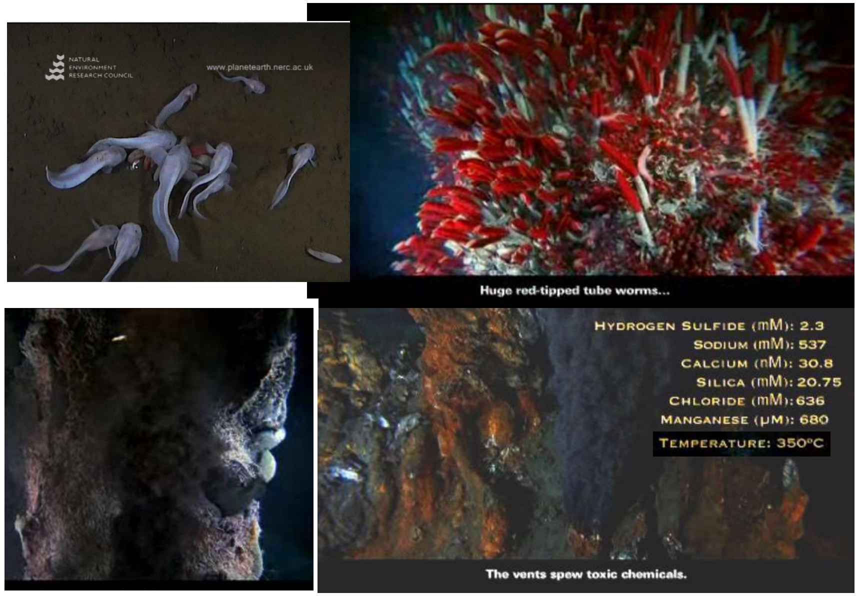
Stability of biological systems

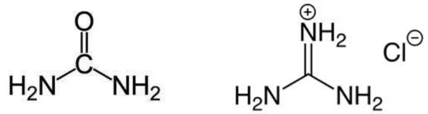
László Smeller



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

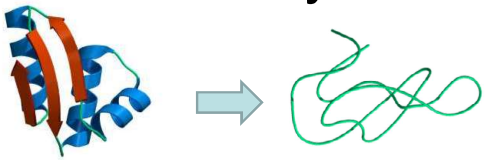


Thermophiles, a type of extremophile, produce some of the bright colors of Grand Prismatic Spring, Yellowstone National Park

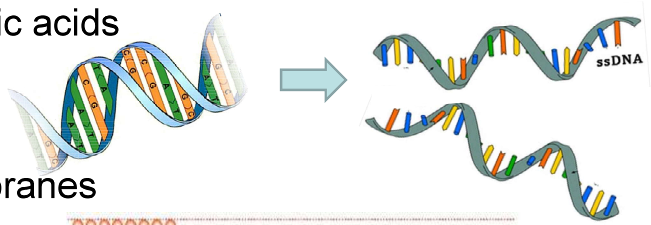
The first extremophile to have its genome sequenced was Methanococcus jannaschii, a microbe that lives near hydrothermal vents 2,600 meters below sea level, where temperatures approach the boiling point of water and the pressure is sufficient to crush an ordinary submarine. Image credit: NOAA

Order and disorder in macromolecular systems

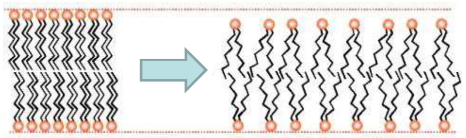
Proteins



Nucleic acids



Membranes



Physical parameter: Temperature

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

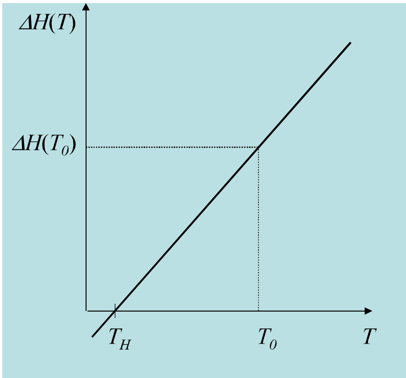
$\Delta G(T_0)=\Delta H_D(T_0)-T_0\Delta S_N(T_0)=0$

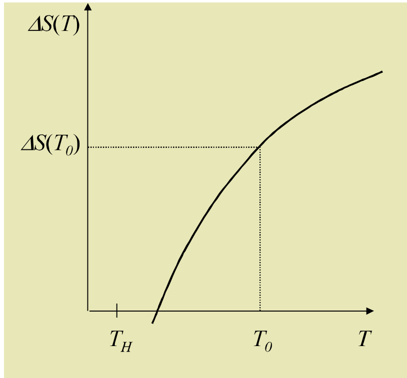
Let us suppose that C_p does not depend on T:

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

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

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



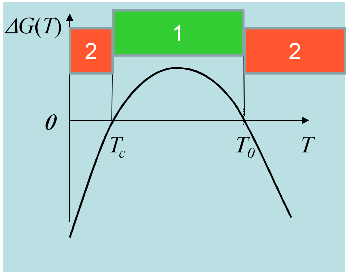


$$\Delta S(T) = \Delta S(T_0) + \int_{T_0}^{T} \frac{\Delta C_p}{T} dT = \Delta S(T_0) + \Delta C_p \ln\left(\frac{T}{T_0}\right)$$

