Putting the Squeeze on Biology: Biomolecules Under Pressure

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A Word on Lamp Posts…

You have had too much to drink and need to drive home.

Question: How can you quickly get sober?

Answer: Quickly pressurize your car to a few hundred atmospheres.

(1 atmosphere = 1 bar = 10^5 Pa)

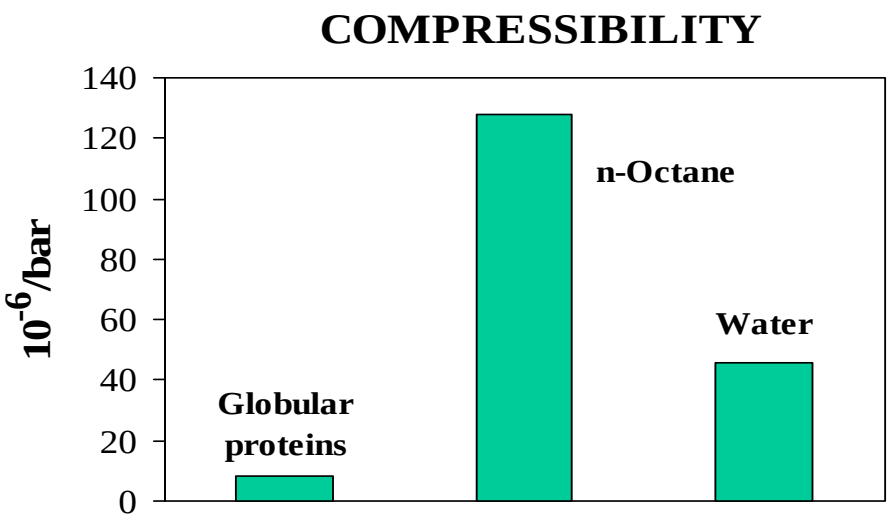
Pressure Affects Many Biomolecular Systems

- Pressure unfolding of proteins (e.g., Bridgman – 1914)
- Multimer association/disassociation
- Changes in ligand binding
- Altered membrane ion transduction
- Changes in transcription of nucleic acids
- Large shifts in chemical kinetic constants
- Changes in conformational states
- Greatly decreased viral infectivity

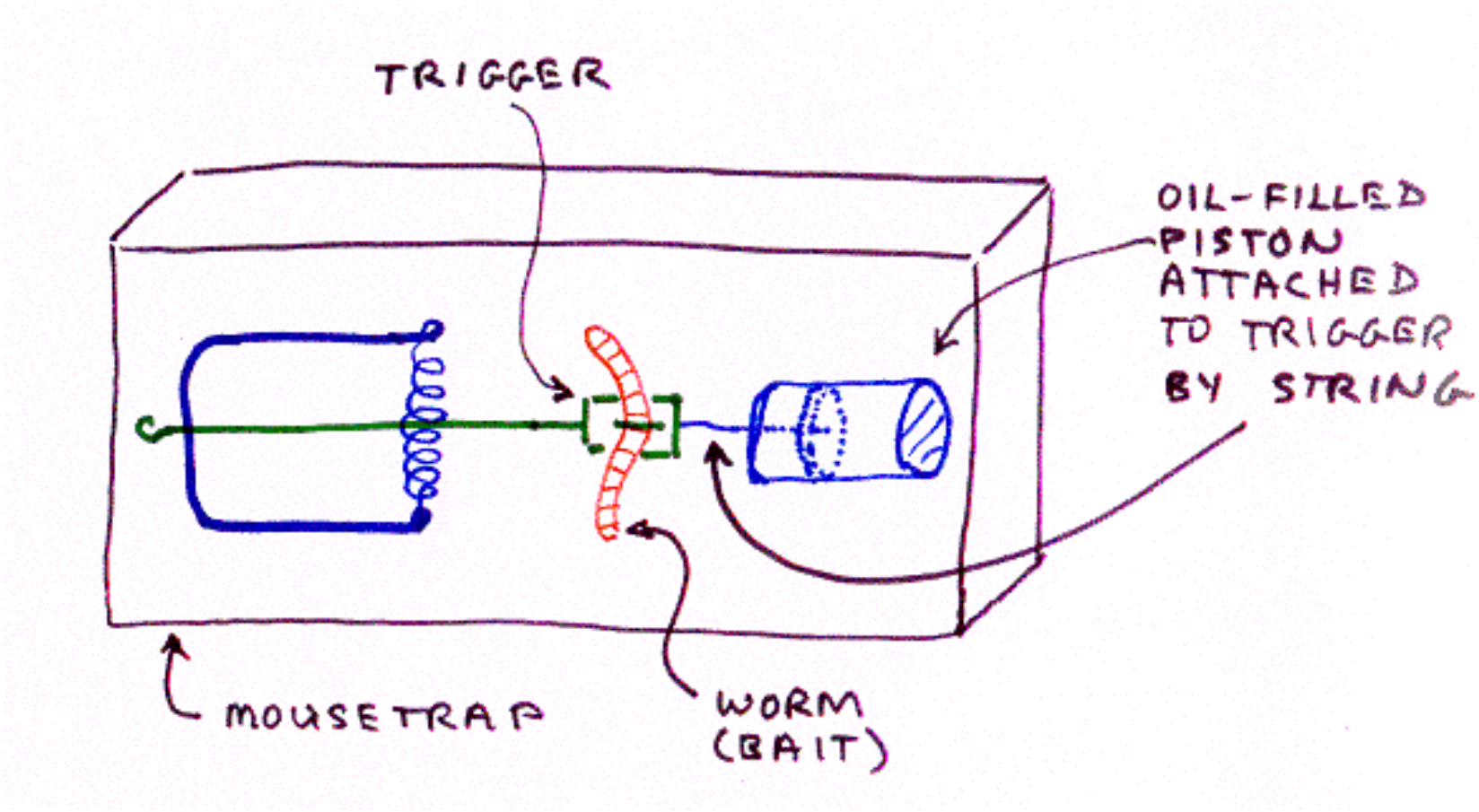
Moreover, many of these effects occur at modest high pressures, such as those encountered in the biosphere.

- **HIGH PRESSURE in the biosphere is <u>not</u> exceptional. In fact, in may ways, it is the norm.**
  - **62% of the biosphere volume is above 100 bar.**
  - **Deepest ocean trenches reach > 1200 bar.**
  - **Pressures and volumes are even more dramatic if bacterial organisms in crustal sediments are included.**

Yet typical biomaterials have low compressibilities. So why do so many effects occur at modest pressures?



FISH TRAPzyme



Pressure Characterization of FISH TRAPzyme

1. $\Delta V/\Delta P$ = very small
2. TRAPzyme seems to exhibit drastic conformational change with modest pressure.
3. $\Delta S/\Delta P$ may be either positive or negative (depending on TRAPzyme materials).
4. …

Does a classic thermodynamic approach fully explain how TRAPzyme works?

No!! Why? Because TRAPzyme is made up of many "parts".

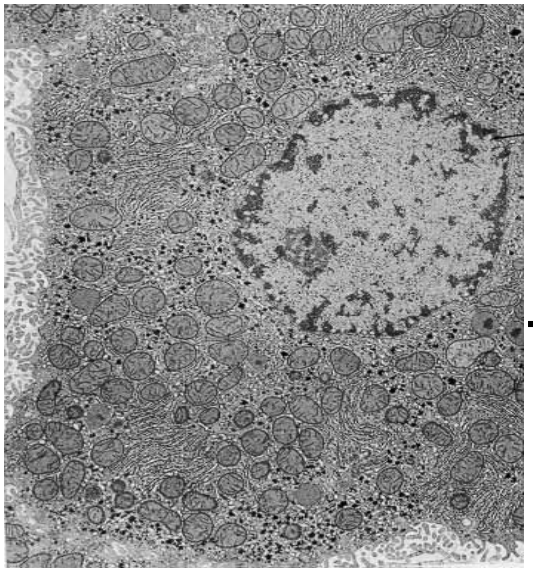
It is first necessary to understand how the parts interconnect and work together, and then to understand the effect of pressure on each part.

In other words, we need structural information.

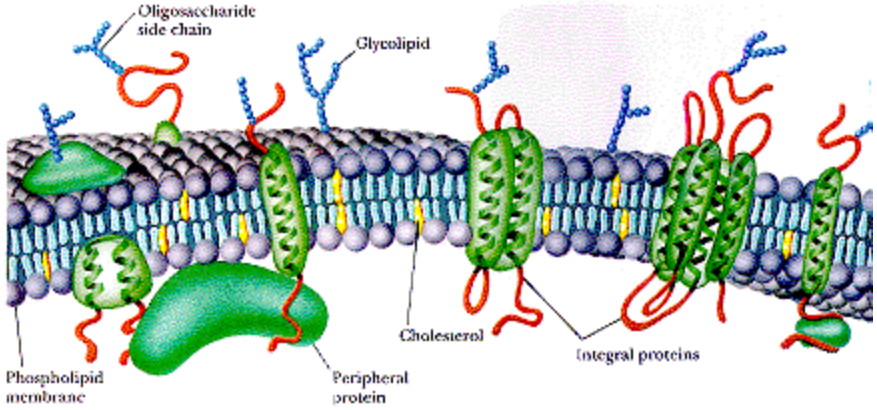
Talk Outline

1. Lipid membrane mesophases
2. Proteins

Membranes make up ~1/3 the dry mass of many eukaryotic cells, the remainder being largely aqueous proteins and carbohydrates. Of the membranes, very roughly half the mass is lipid, half protein and various polymers.

