The Effects of Temperature on Life

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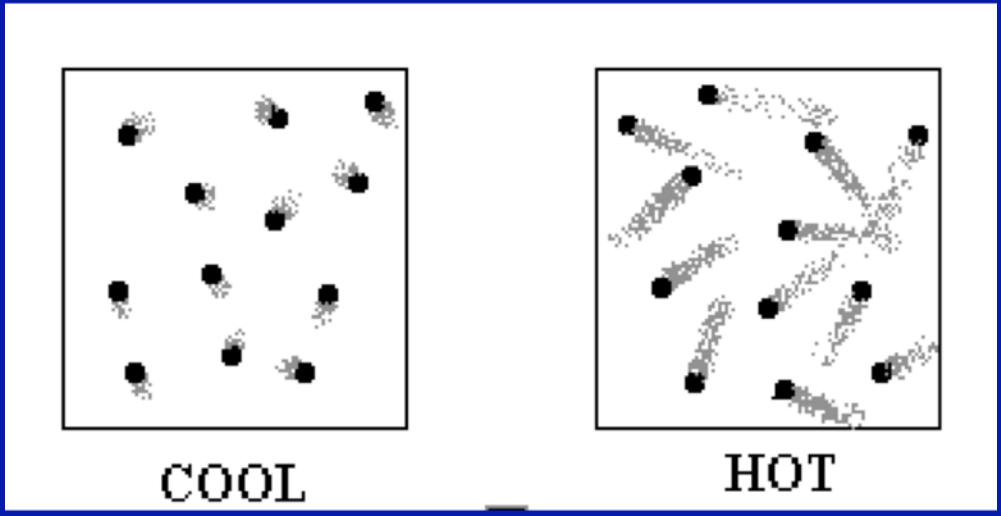
Overview

- Temperature—Limitations
- Limits of Life—Thermophiles and Psychrophiles
- Challenges and Solutions
  - Nucleic Acids
  - Proteins
  - Cell Membranes
  - Miscellaneous
- Summary

What is temperature?

- A measure of the average translational kinetic energy associated with the disordered microscopic motion of atoms and molecules.

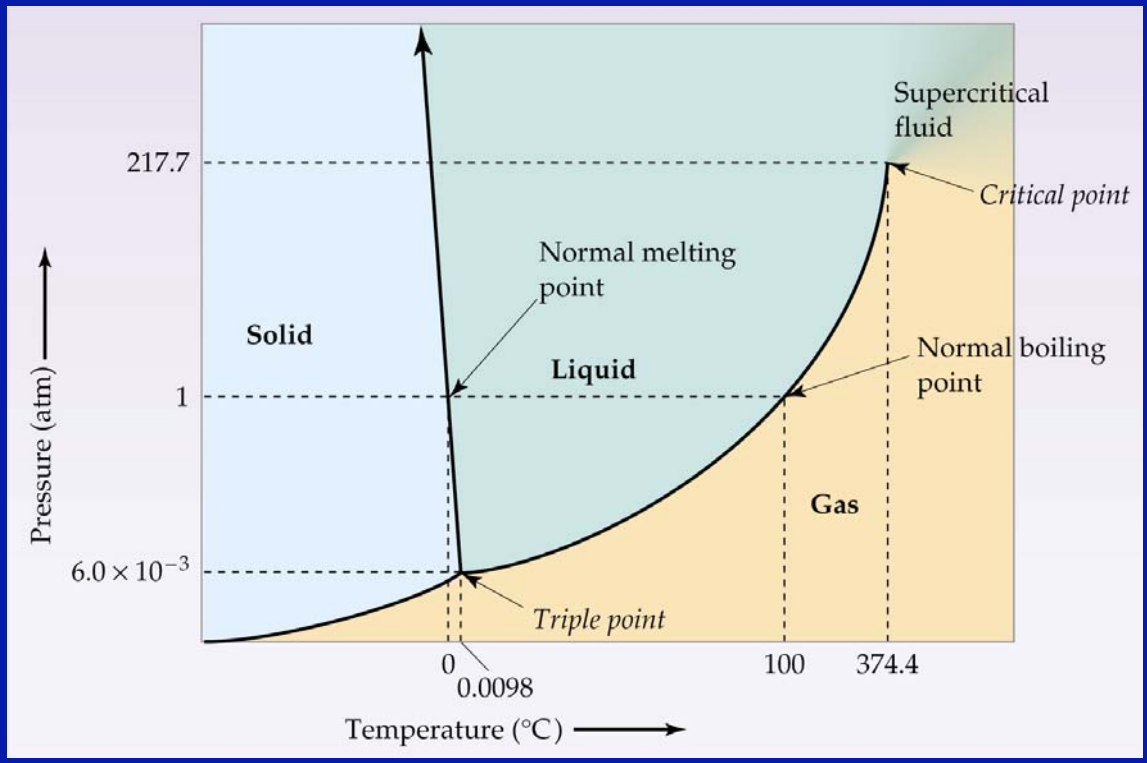


Physical and Chemical Constraints

- High Temperatures: Fast, Fleeting, Flexible
  - Reaction & diffusion rates increase
  - Chemical degradation increases
  - Flexibility/fluidity increase
  - Liquid H_2O → Gas
- Low Temperatures: Slow, Stable, Solid
  - Reaction & diffusion rates decrease
  - Flexibility/fluidity of molecules decrease
  - Liquid H_2O → Solid

