

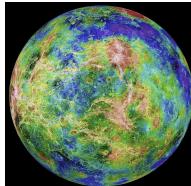
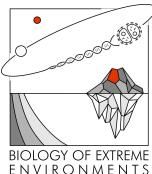
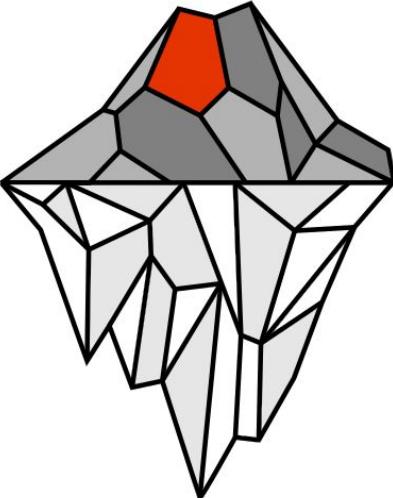
# MICROBIOLOGY OF EXTREME ENVIRONMENTS

## Microbial Life at High Temperatures

Annarita Ricciardelli

PhD in Biotechnology

[annarita.ricciardelli@unina.it](mailto:annarita.ricciardelli@unina.it)





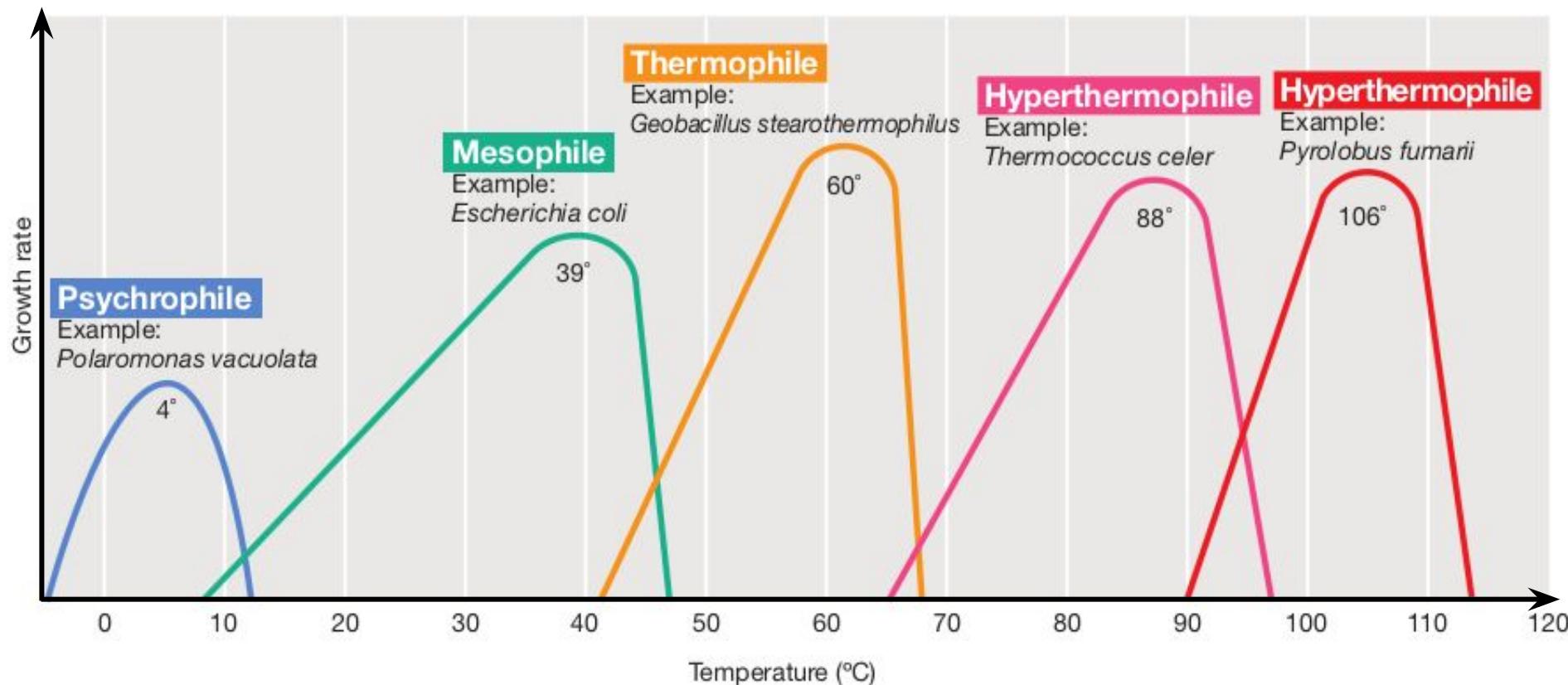
# Microbial Life at High Temperatures

## Environmental Factors affecting microbial growth:

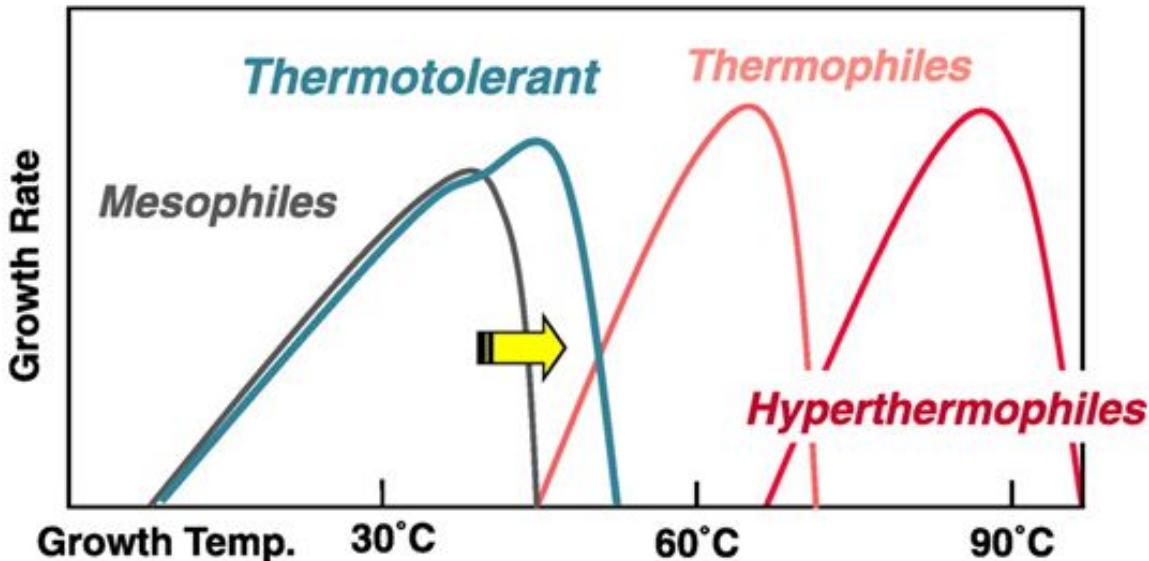
- water availability
- salinity
- pH
- **Temperature**

# Microbial Life at High Temperatures

## Temperature Classes of Organisms



# Microbial Life at High Temperatures



The difference  
is in the *optimum*

**Thermophile:** heat-loving organism that thrives at high temperatures

**Thermotolerant:** organism able to survive at high temperatures

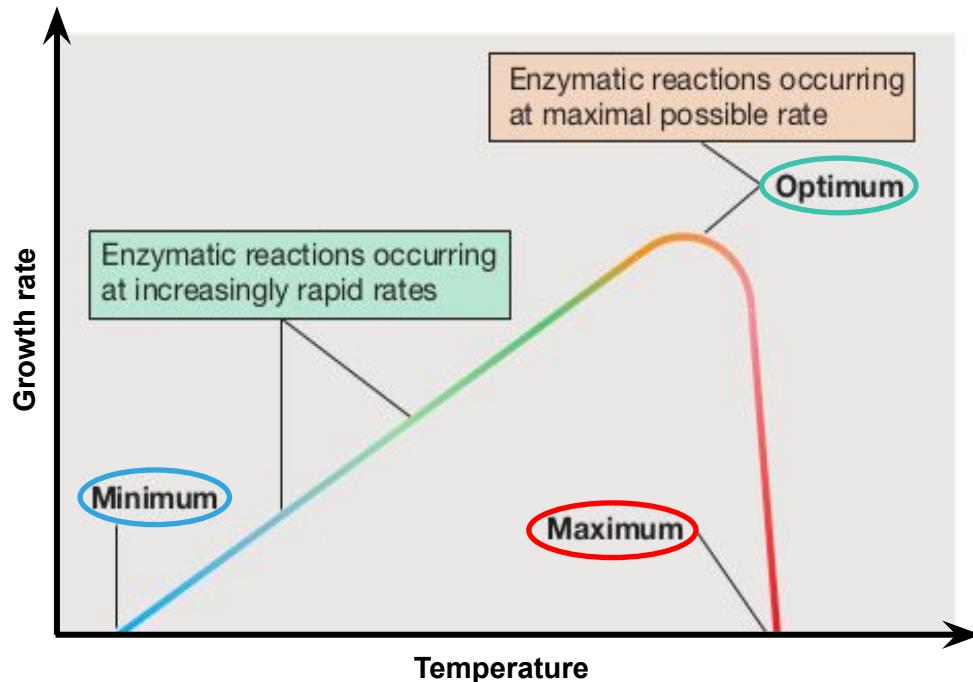
**WHY** is **temperature limiting** for microbial (and presumably all forms of) life?

# Temperature controls:

kinetic energy of molecules

biochemical reactions efficiency

biological cell components stability



## Cardinal Temperatures:

*minimum* temperature

*optimum* temperature

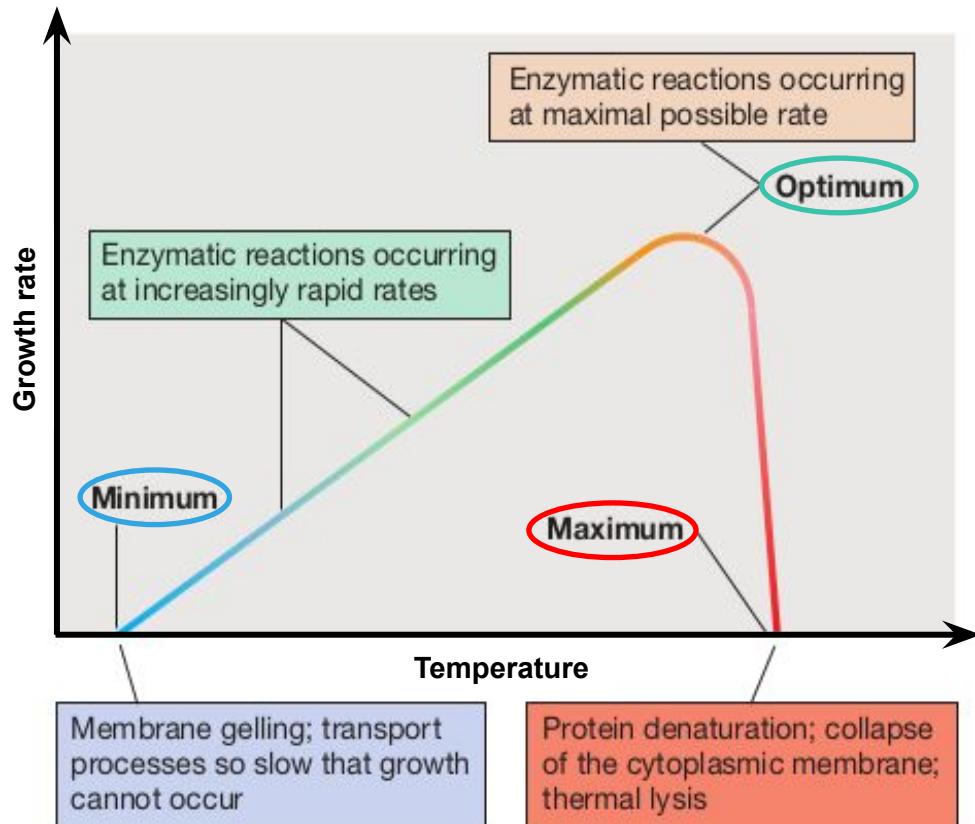
*maximum* temperature

# Temperature controls:

kinetic **energy** of molecules

biochemical reactions efficiency

biological cell components stability



## Cardinal Temperatures:

*minimum* temperature

*optimum* temperature

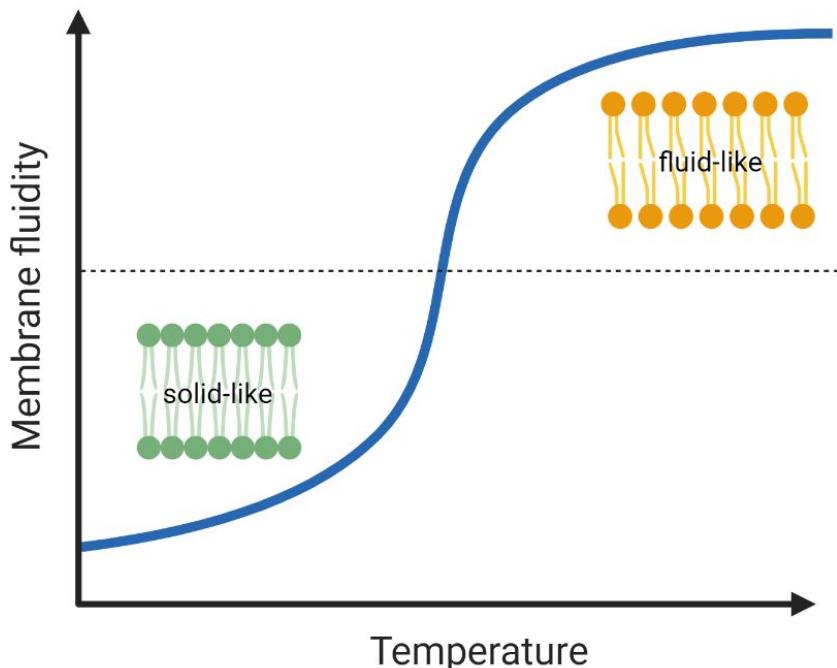
*maximum* temperature

# Temperature controls:

kinetic energy of molecules

biochemical reactions efficiency

biological cell components stability



**solid-like**

rigid state of membrane  
closely packed phospholipids  
low permeability

**fluid-like**

flexible state of membrane  
unpacked and disorganized phospholipids  
high permeability

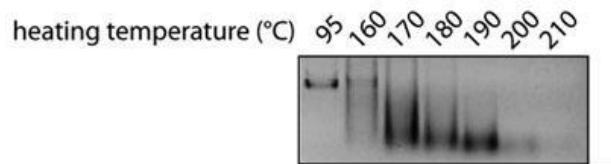
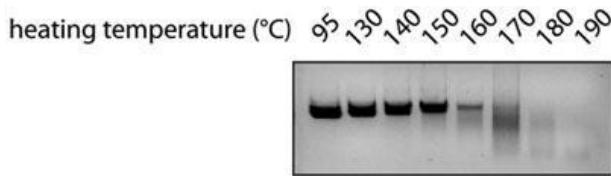
# Temperature controls:

kinetic energy of molecules

biochemical reactions efficiency

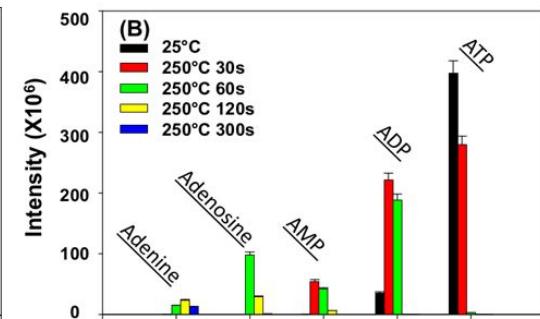
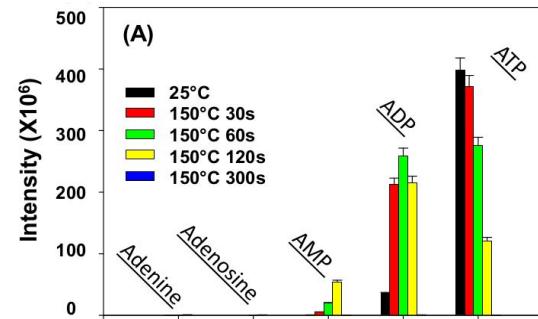
biological cell components stability

## Thermal Degradation of Macromolecules: DNA



- a) 0.75 mg of DNA pUC19 was incubated at the indicated temperatures for 5 min
- b) 0.75 mg of plasmid DNA pGY1 was incubated at the indicated temperatures for 5 min

