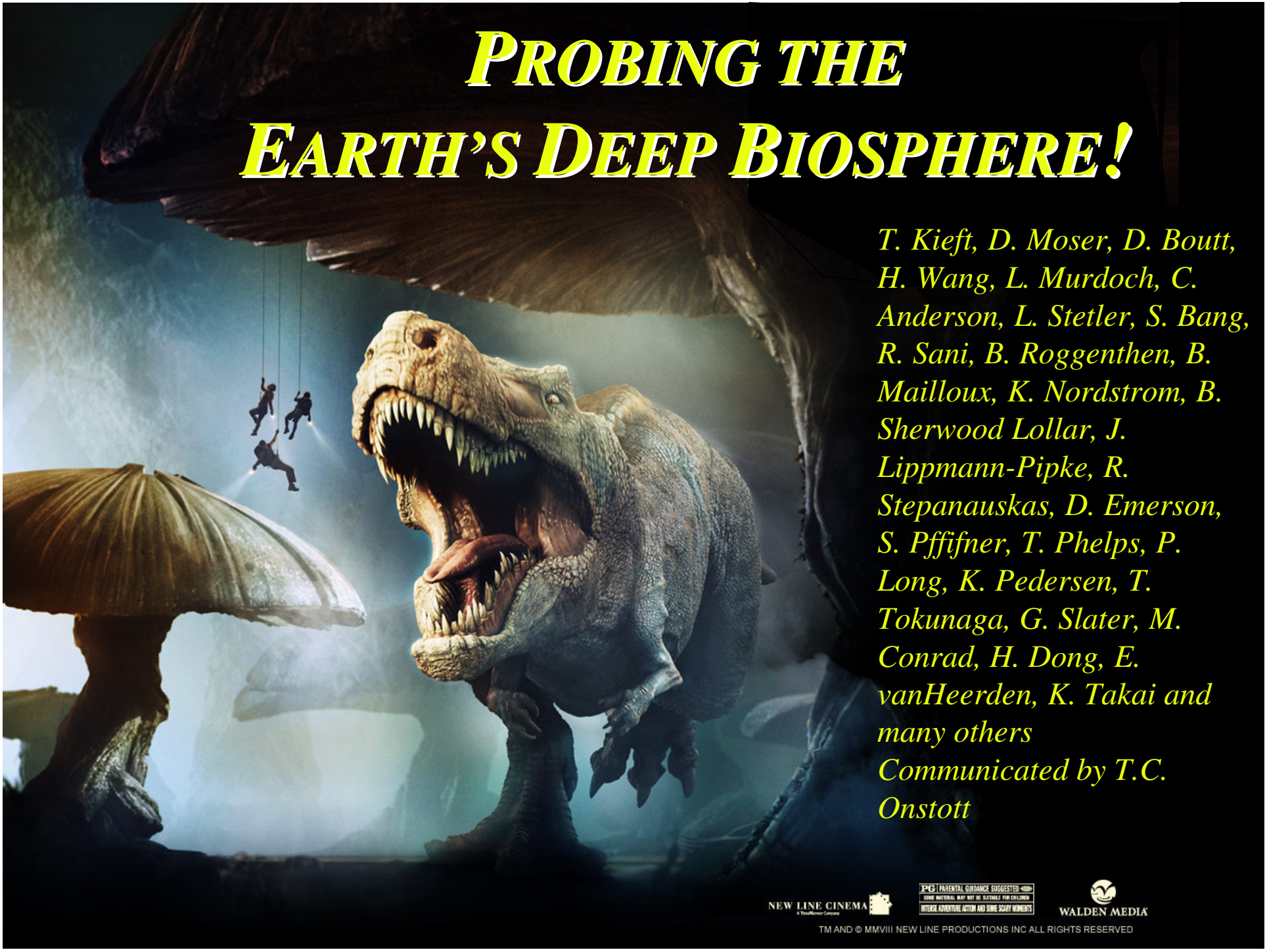

PROBING THE
EARTH'S DEEP BIOSPHERE!
T. Kieft, D. Moser, D. Boutt, H. Wang, L. Murdoch, C. Anderson, L. Stetler, S. Bang, R. Sani, B. Roggenthen, B. Mailloux, K. Nordstrom, B. Sherwood Lollar, J. Lippmann-Pipke, R. Stepanauskas, D. Emerson, S. Pffifner, T. Phelps, P. Long, K. Pedersen, T. Tokunaga, G. Slater, M. Conrad, H. Dong, E. vanHeerden, K. Takai and many others
Communicated by T.C. Onstott
NEW LINE CINEMA
PG PARENTAL GUIDANCE SUGGESTED
SOME MATERIAL MAY NOT BE SUITABLE FOR CHILDREN
INTENSE ADVENTURE ACTION AND SOME SCARY MOMENTS
WALDEN MEDIA
TM AND © MMVIII NEW LINE PRODUCTIONS INC ALL RIGHTS RESERVED

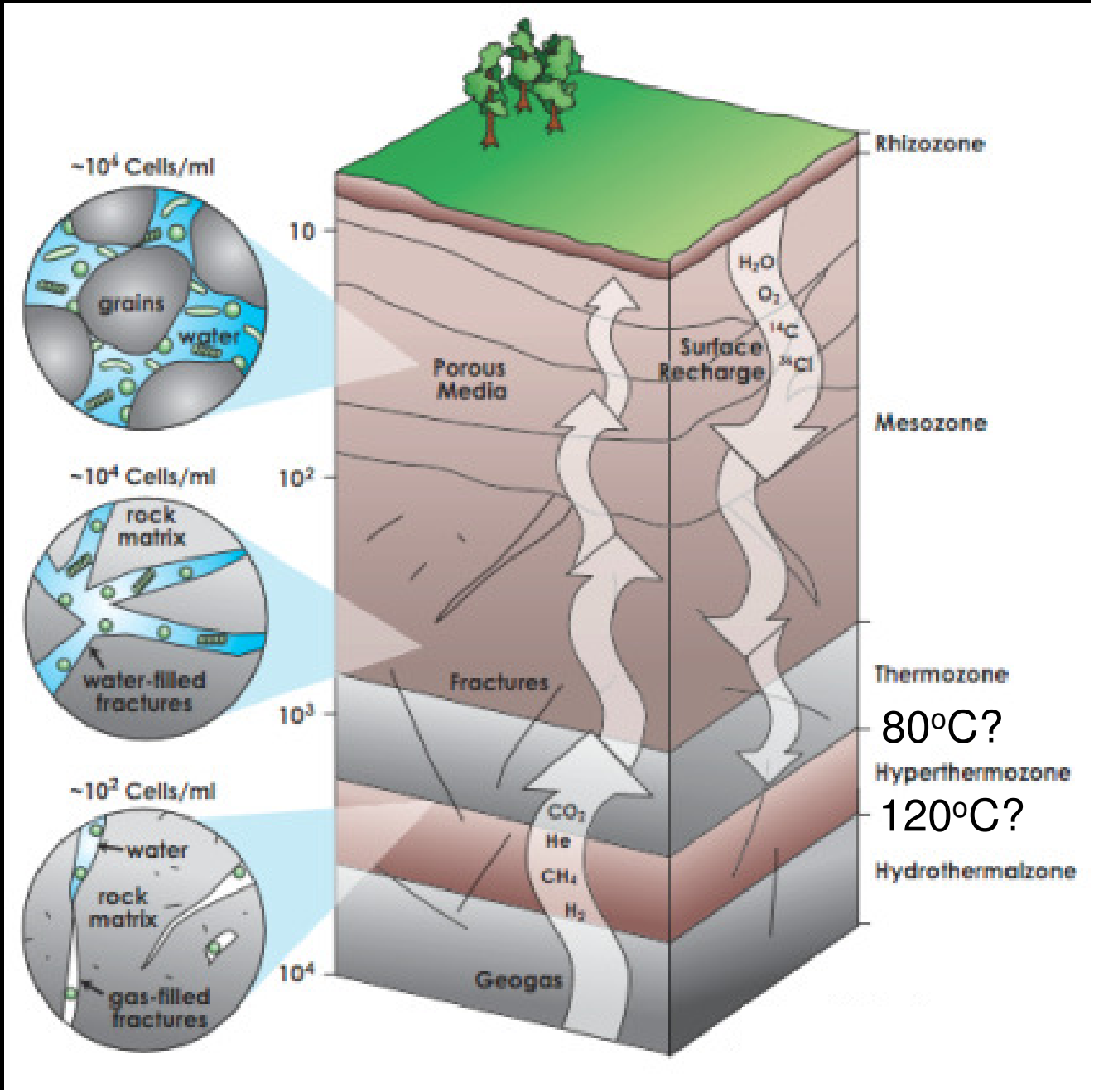
10^{18} g of living C on Earth

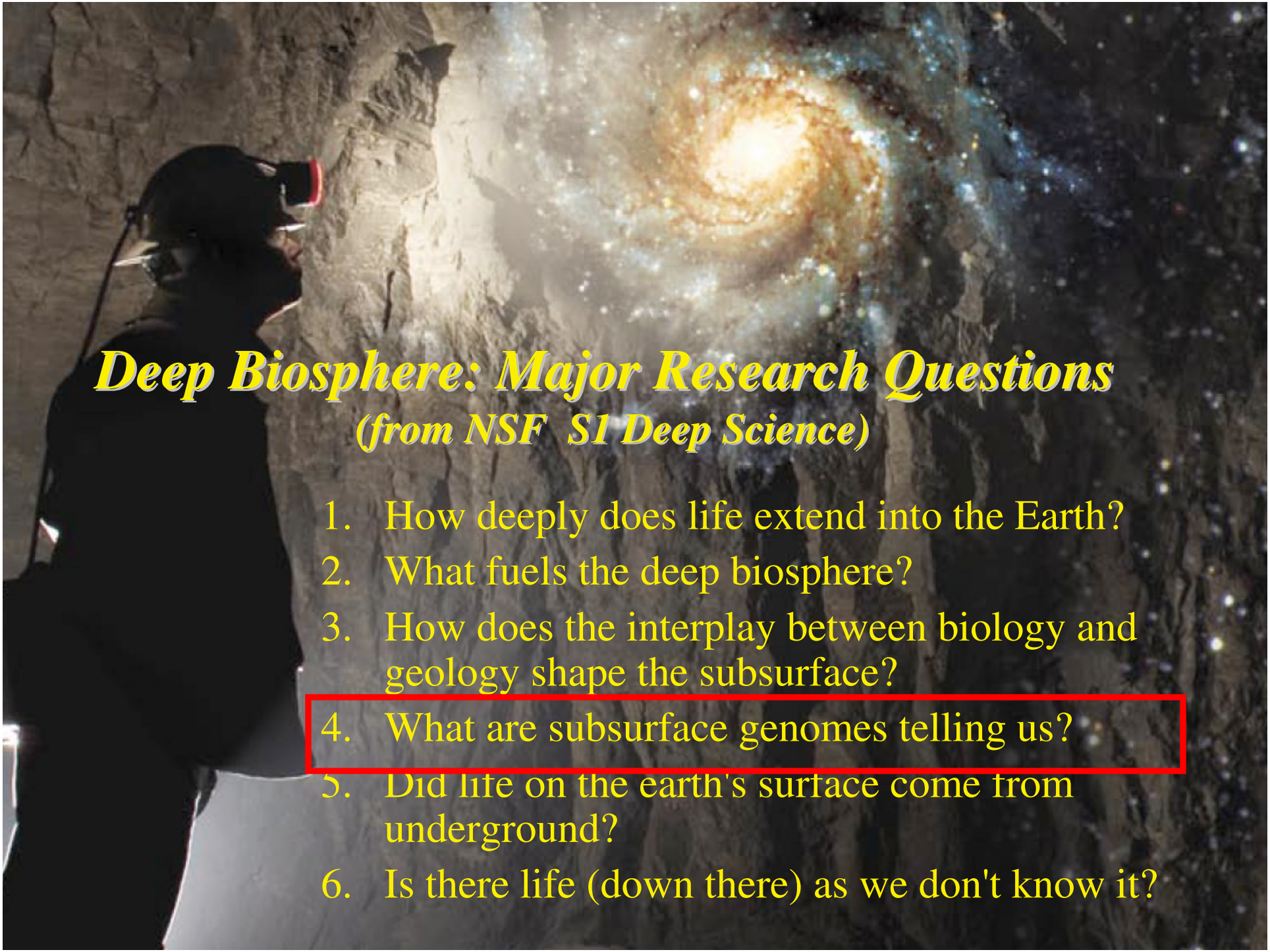
- These microbes control biogeochemical cycling and fluxes between the surface and subsurface.
- Both are crucial to climate change, CO_2 sequestration, long term waste storage and resource extraction.
- The subsurface biosphere is relevant to the origin and evolution of terrestrial life and offers insights to the potential for life on other planets in our solar system.
- The subsurface harbors diverse, novel and demonstrably useful, potentially ancient enzymes.
- Current knowledge of the continental deep subsurface is limited, based on a few cores, fewer boreholes from the surface, still fewer deep mines and one URL.