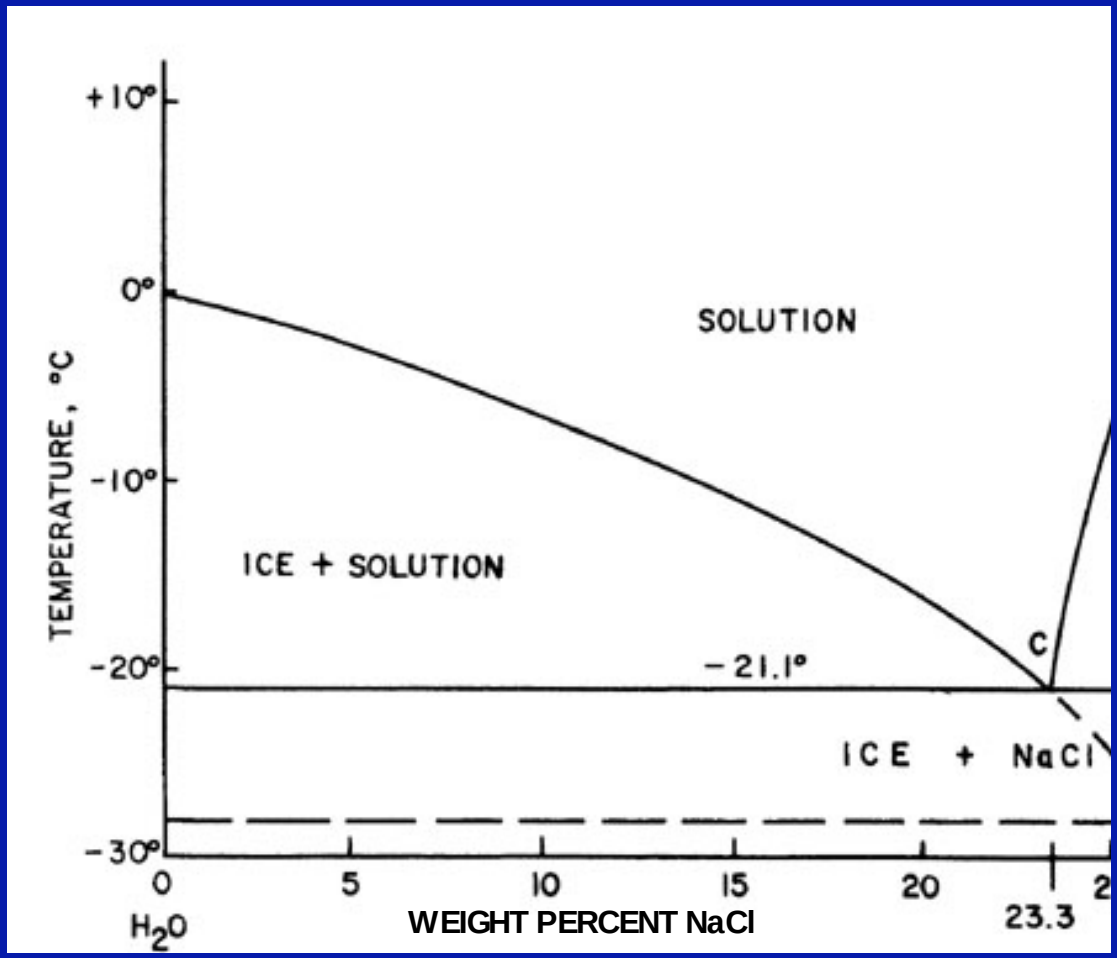
Limits of Liquid H_2O

- Increase boiling point:
  - Increase pressure!
- Decrease freezing point:
  - Add solutes!

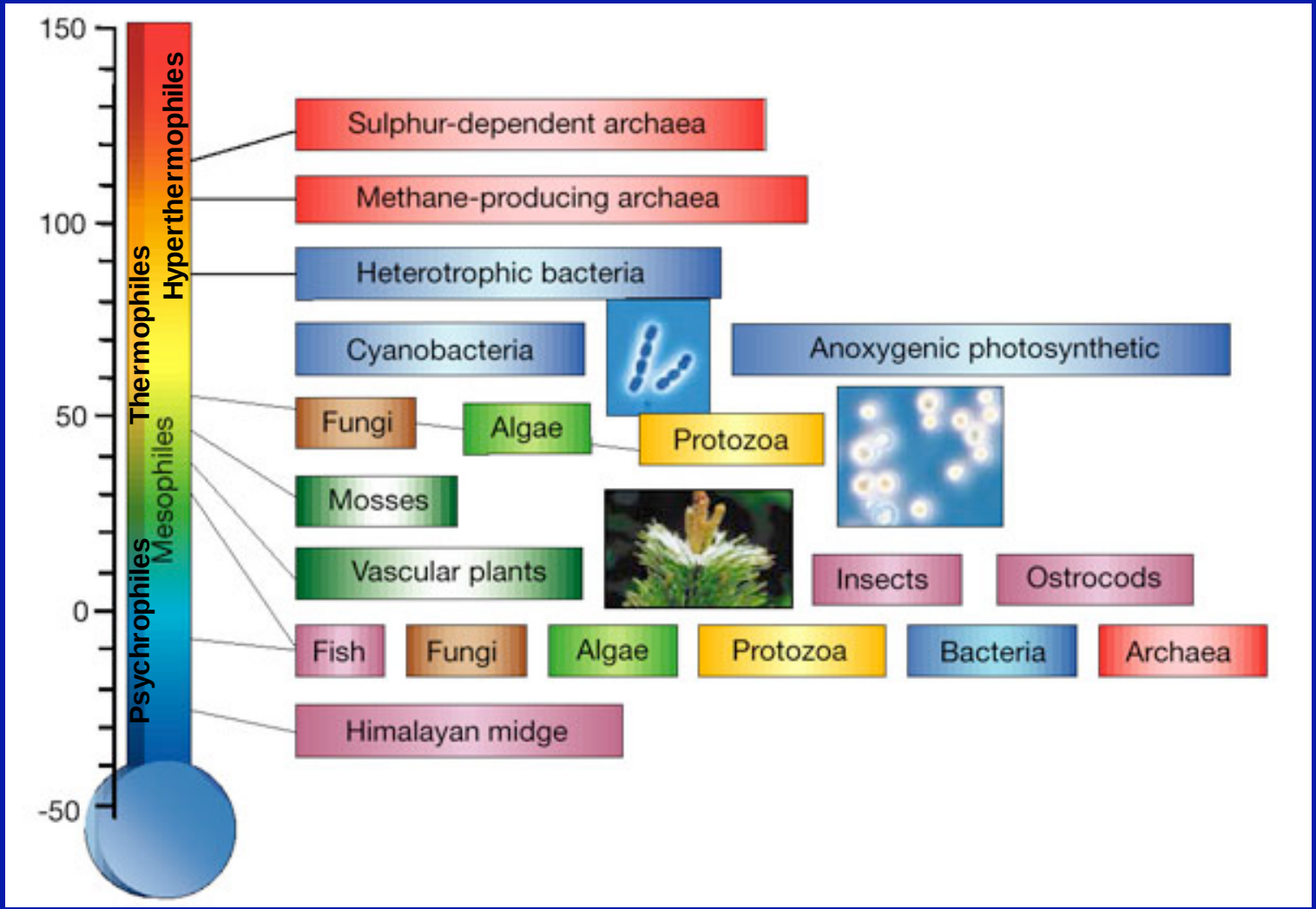


Freezing Point Depression

- Seawater, 3% salinity: T_m = -1.7°C
- Saturated NaCl brine: T_m = - 21°C
- Other Brines
  - Don Juan Pond
    - $CaCl_2$ and NaCl brine
    - T_m = -52°C
  - Mineral acids
    - T_m = -90 °C
- As water solidifies, solutes are excluded from the crystal structure



