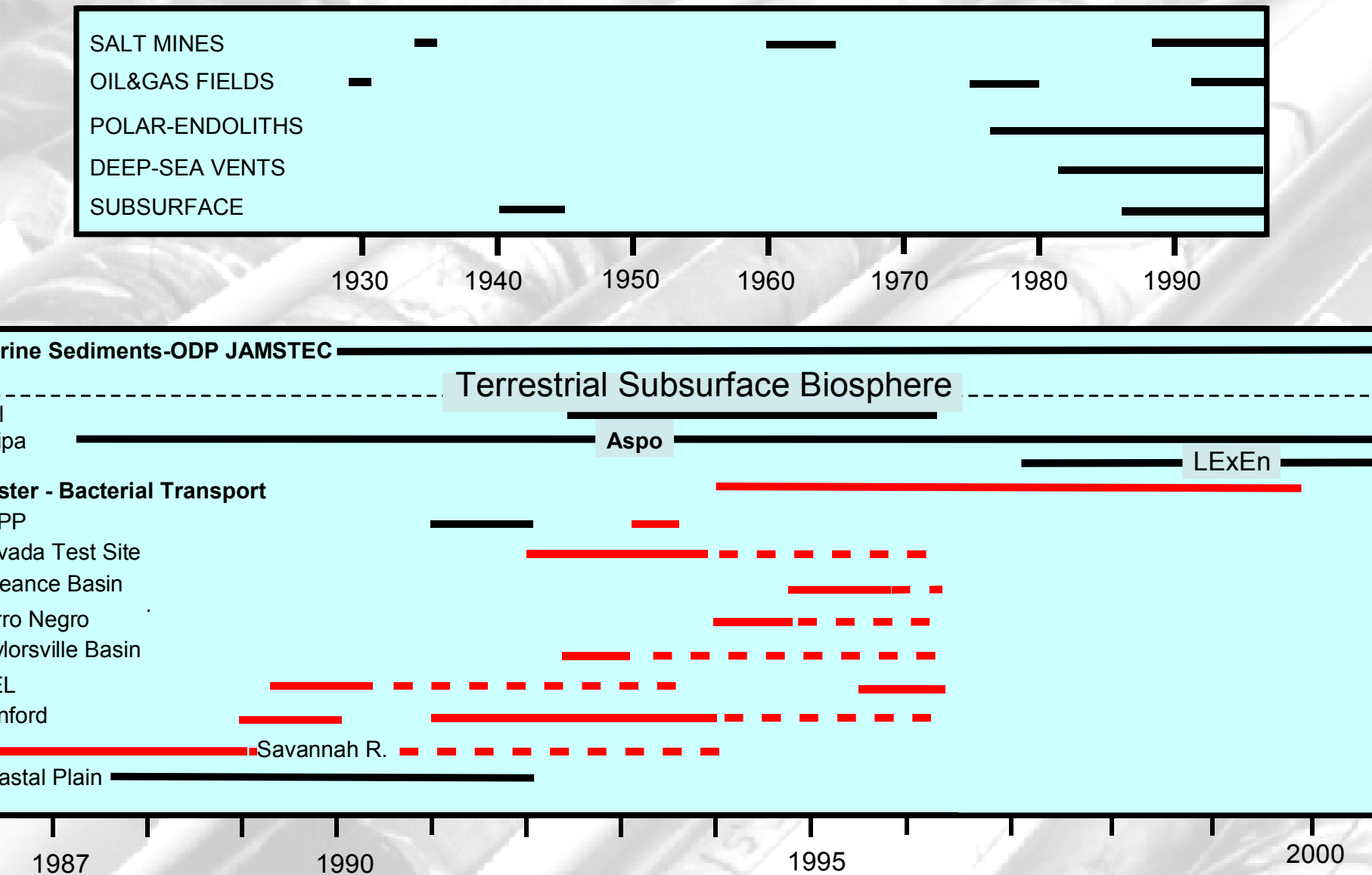
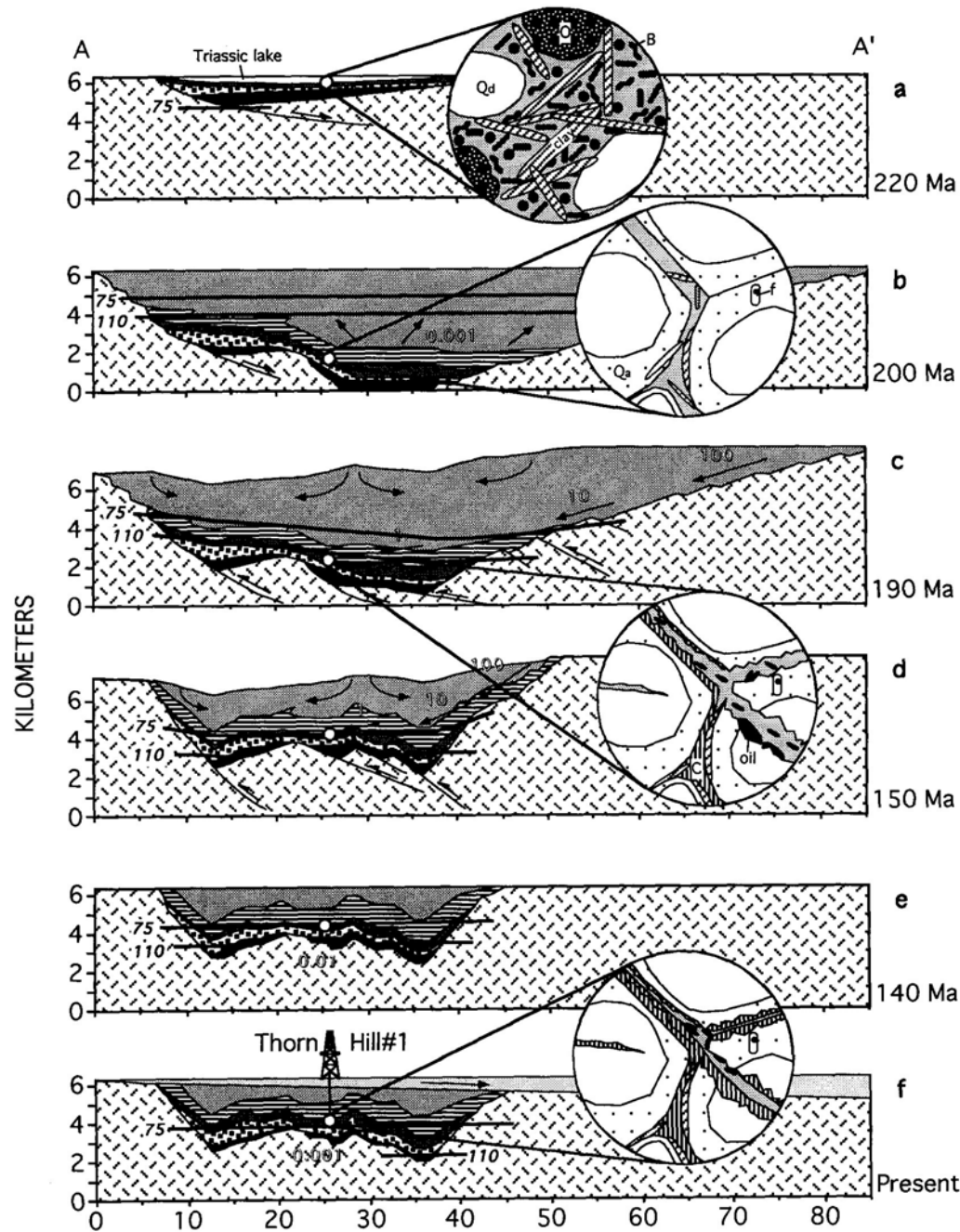


# Deep Subsurface Microorganisms in Tectonically Active Environments:

## A New Frontier?

# CHRONOLOGY OF SUBSURFACE MICROBIOLOGY





## Microbial Origins

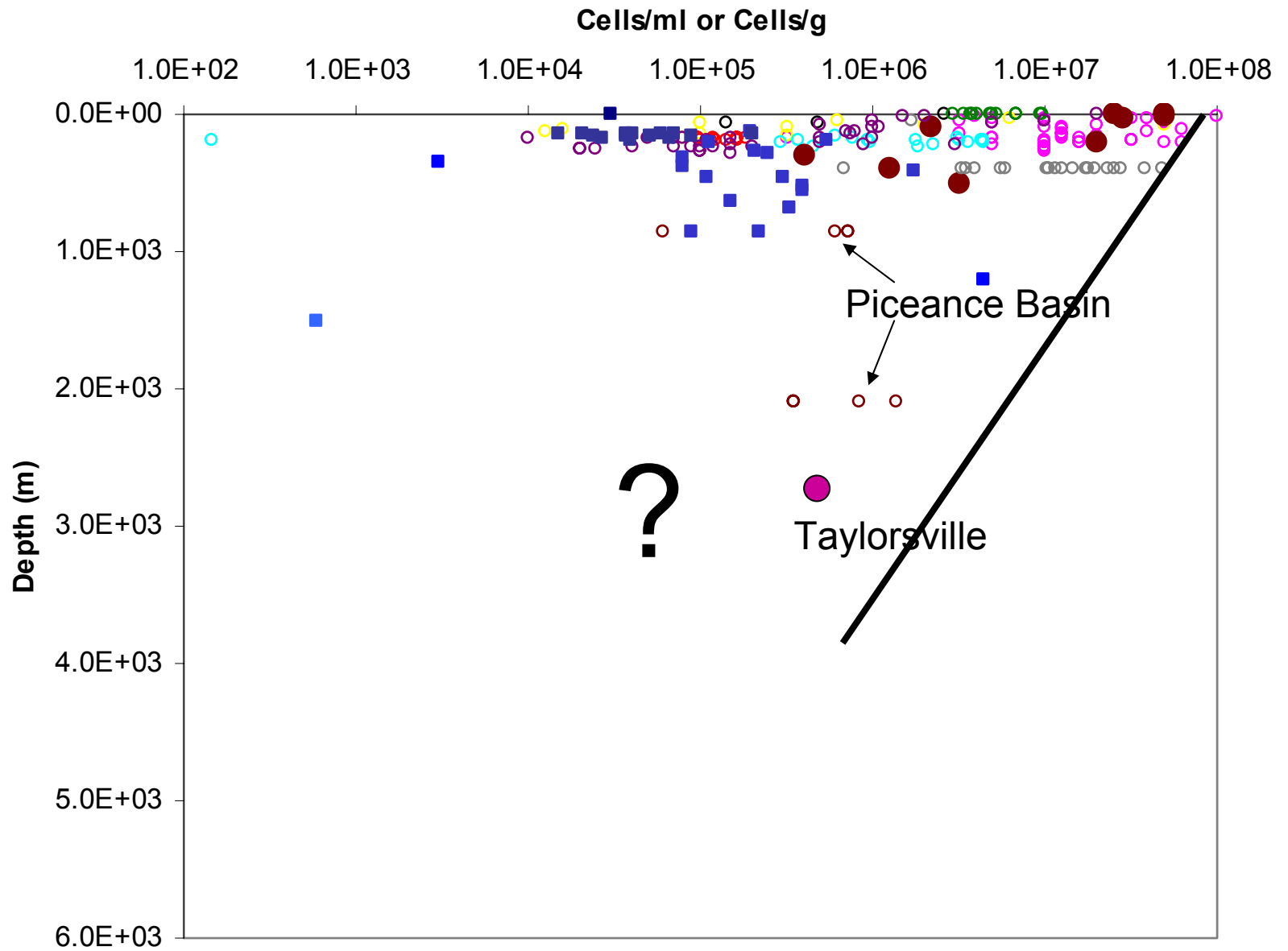
1. Growth in Triassic lacustrine sediments
2. Burial, heating and sterilization at  $T > 150^{\circ}\text{C}$ .
3. Uplift, meteoric fluid infiltration, microbial transport during Jurassic tectonism.
4. Diminishing fluid flow and permeability with increasing cementation.
5. On lap of coastal marine sand and mud hydrologically isolates basin.
6. Deep coring recovers anaerobic microorganisms.

# TECTOGENETIC ORIGIN FOR DEEP SUBSURFACE MICROORGANISMS (Tseng et al. 1996)

Orogenic uplift and cooling leads to:

1. Increased, topographically driven, fluid flow increasing microbial migration.
2. Enhanced hydraulic conductivity due to fracturing increasing microbial migration.
3. Low fluid salinity from meteoric recharge enhances microbial migration.
4. High nutrient availability in sterilized rock promotes microbial growth and hence migration during colonization.