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

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

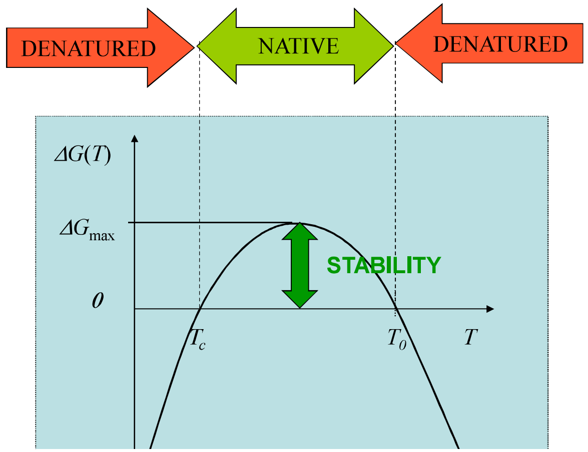
$$T\left(\ln\left(\frac{T}{T_0}\right) - 1\right) + T_0 \approx \frac{(T - T_0)^2}{2T_0}$$



$\Delta G(T)=G_2(T)-G_1(T)$

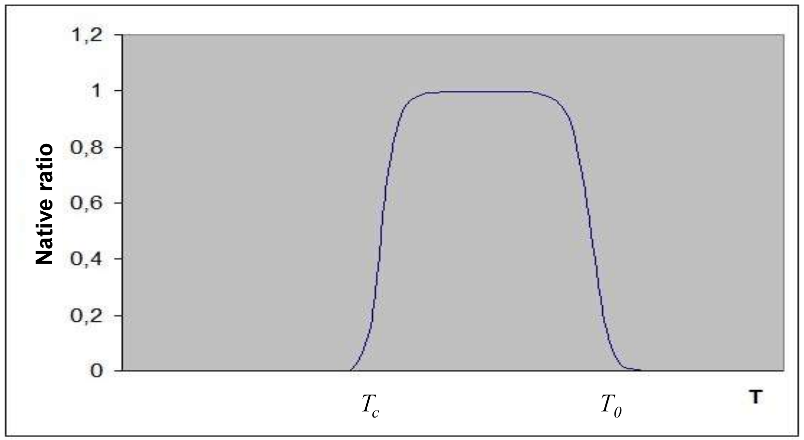
Ha $\Delta G(T)>0$ $G_2(T)>G_1(T)$
Ha $\Delta G(T)<0$ $G_2(T)<G_1(T)$

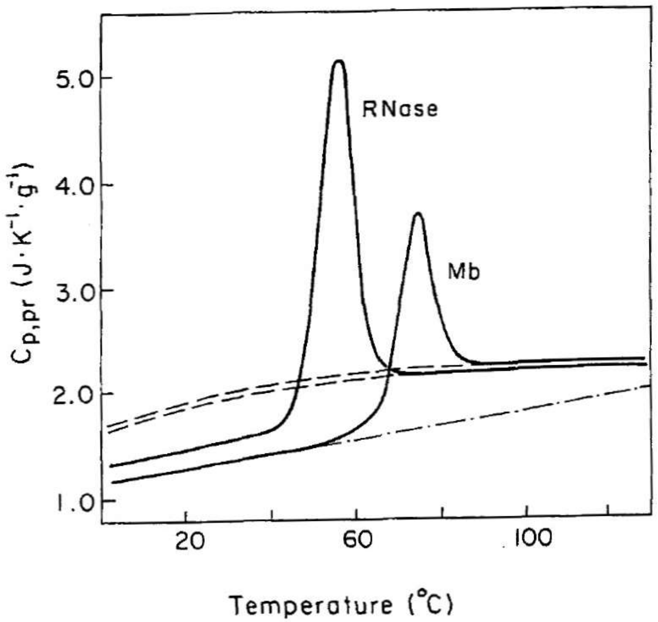
In case of proteins:



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

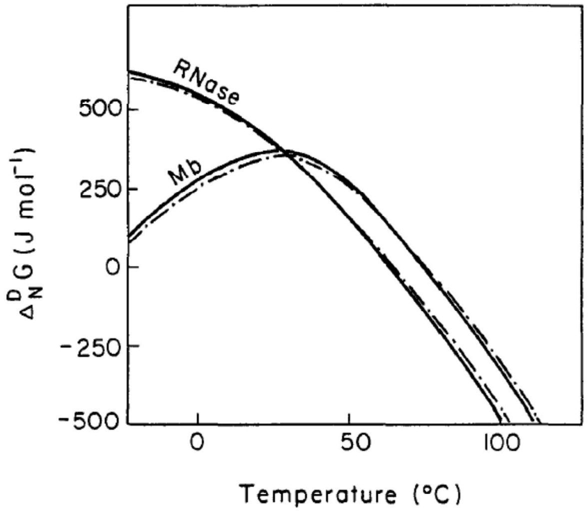


FIGURE 7. The $\Delta_N^D G$ function for RNase and Mb for the same conditions as in Figure 6 calculated from the assumption that $\Delta_N^D C_p$ is temperature independent (dot-dash line) and temperature dependent (dashed line).[114]