Whitman et al. 1998

But none from the surface to the bottom of the biosphere

- **"Subsurface Biosphere"** is a complex ecosystem requiring geological, geophysical, hydrological, geomechanical biogeochemical, molecular and microbiological characterization.
- **Over-arching question**: What are the distributions and activities of subsurface life and what controls them?





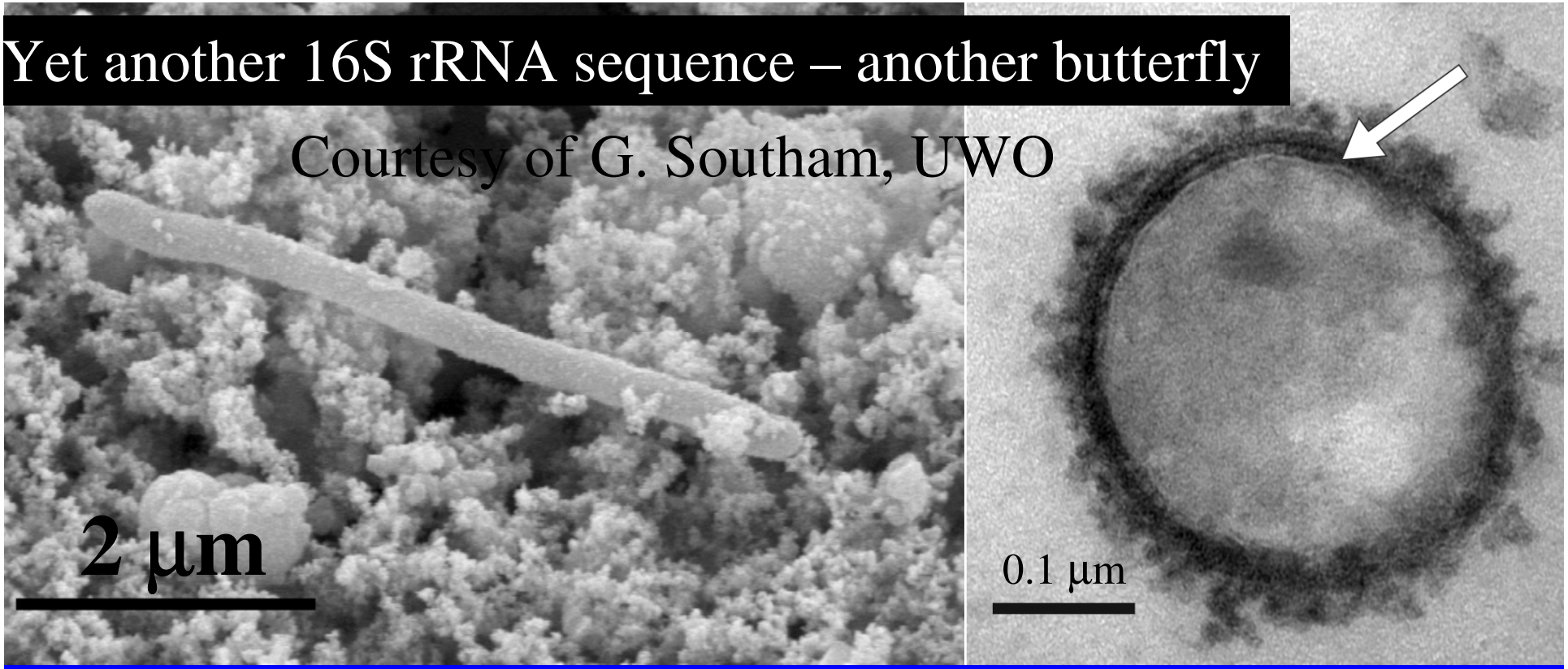
Deep Biosphere: Major Research Questions

(from NSF S1 Deep Science)

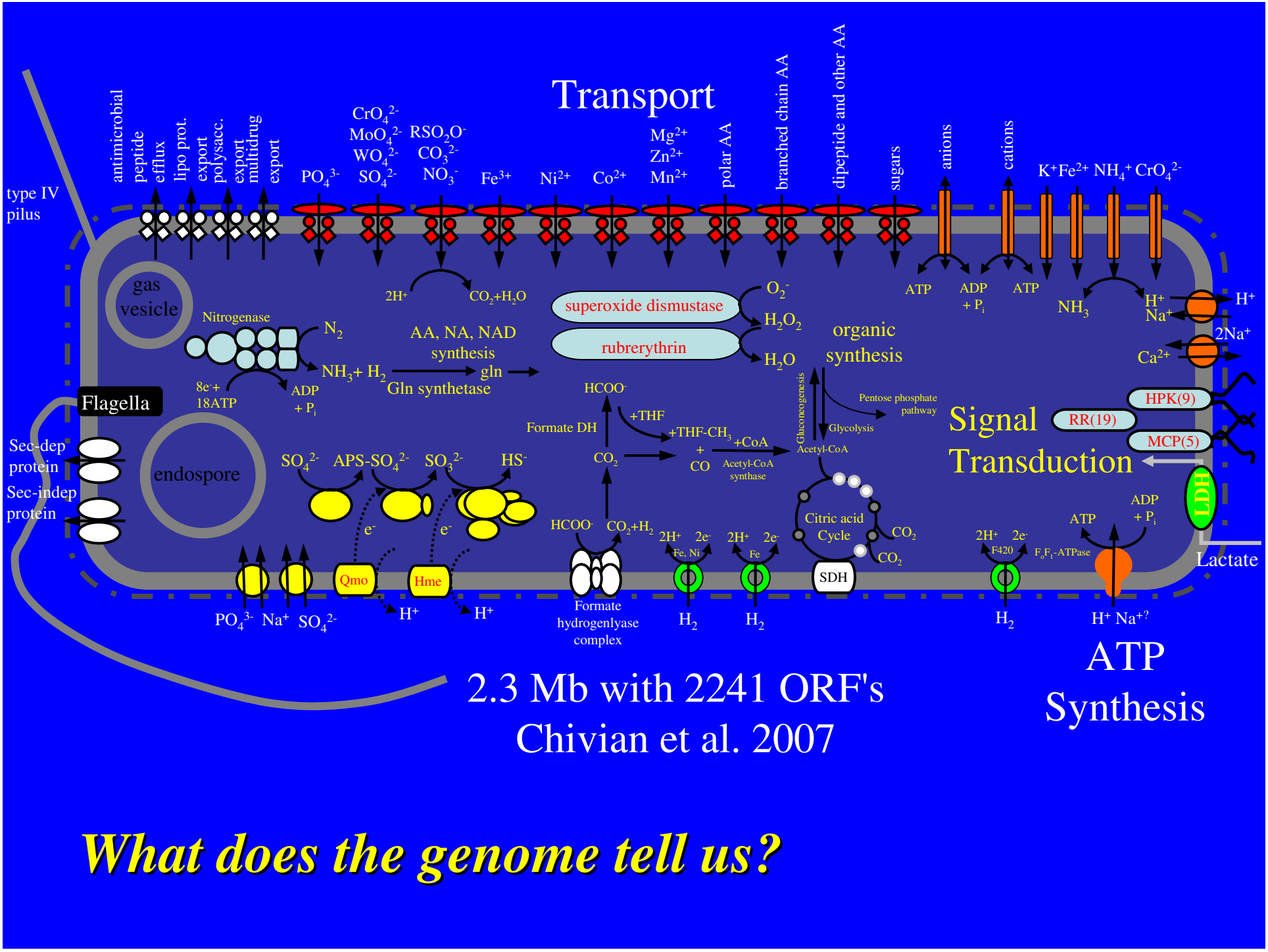
1. How deeply does life extend into the Earth?
2. What fuels the deep biosphere?
3. How does the interplay between biology and geology shape the subsurface?
4. What are subsurface genomes telling us?
5. Did life on the earth's surface come from underground?
6. Is there life (down there) as we don't know it?

