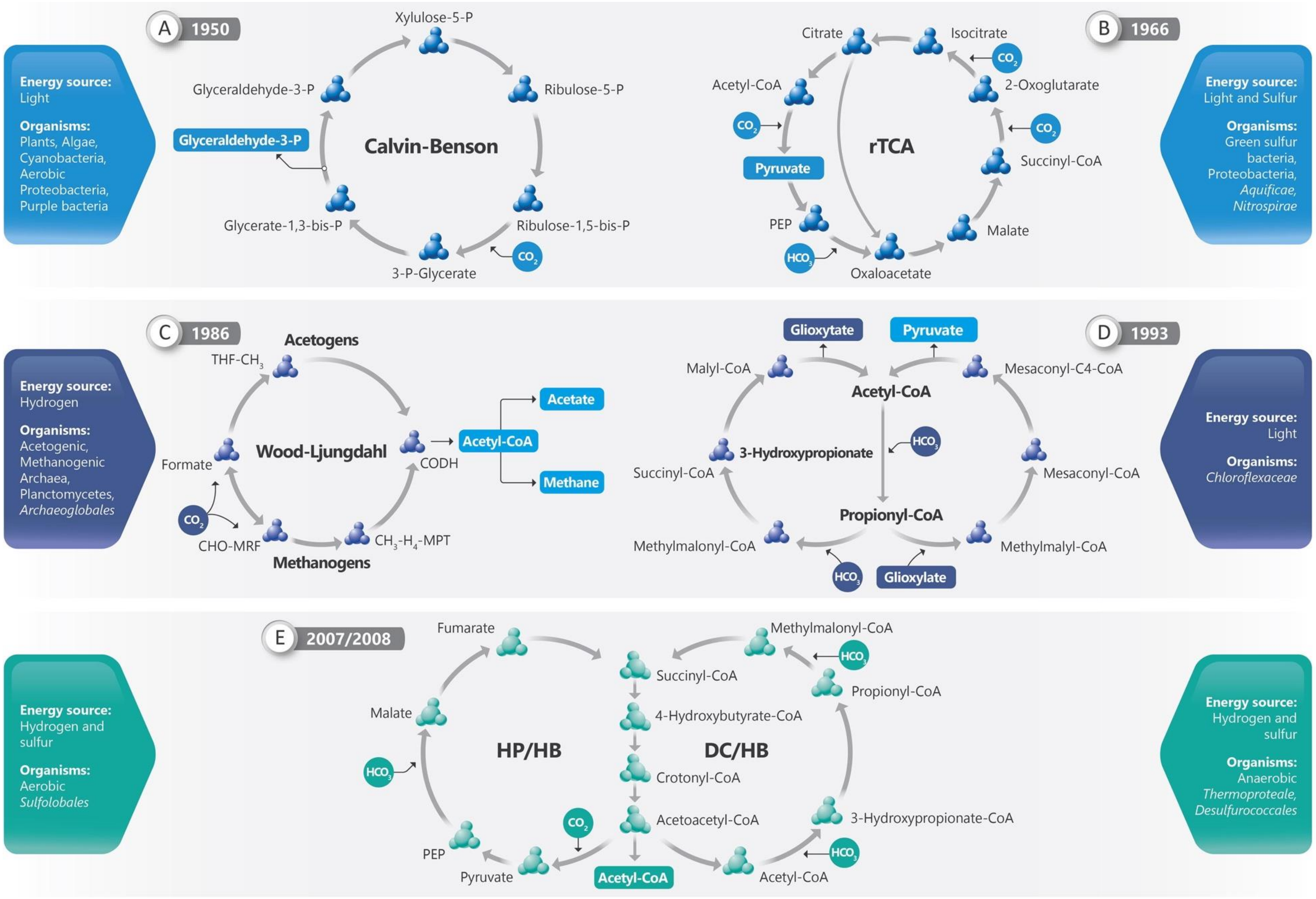


Image credit: Correa et al., J. Adv. Res. **2023**, *47*, 75 (CC BY 4.0)

Six CO_2-Fixation Pathways Used in Nature

The dominant pathway: Calvin-Benson-Bassham (CBB) cycle

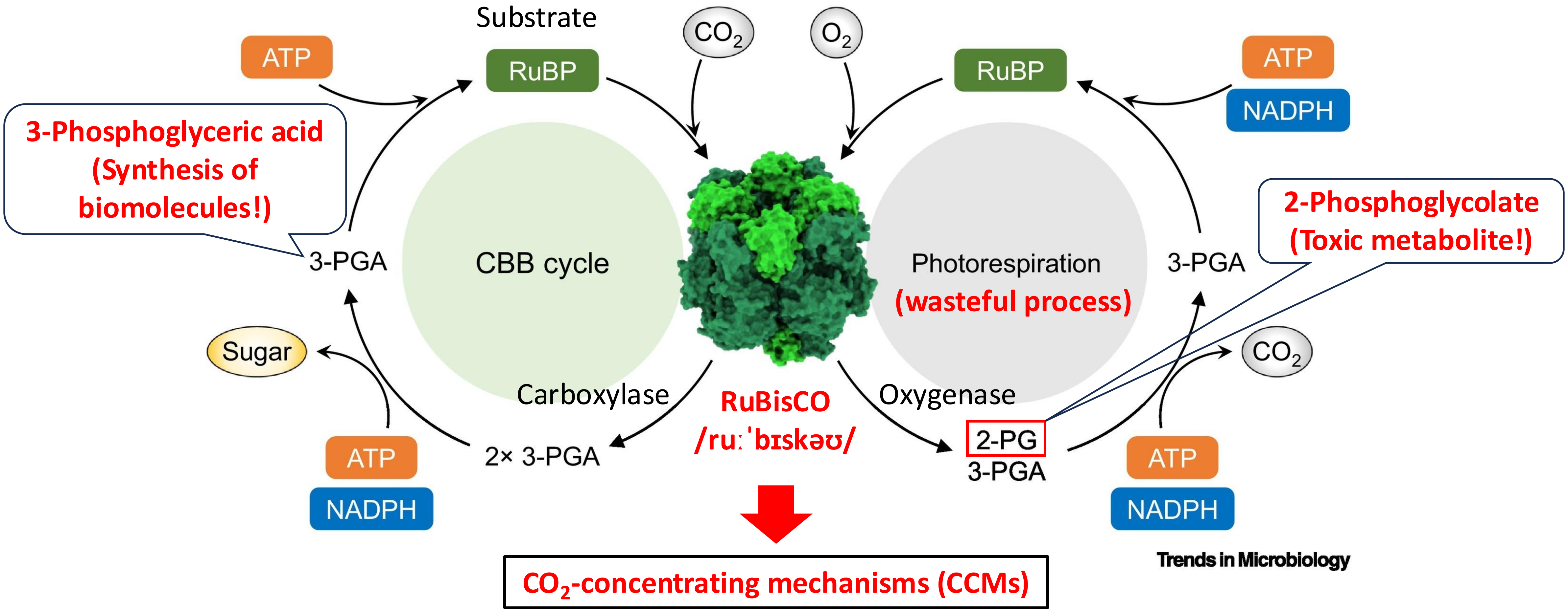
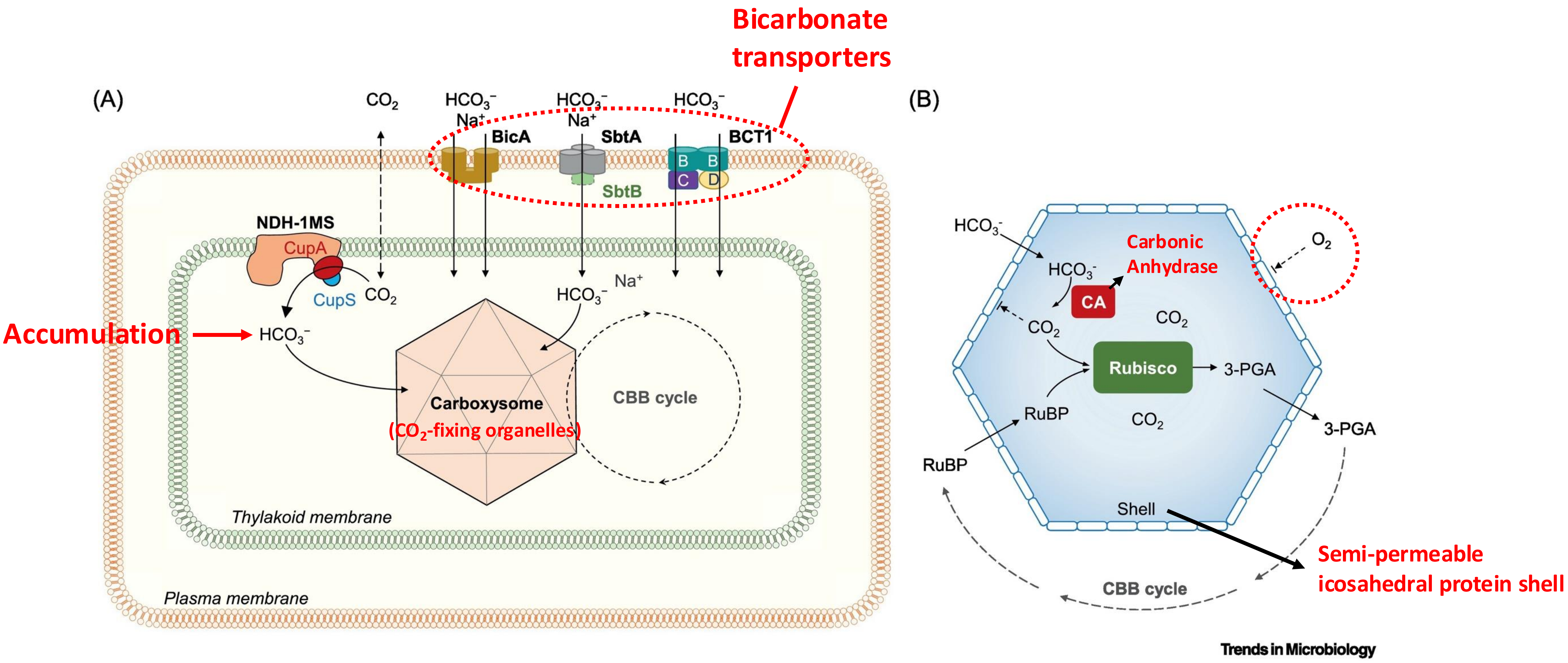


