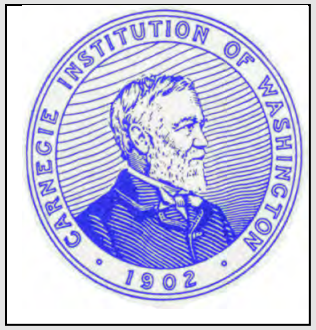


Can High Pressures Enhance The Long-Term Survival of Microorganisms?

Robert M. Hazen & Matt Schrenk
Carnegie Institution & NASA Astrobiology Institute

Pardee Keynote Symposium: Evidence for Long-Term Survival of Microorganisms and Preservation of DNA
Philadelphia – October 24, 2006

Objectives

I. Review the nature and distribution of life at high pressures.

II. Examine the extreme pressure limits of cellular life.

III. Compare experimental approaches to study microbial behavior at high P and T.

IV. Explore how biomolecules and their reactions might change at high pressure?

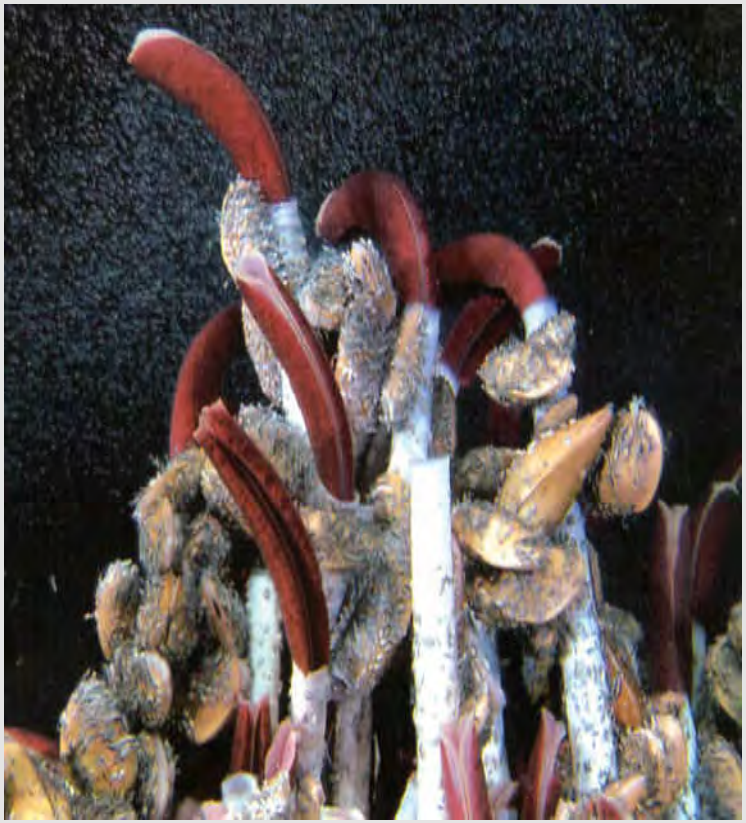
I. High-Pressure Life

PLATE XXIII.—THE "CHALLENGER" AT ST. PAUL'S ROCKS.

HMS Challenger – 1870s

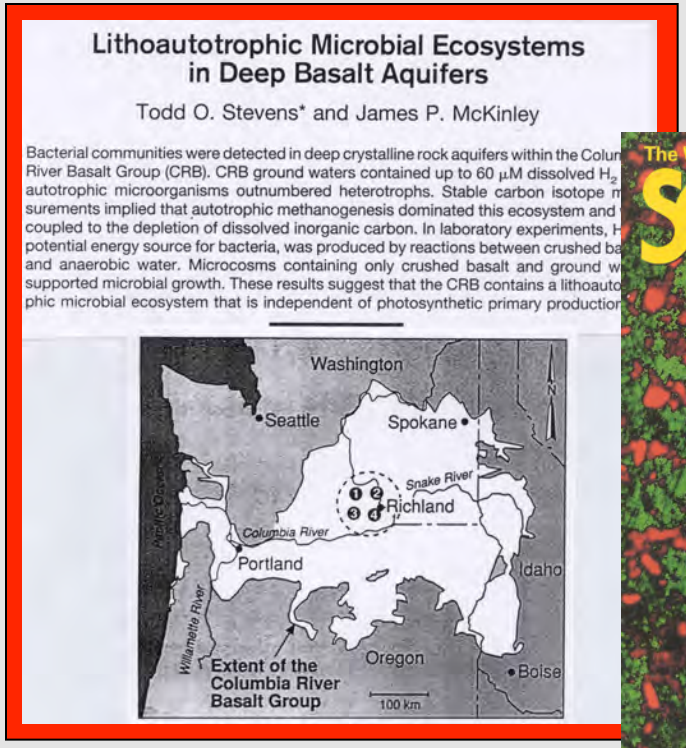
20 MPa (~200 atmospheres)

High-Pressure Life



Deep-Sea Hydrothermal Vents – 1977

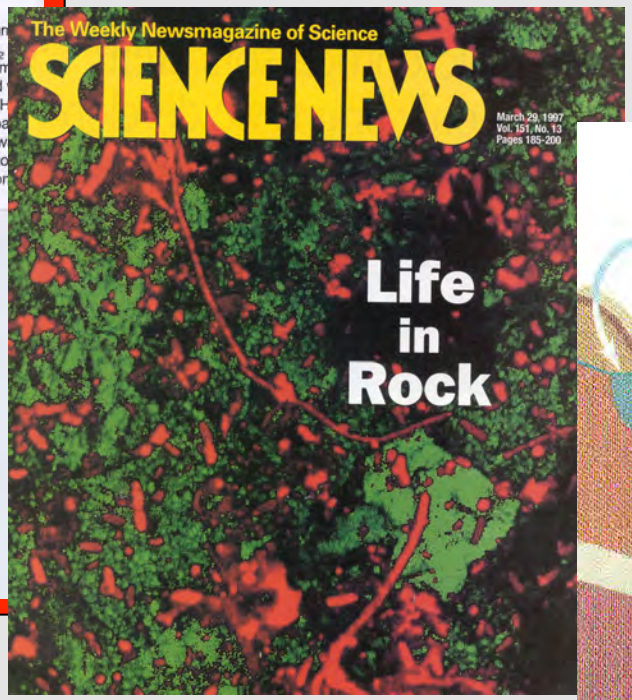
High-Pressure Life

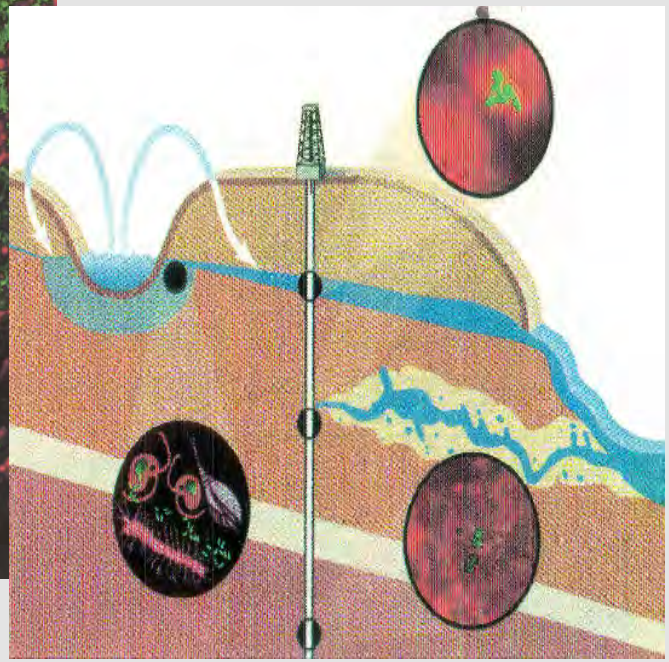


Lithoautotrophic Microbial Ecosystems in Deep Basalt Aquifers

Todd O. Stevens* and James P. McKinley

Bacterial communities were detected in deep crystalline rock aquifers within the Colu River Basalt Group (CRB). CRB ground waters contained up to 60 μM dissolved H_2 autotrophic microorganisms outnumbered heterotrophs. Stable carbon isotope m surements implied that autotrophic methanogenesis dominated this ecosystem and coupled to the depletion of dissolved inorganic carbon. In laboratory experiments, H potential energy source for bacteria, was produced by reactions between crushed ba and anaerobic water. Microcosms containing only crushed basalt and ground w supported microbial growth. These results suggest that the CRB contains a lithoauto phic microbial ecosystem that is independent of photosynthetic primary productio