Yet another 16S rRNA sequence – another butterfly

Courtesy of G. Southam, UWO



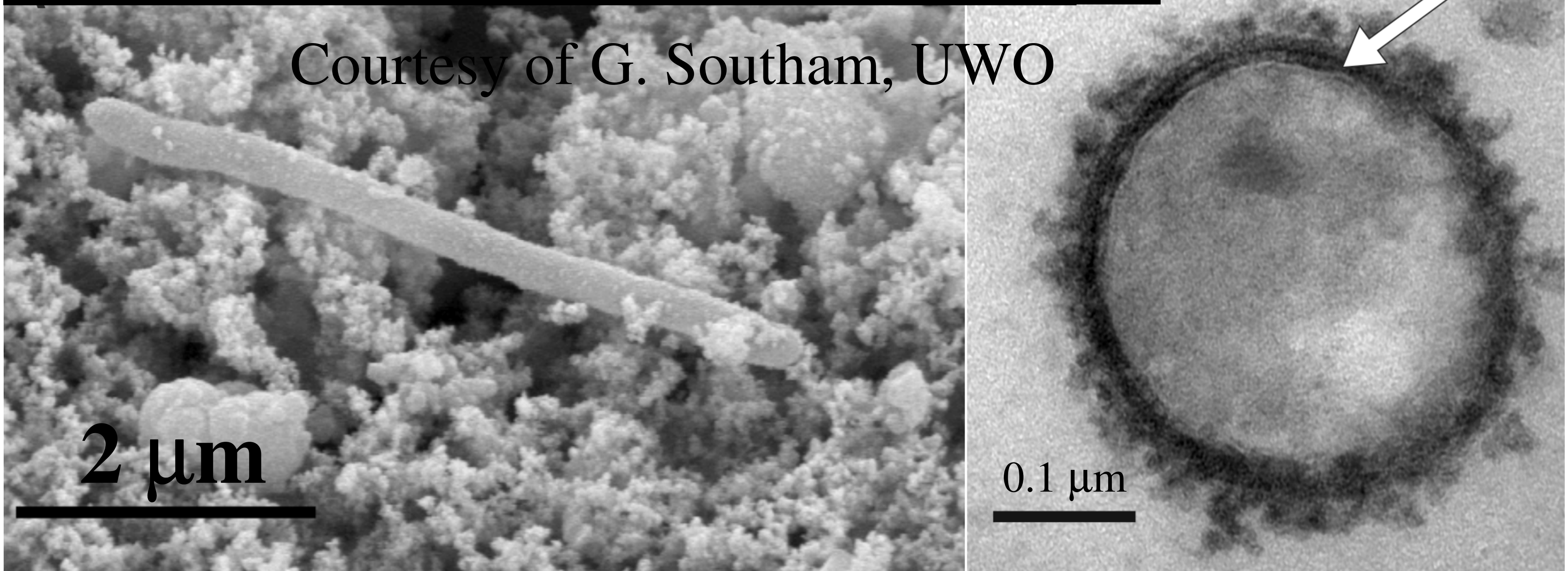
- 16S rRNA gene libraries revealed this new species of bacteria was present only at depths >1.5 km and distributed over a distance of 300 km in the Witwatersrand Basin of South Africa
- In some fractures it is the dominant species or only species.
- You cannot grow it, but the16S rRNA gene sequence is very similar to sequences recovered from a deep sea vent
- This species was remotely related to gram-positive sulfate reducing isolates so it **might** be a sulfate reducer.



2.3 Mb with 2241 ORF's
Chivian et al. 2007

What does the genome tell us?

Candidate species - *Desulforudis audaxviator*

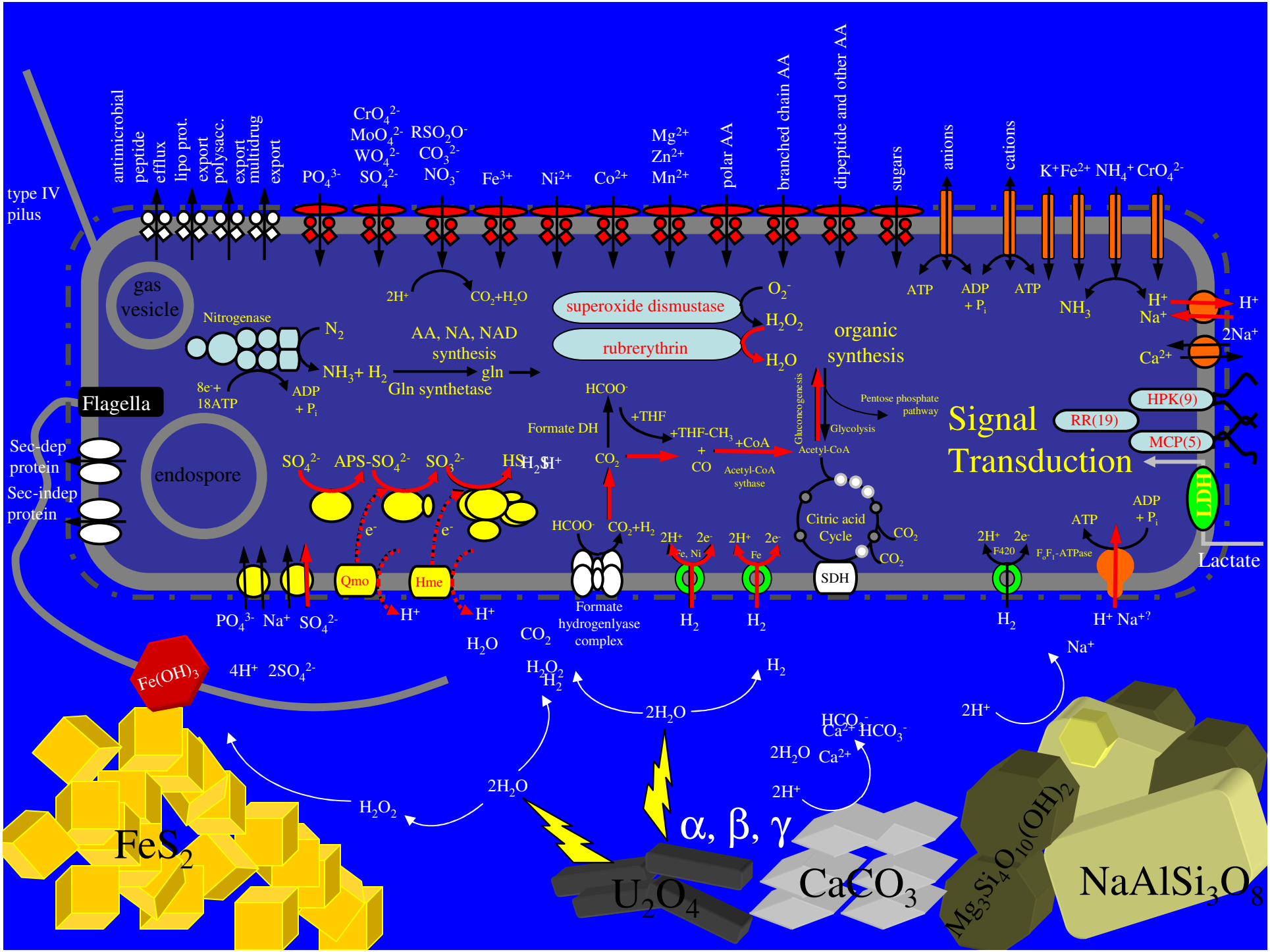


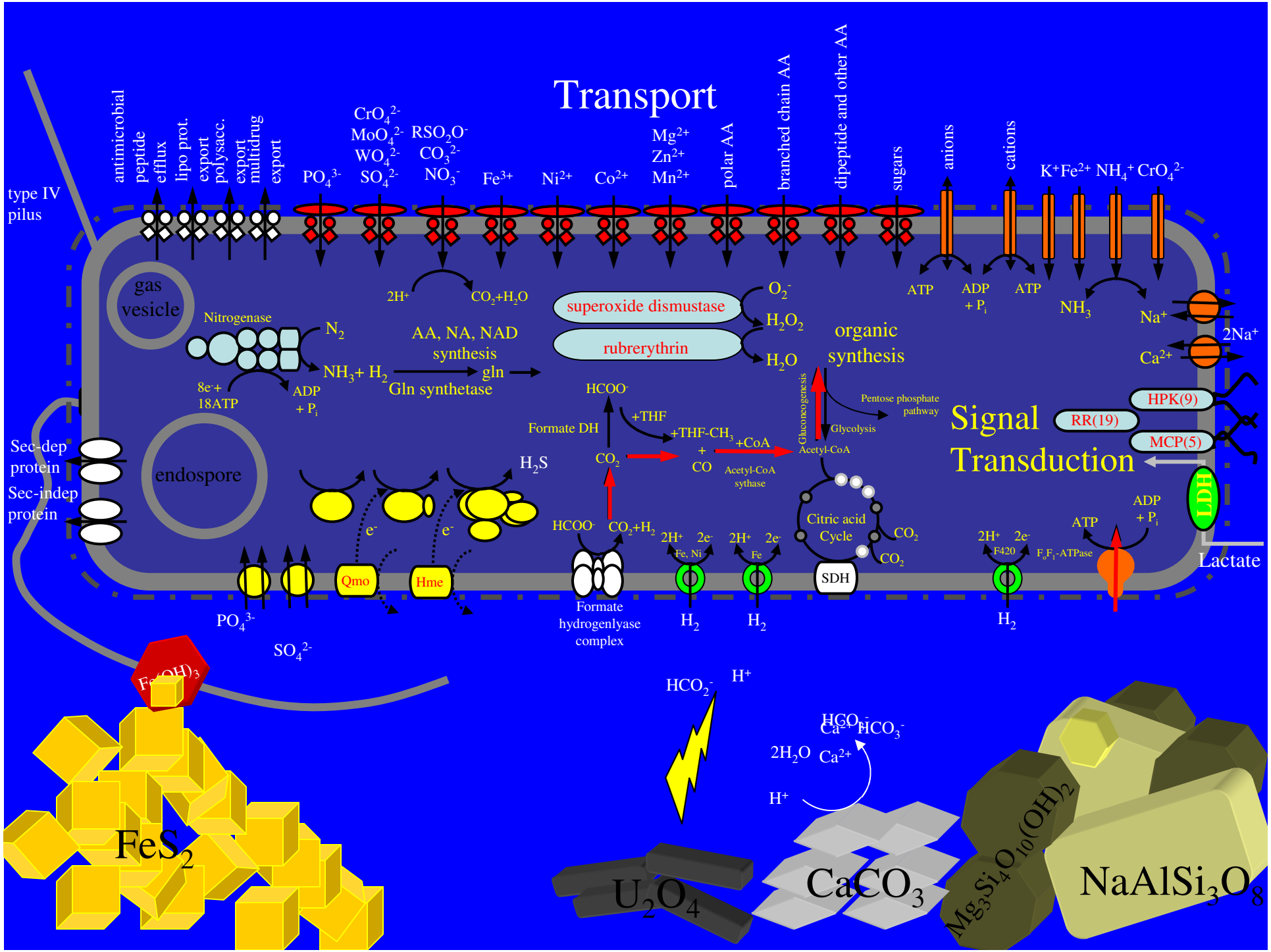
- No protection against O_2 but does against H_2O_2
- Both heterotrophic and autotrophic, adaptable to a changing environment
- Chemotactic, mobile through fractures where environment may vary
- Can fix N_2 if need be, when energy resources are high
- Can sporulate if need be, when energy resources are low
- Possesses all the machinery for a solitary life, self-reliant
- Very low SNP's, almost clonal, suggesting low mutation rates
- Contains genetic elements transferred from anaerobic, thermophilic methanogens (i.e. HGT), did this happen in the subsurface?
- Contains CRISPR sequences, protection against viral infections?