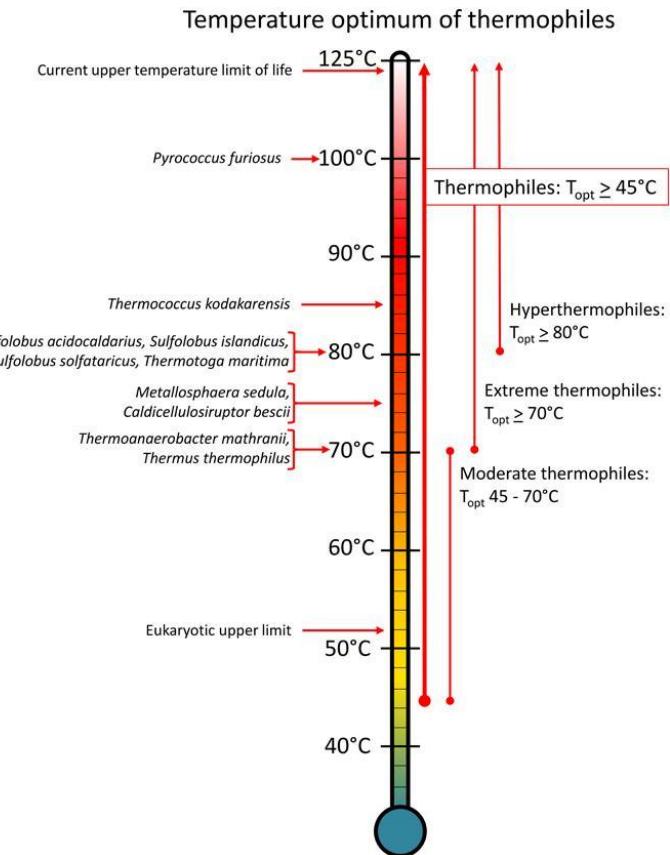
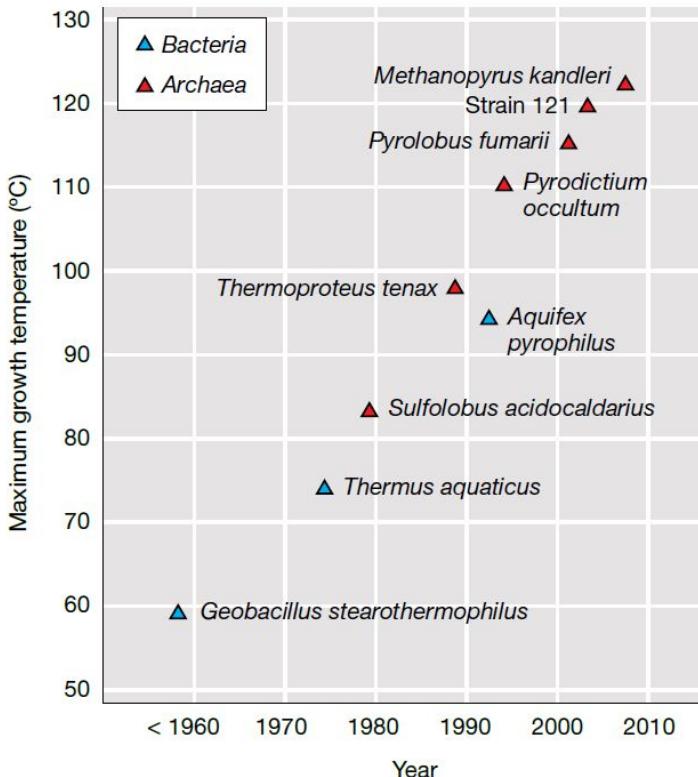
## Thermal Degradation of Small Molecules: ATP



- a) 10 µM ATP was incubated at 150 °C for 30s, 60s, 120s, and 300s
- b) 10 µM ATP was incubated at 250 °C for 30s, 60s, 120s, and 300s

# The unknown upper limit of Temperature

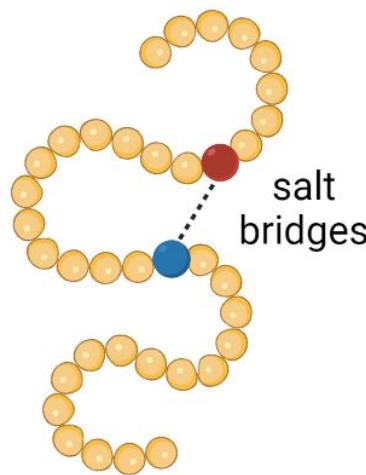
"The upper temperature limit for life in liquid water has not yet been defined, but is likely to be somewhere between 110 degrees and 200 degrees C, since amino acids and nucleotides are destroyed at temperatures over 200 degrees C", Brock TD, 1985.



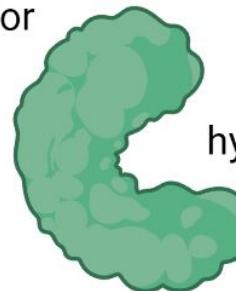
**HOW** do thermophiles and hyperthermophiles  
**thrive** at high temperature?

# Molecular Adaptations to Life at High Temperature

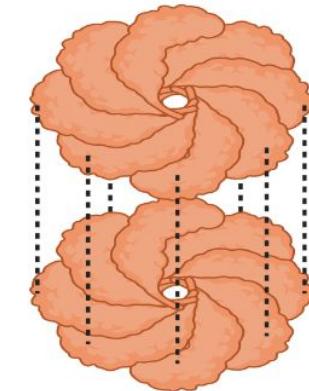
Proteins stability:



hydrophilic exterior

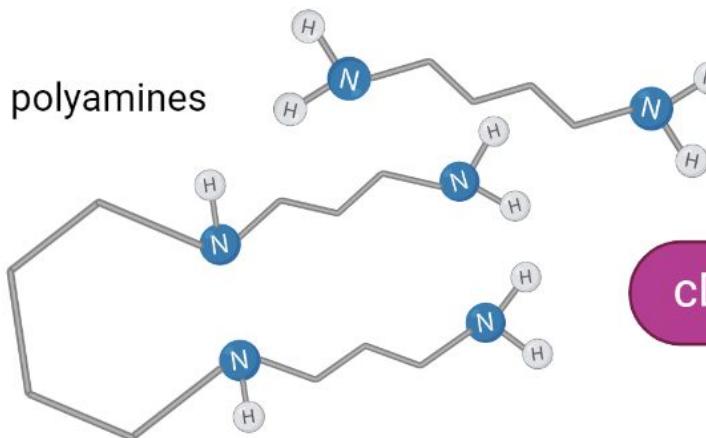


hydrophobic interior



thermosome

DNA/RNA stability:



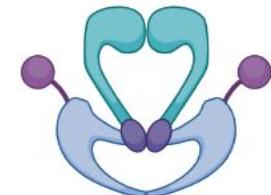
polyamines



cDPG

DIP

osmolytes



reverse gyrase

# Proteins stability: salt bridges (or ion pairs)

## Protein thermostability above 100°C: A key role for ionic interactions

Costantino Vetriani, Dennis L. Maeder, Nicola Tolley, Kitty S.-P. Yip, Timothy J. Stillman, K. Li...  
+ See all authors and affiliations

PNAS October 13, 1998 95 (21) 12300-12305; <https://doi.org/10.1073/pnas.95.21.12300>

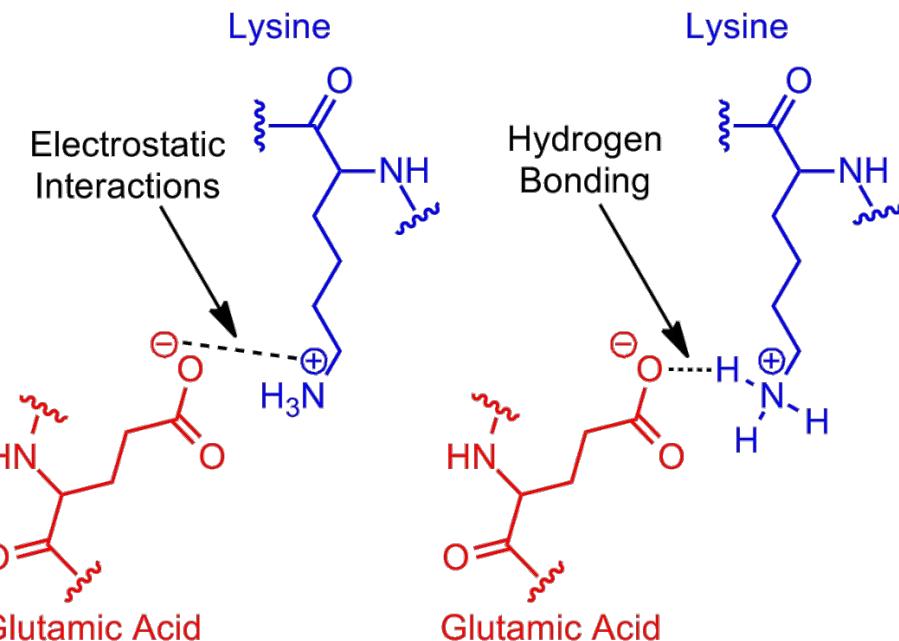
Edited by Max F. Perutz, Medical Research Council, Cambridge, United Kingdom, and approved August 10, 1998  
(received for review April 22, 1998)

Article Figures & SI Info & Metrics PDF

### Abstract

The discovery of hyperthermophilic microorganisms and the analysis of hyperthermostable enzymes has established the fact that multisubunit enzymes can survive for prolonged periods at temperatures above 100°C. We have carried out homology-based modeling and direct structure comparison on the hexameric glutamate dehydrogenases from the hyperthermophiles *Pyrococcus furiosus* and *Thermococcus litoralis* whose optimal growth temperatures are 100°C and 88°C, respectively, to determine key stabilizing features. These enzymes, which are 87% homologous, differ 16-fold in thermal stability at 104°C. We observed that an intersubunit ion-pair network was substantially reduced in the less stable enzyme from *T. litoralis*, and two residues were then altered to restore these interactions. The single mutations both had adverse effects on the thermostability of the protein. However, with both mutations in place, we observed a fourfold improvement of stability at 104°C over the wild-type enzyme. The catalytic properties of the enzymes were unaffected by the mutations. These results suggest that extensive ion-pair networks may provide a general strategy for manipulating enzyme thermostability of multisubunit enzymes. However, this study emphasizes the importance of the exact local environment of a residue in determining its effects on stability.

NOTE: generally, the amino acid composition of thermophilic and hyperthermophilic enzymes is surprisingly similar to that of homologous mesophilic enzymes and have the same catalytic mechanisms

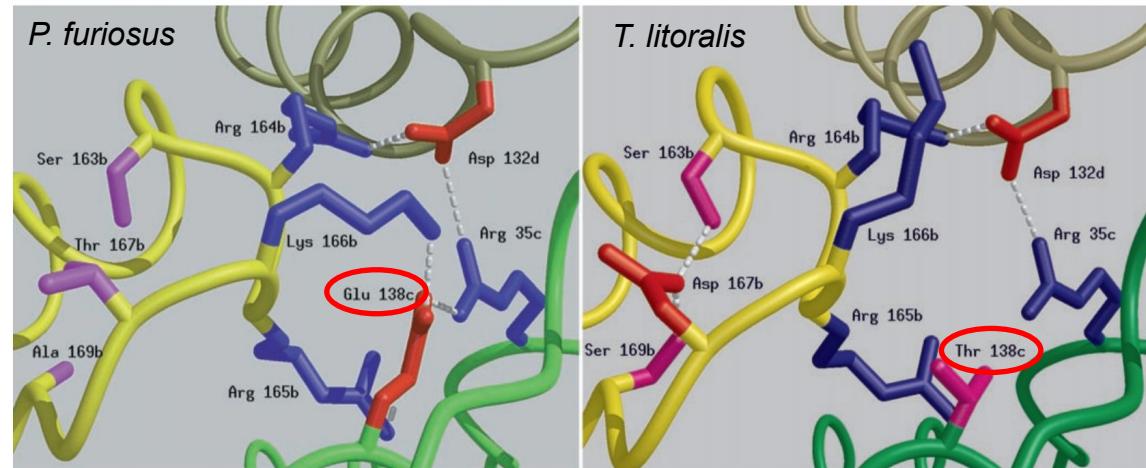


# Proteins stability: salt bridges (or ion pairs)

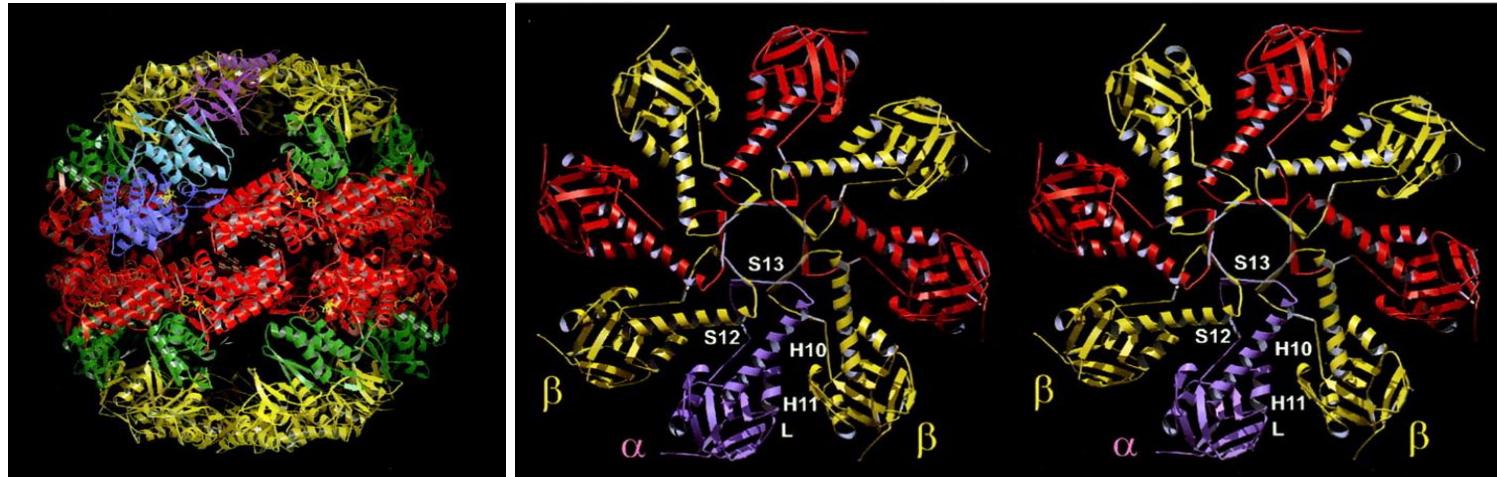
Sequence alignment of archaeal GluDH from *P. furiosus* (upper) and *T. litoralis* (lower) with sequence differences shown in bold and underlined. Intersubunit contacts closer than 3.7 Å within the *P. furiosus* hexamer are indicated above the sequences (X).