Discoveries of Microbial Life in Crustal Rocks – 1990s

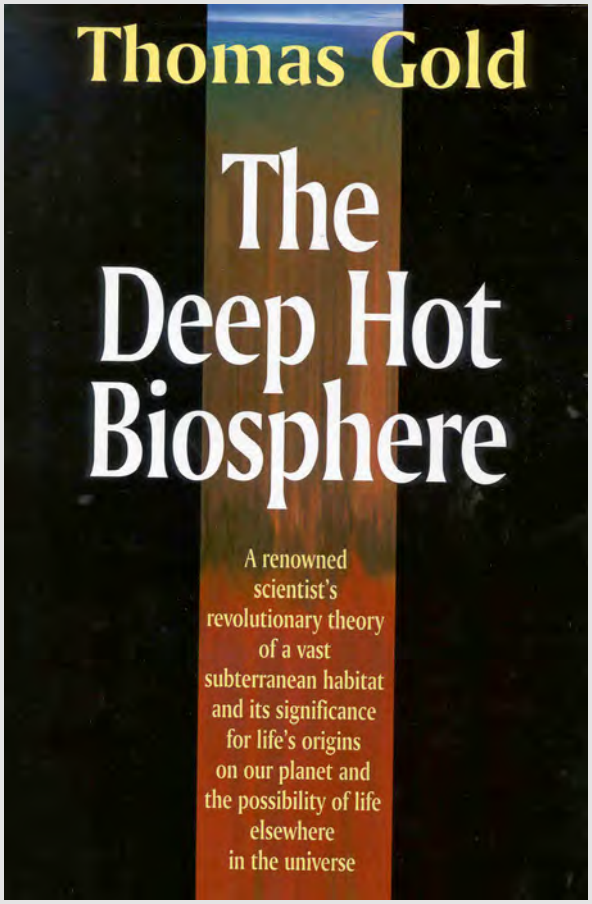
P ~ 100 MPa

Witwatersrand Deep Microbiology Project

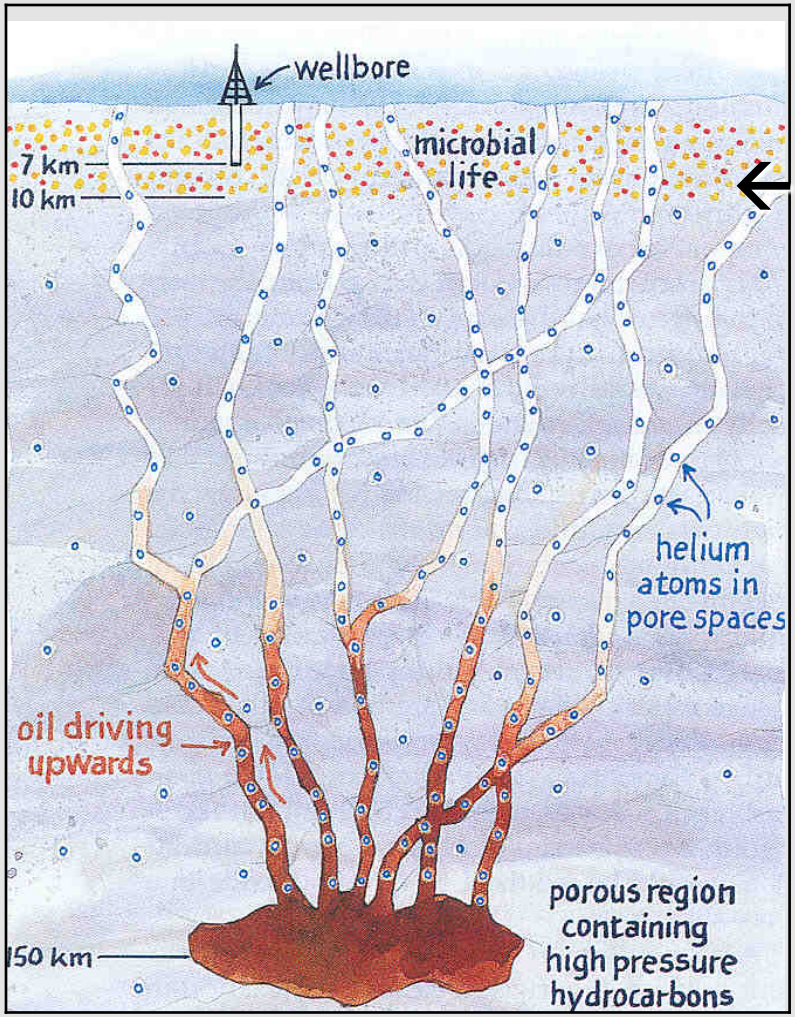
Energy from radiolytic cleavage of water.

Lin, Onstott et al. (2006) *Science*

Thomas Gold's Hypothesis: Organic Synthesis in the Mantle

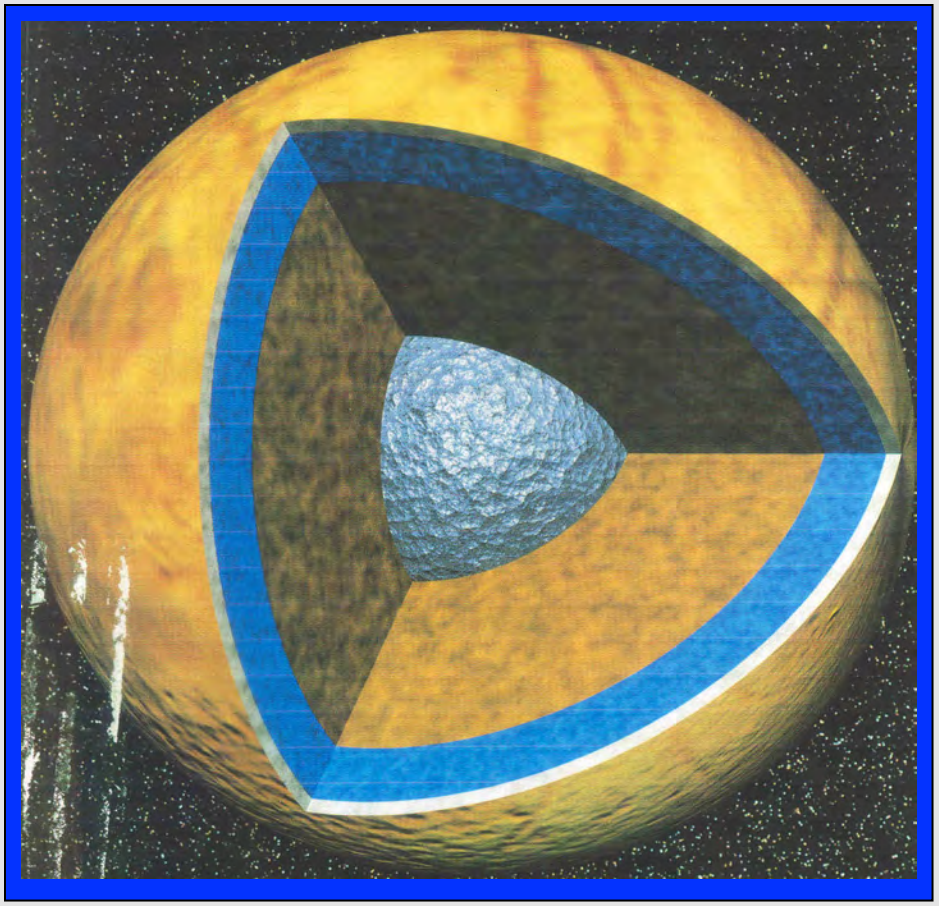


Thomas Gold (1999) NY:Springer-Verlag.



←>300 MPa

Life at High Pressures on other Worlds?



Deep, wet environments may exist on Mars, Europa, etc.

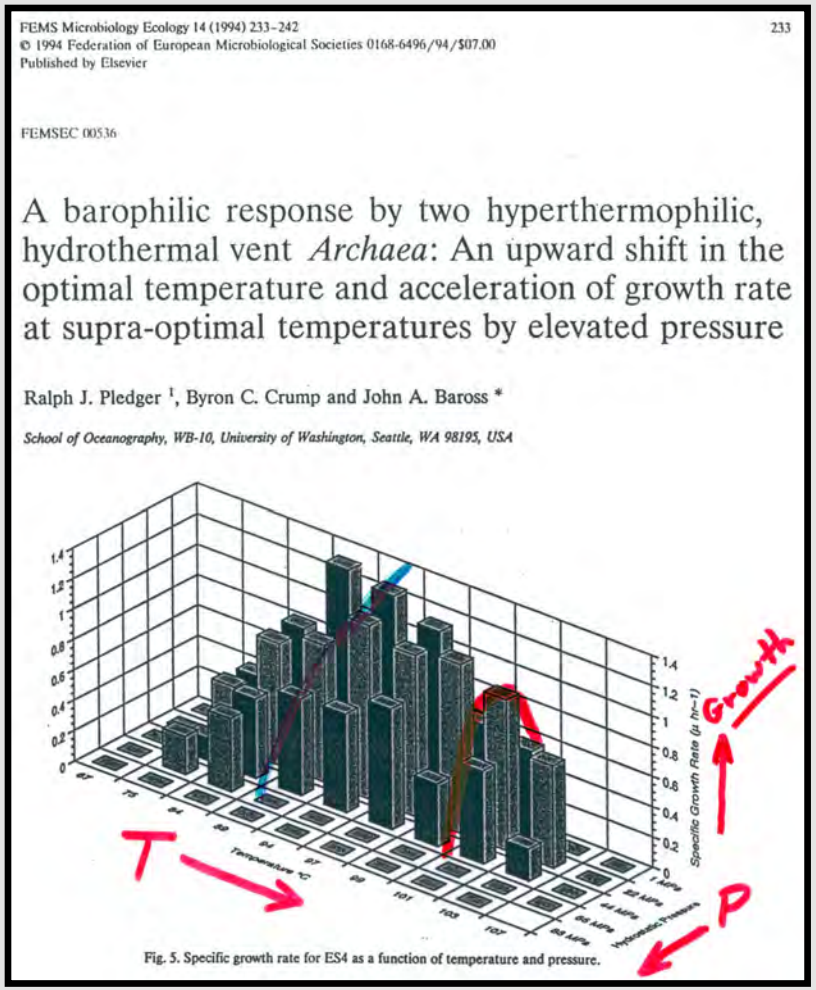
I. What is the Nature and Distribution of Life at High-Pressure?

Deep life, especially microbial life, is abundant throughout the upper crust .

II. What are the Extreme Pressure Limits of Life?

How deep might microbes exist in Earth's crust?

