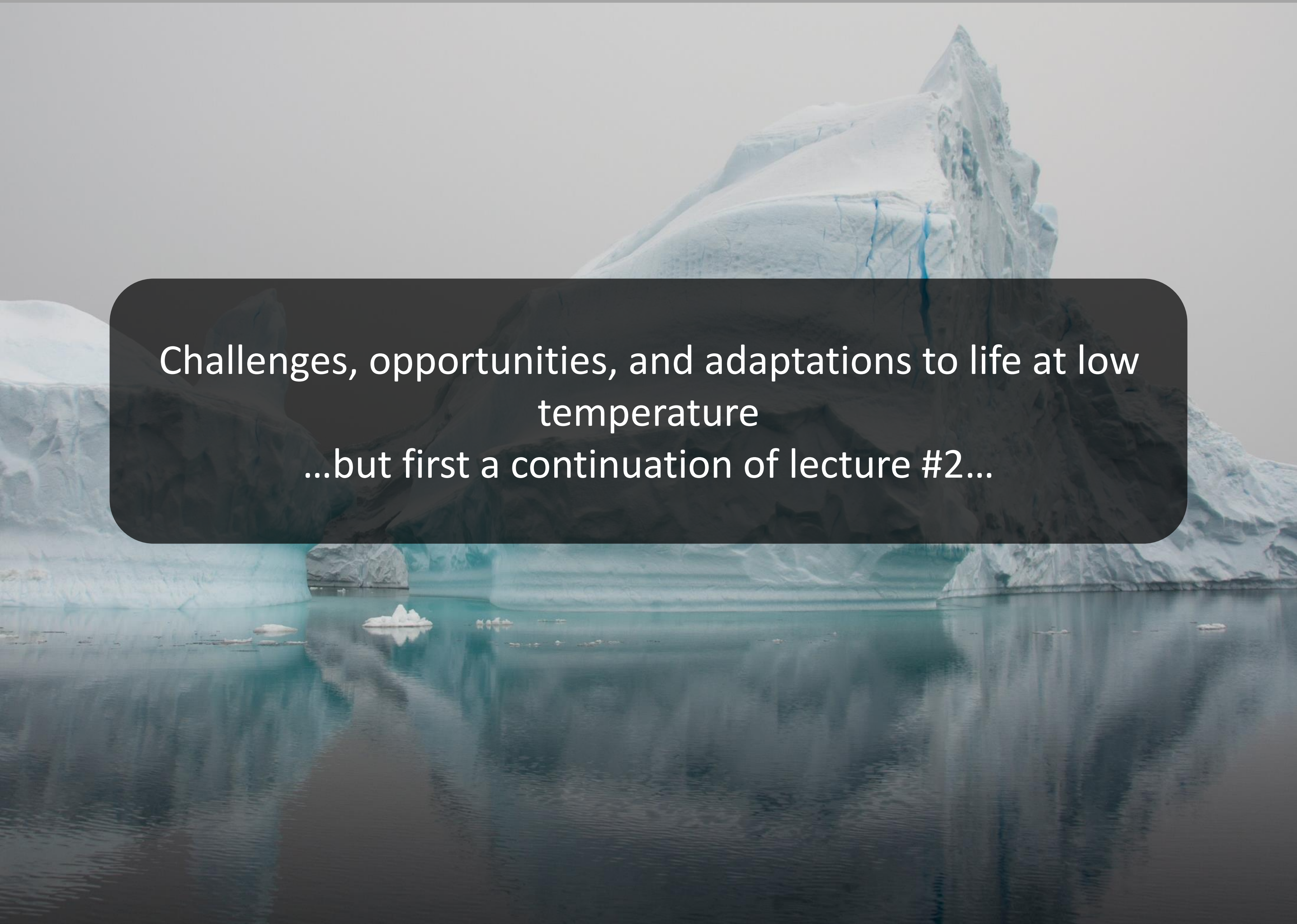
Challenges, opportunities, and adaptations to life at low temperature

…but first a continuation of lecture #2…



Announcements

- Office hours will be held at 1 pm on Thursday
- Will stabilize to 11 am on Thursday after this week

1. What are the three domains of life?

   Bacteria, Archaea, Eukarya

2. In catabolic metabolism involving CH_2O and O_2, which compound is the electron donor?

   CH_2O

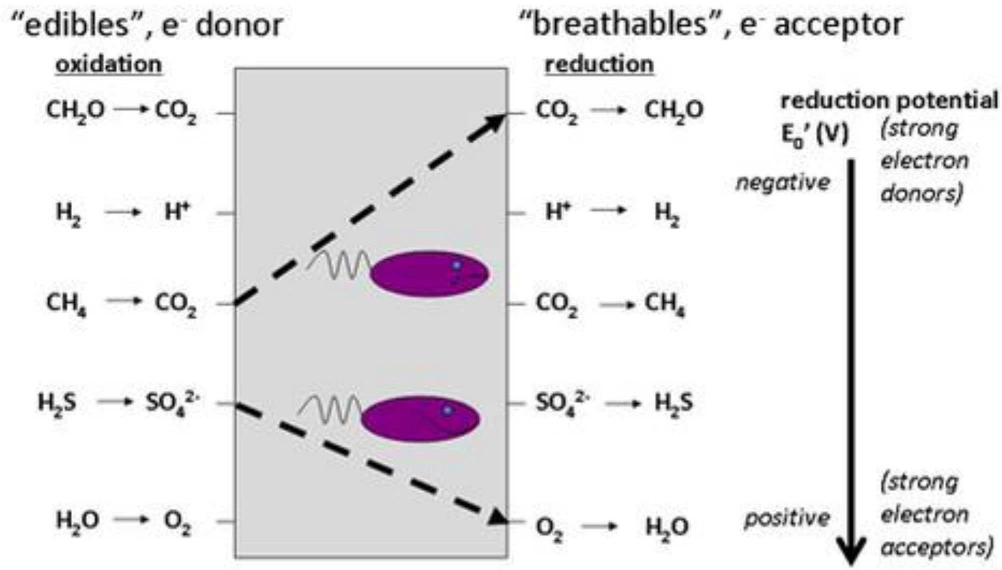
3. List two gases that have had a profound and <u>direct</u> effect on climate over Earth's history.

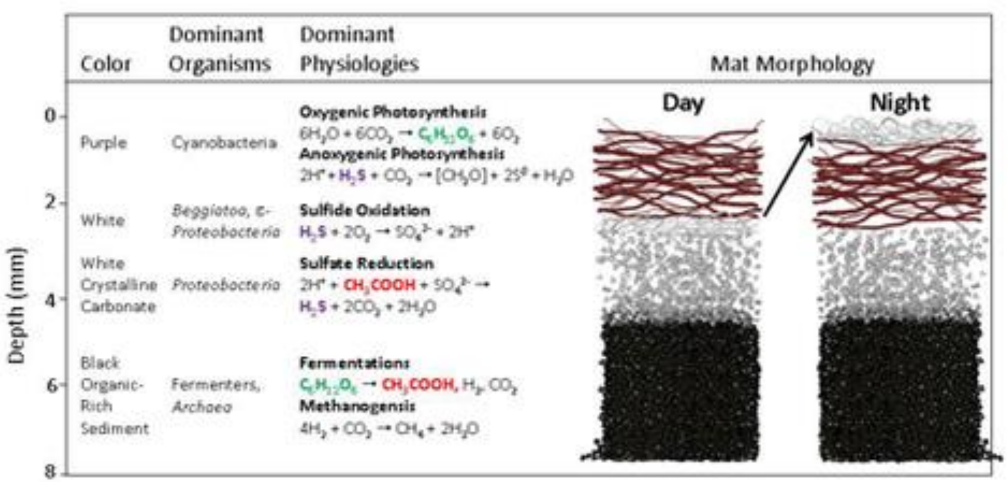
   CO_2, CH_4, NOT O_2

Energy

OIL-RIG

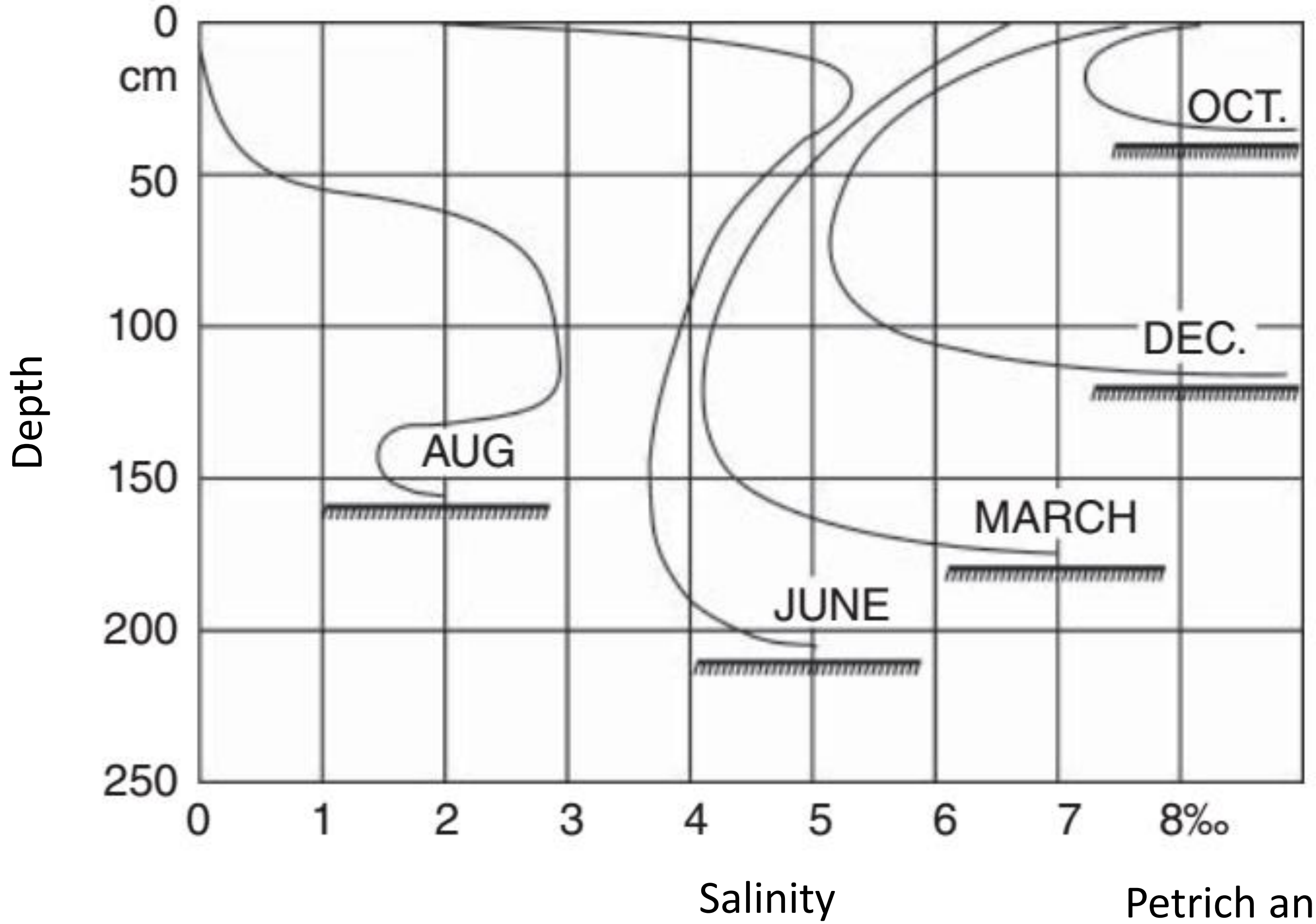
Oxidation involves loss (of electron), reduction involved gain (of electron)



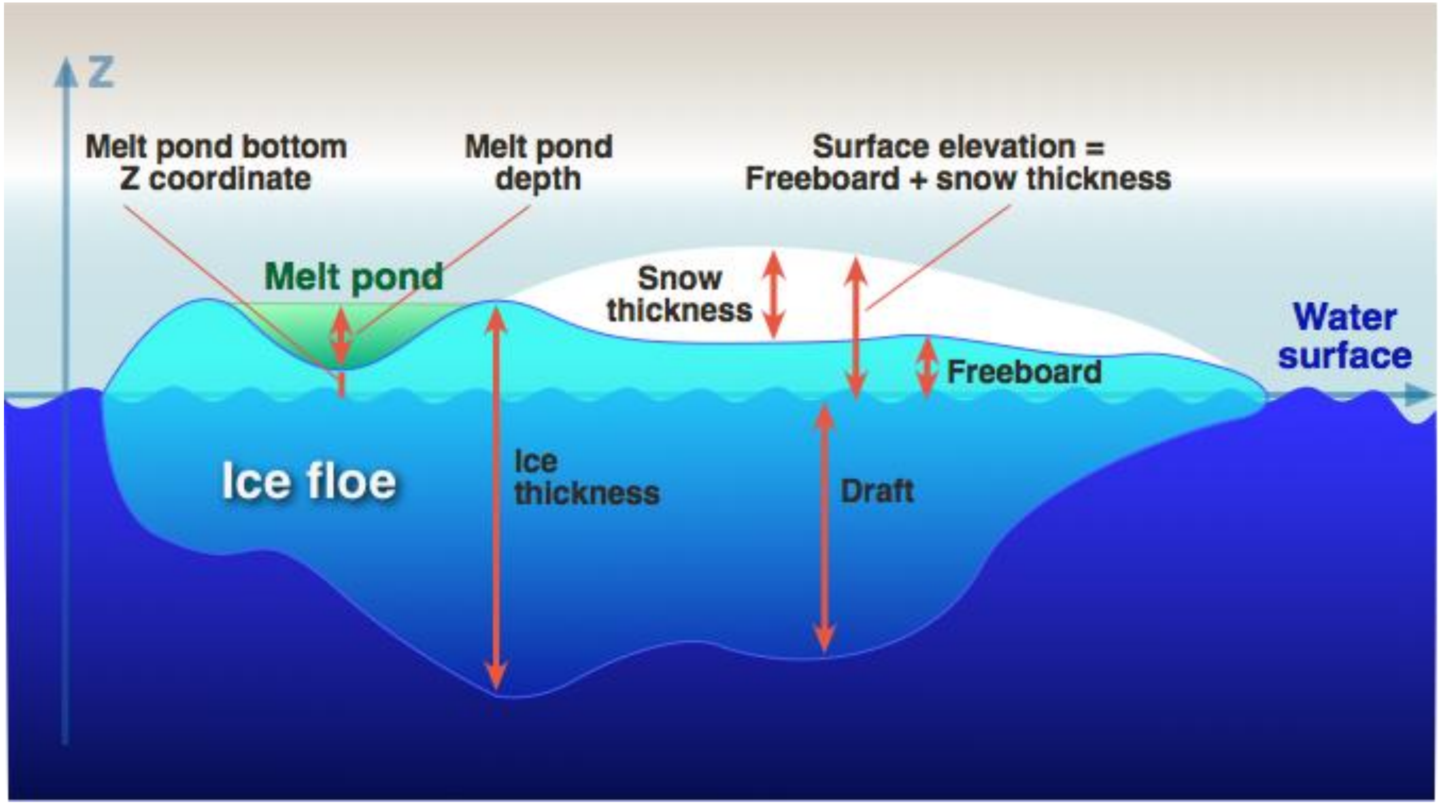


1. Which of the following features likely has the highest brine salinity: frost flowers, columnar sea ice close to the atmosphere, columnar sea ice close to the water.
2. Rank the following in order of highest to lowest albedo: open water, snow, bare sea ice.
3. At what temperature do pore spaces in “typical” sea ice lose connectivity?

