High-Pressure Biology: Protein Crystallography

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Lecture #2

- **Pressure affects many biomolecues.**
- **Examine pressure effects on protein structure.**

Lecture #1 Lessons

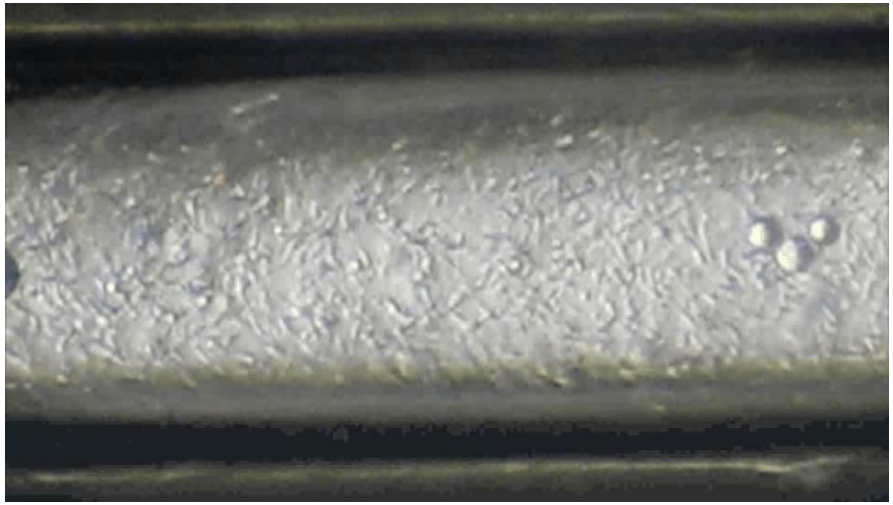
1. There is a paradigm shift going on about life on Earth.
2. Much, if not most of Earth's biomass exists in deep hi-P and often hi-T biomes that were thought to be sterile only a few decades ago.
3. We know little about these biomes, but it is clear that they require revision of much of what be thought we knew about life on Earth.
4. Existence of these biomes raises many profound questions:

Grand Challenge Questions

- **Did life start in Hell, deep down at Hi-P, Hi-T?**
- **When did life start?**
- **How long is a microorganism viable? How long before it reproduces?**
- **What are pressure and temperature limits of life?**
- **Does non-DNA life co-exist on Earth?**
- **How do these extremophiles differ from surface organisms?**
- **What are the biophysics of biomolecules under Hi-P?**

Lecture #1: Example pressure reversal of anaesthesia & alcohol action

(1 atmosphere = 1 bar = 10^5 Pa = 10^5 N/m^2)



1 bar

Drunk bull sperm

1500 bar

Sober bull sperm

What's Going on Here?

- **Pressure reversal of anaesthesia has been known for most of a century. Still not understood.**
- **Understanding pressure effects at the whole organism level is too complicated.**
- **Better: Start by understanding pressure effects on biomolecular structure.**

Pressure Affects Biomacromolecules in Numerous Ways

- Pressure unfolding of proteins (Bridgman – 1914)
- Multimer association/disassociation
- Changes in ligand & small molecule 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
- …. i.e., much of the machinery of life.

Pressure Affects Biomacromolecules in Numerous Ways

- Pres - 1914)
- Mult
- Cha e
- Alte
- Cha
- Larg
- Cha
- Greatly decreased viral infectivity
- …. I.e., much of the machinery of life.

Although the literature contains many papers cataloging these pressure effects, in almost all cases the underlying molecular biophysics is poorly understood.

HIGH PRESSURE in the biosphere is not exceptional. It is the norm. You are the exception.

[Units: 1 atm = 1 bar = 10^5 Pa = 10^5 N/m^2]

Old Paradigm

- 62% of the (ocean + land surface) volume is above 100 atm.
- Deepest ocean trenches reach ~1200 bar.

New Paradigm

- Much, if not most of the planetary biomass is in the deep crust at pressures ranging to at least several kbar: Compelling reason to seek to understand hi-P effects.

Pressure effects predominate in the volume of the biosphere.

How can it be that we understand little about biopressure effects, even though the effects are prevalent, large in magnitude, and affect a great amount of the biomass our planet ??

For the same reasons that we've only recently realized where much of Earth's life exists.

We should be humble: There is much to know that we don't know.

Lecture #2 Outline

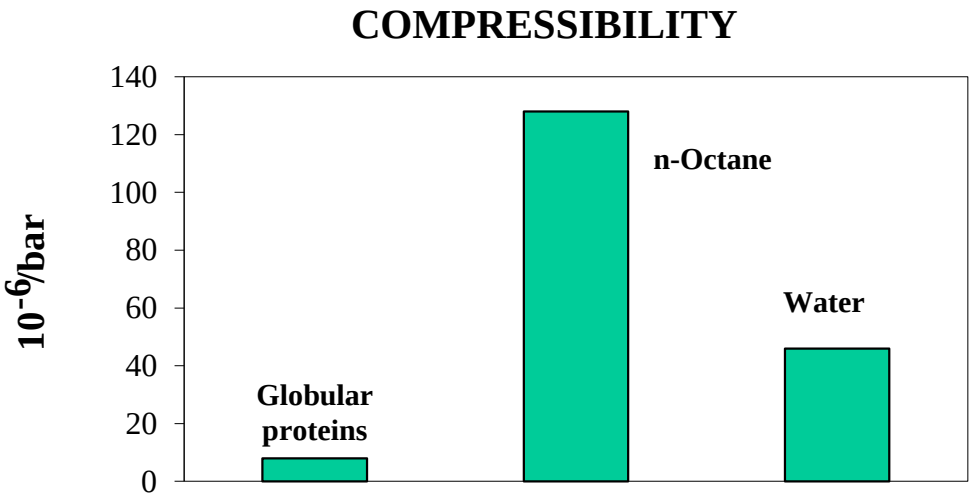
- **Study of proteins under pressure.**
- **Need for hi-P crystallographic tools.**

Hi-P bioscience is not the same as traditional hi-P materials and chemistry

Traditional hi-P science involves PΔV energies on eV scales, typically P >> 10 kbar.

Biopressure effects occur on kT scales at P << 10 kbar.

Biomaterials have low compressibility. So why do so many effects occur << 10 kbar?

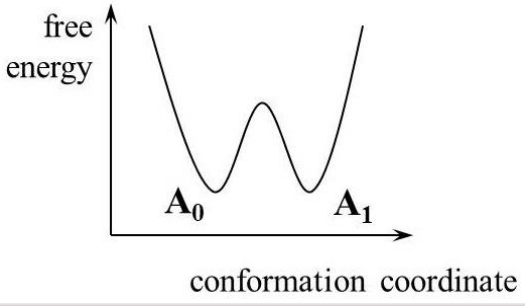


For macromolecules, small ΔV can involve completely different conformational ensembles – think of protein unfolding.

Most Enzymes have Active Sites

PROTEIN STRUCTURE

Scaffold to support and position active site



ACTIVE SITE

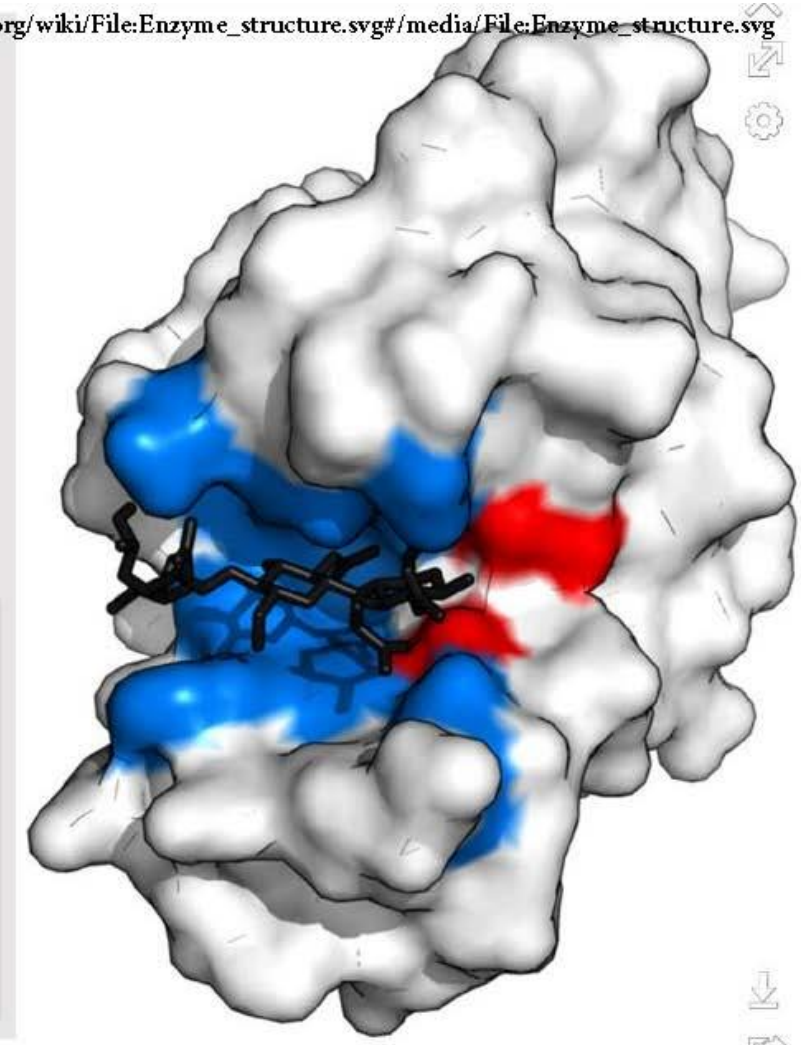
BINDING SITES

Bind and orient substrate(s)

CATALYTIC SITE

Reduce chemical activation energy

commons.wikimedia.org/wiki/File:Enzyme_structure.svg#/media/File:Enzyme_structure.svg



Organisation of enzyme structure and lysozyme example. Binding sites in blue, catalytic site in red and peptidoglycan substrate in black. (PDB: 9LYZ)