Pressure Can Enhance T Stability



FEMS Microbiology Ecology 14 (1994) 233–242 233
© 1994 Federation of European Microbiological Societies 0168-6496/94/$07.00
Published by Elsevier

FEMSEC 00536

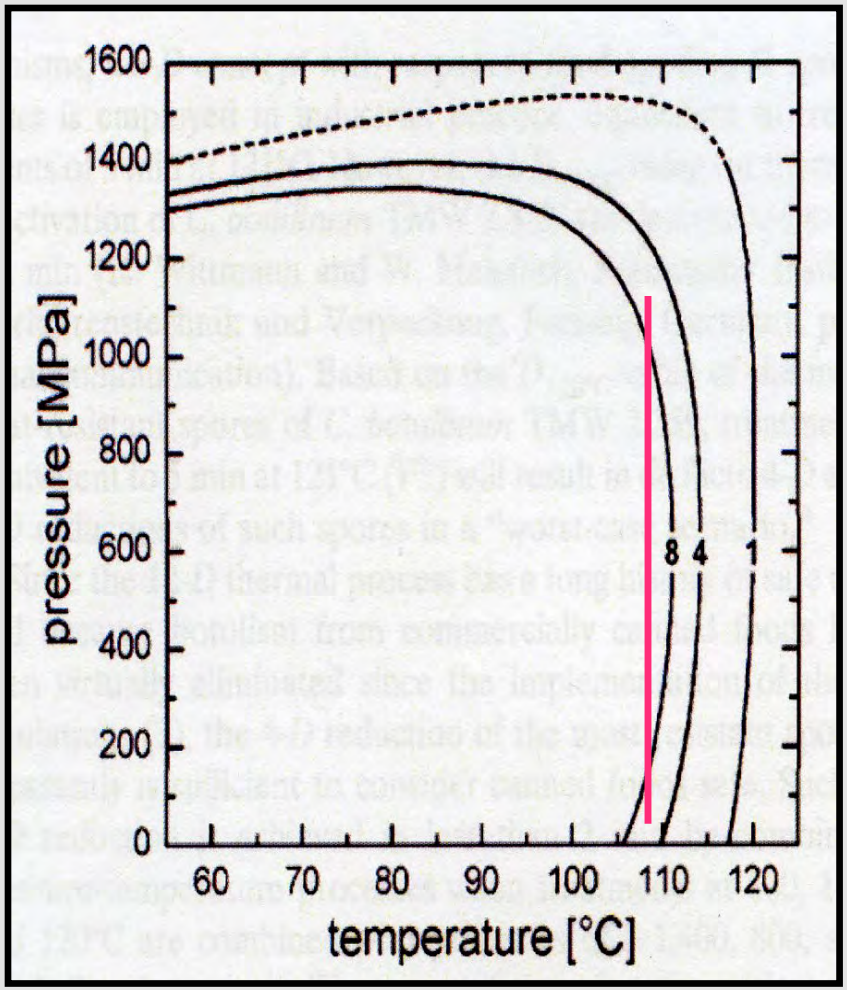
A barophilic response by two hyperthermophilic, hydrothermal vent *Archaea*: An upward shift in the optimal temperature and acceleration of growth rate at supra-optimal temperatures by elevated pressure

Ralph J. Pledger [1], Byron C. Crump and John A. Baross *

School of Oceanography, WB-10, University of Washington, Seattle, WA 98195, USA

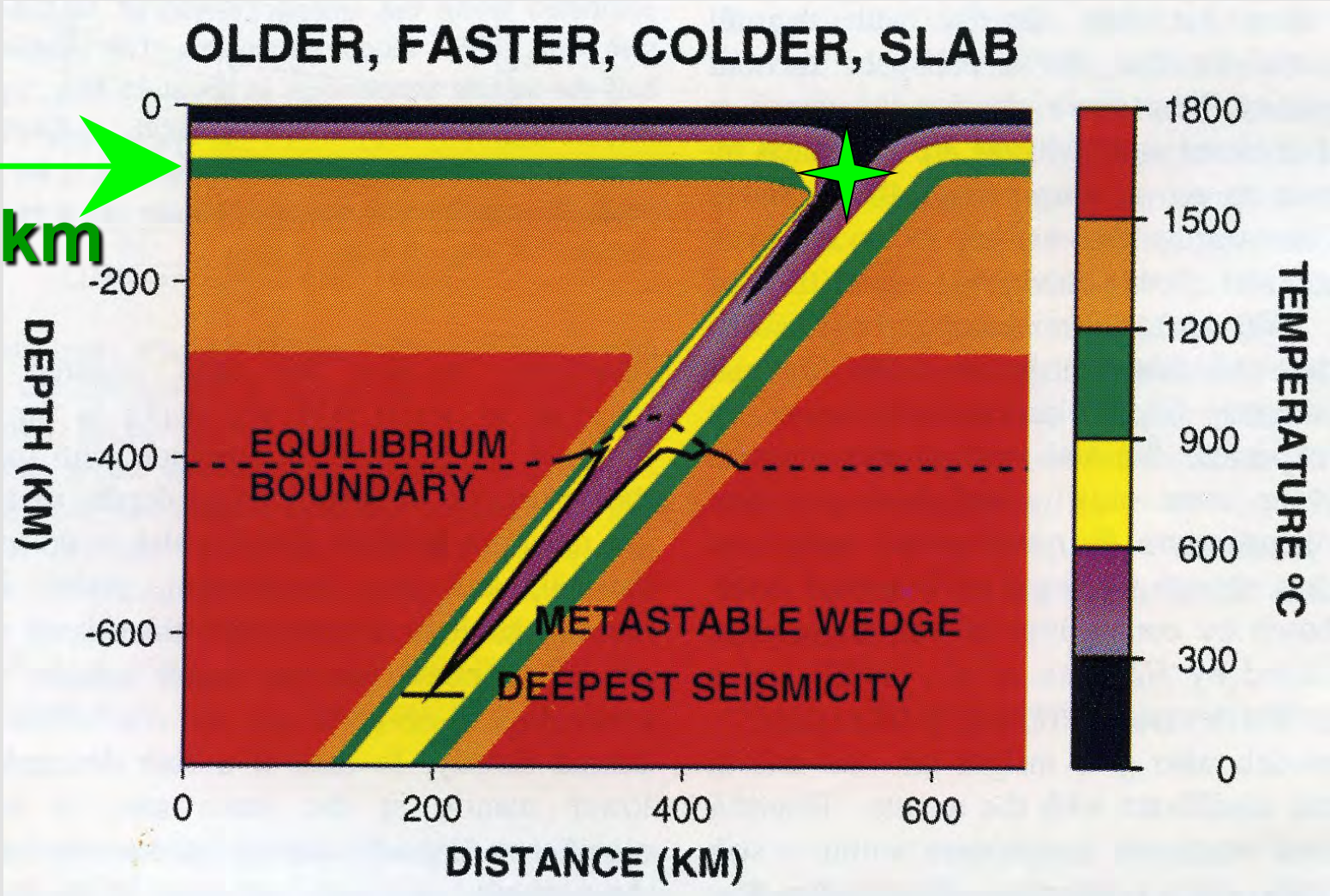
Fig. 5. Specific growth rate for ES4 as a function of temperature and pressure.

[Pledger et al. (1994) *FEMS Microbiology Ecology* 14, 233-242.]



[Margosch et al. (2006) *Appl. Environ. Microbiology* 72, 3476-3481.]

Life's Extreme Pressure Limits

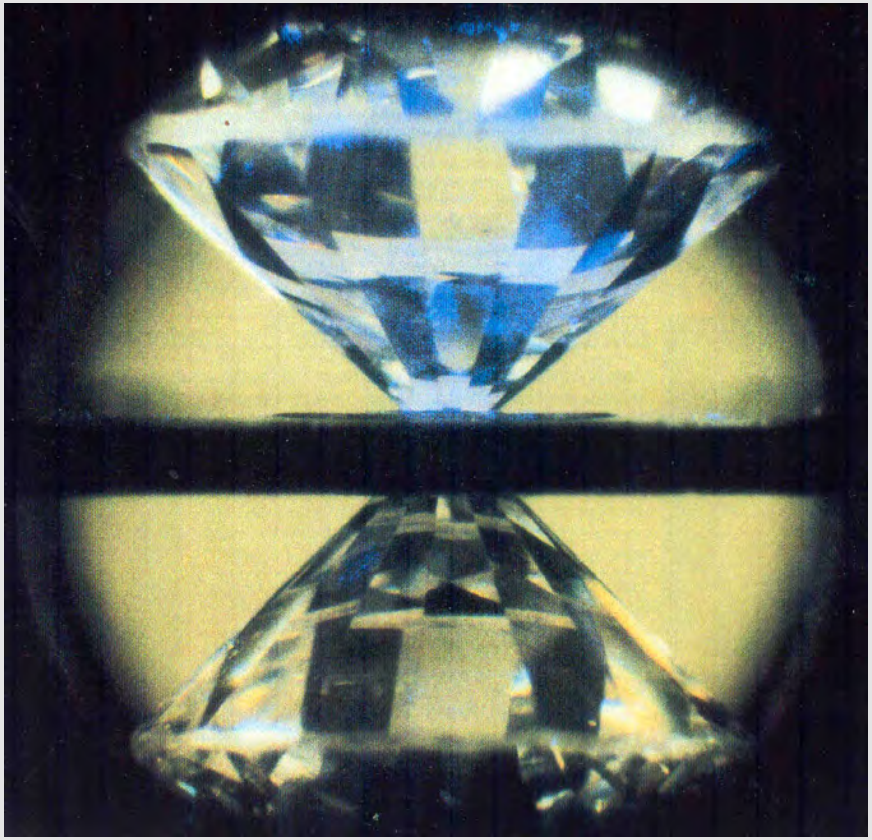


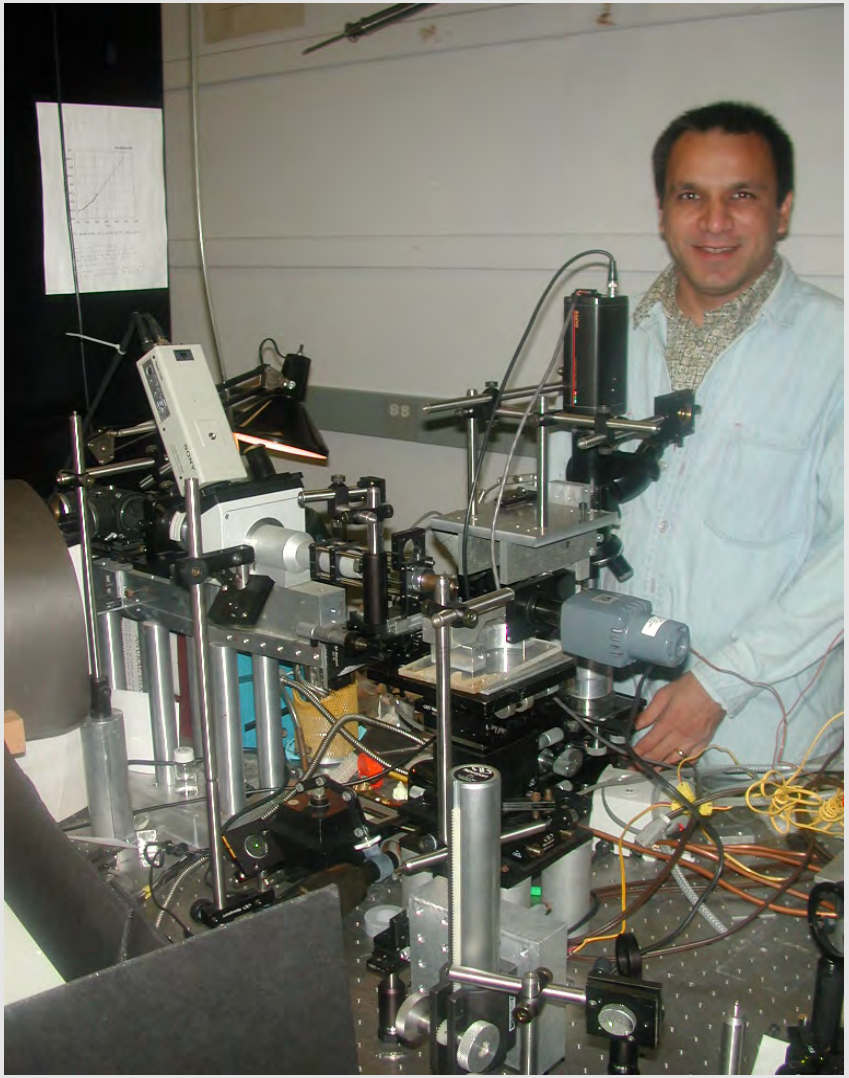
Fast, cold slabs have regions of P > 2 GPa & T < 150°C