Typical salinity distributions in sea ice



Petrich and Eicken, 2017



Density of seawater (approximate) = 1.035 kg L^{-1}
Density of ice (approximate) = 0.90 kg L^{-1}
Q: How much of the ice volume would you expect to find above the surface of seawater?
A: Roughly 10 %
Q: What impact does snow have on sea ice?
A: Decrease freeboard, insulate, increase temperature, increase salinity, increase porosity, potentially flood surface!

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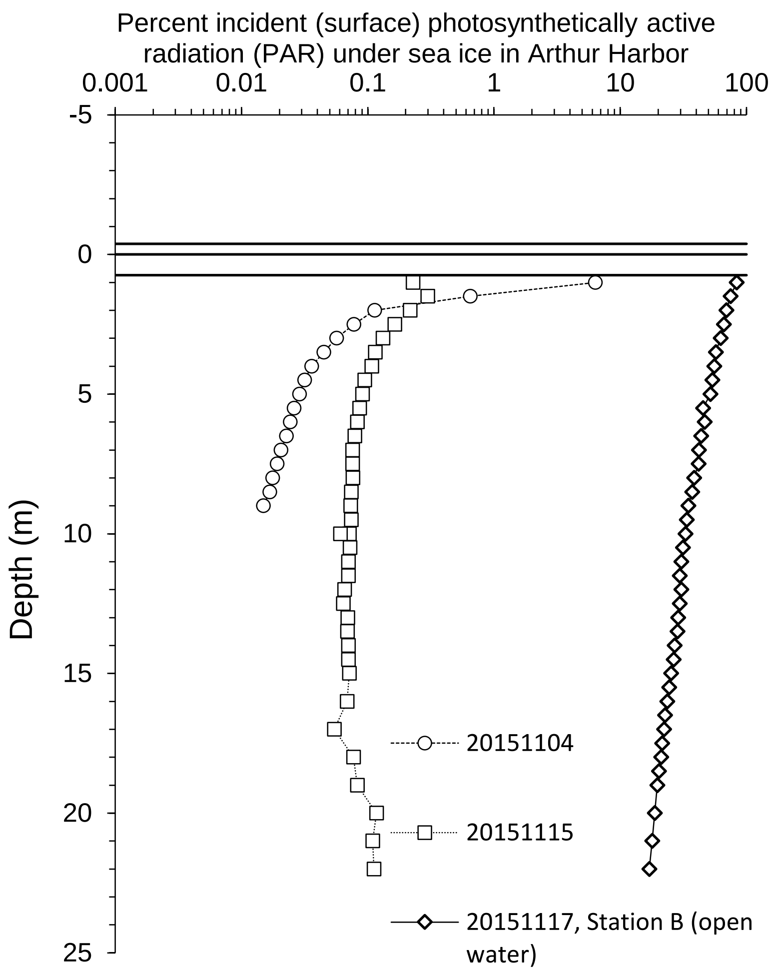
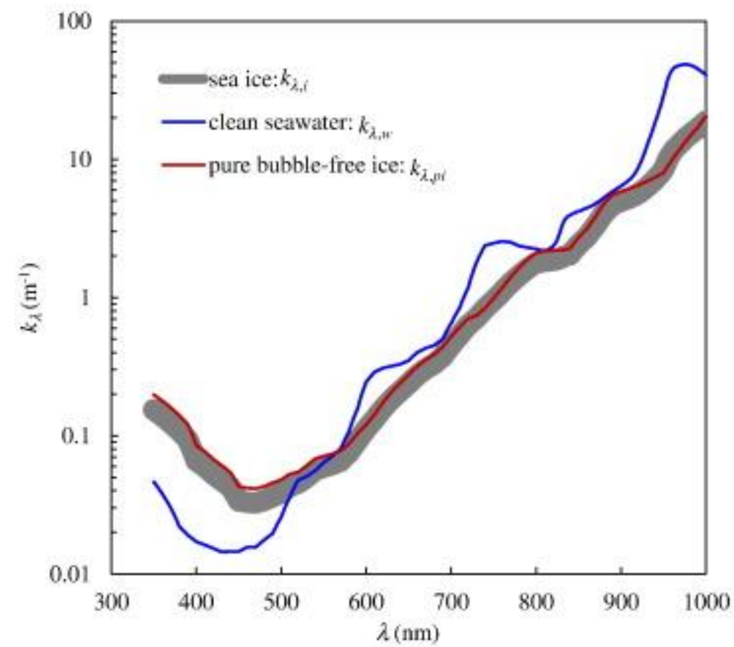
- Snow also impacts the amount of light that penetrates ice
- Q: What other ice features might impact light penetration?





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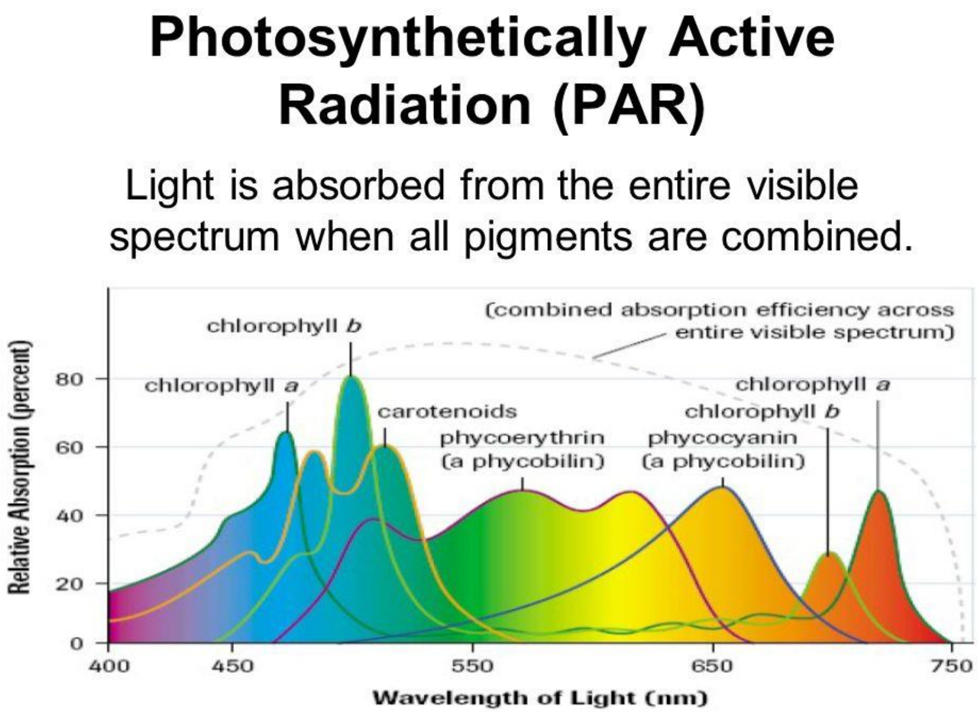

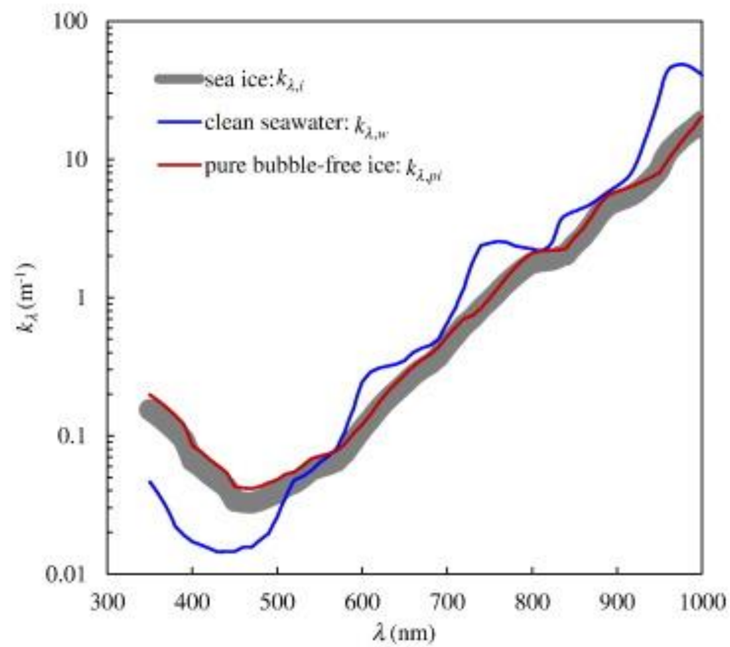


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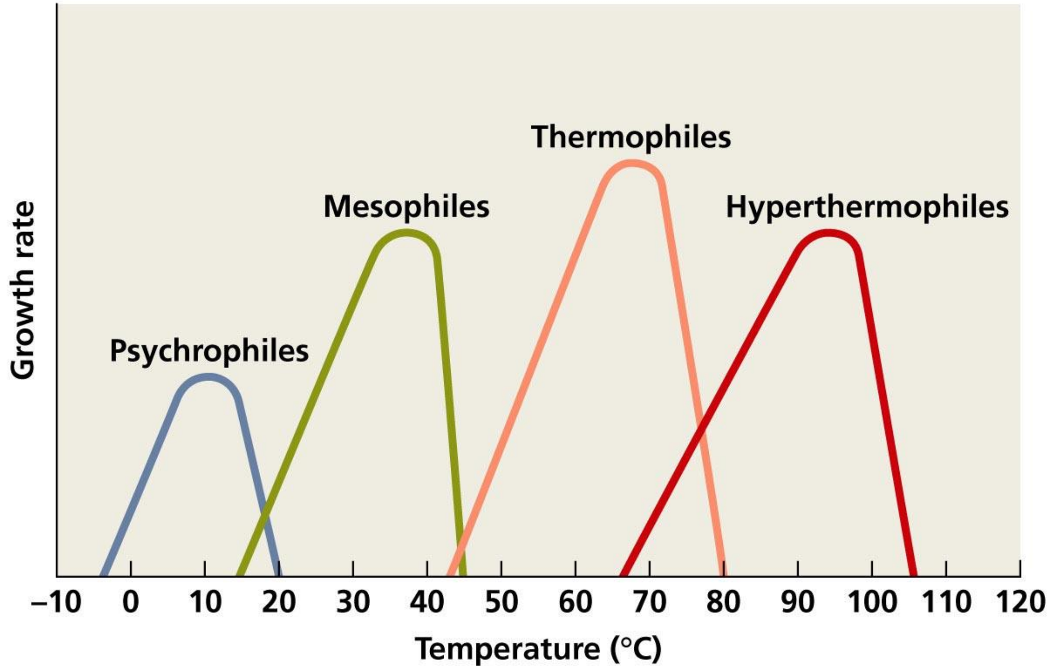
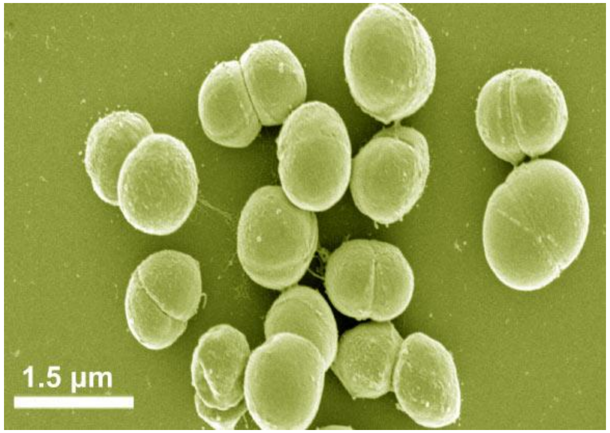
Q: So, what does all that mean for these images?
A: The sea ice-seawater interface is where light and nutrients reach an optimum in an ice covered ocean.