More details

Let's go fishing

Cayuga Lake. 9T liters. Clean.
160m deep. Bottom: 16 bar

Cornell University

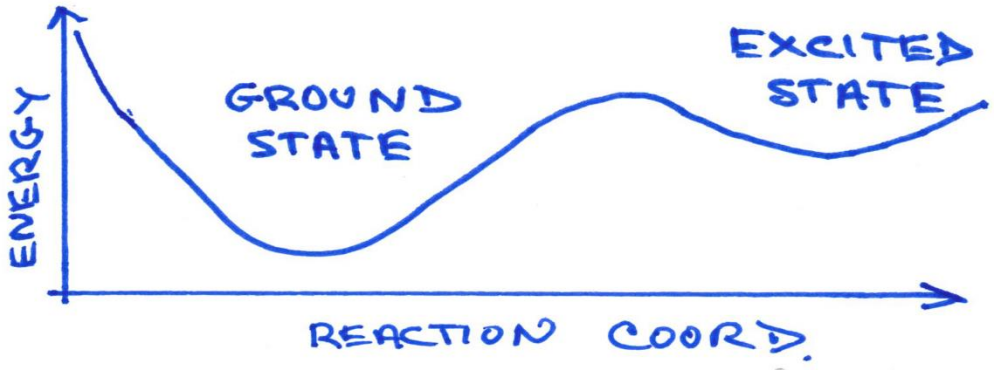
Fish TRAPase

Trapped Dead Fish = Product

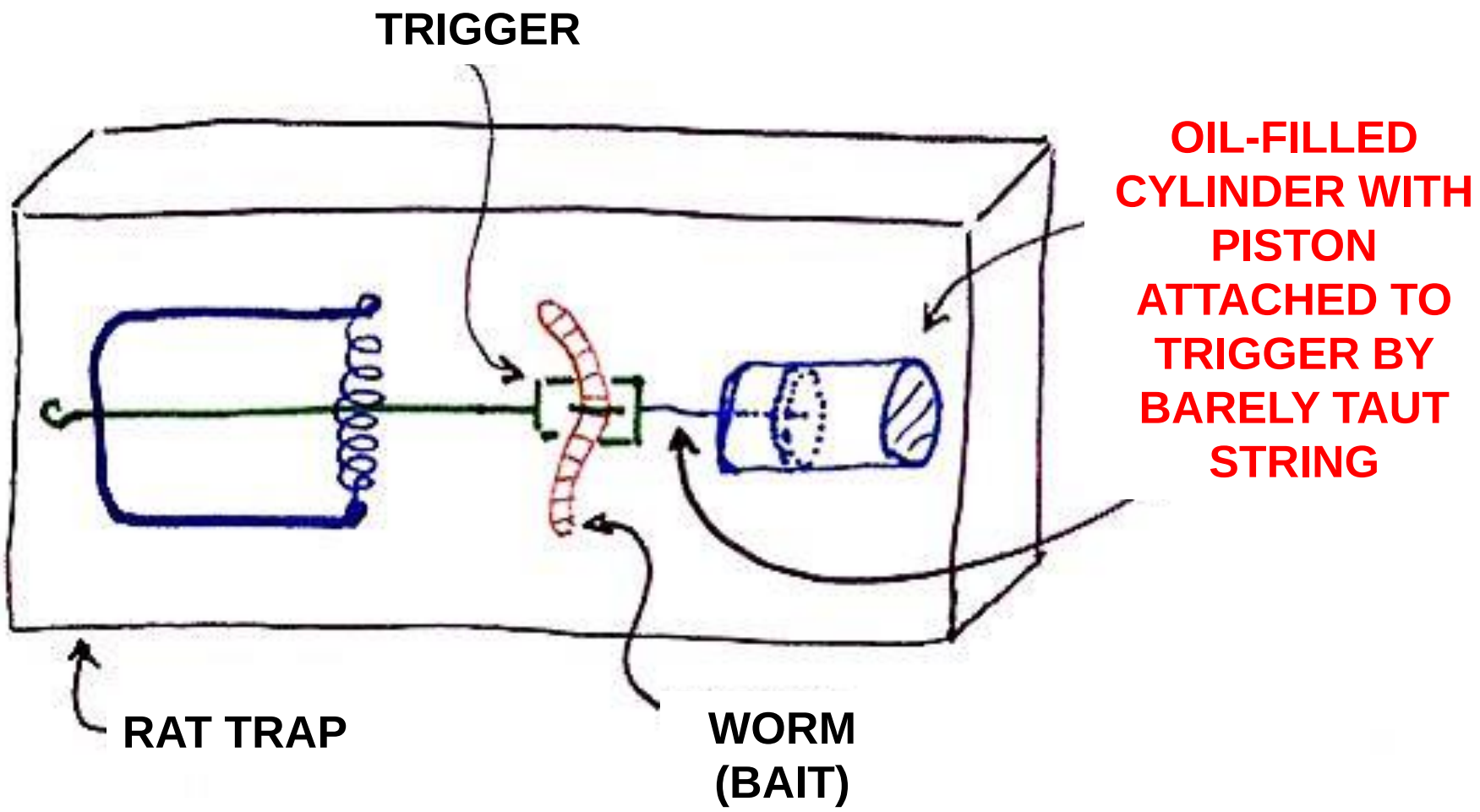
ATP

Active site

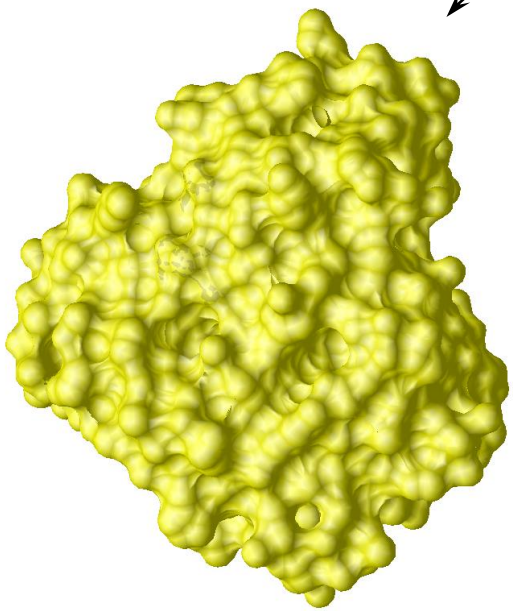
Fish = Substrate



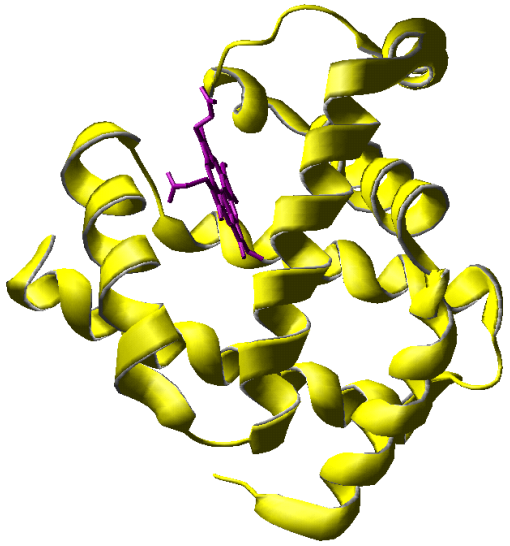
Mutant FISH TRAPase



To understand pressure effects, you need structural information



Beneath the molecular surface, there is a complex internal structure



Structures of Folded Proteins Under Pressure

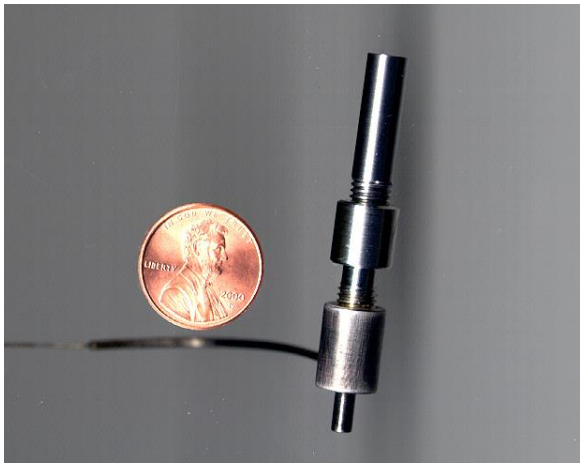
Literature had many papers on pressure effects. To understand these we need structural data.

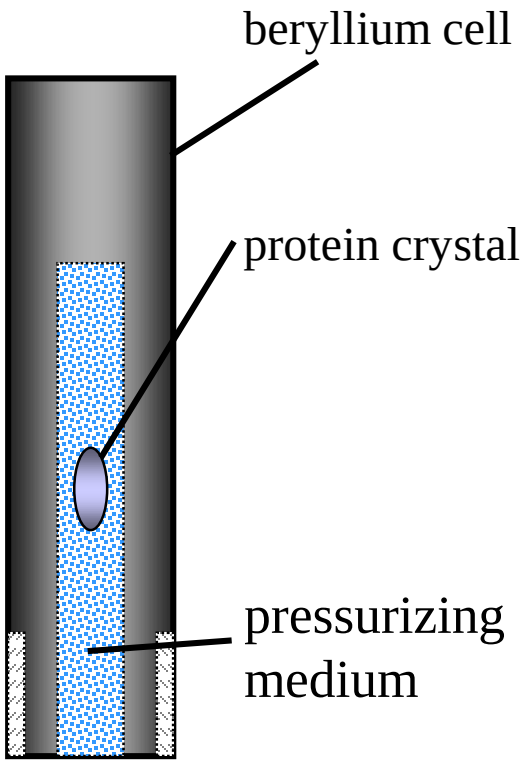
By 2002, there were tens of thousands of protein structures. How many protein structures at atomic resolution at hi-P (≥ 1 kbar)?

Answer: One

Pioneering work was done by Kundrot & Richards

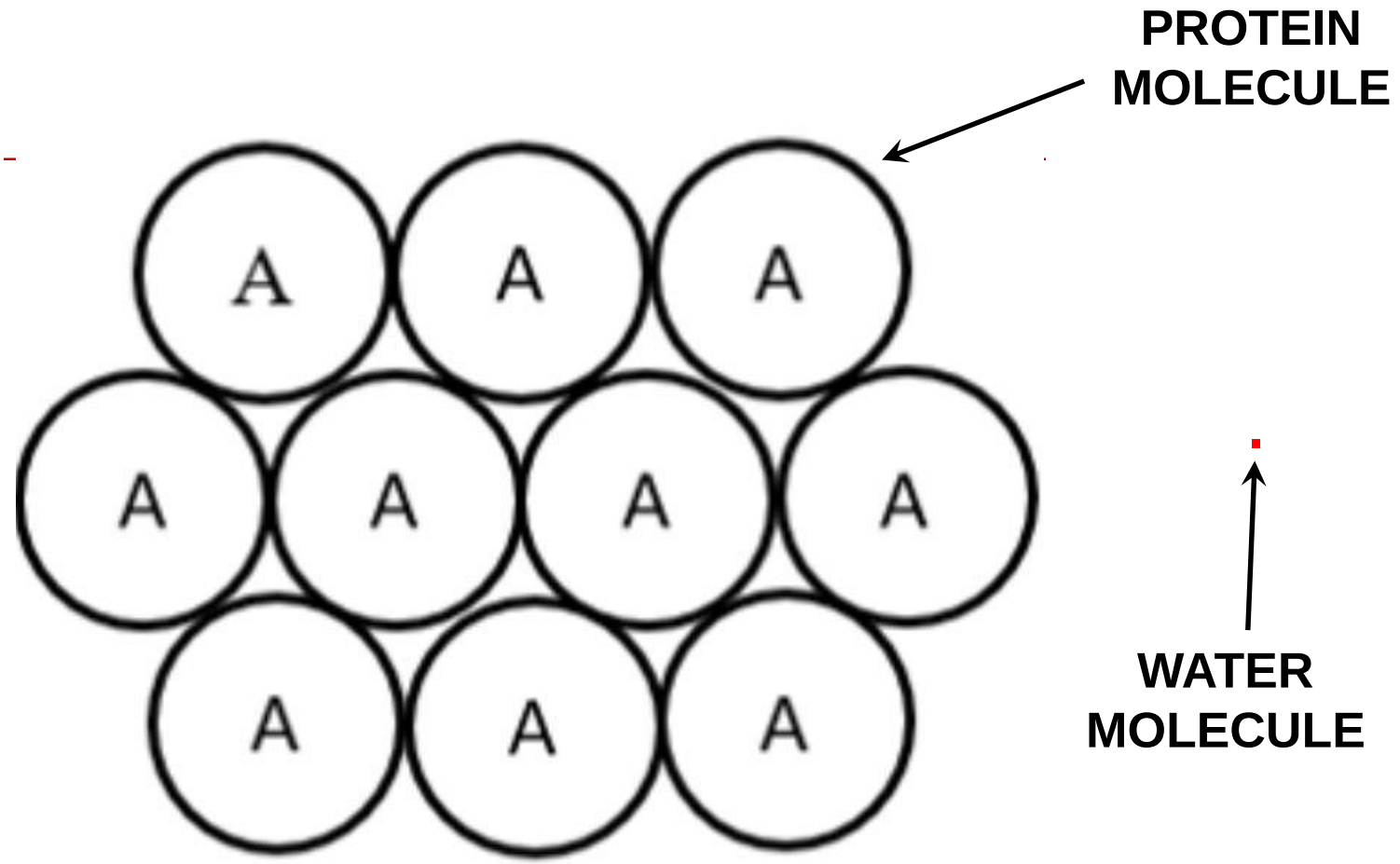
(*J. MOL. BIOL*. 193 (1987) 157)