# Cells density versus depth



# Two Questions

1. How can deep subsurface microbial communities survive tens or hundreds of millions of years separated from the photosphere?
2. Are the deep subsurface microbial and nutrient fluxes greater in tectonically active environments than in quiescent crustal or marine sedimentary environments?

# WITWATERSRAND DEEP MICROBIOLOGY PROJECT

A WINDOW INTO THE EXTREME ENVIRONMENT OF THE DEEP SUBSURFACE

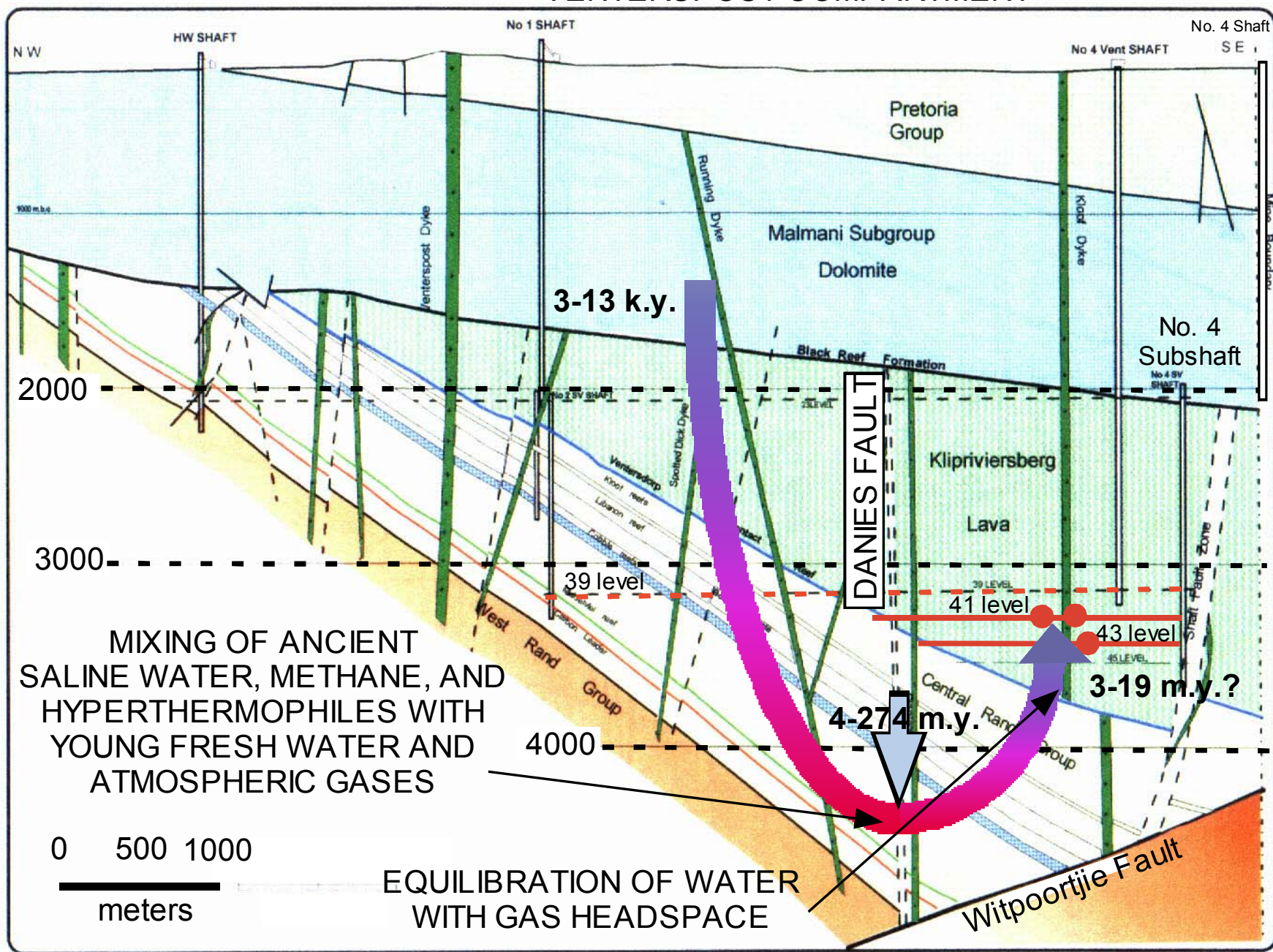


SUPPORTED BY GRANTS FROM:  
THE LEXEN PROGRAM--NATIONAL SCIENCE FOUNDATION  
THE NATIONAL GEOGRAPHIC SOCIETY

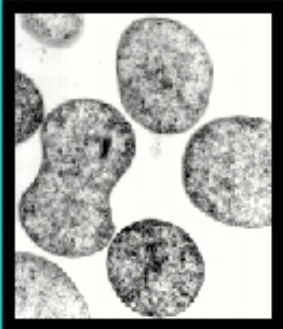


BANK COMPARTMENT

VENTERSPLOST COMPARTMENT







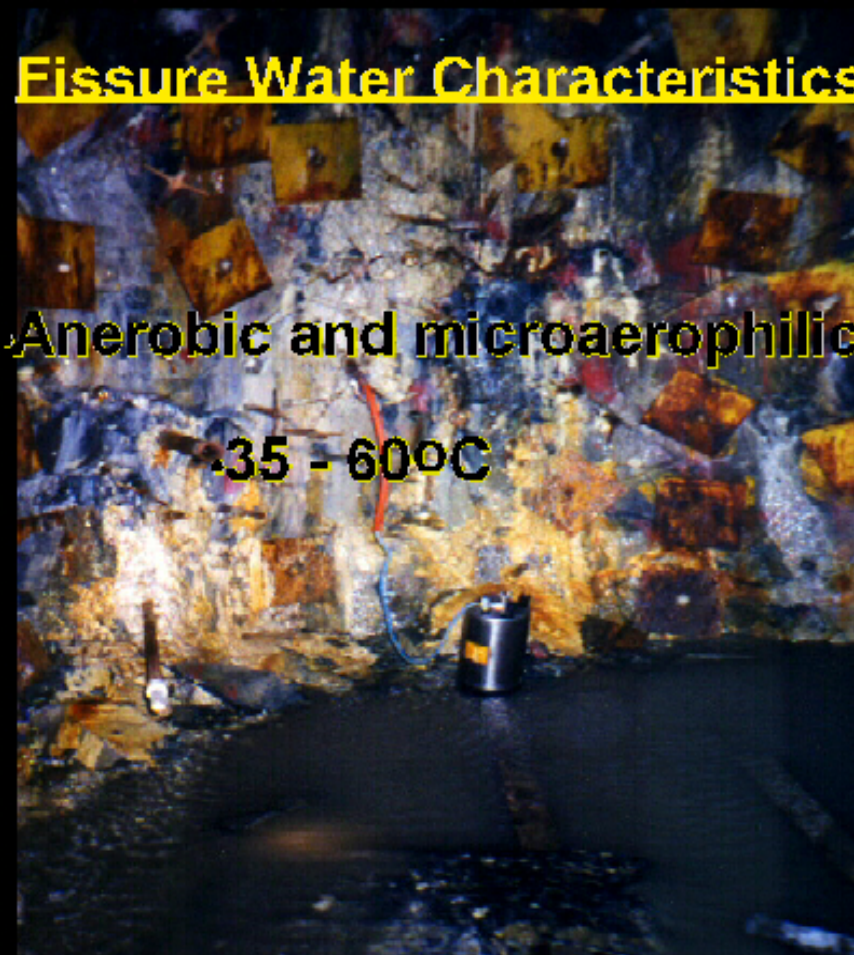
# PYROCOCCUS "FIREBALL" IN MINE FISSURE WATER

A Biological tracer of upwelling geothermal water?

