

Stabilization by H-bonds

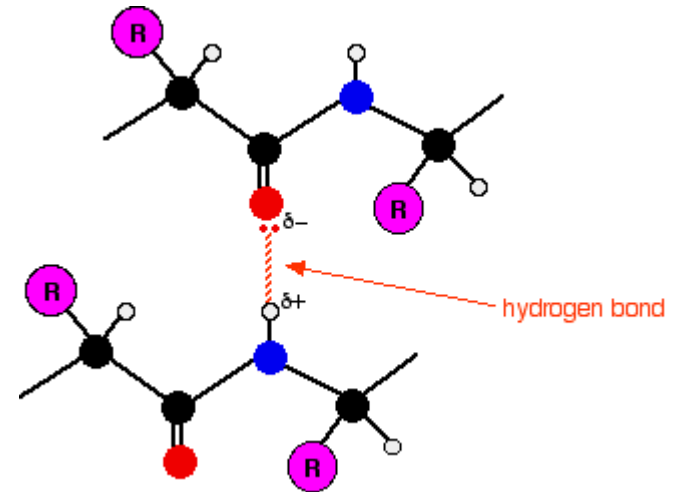
12-30 kJ/mol

Cf: Covalent bond: 200 kJ/mol

van der Waals: 1-2 kJ/mol

thermal energy (RT):

2.5 kJ/mol (T=300K)

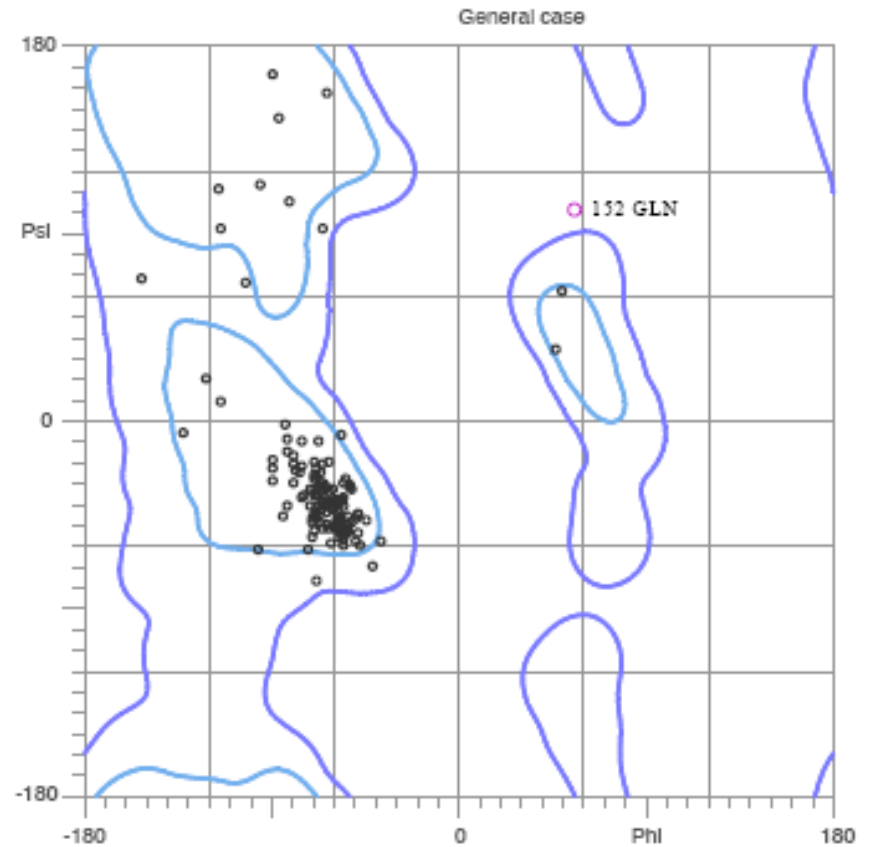
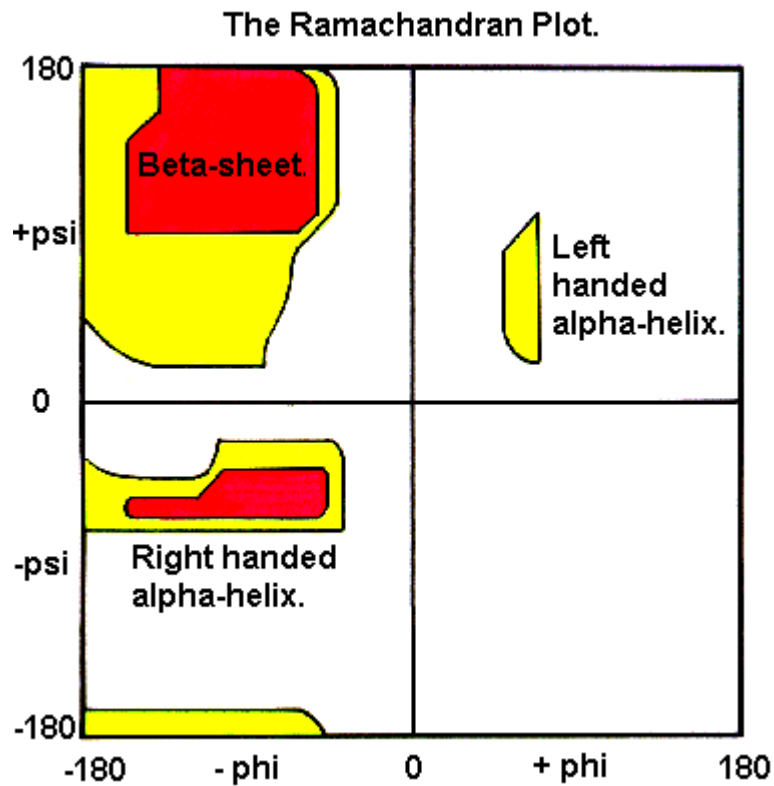


Boltzmann factor: $e^{-\frac{\Delta E}{RT}} = 0.000335 = \frac{1}{2981} \approx \frac{1}{3000}$

($\Delta E=20\text{kJ/mol}$)

Ramachandran plot

1YMB,



Special helices

3_{10} -helix* $i \rightarrow i+3$ (10 atom)

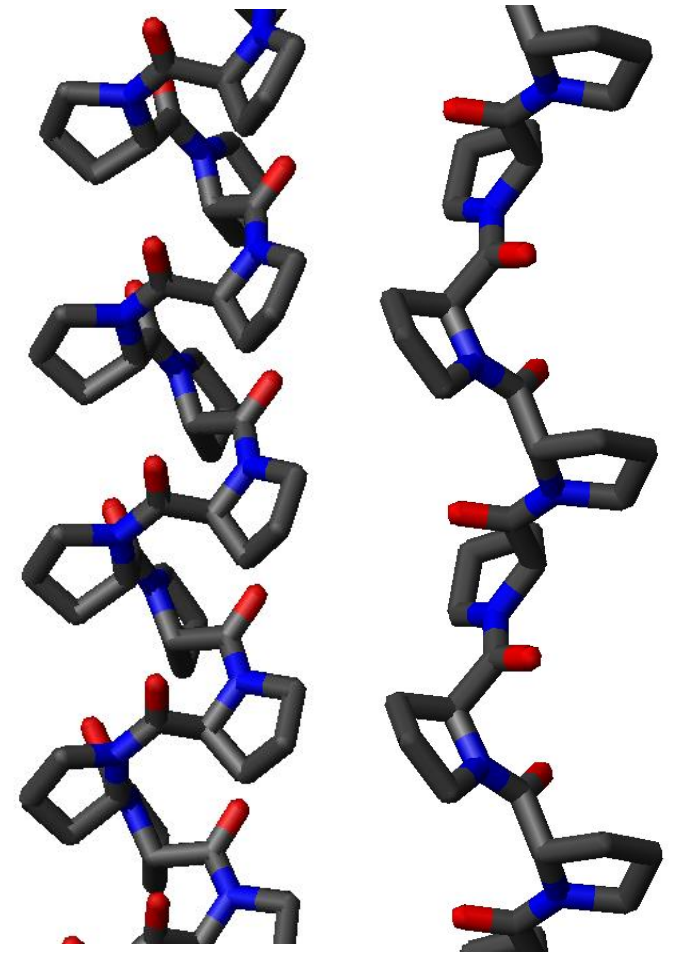
π -helix $i \rightarrow i+5^*$

Polyproline I helix cis

Polyproline II helix** trans

* α -helix: $i \rightarrow i+4$ $3,6_{16}$ helix

** in water



Polyproline

I

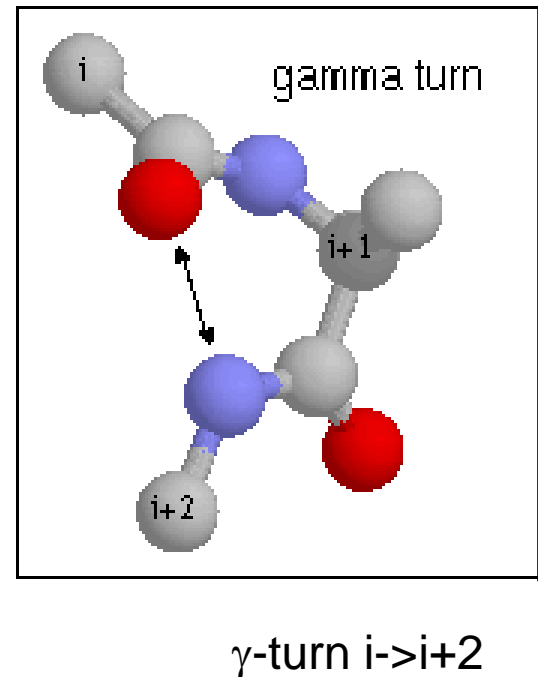
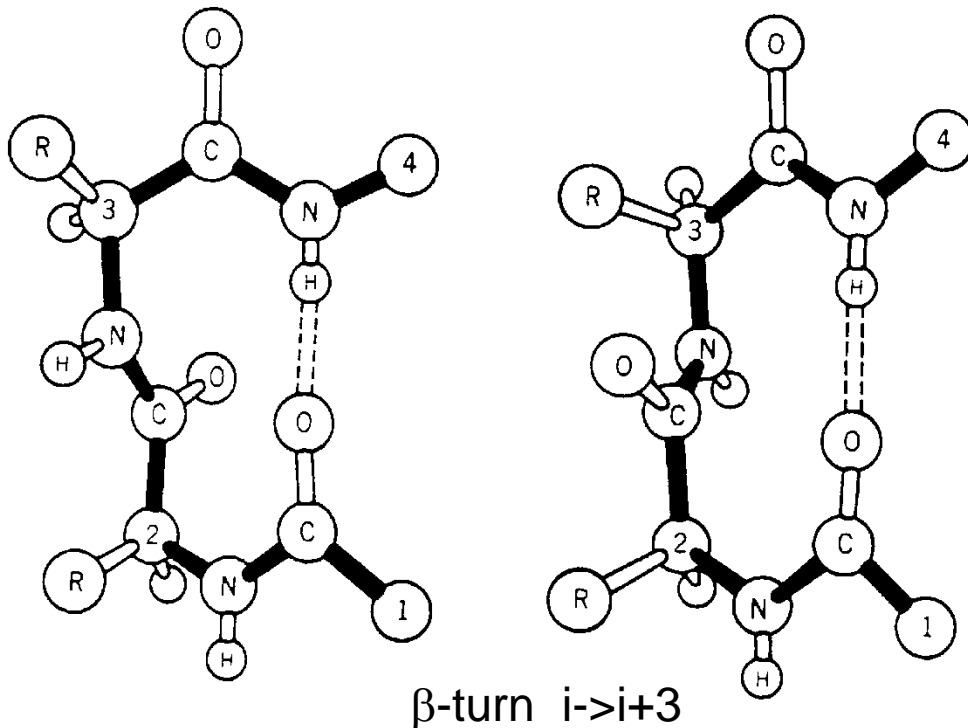
II

Other nonhelical structures

Loops and turns

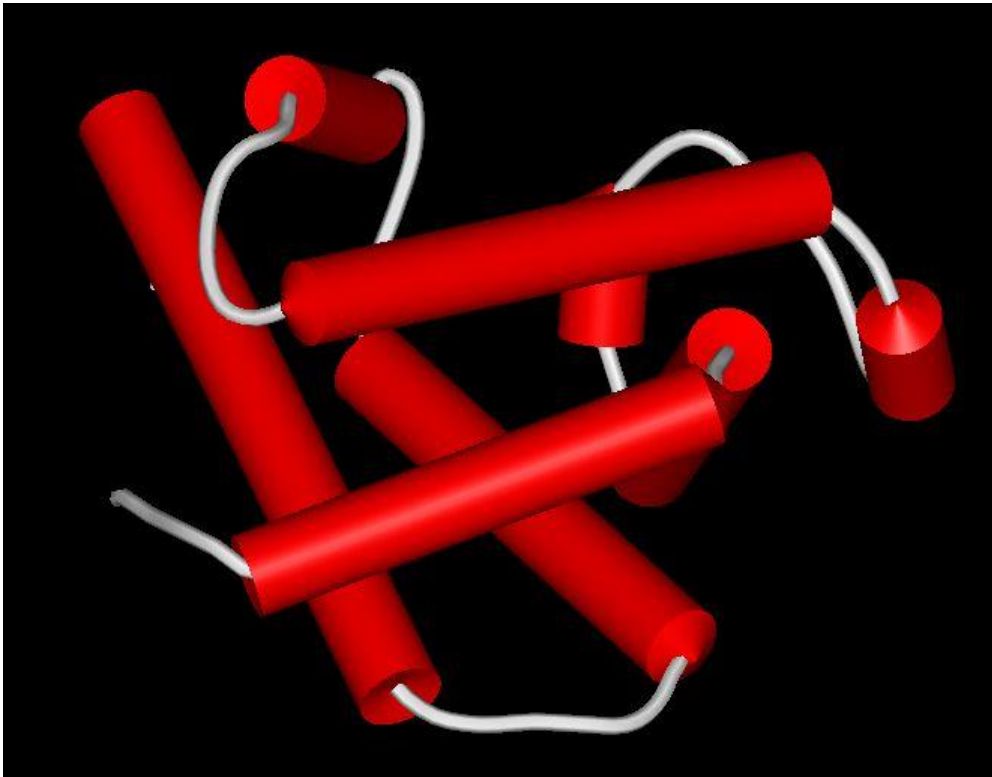
(loop)

(turn)

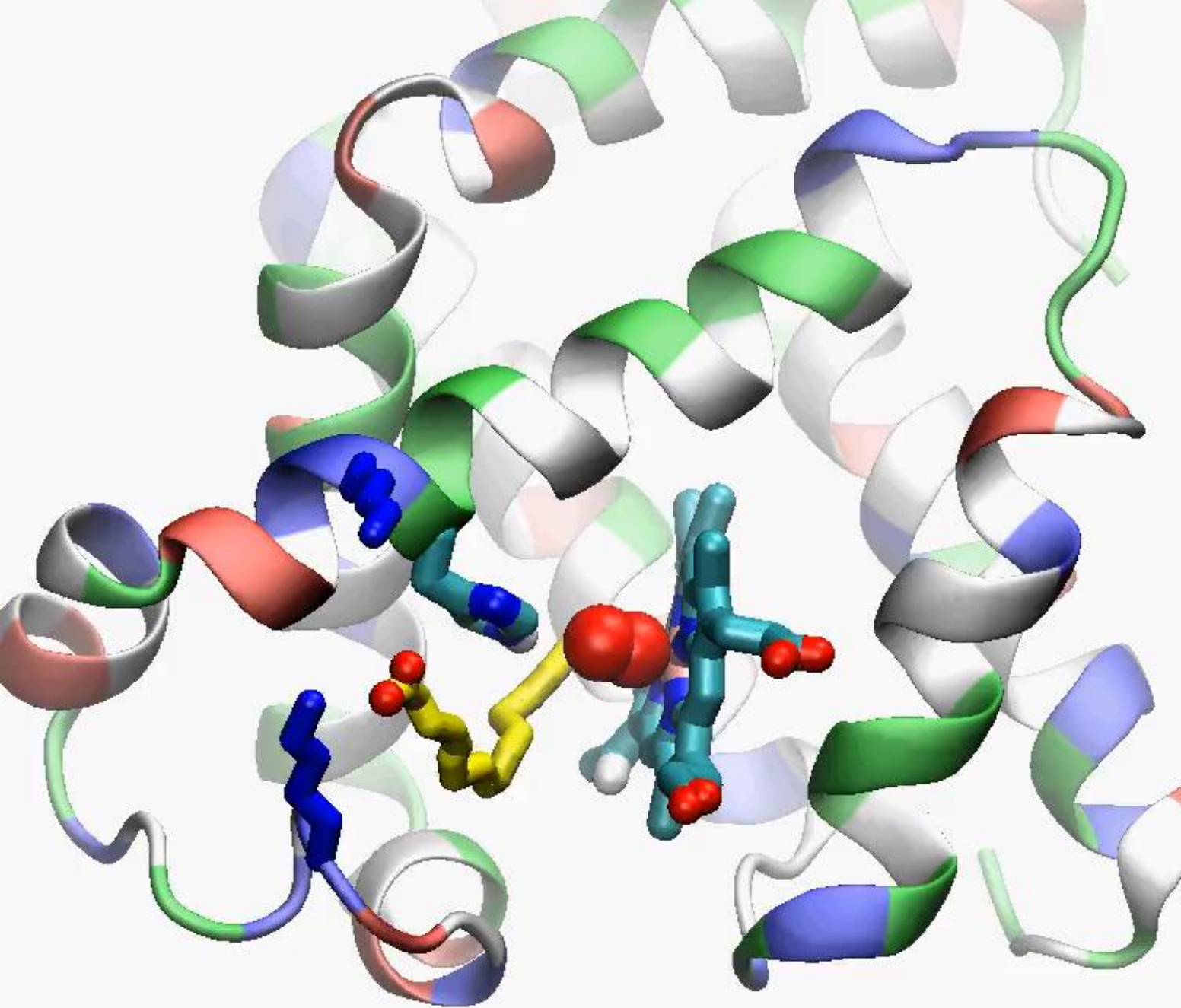


Tertiary structure

Overall topology of the folded polypeptide chain
(Organization of the secondary structure elements)



Myoglobin



Oxy-Myoglobin
+
Palmitic acid

100ns
simulation
slow-motion
video

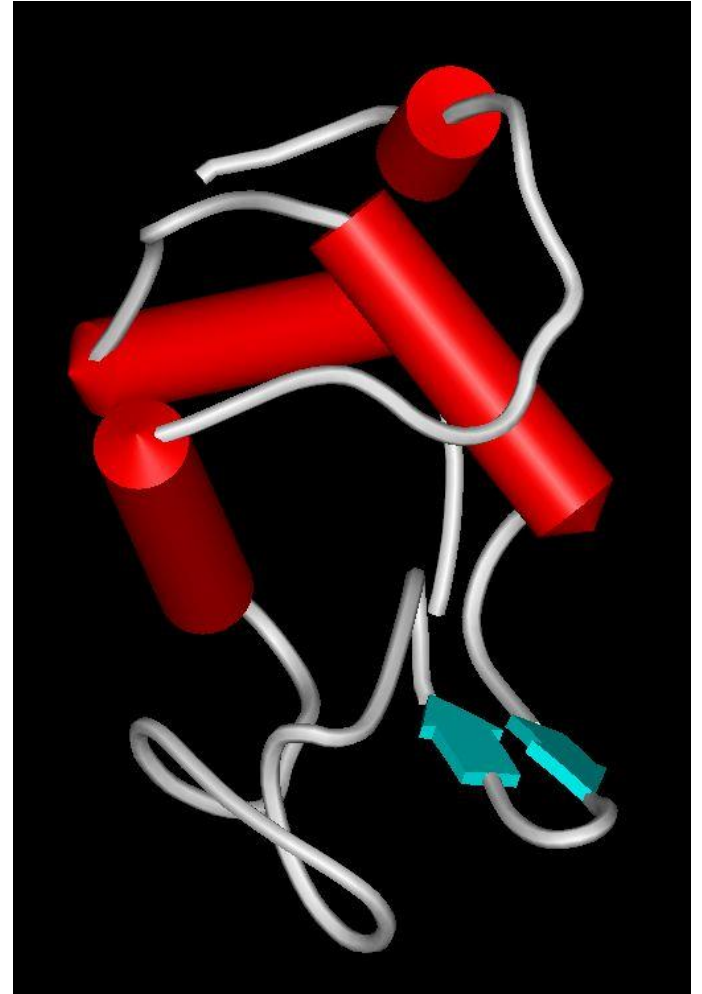
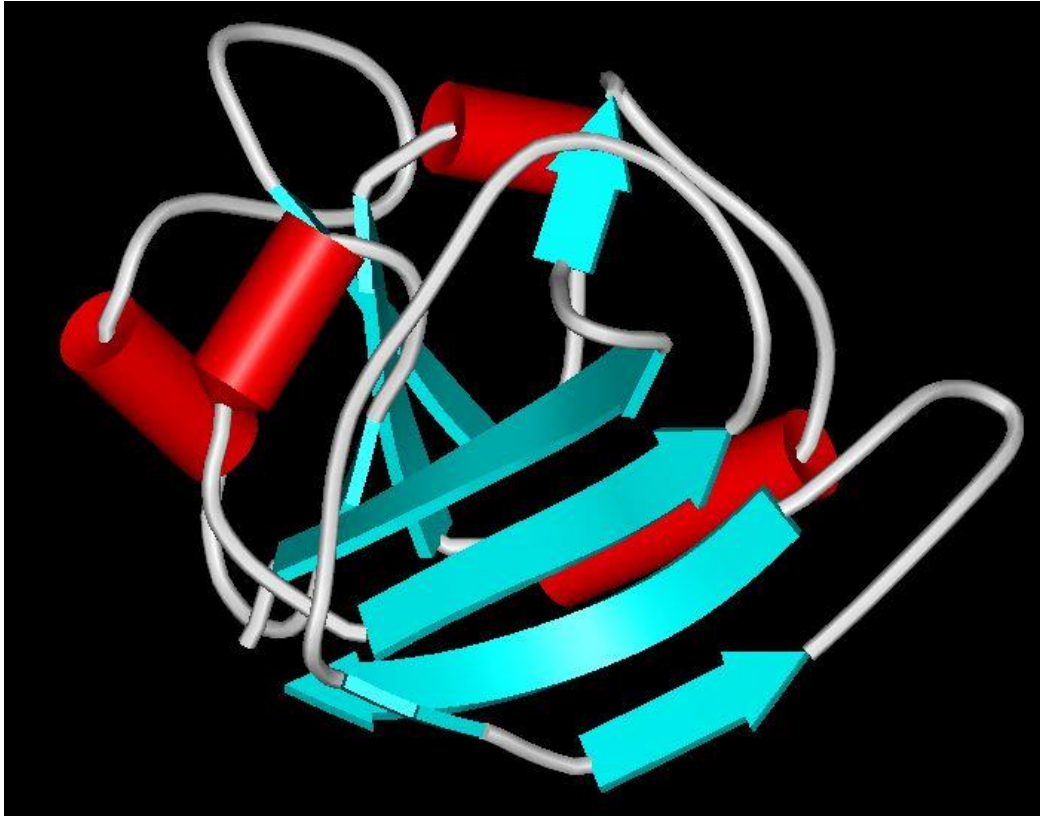
Hierarchy of
time-scales in
motions

ps
ns
μs

Examples

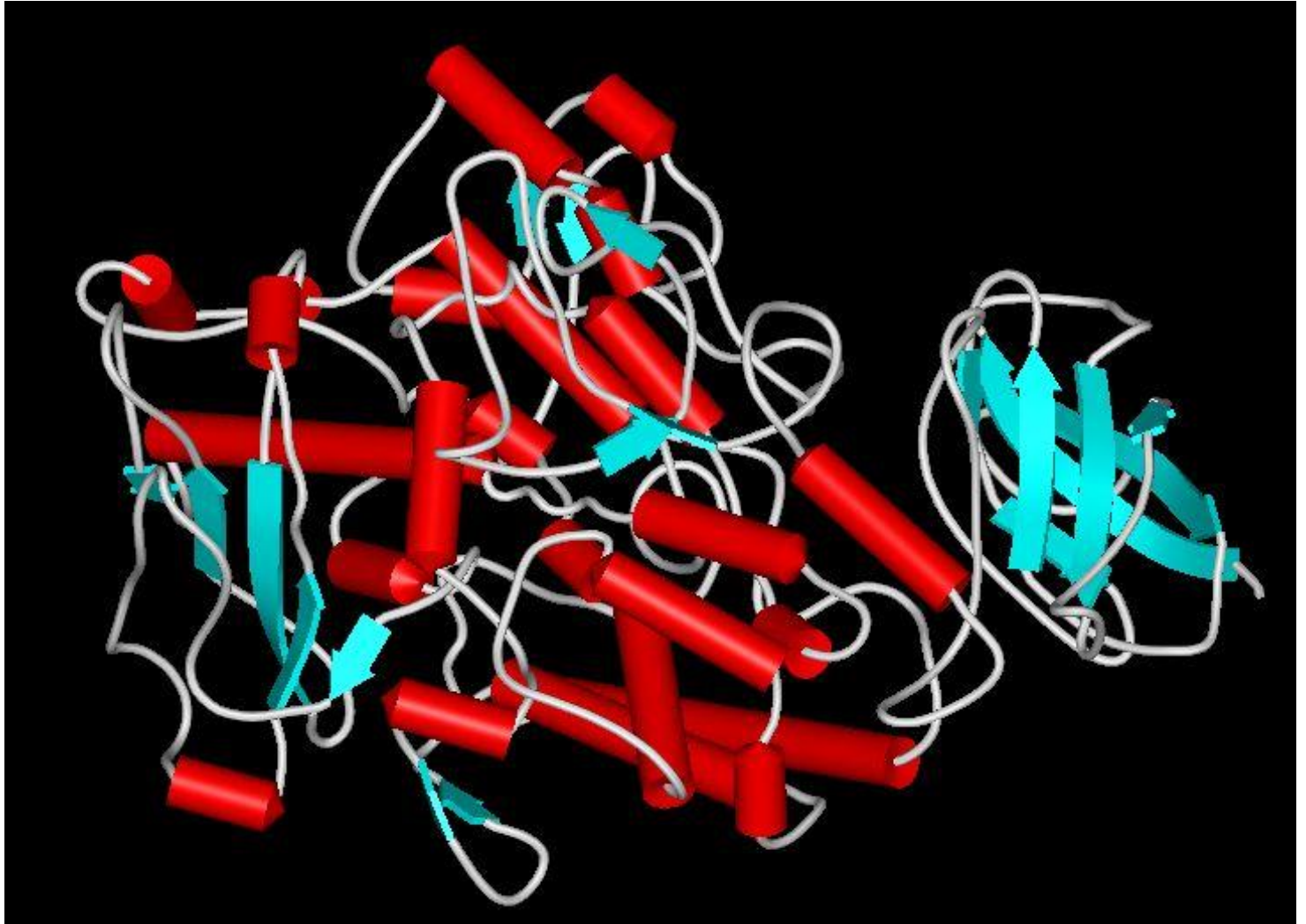
Lysozyme (HEW)

Dihydrofolate reductase

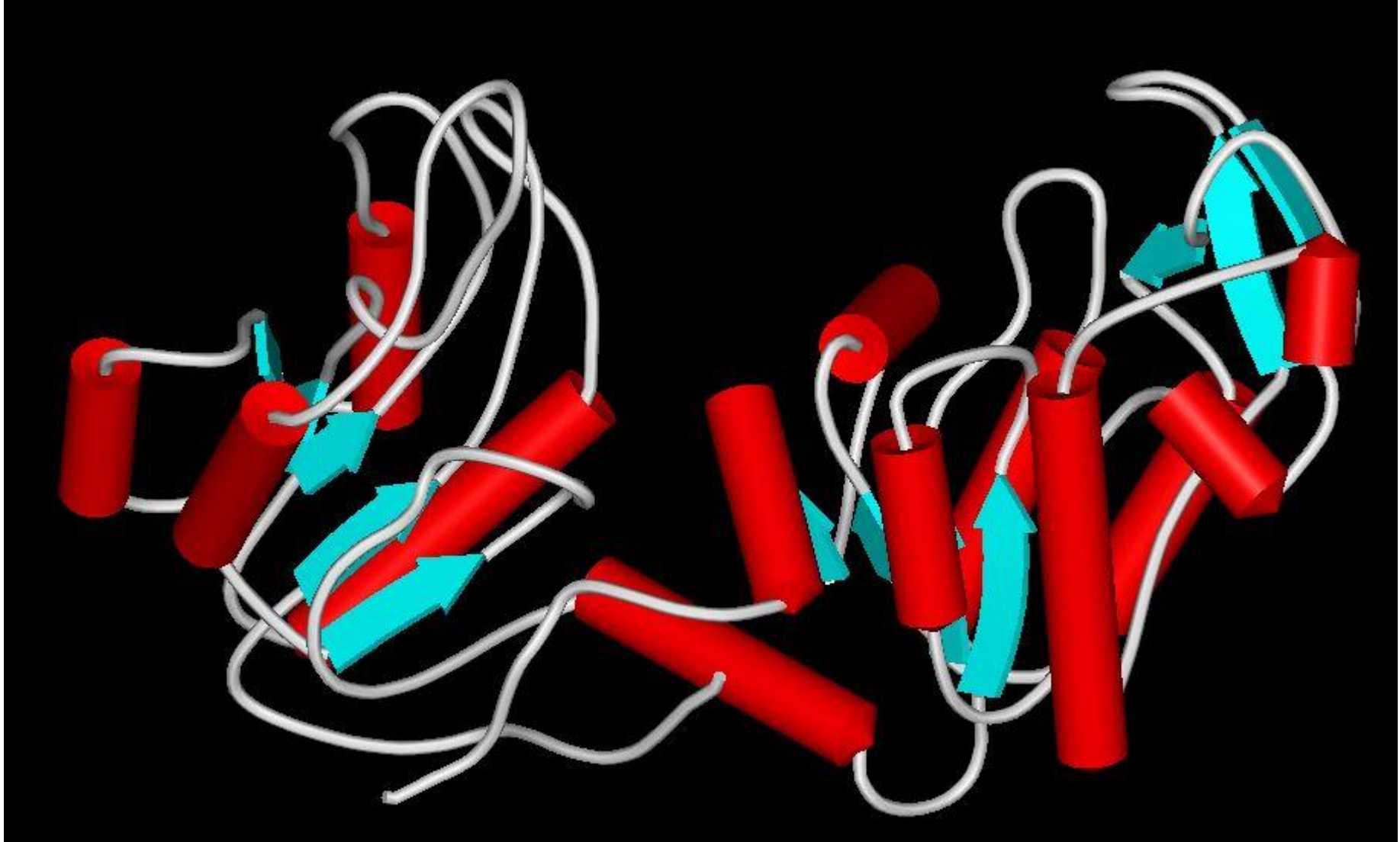


Examples

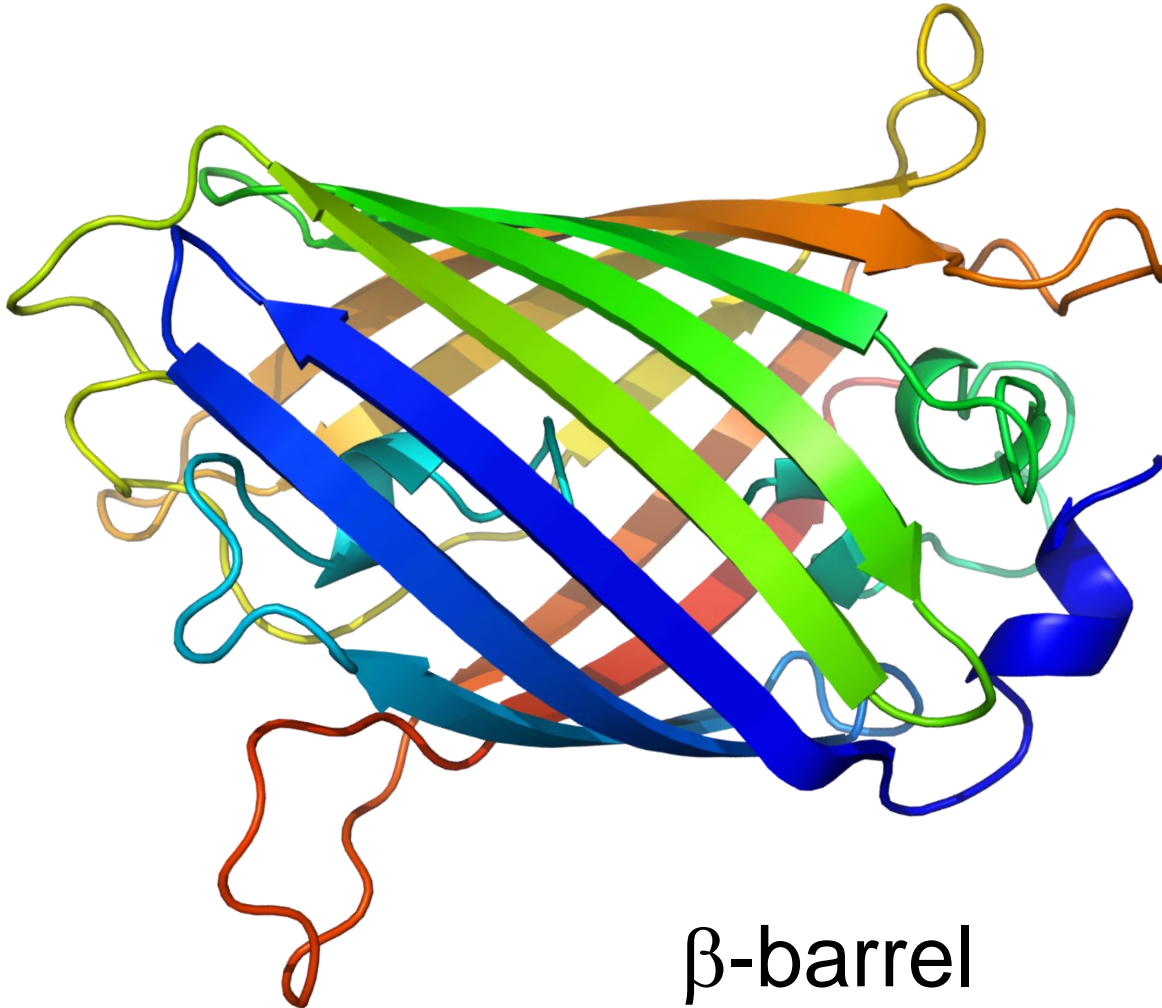
Lipoxygenase



Examples: Phosphoglycerate-kinase (PGK)



Examples: GFP



β -barrel



Stabilization of the tertiary structure

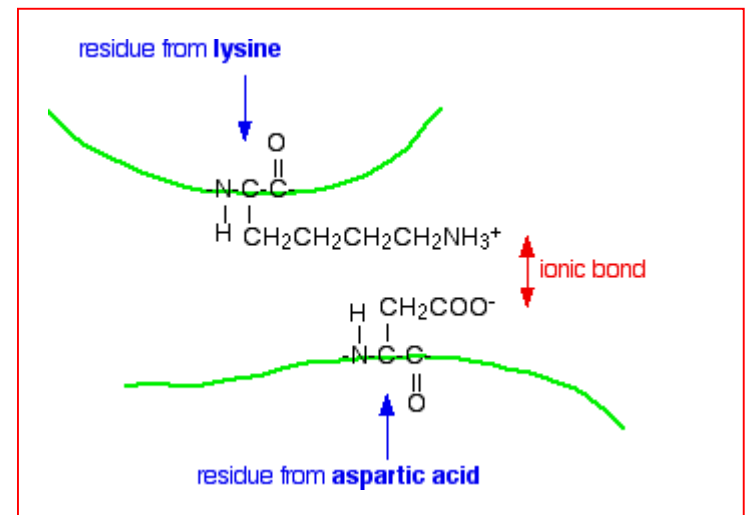
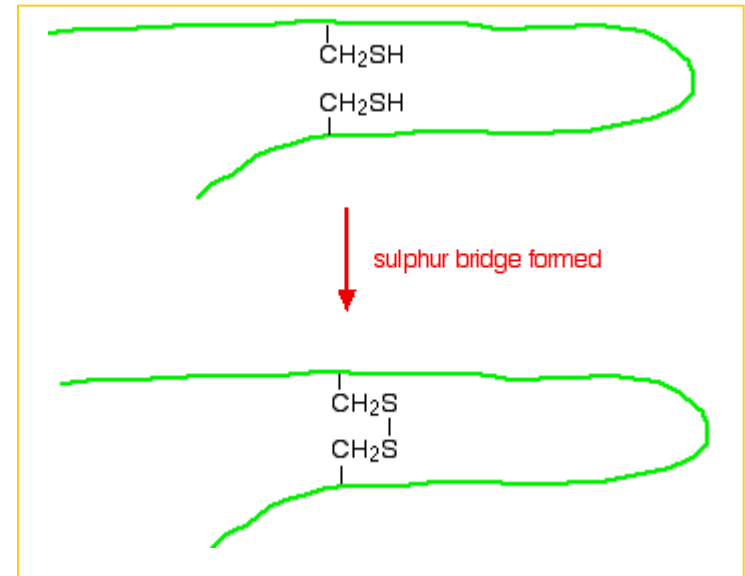
Between the side chains:

disulfide bond

ionic bonds

H-bond

Van der Waals int.