Image credit: Liu, Trends Microbiol. **2022**, *30*, 567

CO_2-Concentrating Mechanisms (CCMs) in Bacteria



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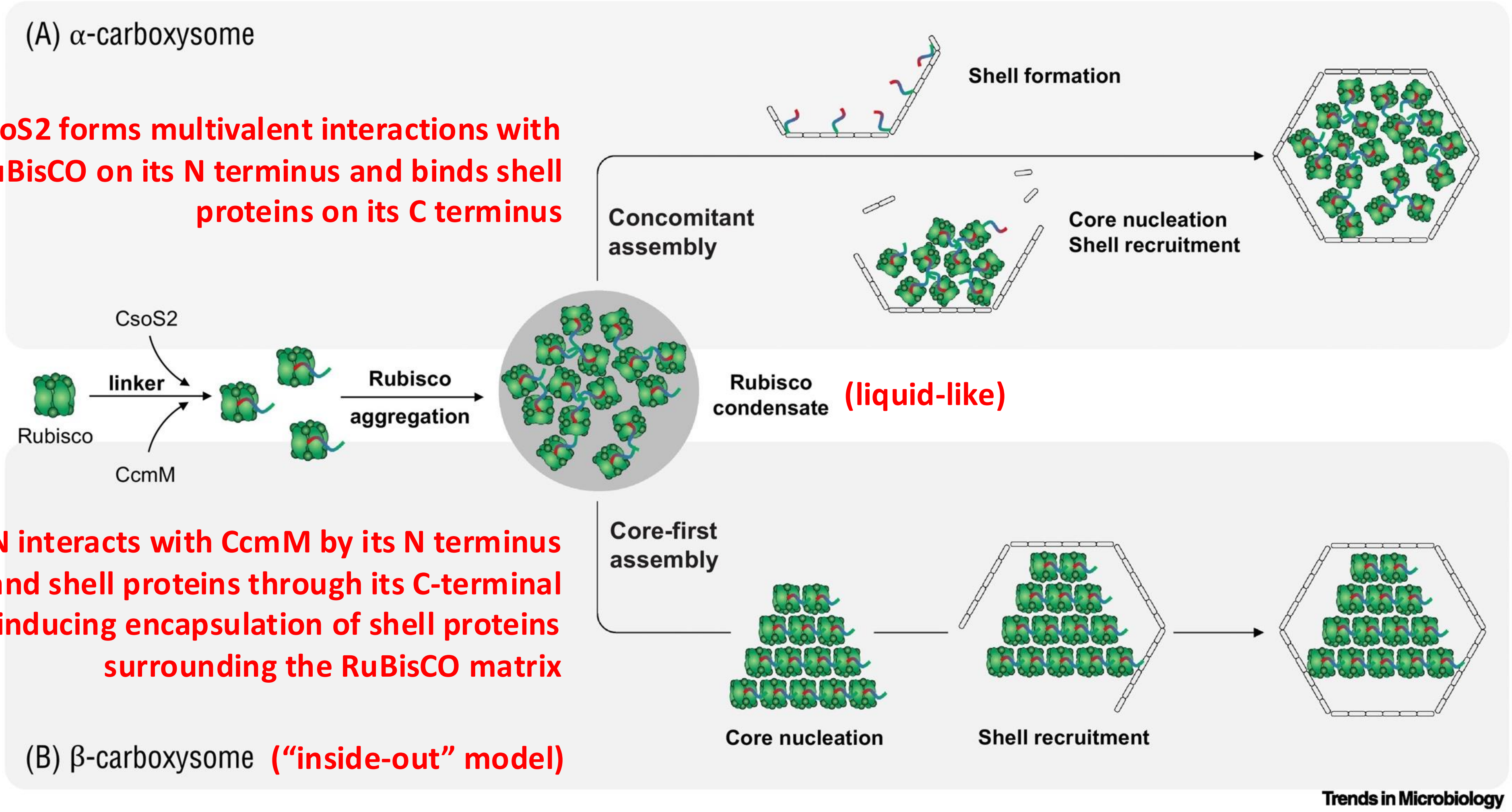
Part II:
Carboxysomes: A Group of Bacterial Microcompartments (BMCs)

Assembly Pathways of Carboxysomes



CsoS2 forms multivalent interactions with RuBisCO on its N terminus and binds shell proteins on its C terminus

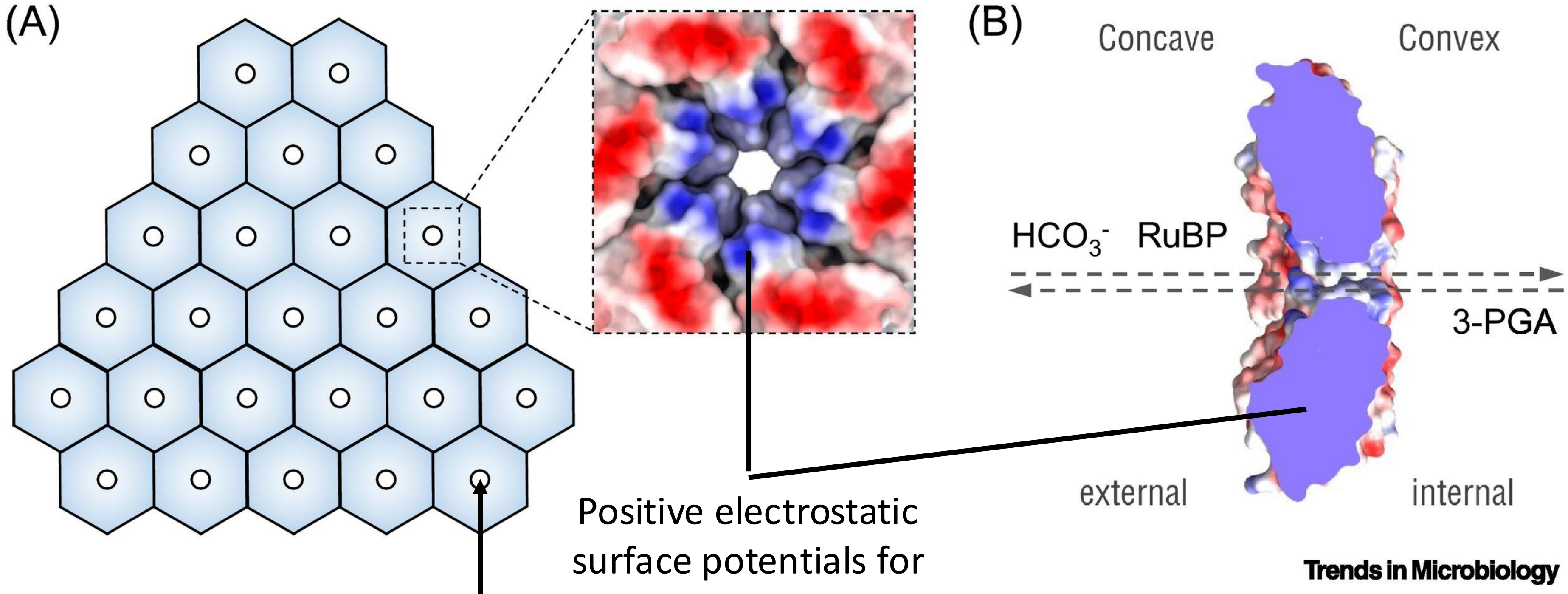
(liquid-like)

