Microbes in "Extreme" Environments

→ Defining microbial life and its diversity
→ Physicochemical boundaries (Temperature / pH / Water activity / Pressure)
→ Energetic boundaries (Metabolism & Flux)

→ Potentially relevant ecologies…
- → Interactions & Symbioses
- → Microhabitats (Surfaces / Boundaries / Gradients)

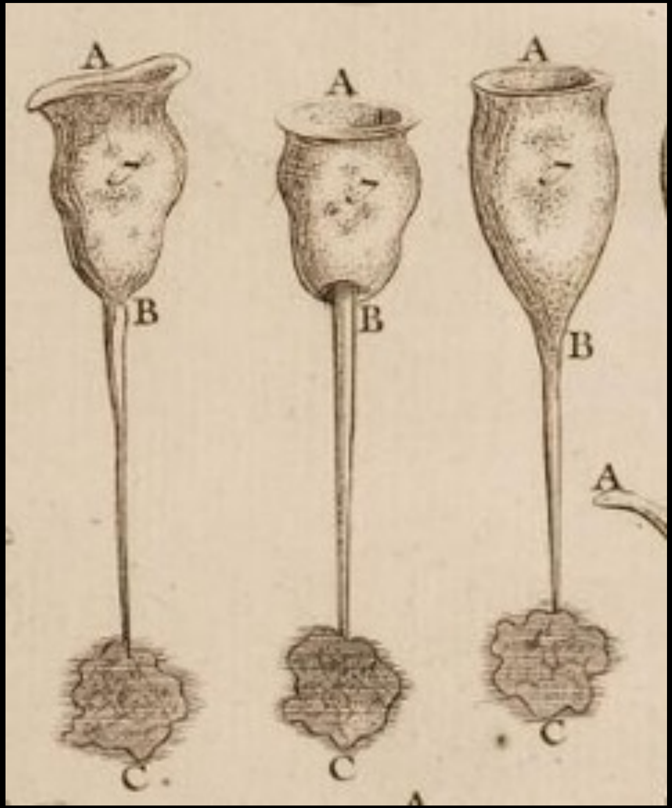
Jeffrey Marlow

BOSTON UNIVERSITY

Microbes in "Extreme" Environments

→ Defining microbial life and its diversity

The first microscopes of the 16th and 17th centuries revealed a new world of tiny, occasionally moving, objects…



Microbes in "Extreme" Environments

→ Defining microbial life and its diversity

= microorganism
= an organism that is microscopic
= an organism that you need a microscope to see

What is life?

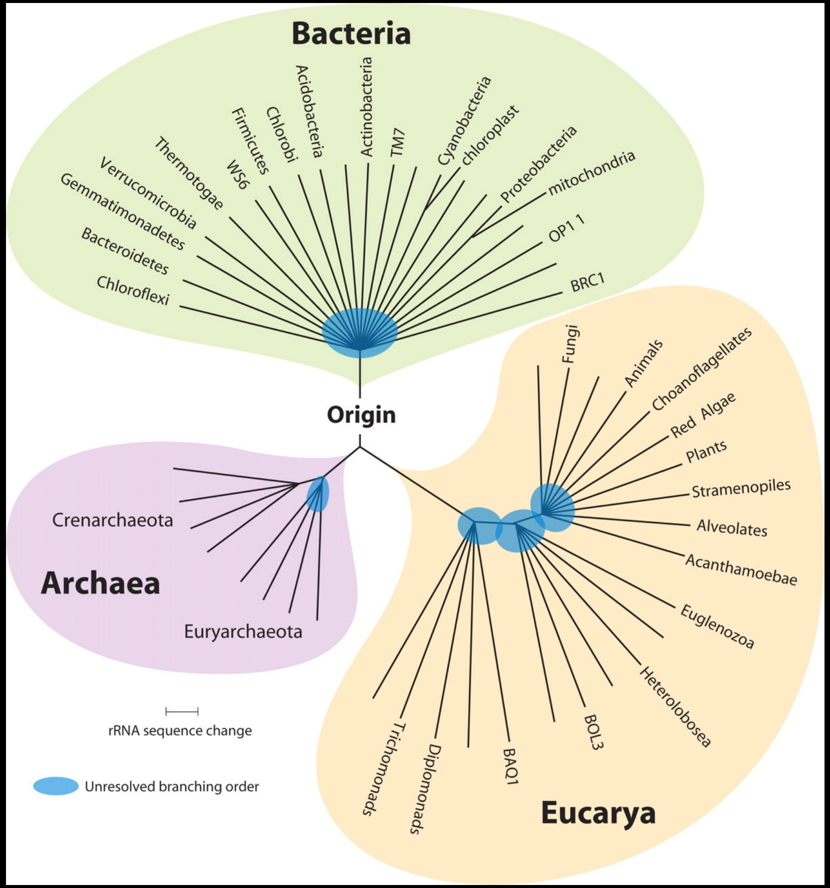
Resolving power of the human eye is ~40-75 μm

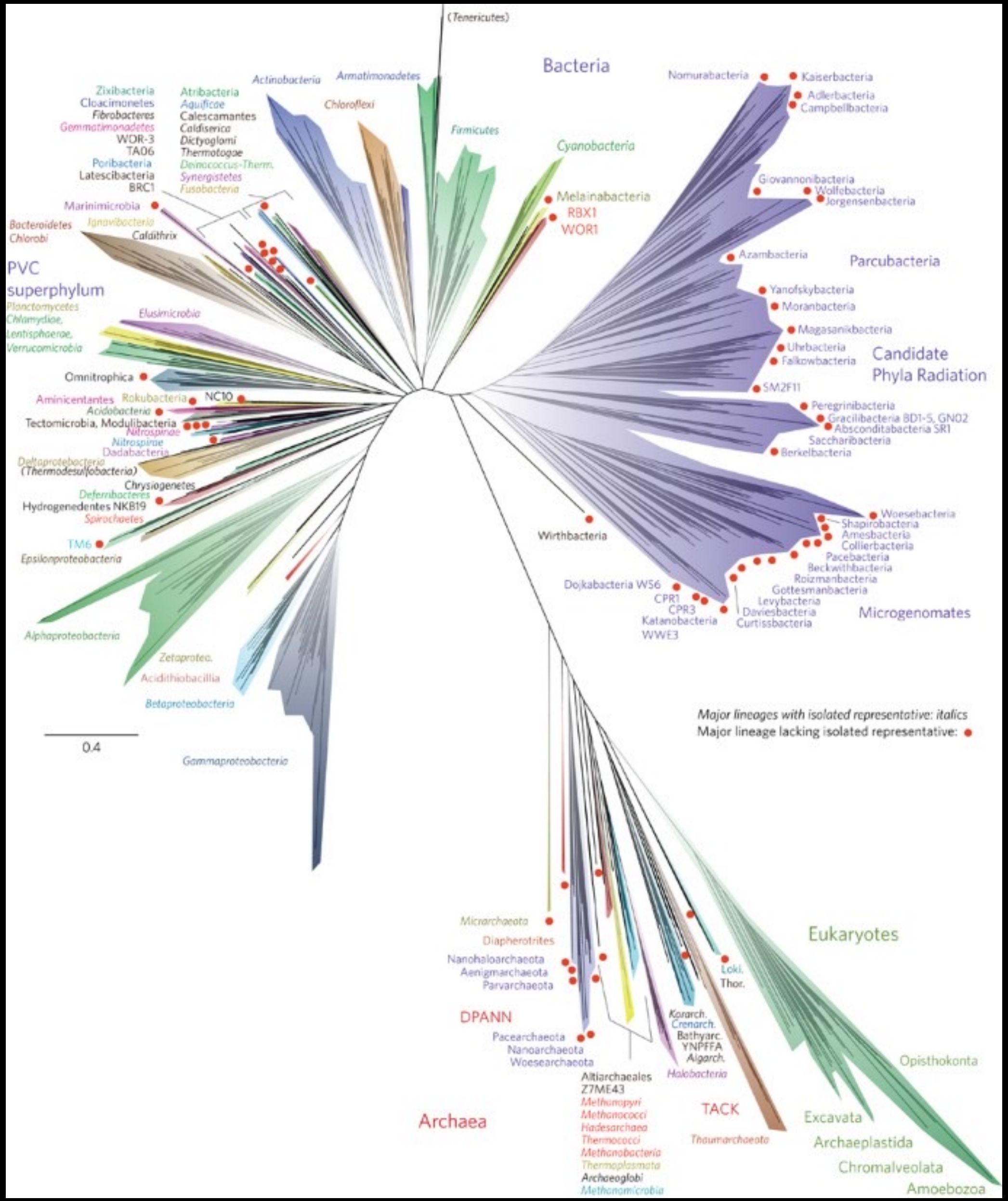
Something that moves? Consumes energy? Replicates?
"A self-sustaining chemical system capable of Darwinian evolution"

Microbes in "Extreme" Environments

→ Defining microbial life and its diversity

Phylogeny, or evolutionary history – the ways in which one type of life evolved from another through genetic modification



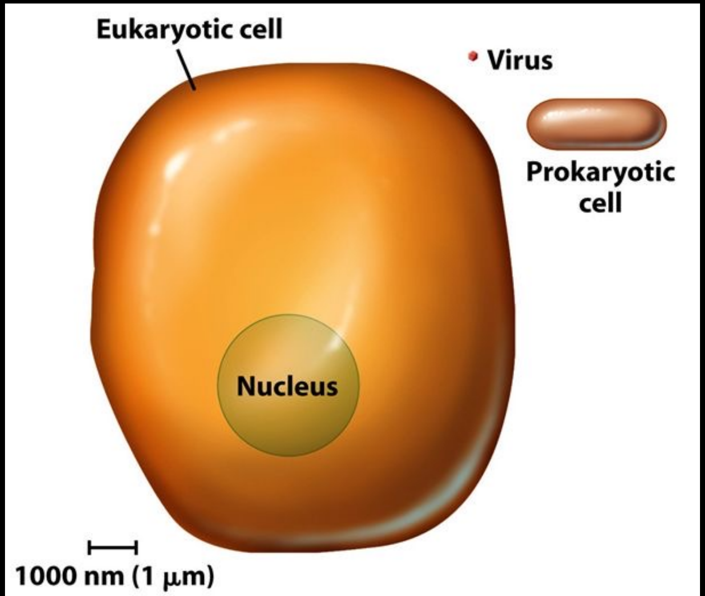


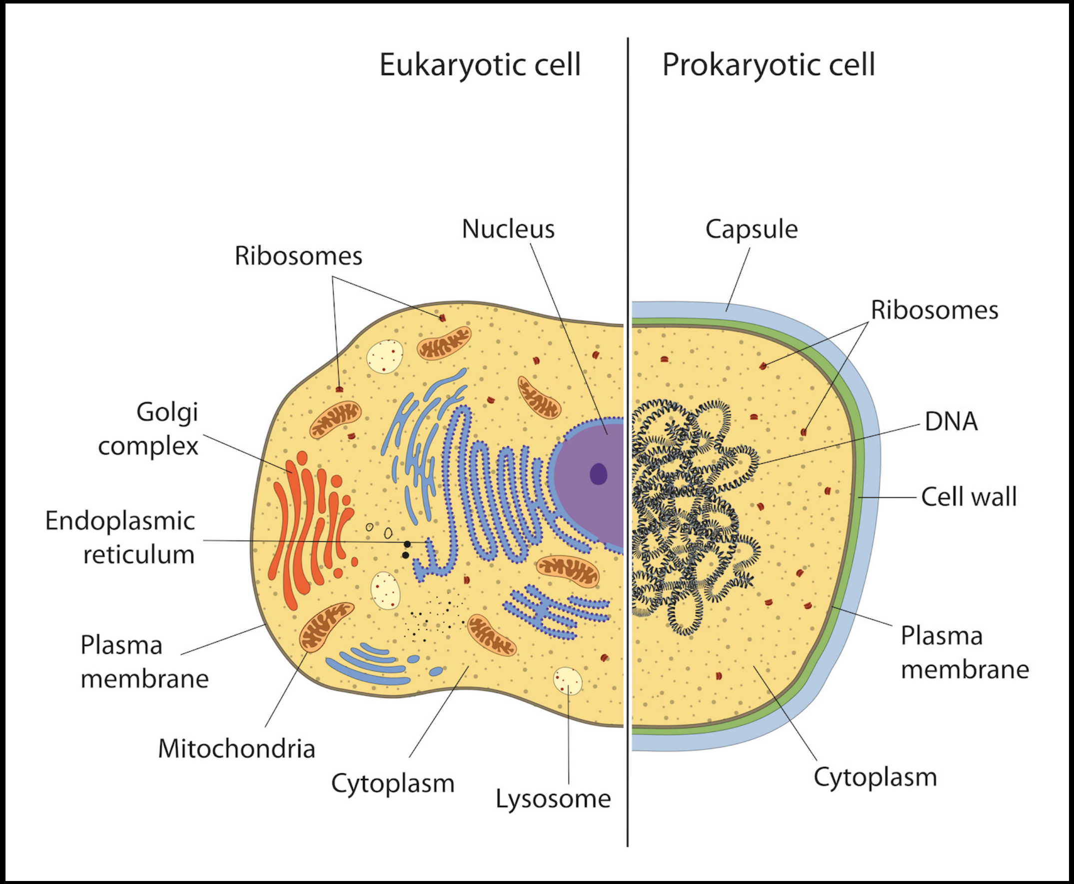
Hug et al., Nature Microbiology, 2016

Microbes in "Extreme" Environments

→ Defining microbial life and its diversity

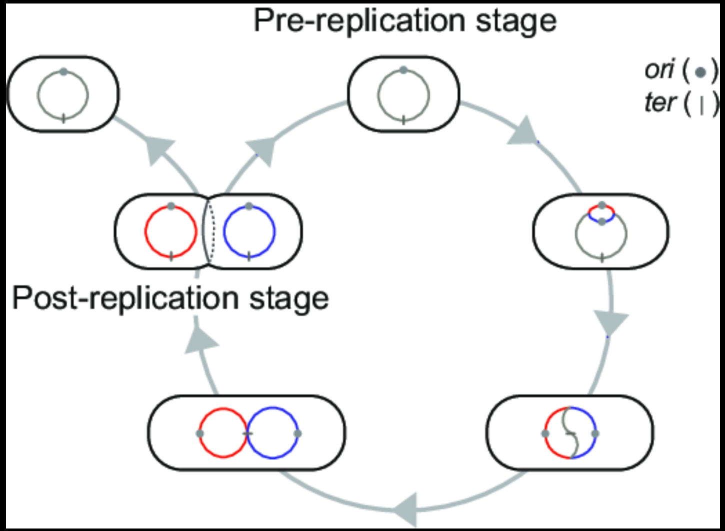
Archaea and Bacteria (Prokaryotes) are generally more 'primitive' and metabolically versatile (i.e., what we're seeking) than Eukaryotes.





Microbes in "Extreme" Environments

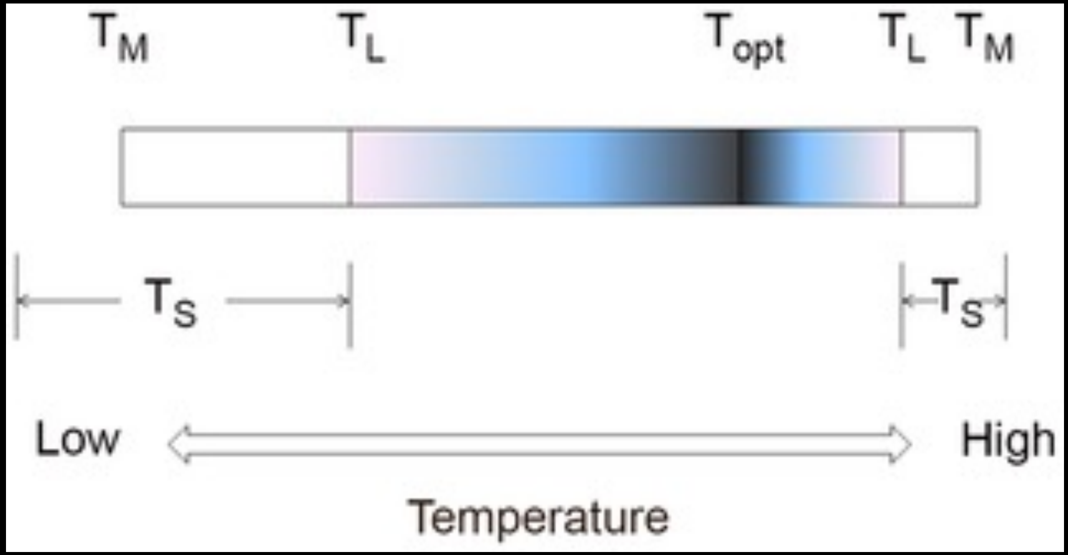
→ Physicochemical boundaries (Temperature / pH / Water activity / Pressure)



T_L = temperature at which an organism can complete its life cycle

T_M = temperature at which an organism can conduct metabolic reactions

T_S = temperature at which an organism can survive, could return to a state of metabolic activity



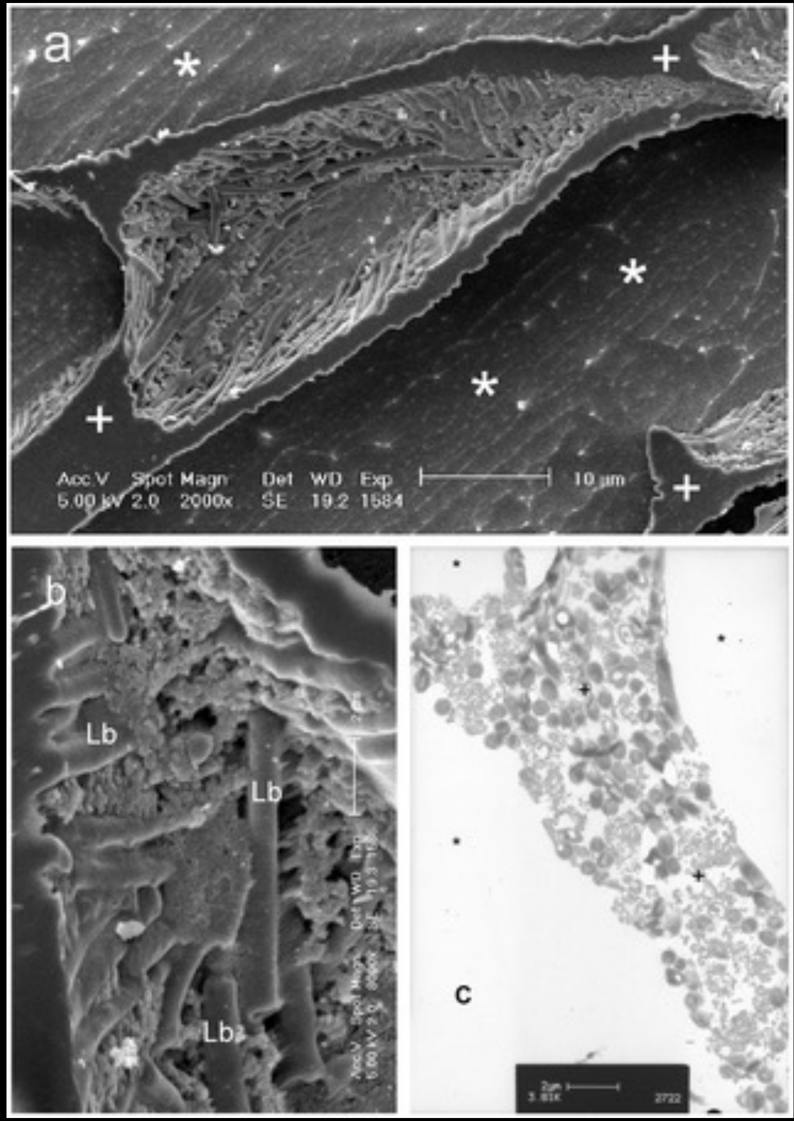
Microbes in "Extreme" Environments

→ Physicochemical boundaries (Temperature / pH / Water activity / Pressure)

Low temperature issues:

- Lower molecular kinetic energy, so all reactions happen slower.
- Lower membrane fluidity, making it harder to transport reactants in and wastes or metabolites out.

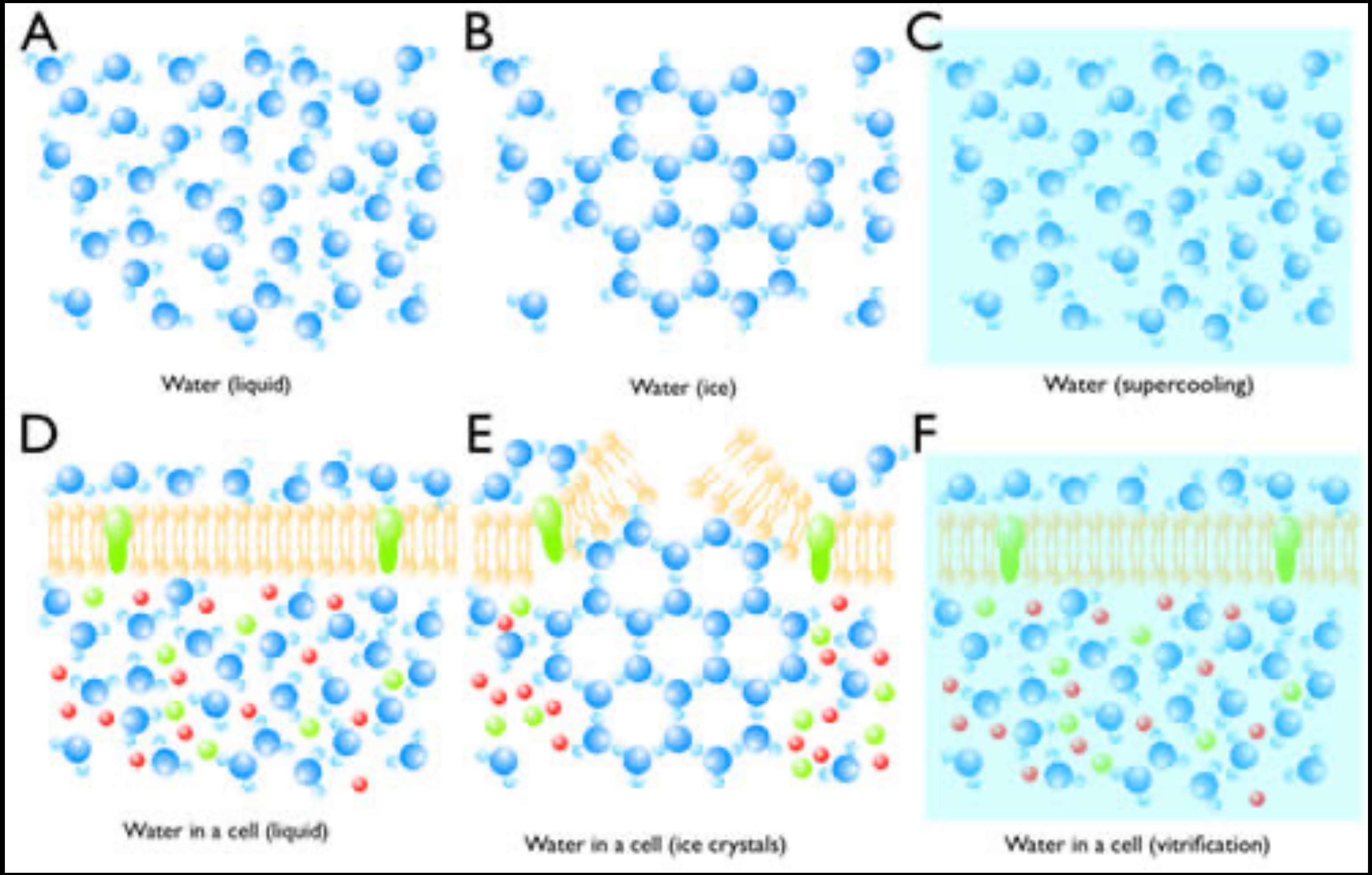
→ Ice is the real enemy. Cells can be mechanically damaged, remaining fluid becomes more concentrated, and ultimately, everything may become solid.



Lactobacillus delbrueckii responding to ice formation
Clarke et al., 2013, PLOS One

Microbes in "Extreme" Environments

→ Physicochemical boundaries (Temperature / pH / Water activity / Pressure)



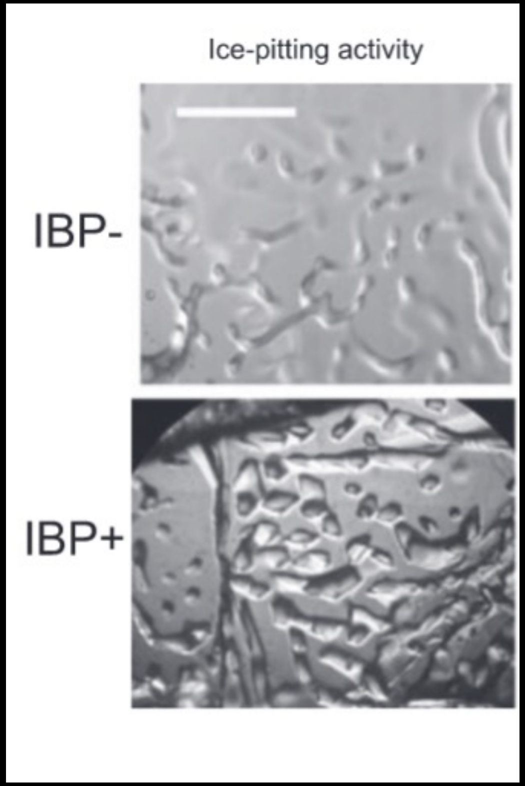
Vitrification can avoid ice nucleation even at sub-zero temperatures.

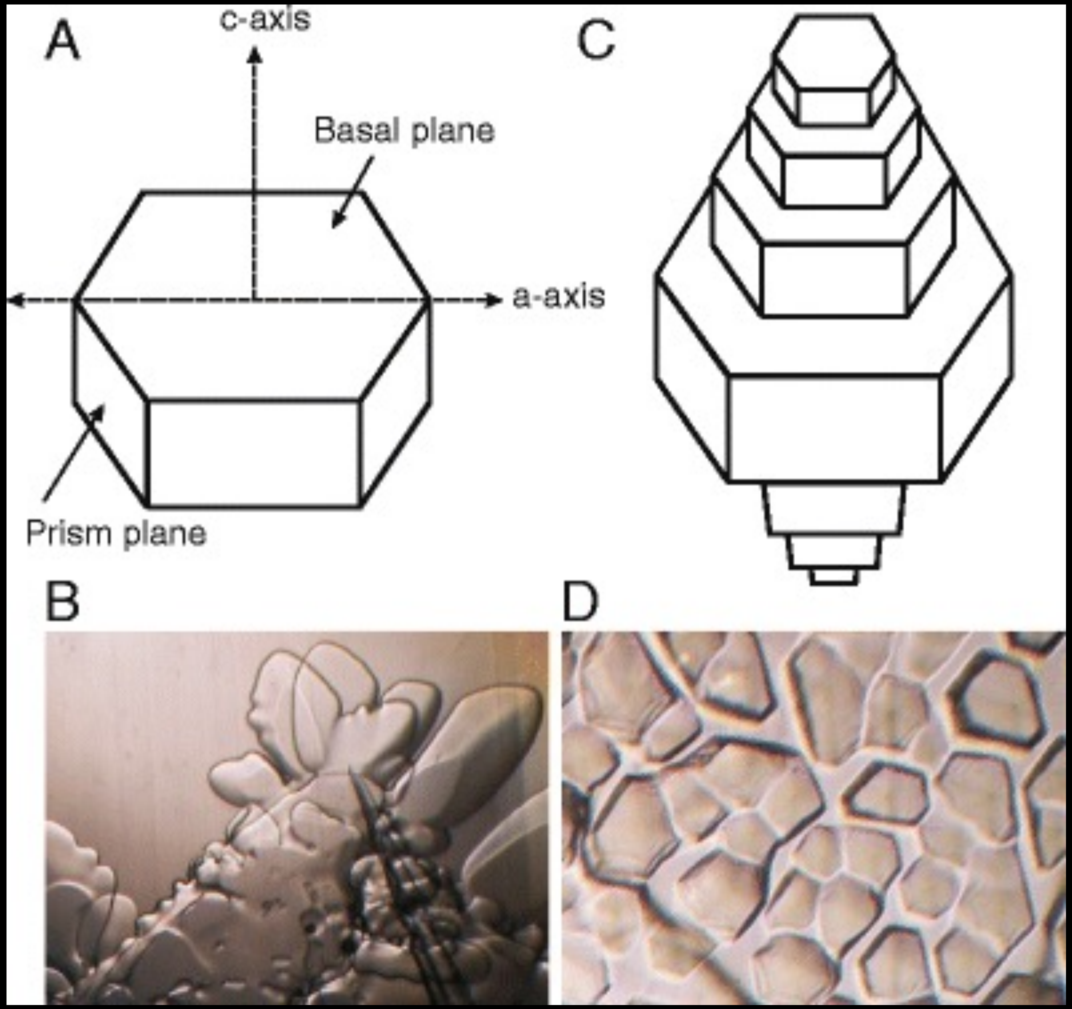
Rapid freezing can maintain an un-ordered state, allowing things to move around and minimizing the threat of physical disruption.