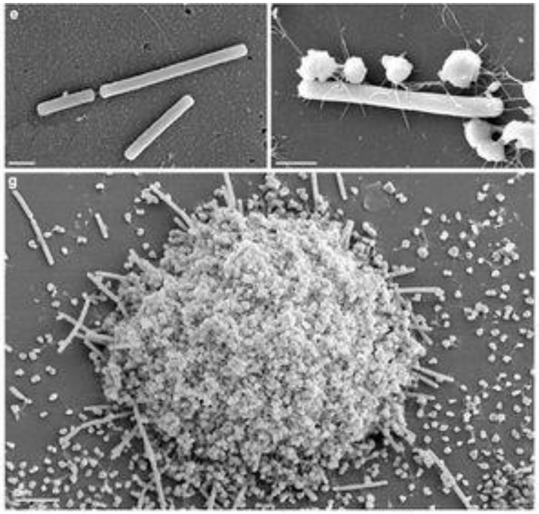
Challenges, opportunities, and adaptations to life at low temperature





Planococcus halocryphilus: Low temperature specialist, can divide at -15 C (5 F)

- Psychrophile growth rates are often lower than for other thermal classes
- Growth is not observed for any organism below about -15 C

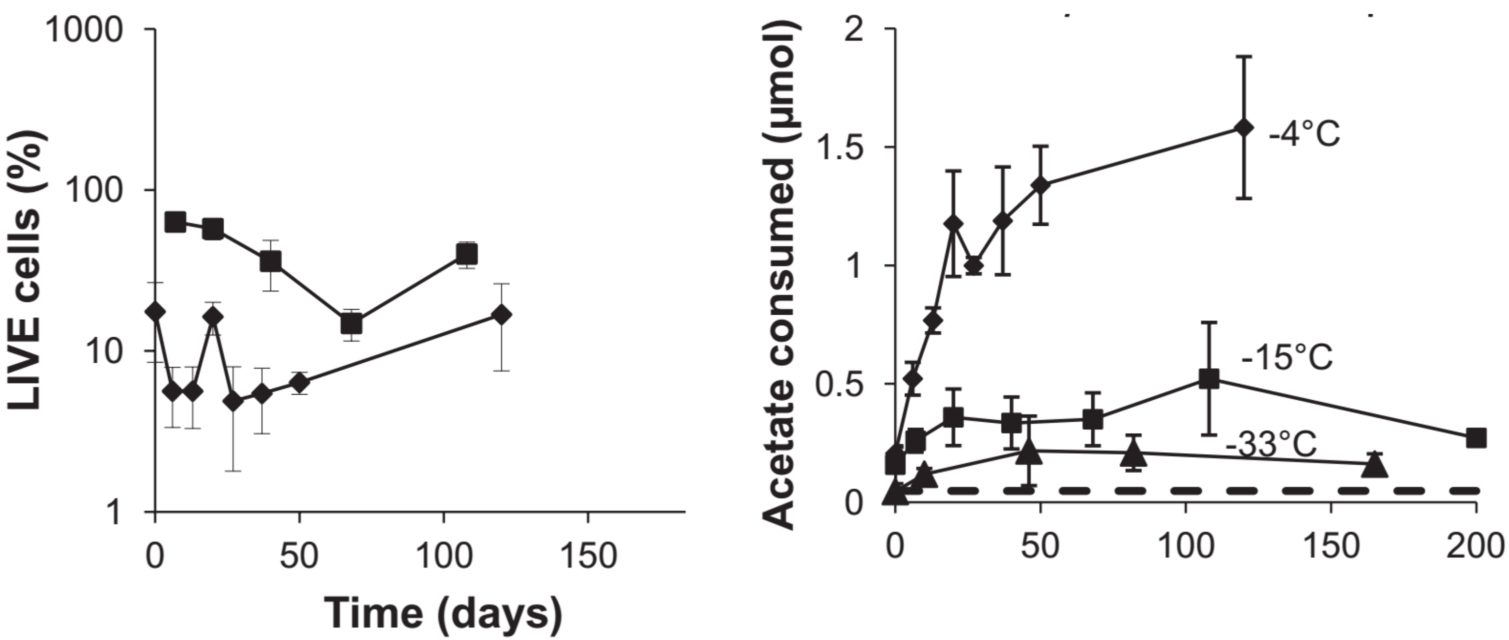


Methanopyrus kandleri: Ultra-high temperature specialist, can divide at 110 C (230 F)

Growth is separate from *activity*!

- Growth: Active cell division, even if it takes a very long time.
- Activity: The cell is metabolizing, but there is not a detectable increase in biomass.

Paenisporosarcina sp. B5

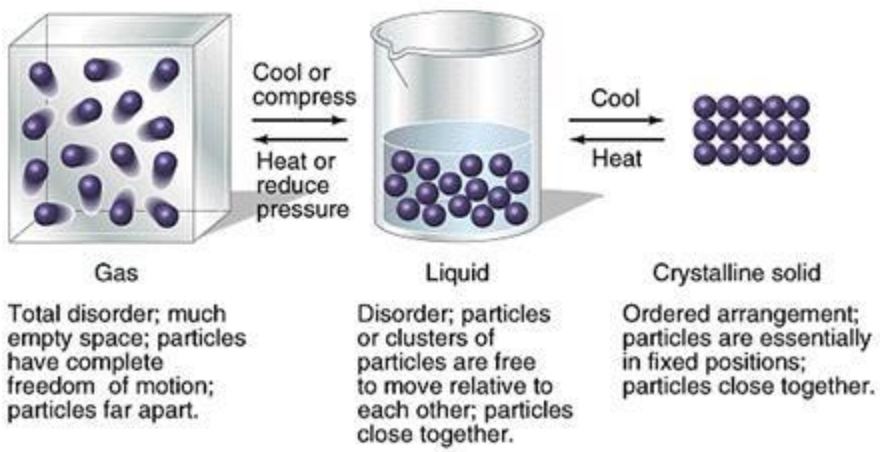


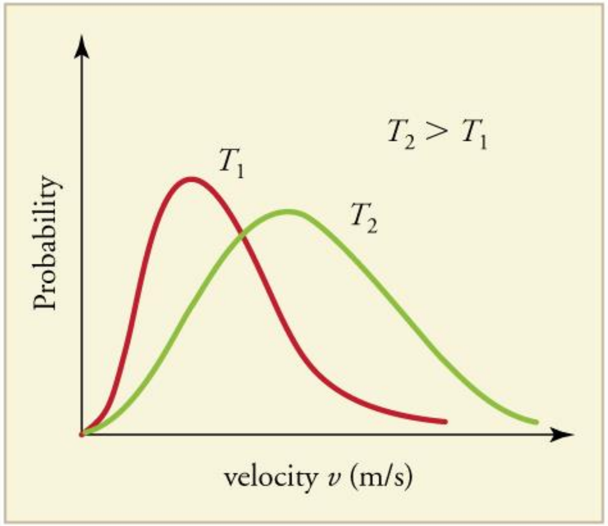
Bakermans et al., 2011

Q: On a fundamental level, what is heat?

A: Heat is molecular motion. When molecular motion is high, molecules react more frequently and macromolecules are more flexible.

- Life is a series of chemical reactions
- Temperature has a direct impact on reaction rate
- Thus temperature has a direct impact on life
- However, biology has evolved some mechanisms to limit this impact





Maxwell-Boltzmann distribution, showing the probability distribution for particles of different energies.

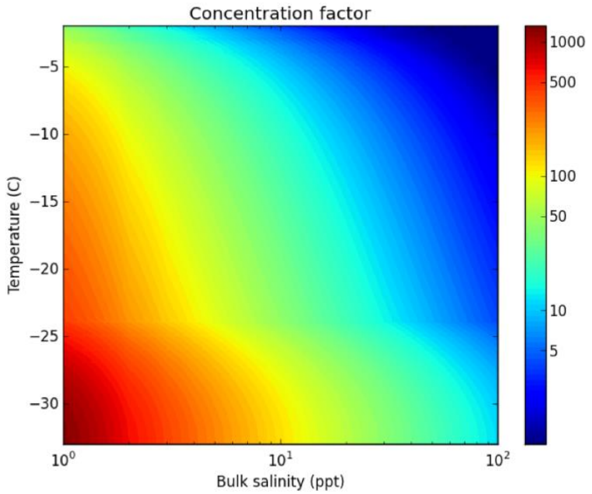
Low temperatures impose some specific challenges on life:

- Proteins are less flexible, leading to lowered enzyme reaction rates
- Lipid membranes are less fluid, leading to a rigid cell surface that resists motion and transport, and that is more prone to damage
- Within ice, solute transport is greatly reduced and ice crystals can damage delicate cellular structures

Q: Giving these, what adaptations do you expect?

- Evolution of more flexible proteins
- Modification of lipid membranes for enhanced flexibility
- Biological alteration of ice structure

Q: Based on this discussion and the previous lecture what opportunities might you expect in low temperature environments?

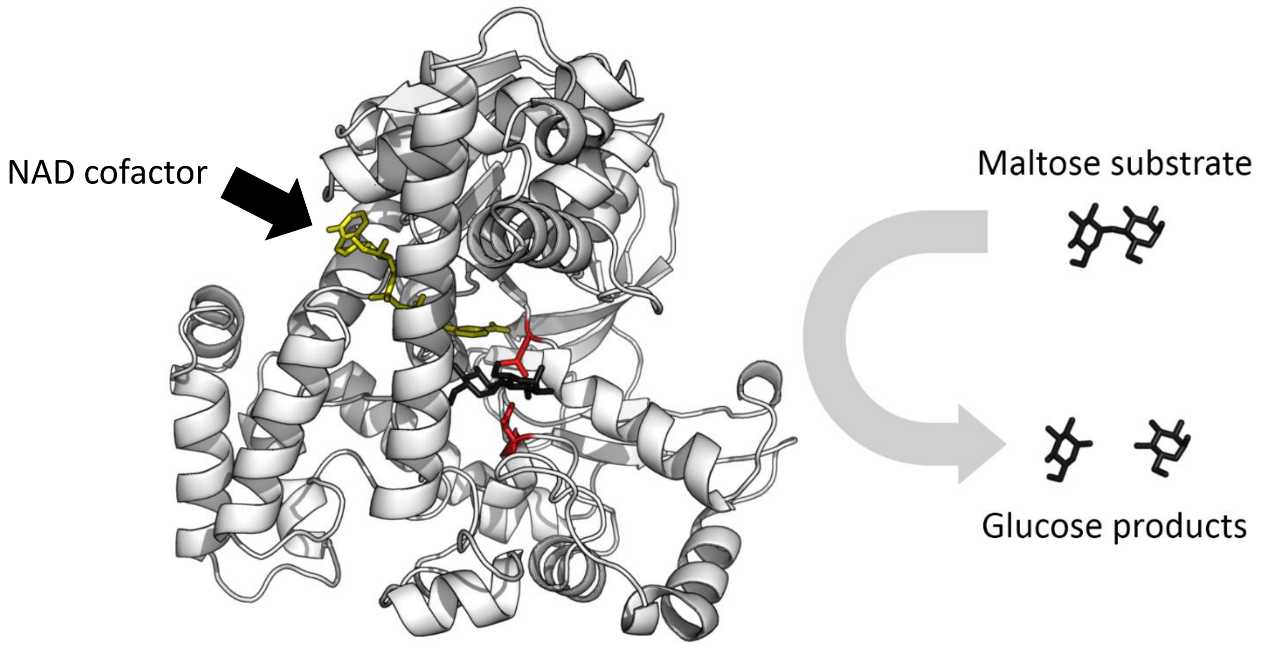


SIO 121, Lecture 3: Challenges – protein flexibility

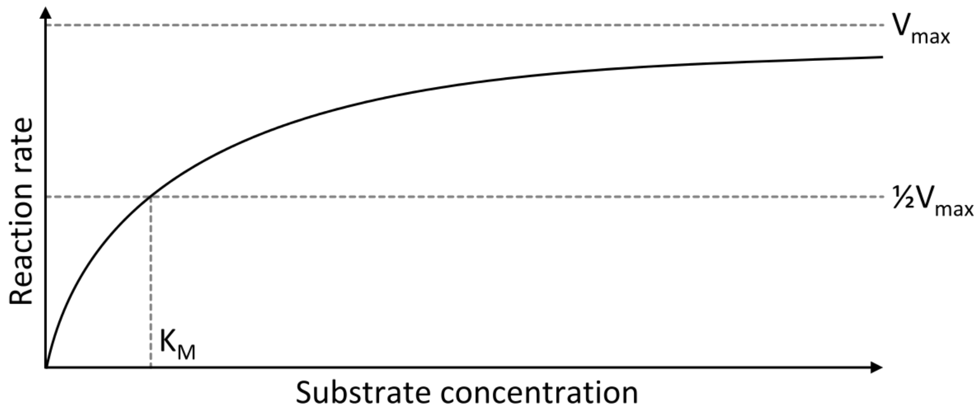
Q: What's an enzyme?

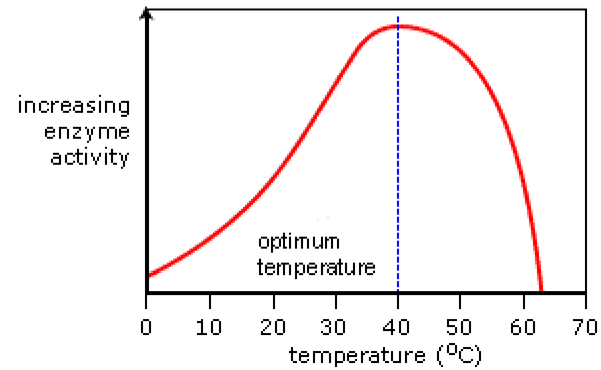
A: An enzyme is a protein that catalyzes a reaction (catalysts must be renewed; cannot undergo permanent change)