CcmN interacts with CcmM by its N terminus and shell proteins through its C-terminal peptide, inducing encapsulation of shell proteins surrounding the RuBisCO matrix

("inside-out" model)

Image credit: [Liu, Trends Microbiol. **2022**, *30*, 567]

Selective Permeability of Carboxysomes Shell Proteins



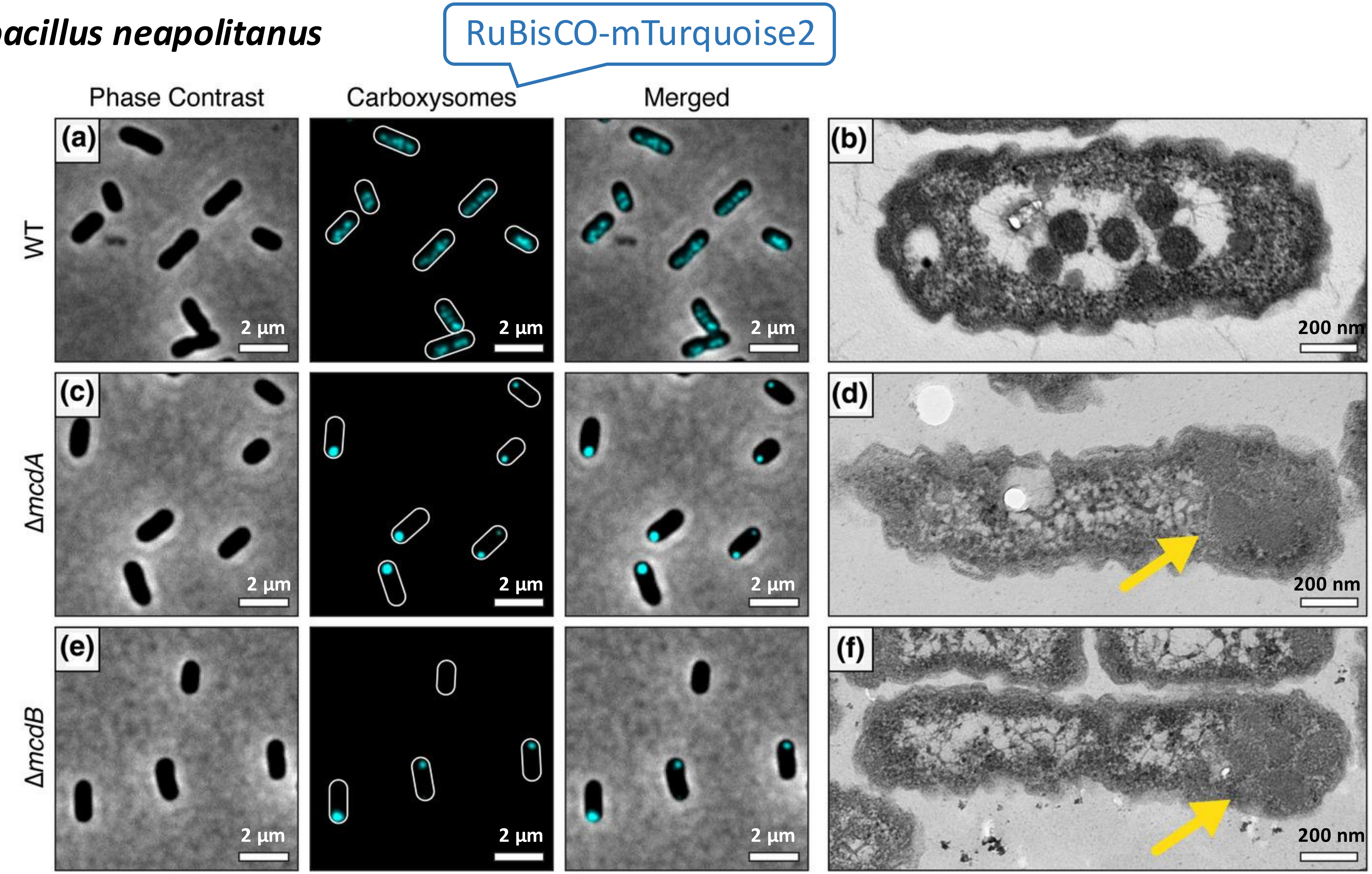
Positive electrostatic surface potentials for HCO_3^-

To mediate permeability

Image credit: Liu, Trends Microbiol. **2022**, *30*, 567

Positioning of Carboxysomes: McdAB Systems

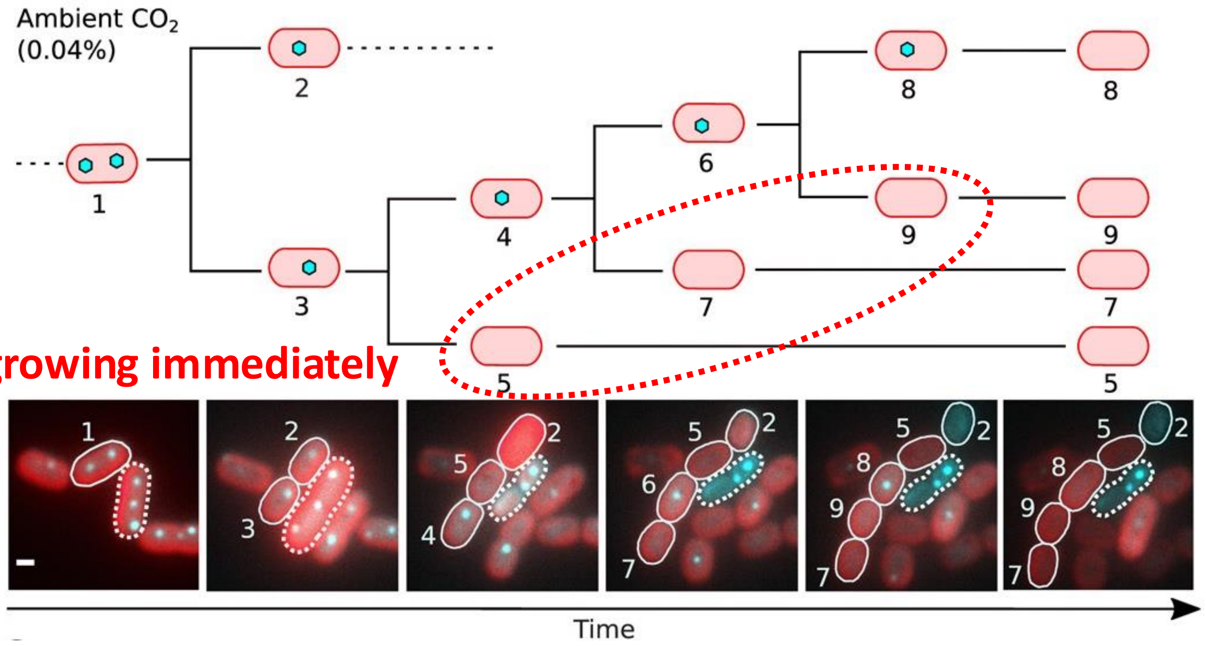
Halothiobacillus neapolitanus



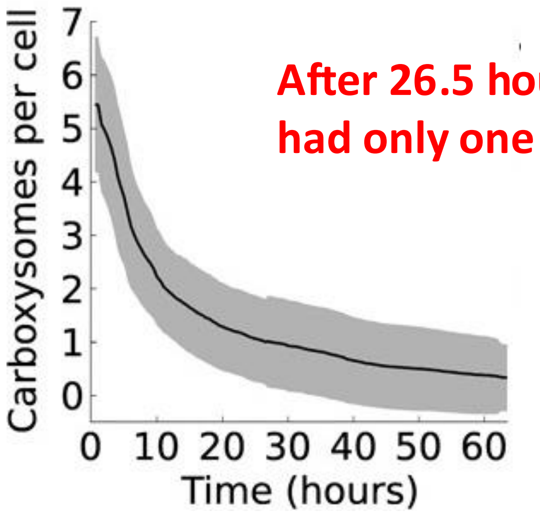
Cell pole region: Carboxysome biogenesis and degradation take place