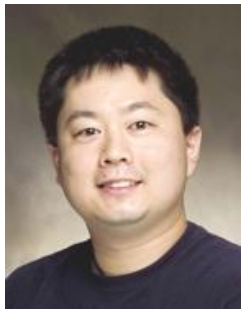


- **HEN EGG WHITE LYSOZYME @ 1 KBAR.**
- **ISOTHERM. COMP.= 4.7×10^{-6}/BAR^{-1}**
- **B-FACTORS CHANGE NONUNIFORM.**
- **CONTRACTION IS NONUNIFORM**
- **a & b AXES CONTRACT 0.6%; c EXPANDS 0.1%**
- **OVERALL STRUCTURE CHANGES LITTLE**

(1) Lack of appreciation of impact of small changes.
(2) Belief that crystals broke under hi-P.
(3) Absence of commercial hi-P cell.

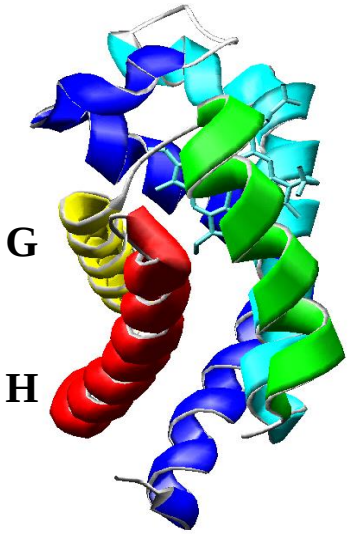

PROTEIN
MOLECULE
A
A
A
A
A
A
A
A
A
A
A
WATER
MOLECULE

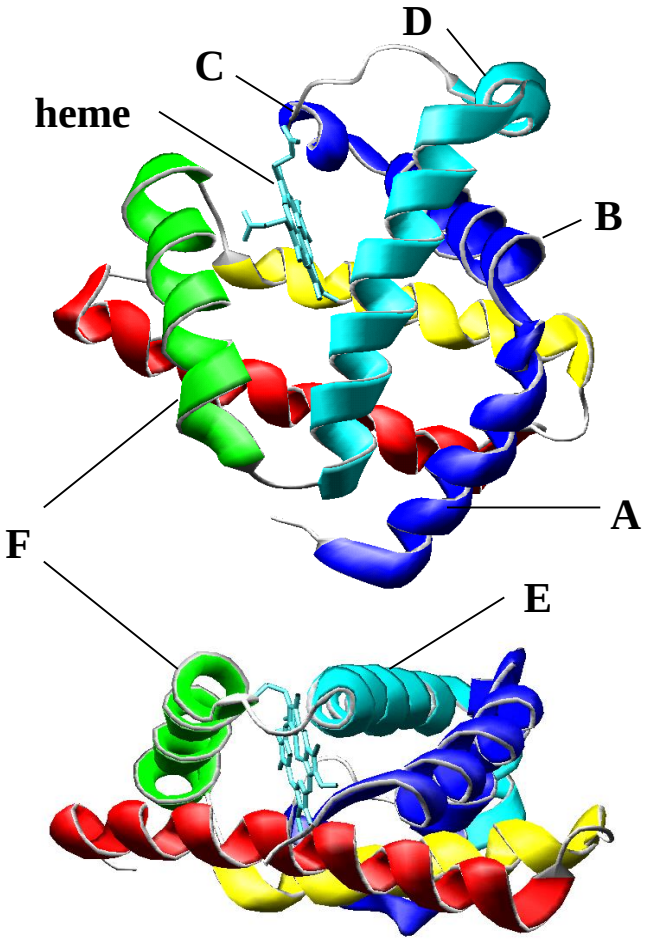
Story #1: Sperm Whale Myoglobin



Paul Urayama

George Phillips



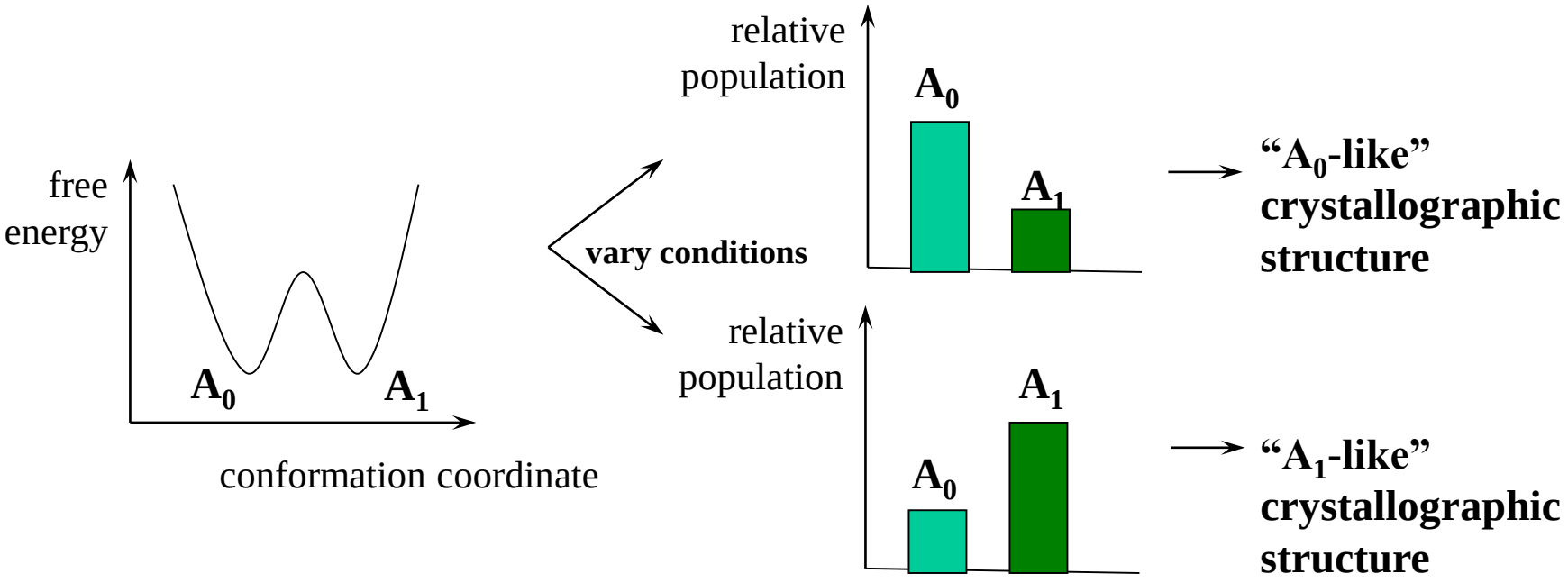


- 153 amino acid residues
- Linear polymer folds into 8 alpha helices joined by loops
- Heme group, an iron tetracoordinated to a porphyrin ring
- O_2 transport/storage in muscle; NO, CO regulation

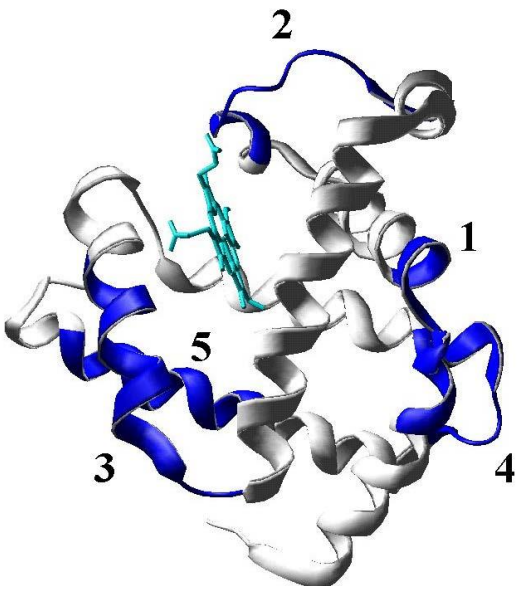
Urayama, Phillips & Gruner, *Structure* 10 (2002) 51.

Conformational Substates

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



Where are Changes Located?

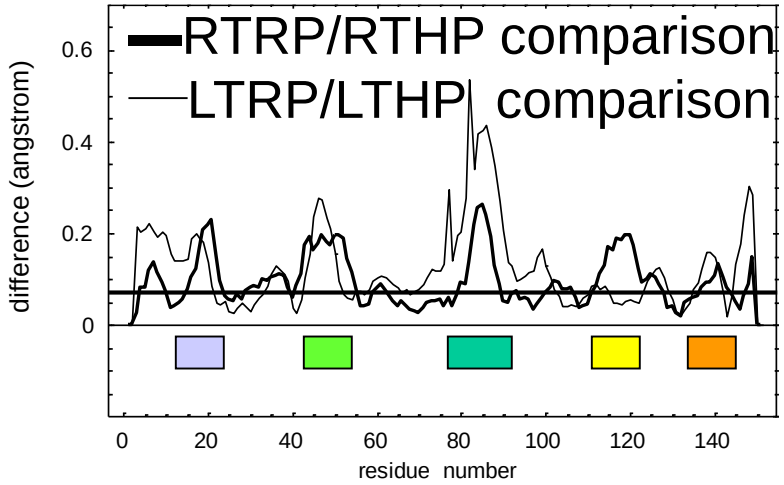


Region #	Location
1	AB loop
2	CD loop
3	F helix
4	GH loop
5	H helix

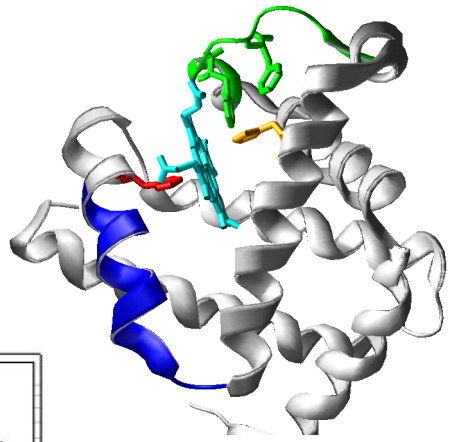
Changes can arise from:

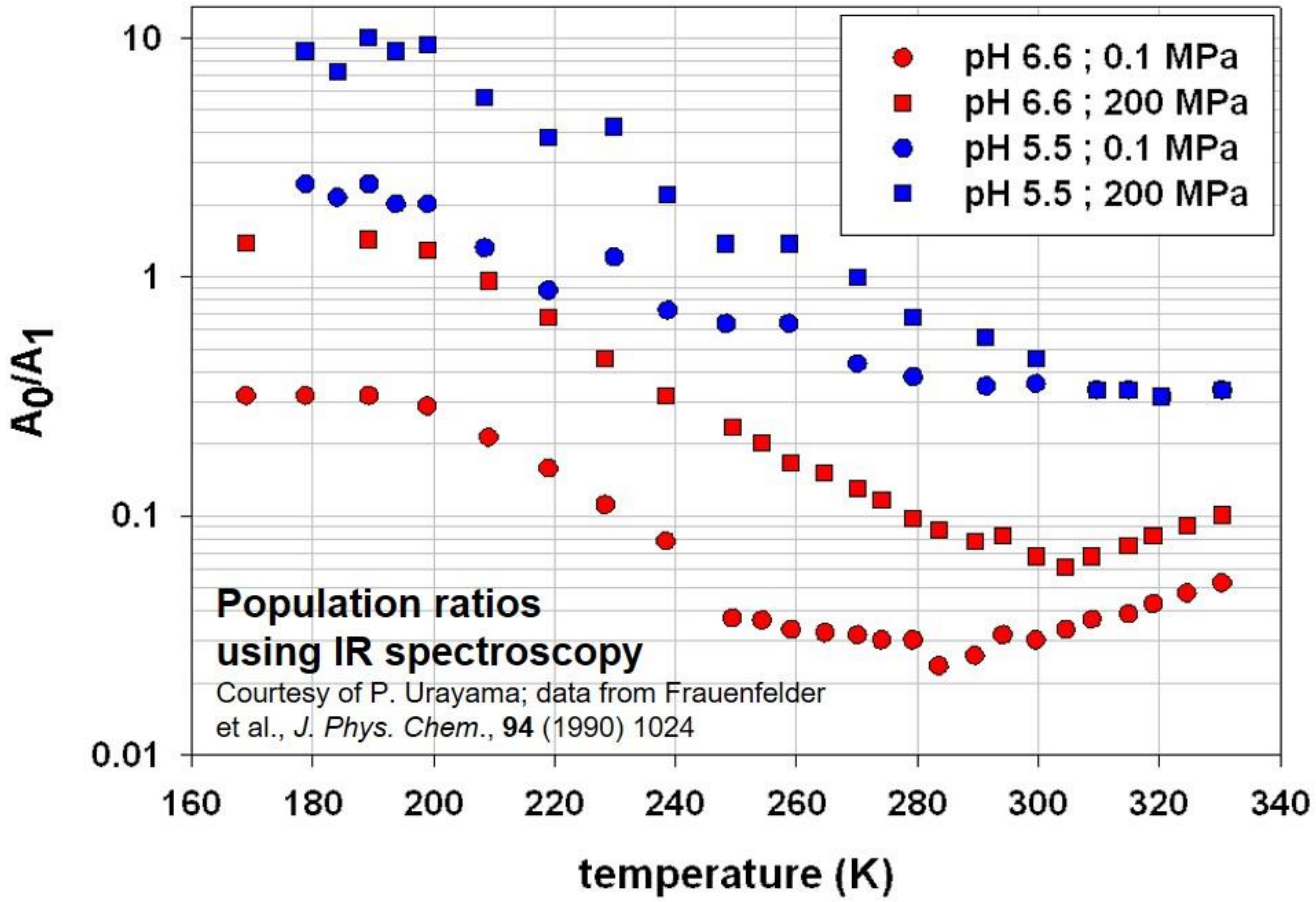
- **Pressure**
- **Temperature**
- **pH**
- **…**

WARNING FOR CRYOCRYSTALLOGRAPHY!



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





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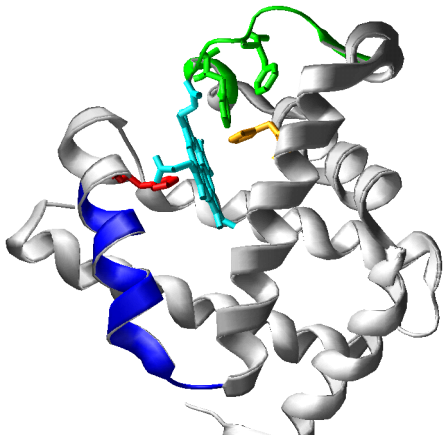

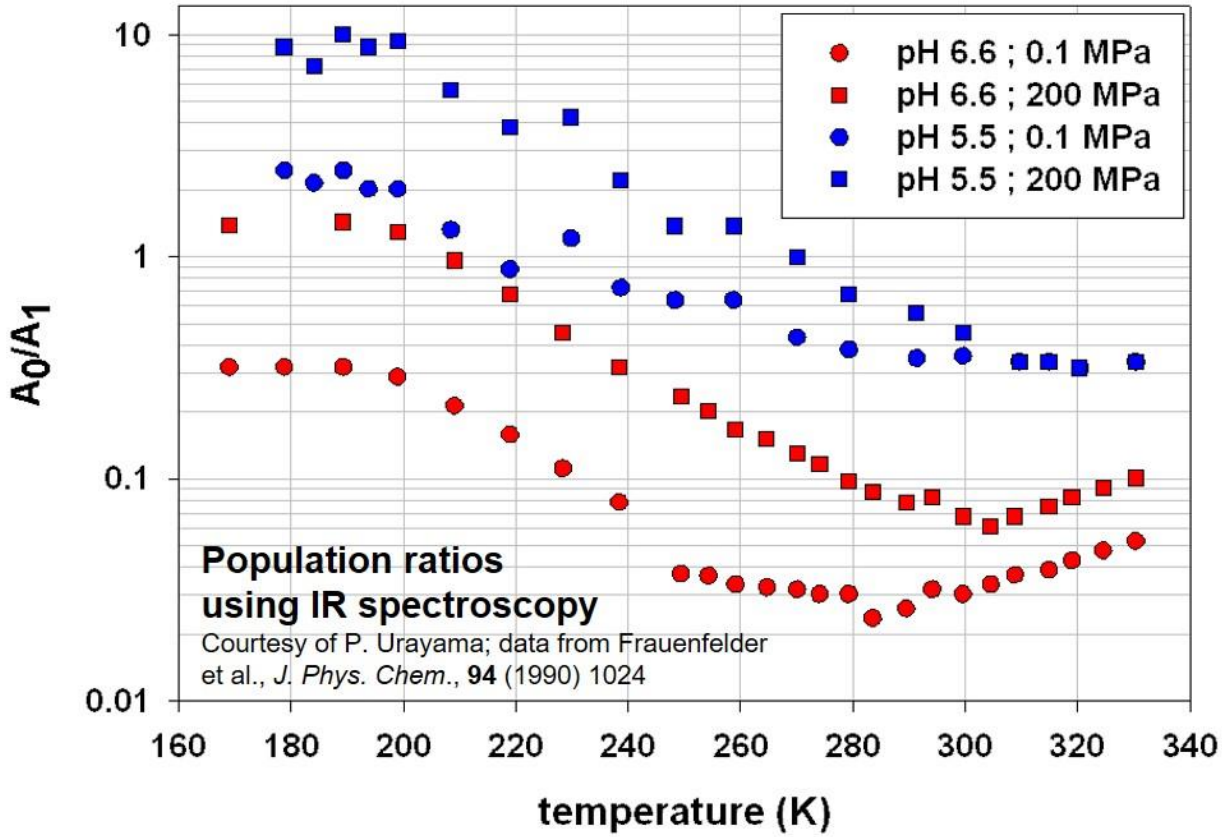

As pH ↓, A_0 ↑

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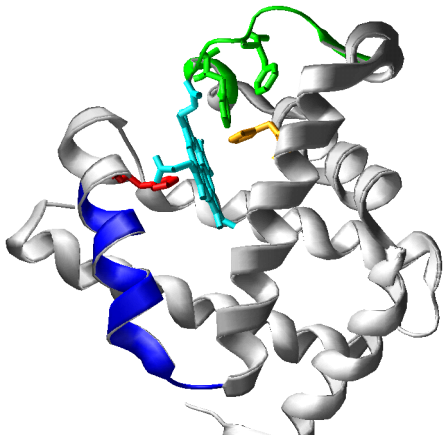

