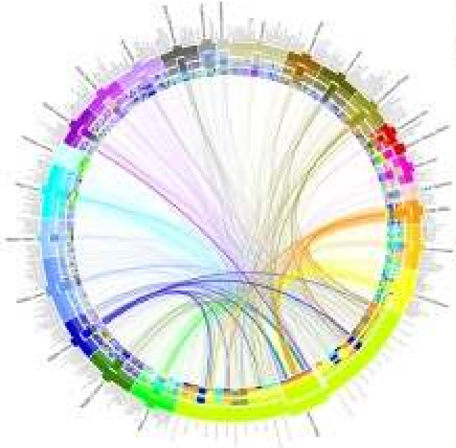
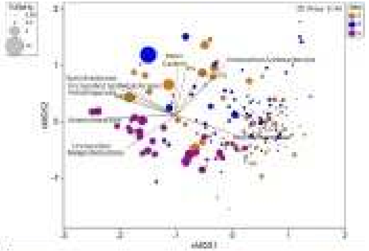
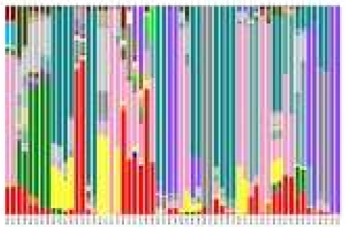
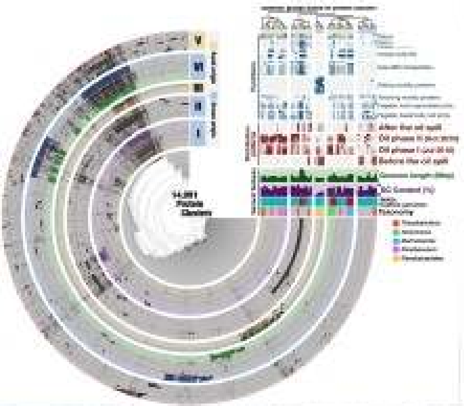
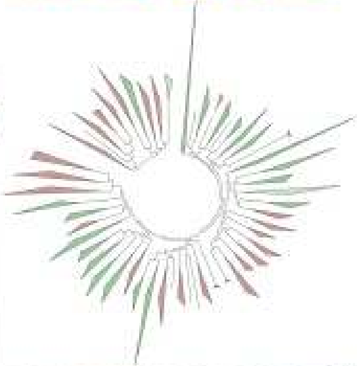


GIOVANNELLI LAB

DIPARTIMENTO DI BIOLOGIA

University of Naples Federico II

Environmental Metagenomic

aa 2020-2021

CARBON FIXATION PATHWAYS AND LIMITS OF LIFE

Donato Giovannelli

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The environment and other organisms as *ecosystem(s)*

ecosystem

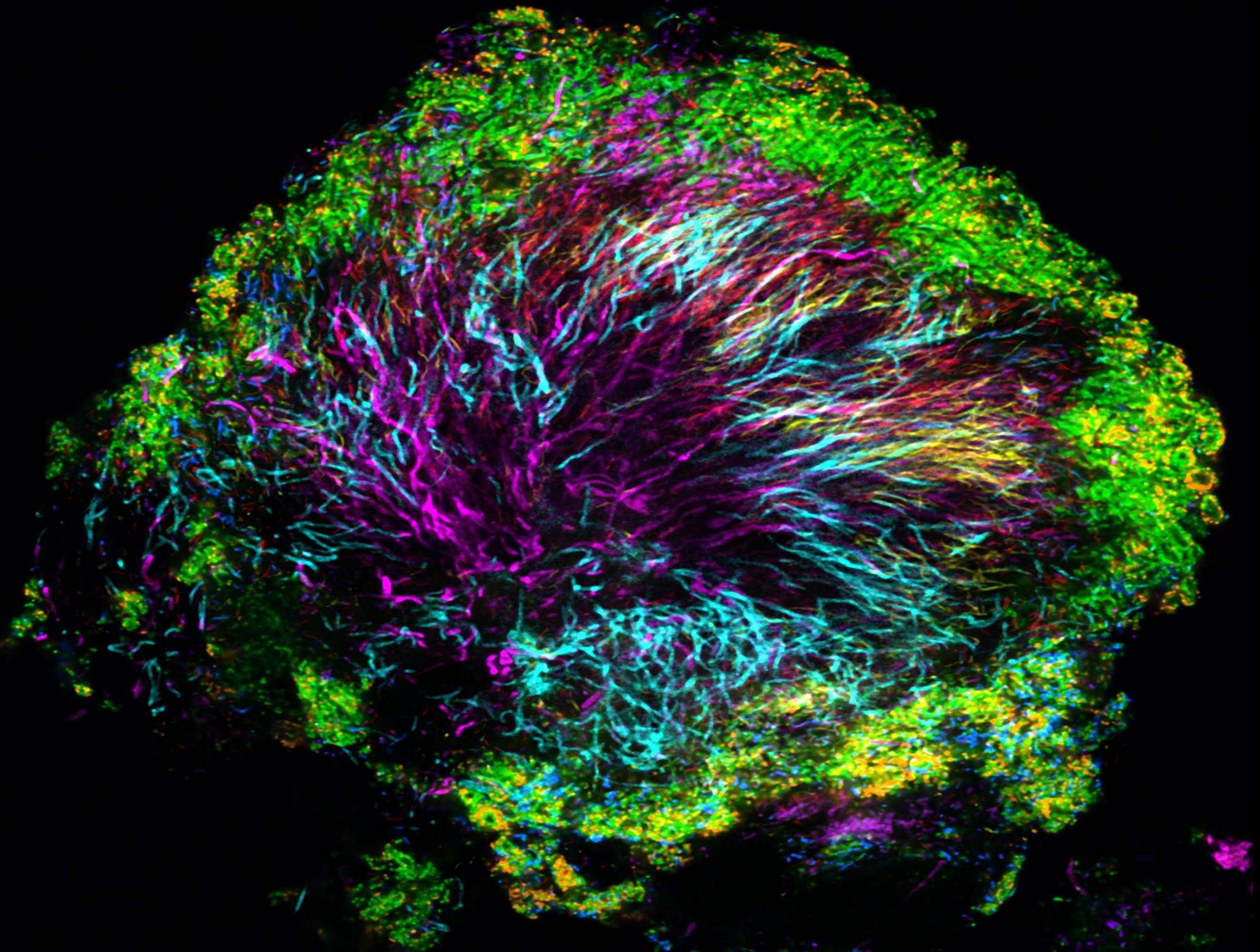
/ˈiːkəʊsɪstəm/

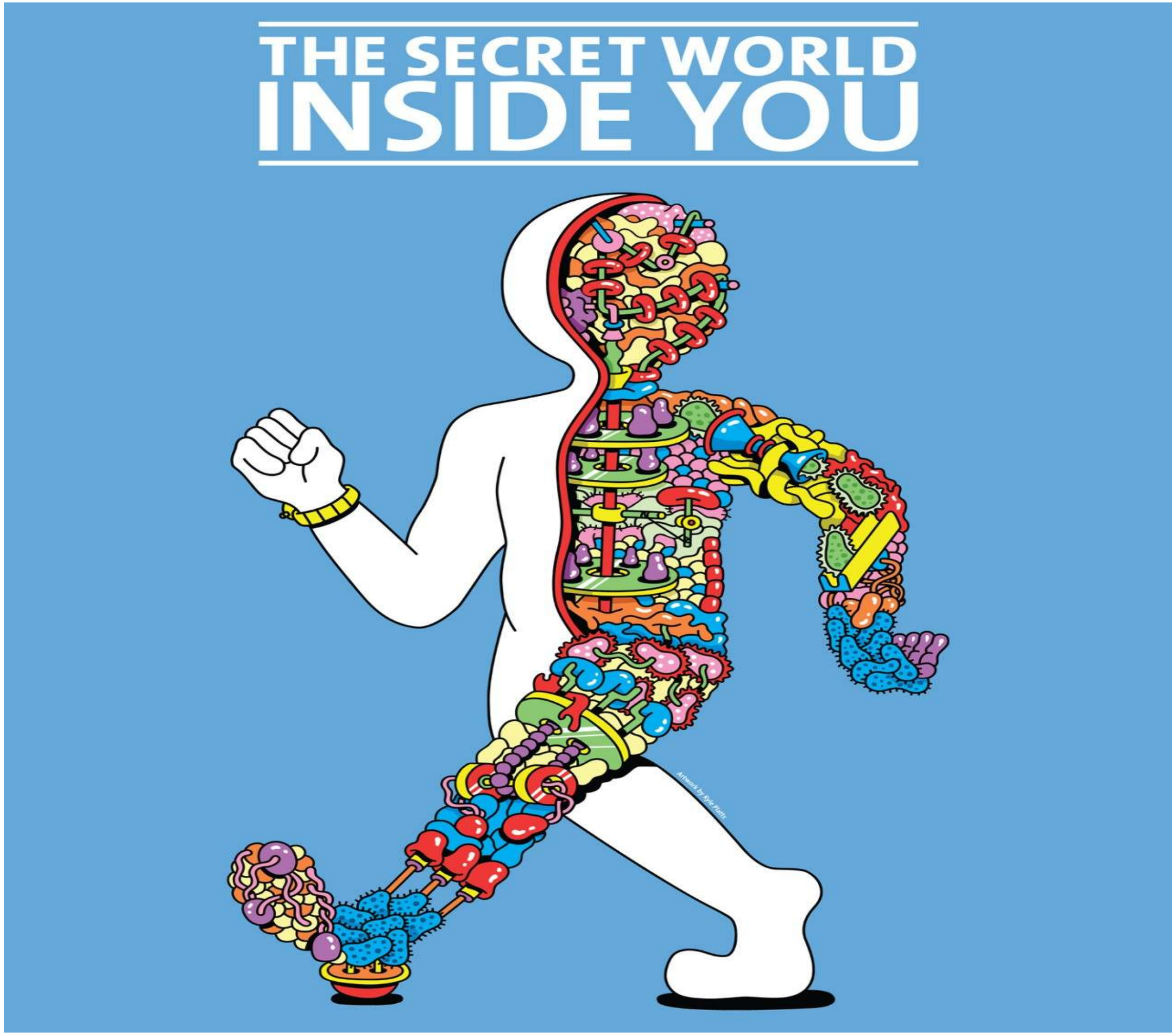
Noun

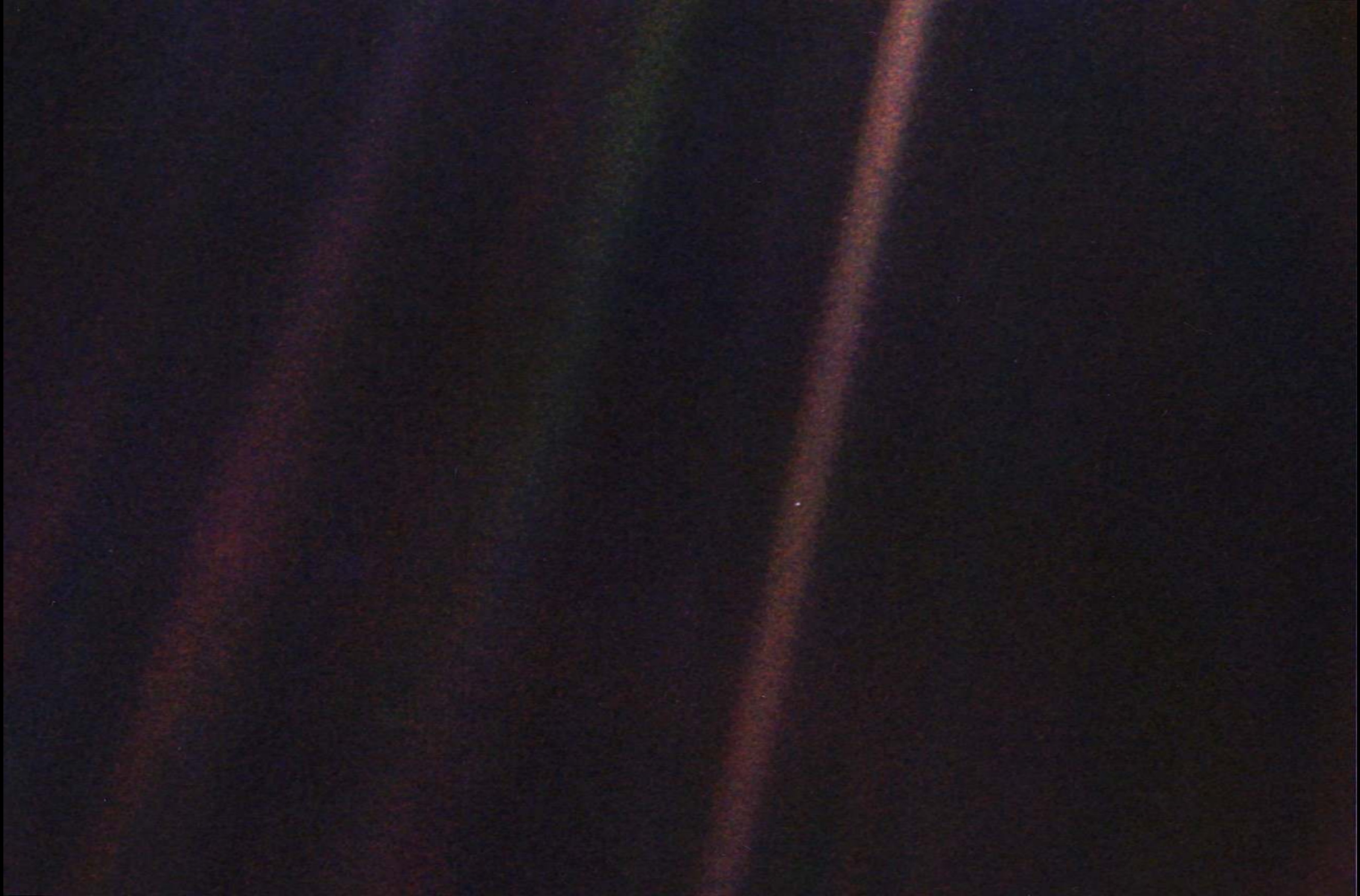
*composite from greek oikos (*οἶκος*, family, family property, house) and the english system. Oikos was the basic unit of greek society.*

