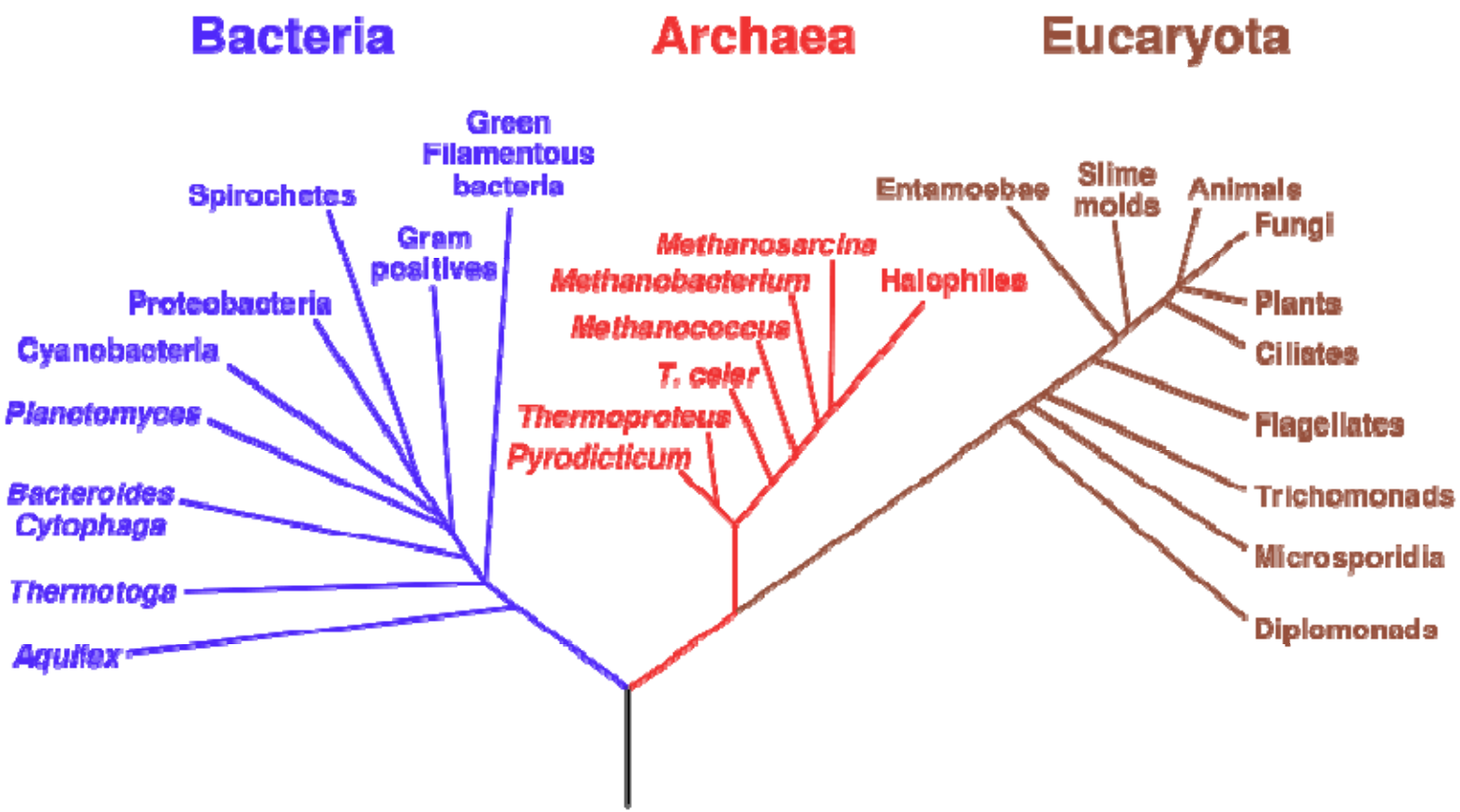
Phylogenetic Tree of Life



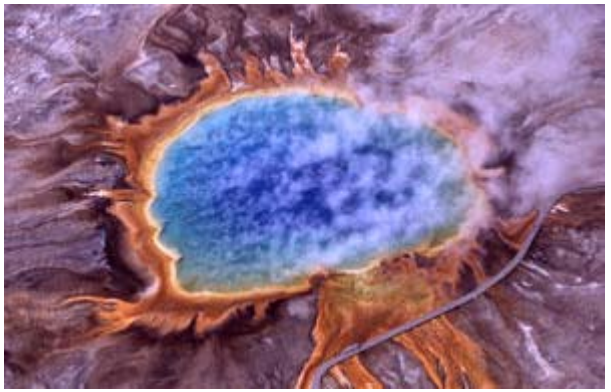
Thermophilic organism

Thermophiles are organisms that grow and thrive at temperatures (60 – 80°C) that are often too high for **mesophiles**—most thermophiles are Archaea

Some organisms grow at even higher temperatures and pressure (> 80 °C)—these are called **hyperthermophiles**—all hyperthermophiles are Archaea

e.g. Pyrococcus furiosus lives at 100 °C

Other extremophiles include organisms that live at extreme pH values, pressure, high doses of ionizing radiation, desiccating conditions, salt concentration, etc



Hyperthermophiles of Yellowstone National Park

Thermozyme

