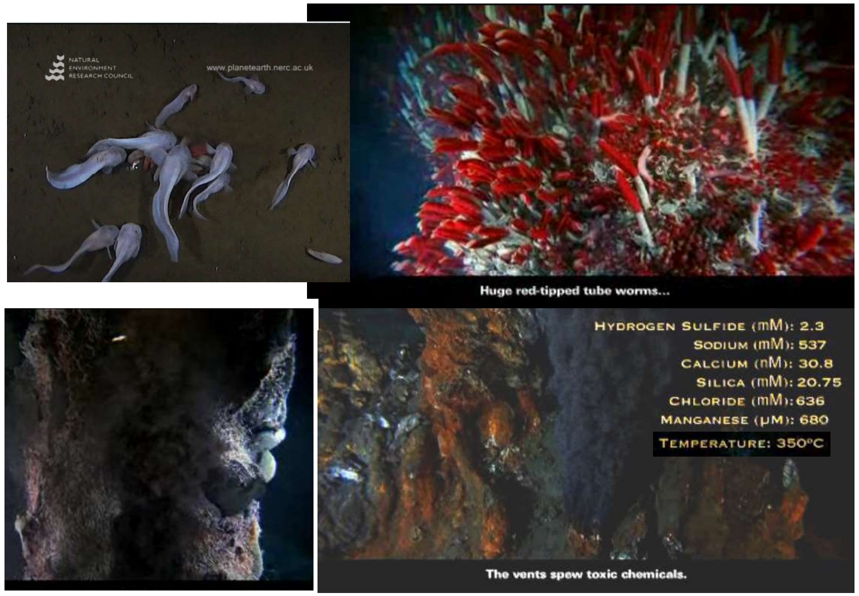
# Stability of biological systems

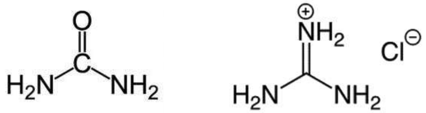
László Smeller




http://ocean.si.edu/ocean-videos/hydrothermal-vent-creatures

# Destabilizing environmental factors

- Physical
  - high temperature
  - low temperature
  - (high) pressure
- Chemical
  - urea (high conc.)
  - GuHCl [guanidinium chloride] (high conc.)
  - extreme pH

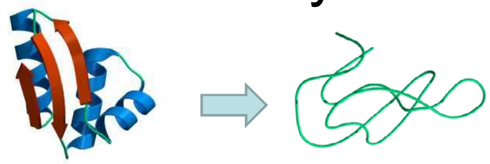



Thermophiles, a type of extremophile, produce some of the bright colors of Grand Prismatic Spring, Yellowstone National Park

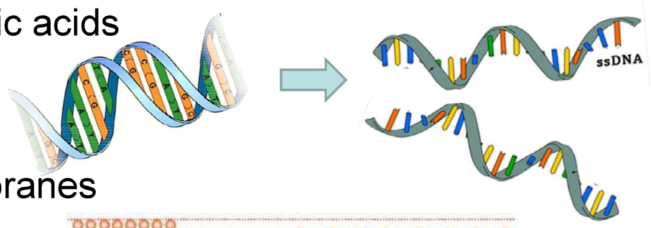
The first extremophile to have its genome sequenced was Methanococcus jannaschii, a microbe that lives near hydrothermal vents 2,600 meters below sea level, where temperatures approach the boiling point of water and the pressure is sufficient to crush an ordinary submarine. Image credit: NOAA

# Order and disorder in macromolecular systems

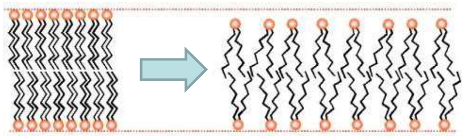
Proteins



Nucleic acids




Membranes



# Physical parameter: Temperature

Two state model: states (1) and (2)
(e.g. ordered and disordered states)

Enthalpy: $H=U+pV$ Gibbs free energy: $G=H-TS$

$\Delta H(T)=H_2(T)-H_1(T)$

$$\left.\frac{\partial \Delta H}{\partial T}\right|_p = \Delta C_p \qquad \left.\frac{\partial \Delta S}{\partial T}\right|_p = \frac{\Delta C_p}{T}$$

$$\Delta H(T) = \Delta H(T_0) + \int_{T_0}^{T} \Delta C_p dT$$

$$\Delta S(T) = \Delta S(T_0) + \int_{T_0}^{T} \frac{\Delta C_p}{T} dT$$

Let $T_0$ be selected on the way that:

$G_1(T_0)=G_2(T_0)$

$\Delta G(T_0)=G_2(T_0)-G_1(T_0)=0$

(I.e. $T_0$ is a phase transition temperature)

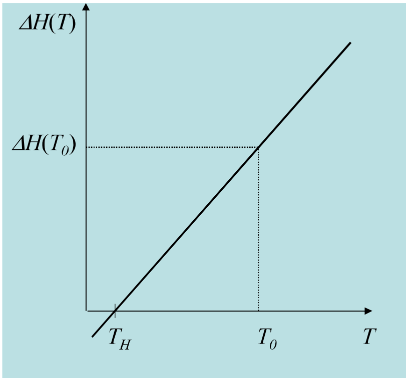
$\Delta G(T_0)=\Delta H_D(T_0)-T_0\Delta S_N(T_0)=0$

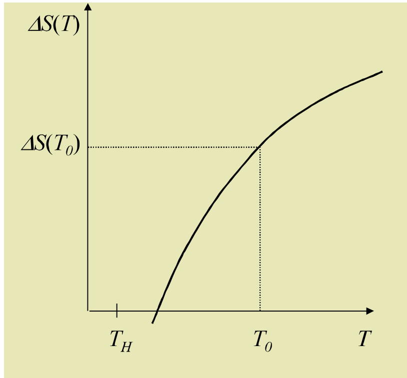
Let us suppose that $C_p$ does not depend on $T$:

$$\Delta H(T) = \Delta H(T_0) + \int_{T_0}^{T} \Delta C_p dT = \Delta H(T_0) + (T - T_0)\Delta C_p$$

$$\Delta S(T) = \Delta S(T_0) + \int_{T_0}^{T} \frac{\Delta C_p}{T} dT = \Delta S(T_0) + \Delta C_p \ln\left(\frac{T}{T_0}\right)$$

$$\Delta H(T) = \Delta H(T_0) + \int_{T_0}^{T} \Delta C_p dT = \Delta H(T_0) + (T - T_0)\Delta C_p$$



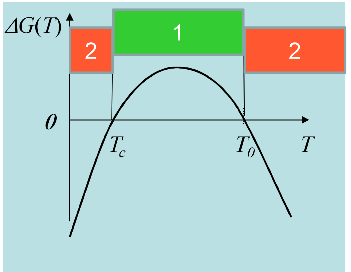




$$\Delta S(T) = \Delta S(T_0) + \int_{T_0}^{T} \frac{\Delta C_p}{T} dT = \Delta S(T_0) + \Delta C_p \ln\left(\frac{T}{T_0}\right)$$

$$\Delta G(T) = \Delta H(T) - T\Delta S(T) =$$
$$\Delta G(T_0) + (T - T_0)\left(\Delta C_p - \Delta S(T_0)\right) - T\,\Delta C_p \ln\left(\frac{T}{T_0}\right)$$
$$= -\Delta S(T_0)(T - T_0) - \Delta C_p\left(T\left(ln\left(\frac{T}{T_0}\right) - 1\right) + T_0\right)$$
$$= -\Delta S(T_0)(T - T_0) - \Delta C_p\frac{(T - T_0)^2}{2T_0}$$

$\Delta G(T_0)=G_2(T_0)\text{-}G_1(T_0)=0$

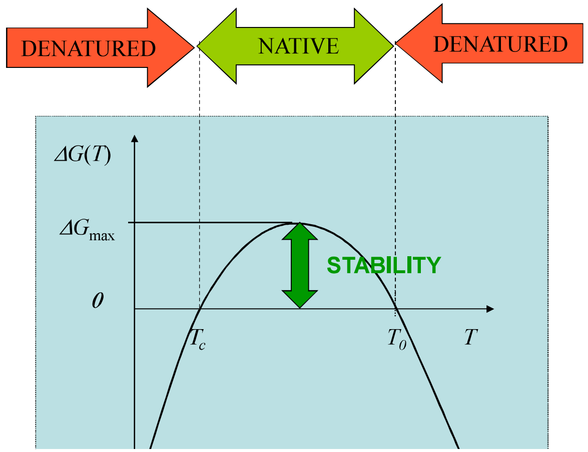
$$T\left(\ln\left(\frac{T}{T_0}\right) - 1\right) + T_0 \approx \frac{(T - T_0)^2}{2T_0}$$