Microbes in "Extreme" Environments

→ Physicochemical boundaries (Temperature / pH / Water activity / Pressure)

Ice binding proteins can direct the location and shape of ice crystal formation, keep more fluid inside the cell



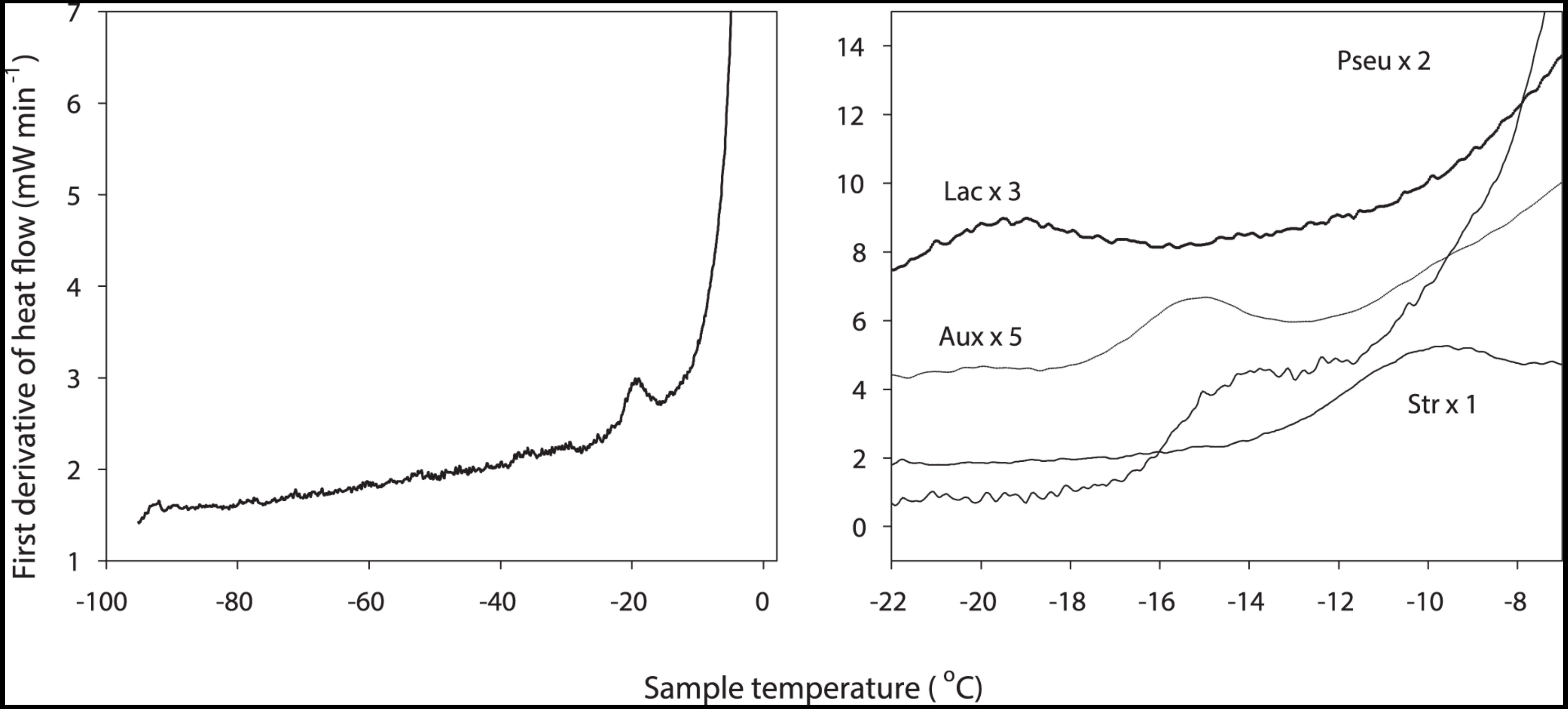


Christner et al., Applied Microbio and Biotech, 2010

Microbes in “Extreme” Environments

→ Physicochemical boundaries (Temperature / pH / Water activity / Pressure)

Calorimetry data showing molecular movement and metabolism below 0 C



Organism	Tg (°C)
Eubacteria	
Lactobacillus delbrueckii ssp. *bulgaricus*	−19.3 (0.9)
Pseudomonas syringae	−13.9 (0.9)
Corynebacterium variabile	−25.6 (0.6)
Arthrobacter arilaitensis	−26.0 (0.8), −21.0 (0.8)
Streptococcus thermophilus	−11.6 (1.0)
Photosynthetic eukaryotes	
Auxenochlorella protothecoides	−15.1 (0.7)
Chlamydomonas nivalis	−24.2 (0.8)
Heteroptrophic eukaryotes	
Debaryomyces hansenii	−11.6 (1.0)
Saccharomyces cerevisiae	−12.3 (1.1)

Intracellular vitrification temperature (Tg) determined by DSC. Tg is the mean of 3–4 independent measurements, with the typical SD of the measurements being 0.8°C. Note that in *Arthrobacter* two vitrification peaks were evident in the DSC traces. Standard deviation from three replicate DSC runs shown in parentheses.

doi:10.1371/journal.pone.0066207.t001

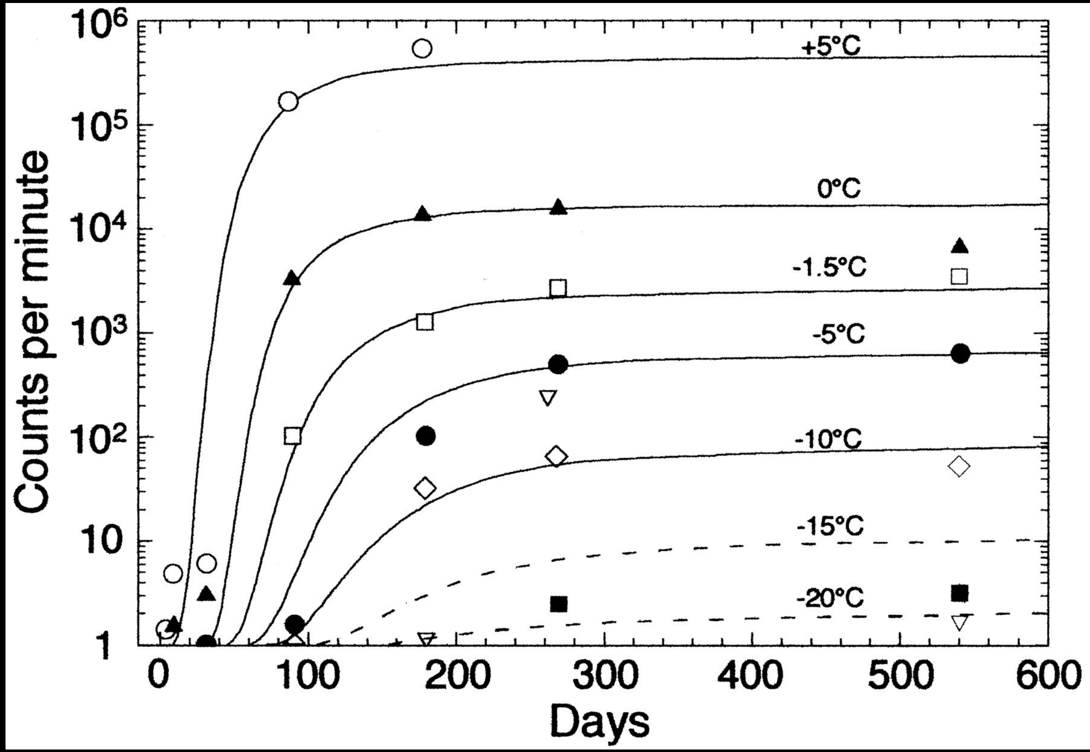
Clarke et al., PLOS One, 2013

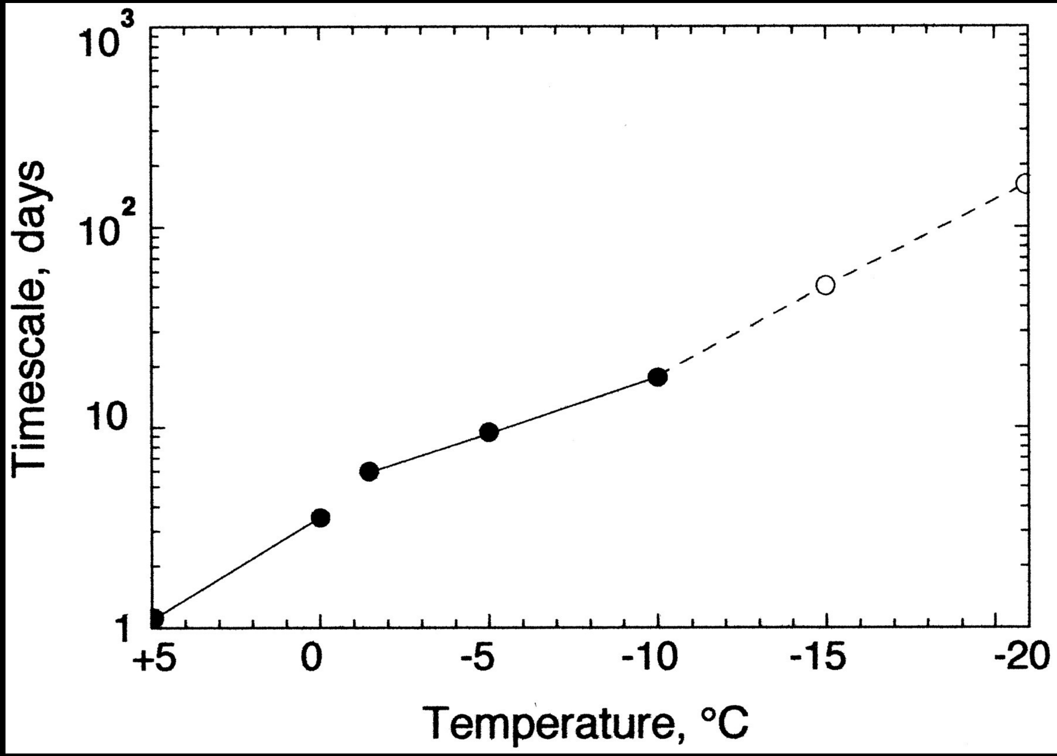
Microbes in "Extreme" Environments

→ Physicochemical boundaries (Temperature / pH / Water activity / Pressure)

Current metabolic limit for life has been measured down to -20 C

^{14}C-acetate was added, which can be metabolized into acetyl-CoA; ^{14}C content of cell lipids was measured

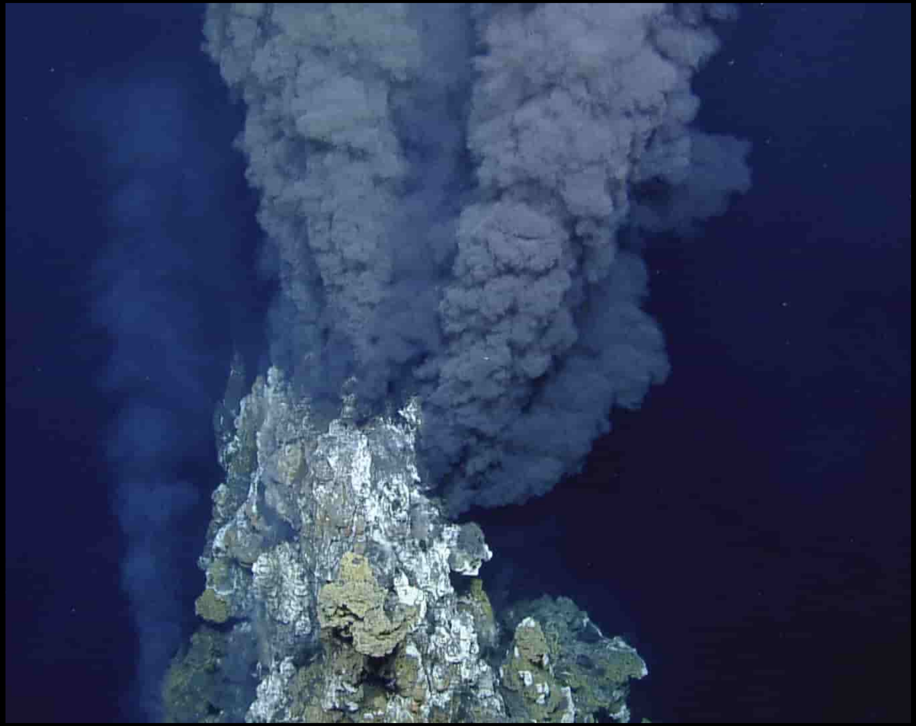


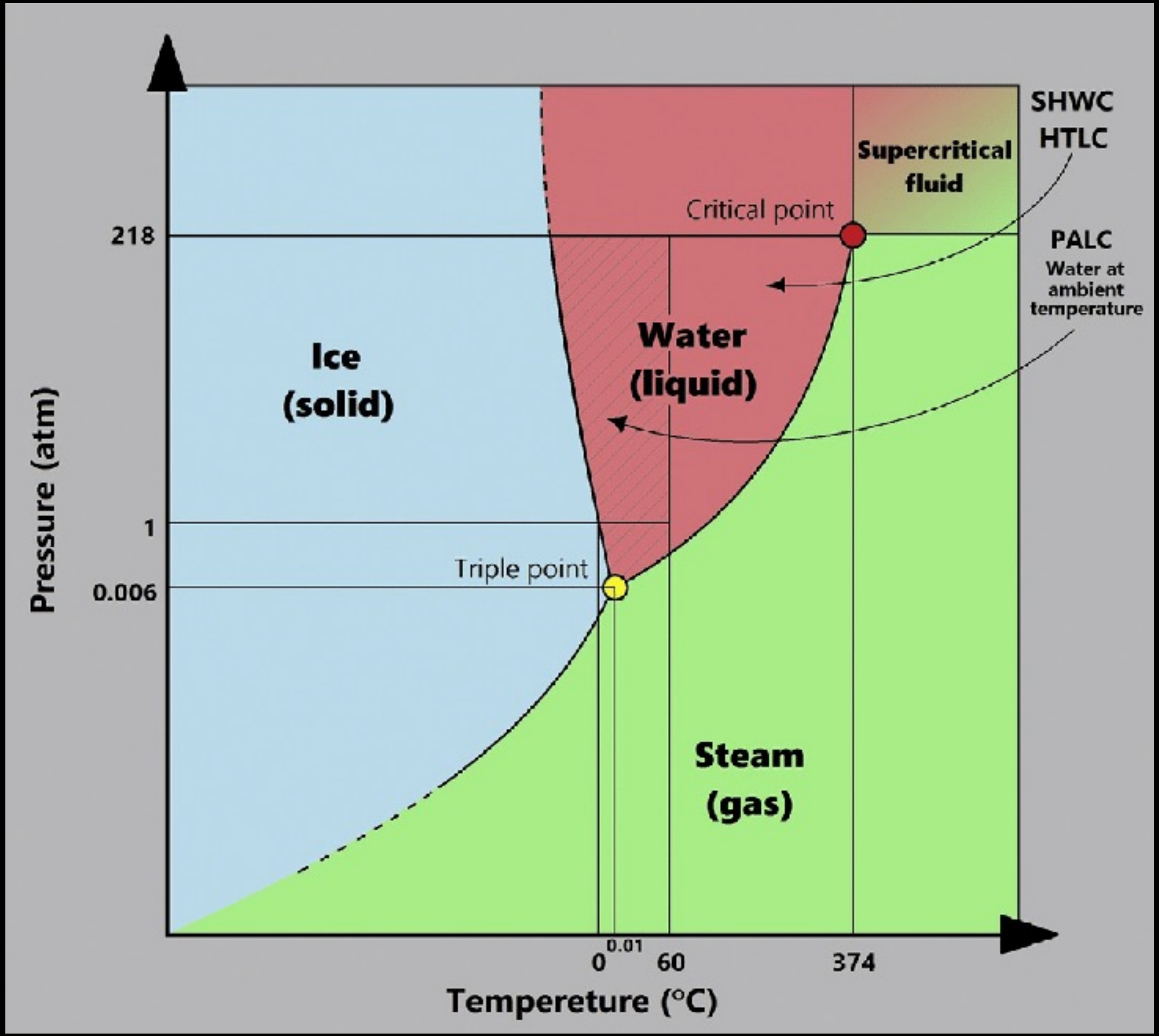


Rivkina et al., 2000

Microbes in "Extreme" Environments

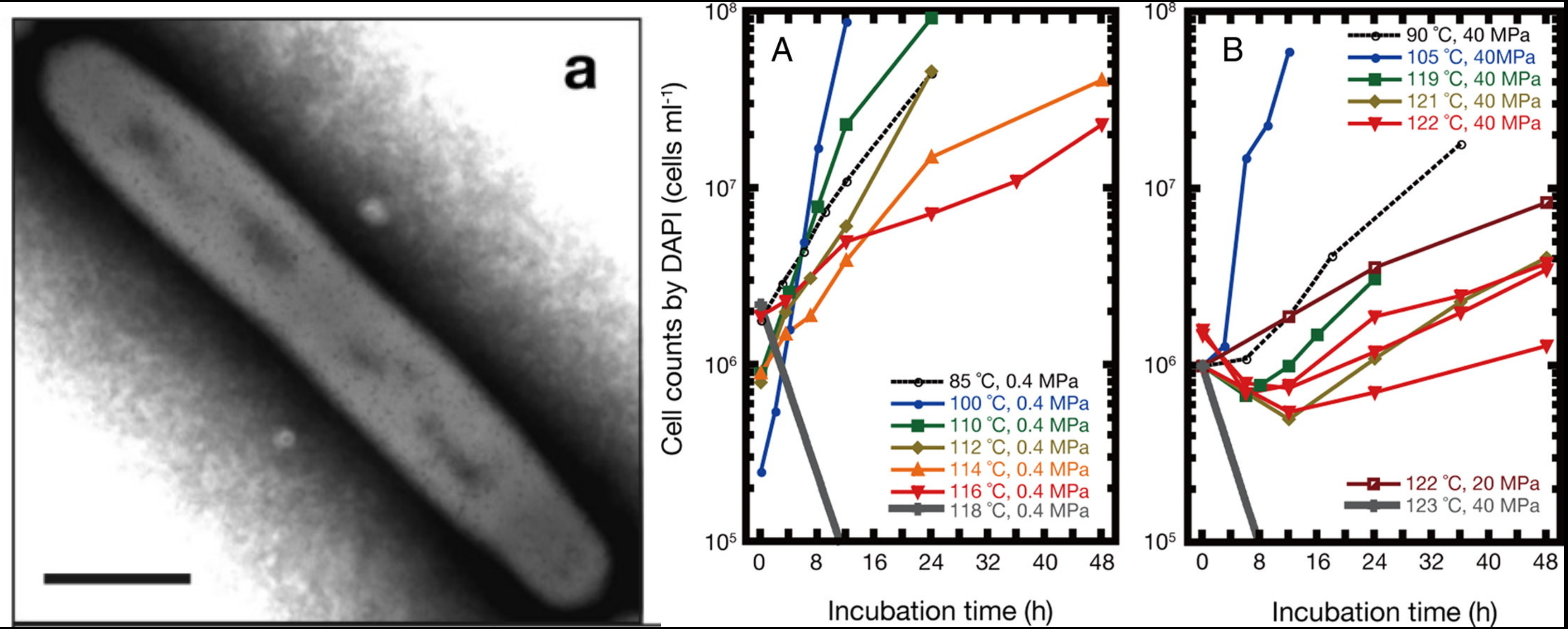
→ Physicochemical boundaries (Temperature / pH / Water activity / Pressure)





Microbes in "Extreme" Environments

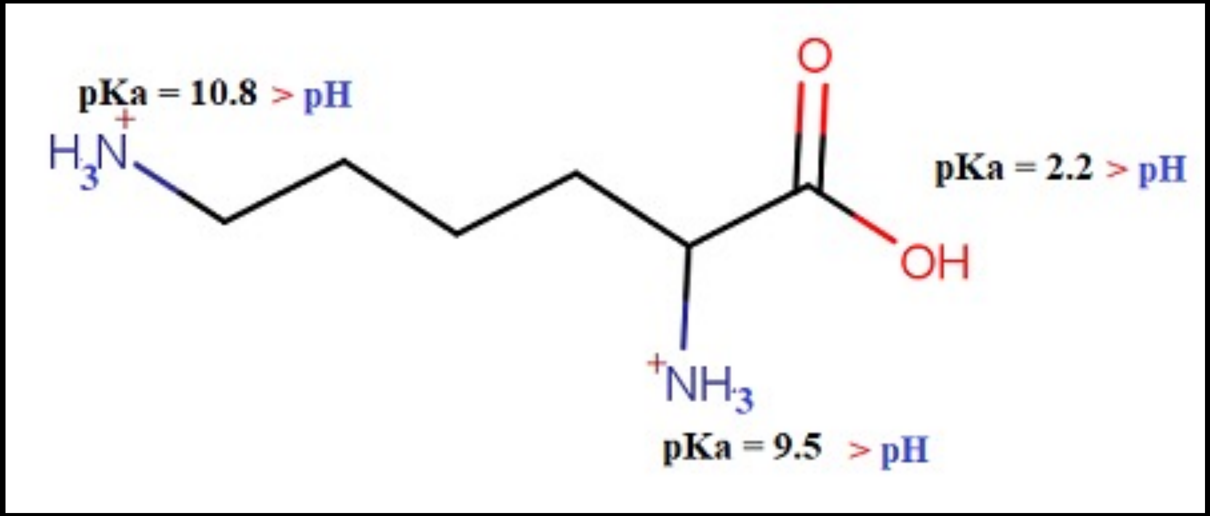
→ Physicochemical boundaries (Temperature / pH / Water activity / Pressure)

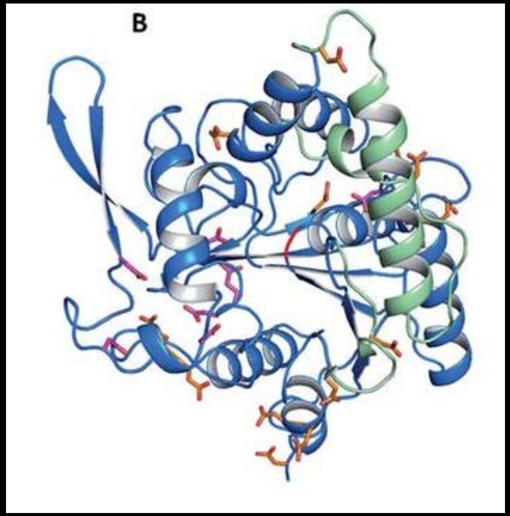


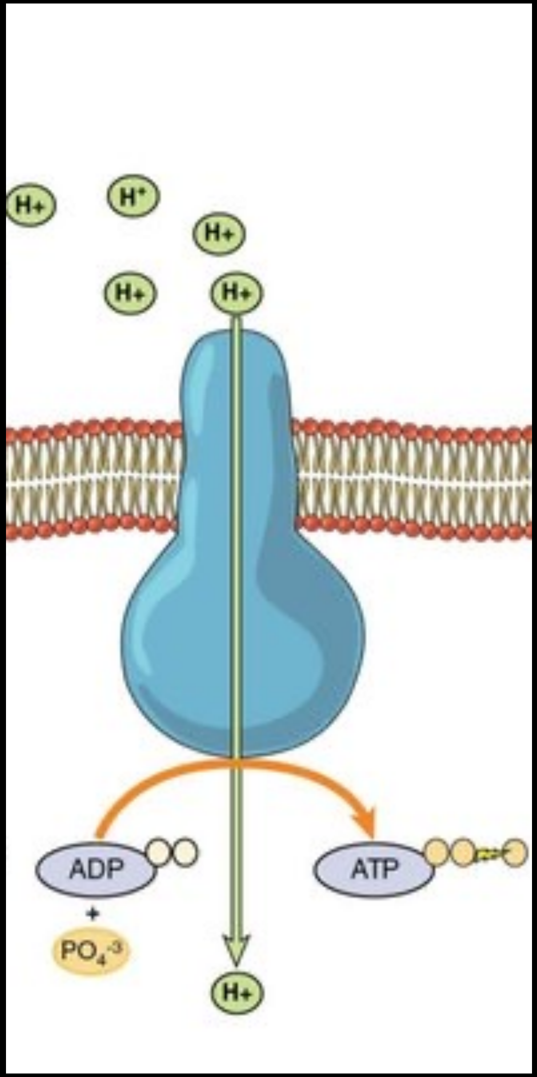
Takai et al., PNAS, 2008

Microbes in "Extreme" Environments

→ Physicochemical boundaries (Temperature / pH / Water activity / Pressure)







Carboxyl (COOH) group typically deprotonates at 2-2.4

Amino (NH_3^+) group typically deprotonates at 9-10.5

Side groups deprotonate between 3.9-13

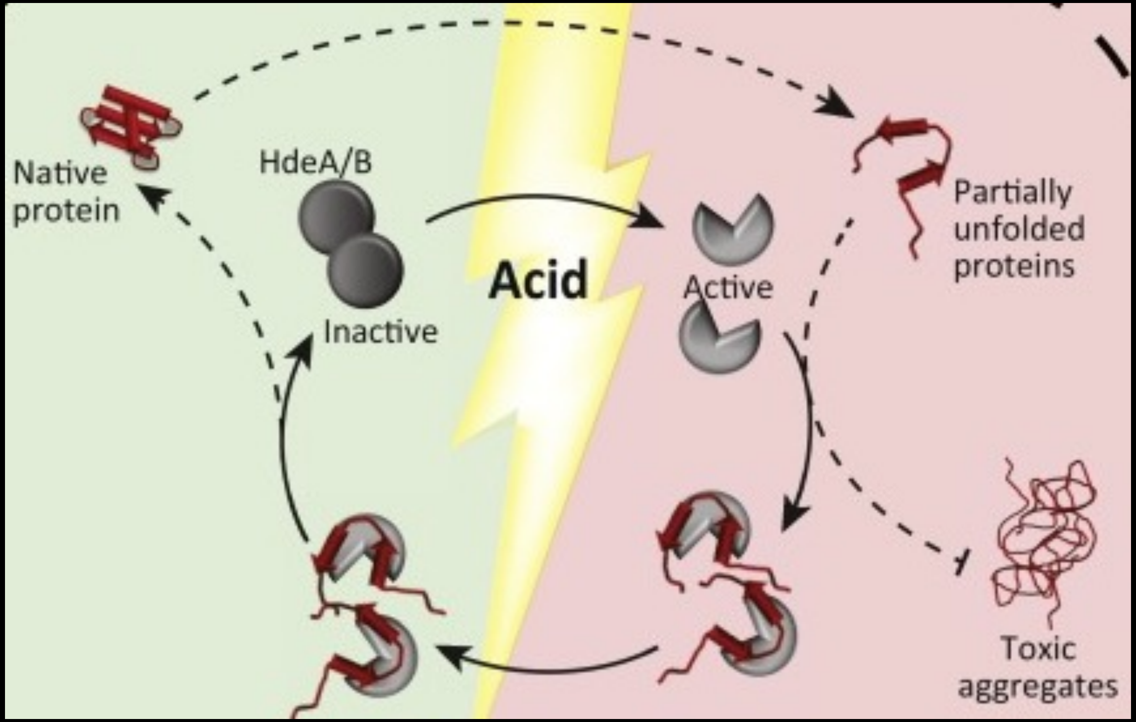
Protein tertiary structure can be disrupted, leading to protein unfolding and aggregations.

