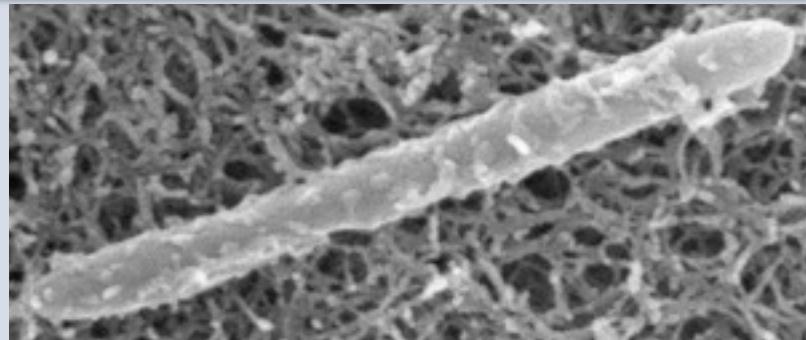


A chassis for survival in energy poor environments.

# **Self-sufficient life below the planet surface**



# Bacteria

- Many short programs for solving the problem of surviving in diverse environments
- Environmentally transformative activities to allow:
  - Extraction of energy from the environment
  - Production of complex chemicals and materials from simple building blocks
  - Transformation of soil and water
  - Self-organizing for producing superstructures
- Relatively “easy” to engineer

Based on.

# Environmental genomics reveals a single, slowly evolving, species ecosystem deep within the Earth

Dylan Chivian, Eric J. Alm, Eoin L. Brodie, David E. Culley, Thomas Gihring, Alla Lapidus, Li-Hung Lin, Steve Lowry, Duane P. Moser, Paul Richardson, Gordon Southam, Greg Wanger, Lisa M. Pratt, Adam P. Arkin, Terry C. Hazen, Fred J. Brockman and T. C. Onstott

# The TIME 100

MAIN

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ENTERTAINERSLEADERS &  
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PIONEERSSCIENTISTS  
& THINKERSBUILDERS &  
TITANS

## SCIENTISTS & THINKERS

70 of 100 [Previous](#) [Next](#)

SEARCH

### Tullis Onstott

By Carolyn Sayre

Wondering

whether

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extraterrestrial life exists? Tullis Onstott, 53, has found it—sort of. The fact that the living things he's discovered make their homes nearly two miles below the earth's surface may disqualify them from the extra part of extraterrestrial. But dig them up and fly them off, and there's no limit to the cosmic places they might survive. That's the beauty of the work Onstott, a professor of geosciences at Princeton University, is doing. Until we collect living aliens, he's showing us they're probably there to be found. Scientists once despaired of discovering even simple otherworldly organisms because they assumed that all life should be like earthly life—built around warmth, light and liquid water. In recent years, however, biology has shown itself to be tougher than that. Investigators have discovered organisms—simple ones—frozen in polar ice and thriving in scalding ocean vents.

Expectations have investigated less thoroughly the regions deep within the earth—places where living things could establish a safe redoubt. Uncover such critters on Earth, and you would prove that they might exist elsewhere too. Last year Onstott and his team published such a find. After years of dust and dirt, piggybacking off gold miners in South Africa, they identified self-sustaining bacteria living in rock deep below the surface that draw their energy from chemicals produced by the radioactive splitting of water, essentially feasting on hydrogen and sulfur compounds. So when we do finally uncover the first alien life-form, you may get the sense that you've seen it before.

[Next: Svante Pääbo >>](#)