Stabilization of the tertiary structure

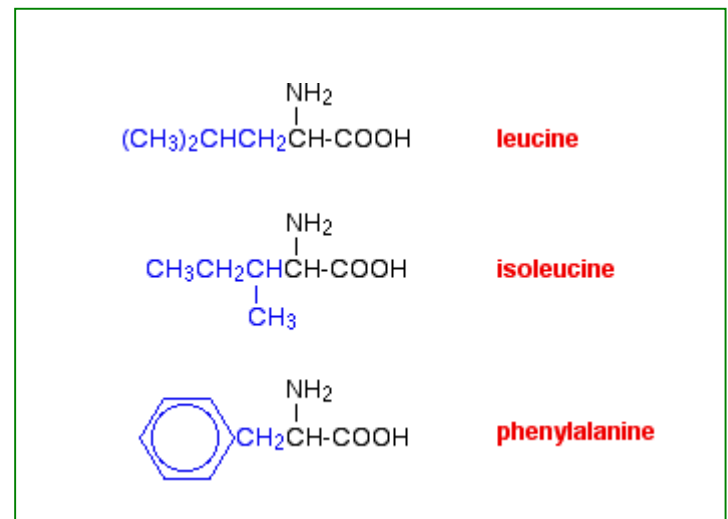
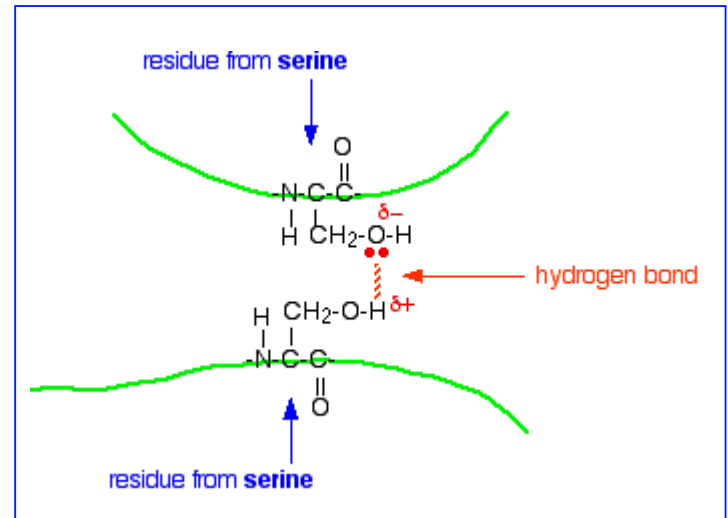
Between the side chains:

disulfide bond

ionic bonds

H-bond

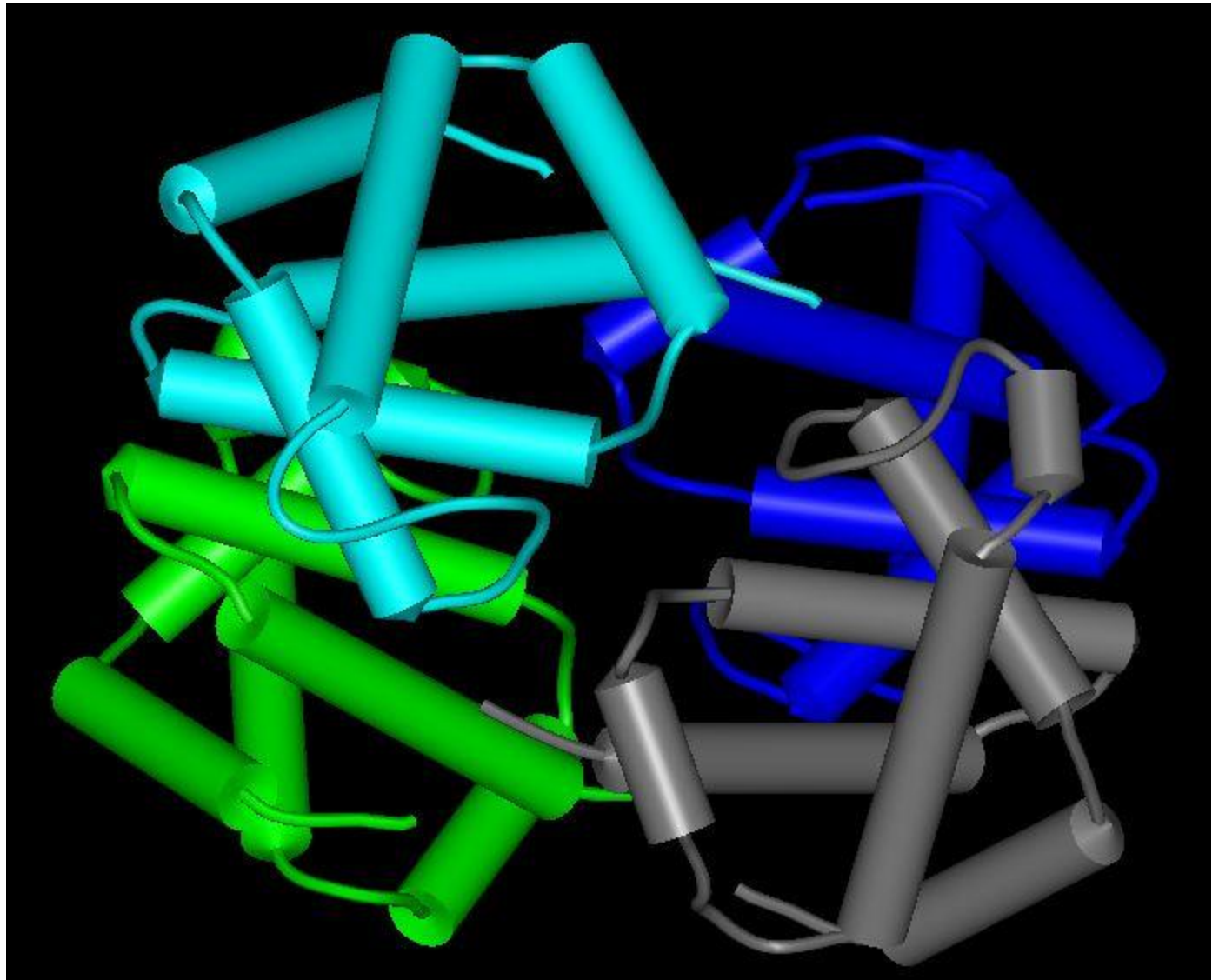
Van der Waals int.

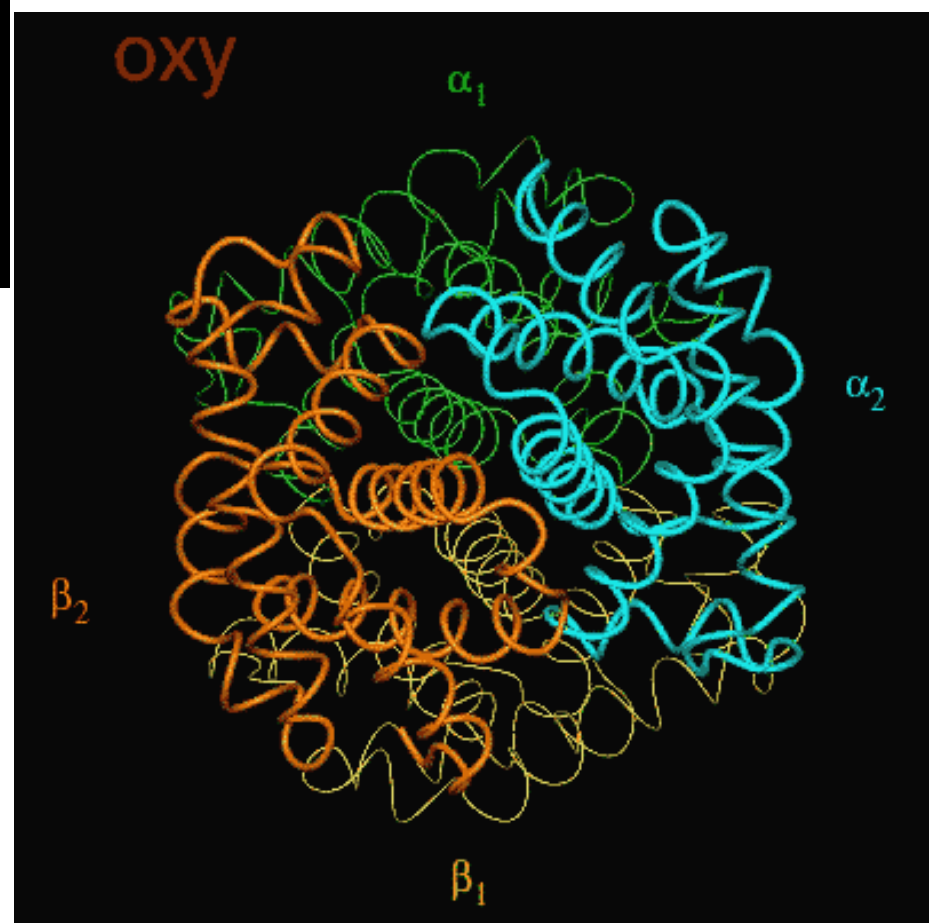
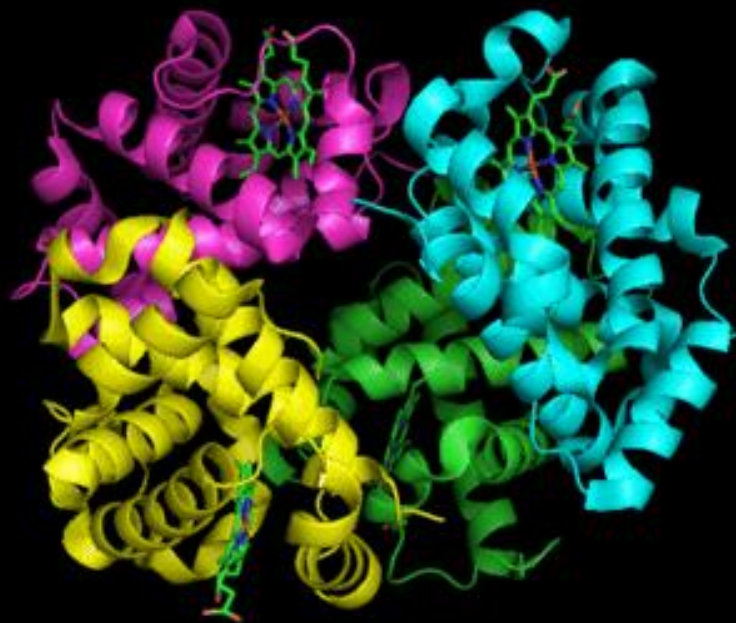


Quaternary structure

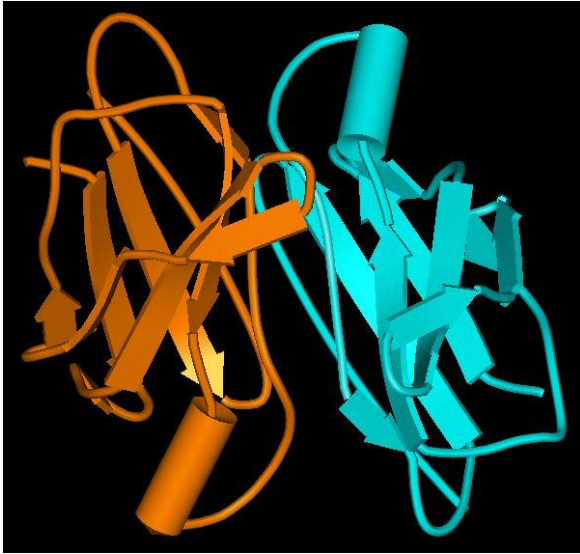
only for
proteins with
more than
one
polypeptide
chains.

E.g.:
Hemoglobin
tetramer

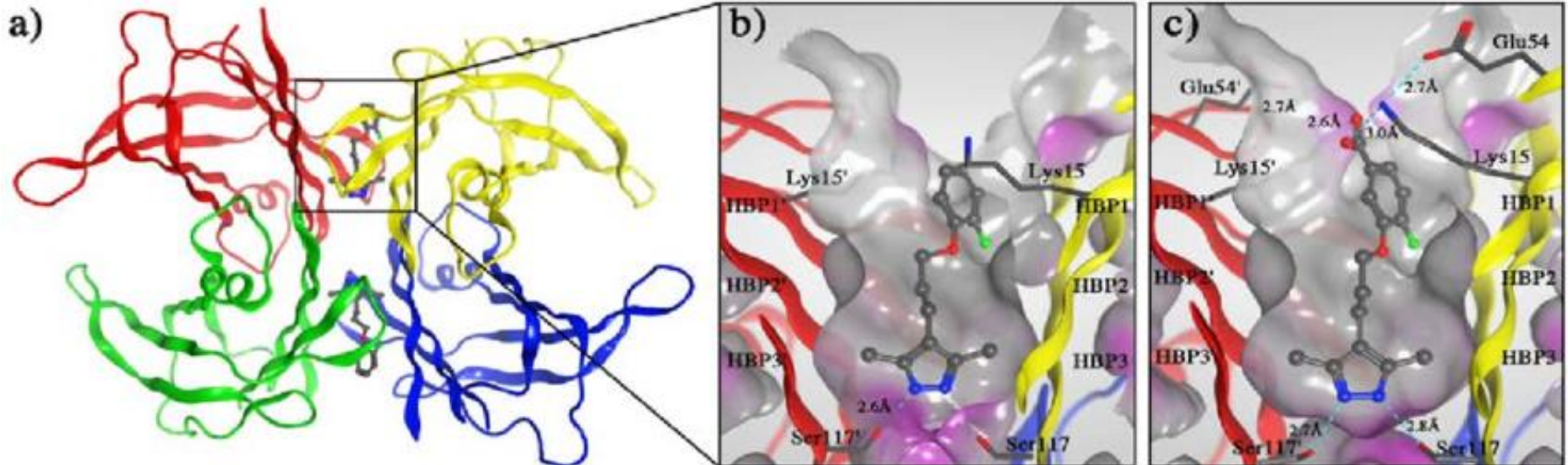




Further examples: Transthyretin

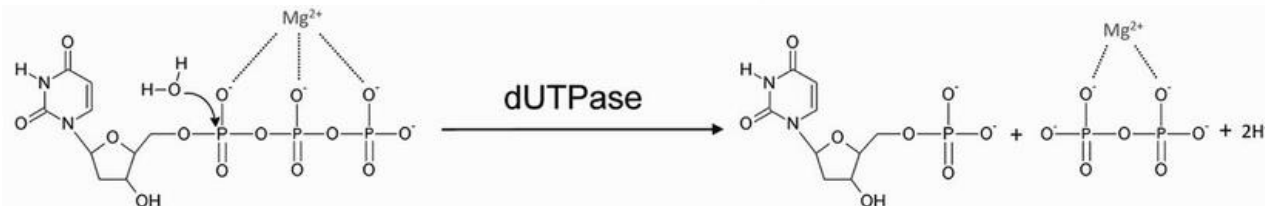
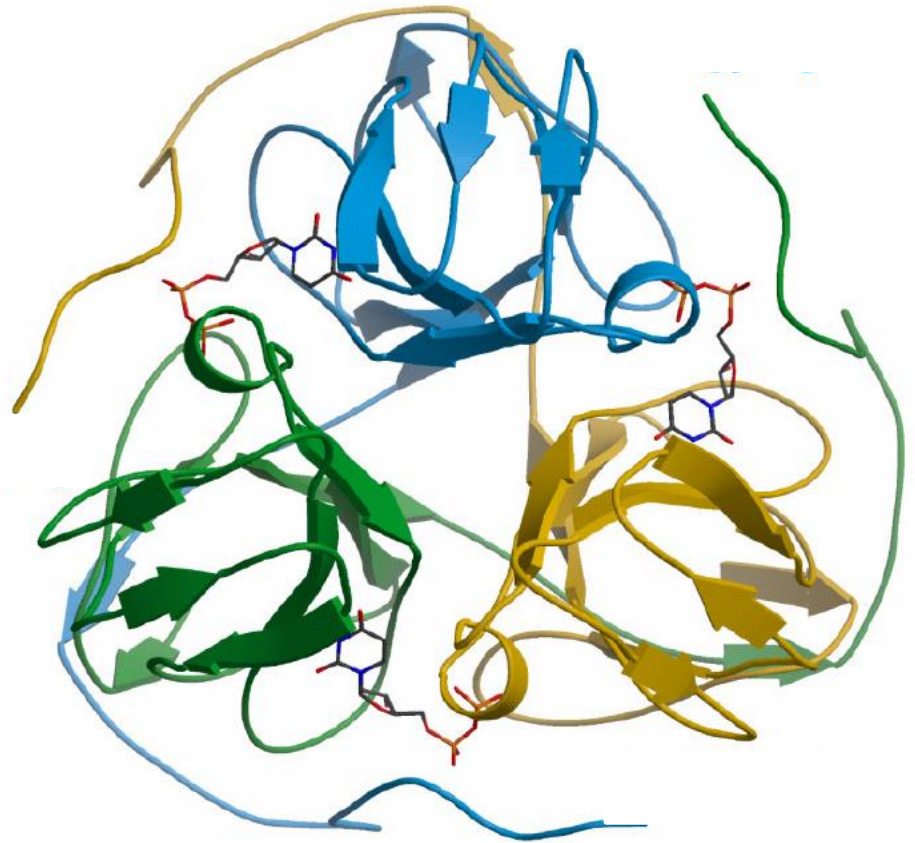


four binding sites;
two for thyroxine and two
for retinol-RBP complex



Further examples: DUTPase

3 subunits



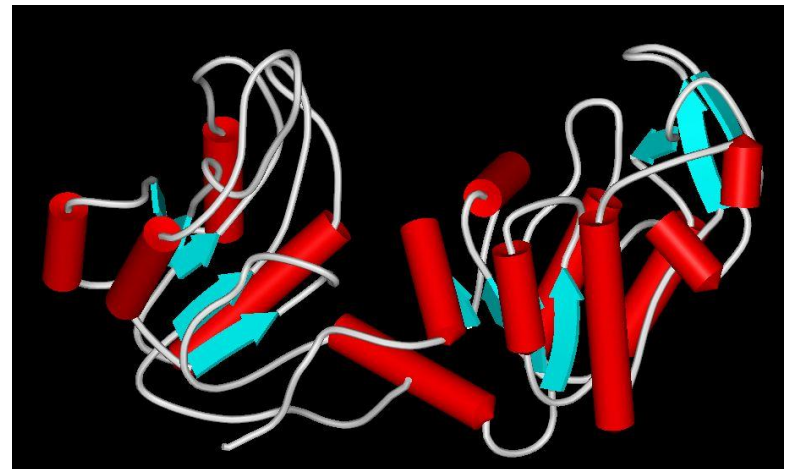
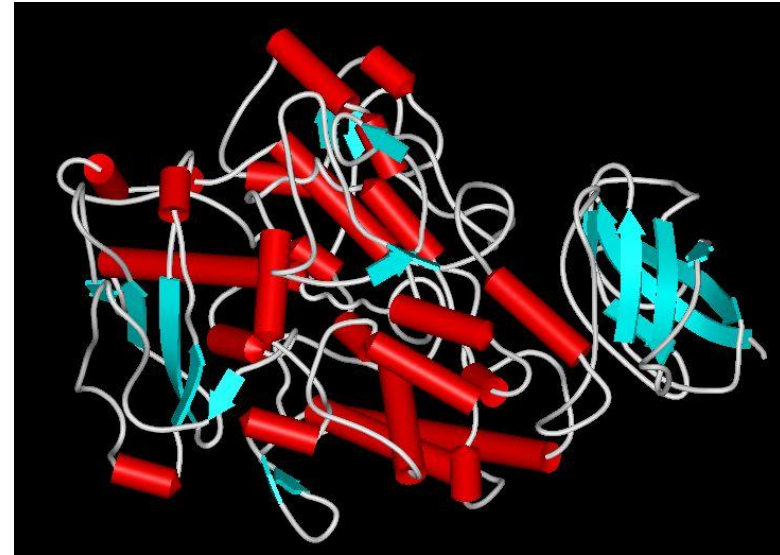
from: http://www.enzim.hu/~vertessy/kovari_phd.pdf

Important further aspects of the protein structure

- Domain
- Prosthetic group
- Posttranslational modifications
- Active site
- Pocket

Domain

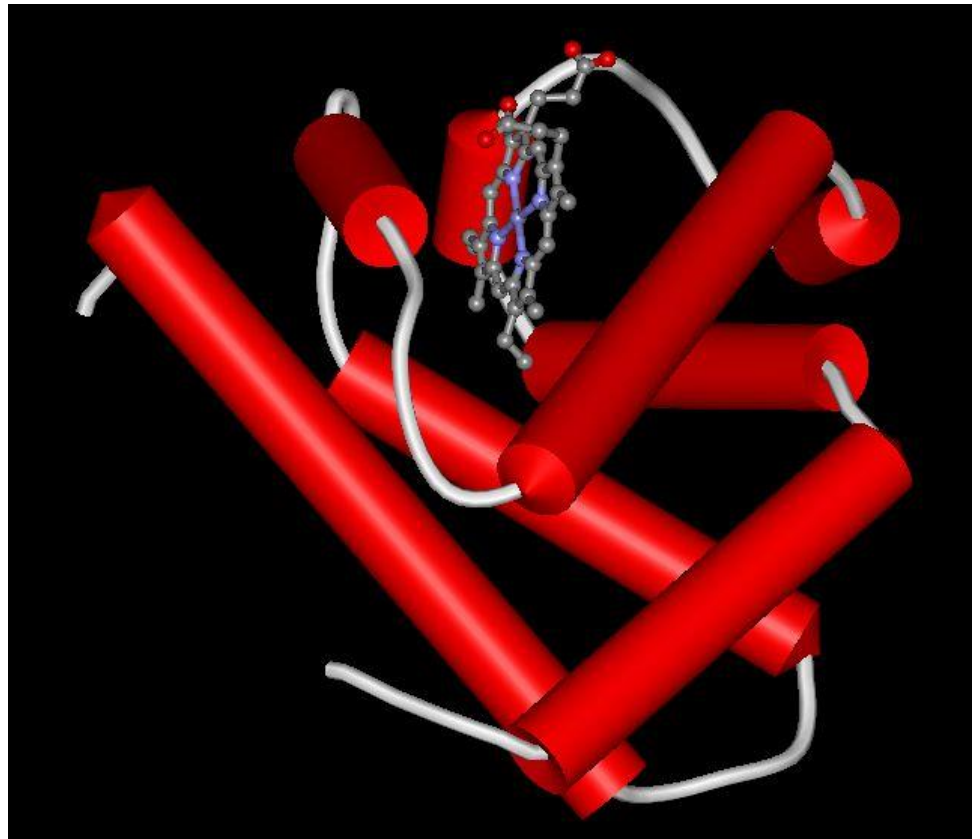
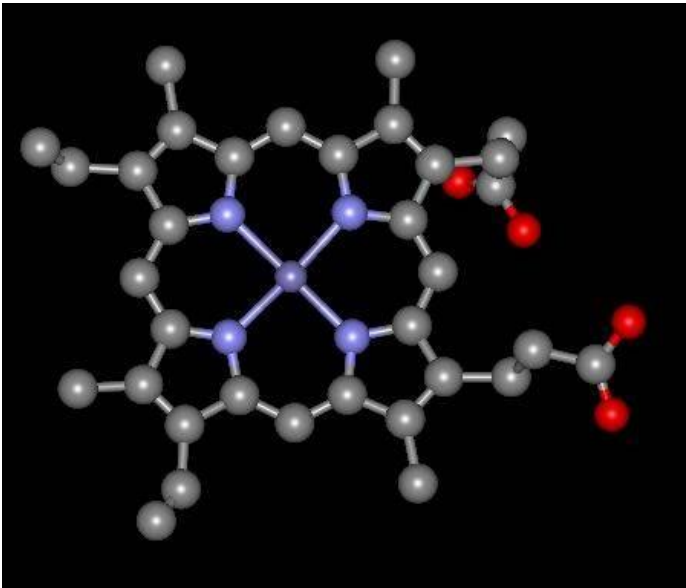
Part of the protein, which can fold into an ordered structure. Its structure is stable, it can function without the presence of the rest of the protein. The different domains of a protein may have different functions: e.g.: ATP binding domain, etc.



Prosthetic group

A non-protein chemical compound that is required for an enzyme's activity. They are bound strongly to the protein.

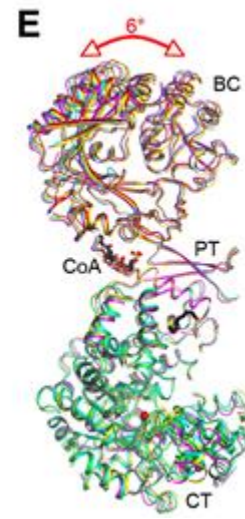
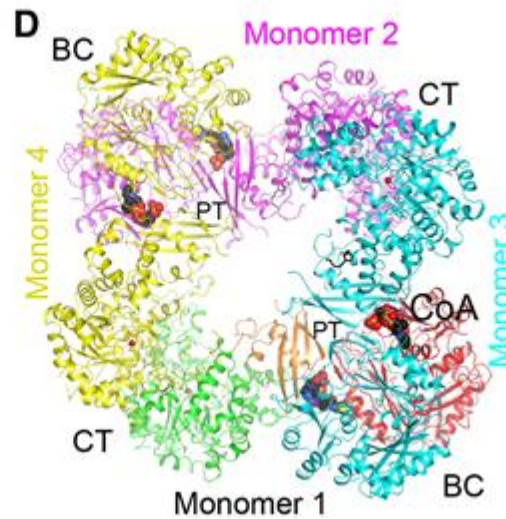
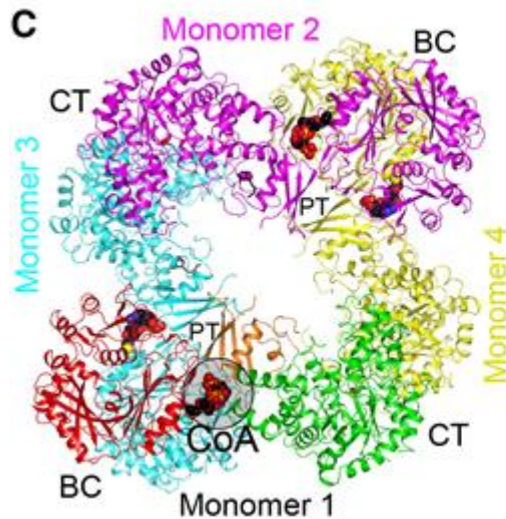
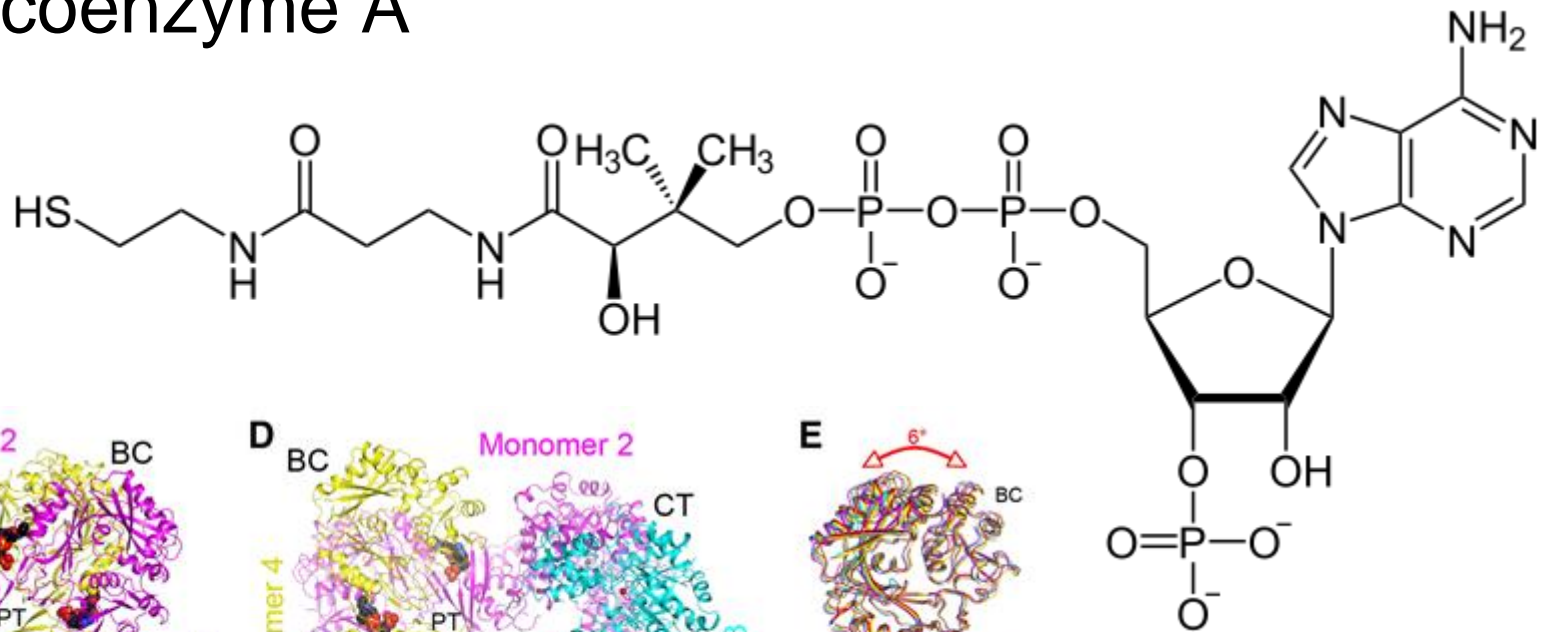
E.g.: hem group



Coenzymes

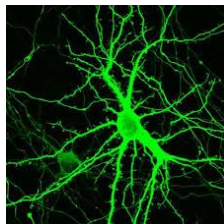
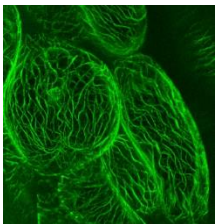
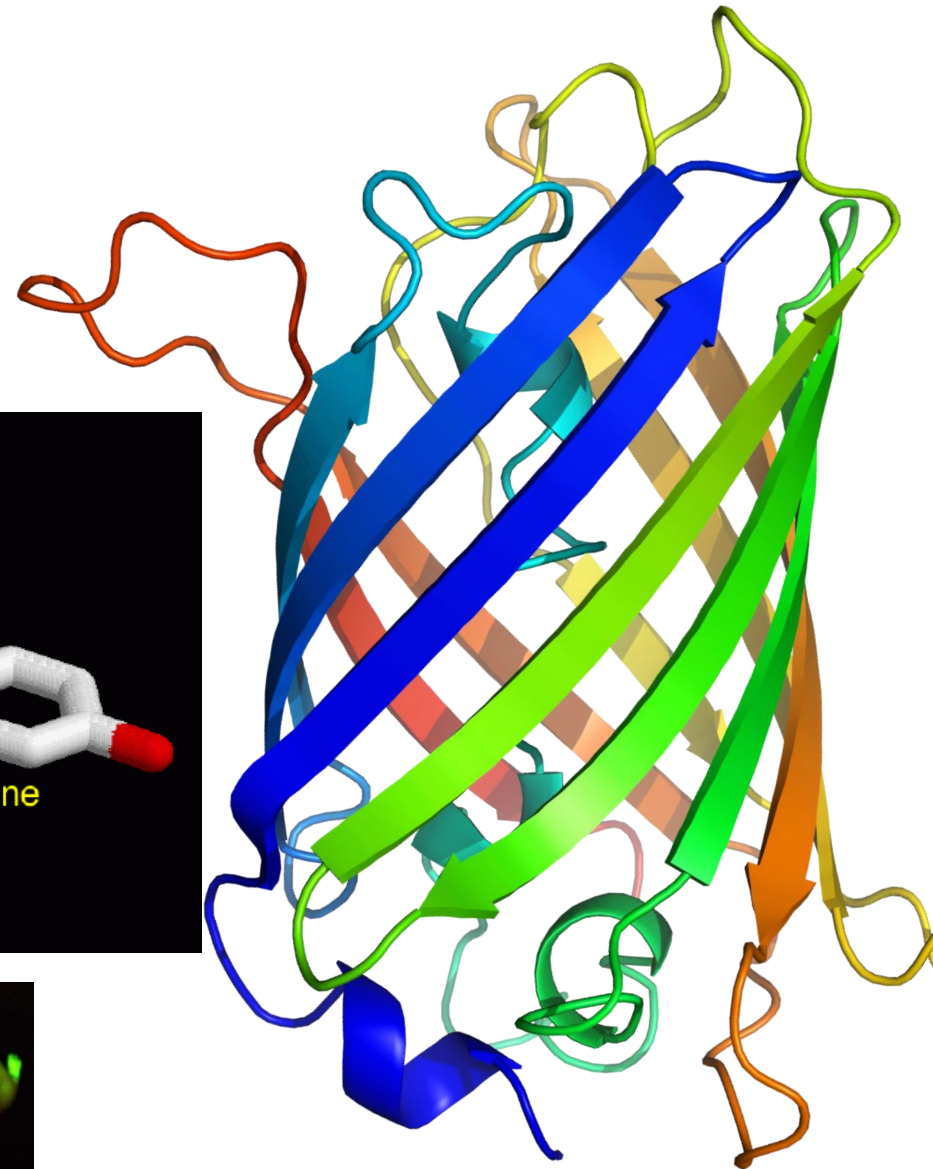
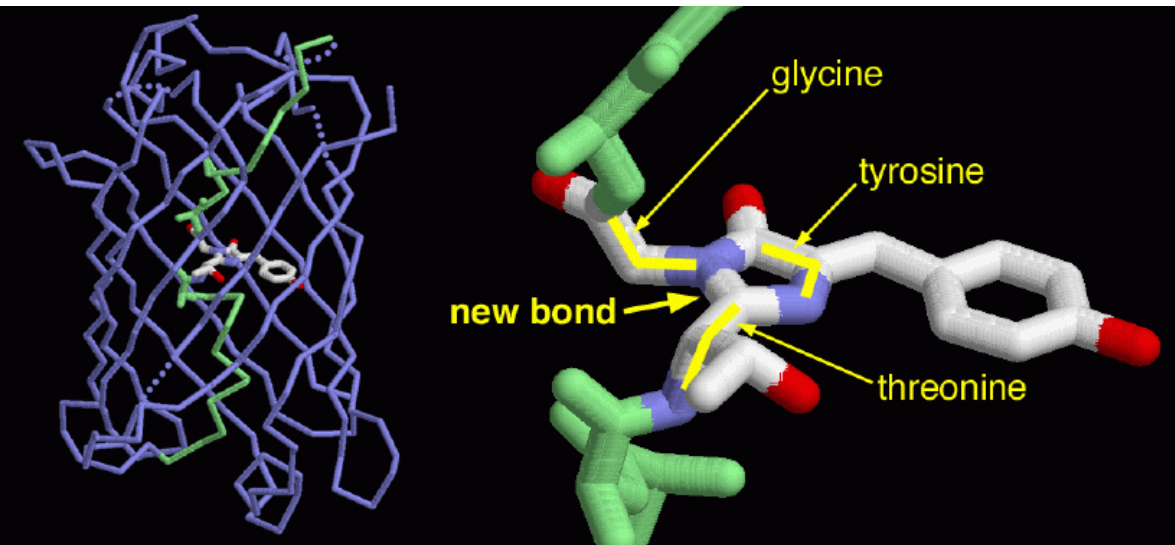
A cofactor is a non-protein chemical compound that is required for an enzyme's activity. They bind weakly and reversible.