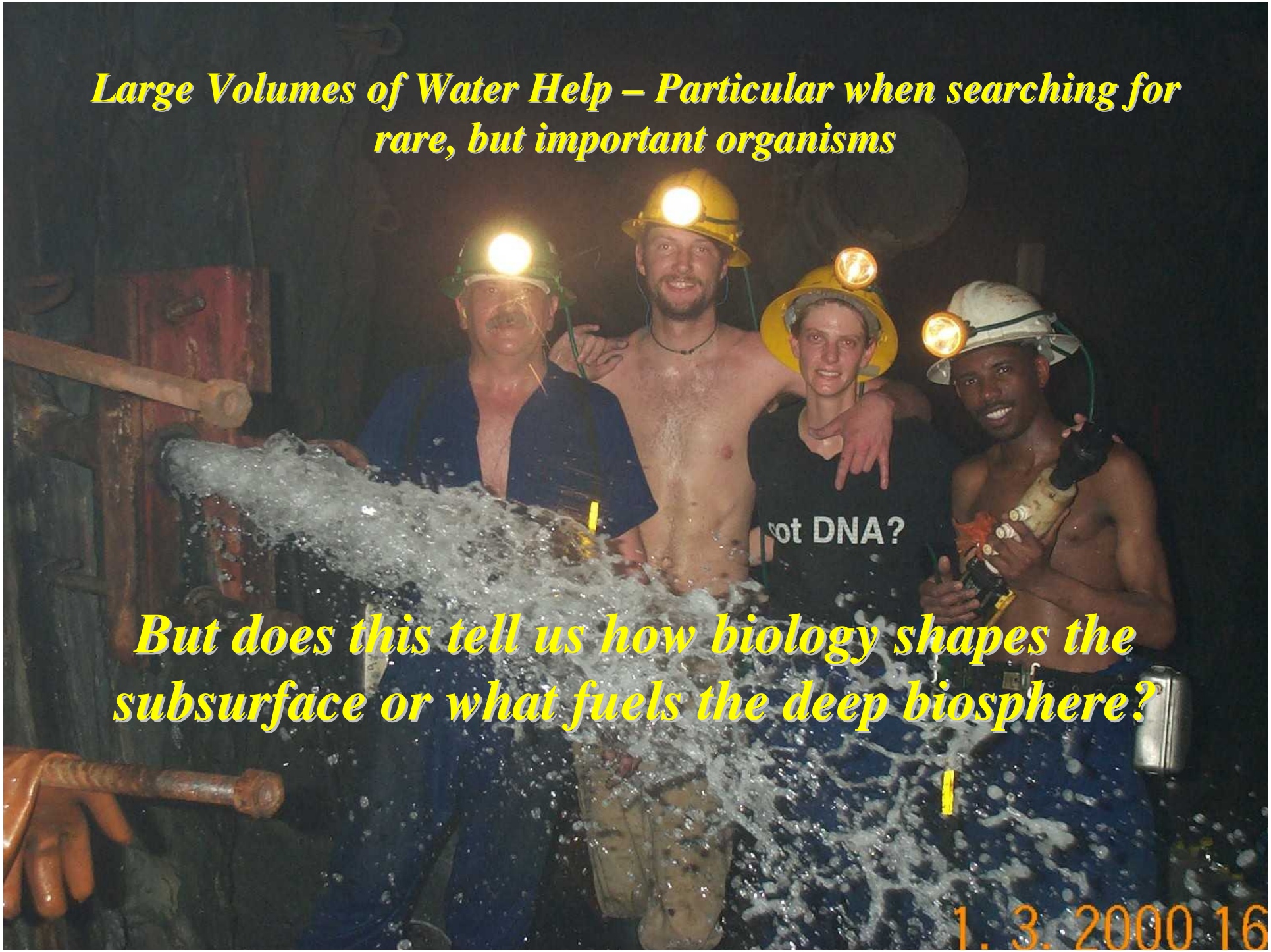
"In Sneffels Joculis craterem quem delibat Umbra Scartaris Julii intra calendas descende, Audax viator, et terrestre centrum attinges."

("Descend, bold traveller, into the crater of the Jokul of Sneffels, which the shadow of Scartaris touches before the kalends of July, and you will attain the center of the earth.")

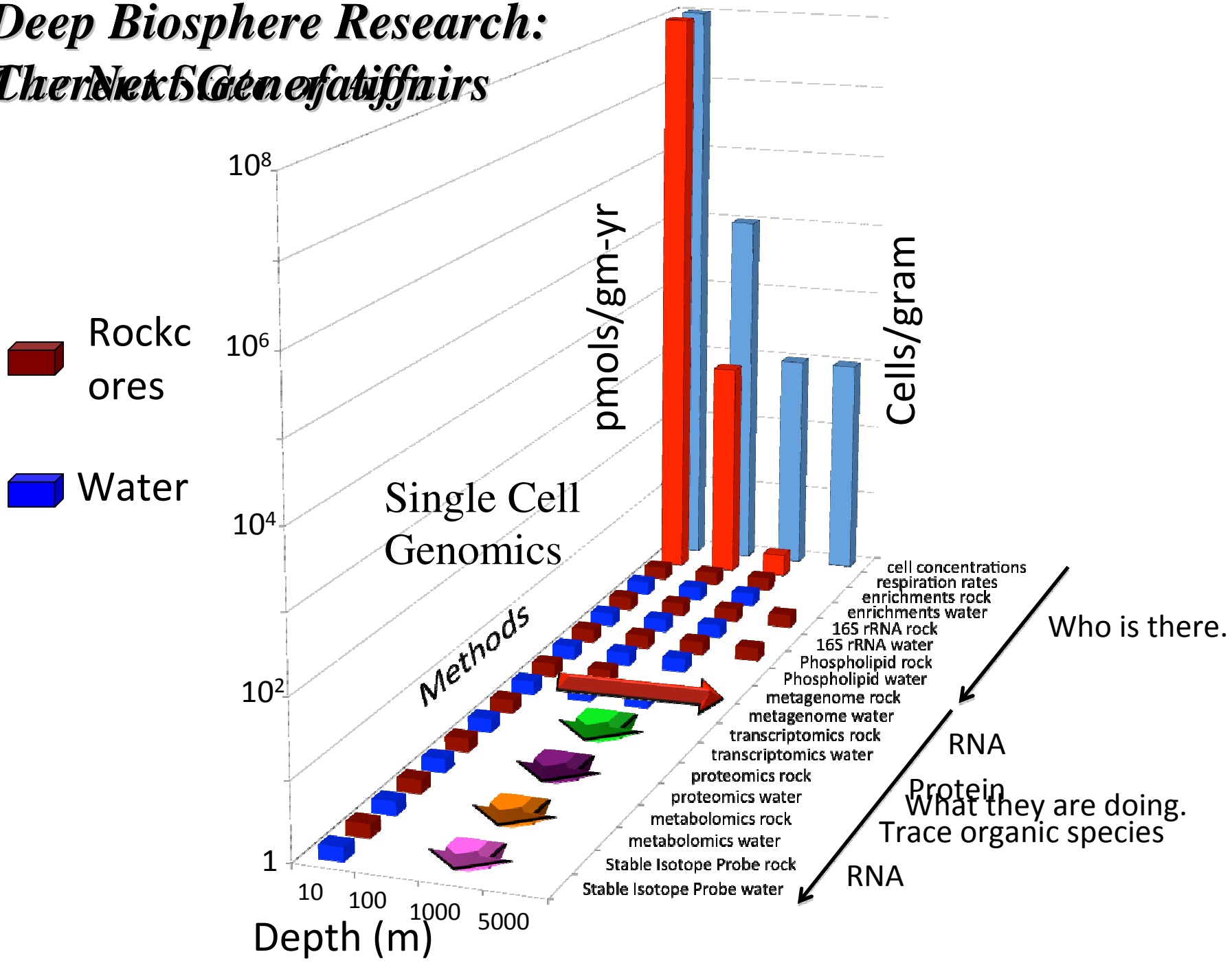
-- Hidden message deciphered from an Icelandic saga that prompts Professor Lidenbrock to undertake his travels in Jules Verne's "Journey to the Center of the Earth"


type IV pilus
antimicrobial peptide efflux
lipo prot. export
polysacc. export
multidrug export
PO_4^{3-}
CrO_4^{2-} MoO_4^{2-} WO_4^{2-} SO_4^{2-}
RSO_2O^- CO_3^{2-} NO_3^-
Fe^{3+}
Ni^{2+}
Co^{2+}
Mg^{2+} Zn^{2+} Mn^{2+}
polar AA
branched chain AA
dipeptide and other AA
sugars
anions
cations
K^+ Fe^{2+} NH_4^+ CrO_4^{2-}
gas vesicle
Nitrogenase
N_2
NH_3+ H_2
8e-+ 18ATP
ADP + P_i
AA, NA, NAD synthesis
gln
Gln synthetase
2H+
CO_2+H_2O
superoxide dismustase
rubrerythrin
O_2^-
H_2O_2
H_2O
ATP
ADP + P_i
ATP
NH_3
H^+ Na^+
H^+
$2Na^+$
Ca^{2+}
organic synthesis
Pentose phosphate pathway
Glycolysis
Gluconeogenesis
Acetyl-CoA
Flagella
Sec-dep protein
Sec-indep protein
endospore
SO_4^{2-} APS-SO_4^{2-} SO_3^{2-} HS^- H_2S H^+
e-
Qmo
Hme
PO_4^{3-} Na^+ SO_4^{2-}
H^+
H^+
H_2O
Formate DH
$HCOO^-$
+THF
+THF-CH_3 + CO
+CoA
Acetyl-CoA sythase
CO_2
$HCOO^-$
CO_2+H_2
Formate hydrogenlyase complex
2H+ 2e-
Fe, Ni
2H+ 2e-
Fe
H_2
H_2
Citric acid Cycle
CO_2
CO_2
SDH
2H+ 2e-
F420
H_2
ATP
ADP + P_i
F_oF_1-ATPase
H^+ Na^+?
Signal Transduction
HPK(9)
RR(19)
MCP(5)
LDH
Lactate
$Fe(OH)_3$
$4H^+$ $2SO_4^{2-}$
FeS_2
H_2O_2
$2H_2O$
H_2O_2
H_2
H_2
$2H_2O$
CO_2
α, β, γ
U_2O_4
HCO_3^-
Ca^{2+} HCO_3^-
$2H_2O$ Ca^{2+}
$2H^+$
$CaCO_3$
$2H^+$
Na^+
$Mg_3Si_4O_{10}(OH)_2$
$NaAlSi_3O_8$


Transport
antimicrobial peptide efflux
lipo prot. export
polysacc. export
multidrug export
PO_4^{3-}
CrO_4^{2-} MoO_4^{2-} WO_4^{2-} SO_4^{2-}
RSO_2O^- CO_3^{2-} NO_3^-
Fe^{3+}
Ni^{2+}
Co^{2+}
Mg^{2+} Zn^{2+} Mn^{2+}
polar AA
branched chain AA
dipeptide and other AA
sugars
anions
cations
K^+ Fe^{2+} NH_4^+ CrO_4^{2-}
type IV pilus
gas vesicle
endospore
Nitrogenase
N_2
NH_3+ H_2
8e-+ 18ATP
ADP + P_i
AA, NA, NAD synthesis
gln
Gln synthetase
2H^+
CO_2+H_2O
superoxide dismustase
rubrerythrin
O_2^-
H_2O_2
H_2O
ATP
ADP + P_i
ATP
NH_3
Na^+
2Na^+
Ca^{2+}
organic synthesis
Pentose phosphate pathway
Glycolysis
Gluconeogenesis
Acetyl-CoA
Signal Transduction
HPK(9)
RR(19)
MCP(5)
LDH
Lactate
Sec-dep protein
Sec-indep protein
H_2S
Formate DH
$HCOO^-$
+THF
+THF-CH_3
+CoA
CO_2
CO
Acetyl-CoA sythase
e-
e-
Qmo
Hme
$HCOO^-$
CO_2+H_2
Formate hydrogenlyase complex
2H^+ 2e- Fe, Ni
2H^+ 2e- Fe
H_2
H_2
Citric acid Cycle
CO_2
CO_2
SDH
2H^+ 2e- F420
H_2
F_oF_1-ATPase
ATP
ADP + P_i
PO_4^{3-}
SO_4^{2-}
$Fe(OH)_3$
FeS_2
HCO_2^-
H^+
HCO_3^-
Ca^{2+} HCO_3^-
$2H_2O$ Ca^{2+}
H^+
U_2O_4
$CaCO_3$
$Mg_3Si_4O_{10}(OH)_2$
$NaAlSi_3O_8$