- *a biological community of interacting organisms and their physical environment*
- *(in general use) a complex network or interconnected system*

First used in 1935 by Sir Arthur George Tansley, ecosystem

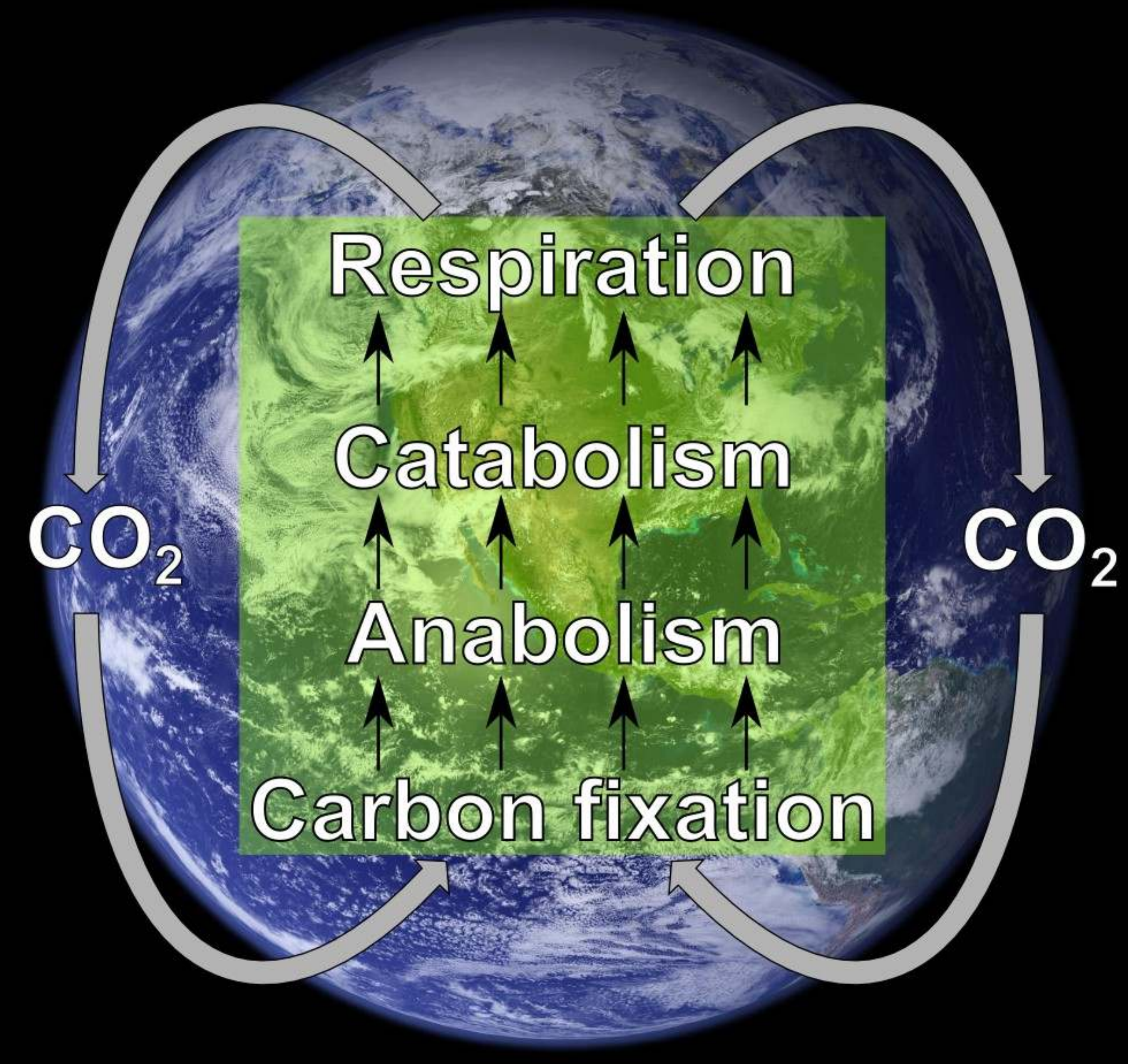


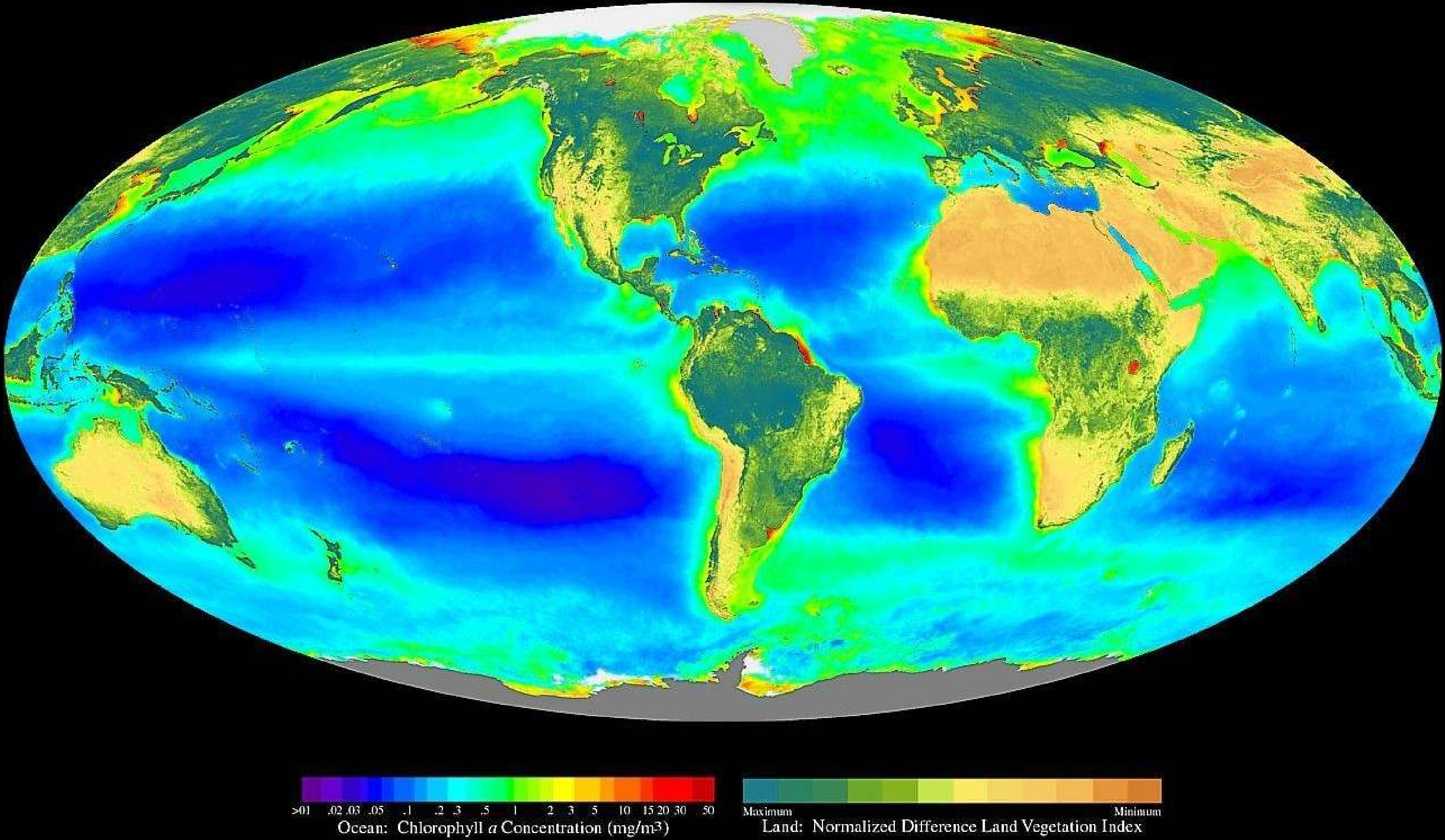

THE SECRET WORLD
INSIDE YOU
Artwork by Kyle Platts



“the Pale Blue Dot” - Voyager 1 1990

OUR PLANET IS GLOBALLY AUTOTROPHIC


Respiration
Catabolism
Anabolism
Carbon fixation
CO_2
CO_2


>01 .02 .03 .05 .1 .2 .3 .5 1 2 3 5 10 15 20 30 50
Ocean: Chlorophyll a Concentration (mg/m3)
Maximum
Minimum
Land: Normalized Difference Land Vegetation Index

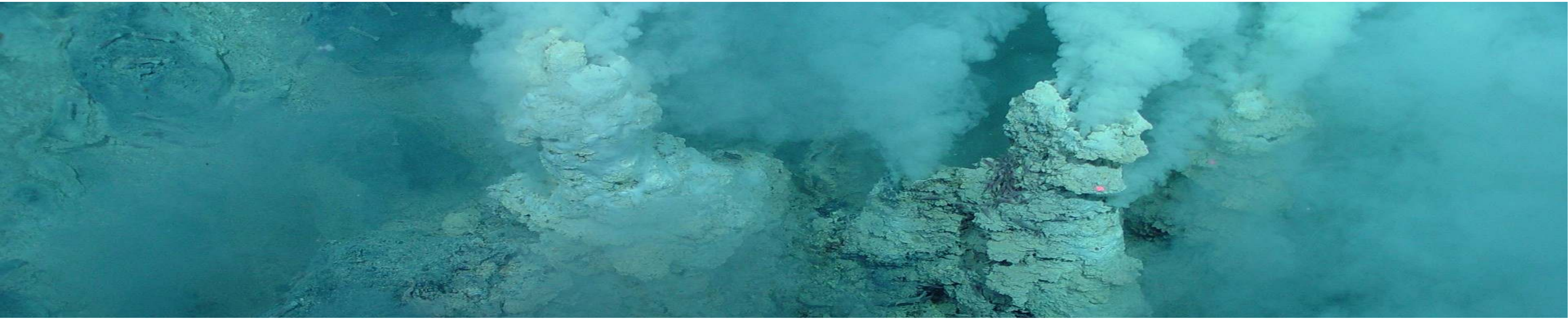
Chemolithoautotrophy

Carbon fixation in the absence of light

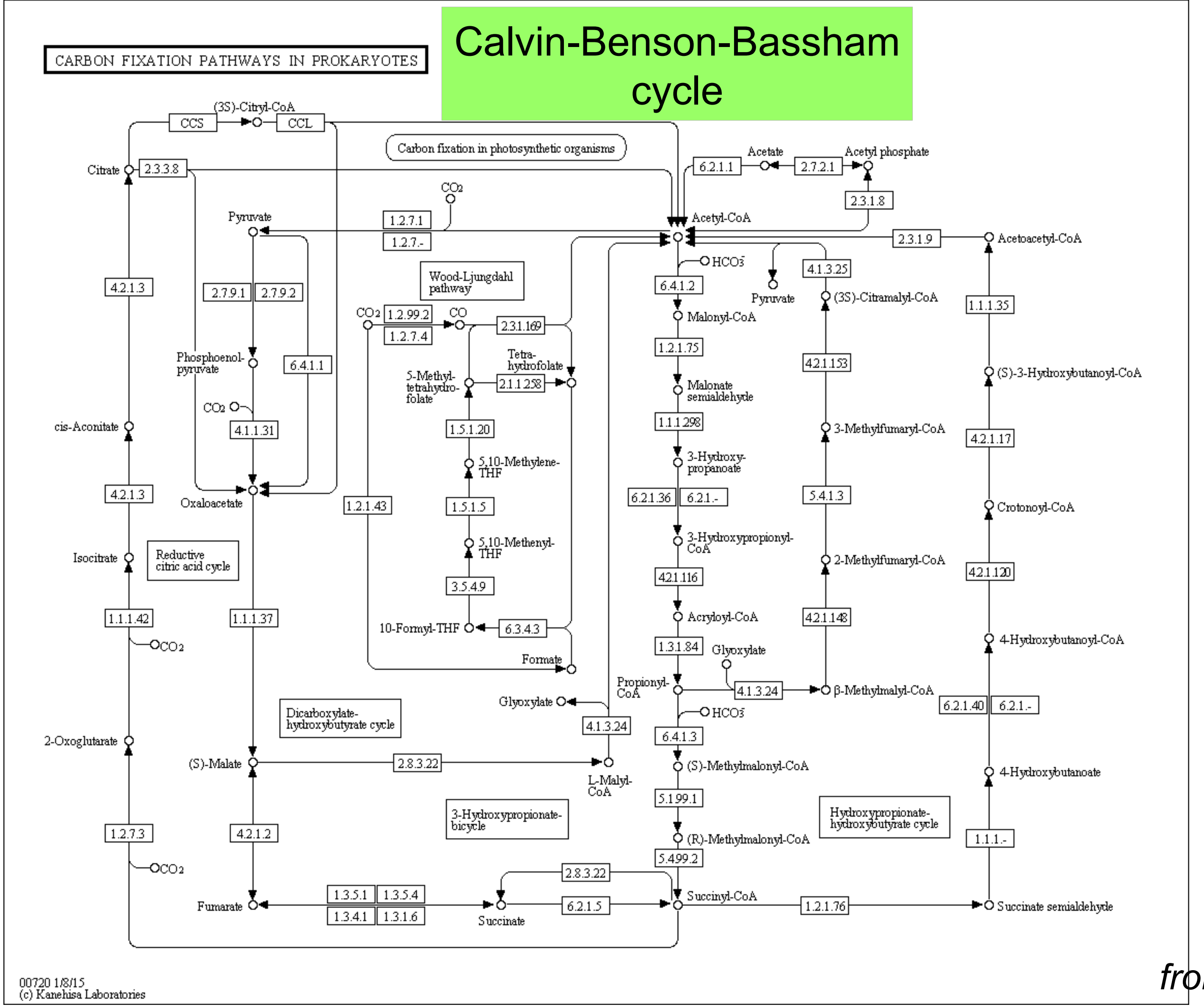
A form of chemotrophic nutrition in which a simple inorganic compound is oxidized in the course of the synthesis of complex organic compounds from carbon dioxide.