## Fissure Water Characteristics

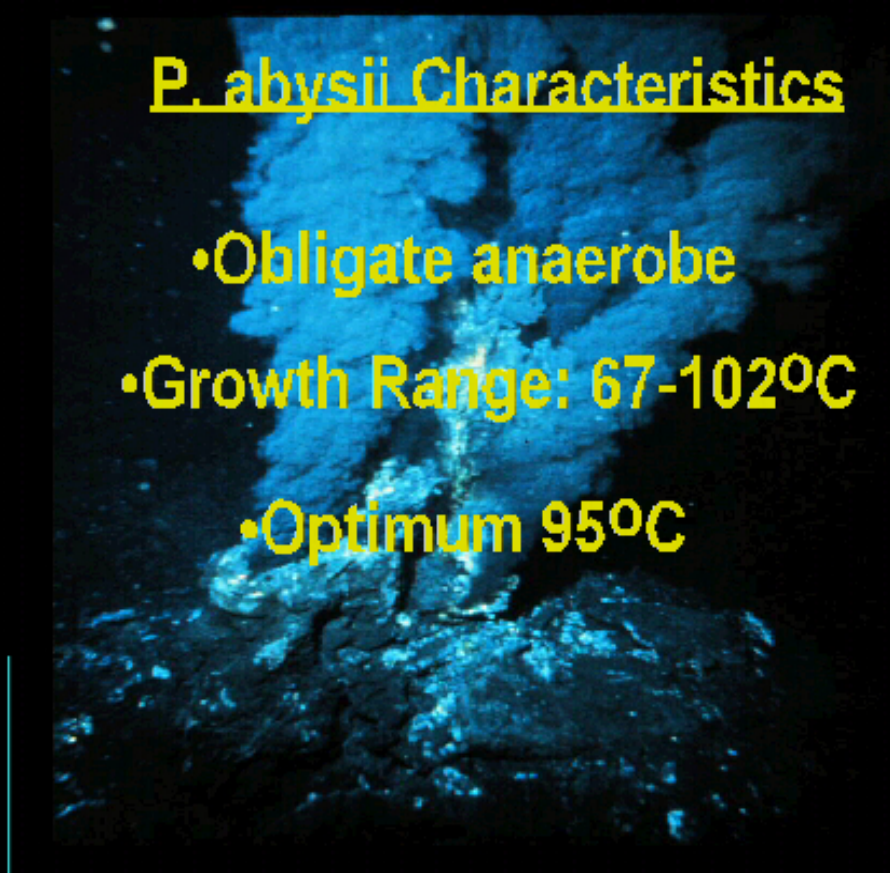
Anerobic and microaerophilic

35 - 60°C



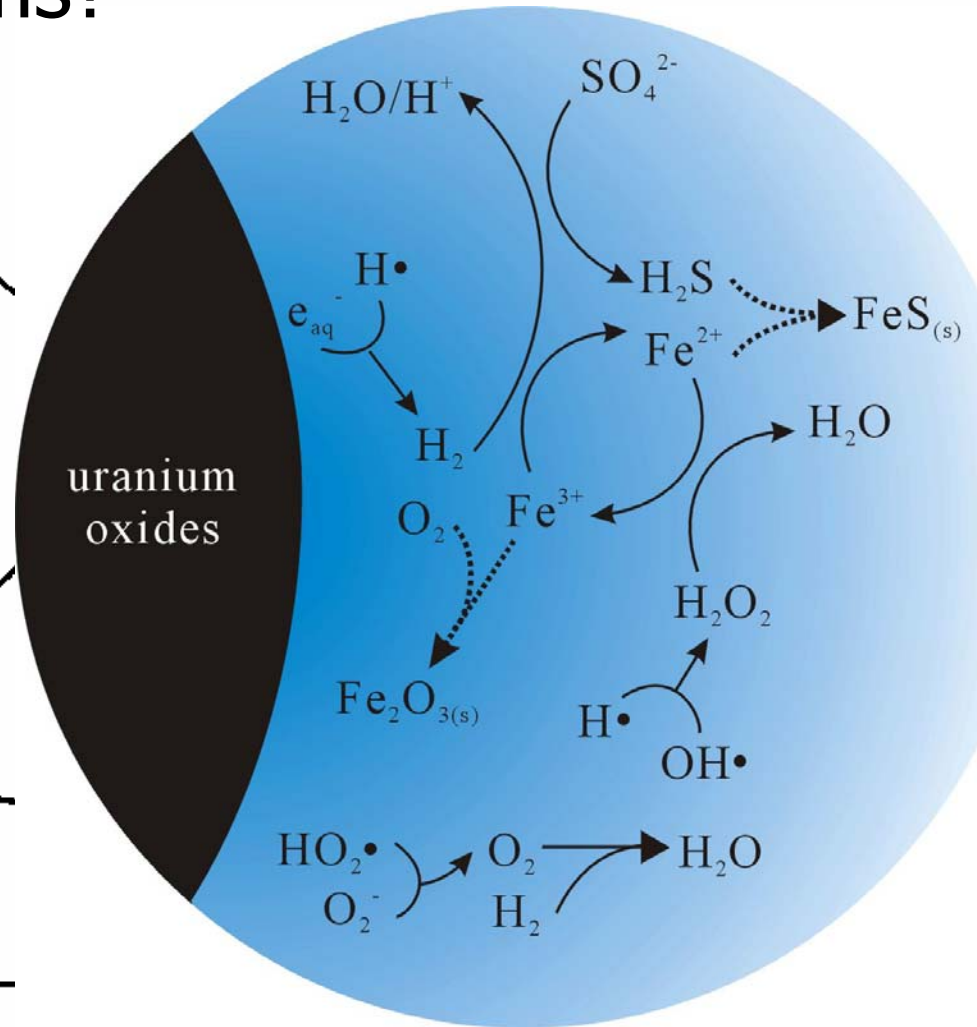
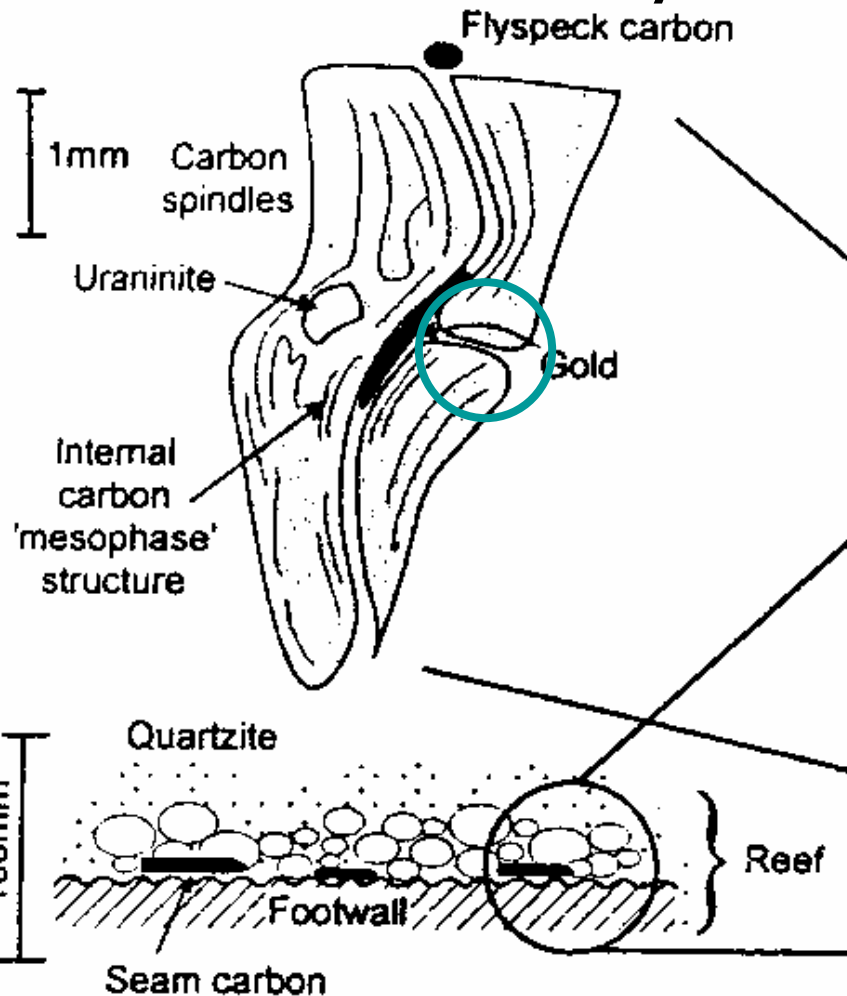
## P. abyssii Characteristics

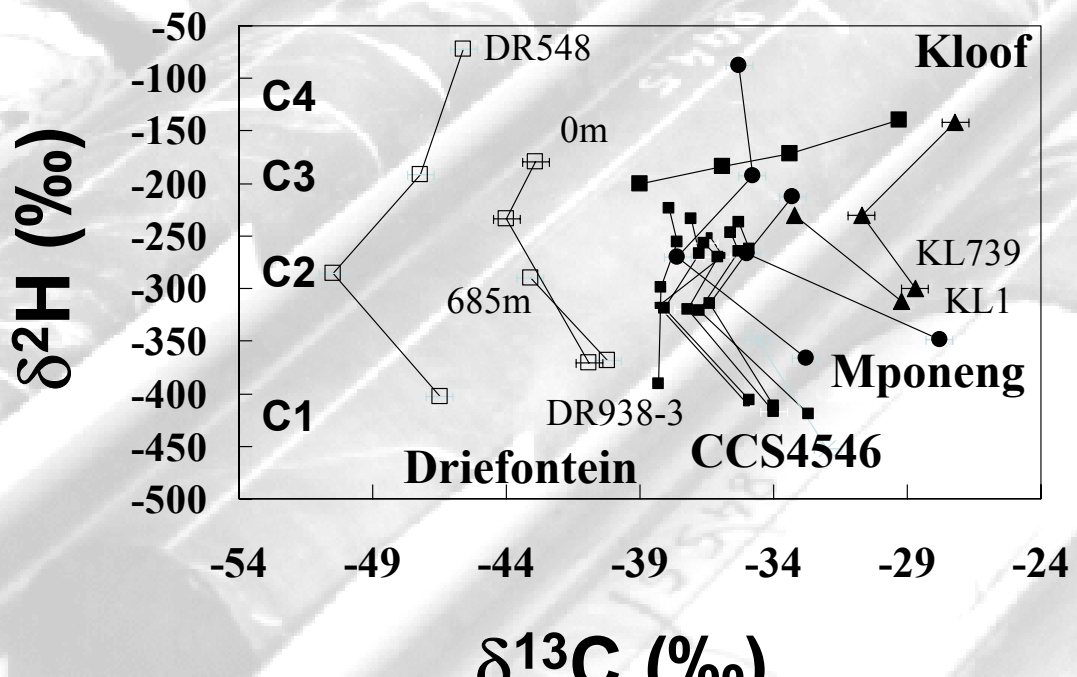
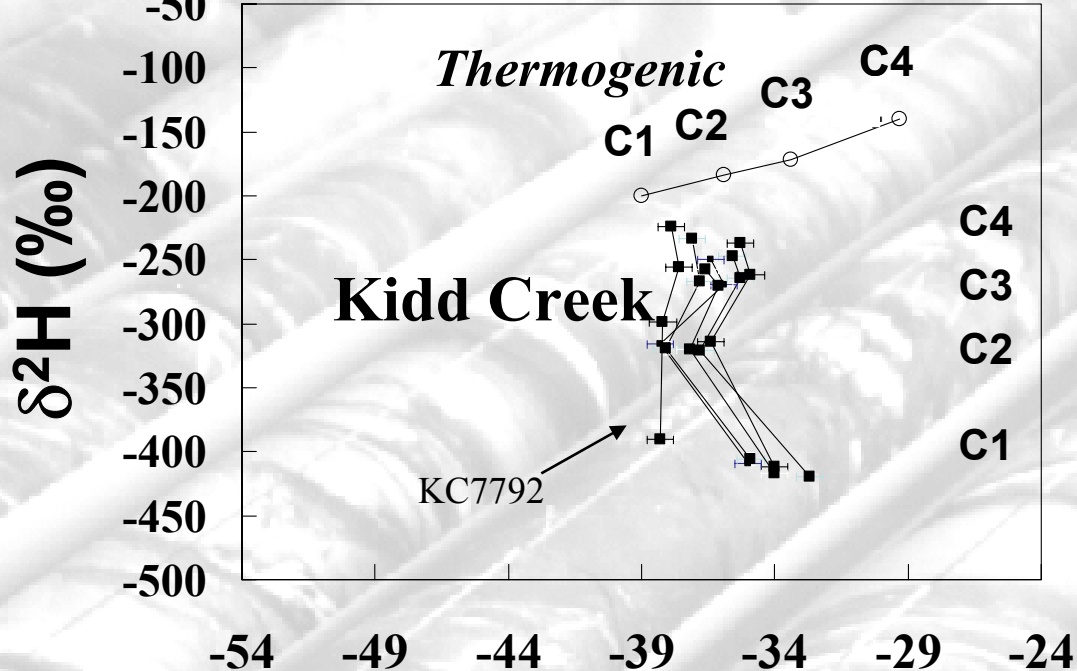
- Obligate anaerobe
- Growth Range: 67-102°C
- Optimum 95°C



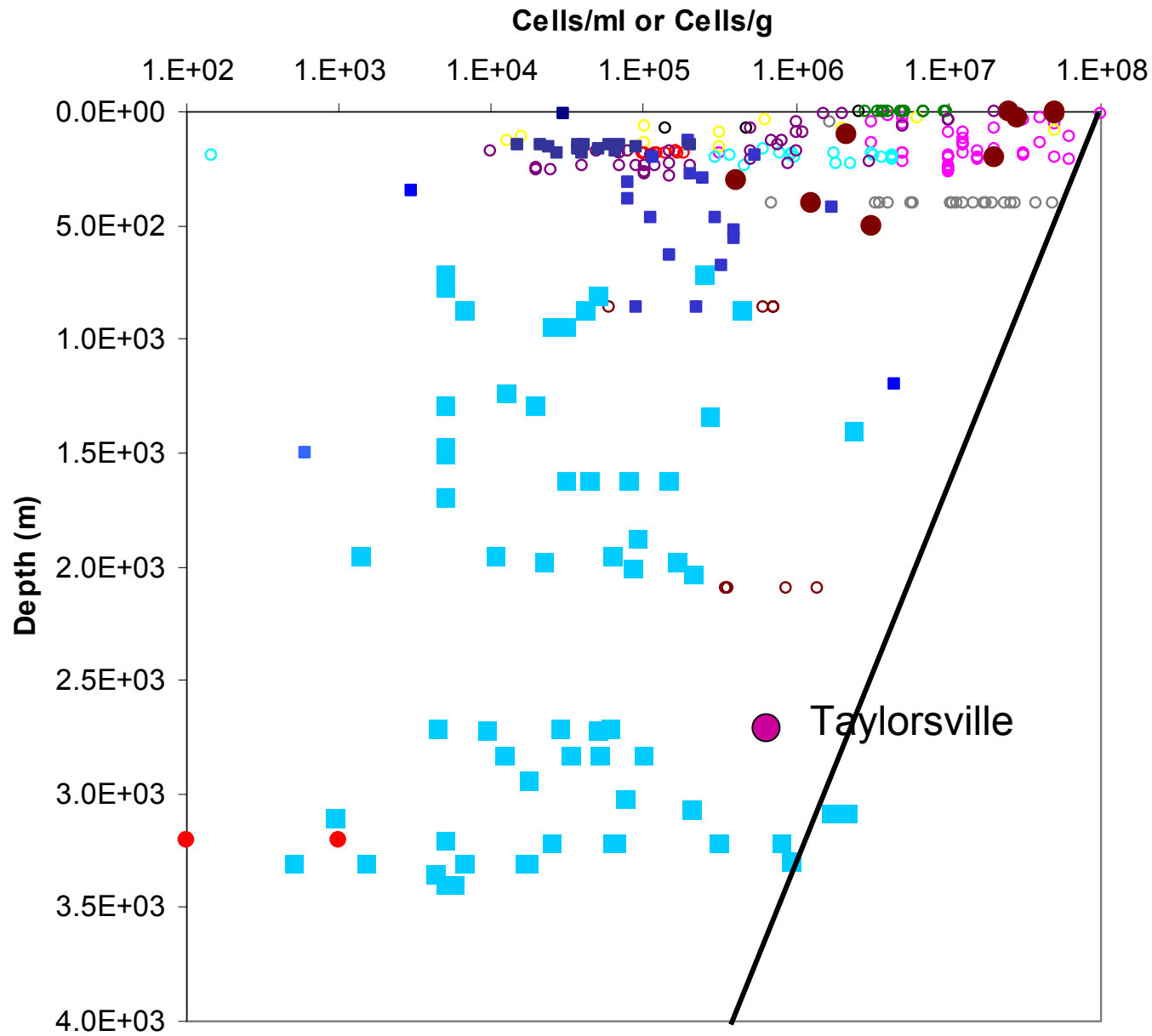
BLACK SMOKER

# Radiolytic production of $\text{H}_2$ , $\text{SO}_4^{2-}$ and $\text{Fe}(\text{OH})_3$ supporting abiogenesis and microbial ecosystems?