example: coenzyme A



Posttranslational modifications

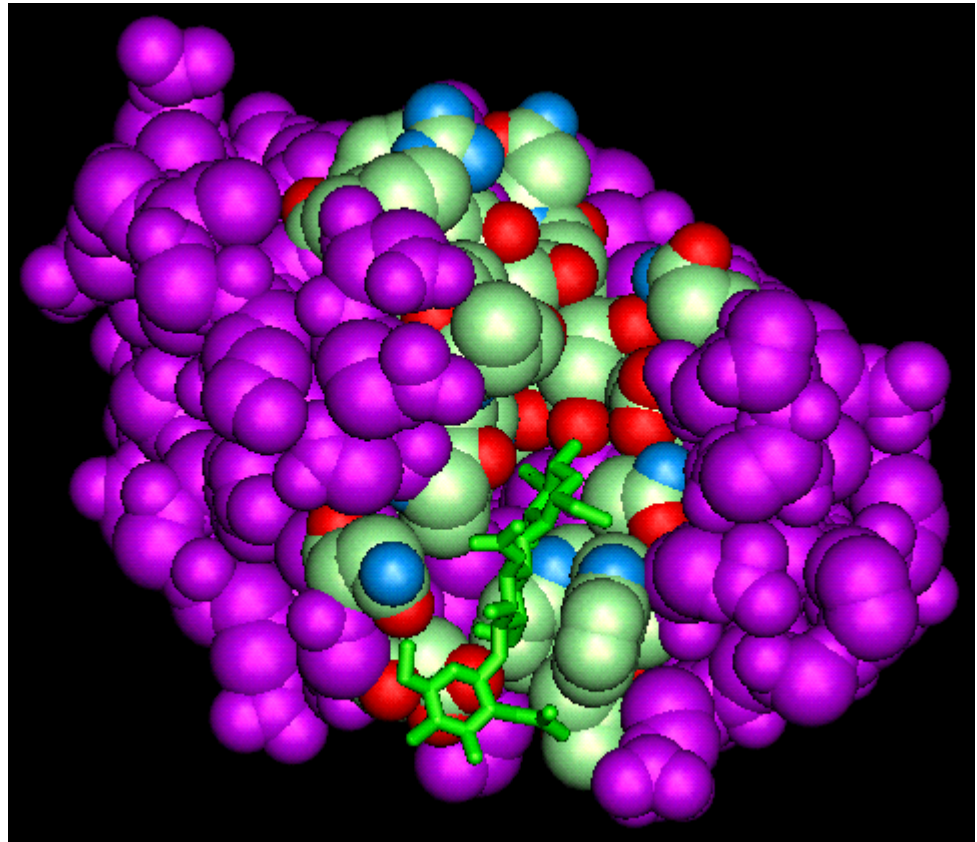
E.g.: formation of the chromophore in GFP



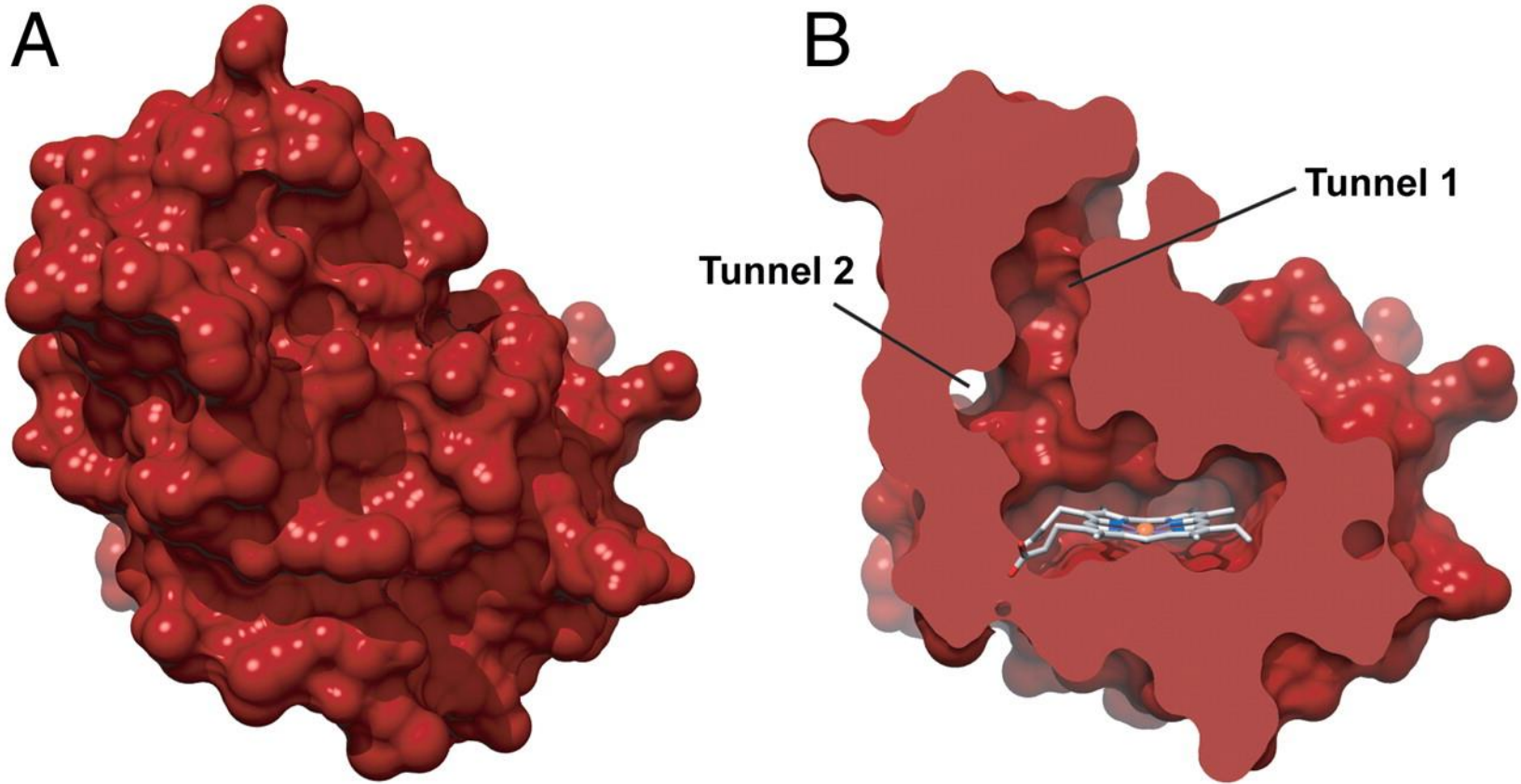
Active site

Active site is the region of an enzyme where substrate molecules bind and undergo a chemical reaction

Binding site
Catalytic site



Hem pocket



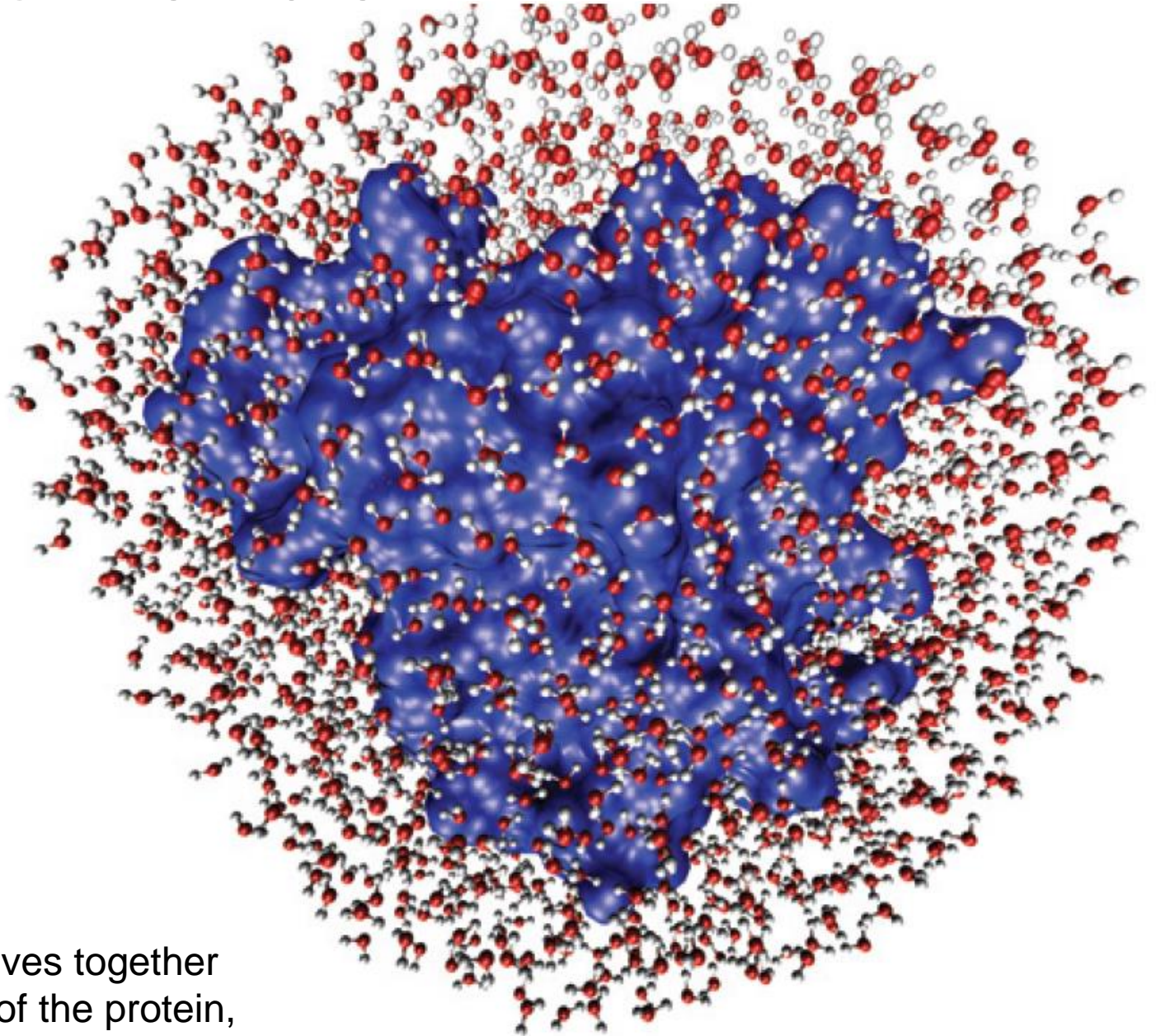
hem nitric oxide/oxygen binding (H-NOX) domain

Winter M B et al. PNAS 2011;108:E881-E889

Role of the water

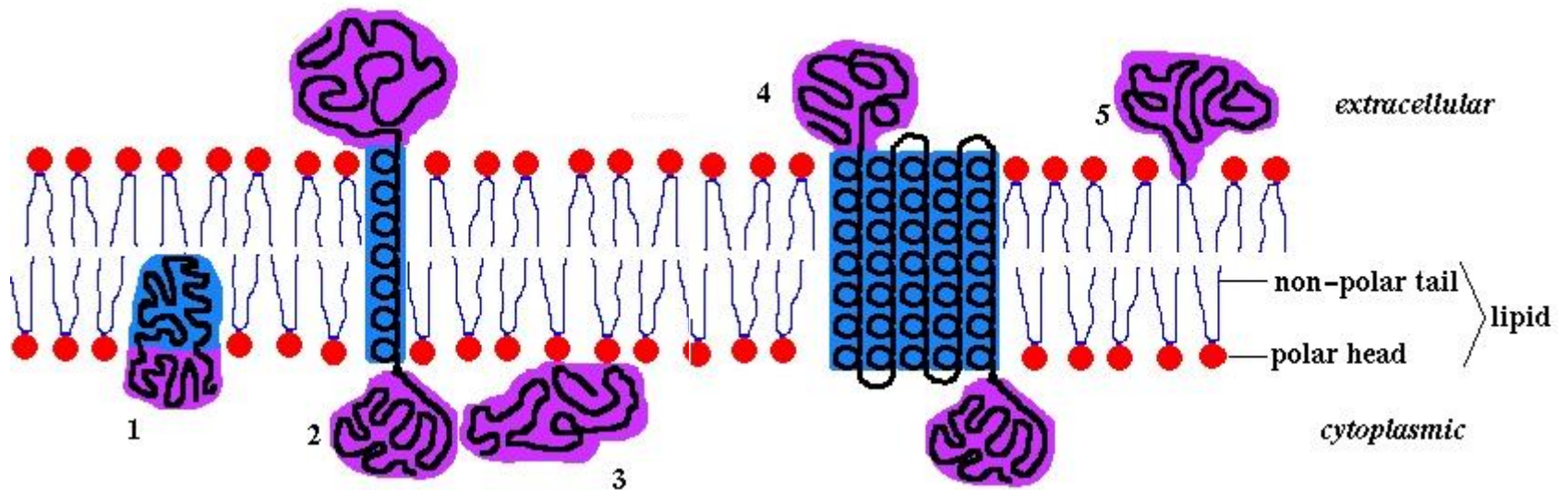
Hydration
layer:

2-3
Water
molecules



This somewhat moves together
with the dynamics of the protein,
couples to the solvent

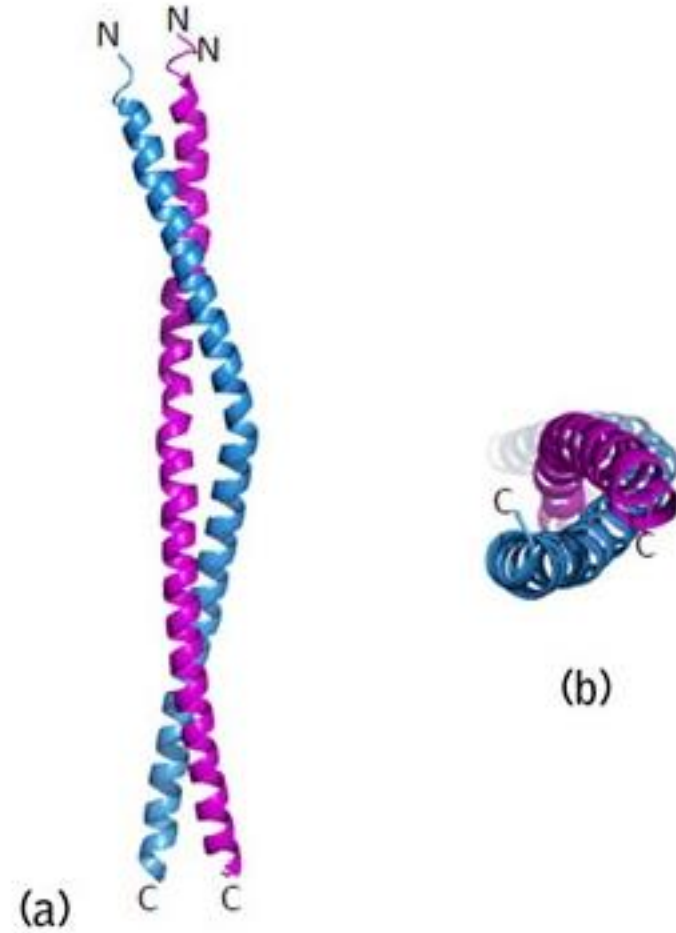
Membrane proteins

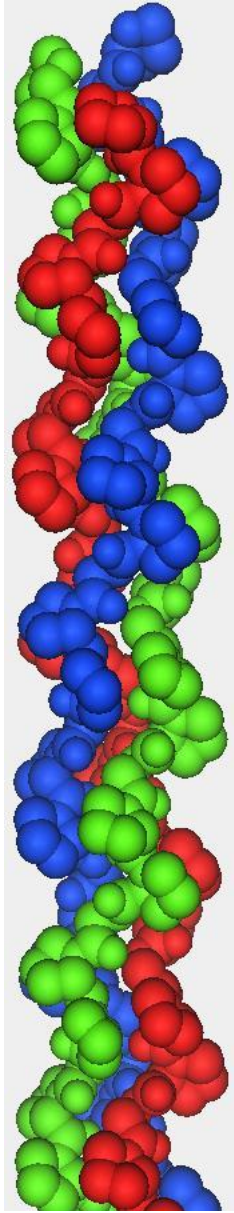


Domains with **hydrophobic** **hydrophilic** surfaces

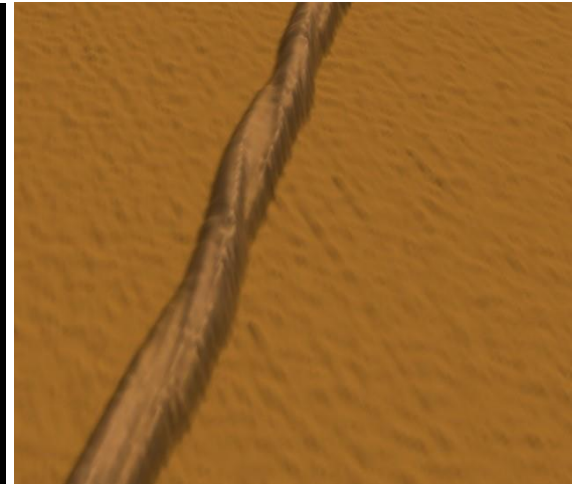
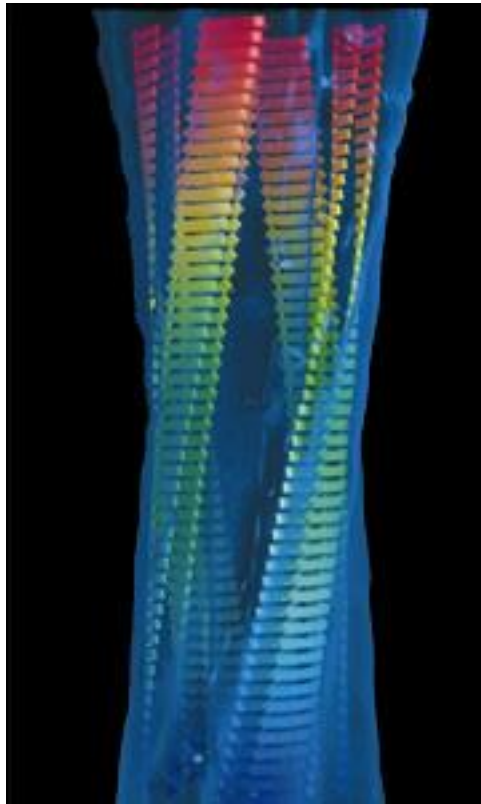
Supramolecular organizations

- Coiled coil
- Collagen
- Fibrillar structures





Collagen



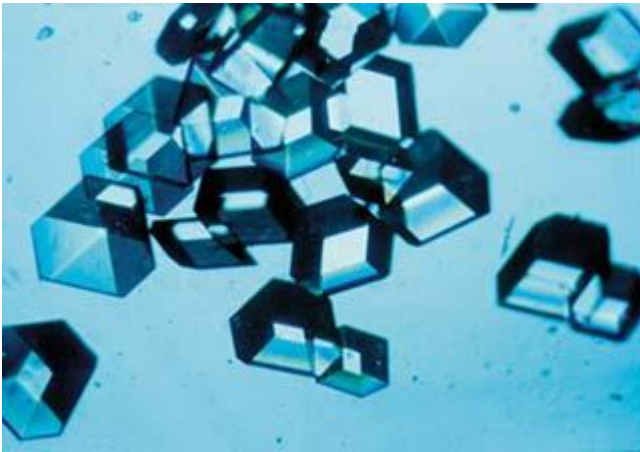
Fibrillar organization

Some methods for determination of the 3D structure of proteins

X-ray crystallography

NMR

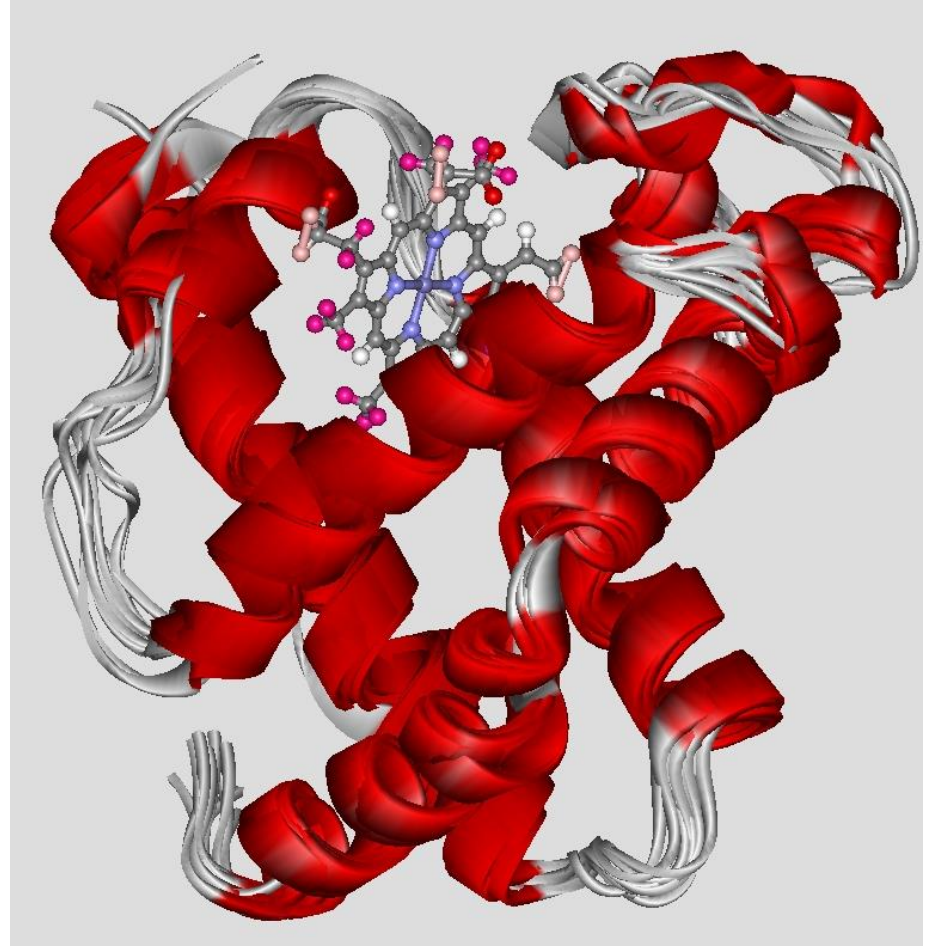
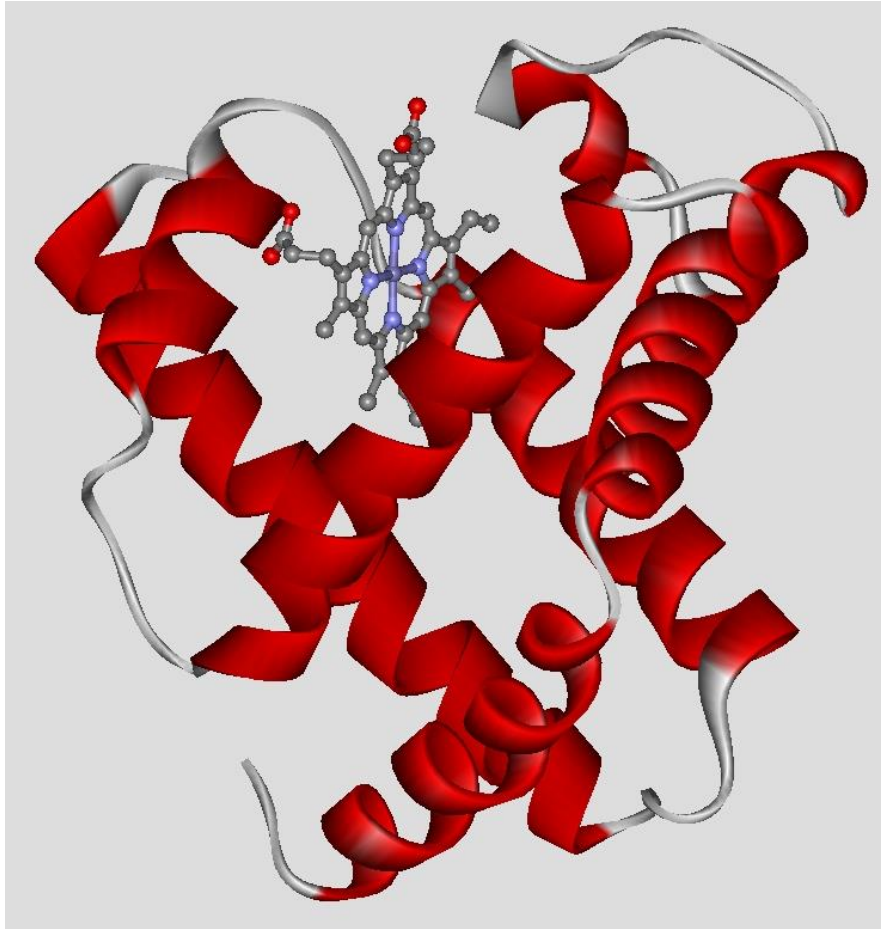
Prediction (homology modelling)



Spectroscopic methods sensitive to the changes of the protein structure

- Circular dichroism (CD)
- Infrared spectroscopy (IR, FTIR)
- Luminescence spectroscopy
- UV absorption spectroscopy
- ...

Crystallography <-> NMR



myoglobin

Protein databases

- PDB

Protein Data Bank

3D Structures (c.a.150 000) from

- X-ray and
- NMR experiments

Swiss-prot

Protein sequences

Proteomics software

Structure prediction (homology modeling)

Calculation/Estimation of the chemical parameters
(e.g. isoelectric point...)

Comparison of the sequences...

Welcome

Deposit

Search

Visualize

Analyze

Download

Learn

A Structural View of Biology

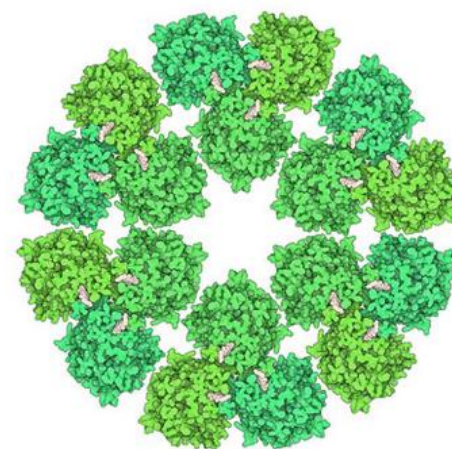
This resource is powered by the Protein Data Bank archive—information about the 3D shapes of proteins, nucleic acids, and complex assemblies that helps students and researchers understand all aspects of biomedicine and agriculture, from protein synthesis to health and disease.

As a member of the wwPDB, the RCSB PDB curates and annotates PDB data.

The RCSB PDB builds upon the data by creating tools and resources for research and education in molecular biology, structural biology, computational biology, and beyond.



February Molecule of the Month



Cellulose Synthase

Latest Entries

As of Tue Feb 09 2021

Features & Highlights



IQB and ERN: Electron Microscopy Community Voice of the Customer
Register for the online February 11 workshop that will solicit feedback from microscopists and facility managers about IT challenges

News

Publications ▾



PDB50: Submit Posters by March 15
Join the wwPDB May 4-5 for a symposium of speakers from around the world who have made tremendous advances in structural biology and bioinformatics » 02/15/2021

26 Feb 2019: 149174 -> 15 Feb 2021: 174507

Stability of biological systems

Destabilizing environmental factors

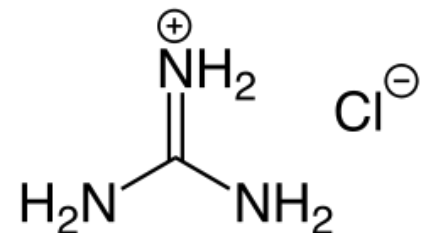
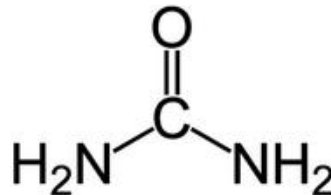
- Physical

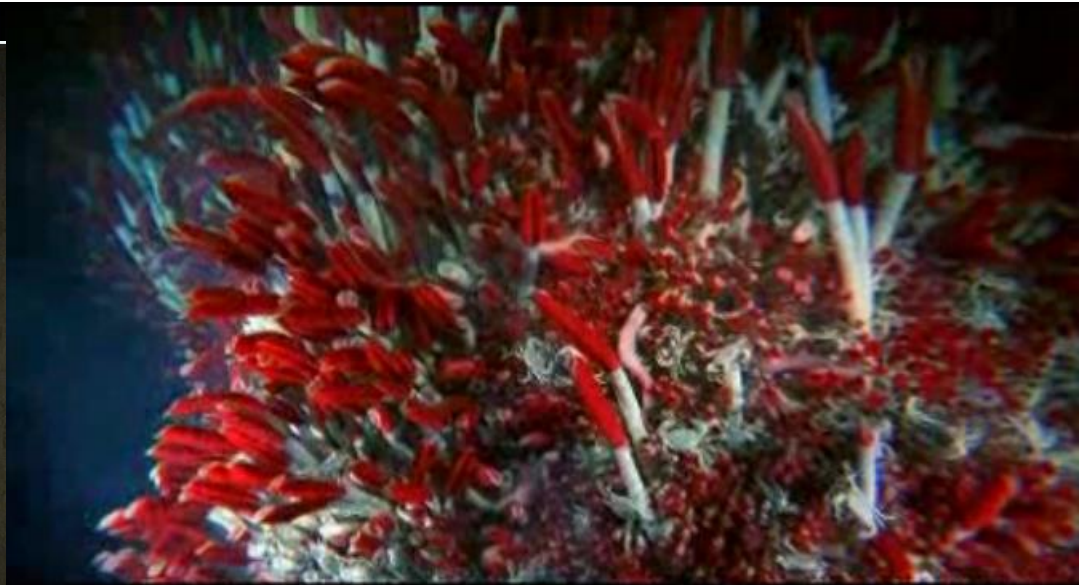
- high temperature
- low temperature
- (high) pressure



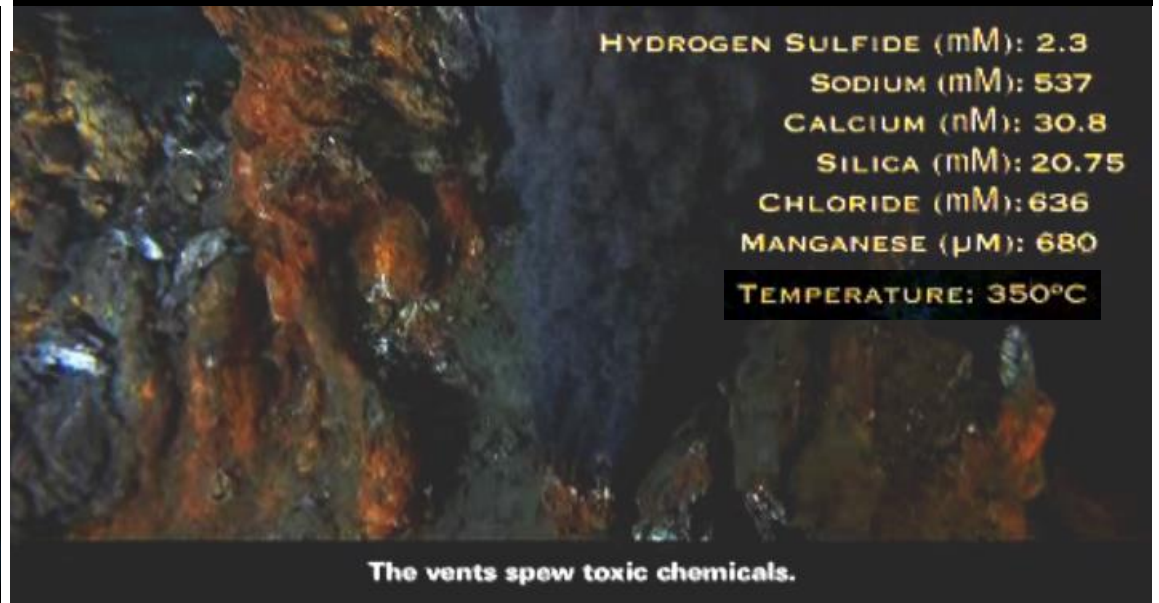
- Chemical

- urea (high conc.)
- GuHCl [guanidinium chloride] (high conc.)
- extreme pH





Huge red-tipped tube worms...



The vents spew toxic chemicals.