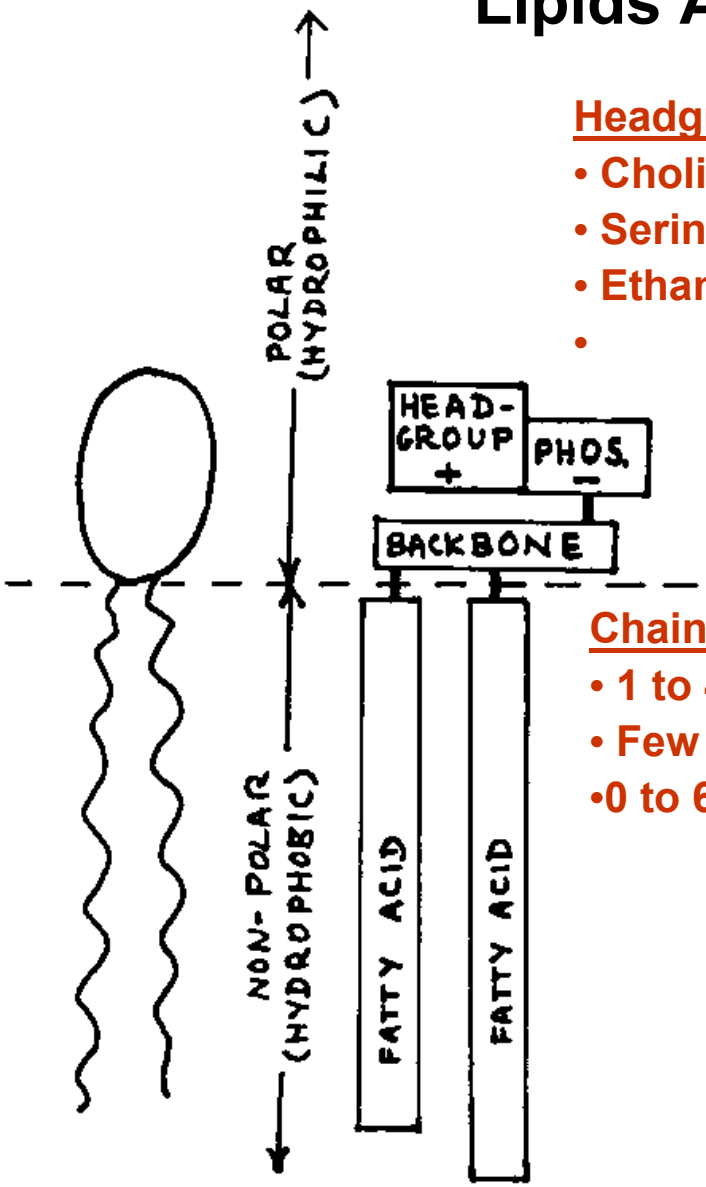


liver cell (fr: Lehninger, Biochem.)



fr: Garrett & Grisham, Biochem.

Lipids Are Tinker Toys



Headgroup

- Choline
- Serine
- Ethanolamine
-

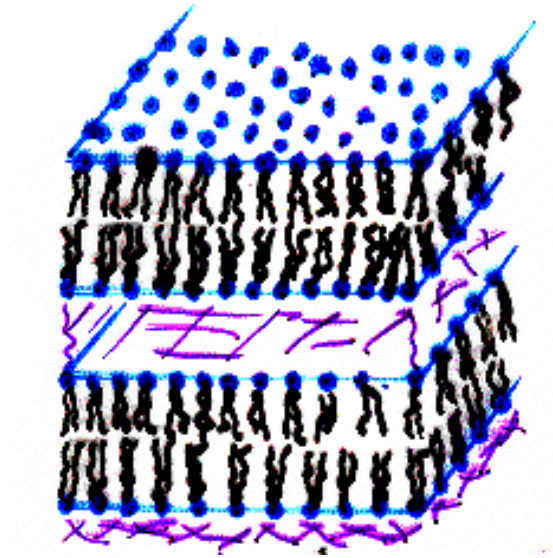
Chains

- 1 to 4 chains
- Few to 24 carbons
- 0 to 6 unsaturated (double) bonds

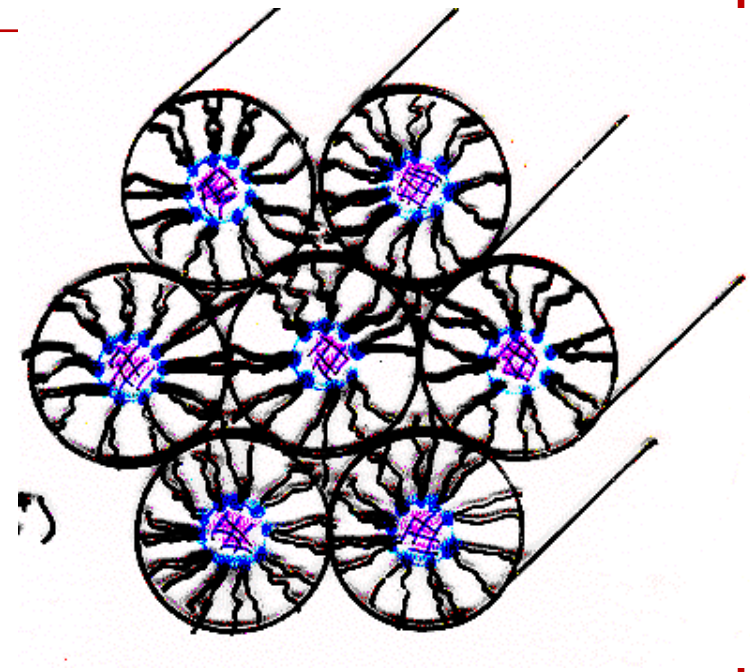
Many water-lipid phases are observed.

H_{II} inverted hexagonal phase

Lα lamellar phase



Raise temperature



KEY

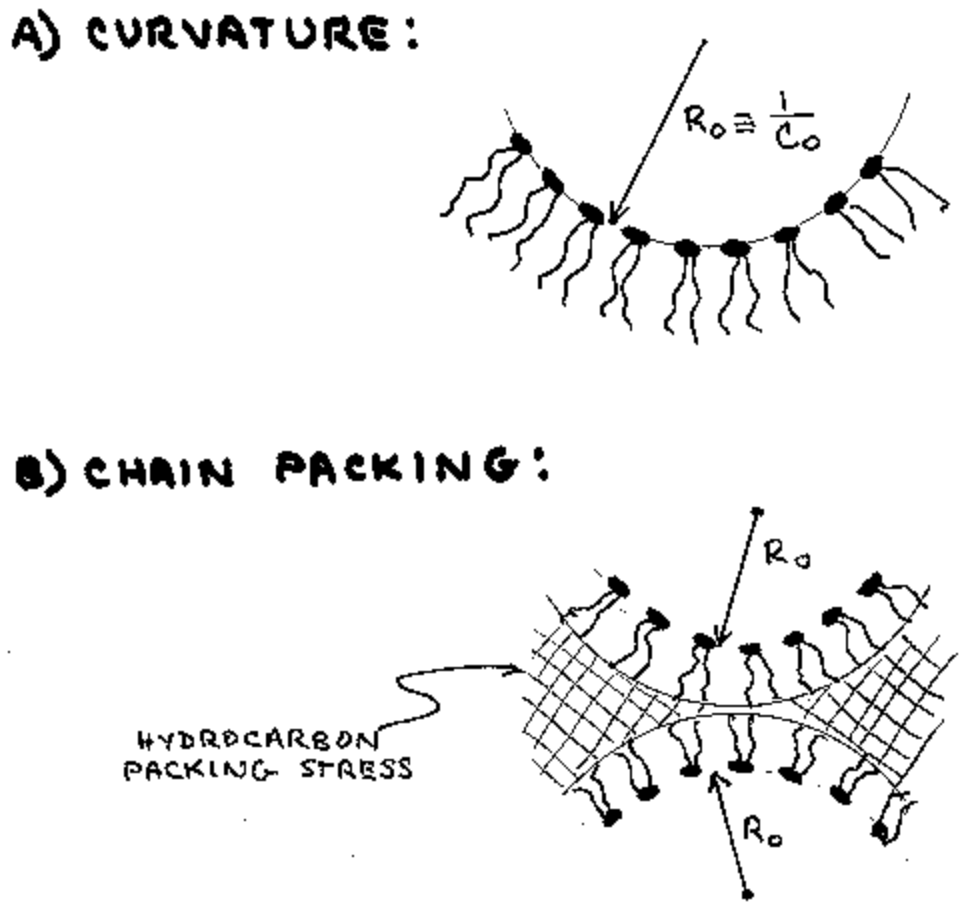
Headgroup

Chains

Lipid

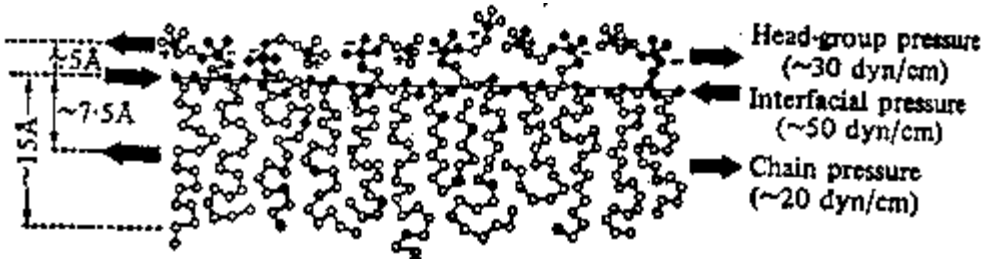
Water

Competition between monolayer curvature and hydrocarbon chain packing dominates the lipid phase behavior.



See: Kirk et al., *Biochem*. 23 (1984) 1093; Gruner, *J. Phys. Chem.* 93 (1989) 7562.

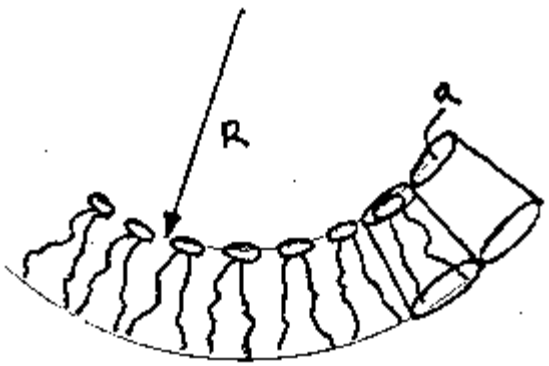
WATER SIDE



OILY SIDE

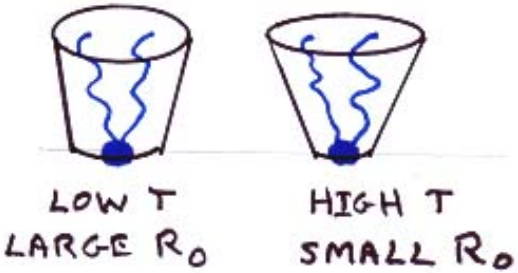
Israelachvili et al., *Quart. Rev. Biophys.* 13 (1980) 121.