$\Delta G(T)=G_2(T)\text{-}G_1(T)$

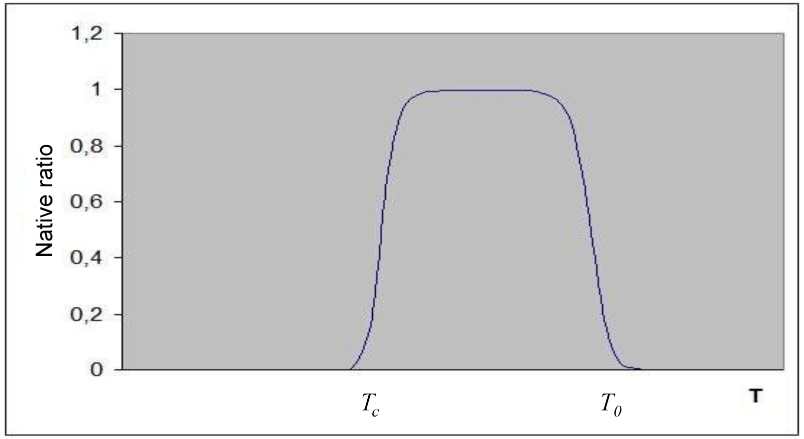
Ha $\Delta G(T)>0 \quad G_2(T)>G_1(T)$
Ha $\Delta G(T)<0 \quad G_2(T)<G_1(T)$

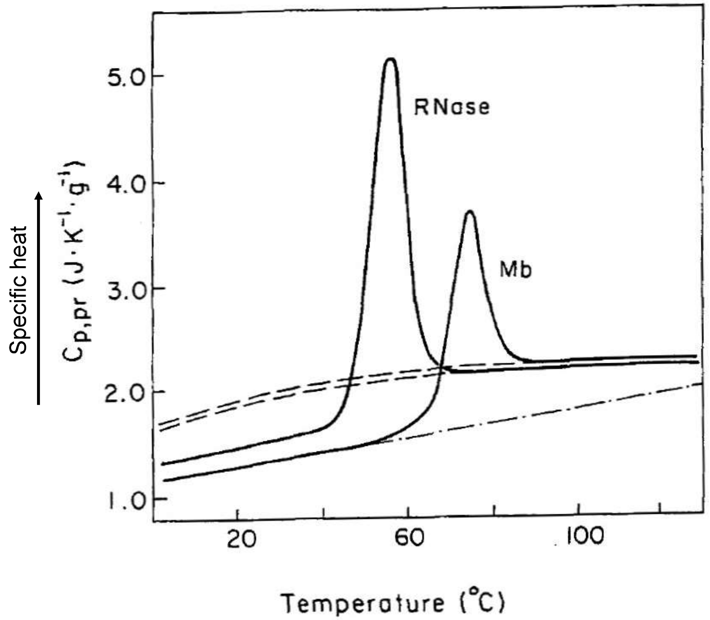
# In case of proteins:




No cold denatruation was observe in case of nucleic acids and memranes.

$$\frac{w_D}{w_N} = e^{-\Delta G/RT}$$



# Cold denaturation

- Often below 0°C
- Technical problems
- Solution:
  - Use of another denaturing agent:
    destabilization: $T_c$ increases.
  - Using the special character of the phase diagram of water:
    water is liquid until -20 °C under pressure

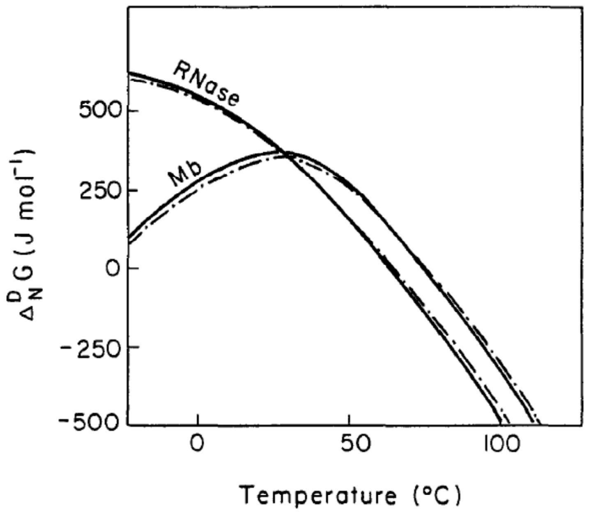



**FIGURE 7.** The $\Delta_N^D G$ function for RNase and Mb for the same conditions as in Figure 6 calculated from the assumption that $\Delta_N^D C_p$ is temperature independent (dot-dash line) and temperature dependent (dashed line).[114]

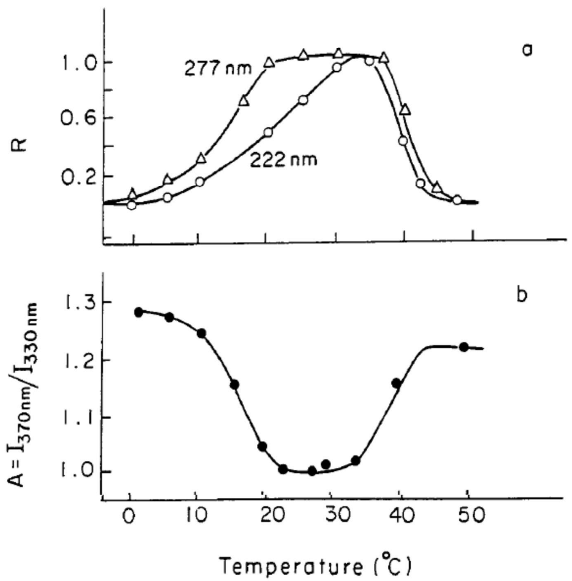
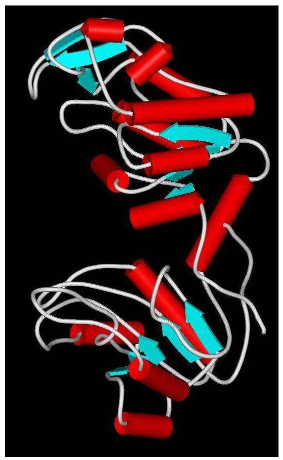
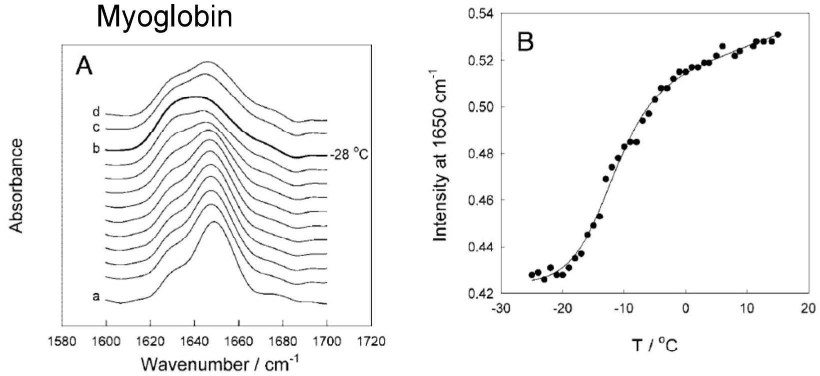



**FIGURE 23.** Temperature dependence of (a) relative changes (R) of phosphoglycerate kinase ellipticity at 222 nm (○) and 277 nm (Δ), (b) tryptophan emission spectrum maximum containing 0.7 *M* GuHCl.[133]

PGK



Myoglobin



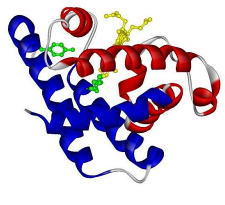



FIGURE 3 (*A*) Stacked plot of the deconvoluted 1600 to 1700 cm$^{-1}$ region of myoglobin upon cold unfolding. The sequence of the spectra is from bottom to top. All spectra were taken at 2 kbar except for the bottom [*a*] and top [*d*] spectrum, which are taken at atmospheric pressure and 20°C before and after the cold unfolding, respectively. The temperatures at which each spectrum was taken are 20, 15, 11, 5, 0, −5, −10, −15, −20, −25, [*b*] −28, [*c*] 10, and [*d*] 20°C. Spectrum [*c*] is taken at 2 kbar and 10°C after the cold unfolding. (*B*) Intensity of the band at 1650 cm$^{-1}$ versus decreasing temperature. Dots are the experimental data, and the full line is the fitted curve.

