Ligand Binding to Myoglobin:
The role of cavities and volume changes

W. Doster

Question:
What do volume changes tell us about biomolecular reactions?
A -> B

1) Measurement by applying pressure, chemical potential:

$$d\Delta\mu_{BA} = -\Delta s_{BA}(T - T_0) + \Delta v_{BA}(P - P_0)$$

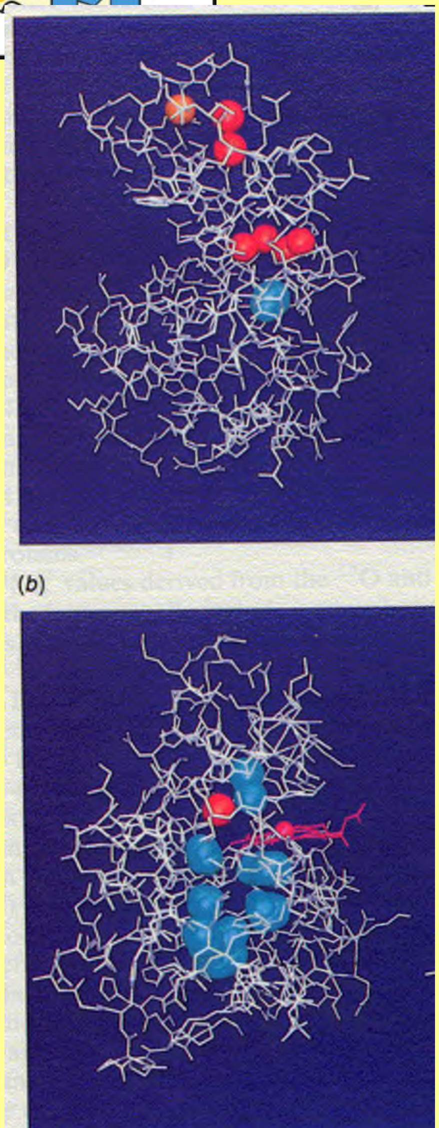
Pressure enhances the energetic relevance of volume changes from << $k_B T$ (1 bar) **to** $\approx$ **$k_B T$** (1 kbar)

2) Do we understand bio-molecular volume changes? model reactions

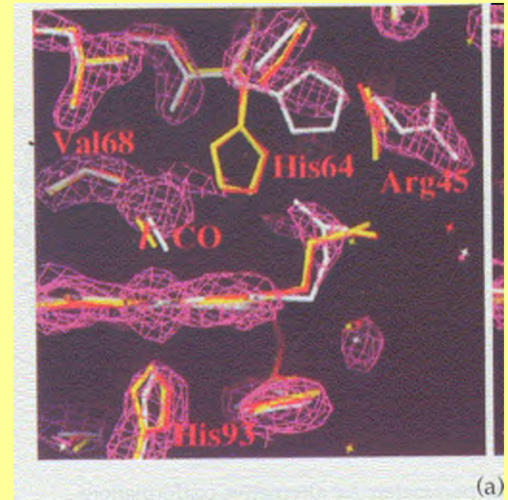
3) Transfer of water in protein processes, protein-internal cavities

Ligand Binding, Protein Unfolding, Dissociation of ProteinComplexes

Water in Protein Cavities

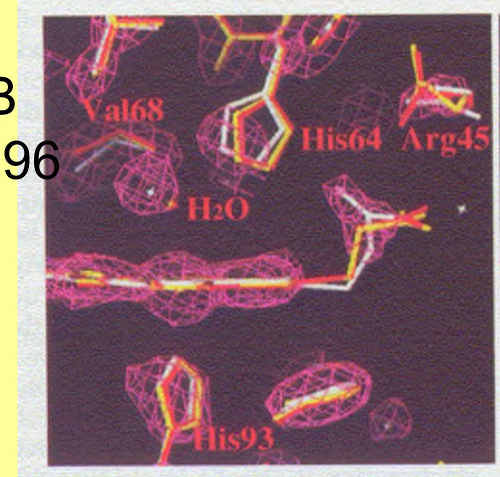


CO-myoglobin
pH 4-6



lysozyme
B. Halle 1996
X-ray/NMRD
7 integral waters
Empty cavity

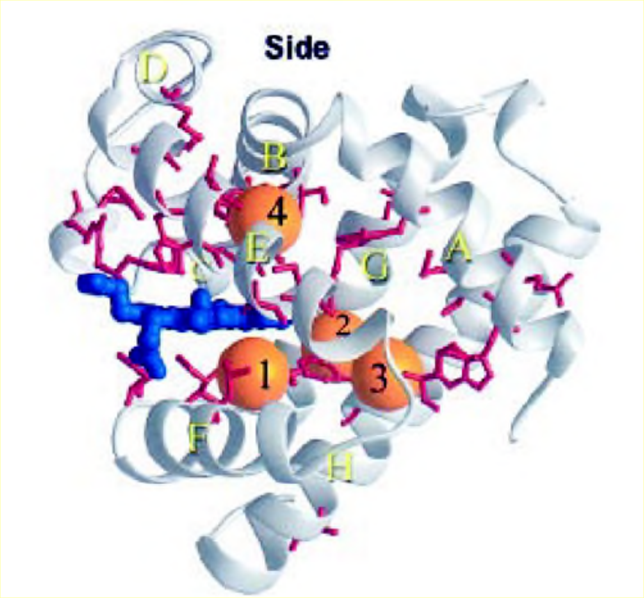
Deoxy-myoglobin
pH 4-6
Takano 1977 JMB
Yang, Philipps 1996