Image credit: [MacCready et al., Mol. Microbiol. **2021**, *116*, 277 (CC BY 4.0)]

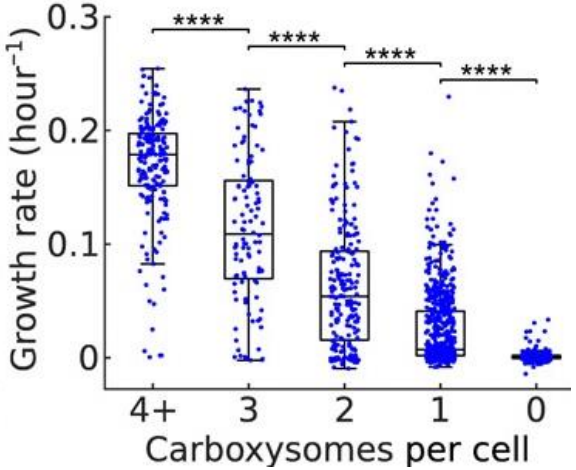
Carboxysomes: Pass from Mother to Daughter Cell



Stop growing immediately



After 26.5 hours, the cells had only one carboxysome

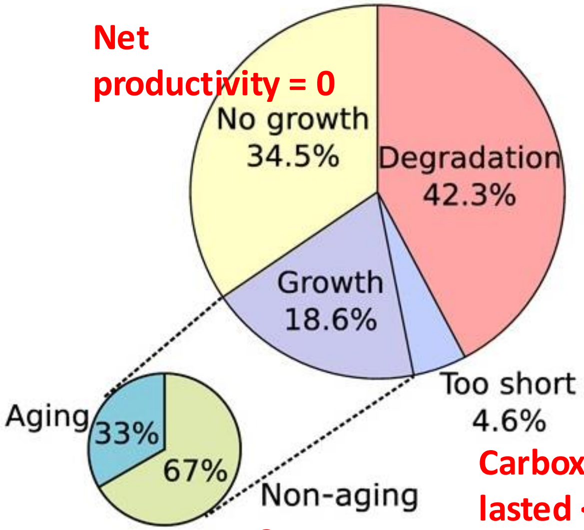


- Decrease in carboxysomes per cell from generation to generation
- The growth rate gradually decreased from generation to generation
- Cells without carboxysome stopped growing immediately

Carboxysomes: Different Activities

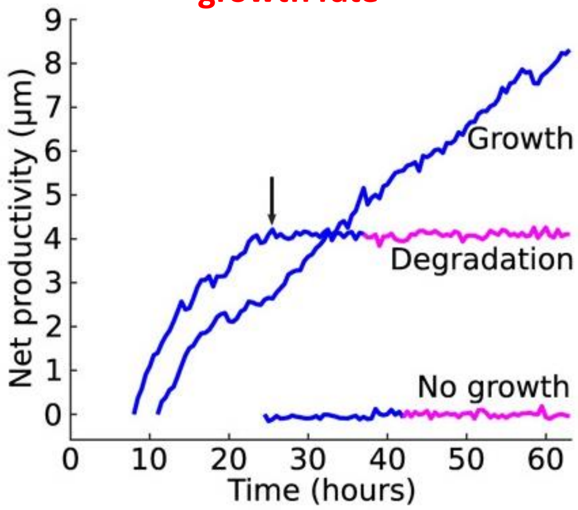
- Cell length was measured
- "Net productivity" was calculated, indicating the activity of a single carboxysome over time
- All biomass accumulation and cell growth can be attributed to a single carboxysome
- Tracked 452 single-carboxysome trees
- Clustered into 4 categories

Net productivity = 0



Carboxysome lasted <13 hours (excluded)

Constant growth rate



<u>An example of an ultra-productive carboxysome</u>

Reached a net productivity of 18 μm in less than 50 hours

Single-handedly supporting 7 generations of growth

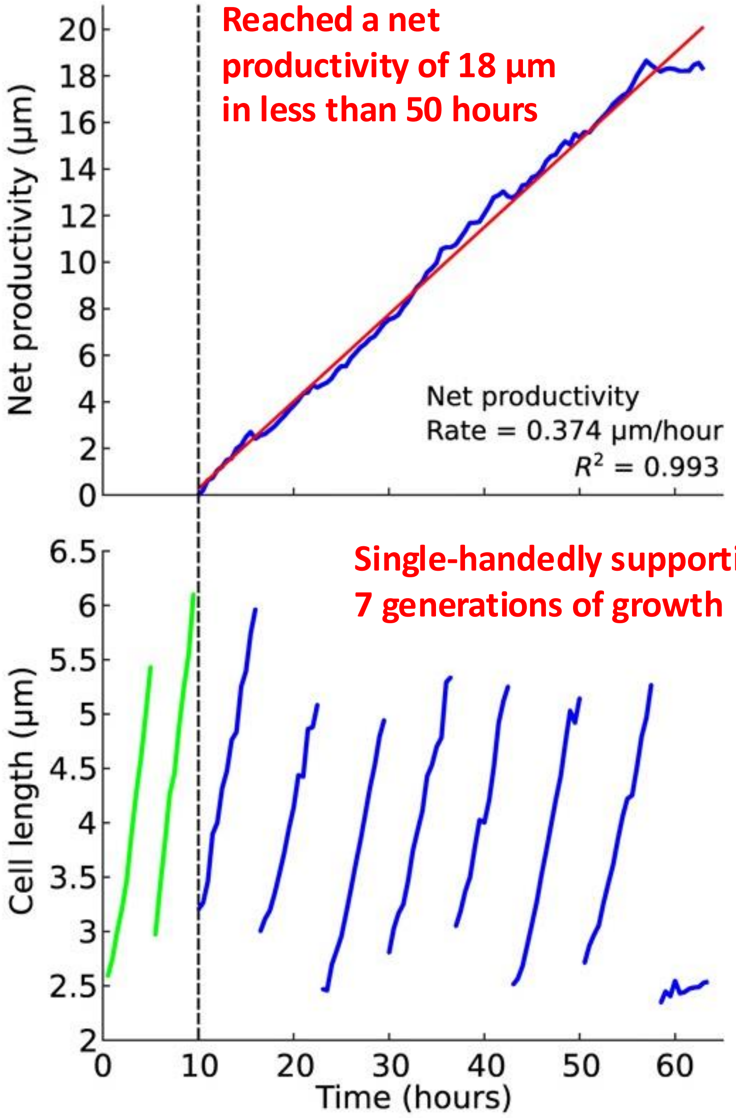
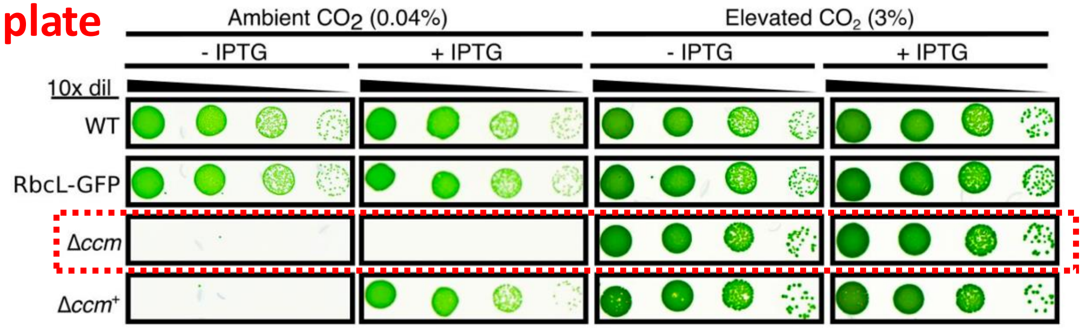
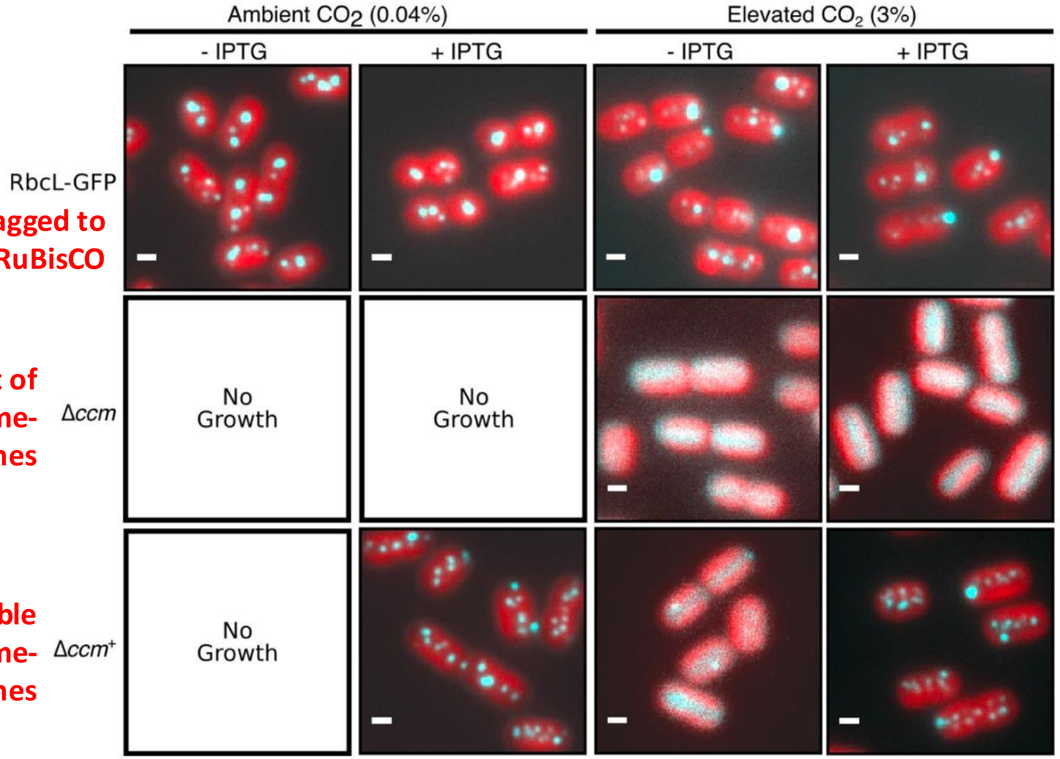