[Stein & Stein (1996) AGU Monograph 96]

Pressure Limits of Life

Hydrothermal Opposed-Anvil Cell

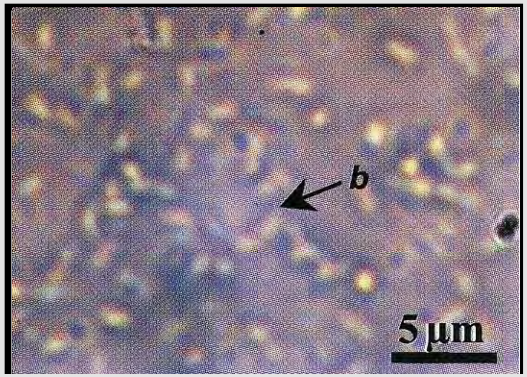




Pressure Limits of Life

E. coli

1 atm



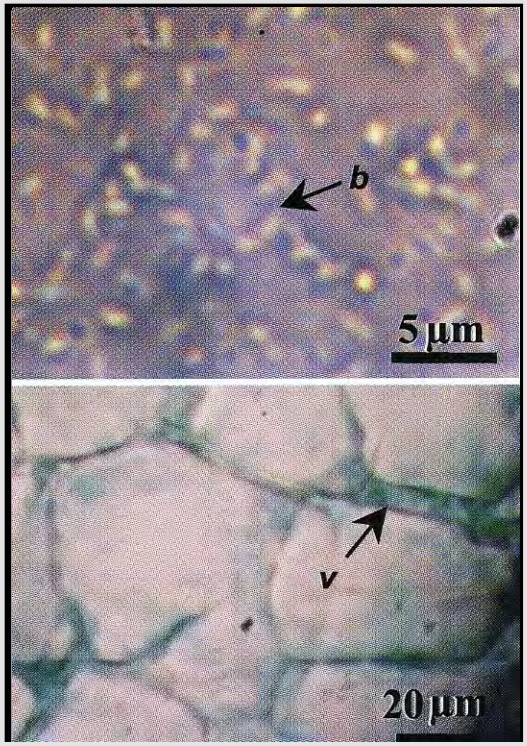
Microbes and their environment can be probed optically at pressure.

[Sharma et al. (2002) Science 295, 1514-1516]

Pressure Limits of Life

E. coli

1 atm



1.4 GPa
1 hour

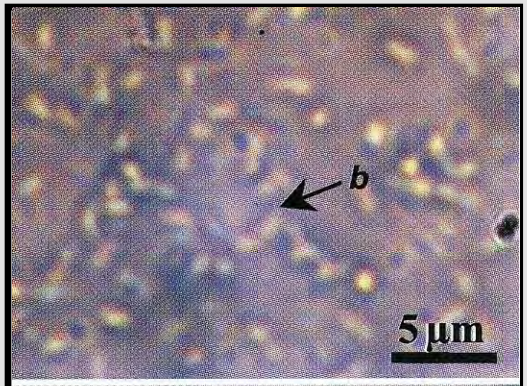
Ice VI forms at P > 1.0 GPa & 25°C. Microbes persist at triple junctions

[Sharma et al. (2002) Science 295, 1514-1516]

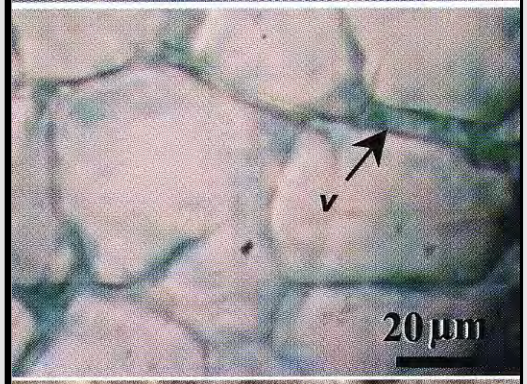
Pressure Limits of Life

E. coli

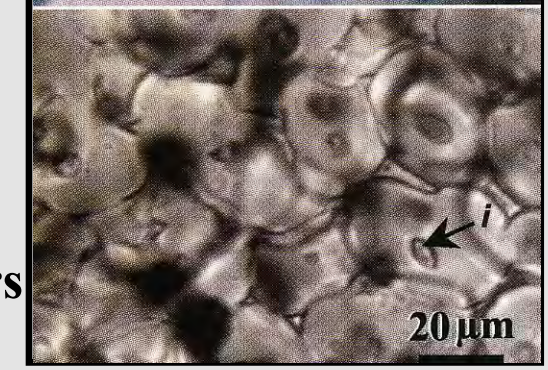
1 atm



1.4 GPa
1 hour



1.4 GPa
>30 hours



Raman measurements show formate reduction to P > 1.4 GPa.

Microbes shape their icy environment and viable microbes are recovered after several days at 1.4 GPa & 25°C.

[Sharma et al. (2002) Science 295, 1514-1516]

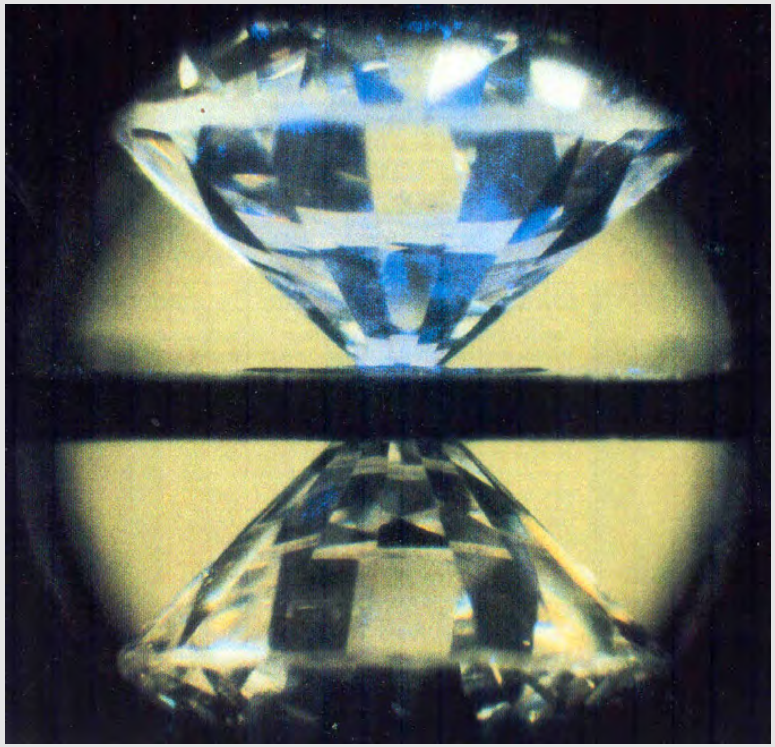
II. What are the Extreme Pressure Limits of Life?

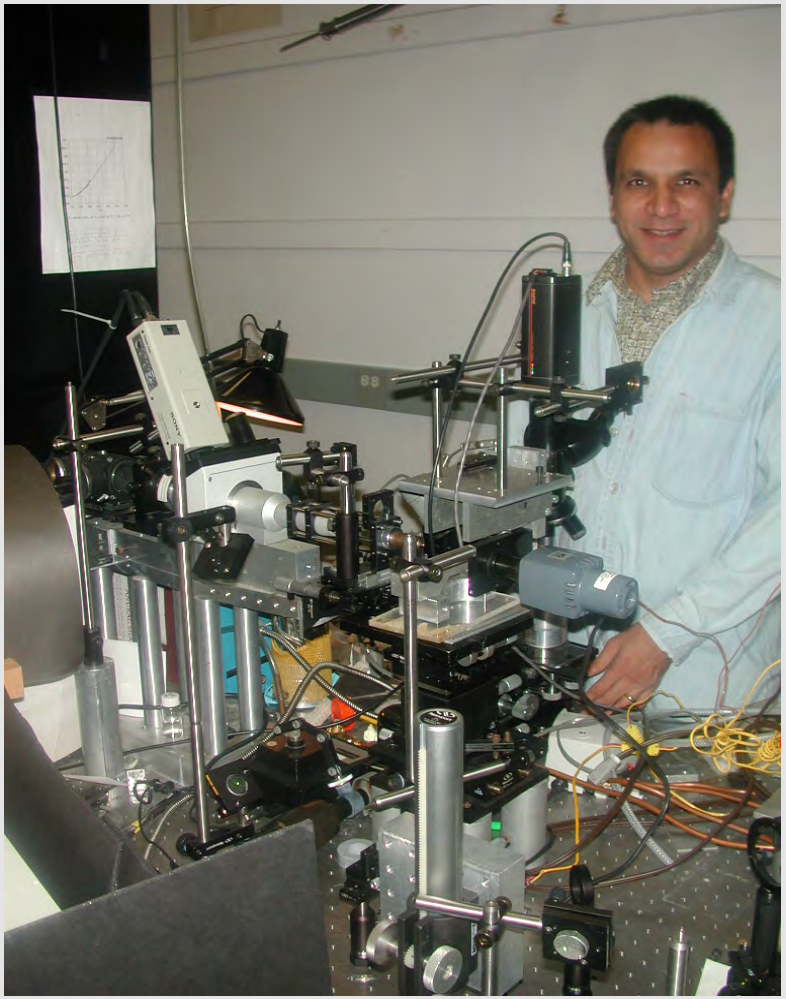
Some microbes survive at P > 1.4 GPa (~14,000 atm).