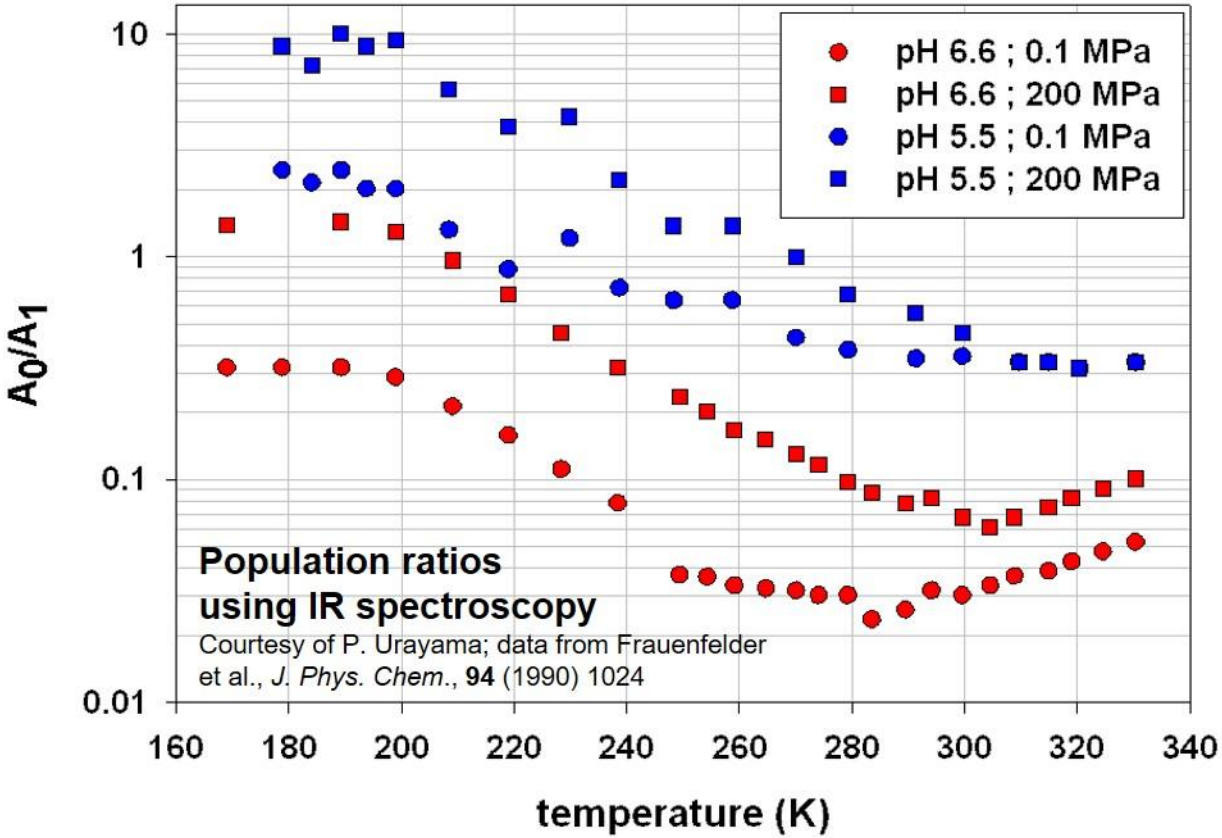

As pH ↓, A_0 ↑

As P ↑, A_0 ↑

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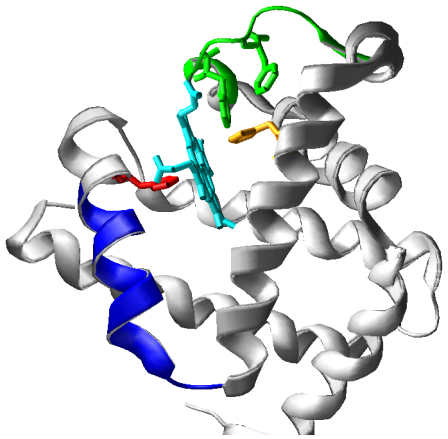
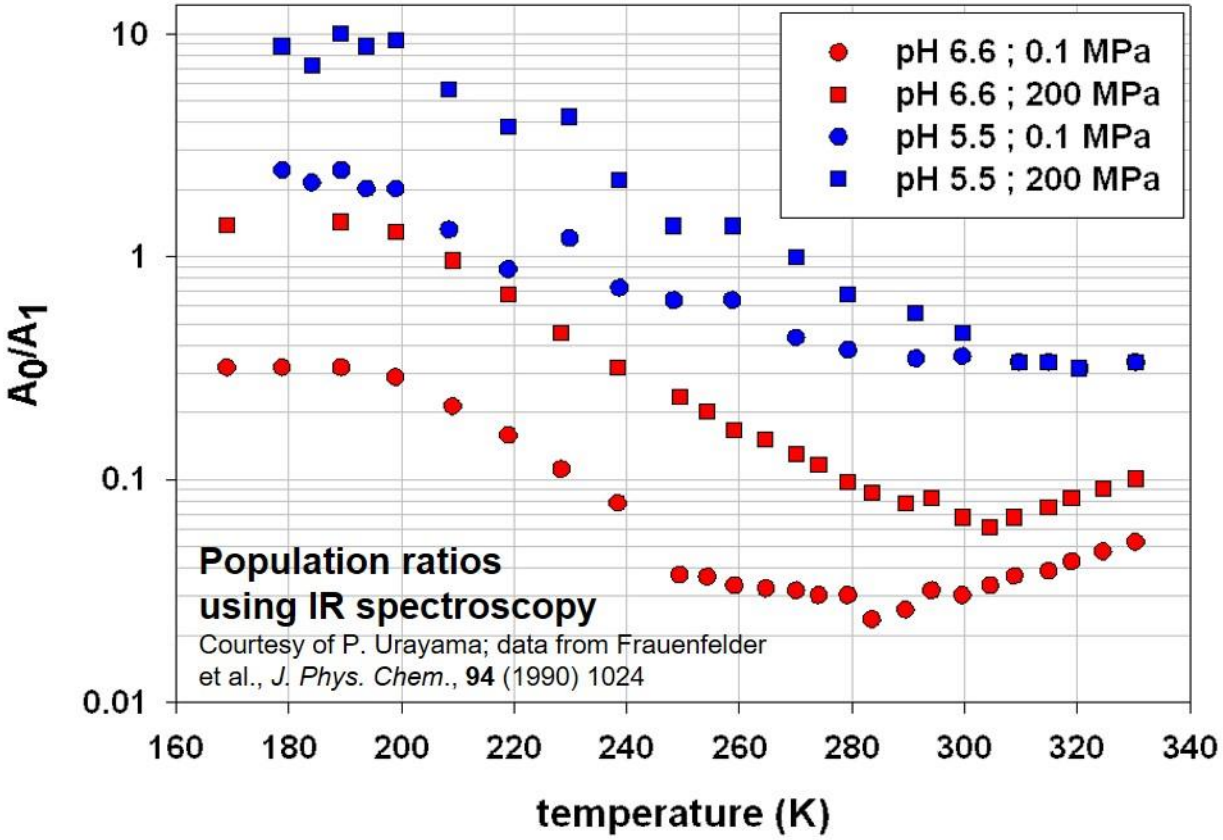
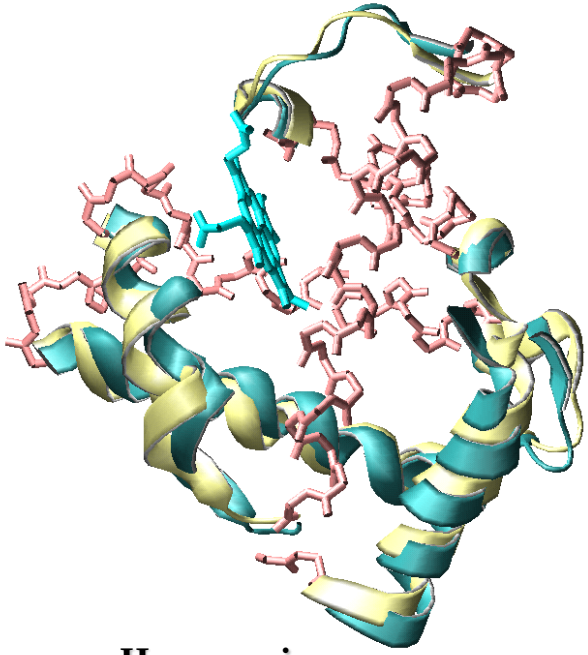




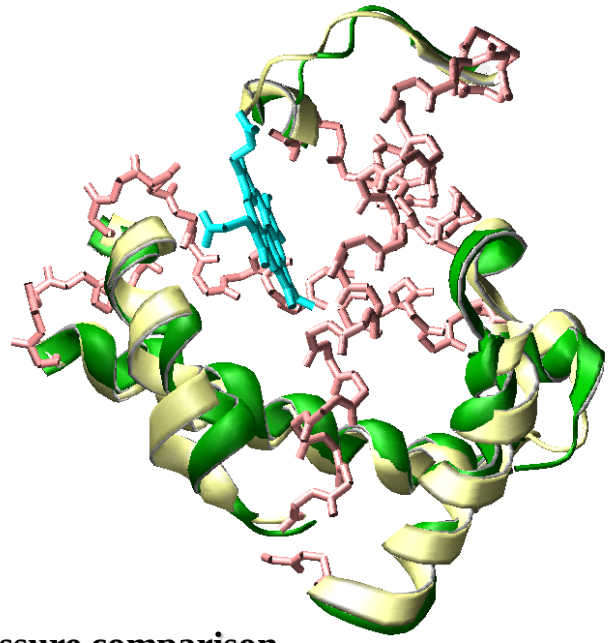
As pH ↓, A_0 ↑

As P ↑, A_0 ↑

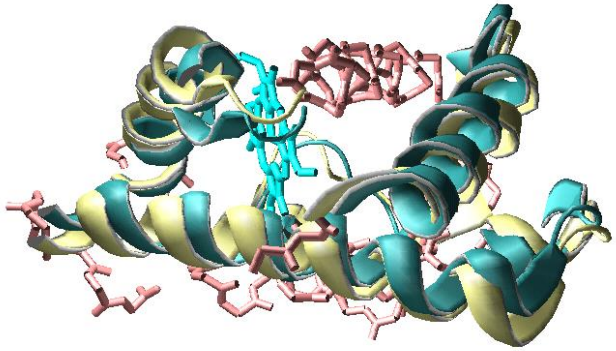
As T ↓, A_0 ↑

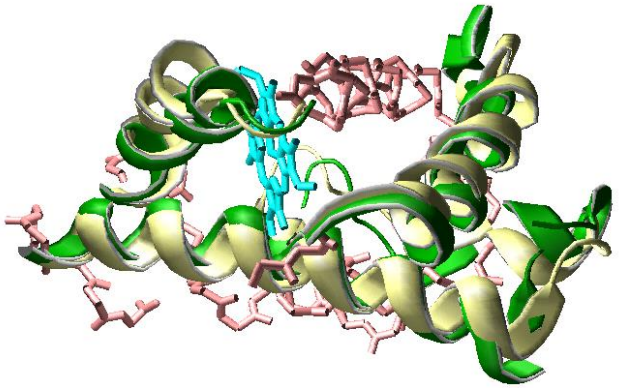


pH comparison



pressure comparison





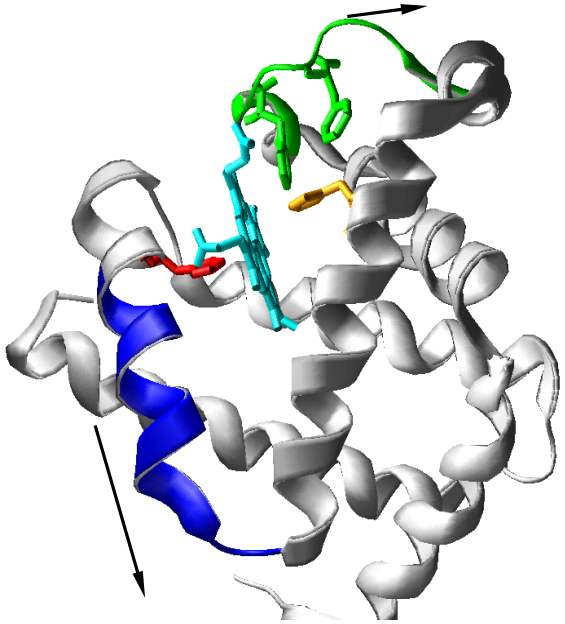
yellow ribbon = ambient pressure, pH 6 position
green ribbon = high pressure position (x 15)
cyan ribbon = low pH position (x 6.2)

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

pH structures solved by Yang and Phillips (1996)

Myoglobin Conclusions

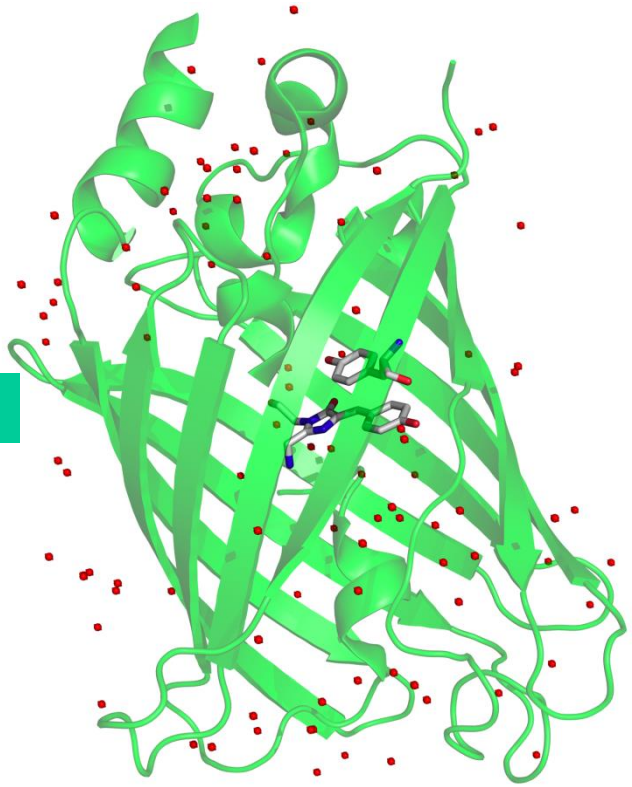
1. Lo pH, Hi P, Lo T all lead to similar structural changes in myoglobin.
2. These all correlate with favoring A_0 substate relative to A_1.
3. "A" substates represent global conformational substates.