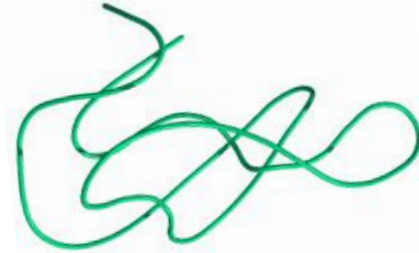
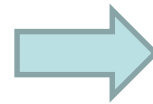
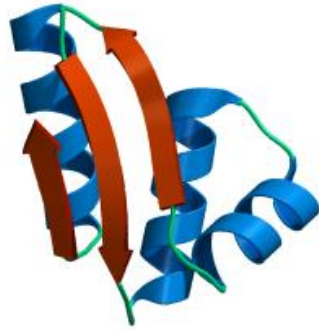
Thermophiles, a type of extremophile, produce some of the bright colors of Grand Prismatic Spring, Yellowstone National Park



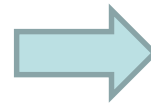
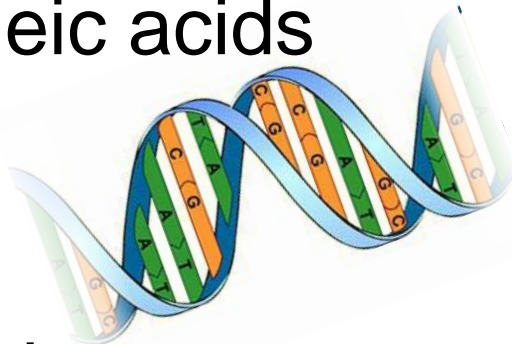
The first extremophile to have its genome sequenced was *Methanococcus jannaschii*, a microbe that lives near hydrothermal vents 2,600 meters below sea level, where temperatures approach the boiling point of water and the pressure is sufficient to crush an ordinary submarine. Image credit: NOAA

Order and disorder in macromolecular systems

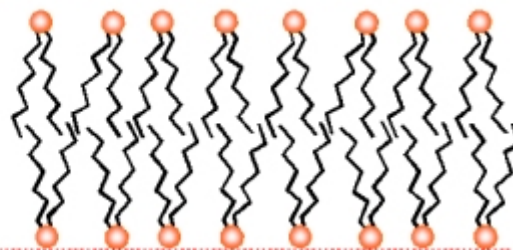
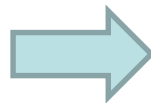
Proteins



Nucleic acids



Membranes



Physical parameter: Temperature

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

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

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

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

Thermodynamic identities

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

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

Let T_0 be selected on the way that:

$$G_1(T_0) = G_2(T_0)$$

Both states have the same gibbs free energy, thus they are in equilibrium at T_0

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

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

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

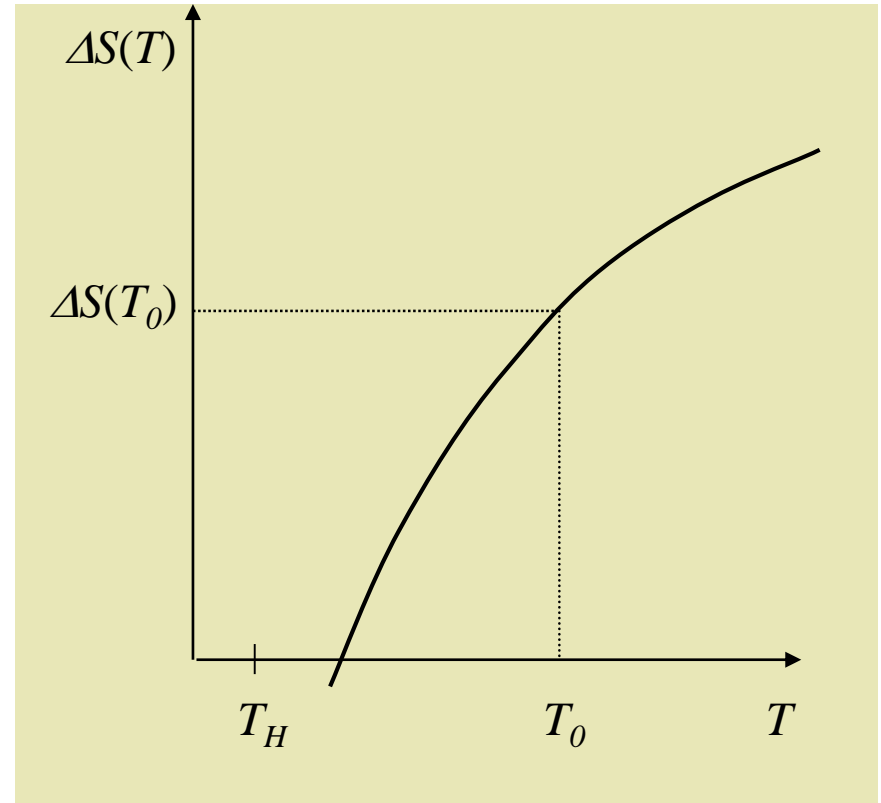
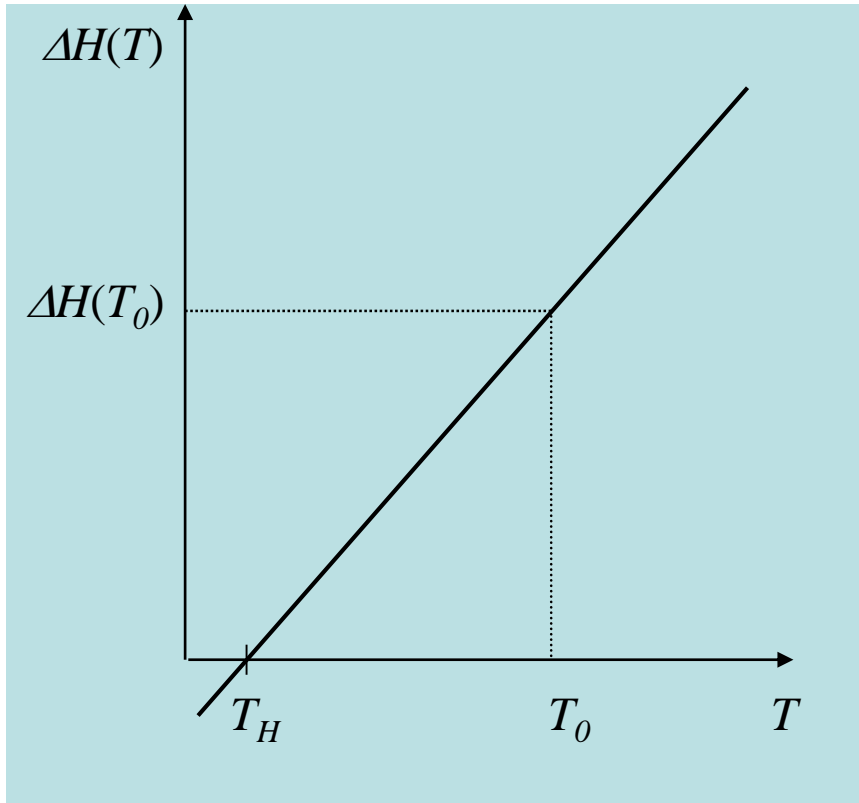
Let us suppose that C_p does not depend on T :

Or very weakly, and can be neglected in the range of $T \dots T_0$

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

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

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



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

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

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

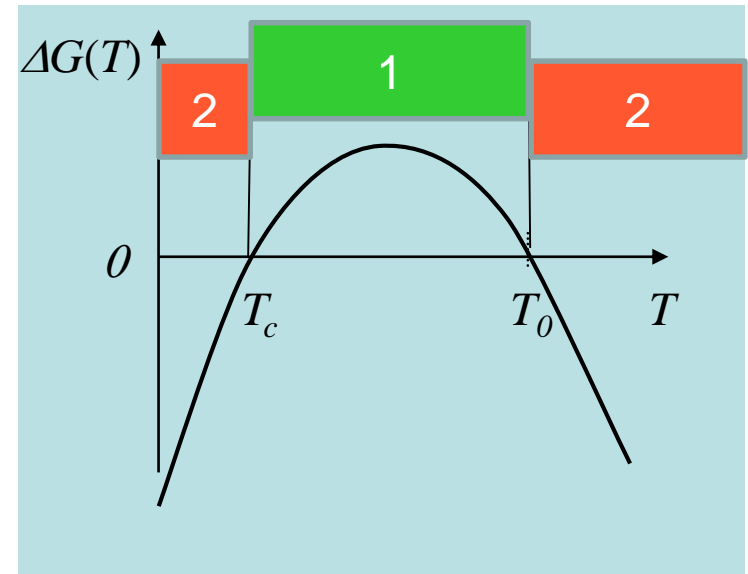
$$T \left(\ln \left(\frac{T}{T_0} \right) - 1 \right) + T_0 \approx \frac{(T - T_0)^2}{2T_0}$$

Taylor series

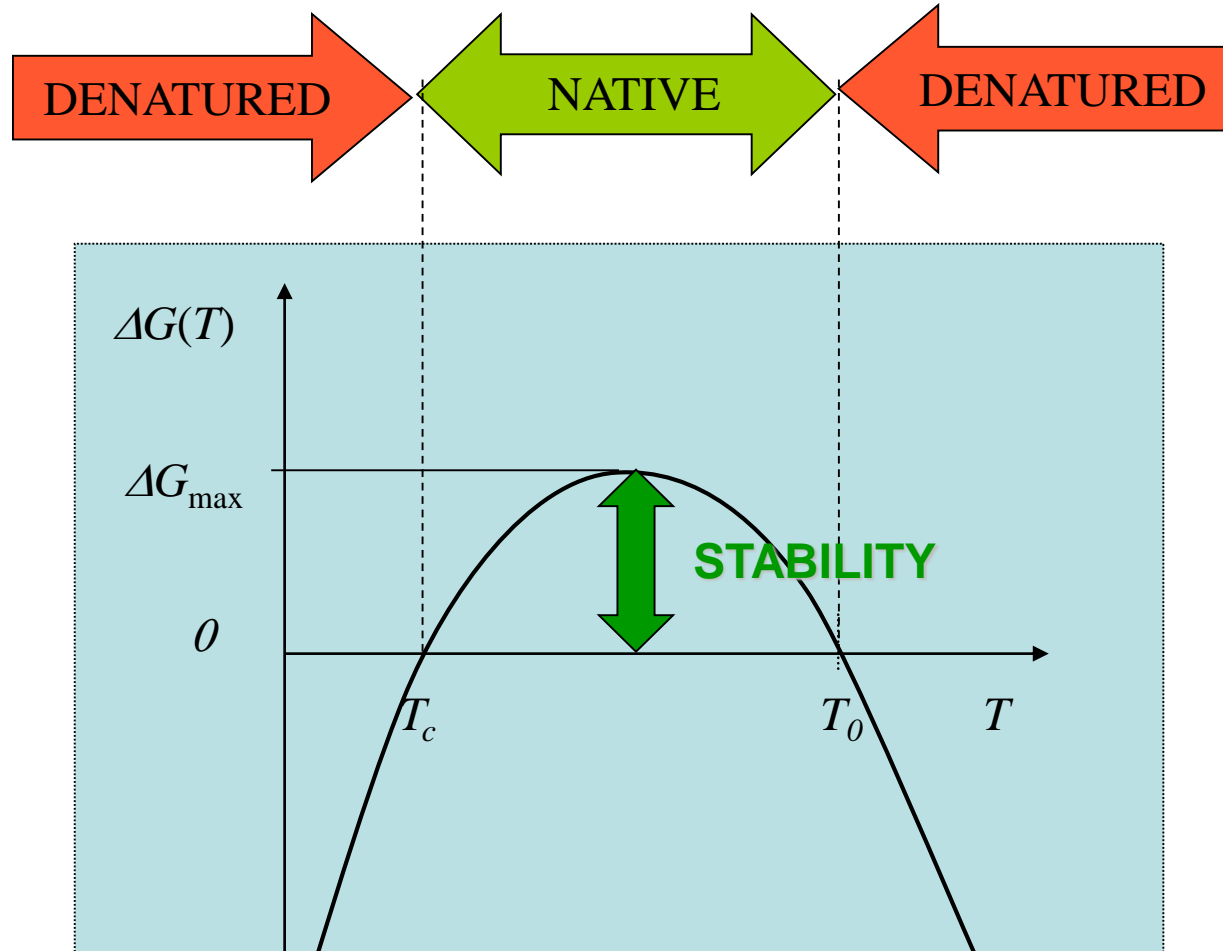
$$\Delta G(T) = G_2(T) - G_1(T)$$

$$\text{IF } \Delta G(T) > 0 \quad G_2(T) > G_1(T)$$

$$\text{IF } \Delta G(T) < 0 \quad G_2(T) < G_1(T)$$



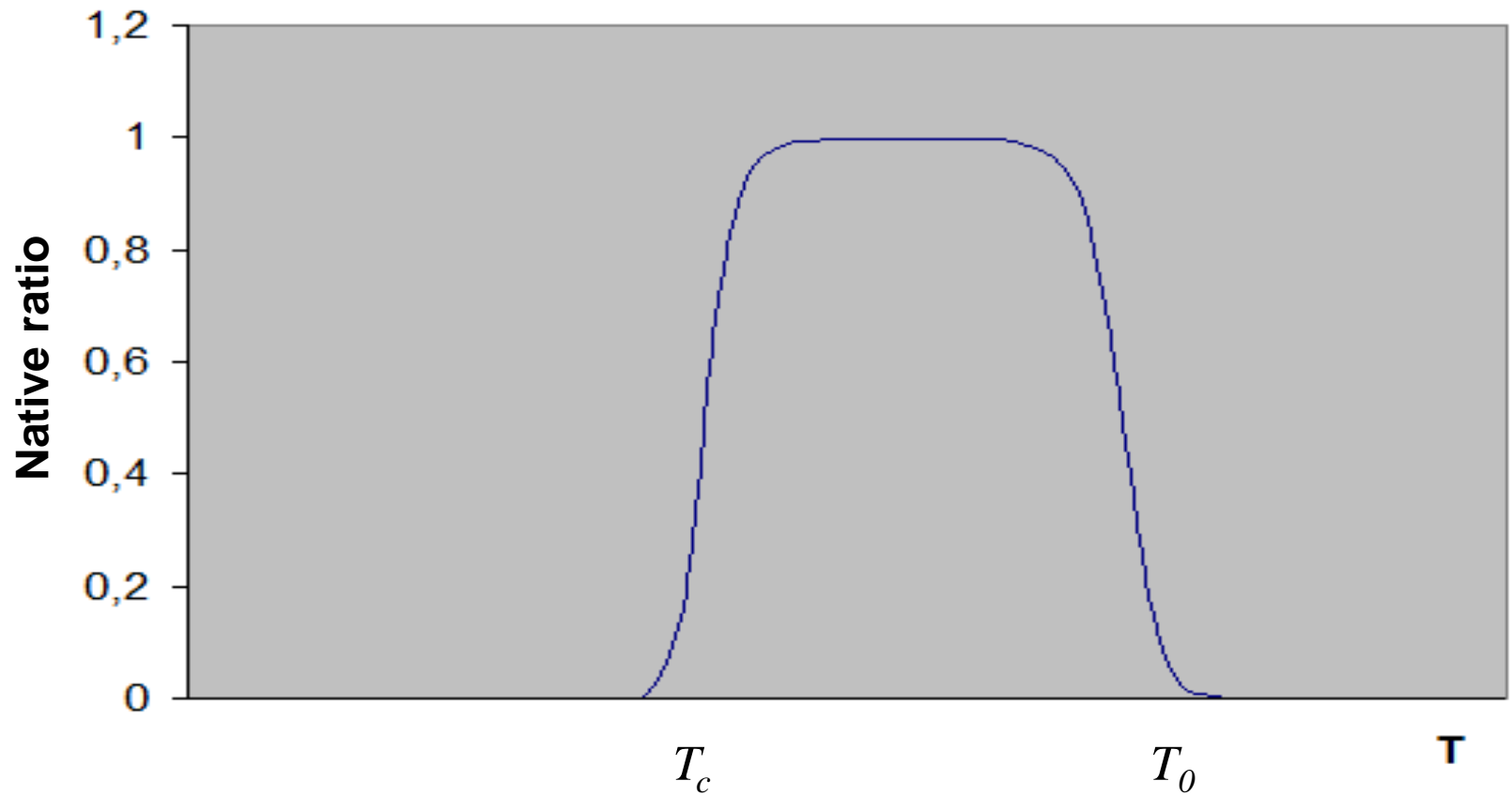
In case of proteins:

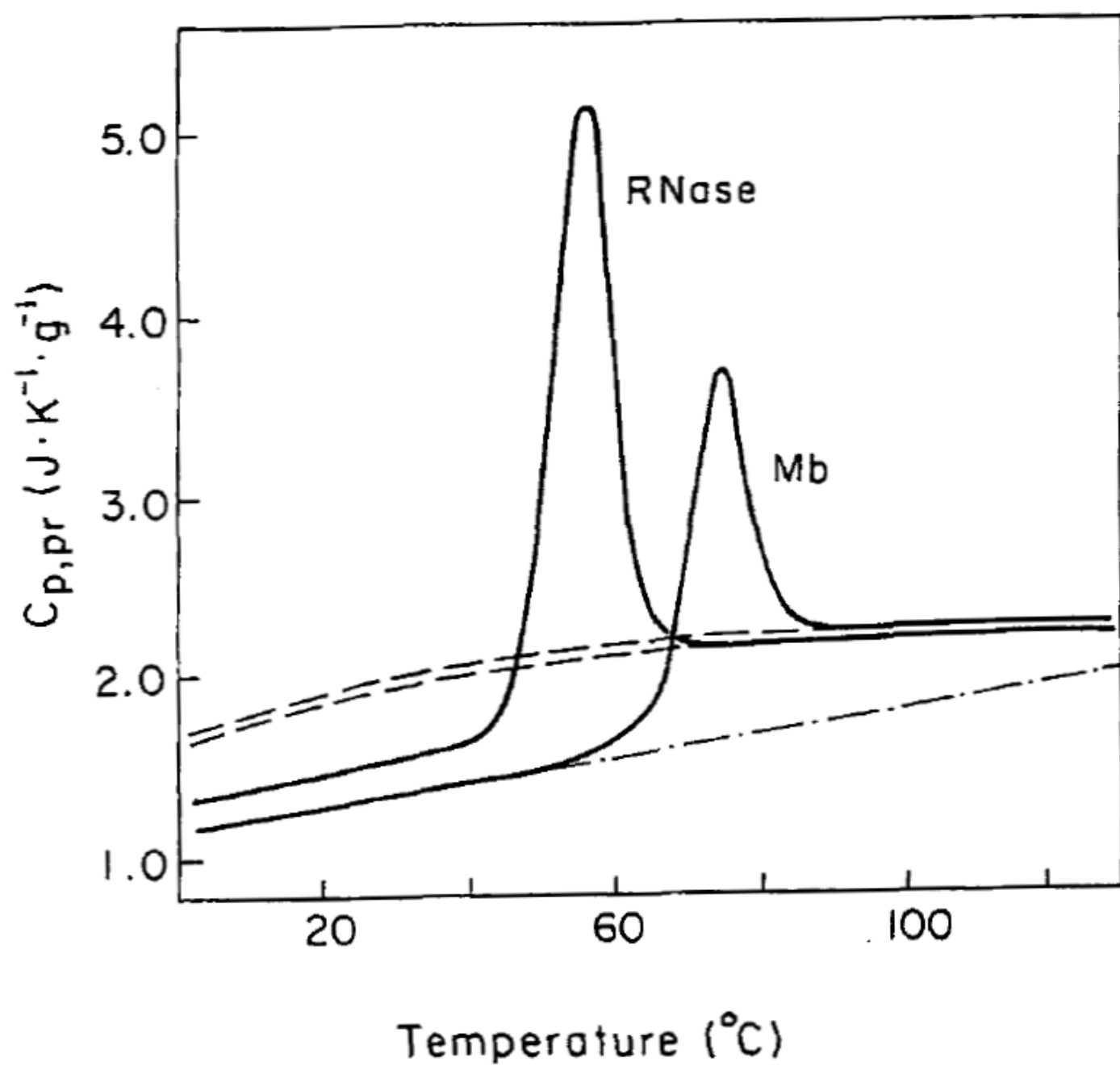


No cold denaturation was observed in case of nucleic acids and membranes.

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

Boltzmann statistics can be applied





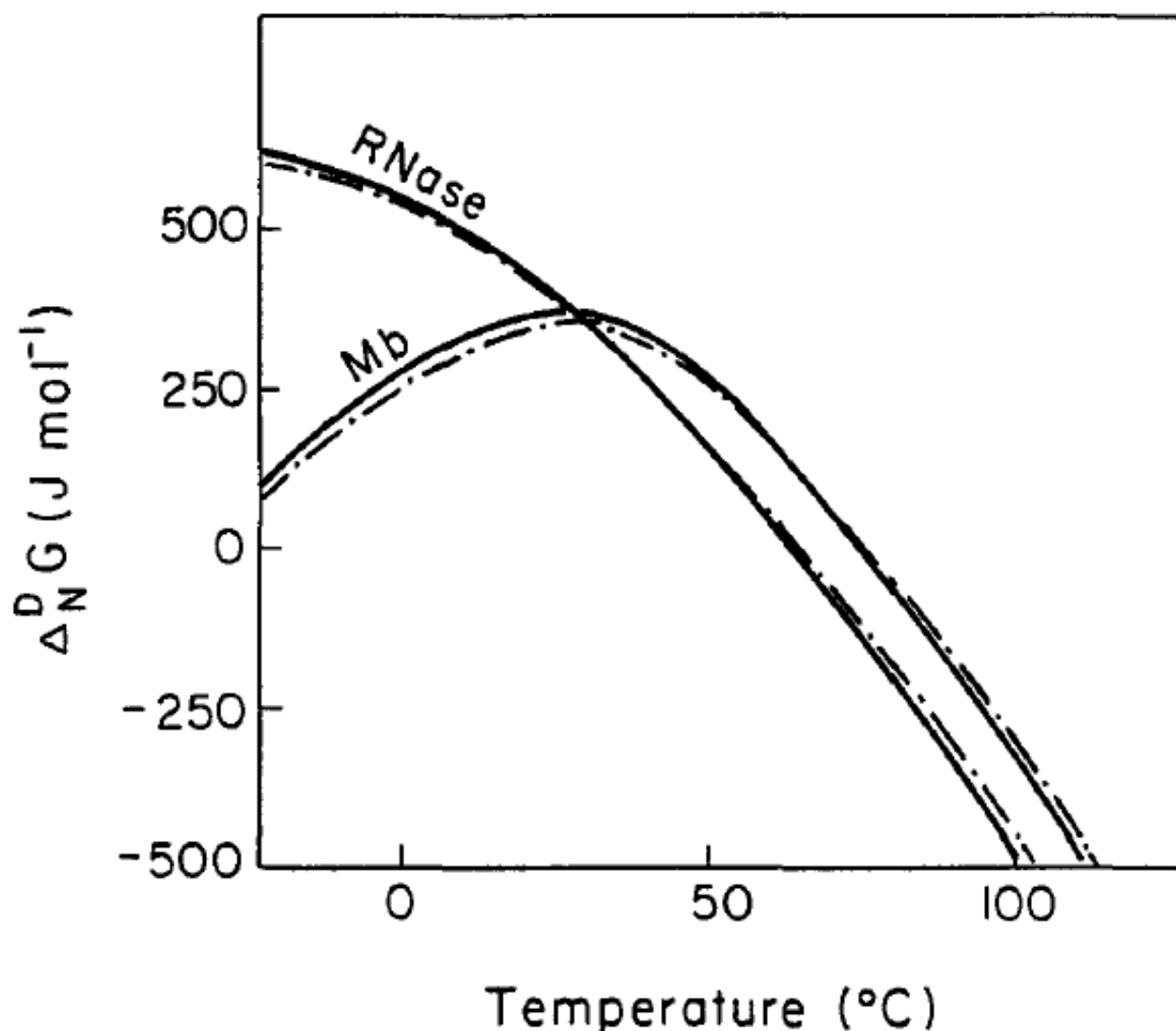
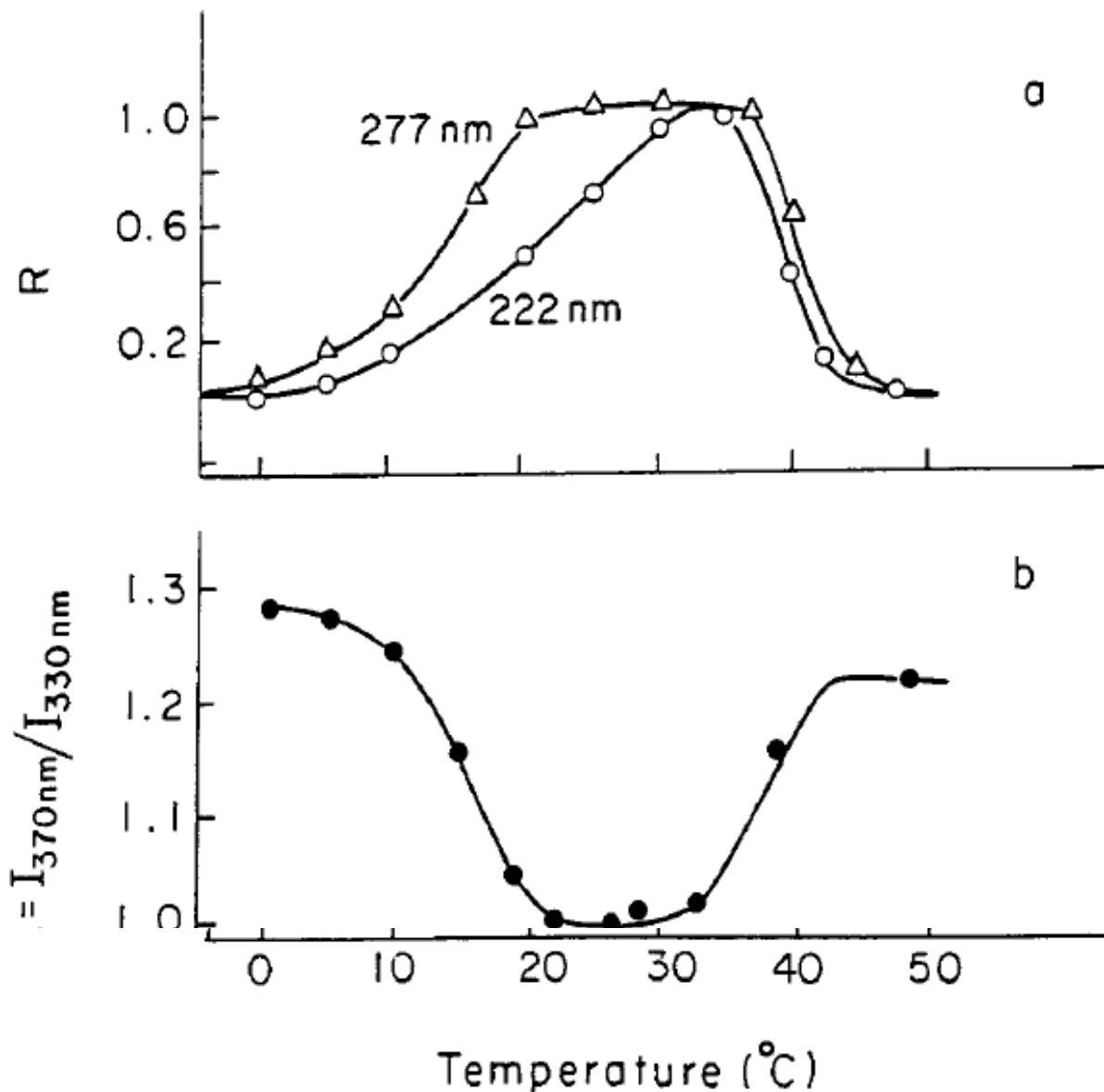


FIGURE 7. The $\Delta_N^D G$ function for RNase and Mb for the same conditions as in Figure 6 calculated from the assumption that $\Delta_N^D C_p$ is temperature independent (dot-dash line) and temperature dependent (dashed line).¹¹⁴

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



PGK

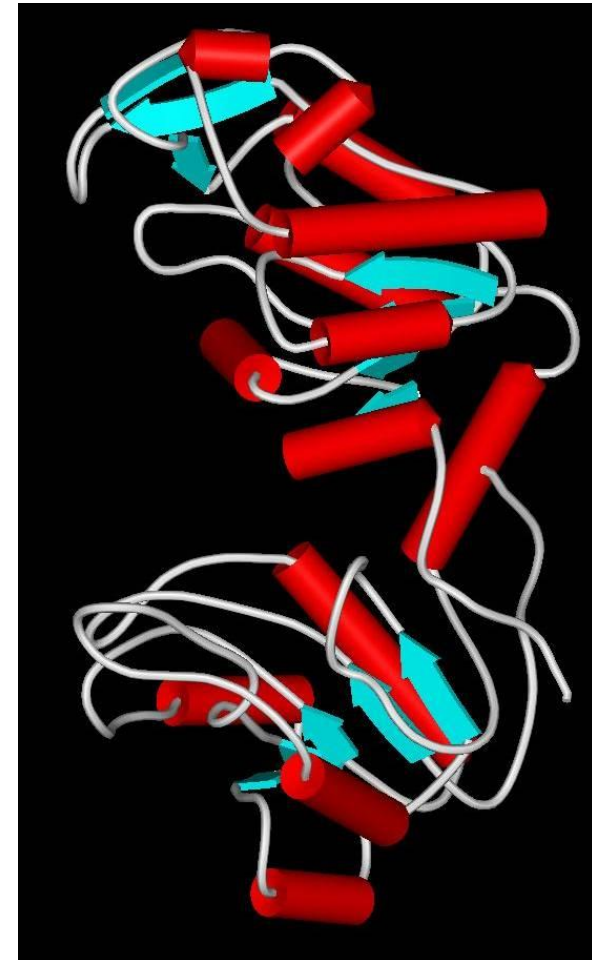


FIGURE 23. Temperature dependence of (a) relative changes (R) of phosphoglycerate kinase ellipticity at 222 nm (○) and 277 nm (Δ), (b) tryptophan emission spectrum maximum, and (c) partial specific heat capacity in solution containing 0.7 M GuHCl.¹³³ containing 0.7 M GuHCl.¹³³

Myoglobin

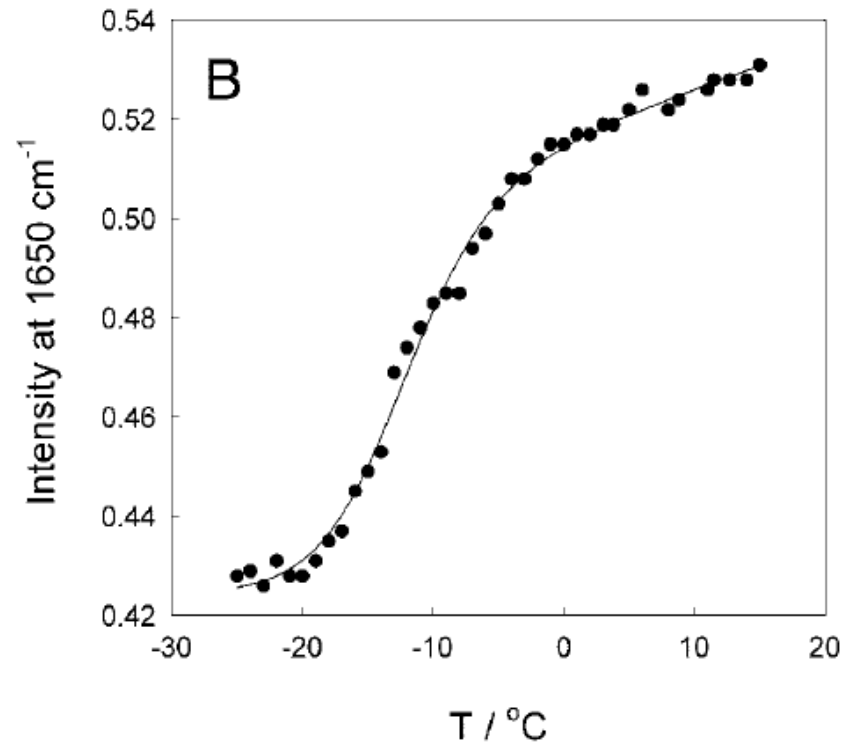
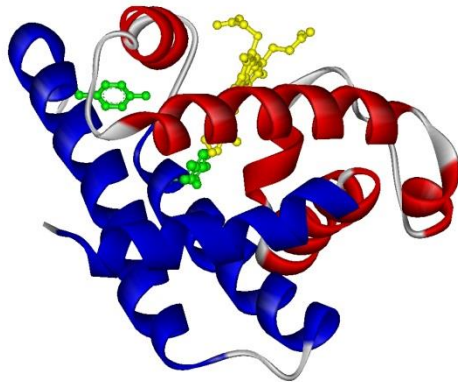
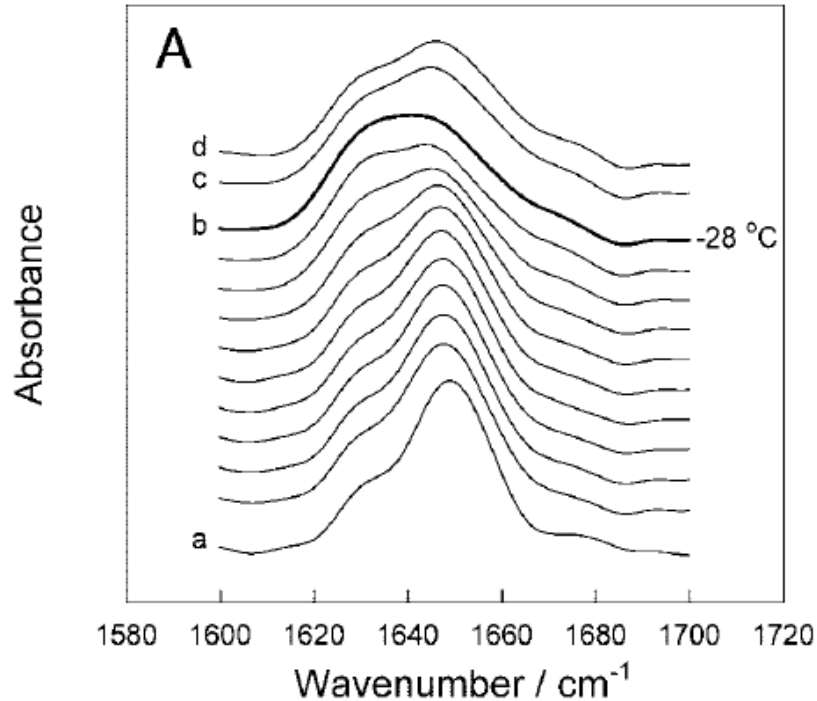


FIGURE 3 (A) Stacked plot of the deconvoluted 1600 to 1700 cm^{-1} region of myoglobin upon cold unfolding. The sequence of the spectra is from bottom to top. All spectra were taken at 2 kbar except for the bottom [a] and top [d] spectrum, which are taken at atmospheric pressure and 20°C before and after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