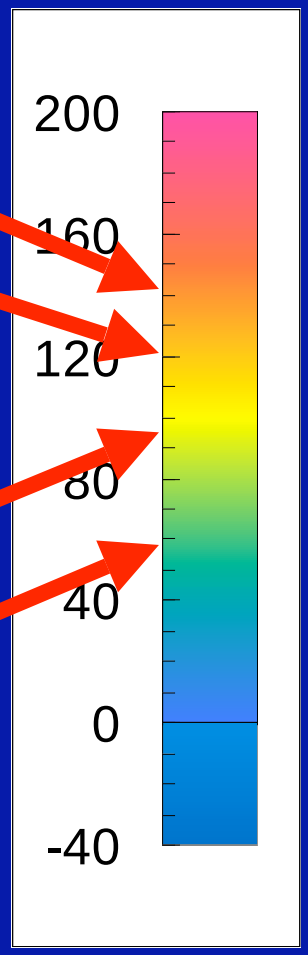
Known T Limits of Life



Rothschild and Mancinelli (2001). "Life in extreme environments." Nature **409**: 1092-1101.

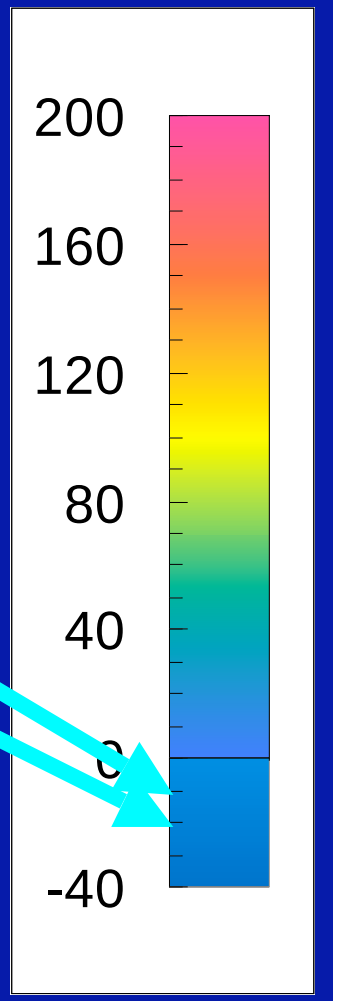
High T Limits of Life

- Enzymes active to 142°C
- Reproduction to 121°C
  - strain 121, family *Pyrodictiaceae* (Kashefi and Lovley, 2003)
- Restricted to prokaryotic life!
  - Bacteria max out at ~95°C
  - Eucaryotes max out at ~60°C