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

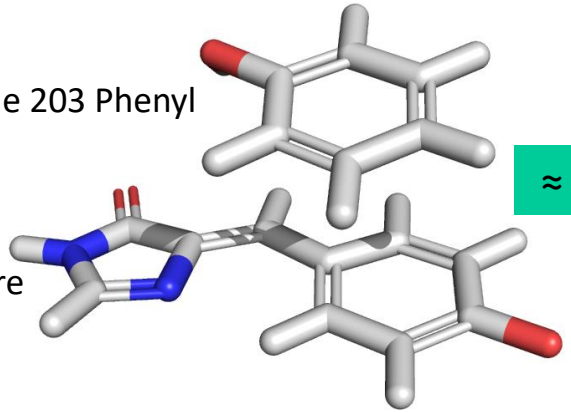
Story #2: Citrine

≈ 50 Å



Tyrosine 203 Phenyl

Wild Type Chromophore



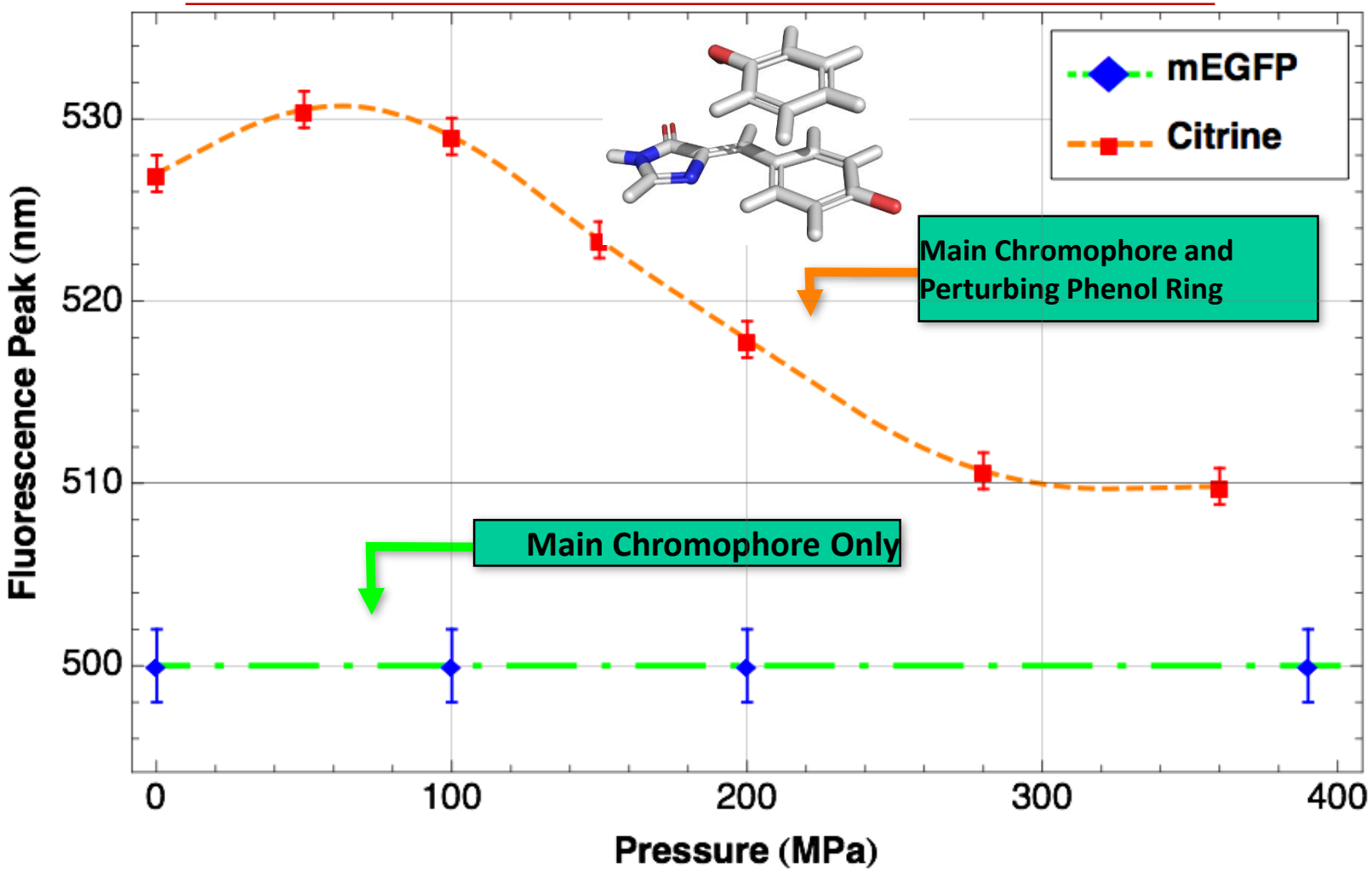
≈ 3.4 Å

Buz Barstow

Nozomi Ando

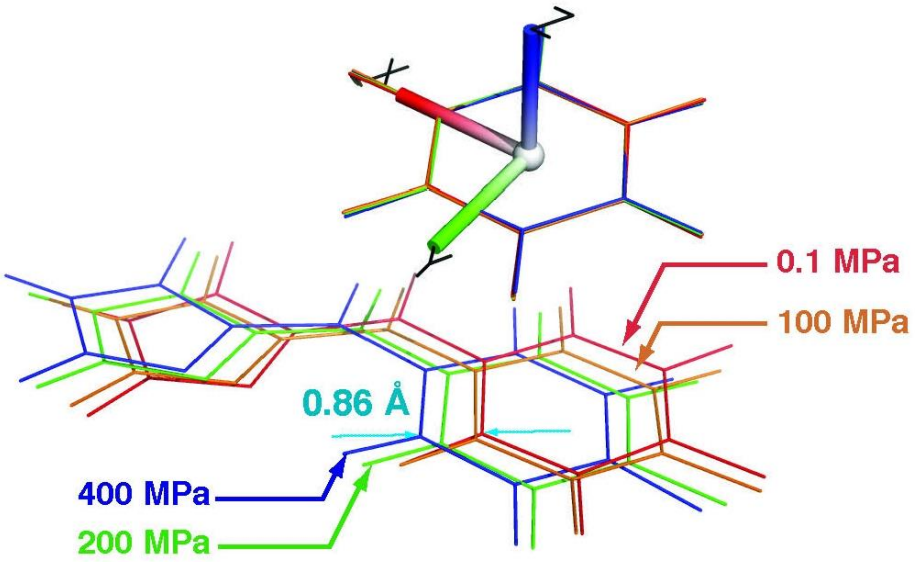
Chae Un Kim

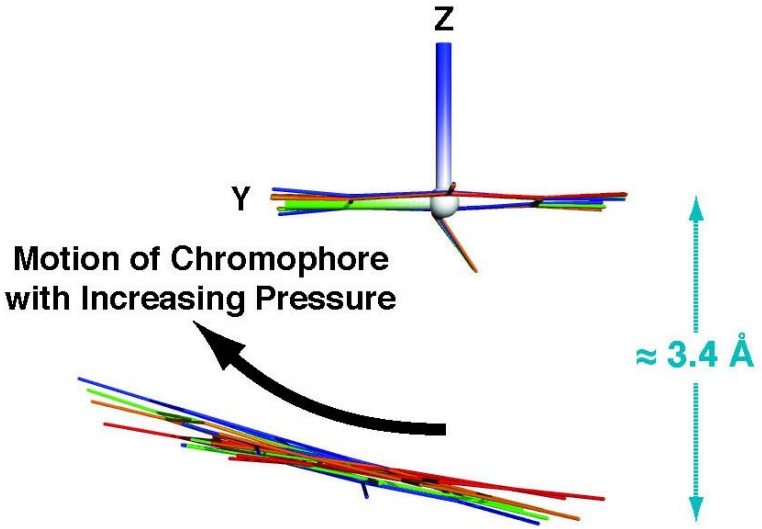
Citrine's "Function": Fluorescence Peak Shift