Large Volumes of Water Help – Particular when searching for rare, but important organisms
ot DNA?
But does this tell us how biology shapes the subsurface or what fuels the deep biosphere?
1. 3. 2000 16

Deep Biosphere Research: The Next Generation



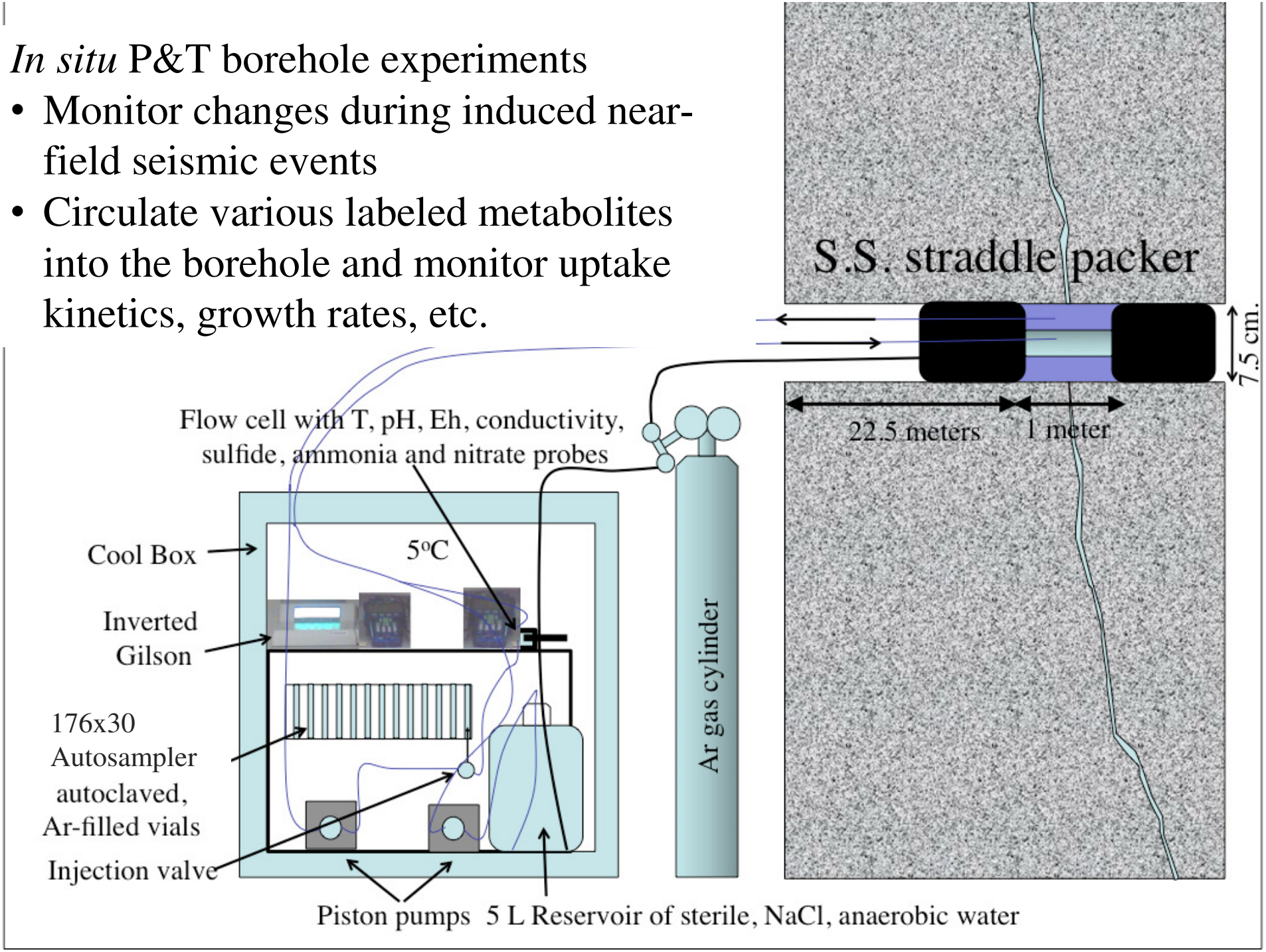
Transformative science goals, why is DUSEL required?

- What are the microbes doing? Perform experiments in a geologically and geochemically well-characterized environment.
- Why are they doing what they are doing? Perform in situ experiments under a variety of environmental stimulus (including changes in temperature, stress and flow rates).
- How did they come to be there? Characterize the ecohydrology from the surface to the base of the biosphere.

This requires a coordinated, multi-institutional, multi-disciplinary, experimental facility at at least one location focused on the biosphere, from the surface to the base, for multiple decades. It is the *in situ* experimentation that will develop a deeper understanding of the complex and interrelated phenomena surrounding subsurface life.

In situ P&T borehole experiments

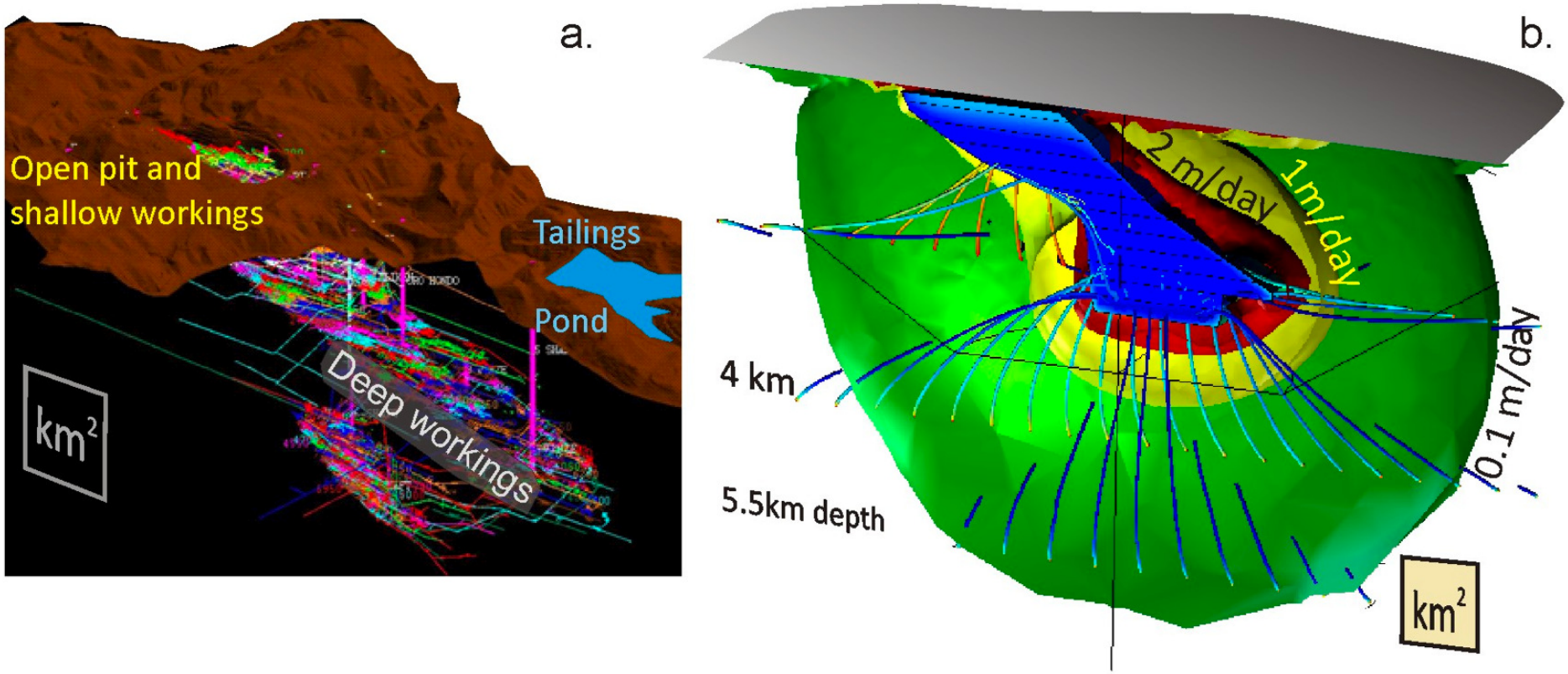
- Monitor changes during induced near-field seismic events
- Circulate various labeled metabolites into the borehole and monitor uptake kinetics, growth rates, etc.



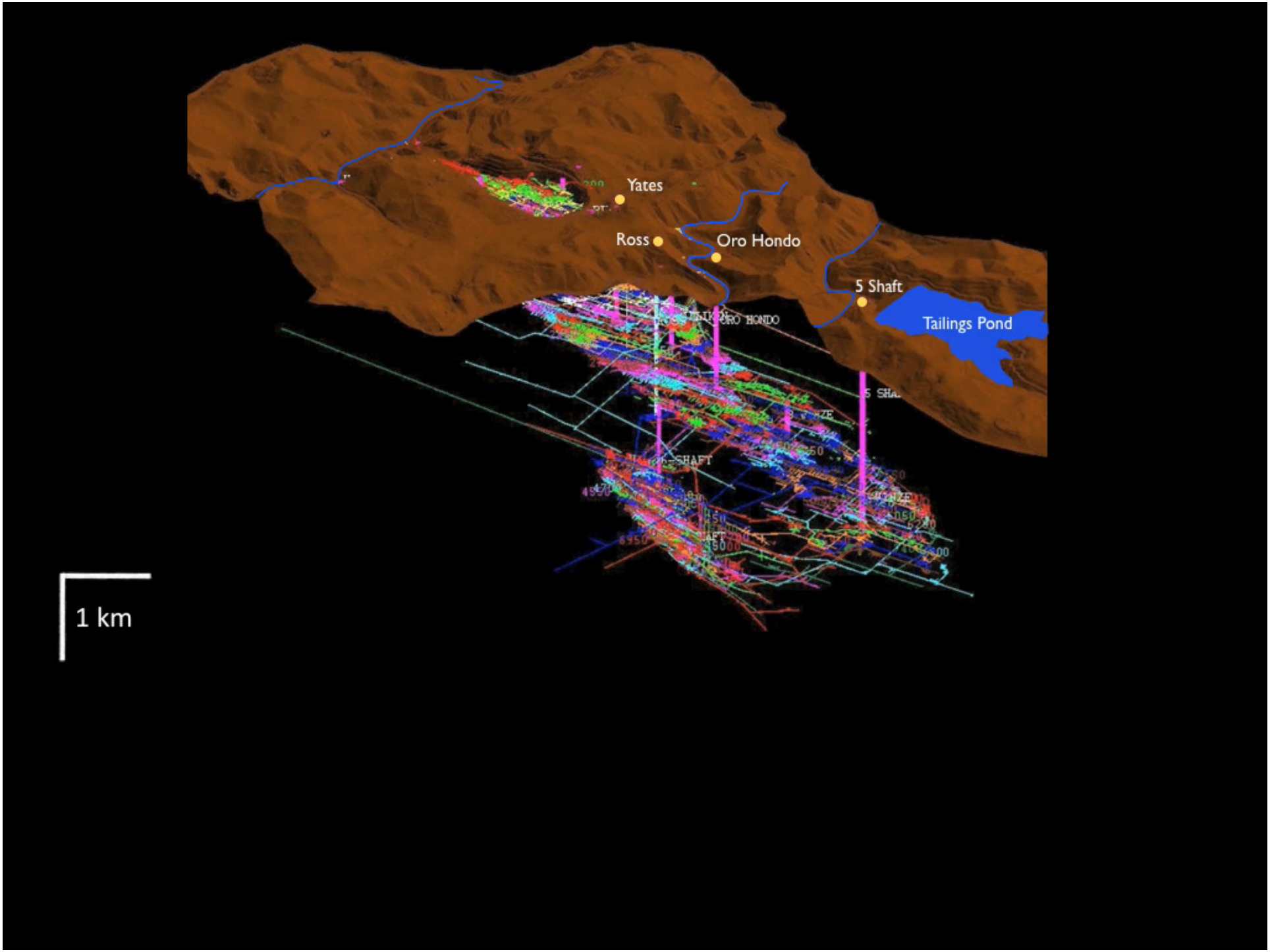
MUL's – Mobile Underground Laboratories

Äspo Hard Rock Laboratory

Why Homestake? – Geologically well-characterized 3D volume to deepest levels in USA.