## Phase diagram of water

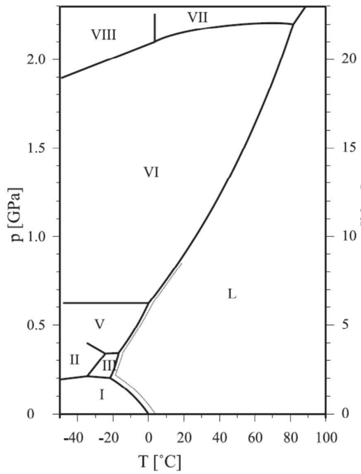
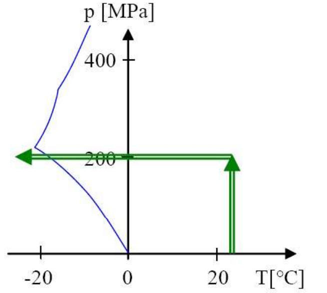
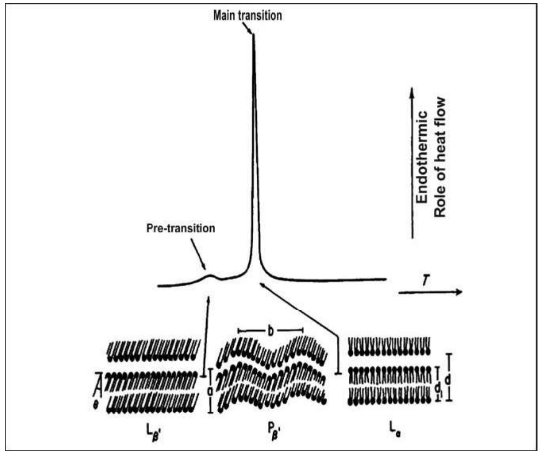



Fig. 1. Phase diagram of water in the temperature range of −50 to +100°C up to a pressure of 2.2 GPa. L refers to the liquid phase; roman numbers (I–VIII) show the different ice phases. The dotted line shows the melting curve of heavy water.

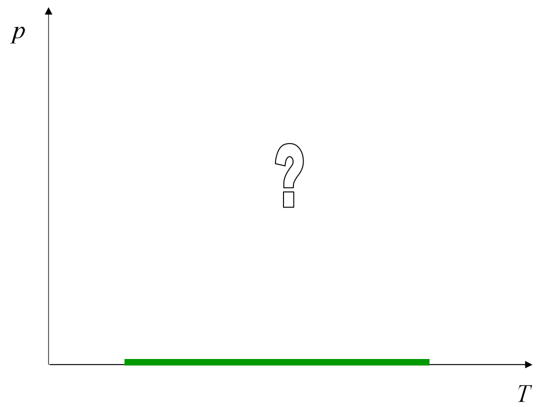



37. ábra. A hideg denaturációs útvonal a *p-T* síkon. A kék görbe jelzi a desztillált víz fagyásgörbéjét, amit Bridgmann (1935) mérései alapján rajzoltunk meg.

# Phase transition of the lipids




# The p-T phase diagram

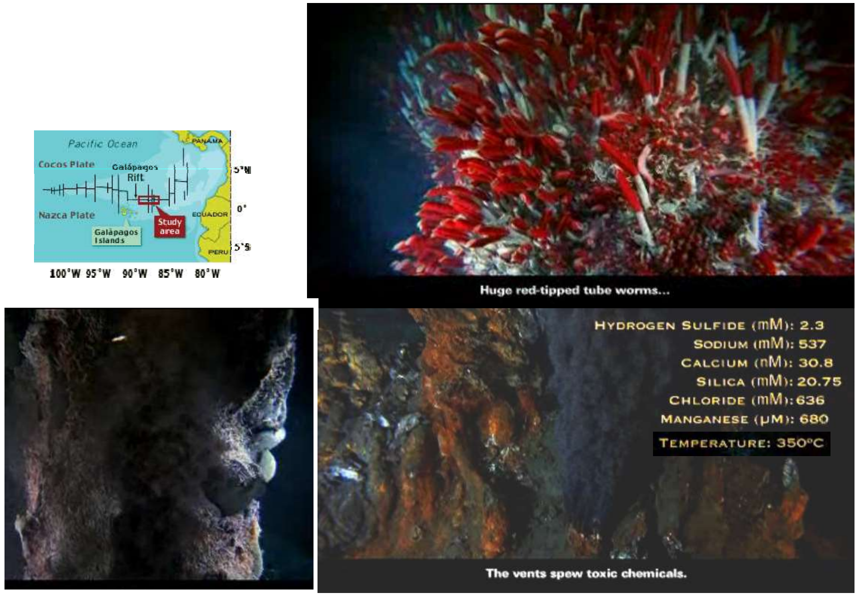



# Why is highly pressure interesting?

- Why not?
  thermodin. parameter
  *T*, *p*,..
- In the biosphere
  p=1 bar…1 kbar
- Data obtained from high pressure experiments can be relevant at atmospheric pressure as well. E.g. $\Delta V$

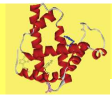
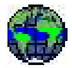
- Technical problems
- we live at *p*=1 bar

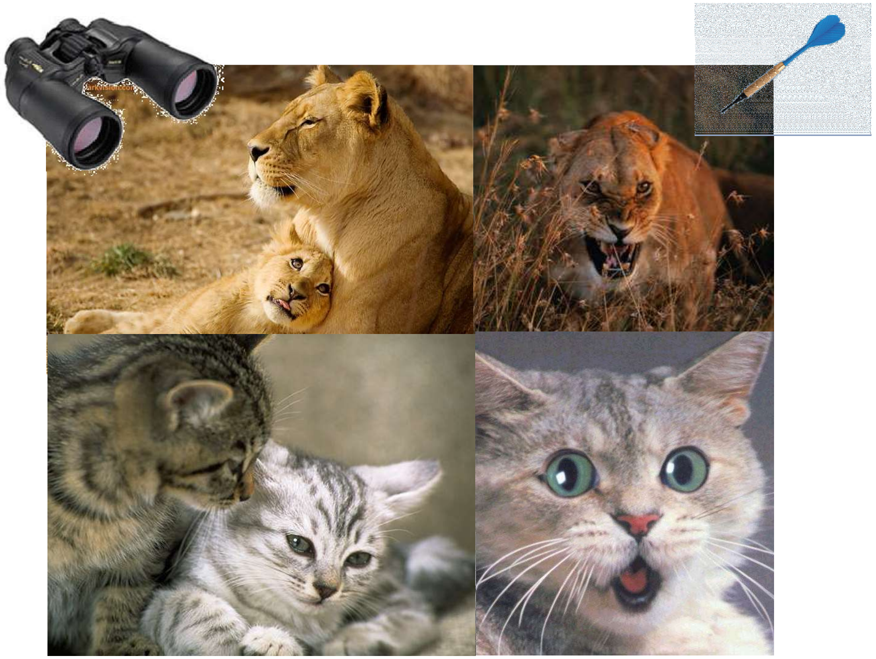

1 bar = 0,1 MPa 1 kbar = 100 MPa 10 kbar = 1GPa 1Mbar=100GPa




http://ocean.si.edu/ocean-videos/hydrothermal-vent-creatures

# The pressure scale

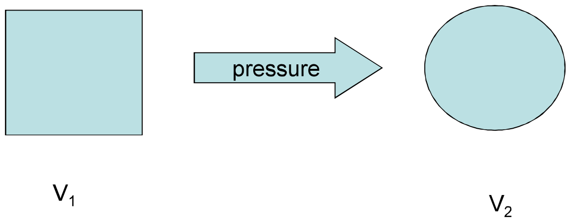
| | | | |
|---|---|---|---|
|  | human life | **1 bar** | **100 kPa** |
|  | max. pressure in the biosphere: (deepsee) | **1 kbar** | **100 Mpa** |
|  | structural changes in proteins: | **5-10 kbar** | **0.5-1 GPa** |
|  | water freezes at room temp.: | **≈10 kbar** | **1GPa** |
|  | in the middle of the planets | **~ Mbar** | **100 GPa** |
|  | max reached in laboratory: | **few Mbar** | **few 100 GPa** |



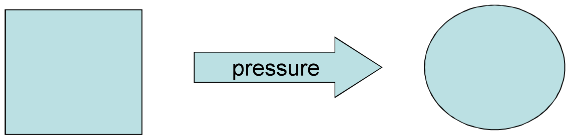
## What is the effect of the pressure?