Barstow, Ando, Kim and Gruner, *Proc. Natl. Acad. Sci. U.S.A.*, 105 (2008) 13362

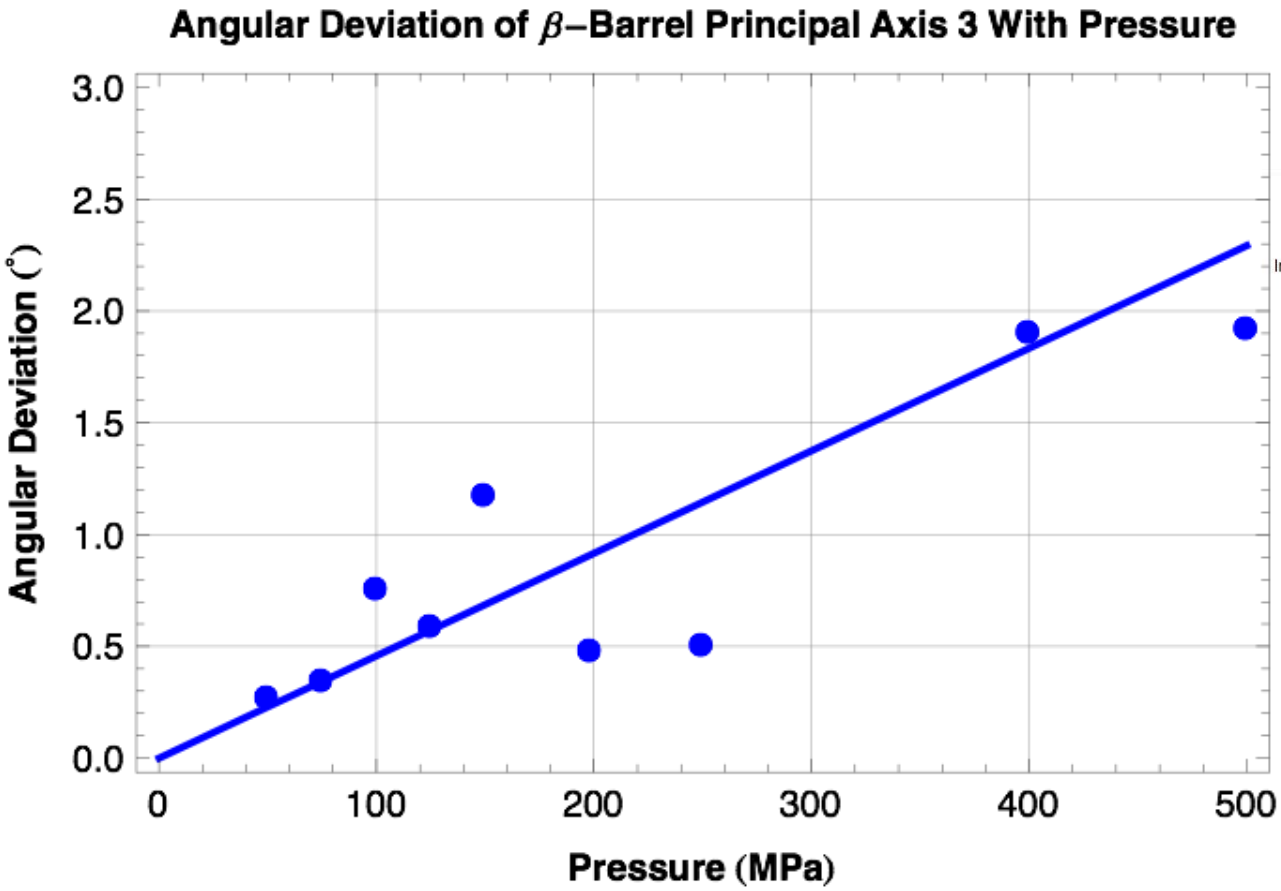
P-Shifts of Citrine Chromaphore

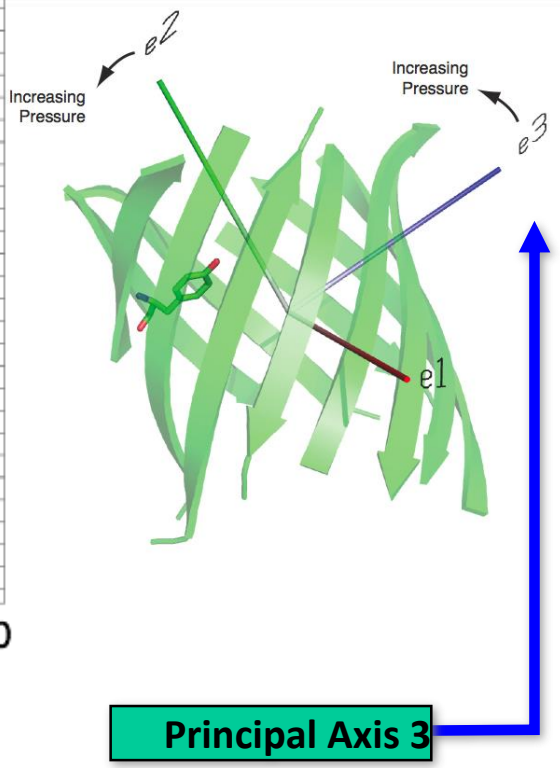




Barstow et al, *PNAS* 105 (2008) 13362.

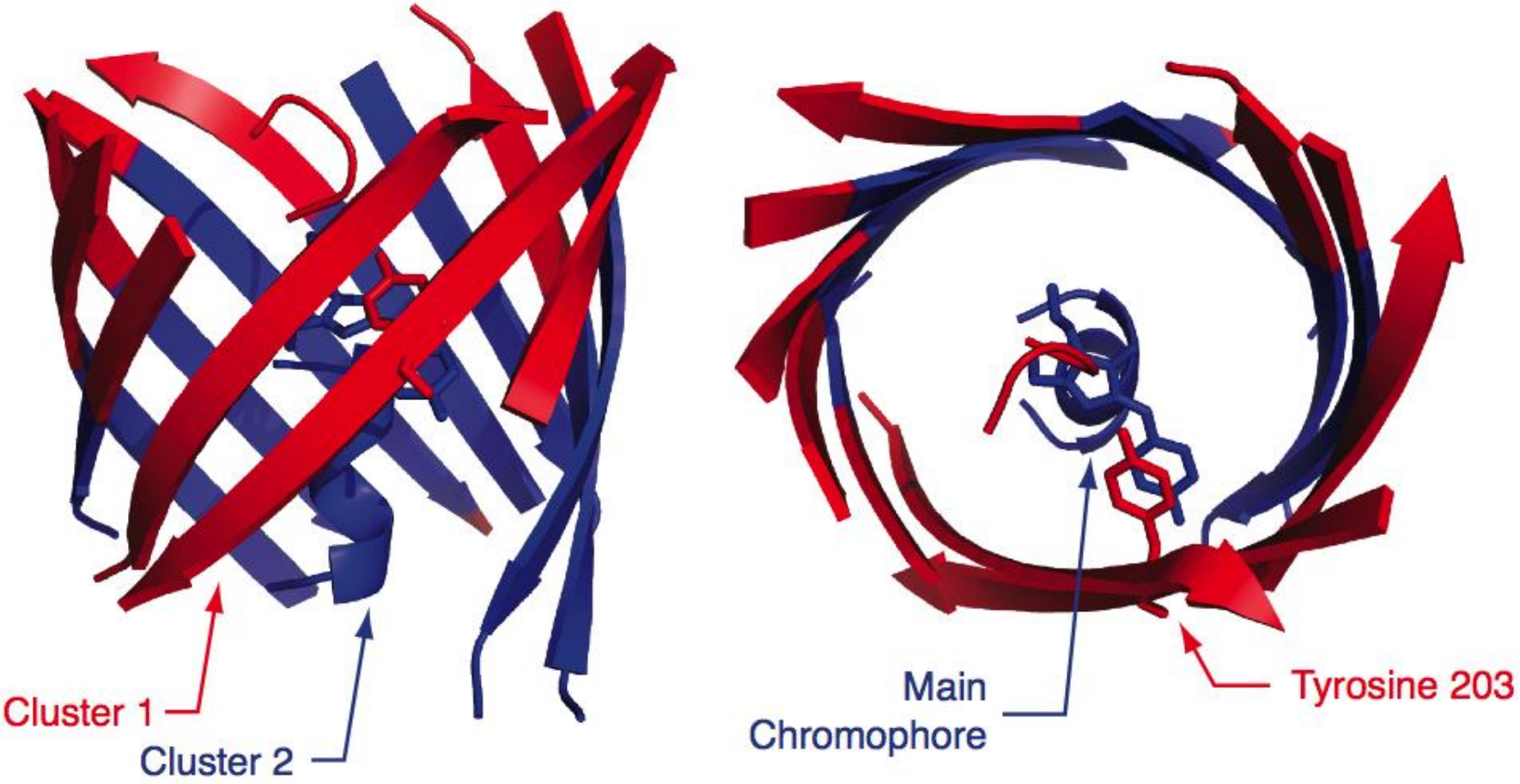
Rotation of β-Barrel Principal Axis 3





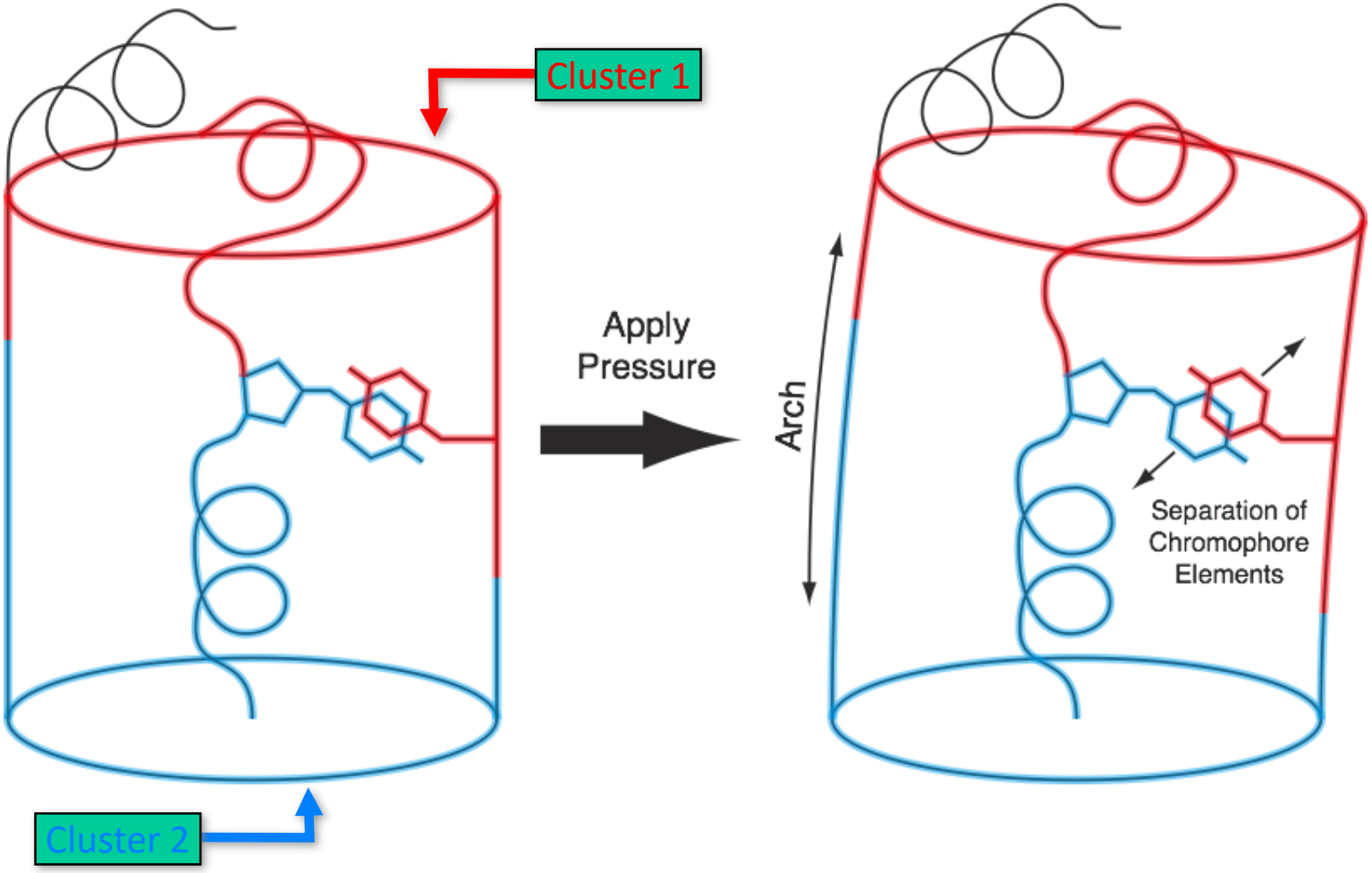
Barstow, Kim, Ando and Gruner, *Biophys. J.* 97 (2009) 1719.

Cluster Analysis of β-Barrel



Barstow, Kim, Ando and Gruner, Biophys. J. 97 (2009) 1719.

How is Pressure Communicated?



Barstow, Kim, Ando and Gruner, Biophys. J. 97 (2009) 1719.

Citrine Conclusions

1. Hi-P changes are colligative: As P increases the whole structure continuously deforms.
2. Deformation is due to many effects: Collapse and water filling of microvoids, changes in hydration and ionization of surface groups, etc.
3. Result is small deformations of “active site”.
4. This mechanism is completely general and likely accounts for much of pressure sensitivity of enzymes.

Wait a minute! You've argued that…

1. High pressure is known to affect a vast number of biochemical functions.
2. Most of the biosphere is at high pressure.
3. Hi-P biostructural studies inform some of the most fundamental questions in biology, such as conditions for the start of life.
4. And yet, with a world full of competitive scientists hungry to win Nobel prizes, the area of high pressure structural biology is barely explored??

How can this be?

- Lack of a coherent hi-P biocommunity.
- Lack of user friendly hi-P biostructural tools.
- Myopia of funding agencies.

RCN NSF Proposal*
6 tools needed for Extreme Biophysics

Cathy Royer

- **Bioinformatics of extremophiles**
- **High pressure NMR**
- **High pressure bacterial culture methods**
- **High pressure microscopy**
- **Computational studies of extreme conditions**
- **High pressure SAXS and crystallography**

* NSF proposal for a Research Coordination Network (RCN).