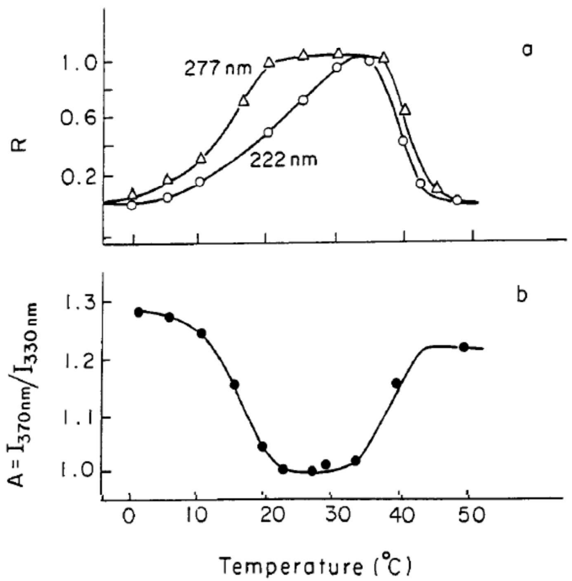
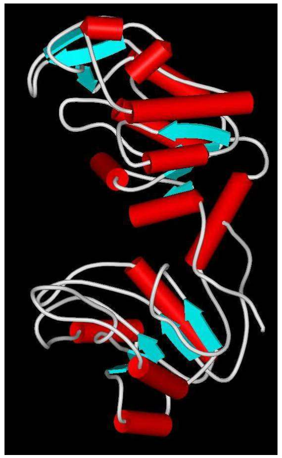
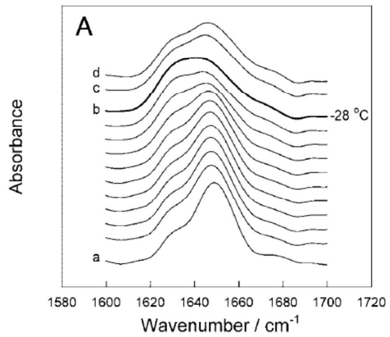


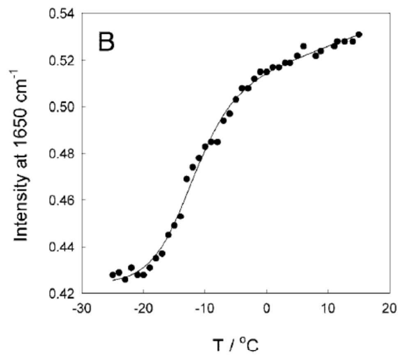
FIGURE 23. Temperature dependence of (a) relative changes (R) of phosphoglycerate kinase ellipticity at 222 nm (○) and 277 nm (Δ), (b) tryptophan emission spectrum maximum containing 0.7 *M* GuHCl.[133]

PGK



Myoglobin





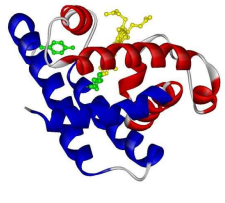


FIGURE 3 (*A*) Stacked plot of the deconvoluted 1600 to 1700 cm^{-1} region of myoglobin upon cold unfolding. The sequence of the spectra is from bottom to top. All spectra were taken at 2 kbar except for the bottom [*a*] and top [*d*] spectrum, which are taken at atmospheric pressure and 20°C before 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.

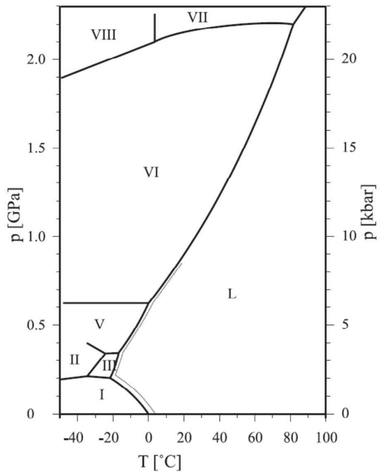
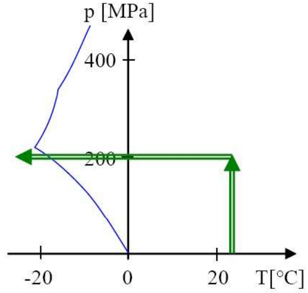


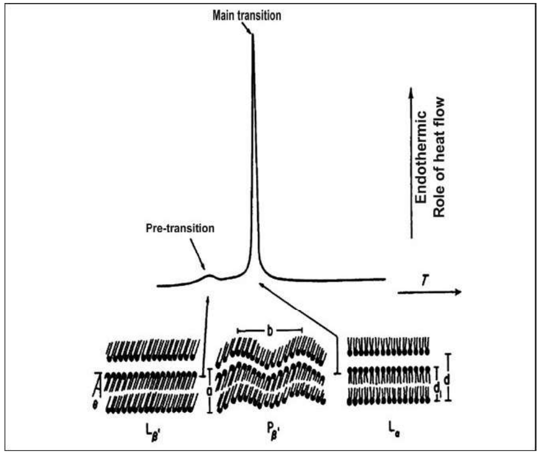
Fig. 1. Phase diagram of water in the temperature range of −50 to +100°C up to a pressure of 2.2 GPa. L refers to the liquid phase; roman numbers (I–VIII) show the different ice phases. The dotted line shows the melting curve of heavy water.

Phase diagram of water

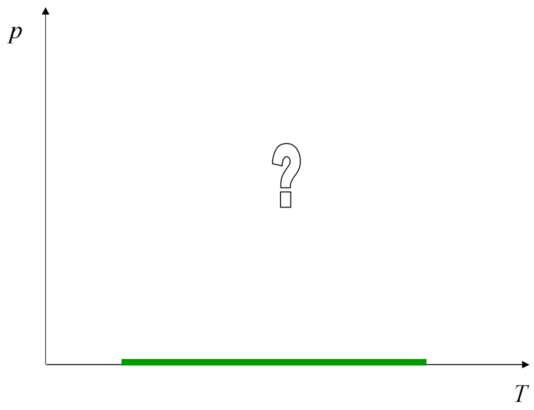


Path of cold denaturation experiment (green). The solidification curve of water is indicated by the blue line.