The Le-Chatelier-Braun principle

pressure <–> volume



## pressure <-> volume

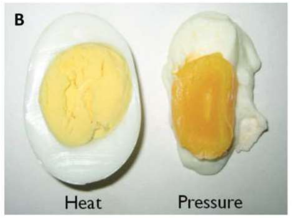



$V_1$ $V_2$

$$\left(\frac{\partial \Delta G}{\partial p}\right)_T = -RT\left(\frac{\partial \ln K}{\partial p}\right)_T = \Delta V$$

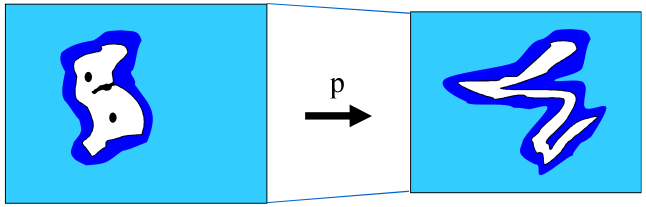
$$\ln K = -\frac{p\Delta V}{RT} + konst.$$

## Effect of pressure on proteins



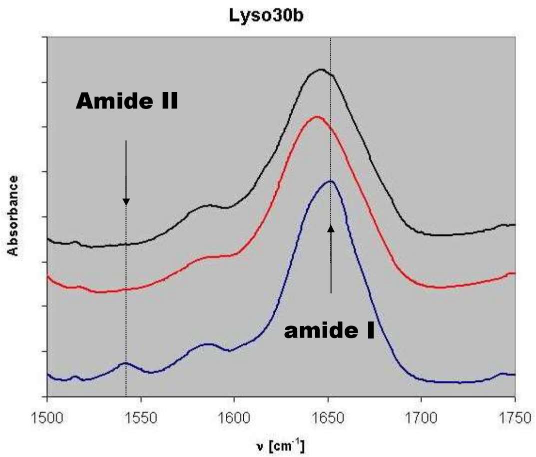

Pressure unfolding

Protein solution




$V_{protein} = V_{atom} + V_{void} + \Delta V_{hydration}$

## Pressure unfolding of proteins: e.g.: lysozyme



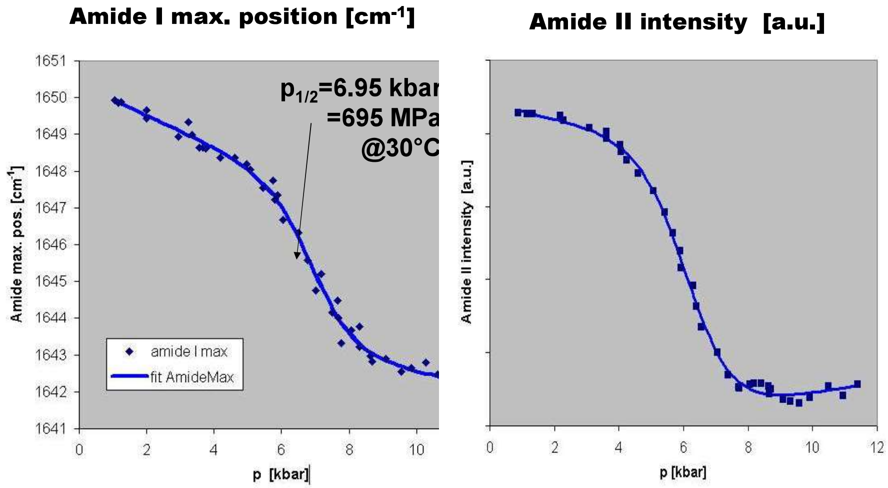

**75mg/ml**

**back to 0.1 MPa**

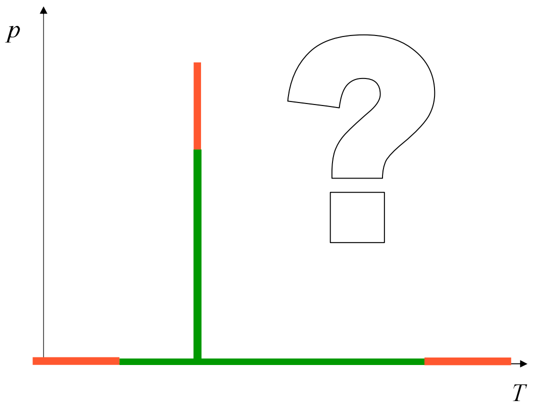
**900 MPa**

**0.1 MPa**

## Pressure unfolding: lysozyme



## Pressure-temperature phase diagram




$$\Delta G = \frac{\Delta\beta}{2}(p-p_0)^2 + \Delta\alpha(p-p_0)(T-T_0) - $$
$$- \Delta C_p\left[T\left(\ln\frac{T}{T_0}-1\right)+T_0\right]$$
$$+ \Delta V_0(p-p_0) - \Delta S_0(T-T_0) + \Delta G_0$$

where: $\beta = (\partial V/\partial p)_T$ compressibility factor,

$\alpha = (\partial V/\partial T)_p = -(\partial S/\partial p)_T$ thermal expansion coeff.

$C_p = T(\partial S/\partial T)_p$ specific heat at const. pressure

## Thermodynamic description of the pressure and temperature denaturation

Two state model: N <-> D

$\Delta G(T) = G_D(T) - G_N(T)$

Let us integrate $d(\Delta G) = -\Delta S dt + \Delta V dp$ starting from a reference point $T_0\ p_0$ until the point $T, p$ :

$$\Delta G(T,p) = \Delta G_0 + \int_{T_0}^{T}\int_{p_0}^{p} -\Delta S dt + \Delta V dp$$

Assuming $T \approx T_0$ :

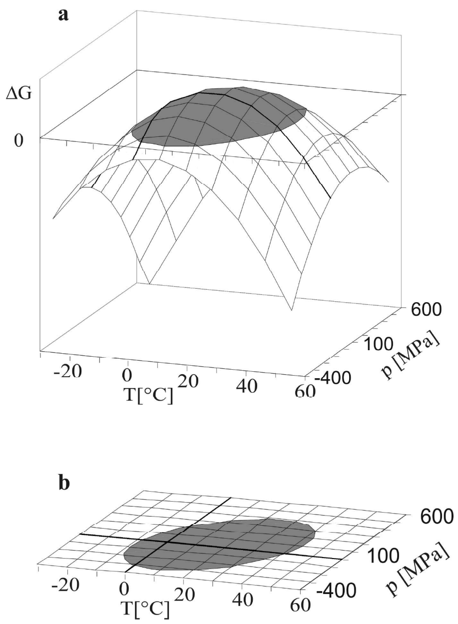
$$T\left(\ln\frac{T}{T_0}-1\right)+T_0 \approx \frac{(T-T_0)^2}{2T_0}$$

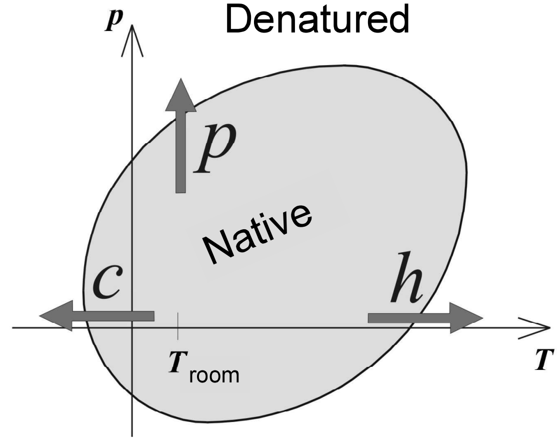
$$\Delta G = \frac{\Delta\beta}{2}(p-p_0)^2 + \Delta\alpha(p-p_0)(T-T_0) - \frac{\Delta C_p}{2T_0}(T-T_0)^2$$
$$+ \Delta V_0(p-p_0) - \Delta S_0(T-T_0) + \Delta G_0$$

Second order function of $T$ and $p$ !

At the middle point of the denaturation: $\Delta G = 0$

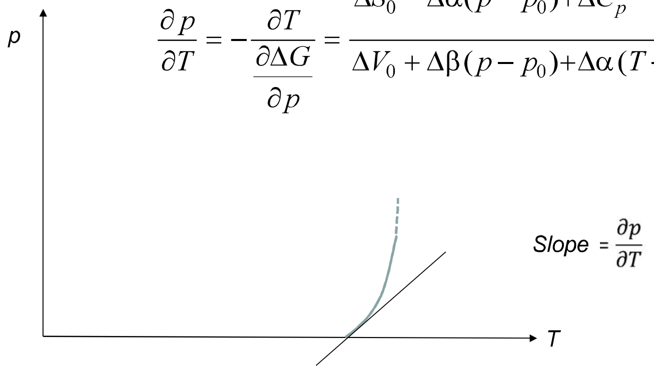
If $\Delta\alpha^2 > \Delta C_p \Delta\beta / T_0$ ,then the points where $\Delta G\ (T,p) = 0$ lie on an ellipse.




## Generalized Clausius-Clapeyron equation

$$\frac{\partial p}{\partial T} = -\frac{\frac{\partial \Delta G}{\partial T}}{\frac{\partial \Delta G}{\partial p}} = \frac{\Delta S_0 - \Delta\alpha(p-p_0) + \Delta C_p \frac{T-T_0}{T_0}}{\Delta V_0 + \Delta\beta(p-p_0) + \Delta\alpha(T-T_0)}$$



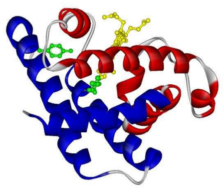

Is the two state model appropriate for the proteins?

Is there only one denatured state?

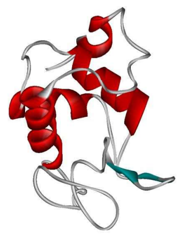
Intermolecular interactions?