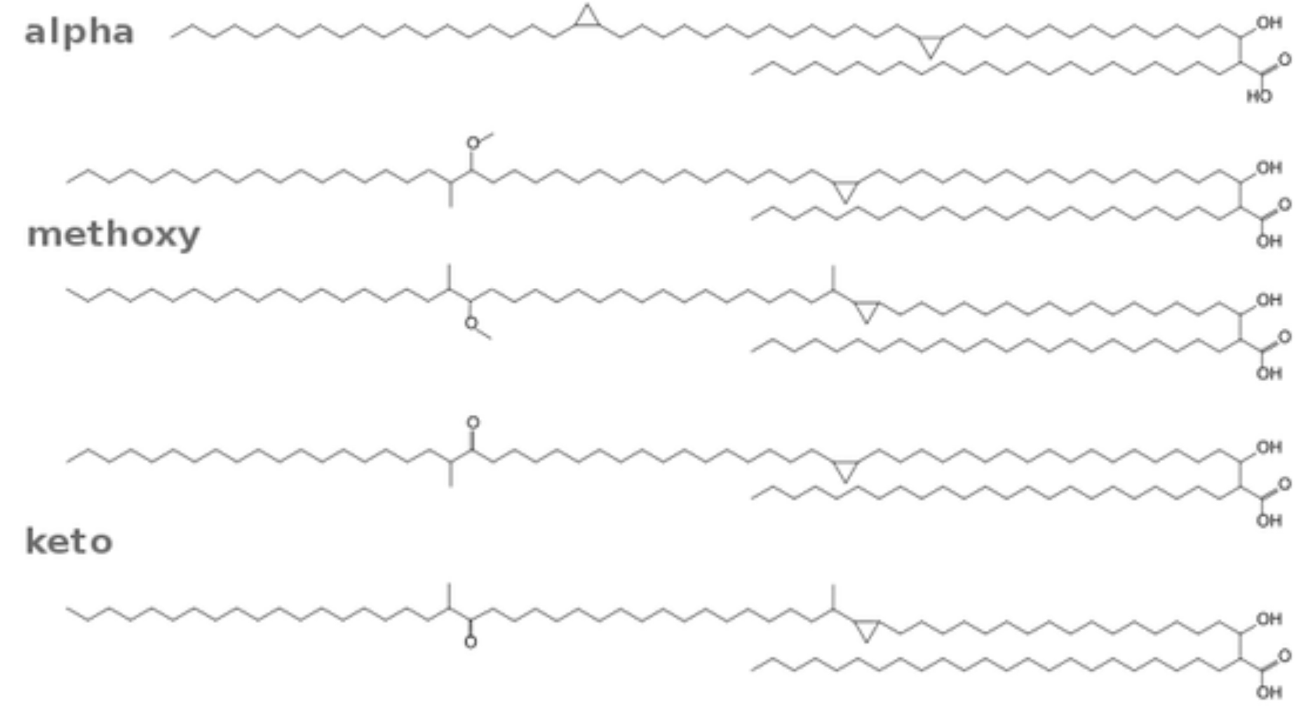
Proton pumps an ATPase can also be affected.

Microbes in "Extreme" Environments

→ Physicochemical boundaries (Temperature / pH / Water activity / Pressure)

1. Change membrane composition – more cyclopropane fatty acids to lower proton permeability
2. DNA repair: Dps and RecA proteins (in *E. coli*)
3. Protein-refolding proteins: chaperones





Microbes in "Extreme" Environments

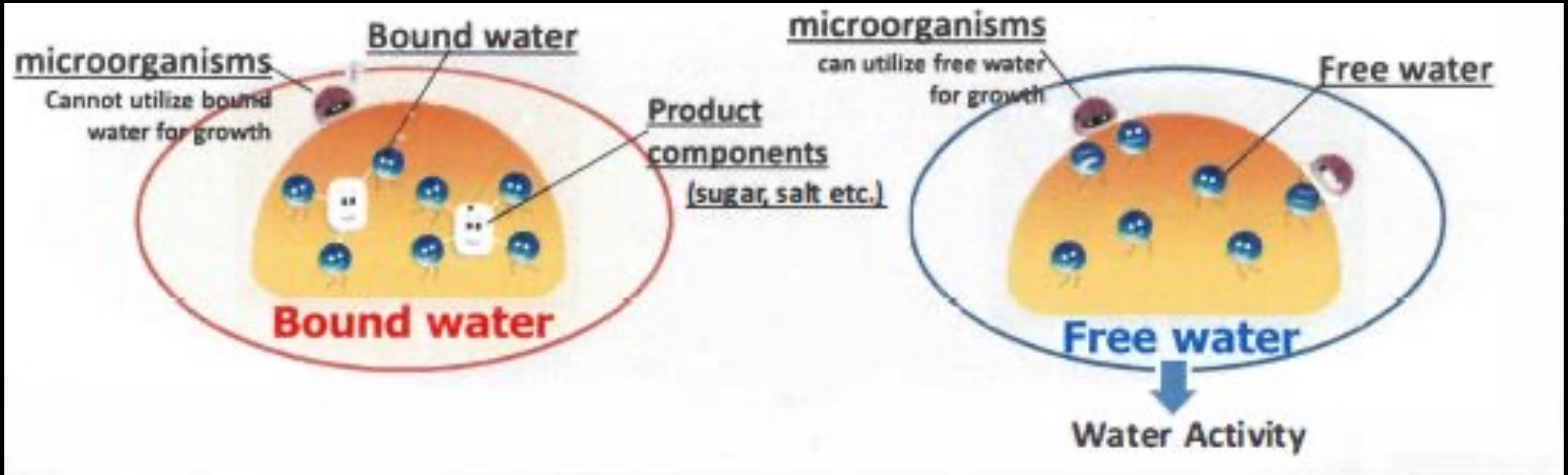
→ Physicochemical boundaries (Temperature / pH / Water activity / Pressure)

Activity is the "felt concentration" of a chemical in a mixture. Activity is not the same as concentration because molecules in solution interact with each other, so just because something is around doesn't mean it's accessible or available for interactions.

$$a_w = x\lambda$$

x = mole fraction of water in solution

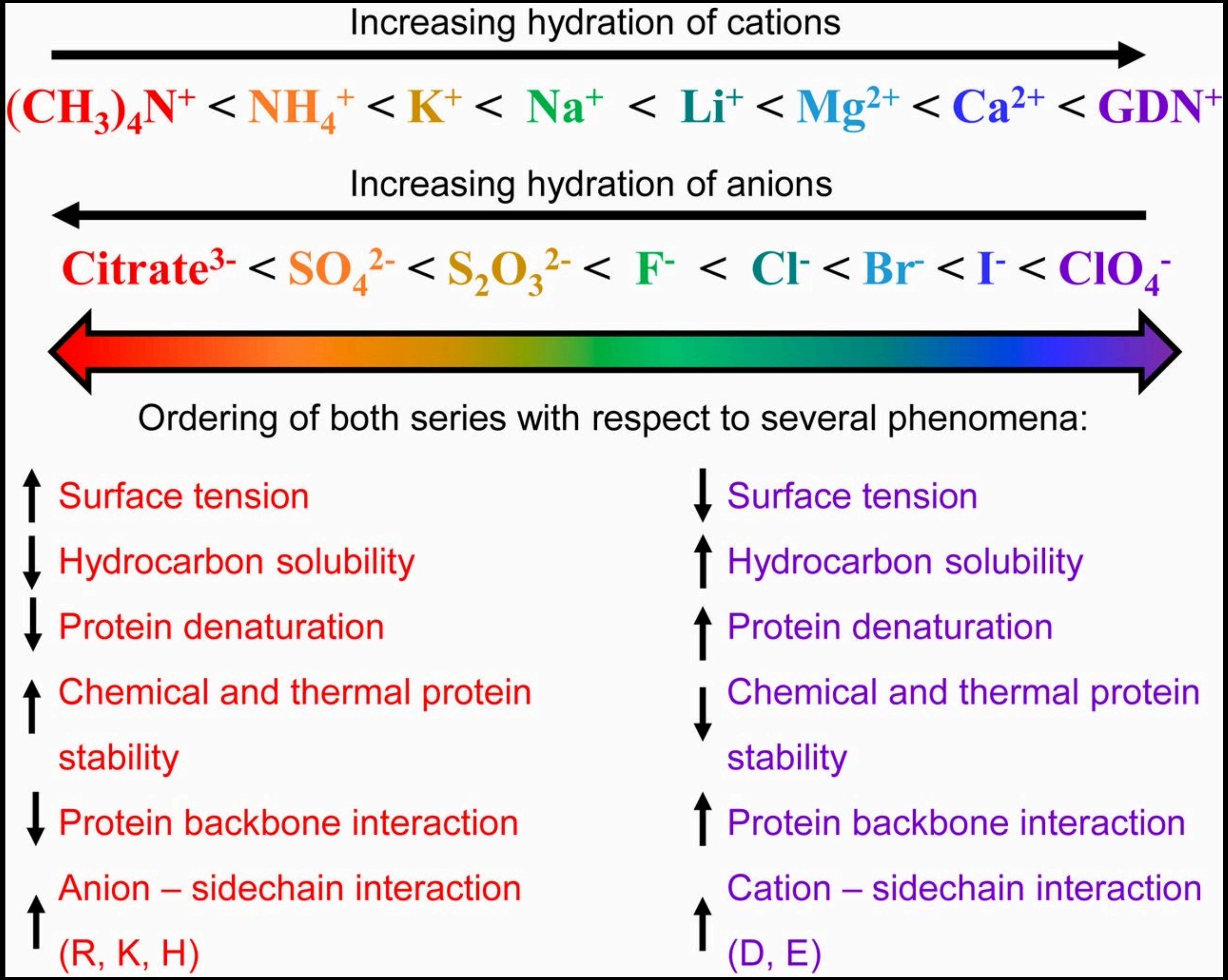
λ = activity coefficient of water



Microbes in “Extreme” Environments

→ Physicochemical boundaries (Temperature / pH / Water activity / Pressure)

kosmotropic chaotropic



Not all salts or ions are equivalent in biological effects

Chaotopic salts disrupt hydrogen bonding better because of electronic structure.

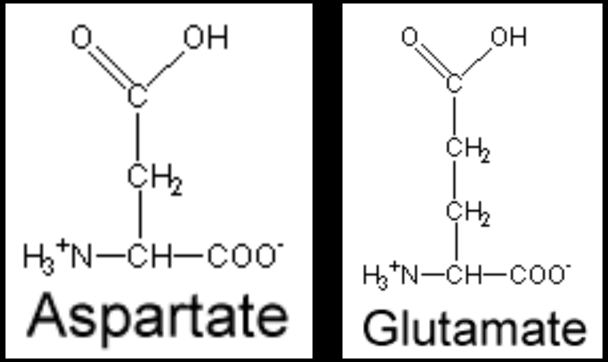
→ Relevant for DNA extraction protocols and for possibility of life on Ocean Worlds

Microbes in "Extreme" Environments

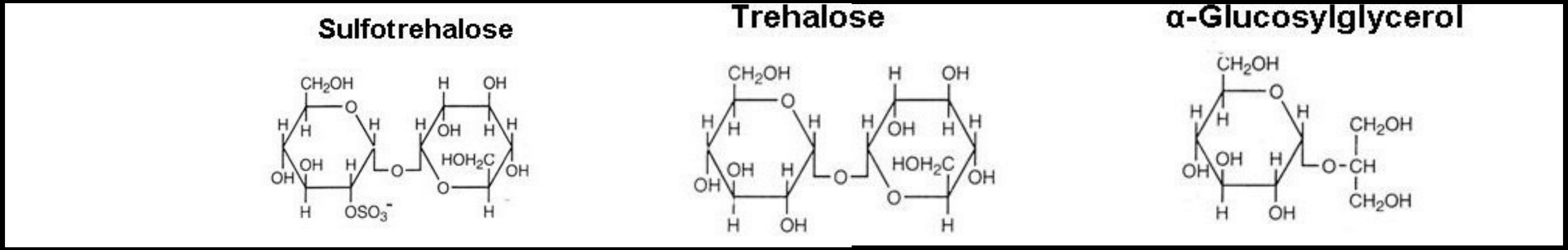
→ Physicochemical boundaries (Temperature / pH / Water activity / Pressure)

Option 1: the "salt-in" strategy – keep lots of KCl inside the cell

Proteome requires significant adjustment – many acidic amino acids to attract more water molecules, encourage fleeting and nonspecific interactions with salt ions. In less salty conditions, these proteins don't work.



Option 2: "compatible solute" strategy – the cell synthesizes a bunch of small organic molecules that decrease the water activity to a level similar to that of the surroundings. This approach is more common.



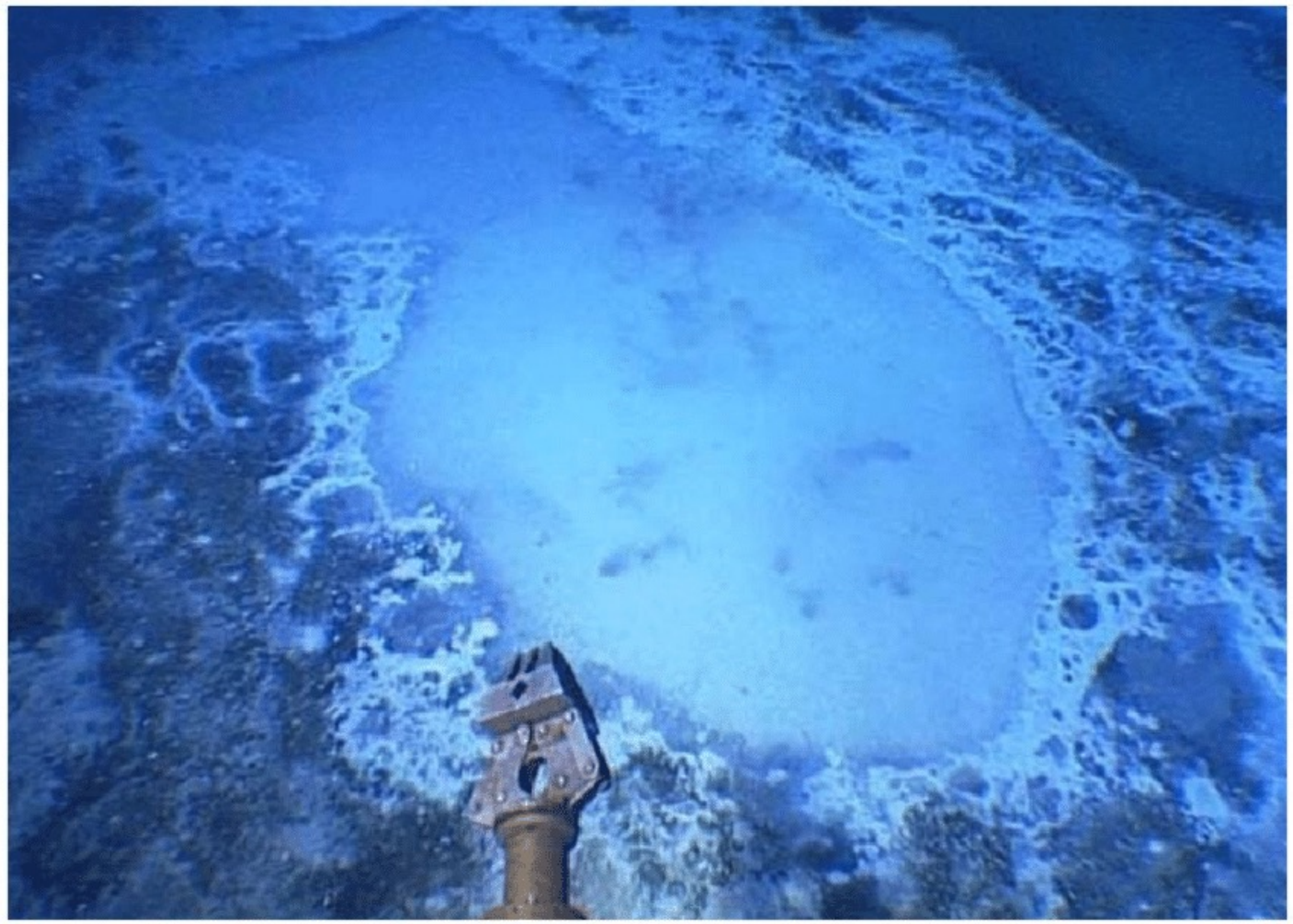
Microbes in "Extreme" Environments

→ Physicochemical boundaries (Temperature / pH / Water activity / Pressure)

Discovery Brine Pool in the Mediterranean Sea – caused by redissolution of magnesium chloride deposits that precipitated out 5.5 Mya; 5M $MgCl_2$

aw < 0.4

Cells recovered, but is there active metabolism? mRNA recovered at a maximum of 2.3 M $MgCl_2$

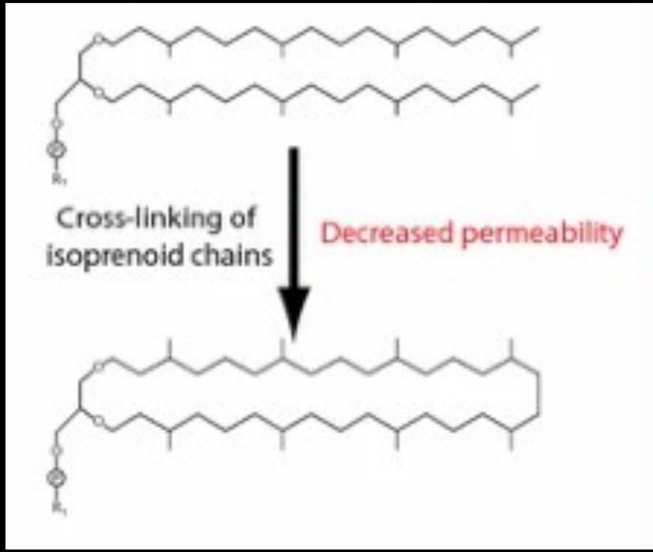


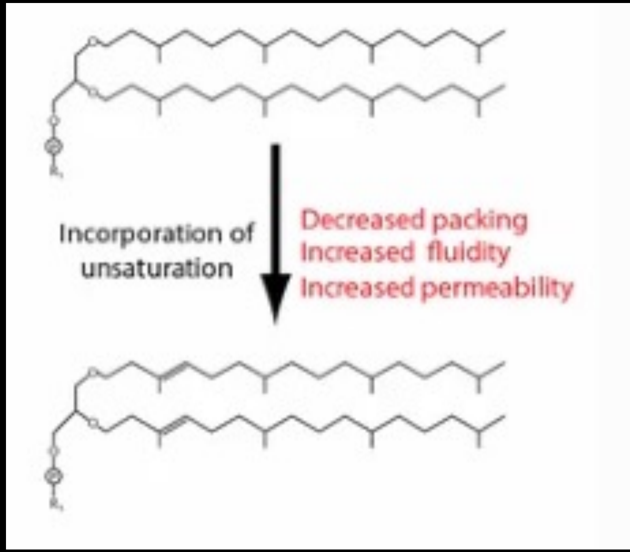
Microbes in "Extreme" Environments

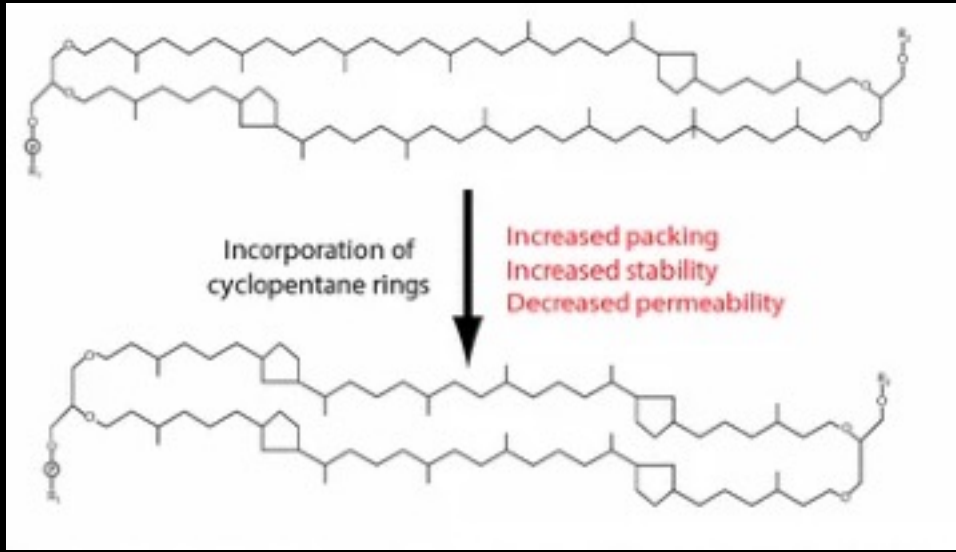
→ Physicochemical boundaries (Temperature / pH / Water activity / Pressure)

Many of the same physiological responses to other stressors are also found in piezophiles.

1 – Compatible solutes
2 – Membrane alterations



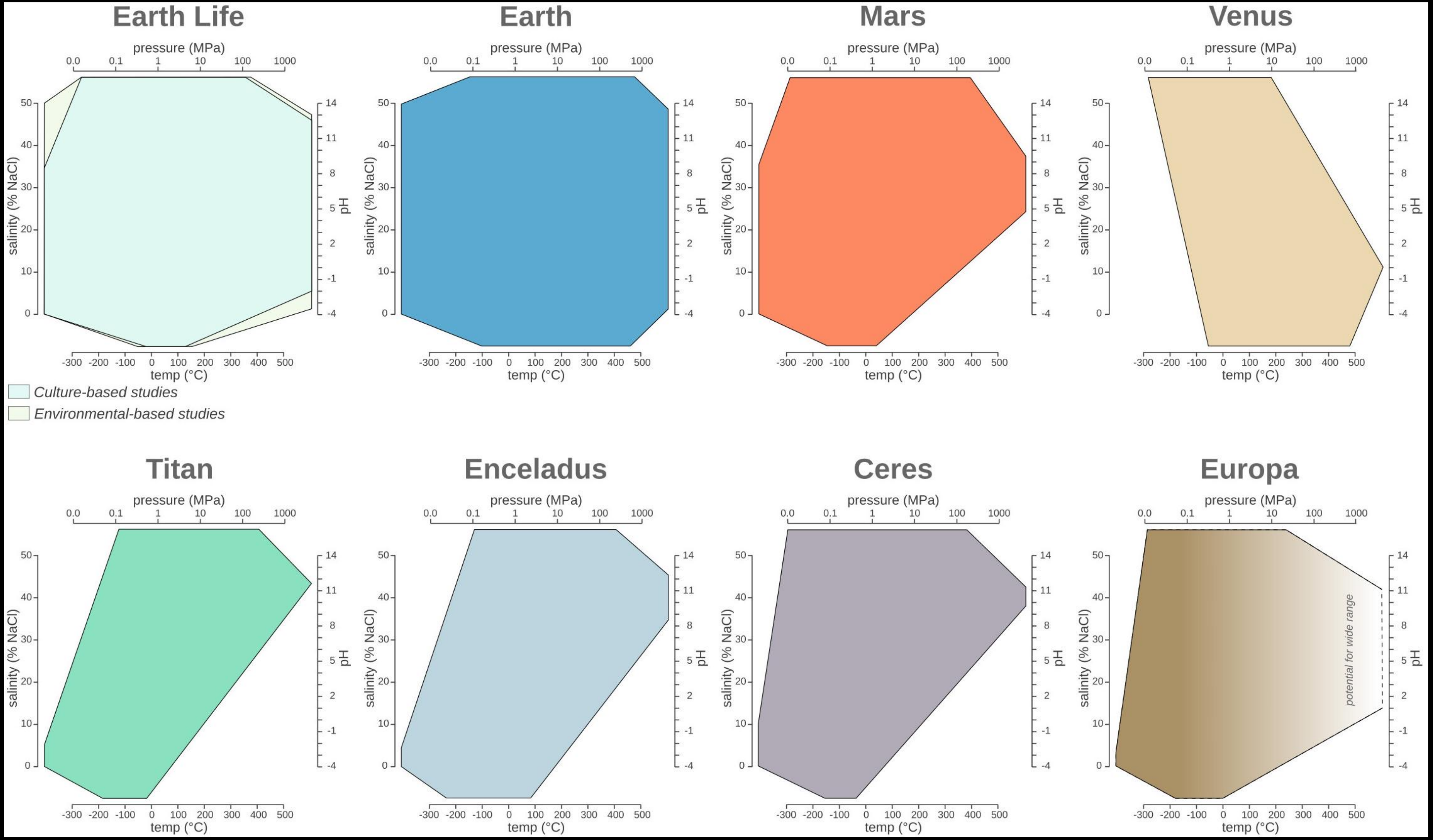




Jebbar et al., Extremophiles, 2015

Microbes in “Extreme” Environments

→ Physicochemical boundaries (Temperature / pH / Water activity / Pressure)