1	10	20	30	40	50	60	70	80
MVEQDP <u>YEIVI</u> KOLERAQAYME <u>I</u> SEEALEFLKR <u>P</u> QRI <u>V</u> E <u>T</u> IPVEMDDGSVKVFTGFRVQ <u>H</u> NWAR <u>G</u> PTKGIRWHPEETLSTVKALAAWM	MVEQDP <u>FEI</u> <u>AV</u> KOLERAQAY <u>M</u> D <u>I</u> SEEALEFLKR <u>P</u> QRI <u>V</u> E <u>S</u> IPVEMDDGSVKVFTGFRVQ <u>Y</u> NWAR <u>G</u> PTKGIRWHPEETLSTVKALAAWM							
90	100	110	120	130	140	150	160	170
		XX	X XX	X XX	X XX	X XX	X XX	X
TWKTAVMDLPYG <u>GGKGG</u> <u>I</u> VD <u>P</u> KK <u>L</u> SDREKERLARG <u>Y</u> R <u>A</u> I <u>D</u> V <u>I</u> SP <u>T</u> ED <u>I</u> P <u>A</u> P <u>D</u> V <u>T</u> NP <u>Q</u> IM <u>A</u> W <u>M</u> DE <u>E</u> T <u>I</u> S <u>R</u> R <u>K</u> <u>T</u> P <u>A</u> G <u>E</u> <u>I</u> T <u>G</u> K <u>P</u> S	TWKTAVMDLPYG <u>GGKGG</u> <u>V</u> <u>C</u> N <u>P</u> <u>K</u> <u>E</u> MSDREKERLARG <u>Y</u> R <u>A</u> I <u>D</u> V <u>I</u> SP <u>T</u> DI <u>P</u> <u>A</u> P <u>D</u> V <u>T</u> NP <u>Q</u> IM <u>A</u> W <u>M</u> DE <u>E</u> T <u>I</u> S <u>R</u> R <u>K</u> <u>D</u> P <u>F</u> G <u>V</u> <u>I</u> T <u>G</u> K <u>P</u> S							
180	190	200	210	220	230	240	250	260
		XX	X XX	X XX	X XX	X XX	X XX	X
<u>I</u> G <u>S</u> <u>I</u> L <u>G</u> R <u>E</u> AT <u>A</u> T <u>R</u> <u>G</u> A <u>S</u> <u>Y</u> T <u>R</u> <u>E</u> A <u>K</u> <u>V</u> <u>L</u> <u>G</u> <u>W</u> <u>D</u> <u>T</u> <u>L</u> <u>K</u> <u>G</u> <u>T</u> <u>I</u> <u>A</u> <u>Q</u> <u>G</u> <u>Y</u> <u>N</u> <u>A</u> <u>G</u> <u>Y</u> <u>L</u> <u>A</u> <u>K</u> <u>I</u> <u>M</u> <u>S</u> <u>E</u> <u>D</u> <u>F</u> <u>G</u> <u>M</u> <u>K</u> <u>V</u> <u>V</u> <u>A</u> <u>S</u> <u>D</u> <u>S</u> <u>K</u> <u>G</u> <u>I</u> <u>Y</u> <u>N</u> <u>P</u> <u>D</u> <u>G</u> <u>L</u> <u>N</u> <u>A</u> <u>D</u> <u>E</u> <u>V</u> <u>L</u> <u>K</u> <u>W</u> <u>N</u> <u>E</u> <u>H</u> <u>G</u> <u>S</u>	<u>V</u> <u>G</u> <u>G</u> <u>T</u> <u>A</u> <u>R</u> <u>M</u> <u>D</u> <u>A</u> T <u>A</u> T <u>R</u> <u>G</u> A <u>S</u> <u>Y</u> T <u>R</u> <u>E</u> A <u>K</u> <u>V</u> <u>L</u> <u>G</u> <u>M</u> <u>D</u> <u>-</u> <u>L</u> <u>K</u> <u>G</u> <u>T</u> <u>I</u> <u>A</u> <u>Q</u> <u>G</u> <u>Y</u> <u>N</u> <u>A</u> <u>G</u> <u>Y</u> <u>M</u> <u>A</u> <u>K</u> <u>I</u> <u>M</u> <u>S</u> <u>E</u> <u>Y</u> <u>G</u> <u>M</u> <u>K</u> <u>V</u> <u>V</u> <u>A</u> <u>S</u> <u>D</u> <u>S</u> <u>K</u> <u>G</u> <u>I</u> <u>Y</u> <u>N</u> <u>P</u> <u>D</u> <u>G</u> <u>L</u> <u>N</u> <u>A</u> <u>D</u> <u>E</u> <u>V</u> <u>L</u> <u>K</u> <u>W</u> <u>N</u> <u>E</u> <u>H</u> <u>G</u> <u>S</u>							
270	280	290	300	310	320	330	340	350
		XX	X XX	X XX	X XX	X XX	X XX	X
V <u>K</u> <u>D</u> <u>F</u> <u>P</u> <u>G</u> <u>A</u> <u>T</u> <u>N</u> <u>I</u> <u>T</u> <u>E</u> <u>E</u> <u>L</u> <u>E</u> <u>V</u> <u>D</u> <u>V</u> <u>L</u> <u>A</u> <u>P</u> <u>A</u> <u>I</u> <u>E</u> <u>E</u> <u>V</u> <u>I</u> <u>T</u> <u>K</u> <u>N</u> <u>A</u> <u>D</u> <u>N</u> <u>I</u> <u>K</u> <u>A</u> <u>I</u> <u>V</u> <u>E</u> <u>V</u> <u>A</u> <u>N</u> <u>G</u> <u>P</u> <u>V</u> <u>T</u> <u>P</u> <u>E</u> <u>A</u> <u>D</u> <u>E</u> <u>I</u> <u>L</u> <u>E</u> <u>F</u> <u>K</u> <u>G</u> <u>I</u> <u>L</u> <u>O</u> <u>I</u> <u>P</u> <u>D</u> <u>F</u> <u>L</u> <u>C</u> <u>N</u> <u>A</u> <u>G</u> <u>G</u> <u>V</u> <u>T</u> <u>V</u> <u>S</u> <u>Y</u> <u>F</u> <u>E</u> <u>W</u> <u>V</u> <u>Q</u> <u>N</u> <u>I</u>	V <u>K</u> <u>D</u> <u>F</u> <u>P</u> <u>G</u> <u>A</u> <u>T</u> <u>N</u> <u>I</u> <u>T</u> <u>E</u> <u>E</u> <u>L</u> <u>E</u> <u>V</u> <u>D</u> <u>V</u> <u>L</u> <u>A</u> <u>P</u> <u>S</u> <u>A</u> <u>I</u> <u>E</u> <u>E</u> <u>V</u> <u>I</u> <u>T</u> <u>K</u> <u>N</u> <u>A</u> <u>D</u> <u>N</u> <u>I</u> <u>K</u> <u>A</u> <u>I</u> <u>E</u> <u>L</u> <u>A</u> <u>N</u> <u>G</u> <u>P</u> <u>T</u> <u>T</u> <u>P</u> <u>E</u> <u>A</u> <u>D</u> <u>E</u> <u>I</u> <u>L</u> <u>E</u> <u>F</u> <u>K</u> <u>G</u> <u>I</u> <u>L</u> <u>O</u> <u>I</u> <u>P</u> <u>D</u> <u>F</u> <u>L</u> <u>C</u> <u>N</u> <u>A</u> <u>G</u> <u>G</u> <u>V</u> <u>T</u> <u>V</u> <u>S</u> <u>Y</u> <u>F</u> <u>E</u> <u>W</u> <u>V</u> <u>Q</u> <u>N</u> <u>I</u>							
360	370	380	390	400	410	420	430	440
		XX	X XX	X XX	X XX	X XX	X XX	X
T <u>G</u> <u>Y</u> <u>W</u> <u>T</u> <u>I</u> <u>E</u> <u>V</u> <u>R</u> <u>E</u> <u>L</u> <u>D</u> <u>K</u> <u>M</u> <u>T</u> <u>K</u> <u>A</u> <u>F</u> <u>D</u> <u>V</u> <u>N</u> <u>I</u> <u>A</u> <u>K</u> <u>E</u> <u>N</u> <u>I</u> <u>H</u> <u>M</u> <u>R</u> <u>D</u> <u>A</u> <u>Y</u> <u>V</u> <u>V</u> <u>A</u> <u>V</u> <u>R</u> <u>Y</u> <u>Q</u> <u>A</u> <u>M</u> <u>I</u> <u>D</u> <u>R</u> <u>G</u> <u>W</u> <u>R</u> <u>H</u> <u>I</u> <u>T</u> <u>G</u> <u>D</u> <u>Y</u> <u>W</u> <u>T</u> <u>V</u> <u>E</u> <u>E</u> <u>T</u> <u>R</u> <u>A</u> <u>K</u> <u>L</u> <u>D</u> <u>K</u> <u>M</u> <u>T</u> <u>K</u> <u>A</u> <u>F</u> <u>D</u> <u>V</u> <u>N</u> <u>I</u> <u>A</u> <u>K</u> <u>E</u> <u>N</u> <u>I</u> <u>H</u> <u>M</u> <u>R</u> <u>D</u> <u>A</u> <u>Y</u> <u>V</u> <u>V</u> <u>A</u> <u>V</u> <u>R</u> <u>Y</u> <u>Q</u> <u>A</u> <u>M</u> <u>I</u> <u>D</u> <u>R</u> <u>G</u> <u>W</u> <u>R</u> <u>H</u> <u>I</u> <u>T</u> <u>G</u> <u>W</u> <u>I</u> <u>K</u> <u>K</u>	T <u>G</u> <u>Y</u> <u>W</u> <u>T</u> <u>I</u> <u>E</u> <u>V</u> <u>R</u> <u>E</u> <u>L</u> <u>D</u> <u>K</u> <u>M</u> <u>T</u> <u>K</u> <u>A</u> <u>F</u> <u>D</u> <u>V</u> <u>N</u> <u>I</u> <u>A</u> <u>K</u> <u>E</u> <u>N</u> <u>I</u> <u>H</u> <u>M</u> <u>R</u> <u>D</u> <u>A</u> <u>Y</u> <u>V</u> <u>V</u> <u>A</u> <u>V</u> <u>R</u> <u>Y</u> <u>Q</u> <u>A</u> <u>M</u> <u>I</u> <u>D</u> <u>R</u> <u>G</u> <u>W</u> <u>R</u> <u>H</u> <u>I</u> <u>T</u> <u>G</u> <u>W</u> <u>I</u> <u>K</u> <u>K</u> <u>I</u> <u>P</u> <u>D</u> <u>F</u> <u>L</u> <u>C</u> <u>N</u> <u>A</u> <u>G</u> <u>G</u> <u>V</u> <u>T</u> <u>V</u> <u>S</u> <u>Y</u> <u>F</u> <u>E</u> <u>W</u> <u>V</u> <u>Q</u> <u>N</u> <u>I</u>							

The positively charged side chains are shown in blue, the negatively charged ones in red, and hydrogen bonds shown as dashed lines.



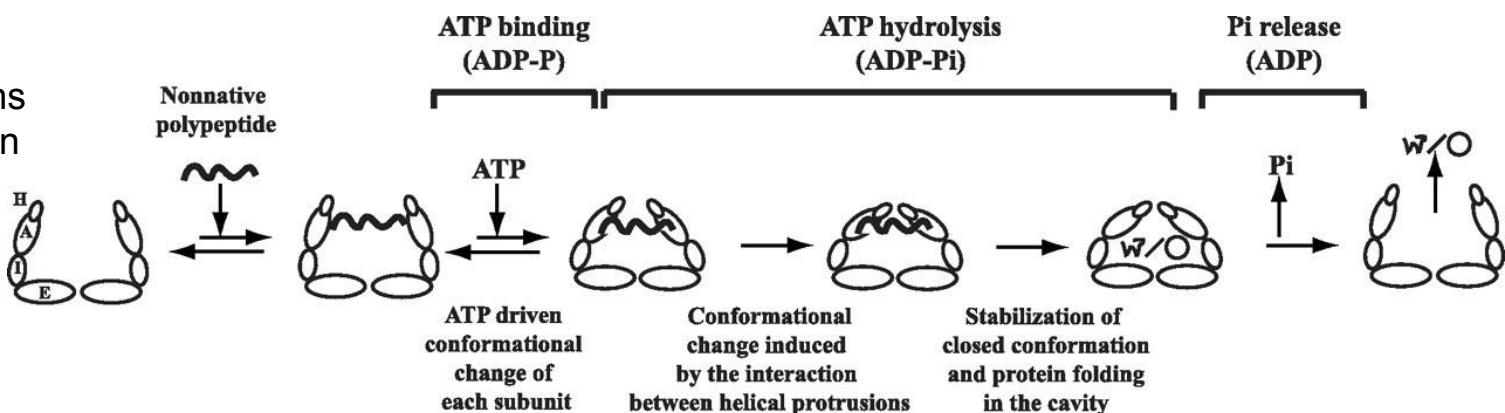
# Proteins stability: thermosome



Crystal structure of the Thermosome, the hexadecameric chaperonin of the archaeon *Thermoplasma acidophilum*

Ditzel L, et al. Crystal structure of the thermosome, the archaeal chaperonin and homolog of CCT. *Cell.* 1998;93(1):125-138.

Group II chaperonins mechanism of action



Kanzaki T, et al. Sequential action of ATP-dependent subunit conformational change and interaction between helical protrusions in the closure of the built-in lid of group II chaperonins. *J Biol Chem.* 2008;283(50):34773-34784.

# Proteins stability: thermosome

## ATP-Induced Structural Change of the Thermosome Is Temperature-Dependent

Irina Gutsche <sup>a</sup>, Jörg Holzinger <sup>b</sup>, Nadine Rauh <sup>a</sup>, Wolfgang Baumeister <sup>a</sup>, Roland P. May <sup>b</sup>

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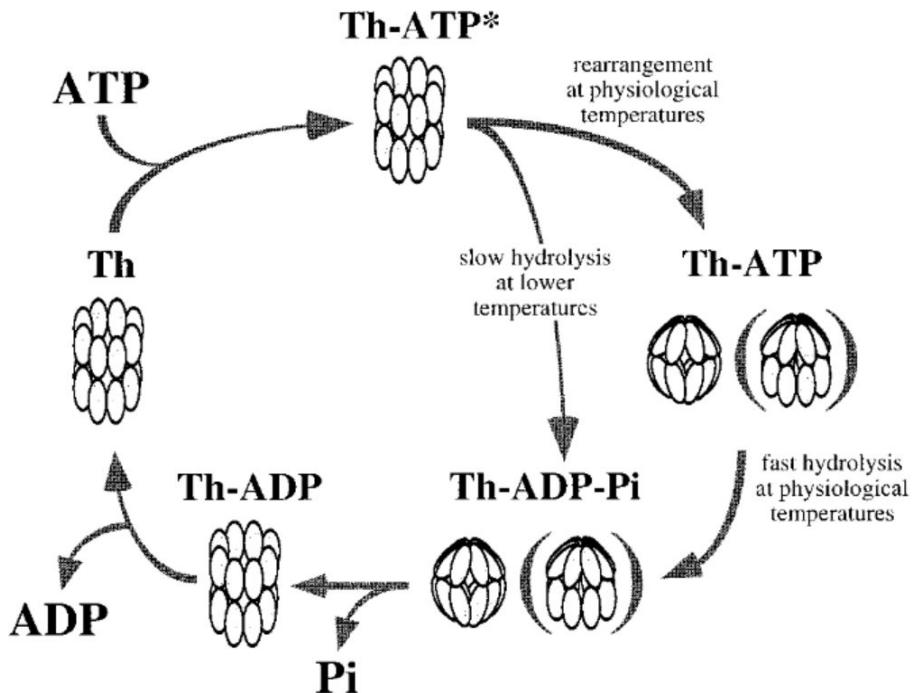
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### Abstract

Protein folding by chaperonins is powered by ATP binding and hydrolysis. ATPase activity drives the folding machine through a series of conformational rearrangements, extensively described for the group I chaperonin GroEL from *Escherichia coli* but still poorly understood for the group II chaperonins. The latter—archaeal thermosome and eukaryotic TRiC/CCT—function independently of a GroES-like cochaperonin and are proposed to rely on protrusions of their own apical domains for opening and closure in an ATP-controlled fashion. Here we use small-angle neutron scattering to analyze structural changes of the recombinant  $\alpha$ -only and the native  $\alpha\beta$ -thermosome from *Thermoplasma acidophilum* upon their ATPase cycling in solution. We show that specific high-salt conditions, but not the presence of MgATP alone, induce formation of higher order thermosome aggregates. The mechanism of the open–closed transition of the thermosome is strongly temperature-dependent. ATP binding to the chaperonin appears to be a two-step process: at lower temperatures an open state of the ATP-thermosome is predominant, whereas heating to physiological temperatures induces its switching to a closed state. Our data reveal an analogy between the ATPase cycles of the two groups of chaperonins and enable us to put forward a model of thermosome action.



# Nucleic acids stability: reverse gyrase

Reverse gyrase is essential for microbial growth at 95 °C

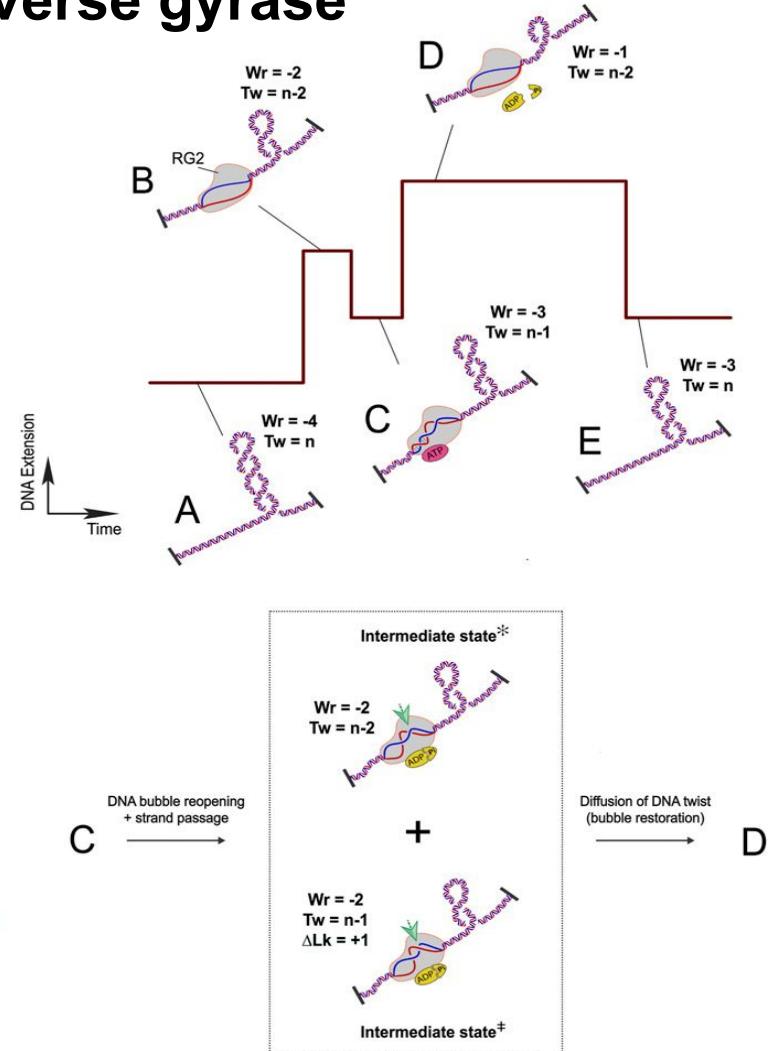
Gina L Lipscomb <sup>1</sup>, Elin M Hahn <sup>1</sup>, Alexander T Crowley <sup>1</sup>, Michael W W Adams <sup>2</sup>

Affiliations + expand

PMID: 28331998 DOI: 10.1007/s00792-017-0929-z

## Abstract

Reverse gyrase is an enzyme that induces positive supercoiling in closed circular DNA in vitro. It is unique to thermophilic organisms and found without exception in all microorganisms defined as hyperthermophiles, that is, those having optimal growth temperatures of 80 °C and above. Although its *in vivo* role has not been clearly defined, it has been implicated in stabilizing DNA at high temperatures. Whether or not it is absolutely required for growth at these high temperatures has yet to be fully determined. In a previous study with an organism that has an optimal growth temperature of 85 °C, it was shown that the enzyme is not a prerequisite for life at extreme temperatures as disruption of its gene did not result in a lethal phenotype at the supraoptimal growth temperature of 90 °C. Herein we show that the enzyme is absolutely required for microbial growth at 95 °C, which in this case is a suboptimal growth temperature. Deletion of the gene encoding the reverse gyrase of the model hyperthermophilic archaeon *Pyrococcus furiosus*, which has an optimal growth temperature of 100 °C, revealed that the gene is required for growth at 95 °C, as well as at 100 °C. The results suggest that a temperature threshold above 90 °C exists, wherein the activity of reverse gyrase is absolutely necessary to maintain a correct DNA twist for any organism growing at such temperature extremes.