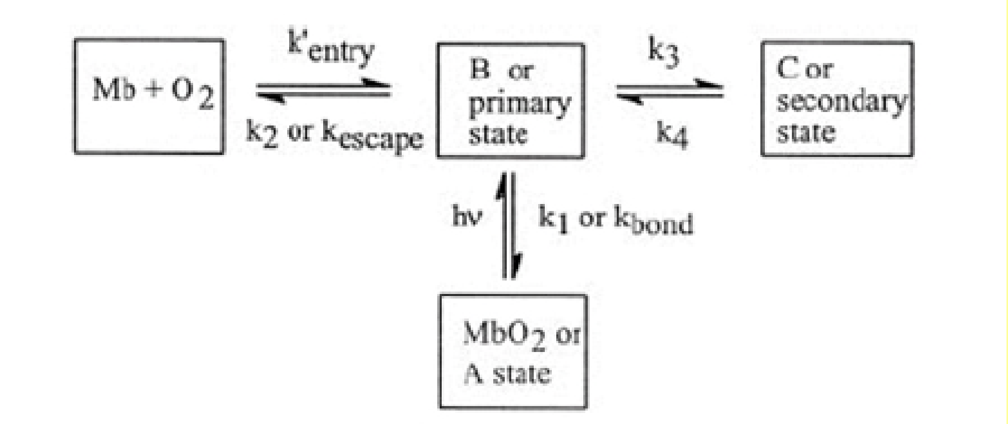


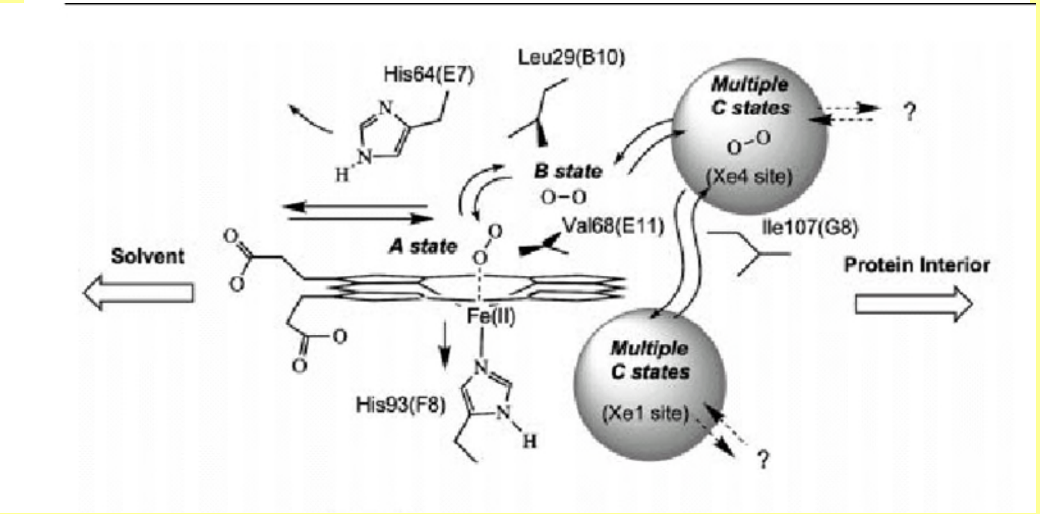
Met-myoglobin
Empty cavities
X-ray: **1 water molecule**
NMRD: several