Net lateral pressure = 0, but not always for a flat surface.



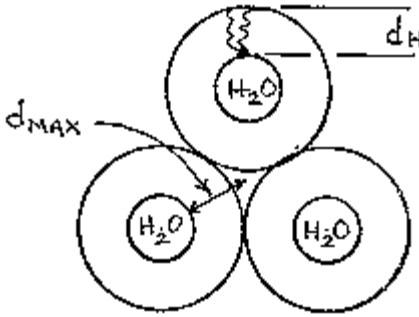
$$\mu_{elastic} = (k/2)(1/R - 1/R_0)^2 + \text{const.}$$

Note: Curvature, C, and radius of curvature, R, are reciprocals of one another: $C = 1/R$. So, $\mu_{elastic} = (k/2)(C - C_0)^2 + \text{const.}$

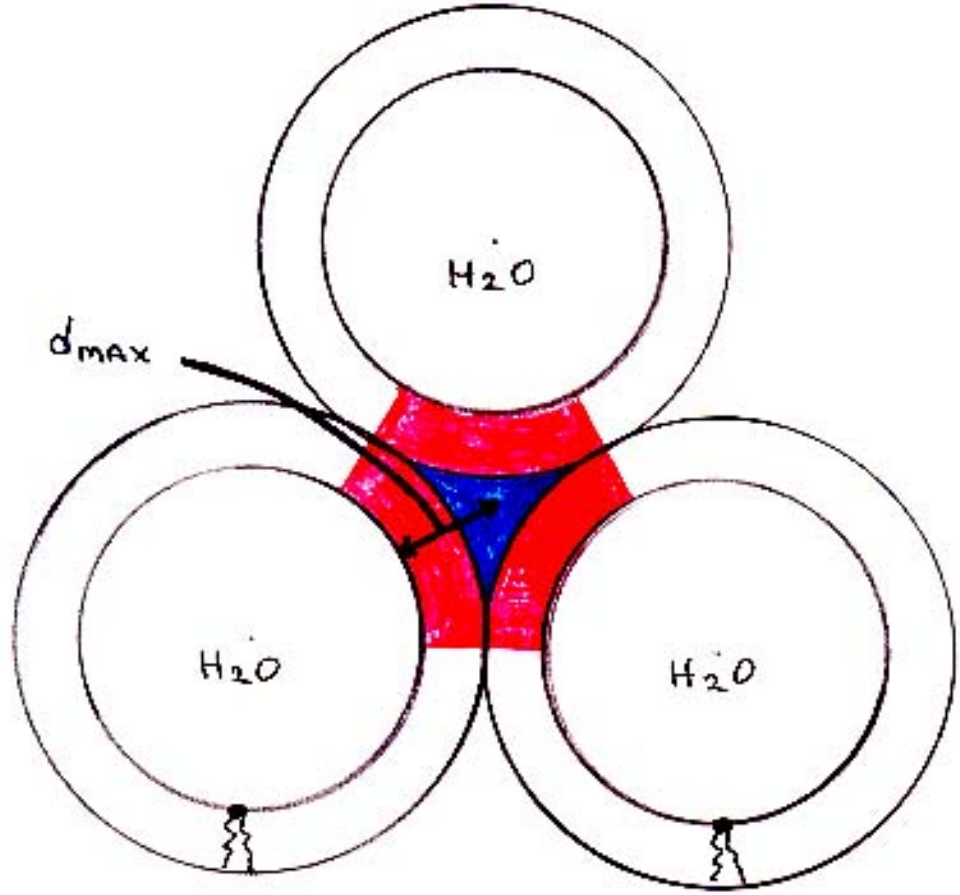


$d_{MAX} \sim R_{water}$

rel. vol. stretched $\sim R_{water}$



T increasing

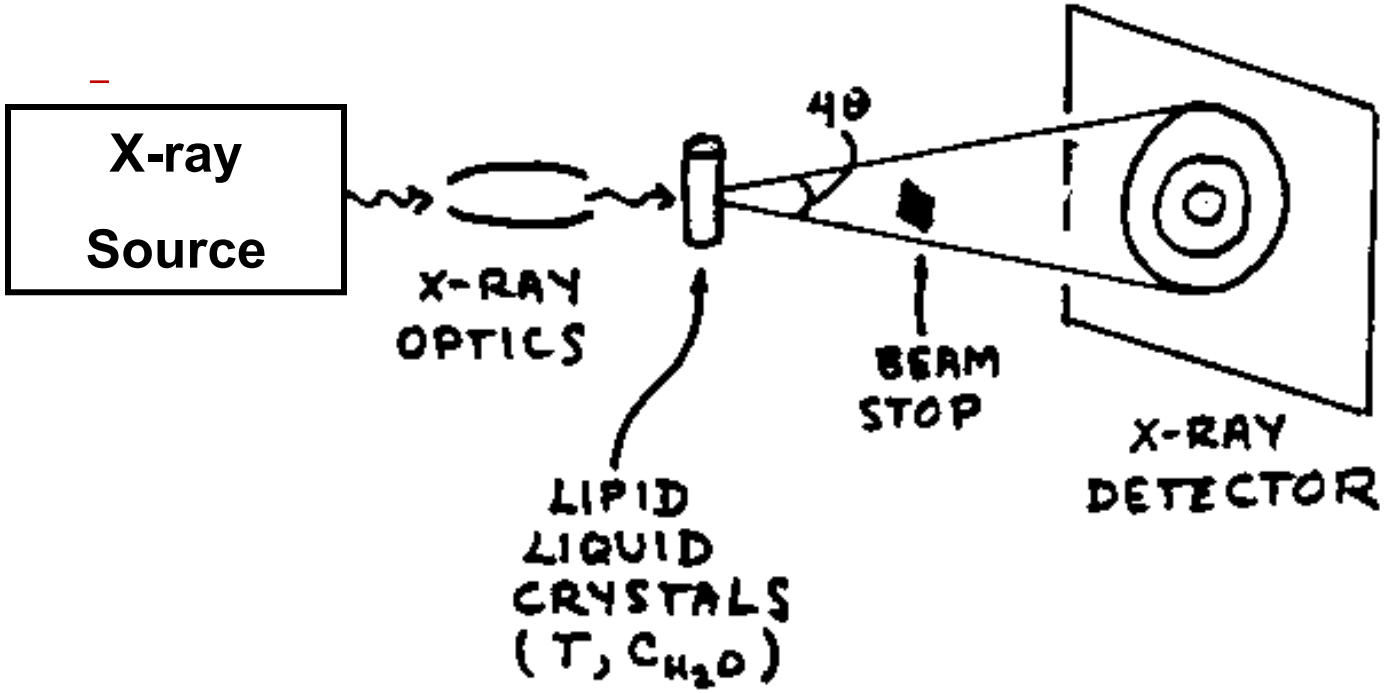


Summary

- L_α – H_{II} transition dominated by competition between monolayer spontaneous curvature & chain packing.
- Larger H_{II} tubes shift equilibrium towards L_α.
- Monolayer spontaneous curvature dominated by chain disorder. Disorder increases with temperature.
  - ►Higher temp. = smaller tubes
  - ►More stable H_{II} phase.

Gruner, Parsegian, Rand, *Faraday Discuss. Chem. Soc.* 81 (1986) 29.

Rand, Fuller, Gruner, Parsegian, *Biochem.* 29 (1990) 76.