Should provide access to a variety of ground water ages from the top to the bottom of the subsurface biosphere.

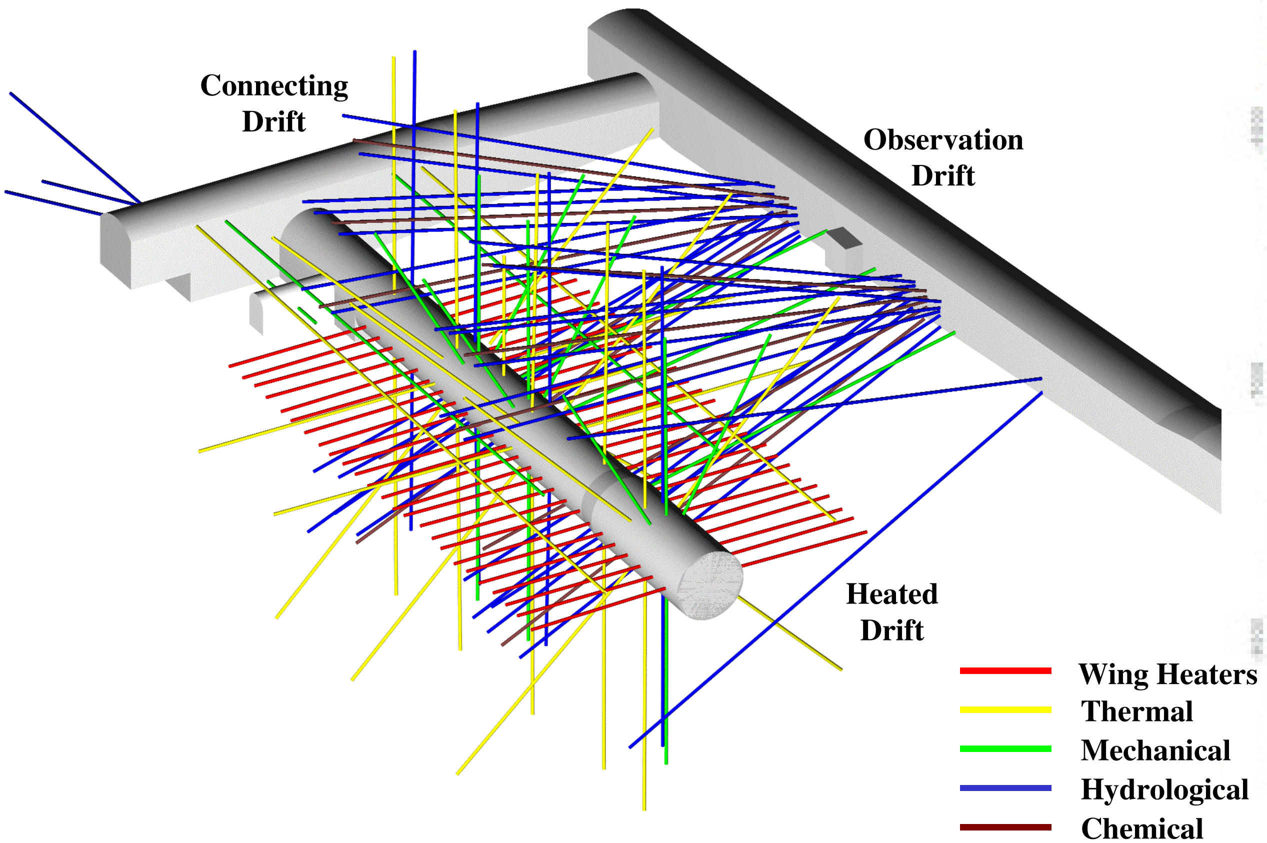

Yates
Ross
Oro Hondo
5 Shaft
Tailings Pond
1 km

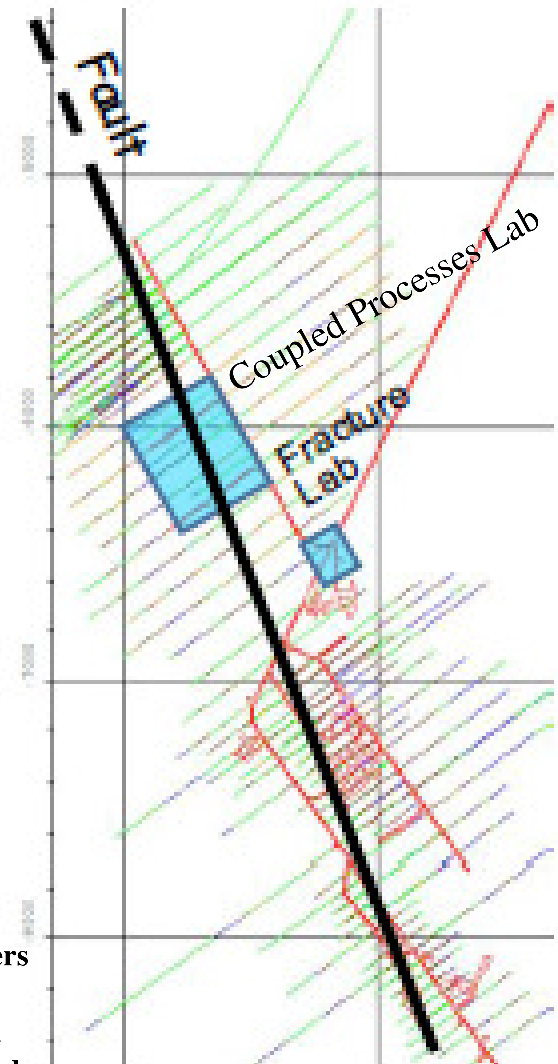
Are Critically Stressed Faults Critical to Subsurface Life?

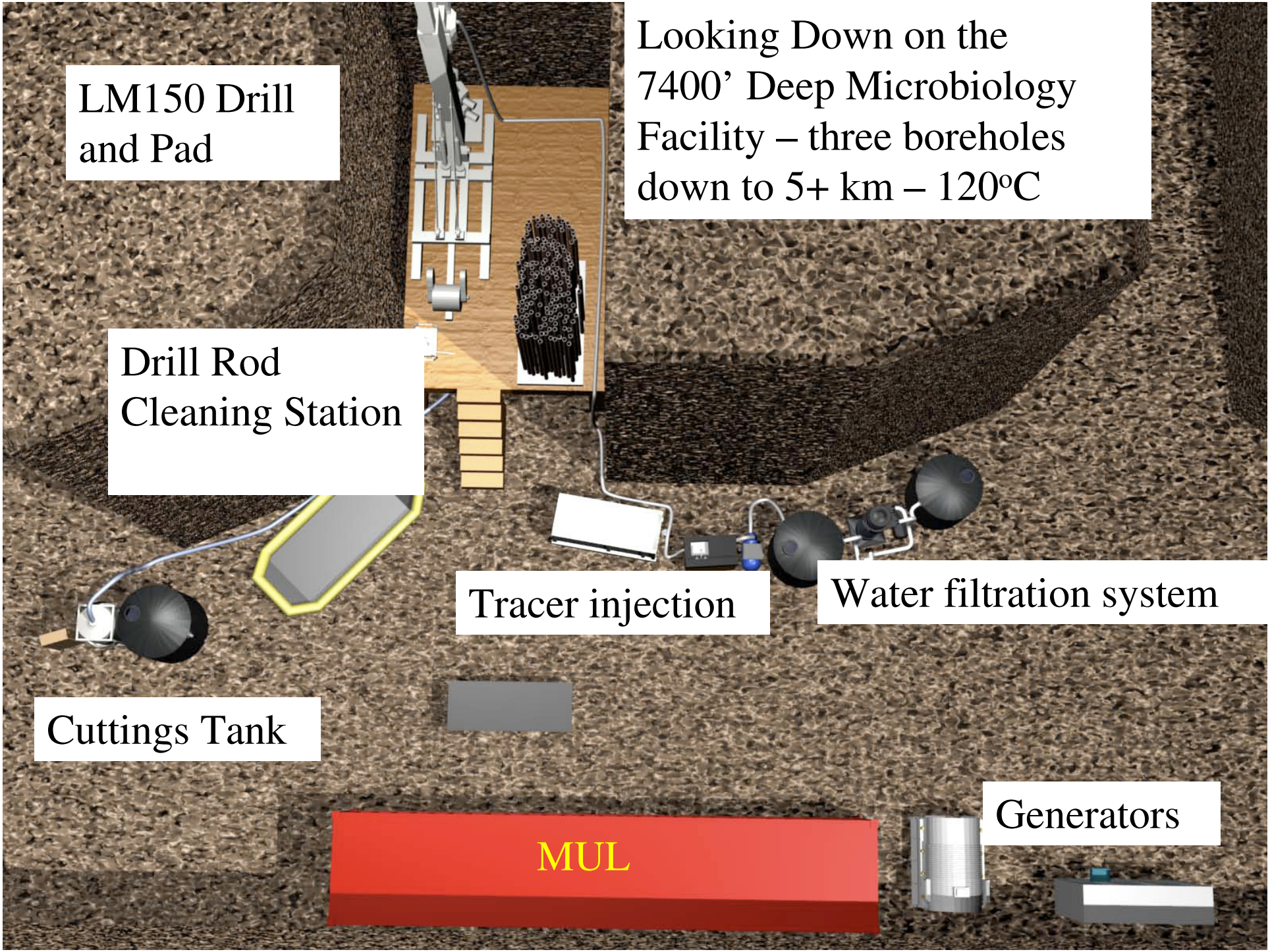
How do Microbial Communities Respond to Hydrothermal Circulation?