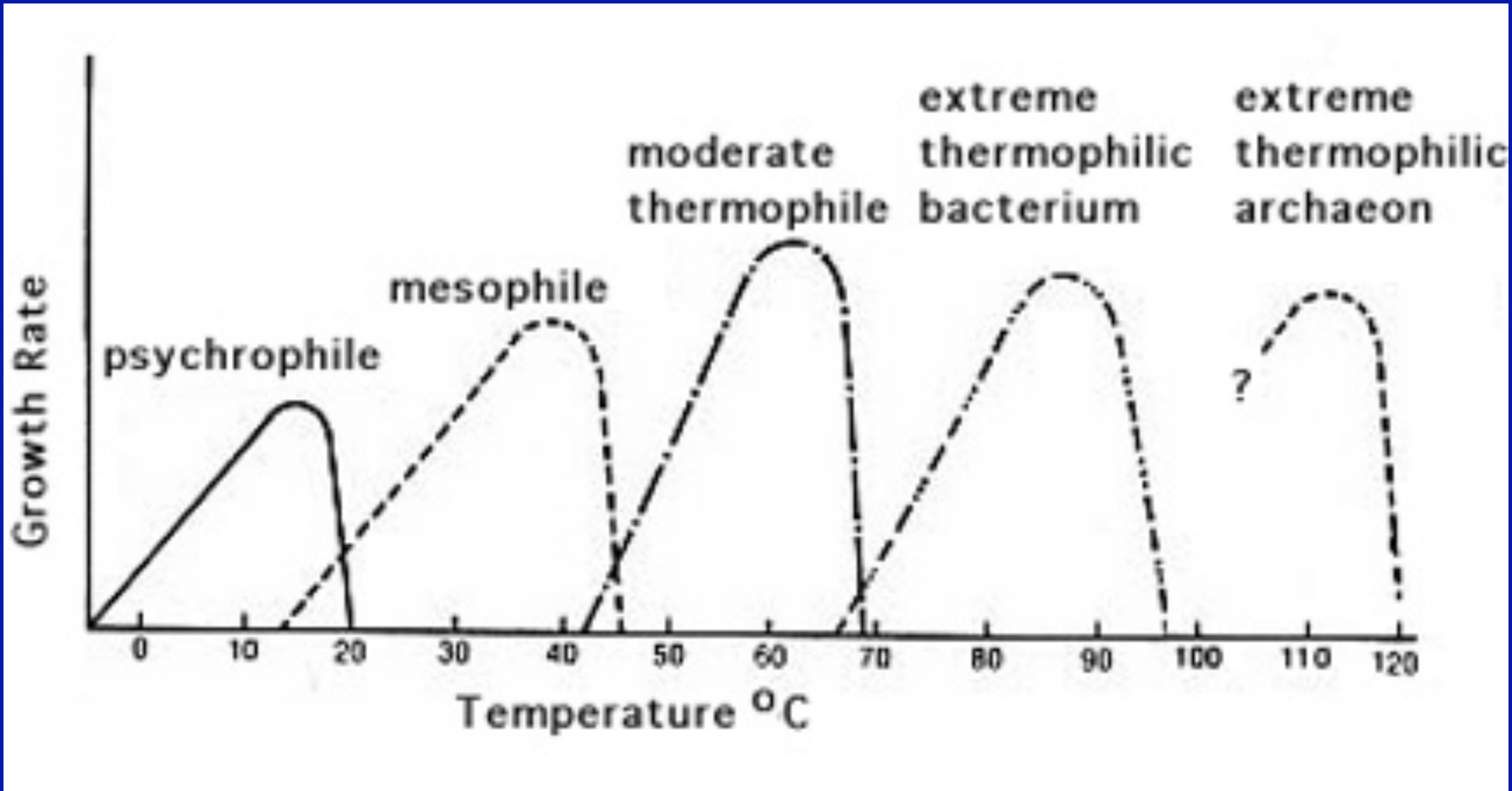


Low T Limits of Life

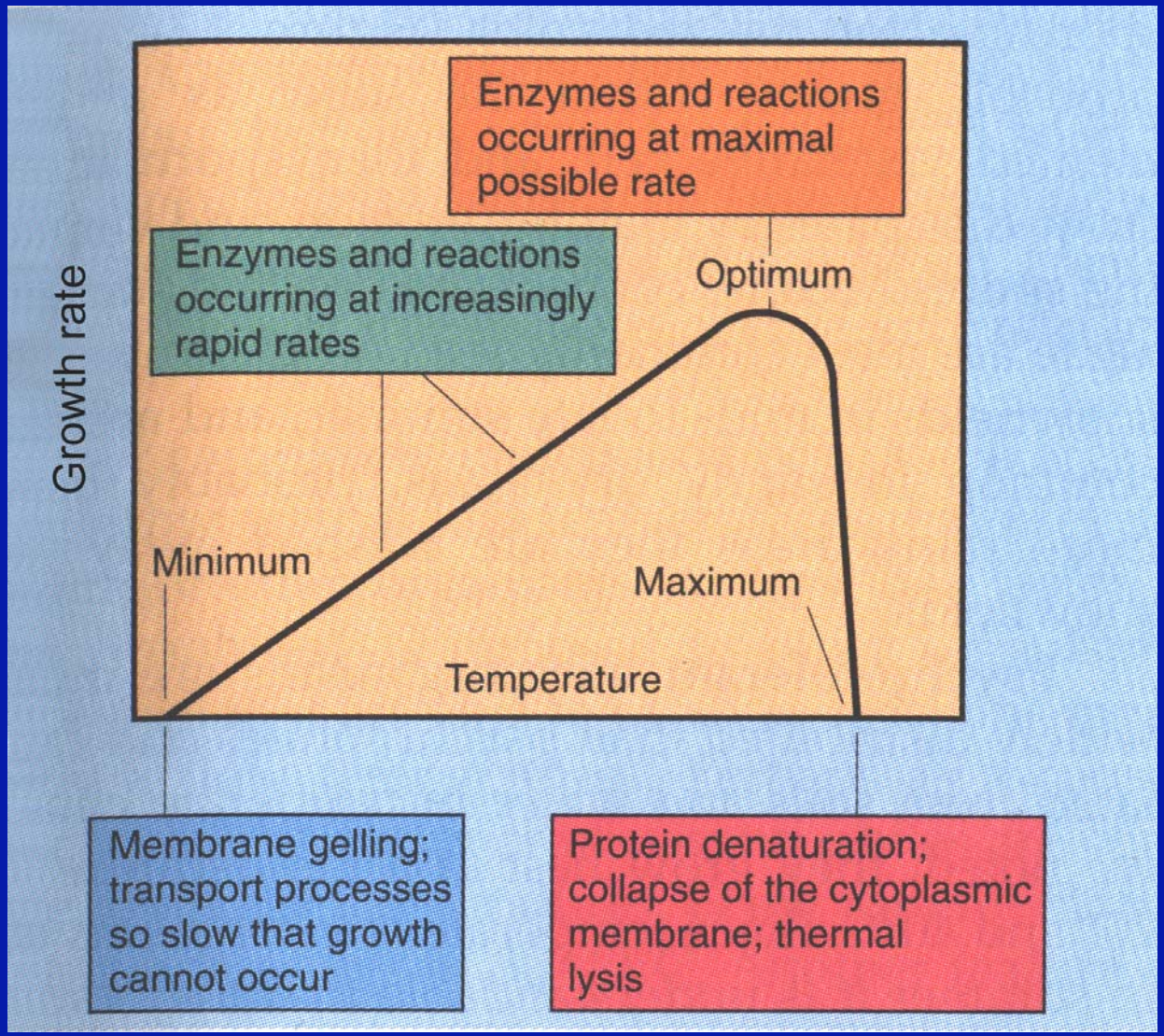
- Reproduction to -12°C
  - Eucaryotes -1.8C (freezing point of seawater)
  - Procaryotes
    - -8°C Permafrost, Frozen Food isolates (Gilichinsky, 1995)
    - -10°C "*Psychrobacter cryohalolentis K5*" (Bakermans, 2003)
    - -12°C "*Psychromonas ingrahamii*" (Breezee, et al. 2004)
- Metabolic activity to -20°C
  - Eucaryotes
    - Himilayan Midge to -18°C (Kohshima, 1984)
    - Antarctic lichens and endoliths (Friedmann, 1993)
  - Bacteria/Archaea
    - Permafrost and South Pole snow (Rivkina, 2000; Carpenter, 2000)
    - Sea Ice (Junge, 2004)
- Can preserve cells at -196°C (liquid N_2)