Glucosidase: catalyzes conversion of maltose to glucose



SIO 121, Lecture 3: Challenges – protein flexibility

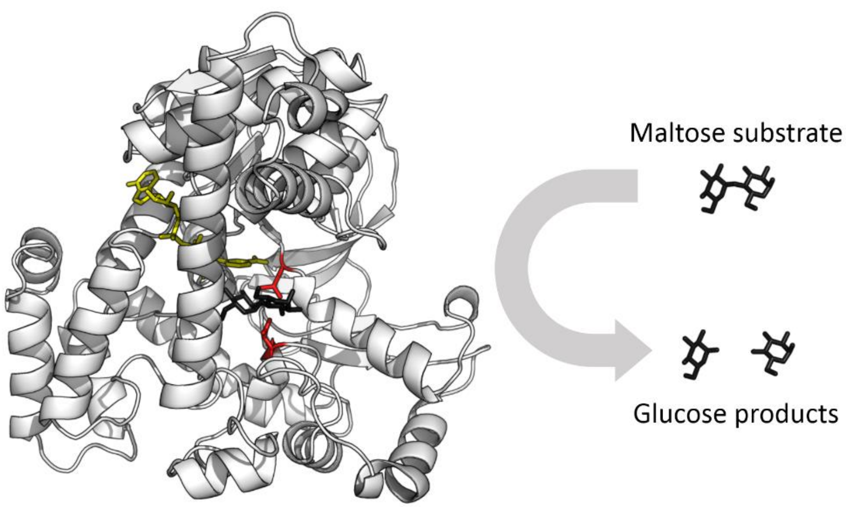


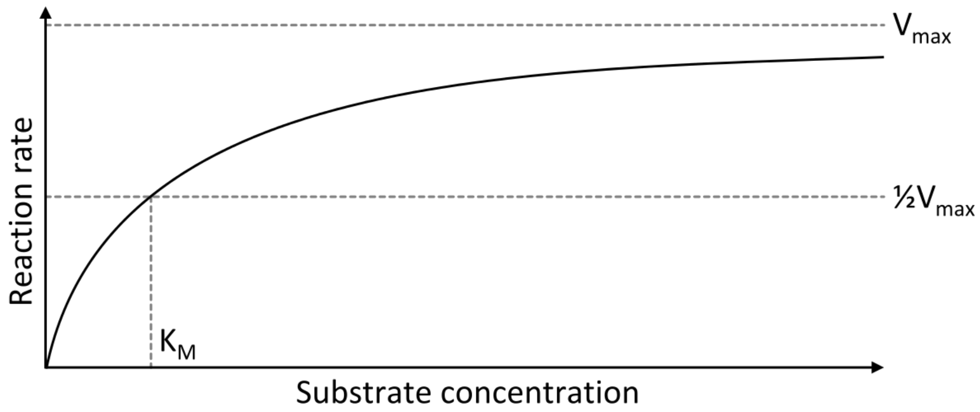


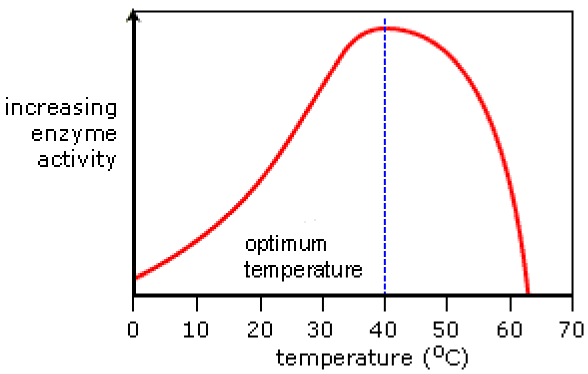
Enzyme reaction rates are described by *Michaelis-Menton kinetics*, and are dependent on *temperature* and *substrate concentration*

V_{max} = maximum reaction rate for a given temperature

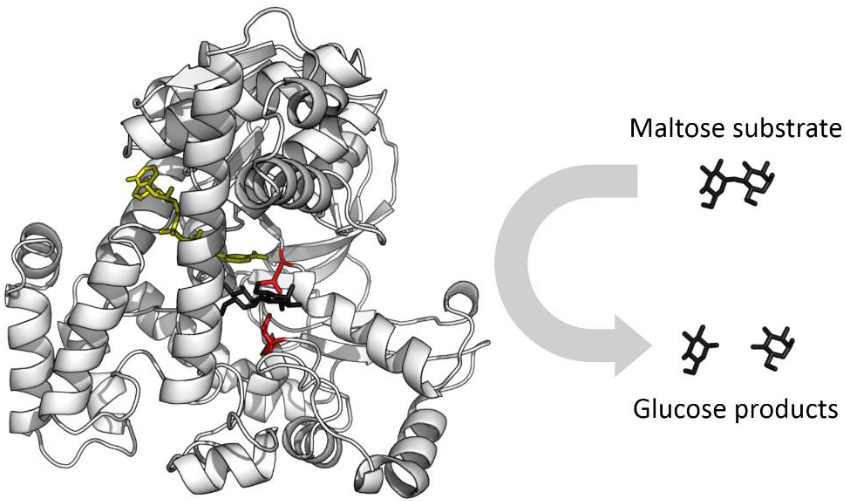
K_M = substrate concentration required to achieve ½ V_{max}





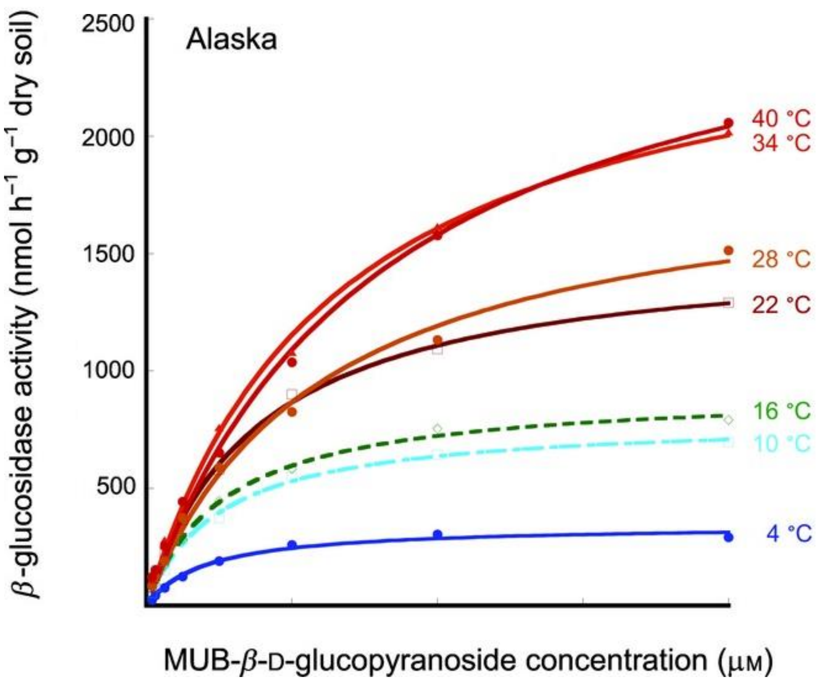


Q: List three ways to increase the rate of this reaction
- Increase temperature
- Increase the concentration of maltose
- Increase the number of glucosidase enzymes



Q_{10} is the temperature sensitivity of an enzyme, enzyme system, or organism, defined as the factor change in activity given a 10 degree shift in temperature.

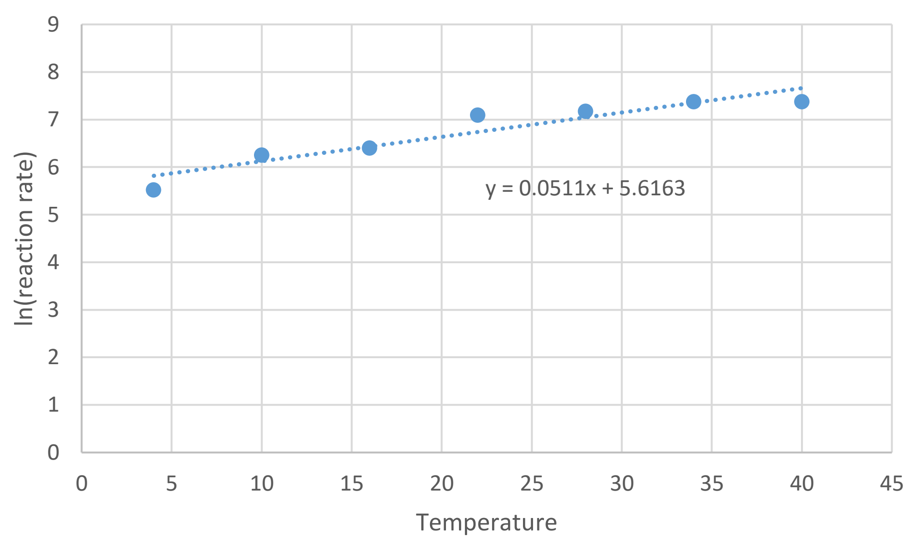
- High Q_{10} indicates high temperature sensitivity
- Low Q_{10} indicates low temperature sensitivity



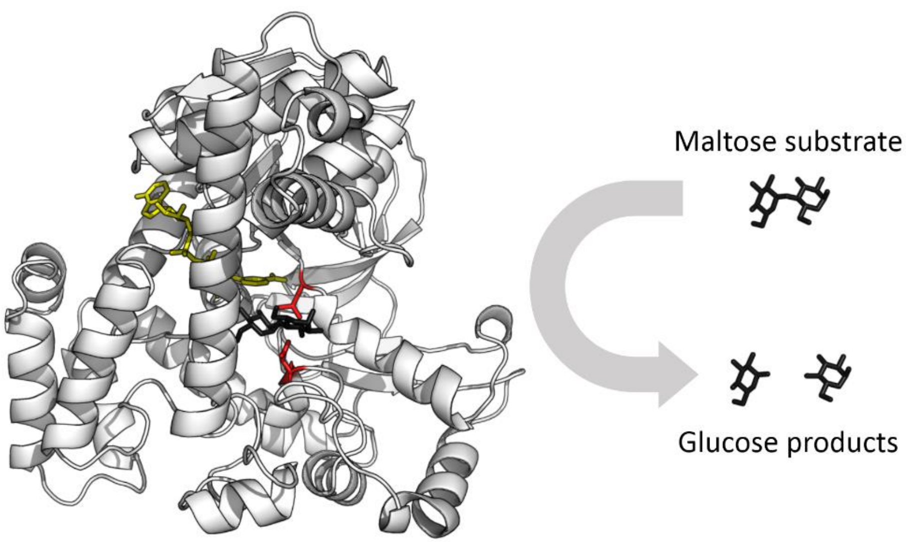
$$Q_{10} = \left(\frac{R_2}{R_1}\right)^{\left(\frac{10}{T_2 - T_1}\right)}$$

$$Q_{10} = e^{(m * 10)}$$

Where m = the slope of ln(reaction rate) as a function of T, for a specific substrate concentration



Enzymes achieve improved flexibility by making modifications at the *active site*, the core of the enzyme that actually interacts with the substrate. Cofactor binding sites are also important.



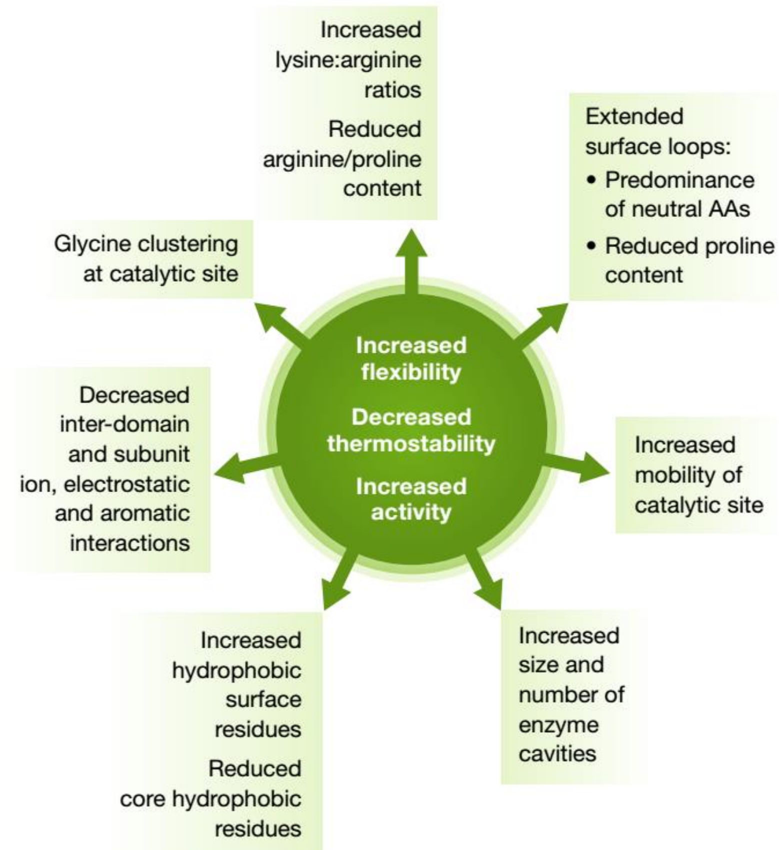


Figure 2. Common structural modifications of psychrophilic enzymes resulting in decreased thermostability, increased flexibility and increased specific activity.

De Maayer et al., 2014

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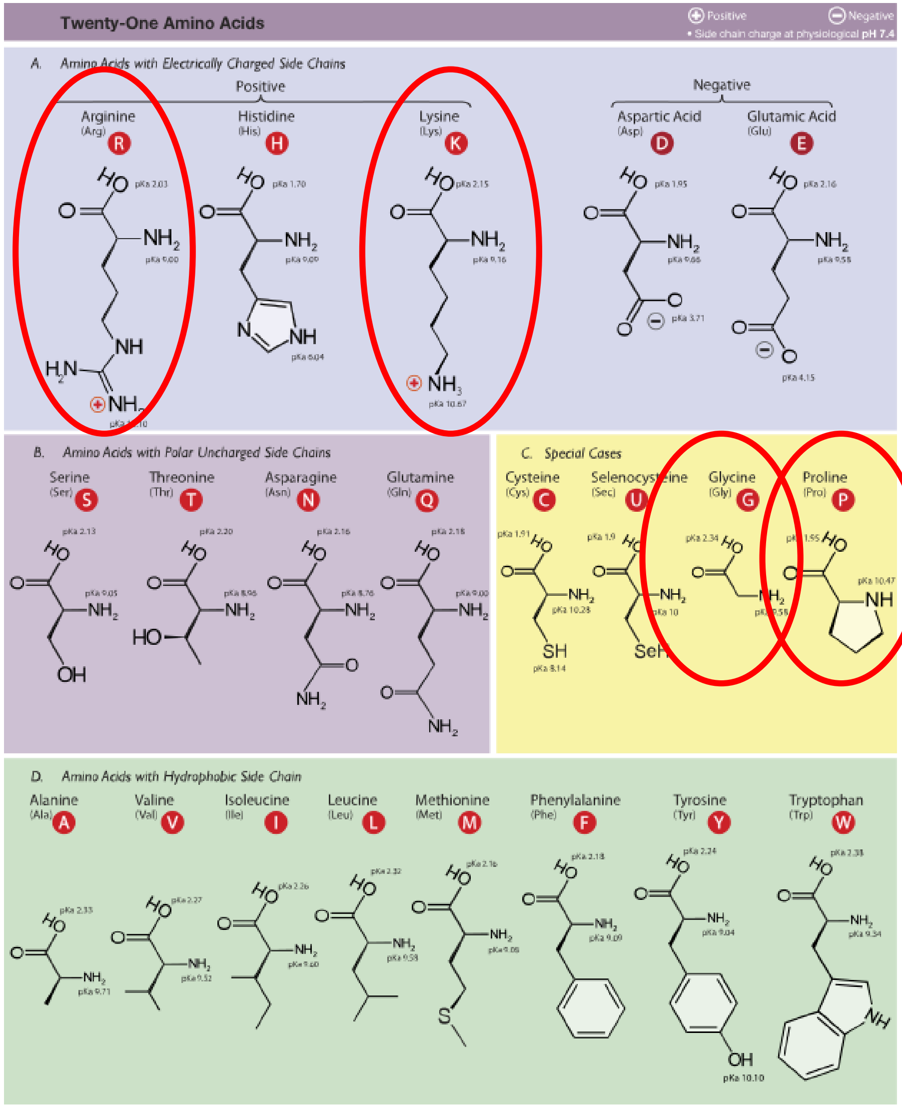