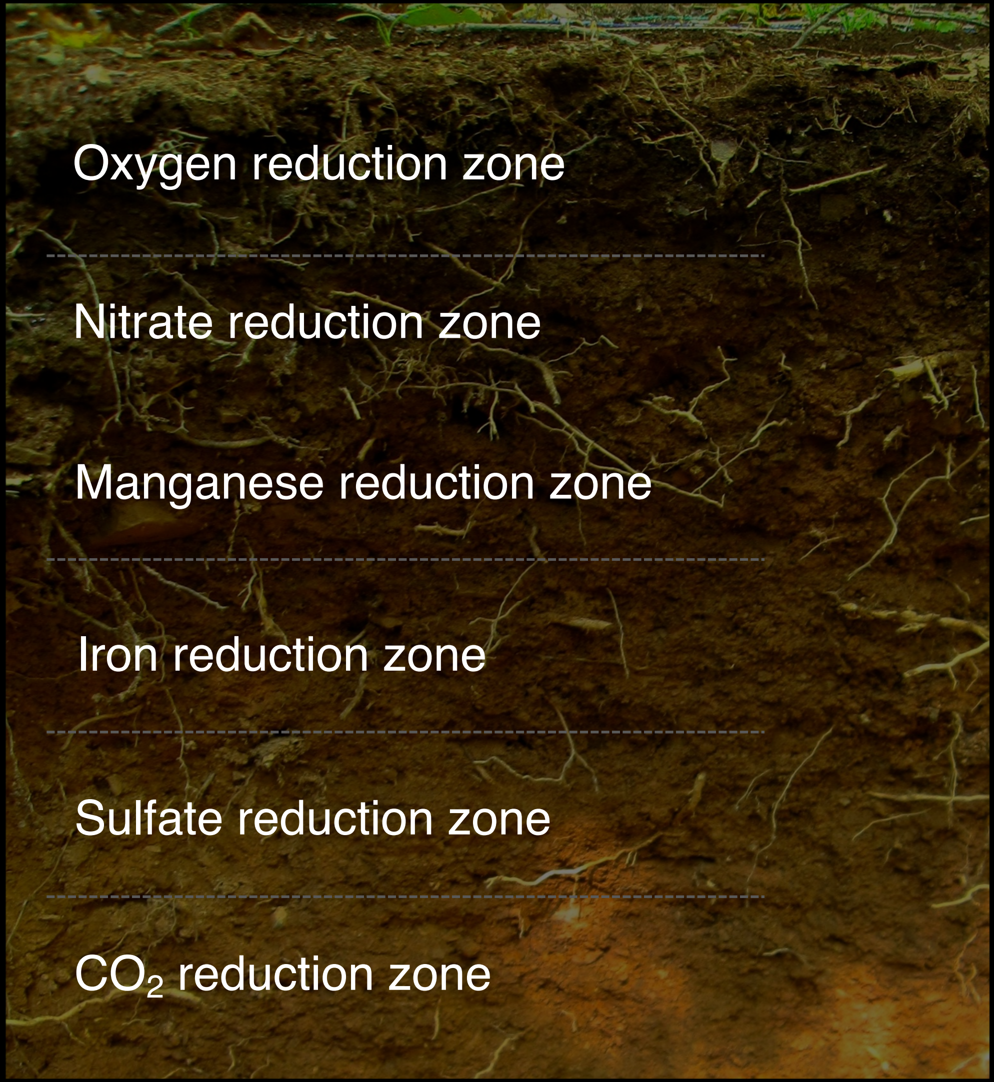


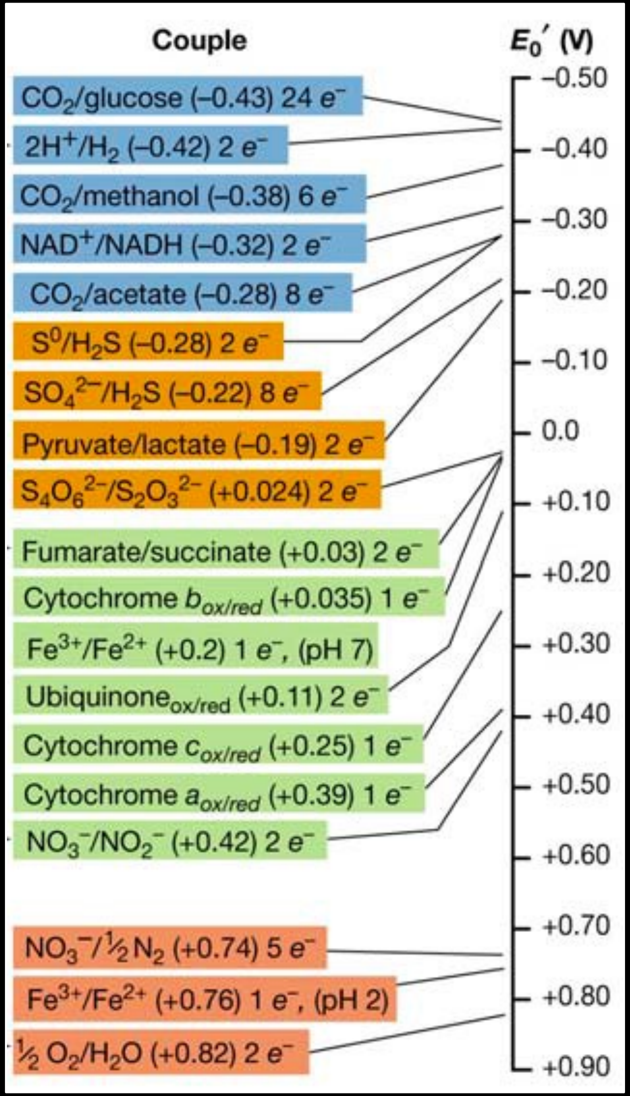
Merino et al., Frontiers in Microbiology, 2019

Microbes in "Extreme" Environments

→ Energetic boundaries (Metabolism & Flux)

Chemical redox energy is transformed into biochemical energy, which can be spent on repair, replication, movement, etc.



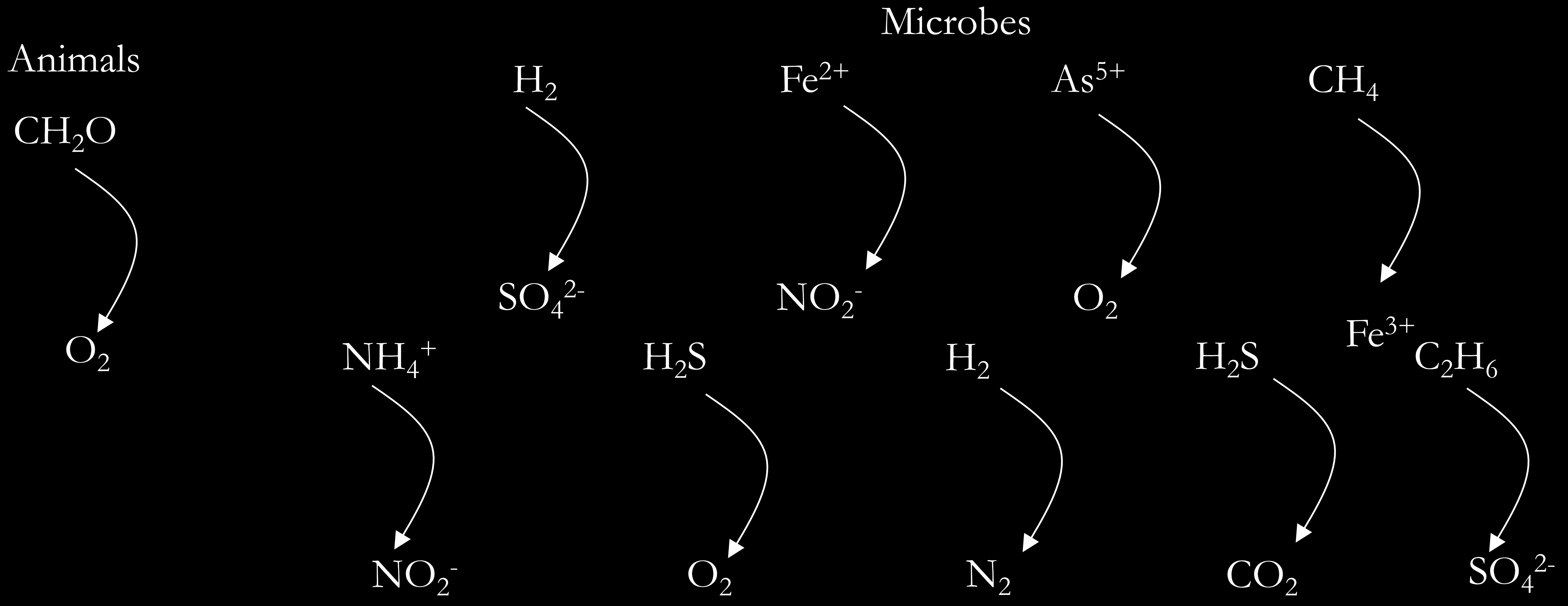


Microbes in "Extreme" Environments

→ Energetic boundaries (Metabolism & Flux)

Metabolic diversity (and environmental range) is what distinguishes microbes from eukaryotes.

Catabolic reactions (electron donor and acceptor pairings)



Microbes in "Extreme" Environments

→ Energetic boundaries (Metabolism & Flux)

Gibbs Energy can be used to determine if a reaction generates energy or consumes energy.
A negative ΔG is necessary but not always sufficient to tell us a reaction will happen.

$$\Delta G_r = RT \ln\left(\frac{Q}{K}\right)$$

$$a\mathrm{A} + b\mathrm{B} \rightleftharpoons c\mathrm{C} + d\mathrm{D} \quad (1)$$

$$Q = \frac{[\mathrm{C}]^c[\mathrm{D}]^d}{[\mathrm{A}]^a[\mathrm{B}]^b} \quad (2a) \qquad K_{eq} = \frac{[\mathrm{C}]^c_{eq}[\mathrm{D}]^d_{eq}}{[\mathrm{A}]^a_{eq}[\mathrm{B}]^b_{eq}} \quad (2b)$$

Reaction Quotient   Equilibrium constant

Microbes in "Extreme" Environments

→ Energetic boundaries (Metabolism & Flux)

Process[‡]	Range[§]	Mid-point[§]	System	Reference
1	−6.2 to −3.8	−5.0	coculture	(Dwyer *et al.*, 1988)
1	−17.5 to −4.5	−11.0	coculture	(Jackson & McInerney, 2002)
2	−11.5 to −6.3	−8.9	chemostat	(Scholten & Conrad, 2000)
3	−14.1 to −9.0	−11.6	chemostat	(Scholten & Conrad, 2000)
4	−10.9 to −9.1	−10.0	marine sediment	(Hoehler *et al.*, 1994)
5	−9.2	−9.2	pure culture	(Elshahed & McInerney, 2001)
3	−11.3 to −9.5	−10.4	marine sediment	(Hoehler *et al.*, 2001)
3	−9.6	−9.6	marine sediment	(Hoehler *et al.*, 1994)
4	−12.8 to −10.5	−11.7	marine sediment	(Hoehler *et al.*, 2001)
6	−16 to −11	−13.5	chemostat	(Seitz *et al.*, 1990)
3	−31.1 to −13.2	−22.2	paddy soil	(Yao & Conrad, 1999)
7	−18 to −14	−16.0	freshwater sediment	(Rothfuss & Conrad, 1993)
3	−15	−15.0	pure culture	(Chong *et al.*, 2002)

Measured limits of minimum energy needed to sustain viable microbes (all in kJ per mol of substrate)

Hoehler et al., Geobiology, 2004

Microbes in "Extreme" Environments

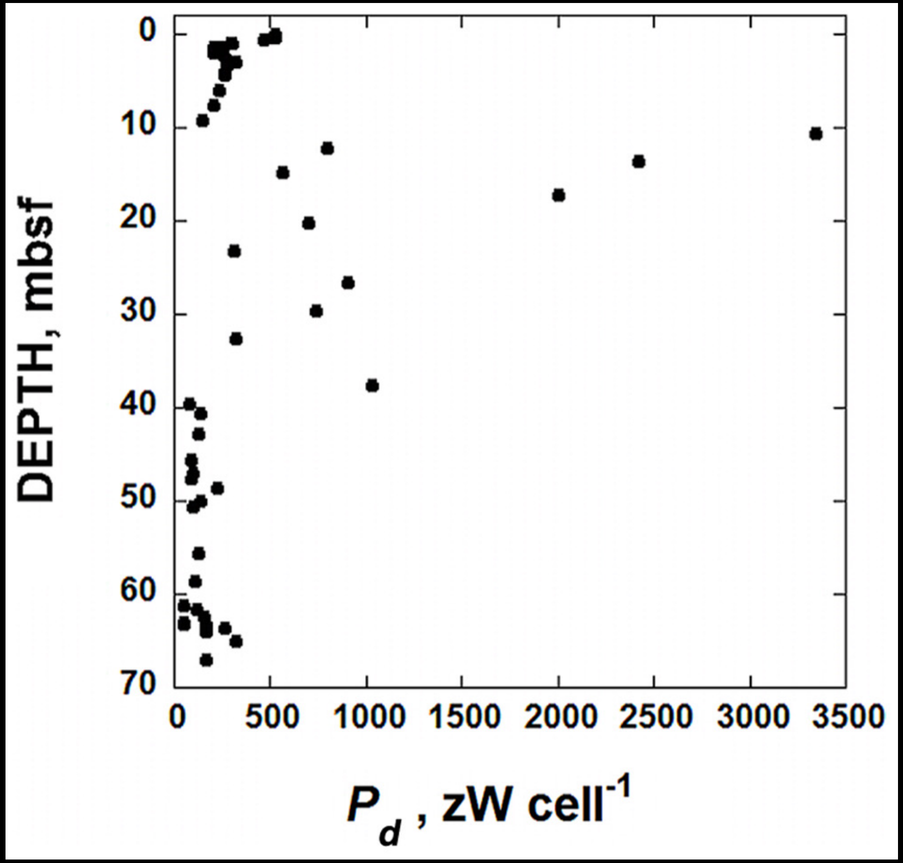
→ Energetic boundaries (Metabolism & Flux)

Power (energy per unit time) is perhaps the more relevant factor in determining energetic limits

$$P_s = \Delta G_r \cdot r$$

Maintenance power depends on how quickly an environment degrades a cell. If there's no breakdown, then viability maintenance power could approach 0.

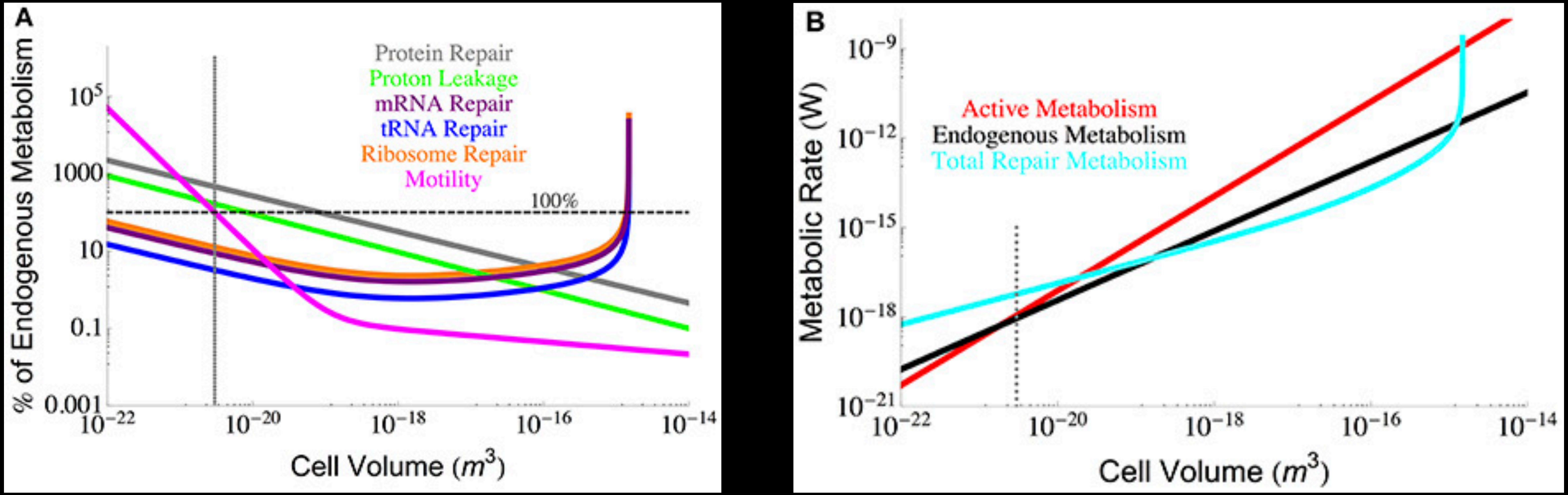
Based on carbon substrate concentration, Gibbs energies, and cell abundances, power values calculated for the subsurface beneath the South Pacific Gyre



LaRowe and Amend, Frontiers in Microbiology, 2015

Microbes in "Extreme" Environments

→ Energetic boundaries (Metabolism & Flux)

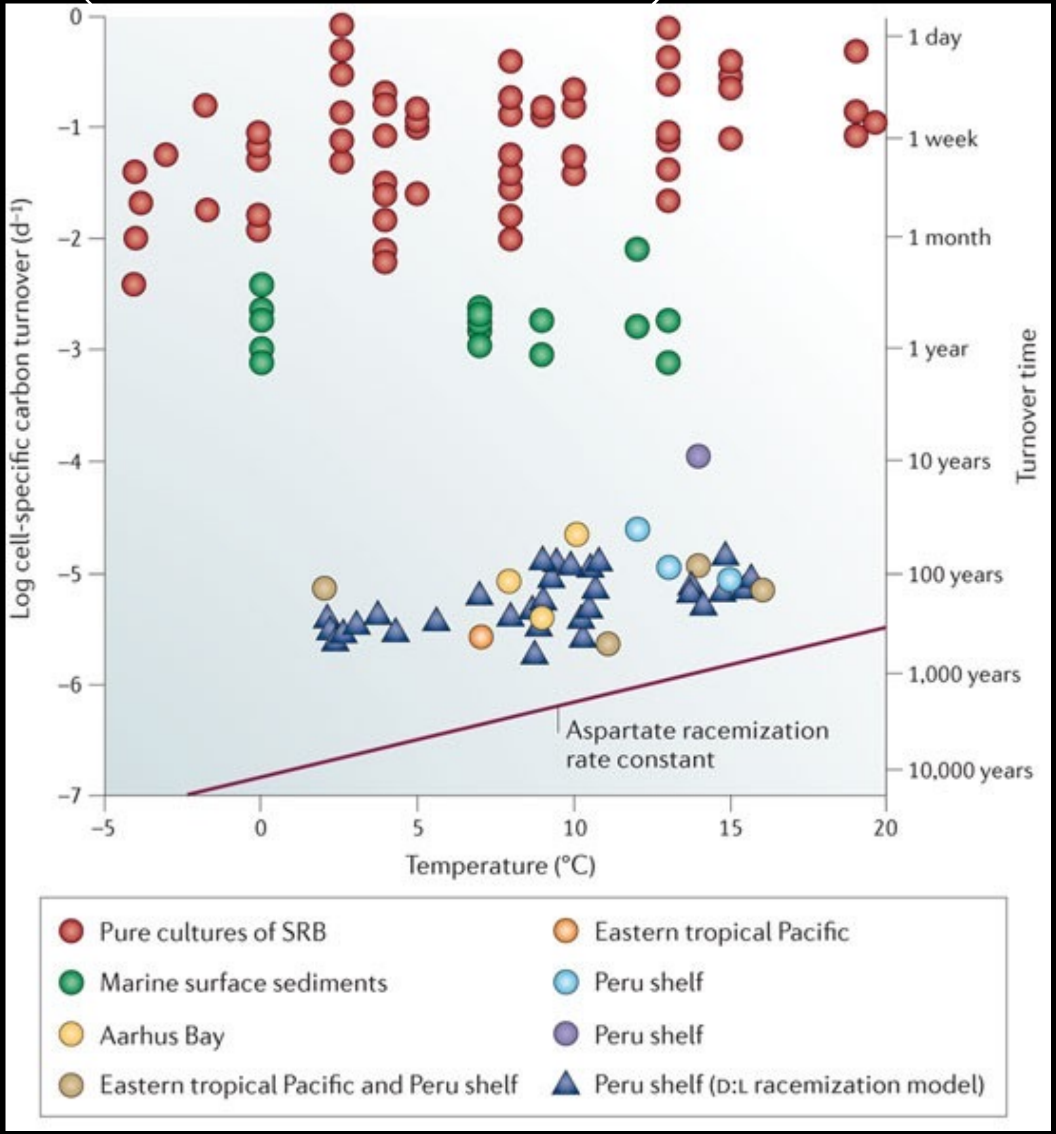


Total metabolism (red)
Maintenance metabolism (black)

Kempes et al., Frontiers in Microbiology, 2017

Microbes in “Extreme” Environments

→ Energetic boundaries (Metabolism & Flux)



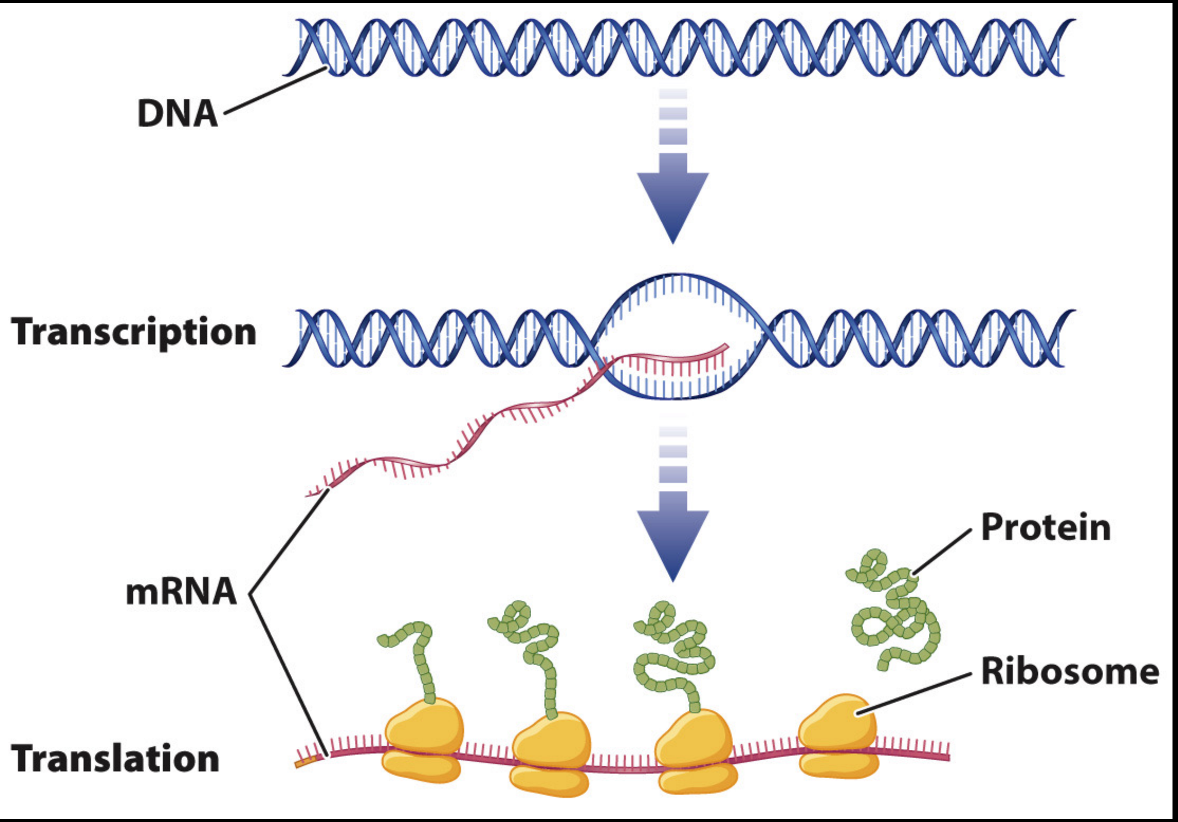
Hoehler and Jorgensen, Nature Reviews Microbiology, 2013

Microbes in "Extreme" Environments

→ Energetic boundaries (Metabolism & Flux)

"Growth" can mean a lot of different things
→ Making new biomolecules?
→ Making more biomass?
→ Making more cells?

"Activity" can also mean different things
→ Metabolic activity (evidence of the process)
→ New biomolecules (evidence of the machinery)



→ Defining microbial life and its diversity
→ Physicochemical boundaries (Temperature / pH / Water activity / Pressure)
→ Energetic boundaries (Metabolism & Flux)

→ Potentially relevant ecologies…
    → Interactions & Symbioses
    → Microhabitats (Surfaces / Boundaries / Gradients)

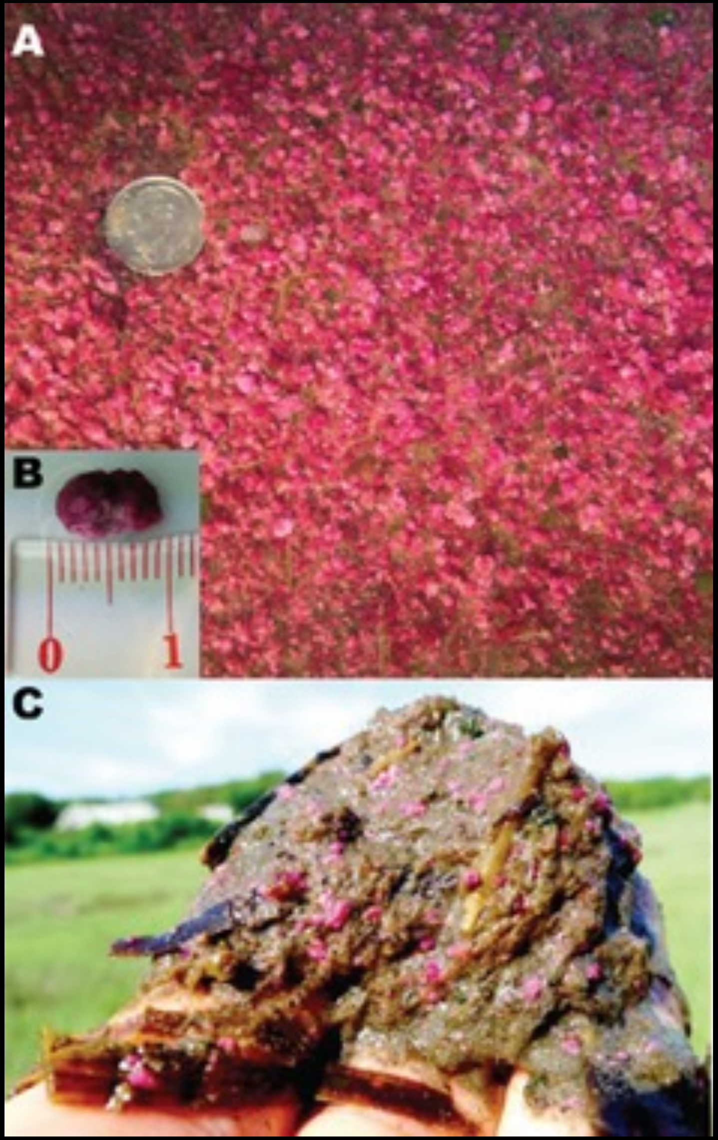
Microbes in "Extreme" Environments

→ Potentially relevant ecologies // Interactions & Symbioses

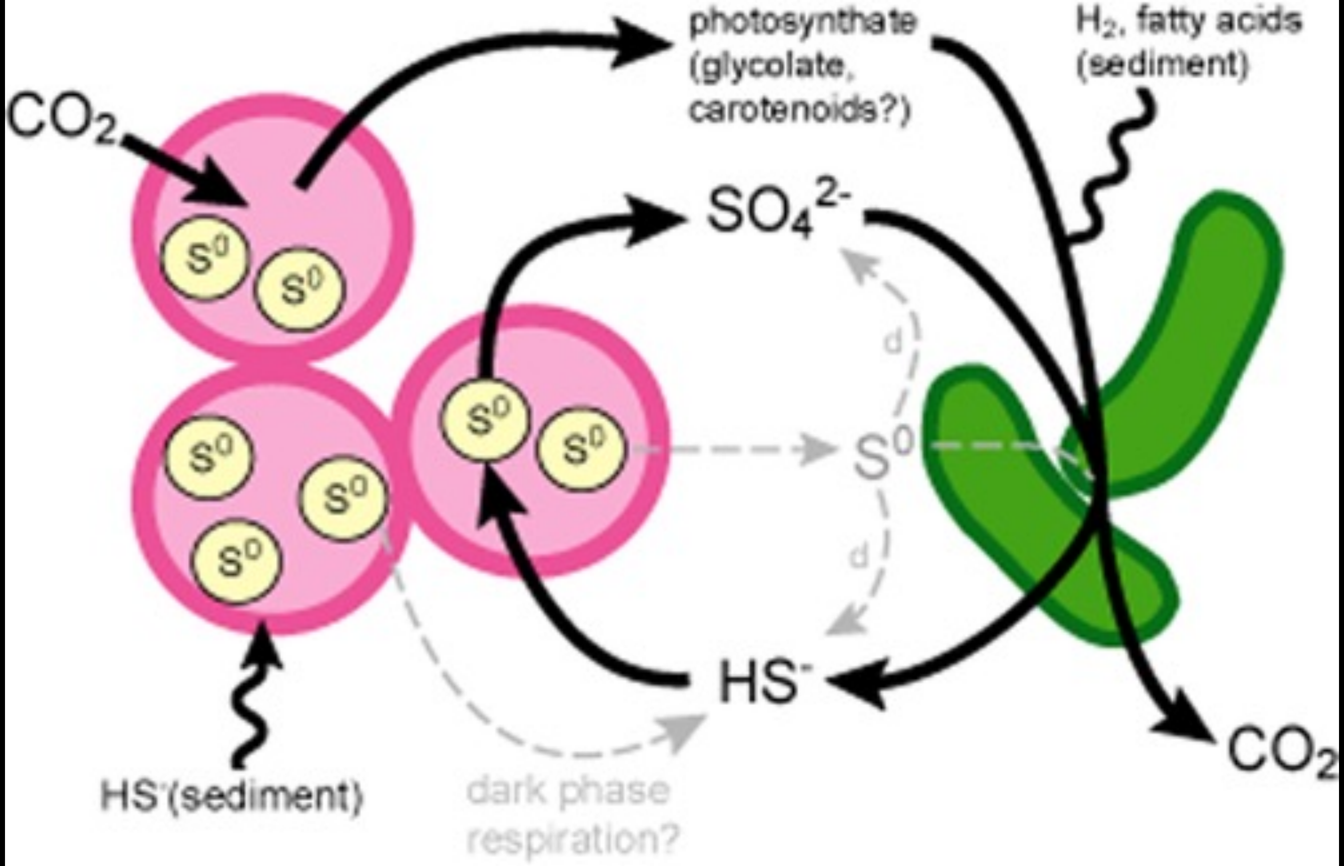
Interactions with minerals / rocks, or with other microbes' metabolic products, can expand the niche space and enhance metabolic activity