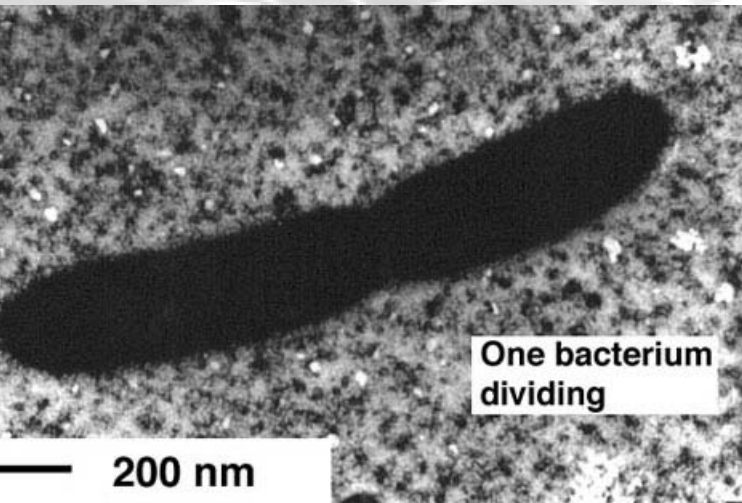
# Cells density versus depth



- Environment stable for past 50 myr. with last major uplift and microbial incursion at ~90 Ma.
- Biodiversity as defined by 16S rDNA diminishes with depth with some fractures dominated by one species of microorganism.
- Methanogens present at < 2 kmbls.
- Sulfate reducing bacteria dominate at > 2 kmbls.

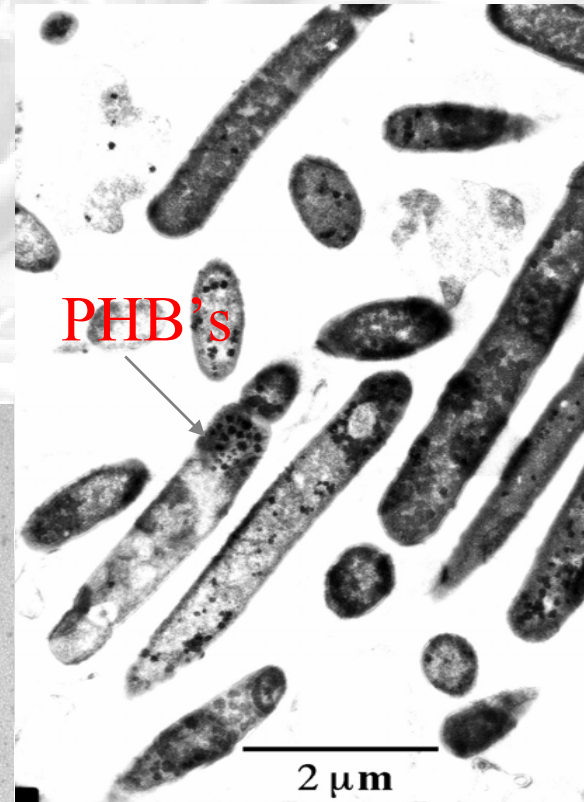
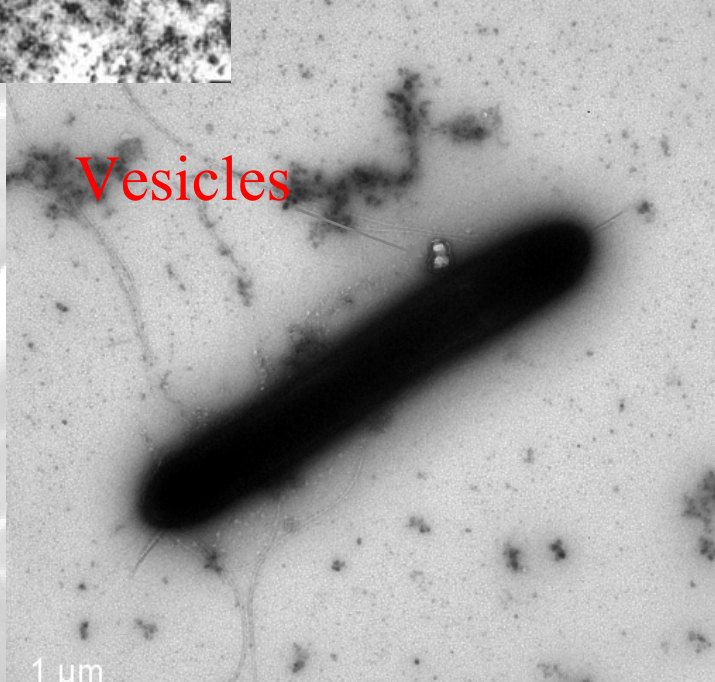


# Economically Beneficial S. African Au Mine Bacterial Isolates



*Thermus  
scotoductus*

*Alkaliphilus  
aruminator*



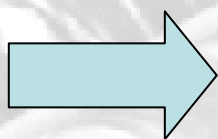
*Bacillus  
thermoaureus*

# What have we learned?

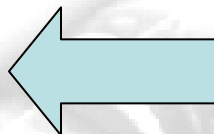
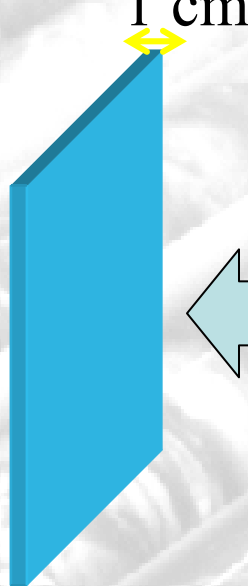
- Radiolysis supplies a radiochemical factory that can support subsurface life over the long term.
- Abundant, non-photosynthetically derived organic carbon sources are present.
- Can the substrate flux sustain the observed subsurface microbial populations?



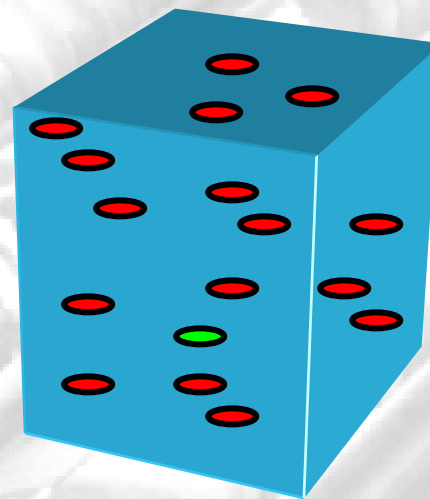
Bulk Production  
Rate In Water  
0.1-1 nM/yr



1m



1 Liter

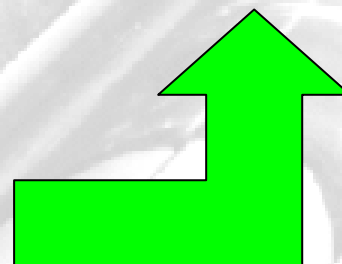
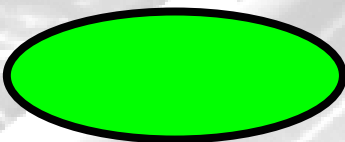


M/yr x cell-yr/moles = #live cells/L  
1 x 1 = 1 cell/L vs.  $10^{6-9}$  observed?

$H_2$

$SO_4^{2-}$

$HS^-$



$$4\pi DrC = 1 \text{ nmole cell}^{-1} \text{ yr}^{-1}$$

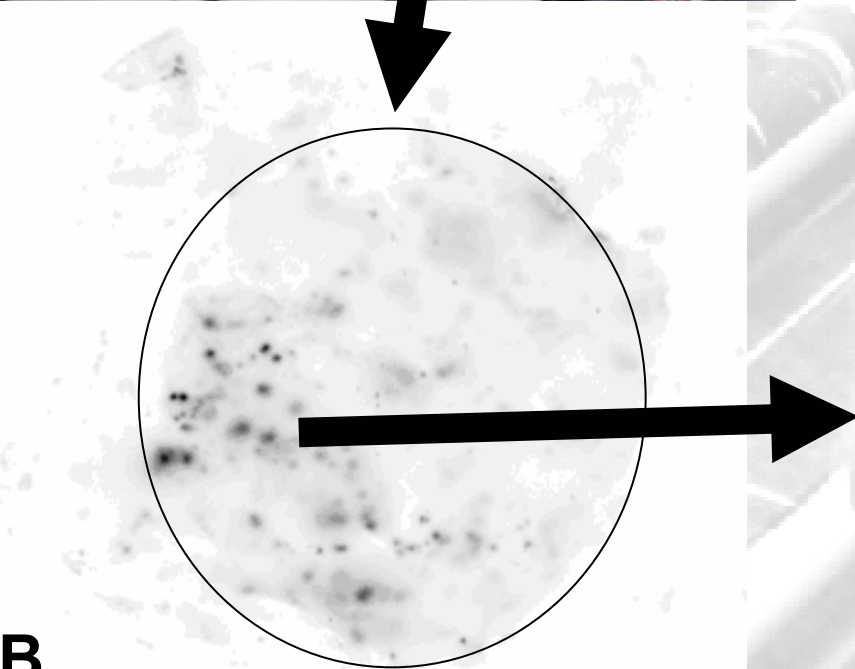
$$4\pi DrC\Delta G = \text{ATP cell}^{-1} \text{ s}^{-1}$$

# Microbial Balance of Power

- Free energy flux = maximum potential power  $\sim 10^{-13} - 10^{-15} \text{ kJ cell}^{-1} \text{ s}^{-1}$
- Assuming 60 kJ required for ATP synthesis this is equivalent to  $10^{-14} - 10^{-16}$  moles of ATP  $\text{cell}^{-1} \text{ s}^{-1}$
- Geochemical estimate of rate  $\sim 10^{-21} - 10^{-23}$  moles of ATP  $\text{cell}^{-1} \text{ s}^{-1}$
- Maintenance power is  $\sim 10^{-19}$  to  $10^{-21}$  moles of ATP  $\text{cell}^{-1} \text{ s}^{-1}$  for mesophilic SRB's and methanogens in culture

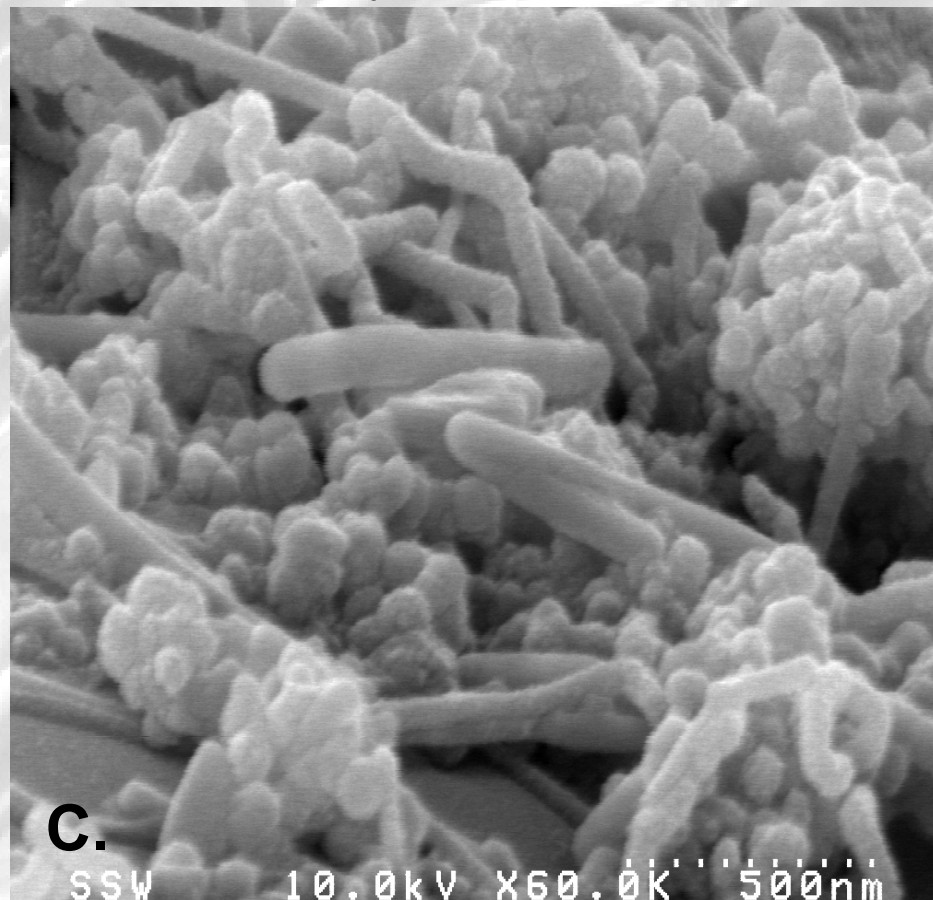


A.