Enzymes isolated from (hyper) thermophiles are called **thermozymes**

Thermozymes have found applications in biotechnology where their unusual thermal stability makes them uniquely qualified for tasks under conditions that would easily denature mesophilic enzymes

Polymerase chain reaction

invented by Kary Mullis (Chemistry Nobel prize 1993)

a biochemical reaction used to amplify nucleic acids

requires cycling between high (~ 95 °C) and medium (~ 50 – 75 °C) temperatures

enzymes that are active at high temperature for many hours

Taq (Thermus aquaticus), Pfu (Pyrococcus furiosus)

Deep Vent (half life 23 hr at 95 °C) polymerases

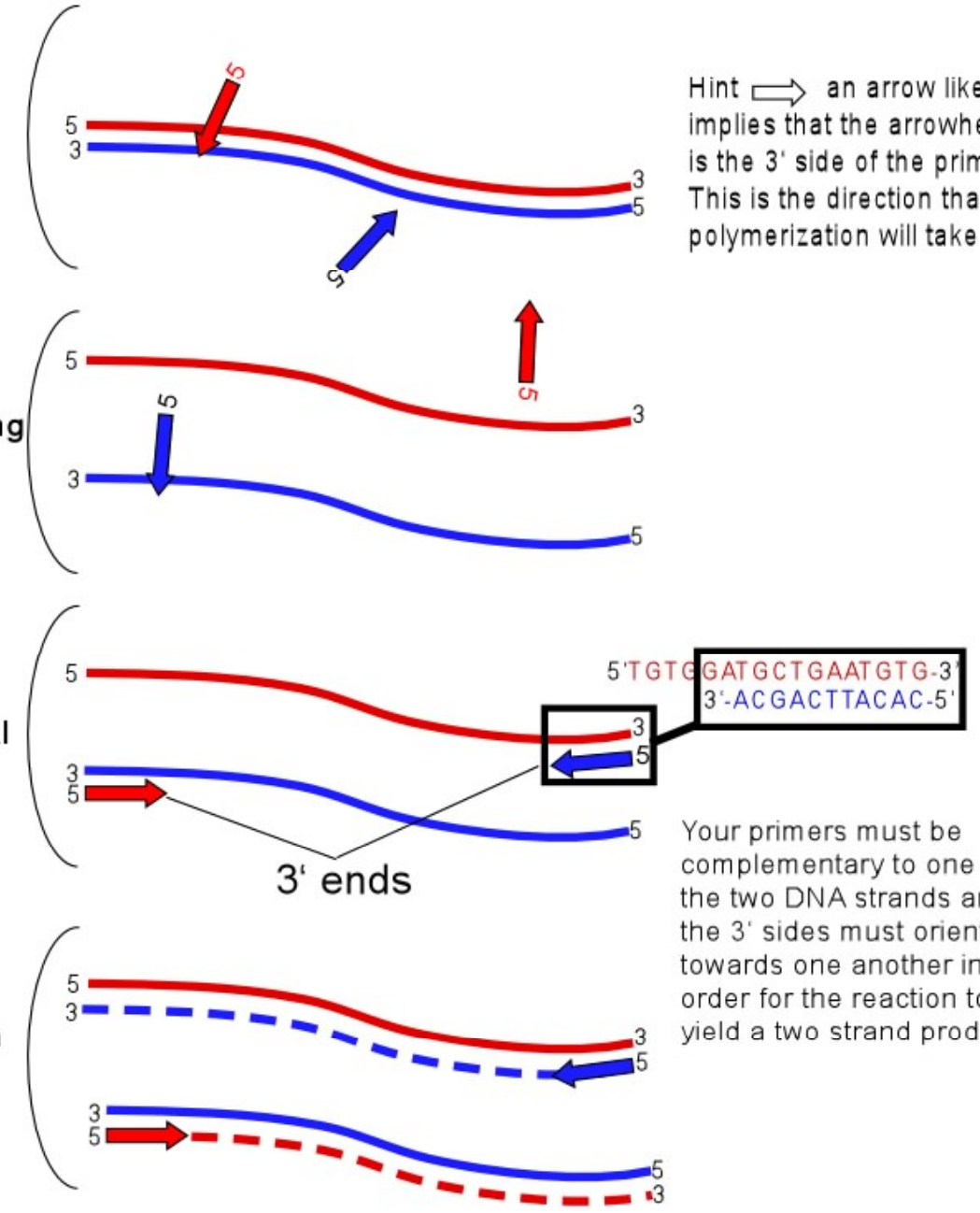
Inside the PCR reaction tube...