Myoglobin: Xe- docking sites, Kinetic model

Scott, Gibson, Olson, JBC 2001

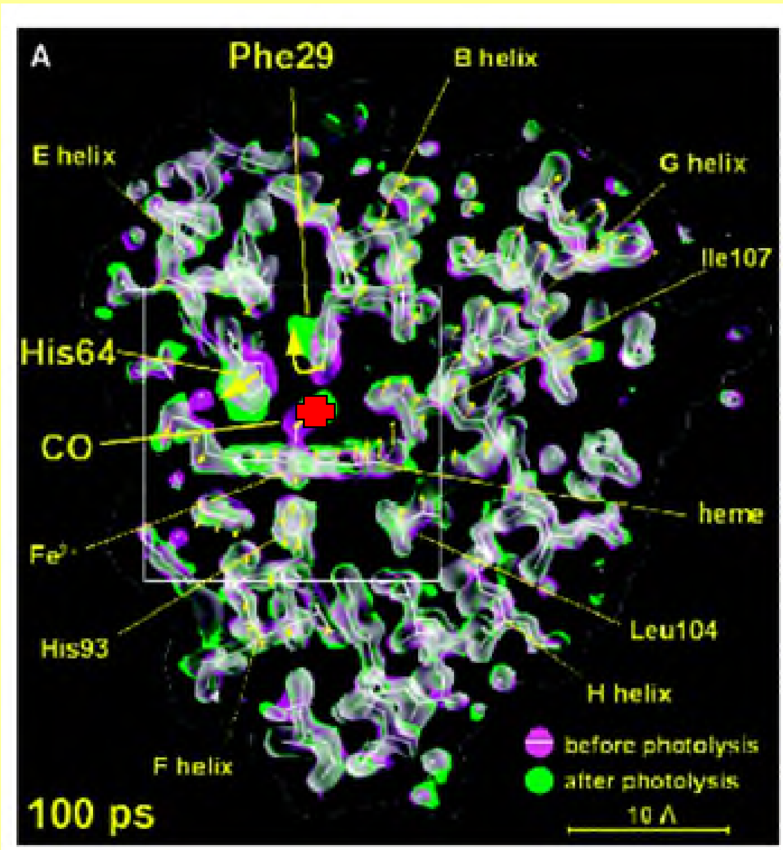




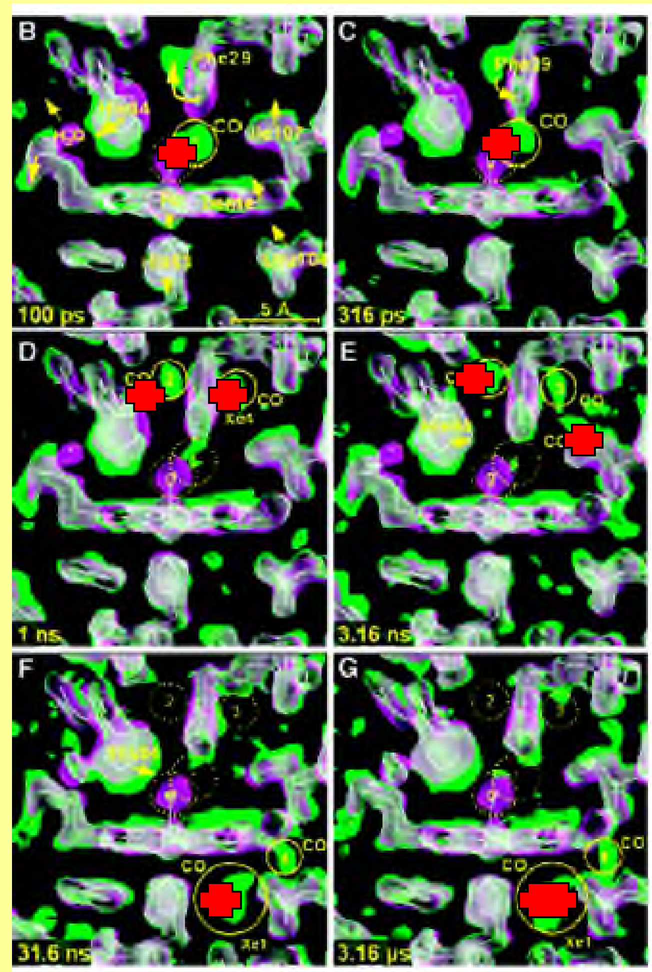


Time-Resolved X-ray of CO binding to myoglobin

Schotte et al. Science (2003) 1944



CO



100 ps

B

3 ns

C

30 ns

3 μs

D

Assignment of intermediates, B, C, D

Time series

TECHNISCHE UNIVERSITÄT MÜNCHEN

Flash Photolysis at high pressure: Myoglobin-CO

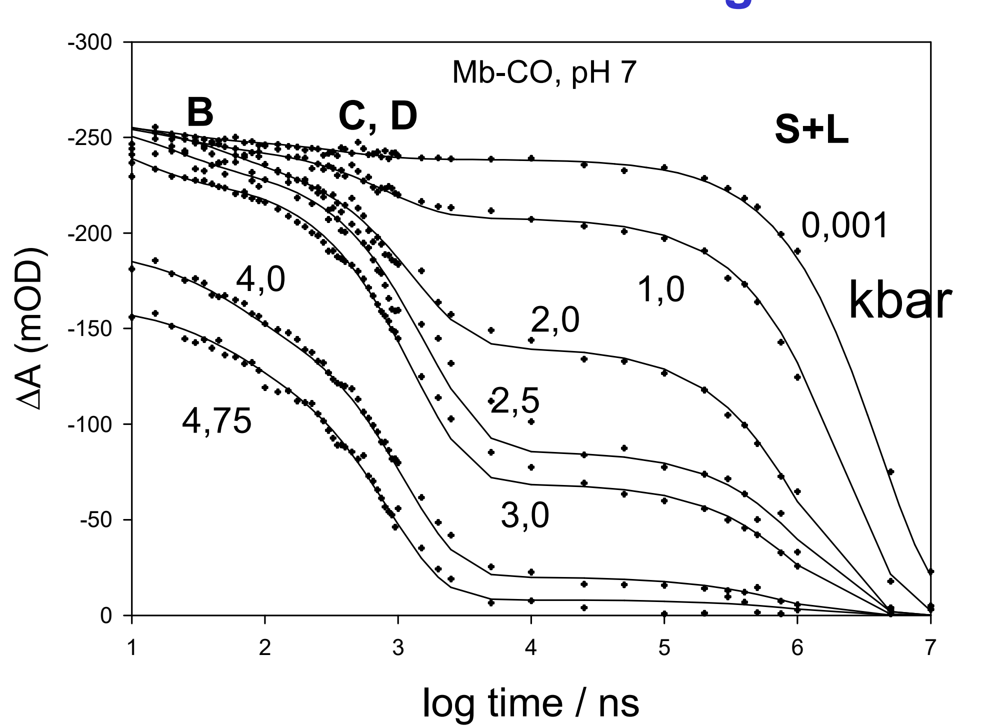
Solvent primary interior sites

S ? B ? C_i

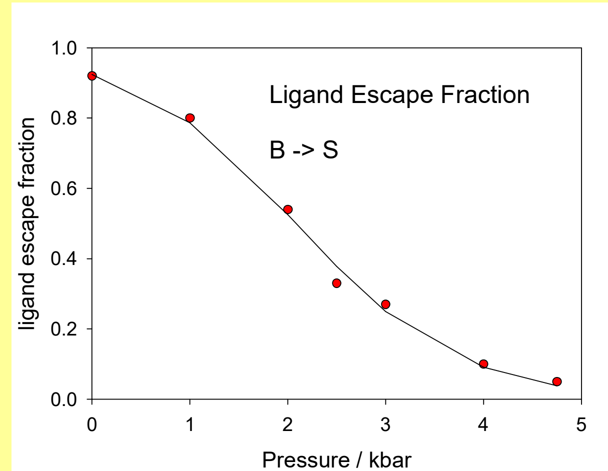
hν ?