# Experimentally determined phase diagrams

Myoglobin

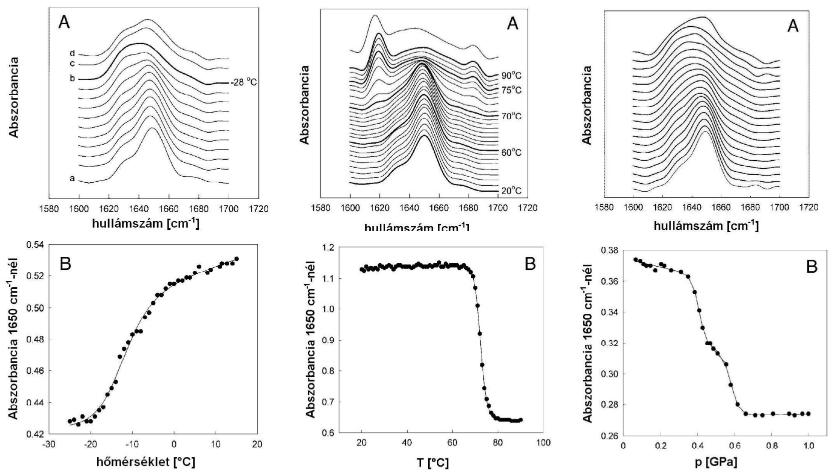


Lysozyme

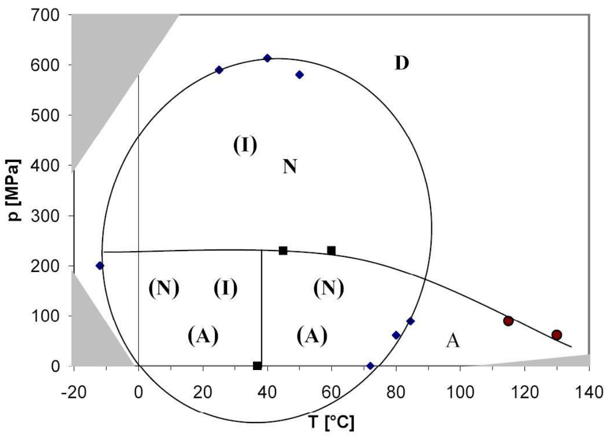


# Myoglobin: FTIR spectroscopy

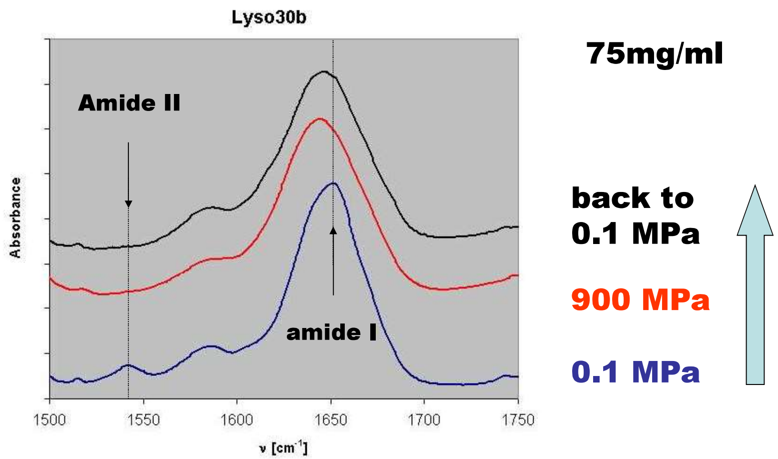
cold heat pressure



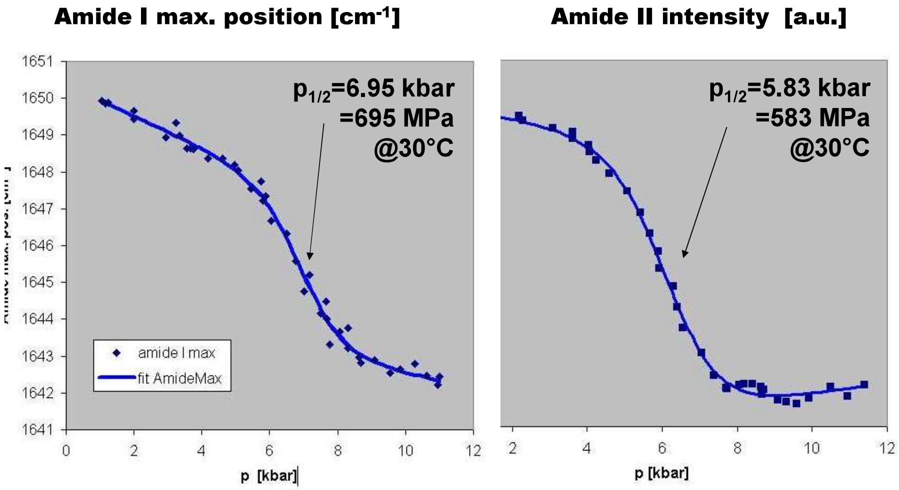

# Phase diagram of myoglobin



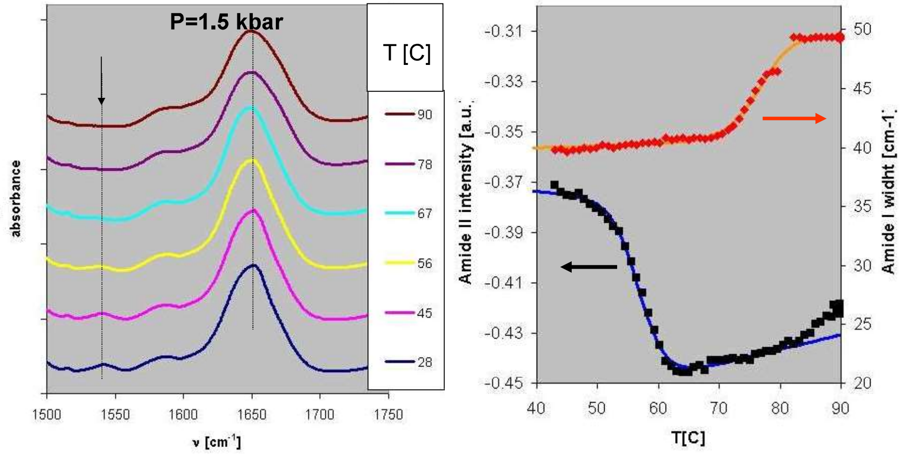

# Lysozyme: FTIR Spectroscopy



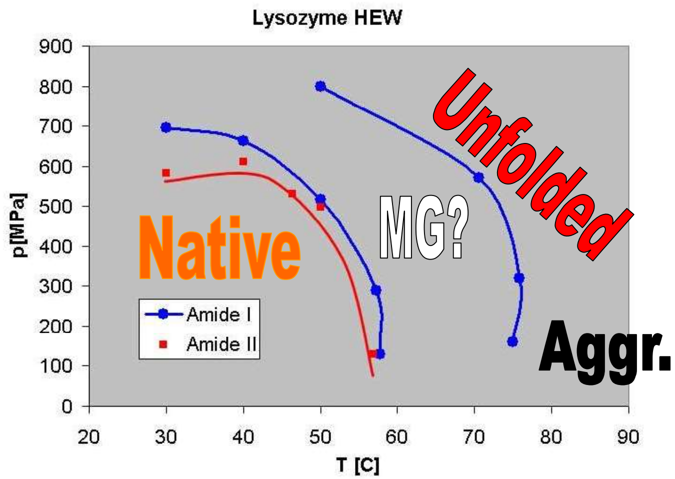
## Lysozyme: pressure denaturation




## Lysozyme: heat denaturation

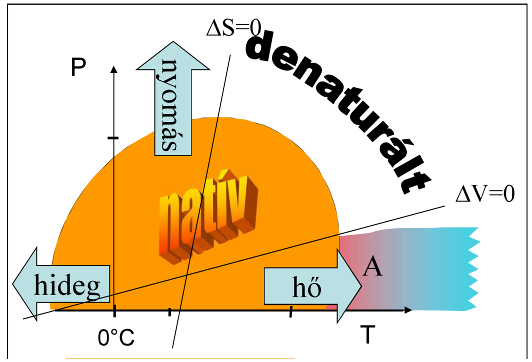



## Lysozyme: T-p phase diagram




## Pressure-temperature phase diagram: the reality

$$\left( \Delta G = \Delta G_0 - \Delta S_0 (T - T_0) - \frac{\Delta C_p}{2T_0}(T - T_0)^2 + \Delta V_0 (p - p_0) + + \frac{\Delta \hat{\beta}}{2}(p - p_0)^2 + \Delta \hat{\alpha}(p - p_0)(T - T_0) + \ldots \right)$$

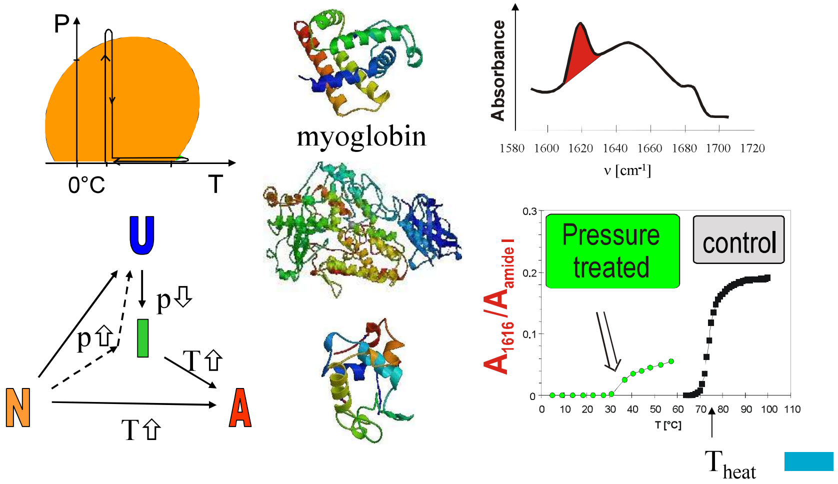



Intermolecular interactions: aggregation (conc!)