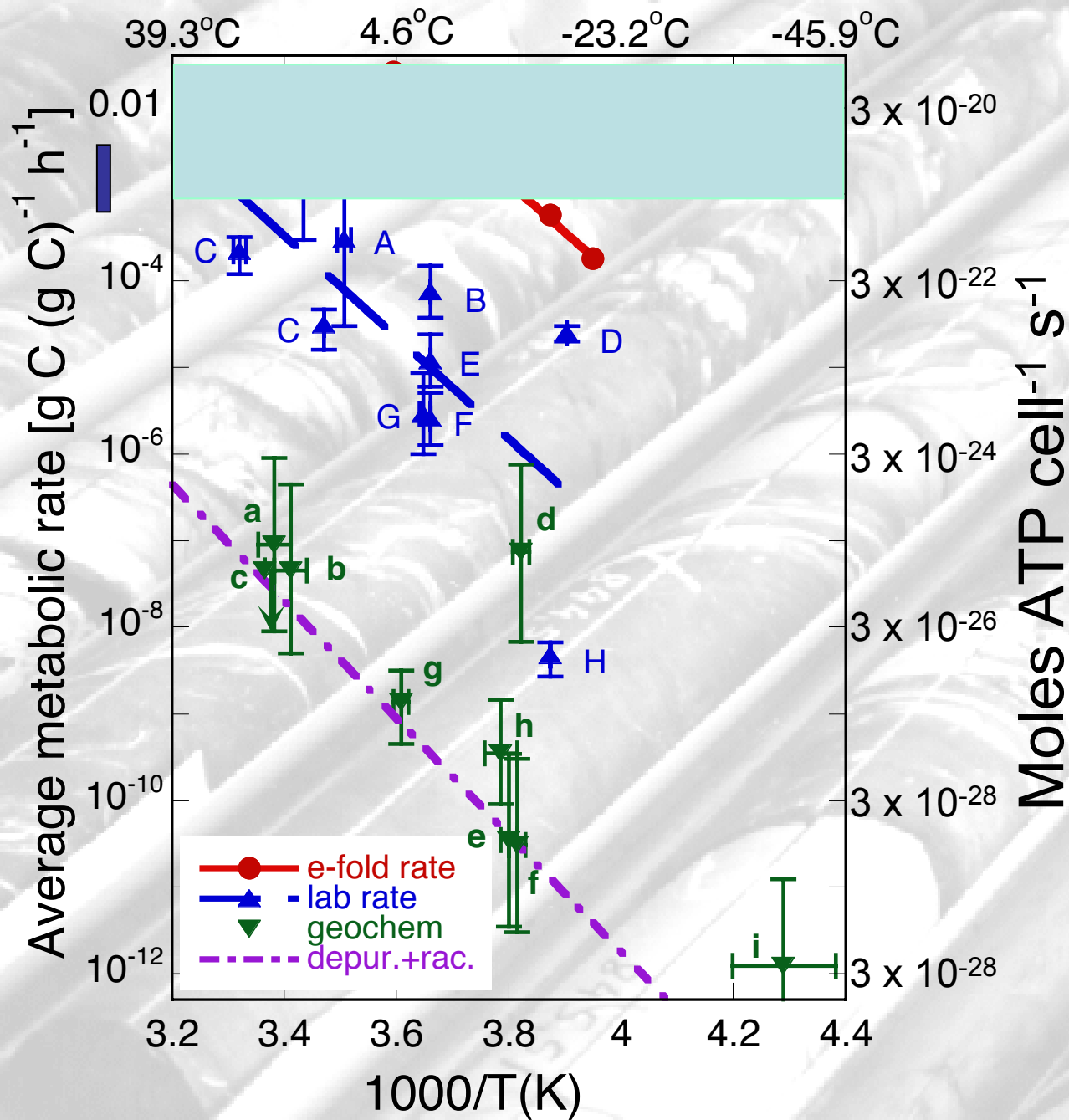


B.

Fig. Xx. A. Witwatersrand quartzite core from 1.95 kilometers beneath land surface. Pink is Rhodamine tracer. B.  $^{35}\text{S}$  auto-radiographic image of core. C. Sulfate reducing bacteria with AgS xtals in pore. Scale bar is 500 nm. Courtesy of Gordon Southam, Univ. of Western Ontario and Mark Davidson, Princeton University.



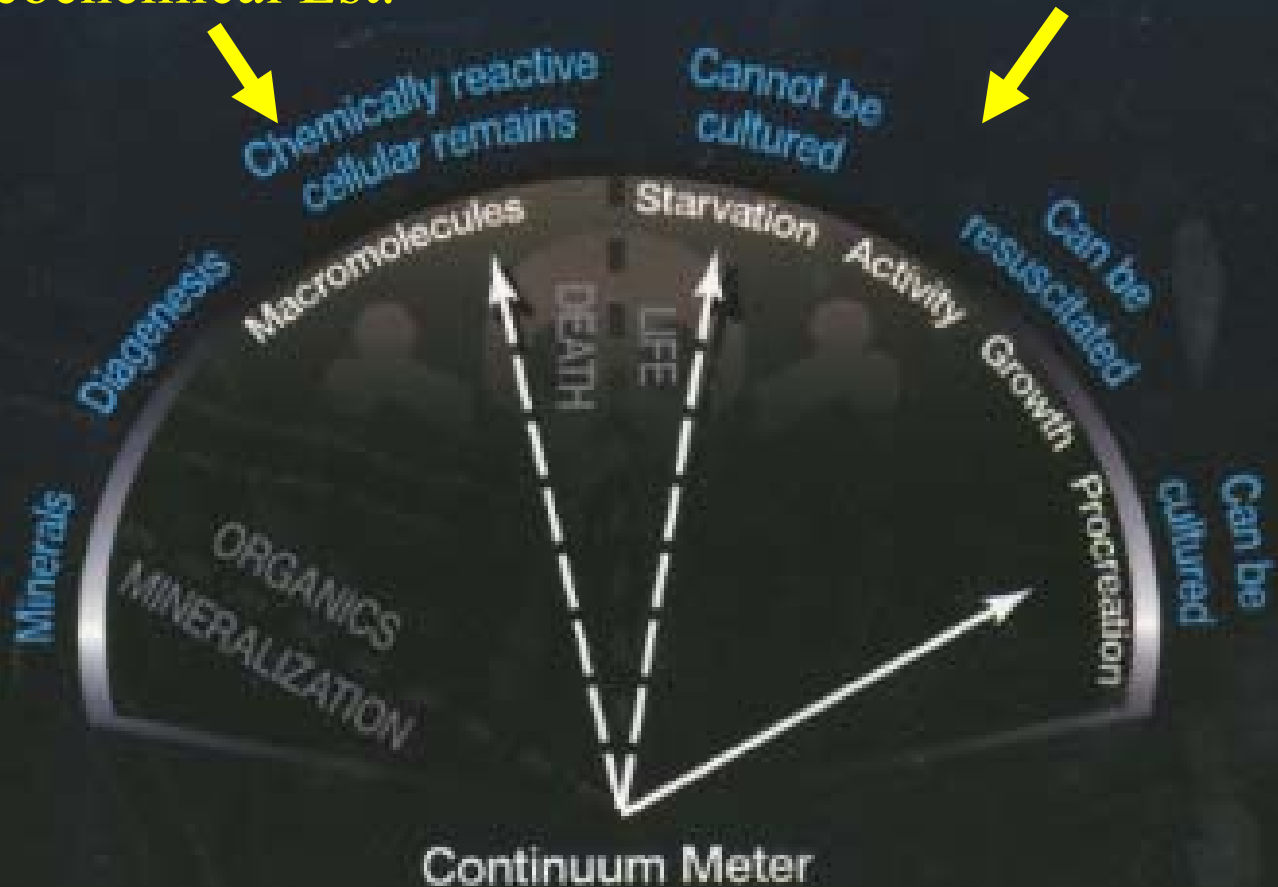
C.



# Death-O-Meter

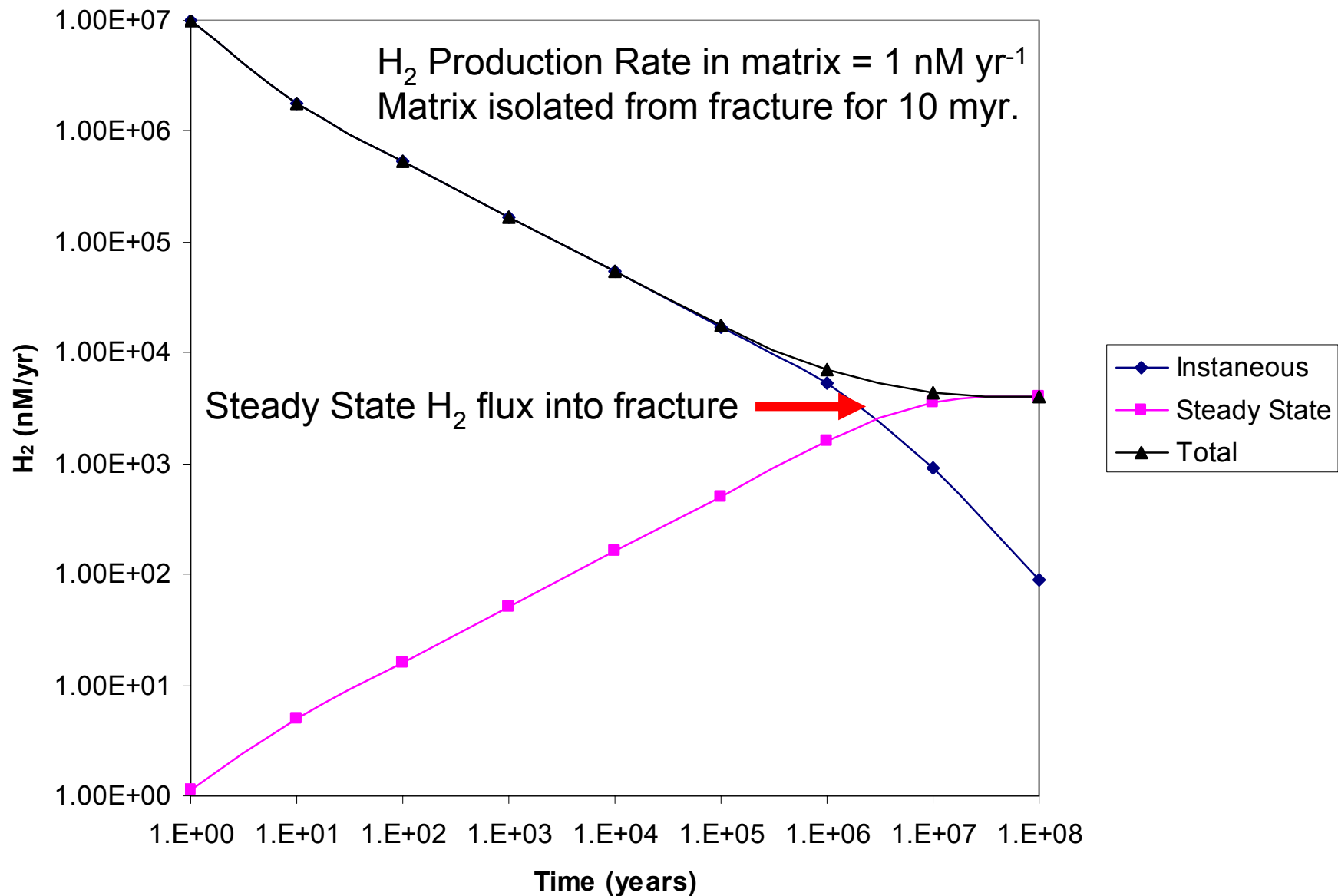
Geochemical Est.