Phase transition of the lipids



The p-T phase diagram

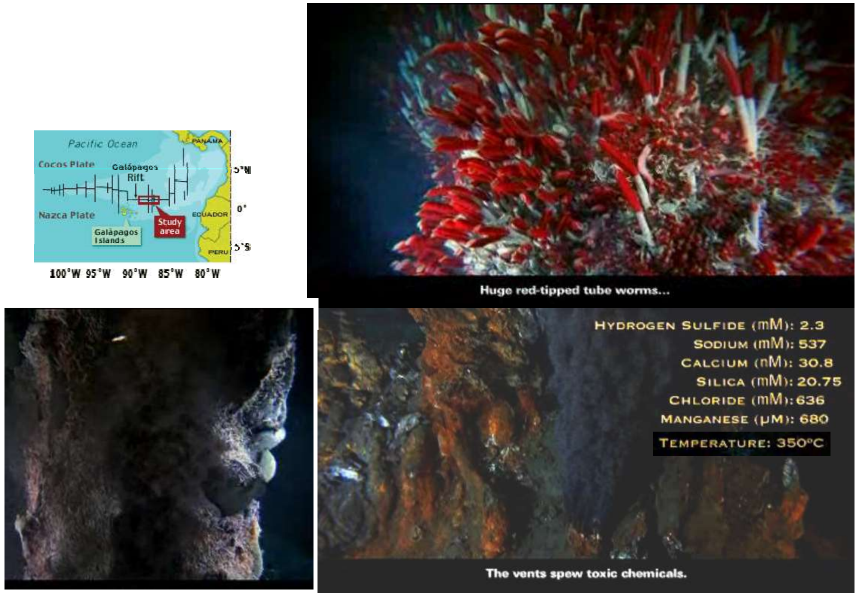


Why is highy pressure interesting?

- Why not?
  thermodin. param.
  T, p,..
- In the biosphere
  p=1 bar…1 kbar
- Data oobtained from high pressure experiments can be relevant at atmospheric pressure as well.

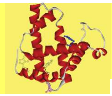
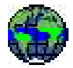
- Technical problems
- we live at p=1 bar

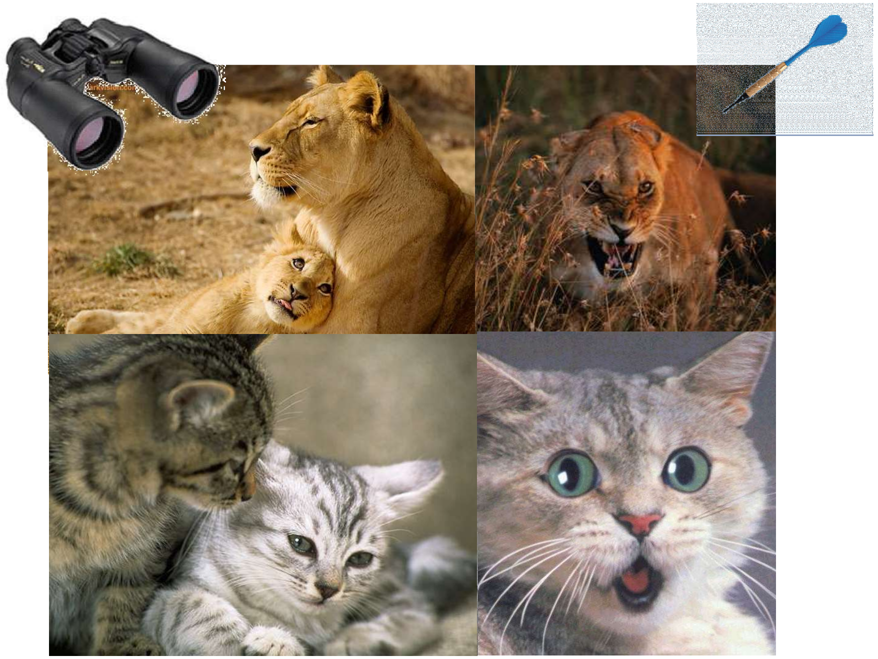
1 bar = 0,1 MPa   1 kbar = 100 MPa   10 kbar = 1GPa   1Mbar=100GPa



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

The pressure scale

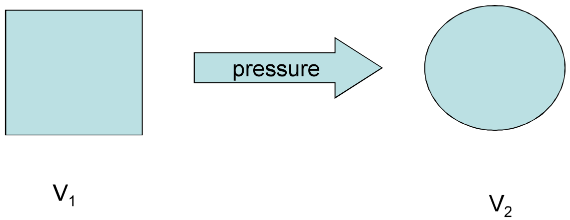
	human life	**1 bar**	**100 kPa**
	max. pressure in the biosphere: (deepsee)	**1 kbar**	**100 Mpa**
	structural changes in proteins:	**5-10 kbar**	**0.5-1 GPa**
	water freezes at room temp.:	**≈10 kbar**	**1GPa**
	in the middle fo the planets	**~ Mbar**	**100 GPa**
	max reached in laboratory:	**few Mbar**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



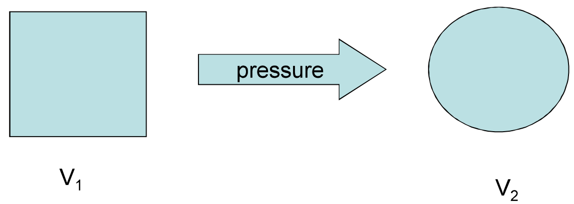
What is the effect of the pressure?