Also know as dark carbon fixation *or* dark primary production*, chemolithoautotrophy is a key metabolic strategy potentially sustaining food webs and higher trophic levels throughout the deep oceans, in the sediments, in extreme environments and in subsurface ecosystems.*

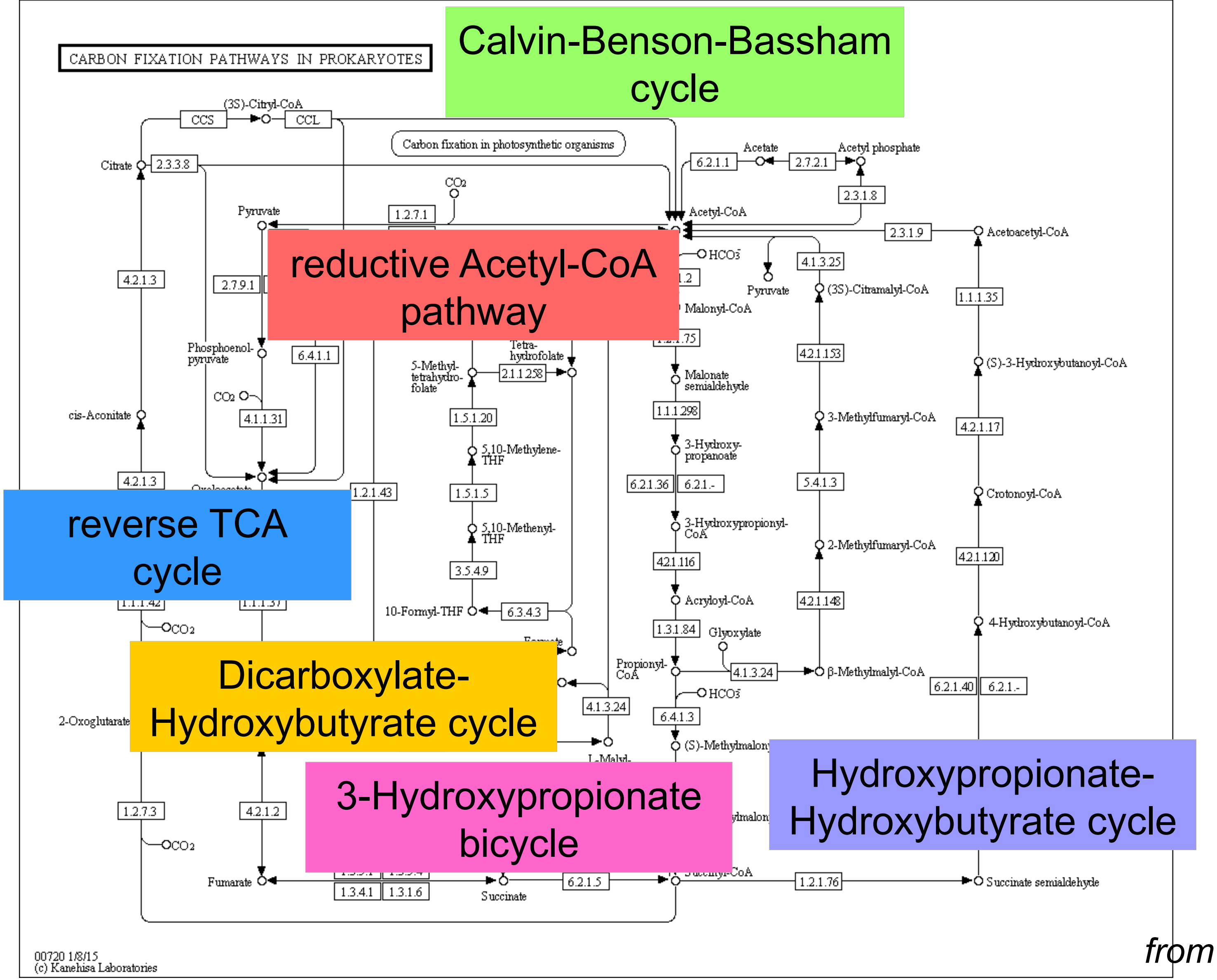
It is a diverse metabolic strategy widespread among Bacteria and Archaea.