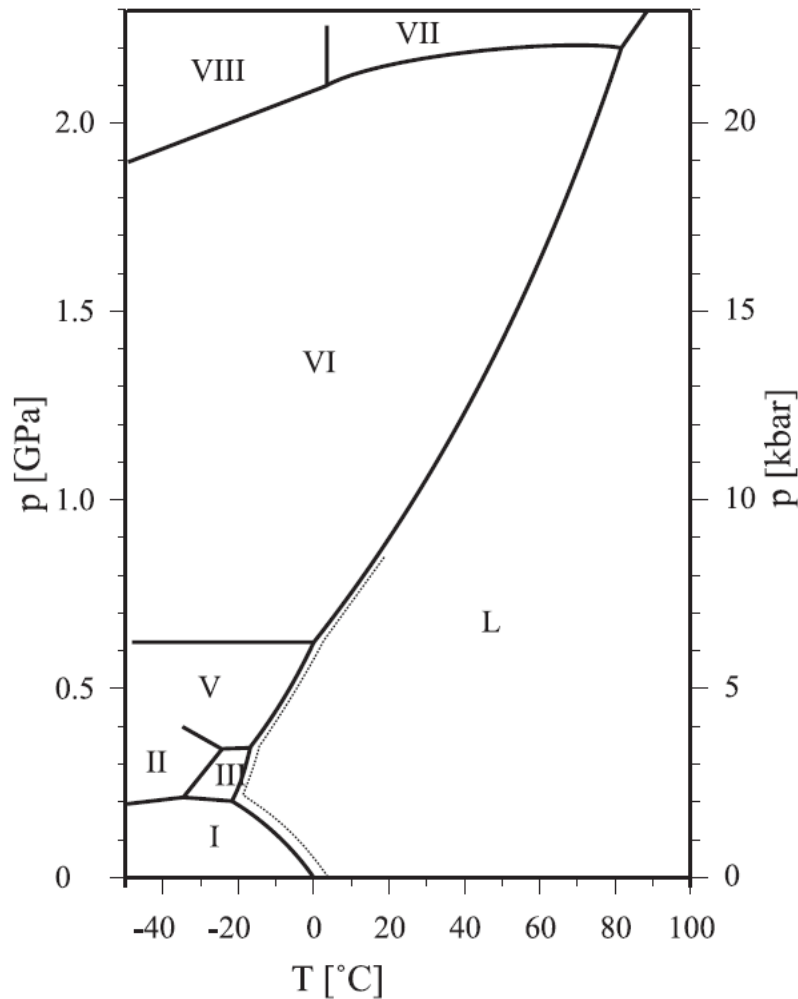
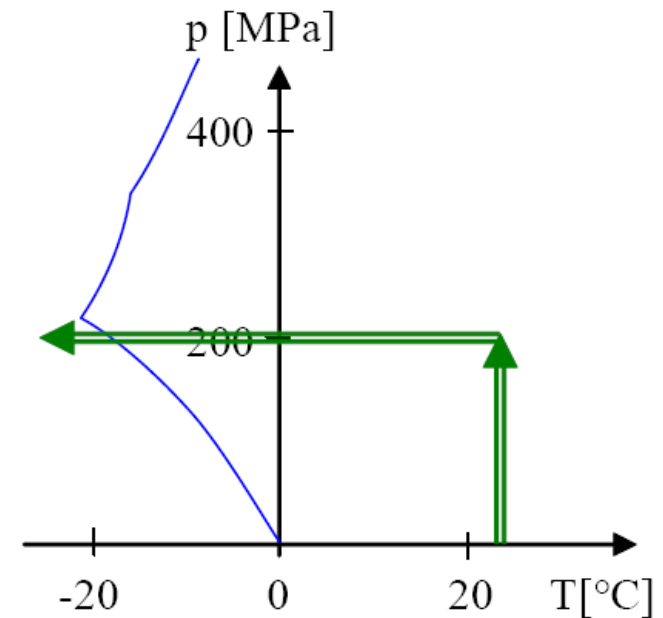
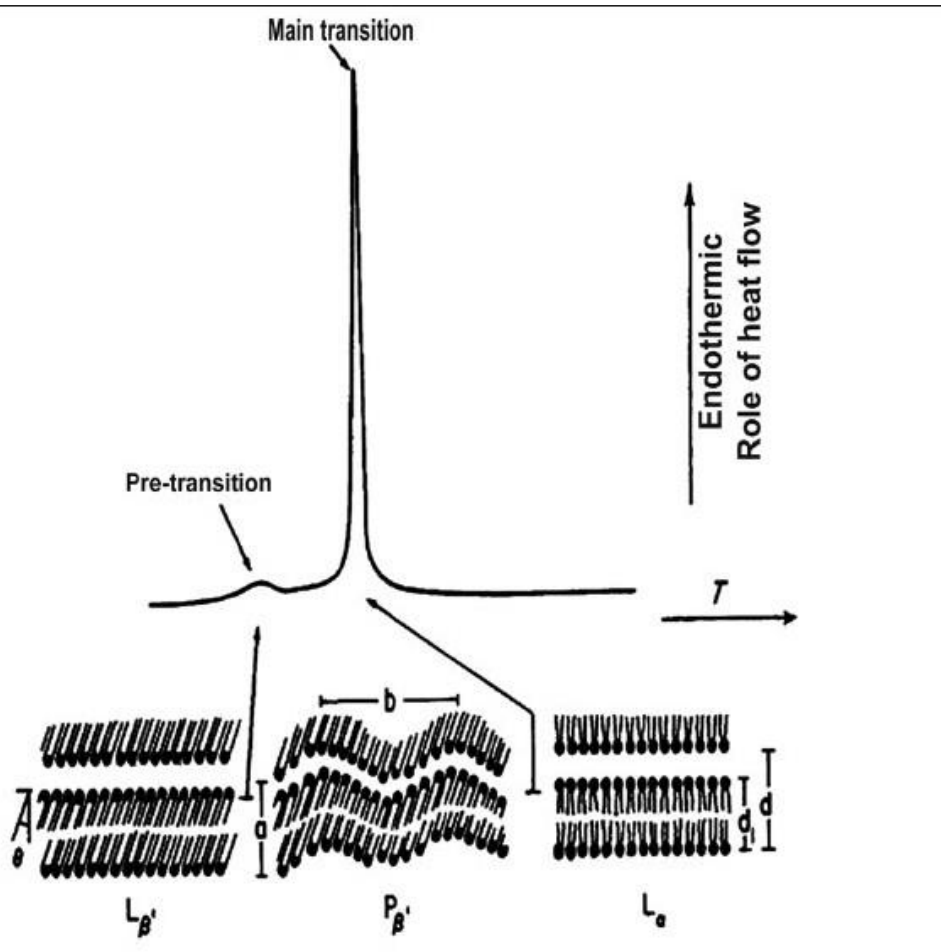


Fig. 1. Phase diagram of water in the temperature range of -50 to $+100^{\circ}\text{C}$ up to a pressure of 2.2 GPa. L refers to the liquid phase; roman numbers (I–VIII) show the different ice phases. The dotted line shows the melting curve of heavy water.

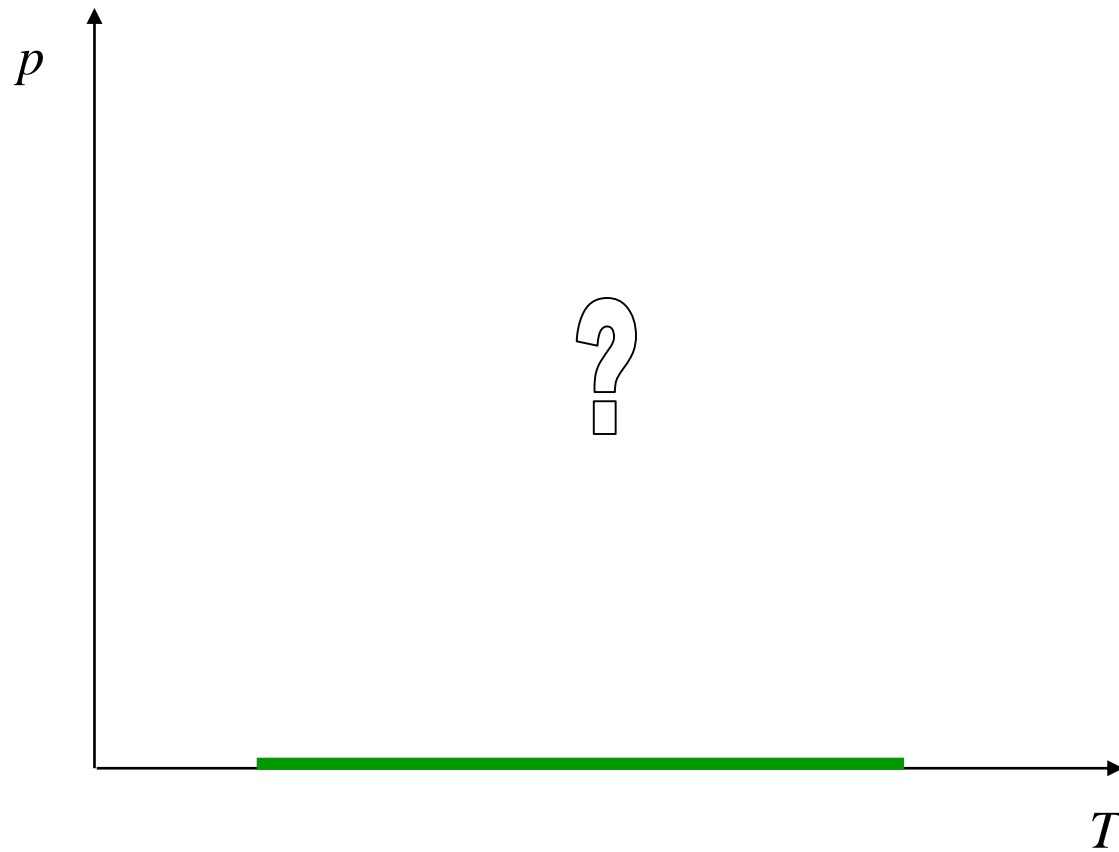


Path of cold denaturation experiment (green). The solidification curve of water is indicated by the blue line.

Phase transition of the lipids



The p-T phase diagram



Why is high pressure interesting?

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

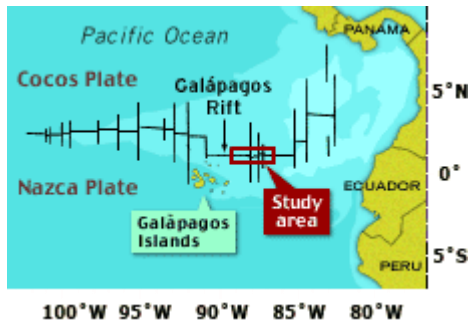


1 bar = 0,1 MPa

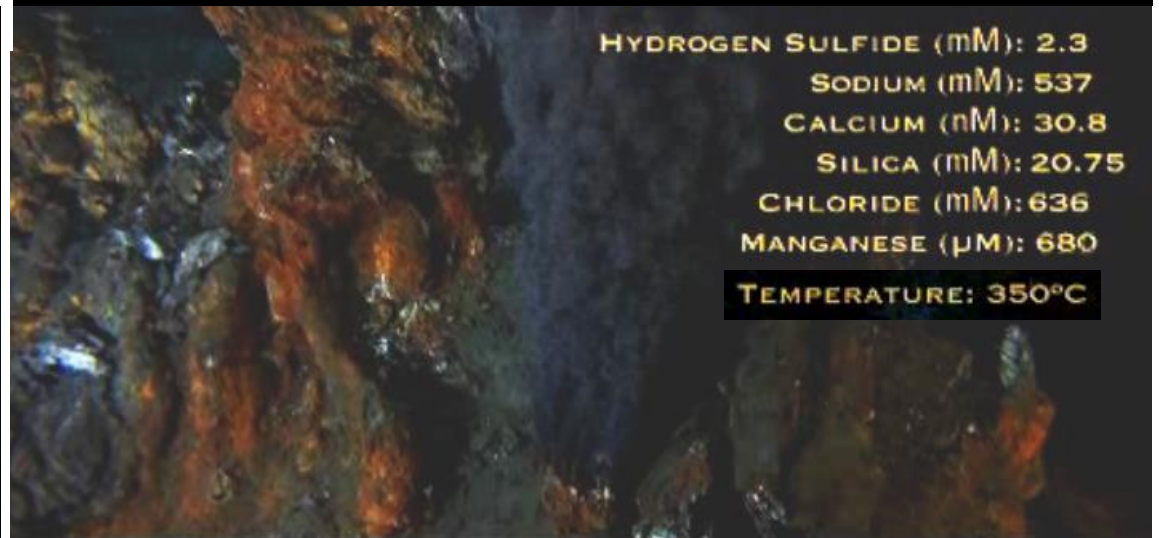
1 kbar = 100 MPa

10 kbar = 1GPa

1Mbar=100GPa

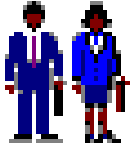


Huge red-tipped tube worms...



The vents spew toxic chemicals.

The pressure scale

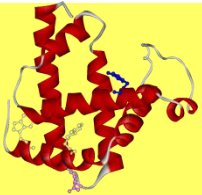


human life

1 bar 100 kPa



max. pressure in the biosphere: **1 kbar 100 Mpa**
(deepsee)



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 **\sim Mbar 100 GPa**

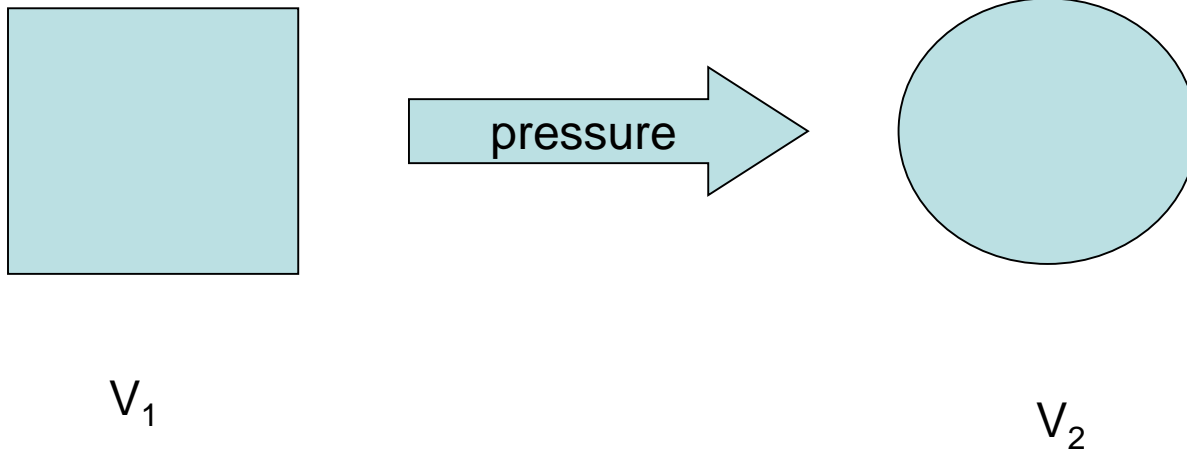


max reached in laboratory: **few Mbar few 100 GPa**

What is the effect of the pressure?

The Le-Chatelier-Braun principle

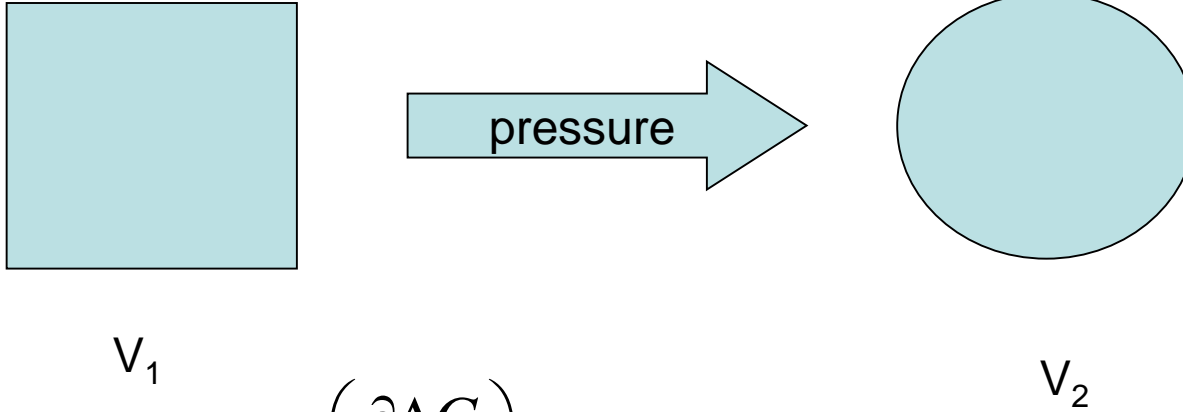
pressure \leftrightarrow volume



pressure \leftrightarrow volume

$$K = e^{-\frac{dG}{RT}}$$

$$dG = -RT \ln(K)$$



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

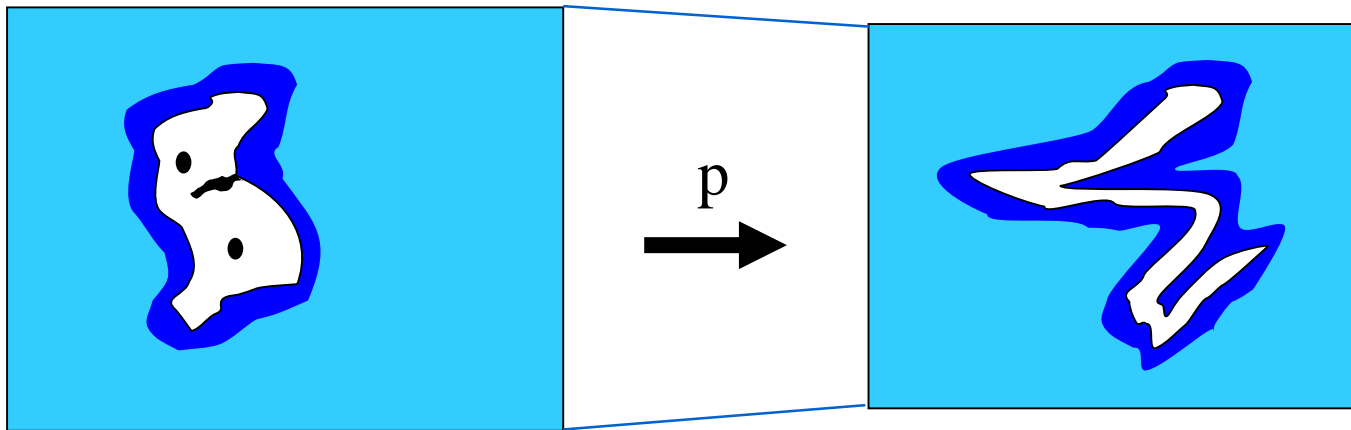
$$-RT \left(\frac{\partial \ln K}{\partial p} \right)_T = \Delta V$$

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

Effect of pressure on the proteins

Pressure unfolding

Protein solution



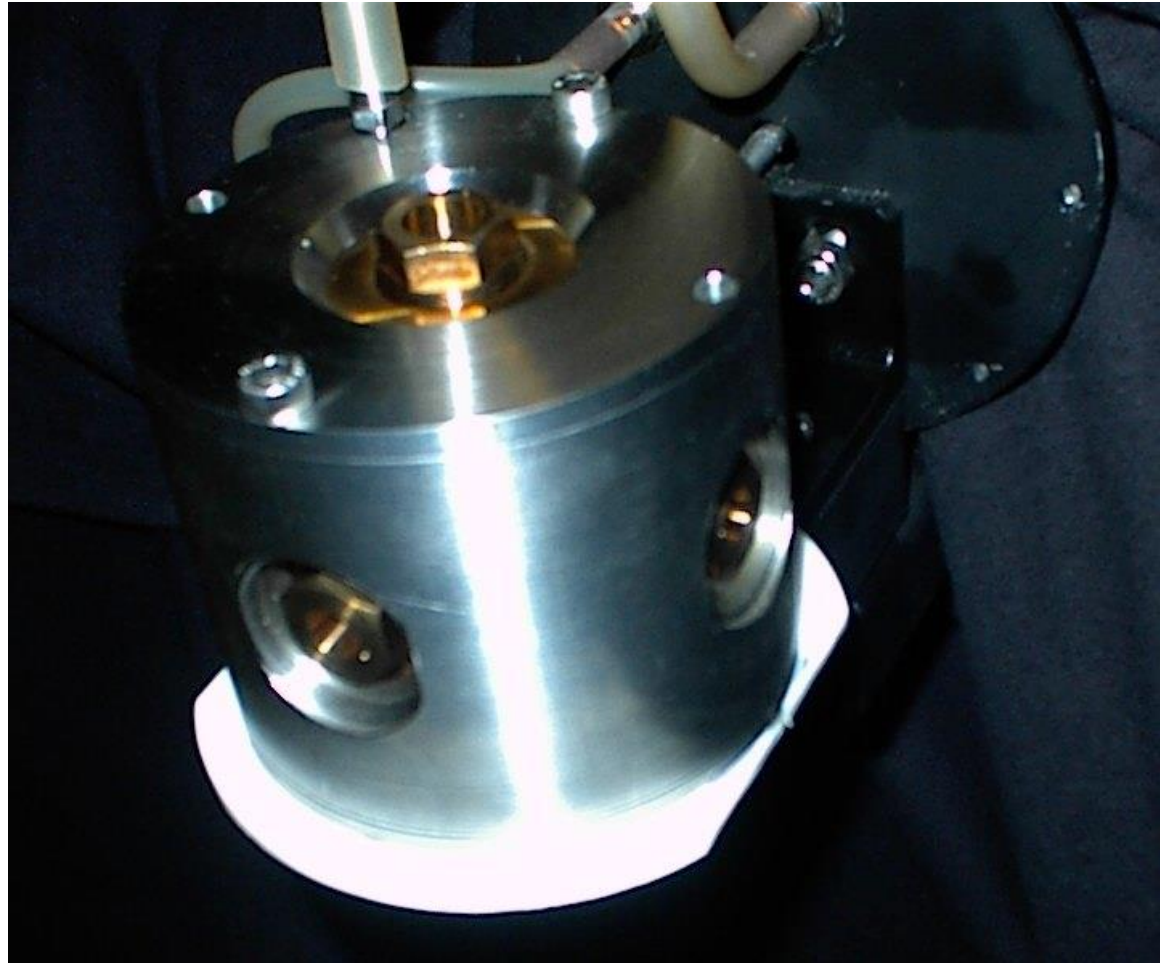
$$V_{\text{protein}} = V_{\text{atom}} + V_{\text{void}} + \Delta V_{\text{hydration}}$$

The high pressure technique

- **Coontainer with very thick and solid wall (bomba)**

outer $\varnothing \approx 10$ cm
sample $\varnothing 10$ mm

3 optical windows

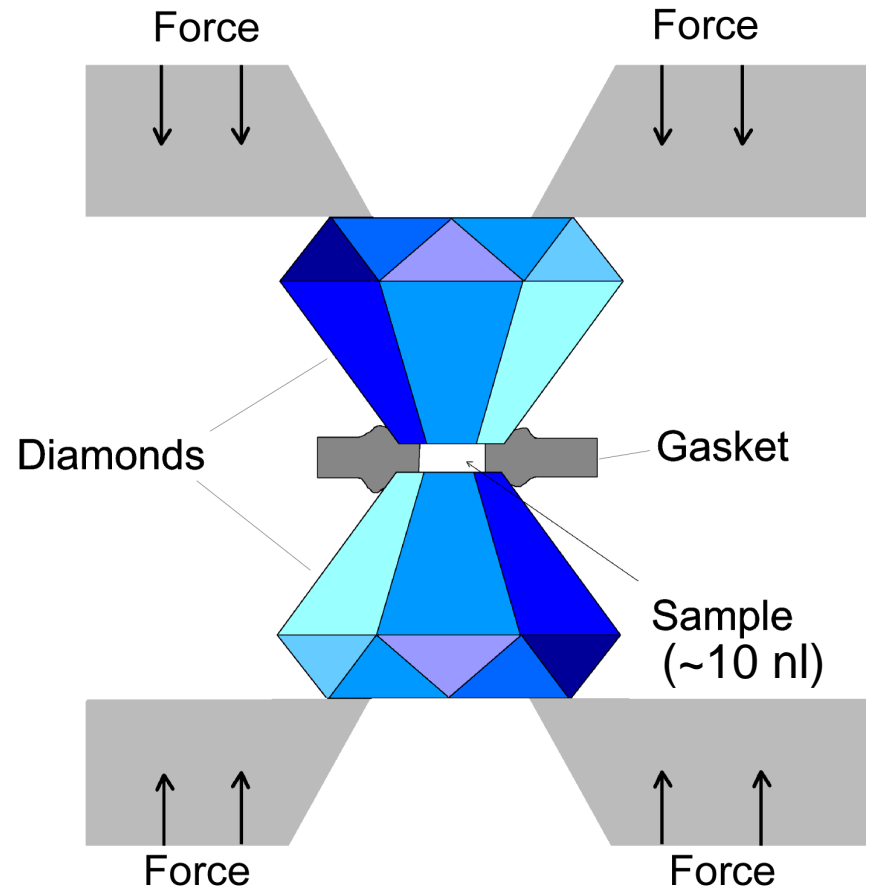


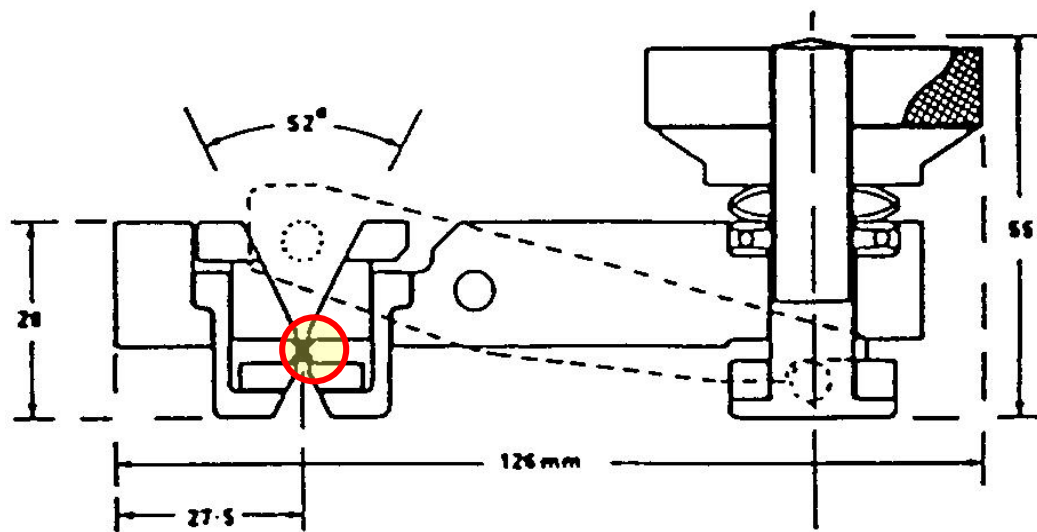
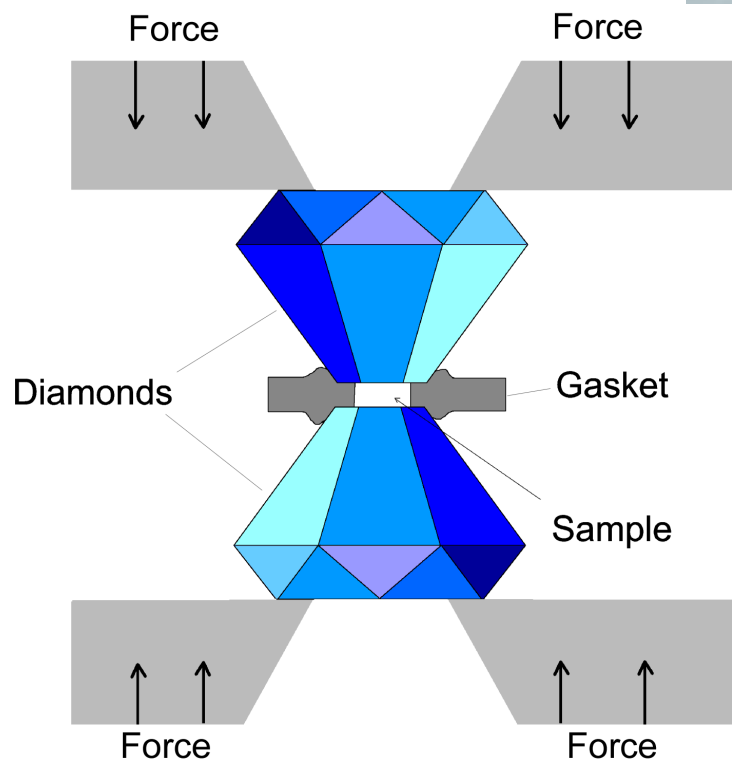


The high pressure technique

DAC
diamond anvil cell

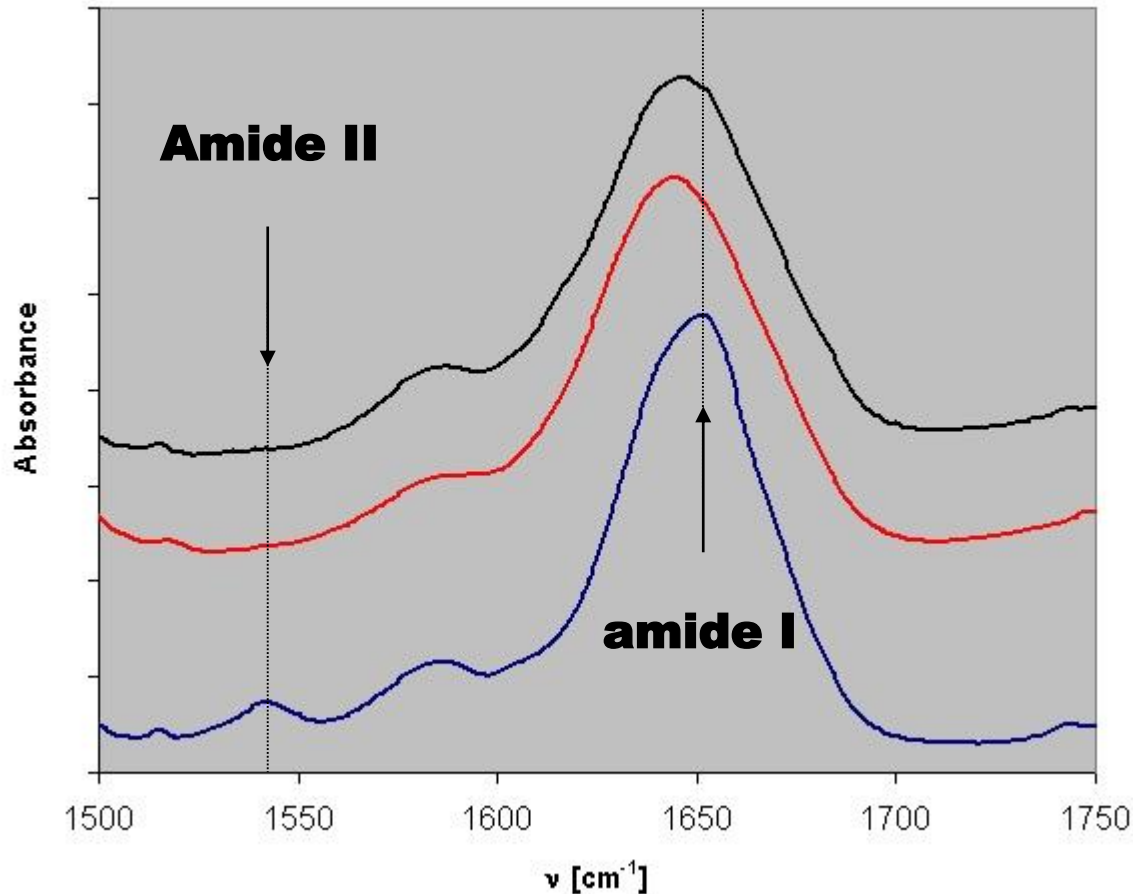
- **Very small surface (and volume)**
Ø0,5mm





Pressure unfolding of proteins: e.g.: lysozyme

Lyso30b

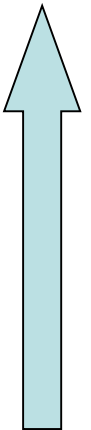


75mg/ml

**back to
0.1 MPa**

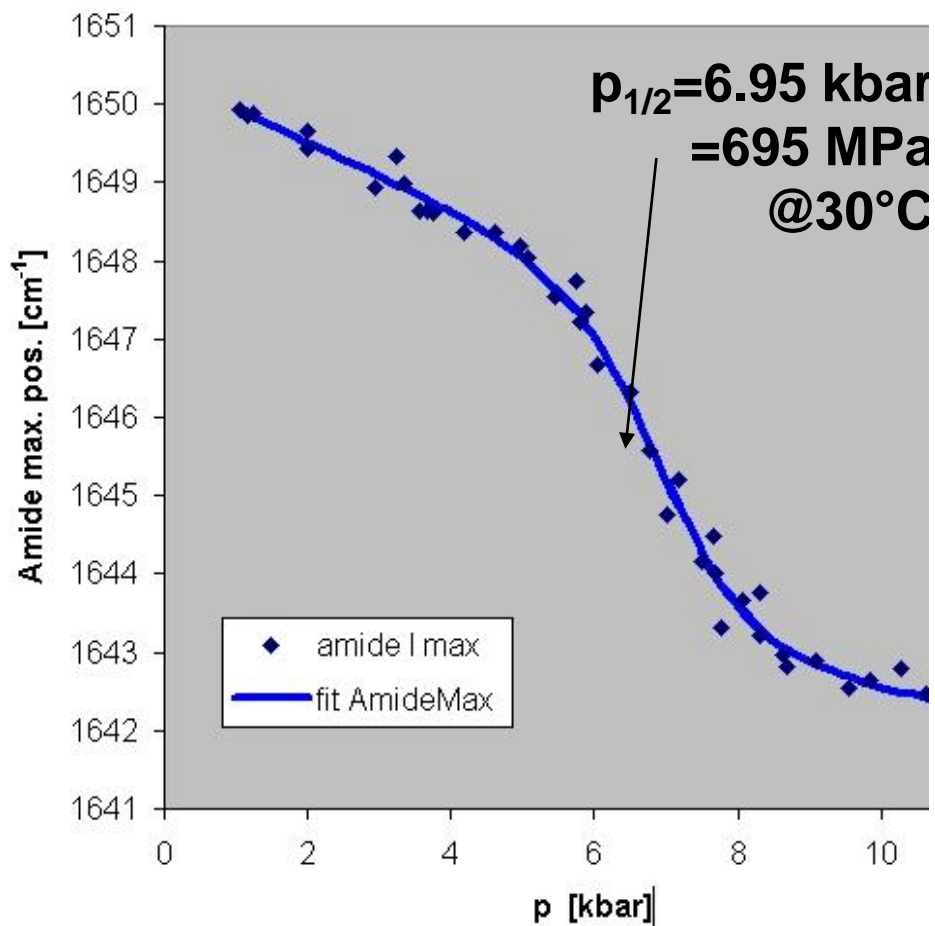
900 MPa

0.1 MPa

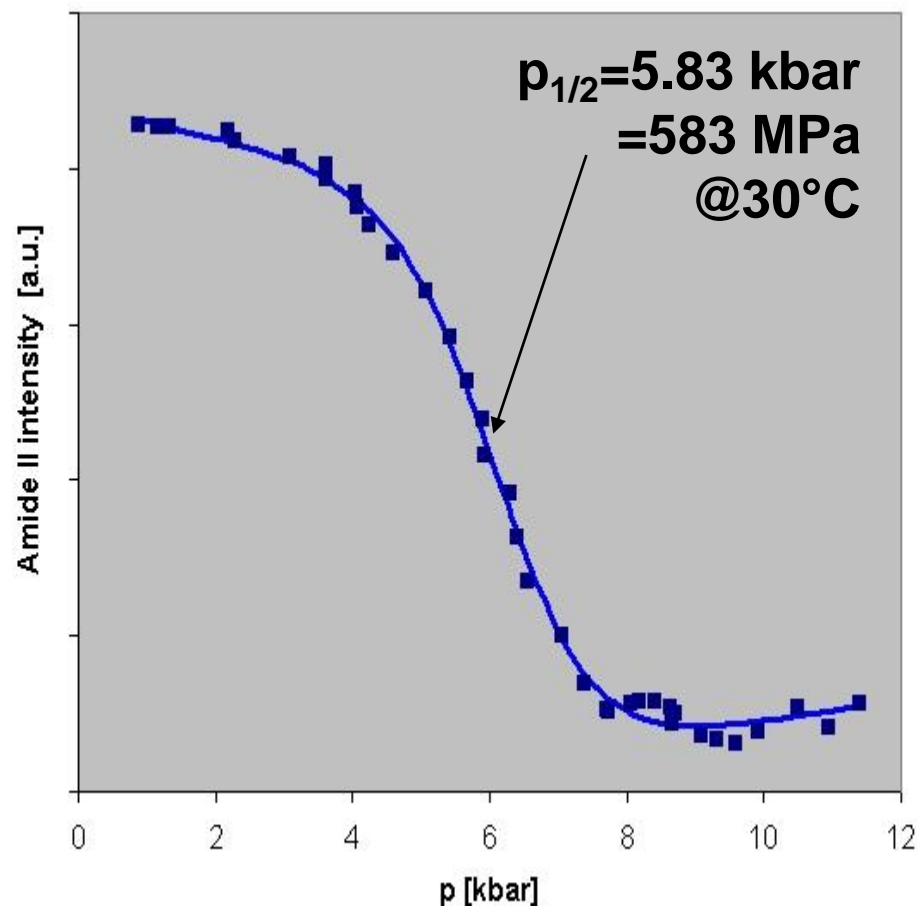


Pressure unfolding: lysozyme

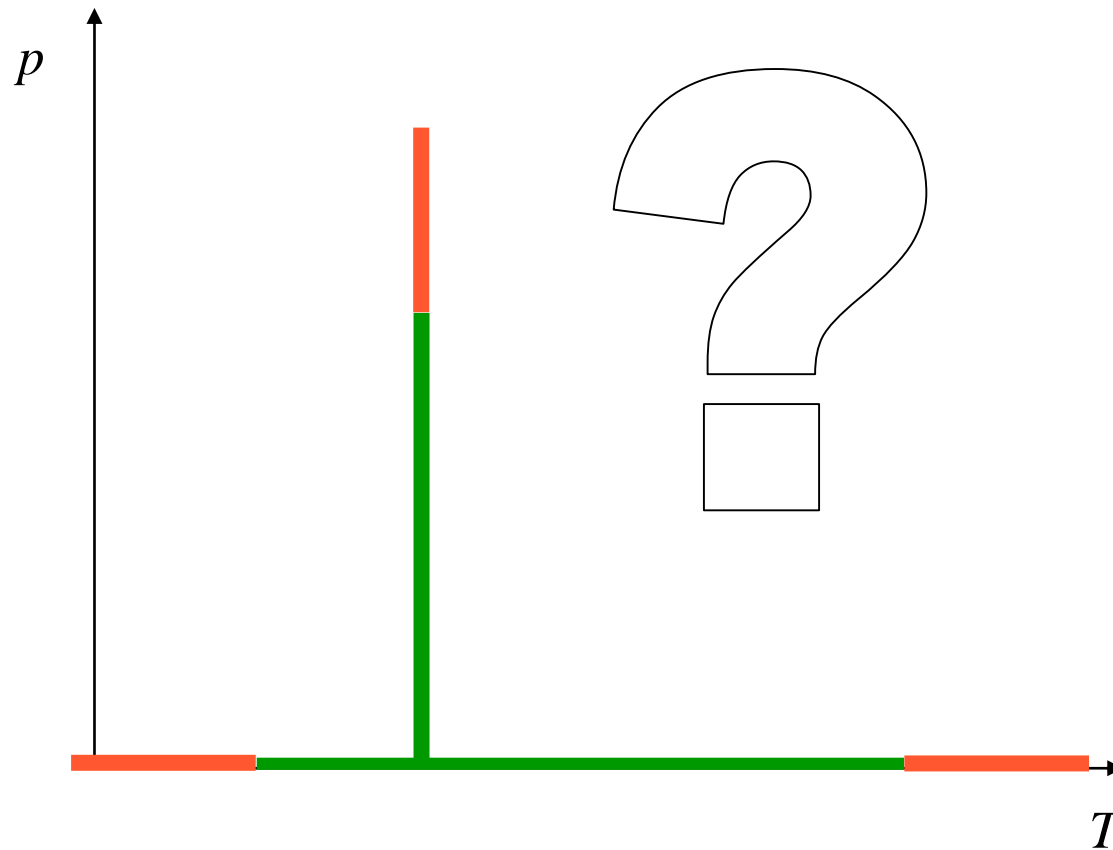
Amide I max. position [cm⁻¹]



Amide II intensity [a.u.]