Microbes in "Extreme" Environments

→ Potentially relevant ecologies // Interactions & Symbioses



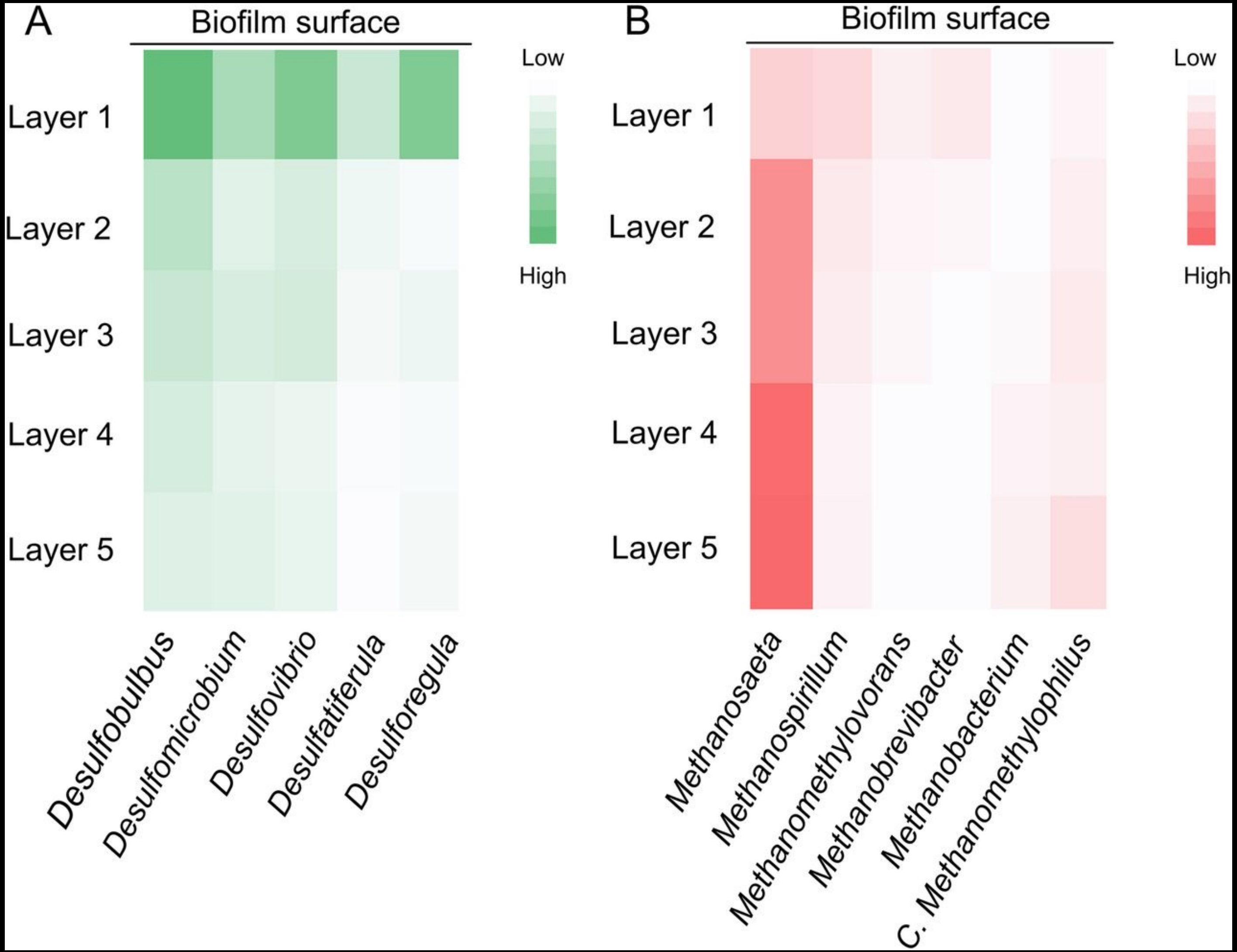
Sulfur cycling aggregates in salt marsh sediments



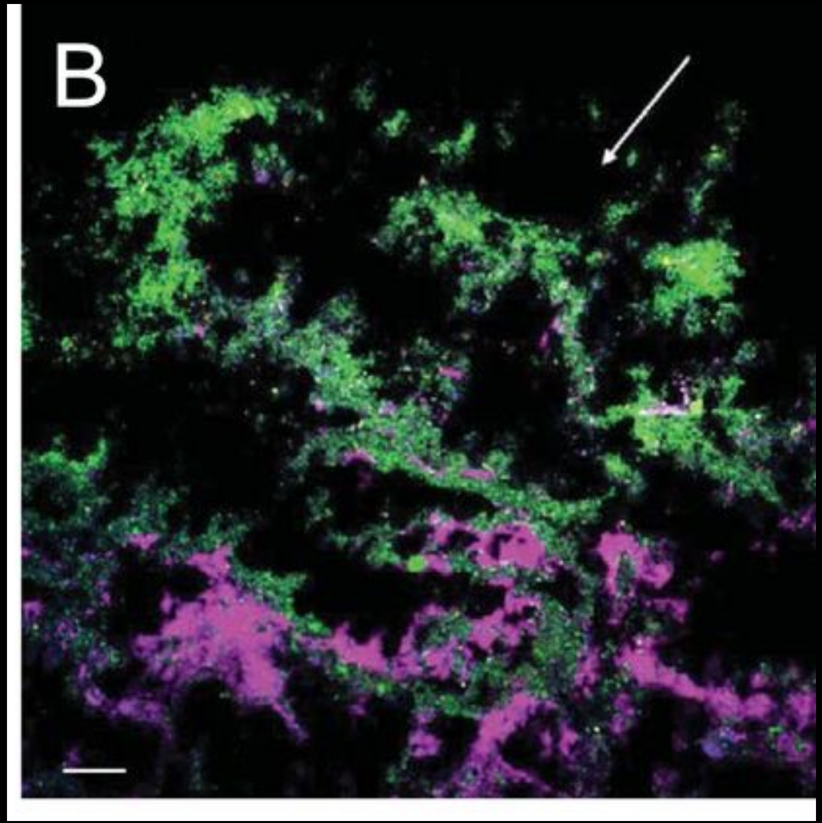
Wilbanks et al., Environmental Microbiology, 2014

Microbes in "Extreme" Environments

→ Potentially relevant ecologies // Interactions & Symbioses



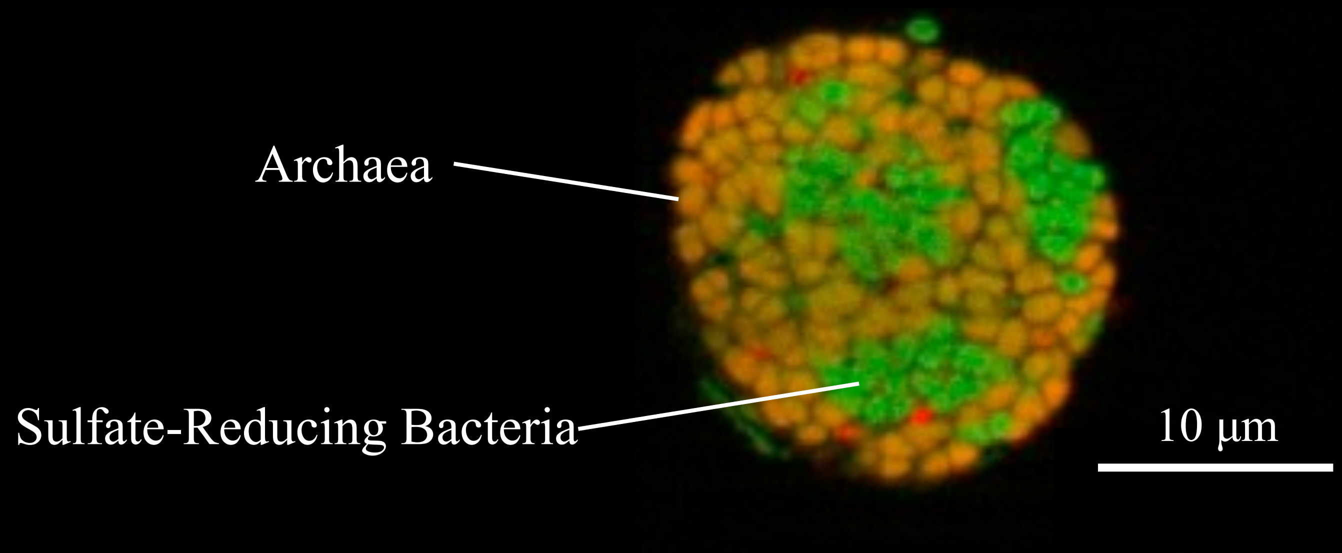
Sulfate-reducing bacteria and methanogens in anaerobic sewer biofilms



Sun et al., AEM, 2014

Microbes in "Extreme" Environments

→ Potentially relevant ecologies // Interactions & Symbioses



Anaerobic methane oxidizing Archaea and sulfate reducing bacteria at methane seeps

Anaerobic methanotroph (ANME) archaea: $CH_4 + 3H_2O \rightarrow HCO_3^- + 8e^- + 9H^+$

Sulfate Reducing bacteria: $SO_4^{2-} + 8e^- + 9H^+ \rightarrow HS^- + 4H_2O$

Net Reaction: $CH_4 + SO_4^{2-} \rightarrow HCO_3^- + HS^- + H_2O$

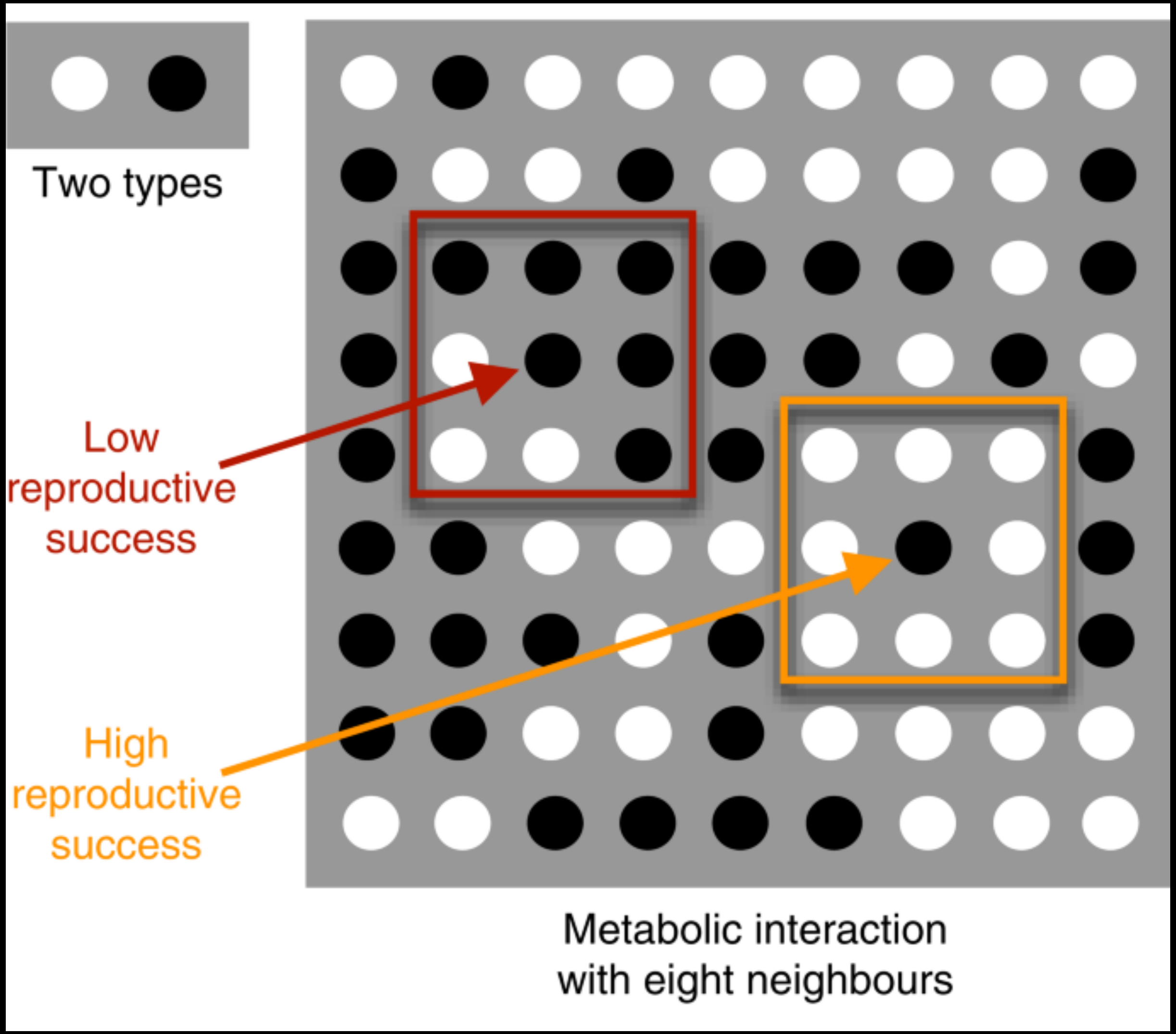
Carbonate Precipitation: $2HCO_3^- + Ca^{2+} \leftrightarrow CaCO_3 + CO_2 + H_2O$

Microbes in “Extreme” Environments

→ Potentially relevant ecologies // Interactions & Symbioses

Simulating spatial implications of interactions

1) Each cell can receive compounds only from cells belonging to the partner species that reside within the interaction range;
2) The growth of individual cells depends on the fraction of the cells of the partner species within the interaction range;
3) If a cell divides, it places an offspring in a neighboring site

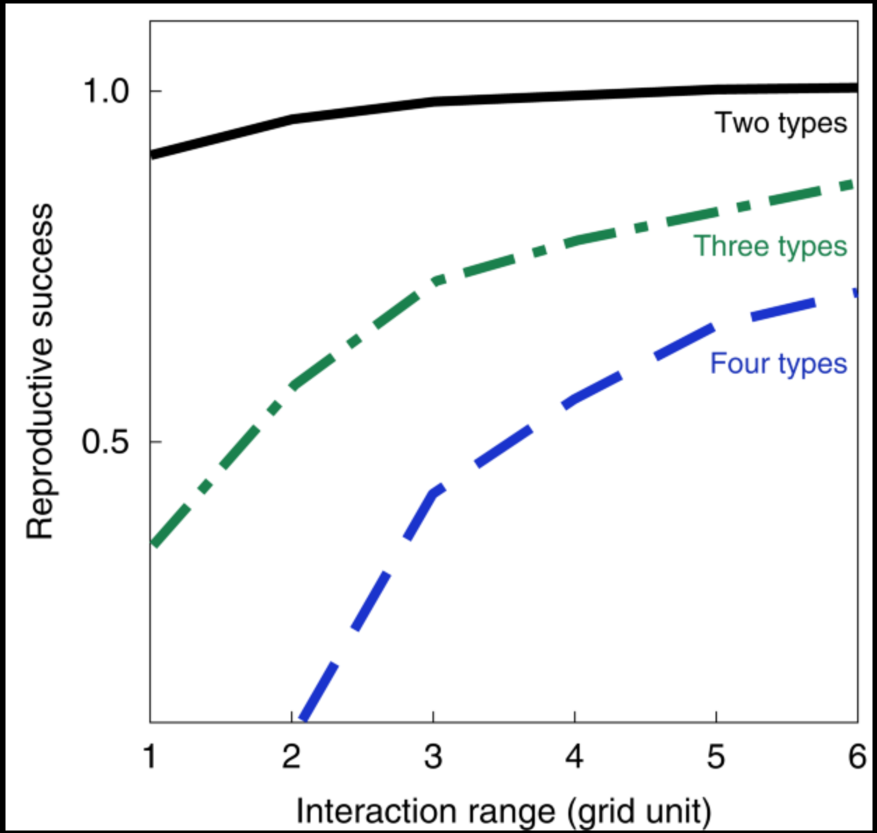


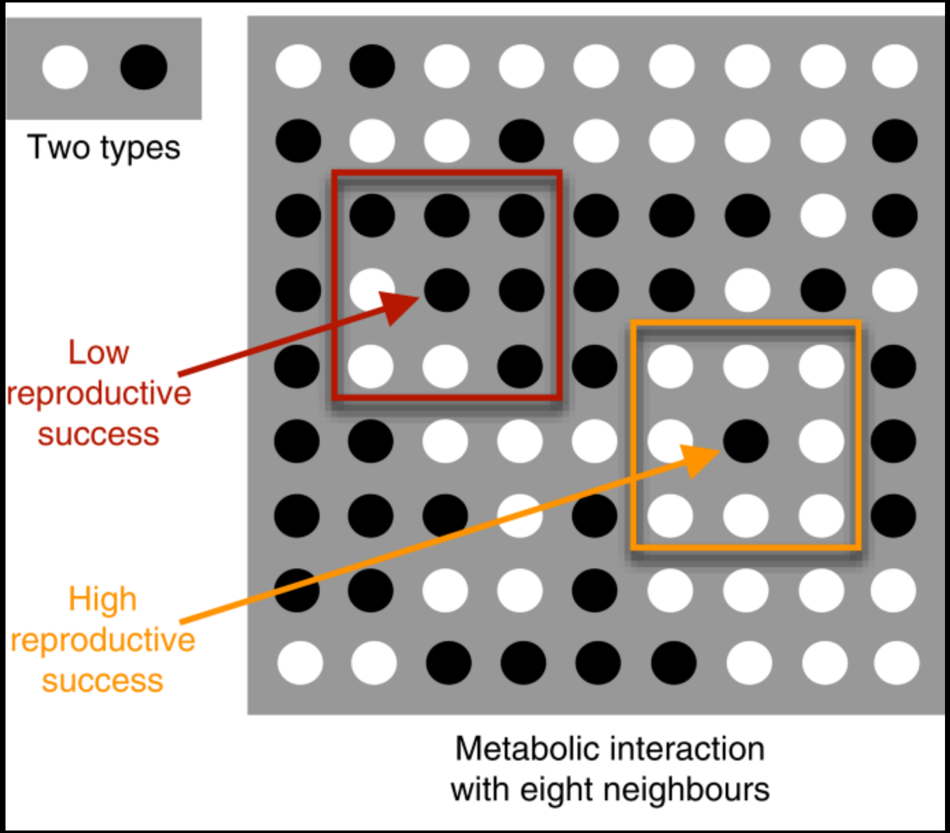
Dal Co et al., Nature Ecology and Evolution, 2020

Microbes in "Extreme" Environments

→ Potentially relevant ecologies // Interactions & Symbioses

When the interaction range is small, growth rates are low…and it gets worse the more different types of cells you need to depend on



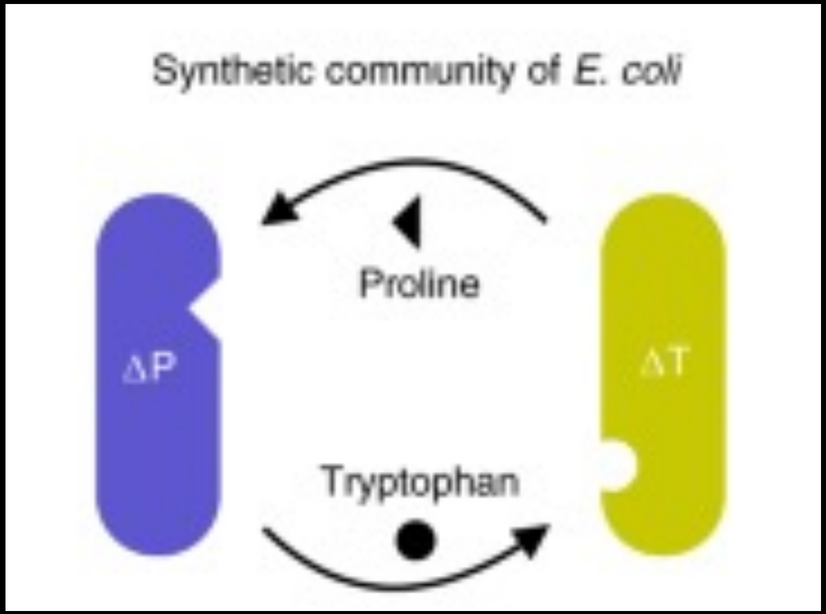


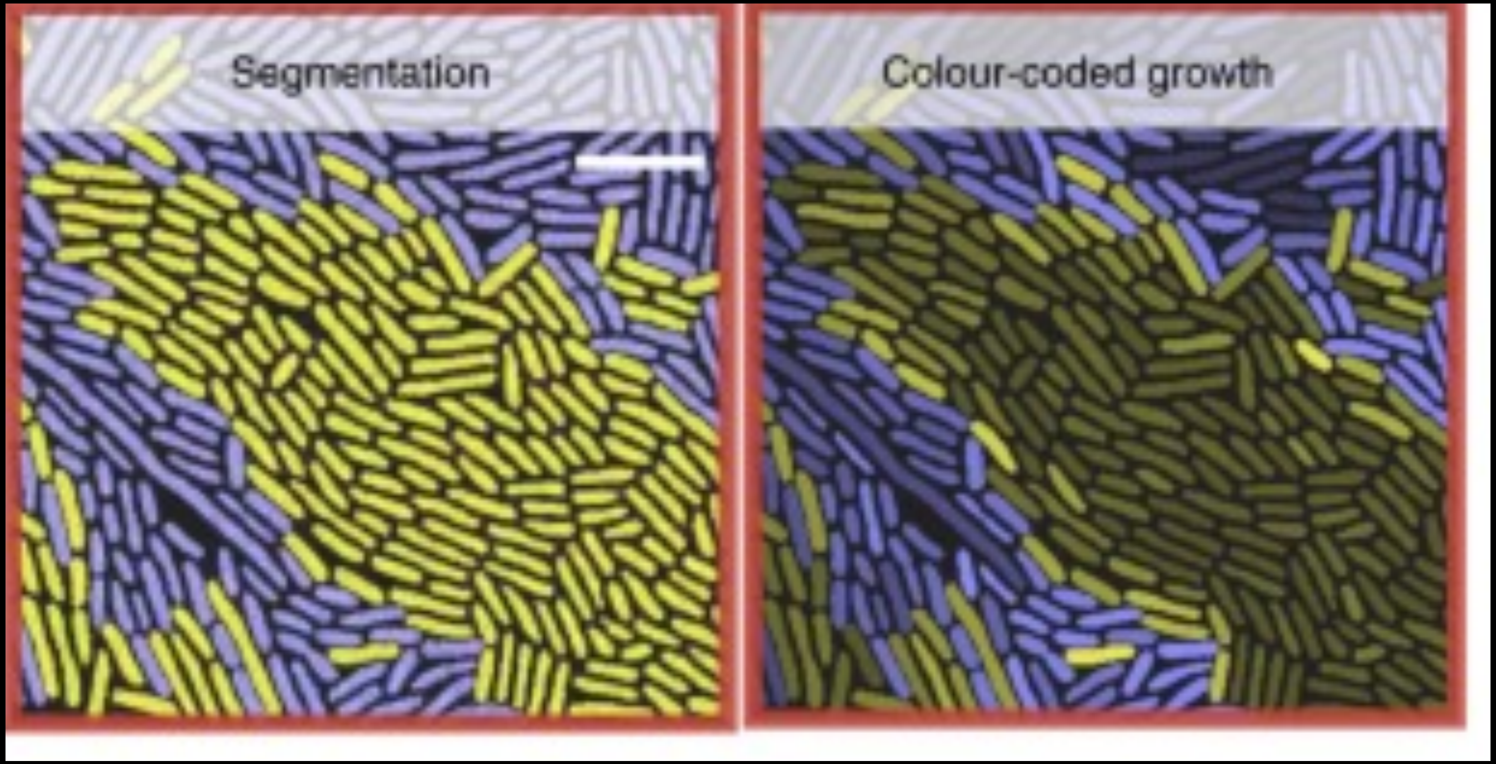
Dal Co et al., Nature Ecology and Evolution, 2020