# Nucleic acids stability: solutes

Osmolytes protect DNA from depurination or depyrimidization due to high temperatures

## Occurrence and Role of Di-myo-Inositol-1,1'-Phosphate in Methanococcus igneus

R A Ciulla <sup>1</sup>, S Burggraf, K O Stetter, M F Roberts

Affiliations + expand

PMID: 16349412 PMCID: PMC201870 DOI: 10.1128/aem.60.10.3660-3664.1994

Free PMC article

DIP

### Abstract

Methanococcus igneus, a hyperthermophilic marine methanogen (optimum growth temperature of 88 degrees C) with a 25-min doubling time, synthesizes an unusual inositol phosphodiester which is present at high intracellular concentrations along with l-alpha-glutamate and beta-glutamate. Identification of this compound as a dimeric inositol phosphodiester (di-myo-inositol-1,1'-phosphate) was provided by two-dimensional nuclear magnetic resonance methods. The intracellular levels of all three negatively charged solutes (l-alpha-glutamate, beta-glutamate, and the inositol phosphodiester) increase with increasing levels of external NaCl, although the inositol compound shows much smaller increases with increasing NaCl levels than the glutamate isomers. The turnover of these solutes was examined by CO(2)-pulse-CO(2)-chase experiments. The results indicated that both the beta-glutamate and the inositol phosphodiester behaved as compatible solutes and were not efficiently metabolized by cells as was l-alpha-glutamate. At a fixed external NaCl concentration, lower ammonium levels increased the fraction of the inositol dimer present in extracts. The most pronounced changes in di-myo-inositol-1,1'-phosphate occurred as a function of cell growth temperature. While the organism grows over a relatively wide temperature range, the phosphodiester accumulated only when *M. igneus* was grown at temperatures of >=80 degrees C. Thus, this unusual compound is a non-nitrogen-containing osmolyte preferentially synthesized at high growth temperatures.

- Ciulla RA, et al. Occurrence and role of di-myo-inositol-1,19-phosphate in *Methanococcus igneus*. *Appl Environ Microbiol.* 1994;60:3660–3664
- Hensel R, König H. Thermoadaptation of methanogenic bacteria by intracellular ion concentration. *FEMS Microbiol Rev.* 1988;49:75–79
- Marguet E, Forterre P. Protection of DNA by salts against thermodegradation at temperatures typical for hyperthermophiles. *Extremophiles.* 1998;2(2):115–122.

"DNA in hyperthermophiles should be strongly protected against depurination by physiological concentrations of salt, especially in those hyperthermophiles with very high intracellular potassium concentrations. Some hyperthermophilic archaea indeed contain high levels of K<sup>+</sup> with various compatible solutes as counter-ions, such as cyclic 2,3-diphosphoglycerate (cDPG), or di-myo-inositol-1,19-phosphate (DIP)" Marguet and Forterre, 1998

## Thermoadaptation of methanogenic bacteria by intracellular ion concentration

cDPG

Reinhard Hensel <sup>1</sup>, Helmut König <sup>2</sup>

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### Abstract

An inter- and intra-species correlation was found between the intracellular potassium concentration and growth temperature within the *Methanobacteriales*, comprising mesophiles as well as moderate (*Methanobacterium thermoautotrophicum*) and extreme thermophiles (*Methanothermus fervidus*, *Mt. sociabilis*). Potassium concentrations in different species were determined at optimal growth temperatures and for the same species cultured at different temperatures. The main anionic component was found to be the unusual trianionic cyclic 2,3-diphosphoglycerate. In vitro experiments with the thermolabile enzymes glyceraldehyde-3-phosphate dehydrogenase and malate dehydrogenase from *Mt. fervidus* indicated that the potassium salt of the cyclic diphosphoglycerate acts as potent thermostabilizer. Thus it appears that, for the methanogens, changes in the intracellular ion concentration are the basis of thermoadaptation.

# Nucleic acids stability: solutes

Polyamines stabilize both ribosomes and nucleic acids at high temperature, help maintain key cellular macromolecules in their active forms

## Stabilization of nucleic acids by unusual polyamines produced by an extreme thermophile, *Thermus thermophilus*

Yusuke Terui <sup>1</sup>, Mio Ohnuma, Kaori Hiraga, Etsuko Kawashima, Tairo Oshima

Affiliations + expand

PMID: 15673283 PMCID: PMC1138949 DOI: 10.1042/BJ20041778

Free PMC article

### Abstract

Extreme thermophiles produce two types of unusual polyamine: long linear polyamines such as caldopentamine and caldohexamine, and branched polyamines such as quaternary ammonium compounds [e.g. tetrakis(3-aminopropyl)ammonium]. To clarify the physiological roles of long linear and branched polyamines in thermophiles, we synthesized them chemically and tested their effects on the stability of ds (double-stranded) and ss (single-stranded) DNAs and tRNA in response to thermal denaturation, as measured by differential scanning calorimetry. Linear polyamines stabilized dsDNA in proportion to the number of amino nitrogen atoms within their molecular structure. We used the empirical results to derive formulae that estimate the melting temperature of dsDNA in the presence of polyamines of a particular molecular composition. ssDNA and tRNA were stabilized more effectively by tetrakis(3-aminopropyl)ammonium than any of the other polyamines tested. We propose that long linear polyamines are effective to stabilize DNA, and tetrakis(3-aminopropyl)ammonium plays important roles in stabilizing RNAs in thermophile cells.

Terui Y, Ohnuma M, Hiraga K, Kawashima E, Oshima T. Stabilization of nucleic acids by unusual polyamines produced by an extreme thermophile, *Thermus thermophilus*. *Biochem J.* 2005;388(Pt 2):427-433.

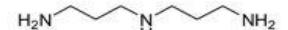
### 1,3-Diaminopropane (3)



### Putrescine (4)



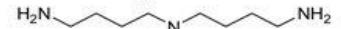
### Norspermidine (33)



### Spermidine (34)



### Homospermidine (44)



### Thermine (333)



### Spermine (343)



### Thermospermine (334)



### Homospermine (344)



### Caldopentamine (3333)



### Thermopentamine (3343)



### Homocaldopentamine (3334)



### Caldohexamine (33333)

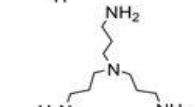


### Homocaldohexamine (33334)

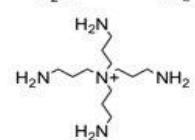


### Tris(3-aminopropyl)amine (3(3)3)

(Mitsubishine)



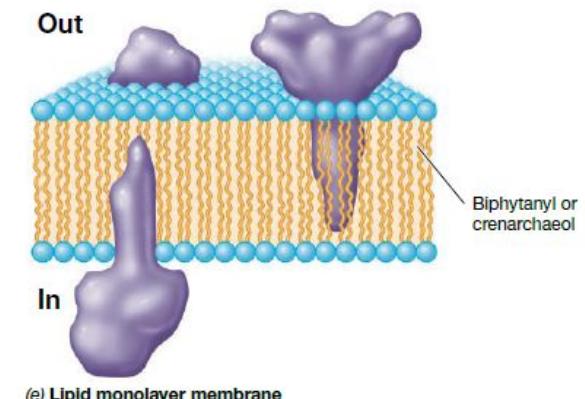
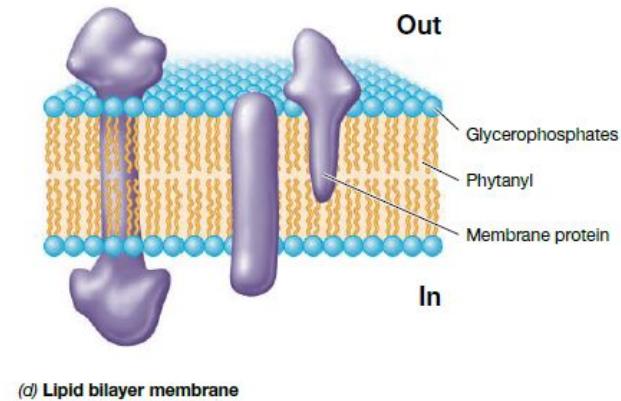
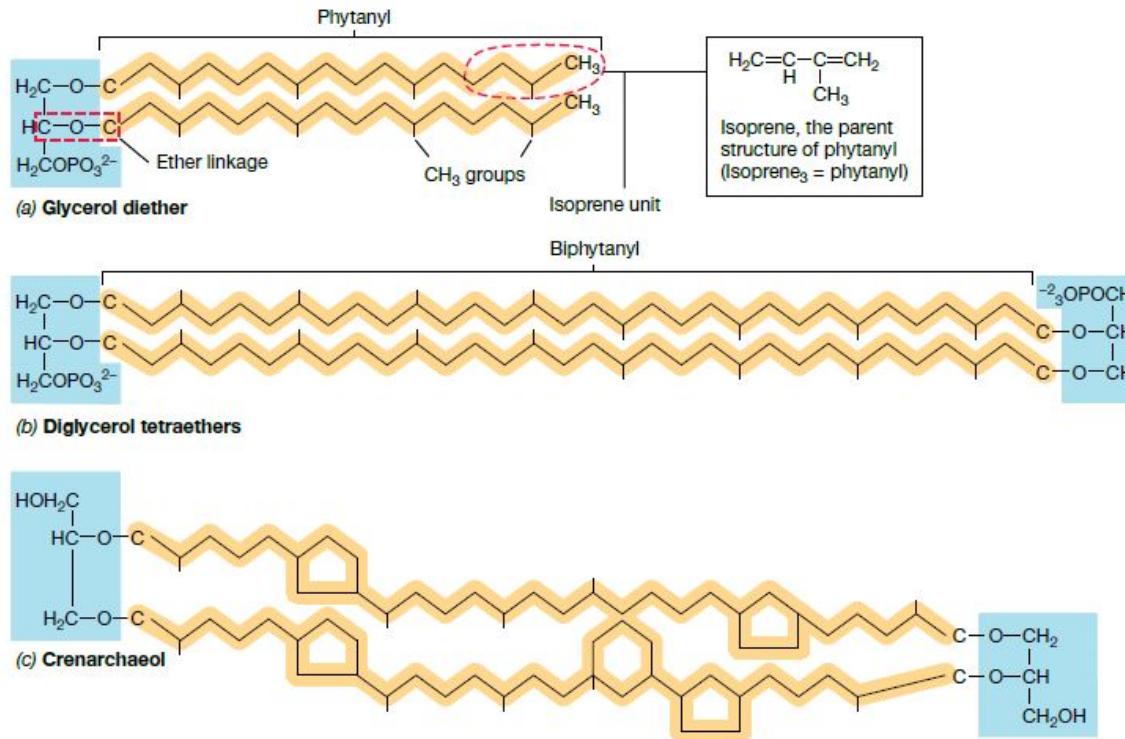
### Tetrakis(3-aminopropyl)ammonium (3(3)(3)3)



# Molecular Adaptations to Life at High Temperature

## Membranes stability:

Major lipids of *Archaea* and the architecture of archaeal membranes



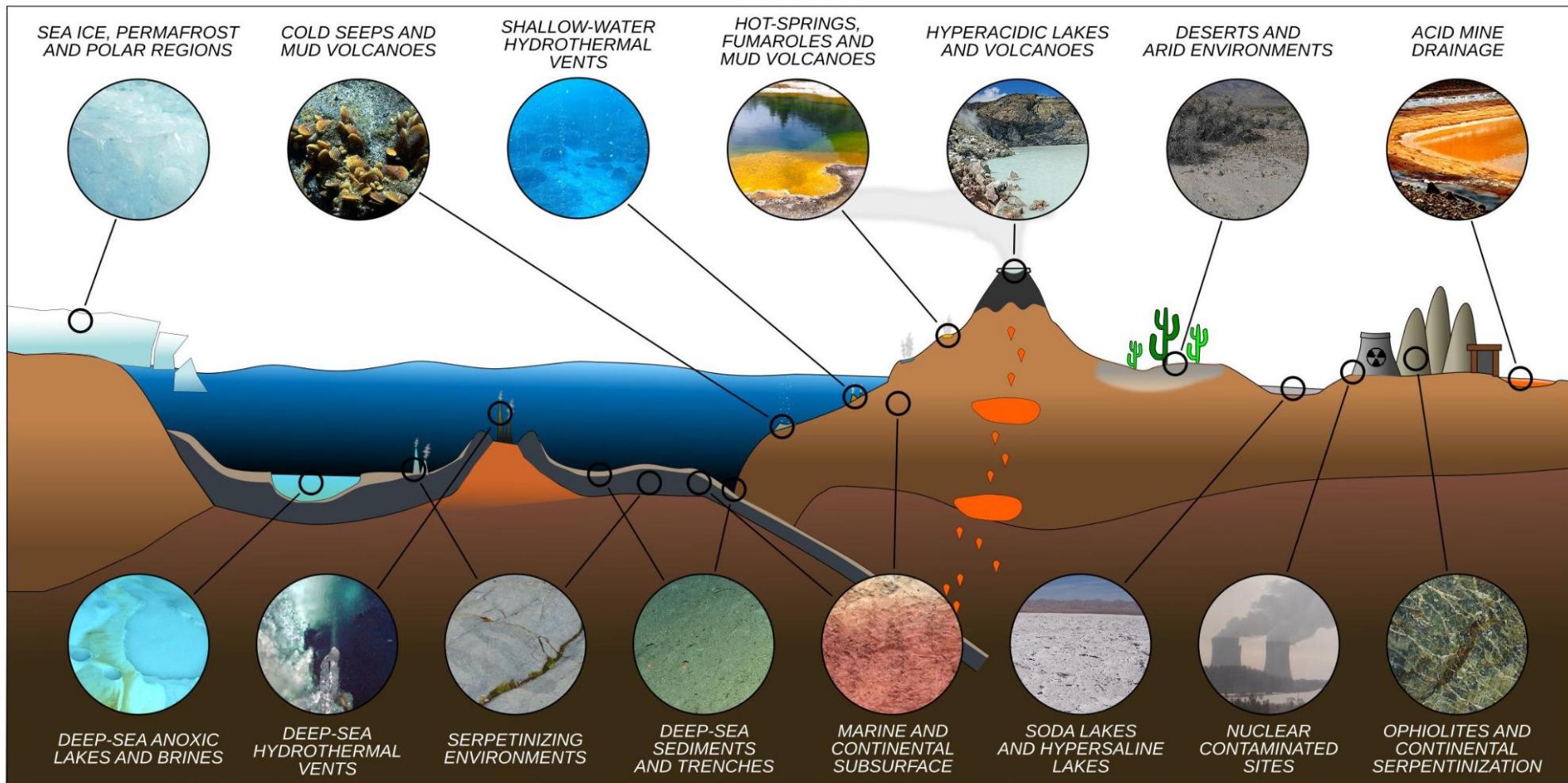
**WHERE** do thermophiles and hyperthermophiles  
**live** at high temperature?