Temperature limits are not known, but pressure enhances T-tolerance for some microbes.

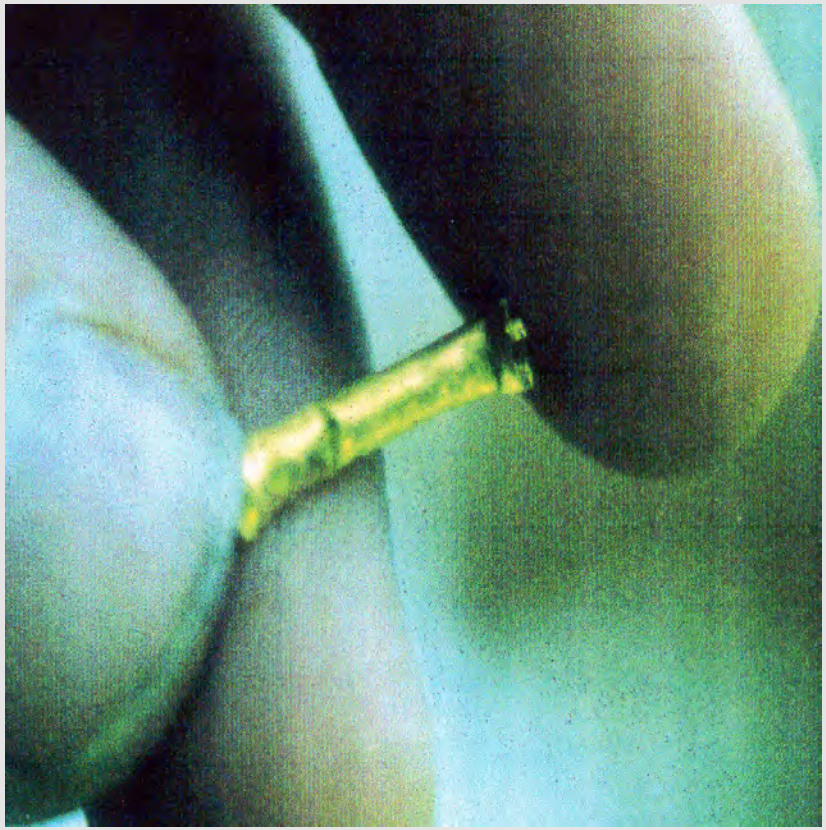
III. Techniques for Studying Microbes at High Pressure

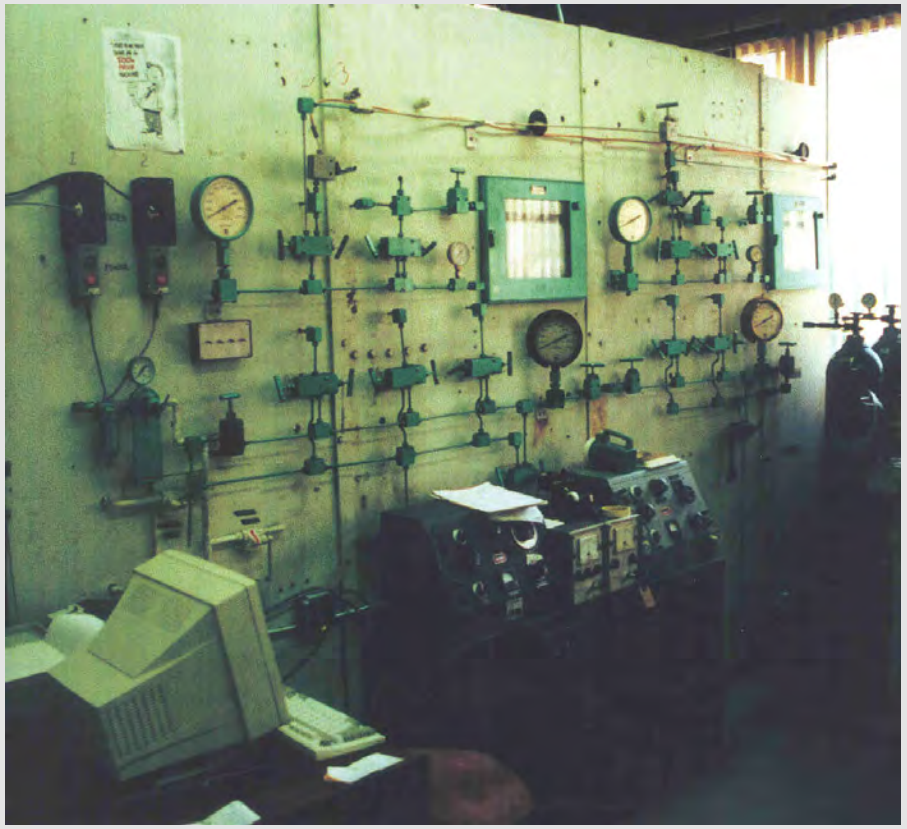
Hydrothermal Opposed-Anvil Cell





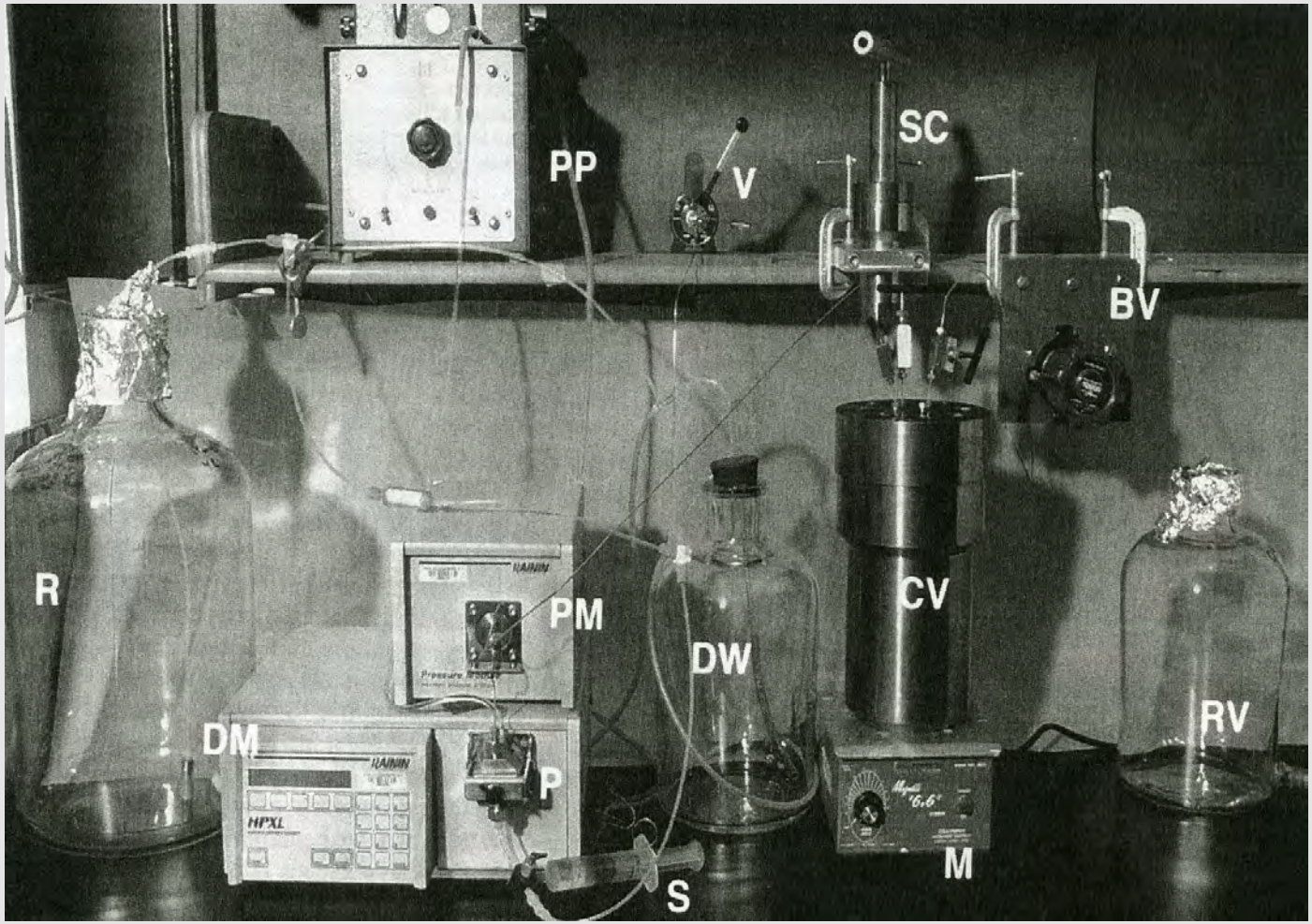
High-Pressure Techniques





Gold tube reactors in hydrothermal bombs

High-Pressure Techniques



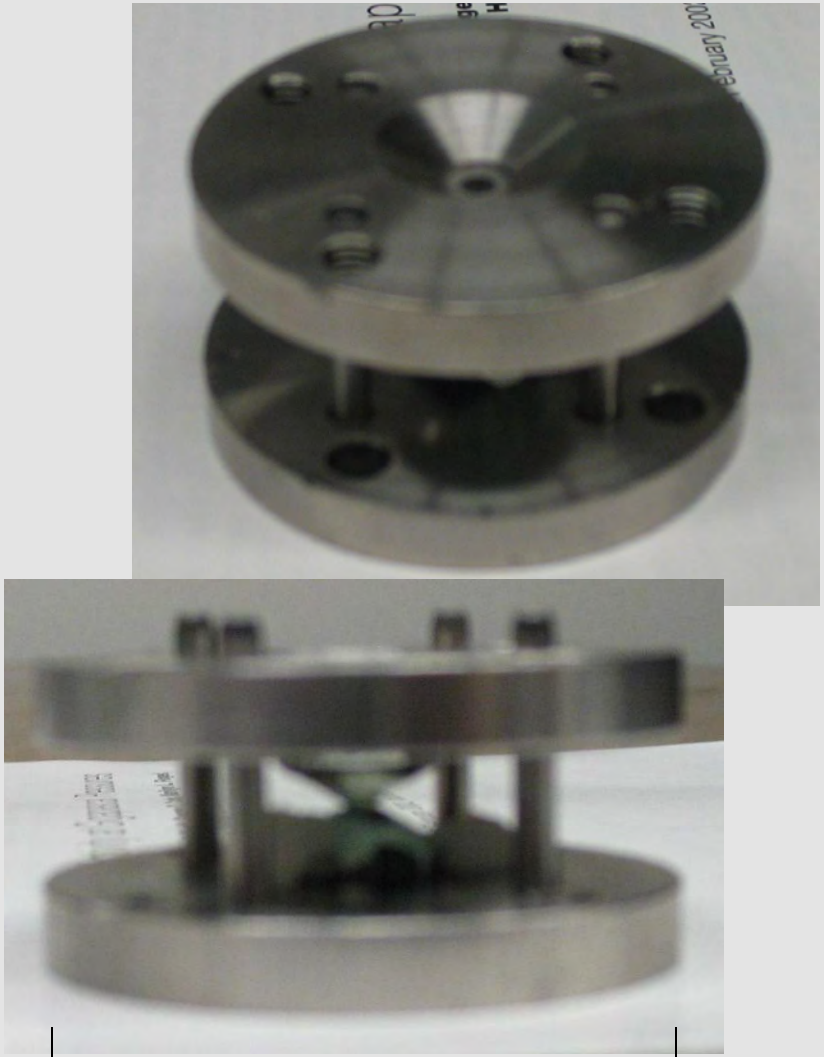
Flow-through Reactor

[Jannasch et al. (1996) Appl. & Environ. Microbiology 62, 1593-1596]