Pressure-temperature phase diagram



Thermodynamic description of the pressure and temperature denaturations

Two state model: $N \rightleftharpoons D$

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

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

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

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

$$- \Delta C_p \left[T \left(\ln \frac{T}{T_0} - 1 \right) + T_0 \right]$$

$$+ \Delta V_0(p - p_0) - \Delta S_0(T - T_0) + \Delta G_0$$

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

So we are not far, and a Taylor expansion works well

Assuming $T \approx T_0$:

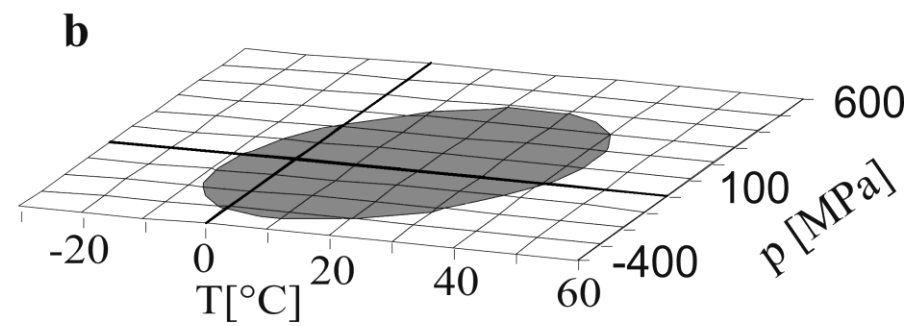
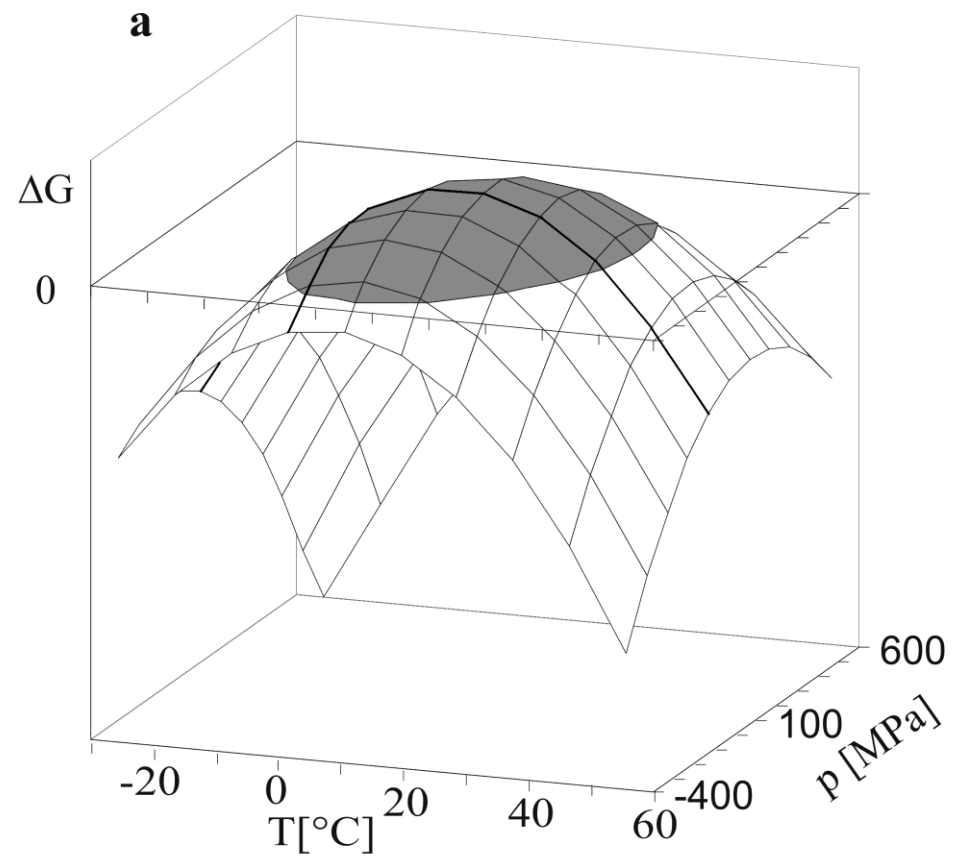
$$T \left(\ln \frac{T}{T_0} - 1 \right) + T_0 \approx \frac{(T - T_0)^2}{2T_0}$$

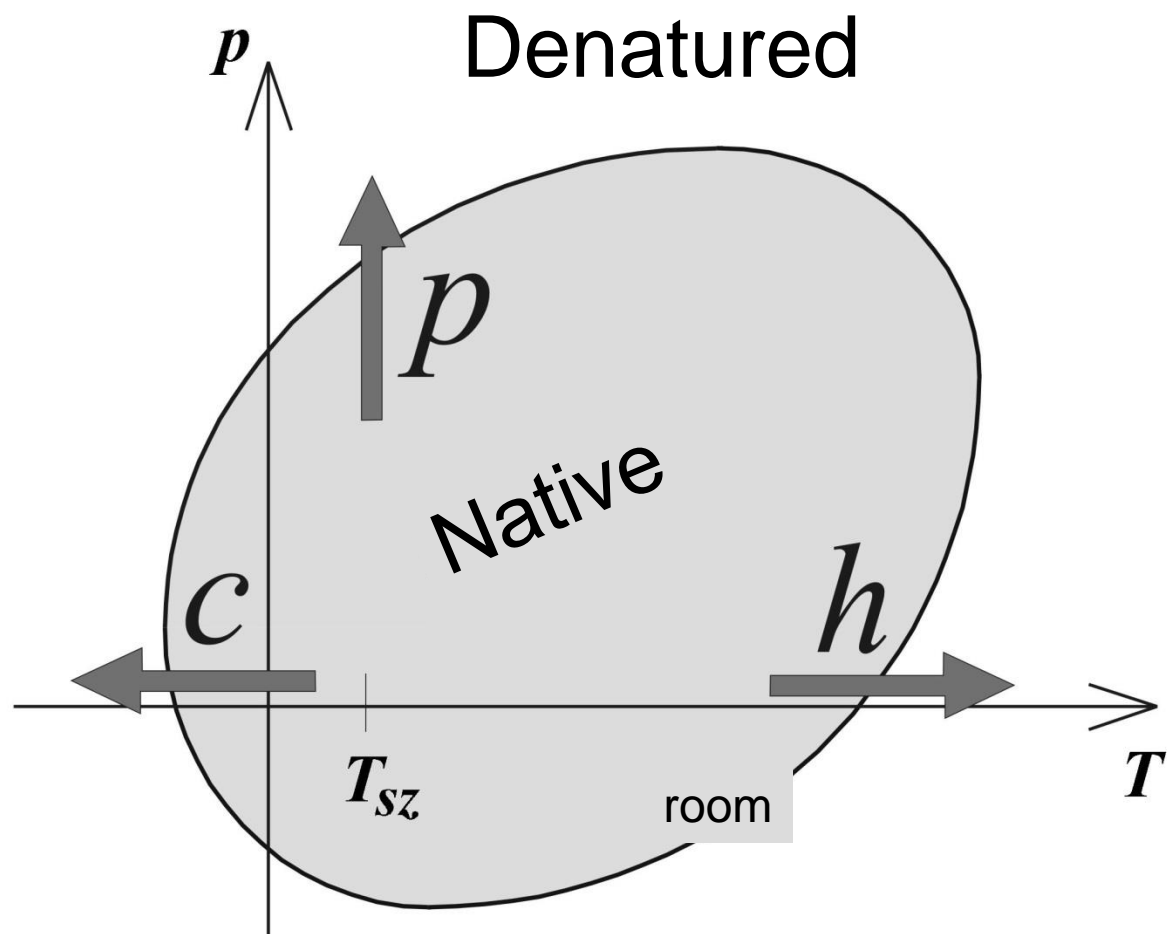
$$\Delta G = \frac{\Delta\beta}{2}(p - p_0)^2 + \Delta\alpha(p - p_0)(T - T_0) - \frac{\Delta C_p}{2T_0}(T - T_0)^2 \\ + \Delta V_0(p - p_0) - \Delta S_0(T - T_0) + \Delta G_0$$

Second order function of T and p !

At the (middle) point of the denaturation: $\Delta G = 0$ (so here is an equilibrium of native \leftrightarrow denatured)

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



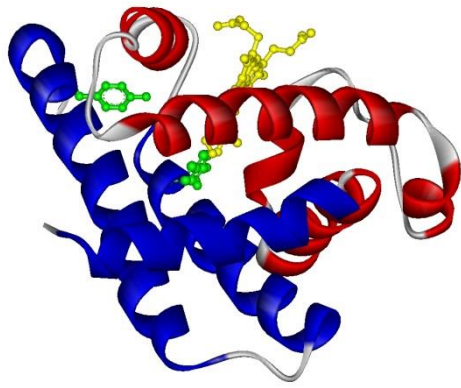


Is the two state model a good description
for the proteins?

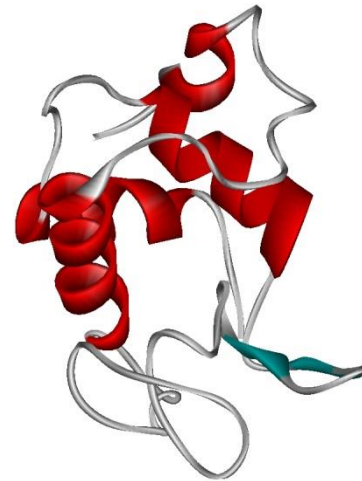
Is there only one denatured state?
Intermolecular interactions?

Experimentally determined phase diagrams

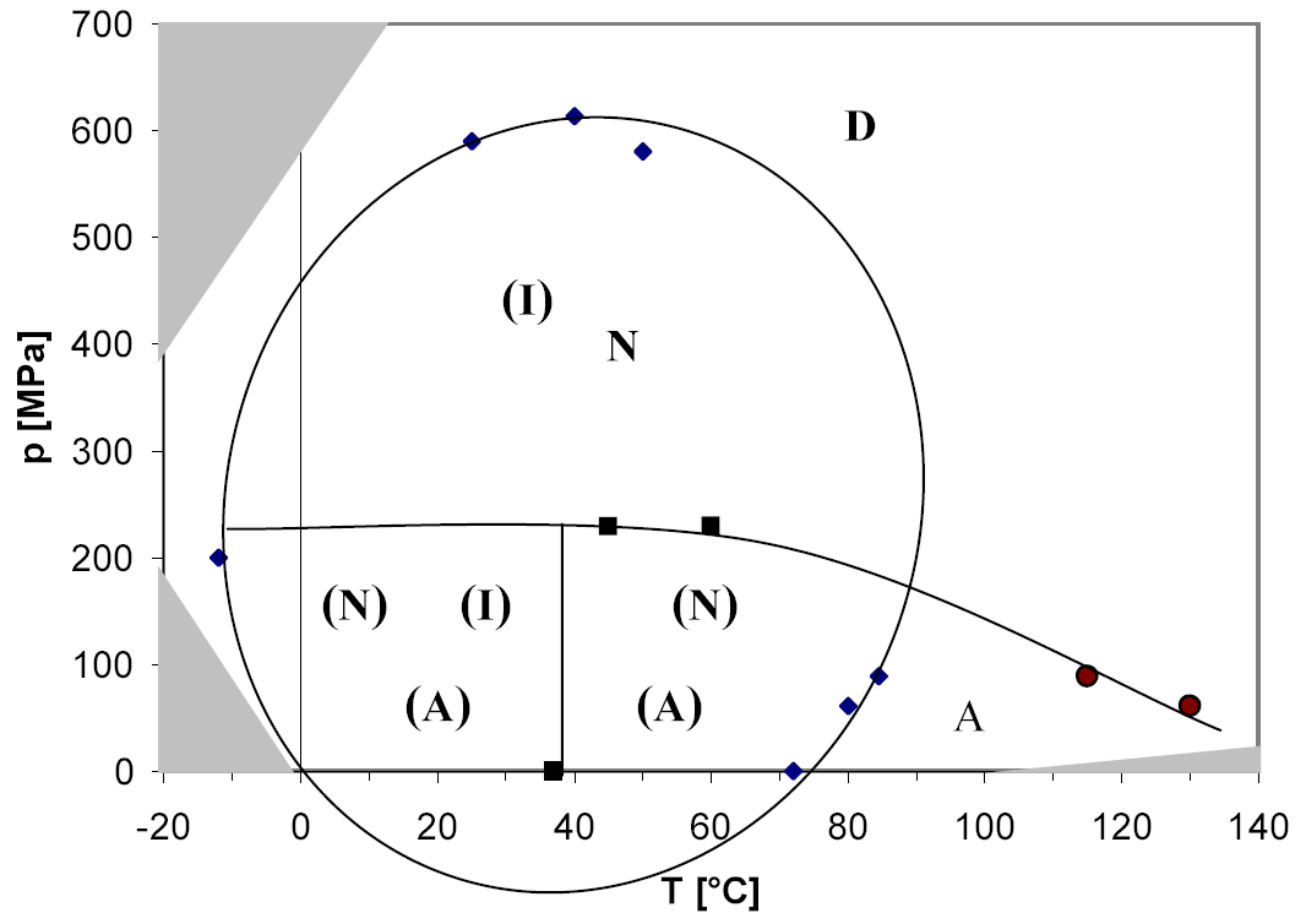
Myoglobin



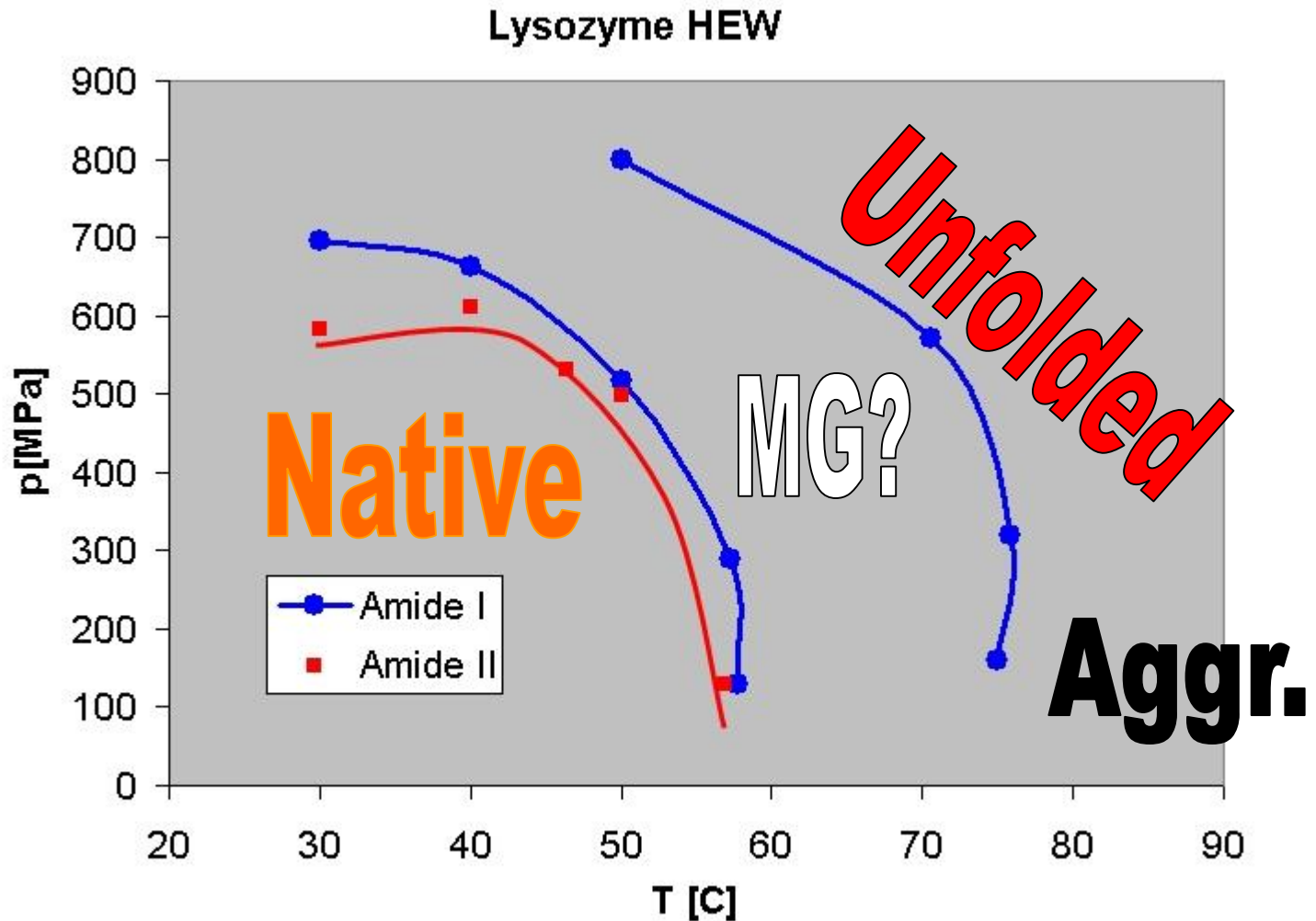
Lysozyme



Phase diagram of myoglobin

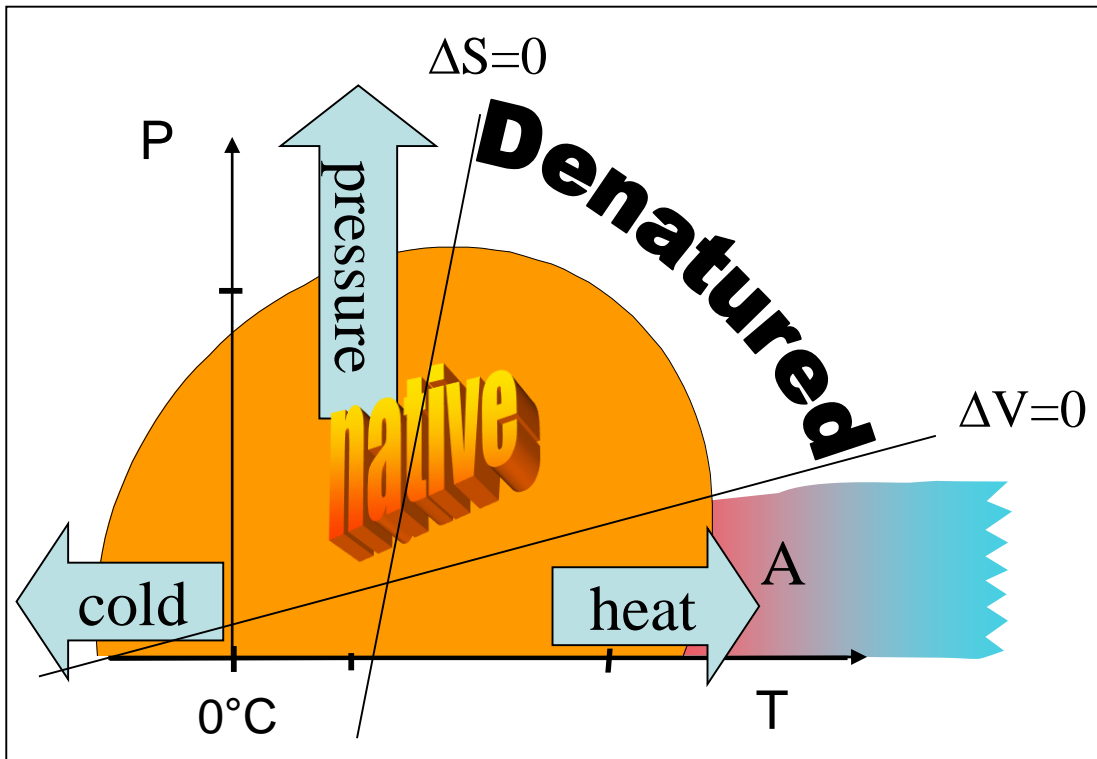


Lysozyme: T-p phase diagram



Pressure-temperature phase diagram: the reality

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

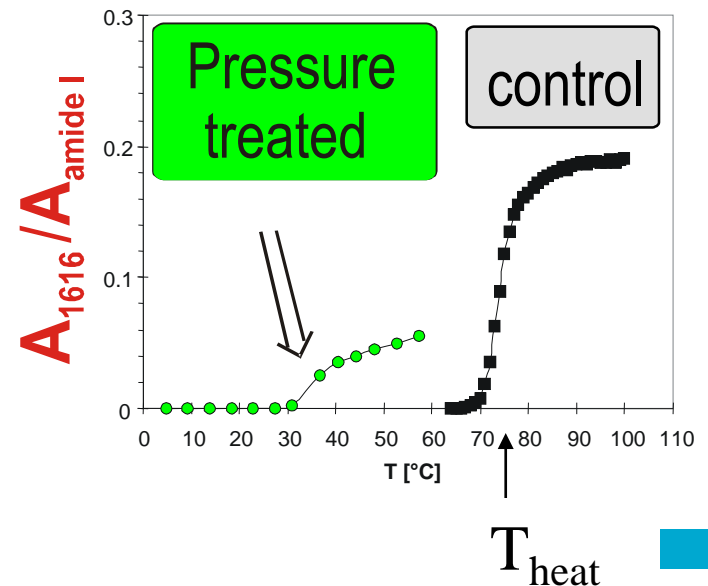
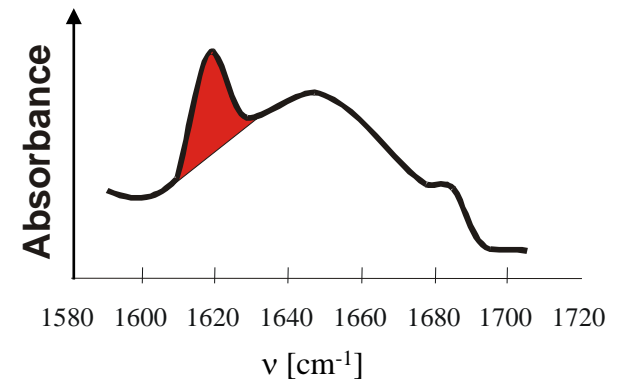
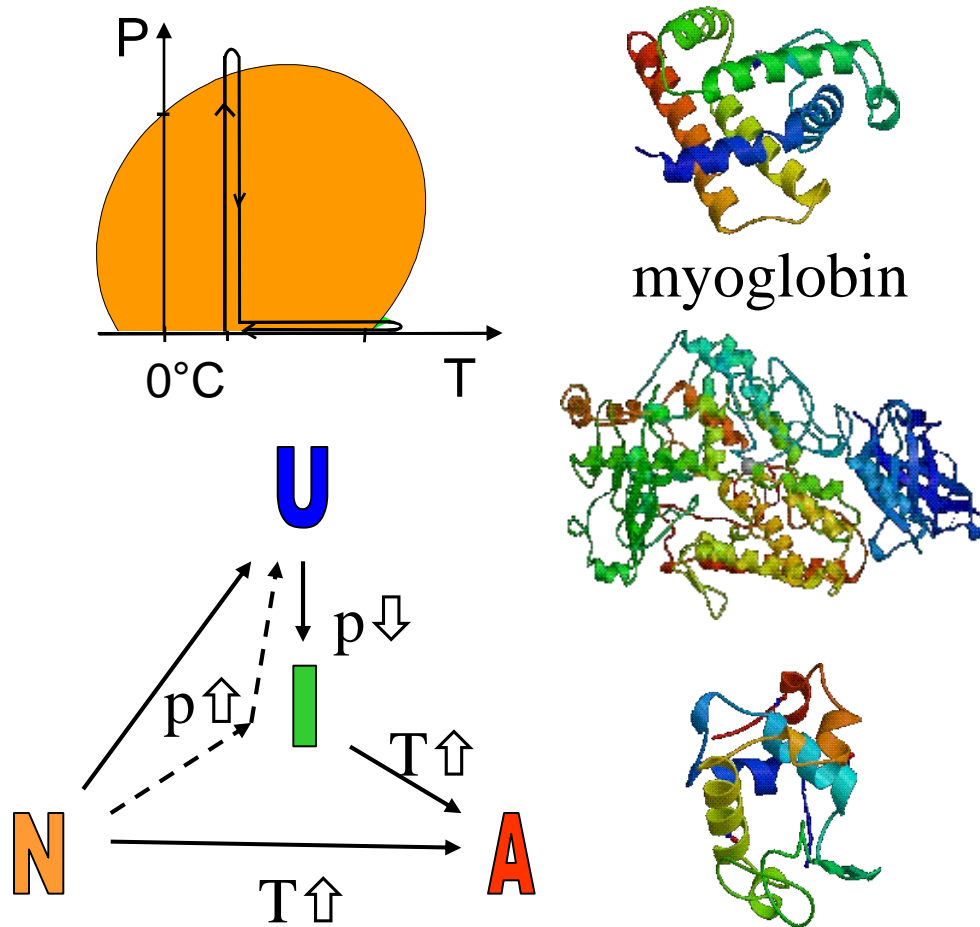


Intermolecular
interactions:
aggregation (conc!)

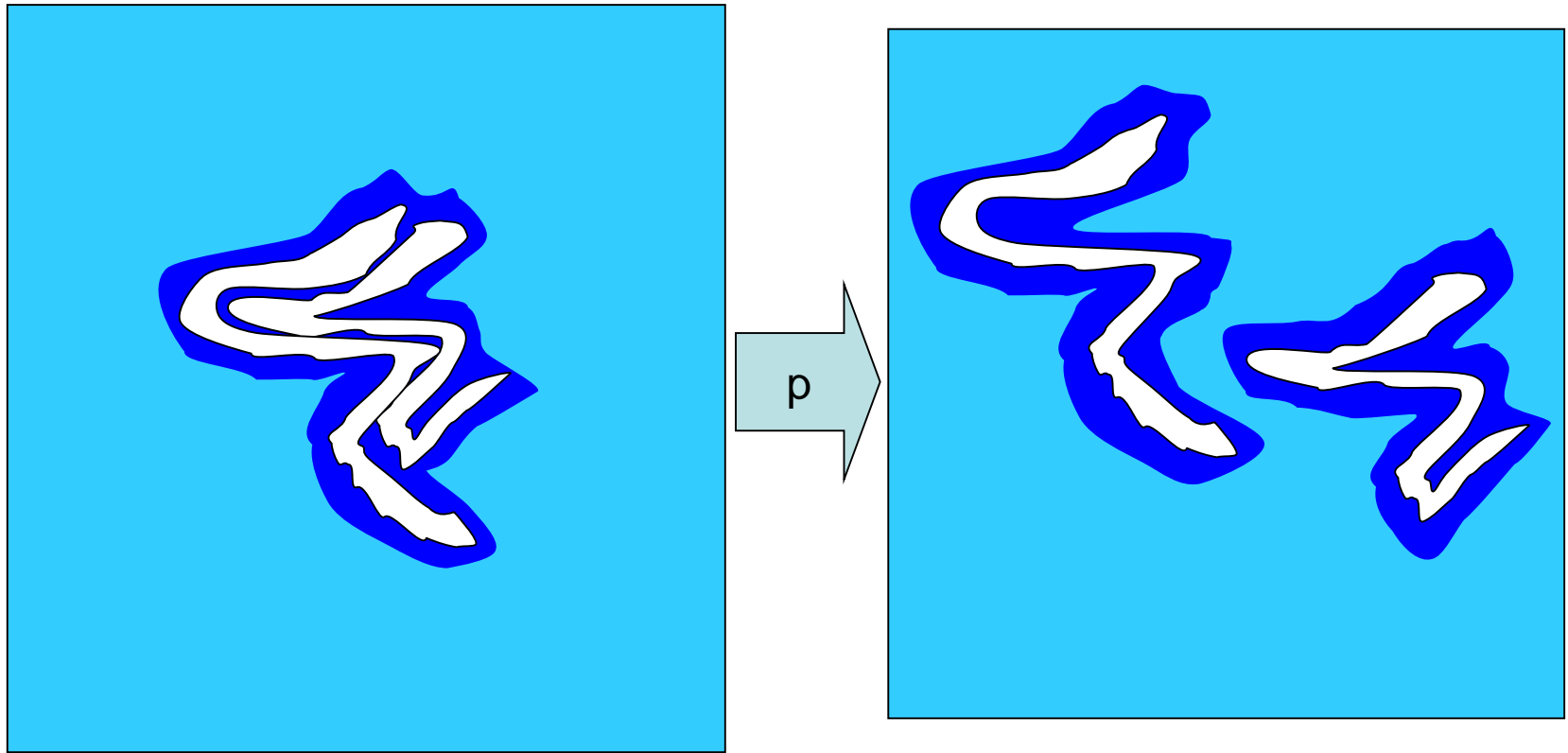
Pressure and cold
denaturation: ΔV

Heat denaturation: ΔS

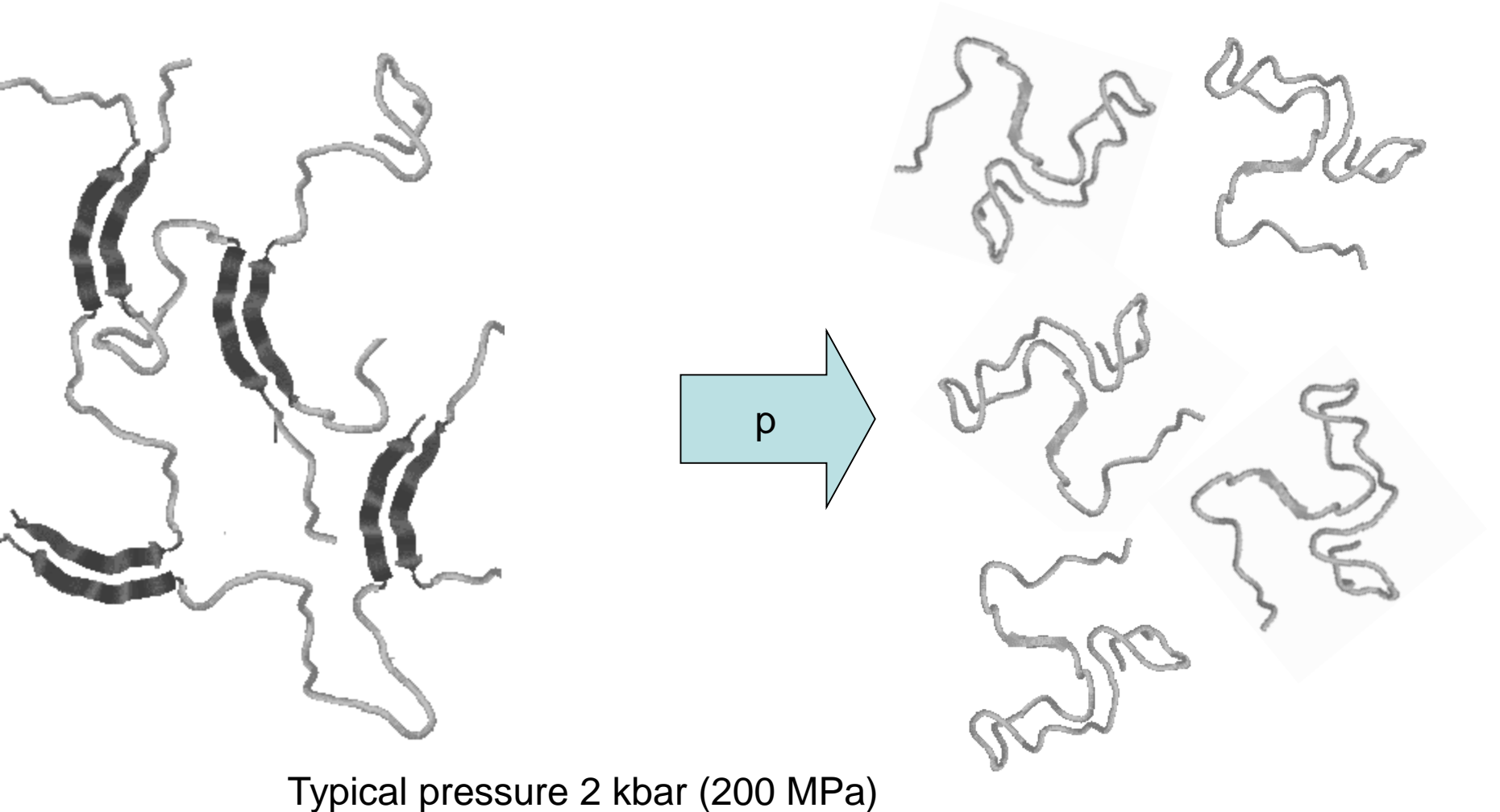
Appearance of aggregation prone intermediates after pressure denaturation