Image credit: Hill et al., Sci. Adv. **2020**, *6*, eaba1269 (CC BY 4.0 NC)

Carboxysomes: Importance in CO_2 Concentration and Cell Growth

Spot plate



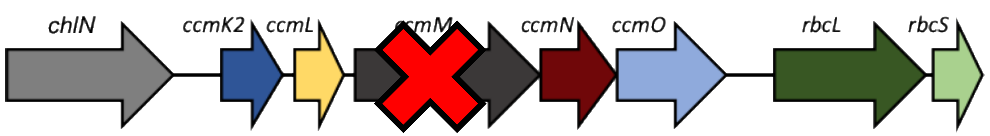


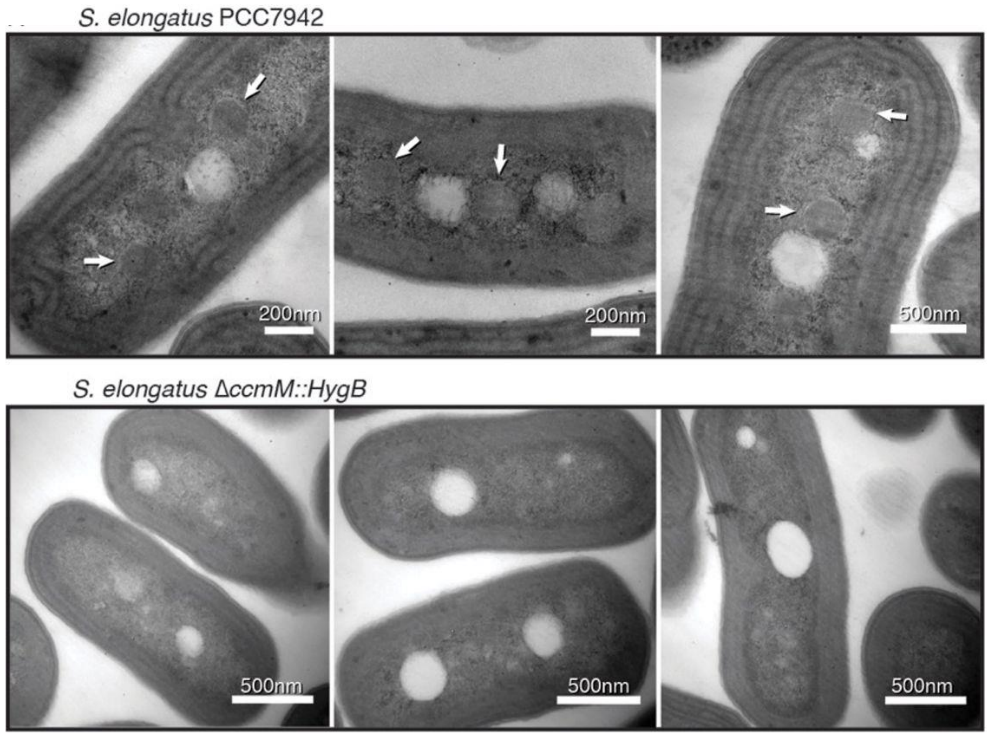
GFP tagged to RuBisCO

Knockout of carboxysome-related genes

IPTG-inducible carboxysome-related genes

Responsible for condensation of RuBisCO





Iconic hexagon shape was gone after knockout

Image credit: (Left) Hill et al., Sci. Adv. **2020**, *6*, eaba1269 (CC BY 4.0 NC)

(right) Niederhuber et al., MBoC. **2017**, *28*, 2734. (CC BY 3.0 NC-SA)

Part III:
Applications of Cyanobacteria in Biotechnology

Potential Applications

Advances in Biochemical Engineering/Biotechnology 183
Series Editors: Thomas Scheper · Roland Ulber

Katja Bühler
Pia Lindberg *Editors*

Cyanobacteria in Biotechnology

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Editorial: Exploring the Growing Role of Cyanobacteria in Industrial Biotechnology and Sustainability

David J. Lea-Smith[1*]   Tina C. Summerfield[2]   Daniel C. Ducat[3]
Xuefeng Lu[4]   Alistair J. McCormick[5]   Saul Purton[6]

Chapter 16

Cyanobacteria: Applications in Biotechnology

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marine drugs   MDPI

Review
Cyanobacteria—From the Oceans to the Potential Biotechnological and Biomedical Applications

Shaden A. M. Khalifa [1,*], Eslam S. Shedid [2], Essa M. Saied [3,4], Amir Reza Jassbi [5], Fatemeh H. Jamebozorgi [5], Mostafa E. Rateb [6], Ming Du [7], Mohamed M. Abdel-Daim [8], Guo-Yin Kai [9], Montaser A. M. Al-Hammady [10], Jianbo Xiao [11], Zhiming Guo [12] and Hesham R. El-Seedi [2,13,14,*]

microorganisms   MDPI

Review
Versatile Applications of Cyanobacteria in Biotechnology

Ewa Żymańczyk-Duda *, Sunday Ocholi Samson *, Małgorzata Brzezińska-Rodak and Magdalena Klimek-Ochab