The Le-Chatelier-Braun principle

pressure <-> volume



pressure <-> volume



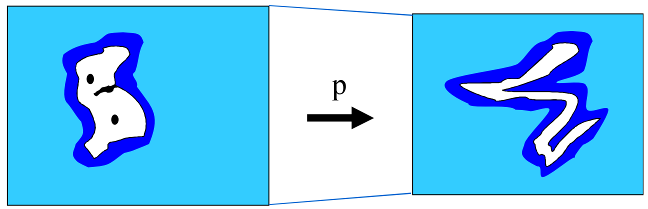
$$\left(\frac{\partial \Delta G}{\partial p}\right)_T = -RT\left(\frac{\partial \ln K}{\partial p}\right)_T = \Delta V$$

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

Efect of pressure on the proteins

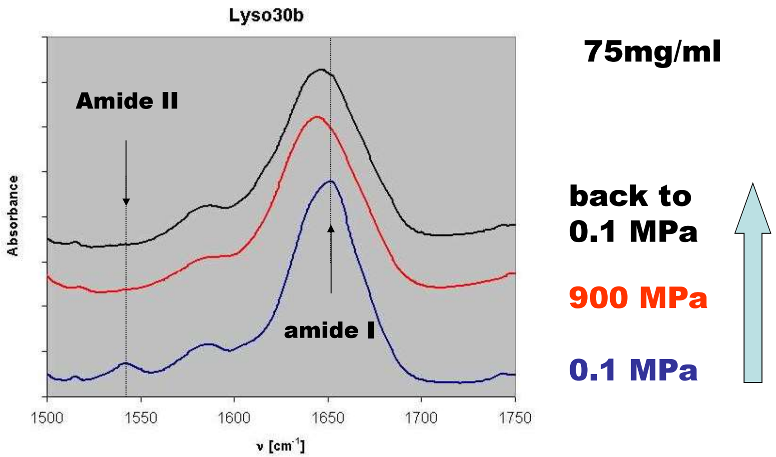
Pressure unfolding

Protein solution

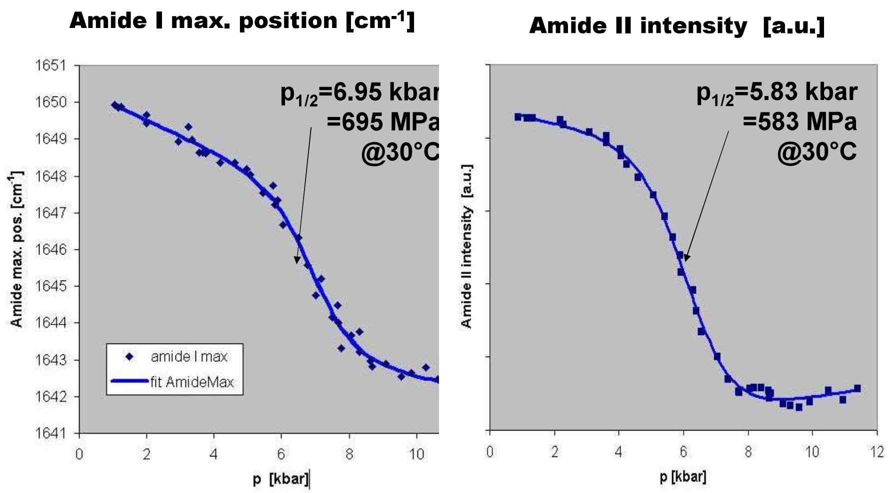


$V_{protein}=V_{atom}+V_{void}+\Delta V_{hydration}$

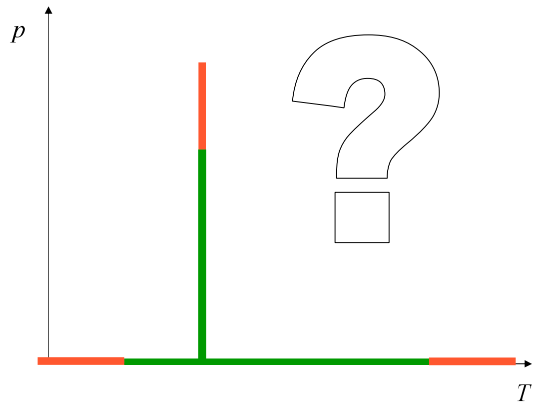
Pressure unfolding of proteins: e.g.: lysozyme



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 temperautre deanaturations

Two state model: N <-> D

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

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

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

Assuming $T \approx T_0$:

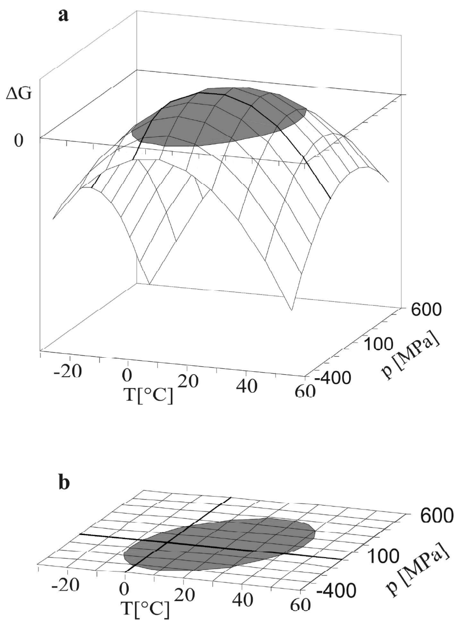
$$T\left(\ln\frac{T}{T_0}-1\right)+T_0 \approx \frac{(T-T_0)^2}{2T_0}$$