A ligated

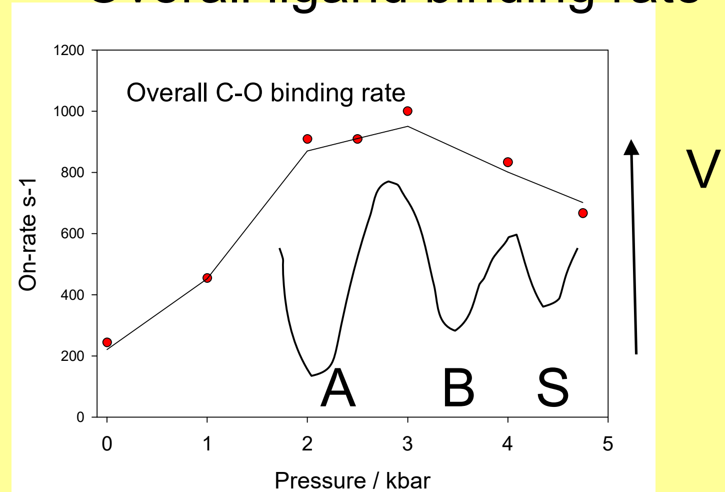
Binding kinetics



Ligand escape fraction

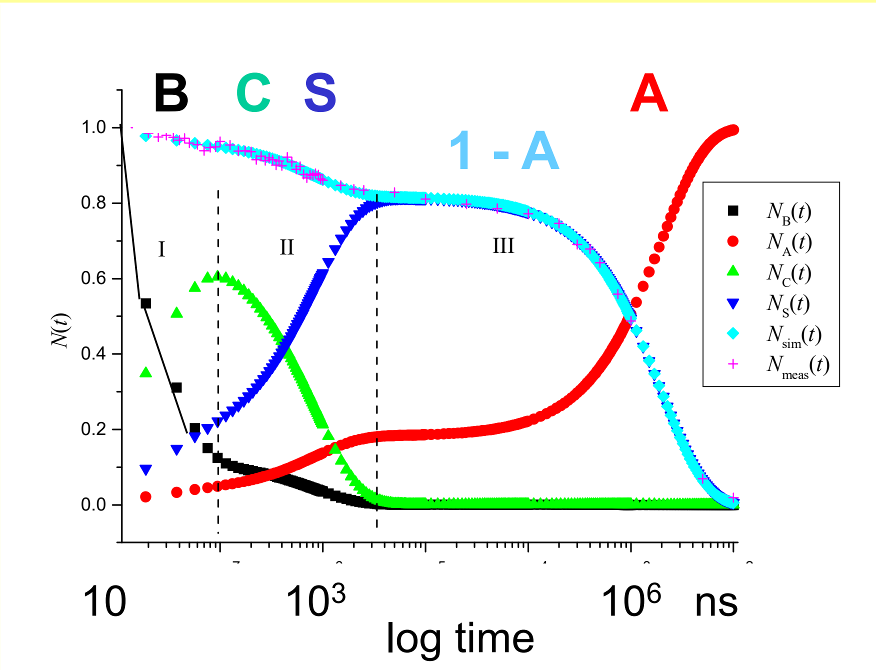


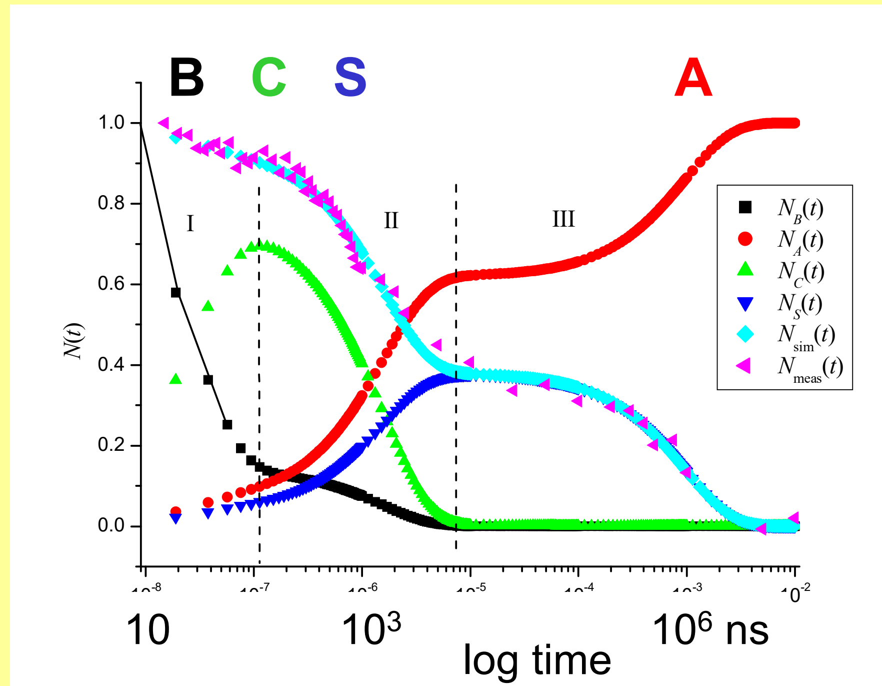
Overall ligand binding rate



Flash photolysis at high pressure: population evolution

1 kbar **2.5 kbar**





Solvent primary interior sites

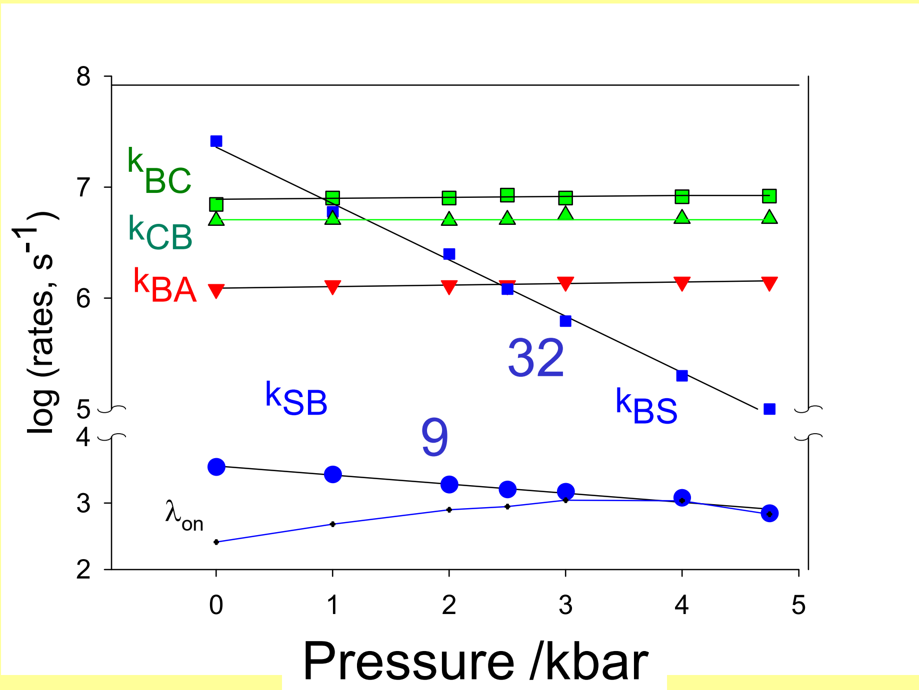
S ? B ? C_i

hν ?

A ligated

Triangles: experiment

Flash Photolysis at high pressure: Mb-CO



Microscopic rates

Solvent primary interior sites

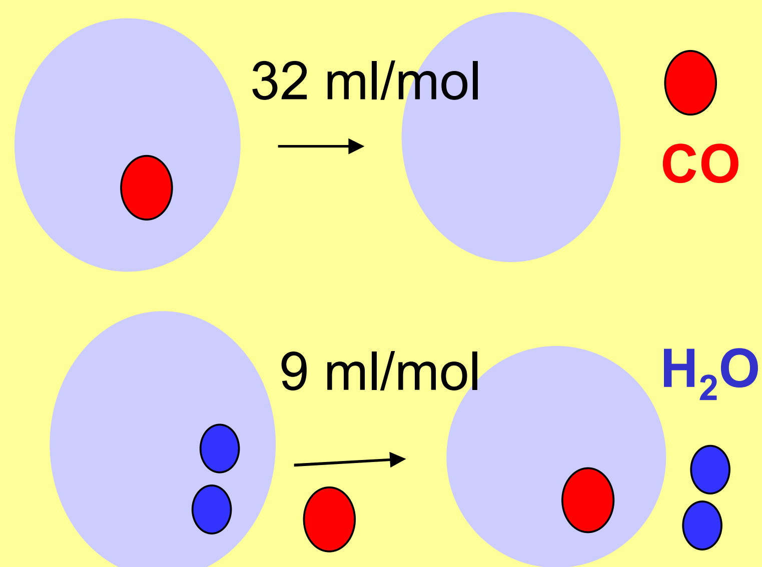
S ? B ? C_i

hν ↑ ?

A ligated

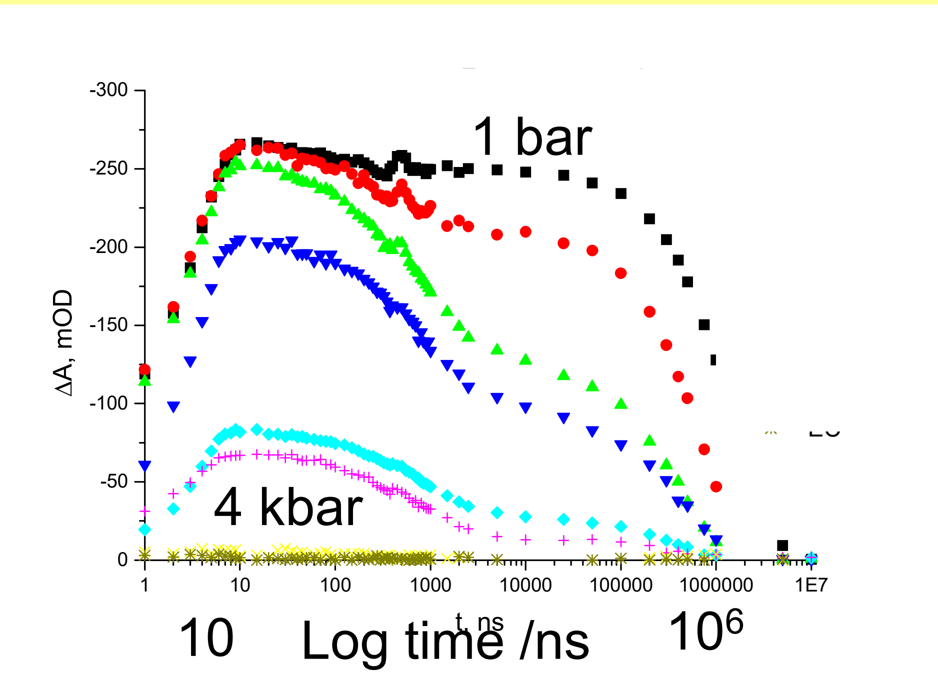
	B -> S	S-> B	B -> C	C -> B	B ->A
ΔV*	**32**	**9**	**0**	**0**	**0 ml/mol**