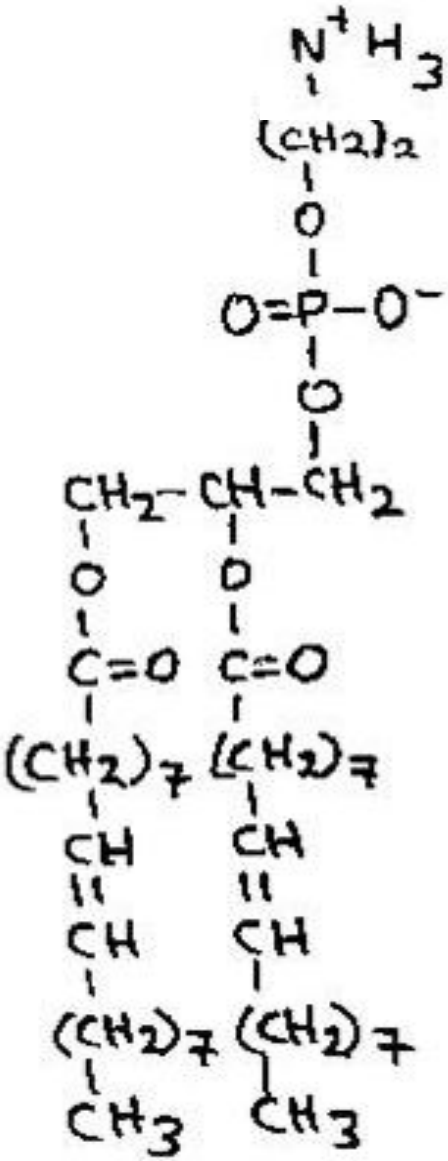


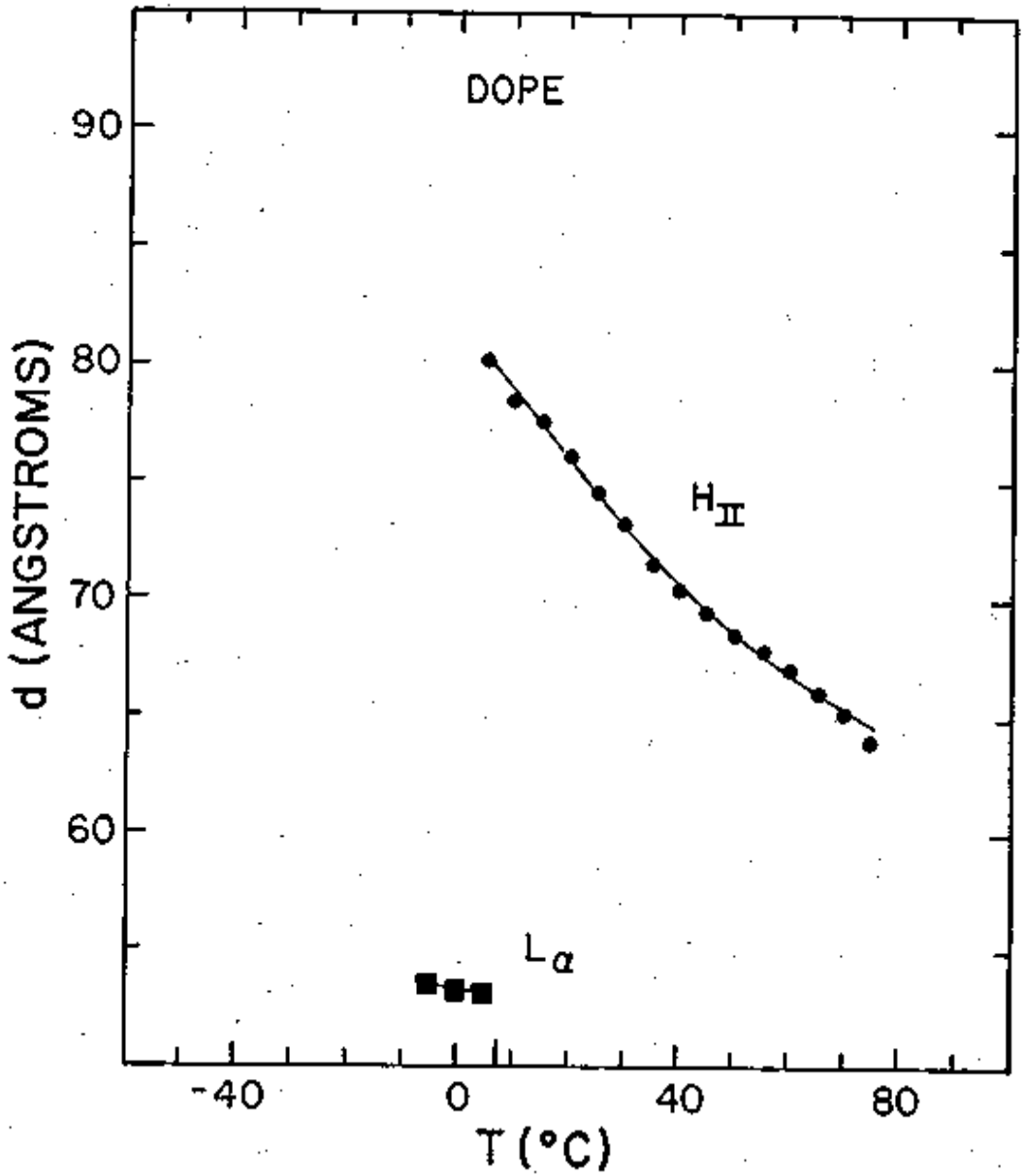
$$k = \frac{2\sin\theta}{\lambda} = \frac{n}{d}$$

FOR $d \sim 100\,\text{Å}$, $n = 1$, $\lambda = 1.54\,\text{Å}$

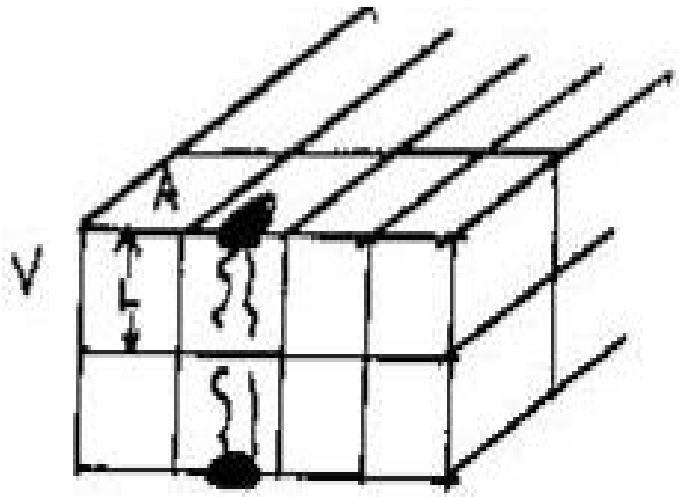
$\theta \sim 0.4°$

DIFFRACTING POWER $\lesssim 10^{-4}$





Kirk & Gruner, *J. Physique* 46 (1985) 761.



As Temp rises,

L shrinks slightly,

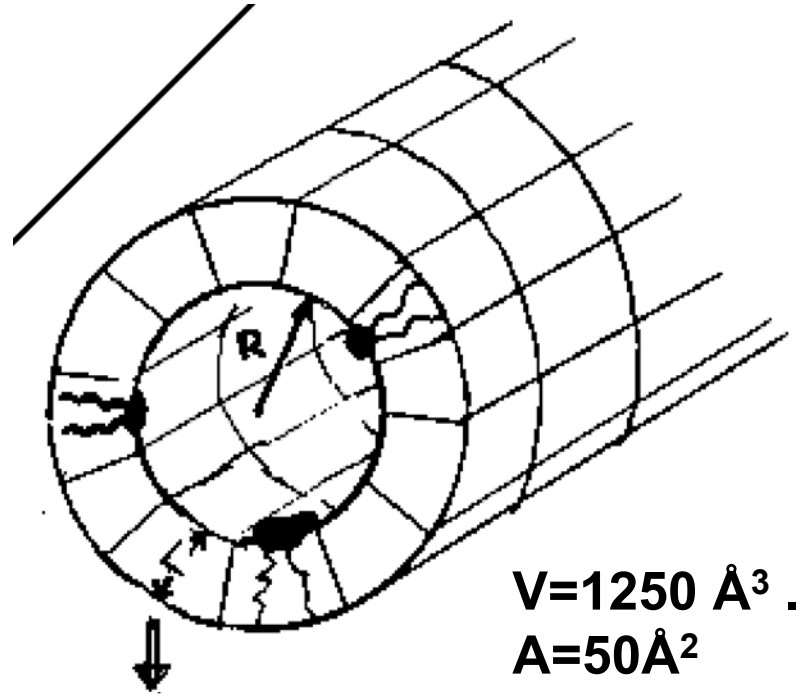
V ≈ const.

V=1250 Å^3

L=20.0 Å → A=62.5 Å^2

L=20.2 Å → A=61.9 Å^2

Δd = 0.2 Å

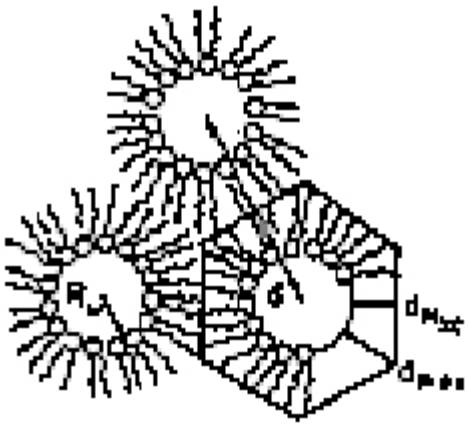


V=1250 Å^3 .
A=50Å^2

L=20.0 Å → R=40.0 Å

L=20.2 Å → R=42.5 Å

Δd = 5 Å !!



H_{II}

$$d = 2(d_{H_{II}} + R_W)$$

$$R_W = \frac{\left[\left(\pi\beta - 2d_{H_{II}}\sqrt{3}\right) - \left\{\left(\pi\beta - 2d_{H_{II}}\sqrt{3}\right)^2 - 2\sqrt{3}\left(2\sqrt{3} - \pi\right)d_{H_{II}}{}^2\right\}\right]^{1/2}}{\left(2\sqrt{3} - \pi\right)}$$

$$\beta = v/a = 25.6 \text{ Å} \quad \text{(DOPE)}$$

$$d_{H_{II}}\left(\text{Å}\right) = 16.44(\pm 0.15) - 0.0139(\pm 0.0013) \text{ x } T(°\text{C})$$

Tate & Gruner, *Biochem.*, 28 (1989) 4245

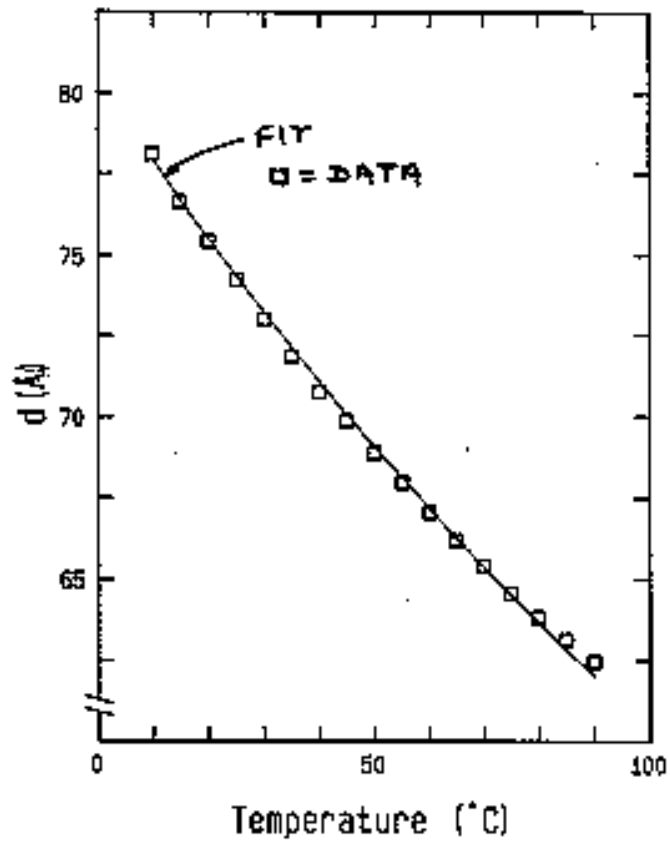
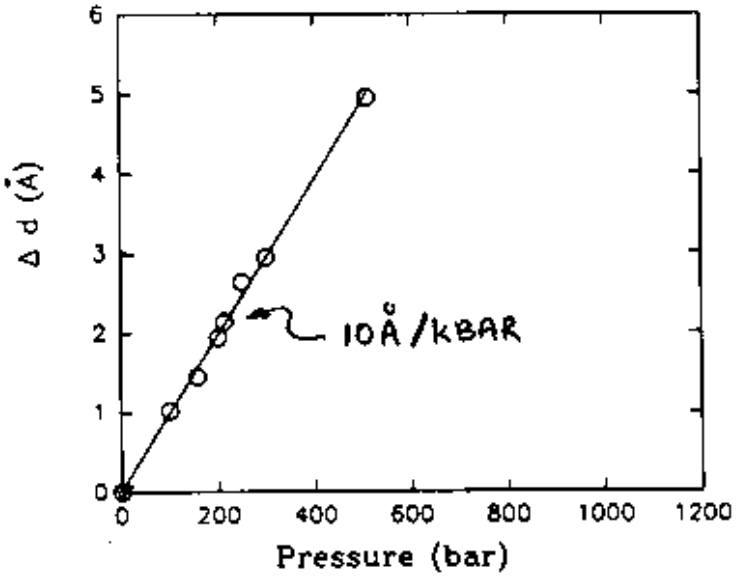


FIGURE 6: Temperature dependence of a fully hydrated H_{II} phase in the DOPE–water system. The solid curve represents spacings calculated by assuming $d_{H_{II}}$ has a linear dependence on temperature and v/a is constant, and equal to the average of the measured values.