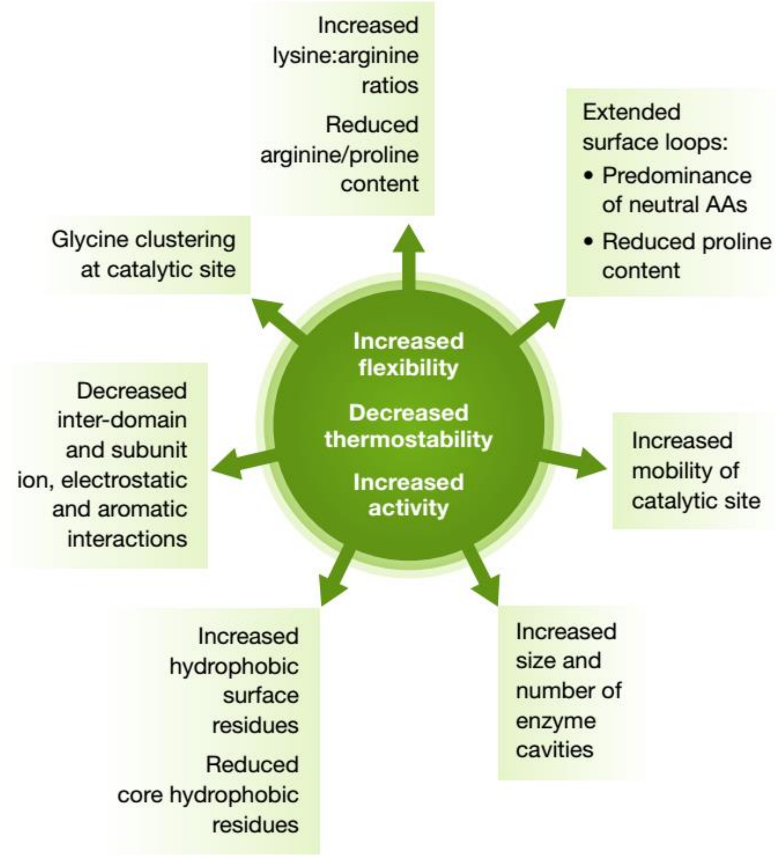

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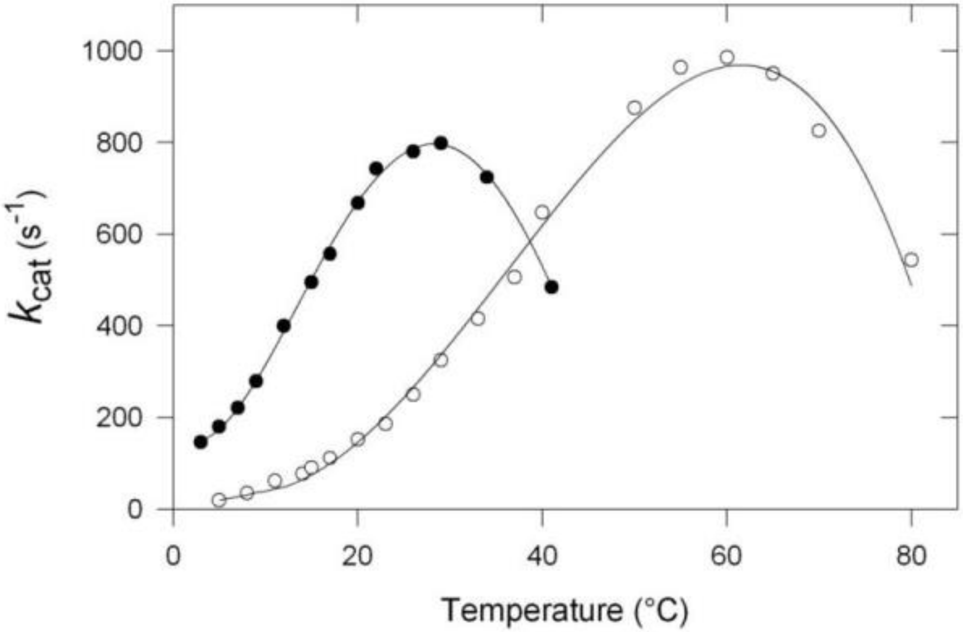
De Maayer et al., 2014



Gerday, 2013

Dark circles: enzyme from the psychrophile *Pseudoalteromonas planktomarina*
Open circles: related enzyme from the mesophile *Bacillus amyloliquiefaciens*

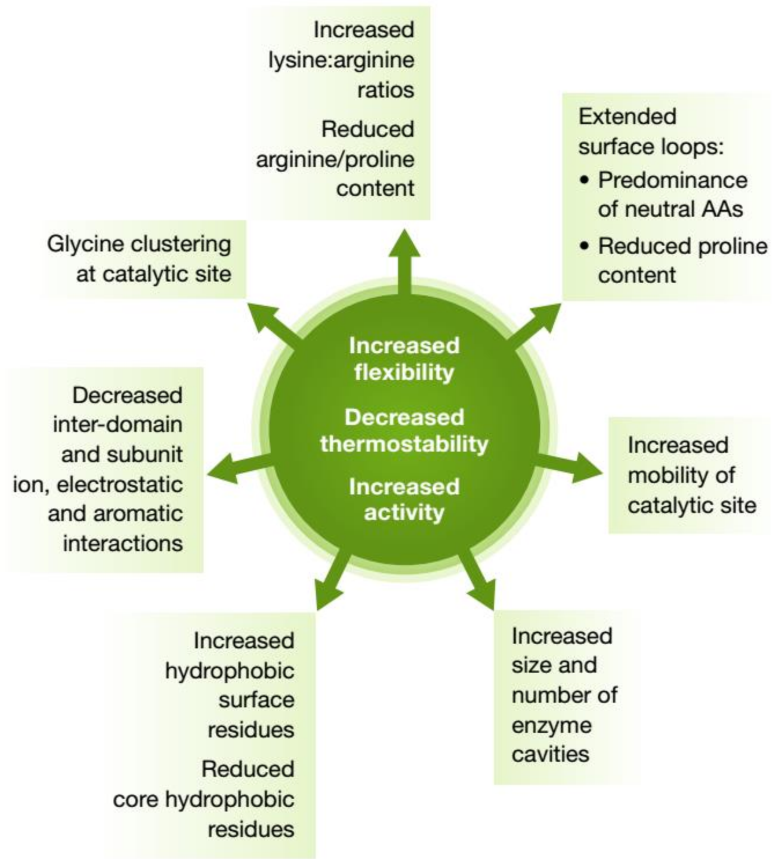
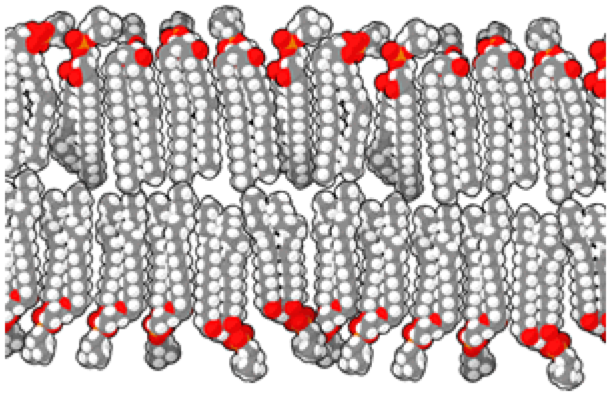


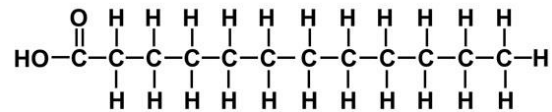
Figure 2. Common structural modifications of psychrophilic enzymes resulting in decreased thermostability, increased flexibility and increased specific activity.

De Maayer et al., 2014

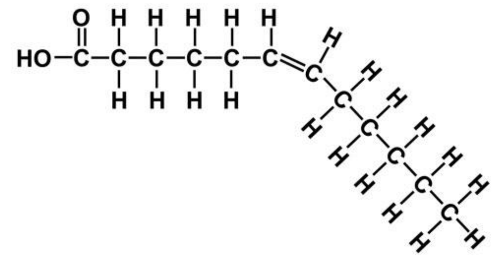
Organisms can alter the composition of the lipid membrane to have more saturated phospholipids (tightly packed, more *hydrophobic interactions*) or fewer (loosely packed, fewer interactions)



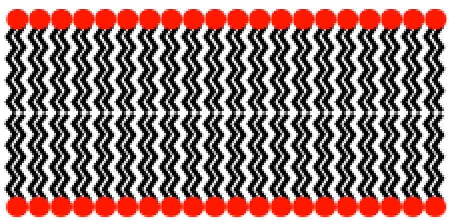
Saturated Fatty Acid



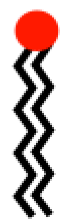
Unsaturated Fatty Acid



Not cold adapted

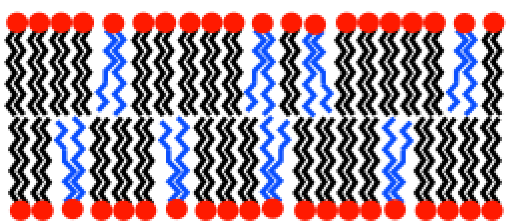


Saturated lipids only

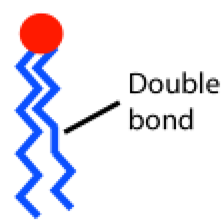


Saturated

Cold adapted

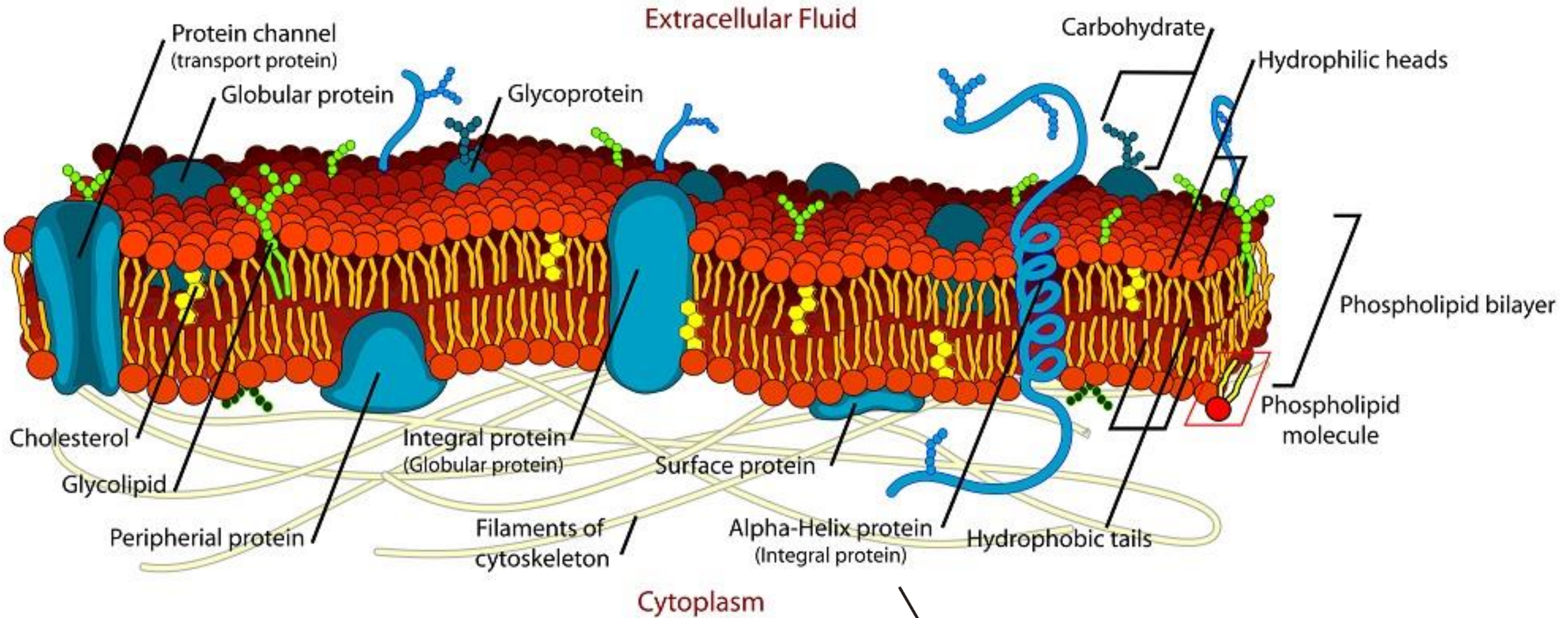


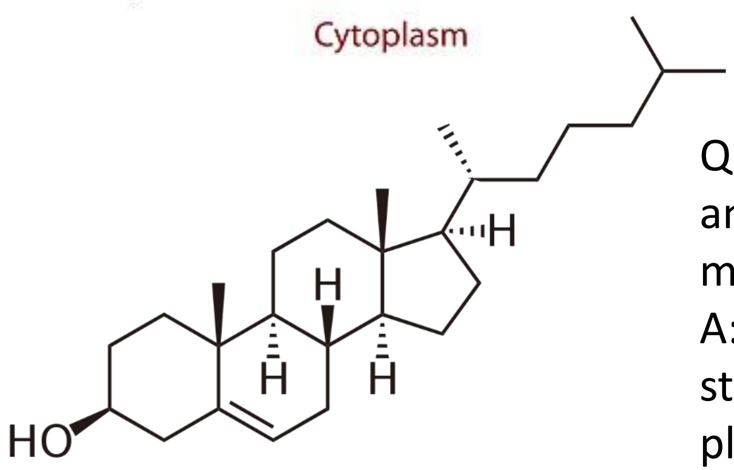
Mixed saturated and unsaturated



Monounsaturated

Other membrane components can also influence fluidity. *Sterols* for example, decrease membrane fluidity by encouraging tighter packing of phospholipids

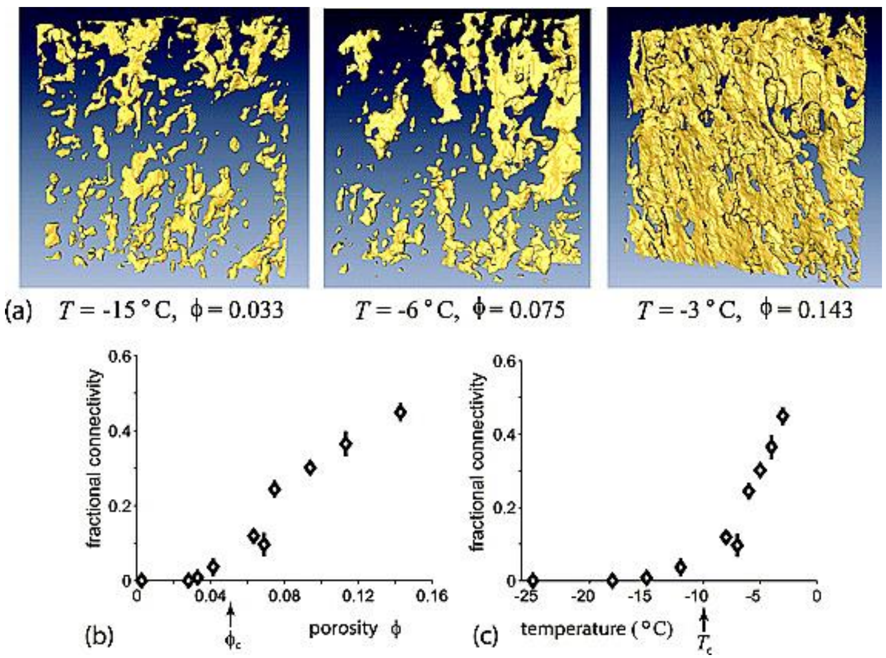




Q: Can you identify this molecule and guess its influence on the lipid membrane?