High-Pressure Techniques

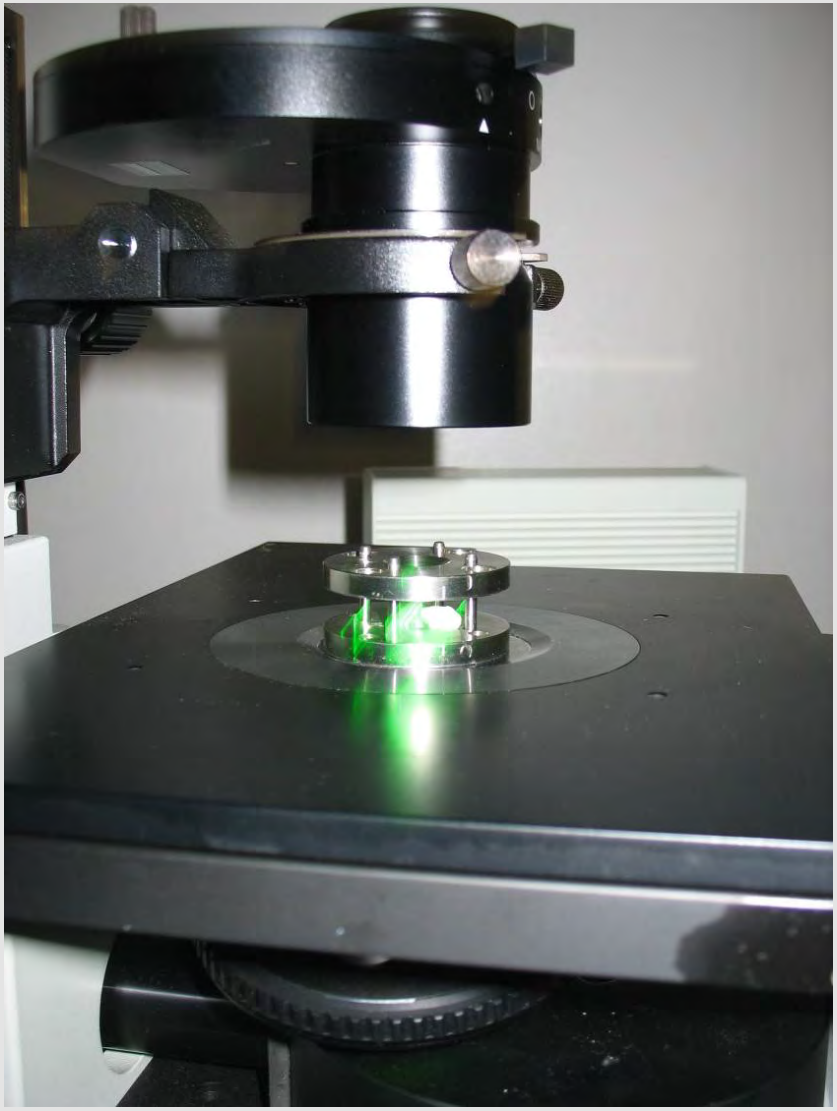
Flow-through Reactor

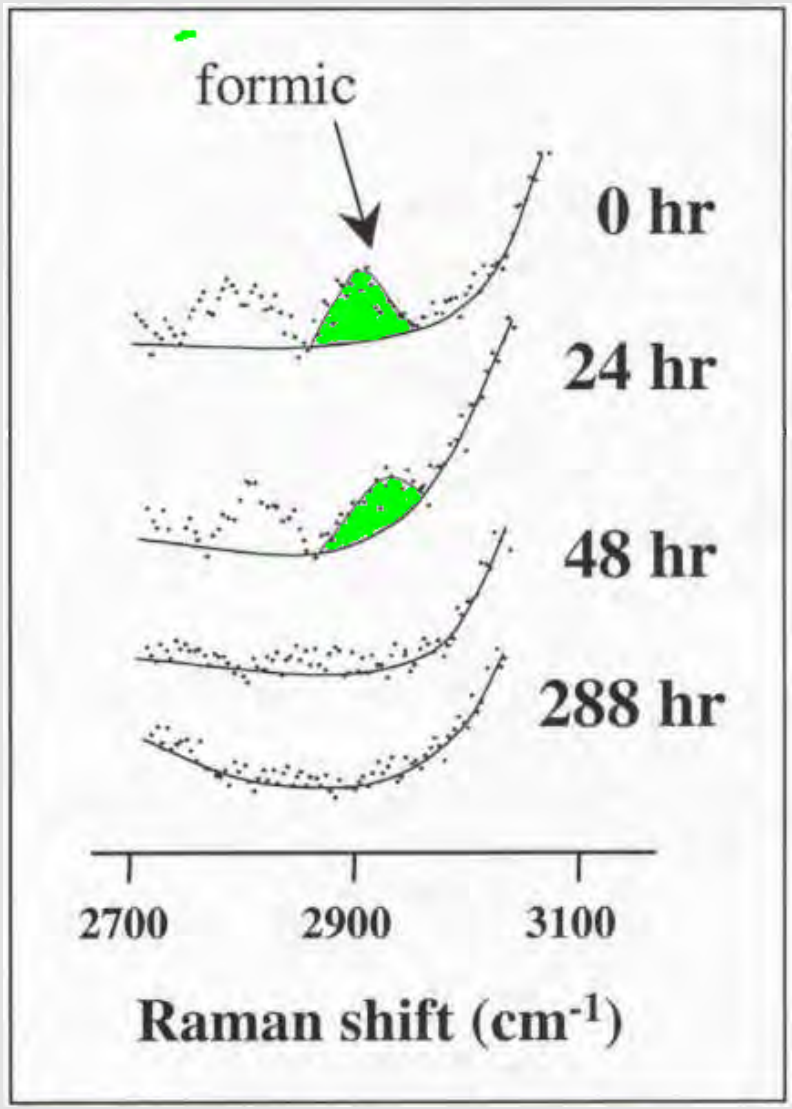
New Opposed Anvil Cell



- "Large" volume:
  - 100 mm^3 at 200 MPa
  - 10 mm^3 at 500 MPa
- Easy optical access
- Rapid loading
- Heating options
- Cryo-quenching
- Inexpensive
  - Moissanite (SiC) anvils
  - Four-screw design
  - Aluminum gaskets

New Opposed Anvil Cell





Microbe Pressure Cell Array

Studies employ ~50 pressure cells

Microbe Pressure Cell Array

Pressure ↓

Pressure ↓

Redox Potential →

Microbe Pressure Cell Array

Pressure ↓

Pressure ↓

pH →

Microbe Pressure Cell Array

Pressure ↓

Pressure ↓

Temperature →

Advantages of Pressure Array

In situ **observations: optical, Raman**

Significant sample volume

Multiple variables: nutrients, redox, pH, temperature, minerals, consortia

The anvil cell environment may mimic deep pockets (essentially a closed system).

IV. What Pressure Effects on Biomolecules Might We Study?

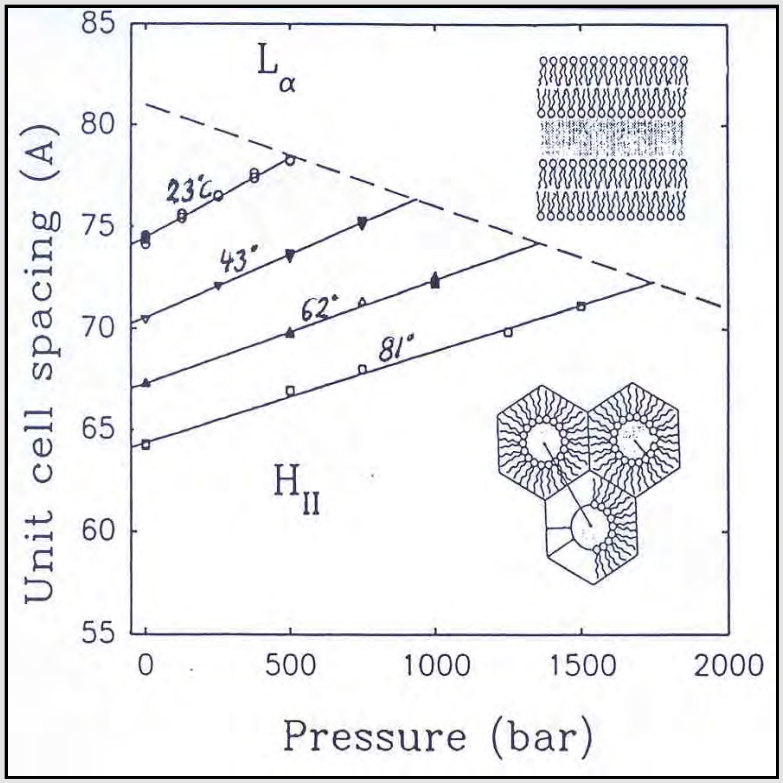
1. Pressure induced phase transitions
2. Volumes of reactions
3. Activation volumes
4. Pressure effects on enzymes
5. Diffusion rates and membrane permeability