Microbes in “Extreme” Environments

→ Potentially relevant ecologies // Interactions & Symbioses

Microfluidic growth chamber of two auxotrophic E. coli strains



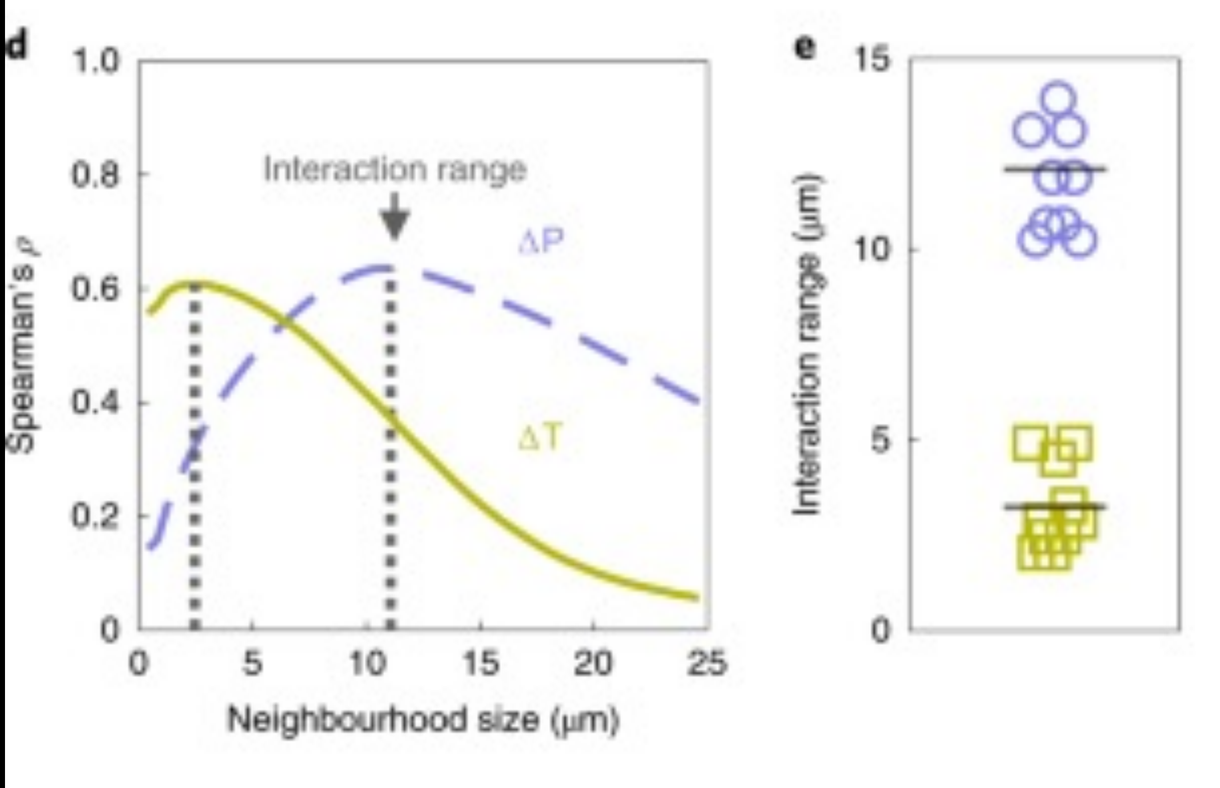


Dal Co et al., Nature Ecology and Evolution, 2020

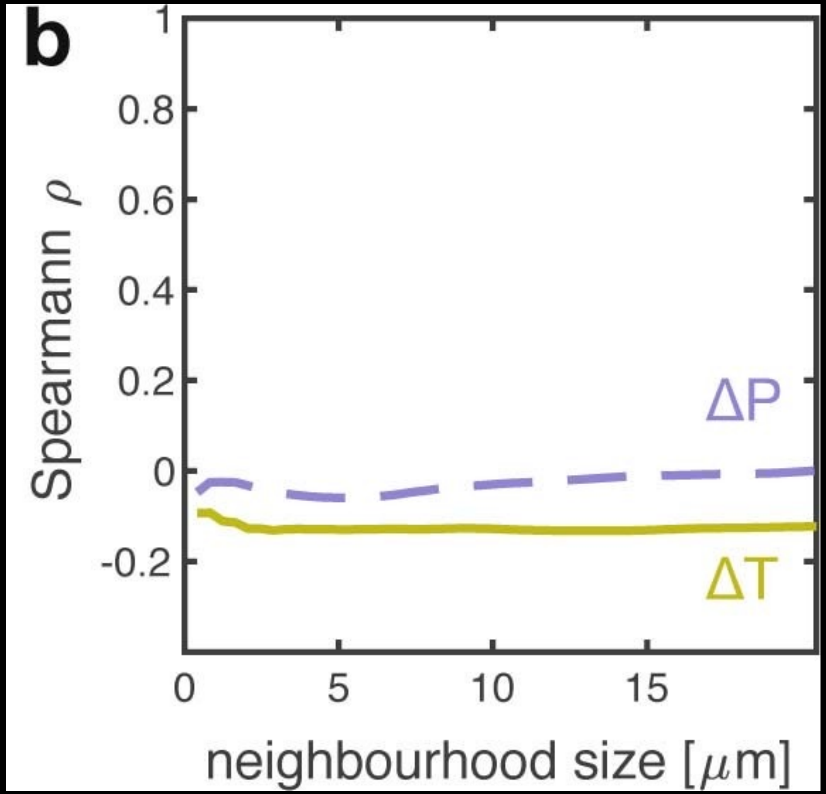
Microbes in "Extreme" Environments

→ Potentially relevant ecologies // Interactions & Symbioses

Looked at correlation between growth rate and potential interaction range



When all necessary amino acids are provided, no spatial dependence



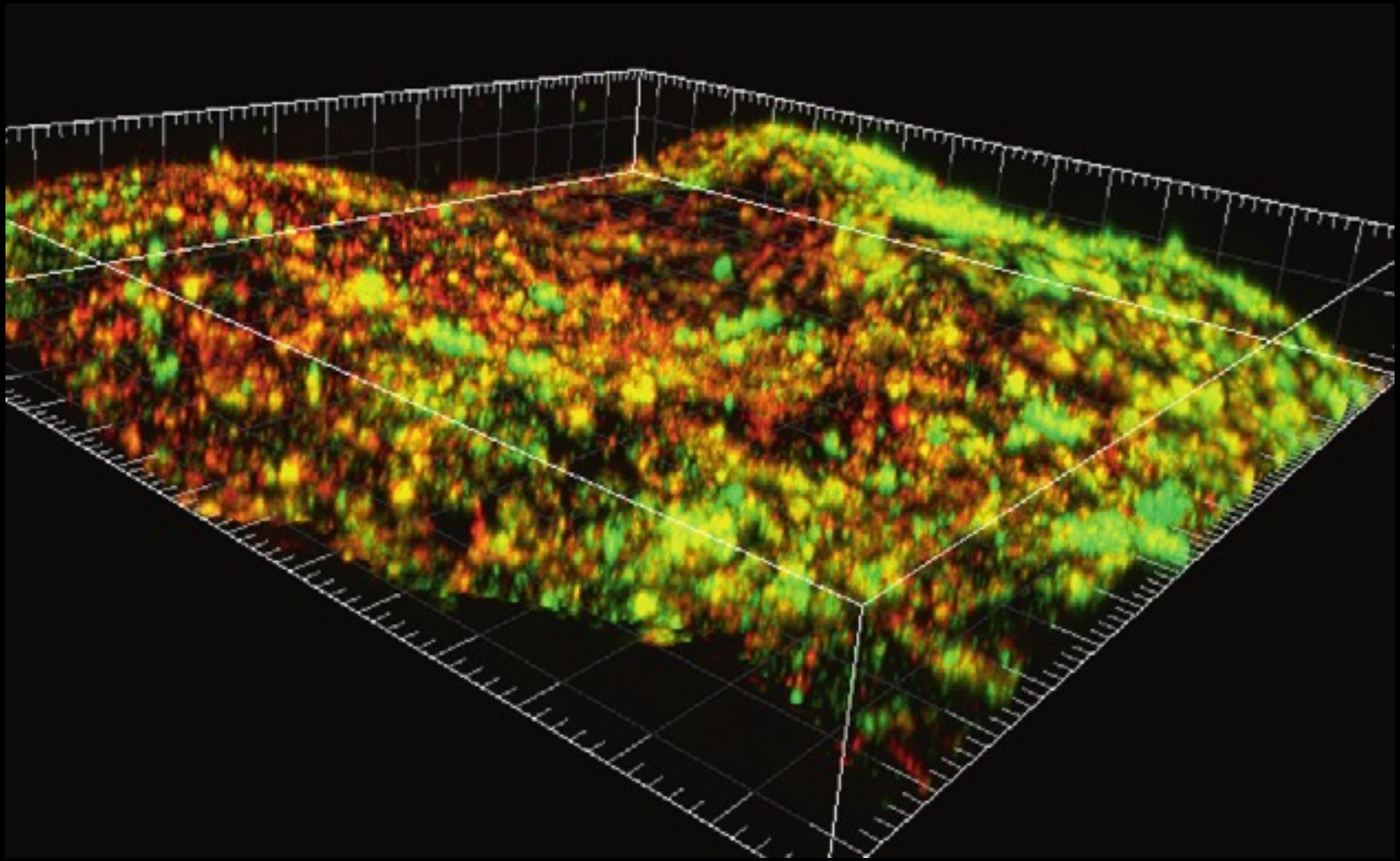
Dal Co et al., Nature Ecology and Evolution, 2020

Microbes in “Extreme” Environments

→ Potentially relevant ecologies // Microhabitats

Microbes love surfaces, boundary zones, and chemical gradients.

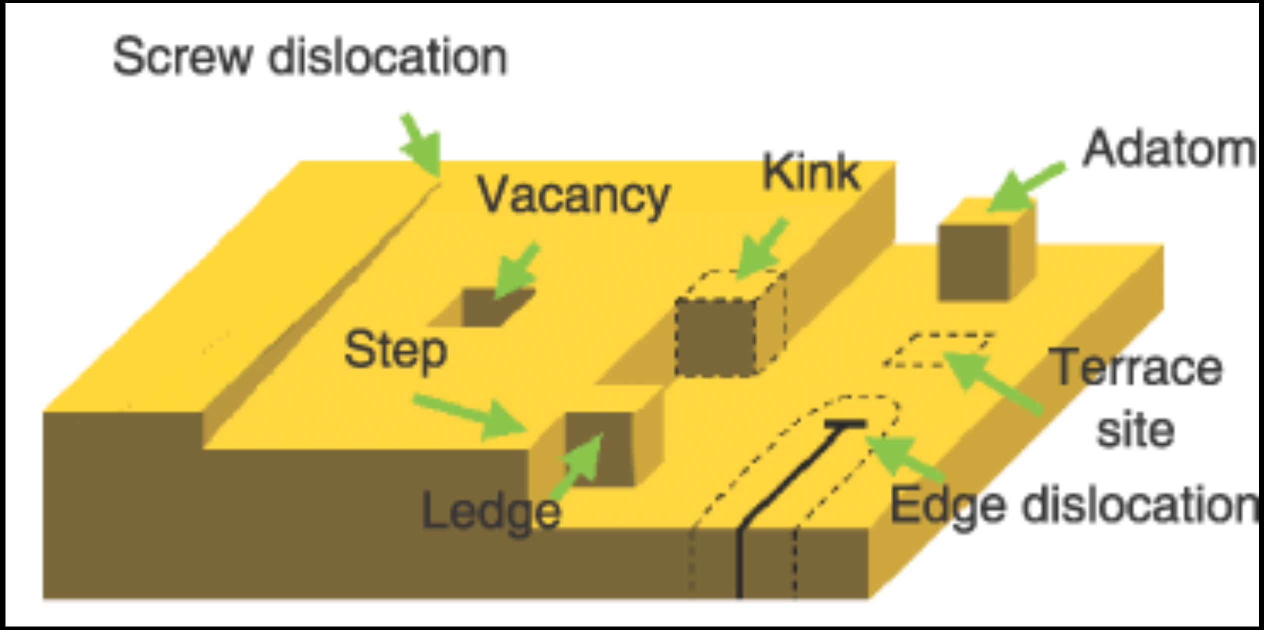
But it can be tough to separate generic surfaces from chemically reactive materials

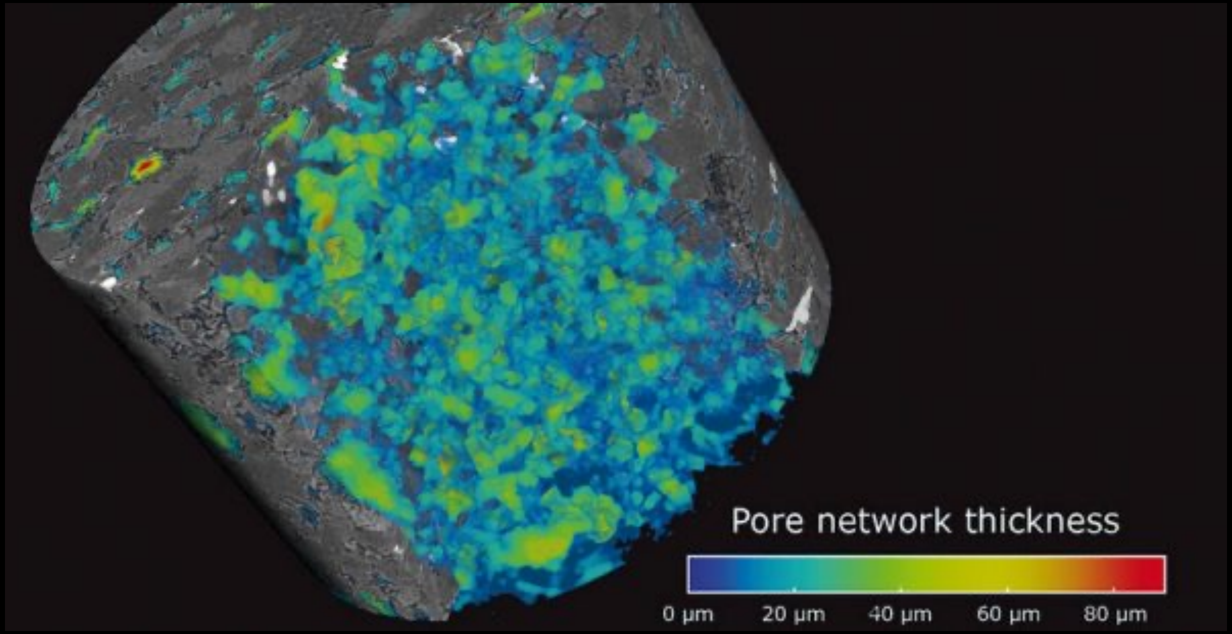


Hammes et al., 2011

Microbes in "Extreme" Environments

→ Potentially relevant ecologies // Microhabitats



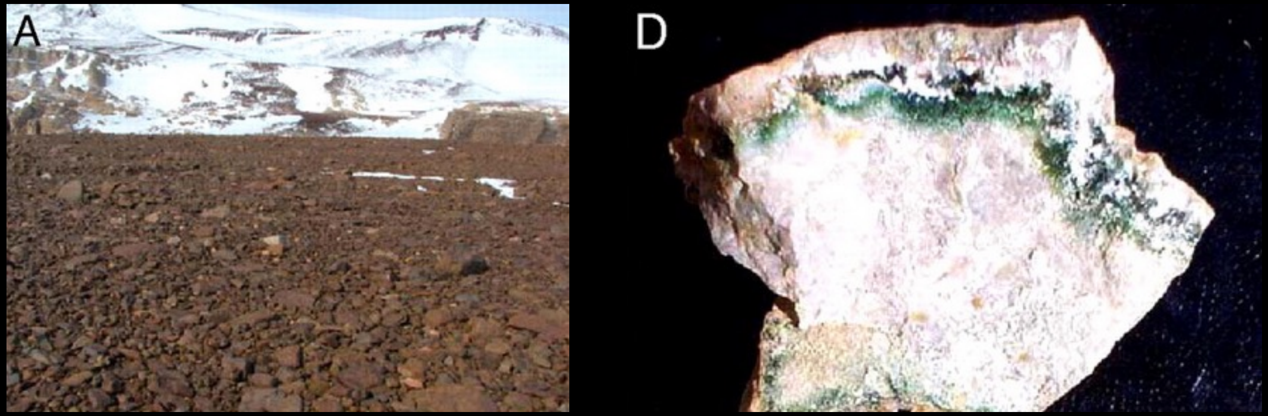


Lu et al., Nature Communications, 2012

Microbes in "Extreme" Environments

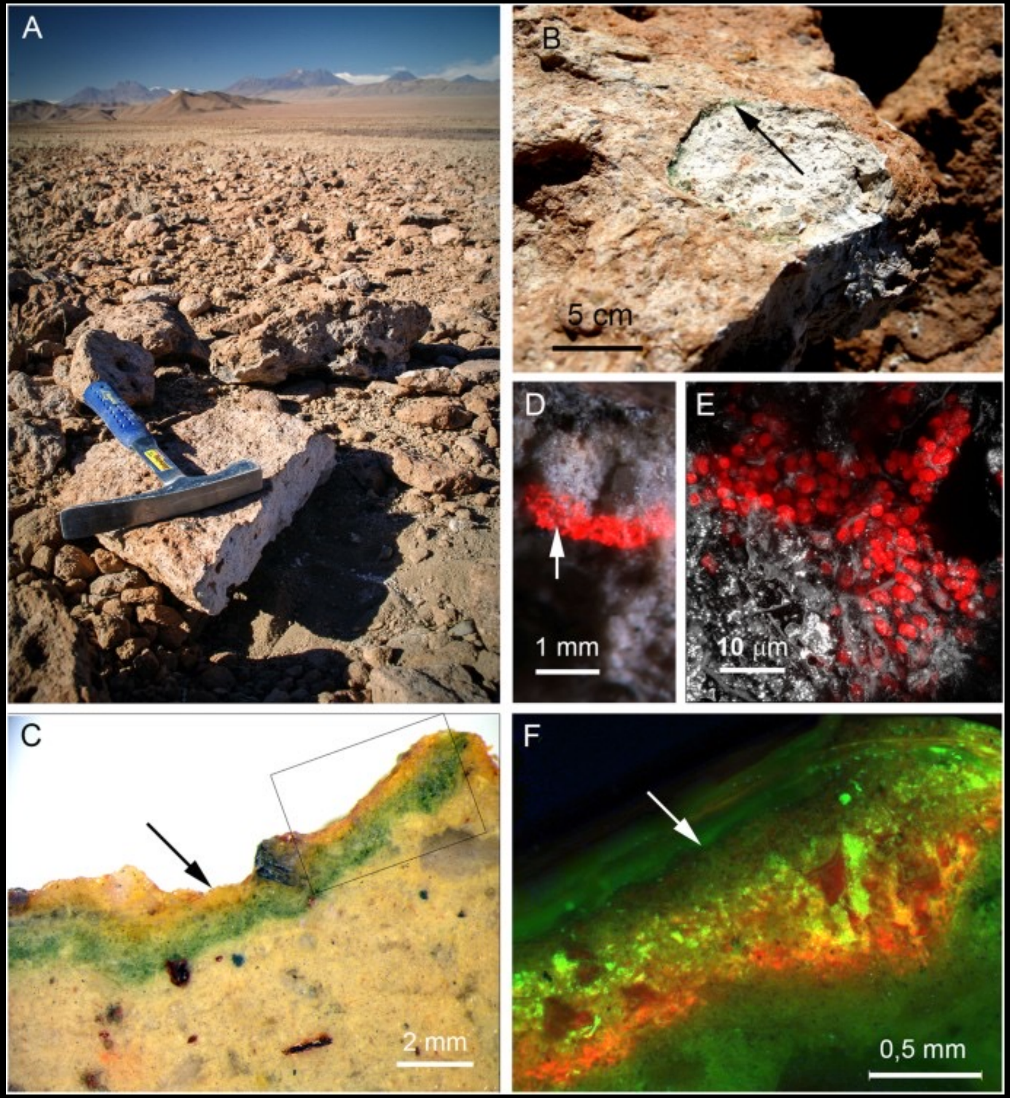
→ Potentially relevant ecologies // Microhabitats

Antarctic endolithic cyanobacteria



Different absorptive pigments at different depths in the rock: longer wavelengths can get deeper (but have less energy)

Atacama Desert →

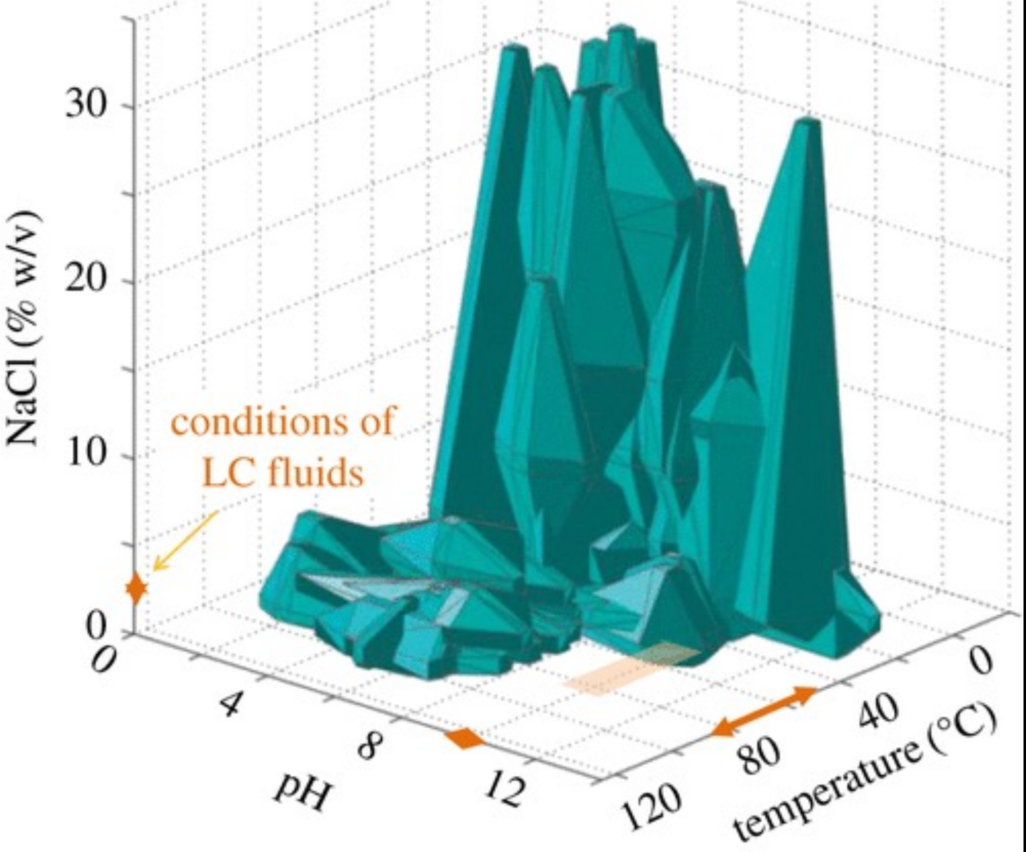


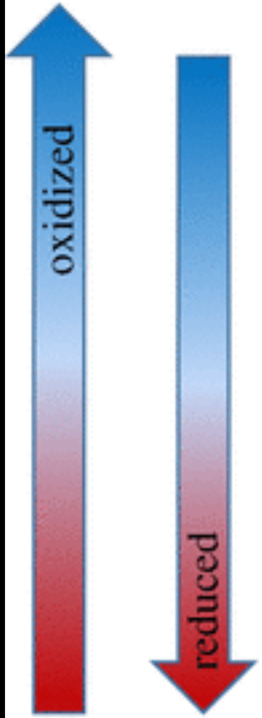
Vitek et al., Scientific Reports, 2017

Microbes in “Extreme” Environments

→ Potentially relevant ecologies // Microhabitats

→ Lost City, active serpentinization site with high H_2, CH_4, formate





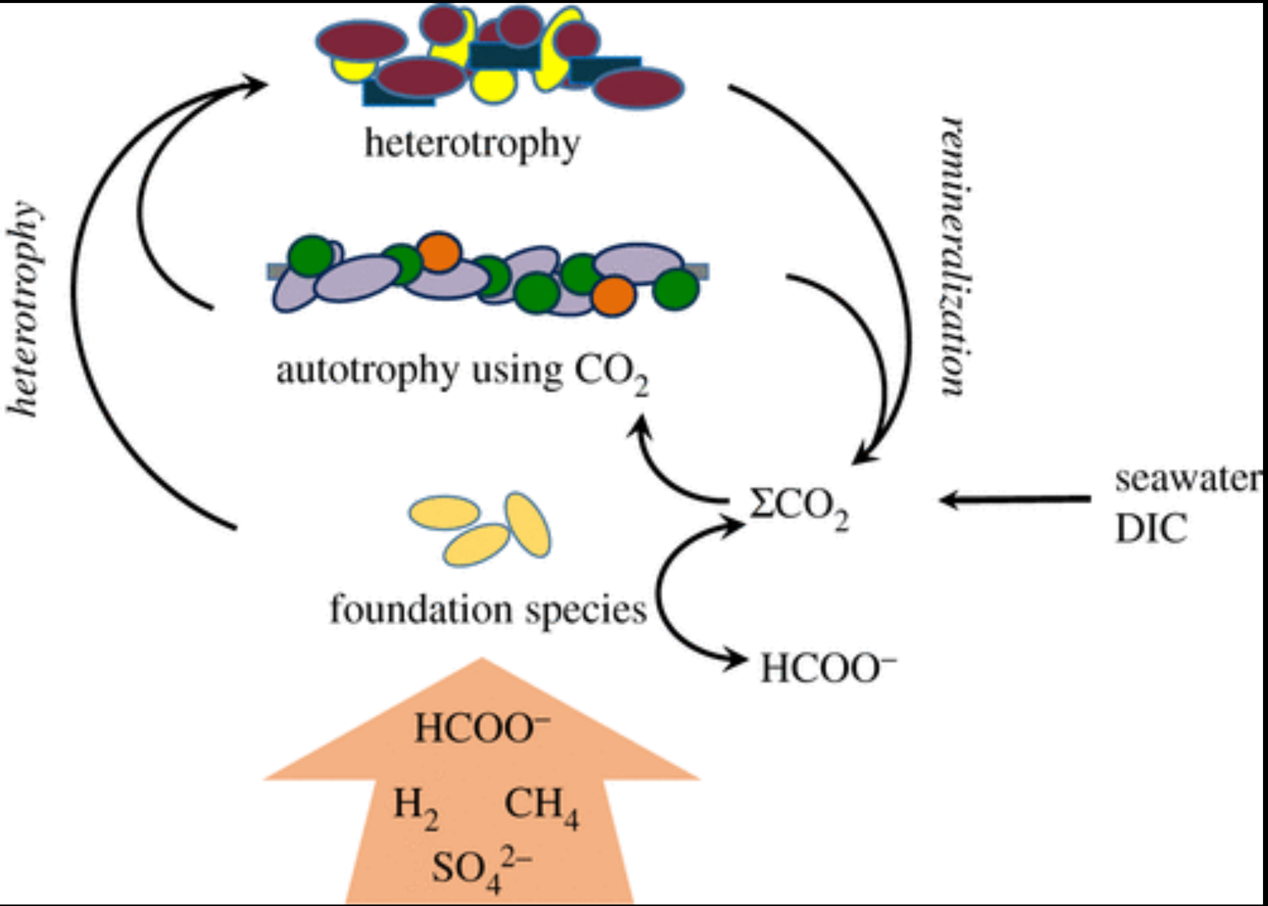
chemical species	conc. (μM)
CO_2 (carbon dioxide)	0.1–0.6
$HCOO^-$ (formate)	34–140
CO (carbon monoxide)	below detection
CH_3COO^- (acetate)	5–35
Hydrolizable amino acids	0.7–2.3
CH_3SH (methanethiol)	0.0014–0.0019
CH_4 (methane)	890–1980