A: Cholesterol, sterols have a stabilizing effect (think arterial plaque).

Remember that sea ice is a *porous matrix*, and sea ice organisms are partitioned into the *brine phase* of this matrix.



X-ray tomography of laboratory sea ice at different temperatures, from Golden et al., 2007. Gold = brine.

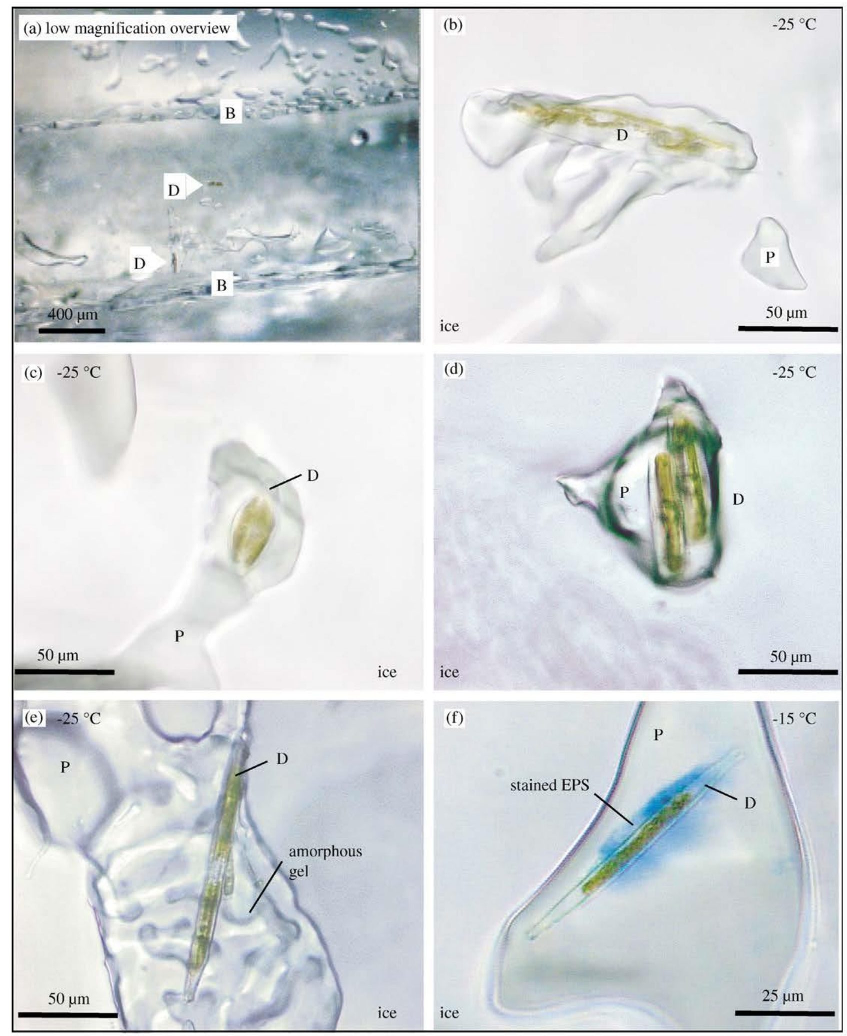
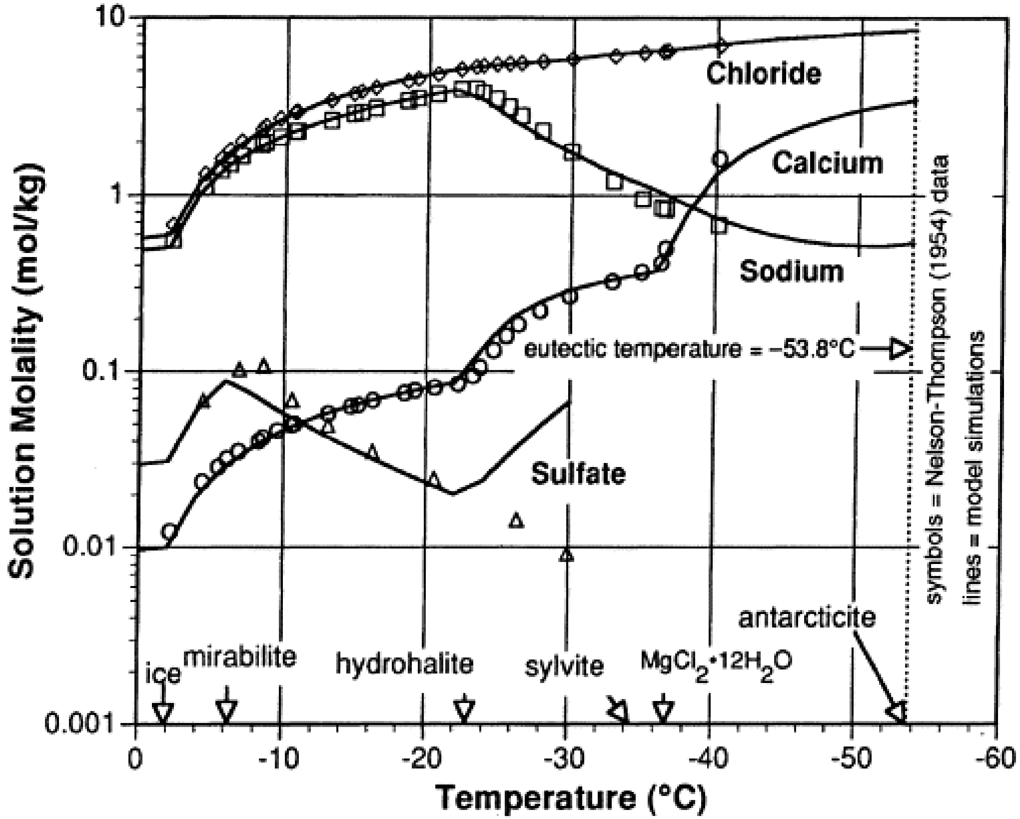


Fig. 5. Microphotographs of pennate diatoms residing within pore spaces at a depth of 112 cm in an ice core collected in March of 1999 after cooling from in situ temperature to −25°C: (a) low-magnification image showing ice texture, brine layers (B) and diatoms (D); (b) damaged diatom cell in a pore with encroaching ice crystals and an empty pore space (P); (c) diatom in a pore connected to a brine layer; d) two diatoms in an isolated pore; (e) diatom in a pore with indications of amorphous transparent gel-like exopolymeric material; and (f) diatom surrounded by an EPS matrix successfully stained with Alcian blue (at −2°C prior to chilling to −15°C).

This brine presents two challenges for life:

- Reduced water activity
- Heightened ion concentration



Mirabilite = $Na_2SO_4 \bullet 10H_2O$
Hydrohalite = $NaCl \bullet 2H_2O$
Sylvite = KCl
Antarcticite = $CaCl_2 \bullet 6H_2O$
1 mol Na kg^{-1} = 23 ppt

Marion et al., 1999

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- Reduced water activity
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Water activity: The vapor pressure of water in a substance, divided by the vapor pressure of pure water at that temperature. Think of it as the ability of water to move across a membrane. The maximum value is 1, which would be pure H_2O. Any solute will help water molecules resist evaporation (or transport across a membrane), and thus lowers water activity.

$$A_w = \frac{V_s}{V_{H_2O}}$$

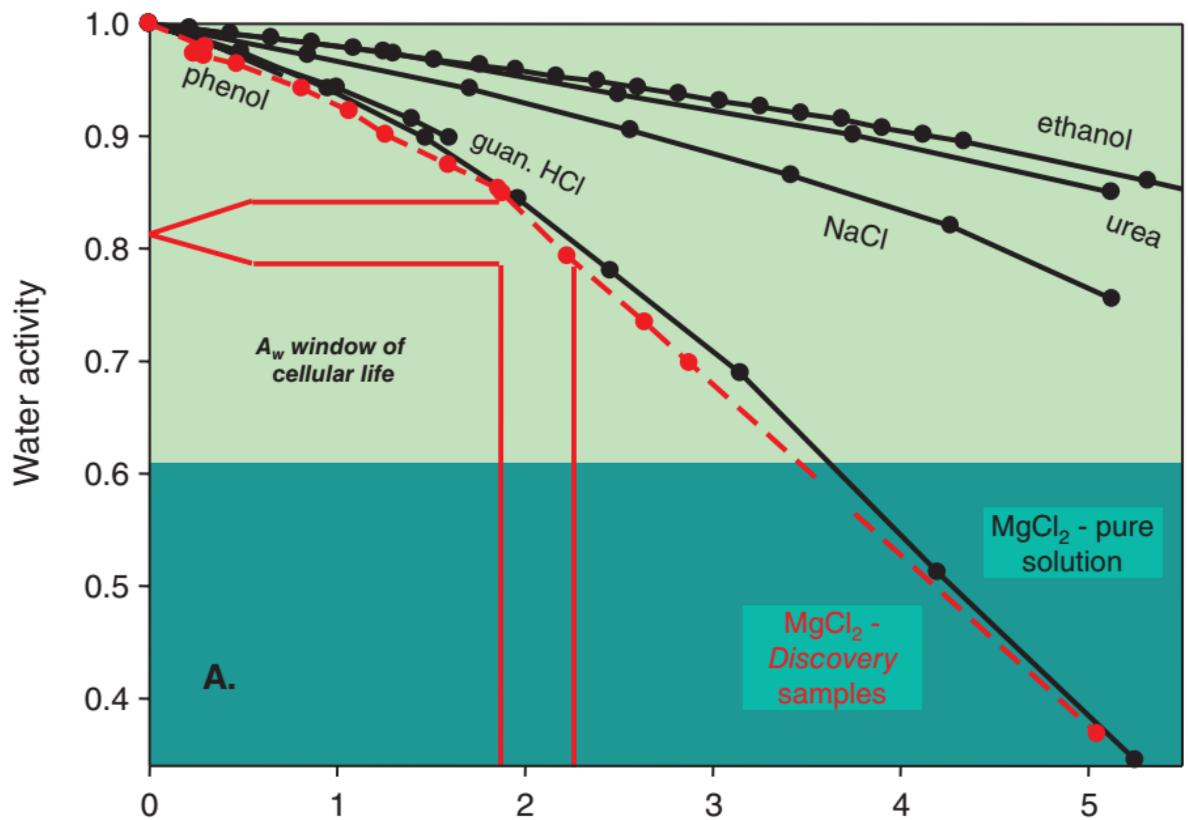
Species	Lowest water activity (a_w) for cell division	Environmental source	Refs
Bacteria and archaea			
Haloarchaea GN-2	0.635	Solar salterns, Mexico	3
Haloarchaea GN-5	0.635	Solar salterns, Mexico	3
Halorhabdus utahensis DSM 12940	0.647	Salt Lake, USA	3
Halobacterium strain 004.1	0.658	Brine pool, UK	3
Halorhodospira halophila DSM 244	0.66	Salt lake, USA	3
Salinibacter ruber DSM13855	0.725	Solar salterns, Spain	3
Salisaeta longa DSM 21114	0.747	Dead Sea, Israel	3
Fungi			
Aspergillus penicillioides	0.585	Raisins, Australia	7,106
Xeromyces bisporus	0.637	Antique wood, Thailand	3,6,7,107
Aspergillus amstelodami FRR2792	0.656	Dates, Australia	7
Xerochrysium xerophilum FRR 0530 (formerly known as *Chrysosporium xerophilum*)	0.686	High-moisture prunes, Australia	7
Aspergillus chevalieri PIL 119	0.71	Soiled prunes, Australia	7

Libre et al., 2017

Q: Dry room air is about 0.5 and is full of bacteria. So how does that work?
A: Microorganisms can buffer themselves from their environment, and can make use of local moisture sources.