Intermolecular interactions and the pressure

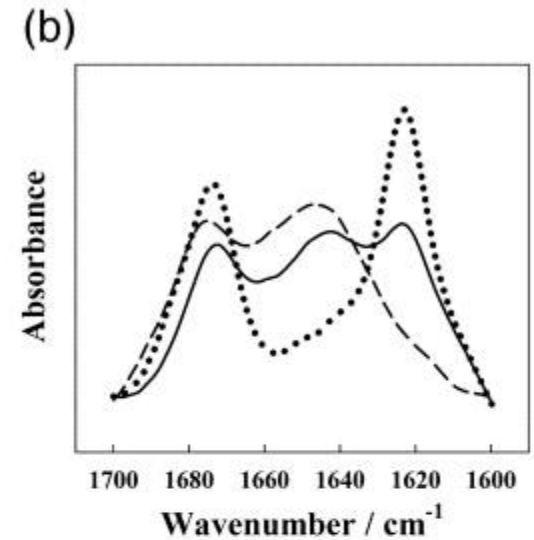
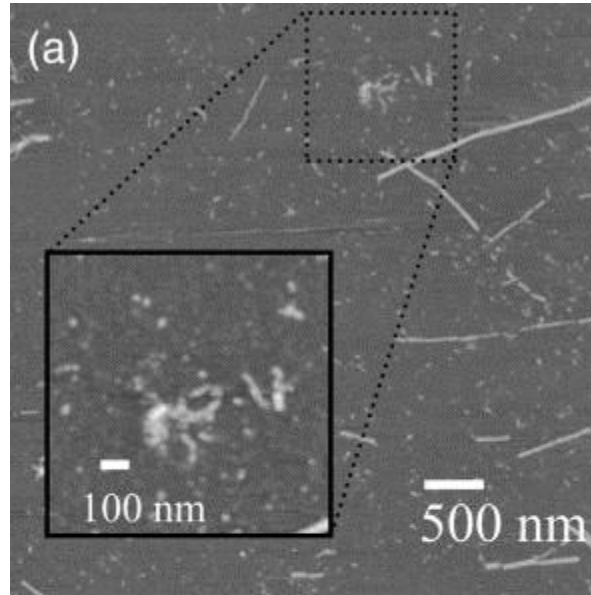


Intermolecular interactions and the pressure

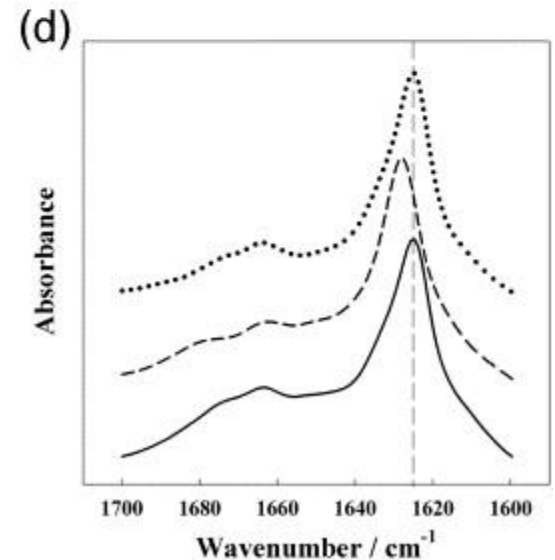
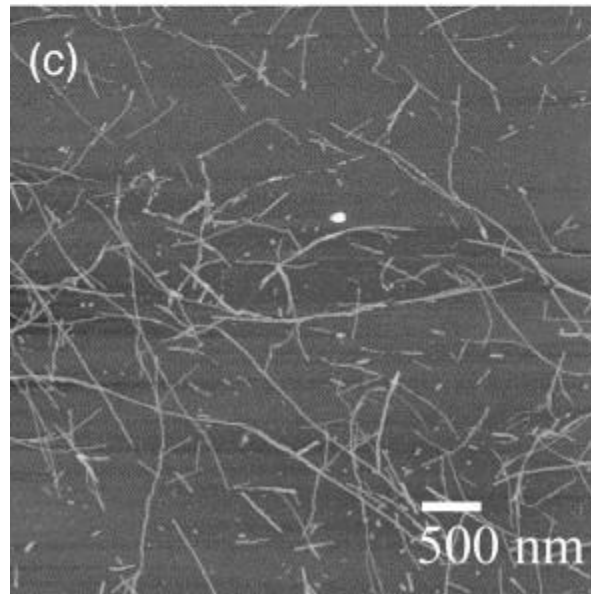


Aggregates and fibers

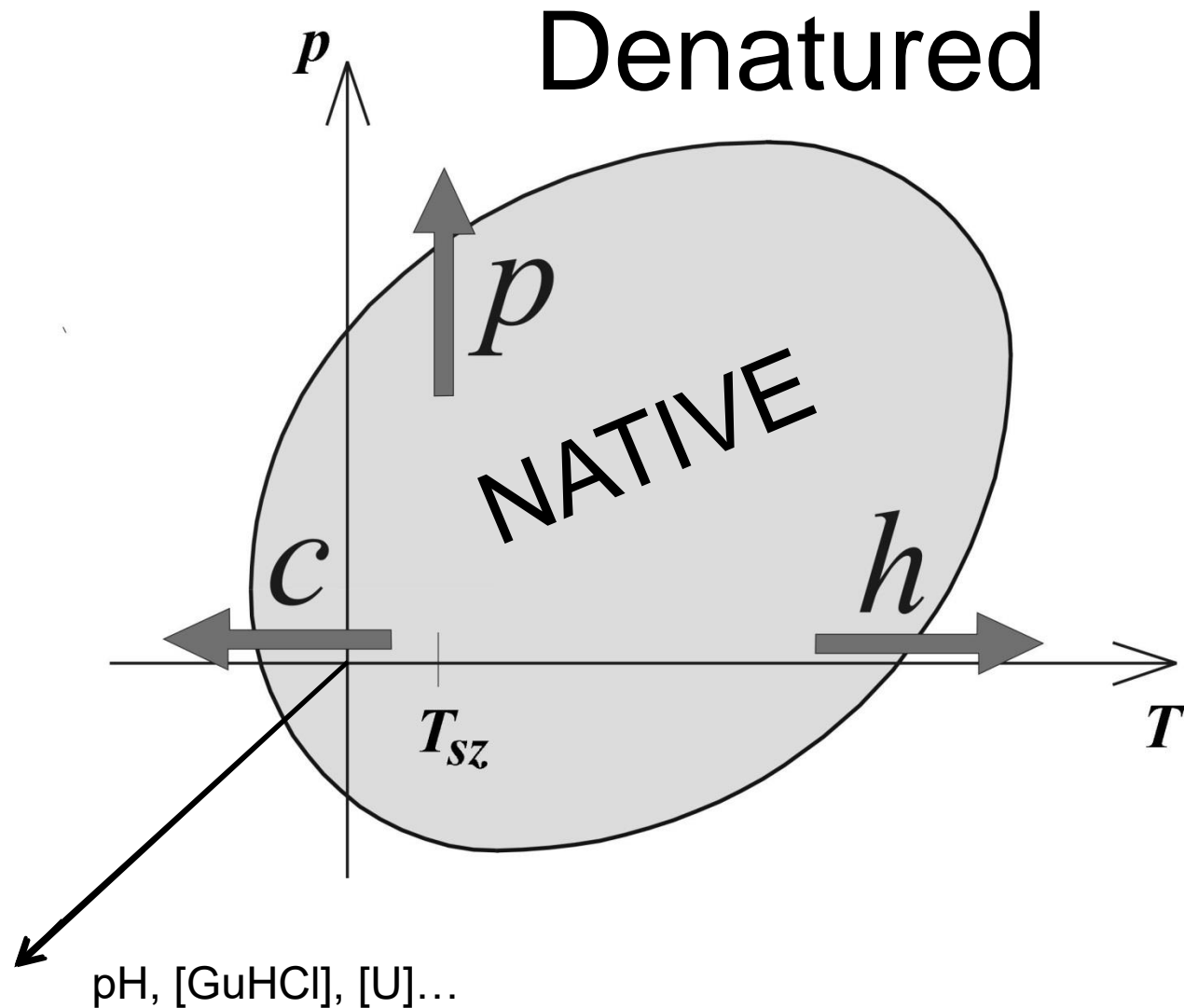
Day 1: (a) AFM (b) amide I band of TTR105–115 at 0.1 MPa (full line), 550 MPa (broken line) and 0.1 MPa after decompression (dotted line).



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).



The third (fourth...) dimension



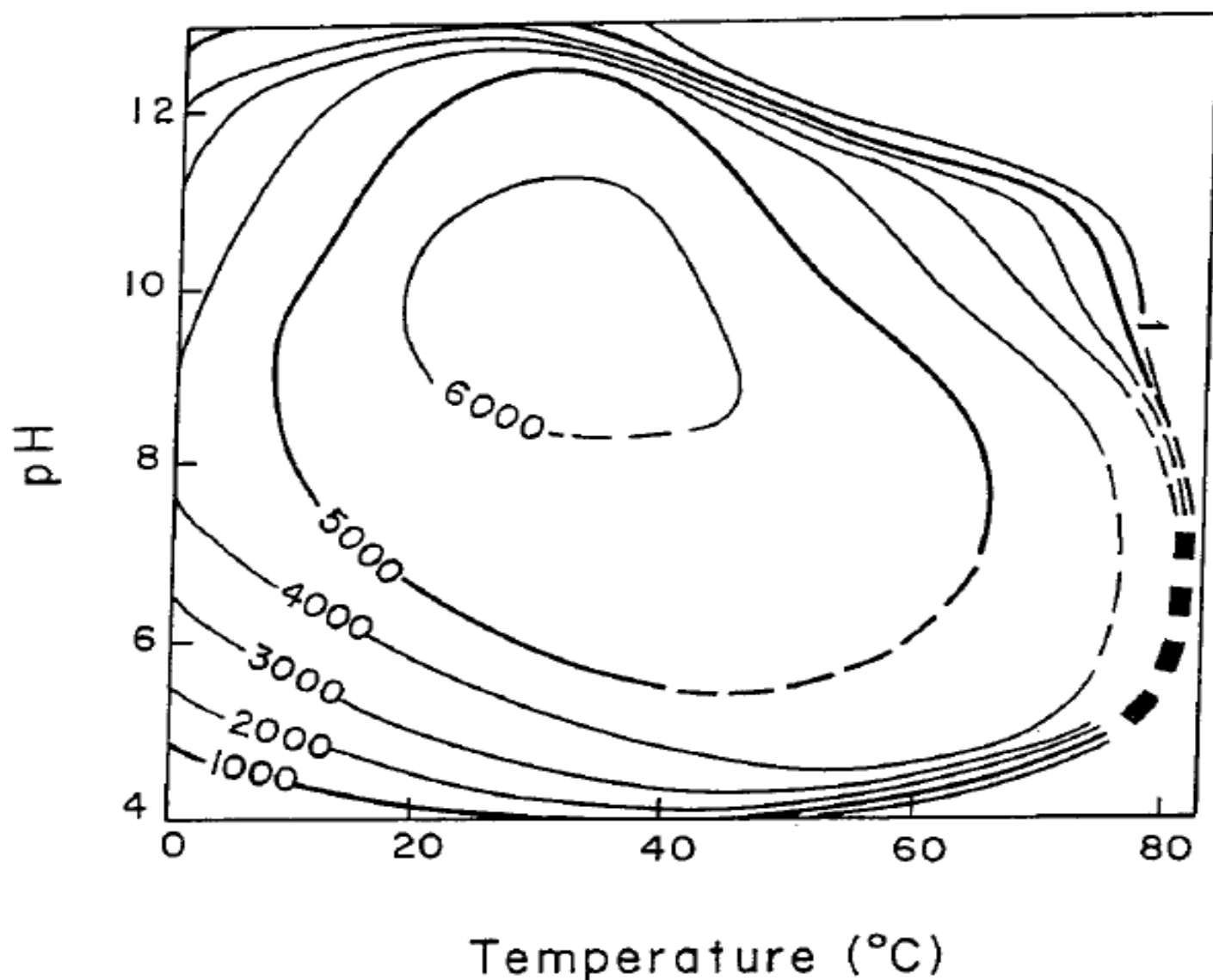


FIGURE 3. Contours of the constant pressure in the pH-temperature plane at which $\Delta_N^D G = 0$ for denaturation of metmyoglobin. The native state is more stable than the denatured one inside each contour.⁹⁶

The phase diagram of DNA

The double helix form is pressure independent

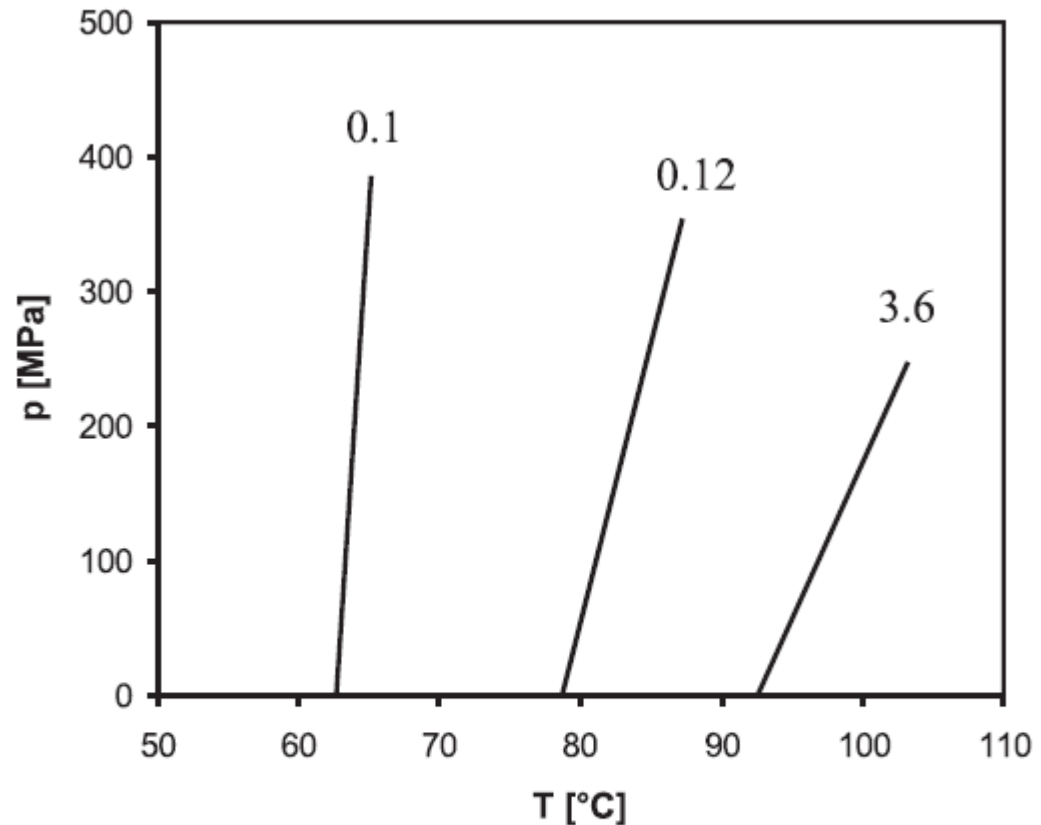
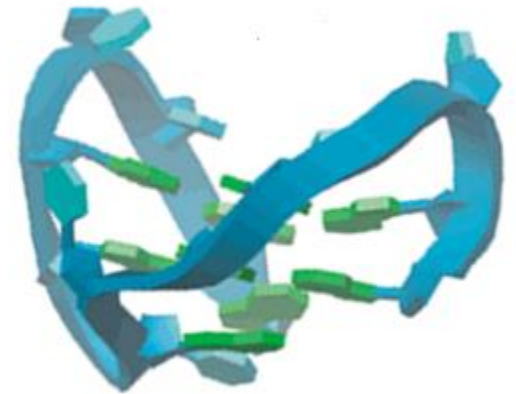
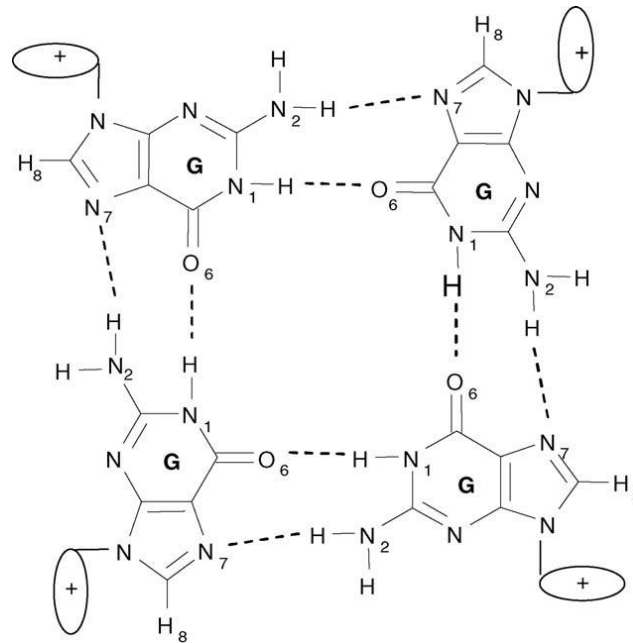
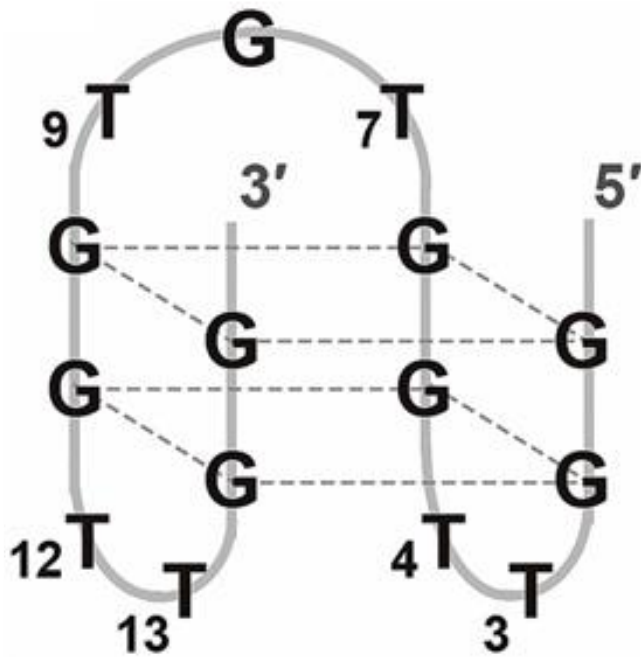


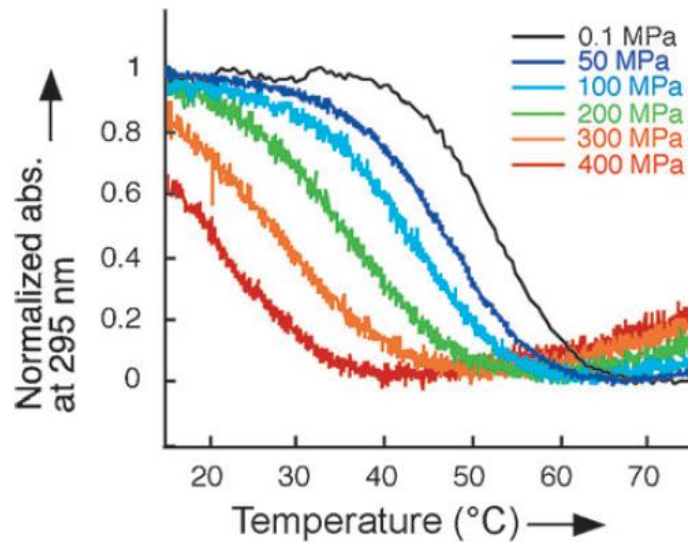
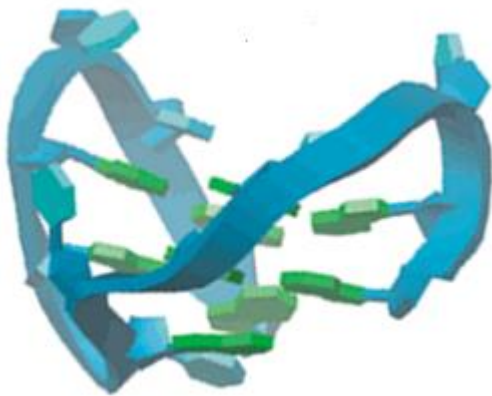
Fig. 10. Pressure-temperature diagram of DNA helix-coil transformation. Unlike the heat unfolding temperature of proteins, the melting temperature of DNA does not show any curvature, but a purely linear pressure dependence. The numbers refer to the molar concentration of neutral salts. Drawn after [84].

Exotic DNA structures: G-quadruplex



Phase diagram of DNA

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



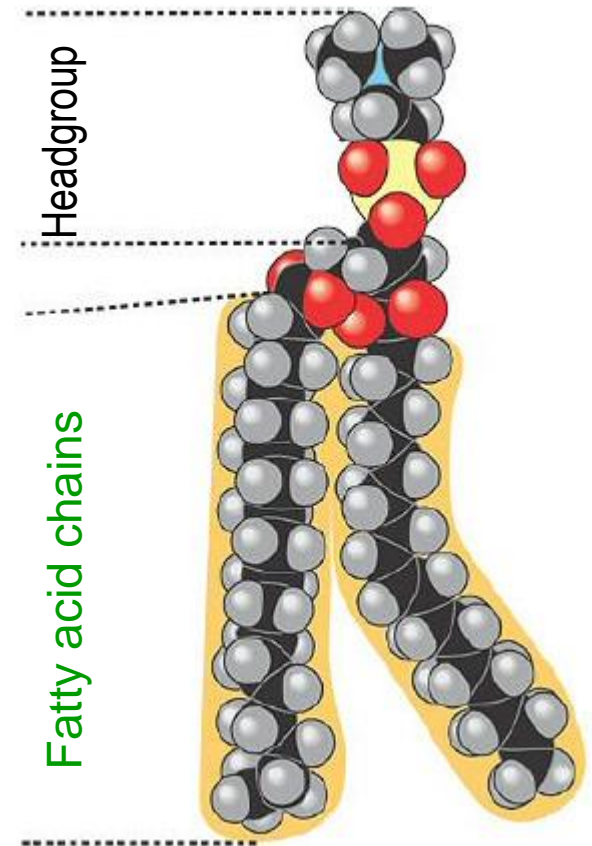
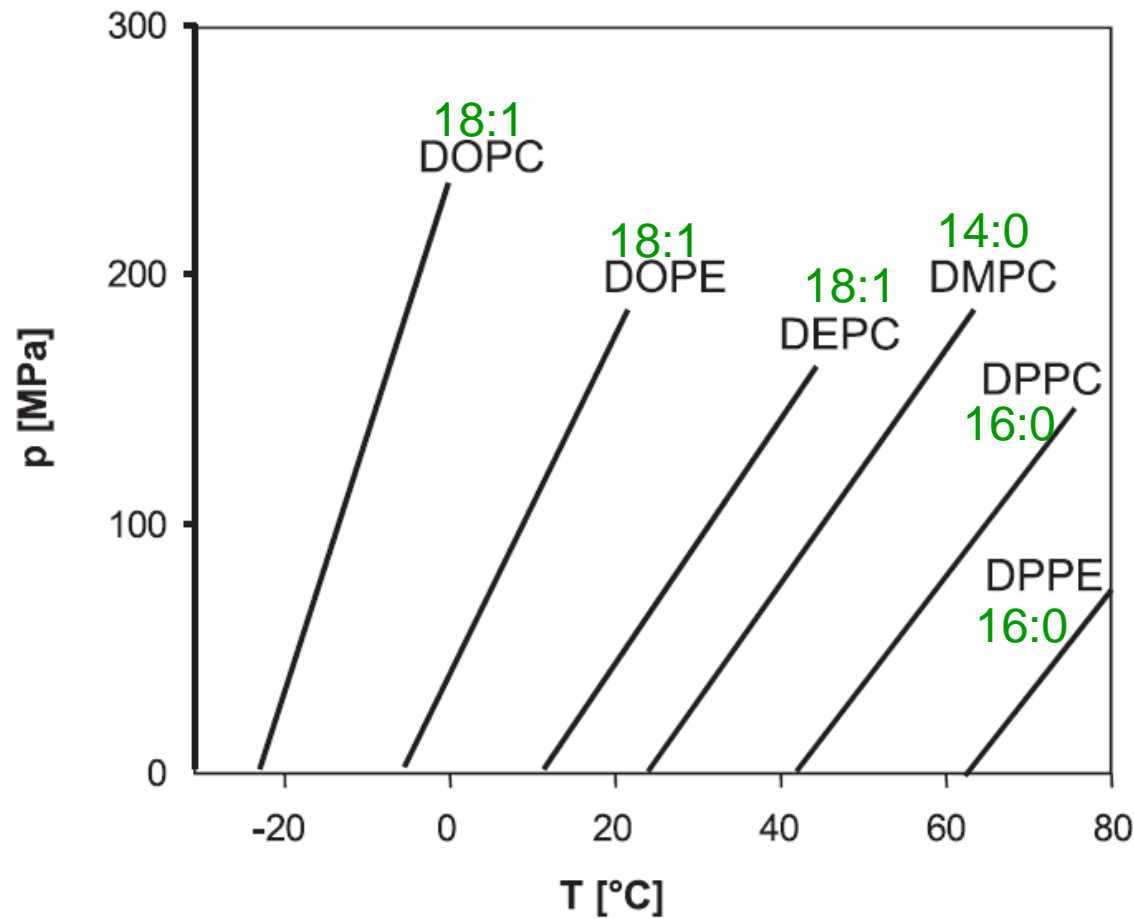
G-quadruplex



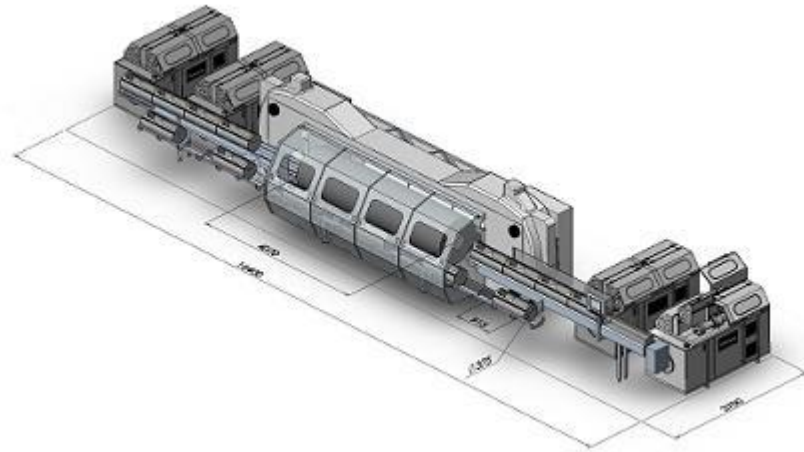
ss DNS

$\Delta V!$

Phase diagram of membranes



Applications

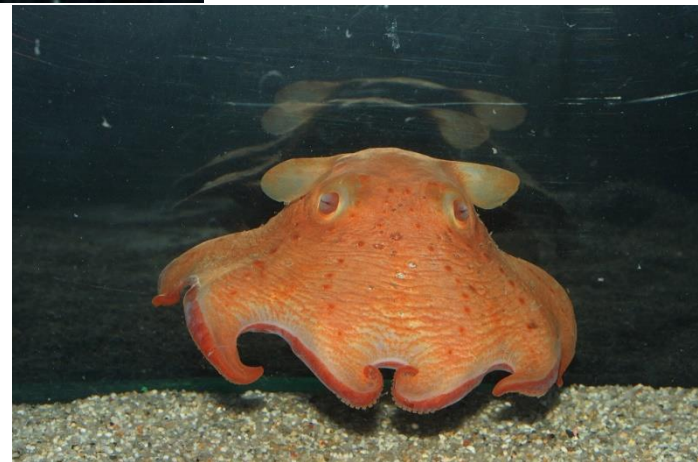
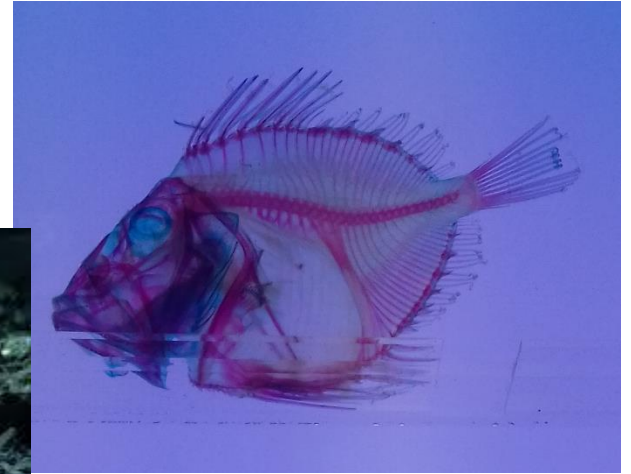




Pressurized pizza at a high pressure conference

Pressurized food in a japanese supermarket

Deepsee organisms



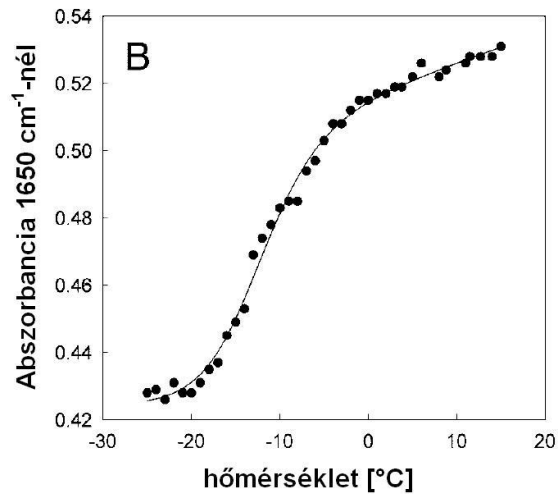
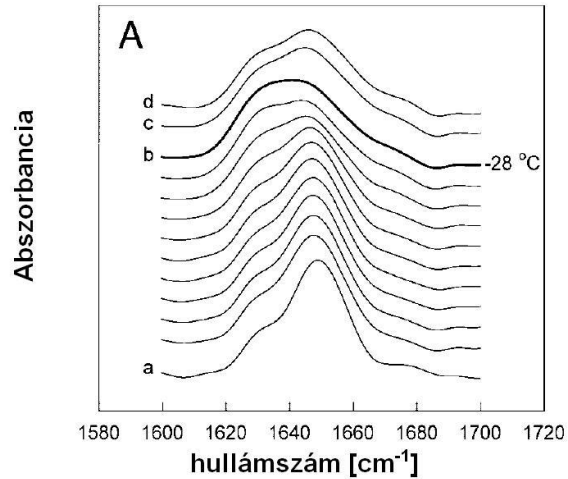
End

References

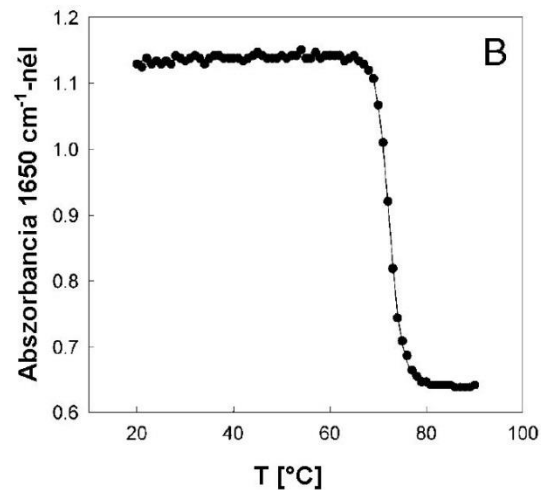
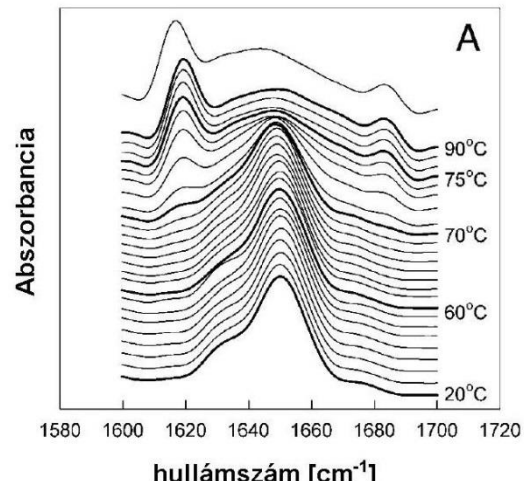
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- <https://matome.naver.jp/>
- <https://rr.img.naver.jp>

Myoglobin: FTIR spectroscopy

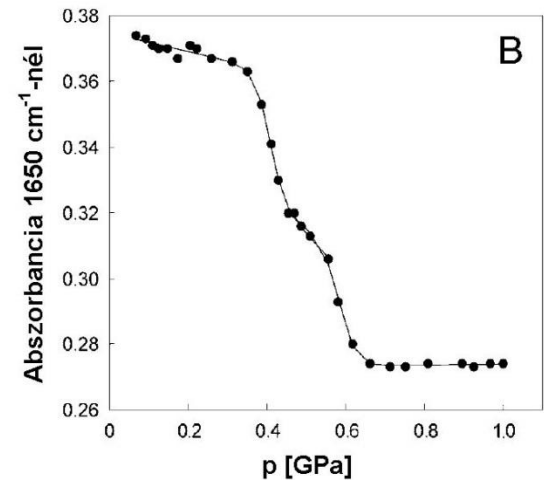
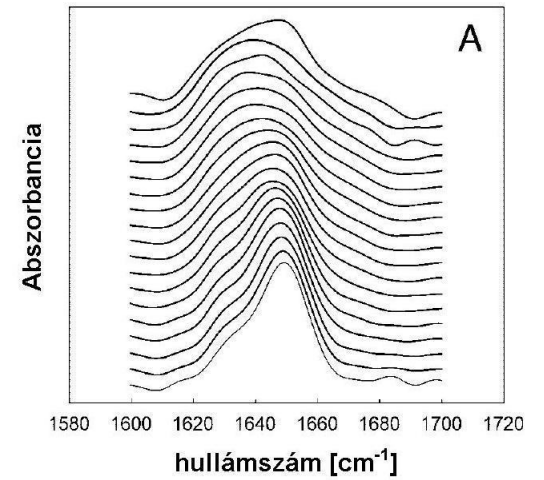
cold