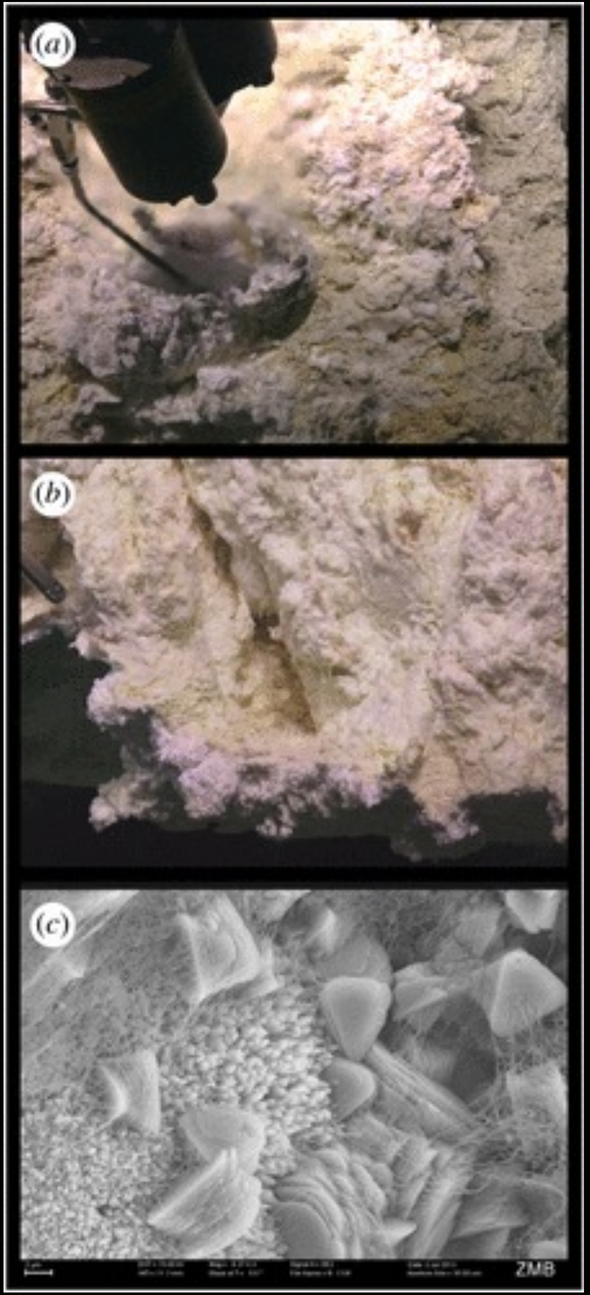
Lang & Brazelton, Philosophical Transactions of the Royal Society, 2020

Microbes in "Extreme" Environments

→ Potentially relevant ecologies // Microhabitats

→ Lost City, active serpentinization site with high H_2, CH_4, formate

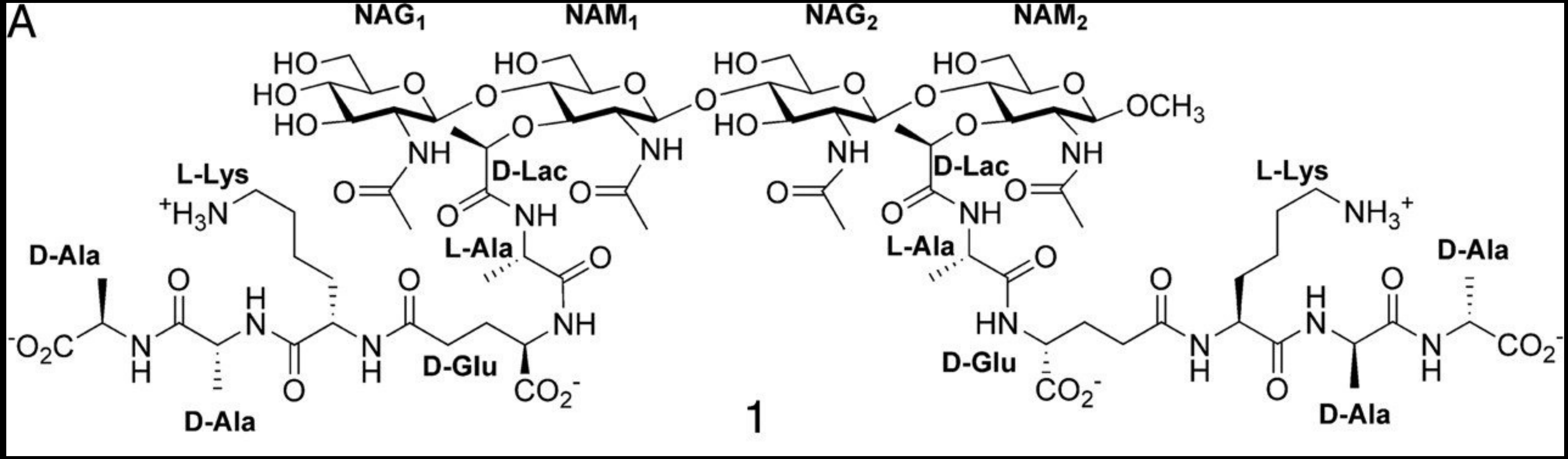




Lang & Brazelton, Philosophical Transactions of the Royal Society, 2020

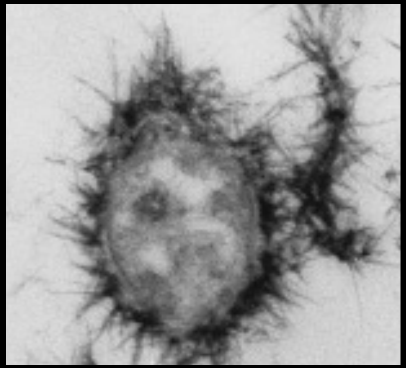
Microbes in "Extreme" Environments

→ Potentially relevant ecologies // Microhabitats

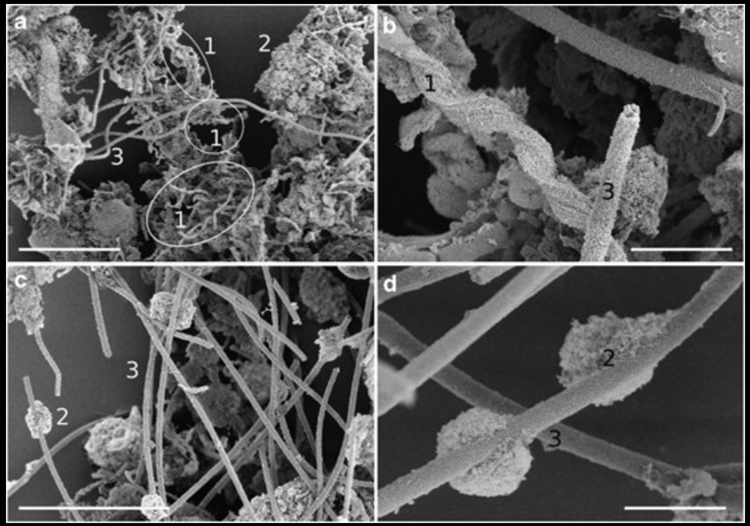


Peptidoglycan in cell walls is negatively charged, and it attracts positively charged molecules (metal ions and metal-organic complexes)

Iron oxidation…
Passive nucleation sites
- Fe^{2+} adsorbs to negatively charged cell

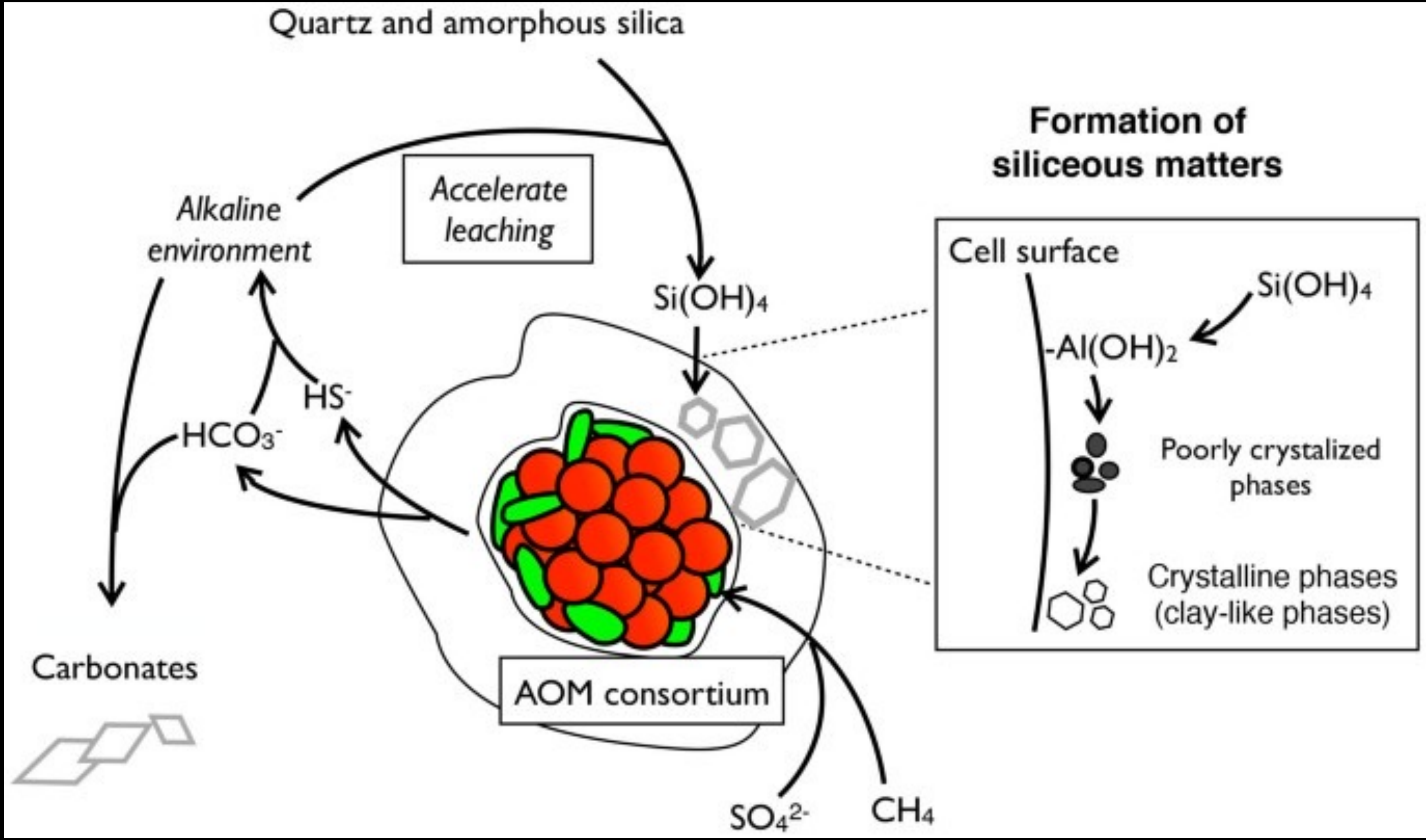


Active molecular involvement
- Ligands promote Fe^{2+} oxidation



Microbes in "Extreme" Environments

→ Potentially relevant ecologies // Microhabitats

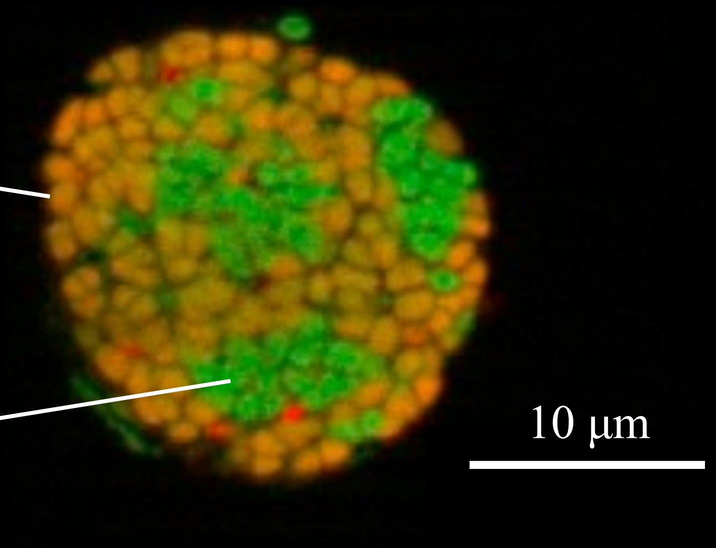


Silicate and aluminum adsorb to negatively charged cell membrane groups. After nucleation of these minerals, they form a template upon which other minerals grow

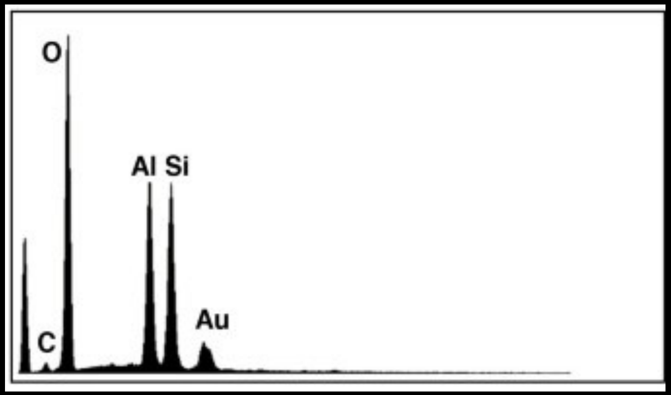
Chen et al., Scientific Reports, 2014

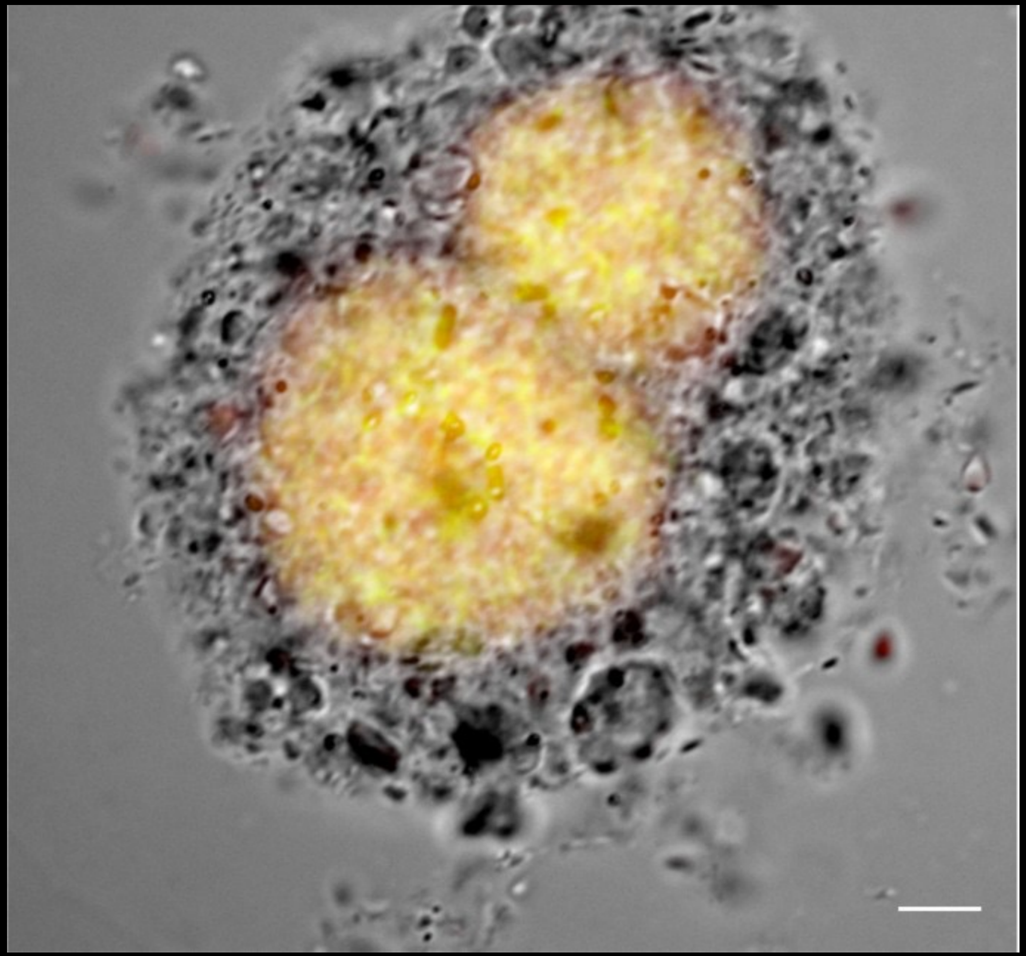
Microbes in "Extreme" Environments

→ Potentially relevant ecologies // Microhabitats



Elemental compositions confirm aluminum silicon oxide clays →



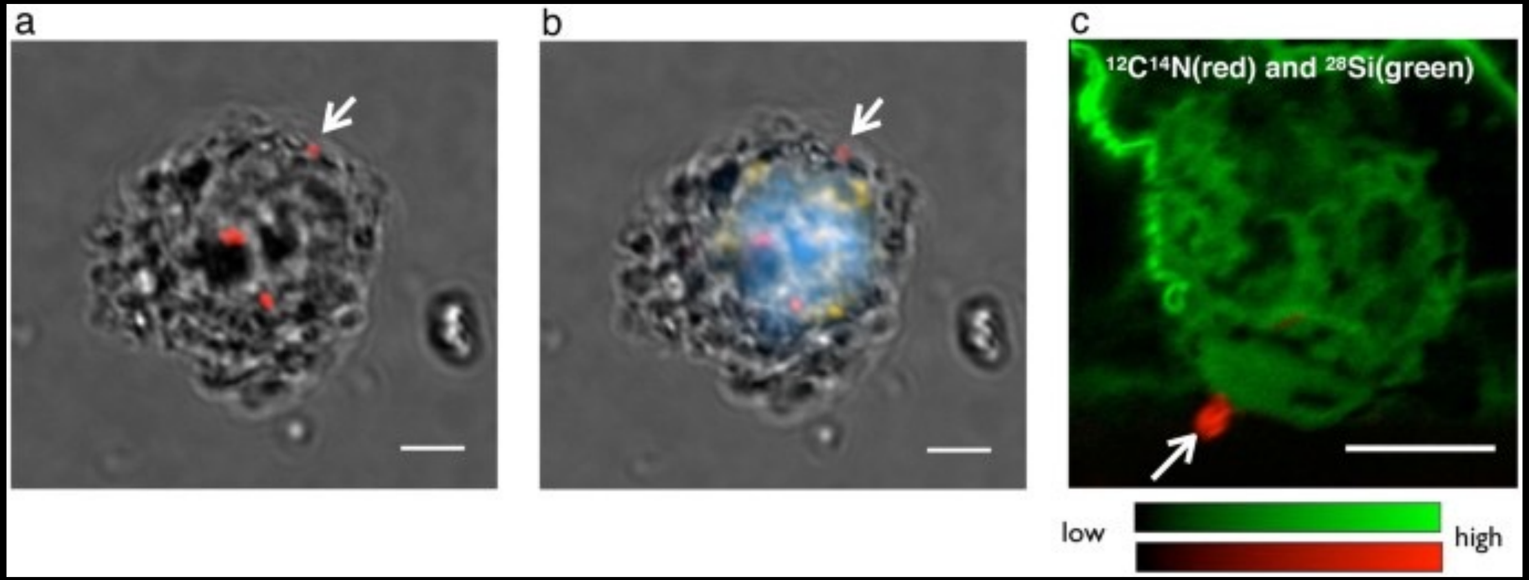


Chen et al., Scientific Reports, 2014

Microbes in "Extreme" Environments

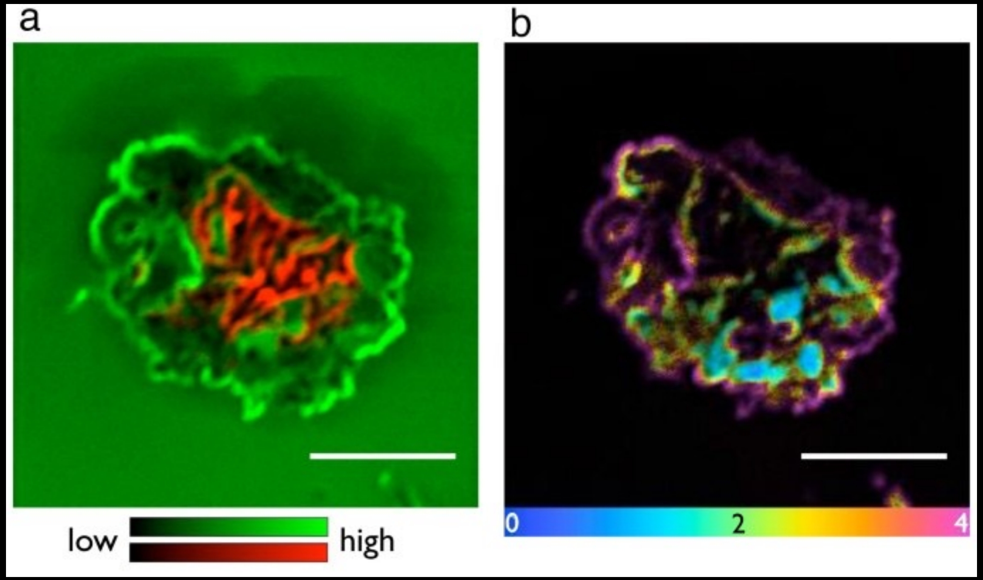
→ Potentially relevant ecologies // Microhabitats

Clay coatings provide a secondary surface for adherent microbes to attach, generally betaproteobacteria