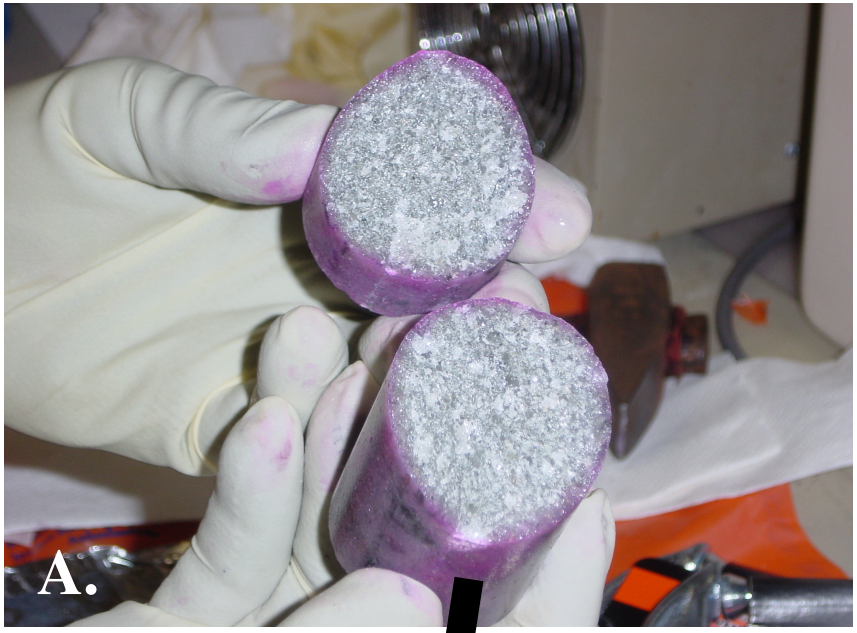
- During fault rupture do changes in fluid flux induce changes in chemical flux which in turn alter microbial dynamics?
- If so, then is this an important process for the

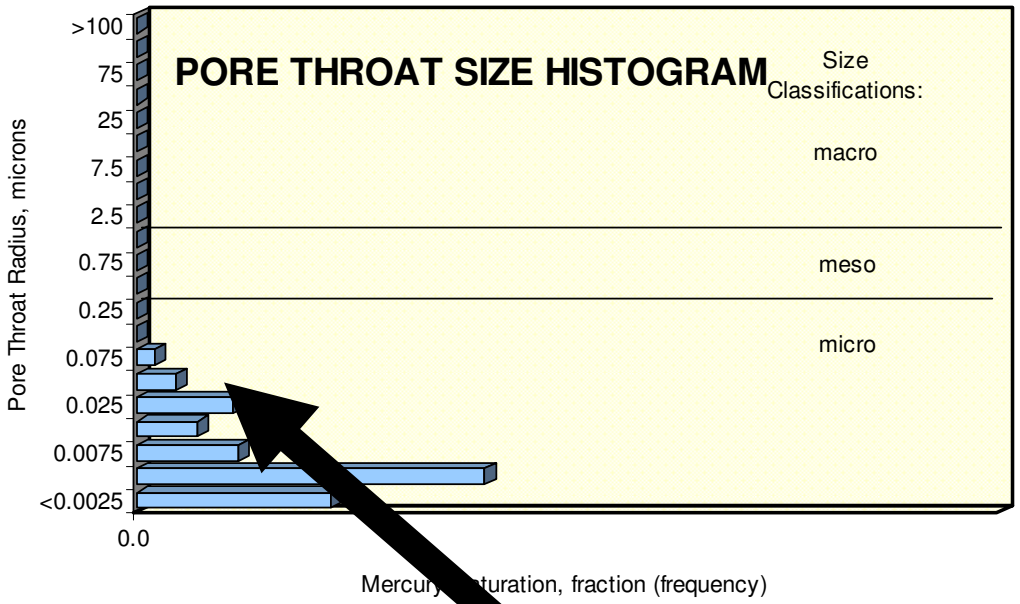
- Are the thermophiles enriched in fault zones?
- Does enhanced fluid flux lead to enhanced activity and growth rates?



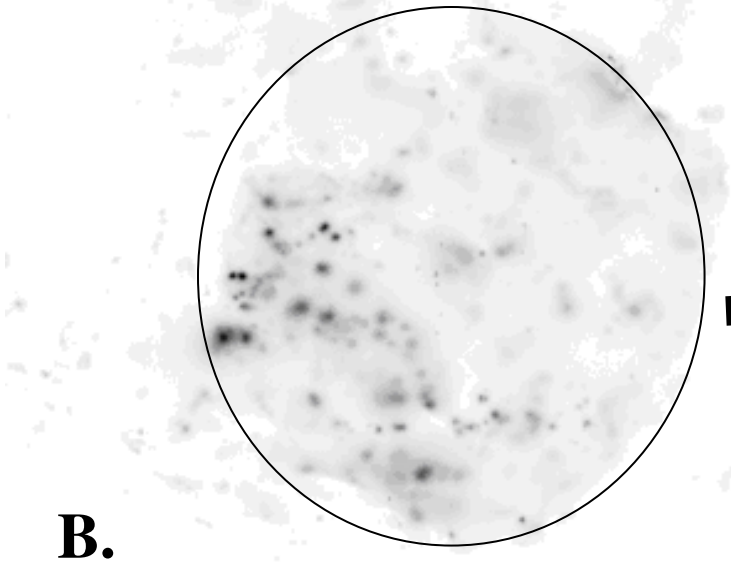


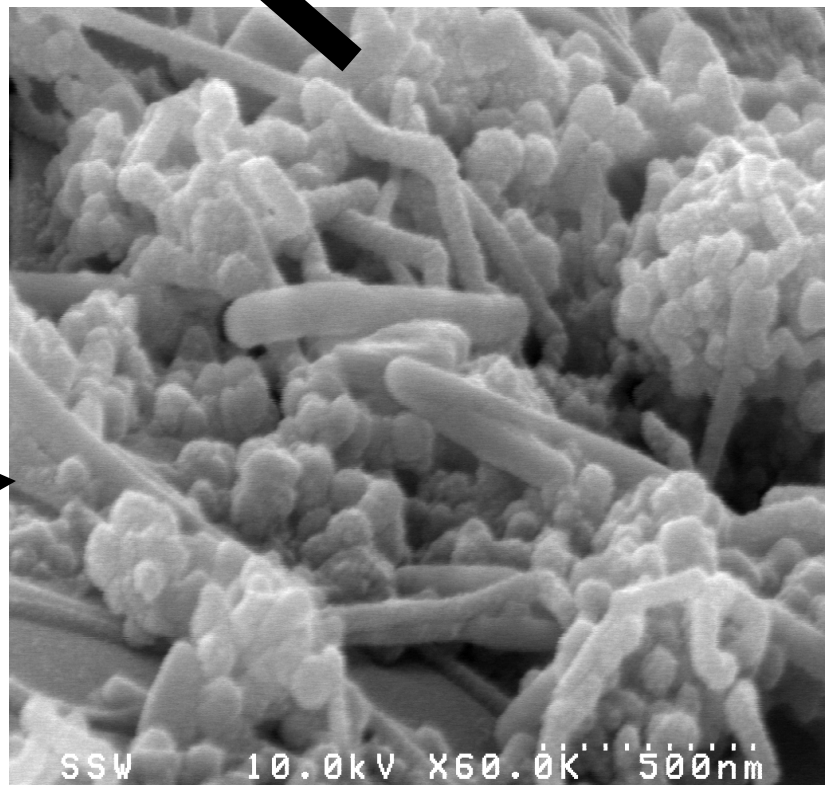

LM150 Drill and Pad
Looking Down on the 7400' Deep Microbiology Facility – three boreholes down to 5+ km – 120°C
Drill Rod Cleaning Station
Tracer injection
Water filtration system
Cuttings Tank
MUL
Generators


A.


PORE THROAT SIZE HISTOGRAM
Size Classifications:
macro
meso
micro
Pore Throat Radius, microns
>100
75
25
7.5
2.5
0.75
0.25
0.075
0.025
0.0075
<0.0025
0.0
Mercury Saturation, fraction (frequency)

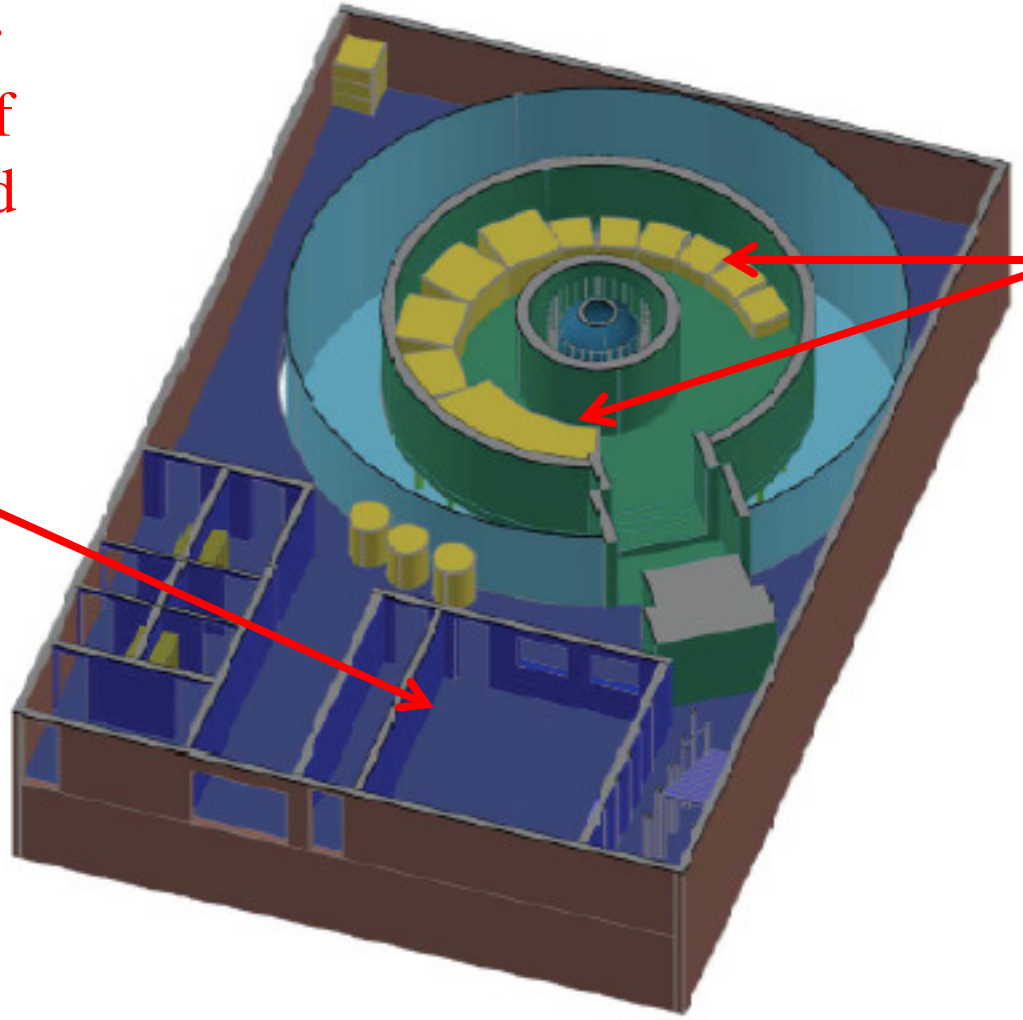
D.