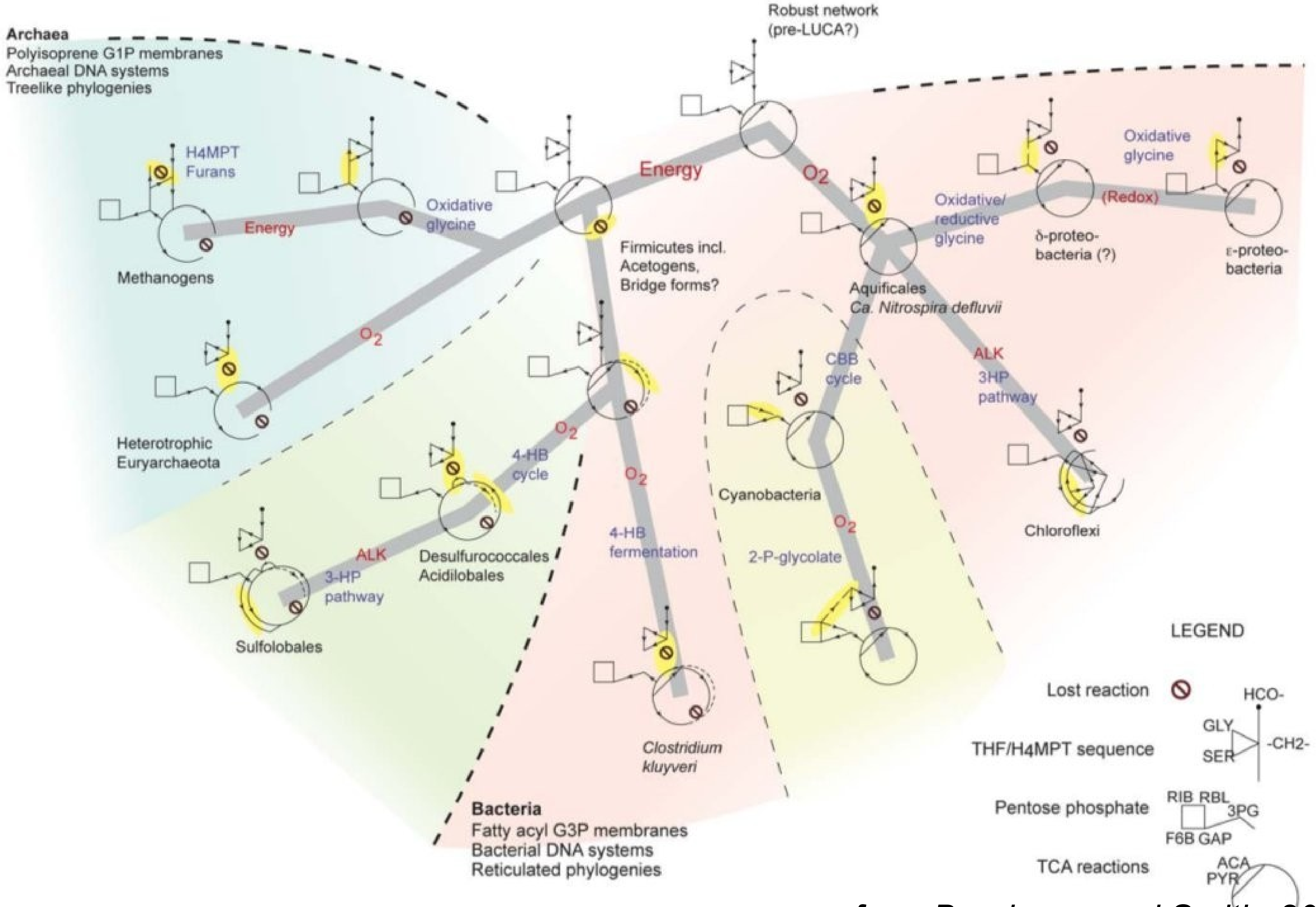
Calvin-Benson-Bassham cycle



from http://www.genome.jp/kegg/

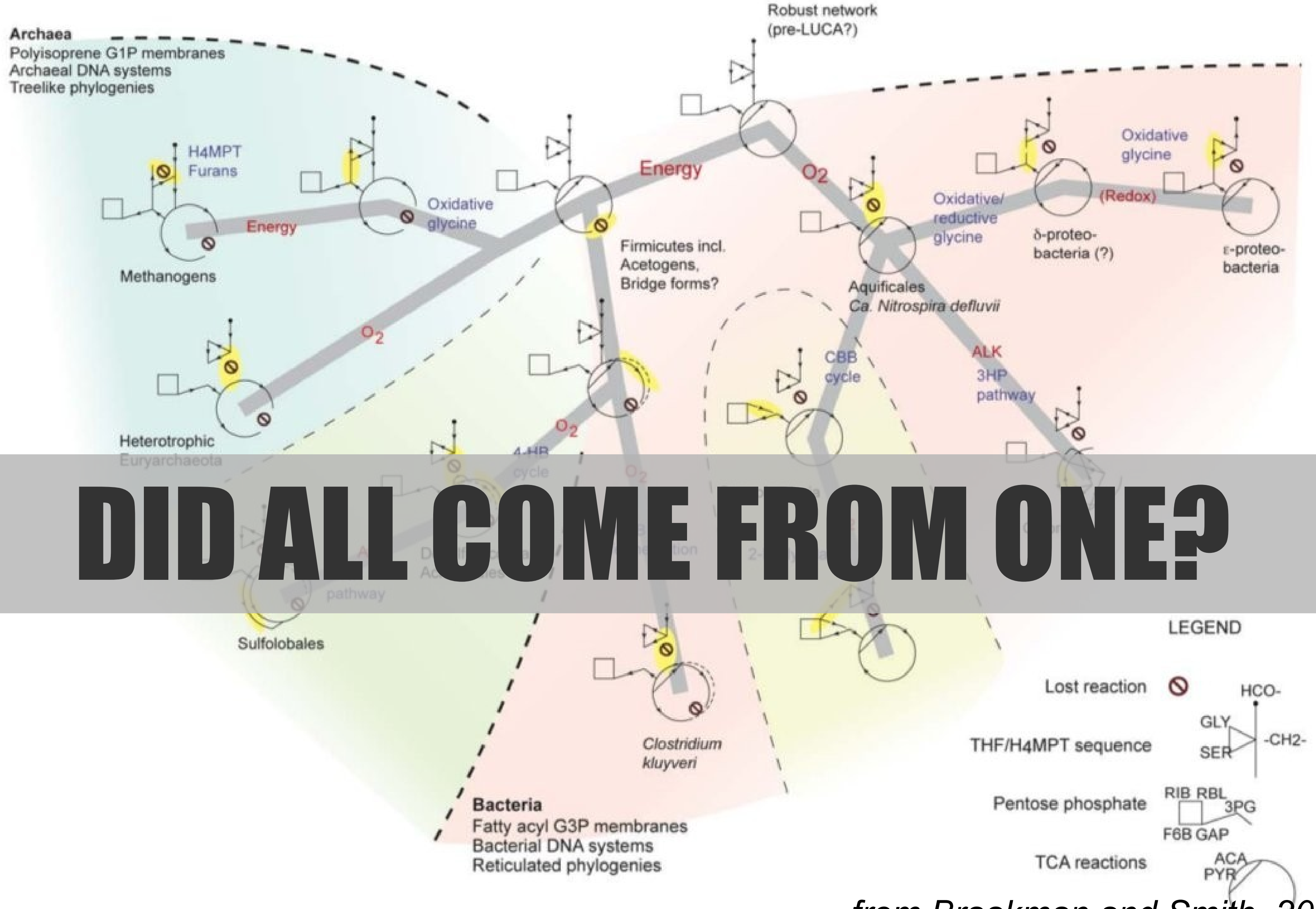


from http://www.genome.jp/kegg/



from Braakman and Smith, 2012 Plos Biol

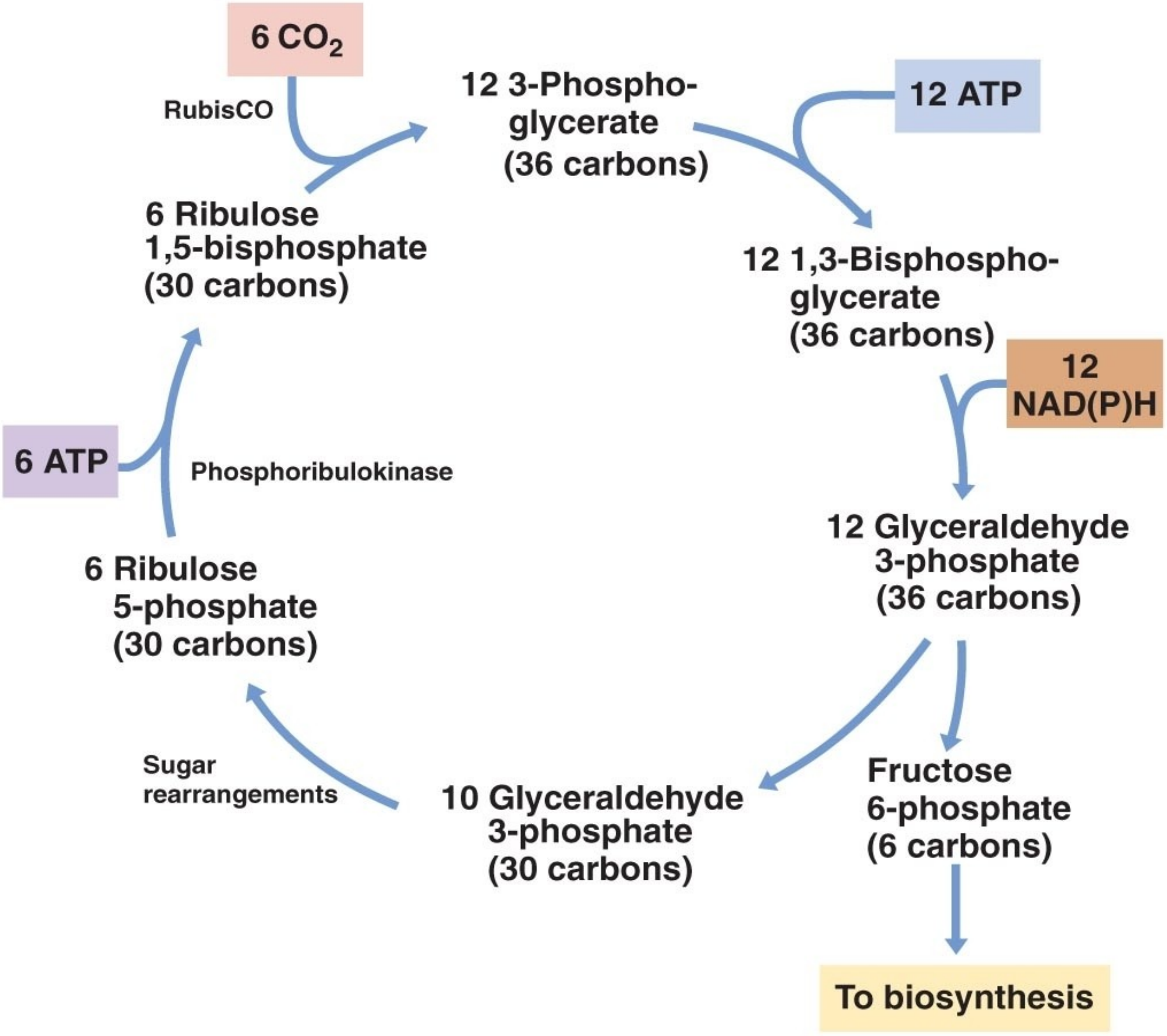
DID ALL COME FROM ONE?



from Braakman and Smith, 2012 Plos Biol

The Calvin Cycle

- The Calvin-Benxon-Bassan Cycle, known as the Calvin Cycle or the Reductive Pentose Phosphate Cycle is the major pathway of carbon fixation in the extant biosphere
- It is used by oxygenic photoautotrophs and numerous aerobic chemolithoautotrophs
- Requires NADPH, ATP, ribulose 1,5-bisphophate_carboxylase (RubisCO), and phosphoribulokinase
- 6 molecules of CO_2 and 18 ATP are required to make 1 molecule of glucose



Overall stoichiometry:

$$6\ CO_2 + 12\ NADPH + 18\ ATP \longrightarrow$$
$$C_6H_{12}O_6(PO_3H_2) + 12\ NADP^+ + 18\ ADP + 17\ P_i$$

RUBISCO

ribulose 1,5 bisphosphate carboxylase oxygenase

A large enzyme 500 000 Daltons

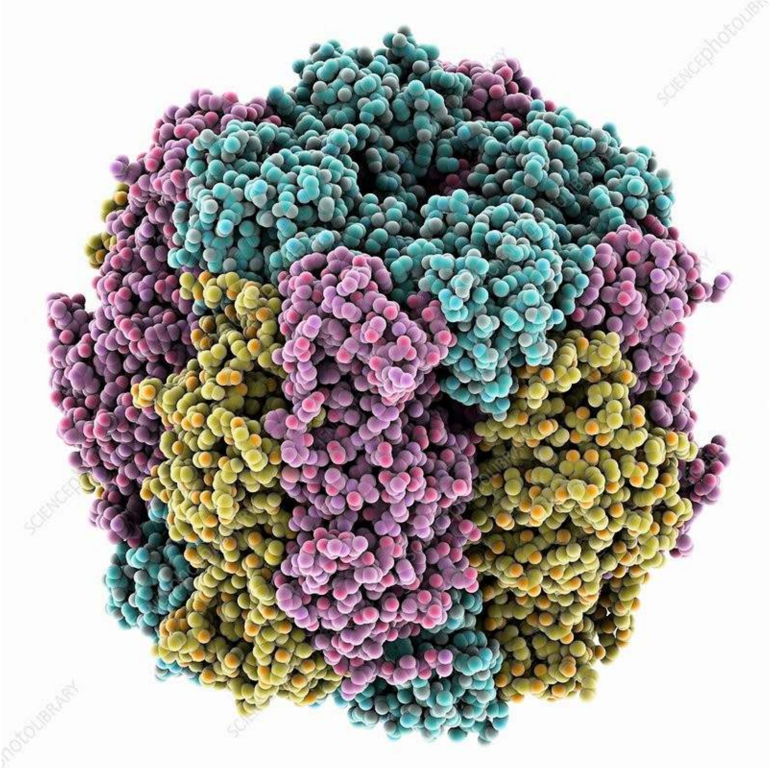
Inhibited by oxygen

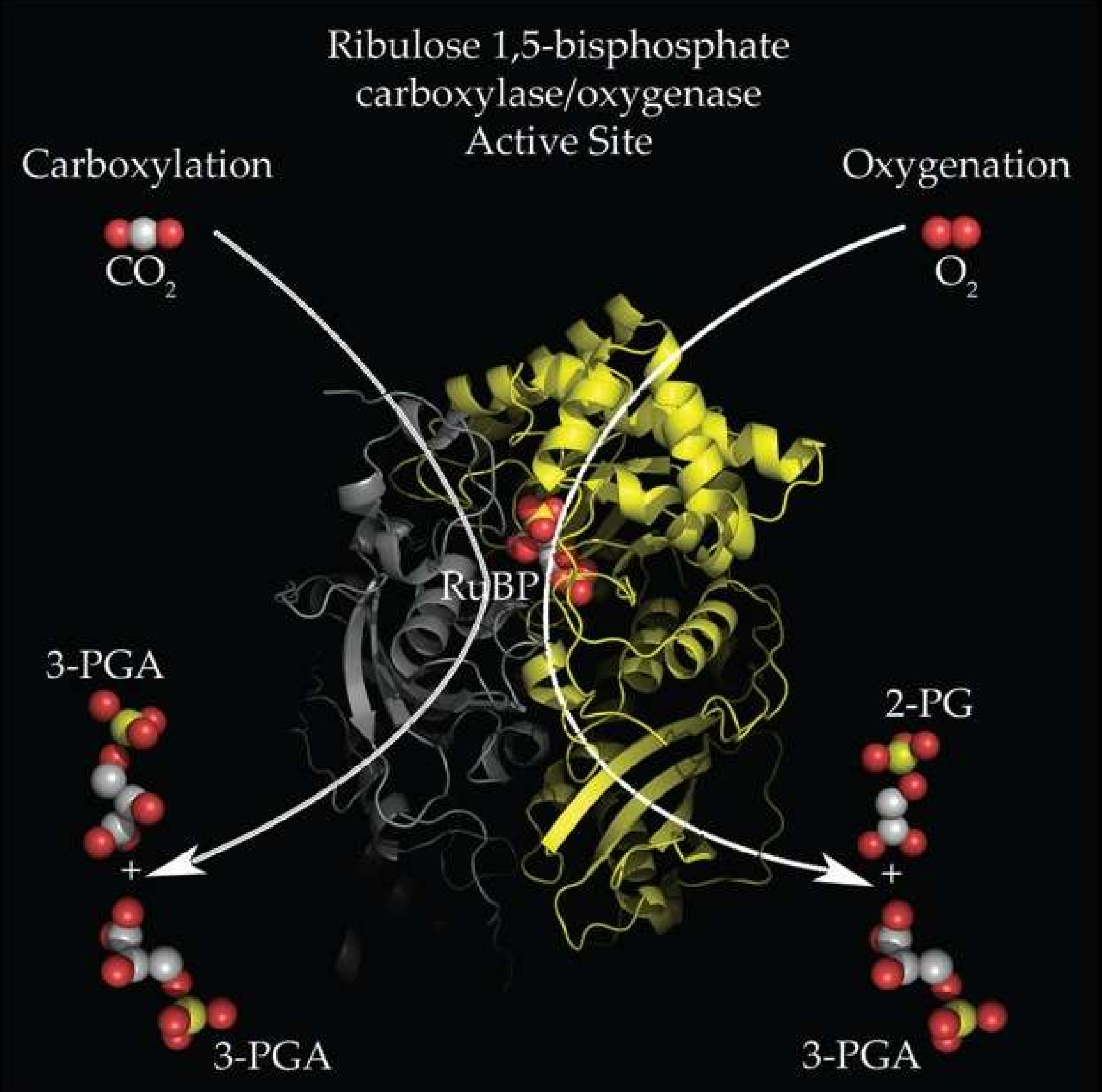
A relatively slow enzyme

50% of chloroplast protein

Probably the most abundant protein on Earth

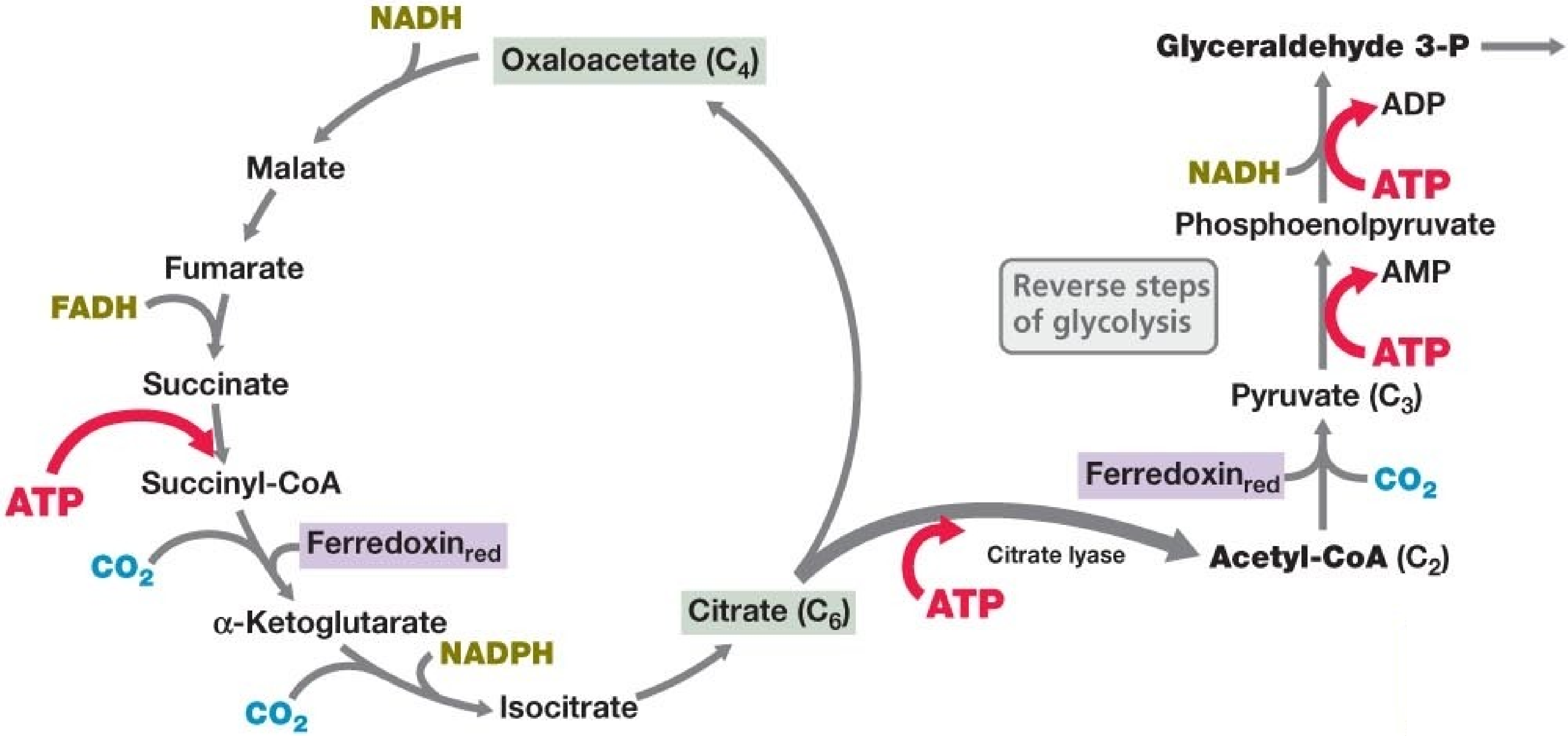
Consists of two subunits (1 large + 1 small)