This brine presents two challenges for life:

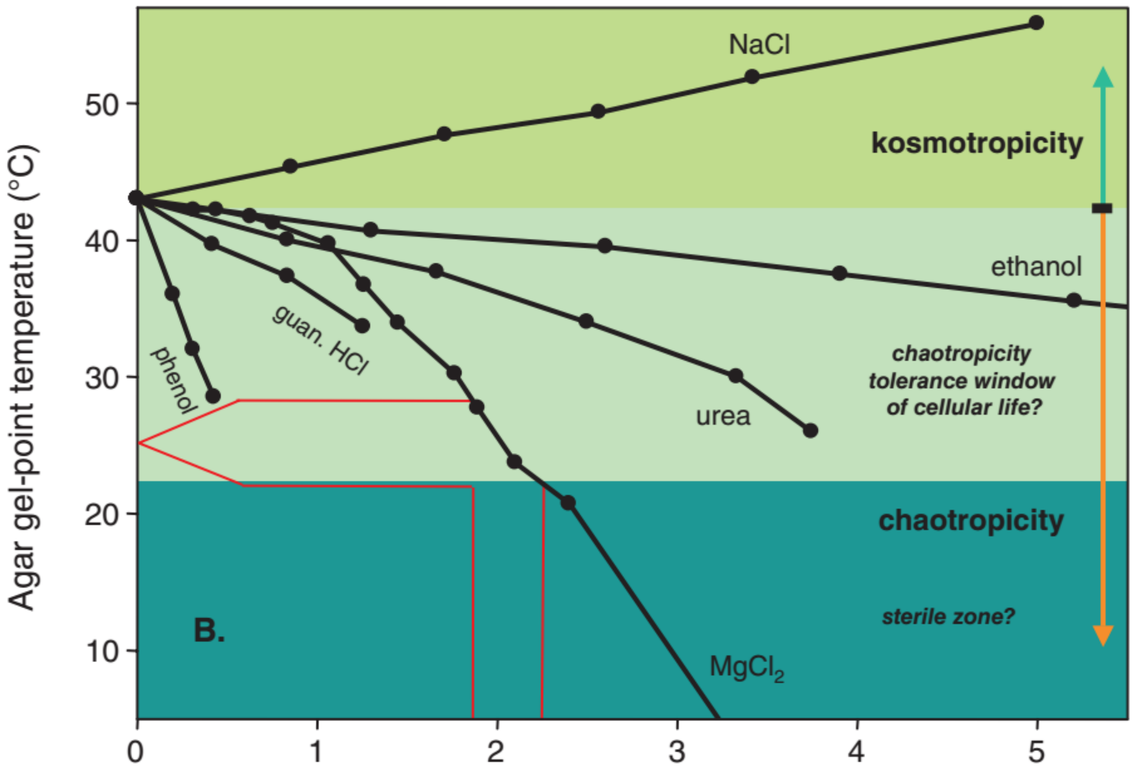
- Reduced water activity
    - Water activity differs for different solutes at the same concentration
    - 16 % NaCl = 0.9, 16.7 % sucrose = 0.998



Hallsworth et al., 2007

This brine presents two challenges for life:

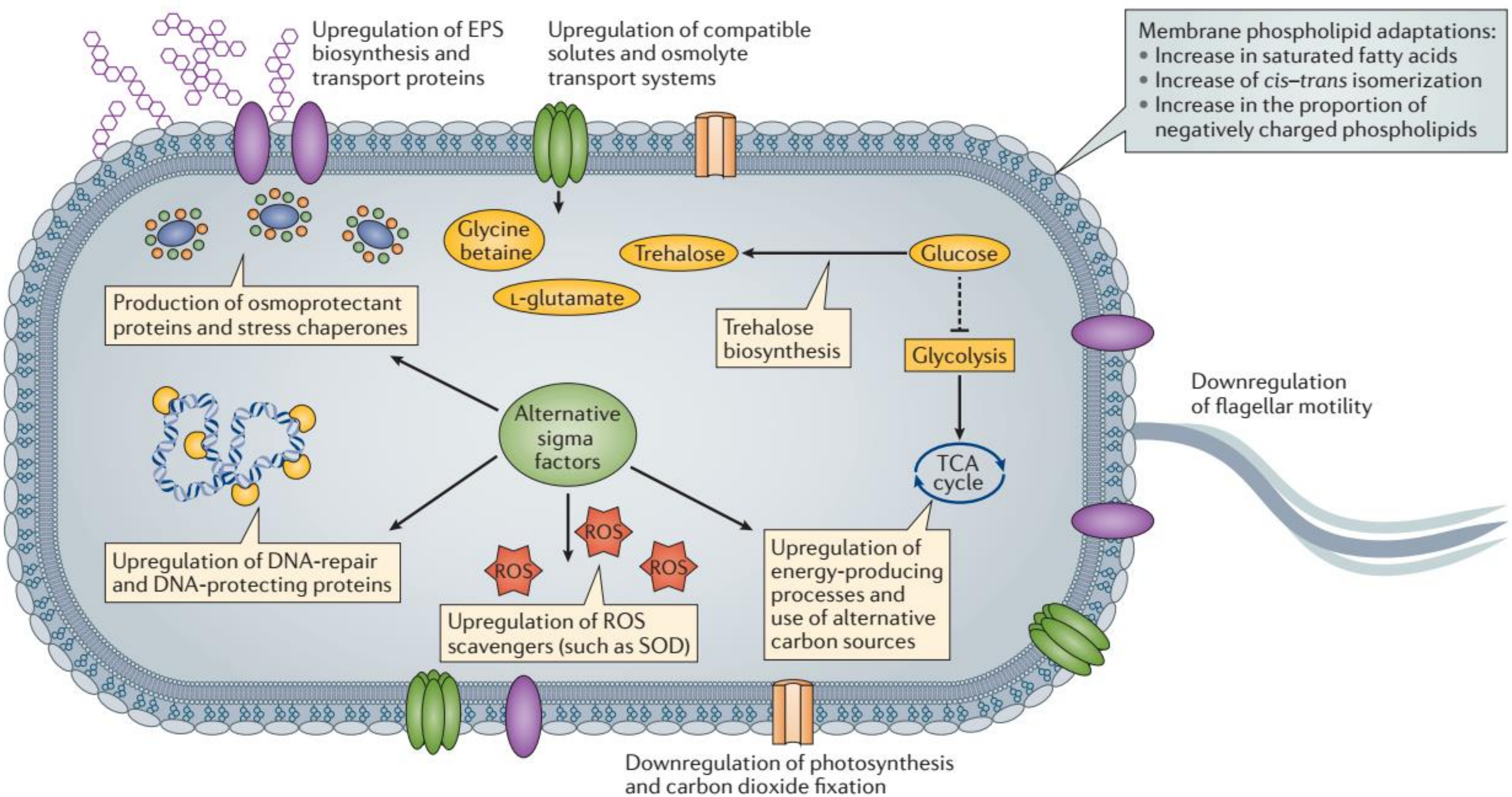
- Reduced water activity
  - Water activity differs for different solutes at the same concentration
  - 16 % NaCl = 0.9, 16.7 % sucrose = 0.998
- Heightened ion concentration
  - Not all ions interfere with cellular function to the same extent
  - *Chaotropic* (destabilizing) and *kosmotropic* (stabilizing) salts are one example of this



Hallsworth et al., 2007

Three general strategies for dealing with high salt concentrations:

- Buffer cell from environment
- Accumulate non-harmful salts (e.g., K: the “salt-in approach”)
    - Require specially adapted internal proteins
- Accumulate compatible organic solutes (e.g., trehalose: the “salt-out approach”)
    - Does not require specially adapted internal proteins, but does require modifications to membrane and membrane proteins

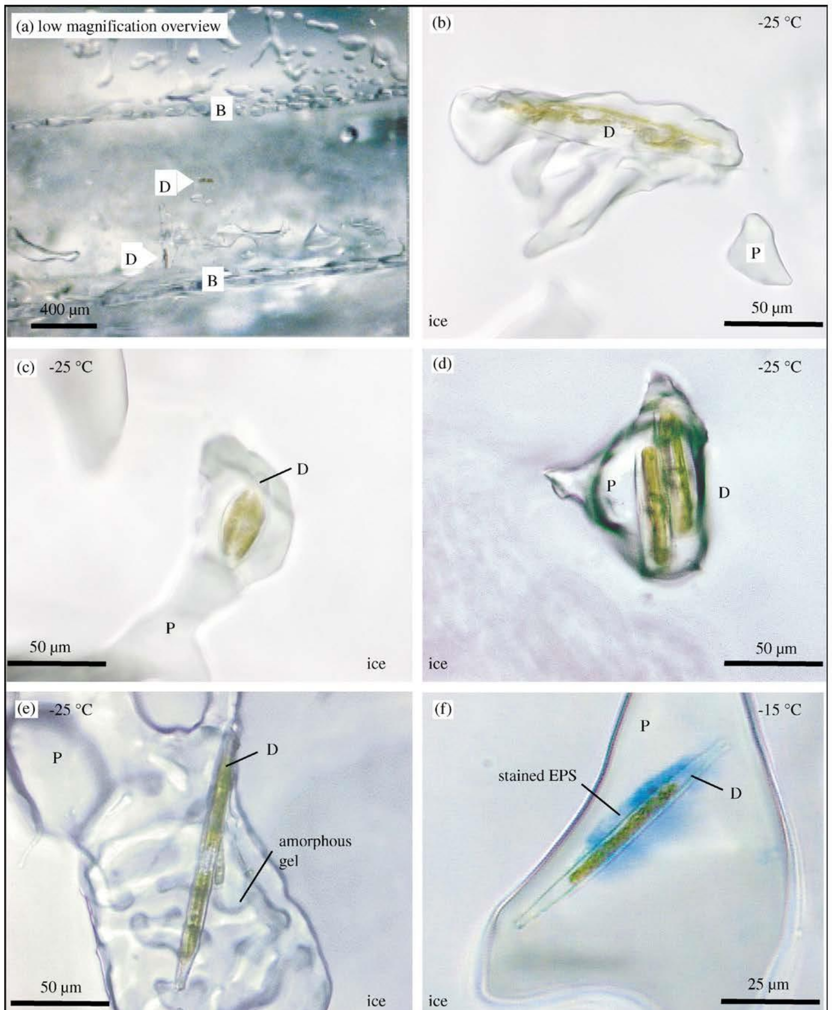


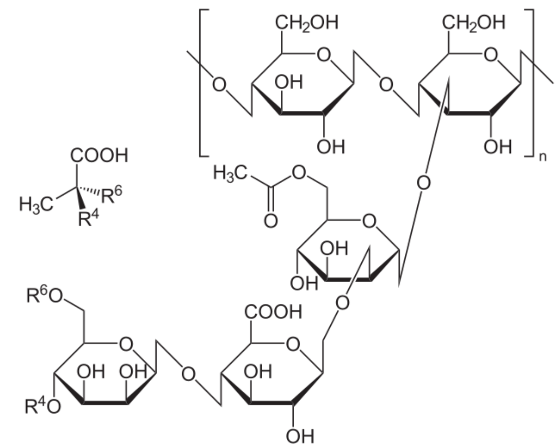
Libre et al., 2017

Cells buffer themselves from the environment by producing exopolymers (EPS)

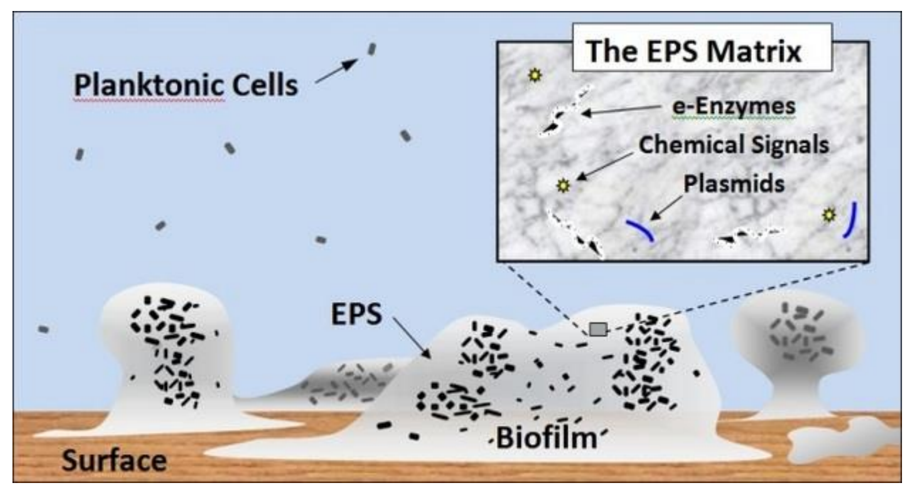
- EPS are hydrated gels composed of polysaccharides, proteins, and other material
- EPS are ubiquitous in nature and plays a major role in disease, ecology, and industry

2172 C. Krembs et al. / Deep-Sea Research I 49 (2002) 2163 2181





Xantham gum



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- EPS are hydrated gels composed of polysaccharides, proteins, and other material
- EPS are ubiquitous in nature and plays a major role in disease, ecology, and industry

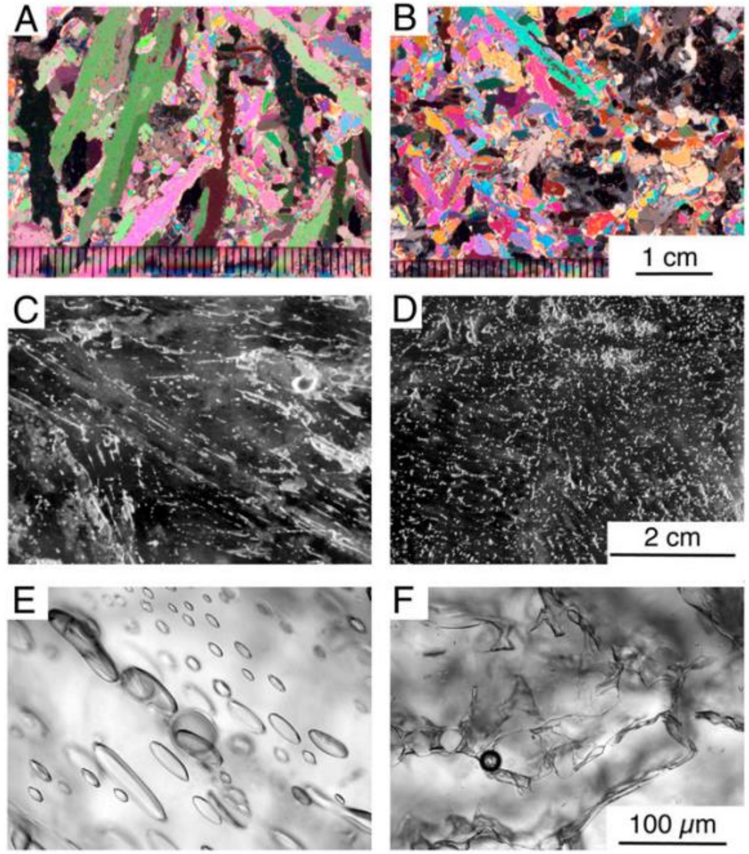


Fig. 2. Photomicrographs at −10 °C of artificial sea ice, with and without EPS. Images show ice texture in horizontal sections (5-cm depth) at low magnification under polarized light (*A* and *B*) and with contrast staining (*C* and *D*), and pore structure at high magnification (*E* and *F*). The ice was grown from saline solutions with no added EPS (*A*, *C*, and *E*) or with *Melosira* EPS (*B*, *D*, and *F*).