0) 55 C Before Cycle

1) 90 C DNA Denaturing

2) 55 C Primers anneal

3) 72 C Polymerization



Hint ⇨ an arrow like this implies that the arrowhead is the 3' side of the primer. This is the direction that the polymerization will take place.

Your primers must be complementary to one of the two DNA strands and the 3' sides must orient towards one another in order for the reaction to yield a two strand product.

Why study thermophilic proteins

Extremely high thermostability can be achieved in some proteins through a combination of van der Waals, H-bond, and electrostatic interaction

Evaluate what factors contribute to their high stability and thus learn to correlate **structural** properties with **thermodynamic** properties

Practical concerns: When engineering proteins for **therapeutic** use, we want to maximize (thermal) stability while maintaining activity

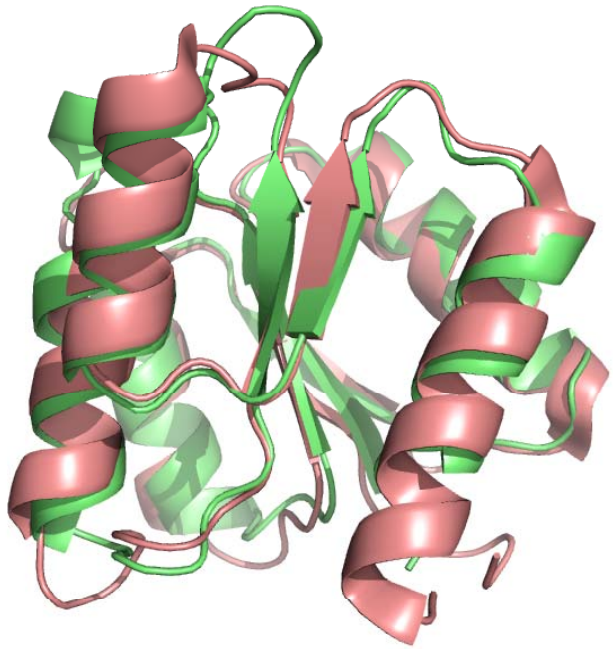
General observations

(Hyper)Thermophilic proteins are highly homologous to their mesophilic counterparts both in sequence (40 – 85%) and structure

The catalytic mechanism is conserved in both classes of proteins

Thermophilic proteins expressed in mesophilic organisms are functional—i.e. these proteins are intrinsically thermostable and do not require extrinsic factors such as glycosylation