Free Energy Flux



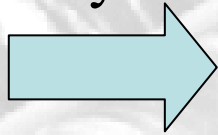
## H<sub>2</sub> diffusion into fracture

H<sub>2</sub> Production Rate in matrix = 1 nM yr<sup>-1</sup>  
Matrix isolated from fracture for 10 myr.

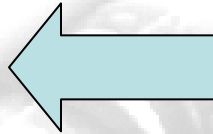
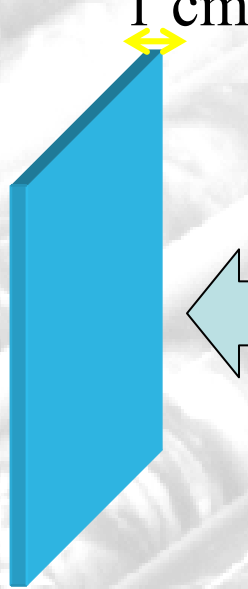


Diffusive Flux  
Into Fracture

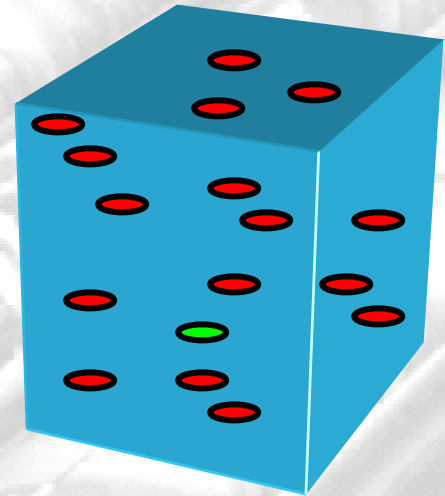
$10^4$  nM/yr



1m



1 Liter

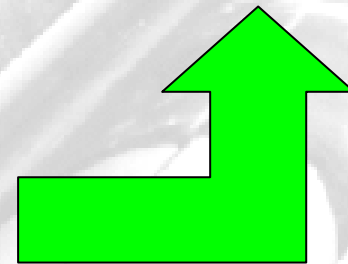
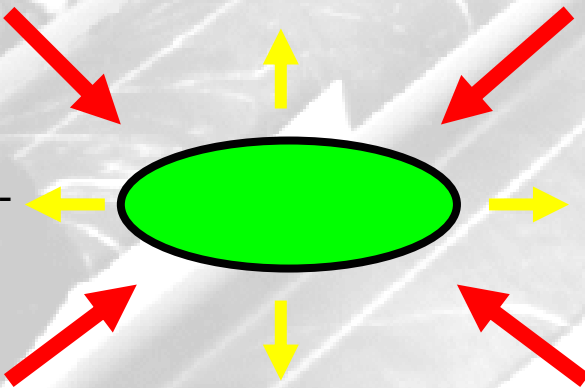


nM/yr x cell-yr/nmoles = #live cells/L  
 $10^4 \times 1 = 10^4$  cells/L vs.  $10^{6-9}$  observe

$H_2$

$SO_4^{2-}$

$HS^-$



$$4\pi DrC = 1 \text{ nmole cell}^{-1} \text{ yr}^{-1}$$

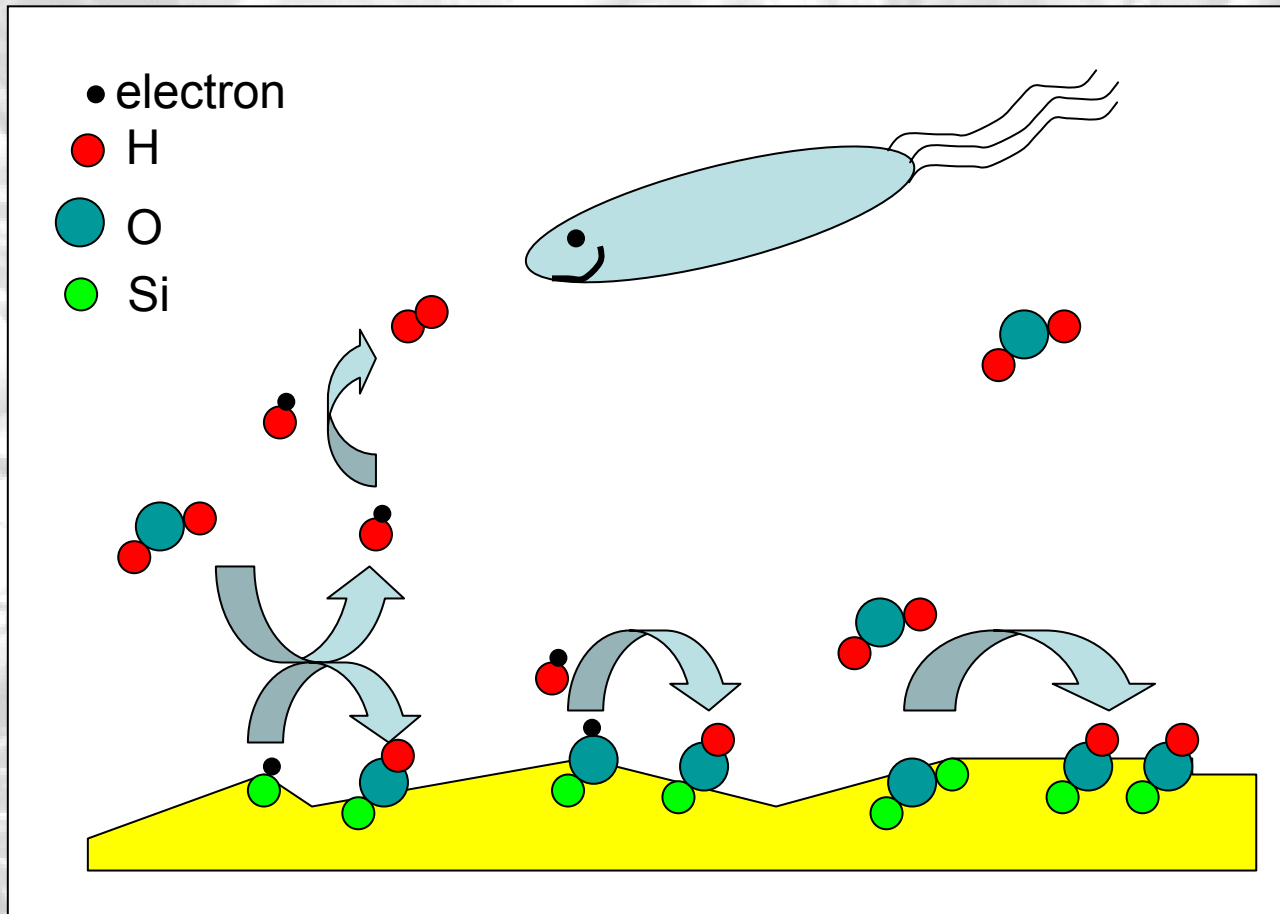
$$4\pi DrC\Delta G = \text{ATP cell}^{-1} \text{ s}^{-1}$$



# What's going on?

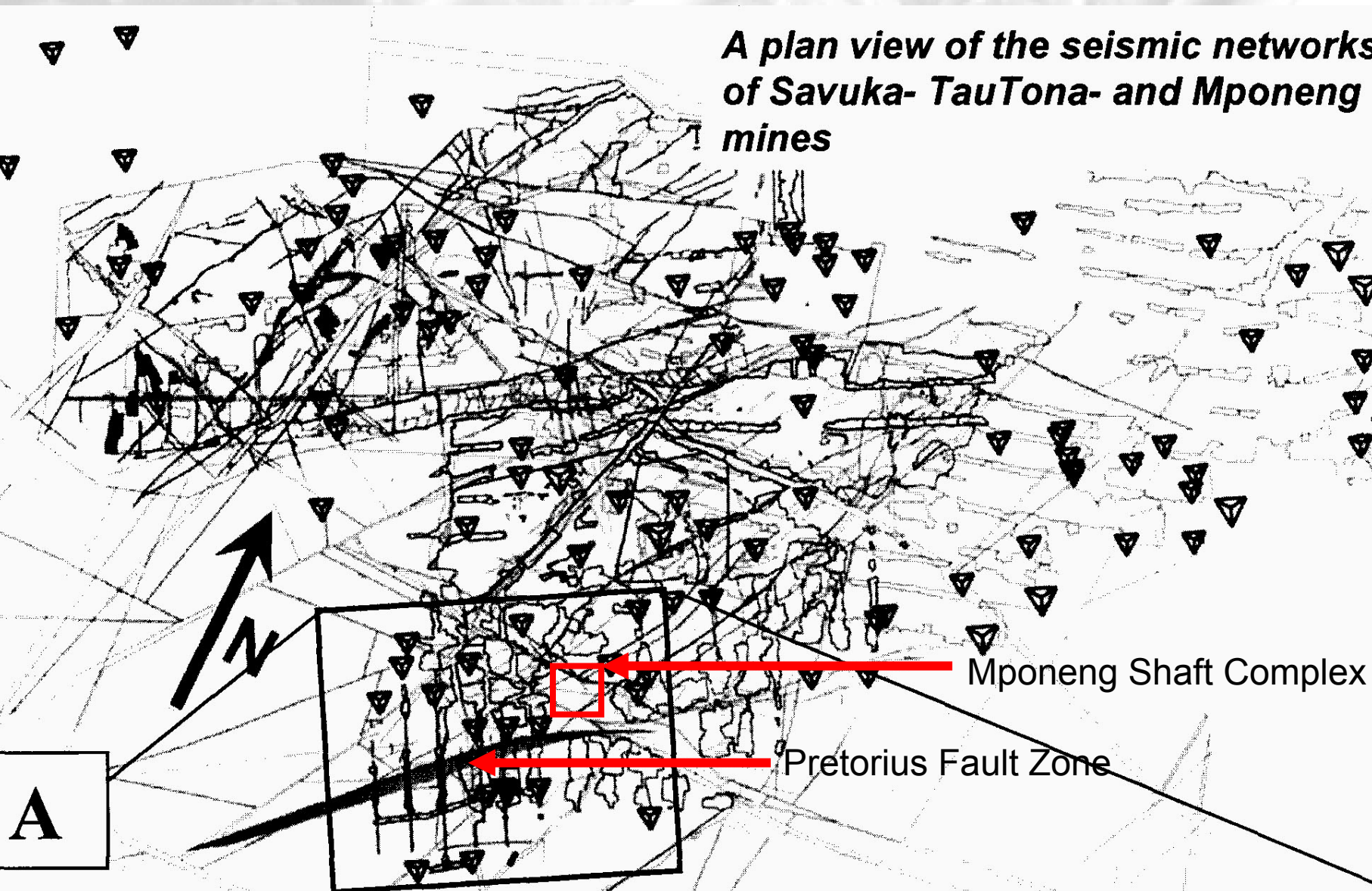
- Are most of the cells that we see essentially dead (moribund)?
- Is the origin of the substrate more complex, i.e. are the microorganisms switching metabolisms when the  $\Delta G$  approaches 0?
- Is mining induced fracturing enhancing substrate flux rates-i.e.  $10^7$  nM yr<sup>-1</sup>, that cannot be sustained in the long haul?