Ribulose 1,5-bisphosphate
carboxylase/oxygenase
Active Site
Carboxylation
Oxygenation
CO_2
O_2
RuBP
3-PGA
2-PG
+
+
3-PGA
3-PGA

The reverse TCA Cycle

- The reverse TCA cycle (rTCA) is also known as the Arnon-Buchanan Cycle
- It is abundant in numerous anaerobic chemolithoautotrophic bacteria, some anaerobic chemolithoautotrophic archaea and some anoxygenic photoautotrophs
- Two major variants exist
- ATP-citrate lyase, Succinyl-CoA carboxylase and 2-Oxoglutarate synthase are the key enzyme for its functioning in reverse and are extremely oxygen sensitive
- 3 CO_2 and 2-3 ATP are used to generate 1 molecule of Pyruvate



(a) **Reverse citric acid cycle**

The Acetyl-CoA pathway

- *The Acetyl-CoA pathway is also known as the Wood-Ljungdale pathway and it is not a cycle, but a linear pathway*
- *It is used by methanogens, acetogens and several anaerobic chemolithoautotrophs*
- *The pathway is divided in two branches, producing the methyl group and the carbonyl group of the Acetyl-CoA respectively*
- *The methyl branch is functionally similar but not homologous between Bacteria and Archaea, while the carbonyl branch is*
- *The key enzymes is a bifunctional heterotetramer formed by the carbon monoxide dehydrogenase and the acetyl-CoA synthase*
- *It has been proposed as the possible ancestral c-fixation pathway*

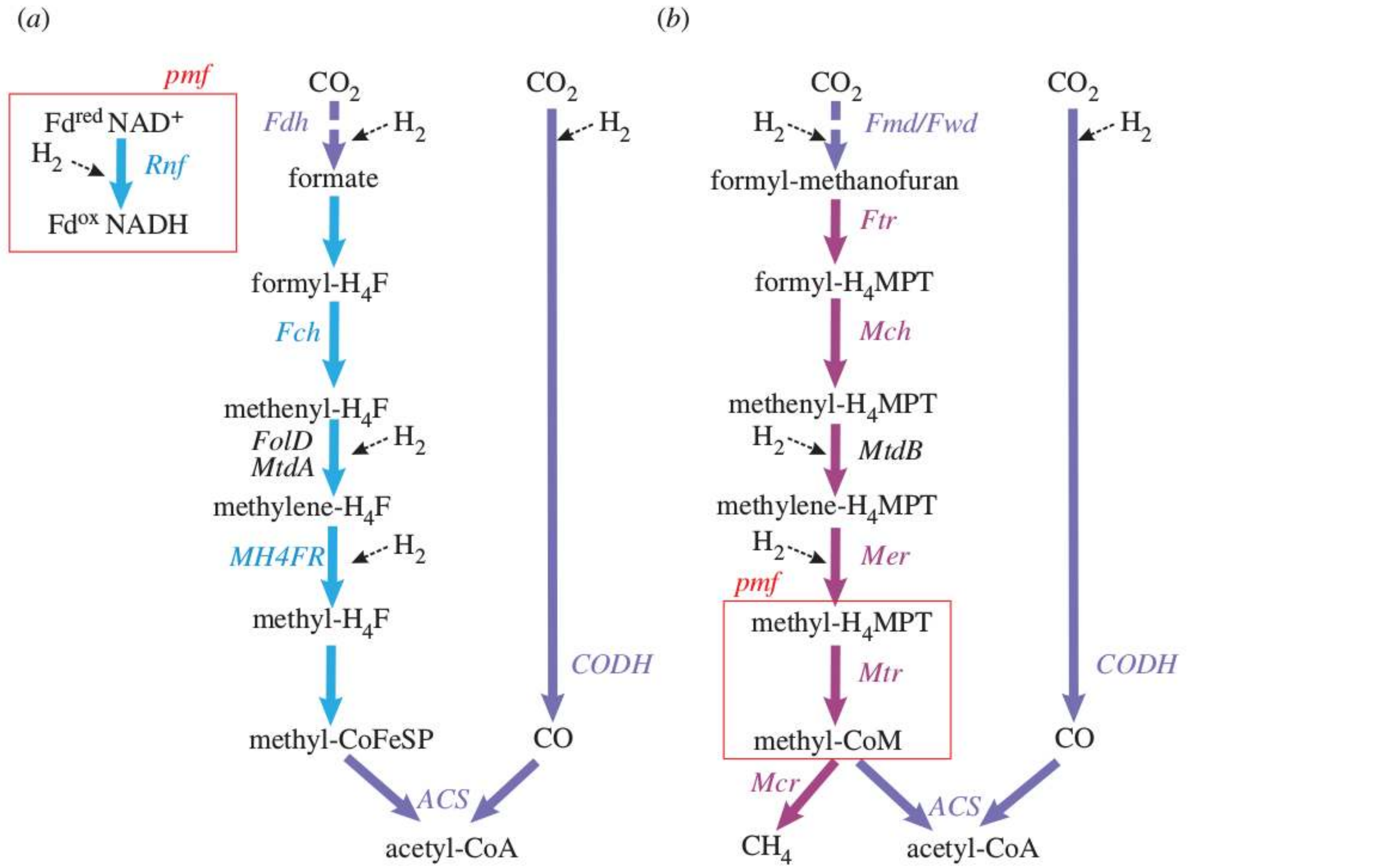
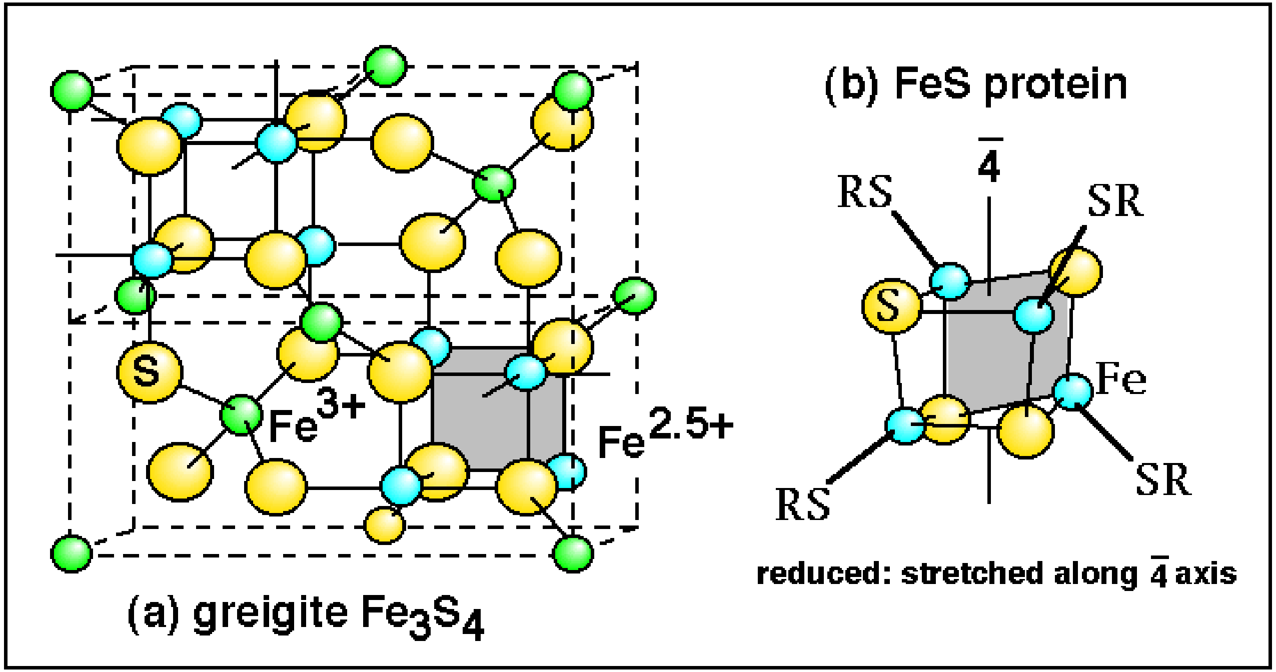


Figure 1. Schematic of the main reaction steps and enzymes involved in acetogenic (*a*) and methanogenic (*b*) WL-type pathways. Steps restricted to Bacteria are marked in bright blue colour, whereas those only found in Archaea are shown in violet. Dark blue stands for reactions and enzymes observed in both prokaryotic domains. Proton motif force (pmf)-generating steps are boxed in red in both reactions.

The Rocky Roots of Metal Cofactors



Other Carbon Fixation Pathways

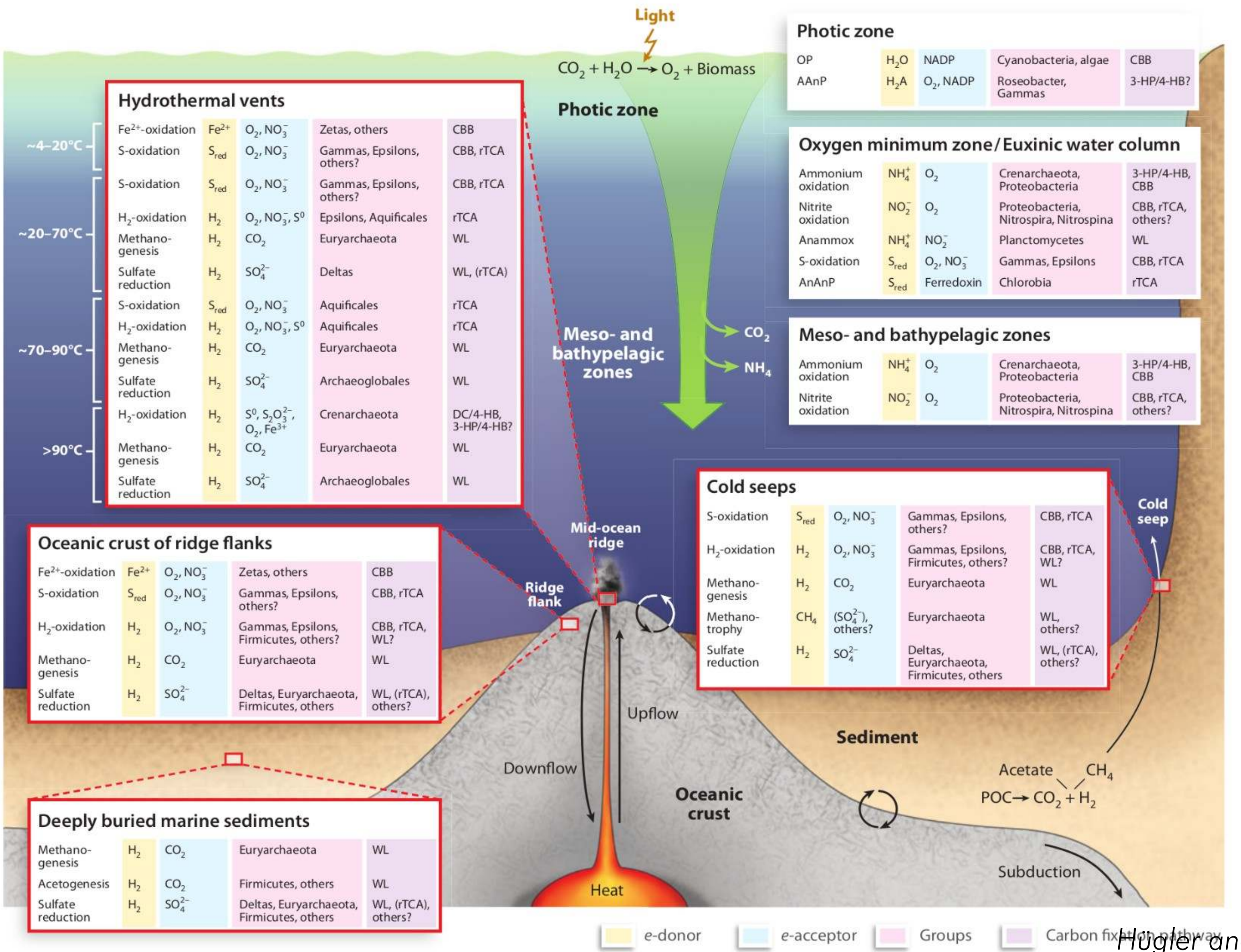
- The 3-Hydroxypropionate Bycycle, the Dicarboxylate / 4-Hydroxybutyrate pathways and the 3-Hydroxypropionate / 4-Hydroxybutyrate Cycle are alternative pathways of c-fixation
- They are used by some aerobic and anaerobic chemolithotrophic archaea and the bacterial group Chloroflexaceae
- They have been discovered relative recently and their ecosystemic relevance is not clear
- They overlap with the other major c-fixation pathways in several key steps