Given the temperature dependence, what can we predict about pressure dependence?

DOPE + excess water

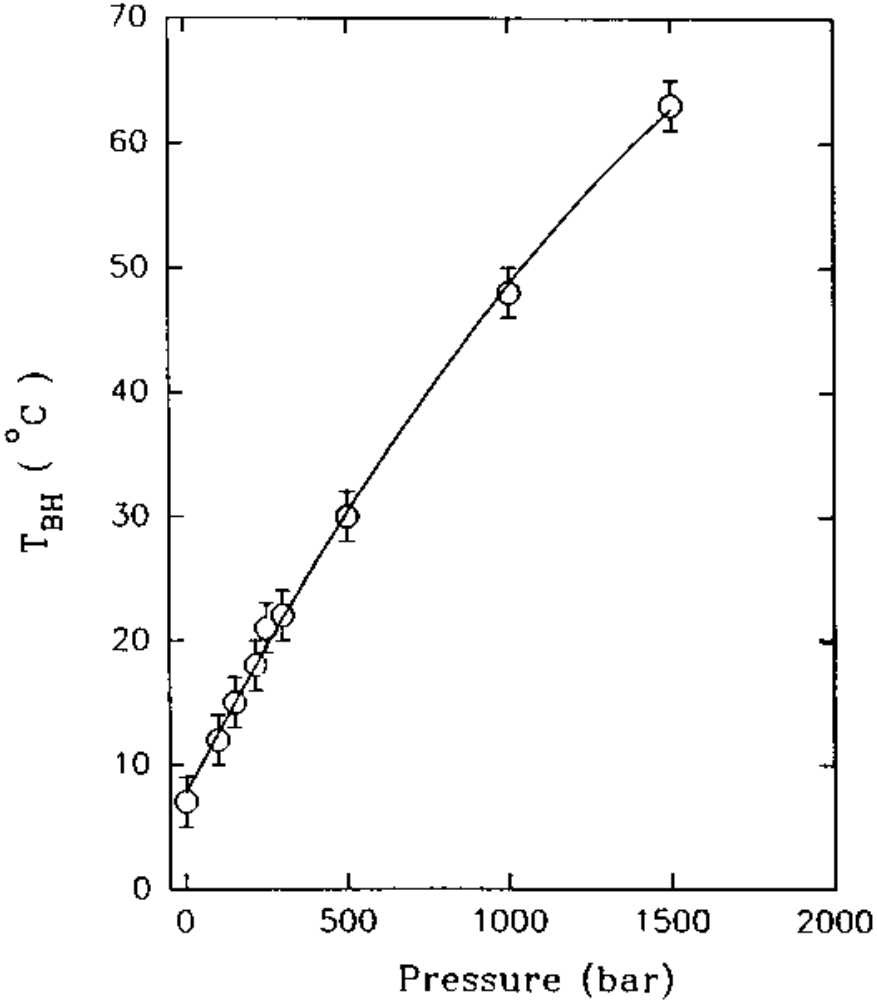
H_{II} BASIS VECTOR LENGTH, d, vs. PRESSURE



$$\left.\frac{\partial l_{H_{II}}}{\partial P}\right|_T \approx +0.3\ \text{Å}/kbar$$

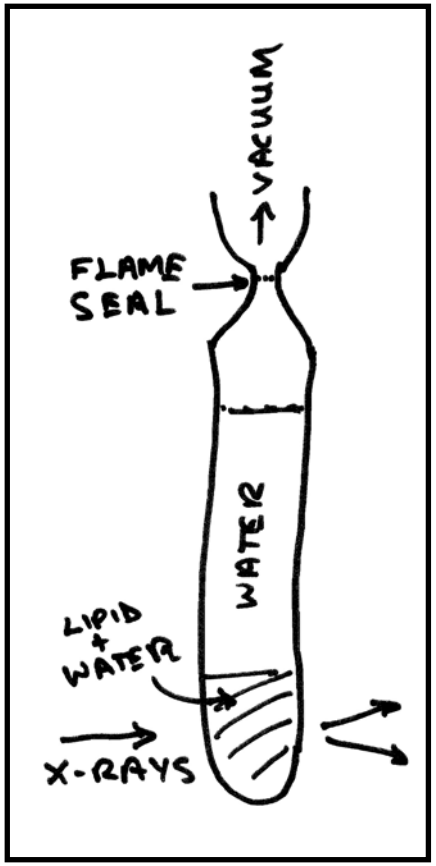
$$\left.\frac{\partial A_{H_{II}}}{\partial P}\right|_T \approx -1.3\ \text{Å}^2/kbar$$

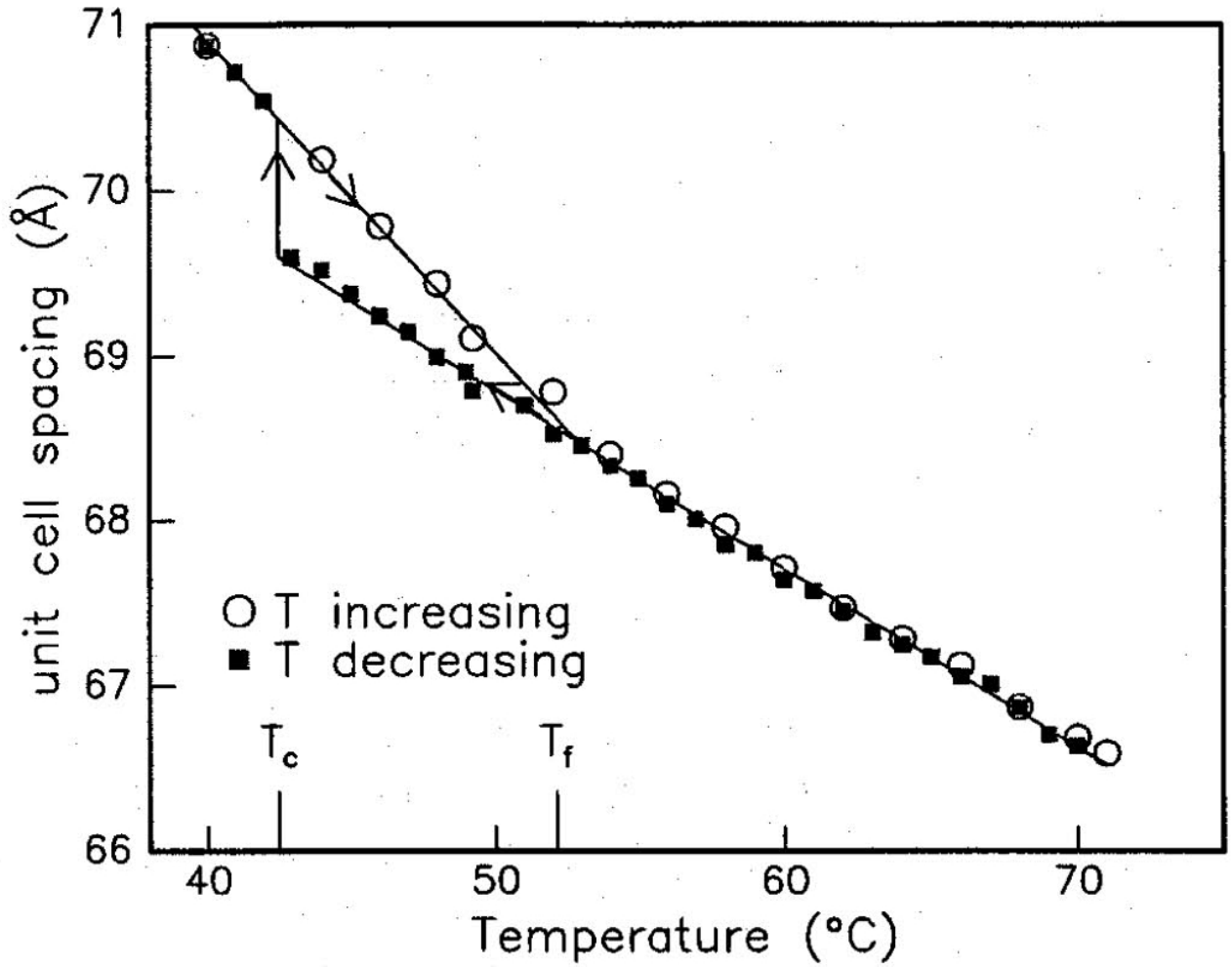
$$\left.\frac{1}{V}\frac{\partial V}{\partial P}\right|_T \approx +0.04/kbar$$



1. P. So, Ph.D. thesis, Phys. Dept., Princeton Univ.
2. So et al., *Phys.Rev. Lett*, 70 (1993) 3455

Works with Negative Pressure too!





What have we learned so far?