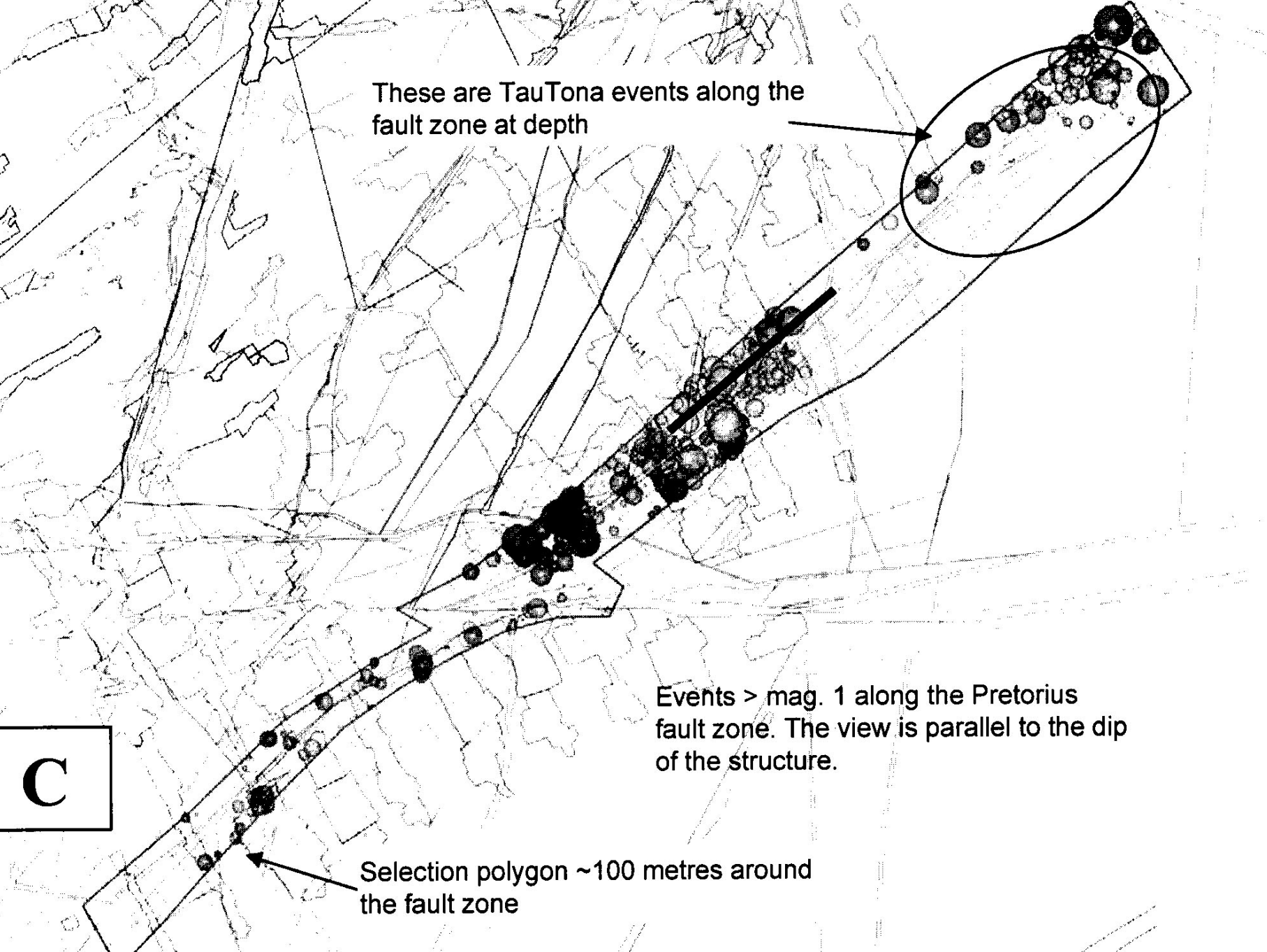
Can fracturing enhance redox flux by other mechanisms?



$\text{H}_2$  Generation from  $\text{H}_2\text{O}$  reacting with fractured  $\text{SiO}_2$

# Drilling and Monitoring an Active Fault in a South African Mine



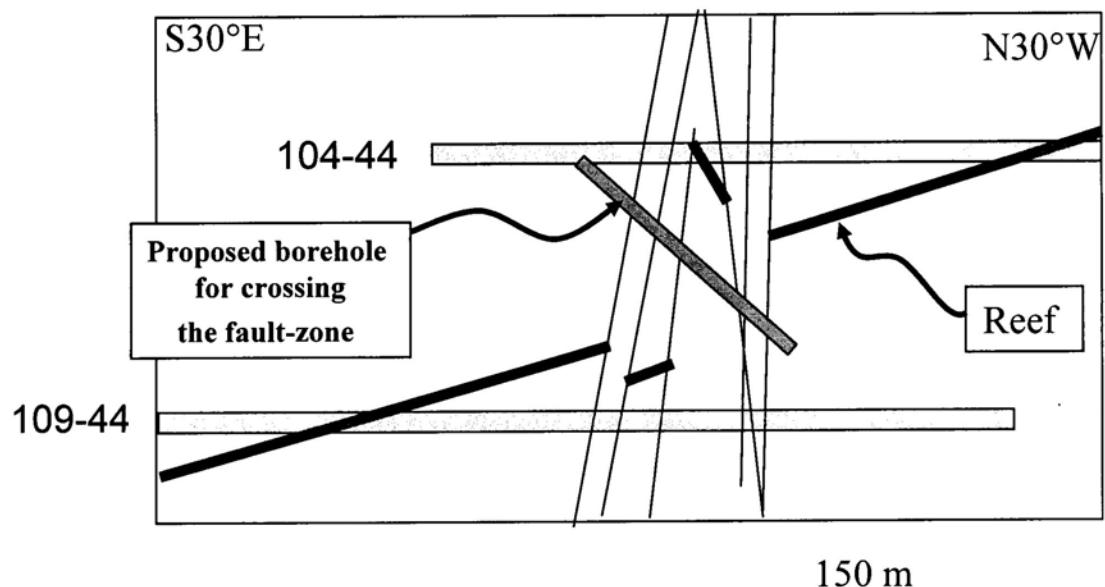
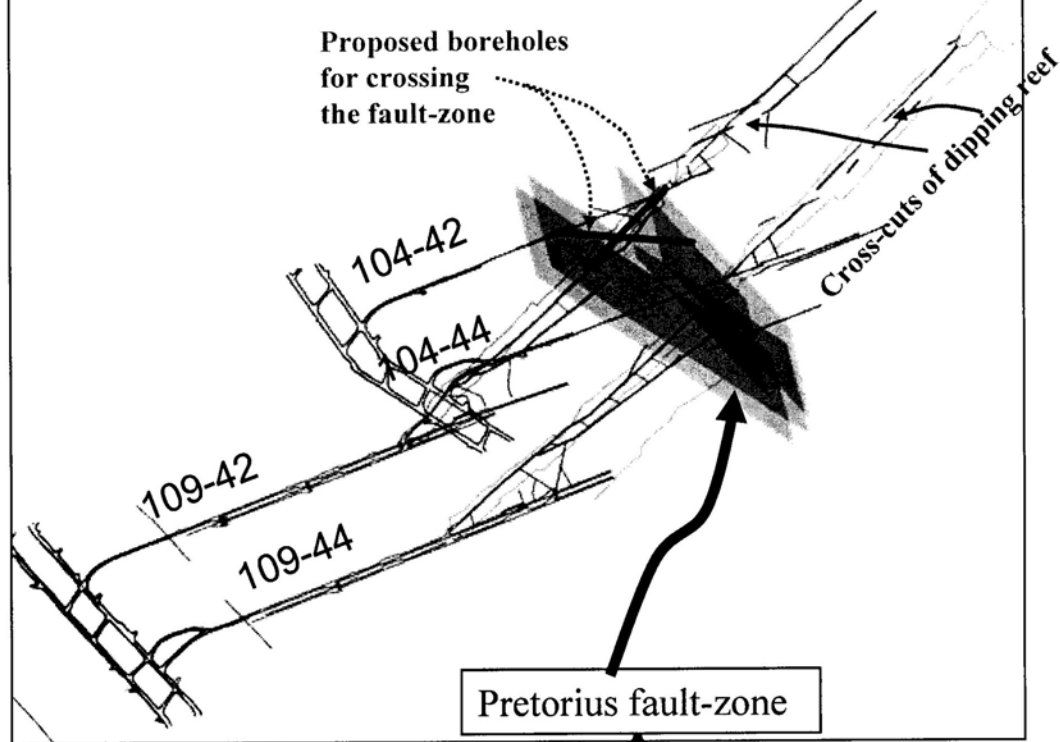


These are TauTona events along the fault zone at depth

Events > mag. 1 along the Pretorius fault zone. The view is parallel to the dip of the structure.

Selection polygon ~100 metres around the fault zone

C



## Core analysis

- On-site sampling of ~10 freshly drilled core segments from across the fault
- sealing cores in evacuated containers for lab analysis
- Stepwise heating of core material and mass spec analysis of released noble gas isotopes (room temp – 1800°C, routine procedure)

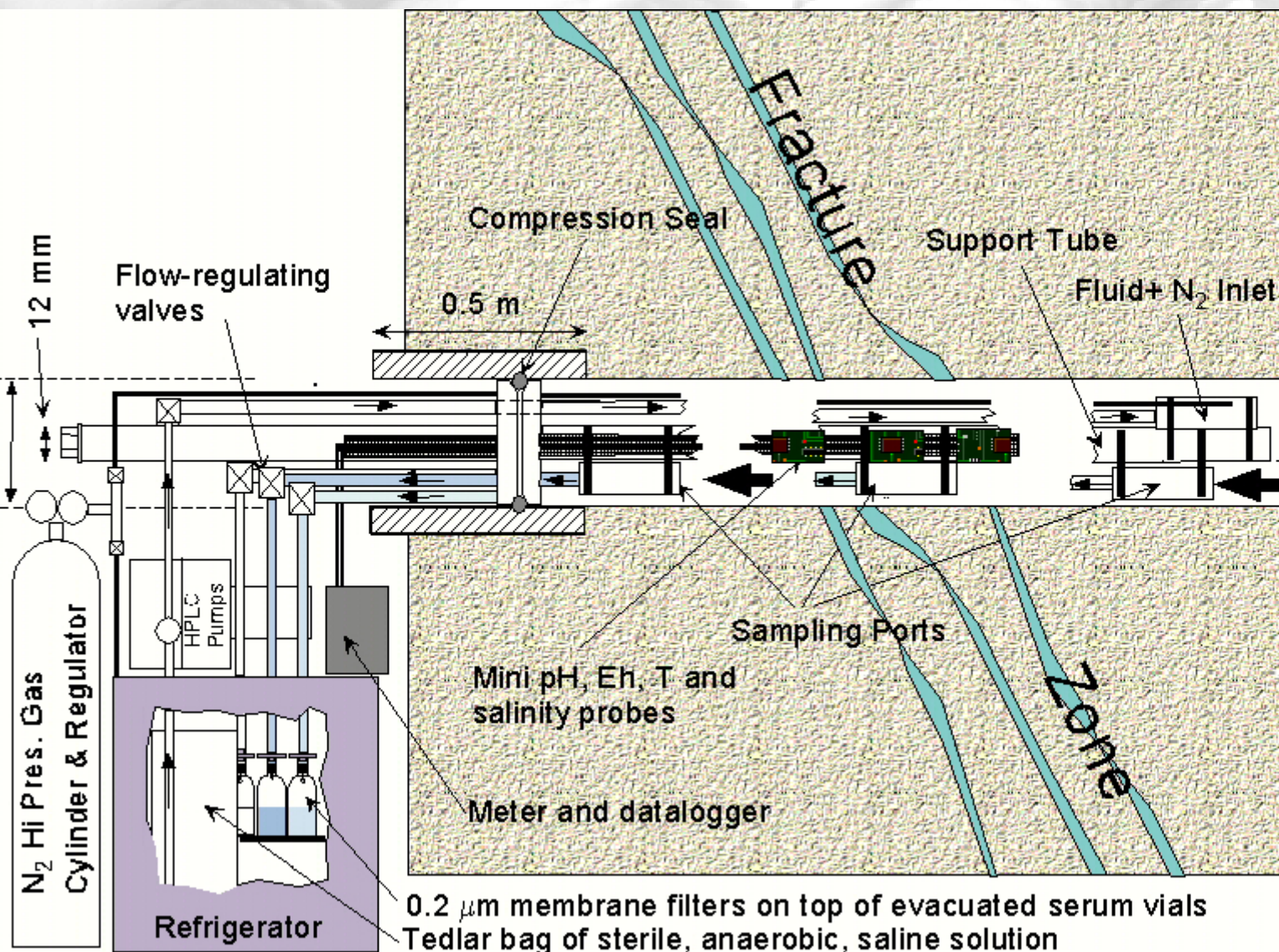
## **Thermal characterization of outgassing processes (field+lab)**

### • Online gas monitoring

- Continuous analysis of borehole gas by a quadrupole mass spectrometer (He, Ne, Ar, CO<sub>2</sub>, CH<sub>4</sub>, N<sub>2</sub>, O<sub>2</sub>, H<sub>2</sub>)
- Gas is sucked through multi tube system from ~5 locations inside borehole
- Threshold signal (He, CH<sub>4</sub>) triggers special gas sampling device (glass vials) for off-line analysis in lab