Genes from hyperthermophilic archaea can complement yeast mutations



(green) E.coli CheY
(pink) T. maritima CheY

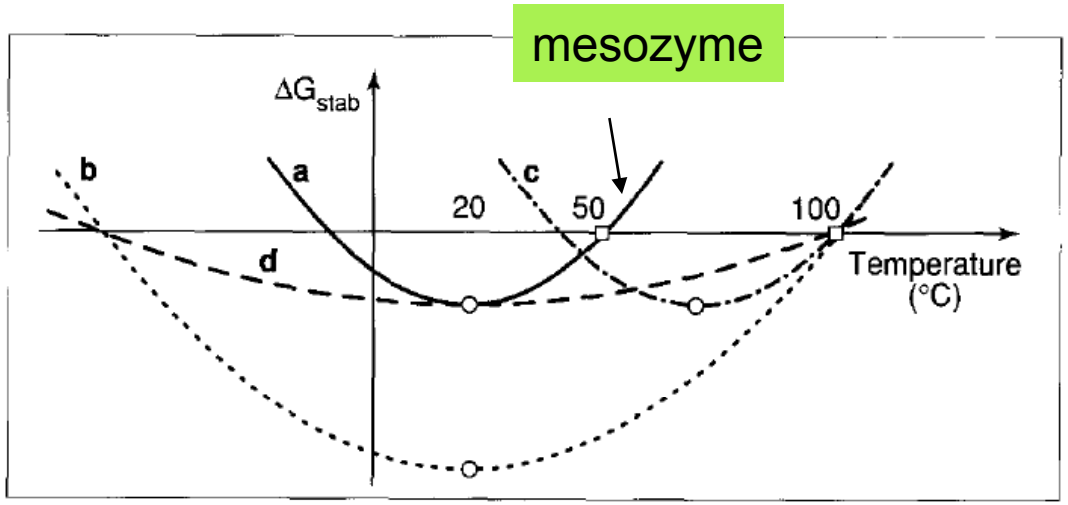
CheY: response regulator in the chemotaxis pathway

Fundamentals of protein stability

Stabilization may require contributions from all levels of hierarchy: amino acid identity, packing, secondary and super secondary structure, domain and subunit

Free energy of folding changes parabolically as a function of temperature

$$U \longrightarrow F \qquad \Delta G$$



Need a mechanism of stabilizing a protein without raising the temperature at which it becomes fully active

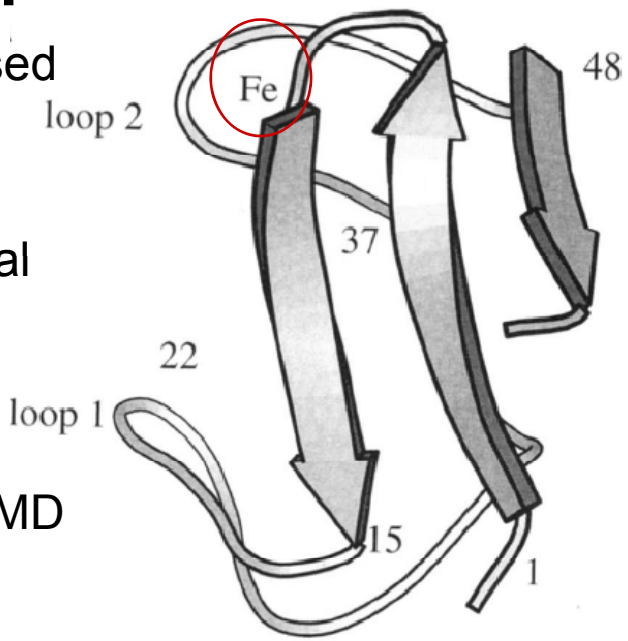
Computer simulation

Rubredoxin is a small model protein containing a Fe-based redox center that participates in electron transfer

Small size renders the protein amenable to computational studies

Unfolding of rubredoxin from mesophilic and hyperthermophilic organisms has been simulated using MD