TRAVIS PITTZ / LEVINE AND LEVINE FOR TIME  
Tullis Onstott

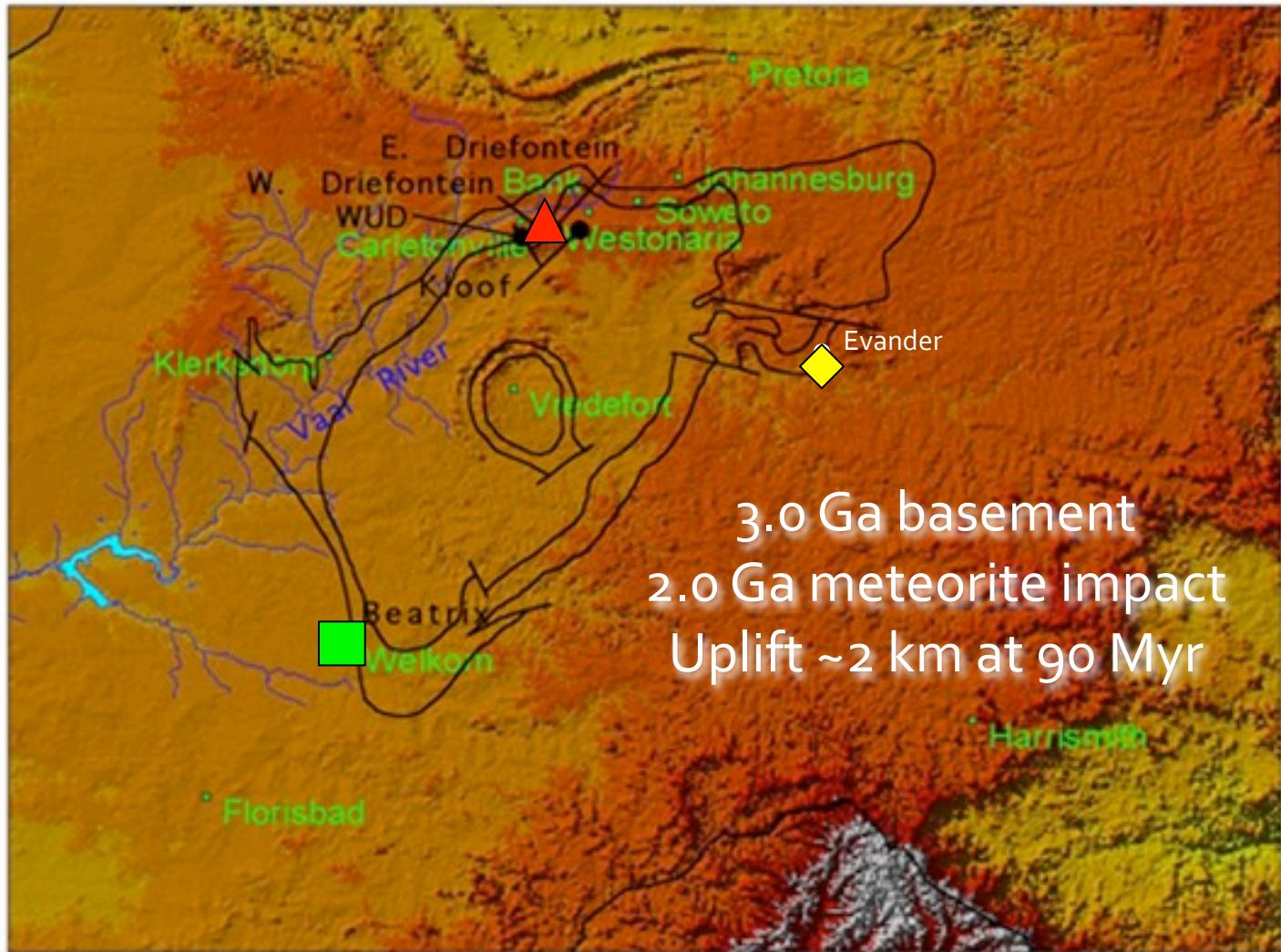
## The People Who Shape Our World

Here's our list of the 100 men and women whose power, talent or moral example is transforming the world



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Do deep, sequestered microbial communities  
 evolve? If so, how?