Distribution of C Fixation Pathways

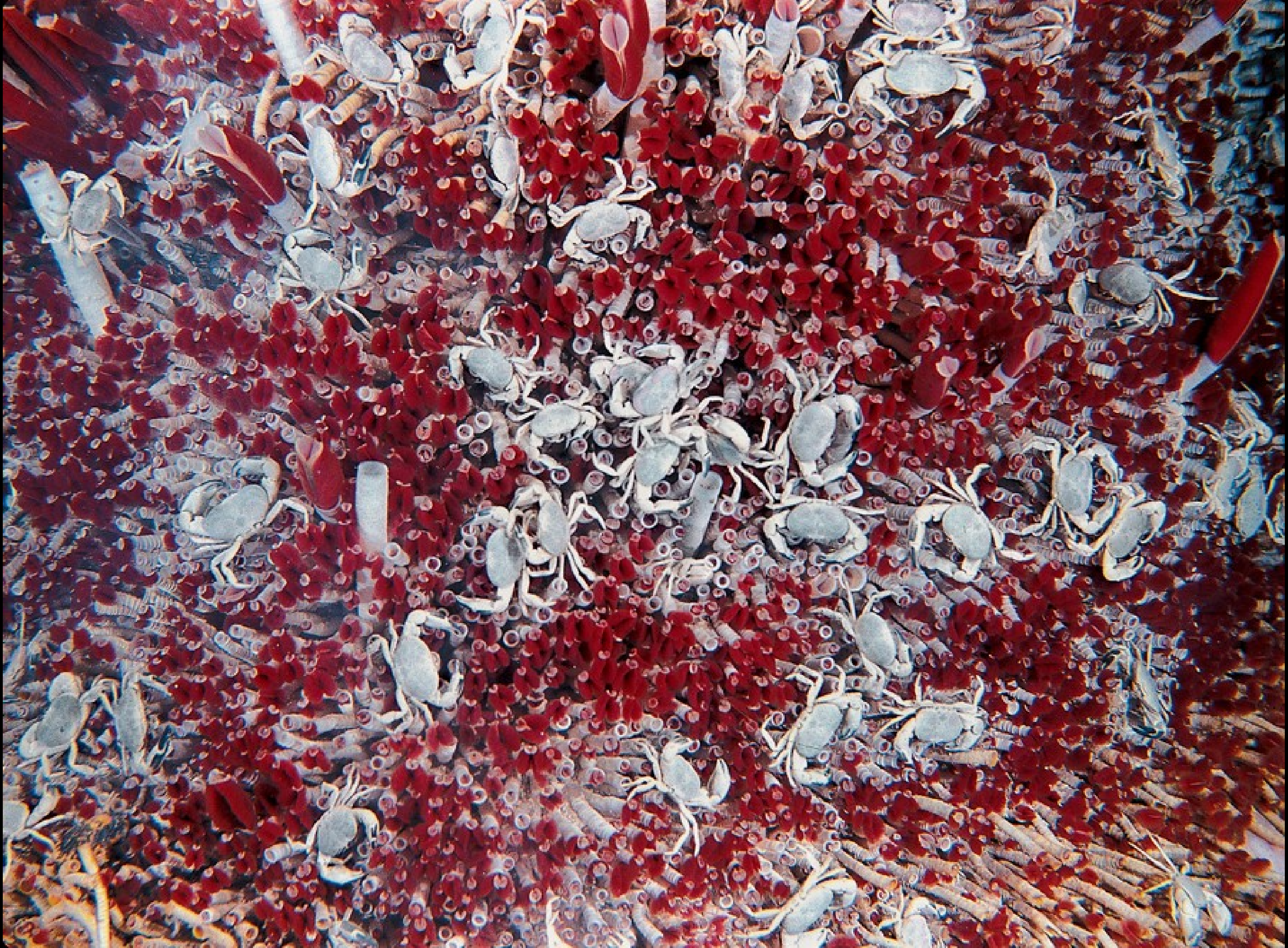
Pathway	Distribution
Calvin Cycle	Plants, algae, Cyanobacteria, most aerobic or facultative aerobic chemolithoautotrophic Bacteria
rTCA Cycle	Chlorobiales, Aquificae, Epsilonproteobacteria, some Deltaproteobacteria, few Alphaproteobacteria (Magnetococcus), one Gammaproteobacteria, Nitrospirae
rAcetyl-CoA	Methanogenic and sulfate reducing Euryarchaeota, acetogenic Firmicutes, some Spirochaetes, many Deltaproteobacteria, Annamox bacteria of Plancomycetes
3-hydroxyproprionate Bycycle	Chloroflexaceae
4-Hydroxybutyrate Cycle	Anaerobic Thermoproteales, Desulfurococcales (Crenarchaeota)
3H/4H Cycle	Aerobic Sulfolobales (Crenarchaeota), possibly marine and soil ammonia oxidizing Thaumarchaeota

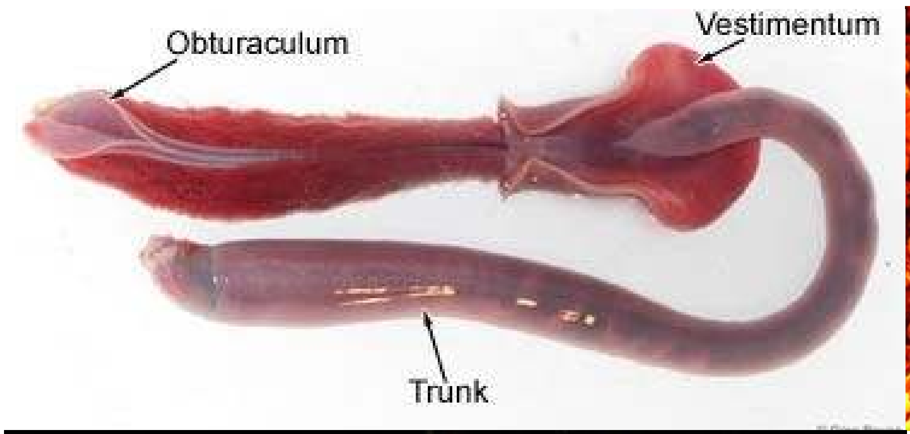
Cost of different C Fixation Pathways

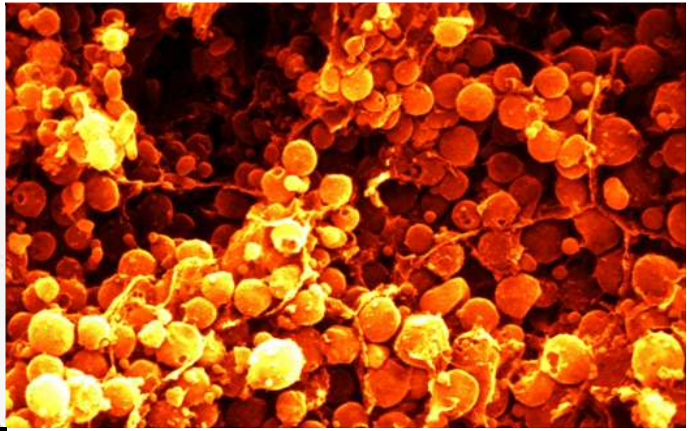
Pathway	ATP for synthesis of 1 Pyruvate	Key enzymes
Calvin Cycle	7	RubisCO; phosphoribulokinase
rTCA Cycle	2-3	2-Oxoglutarate synthase, ATP-citrate lyase
rAcetyl-CoA	1	Acetyl-CoA synthase/ CO dehydrogenase
3-hydroxyproprionate Bycycle	5	Malonyl-CoA reductase, propionyl-CoA synthase, malyl-CoA lyase
4-Hydroxybutyrate Cycle	7	4-Hydroxybutyryl-CoA dehydratase
3H/4H Cycle	9	Acetyl-CoA/propionyl-CoA carboxylase

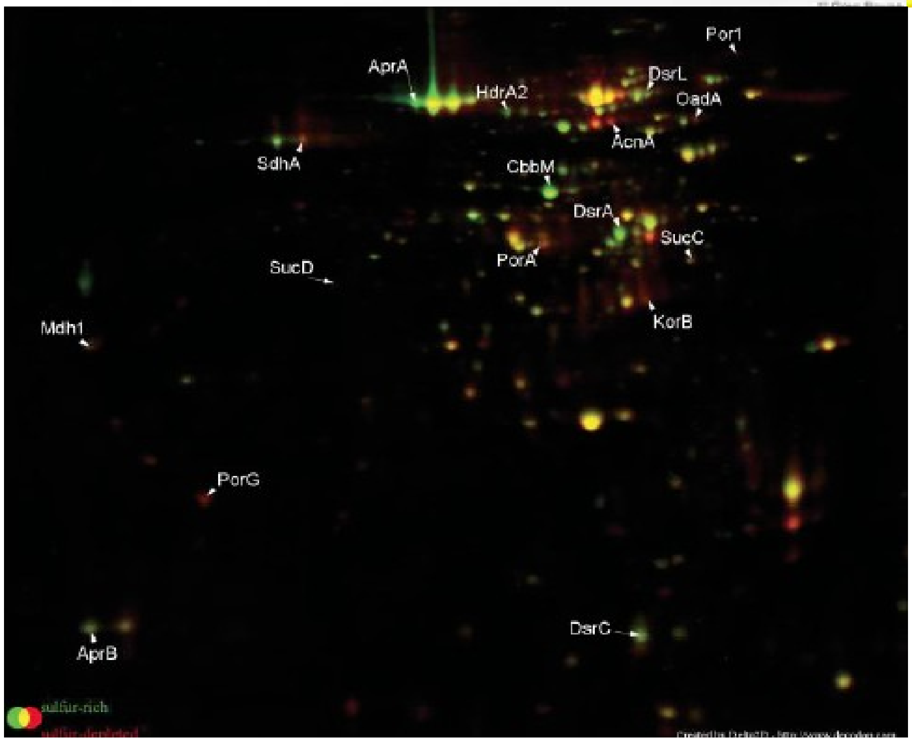


Hügler and Sievert, 2011 Ann. Rev. Mar. Sci.









***Riftia pachyptila* symbiont**

Thiotrophic symbionts

$H_2S + 2O_2 \rightarrow H_2SO_4$

$CO_2 + H_2O + H_2S + O_2 \rightarrow [CH_2O] + H_2SO_4$

Phylum ***Proteobacteria***

Class ***Gammaproteobacteria***

Can use either the ***Calvin-Benson***

cycle and the ***rTCA*** *cycle in response to different sulfide levels in the environment*

Markert et al., 2007 Science

from NASA

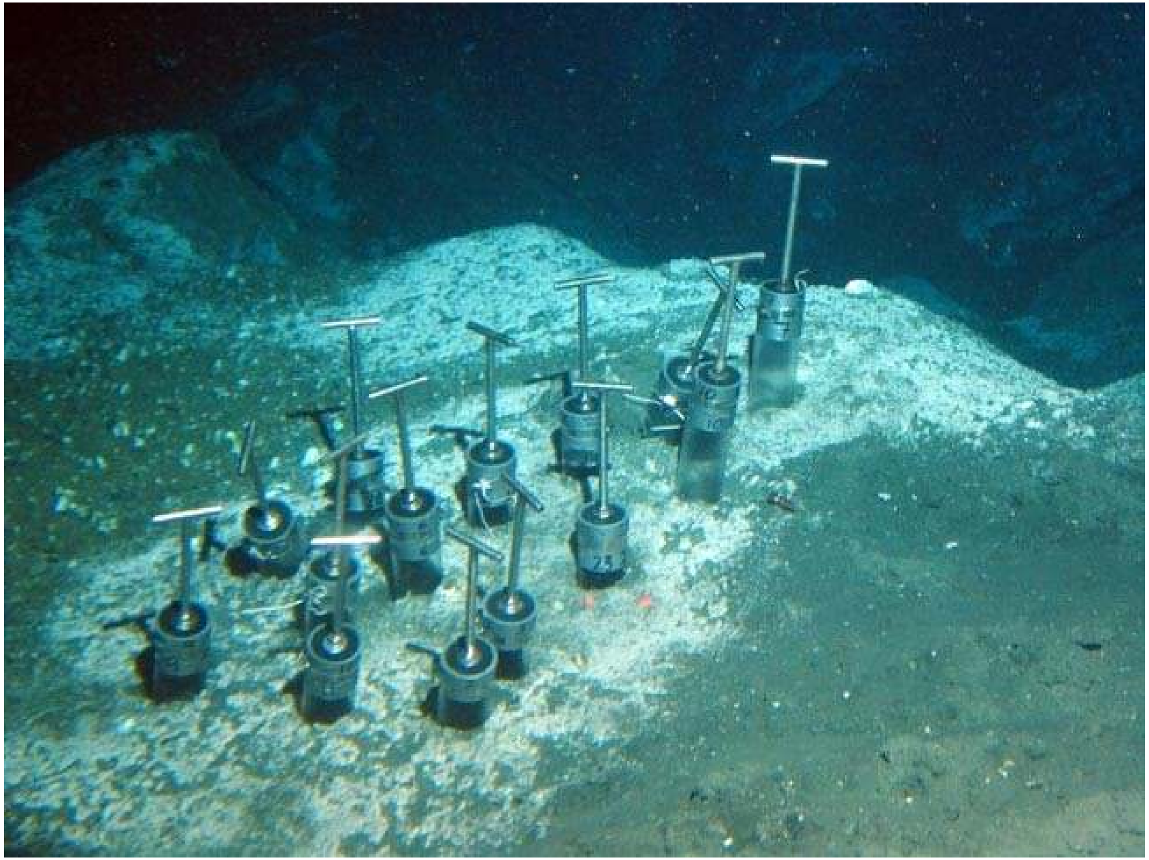
Sergei Nikolaievich Winogradsky

Winogradsky (1856 – 1953) was a Russian microbiologist, ecologist and soil scientist who pioneered the cycle-of-life concept.

Winogradsky discovered the first known form of lithotrophy during his research with Beggiatoa in 1887. He reported that Beggiatoa oxidized hydrogen sulfide (H2S) as an energy source and formed intracellular sulfur droplets. This research provided the first example of chemolithotrophy.

His research on nitrifying bacteria would report the first known form of chemoautotrophy, and paved the way to the idea of bioegeochemical cycles.

Dworkin and Gutnick, 2012 FEMS Microbiol. Rev.