X-ray Based Structural Information

Crystallography: Angstrom (atomic) scales (LECTURE #2)

- Atomic & residue displacements; conformational changes
- Binding site distortions
- Ligand & small molecule binding & unbinding
- Hydrogen bonding
- Bound waters
- …

SAXS: Nanometer (molecular) length scales (LECTURE #3)

- Multimerization
- Large shape changes
- Lipid phases
- Folding/unfolding
- …

CORNELL CHRONICLE
July 22, 2019

Lindsay France/Cornell University

Guebre Tessema, right, NSF materials research program director, tours the CHESS facility June 3 with CHESS director Joel Brock.

Cornell announces $54M from NSF for new CHESS subfacility

Supports 4 beamlines: One is devoted to Hi-P structural biology.

HP Macromolecular Crystallography

J. Appl. Cryst. (1968). **1**, 23

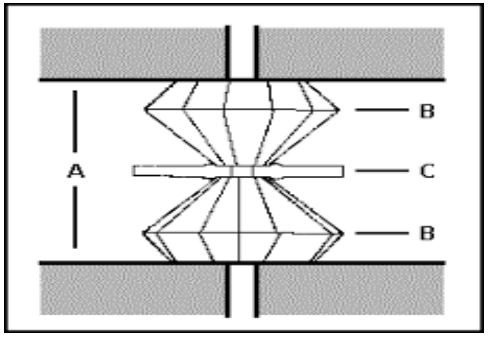
Appareillage pour Etudes Radiocristallographiques sous Pression et à Température Variable

PAR ROGER FOURME

Laboratoire de Chimie Physique de la Faculté des Sciences de Paris, 91 *Orsay*, *France*

(*Reçu le* 15 *novembre* 1967)

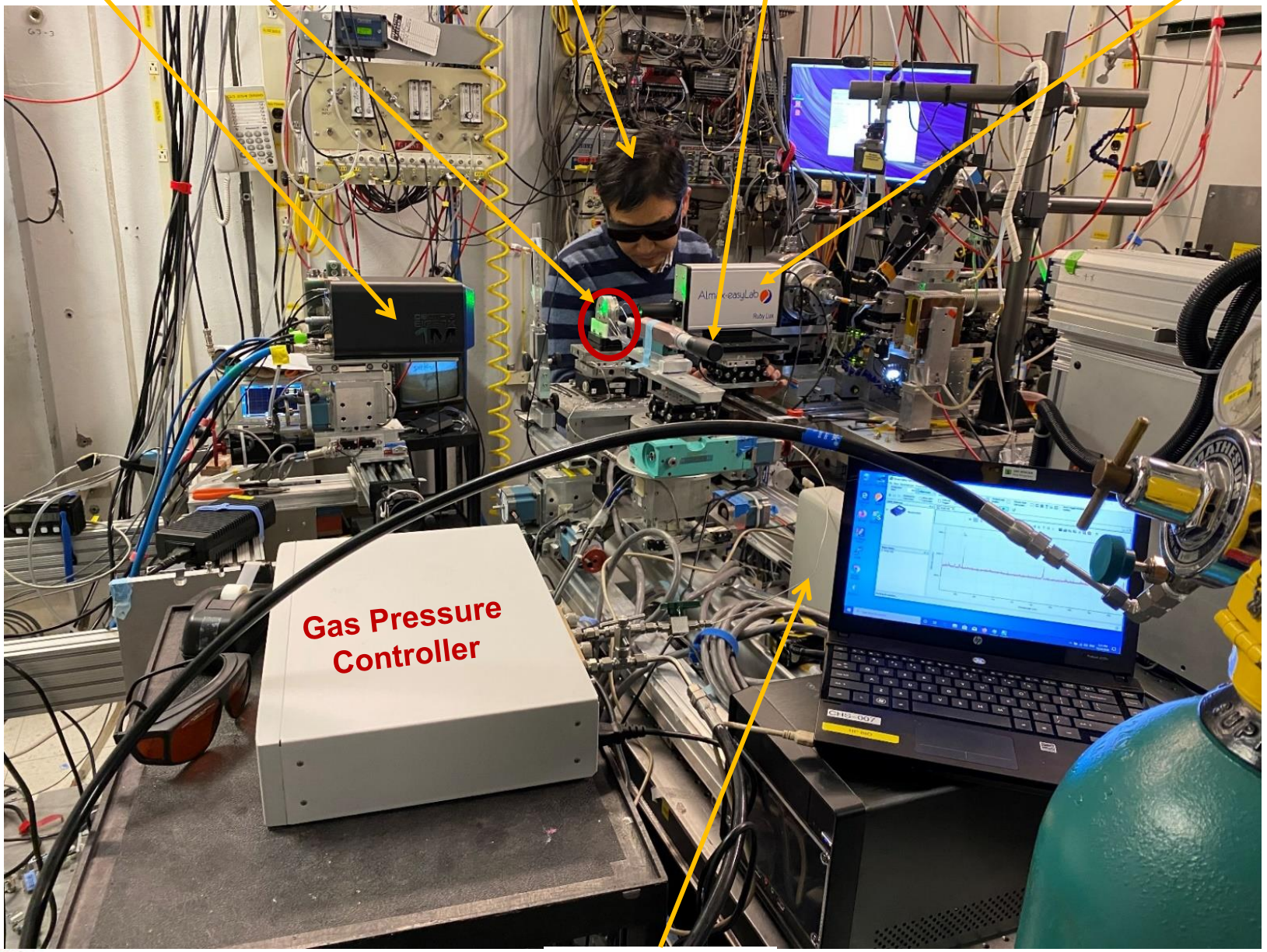
A diamond-anvil high-pressure cell especially designed for a standard precession camera and its operation are described. The applied force is generated by means of a pneumatic device. Single crystals grown by Van Valkenburg's technique are maintained under high pressure (1–30000 bars) at adjustable temperature (20–220°C). A steel goniometer head provides facility of centring and orienting the crystal. Some results are given, including crystal growth, optical observation of the phase transition in cyclohexane and crystal structure of chloroform at ~7000 bars. Conditions for gathering precise intensity data are discussed.



R. Letoullec, et al., **The membrane diamond anvil cell**: A new device for generating continuous pressure and temperature variations. *High Pressure Research*, **1** (1988) 77

DAC Protein Crystallography at CHESS

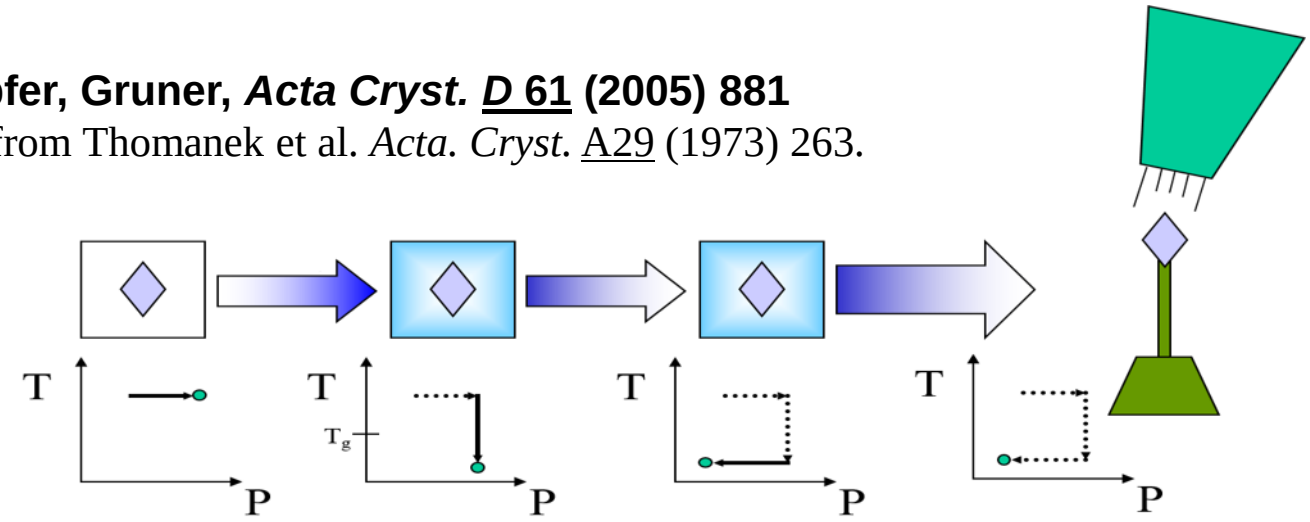
- DAC: Almax Easy Lab (Belgium)
- Pressures: Low to 1.5 GPa
- Force control: Gas filled toroidal membrane.
- Continuous ruby fluorescence feedback controls toroidal membrane gas pressure.
- 100 degree opening angle via Boehler Almax diamond mount.
- Small exit diamond of 0.8 mm thickness.

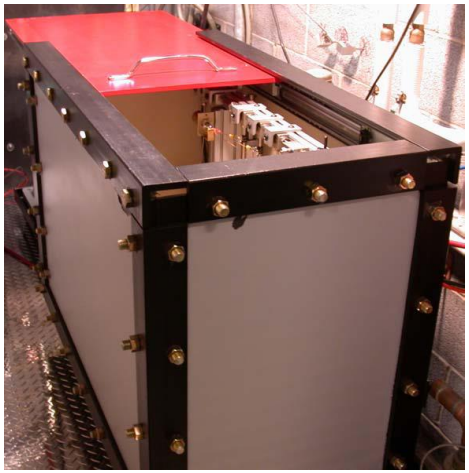

Detector
DAC
Zhongwu Wang
Alignment Camera
Fluorescence laser
Gas Pressure Controller
Spectrometer

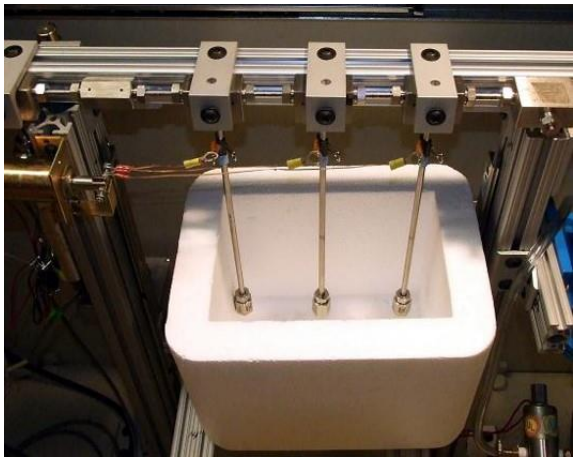
High Pressure Gas Cooling Method

Pressurize with He then cryocool to high density amorphous water.

Kim, Kapfer, Gruner, *Acta Cryst.* D 61 (2005) 881
Modified from Thomanek et al. *Acta. Cryst.* A29 (1973) 263.







Procedural modifications allow loading crystal with Kr, O_2, etc.

Summary

- **Much about life on Earth is unknown. Be humble. The paradigm is shifting.**
- **Much life on Earth is at hi-P.**
- **High pressure biostructural science provides a path to the unknown for those brave enough to go there.**
- **The requisite tools are becoming available. DESY should implement them:**

Hi-P bio is the science of the future.

Three Lectures

Lecture #1: Why we should study high pressure biology.

Lecture #2: How to use WAXS for structures under pressure.

Lecture #3: How to use SAXS for high-pressure biomolecular structure

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- Many other people at CHESS & MacCHESS

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https://desy.zoom.us/j/68572076649
Code: 039470



END OF LECTURE #2