# Mining biological gold?



# Witwatersrand Deep Microbiology Project

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Dec. 2001, p. 4793-4793  
0899-9005/01/\$04.00+0 34100112/0/AEM/71/12/4793-4793\$15.00

Ned W. Beck

*Desulfotomaculum* and *Methanobacterium* spp. Dominate a 4- to 5-Kilometer-Deep Fault

Duane P. Moser,<sup>1,\*</sup> Thomas M. Gihring,<sup>†</sup> Fred J. Brockman,<sup>1</sup> James K. Fredrickson,<sup>1</sup> David L. Balkwill,<sup>1</sup> Michael E. Dolhopf,<sup>‡</sup> Barbara Sherwood Lollar,<sup>§</sup> Lisa M. Pratt,<sup>§</sup> Erik Boice,<sup>1</sup> Gordon Southam,<sup>§</sup> Greg Wanger,<sup>§</sup> Brett J. Baker,<sup>§</sup>

*Environmental Microbiology Group, Pacific Northwest National Laboratory, Richland, Environmental Sciences Division, The Florida State University, Tallahassee, Florida 32306-4350;*  
*University of Toronto, Toronto, Ontario M5S 3H6, Canada;* *Department of Geodesy and*  
*Geomatics, Indiana University, Bloomington, Indiana 47406-5200;* *Department of Geodesy and*  
*Geomatics, Western Ontario, London, Ontario N6A 3H7, Canada;* *Earth and Planetary*  
*California, Berkeley, California 94720-2470;* *Center of Biomolecular Analysis,*  
*Tennessee, Knoxville, Tennessee 37992-3432;* *and Department of Geosciences,*  
*Geophysical Sciences, Princeton University, Princeton, New Jersey 08544.*

Received 6 April 2005; Accepted 15 August 2005

Alkaline, sulfide, 54 to 60°C, 4 to 53 million-year-old meteoric water emanating from quartzite-hosted fractures >2.5 km beneath the surface supported a microbial bacterial species affiliated with *Bacillus*-like spp., and an archaeal species spp. The geochemical homogeneity over the 670-m length of the borehole, the absence of these microorganisms in nitrified water support an indigenous community. The coexistence of these two microorganisms is consistent with a  $\text{H}_2$  and  $\text{SO}_4^{2-}$  in the presence of high pH, high concentrations of  $\text{H}_2$ , and  $\text{CH}_4$  autotrophic methanogens. Sulfide isotopic compositions were highly enriched  $\text{SO}_4^{2-}$  reduction under hydrologic isolation. An analogous microbial couple and have been reported recently for hydrothermal carbonate veins of the Lucy Ridge (D. B. Kelly et al., *Science* 307:1428–1434, 2005), suggesting that these form subsurface habitats (continental and marine) bearing this geochemical signatures. Microbial communities described here are notably different from microbial communities in continental subsurface environments.

Numerous studies have revealed the presence of microbial communities occupying oceanic and terrestrial deep subsurface settings (12, 14, 23, 25, 53, 54). Due to its enormous volume, this habitat may host the majority of Earth's prokaryotes (76), and according to some estimates (25, 76), the collective biomass of subsurface microbiota may rival that of flora and fauna at the surface. It is generally accepted that life on Earth requires liquid water (9). The upper 4 km of the terrestrial crust contains  $9.3 \times 10^9 \text{ km}^3$  of groundwater, of which about 56% lies below 0.75 km in depth (6). Since requirements of microorganisms over this depth interval confine (4, 53, 54, 76) and the basic requirements for life appear to be met (e.g., liquid water, habitable space, and permissive temperatures), it follows that a significant proportion of the biosphere may be microbial and associated with deep terrestrial hydrologic systems (76).

Although a reasonable database for marine and estuarine systems to a depth of 30 m exists for the euryhaline rock systems remain in igneous rock-hosted a chemolithotrophic/biochemical methanogenesis geological H<sub>2</sub> production limited in photophytes. Relatively little is known about such communities at intermediate depths of other deep-sea metagenomes.

## **Environmental Genomics Reveals a Single-Species Ecosystem Deep Within Earth**

Dylan Christian,<sup>1,2</sup> Enikő L. Brodsky,<sup>1,2</sup> Erik J. Adam,<sup>1,2</sup> David E. Colley,<sup>5</sup> Paramoreli S. Detwiller,<sup>1,2</sup> Todd B. Durstantic,<sup>1</sup> Thomas M. Gilfrich,<sup>6</sup> Alia Lapidus,<sup>7</sup> Li-Hsiang Lin,<sup>8</sup> Stephen R. Lossey,<sup>1</sup> Suzanne P. Massie,<sup>2</sup> Paul W. Richardson,<sup>2</sup> Gordon Southam,<sup>1</sup> Greg Wanger,<sup>1</sup> Lisa H. Pratt,<sup>1,11,12</sup> Lars J. L. Andersen,<sup>2,3</sup> Karen C. Hanson,<sup>1,3,14</sup> and Eric J. Wickstrom<sup>1,2</sup> *Adam B. Aebischer,<sup>1,2</sup> Gisela M. Kudla,<sup>1,2</sup> Christopher J. Donahue<sup>1,2,13</sup>*