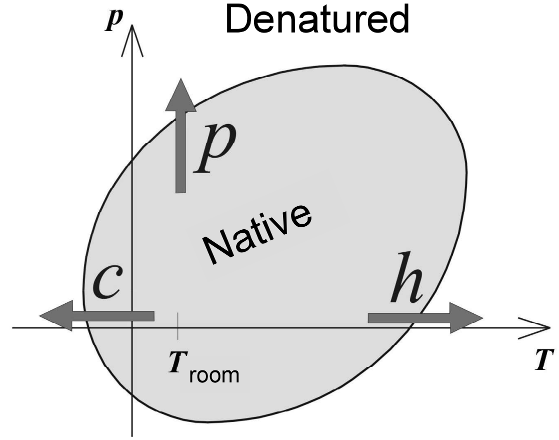
$$\Delta G = \frac{\Delta\beta}{2}(p-p_0)^2 + \Delta\alpha(p-p_0)(T-T_0) - \frac{\Delta C_p}{2T_0}(T-T_0)^2$$
$$+\Delta V_0(p-p_0) - \Delta S_0(T-T_0) + \Delta G_0$$

Seond 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 ellpse.





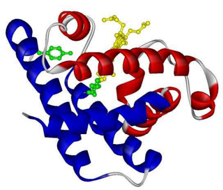
Experiementally determinedphase diagrams

Is the two state model a good description for the proteins?

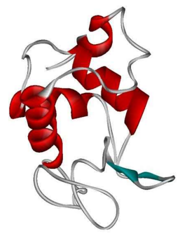
Is there only one denatured state?

Intermolecular interactions?

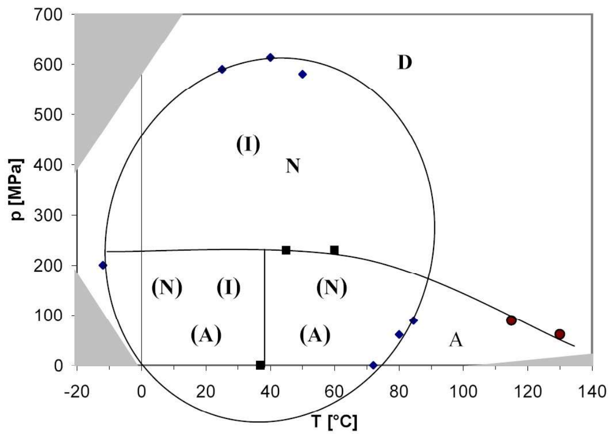
Myoglobin



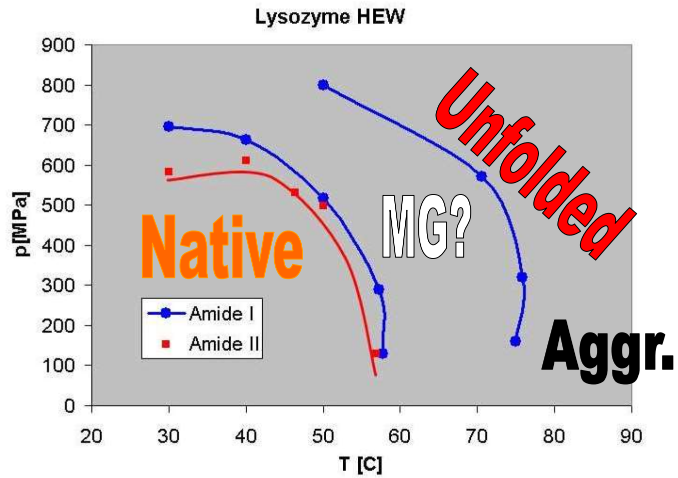
Lysozyme



Phase diagram of myoglobin

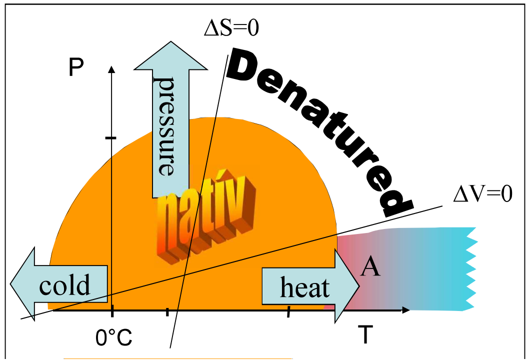


Lysozyme: T-p phase diagram



Pressure-temparature phase diagram: the reality

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

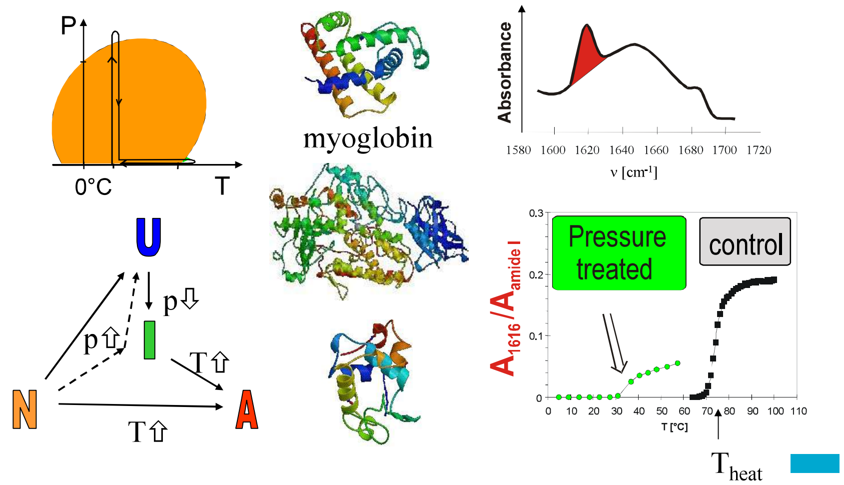


Intermolecular interacions: aggragation (conc!)