ΔV(CO) = 34 ml/mol ΔV(H_2O) = 18 ml/mol

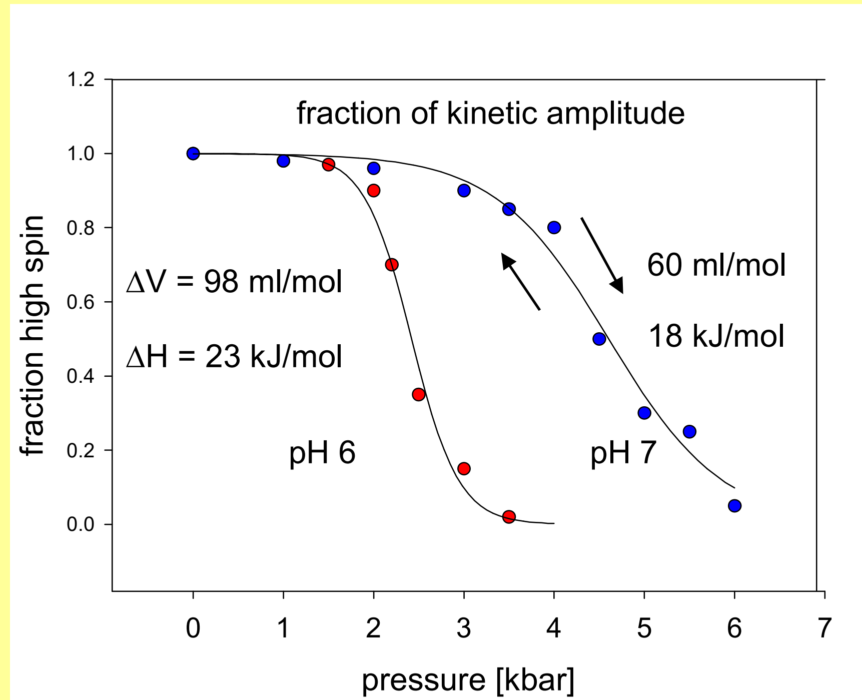


Effect of pressure on kinetic amplitude

Nano-second kinetics, **pH 6**



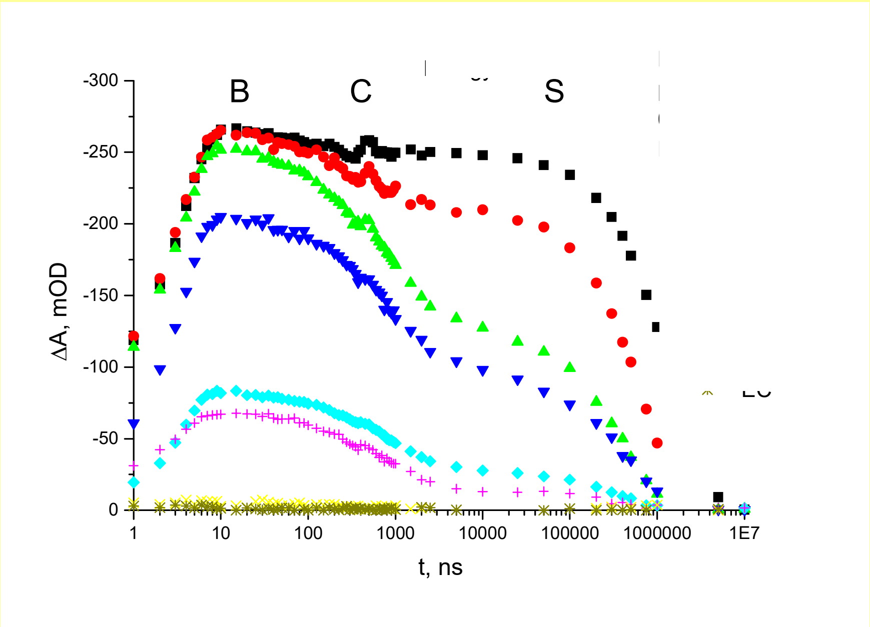
Kinetic amplitude

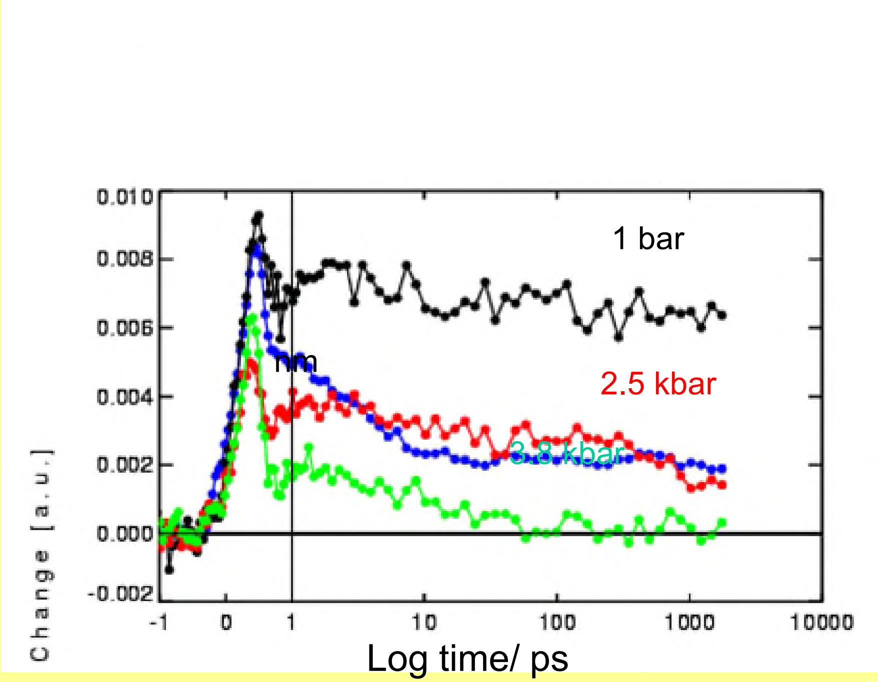


Kinetic amplitude decreases with pressure

Two-state model N/D
ΔV = 98 and 60 ml/ mol
unfolding

Ultra-fast ligand binding





pH 6

Nano-second kinetics
Amplitude decreases
aborve 2 kbar

pico-second
Kinetics:
Decreasing
amplitude

Loss in nano-second amplitude: ultra-fast rebinding

Conclusions

1) **bond formation:** Mb+CO -> Mb-CO
native structure: rate independent of pressure
Unfolding: ultra- fast rebinding (low spin state)

2) **Inter-cavity transitions** independent of pressure: B -> C, C-> B
protein incompressible, no change of water occupancy in cavities

3) **Protein-solvent interface:**
$\Delta V^*_{B\text{->}S} = +32$ ml/mol $\approx V_{CO.}$ CO- cavity created, ΔV_{prot} small !!
$\Delta V^*_{S\text{->}B} = +9$ ml/mol $\approx V_{CO} - V_{H2O,}$ 2 intra-molecular water molecules replaced
Association reaction is different from dissociation reaction!
Doster et al. Biochem. 37 (1998)

4) **Unfolding: 2-state system,** regular kinetics with loss in amplitude (reversible)
$\Delta V_{unf} = 60 - 100$ ml/mol

Collaborators

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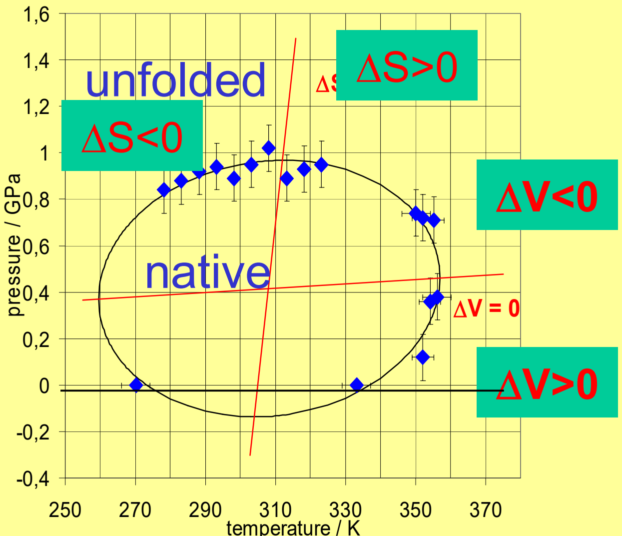
Thermodynamics of Unfolding
Phase diagram and unfolding volume

Why does a compact protein structure unfold under pressure?