# Global distribution of representative extreme environments

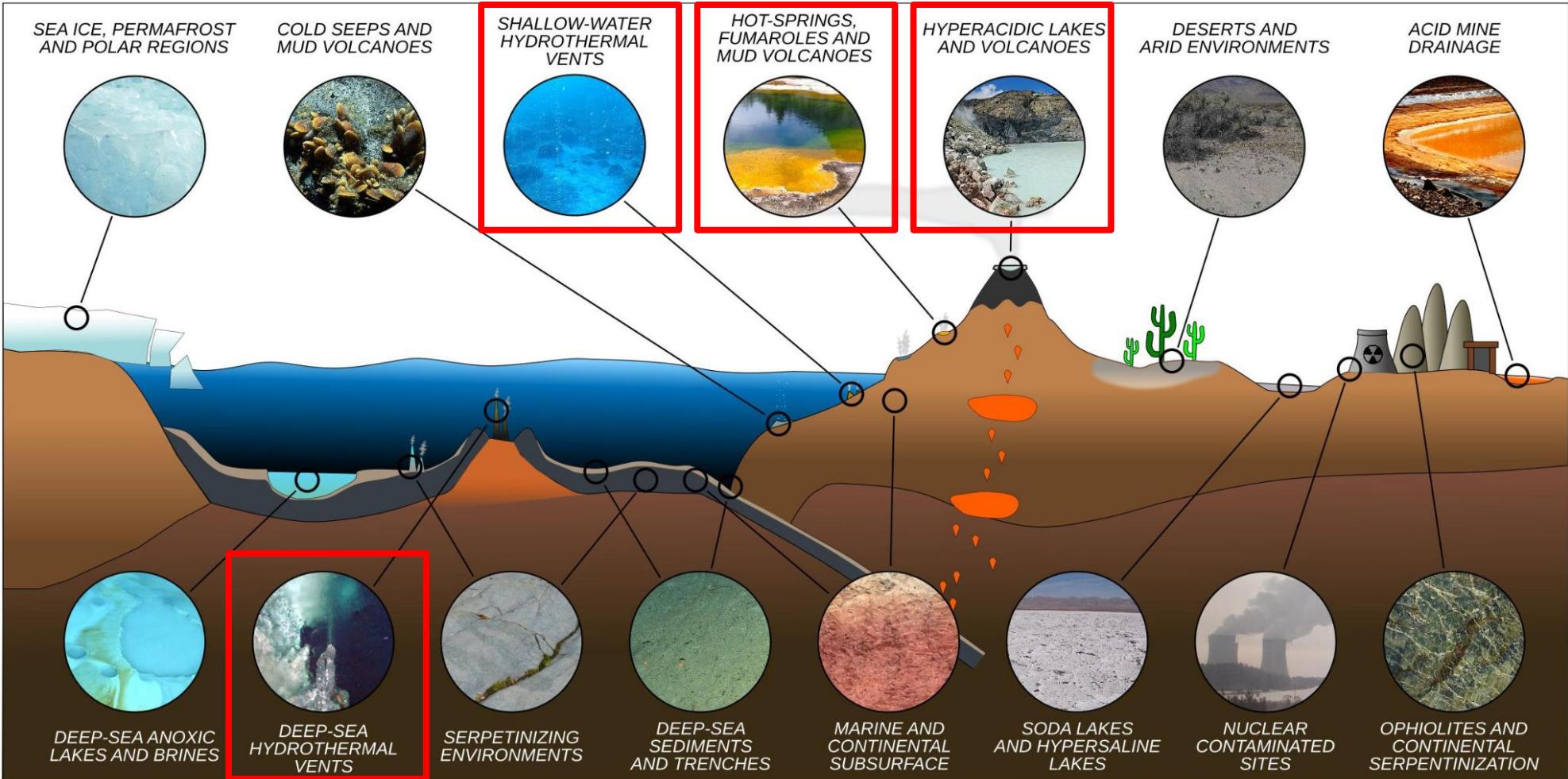


# Hyperthermophilic Environments

cross section of Earth's crust



# Hyperthermophilic Environments



# Shallow Water Hydrothermal Vents

- They are fissures on the seafloor (<200 m in depth), from which geothermally heated water discharges from below the seafloor and mix with water and gases from the overlying ocean
- commonly found near volcanically active places, areas where tectonic plates are moving apart at spreading centers, ocean basins, and hotspots
- fluid temperature: 10–119 °C
- high concentrations of compounds and metal elements involved in geochemical cycles, including carbon, sulfur, methane, and iron



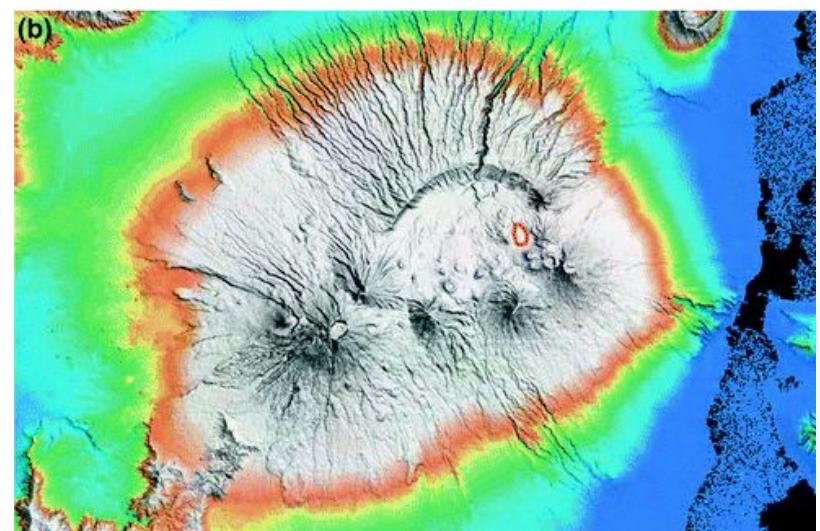
# Hot Springs, Fumaroles and Mud Volcanoes

- hot springs range from acid sulfate springs with a pH < 1, to alkaline chloride springs saturated with silica, to bicarbonate springs saturated with carbon dioxide and carbonate minerals
- fumaroles emit volcanic gases generally including sulfur compounds, such as various sulfur oxides and hydrogen sulfide, for which they are also called solfataras
- mud volcanoes ejected materials are most often a slurry of fine solids suspended in water that may contain a mixture of salts, acids and various hydrocarbons, with the 86% of gas released as methane



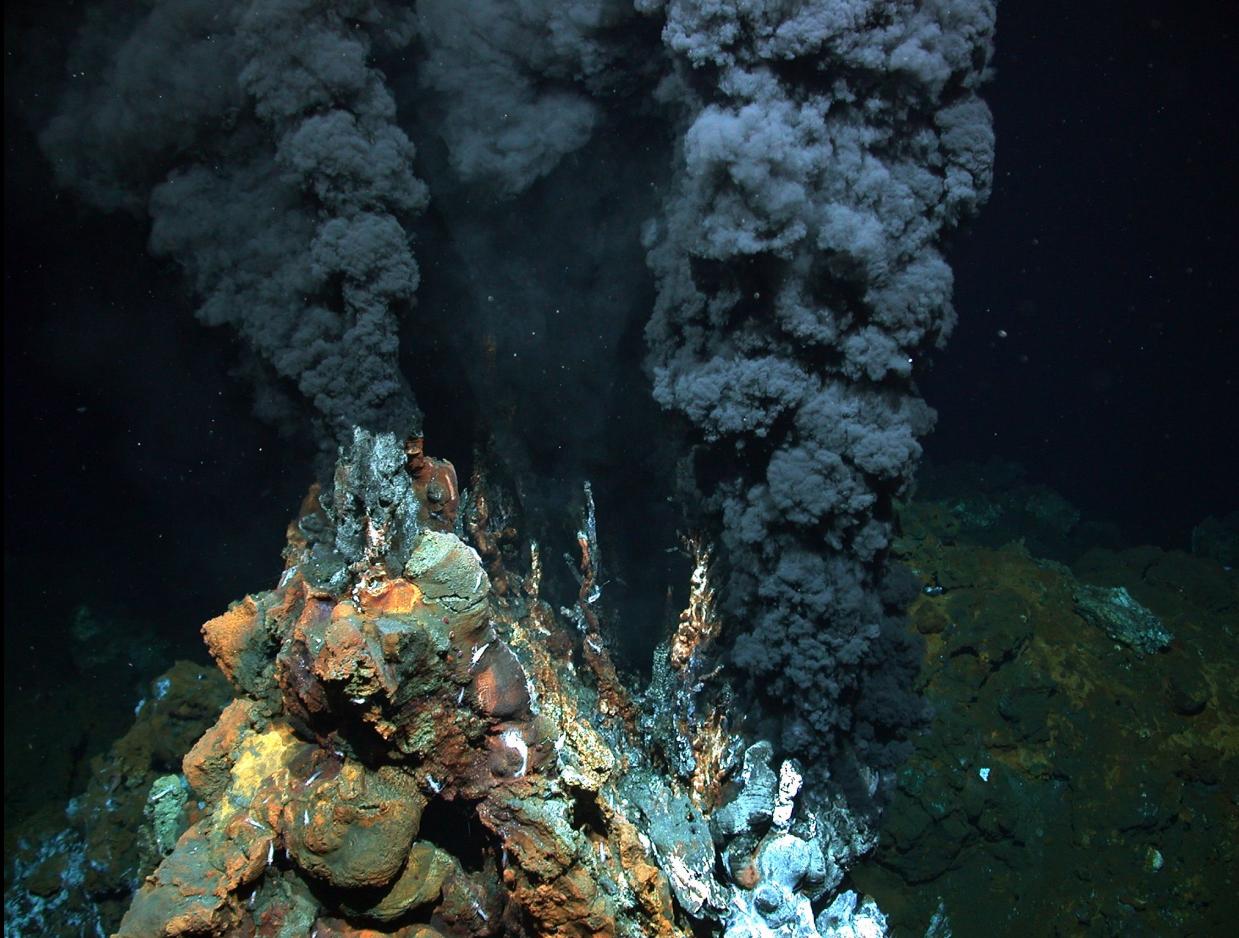
# Hyperacidic Volcanic Lakes

- They form when volcanic hot and acidic gases meet the atmospheric water at the Earth's surface
- temperature and acidity of water are sustained by the capture of heat and SO<sub>2</sub> (and HCl) and a suite of metals and metalloids, including Cu, Fe, As, Au, Bi, Se, Te, and Sb, from the magmatic gas



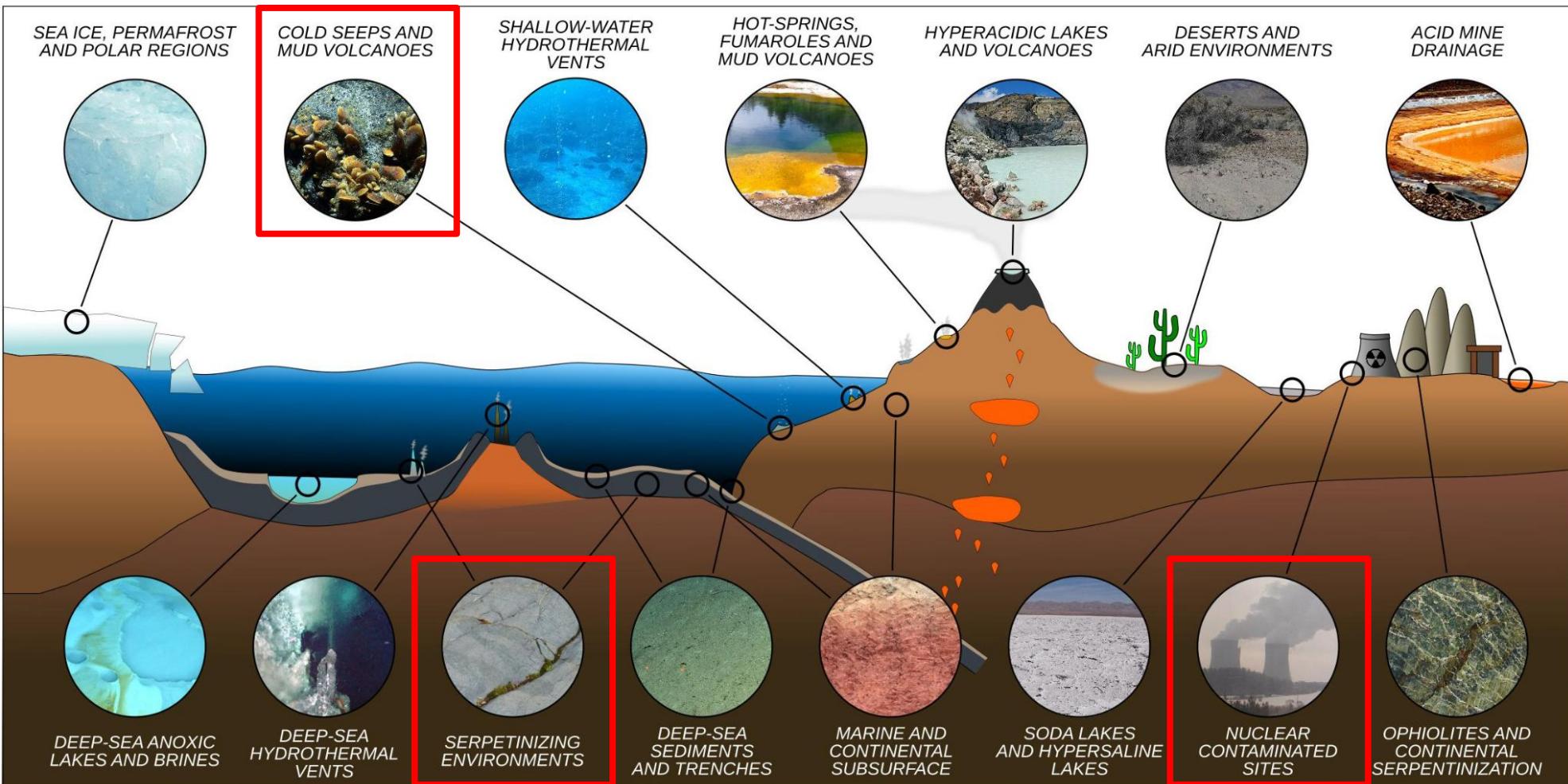
Hyperacidic crater lake at Kawah Ijen, Java, Indonesia  
**Temperature > 800 °C**

# Deep Sea Hydrothermal Vents

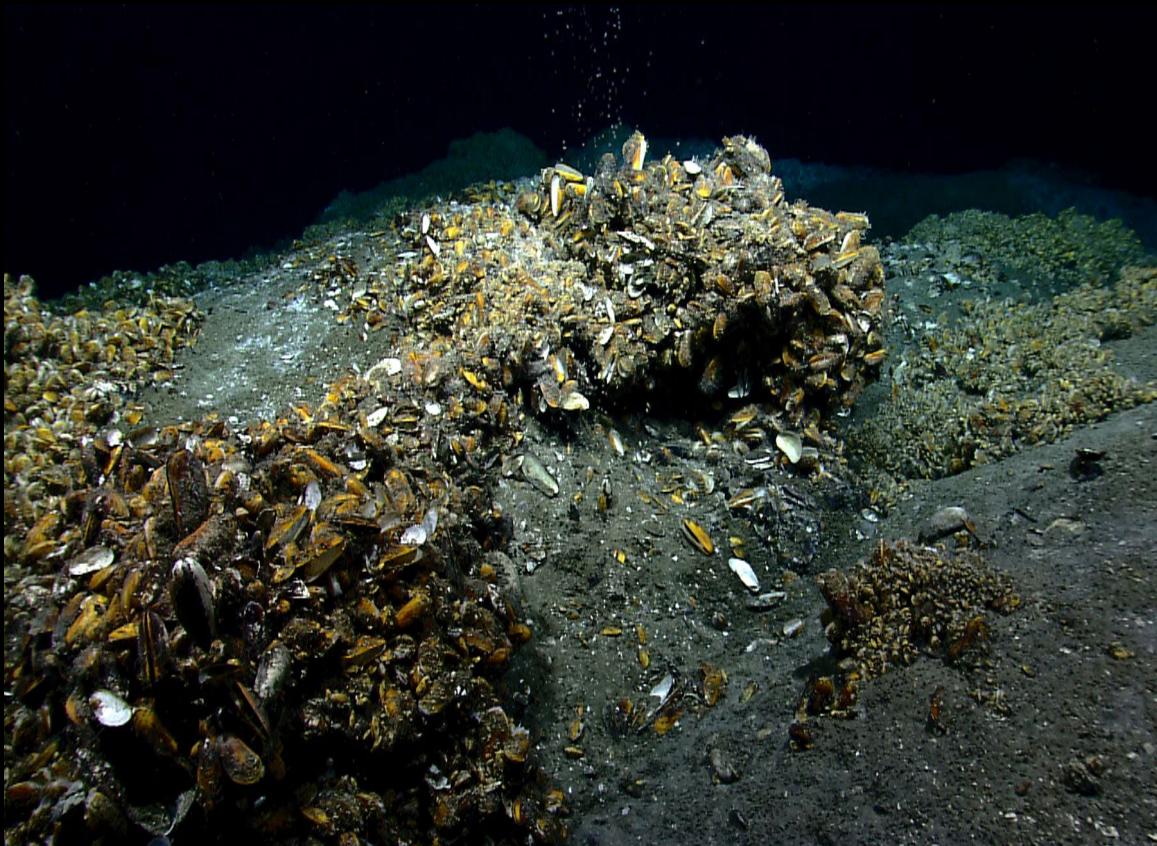


- they are hot springs on the ocean floor (2000–5000 m in depth)
- form along mid-ocean ridges where tectonic plates spread apart, magma rises and cools to form new crust and volcanic mountain chains
- seawater circulates deep in the ocean's crust and becomes super-heated by hot magma