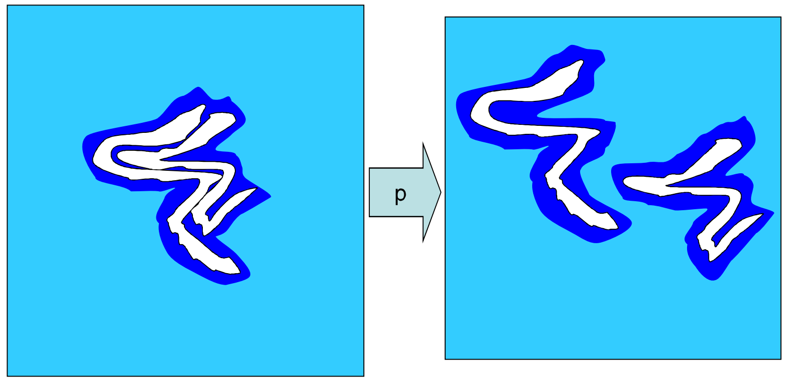
Pressure and cold denaturation: ΔV

Heat denaturation: ΔS

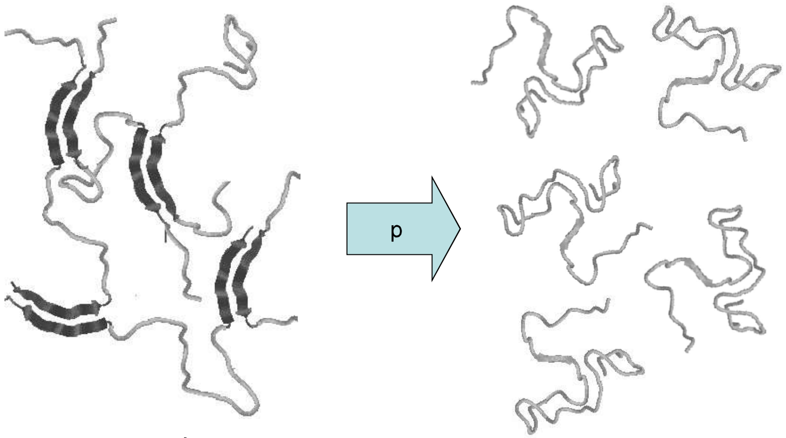
# Appearance of aggregation prone intermediates after pressure denaturation




# Intermolecular interactions and the pressure

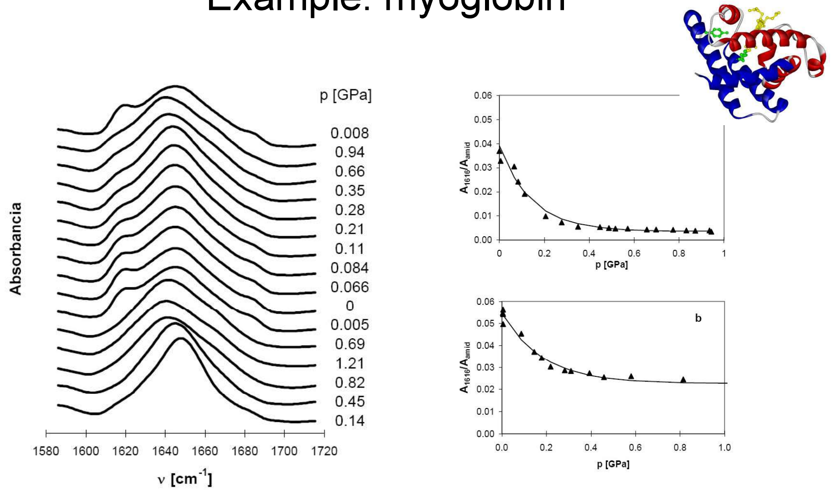



# Intermolecular interactions and the pressure

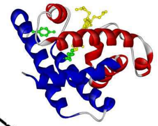


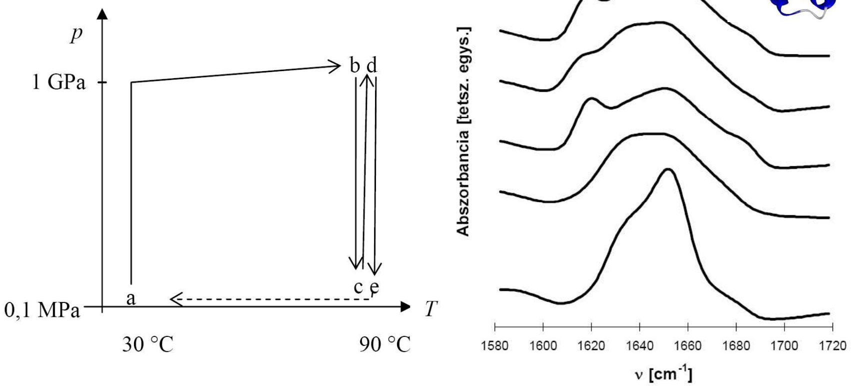

Typical pressure 2 kbar (200 MPa)

# Dissociation of aggregates using pressure. Example: myoglobin



## Dissociation of aggregates using pressure. Example: myoglobin



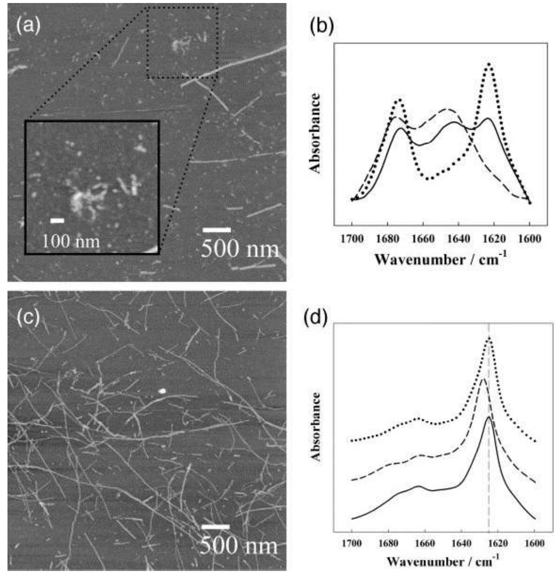



## Aggregates and fibers

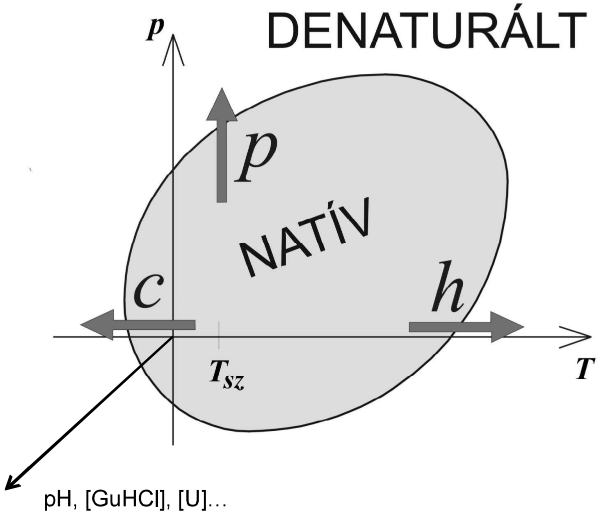
Day 1: (a) AFM (b) amide I band of TTR105–115 at 0.1 MPa (full line), 550 MPa (broken line) and 0.1 MPa after decompression (dotted line).

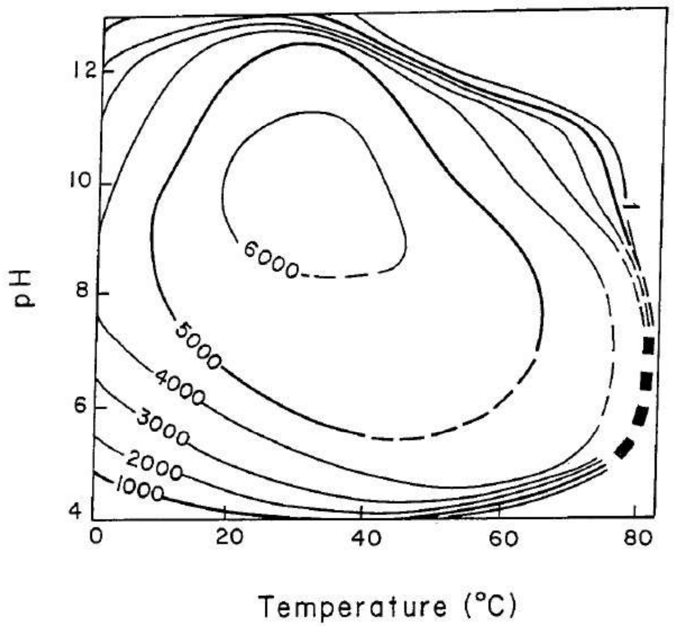
Day 4: (c) AFM (d) amide I band of TTR105–115 fibrils at 0.1 MPa (lower), 1.3 GPa (middle) and 0.1 MPa after decompression (upper).