Pressure



temperature

Cytochrome C
(**Lesch et al. Biophys.J. 2001**)

Le Chatelier Principle: $\Delta V < 0$

Expected: stabilisation of native state: **$\Delta V>0$**

$$d(\Delta\mu^{DN}) = -(\Delta S)\,dT + (\Delta V)\,dP = 0$$

$dP/dT = \Delta S / \Delta V$ Clausius-Claperon

ΔS, ΔV depend on temperature and pressure

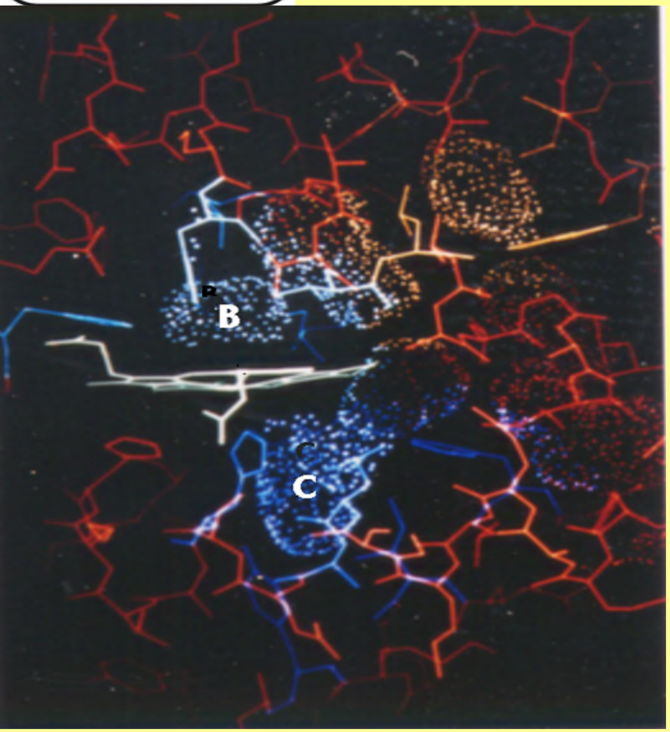
$$\Delta S = \Delta S_0 + \Delta c_P (T-T_0)/T$$

$$\Delta V(P,T) = \Delta V_0 - \Delta\beta \cdot P + \Delta\alpha \cdot (T - T_0)$$

compressibility thermal expansion

The Protein-Unfolding Volume Puzzle

Experiment: ΔV/V ≈ - 0.5 %



$$V = V(\text{atom}) + V(\text{cavities}) + \Delta V(\text{surface}) + \Delta V(\text{hydr})$$

ΔV_{DN}:   0 %   - 2 %   + 2.2 %   -2.5 %

Theory: ΔV/V ≈ -2.3 %

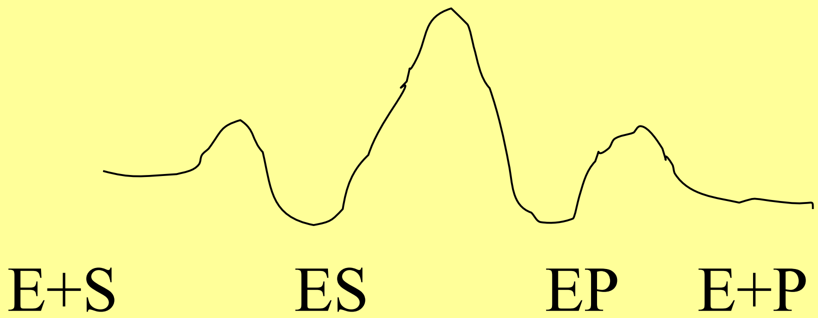
Cavities:

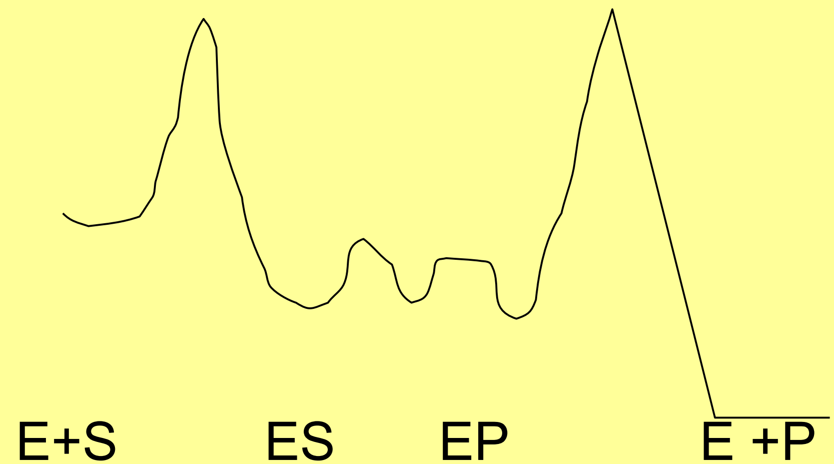
Myoglobin: V = 12 500 ml/mol,
Vcav = 260 ml/mol

	ΔV(theor)	ΔV(exp) ml/mol
Myoglobin	-260	-60 (Prehoda et al.)
Ribonuclease	-225	-35
Lysozyme	-237	-20

Kinetic volume changes:
activation volumes

Association-dissociation reactions



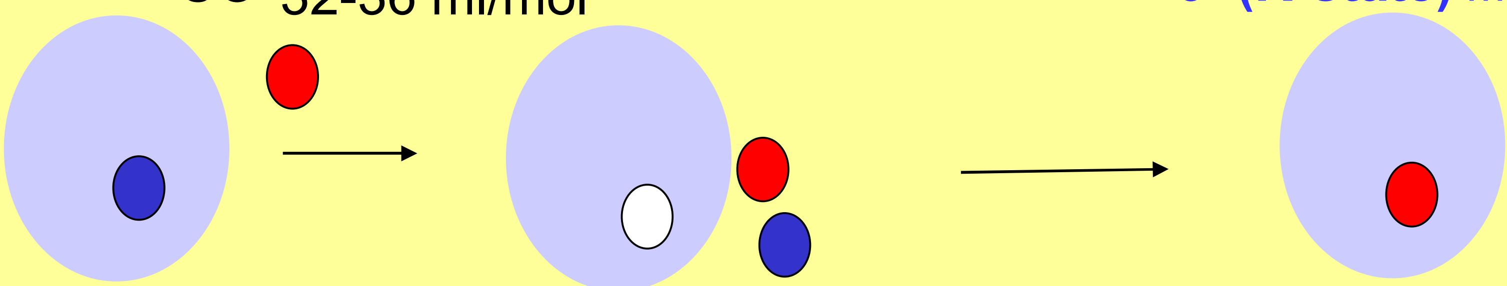


$\partial (\ln k_{ij}^*) / \partial P = -\Delta V_{ij}^*/RT$

$\Delta V^*_{ass}(Hb+CO)$ = - 32 ml/mol (T-state)