1. Pressure Induced Phase Transitions

VOLUME 70, NUMBER 22 PHYSICAL REVIEW LETTERS 31 MAY 1993

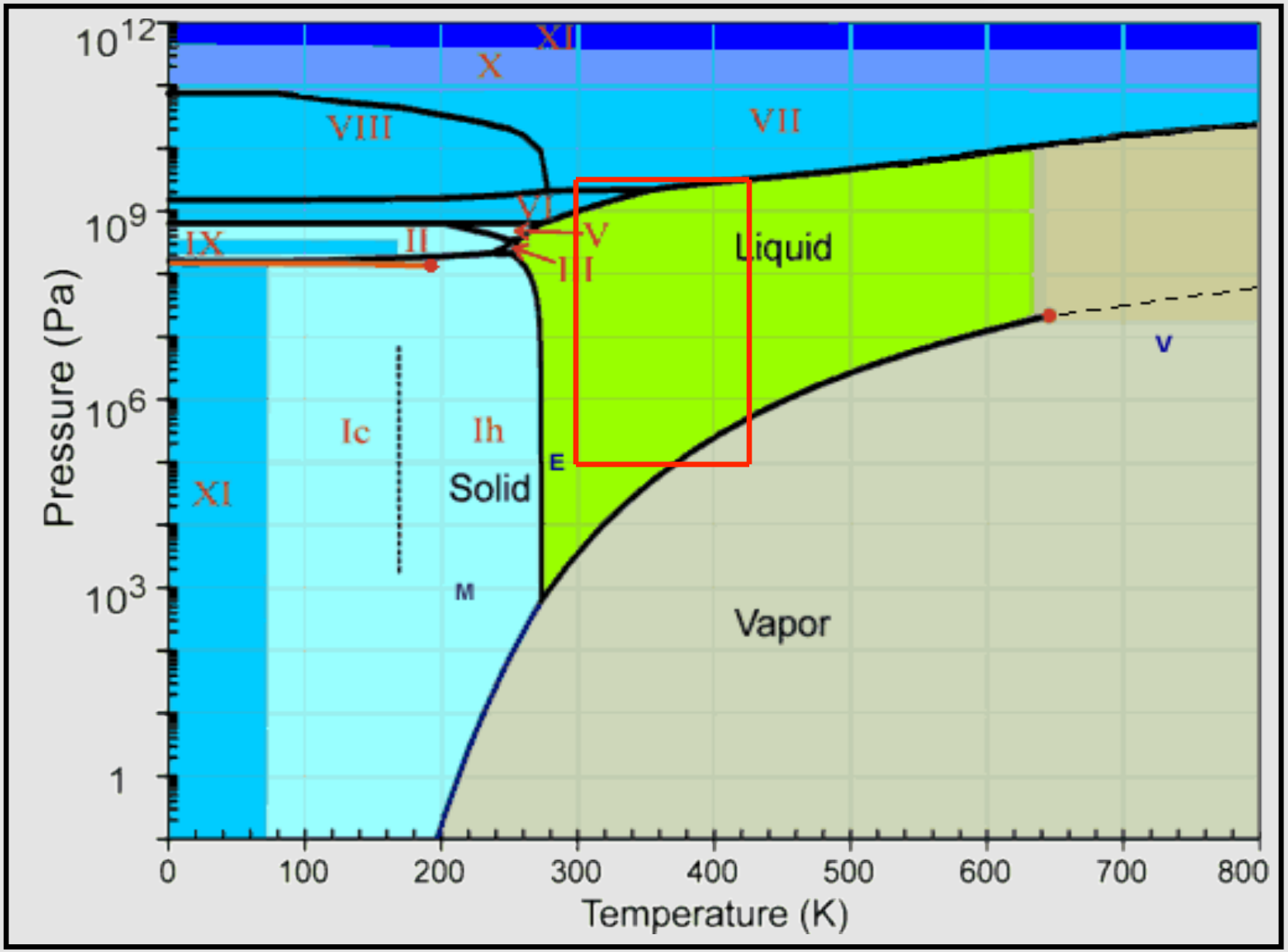
Pressure-Induced Topological Phase Transitions in Membranes

P. T. C. So,[a] Sol M. Gruner, and Shyamsunder Erramilli[b]
Department of Physics, Joseph Henry Laboratories, Princeton University, P.O. Box 708, Princeton, New Jersey 08544
(Received 16 October 1992)



Pressure induces phase transitions in membrane-forming lipid molecules.

Ice VI and VII



2. Volumes of Reaction, ΔV

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

Pressure may suppress reactions if $\Delta V > 0$.

2. Volumes of Reaction, ΔV

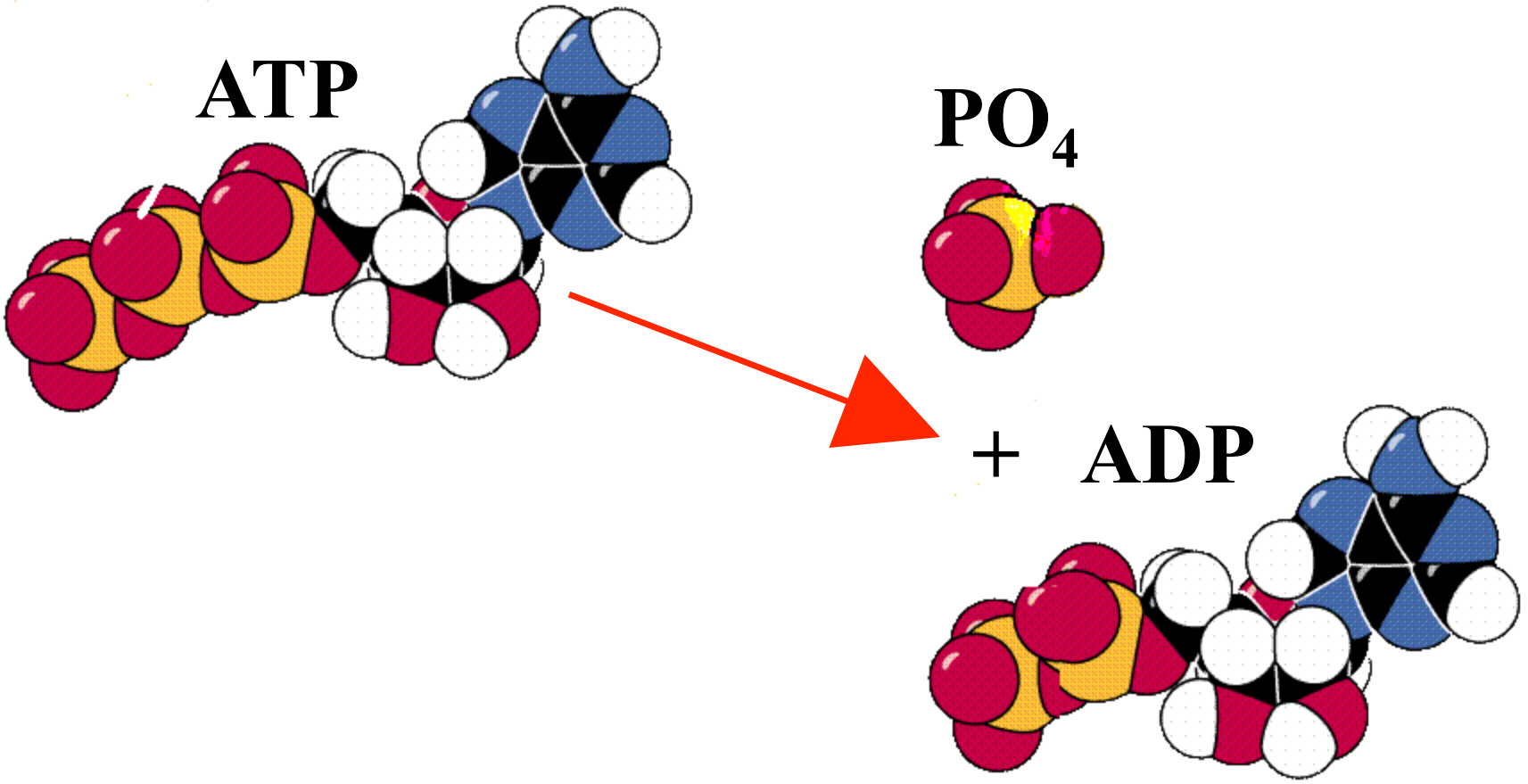
Polyphosphate cleavage (ATP → ADP)

$$H_4P_2O_7 + H_2O \rightarrow 2H_3PO_4$$

$$\Delta V \sim +15 \text{ cc/mol}$$

for $P < 1$ GPa and $T < 200°C$

2. Volumes of Reaction, ΔV



2. Volumes of Reaction, ΔV

Pressure may enhance reactions if $\Delta V < 0$.