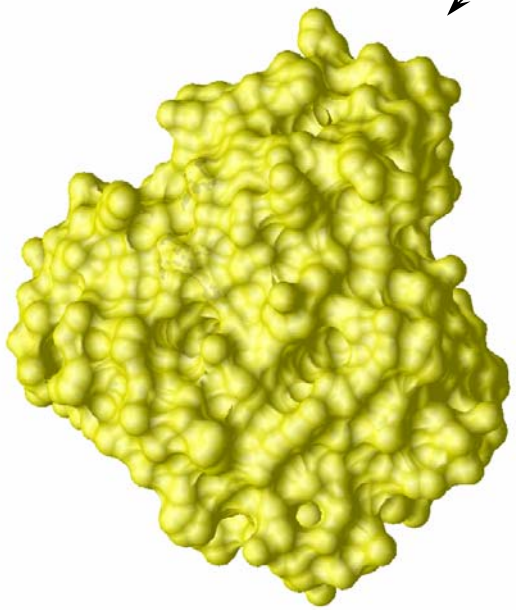
- **Macromolecules have "parts" (Lipid: Heads, chains, water)**
- **Parts are subject to many interactions (e.g., H-bonds, bond rotation, charge)**
- **Interactions are pressure sensitive (*cis* to *trans* rotamers)**
- **Alternative language: Macromolecules have many conformations. The ensemble of conformations present depends on pressure.**

What about proteins?

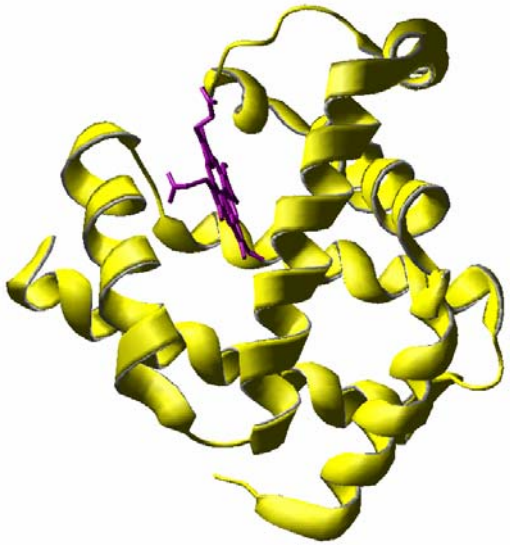
- **Proteins also have "parts" (α-helices, β-sheets, water, tertiary and quaternary domains) and ensembles of conformations.**
- **Protein function depends on conformations, which depends on relative position of residues and larger parts.**

Structural Information is Important

- **To understand pressure effects, you need STRUCTURAL INFORMATION**



Beneath the molecular surface, there is a complex internal structure



Structure of Proteins Under Pressure

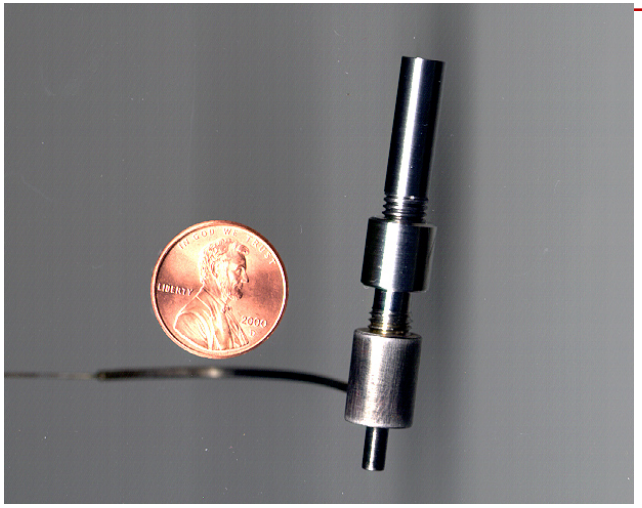
Literature has many hundreds of papers on pressure effects. <u>To understand these we need structural data.</u>

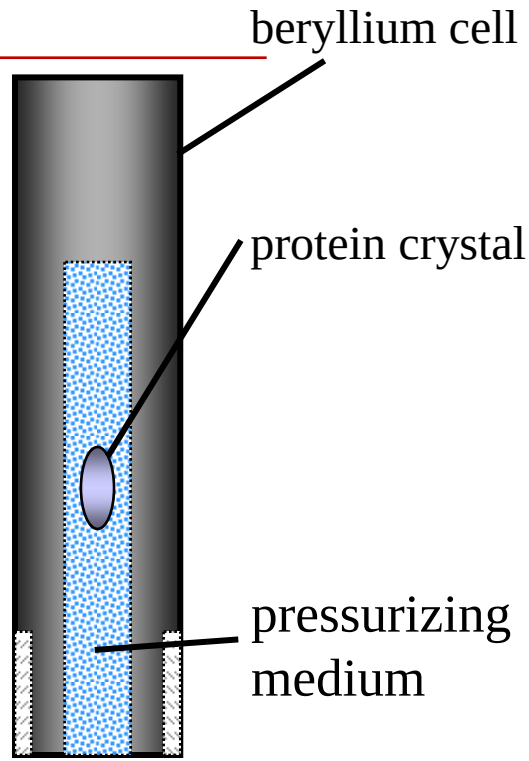
By 2002, there were many thousands of protein structures. How many at hi pressure (≥ kbar)?

Answer: One

Pioneering work was done by Kundrot & Richards

(*J. MOL. BIOL*. 193 (1987) 157)

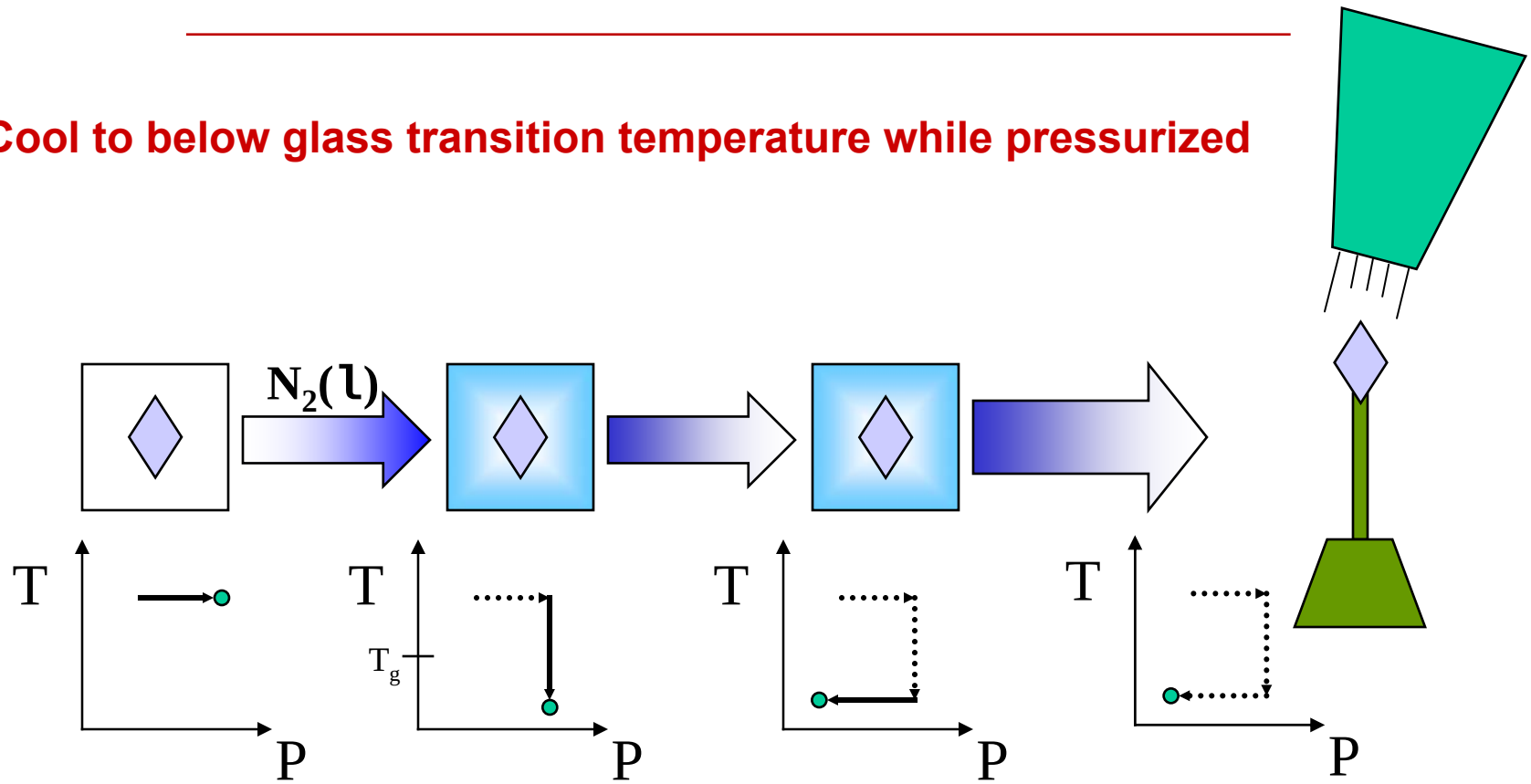




- HEN EGG WHITE LYSOZYME @ 1 KBAR.
- ISOTHERM. COMP.= 4.7×10^{-3}/kBAR^{-1}
- CONTRACTION IS NONUNIFORM
- B-FACTORS CHANGE NONUNIFORM.
- a & b AXES CONTRACT 0.6%; c EXPANDS 0.1%
- OVERALL STRUCTURE CHANGES LITTLE