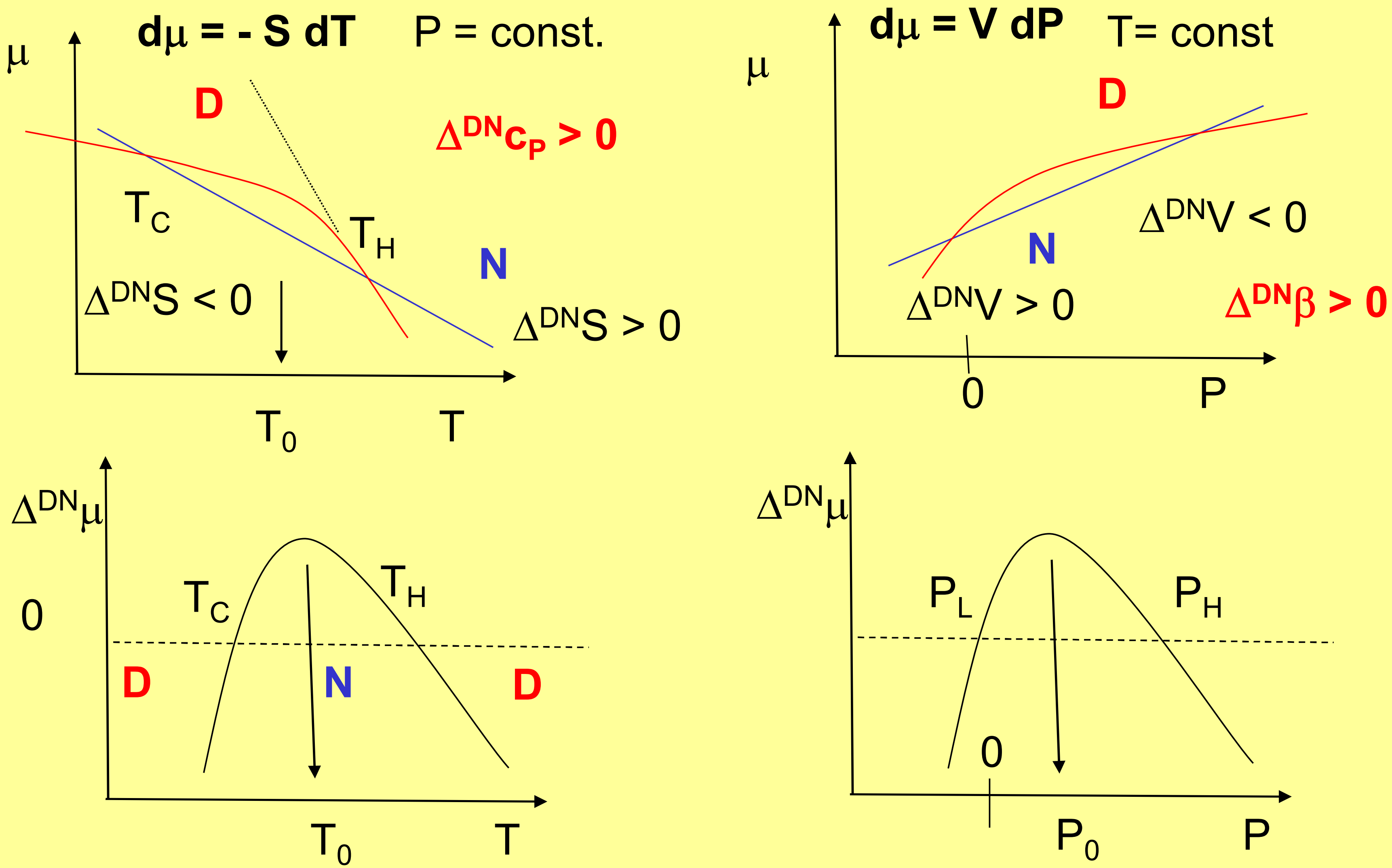
- 9 (R-state) Morishima 1996

CO 32-36 ml/mol

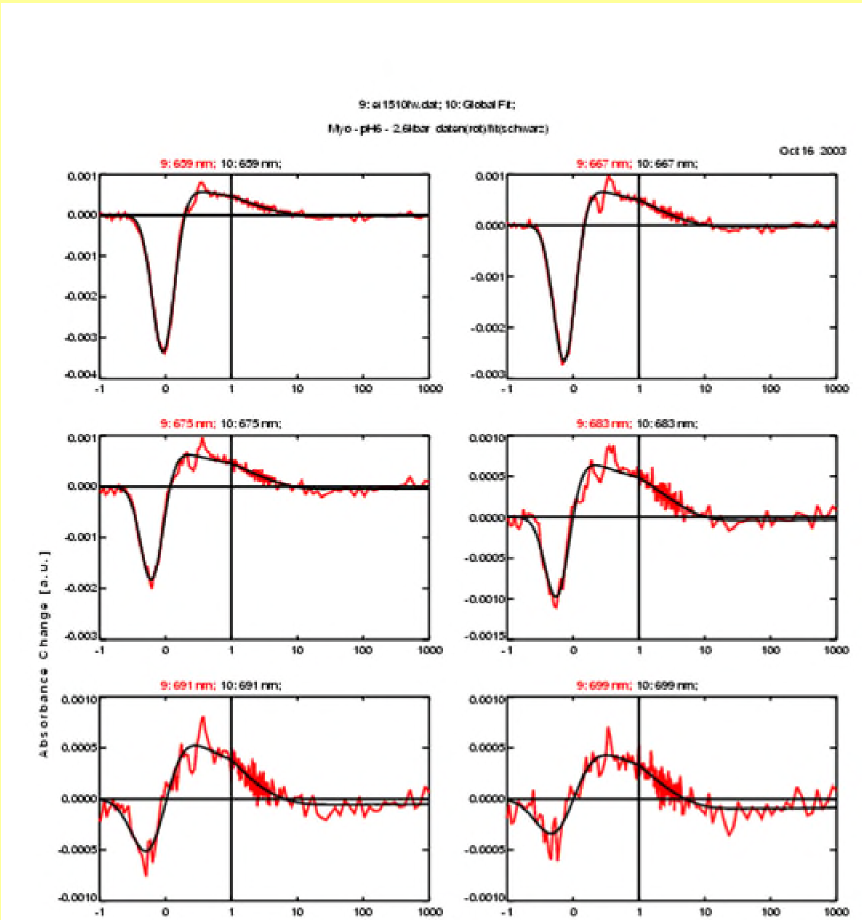


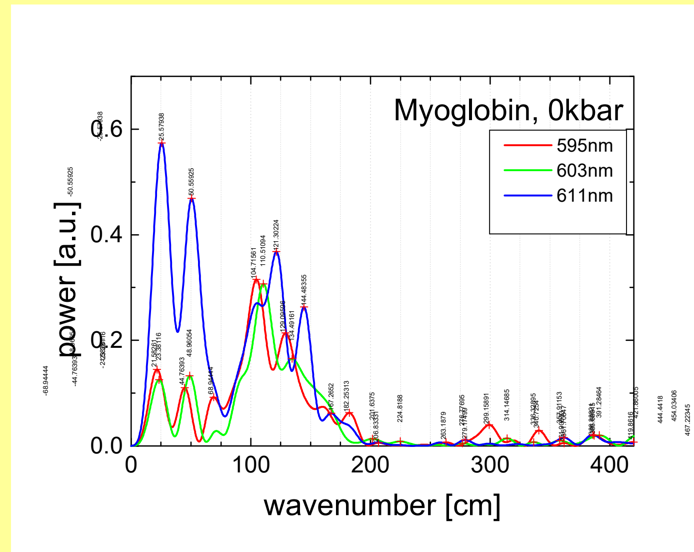
Protein
Chemical Potential

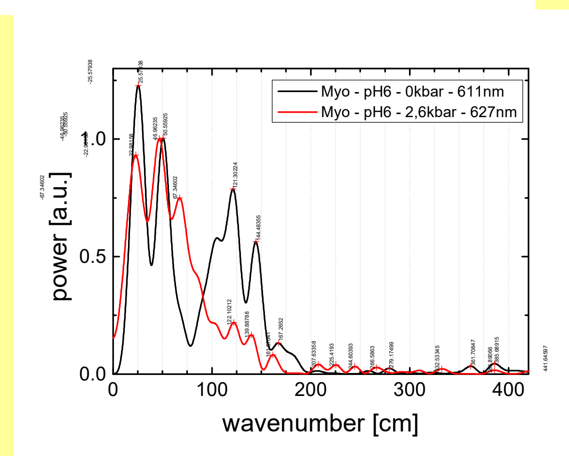
W. Doster and J. Friedrich in: Handbook of Protein Folding (1994):
Pressure-temperature phase diagram of proteins