From Dirix et al.




## The third (fourth…) dimension



# The phase diagram of DNA

The double helix form is pressure independent

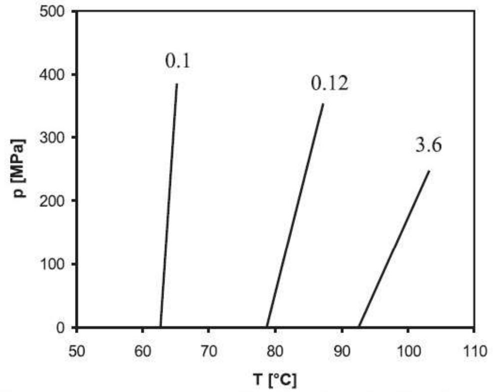
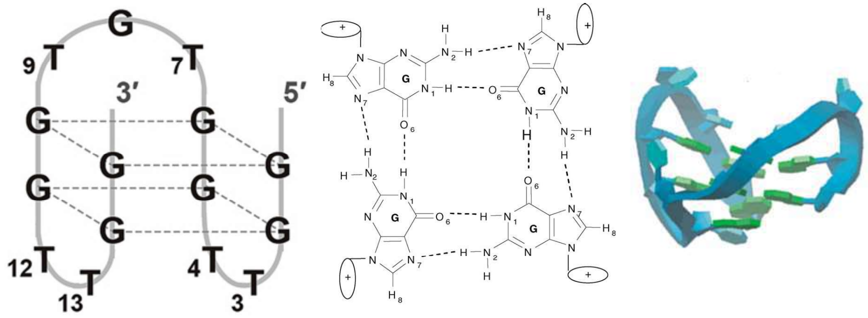


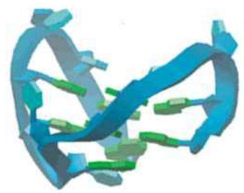

Fig. 10. Pressure–temperature diagram of DNA helix–coil transformation. Unlike the heat unfolding temperature of proteins, the melting temperature of DNA does not show any curvature, but a purely linear pressure dependence. The numbers refer to the molar concentration of neutral salts. Drawn after [84].

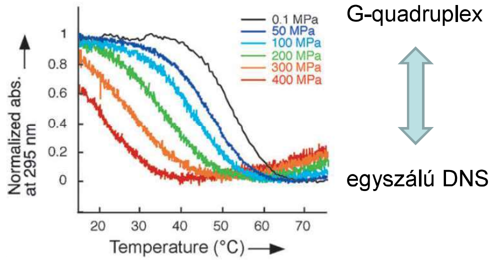
# Exotic DNA structures:
## G-quadruplex




# Phase diagram of DNA

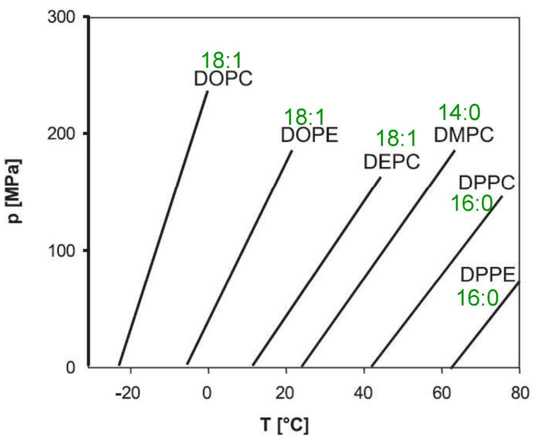
Exotic DNS structures, like G-quadruplex are pressure sensitive.

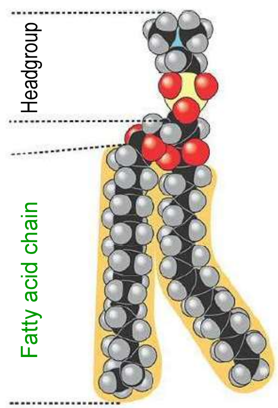





ΔV!

# Phase diagram of membranes



## The high pressure technique

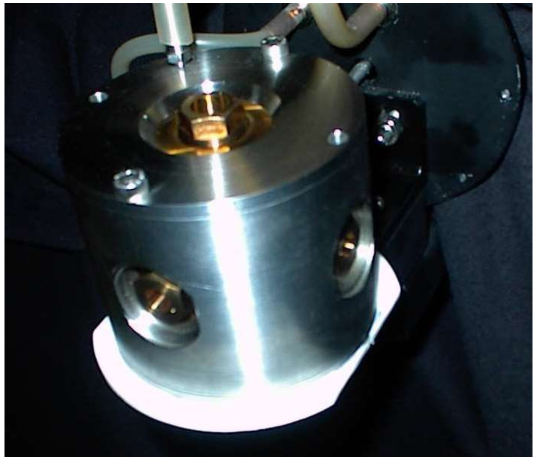
- Thick wall container
- A small volume diamond cell

## The high pressure technique

- **Container with very thick and solid wall (bomb)**

outer ∅ ≈ 10 cm
sample ∅ 10 mm

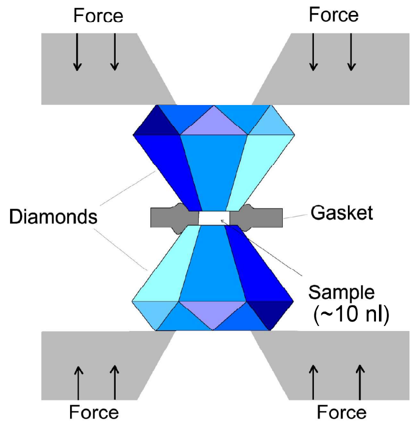
3 optical windows

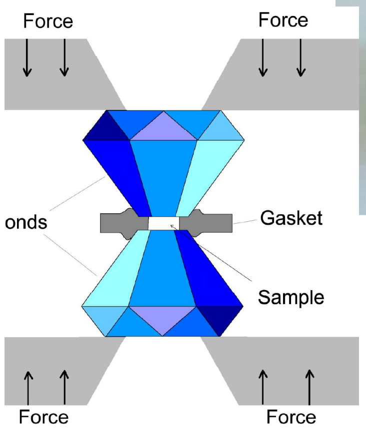


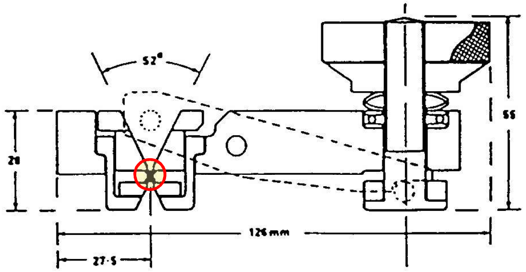
## The high pressure technique

***DAC***
***diamond anvil cell***

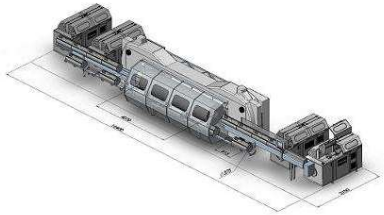
- **Very small surface (and volume)**
  **∅0,5mm**





## Applications



Pressurized pizza at a high pressure conference

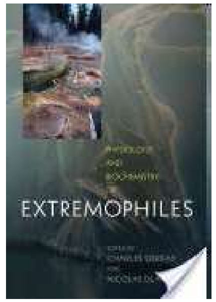
Pressurized food in a Japanese supermarket

## Thermophile and piesophile proteins

**Extremophile microorganisms**

They live under

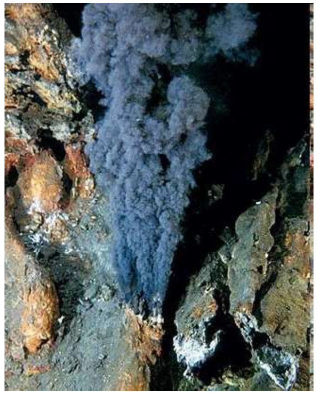
- extreme temperature (T>100 C)
- extreme pressure  (few 100 bar)
- extreme chemical environment
  (e.g. highly acidic)