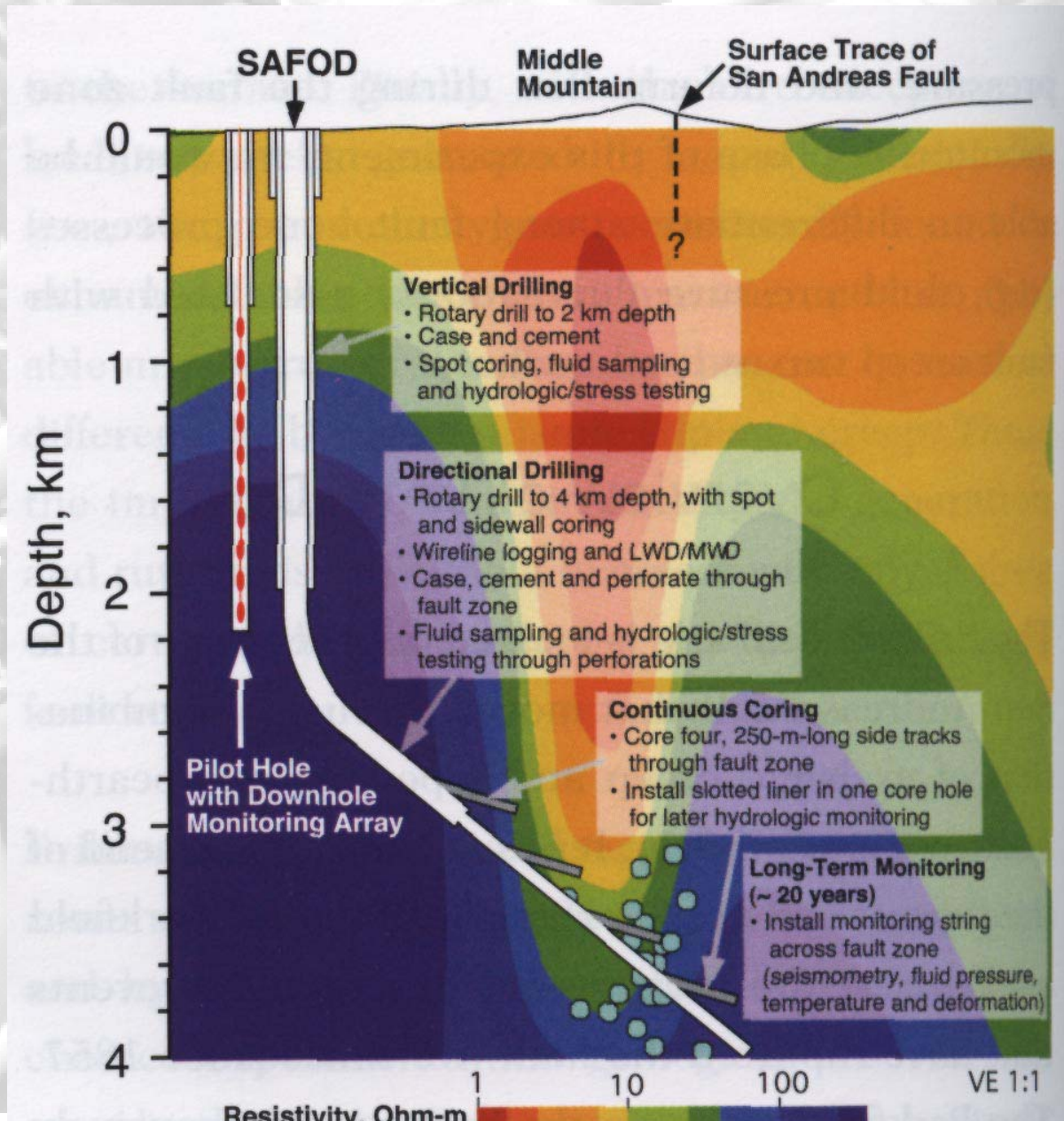
## **Earthquake induced outgassing process (field)**







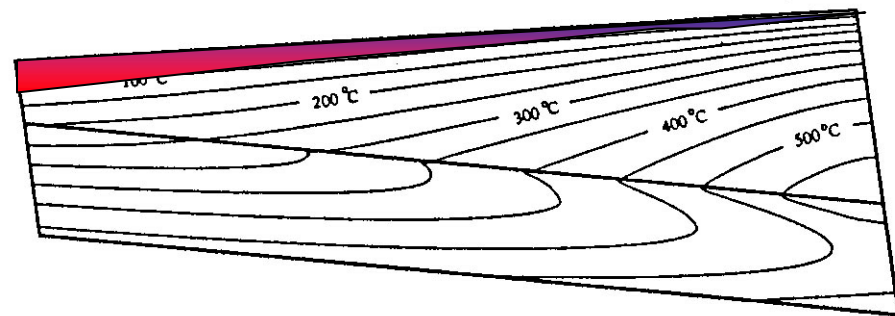
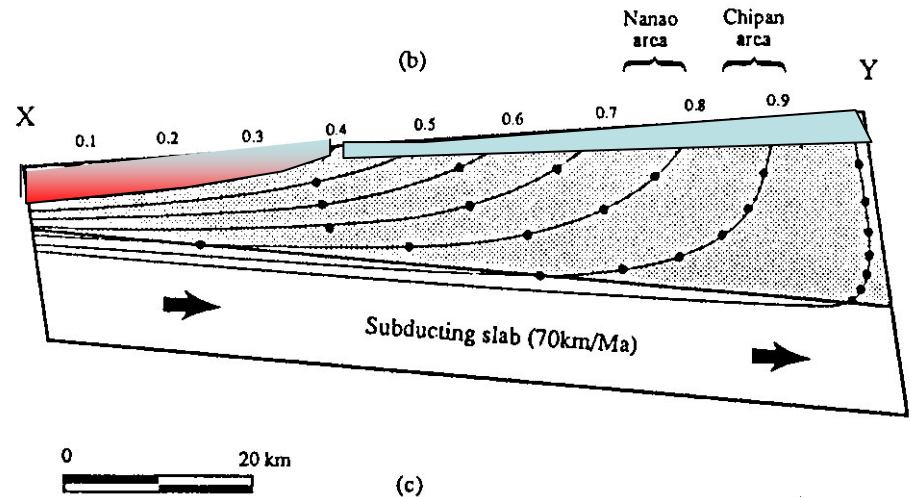
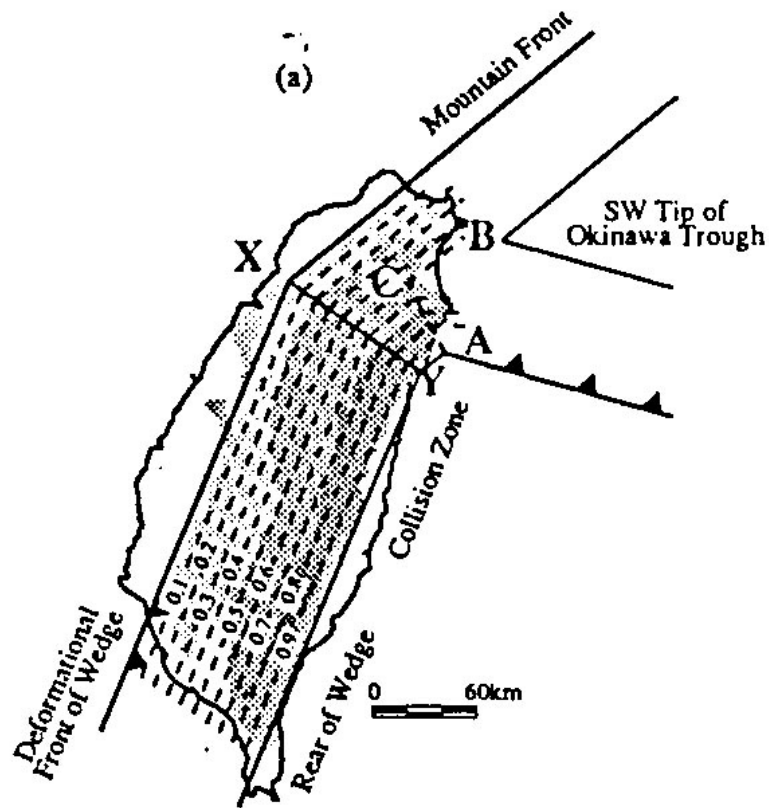
# San Andreas Fault Zone Coring and Monitoring



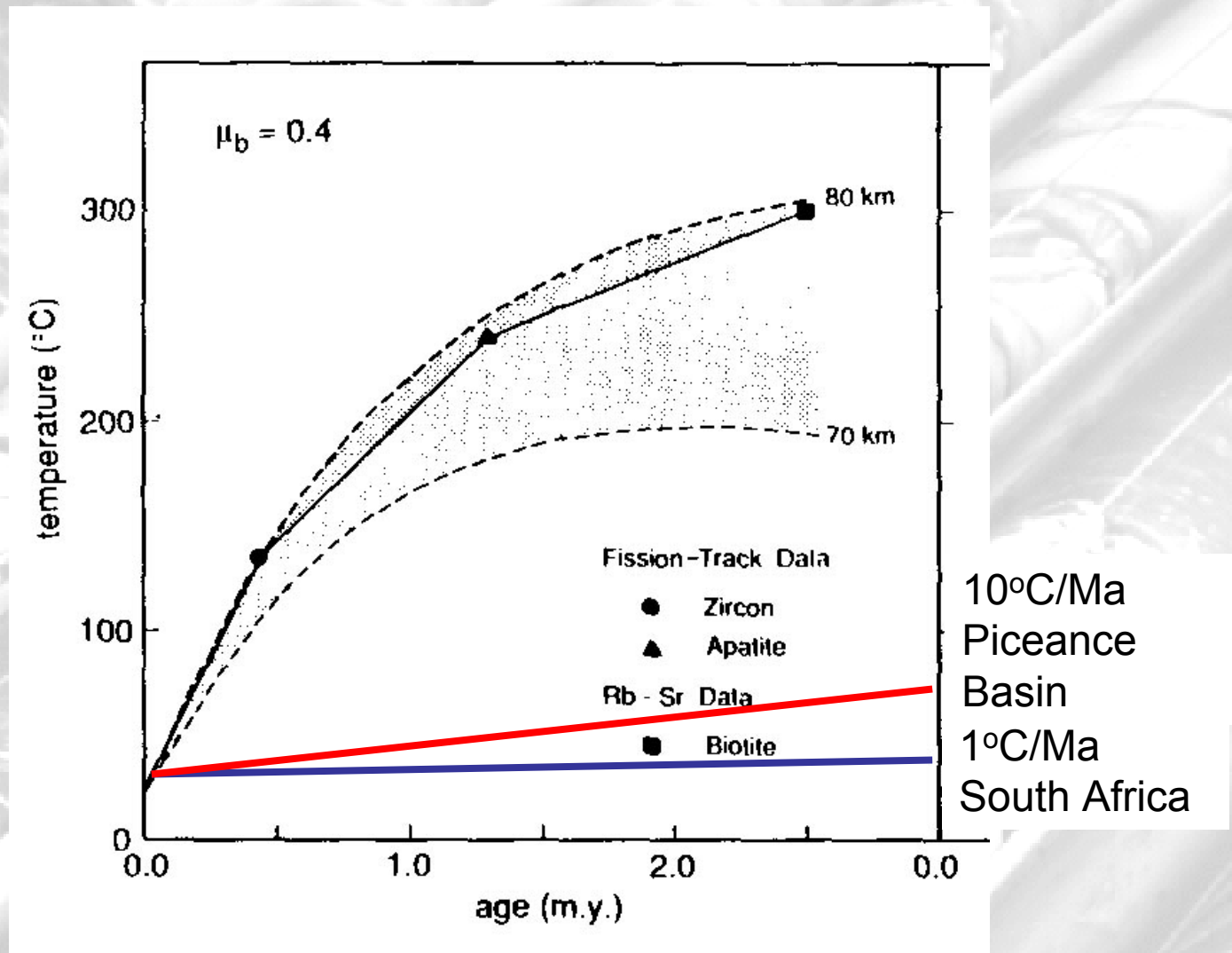
Temperatures of 80° to 200°C.

High conductivity (salinity?) anomaly within fault zone.

Will the origin of deep subsurface microorganisms vary across Taiwan?



# Can microbial colonization keep pace with tectonic uplift?



# Two Questions

1. How can deep subsurface microbial communities survive tens or hundreds of millions of years separated from the photosphere?
2. Are the deep subsurface microbial and nutrient fluxes greater in tectonically active environments than in quiescent crustal or marine sedimentary environments?