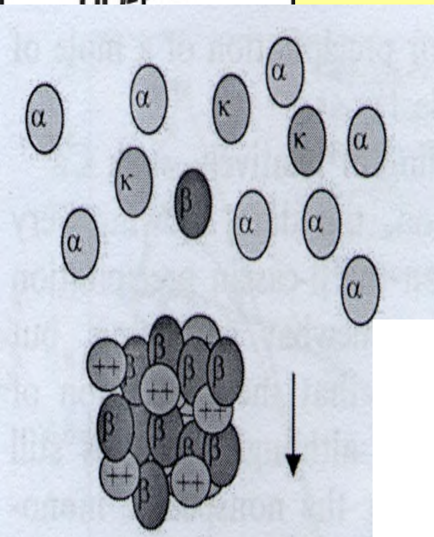
real time observation of low frequency vibrations



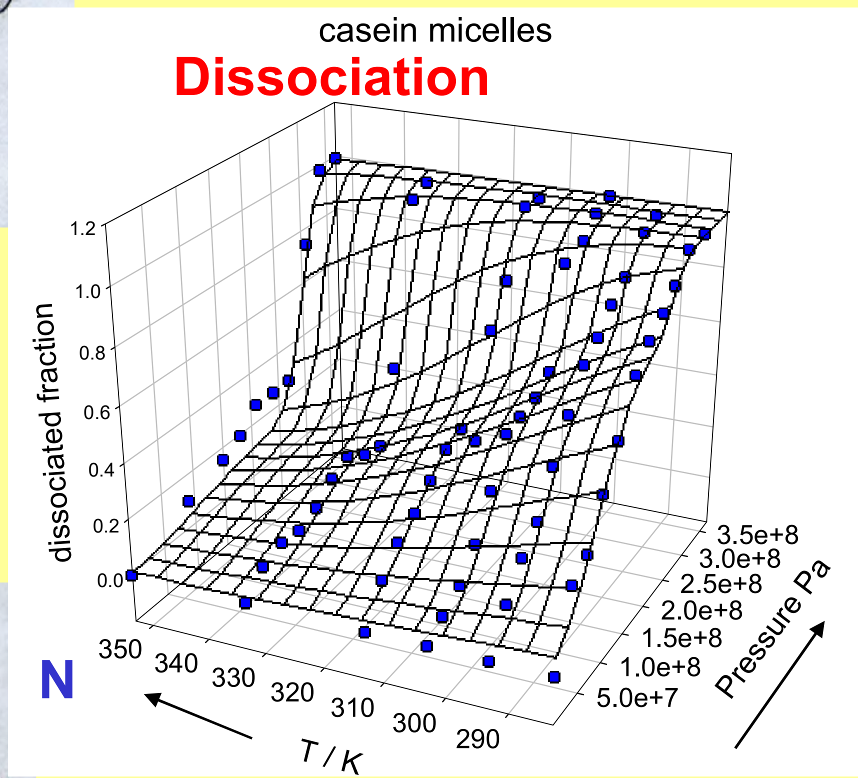


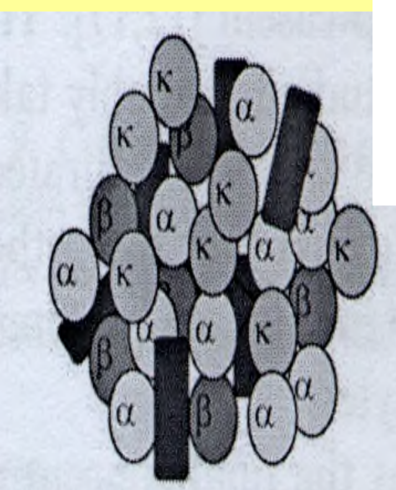


Dissociation of Protein Complexes



$$\Delta^{DN}\mu = \Delta^{DN}\mu_0 - \Delta^{DN}S\,(T-T_0) + \Delta^{DN}V\,(P-P_0)$$





$$\partial(\ln K_D)/\partial P = -\Delta V^{DN}/RT$$

$$\partial(\ln K_D)/\partial T = \Delta S^{DN}/RT$$

ΔV = 220 ml/mol

$V(H_2O)$ = 18 ml/mol

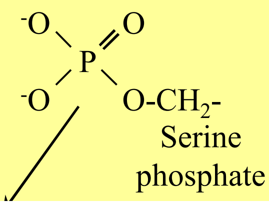
Hydrophobic Solvation
10 water molecules

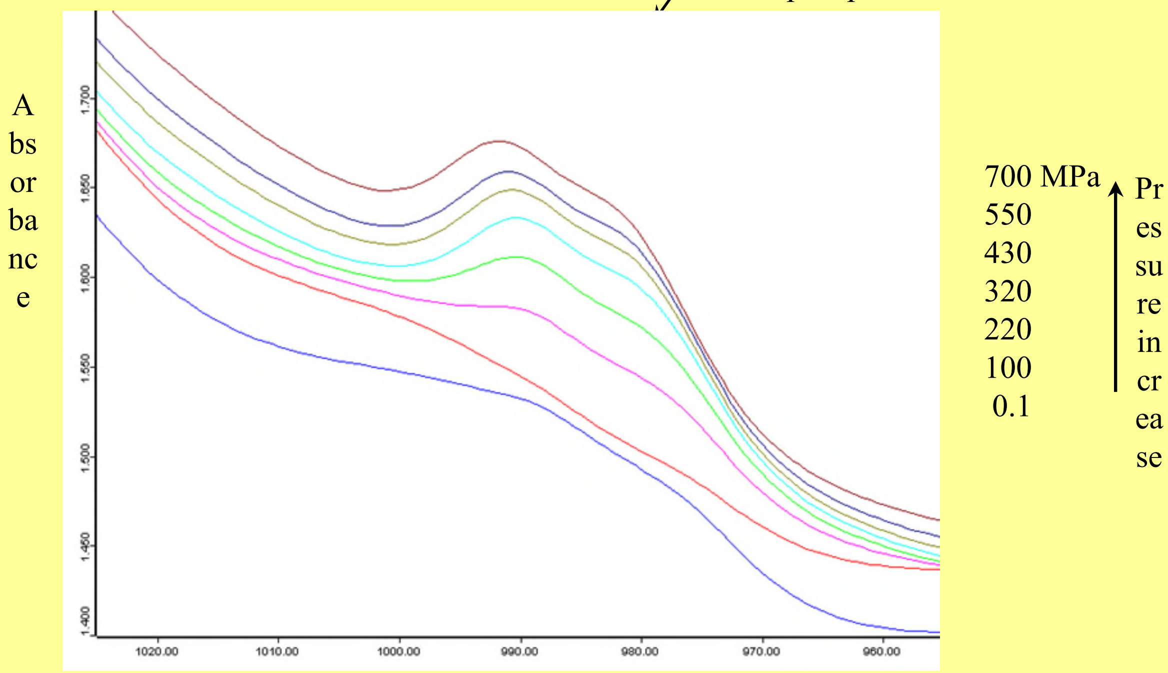
2 Posters: Casein/Hemocyanin (R. Gebhardt)

Pressure-induced dissociation of charges

FTIR- Spectrum of Ca-Casein in diamond-anvil cell

N. Takeda





Ca-PO$_4$-Ser - > Ca^{2+} + PO$_4^{2-}$ Ser

pressure