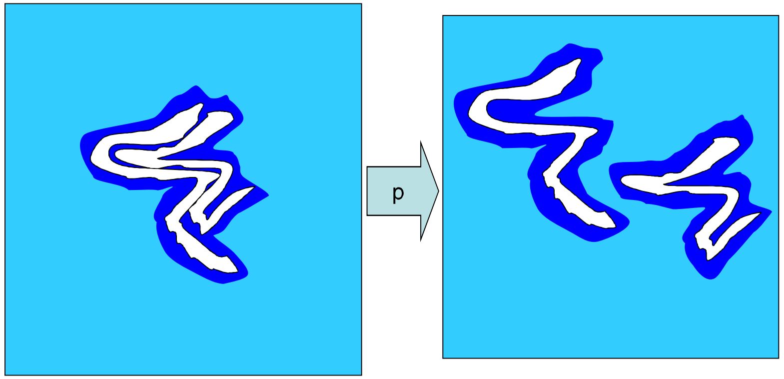
Pressure and cold denatruation: ΔV

Heat denatruation: ΔS

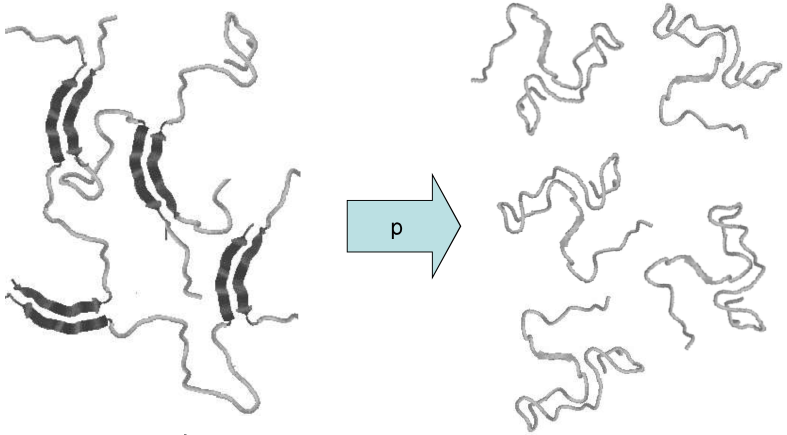
Appearence of aggregation prone intermediates after pressure denaturation



Intermolecular interactions and the pressure



Intermolecular interactions and the pressure



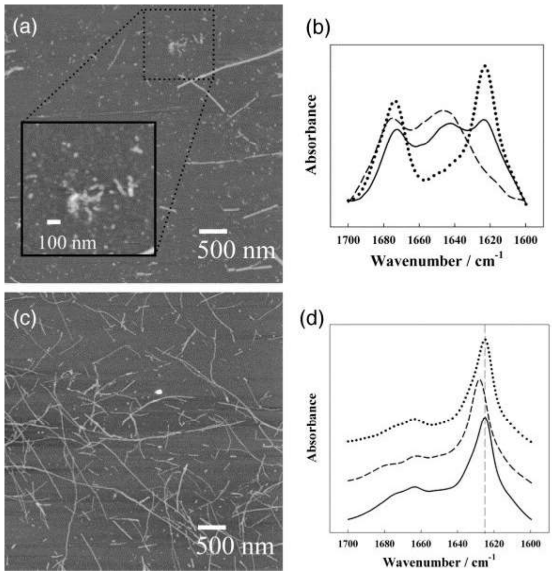
Typical pressure 2 kbar (200 MPa)

Aggregates and fibers

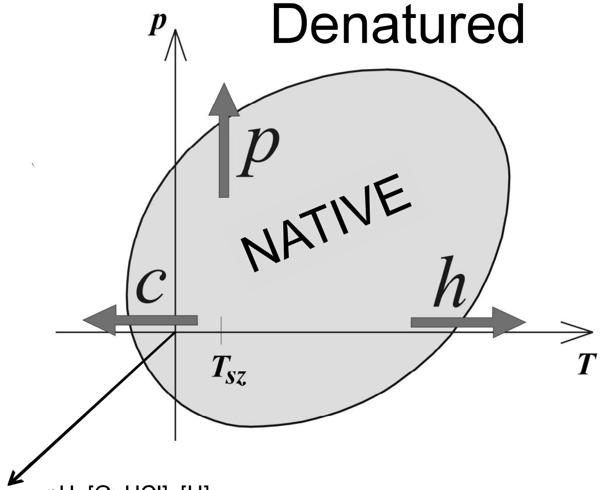
Day 1: (a) AFM (b) amide I band of TTR105–115 at 0.1 MPa (full line), 550 MPa (broken line) and 0.1 MPa after decompression (dotted line).