Thermal Classes of Organisms



Temperature Effects on Growth

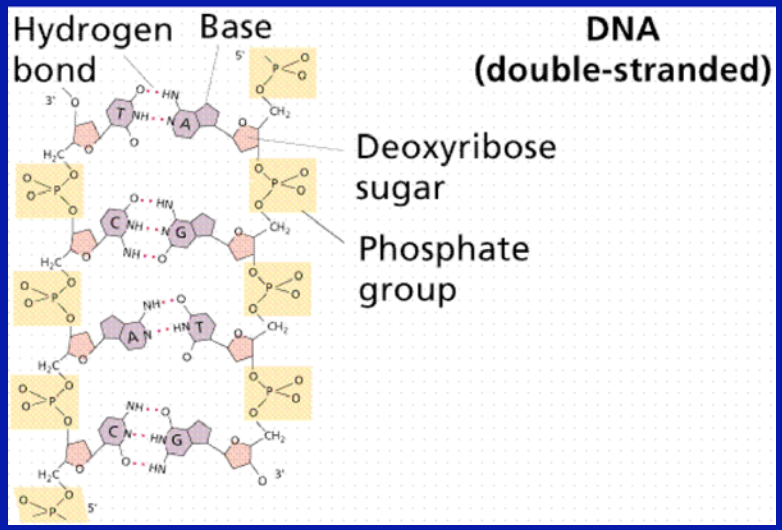


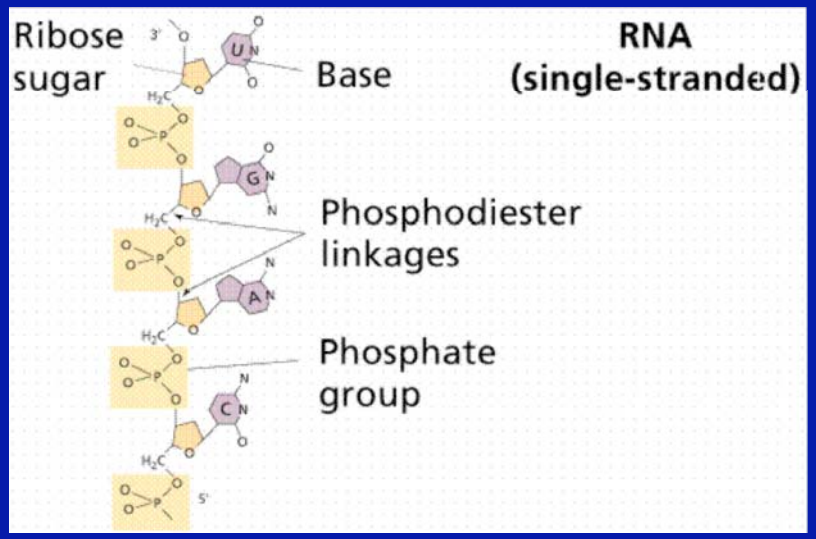
Brock et al., 1994

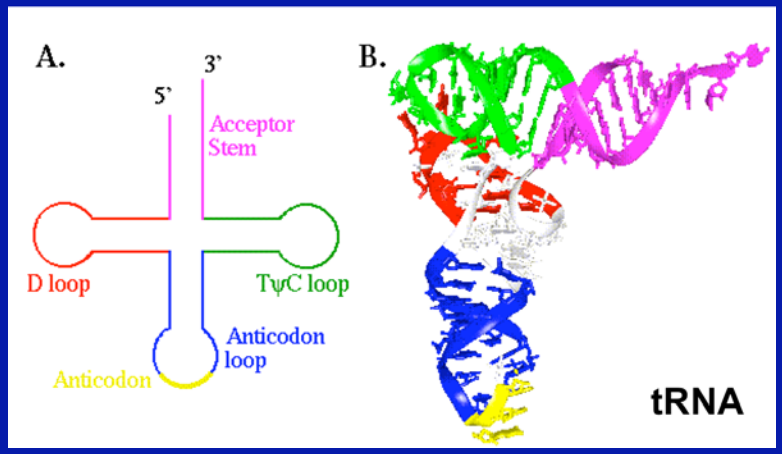
	High T	Low T
Nucleic Acids	Denaturation by strand separation Degradation of bases (loss & deamination)	Unfavorable secondary structures Difficult to unwind and access
Proteins	Denature and aggregate→ metabolism collapses Deamidation of Asn & Gln Glu & Asp form succinimide His, Met, Cyst, Trp, & Tyr oxidized	Cold denature (driven by hydration of polar and non-polar residues) Slowed reaction rates
Lipids	Too fluid	Too viscous, crystallize Ruptured by ice crystals
OTHER	Metabolites degrade	Energy costs high

Nucleic Acids

- DNA
- RNA
  - Ribosomal
  - Messenger
  - Transfer







Nucleic Acids at High Temperatures

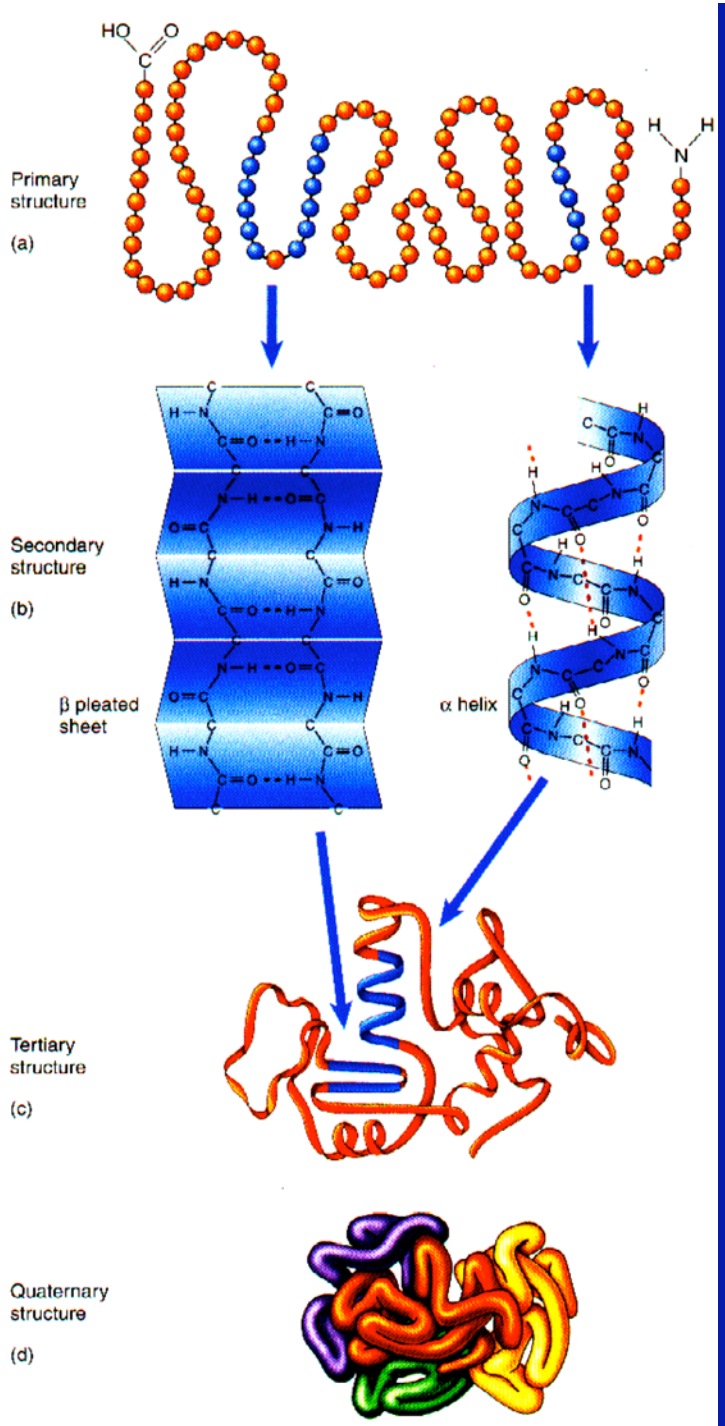
- Problems:
  - Strand separation
  - Degradation of bases (loss and deamination)
- Solutions:
  - Increase concentration of salts or polycationic polyamines (also help stabilize proteins)
  - Introduce supercoils
  - Cationic DNA-binding proteins
  - Post-translational modification of tRNA
  - DNA repair systems