High Pressure Cooling Method

Cool to below glass transition temperature while pressurized

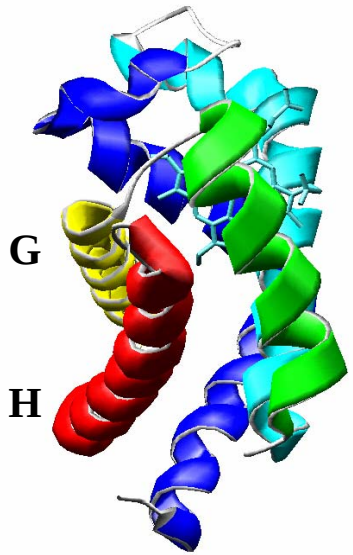


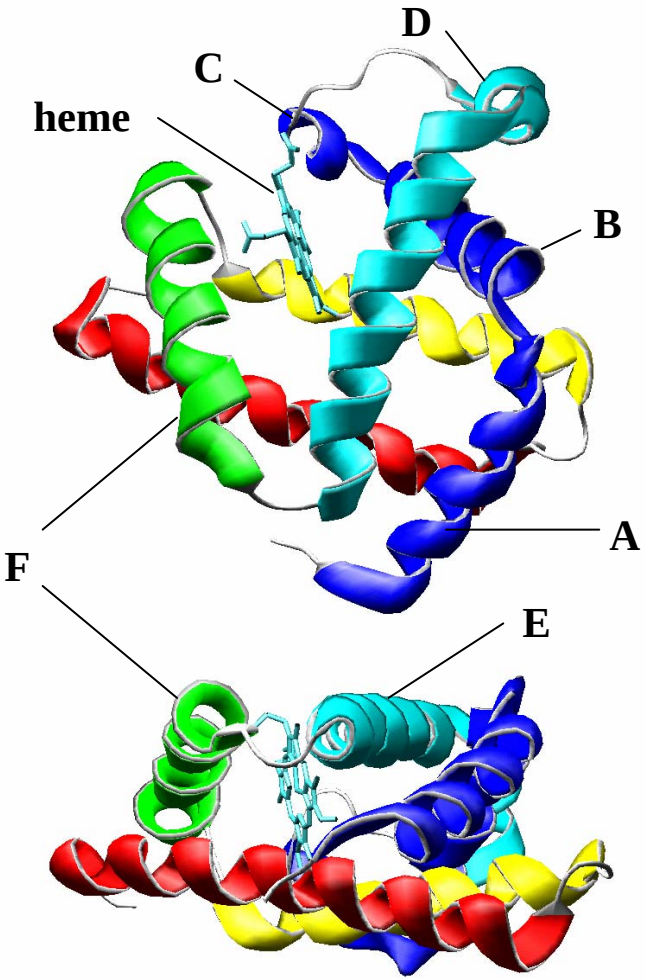
Modified from Thomanek et al. *Acta. Cryst.* A29 (1973) 263.

Story #1: Citrine

Note: Two slides describing pressure effects on Citrine have been removed pending publication.

Story #2: Sperm Whale Myoglobin



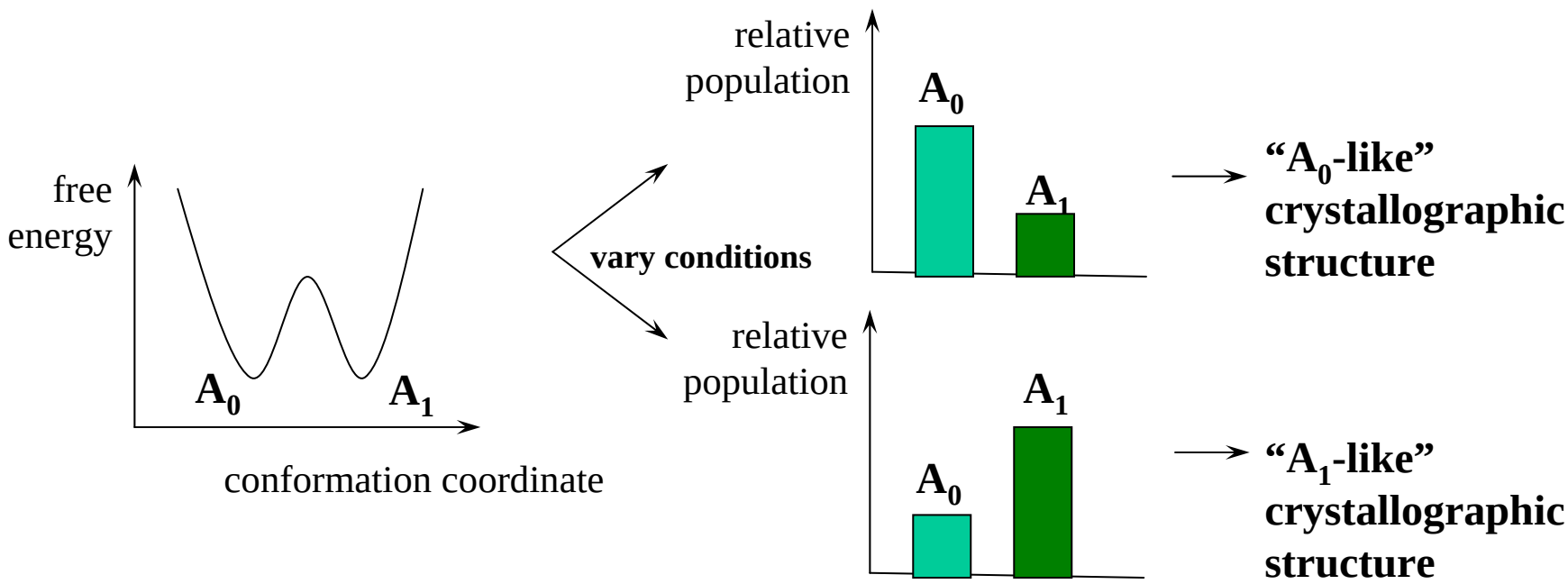


- 153 amino acid monomers, or residues
- Linear polymer folds into 8 alpha helices joined by loops
- Heme group, an iron tetracoordinated to a porphyrin ring
- O_2 transport in muscle; NO, CO regulation

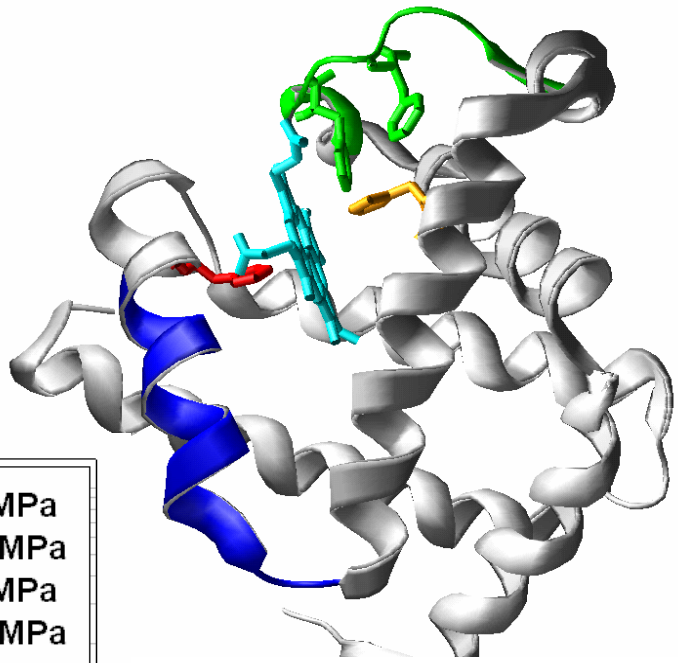
Urayama, Phillips & Gruner, Structure **10** (2002) 51.