# Thermophilic Environments



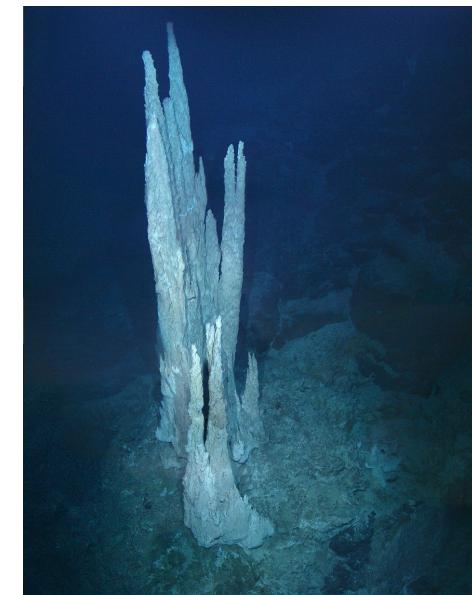
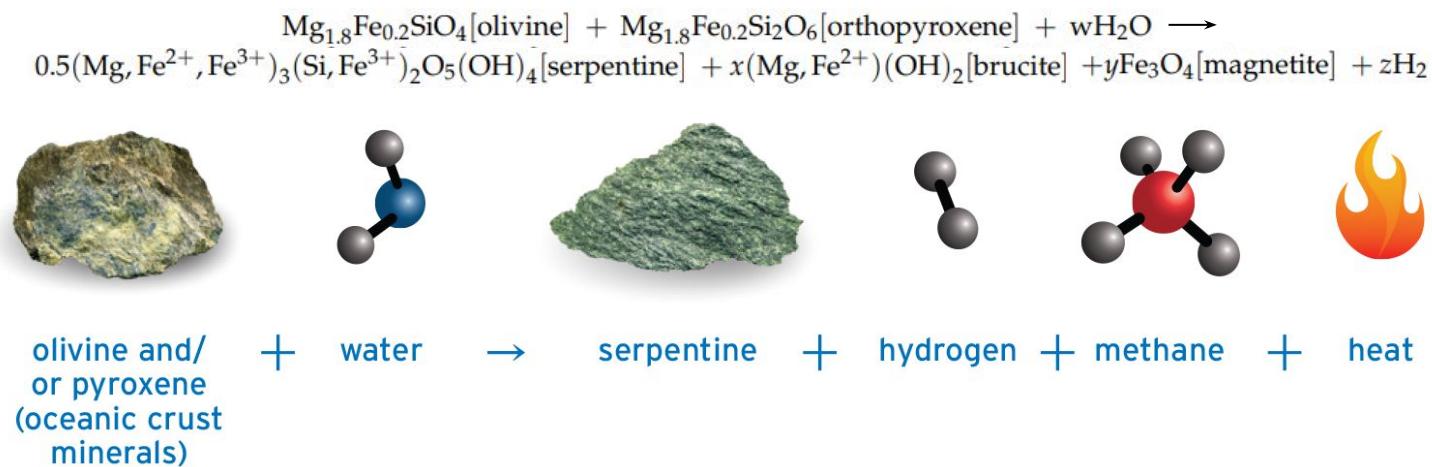
# Cold Seeps



- cold seeps occur over fissures on the seafloor caused by tectonic activity
- hydrogen sulfide, methane and other hydrocarbon-rich fluid "seep" out of those fissures, get diffused by sediment, and emerge over an area several hundred meters wide
- cold seeps develop unique topographical features, as the reactions between methane and seawater create carbonate rock formations and reefs over time
- indicated as "cold" because they do not exhibit the boiling temperatures of hydrothermal vents, they are not colder than the surrounding seafloor

# Serpentinized Environments

- Serpentinization is a hydrothermal alteration of ultramafic rocks of the mantle, occurring in submarine environment, such as slow- and ultraslow-spreading mid-ocean ridges, continental margins and forearc settings of subduction zones
- this phenomenon affects the chemical composition, rheology, magnetic properties, seismic structure and habitability of the shallow lithosphere, because of the high temperature and pH, limited availability of terminal electron acceptors, and low concentrations of inorganic carbon



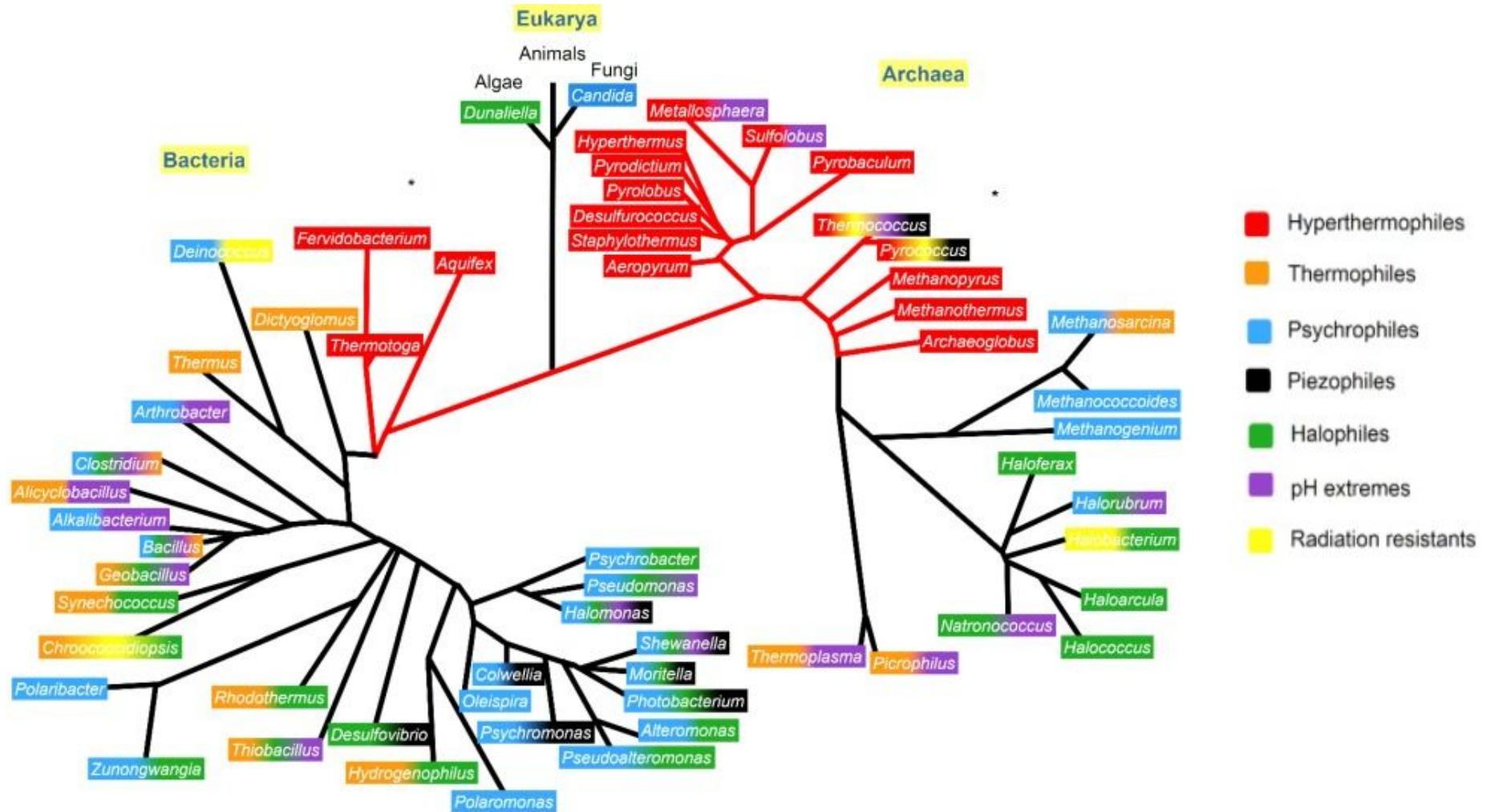
# Nuclear Contaminated Sites



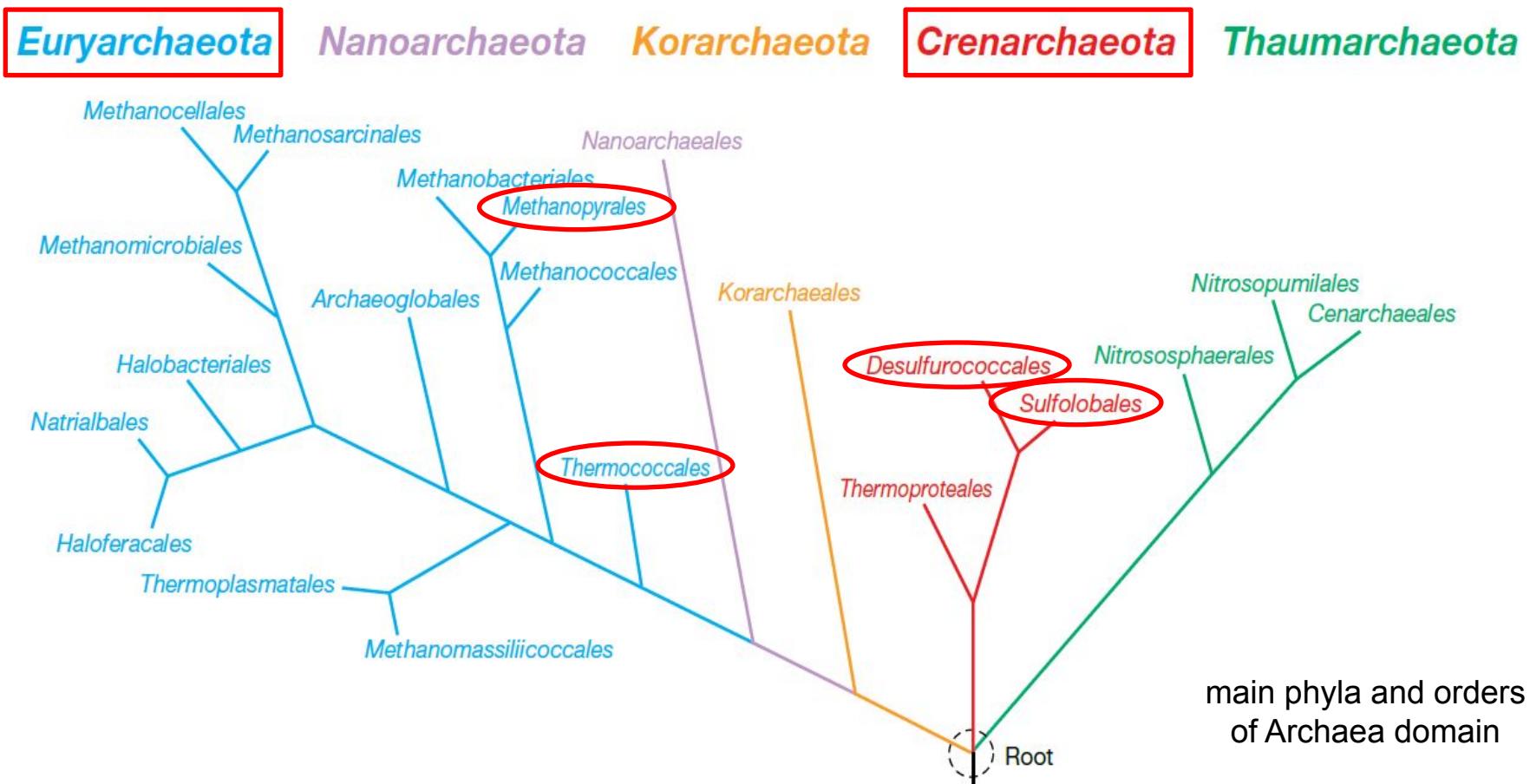
high temperatures  
and high radiations

**Which** are the most representative groups  
of **Thermophiles** and **Hyperthermophiles**?

# Phylogenetic tree of extremophiles

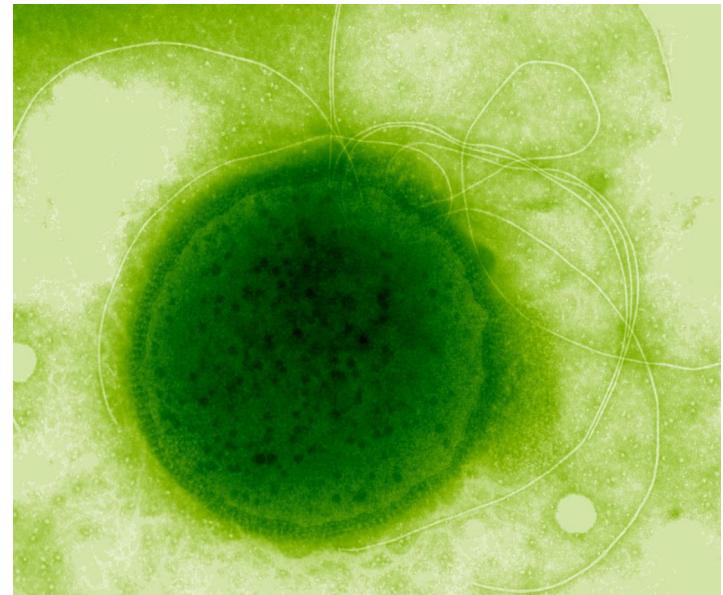


# Thermophilic and Hyperthermophilic Archaea



# Euryarchaeota phylum: *Thermococcus*

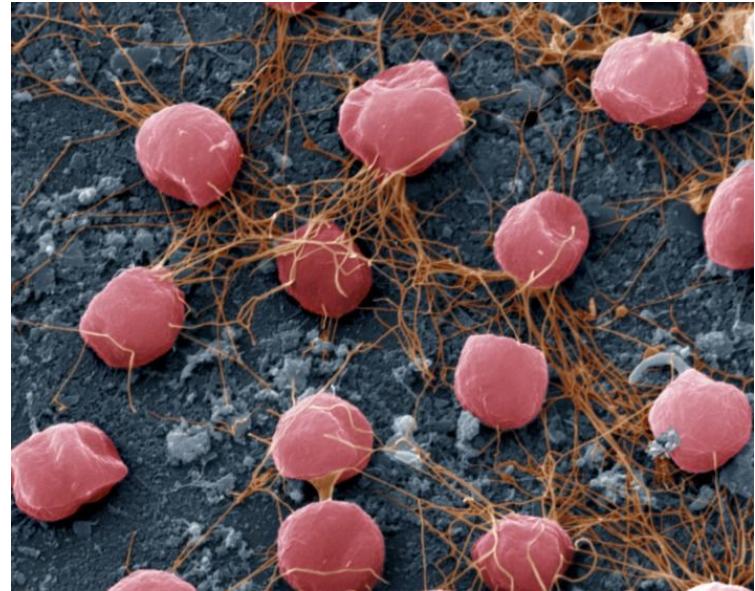
- spherical hyperthermophilic euryarchaeote indigenous to anoxic thermal waters
- obligate anaerobic chemoorganotroph that metabolizes proteins and other complex organic mixtures (starch and piruvate)
- elemental sulfur ( $S_0$ ) as terminal electron acceptor, generating hydrogen sulfide ( $H_2S$ ) and only  $H_2$  when  $S_0$  is absent
- grows at temperatures ranging from 60 to 102°C, with an optimum at 85°C, and between 5-9 pH, with an optimum at 6.7
- *T. kodakarensis* was first isolated from a solfatara near the shore of Kodakara Island, Kagoshima, Japan
- *T. kodakarensis* cells are irregular cocci 1–2  $\mu m$  in diameter, often occurring in pairs, and are highly motile by means of lophotrichous archaella



Domain:	Archaea
Phylum:	Euryarchaeota
Class:	Thermococci
Order:	Thermococcales
Family:	Thermococcaceae
Genus:	<i>Thermococcus</i>
Species:	<i>T. kodakarensis</i>

# Euryarchaeota phylum: *Pyrococcus*

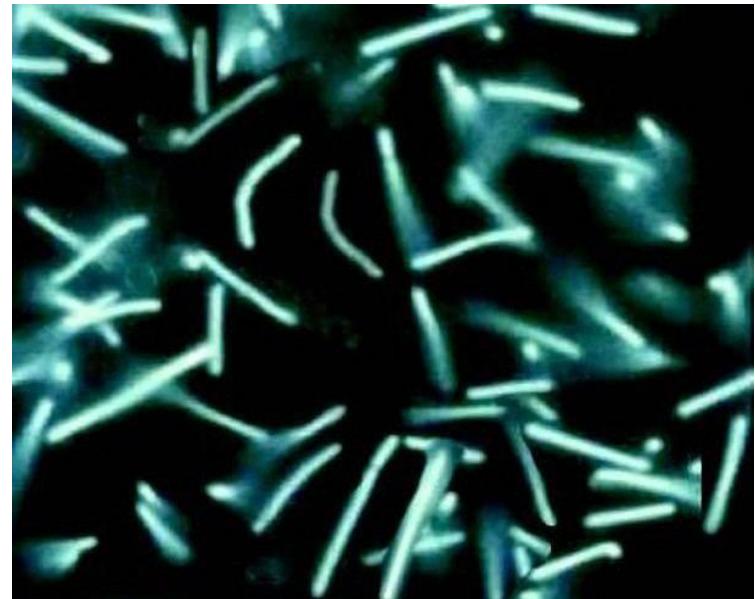
- strictly anaerobic and heterotrophic, it grows well on complex organic mixtures (yeast extract, maltose, starch) and protein sources
- elemental sulfur ( $S_0$ ) as terminal electron acceptor, generating hydrogen sulfide ( $H_2S$ ) and only  $H_2$  when  $S_0$  is absent
- grows at temperatures ranging from 70 to 106°C, with an optimum at 100°C, and between 5-9 pH, with an optimum at 7
- one of the few organisms identified as possessing aldehyde ferredoxin oxidoreductase enzymes containing tungsten, an element rarely found in biological molecules
- *P. furiosus* was first isolated from a hydrothermal vent near Vulcano Island, Italy
- *P. furiosus* cells appears as mostly regular cocci of 0.8  $\mu m$  to 1.5  $\mu m$  diameter with monopolar polytrichous flagellation