B.


C.
SSW 10.0kV X60.0K 500nm

Low Background Counting Laboratory Located at the 4850' Level

Lab space for preparation of biological and hydrological samples (e.g. ^{210}Pb, ^{137}Cs, ^{39}Ar, etc.)



α, β and γ counters behind water shield, including large volume β cage.

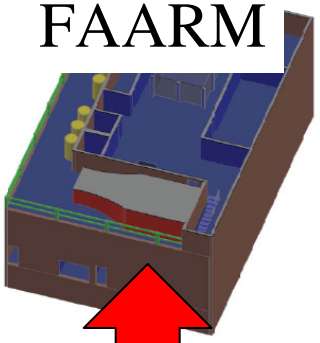
Ecohydrology would produce ~60,000 samples for counting over 5 years

Conceptual design of the DUSEL biology program

1. MREFC - Clean coring and core hole completions from surface to base of biosphere with metal-free packers isolating individual fractures.
   a. Employ proven and new technologies to characterize geochemistry and microbiology of rock matrix, fracture surfaces and fracture water.
   b. Monitor fractures over time until they stabilize.
2. Post MREFC - Once core hole has recovered from drilling impact initiate experimental phase.
   a. Perform single cell DNA sequencing to generate reference genomes for community DNA, RNA and protein studies
   b. Perform population genomics analyses to determine evolutionary histories, current selective pressures and gene flow.
   c. Utilize MUL's to perform microcosm and *in situ* experiments that employ isotope labels and isotope microarrays to quantify metabolite flow, respiration rates, replication rates, the effects of nutrient limitation, quorum sensing, viral populations, and predation.

Integration of biology program with other activities at DUSEL

FAARM

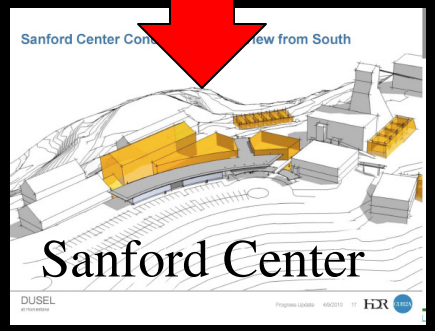


Geophysics

LUCI – CO_2

Ecohydrology

Induced Fracture Lab

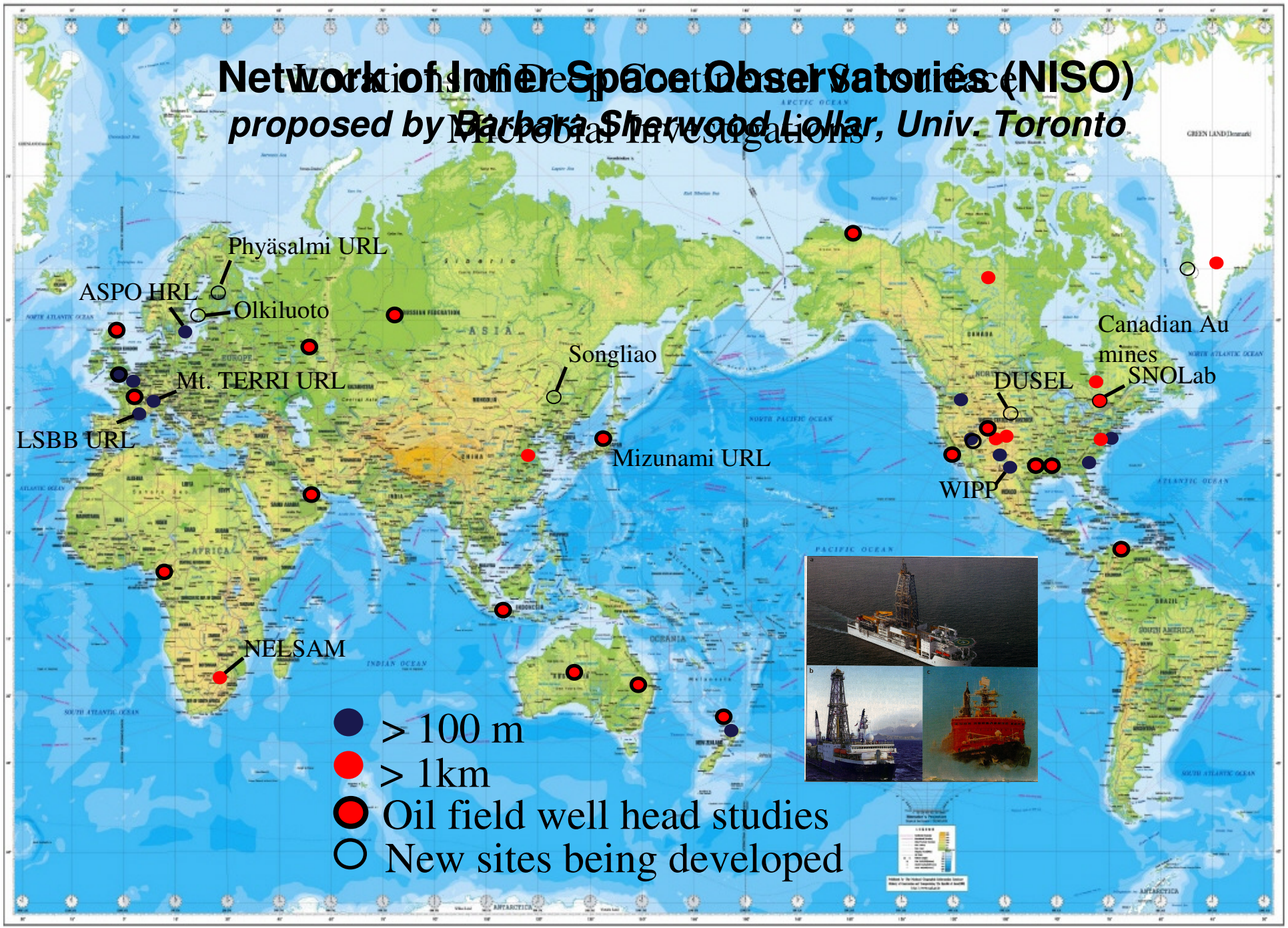


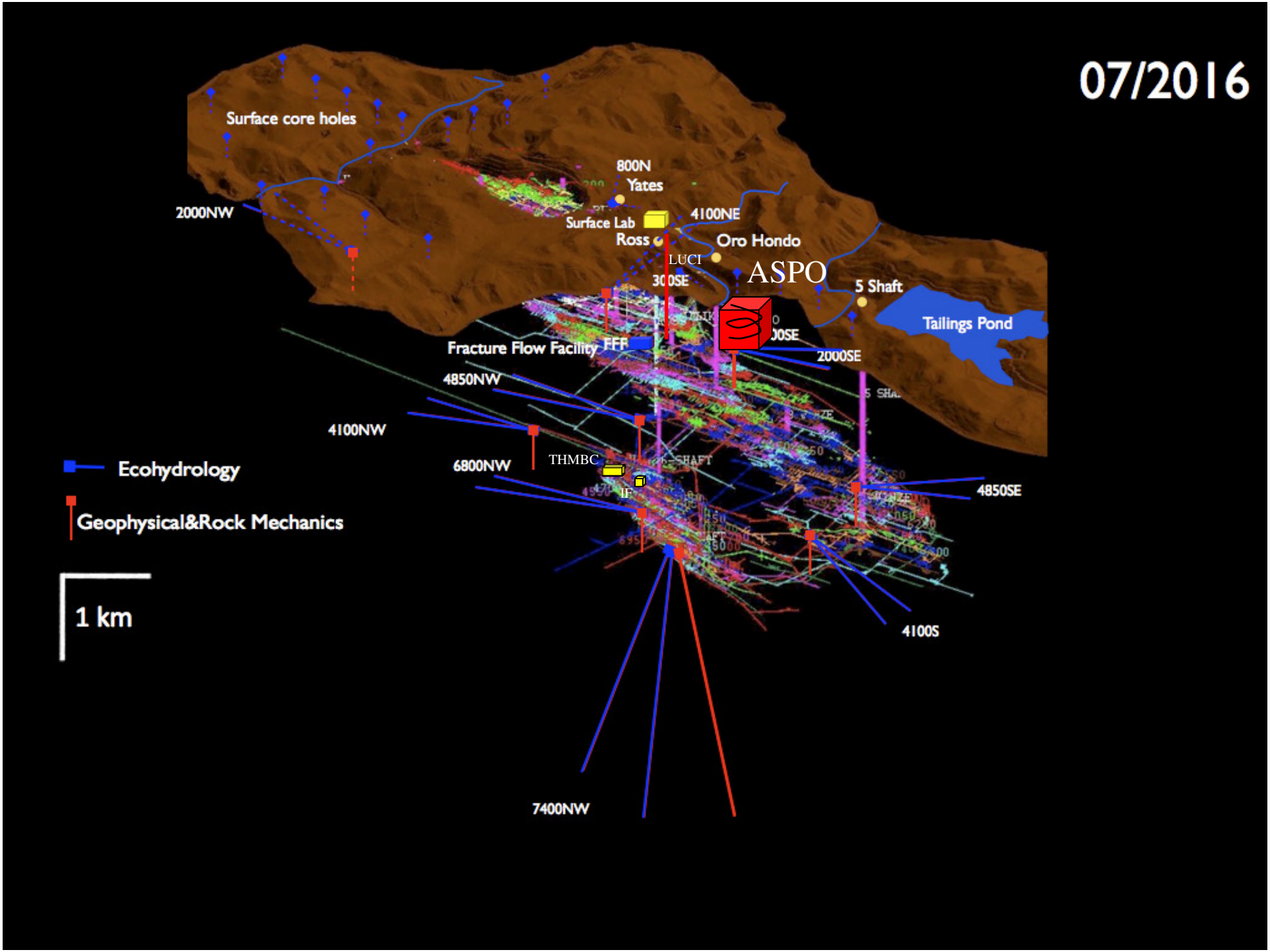
THMCB

Network of Inner Space Observatories (NISO)

proposed by Barbara Sherwood Lollar, Univ. Toronto

Microbial Investigations




07/2016
Surface core holes
800N
Yates
2000NW
4100NE
Surface Lab
Ross
Oro Hondo
LUCI
ASPO
5 Shaft
Tailings Pond
Fracture Flow Facility
FFF
2000SE
4850NW
4100NW
THMBC
6800NW
IF
4850SE
4100S
7400NW
Ecohydrology
Geophysical&Rock Mechanics
1 km

Anticipated Biological Results from DUSEL in the Coming Decade

- **Discovery of the ultimate high temperature limit for life in the crust and what controls it.**

- **Discovery of what controls the "average age" of microorganisms in the deep subsurface.**

- **Discovery of what other factors control subsurface biomass concentration, diversity, migration rates and evolution.**

- **Discovery of how microorganisms impact rock strength, hydrothermal circulation and CO_2 storage by mineral dissolution and precipitation.**

- **Discovery of new geophysical sources of energy for deep subsurface microbial communities.**