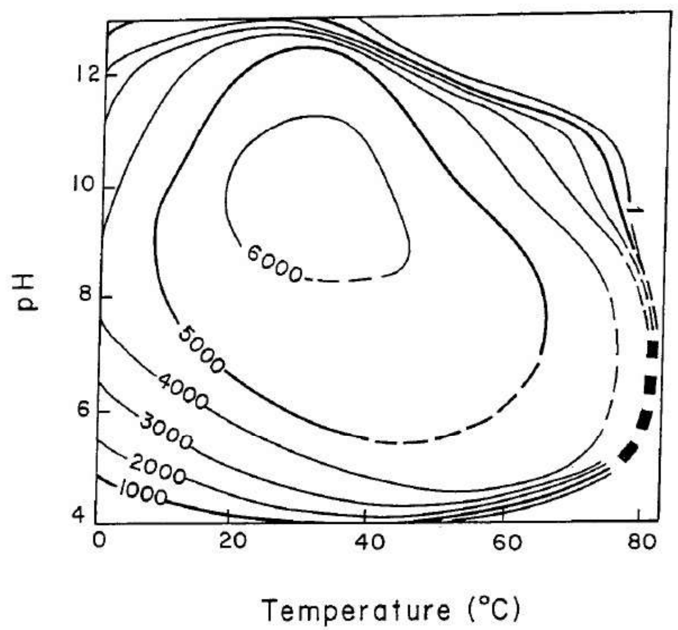
Day 4: (c) AFM (d) amide I band of TTR105–115 fibrils at 0.1 MPa (lower), 1.3 GPa (middle) and 0.1 MPa after decompression (upper).

From Dirix et al.



The third (fuorth…) dimension





The phase diagram of DNA

The duoble helix form is pressure indepndent

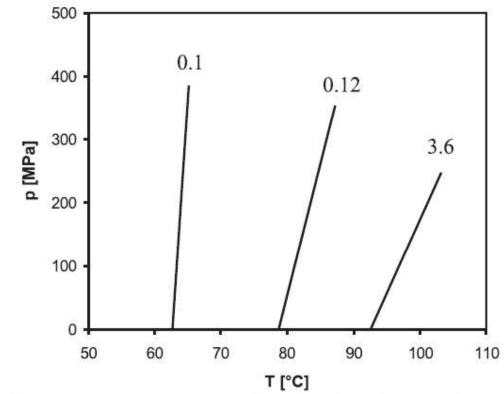
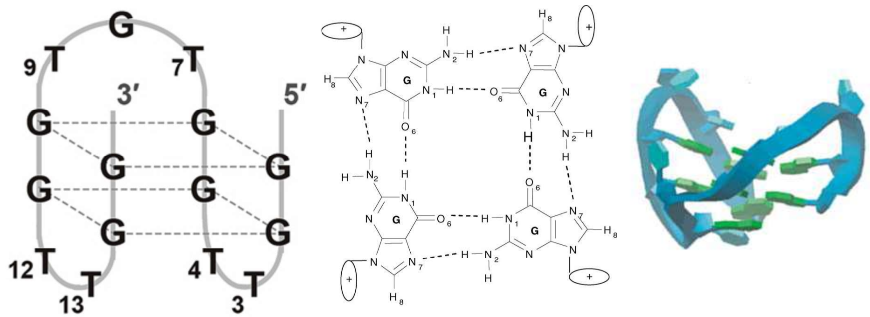


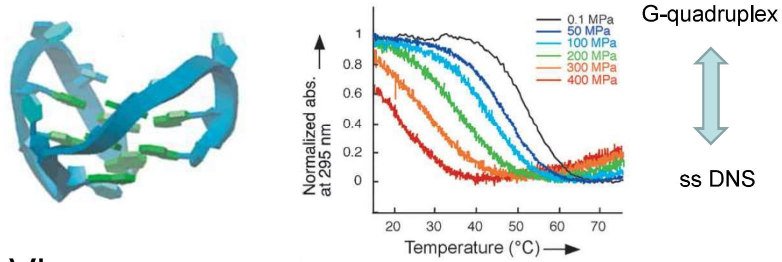
Fig. 10. Pressure–temperature diagram of DNA helix–coil transformation. Unlike the heat unfolding temperature of proteins, the melting temperature of DNA does not show any curvature, but a purely linear pressure dependence. The numbers refer to the molar concentration of neutral salts. Drawn after [84].

Exotic DNA structures:
G-quadruplex



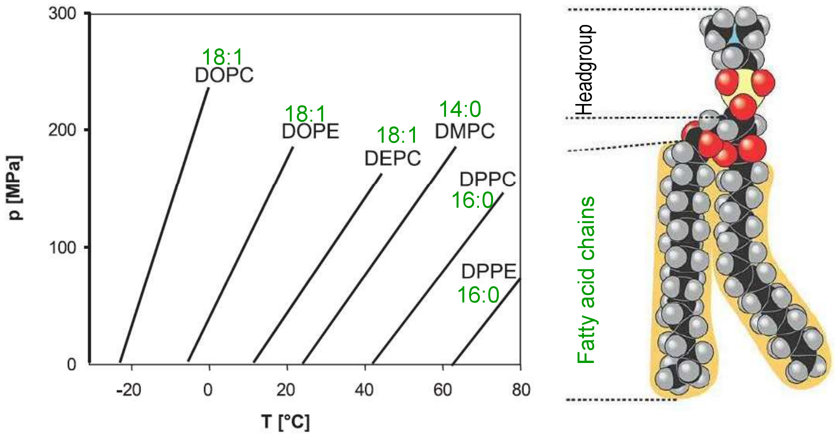
Phase diagram of DNA

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

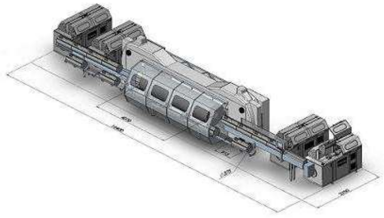


ΔV!

Phase diagram of membranes



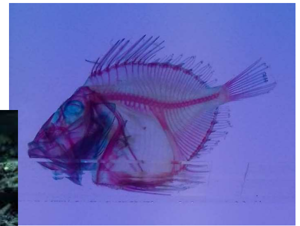
Applications



Pressurized pizza at a high pressure conference

Pressurized food in a japanese supermarket

Deepsee organisms



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