Lazaridis et al, Protein Sci 6, 2589 (1997)



meso →
thermo →

```
                1                  11                 21                 31                 41                 51
                |                  |                  |                  |                  |                  |
RdDv  MKKYVCTVCG YEYDPAEGDP DNGVKPGTSF DDLPADWVCP VCGAPKSEFE AA
RdPf    AKWVCKICG YIYDEDAGDP DNGISPGTKF EELPDDWVCP ICGAPKSEFE KLED
                  *                  * *                *                  *    *                  * *
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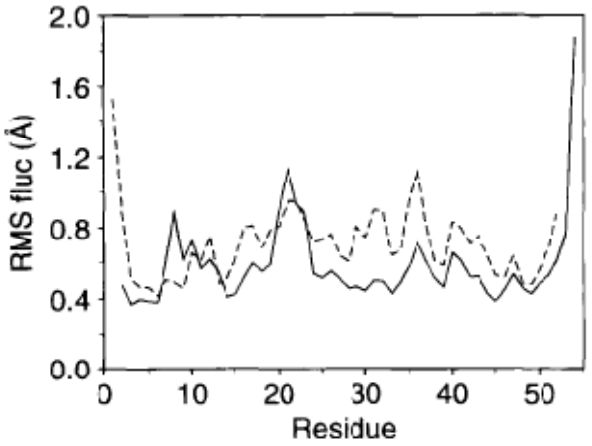
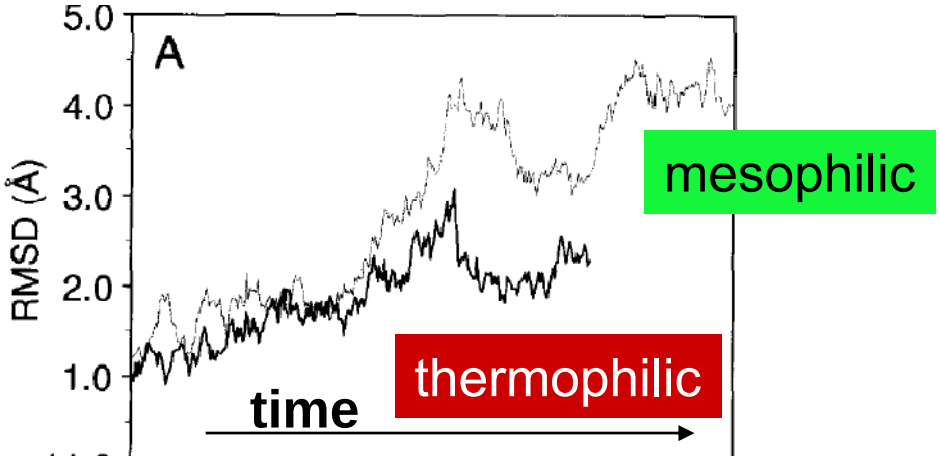




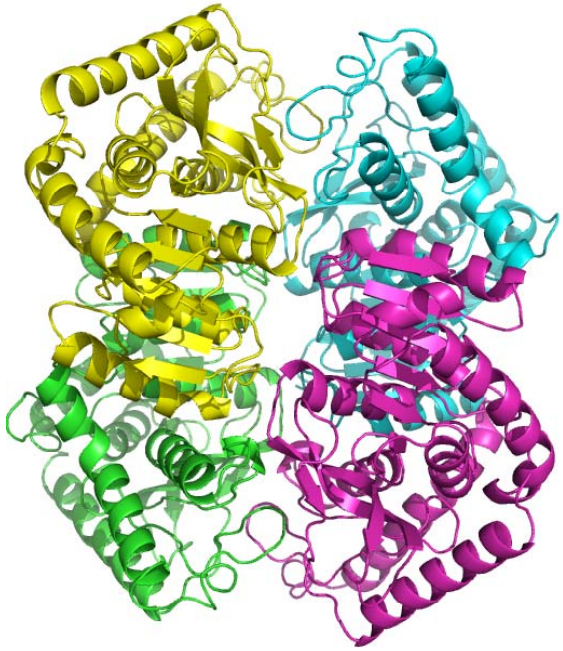
Lactate dehydrogenase (LDH)

Comparative study of **homologous proteins** from mesophilic and thermophilic organisms can help reveal the mechanism by which thermal stability of a protein is achieved

LDH catalyzes the last step in anaerobic glycolysis by converting pyruvate to lactate

High resolution structures are available for homologous LDH from different organisms

Tm: *Thermotoga maritima*
Bs: *Bacillus stearothermophilus*
Bl: *Bifiobacterium longum*
Lc: *Lactobacillus casei*
Ss: *Sus scrofa*
Sa: *Squalus acanthia*



Auerbach et al. Structure 6, 769 (1998)

	Tm	Bs	Bl	Lc	Ss	Sa
Growth temperature (°C)	50-90	40-65	37-41	30-40	37	20
Hydrophobic/charged surface area	0.57	0.66	0.76	0.73	0.89	0.86
Intrasubunit on pairs (4 Å cutoff)	17	16.5	8	8	10.5	13
Alpha helix (%)	46	42	42	42	40	=
Beta strand (%)	23	22	19	20	19	=

Tm LDH (*Thermotoga maritima*, most stable)

- has the fewest and smallest cavities
- has the most intrasubunit ion pairs (salt bridges)
- has the highest percentage of Arg
- increased hydrophobic intersubunit interaction (three Phe per monomer)

No significant increase in the number of “intersubunit” ion pairs

No clear correlation with the core packing (fraction of atoms with zero ASA)

No correlation with the volume of the monomer and tetramer

No correlation with the surface to volume ratio

No correlation with the abundance of Lys/Arg near the C-terminus of helix or of Asp/Glu near the N-terminus