(E.g. in the deepsee, near hydrothermal wents

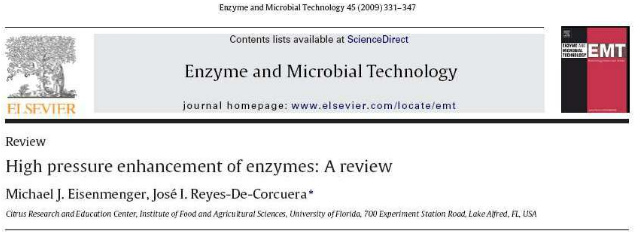
**They are sources of thermophile and piezophile proteins**

Thermophiles, a type of extremophile, produce some of the bright colors of Grand Prismatic Spring, Yellowstone National Park



The first extremophile to have its genome sequenced was *Methanococcus jannaschii*, a microbe that lives near hydrothermal vents 2,600 meters below sea level, where temperatures approach the boiling point of water and the pressure is sufficient to crush an ordinary submarine. Image credit: NOAA

# Increased enzymatic activity under pressure



Enzyme and Microbial Technology 45 (2009) 331–347

Contents lists available at ScienceDirect

Enzyme and Microbial Technology

journal homepage: www.elsevier.com/locate/emt

Review

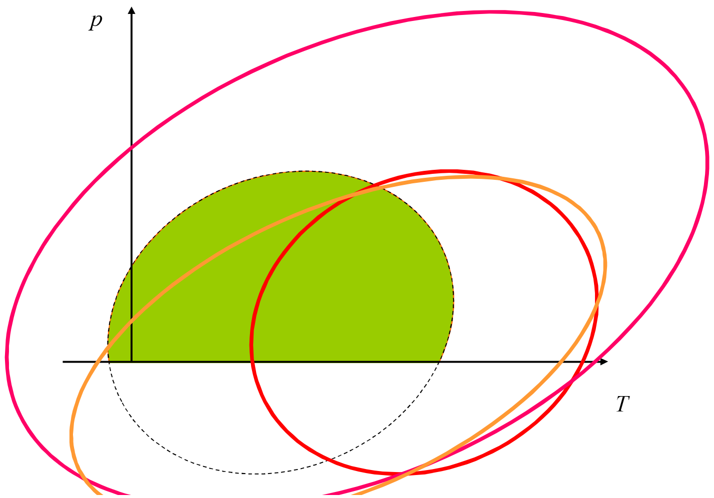
High pressure enhancement of enzymes: A review

Michael J. Eisenmenger, José I. Reyes-De-Corcuera*

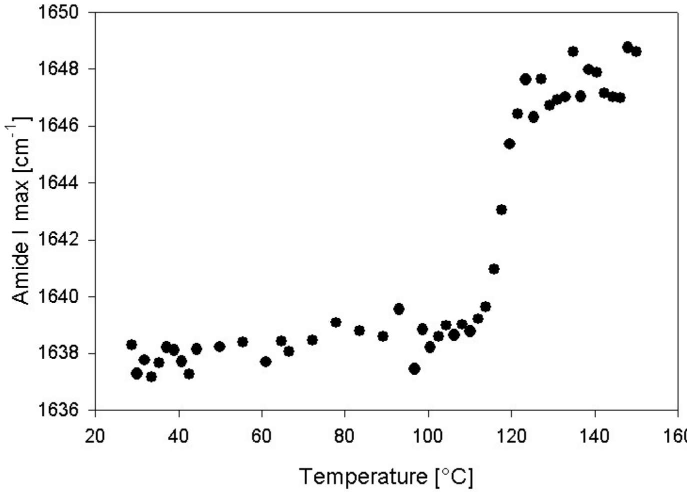
Citrus Research and Education Center, Institute of Food and Agricultural Sciences, University of Florida, 700 Experiment Station Road, Lake Alfred, FL, USA

| | | | | |
|---|---|---|---|---|
| α-cymotrypsin | 0,1 MPa | => | 20°C 480 Mpa | 6,5x |
| polyphenyl oxydase | 0,1 MPa | => | 500 MPa | +40% |
| α-amylase | 59°C atm.p. | => | 64°C 152 MPa | +25% |
| pepsin | 0,1 MPa | => | 300 MPa | 270x |
| … | | | | |

# How does the phase diagram change?




# An example: SHSP16,5 protein from *Methanococcus jannaschii*



# Pressure tolerance in the biosphere

For proteins:

p in the biosphere is max. 1 kbar

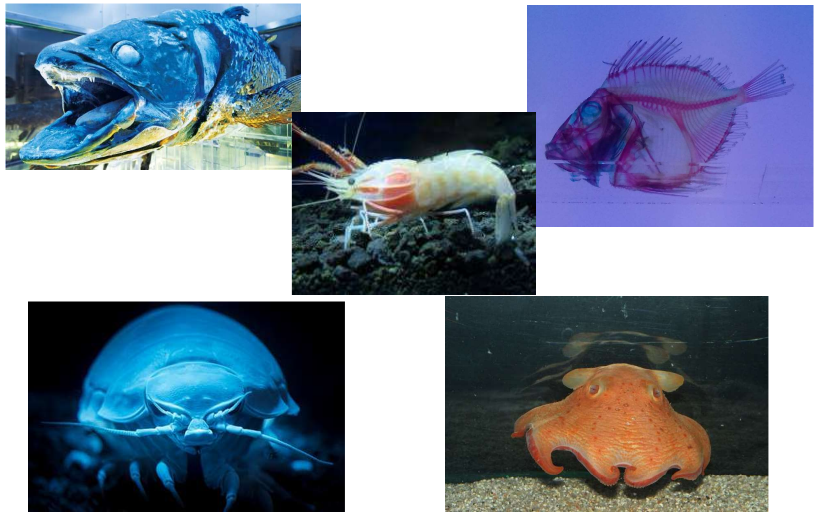
typical protein stability is ca. 5 kbar

For membranes:

$T_m$ increases by 20 °C under 1 kbar

Deepsee organisms

# Deepsee organisms



# Enzymatic activity in extreme environment

PI:

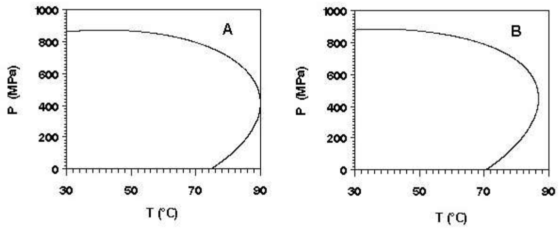



**Figure 3.** Heat-pressure iso-rate contour plots of 95 % inactivation of purified banana PME (in 20 mM Tris-HCl buffer, pH 7.0) for a total process time of 30 min (k = 0.099858 min-1) based on (A) the 'classical' thermodynamic model (eq 4); (B) the third-degree thermodynamic model (eq 6);
From: *Binh Ly-Nguyen1 et al,* Effect of Mild-Heat and High-Pressure Processing on Banana Pectin Methylesterase: A Kinetic Study

# References

Privalov PL. Cold denaturation of proteins. Crit Rev Biochem Mol Biol. 1990;25(4):281-305.

Meersman, F., Smeller, L., Heremans, K (2005) Extending the pressure-temperatura state diagram of myoglobin Helv. Chim. Acta 88, 546-556

Tölgyesi, F., Böde Cs., Smeller, L., Kim, K. K., Heremans, K., Fidy, J. (2004) Pressure activation of the chaperone function of small heat-shock proteins Cell. Mol. Biol. 50, 361-369.

F. Meersmann, L. Smeller, K. Heremans (2002) A comparative study of cold-, pressure- and heat-induced unfolding and aggregation of myoglobin Biophys. J. 82 2635-2644.

L. Smeller (2002) Pressure-temperature phase diagram of biomolecules Biophys. Biochim. Acta 1595 11-29.

L. Smeller, P. Rubens, K. Heremans (1999) Pressure effect on the temperature induced unfolding and tendency to aggregate of myoglobin Biochemistry 38 3816-3820.

Smeller László: A fehérjék konformációs és dinamikai tulajdonságai. Új eredmények nagy nyomással kombinált infravörös és fluoreszcencia spektroszkópiai módszerekkel. MTA doktori értekezés

Ly-Nguyen B, Van Loey AM, Smout C, Verlent I, Duvetter T, Hendrickx ME. Effect of Mild-Heat and High-Pressure Processing on Banana Pectin Methylesterase: A Kinetic Study J Agric Food Chem. 2003 Dec 31;51(27):7974-9.

http://bartlettlab.ucsd.edu/Research.html

http://ocean.si.edu/ocean-videos/hydrothermal-vent-creatures

http://www.hiperbaric.com/en

Dirix, C; Meersman, F; MacPhee, CE; Dobson, CM; Heremans, K High hydrostatic pressure dissociates early aggregates of TTR105-115, but not the mature amyloid fibrils J. Mol Biol. 347 (2005) 903-909

Eisenmenger, Michael J.; Reyes-De-Corcuera, Jose I. High pressure enhancement of enzymes: A review Enzmye Microbial Technol. 45 (2009) 331-347

https://japan-magazine.jnto.go.jp/en/1406_aquarium.html

https://matome.naver.jp/

https://rr.img.naver.jp