Domain:	Archaea
Phylum:	Euryarchaeota
Class:	Thermococci
Order:	Thermococcales
Family:	Thermococcaceae
Genus:	<i>Pyrococcus</i>
Species:	<i>P. furiosus</i>

# Euryarchaeota phylum: *Methanopyrus*

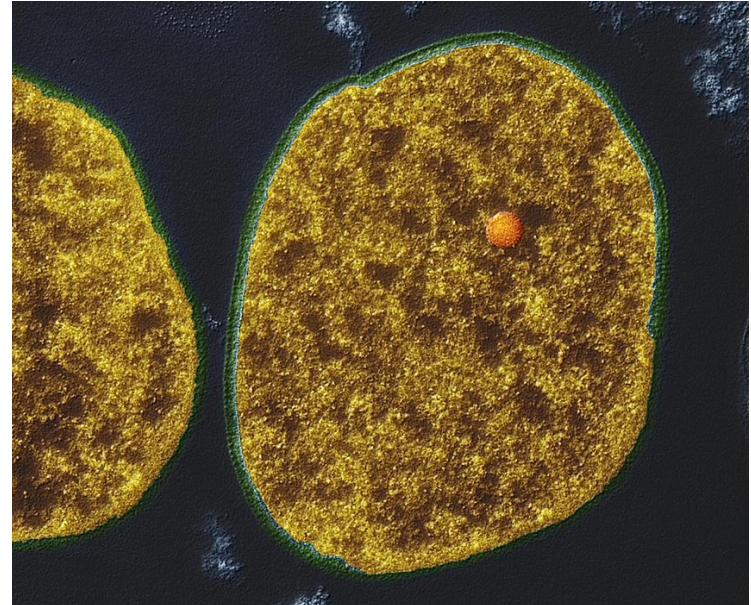
- rod-shaped methanogen euryarchaeote, obligate anaerobic, chemolithoautotroph
- utilizes hydrogen as an electron source and reduces carbon dioxide from the environment into methane  
$$4\text{H}_2 + \text{CO}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O}$$
- grows at temperatures ranging from 84 to 110°C, with an optimum at 98°C, and between 5.5-7 pH, with an optimum at 6.5
- *M. kandleri* was first isolated from the wall of “black smoker” hydrothermal vent chimneys from the Gulf of California at a depth of 2000 m
- strain 116 was reported to grow at 122°C, the highest temperature yet shown to support microbial growth, in particular pressure conditions
- *M. kandleri* cells have approximate length of 2-14 µm and diameter of 0.5 µm



Domain:	Archaea
Phylum:	Euryarchaeota
Class:	Methanopyri
Order:	Methanopyrales
Family:	Methanopyraceae
Genus:	<i>Methanopyrus</i>
Species:	<i>M. kandleri</i>

# Crenarchaeota phylum: *Sulfolobus*

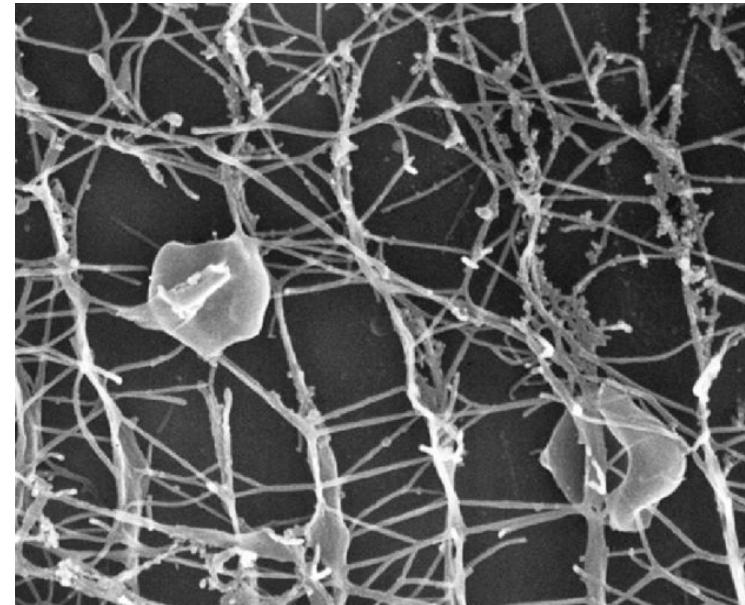
- thermoacidophilic archaeon, it was transferred from the genus *Sulfolobus* to the new genus *Saccharolobus* in 2018
- aerobic chemolithoautotroph that oxidizes  $\text{H}_2\text{S}$  or  $\text{S}_0$  to  $\text{H}_2\text{SO}_4$  and fixes  $\text{CO}_2$  as a carbon source, but can also grow chemoorganotrophically
- grows at temperatures ranging from 65 to 95°C, with an optimum at 80°C, and between 2-4 pH
- used as a model organism in archaeal research in cellular and molecular biology; well characterized reverse gyrase
- *S. solfataricus* was first isolated from Solfatara volcano in Pozzuoli, Italy
- *S. solfataricus* cells have a spherical shape and a clockwise flagellum, and are endowed with a peculiar monolayer membrane, composed by ether-linked lipids, joined covalently to form tetraethers



Domain:	Archaea
Phylum:	Thermoproteota
Class:	Thermoprotei
Order:	Sulfolobales
Family:	Sulfolobaceae
Genus:	<i>Saccharolobus</i>
Species:	<i>S. solfataricus</i>

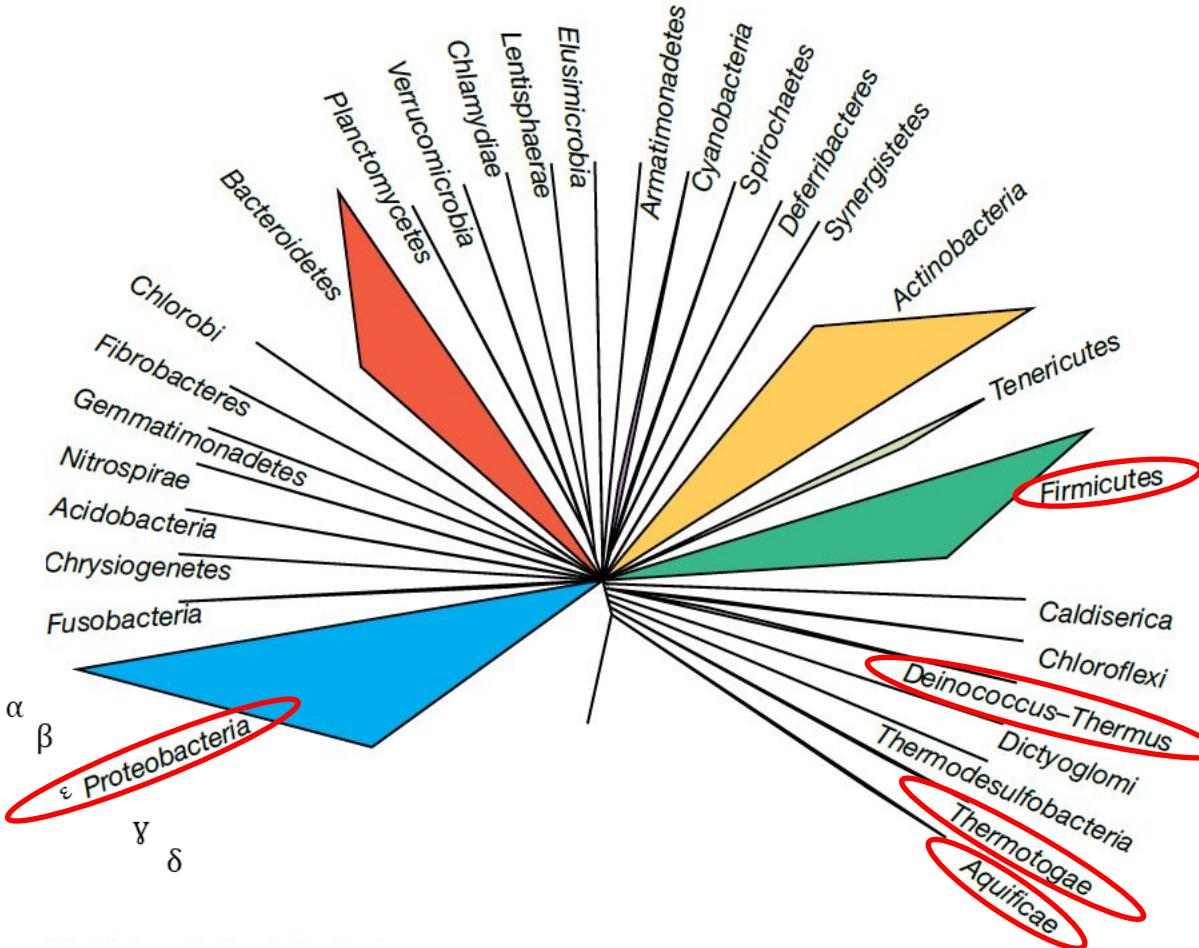
# Crenarchaeota phylum: *Pyrodictium*

- submarine hyperthermophilic archaea, strict anaerobe that grows chemolithotrophically on H<sub>2</sub> as an electron donor and S<sub>0</sub> as an electron acceptor, but can also grow chemoorganotrophically
- grows at temperatures ranging from 80 to 110°C, with an optimum at 105°C
- well studied organism for the peculiar thermostability molecular adaptations
- *Pyrodictium* are generally found in the porous walls of deep-sea vents where the inside temperatures get as high as 400°C, while the outside marine environment is 3°C
- *Pyrodictium* cells appear as flat disks, with a large size range (300 to 2500 nanometres in diameter) which may allow *Pyrodictium* to inhabit a variety of pores
- cells grow in unique flake-like shapes held together by a network of hollow cannulae, branching out and connecting with other cells



Domain:	Archaea
Phylum:	Thermoproteota
Class:	Thermoprotei
Order:	Desulfurococcales
Family:	Pyrodictiaceae
Genus:	<i>Pyrodictium</i>
Species:	<i>P. abyssi</i>

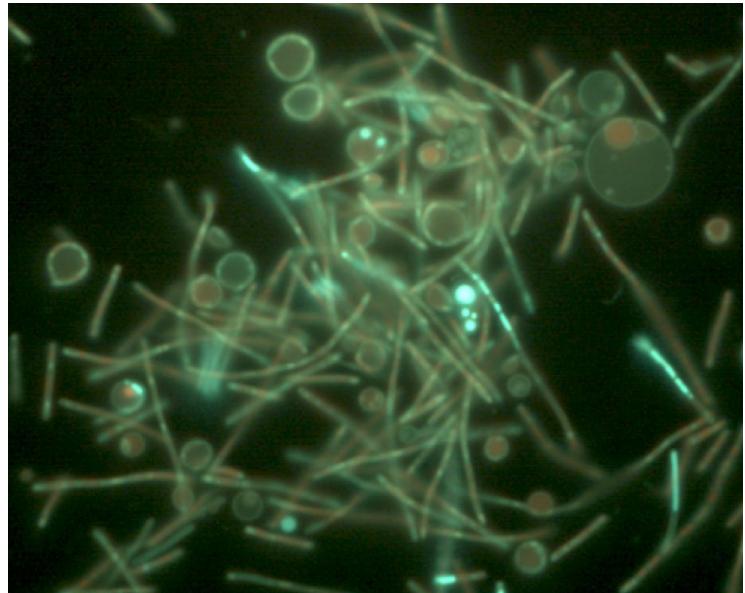
# Thermophilic and Hyperthermophilic Bacteria



main phyla of  
Bacteria domain

# Deinococcus-Thermus phylum: *Thermus*

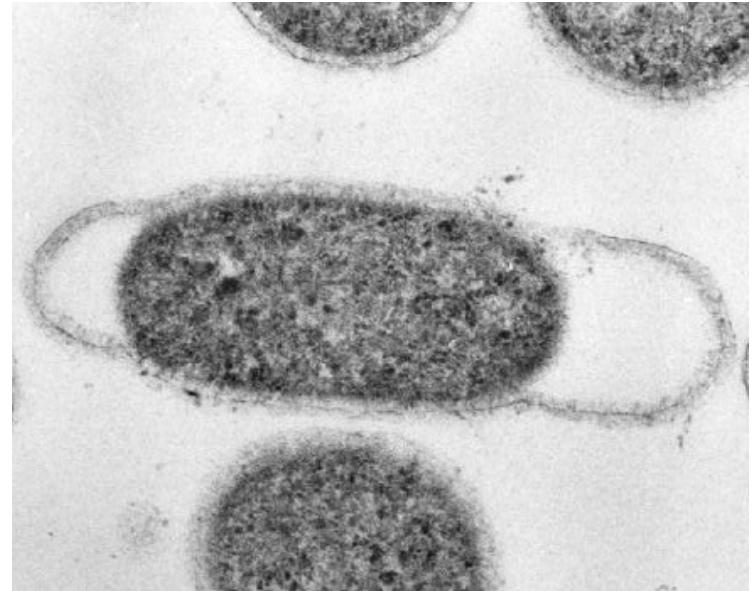
- thermophilic or hyperthermophilic bacteria, typically aerobic chemoorganotrophs that metabolize sugars, amino and organic acids, or various complex mixtures
- are distinguished by the presence of conserved signature indels (CSIs) and conserved signature proteins (CSPs) assumed to be related to the thermostability
- grows at temperatures ranging from 40 to 80°C, with an optimum at 70°C, and at a pH optimum of 7.5 to 7.8
- *T. aquaticus* was first isolated from a hot spring in the Lower Geyser Basin of Yellowstone National Park, USA
- precious source of thermostable enzymes, for example the Taq DNA polymerase
- *T. aquaticus* cells are generally of cylindrical shape with a diameter of 0.5 µm to 0.8 µm and a length of around 200 µm; in some cases it showed different morphologies, such as peculiar spherical bodies probably generated as temporary food and nucleotide storage



Domain:	Bacteria
Phylum:	Deinococcota
Class:	Deinococci
Order:	Thermales
Family:	Thermaceae
Genus:	<i>Thermus</i>
Species:	<i>T. aquaticus</i>

# Termotogae phylum: *Thermotoga*

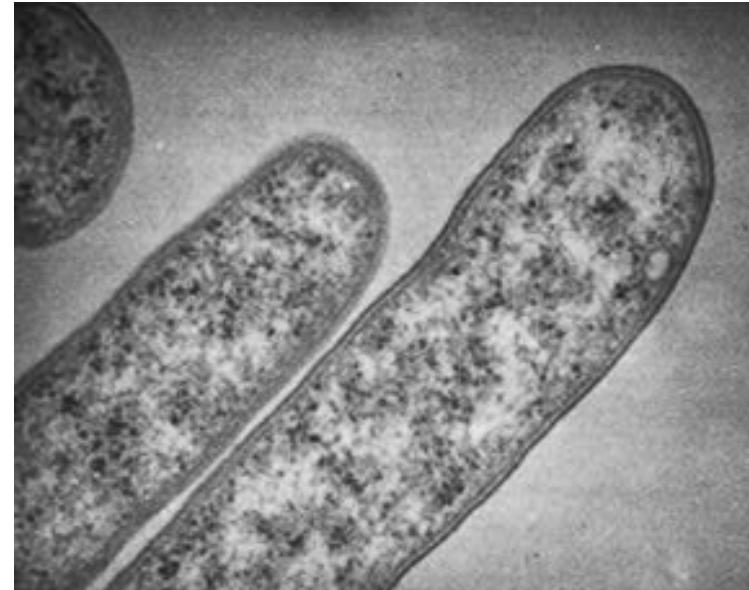
- rod-shaped thermophiles and hyperthermophiles, that form a sheathlike envelope (called *toga*)
- fermentative anaerobes, chemoorganotroph, catabolizing sugars or starch and producing lactate, acetate, CO<sub>2</sub>, and H<sub>2</sub> as fermentation products; can also grow by anaerobic respiration using H<sub>2</sub> as an electron donor and ferric iron (Fe<sup>+3</sup>) as acceptor
- over 20% of the genes of *Thermotoga* genus probably originated from Archaea by horizontal gene transfers
- grows at temperatures ranging from 55 to 90°C, with an optimum at 80°C, and at a pH optimum of 6-7
- *T. maritima* was first isolated from a geothermally heated shallow marine sediment in Vulcano island, Italy
- under investigation as cell factory for bio-hydrogen production, because of its potential to ferment a wide variety of sugars with the highest theoretical H<sub>2</sub>/glucose yields



Domain:	Bacteria
Phylum:	Thermotogota
Class:	Thermotogae
Order:	Thermotogales
Family:	Thermotogaceae
Genus:	<i>Thermotoga</i>
Species:	<i>T. maritima</i>