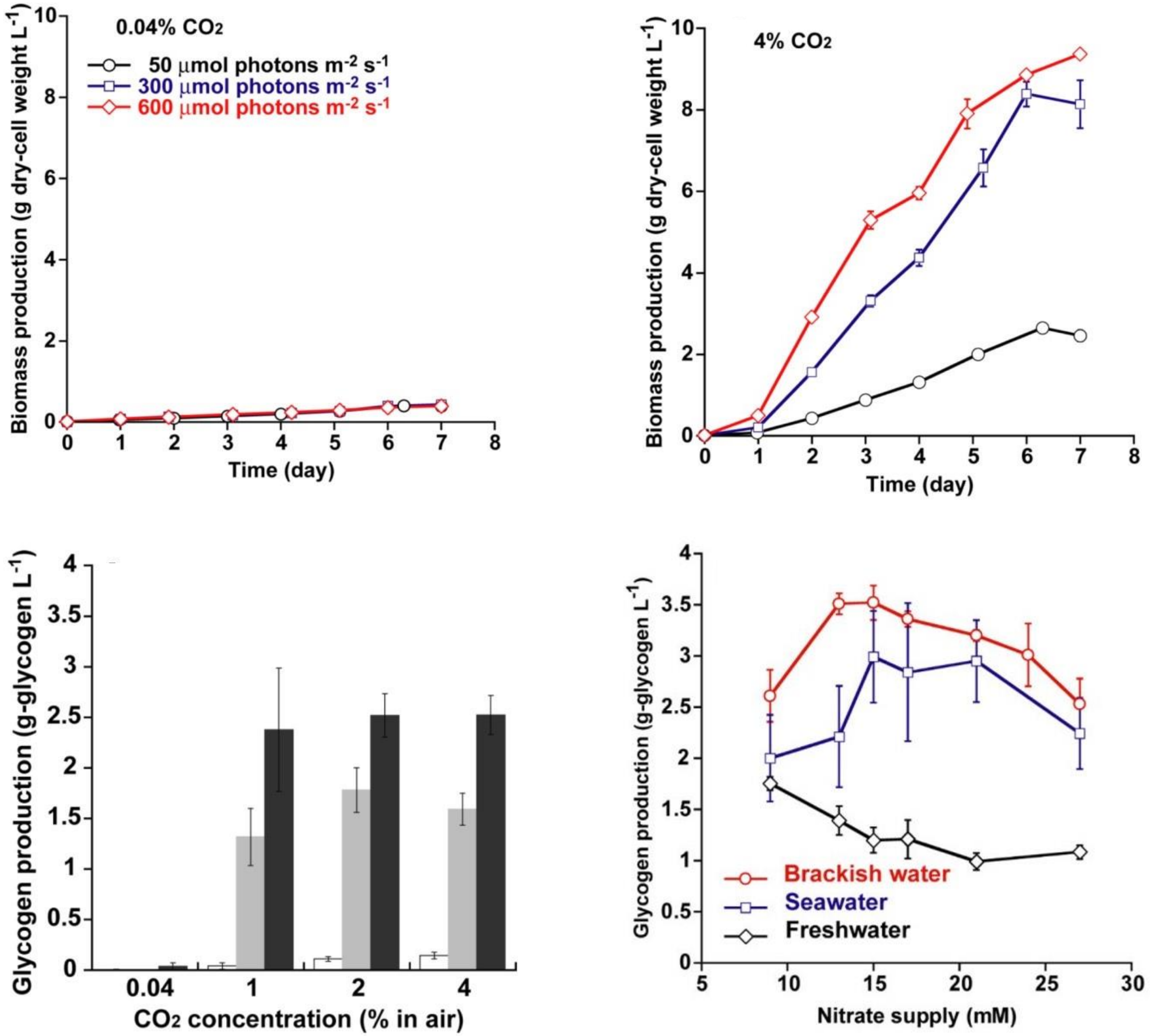
Example 1: Engineering Cyanobacteria for Carbohydrates Biosynthesis

- In comparison to heterotrophic hosts (i.e., *E. coli*, and yeast), native cyanobacteria are usually less tolerant to chemicals

- Synthesising soluble sugars are less or not toxic

- One example is glycogen which accumulated in cyanobacterial strains for carbon storage naturally, and is an important and promising feedstock material for producing biofuel

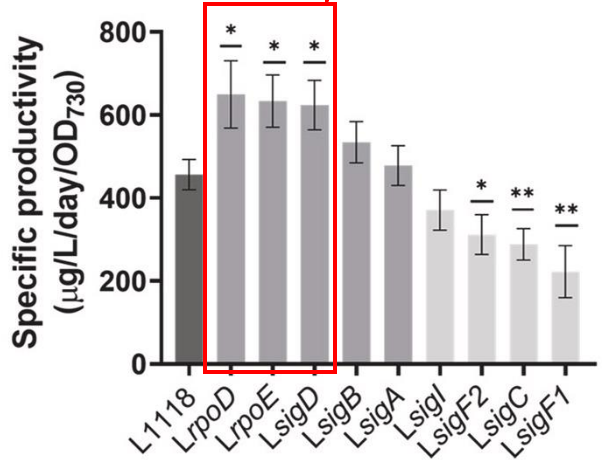
- Strain used: *Synechococcus* sp. strain PCC 7002



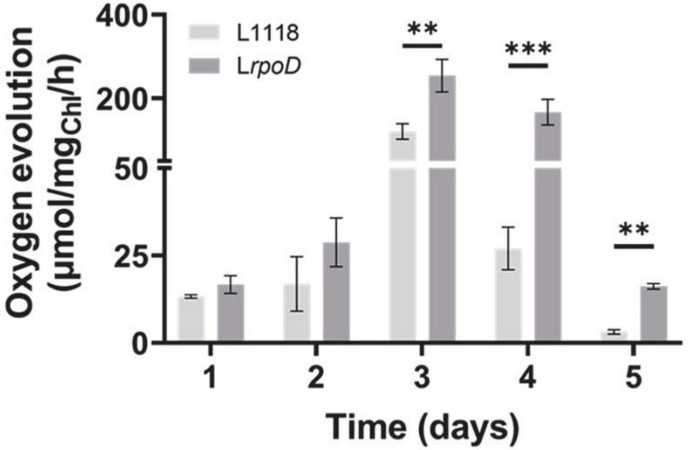
Example 2: Increasing Limonene Yield in Cyanobacteria for Bioeconomy

- Pathway enzyme engineering marginally increases cyanobacterial terpene (/ˈtɜːrpiːn/) production
- Sigma factor overexpression improves photosynthetic efficiency in cyanobacteria
- Enhanced photosynthesis results in high limonene production in cyanobacteria

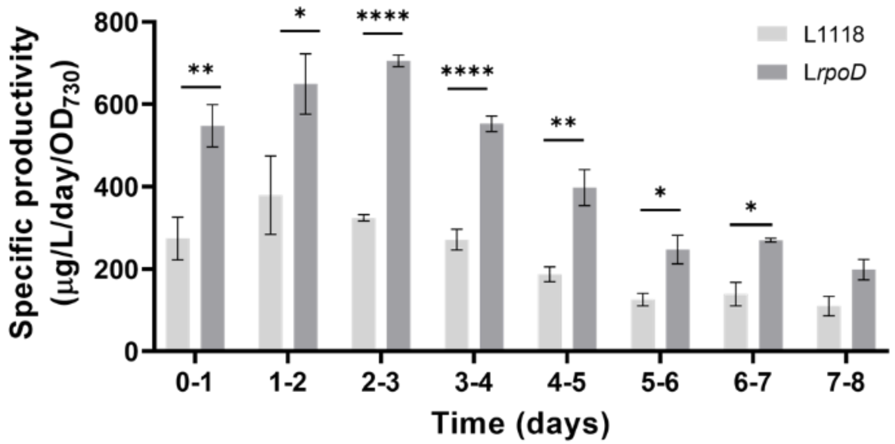
Limonene (/'lɪmənˌiːn/)



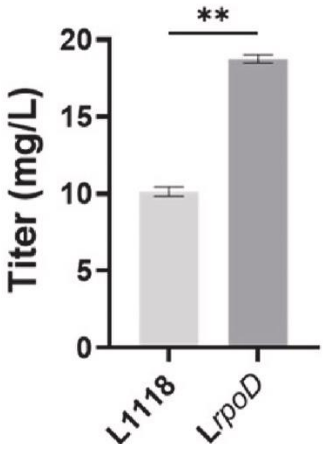
Higher oxygen evolution



Under 5% CO_2 environment



19 mg/L in 7 days



Credit: Shinde et al., Metab. Eng. Commun. **2022**, *14*, e00193 (CC BY 4.0 NC-ND)

Conclusion

1

Roles of Autotrophs in Carbon Cycle

- Autotrophs play an important role in carbon cycle
- Cyanobacteria are responsible for 20~30% global carbon fixation
- Achievable because of their CCMs

2

Carboxysomes: A Group of Bacterial Microcompartments

- Carboxysomes trap HCO_3^- for RuBisCO with the selective pore
- The role of McdAB system in positioning carboxysomes
- It passes from mother to daughter cell with varying activity dynamics

3

Applications of Cyanobacteria in Biotechnology

- Cyanobacteria are abundant sources of bio-active compounds
- Recent studies focus on identifying the strains and growth conditions suitable for large-scale cultivation
- It has huge potential for bio-economy

Thank you!