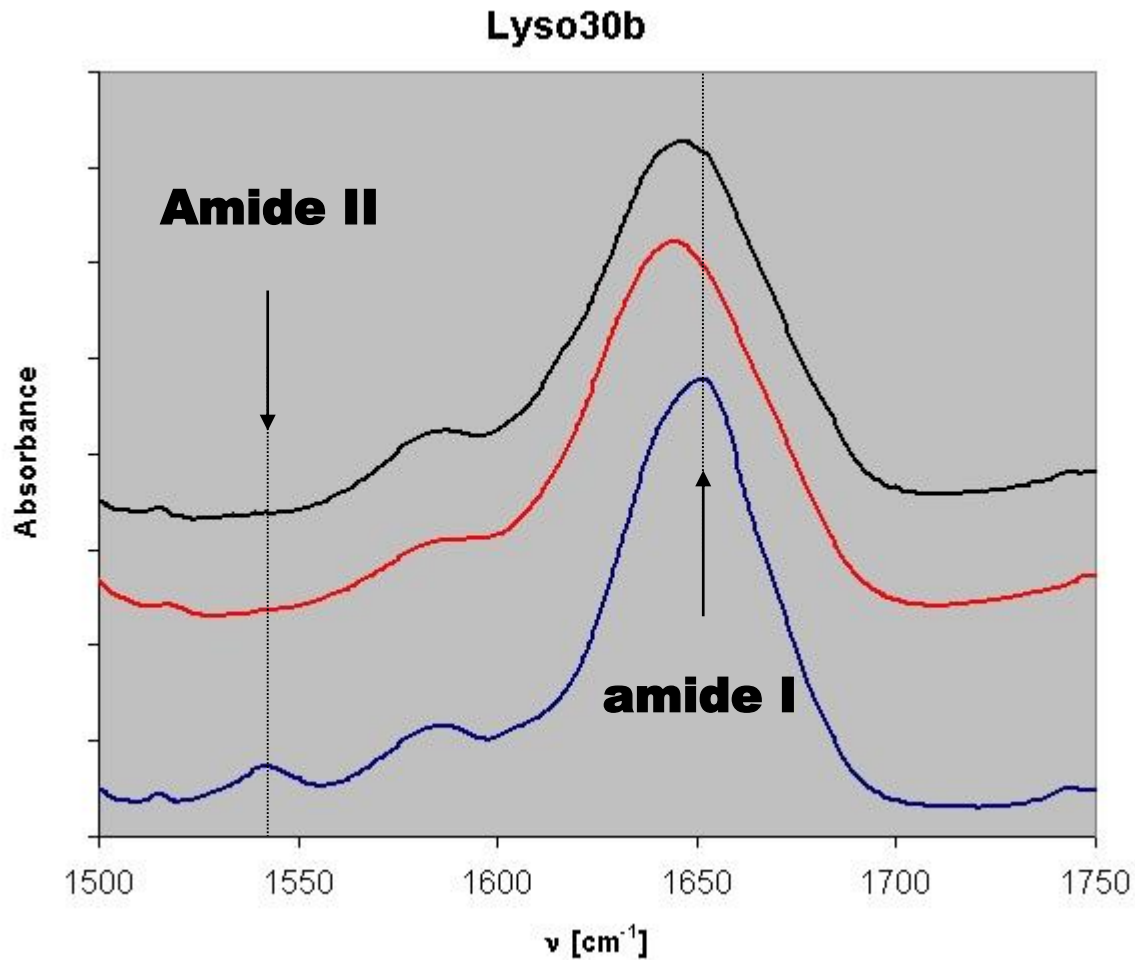
heat



pressure



Lysozyme: FTIR Spectroscopy

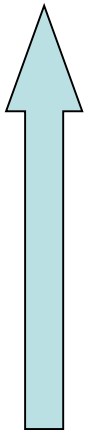


75mg/ml

**back to
0.1 MPa**

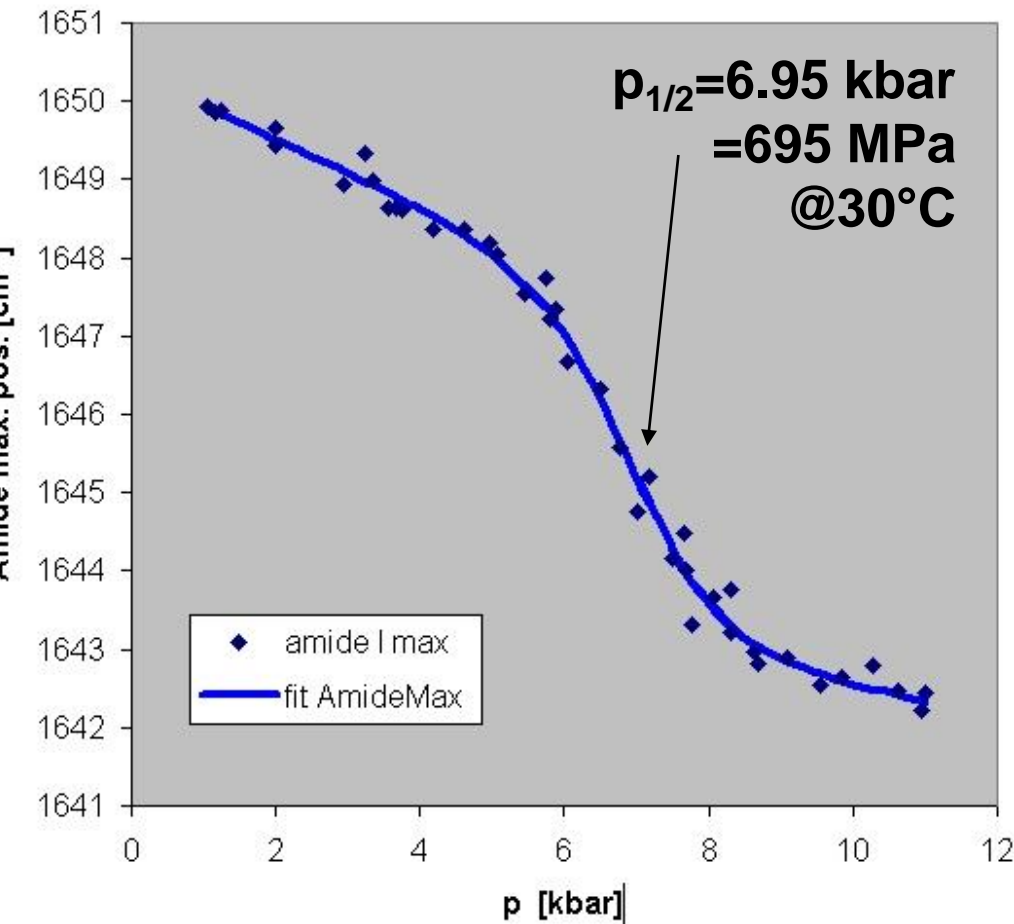
900 MPa

0.1 MPa

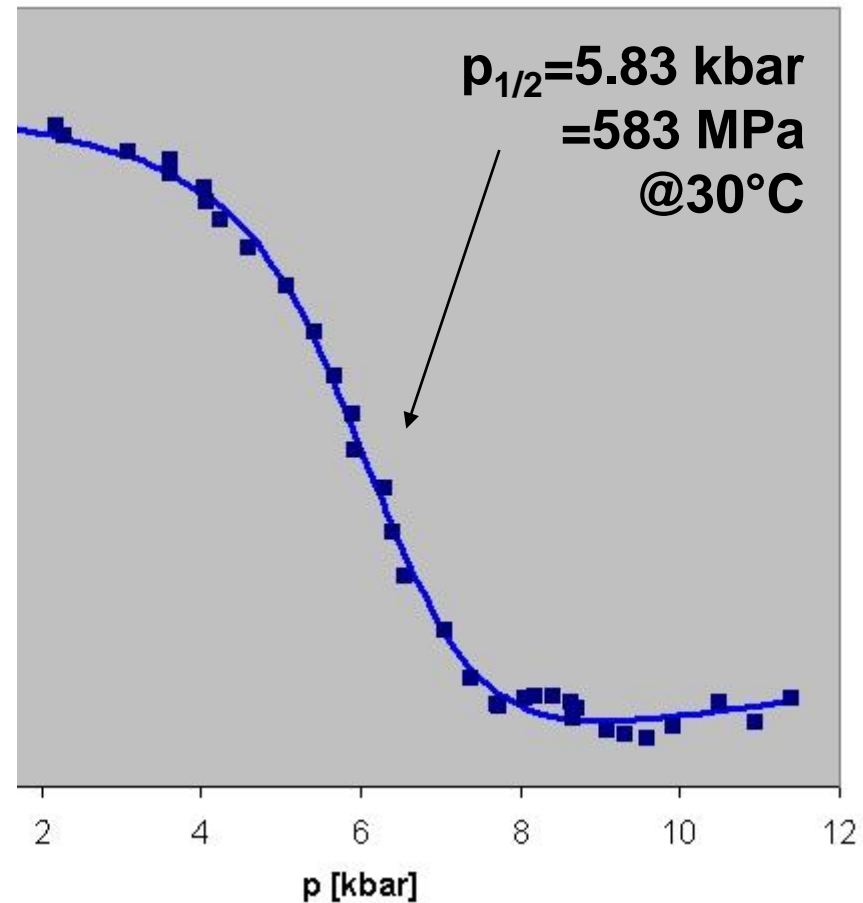


Lysozyme: pressure denaturation

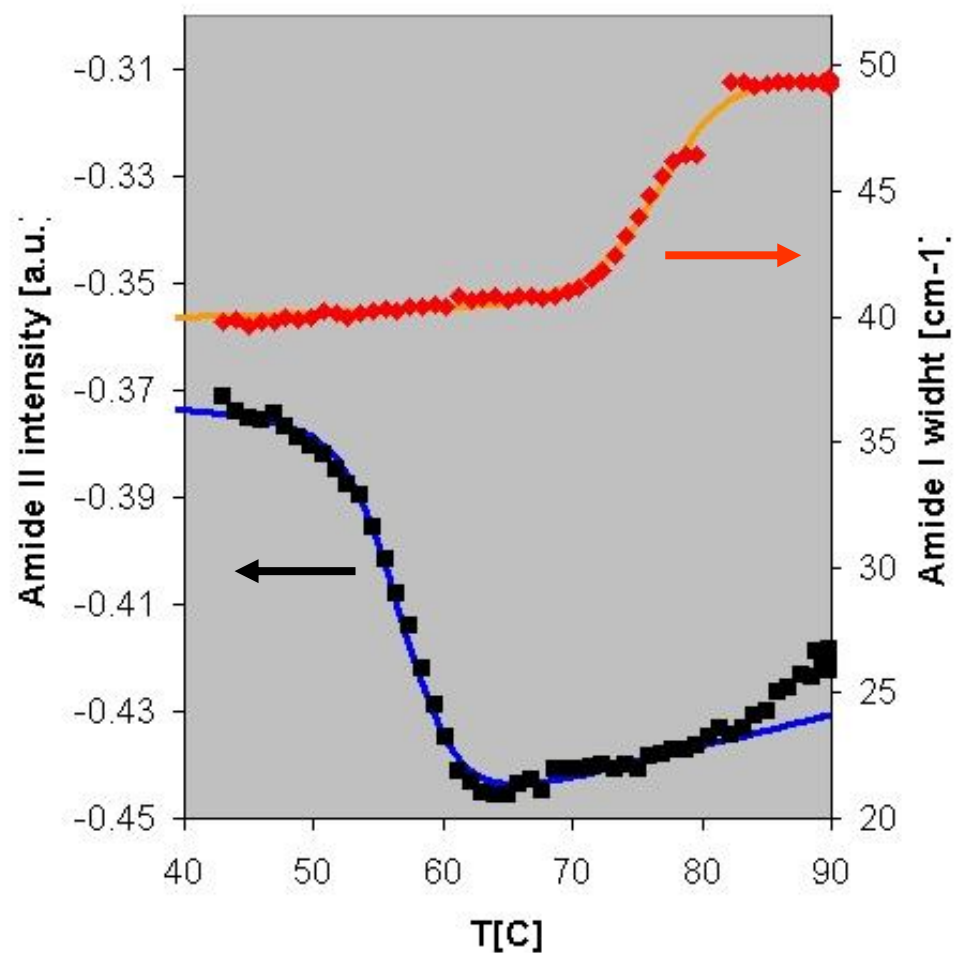
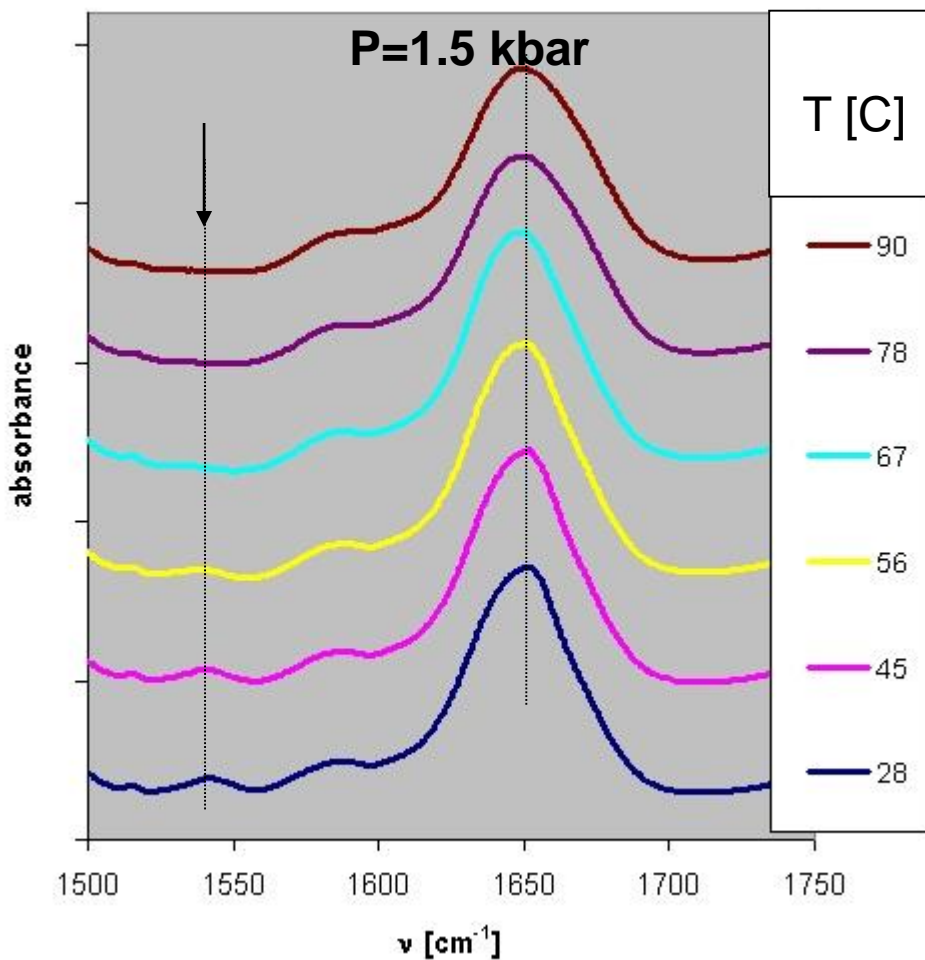
Amide I max. position [cm⁻¹]



Amide II intensity [a.u.]

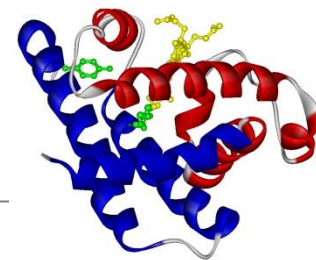
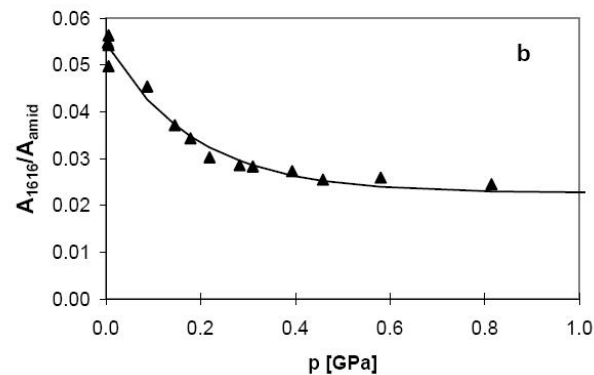
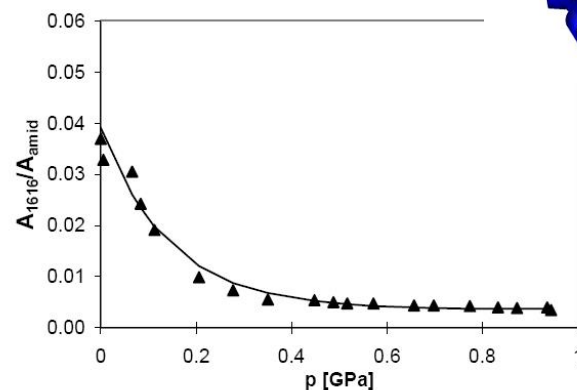
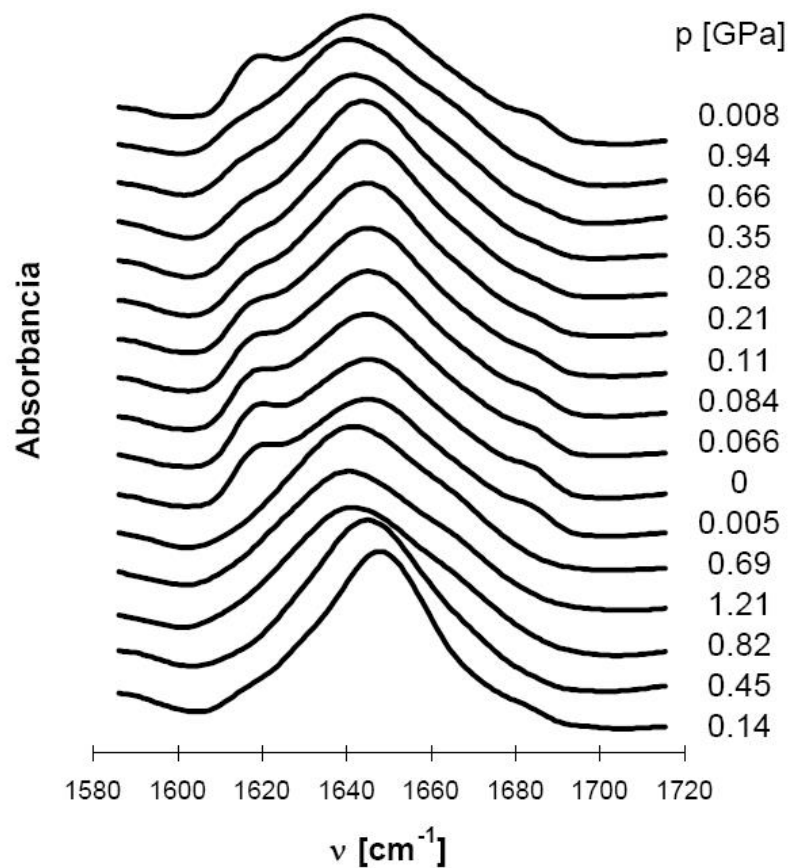


Lysozyme: heat denatruation



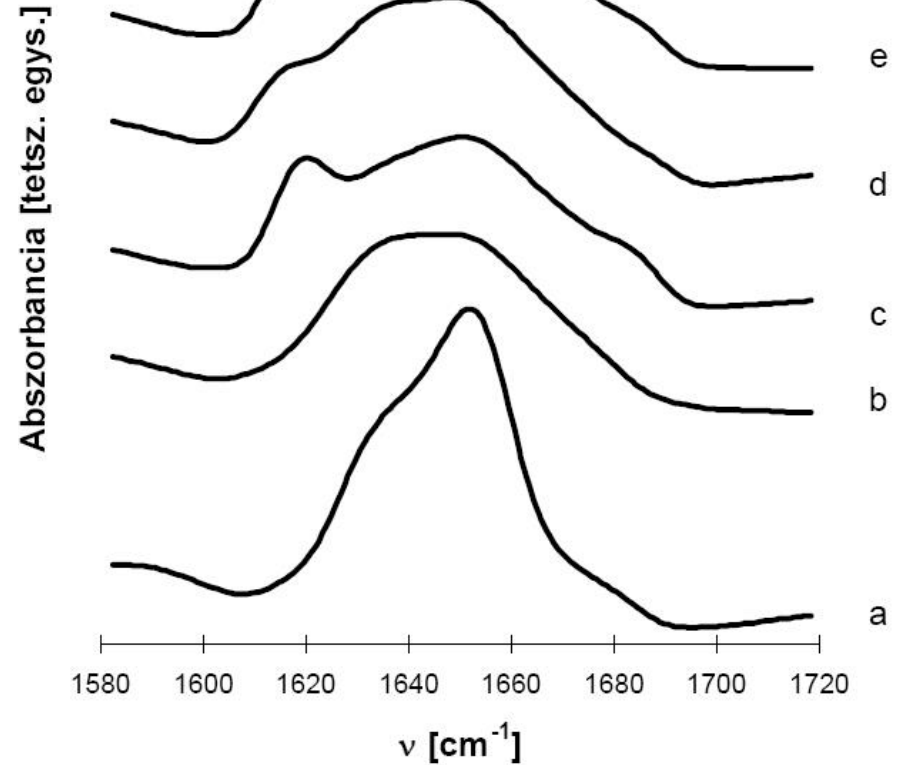
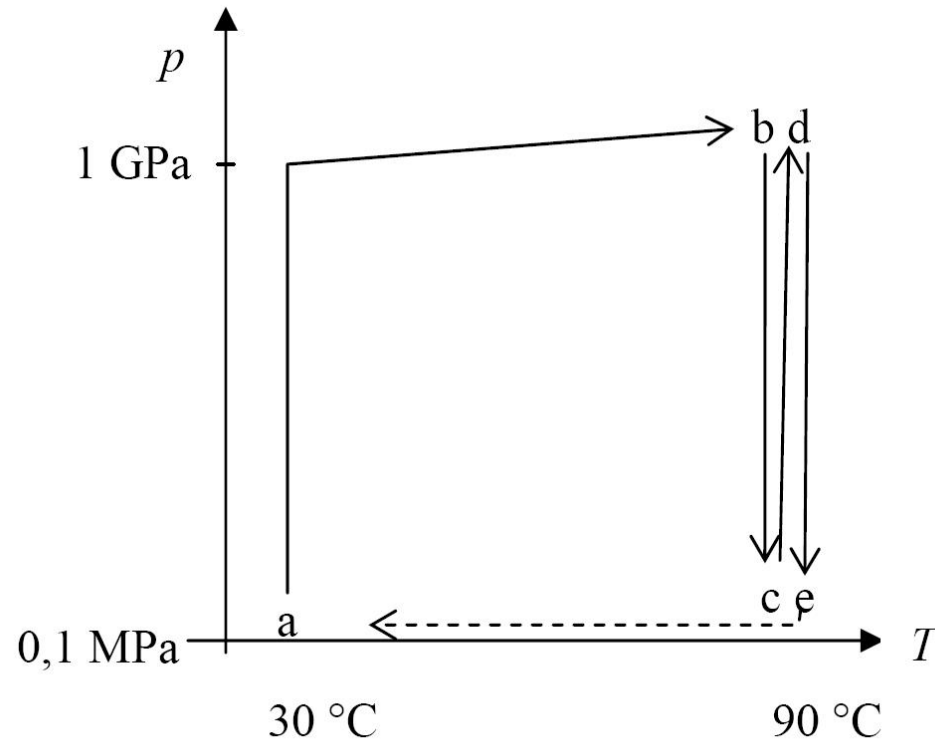
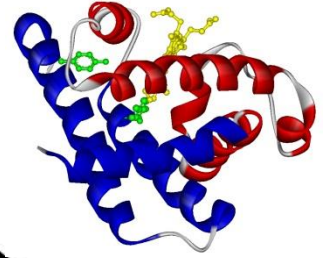
Dissociation of aggregates using pressure.

Example: myoglobin



Dissociation of aggregates using pressure.

Example: myoglobin



The high pressure technique

- Thick wall container
- A small volume diamond cell

Termophile and piesophile proteins

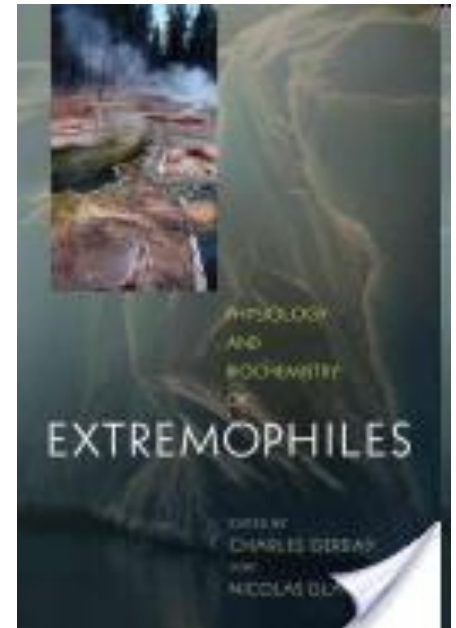
Extremophyle microorganisms

They live under

- extreme temperaure ($T > 100\text{ C}$)
- extreme pressure (few 100 bar)
- extreme chemical environment
(e.g. highly acidic)

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

They are sources of hermophile and piezophile proeins





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

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



Review

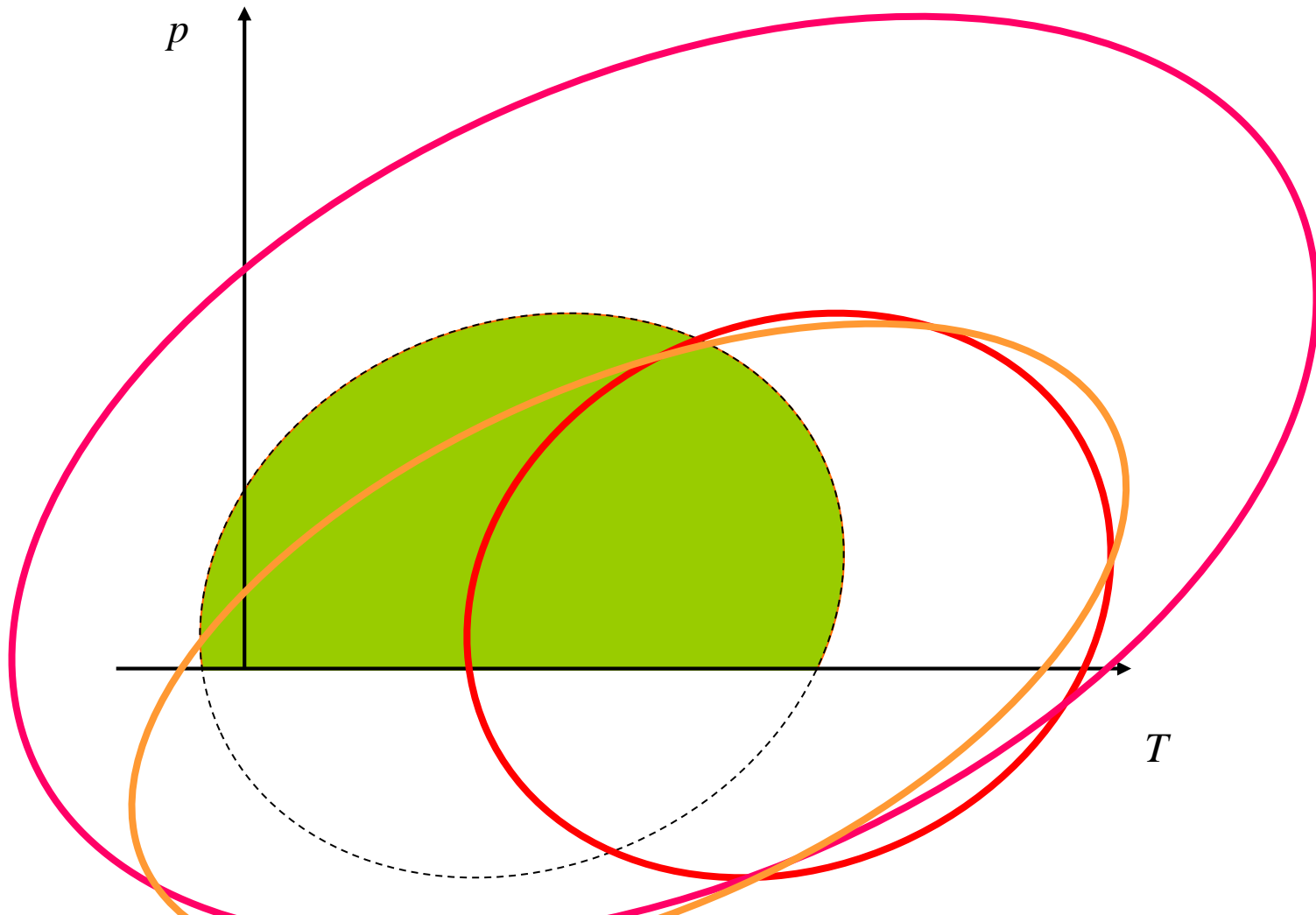
High pressure enhancement of enzymes: A review

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

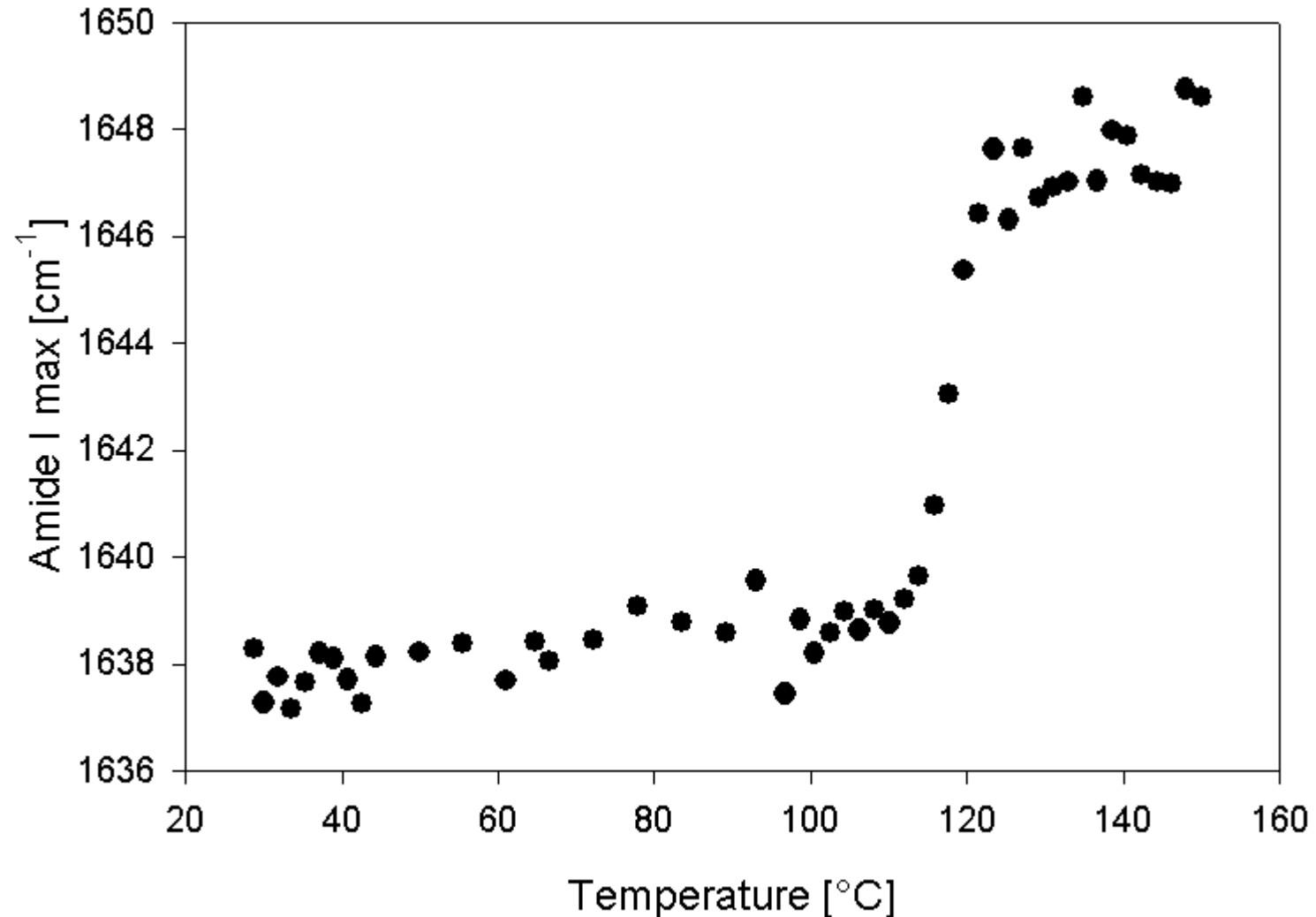
Citrus Research and Education Center, Institute of Food and Agricultural Sciences, University of Florida, 700 Experiment Station Road, Lake Alfred, FL, USA

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



Enzymatic activity in extreme environment

PI:

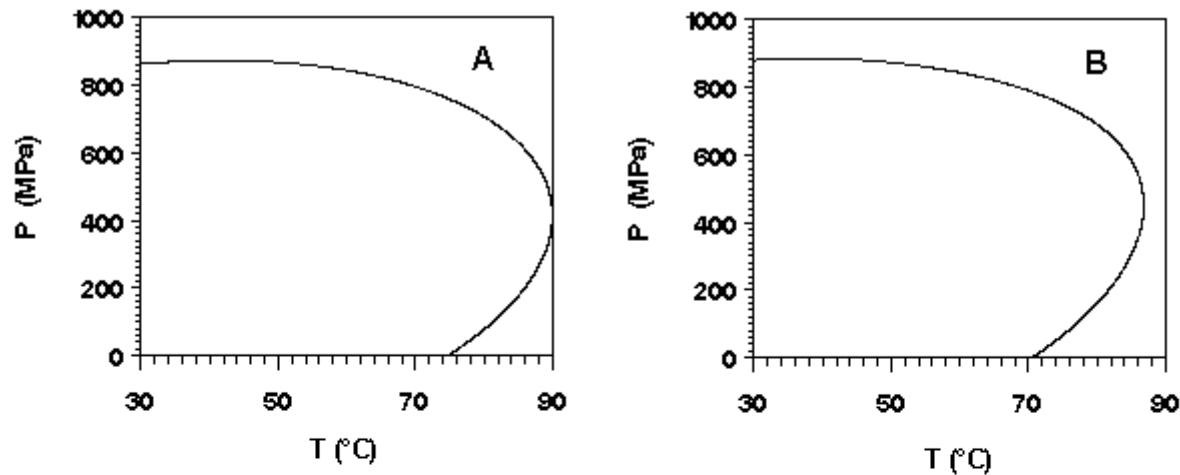


Figure 3. Heat-pressure iso-rate contour plots of 95 % inactivation of purified banana PME (in 20 mM Tris-HCl buffer, pH 7.0) for a total process time of 30 min ($k = 0.099858 \text{ min}^{-1}$) based on (A) the 'classical' thermodynamic model (eq 4); (B) the third-degree thermodynamic model (eq 6);

From: *Binh Ly-Nguyen1 et al*, Effect of Mild-Heat and High-Pressure Processing on Banana Pectin Methyltransferase: A Kinetic Study

Generalized Clausius Clapeyron equation

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