Red: $^{12}C^{14}N$
Green: ^{28}Si

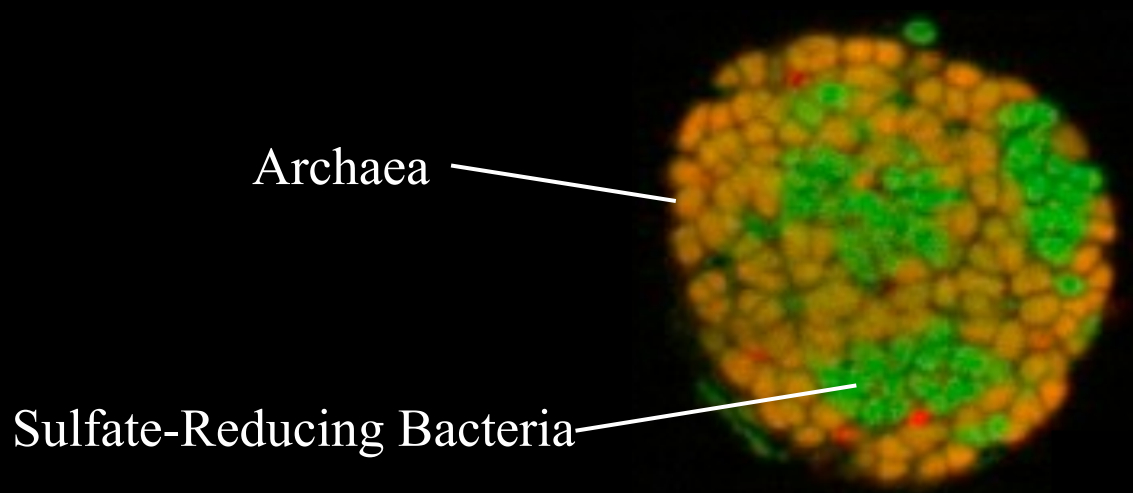
Si/Al ratio

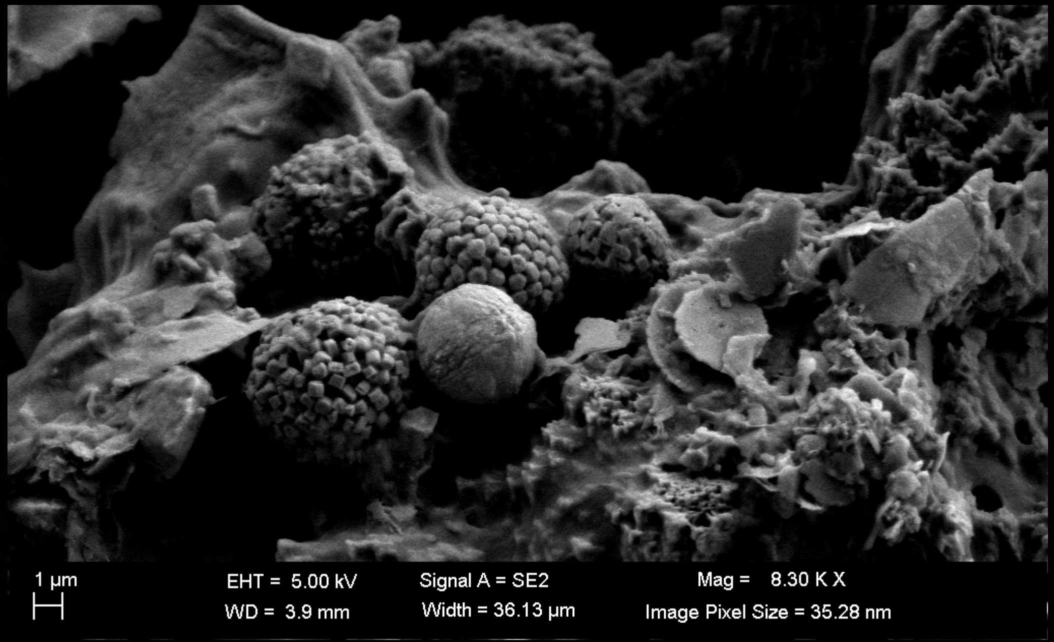


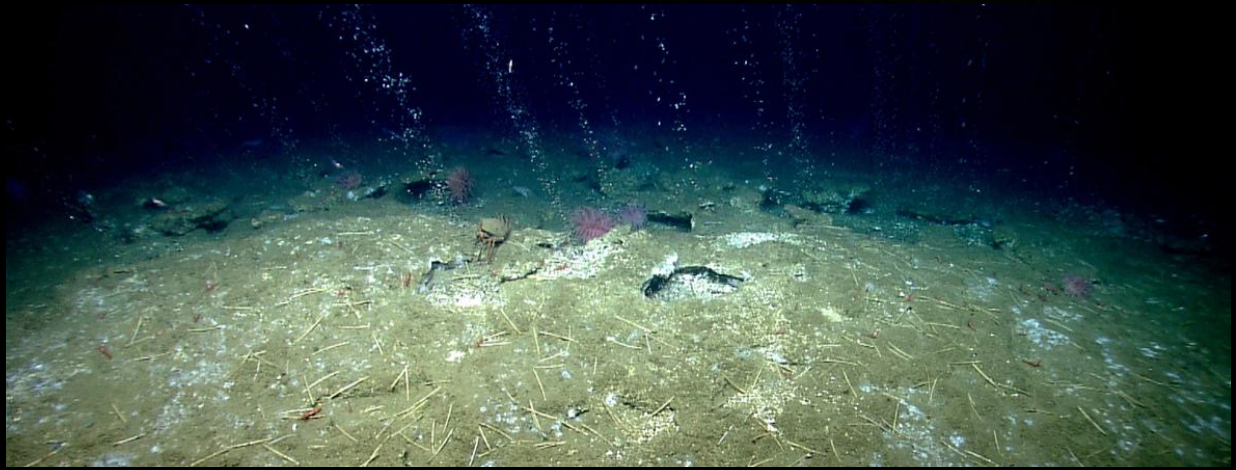
Chen et al., Scientific Reports, 2014

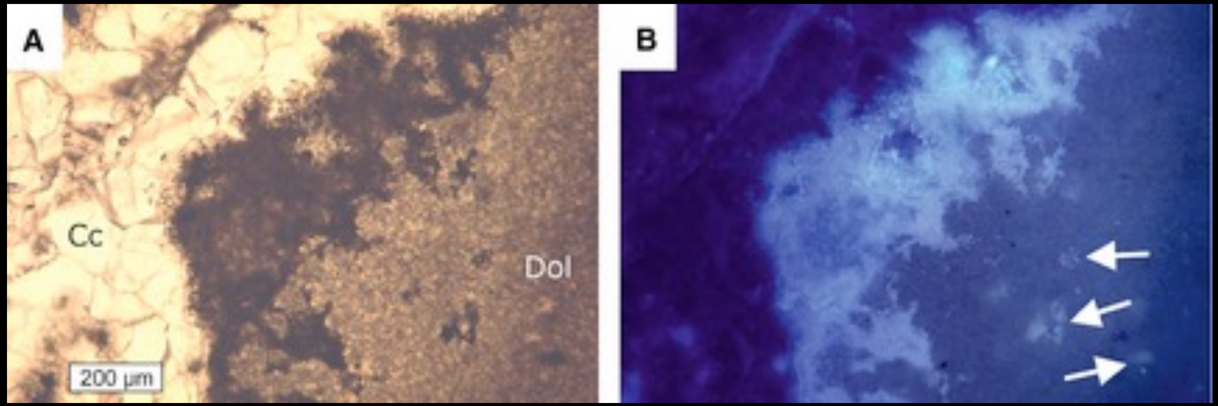
Microbes in "Extreme" Environments

→ Potentially relevant ecologies // Microhabitats







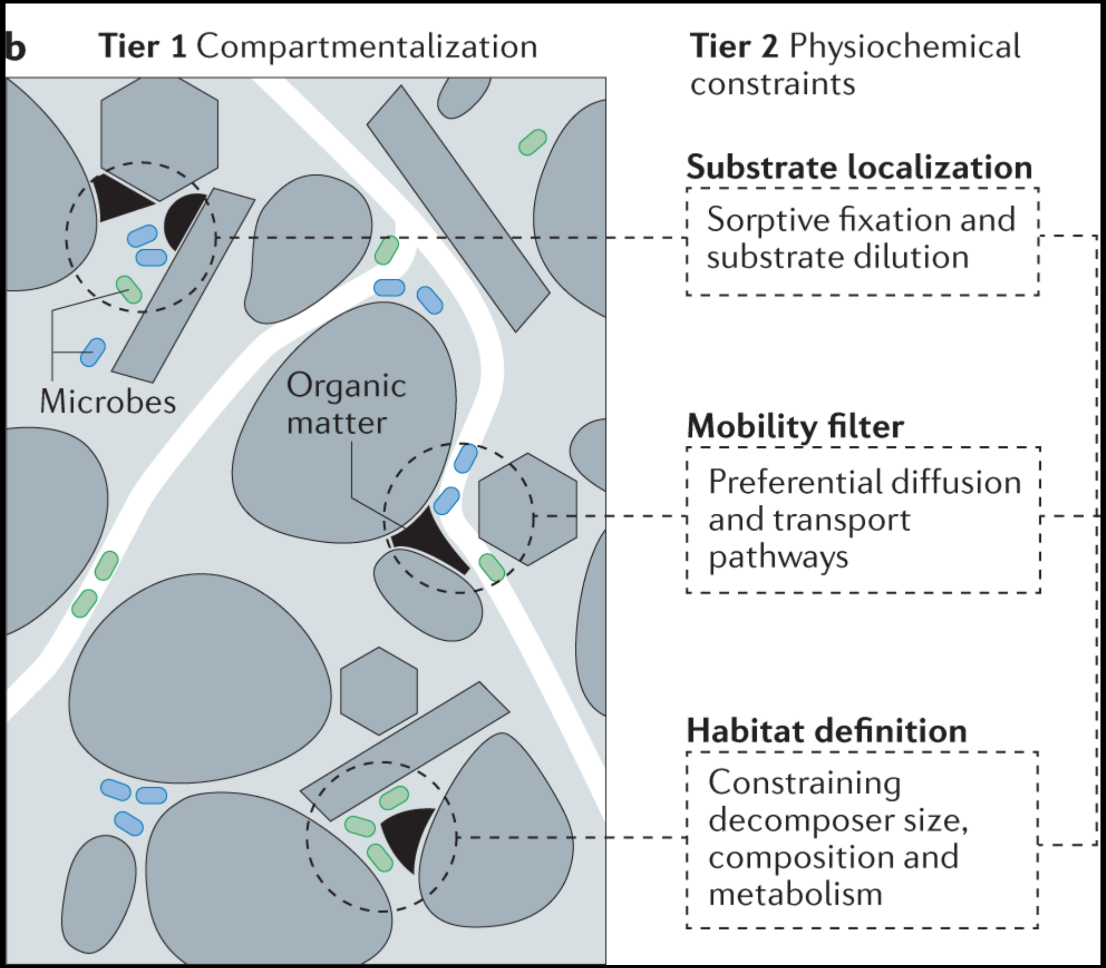


Marlow et al., Geobiology, 2015

Microbes in "Extreme" Environments

→ Potentially relevant ecologies // Microhabitats

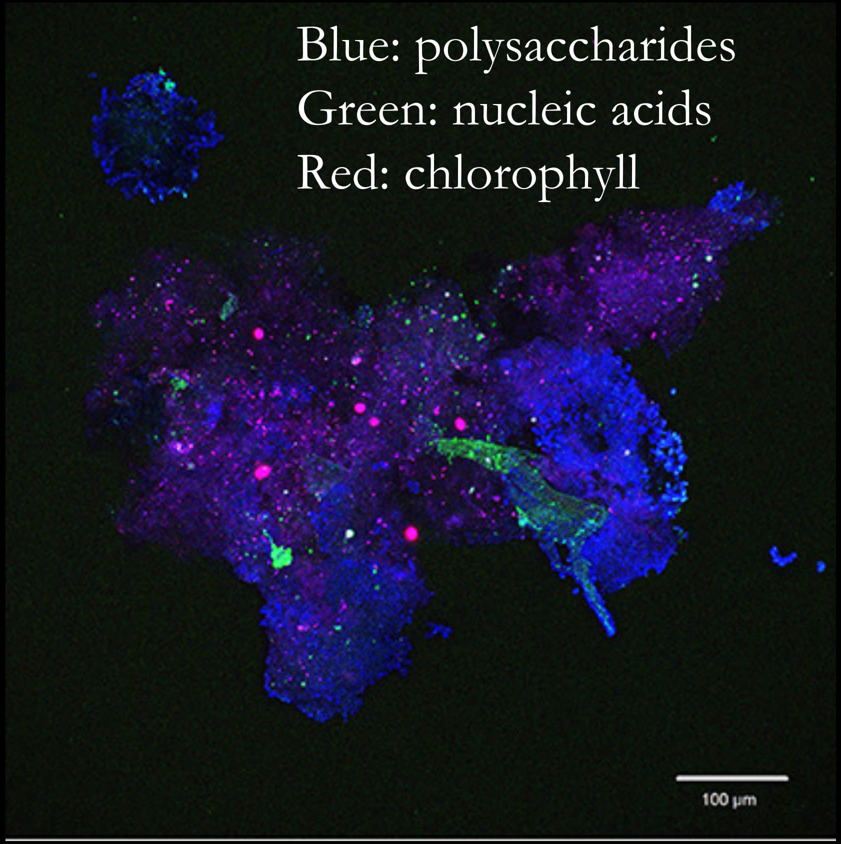
→ Otherwise labile organic molecules can avoid consumption if adsorbed onto mineral surfaces with tiny (nanoscale) pockets, or armored with mineral grains

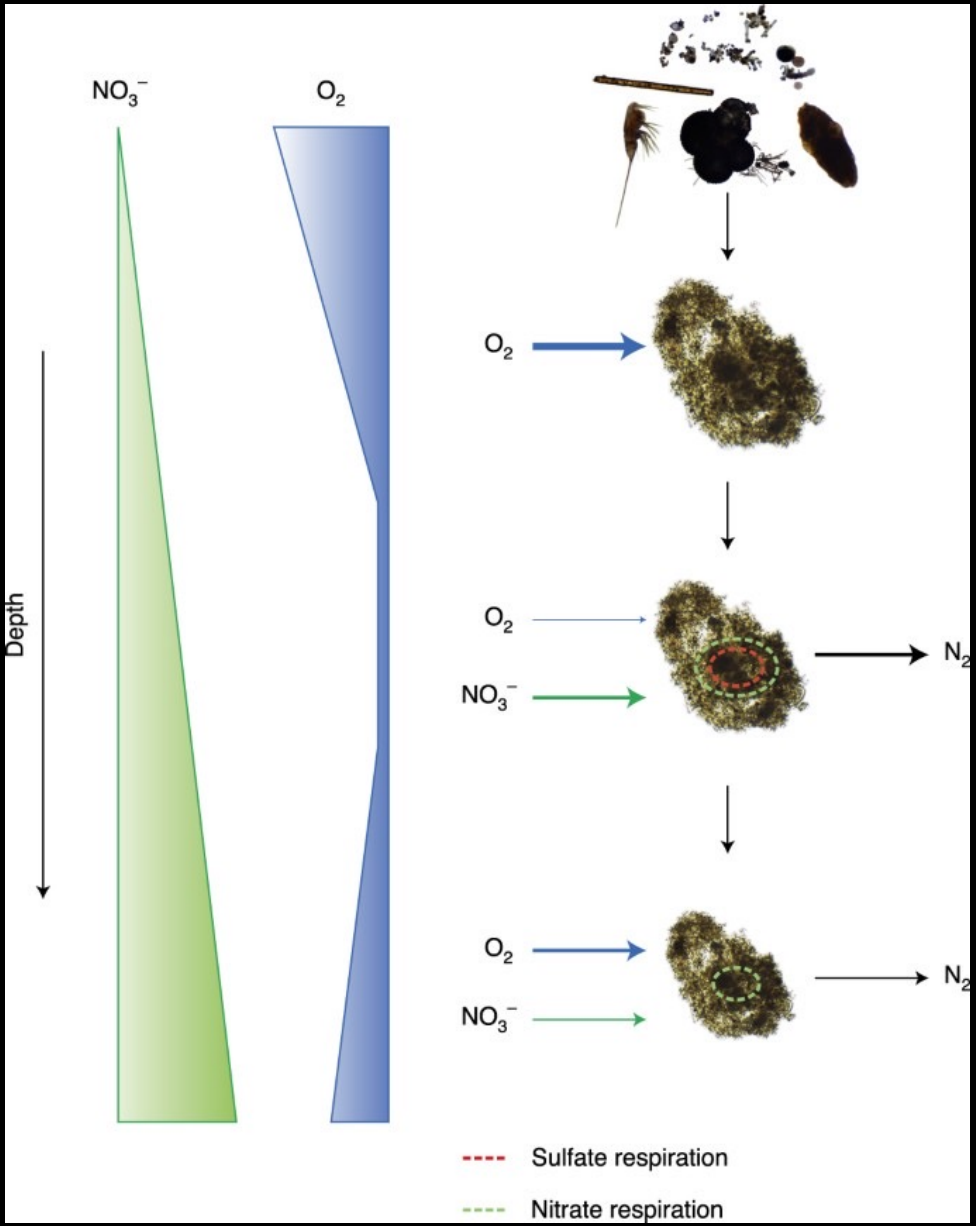


Microbes in “Extreme” Environments

→ Potentially relevant ecologies // Microhabitats

→ Clumps of organic detritus can form anoxic zones that could prevent some microbes from accessing them





Bristow, Nature Geoscience, 2018

Microbes in "Extreme" Environments

→ Potentially relevant ecologies // Microhabitats

→ Sulfuric acid dissolution of carbonate to make gypsum

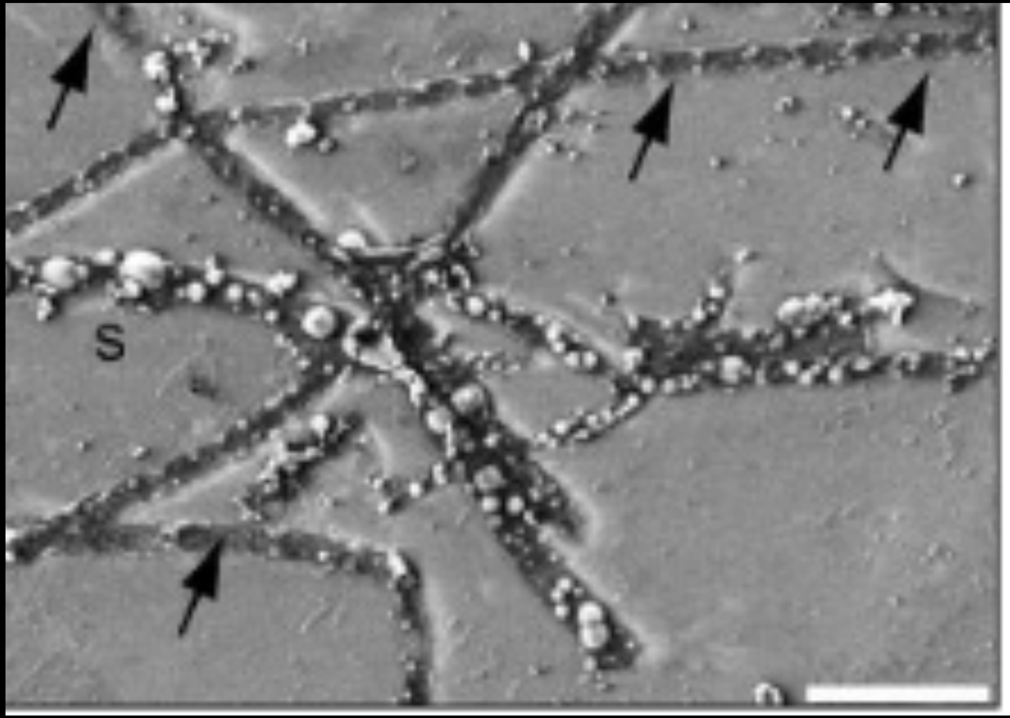
Sulfide oxidation produces acid

$$H_2S + 2O_2 \rightarrow SO_4^{2-} + 2H^+$$

$$(-798\ \text{kJ/mol S}).$$

$$HS^- + \tfrac{1}{2}O_2 + H^+ \rightarrow S^0 + H_2O \quad (-209\ \text{kJ/mol S})$$

$$S^0 + H_2O + 1\tfrac{1}{2}O_2 \rightarrow SO_4^{2-} + 2H^+ \quad (-587\ \text{kJ/mol S}).$$



Leprich et al, ISME Journal, 2021

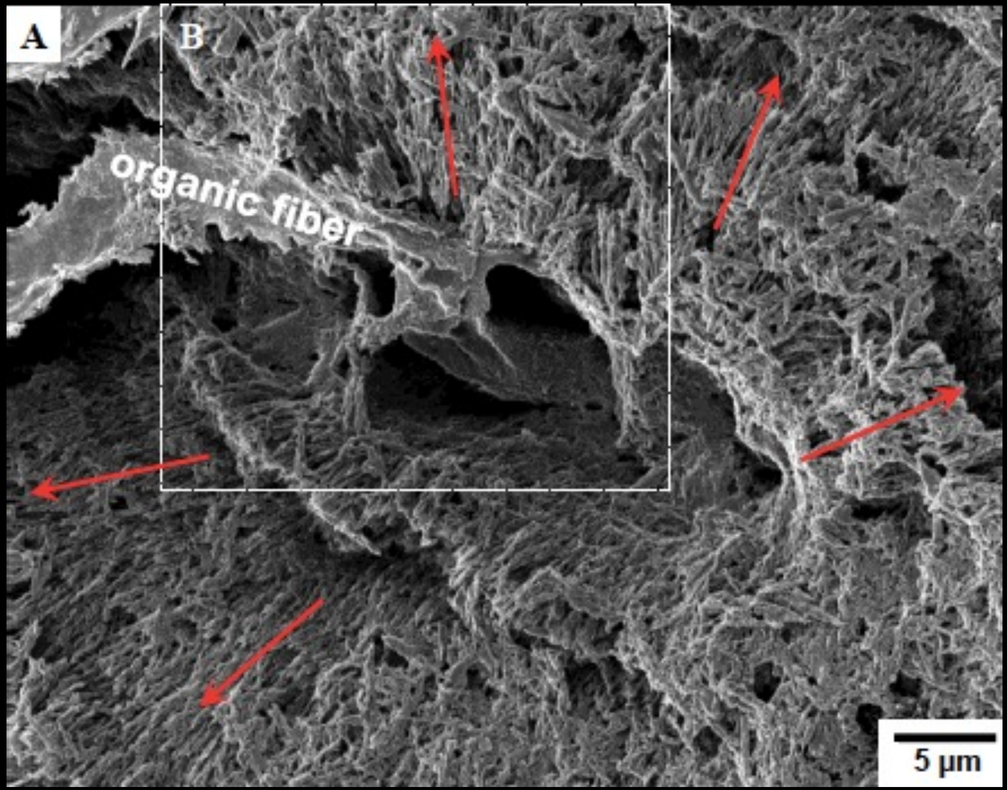
Microbes in “Extreme” Environments

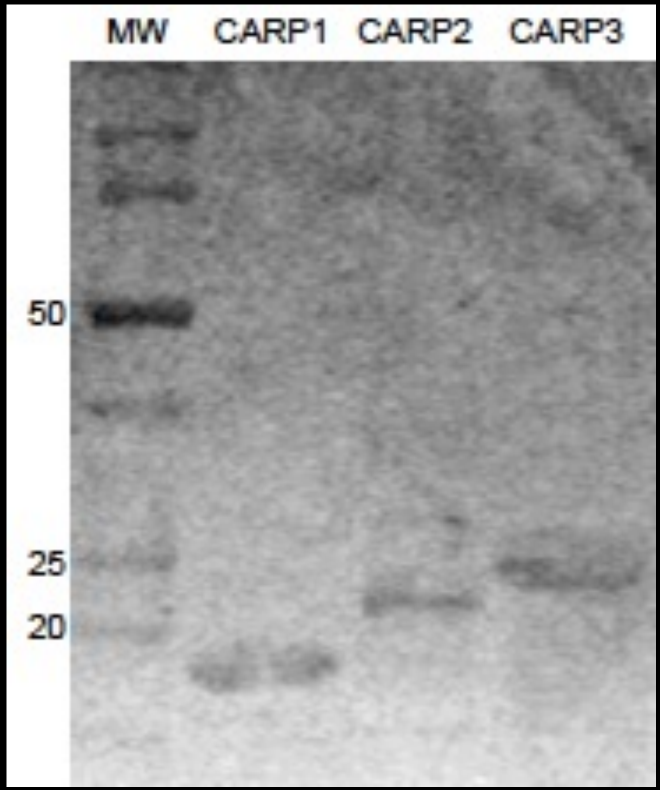
→ Key Questions

Do microbes “control” mineral composition, and if so, how / why?

Organic substrates form nucleation sites for aragonite-concentrating nanoparticles

Acid-rich proteins can precipitate aragonite from seawater



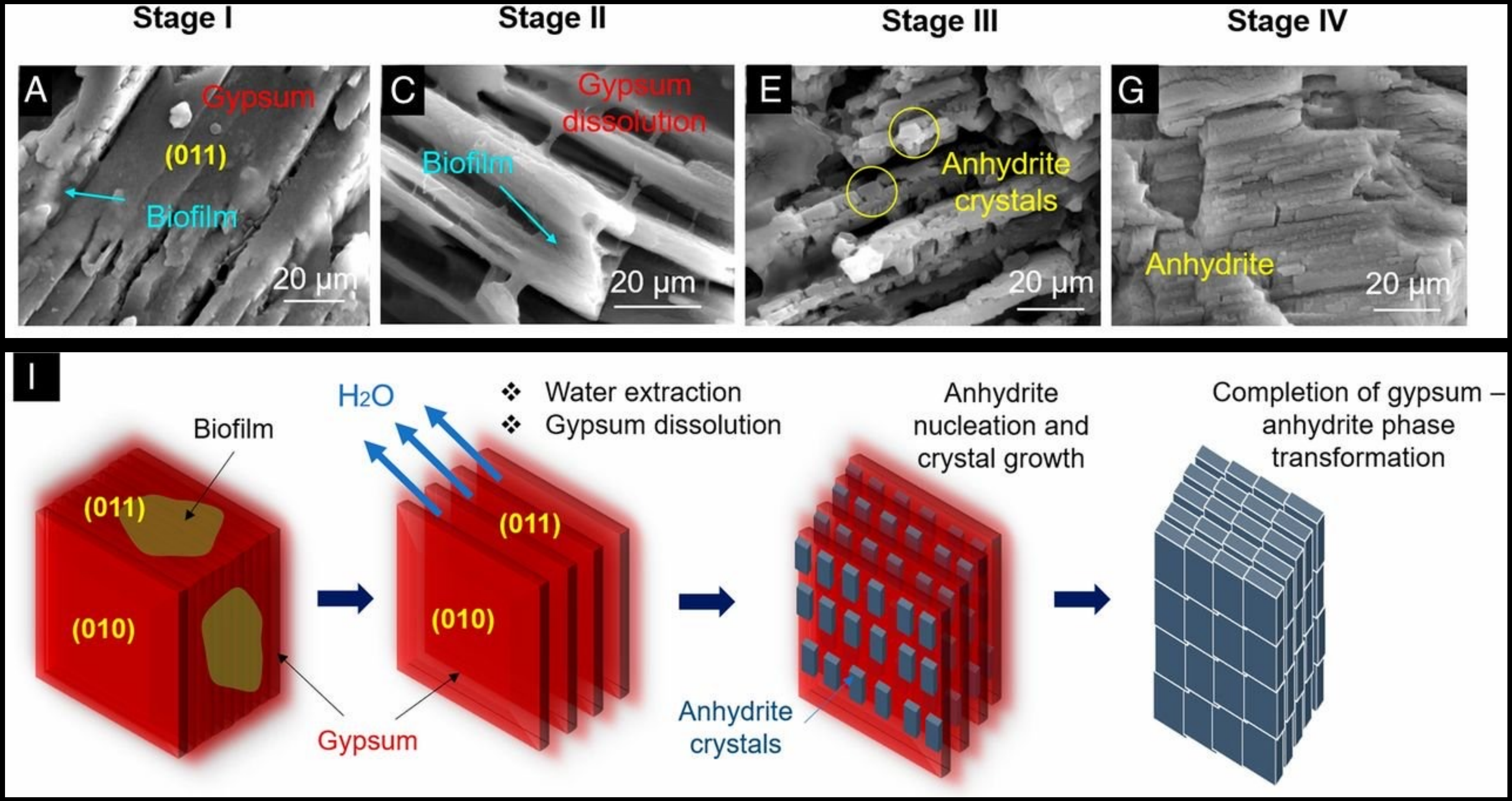


Von Euw et al., Science, 2017; Mass et al., Current Biology, 2013

Microbes in "Extreme" Environments

→ Key Questions

Can microbes access "consumables" in the mineral structure?



Microbial <u>water</u> extraction from gypsum can change the mineralogy

Huang et al., PNAS, 2020

Microbes in "Extreme" Environments

→ Key Questions

Can we see microbes in their sediment or rock-hosted habitats non-invasively and non-destructively?

$$I_1 = I_0 \cdot e^{(-\mu t)}$$

- I_1: X-ray intensity measured at detector
- I_0: Initial X-ray intensity
- μ: X-ray attenuation coefficient
- t: Thickness of attenuating material

Microbes in "Extreme" Environments

→ Key Questions

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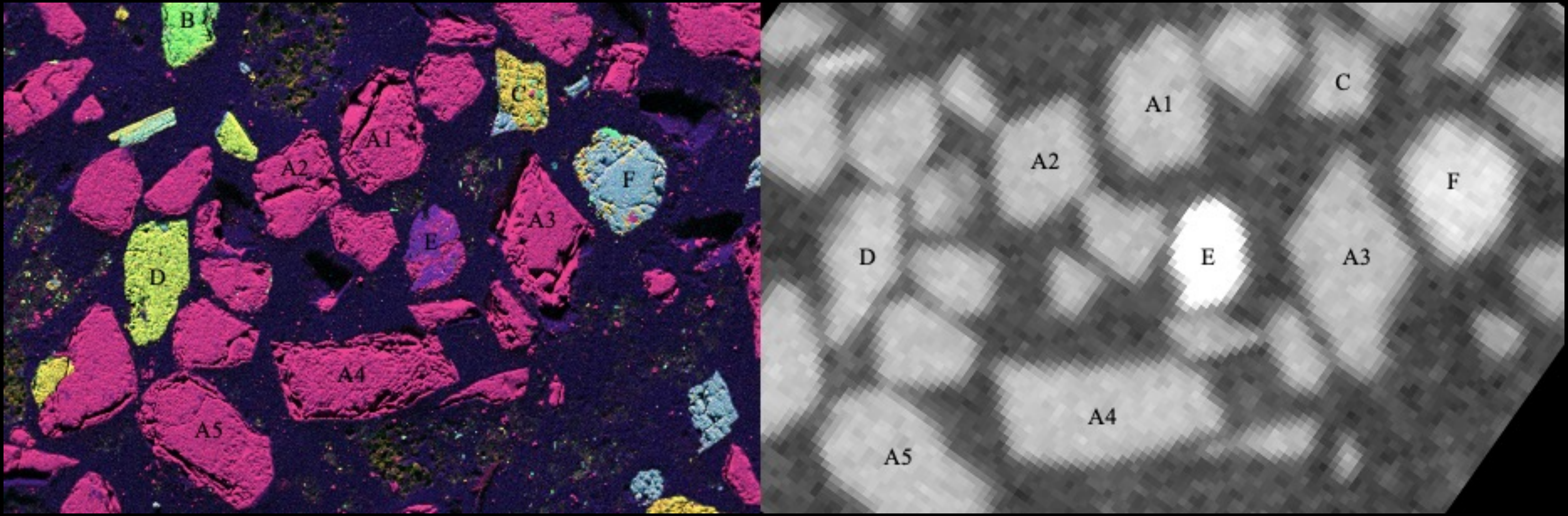

Microbes in "Extreme" Environments

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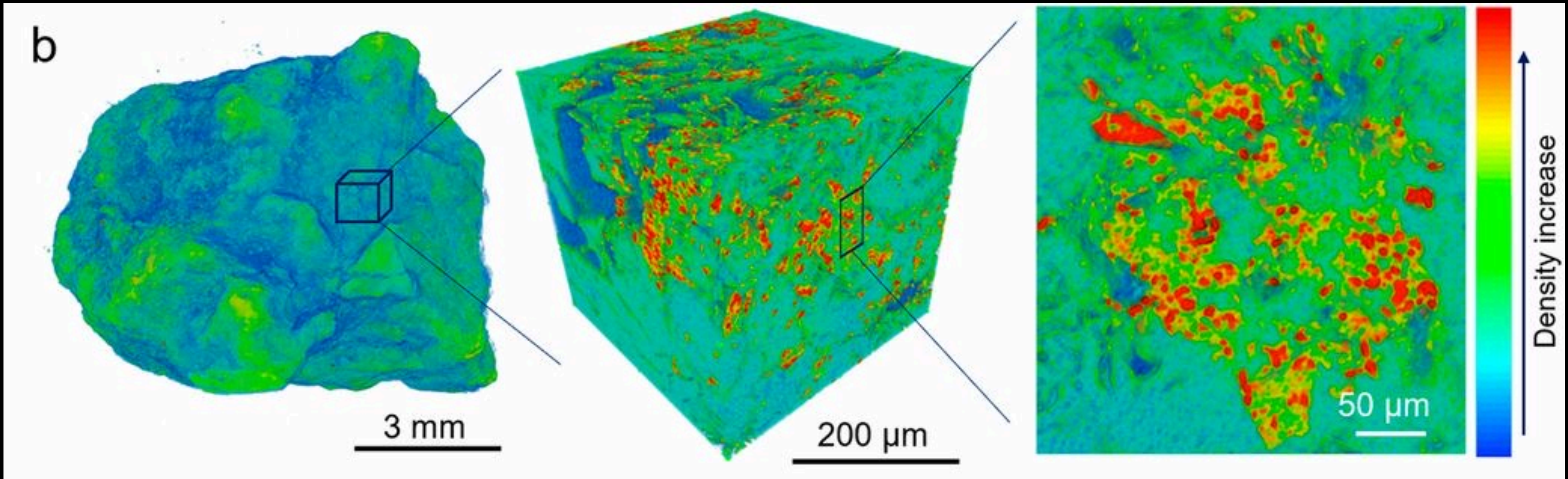

Huang et al., PNAS, 2020