Krembs et al., 2011

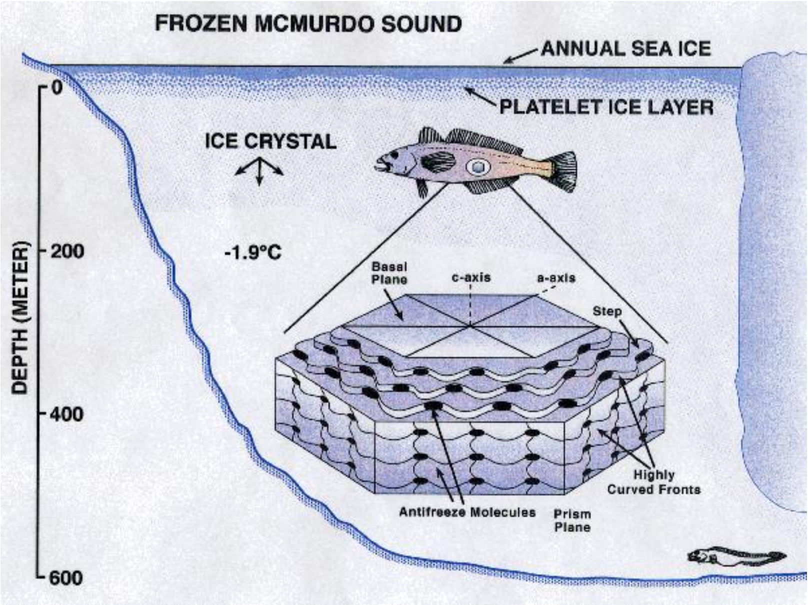
Ice crystal formation also presents challenges for life

- If cytosol freezes the cell will lyse due to expansion
- The cell surface can be impinged by growing ice crystals
- Some organisms have evolved the ability to control the formation of ice
  - Depress the intracellular or extracellular freezing point
  - Nucleate ice crystals to control the formation process

Production of antifreeze proteins and compounds

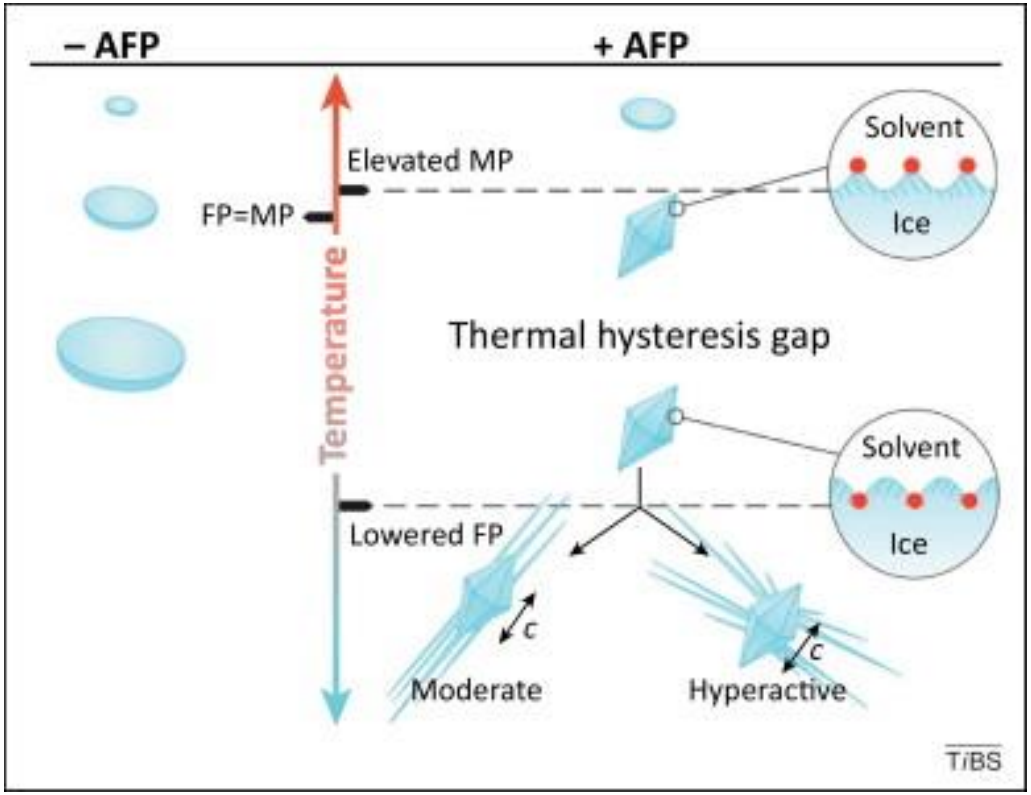
- Ice binding proteins such as those produced by icefishes of the family Channichthyidae bind to newly formed ice crystals to halt their growth

Q: Icefish are the only vertebrates that lack the protein hemoglobin. Why?
A: Oxygen is very soluble in cold water, reducing the selective pressure to maintain an oxygen-binding protein.



Production of antifreeze proteins and compounds

- Ice binding proteins such as those produced by icefishes of the family Channichthyidae bind to newly formed ice crystals to halt their growth
- Ice binding proteins create a *thermal hysteresis gap* by elevating the melting point and depressing the freezing point
- This allows them to serve multiple (contrasting) ecological functions.



Davies et al., 2014

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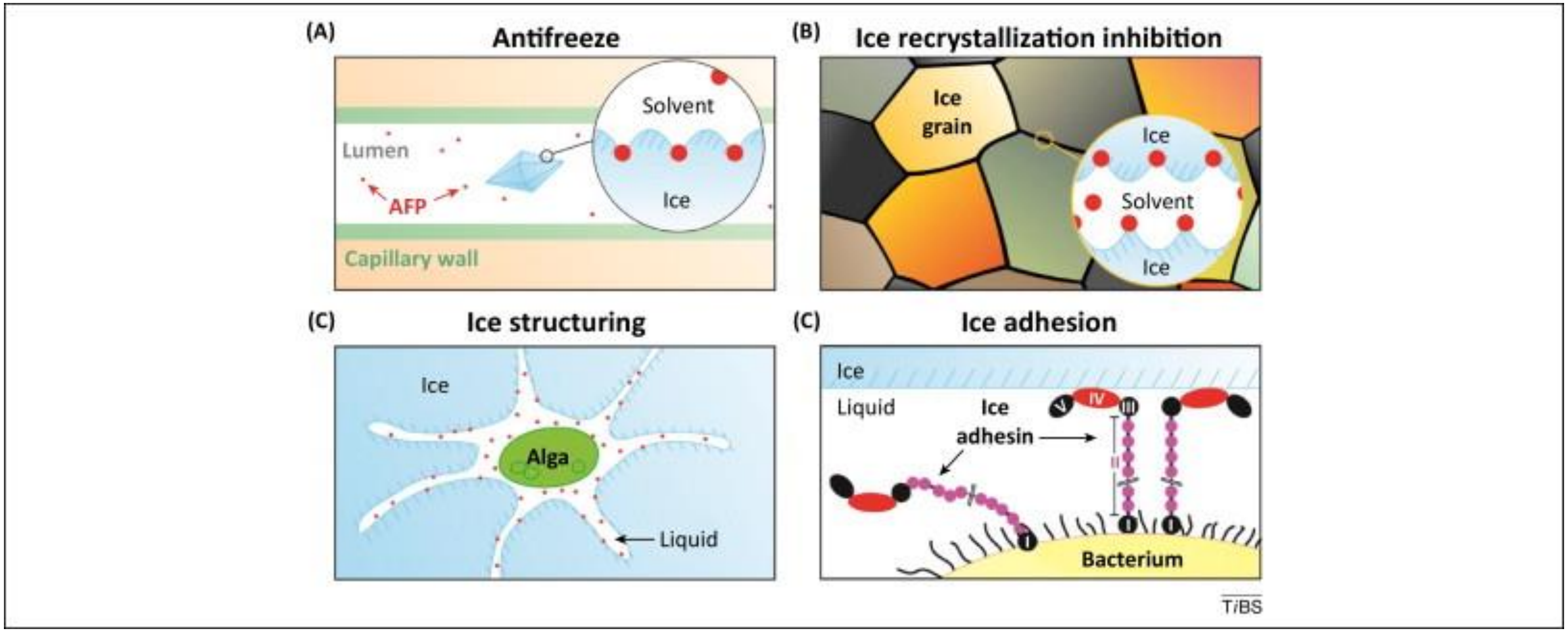

Davies et al., 2014

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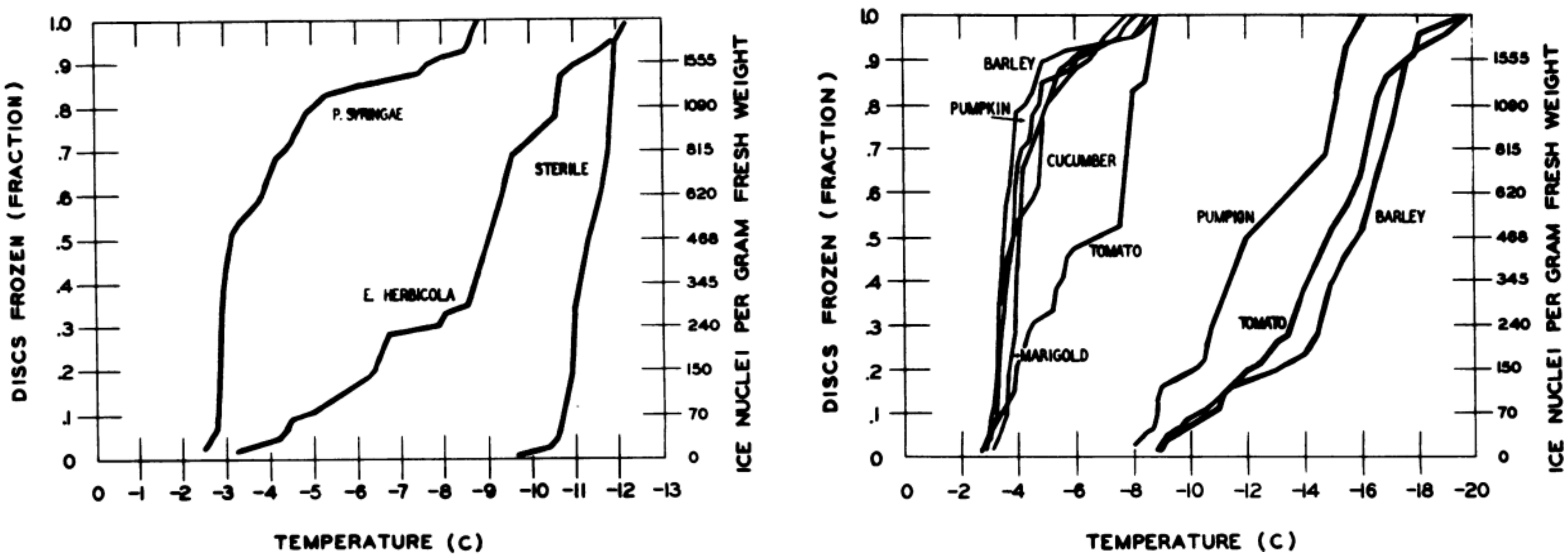

Lindow et al., 1982

Water does not spontaneously freeze until -40 C. Freezing above this point is caused by *nucleation*.

- Some common, temperate, plant-associated bacteria have ice binding proteins
- This may be an effective mechanism for them to attack plant cells

Production of antifreeze proteins and compounds

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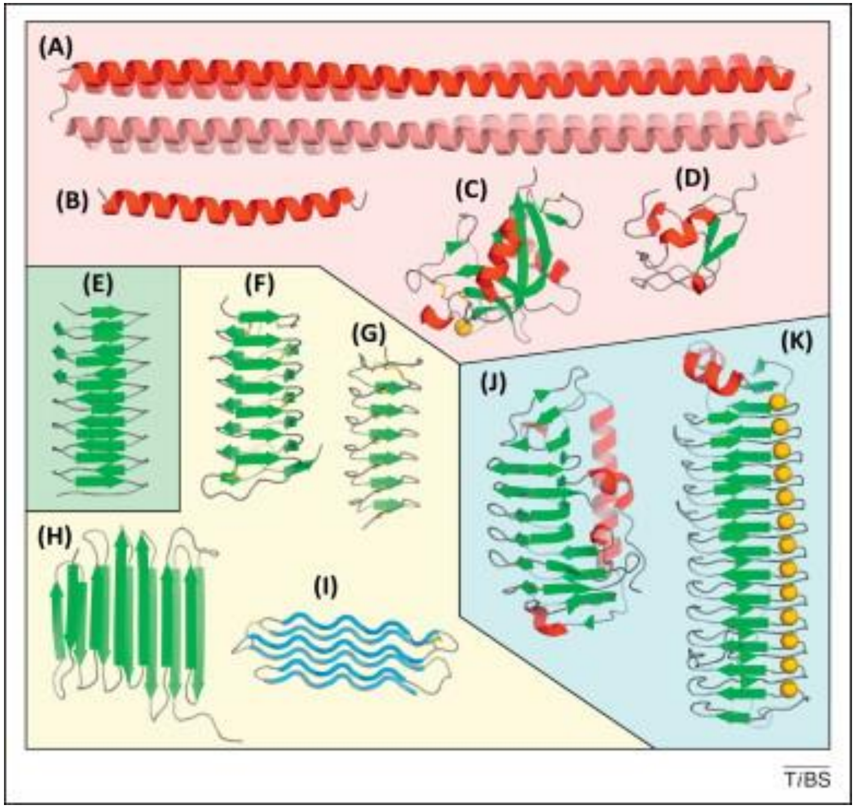
- This allows them to serve multiple (contrasting) ecological functions.
- Structural diversity suggests recent evolution in response to freezing conditions.



Davies et al., 2014