No correlation with the number of hydrogen bonds in the monomer and tetramer

growth temp

31 °C

37 °C

55 °C

85 °C

100 °C

Citrate synthase

acetyl-CoA + oxaloacetate + H_2O → citrate + CoA-SH

The structure of citrate synthase from organisms that live at five different temperatures has been determined

Source organism	Optimum growth temperature (°C)
Arthrobacter DS23R	31 [a]
Pig	37
Thermoplasma acidophilum	55
Sulfolobus solfataricus	85
Pyrococcus furiosus	100

All CS are alpha helical homodimeric proteins with the active site located at the interface with residues from both subunits—maintaining the dimer structure is essential for enzymatic activity

Bell et al, Eur J Biochem 269, 6250 (2002)

- Bacterial CS (including ArCS) have smaller surface areas than pigCS
- Psychrophilic (cold) CS has the smallest volume

- Total amount of exposed hydrophobic area decreases as the thermostability increases
- Number and size of internal cavities decreases with thermostability
- Hyperthermophilic CS has the highest percentage of buried atoms

- Conflicting results for the number of ion pairs
  - psychrophilic has the most overall
  - thermophilic has the most interfacial ion pairs
  - mesophilic has more intrasubunit interactions

- Loops are stabilized through ionic interactions in thermophilic and psychrophilic CS
- Loop length does not correlate with thermostability—for **this** example

No simple rules for thermostability

Thermostability cannot be explained in terms of amino acid composition as the trends are weak and there are too many exceptions

Distribution of the residues and their interactions in the protein are important

No universally applicable generalizations have been found

Factors that contribute toward thermostability include:

- higher oligomeric state to modulate surface to volume ratio
- increased number of stabilizing interactions
- reduction in size of surface loops to minimize entropy
- increased hydrophobic interaction (e.g. burial of hydrophobic residues)
- remove factors that could lead to irreversible modifications—e.g. fewer Asn residues as Asn may catalyze peptide chain hydrolysis

Possible mechanisms of thermostability

- More stable protein core