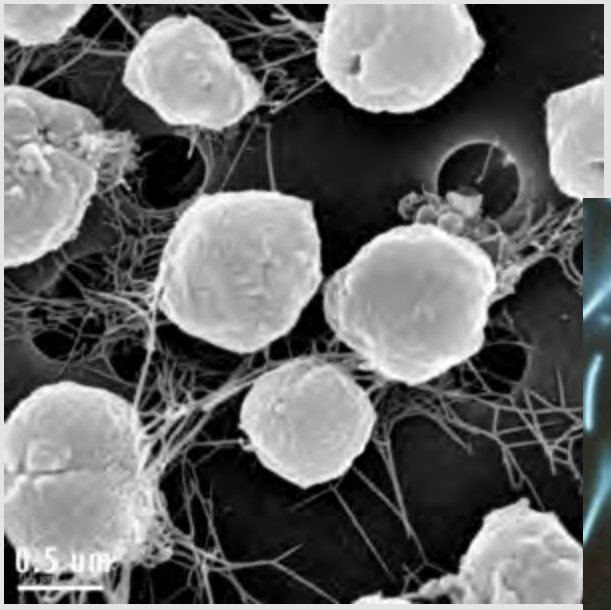
Methanogenesis:

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$$

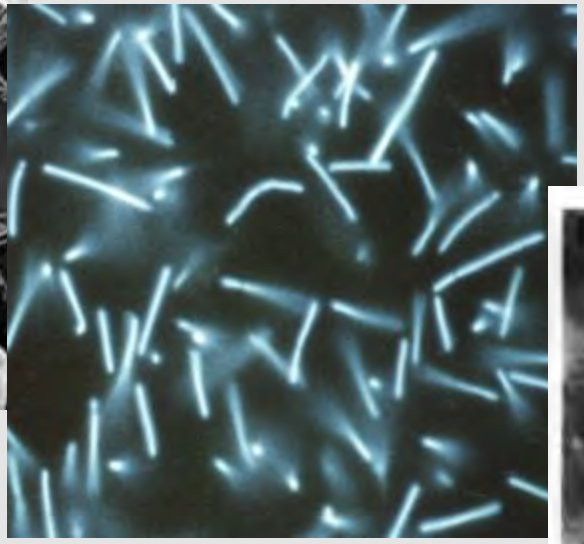
$$\Delta V \sim -60 \text{ cc/mol}$$

for $P < 1$ GPa and $T < 200°C$

2. Volumes of Reaction, ΔV



Methanococcus jannaschii



Methanopyrus kandleri

Methanothermobacter thermoautotrophicum

Methanogenesis may be enhanced by P

3. Activation Volumes

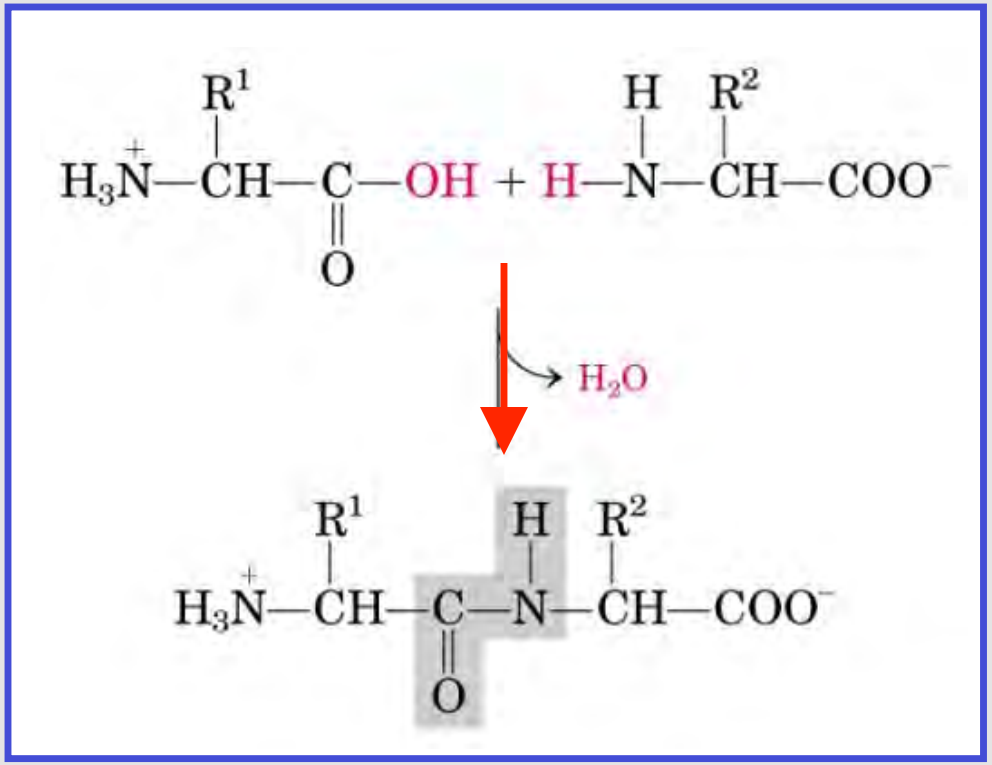
Activation volume is defined as the difference between the partial molar volumes of the initial state and the transition state:

$$\Delta V^{\ddagger} = -RT\frac{\delta \ln k}{\delta P}$$

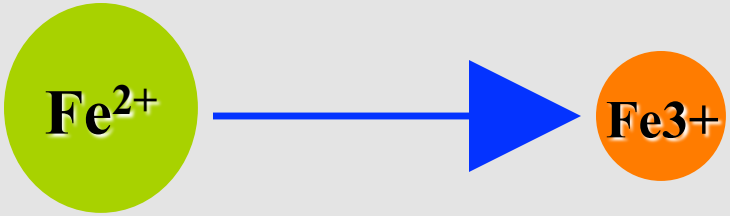
3. Activation Volumes

If activation volume is negative, then reaction rates increase with P.

Bond formation; condensation



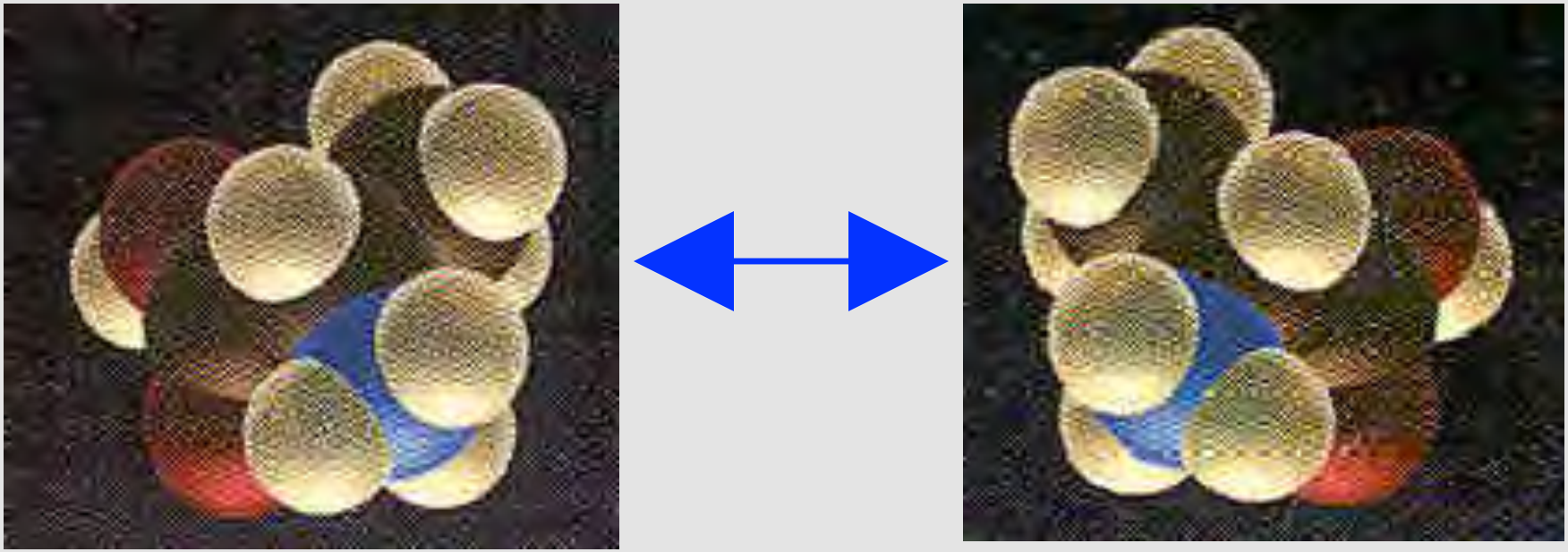
Charge concentration



3. Activation Volumes

If activation volume is positive, then reaction rates decrease with P.

Unfolding; racemization



L-ASP **D-ASP**

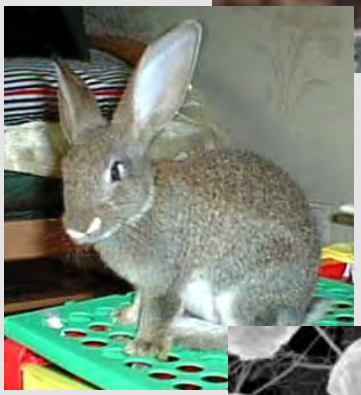
4. Pressure Effects on Activity and Denaturation of Enzymes

Fructose diphosphatase (FDPase) at 150 MPa (fish)

Hochachka et al. (1970) *Marine Biology* 7, 285-293.

Lactic dehydrogenase deactivation (rabbits)

Schmid et al. (1975) *Biophys. Chem*. 3, 90-98.

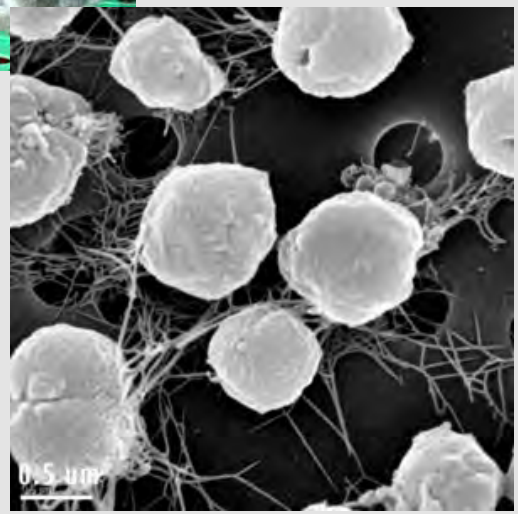


Pyrophosphatase activity (*Bacillus*)

Morita & Mathemeier (1964) *J. Bacteriology* 88, 1667-1671.

Protease activity (*Methanococcus*)

Michels & Clark (1997) Appl. Environ. Microbiology 63, 3985-3991.



4. Pressure Effects on Microbial Enzymes

Use pressure cell array to:

- **Identify and analyze stress proteins**
- **Measure metabolic rates**
- **Survey gene expression (using mRNA)**

5. Diffusion rates and Membrane Permeability

Pressure decreases diffusion rates and membrane permeability.

Pressure increases relative diffusion rate of hydrogen

Hydrogen may be an optimal energy source for long-term survival of microbes at high P.

[Morita (2000) *Microbial Ecology* 38:307-320]

Conclusions

Pressure has the potential to slow microbial metabolism by several mechanisms:

- **Positive volumes of reactions.**
- **Positive activation volumes of reactions**
- **Reduced enzyme activites**
- **Reduced diffusion rates**
- **Reduced membrane permeability**

More experimental studies are needed.

With thanks to:

NASA Astrobiology Institute
National Science Foundation
Carnegie Institution of Washington