Do you have any questions?

References

Aikawa, S., Nishida, A., Ho, S. H., Chang, J. S., Hasunuma, T., & Kondo, A. (2014). Glycogen production for biofuels by the euryhaline cyanobacteria *Synechococcus* sp. strain PCC 7002 from an oceanic environment. Biotechnology for Biofuels, *7*, 1-8. https://doi.org/10.1186/1754-6834-7-88

Berkshire Community College Bioscience Image Library. (n.d.). *Video: The Cyanobacteria: Oscillatoria and Gleocapsa*. Flickr. https://www.flickr.com/photos/146824358@N03/33595507874

Correa, S. S., Schultz, J., Lauersen, K. J., & Rosado, A. S. (2023). Natural carbon fixation and advances in synthetic engineering for redesigning and creating new fixation pathways. *Journal of Advanced Research*, *47*, 75-92. https://doi.org/10.1016/j.jare.2022.07.011

Dadheech, P. K., Mahmoud, H., Kotut, K., & Krienitz, L. (2012). *Haloleptolyngbya alcalis* gen. et sp. nov., a new filamentous cyanobacterium from the soda lake Nakuru, Kenya. *Hydrobiologia*, *691*, 269-283. https://doi.org/10.1007/s10750-012-1080-6

Earth Science Education (ERESE) (n.d.) *Difference Between Photosynthesis and Chemosynthesis*. https://earthref.org/ERDA/582/

Hill, N. C., Tay, J. W., Altus, S., Bortz, D. M., & Cameron, J. C. (2020). Life cycle of a cyanobacterial carboxysome. *Science Advances*, *6*(19), eaba1269. https://doi.org/10.1126/sciadv.aba1269

Kalaitzidou, M., Filliousis, G., Petridou, E., Economou, V., Theodoridis, A., & Aggelidis, P. (2015, September). Isolation of Toxic Marine Cyanobacteria and Detection of Microcystins in Thermaikos Gulf in Central Macedonia in Greece. In *HAICTA*(pp. 832-841).

References

Liu, L. N. (2022). Advances in the bacterial organelles for CO_2 fixation. *Trends in Microbiology*, *30*(6), 567-580. https://doi.org/10.1016/j.tim.2021.10.004

MacCready, J. S., Tran, L., Basalla, J. L., Hakim, P., & Vecchiarelli, A. G. (2021). The McdAB system positions α-carboxysomes in proteobacteria. *Molecular Microbiology*, *116*(1), 277-297. https://doi.org/10.1111/mmi.14708

NASA Earth Observatory. (n.d.). *The Carbon Cycle*. https://earthobservatory.nasa.gov/features/CarbonCycle

Niederhuber, M. J., Lambert, T. J., Yapp, C., Silver, P. A., & Polka, J. K. (2017). Superresolution microscopy of the β-carboxysome reveals a homogeneous matrix. *Molecular Biology of the Cell*, *28*(20), 2734-2745. https://doi.org/10.1091/mbc.e17-01-0069

Norena-Caro, D., & Benton, M. G. (2018). Cyanobacteria as photoautotrophic biofactories of high-value chemicals. *Journal of CO2 Utilization*, *28*, 335-366. https://doi.org/10.1016/j.jcou.2018.10.008

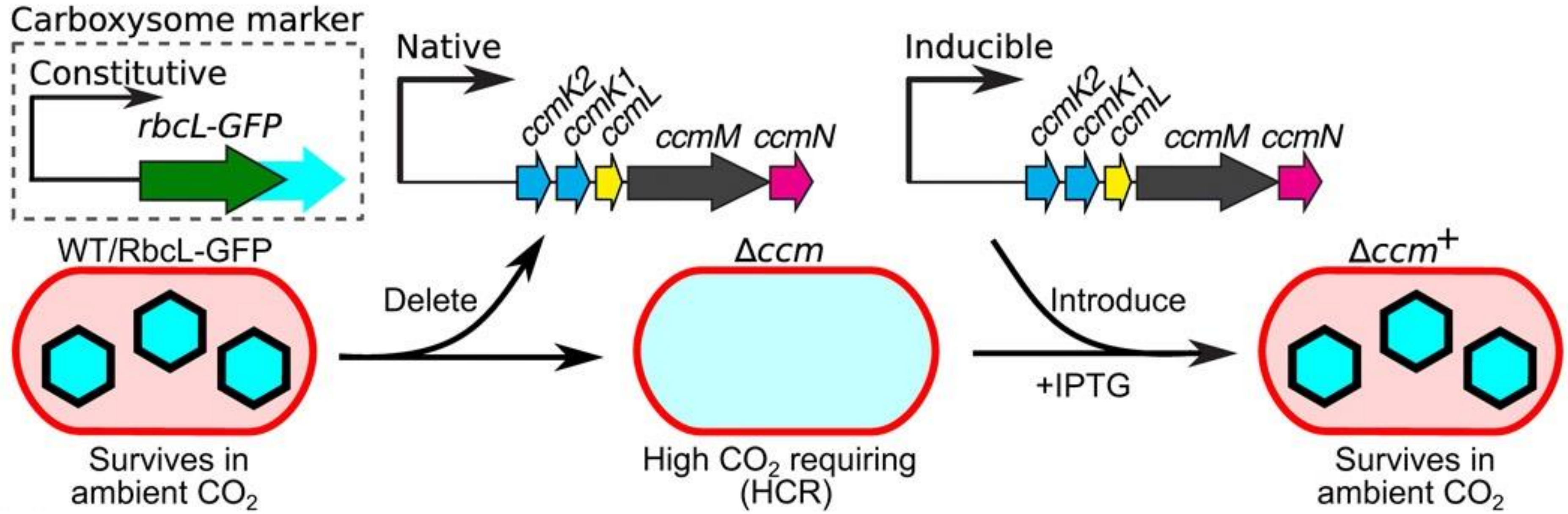
Shinde, S., Singapuri, S., Jiang, Z., Long, B., Wilcox, D., Klatt, C., . . . & Wang, X. (2022). Thermodynamics contributes to high limonene productivity in cyanobacteria. *Metabolic Engineering Communications*, *14*, e00193. https://doi.org/10.1016/j.mec.2022.e00193

Thompson & Watsons (n.d.). *MED4_artificial green coloring*. Flickr. https://www.flickr.com/photos/prochlorococcus/33750937901

University Corporation for Atmospheric Research (n.d.). *Biogeochemical Cycles | Center for Science Education*. UCAR. https://scied.ucar.edu/learning-zone/earth-system/biogeochemical-cycles