  - reduction in number and volume of cavities
  - increased number of hydrophobic residues because hydrophobic effects increase with temperature
- Number of hydrogen bonds
- Tendency to form higher order oligomers
  - optimized intersubunit contacts within oligomers
- Increased secondary structure
  - stabilization of alpha helices using residues with higher helix propensity
  - redesign of the helix caps with Gly
  - but contribution from beta sheets is uncertain
  - number of helix and sheet doesn't correlate with stability
- Decrease in conformational degrees of freedom
  - increased number of Pro
- Improved electrostatic interactions, i.e. salt bridges
  - intrahelical, interhelical, intrasubunit, intersubunit, surface, buried

Petsko, Meth Enzy 334, 469 (2001)

Evolution of thermostability

How did thermostability evolve?

what are potential physical mechanisms and how do organisms apply these mechanisms to adapt

Some organisms started inhabiting hot habitats from the very beginning

mostly archae

their enzymes are more compact and hydrophobic

“designed” their proteins to probe alternate structural repertoire

Others started out as mesophilic organisms but relocated to hot environments

mostly bacteria

these organisms had to make adjustments at the amino acid level to achieve

Thermostability may be **structural** or **sequential**

- Berezovsky and Shakhnovich PNAS 102, 12742 (2005)
- MD simulated unfolding and sequence analysis
- structural: increase in overall contacts and interactions; also more compact
- sequential: a few exceptionally strong interactions, e.g. stabilizing ionic interaction

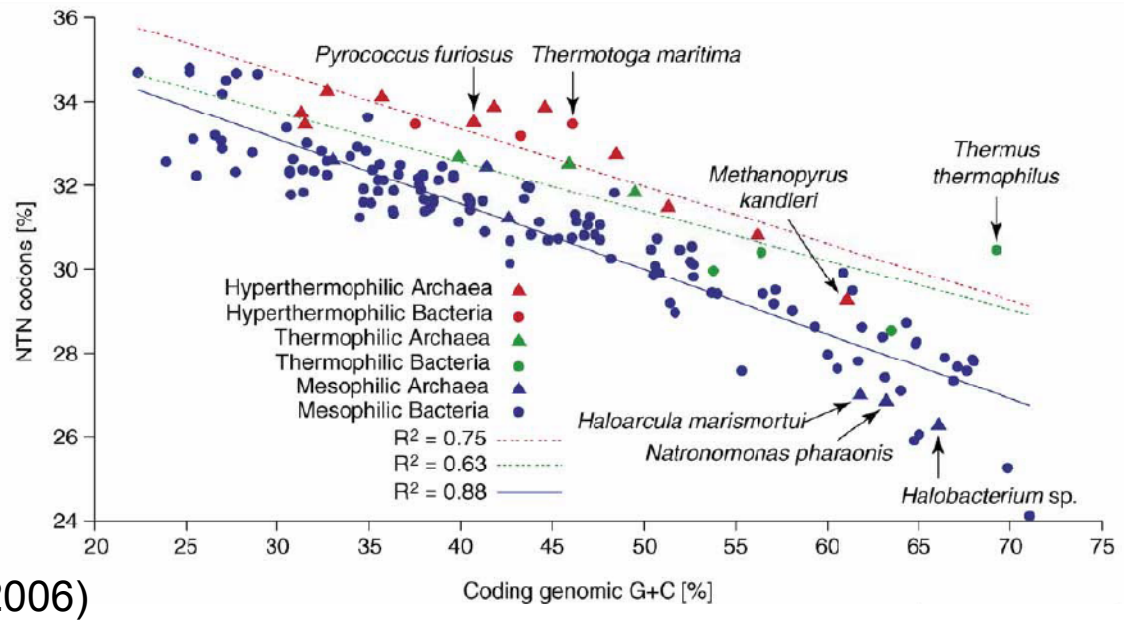
NTN hypothesis

The codon NTN codes for hydrophobic residues: F, I, V, L, M

Genomic analysis of 14 hyperthermophiles, 7 thermophiles, and 142 mesophiles shows an increase in hydrophobic residues in thermophilic organisms by three for every 100 amino acids

An increased frequency of NTN is observed both in archae and bacteria

- use of hydrophobic residues for thermostability deviates from the mechanistic distinctions based on phylogenetic history



Lieph, et al Trends in Microbio 14, 423 (2006)