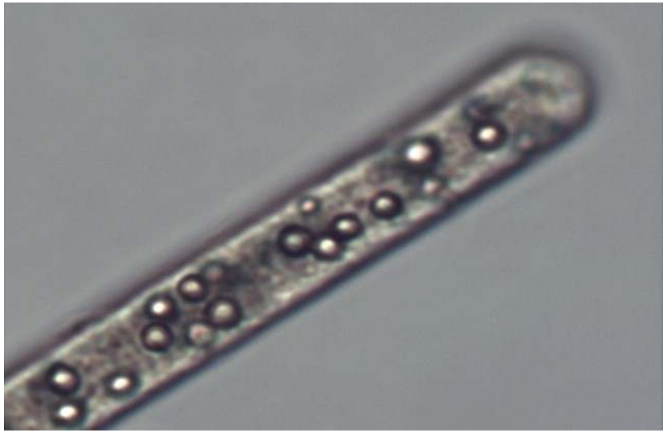


Beggiatoa is a genus of bacteria in the class Gammaproteobacteria, in the Proteobacteria phylum.

They are named after the Italian medic and botanist F. S. Beggiato.

Beggiatoa oxidized hydrogen sulfide (H2S) as an energy source, forming intracellular sulfur droplets. Oxygen is the terminal electron acceptor and CO2 is used as carbon source.

Winogradsky referred to this form of metabolism as inorgoxidation (oxidation of inorganic compounds).



$$2H_2S + O_2 \rightarrow 2S + 2H_2O$$

HHMI
HOWARD HUGHES MEDICAL INSTITUTE

Winogradsky Column: Microbial Ecology in a Bottle

BioInteractive.org

Sergei N. Winogradsky
1856 – 1953

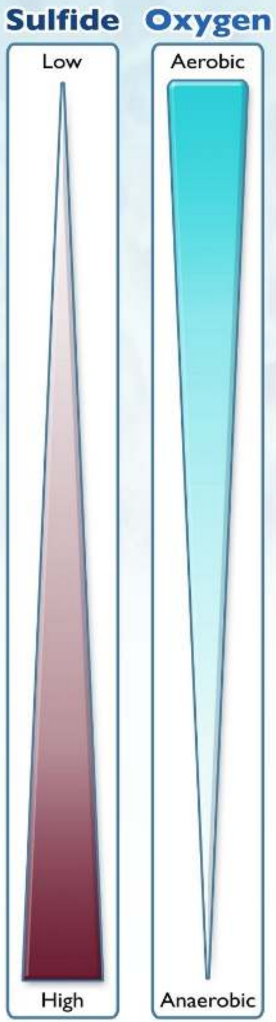
Sergei N. Winogradsky was one of the first microbiologists to study the organisms found in complex biofilm communities. One of the strategies he used to isolate organisms from nature was a miniature model of a pond cross section that is now called a Winogradsky column. It is a simple device for constructing a stratified ecosystem and provides a visual example of various modes of metabolism and zonation in the microbial world. It is a classic demonstration of the metabolic diversity of prokaryotes.

HHMI

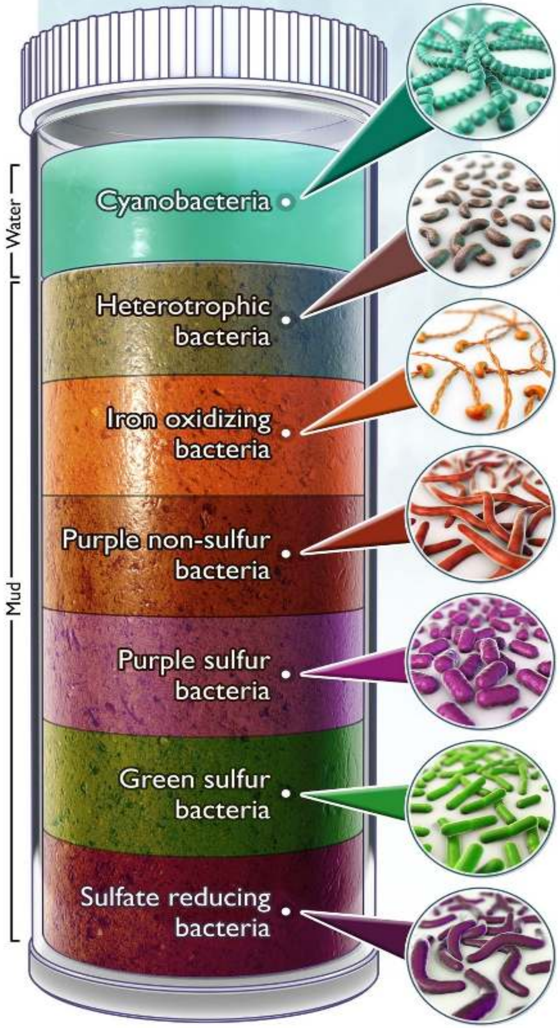
H. Olins

A soil or sediment sample is collected from nearly any source and amended with a variety of compounds such as carbon, sulfur, iron, and/or calcium. The mixture

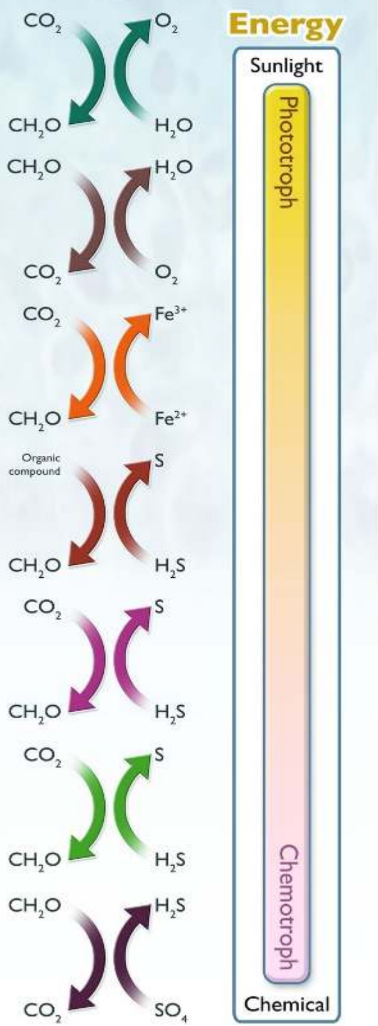
Gradients



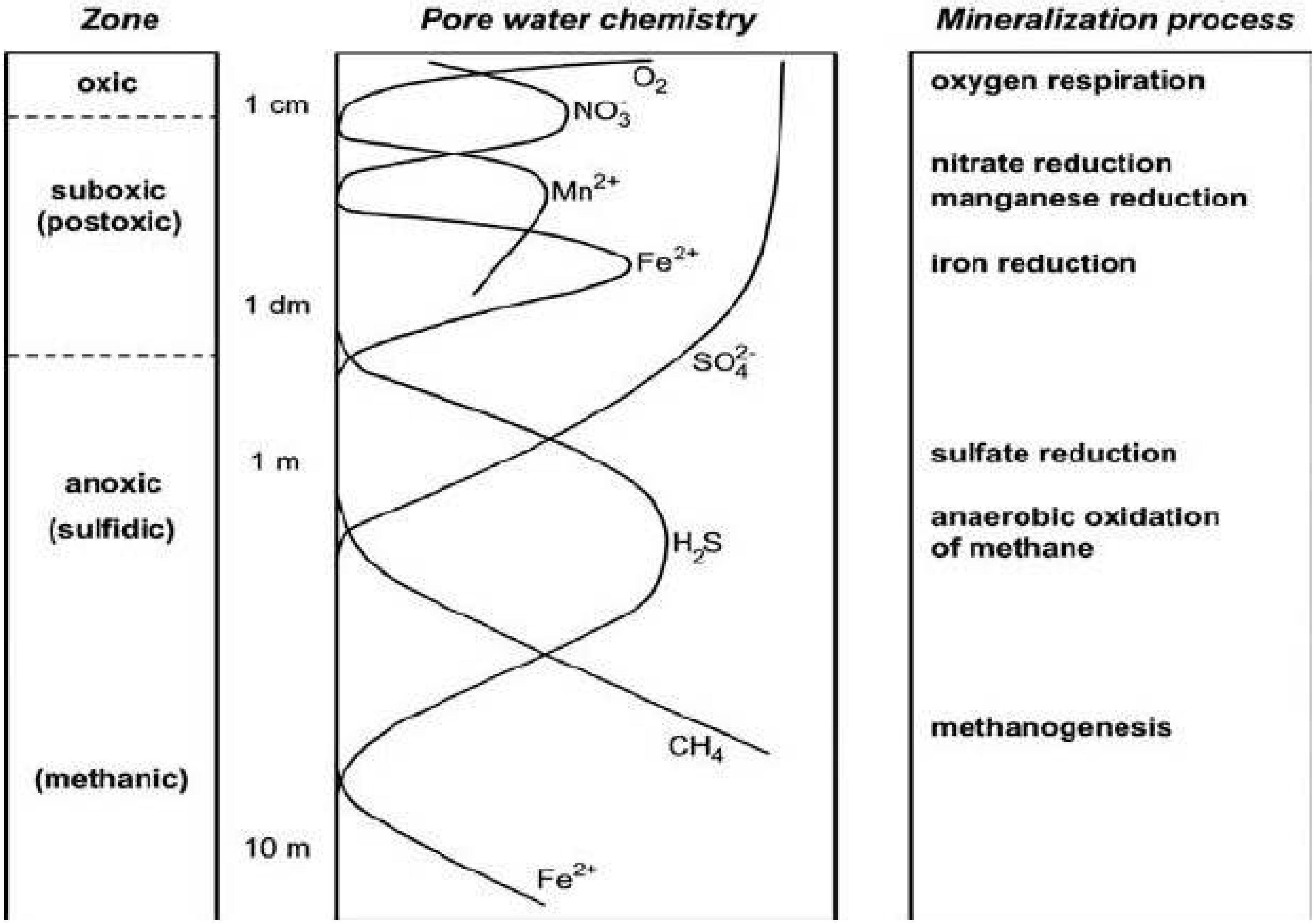
Zonation

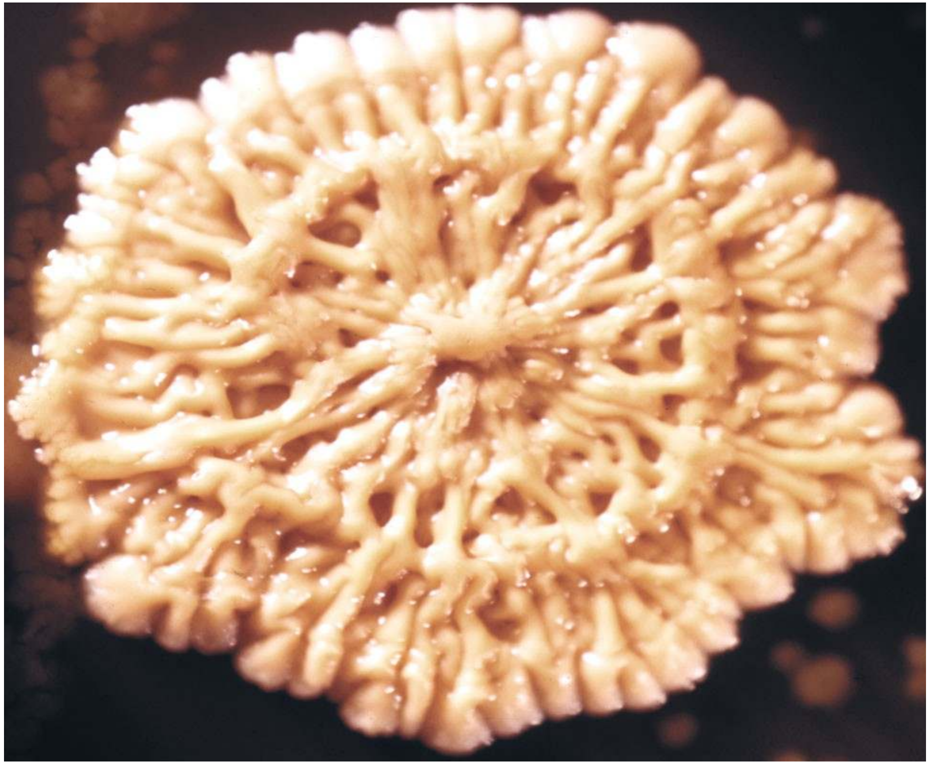


Metabolic Niches



Down the thermodynamic ladder






“[…] Invented almost exactly 130 years ago by their respective eponyms, the German Julius Richard Petri and the Russian-French Sergei N. Winogradsky, [these two techniques] embody two different, even contrary approaches to study the microbial world – one, Petri's, based on pure cultures, synthetic media and medicine, the other, Winogradsky's inspired by natural environment and ecology.”