Nucleic Acids at Low Temperatures

- Problems:
  - Unfavorable secondary structures
  - Difficult to unwind and access
- Solutions:
  - DNA binding proteins
  - RNA helicases to unwind secondary structure
  - RNA chaparones
  - Post-transcriptional modification of tRNA (increase dihydrouridine)

Proteins

- GOAL: balance molecular stability and structural flexibility in order to maintain activity



Protein Modifications

	High T (thermophiles)	Low T (psychrophiles)
Uncharged polar residues	↓	↑ (on surface)
Charged residues	↑	↓ (neg. charges ↑ on surface)
Residue hydrophobicity	↑	↓ (but…)
Other	β sheets more likely ↓ residue volume	↓ ion pairs & H bonds ↑ gly and ↓ pro
Genome Level	↓ size of proteins ↓ gln ↑ glu (H only)	↑ gln ↑thr ↓leu ??? ↑ hydrophobic res on surface ???

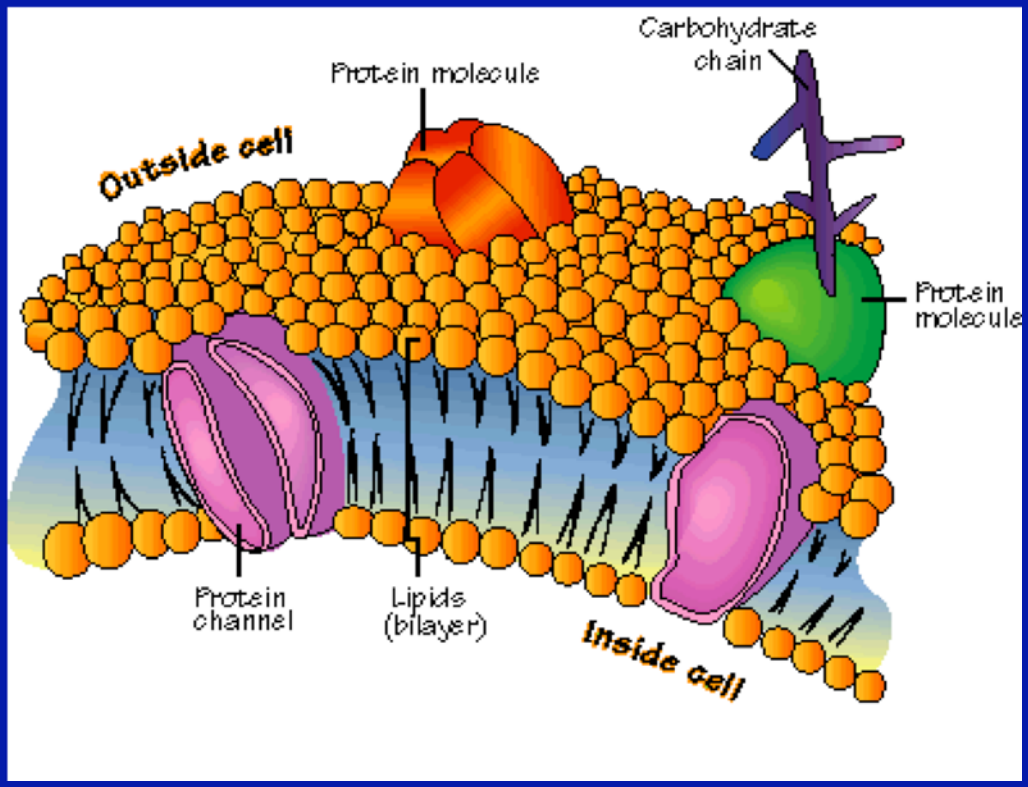
Fields, 2001; Haney, PNAS, 1999; Chakravarty, 2000; Tekaia, 2002; Feller 1997; Saunders, 2003

Protein Stabilization

- Compatible solutes (aka ‘extrinsic stabilizers’)
  - Advantages:
    - Can occupy broader thermal niches (simply vary concentration of solute)—good for transient changes in T
    - Affects all proteins in cell
  - Examples:
    - Increase with high temperature:
      - 2,3-phosphoglycerate (at high T in methanogens)
      - Myo-inositol phosphate derivatives, diglycerol phoshpate, α-mannosyl glycerate, inorganic ions, stress proteins
    - Increase with low temperature:
      - Stress proteins
      - Glycine betaine, glycerol,trehalose, sucrose, proline, dimethylsulonioпropionate (Welsh, 2000)