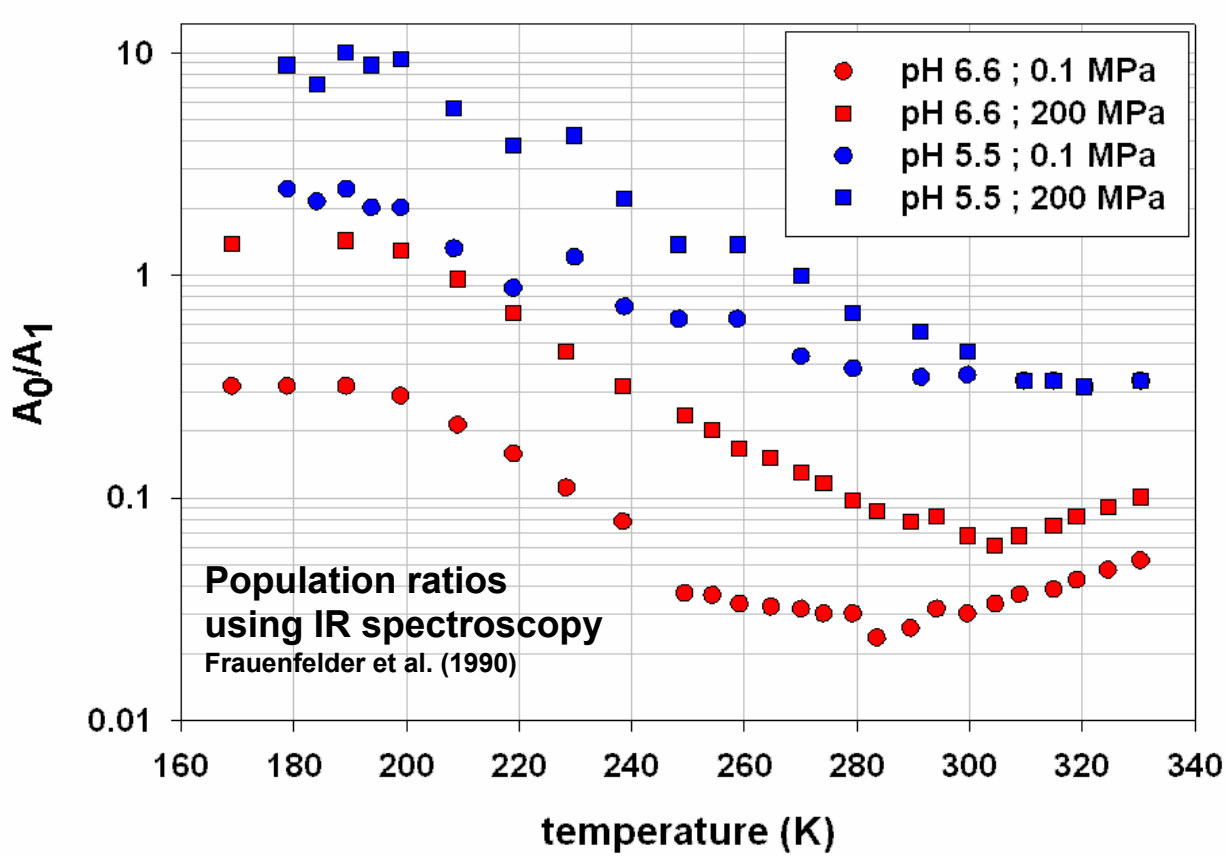
Conformational Substates

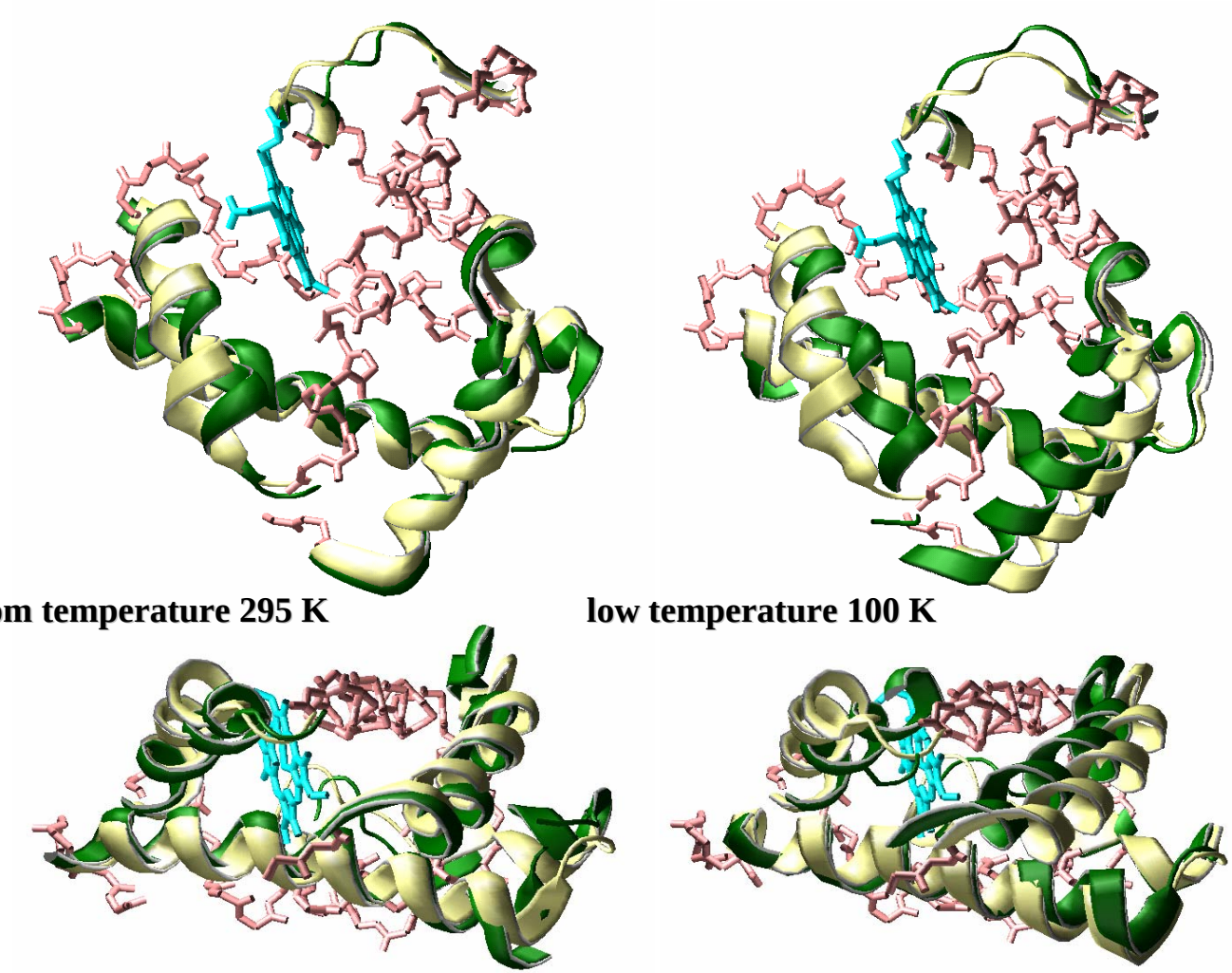
- A_0 and A_1 substates found by IR spectroscopy of stretch modes of bound CO
- Crystal structure is ensemble average of protein population



- **Heme pocket well characterized**
  - state of distal histidine
  - A_0 = "open" conformation
  - A_1 = "closed" conformation
- **Population ratios from IR spectroscopy**

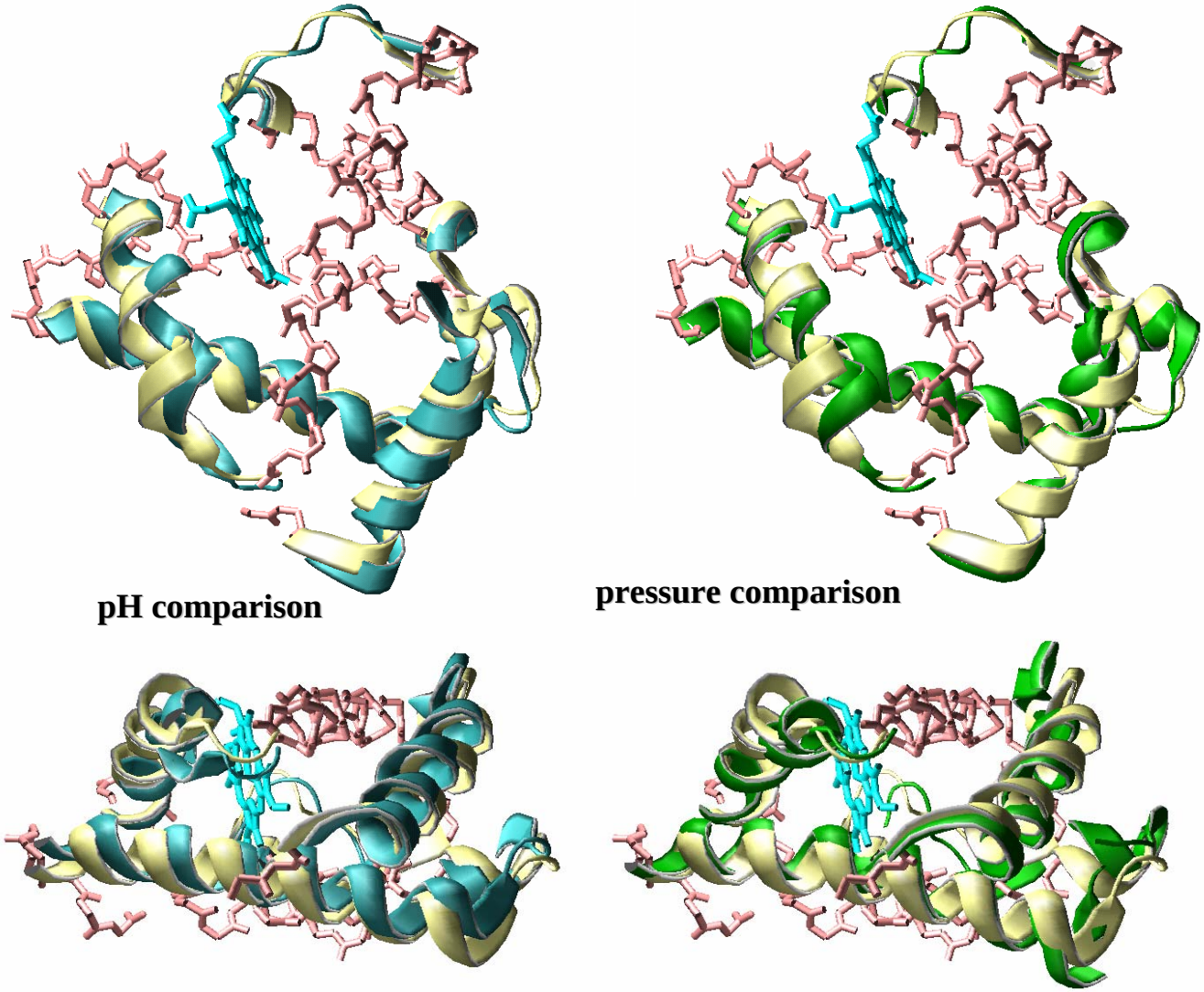






yellow ribbon = ambient pressure position
green ribbon = high pressure position (x 15)

Major displacements show similarities

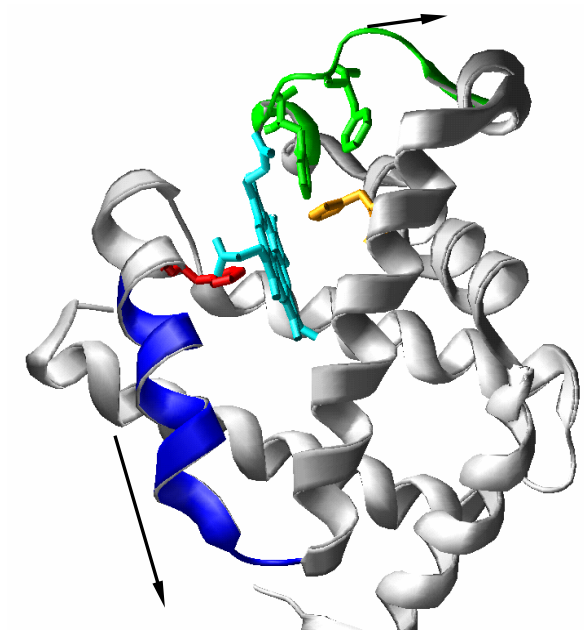


yellow ribbon = ambient pressure, pH 6 position
green ribbon = high pressure position (x 15)
cyan ribbon = low pH position (x 6.2)

pH structures solved by Yang and Phillips (1996)

Story #2 Conclusions

1. Lo pH, Hi P, Lo T all lead to similar structural changes in myoglobin.
2. These all correlate with favoring A_0 substate relative to A_1.
3. "A" substates represent global conformational substates.



Summary

1. Proteins truly are “nanomachines” built of interlinked parts.
2. Movement of the parts can be achieved by pressure, pH, temperature, etc.
3. Movements couple to function.
4. Pressure effects, as well as pH and Temp effects can be understood in a unified way.

Acknowledgements

I've been fortunate to enjoy working with many students, colleagues and post-docs. In the pressure area, I thank:

- Peter So (now @ M.I.T.)
- Shyamsunder Erramilli (now Boston @ Univ.)
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END