Supplementary Information

Carboxysomes: Pass from Mother to Daughter Cell



Remove IPTG to stop the biogenesis of carboxysome

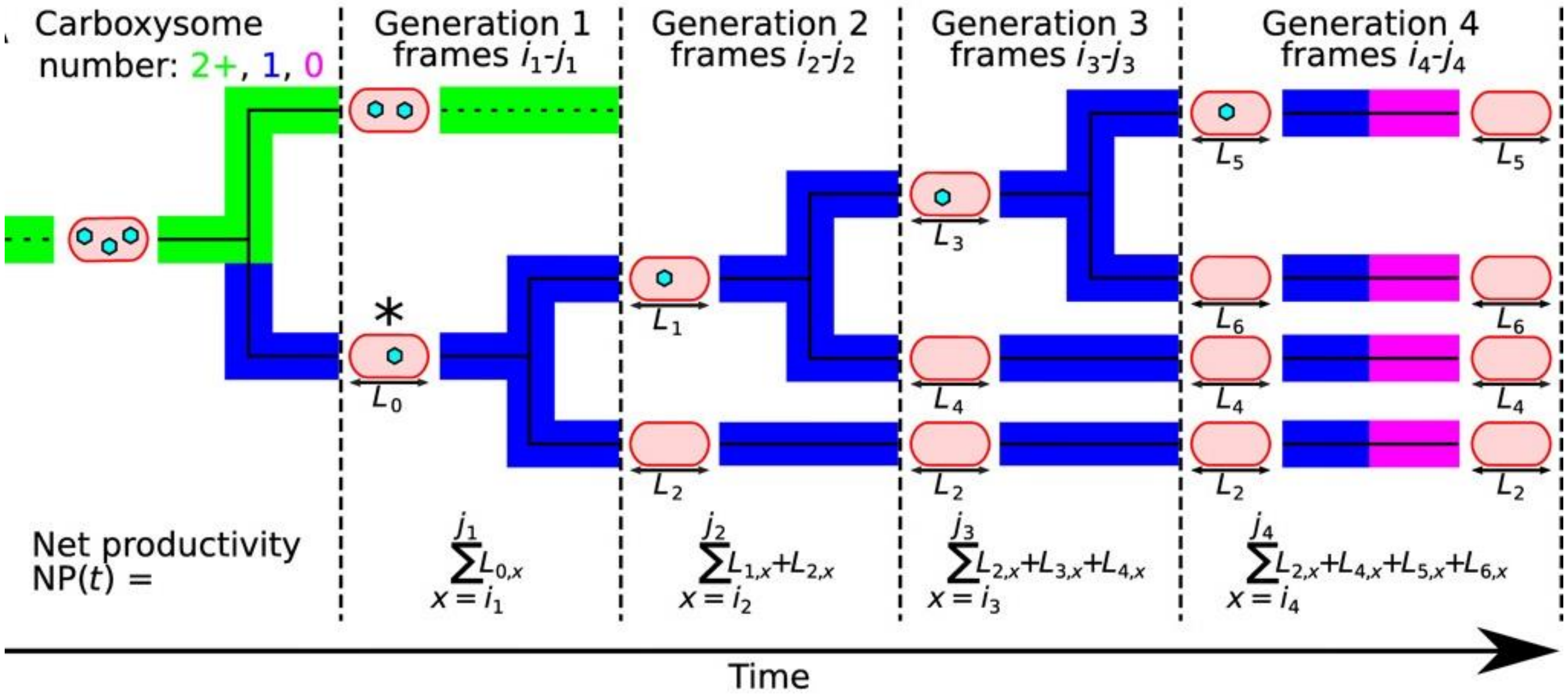
Constitutively expressed RbcL-GFP allows for RuBisCO visualisation

The native *ccm* operon (*ccmK2K1LMN*) was knocked out, producing the HCR strain *Δccm*

An IPTG-inducible version of the *ccm* operon was reintroduced to create the *Δccm*$^+$ strain, resulting in IPTG-dependent carboxysome expression and growth rescue in ambient CO_2

Carboxysomes: Different Activities

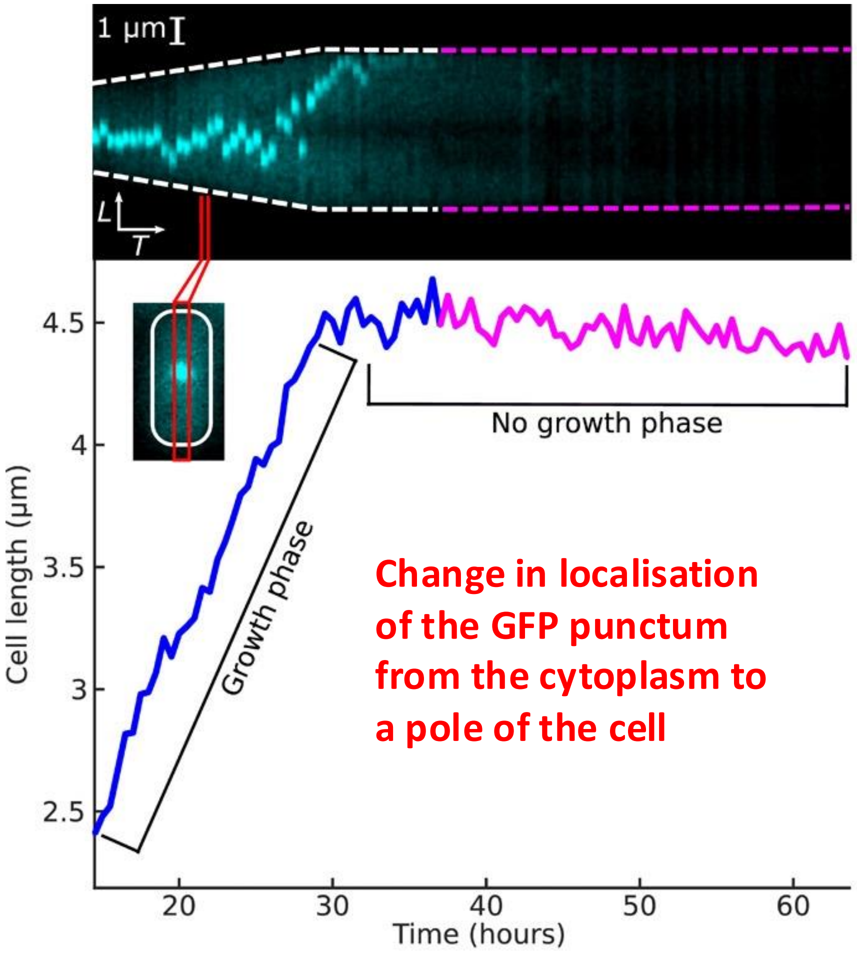
Diagram of a Δ*ccm*⁺ family tree



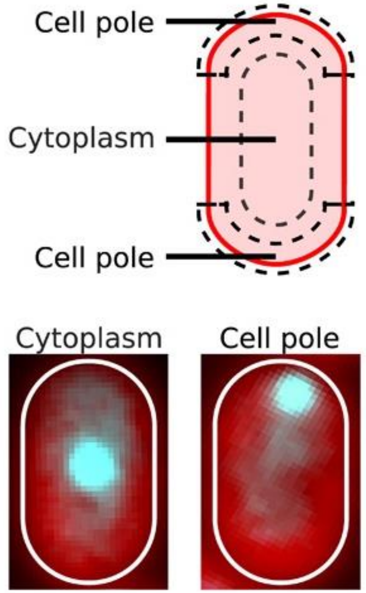
- The single-carboxysome tree starts at the cell indicated with an asterisk
- Net productivity is calculated for each frame of the single-carboxysome tree
- Green, blue, and magenta colours indicate 2+, 1, or 0 carboxysomes, respectively, present at that time in the tree

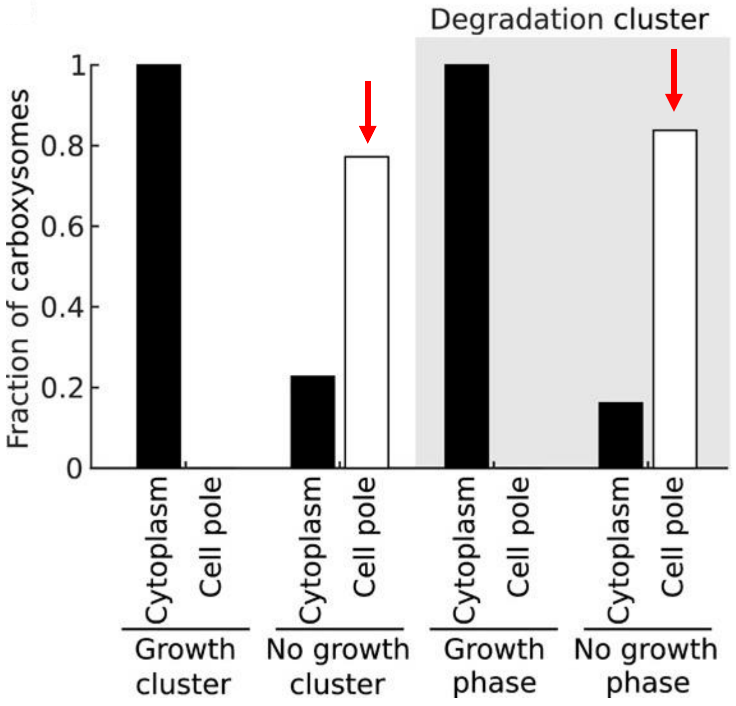
Image credit: Hill et al., Sci. Adv. **2020**, *6*, eaba1269 (CC BY 4.0 NC)

Loss of Carboxysome Activity: Shell Breakage



Change in localisation of the GFP punctum from the cytoplasm to a pole of the cell





- Rapid diffusion of CO_2 into the cytoplasm
- Carboxysome lumen is no longer distinct from the cytoplasm
- In other words, the CCMs are abolished

Image credit: Hill et al., Sci. Adv. **2020**, *6*, eaba1269 (CC BY 4.0 NC)