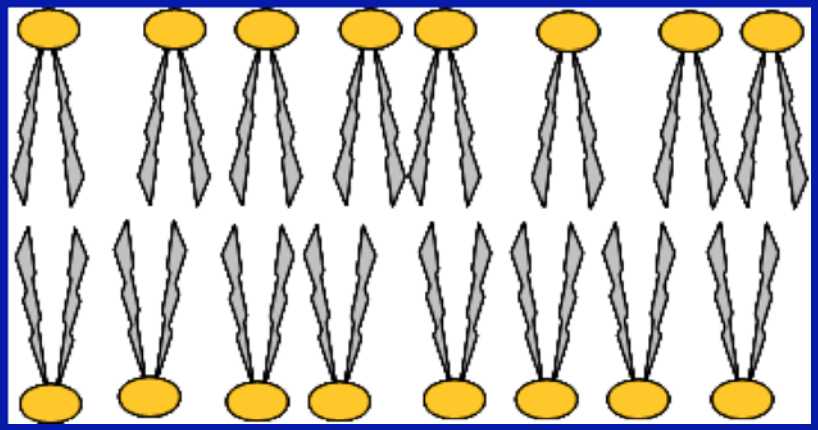
Cell Membranes

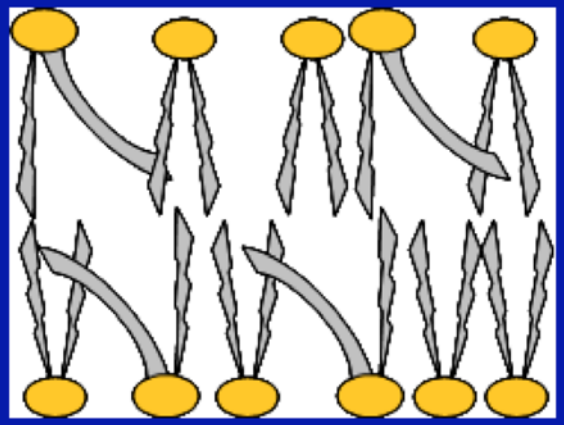
- Fluidity is important for metabolic function.
- Fluidity affected by:
  - Temperature
  - Composition



Saturated→Viscous

Unsaturated→Fluid

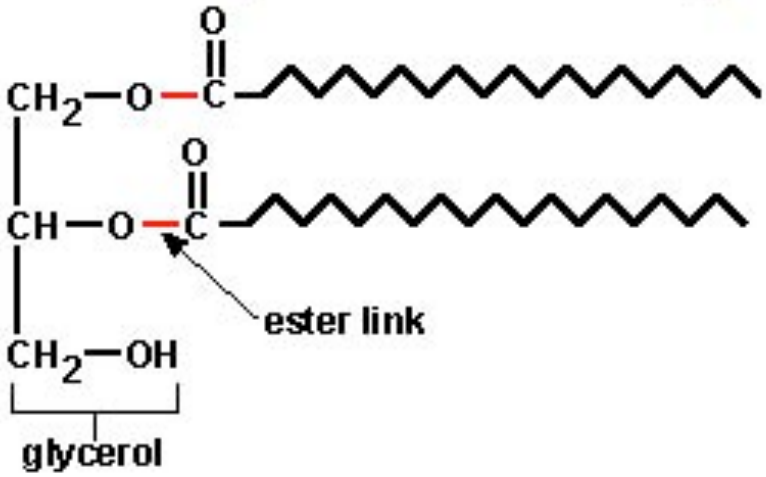




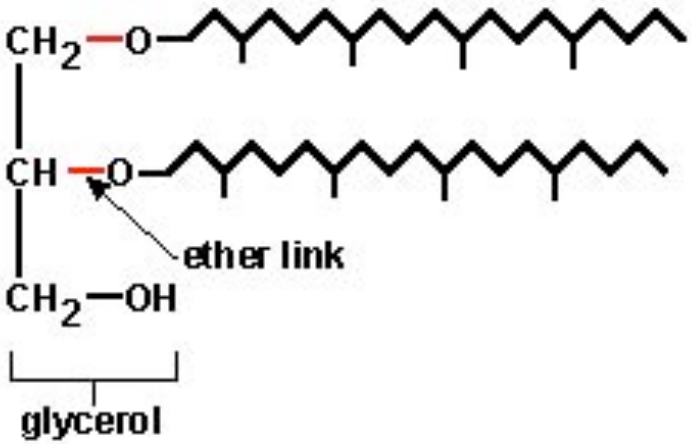
Maintaining Membrane Viscosity at High Temperatures (thermophiles)

- Archaea
  - Ether bonds (vs. ester bonds)
  - Enhance membrane packing to reduce fluidity:
    - Caldarchaeols & nonitolcaldarchaeols
    - Cyclic structures in transmembrane spanning fatty acids
- Bacteria
  - Mimic archaeal ether & diether linkages with:
    - Tetraesters
    - Fatty alcohols
    - Long-chain aliphatic diols
  - Increase acyl-chain length, saturation, branching and/or cyclization