Grote, 2018 - History and Philosophy of the Life Sciences


#GiovannelliLab
http://dgiovannelli.github.io

Make your own Winogradsky column

Tools + Materials

- *Buckets*
- *Shovel*
- *Large mixing bowl*
- *Mixing spoon*
- *1/4-page of shredded newspaper*
- *1 egg yolk*
- *Scissors*
- *1 two-liter soda bottle with top cut off*
- *Plastic wrap*
- *Rubber bands*

Instructions

- *Collect half a gallon of mud from a pond or stream, adding water until it has the consistency of a milkshake.*
- *Mix a quarter of the mud with egg yolk and shredded newspaper. Spoon the mixture into the bottle. Keep filling the bottle with mud, and tap it periodically to remove air pockets.*
- *Add an inch of pond water, leaving a little air at the top. Seal the bottle with plastic wrap and rubber bands, and place it near a window, out of direct sunlight.*
- *Layers will develop over two months. Look for dark green, purple, and black sulfur-eating bacteria at the bottom; red, orange, brown, and purple carbon-eating ones in the middle; and green photosynthesizing microbes at the top.*

Make your own Winogradsky column

Nutrient/Element	Common household
Sulfur	Egg yolk, matches
Nitrogen	Fertilizer,
Phosphorus	Fertilizer, matches
Vitamins	Multivitamins
Trace elements	Multivitamins
Carbon (inorganic)	Egg shell, bicarbonate
Carbon (organic)	Newspaper, meat broth, dead leaves

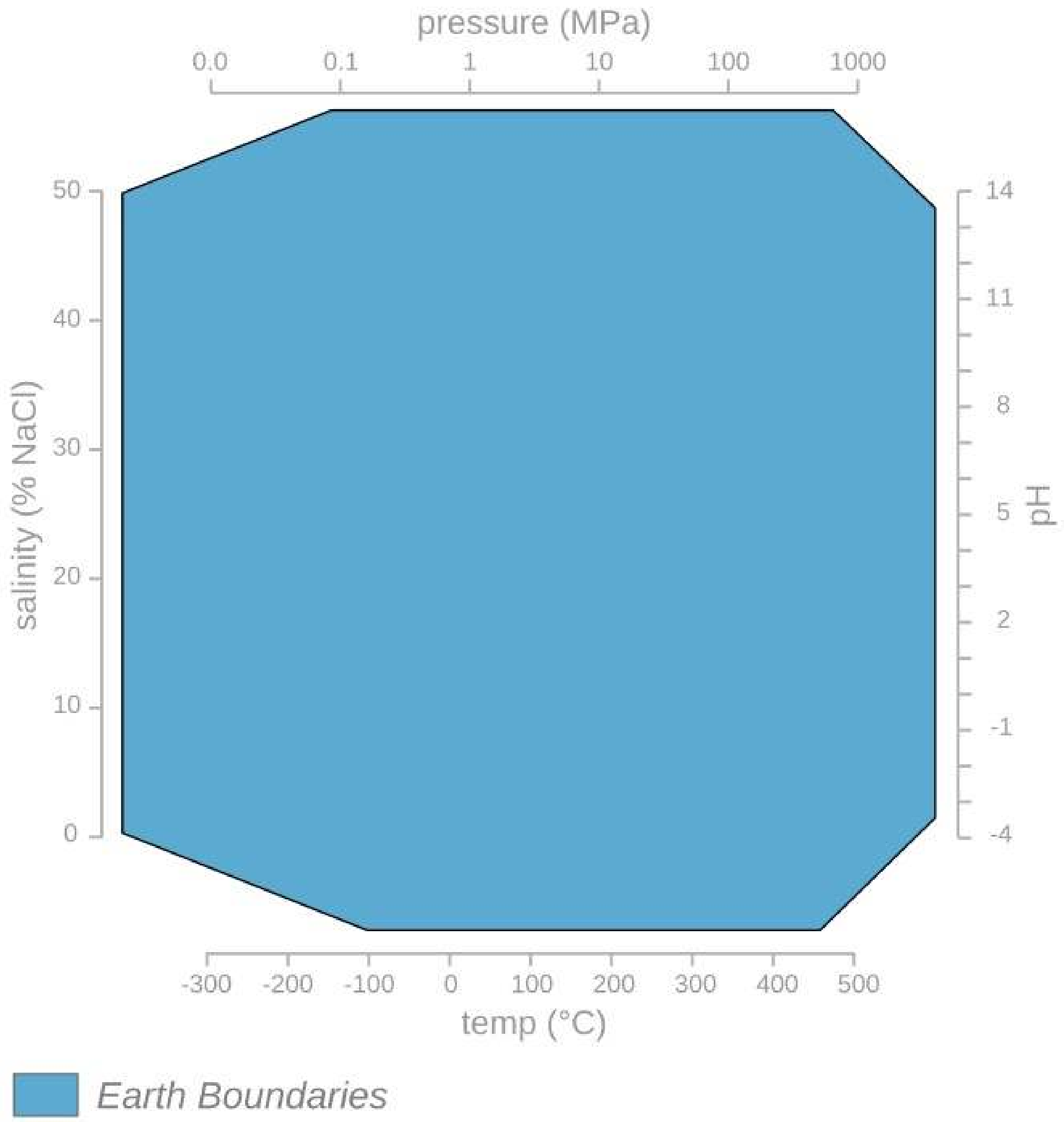
Limits of life

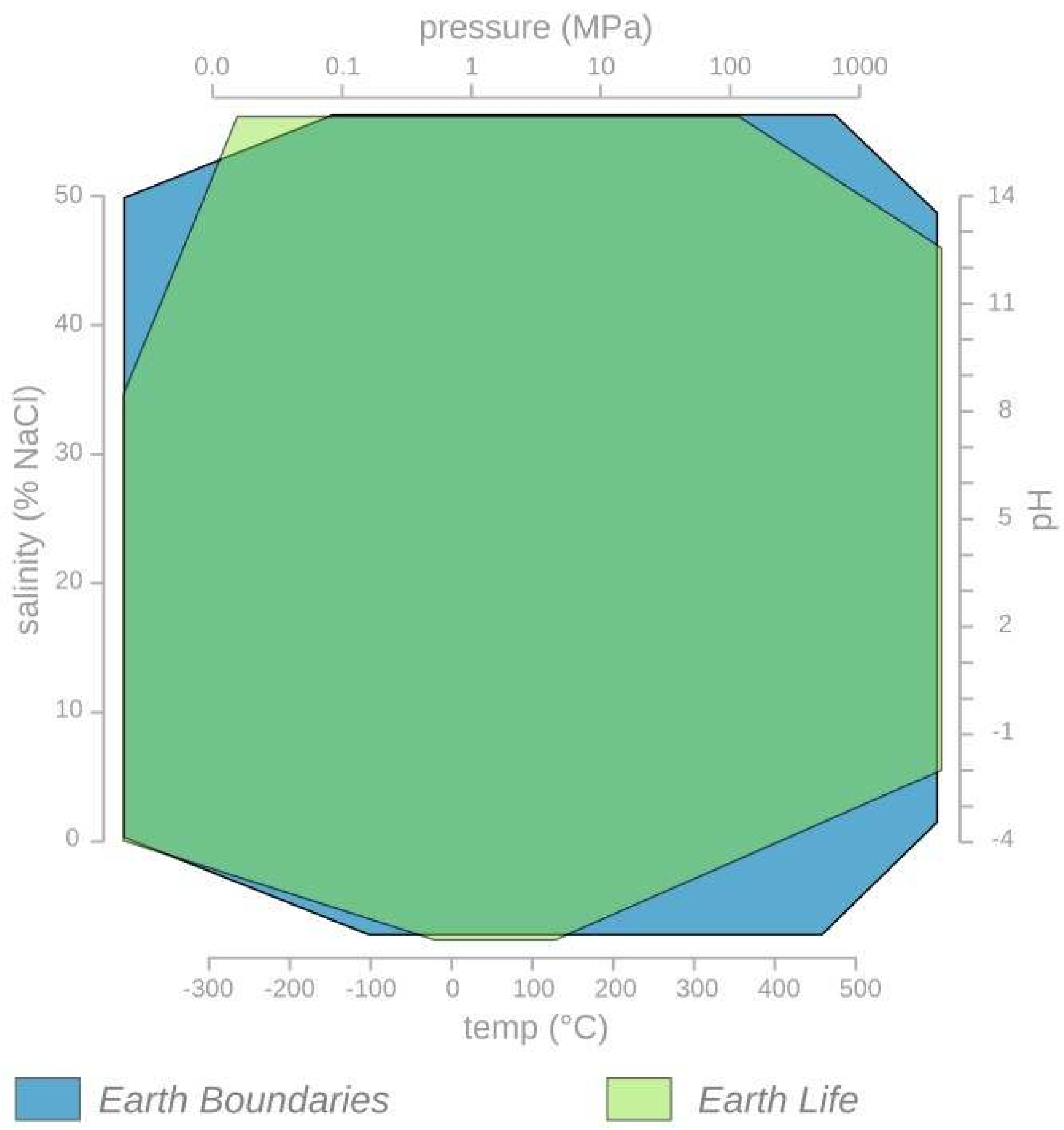
Limits of life

TABLE 3 | Limits of life as identified by (poly)extremophilic organisms in pure cultures.

Strain	Domain	Extremophile Type	Isolation ecosystem	Temperature (°C)	pH	Pressure (Mpa)	Salinity (%)	Water activity (a_w)
Picrophilus oshimae KAW 2/2	*Archaea*	Hypercidophile	Hot springs, Solfataras	47–65 (60)[a]	**−0.06**–1.8 (0.7)	nr	0–20	nr
Serpentinomonas sp. B1	*Bacteria*	Alkaliphile	Serpentinizing system (water)	18–37 (30)	9–**12.5** (11)	nr	0–0.5 (0)	nr
Methanopyrus kandleri 116	*Archaea*	Hyperthermophile	Deep-sea hydrothermal vent	90–**122** (105)	(6.3–6.6)	0.4–40	0.5–4.5 (3.0)	nr
Planococcus halocryophilus Or1	*Bacteria*	Halopsychrophile	Sea ice core	**−18**–**37** (25)	nr (7–8)	nr	0–19 (2)	nr
Halarsenatibacter silvermanii SLAS-1	*Bacteria*	Haloalkaliphile	Soda lake	28–55 (44)	8.7–9.8 (9.4)	nr	20–35 (**35**)	nr
Thermococcus piezophilus CDGS	*Archaea*	Piezothermophile	Deep-sea hydrothermal vent	60–95 (75)	5.5–9 (6)	0.1–**125** (50)	2–6 (3)	nr
Haloarchaeal strains GN-2 and GN-5	*Archaea*	Xerophile	Solar salterns (brine)	nr	nr	nr	nr	**0.635**

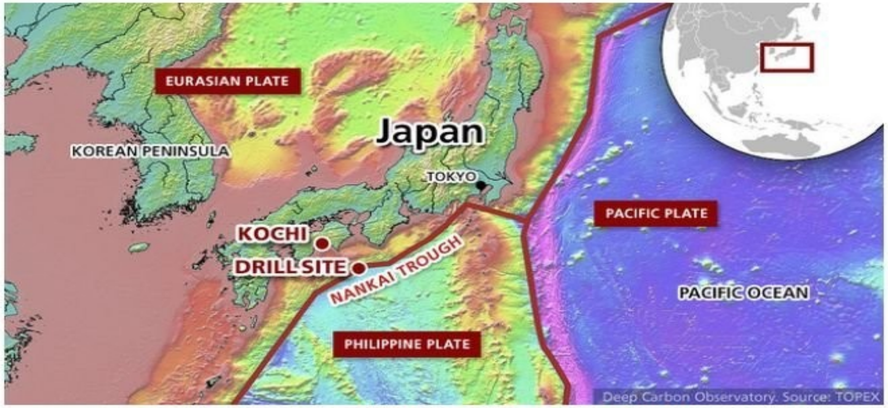
[a]*Data presented as range (optimum) for each parameter. nr, not reported in the original publication. Current limits are highlighted in bold.*


pressure (MPa)
0.0
0.1
1
10
100
1000
salinity (% NaCl)
50
40
30
20
10
0
pH
14
11
8
5
2
-1
-4
-300
-200
-100
0
100
200
300
400
500
temp (°C)
Earth Boundaries



pressure (MPa)
0.0
0.1
1
10
100
1000
salinity (% NaCl)
50
40
30
20
10
0
pH
14
11
8
5
2
-1
-4
-300
-200
-100
0
100
200
300
400
500
temp (°C)
Earth Boundaries
Earth Life

How hot is too hot?



Expedition 370 will head to the Nankai Trough (latitude/longitude 32.3423, 134.9564) off the coast of Japan to find the temperature limit of Earth-style life. Credit: Deep Carbon Observatory

D/V *Chikyu*, the world's largest scientific research vessel, at sea during International Ocean Discovery Program (IODP) Expedition 337. Credit: Luc Riolon/JAMSTEC

HOW HOT IS TOO HOT?

Guess the temperature limit of Earth-style life

What temperature is too hot for life to exist deep beneath the seafloor? An international research team is answering this question. What they learn will help define the borders of life inside Earth. Join their scientific mission of discovery by submitting your own guess about the temperature that is too hot for life deep inside Earth. Submit your estimate in degrees Celsius. The winner(s) will receive a Deep Carbon Observatory baseball cap and a sticker commemorating the T-Limit expedition.

https://goo.gl/oszxNd

This week read

Fuchs, G. (2011). Alternative Pathways of Carbon Dioxide Fixation: Insights into the Early Evolution of Life? Annual Review of Microbiology 65, 631–658. doi:10.1146/annurev-micro-090110-102801.

Berg, I. A. (2011). Ecological Aspects of the Distribution of Different Autotrophic CO2 Fixation Pathways. Appl. Environ. Microbiol. 77, 1925–1936. doi:10.1128/AEM.02473-10.

Braakman, R., and Smith, E. (2012). The Emergence and Early Evolution of Biological Carbon-Fixation. PLoS Comput Biol 8, e1002455. doi:10.1371/journal.pcbi.1002455.

Grote, M. (2017). Petri dish versus Winogradsky column: a longue durée perspective on purity and diversity in microbiology, 1880s-1980s. Hist Philos Life Sci 40, 11. doi:10.1007/s40656-017-0175-9.

Merino, N., Aronson, H. S., Bojanova, D. P., Feyhl-Buska, J., Wong, M. L., Zhang, S., & Giovannelli, D. (2019). Living at the Extremes: Extremophiles and the Limits of Life in a Planetary Context. Frontiers in Microbiology, 10. https://doi.org/10.3389/fmicb.2019.00780