# Aquifae phylum: *Aquifex*

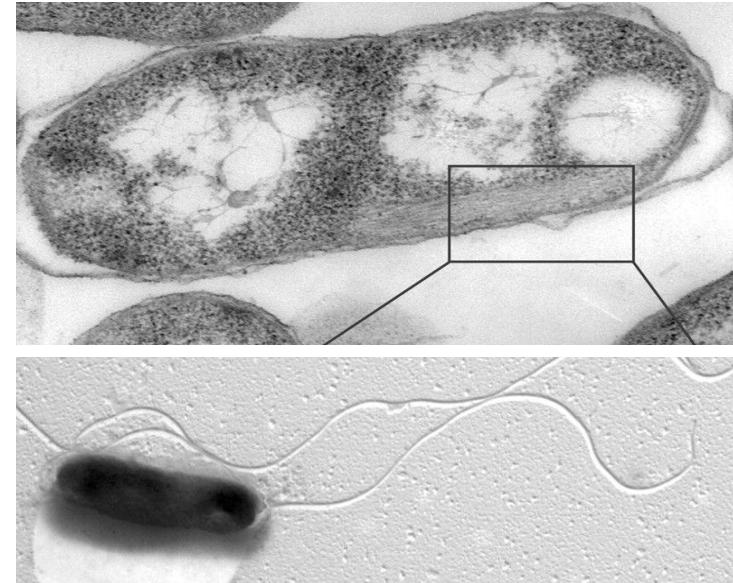
- obligately chemolithotrophic and autotrophic hyperthermophile, is the most thermophilic genus of all known Bacteria domain
- utilize H<sub>2</sub>, sulfur (S<sub>0</sub>), or thiosulfate (S<sub>2</sub>O<sub>3</sub><sup>2-</sup>) as electron donors and O<sub>2</sub> as electron acceptors, forming water
- sulfur-grown cells often contain inclusions of elemental sulfur
- generally strictly aerobic, with some exceptions, such as *A. pyrophilus*, an anaerobic nitrogen-reducing species (forming molecular nitrogen (N<sub>2</sub>) as end product instead of water)
- grows at temperatures ranging from 70 to 95°C (upper limit for bacteria), with an optimum at 85°C
- *A. pyrophilus* was first isolated from hot marine sediments (depth: 106 m) at the Kolbeinsey Ridge, Iceland
- cells are rod-shaped, with a length of 2 to 6 µm, and a diameter of around 0.5 µm, and are endowed with polytrichously inserted flagella for motility



Domain:	Bacteria
Phylum:	Aquificota
Class:	Aquificia
Order:	Aquicales
Family:	Aquicaceae
Genus:	<i>Aquifex</i>
Species:	<i>A. pyrophilus</i>

# $\epsilon$ -proteobacteria phylum: *Caminibacter*

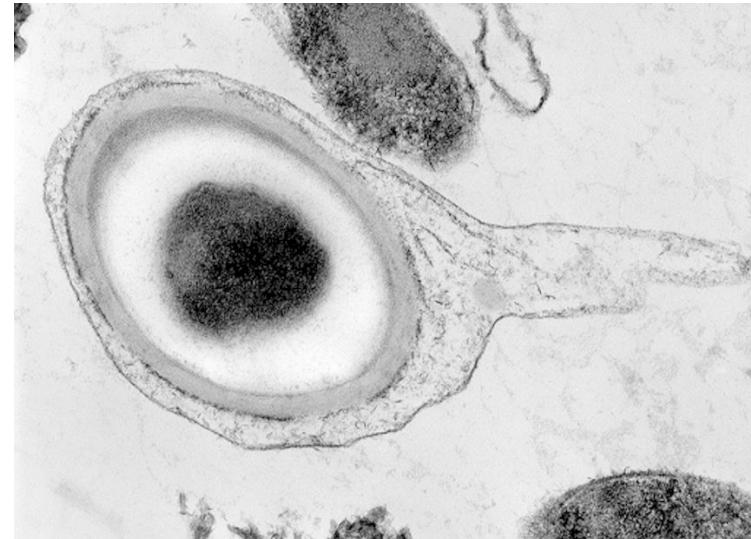
- $\epsilon$ -proteobacteria were initially defined by only a few pathogenic species belonging to *Campylobacter* and *Helicobacter* genus
- lately discovered environmental species, are anaerobic, chemolithoautotrophic thermophiles, especially abundant in anoxic sulfur-rich environments
- uses H<sub>2</sub> as the energy source and CO<sub>2</sub> as the carbon source, whereas nitrate (NO<sup>3-</sup>) or (S<sub>0</sub>) are used as the electron acceptor, with resulting production of ammonium (NH<sup>4+</sup>) and hydrogen sulfide (H<sub>2</sub>S)
- grows at temperatures ranging from 45 to 70°C, with an optimum at 55°C
- *C. mediatlanticus* was isolated from the walls of an active deep-sea hydrothermal vent chimney on the Mid-Atlantic Ridge
- AI-2/luxS quorum sensing system involved in regulation of flagellar motility, adhesion and biofilm formation



Domain:	Bacteria
Phylum:	Campylobacterota
Class:	Nautilia
Order:	Nautiales
Family:	Nautiliaceae
Genus:	<i>Caminibacter</i>
Species:	<i>C. mediatlanticus</i>

# Firmicutes phylum: *Carboxydothermus*

- the phylum is divided into the Clostridia, which are anaerobic, and the Bacilli, which are obligate or facultative aerobes
- Belonging to the Clostridia, *Carboxydothermus hydrogenoformans* is extremely thermophilic, strictly anaerobic, chemolithotroph
- uses carbon monoxide (CO) as their sole carbon source and water as an electron acceptor, producing carbon (CO<sub>2</sub>) dioxide and hydrogen (H<sub>2</sub>) as waste products
- C. hydrogenoformans* was first isolated from a hot springs on the Russian volcanic island of Kunashir
- grows at temperatures ranging from 40 to 78°C, with an optimum at 70°C, and pH optimum at 6.8-7.0
- round cells of about 1.3-2.4 µm and a diameter of 0.5 µm, under stressful growth conditions, form endospore-like structures

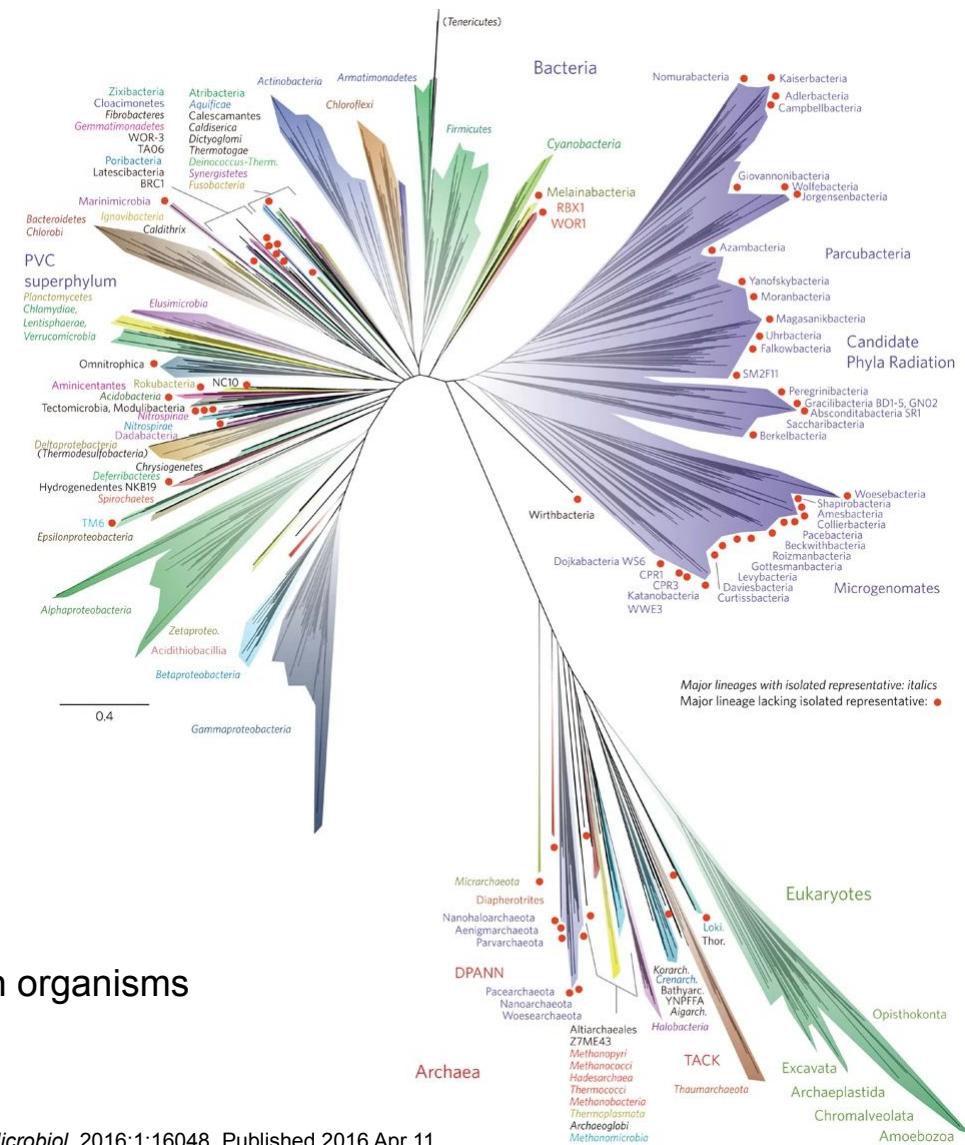


Domain:	Bacteria
Phylum:	Bacillota
Class:	Clostridia
Order:	Thermoanaerobacterales
Family:	Thermoanaerobacteraceae
Genus:	<i>Carboxydothermus</i>
Species:	<i>C. hydrogenoformans</i>

# Tree of life

A current view of the tree of life, encompassing the total diversity represented by sequenced genomes

over 1,000 uncultivated and little known organisms



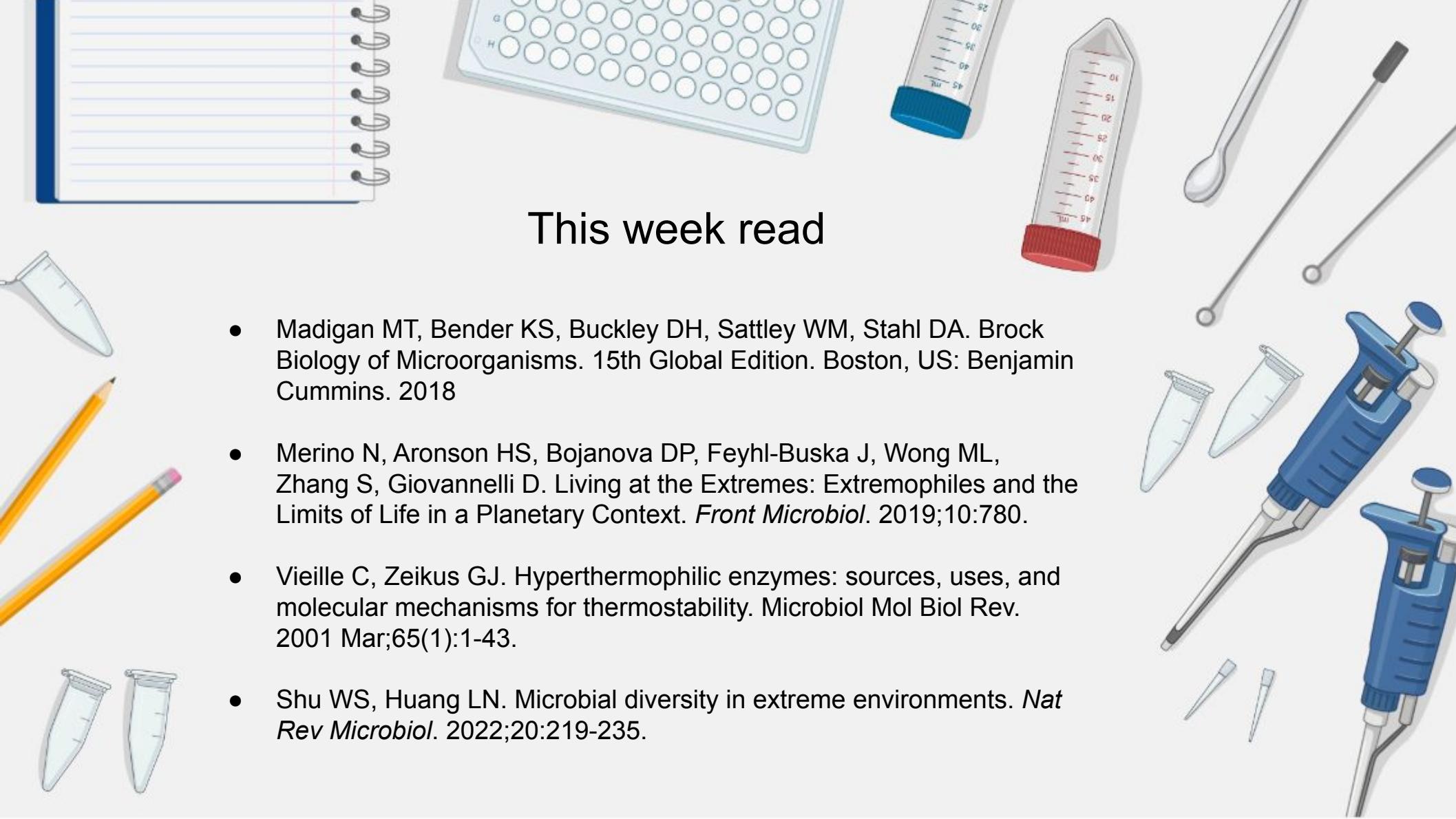


# **Thermophiles and Hyperthermophiles**

## biotechnological applications

# Hyperthermophilic Enzymes

Enzyme	Origin	Applications	Properties	Source or reference(s)
<i>Taq</i> polymerase	<i>T. aquaticus</i>	PCR technologies	Optimal activity at 75°C, pH 9.0	26, 199
Vent DNA polymerase	<i>T. litoralis</i>		Optimal activity at 75°C, proofreading activity	2, 271
Deep Vent DNA polymerase	<i>P. furiosus</i>		Optimal activity at 75°C, proofreading activity; $t_{1/2}$ , 23 h (95°C)	2, 343
<i>C. therm</i> DNA polymerase	<i>Carboxydothermus hydrogenoformans</i>	Reverse transcription-PCR	Reverse transcriptase activity, 3'→5' proofreading activity; optimal activity as 60–70°C	Roche Molecular Biochemicals
DNA polymerase	<i>Thermus thermophilus</i>		Reverse transcriptase activity	Roche Molecular Biochemicals
<i>Pfu</i> DNA ligase	<i>P. furiosus</i>	Ligase chain reaction and DNA ligations	Active at 45–80°C; $t_{1/2} > 60$ min (95°C)	Stratagene
<i>Tcs</i> DNA ligase	<i>Thermus scodductus</i>	Ligase chain reaction	Optimal activity at 45°C	Roche Molecular Biochemicals
DNA binding protein Ssod7	<i>S. solfataricus</i>	Time-reducing and specificity-enhancing in DNA-DNA hybridizations; locking of antisense oligonucleotide to target sequence	Sequence-aspecific DNA binding; ATP-independent, homology-dependent DNA annealing at 60°C	123
Serine protease (PRETAQ) Protease S	<i>Thermus</i> strain Rt41A <i>P. furiosus</i>	DNA and RNA purifications; cellular structures degradation prior to PCR Protein fragmentation for sequencing	Optimal activity at 90°C, pH 8.0; $t_{1/2}$ , 20 min (90°C) (+CaCl <sub>2</sub> ) Optimal activity at 85–95°C, pH 6.0–8.0; 80% active after 3 h (95°C)	Life Technologies TaKaRa Biomedicals
Methionine aminopeptidase	<i>P. furiosus</i>	Cleavage of the N-terminal Met in proteins	Optimal activity at 85–95°C, pH 7.0–8.0; stable for 1 h (75°C)	TaKaRa Biomedicals
Pyroglutamate aminopeptidase	<i>P. furiosus</i>	Cleavage of the N-terminal L-pyroglutamate in proteins	Optimal activity at 95–100°C, pH 6.0–9.0; 95% active after 2.5 h (75°C)	TaKaRa Biomedicals
Carboxypeptidase	<i>S. solfataricus</i>	C-terminal sequencing	Broad specificity (can release basic, acidic, and aromatic residues); stable in solvents at 40°C	66
Alkaline phosphatase	<i>T. neapolitana</i>	Enzyme-labeling applications where high stability is required	Optimal activity at 85°C, pH 9.9; $t_{1/2}$ , 4 h (90°C) (+ Co <sup>2+</sup> )	87

A background collage of various laboratory glassware and tools, including a spiral-bound notebook, a 96-well plate, test tubes, a graduated cylinder, a dropper, a pencil, and several blue pipettes.

## This week read

- Madigan MT, Bender KS, Buckley DH, Sattley WM, Stahl DA. Brock Biology of Microorganisms. 15th Global Edition. Boston, US: Benjamin Cummins. 2018
- Merino N, Aronson HS, Bojanova DP, Feyhl-Buska J, Wong ML, Zhang S, Giovannelli D. Living at the Extremes: Extremophiles and the Limits of Life in a Planetary Context. *Front Microbiol.* 2019;10:780.
- Vieille C, Zeikus GJ. Hyperthermophilic enzymes: sources, uses, and molecular mechanisms for thermostability. *Microbiol Mol Biol Rev.* 2001 Mar;65(1):1-43.
- Shu WS, Huang LN. Microbial diversity in extreme environments. *Nat Rev Microbiol.* 2022;20:219-235.