Membrane Lipids of *Bacteria* and *Eukarya*



Membrane Lipids of *Archaea*



Membrane Lipids of Thermophilic *Archaea*

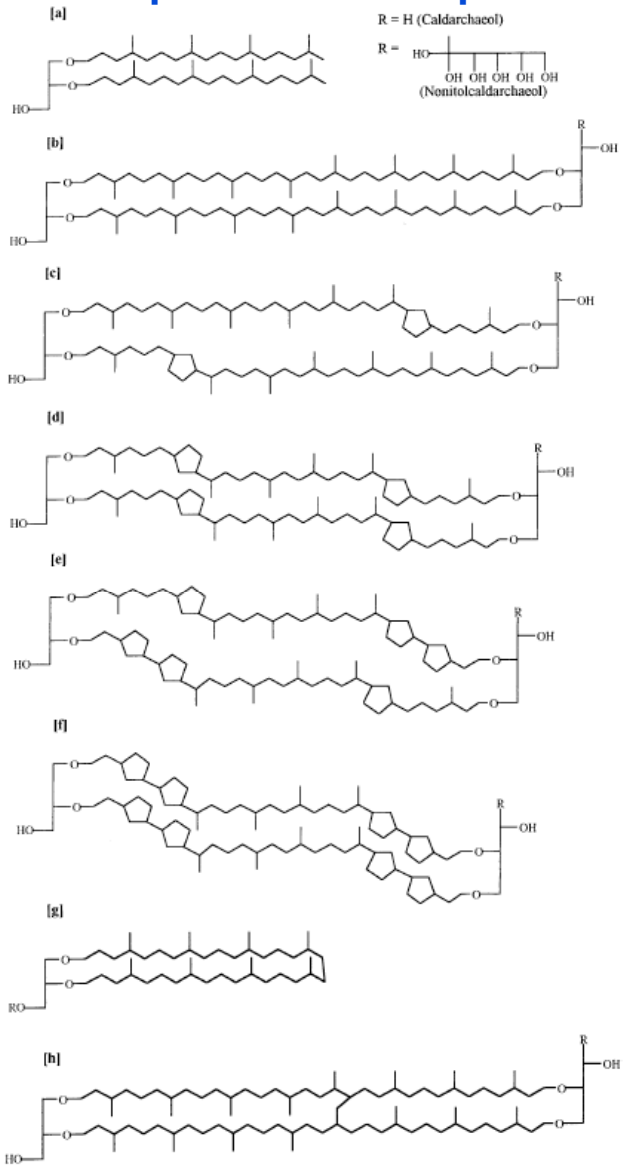
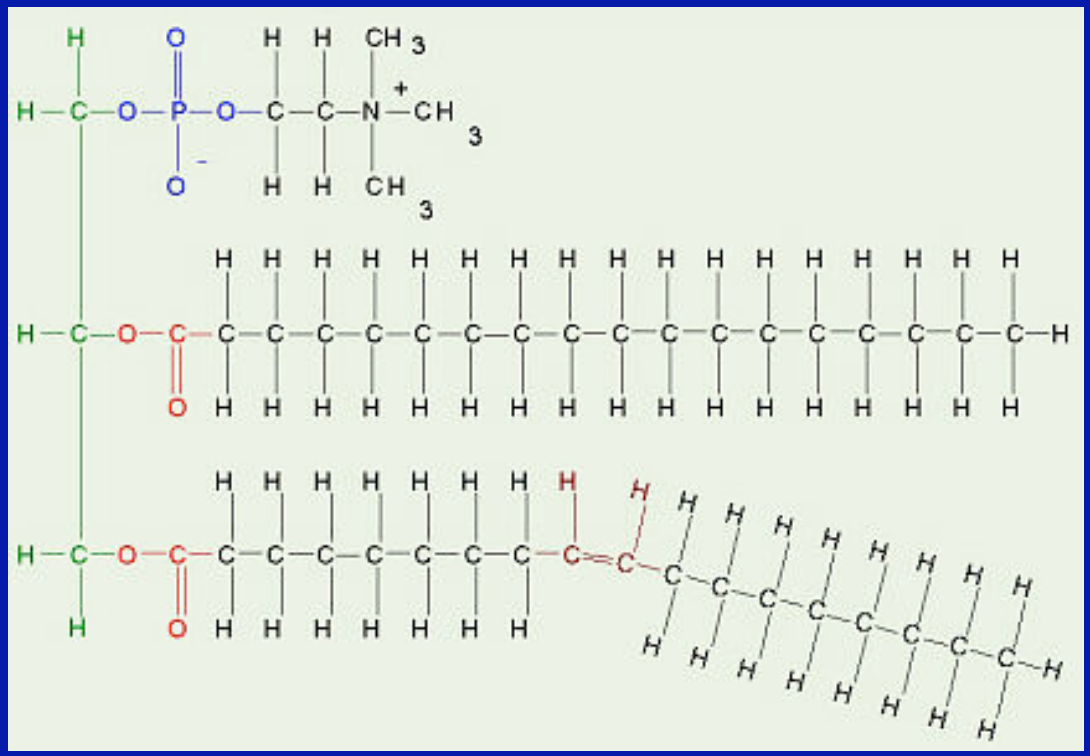


Figure 3. Archaeal lipid architecture. (a) Diphytanyl glycerol diethers, (b) dibiphytanyl diglycerol tetraethers, (c–f) internal cyclisation in dibiphytanyl diglycerol tetraethers, (g) macrocyclic diphytanyl glycerol diether, (h) internal covalent cross-linking in dibiphytanyl diglycerol tetraether.

Maintaining Membrane Fluidity at Low Temperatures (psychrophiles)

- Reduce membrane packing to enhance fluidity
  - Decrease acyl-chain length, saturation, and/or branching



Saturated

Unsaturated

Additional High Temperature Concerns

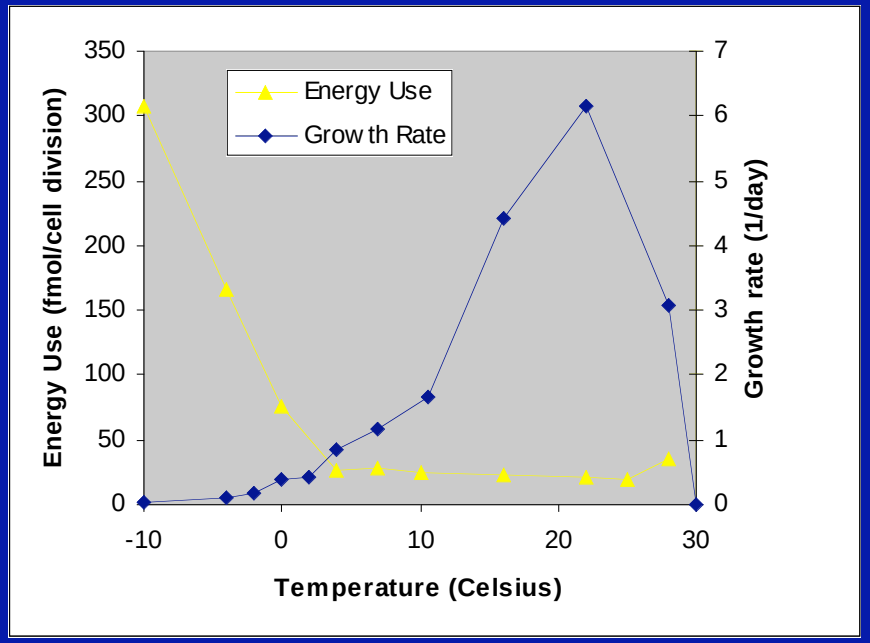
Metabolites

- Problem: stability
  - Very short half-lives at 95, 105°C
  - FMN, pyridoxal phosphate, glucose-1,6-diphosphate, acetyl phosphate, CoASH, ATP, ADP
- Solutions:
  - Protective microenvironment—control pH, metal ion concentration, etc.
  - Metabolic channeling—between enzymes that are physically next to each other
  - Alternate pathway or compound

Additional Low Temperature Concerns

- Ice crystals
  - Live in a salty environment
  - Produce cryoprotectants
    - Antifreeze proteins
    - Glycine betaine, glycerol, ectoine, proline, glutamate, alanine, trehalose (a.k.a. compatible solutes)
- Salt
  - Produce compatible solutes
- Low diffusion rates
  - More transporters in membranes
  - Enzymes with higher affinity

- Energy
  - More required at low T



Summary/Conclusions

- High Temperatures—Fast, Fleeting, Flexible
- Low Temperatures—Slow, Stable, Solid

- Have we identified the actual limits of terrestrial life and biomolecules?
- What factors will define the actual limits?
  - High—chemical degradation?
  - Low—presence of liquid H_20?