DNA from low-biodiversity fracture water collected at 2.8-kilometre depth in a South African gold mine was sequenced and assembled into a single, complete genome. This bacterium, *Candidatus Desulfovibrulli oxidoreducens*, comprises >99.9% of the microorganisms inhabiting the fluid phase of this particular fracture. Its genome indicates a motile, sporulating, sulfate-reducing chemolithotrophic thermophile that can fix its own nitrogen and carbon by using machinery shared with archaea. *Candidatus Desulfovibrulli oxidoreducens* is capable of an independent life-style well suited to long-term isolation from the phosphoreiche deep within Earth's crust and offers an example of a natural ecosystem that appears to have its biological component entirely encoded within a single genome.

A more complete picture of life on, or even in, Earth has recently become possible by extracting and sequencing DNA from an environmental sample, a process called environmental genomics or metagenomics (1–6). This approach allows us to identify members of microbial communities and characterize the abilities of the dominant members even when isolation of those organisms has proven intractable. However, with a few exceptions (7), assembling complete or even near-complete genomes for a substantial portion of the microbial species is usually hampered by the complexity of mixed microbial communities.

In addition to elevated temperatures and lack of O<sub>2</sub>, conditions within Earth's crust depths >1 cm are fundamentally different from those of the surface and deep ocean environments. Severe nutrient limitation is believed to result in cell doubling times ranging from 10 to 1000s of years (P-1), and as a result, surface microorganisms might be expected to reduce their reproductive burden and exhibit slow-strained genomes of specialists or spend most of their time in a state of semi-senescence waiting for the return of favorable conditions.

## **Long-Term Sustainability of a High-Energy, Low-Diversity Crustal Biome**

Li-Hwang Lin,<sup>1,2\*</sup> Pei-Ling Wang,<sup>1</sup> Douglas Rumble,<sup>2</sup> Johanna Lippmann-Pike,<sup>1</sup> Erik Boen,<sup>4</sup> Lisa M. Pratt,<sup>4</sup> Barbara Sherwood Lollar,<sup>5</sup> Edie L. Brodin,<sup>1</sup> Terry C. Kazem,<sup>1</sup> Gary L. Andersen,<sup>6</sup> Todd D. MacKenzie,<sup>7</sup> Dennis P. Moran,<sup>8</sup> David J. Stevenson<sup>1,2,3</sup> (Received April 2, 2007)

Geochemical, microbiological, and molecular analysis of alkaline saline groundwater at 2.8 kilometers depth in Archanas metabasalt revealed a microbial biome dominated by a single *glycolysate* affiliated with thermophilic sulfate reducers belonging to *Fimbricutes*. These sulfate-

genome of this organism appeared to possess all of the metabolic capabilities necessary for an independent life-style. This gene complement was consistent with the previous geochemical and thermodynamic analyses at the anoxic,  $-60^{\circ}\text{C}$  temperature and pH of 4.3, which indicated metabolically generated chemical species as providing the energy and nutrients to the system (65% with fumarate and 6% with propionate) having greatest potential among candidate electron donors, and sulfate ( $\text{SO}_4^{2-}$ ) reduction as the dominant electron-acceptor process (22).

DNA was extracted from ~5000 litres of filtered surface water by using a protocol that has been demonstrated to be effective on a broad range of bacterial and archaeal species, including noncultivable organisms (16). A single, complete, 2.3-megabase pair (Mbp) genome was assembled with a combination of shotgun Sanger sequencing and 454 pyrosequencing (16). Similar to other studies that obtained non-competition genomes from environmental samples (3,5,7,17), heterogeneity in the population of the dominant species as measured with single nucleotide polymorphism (SNP) was evaluated by

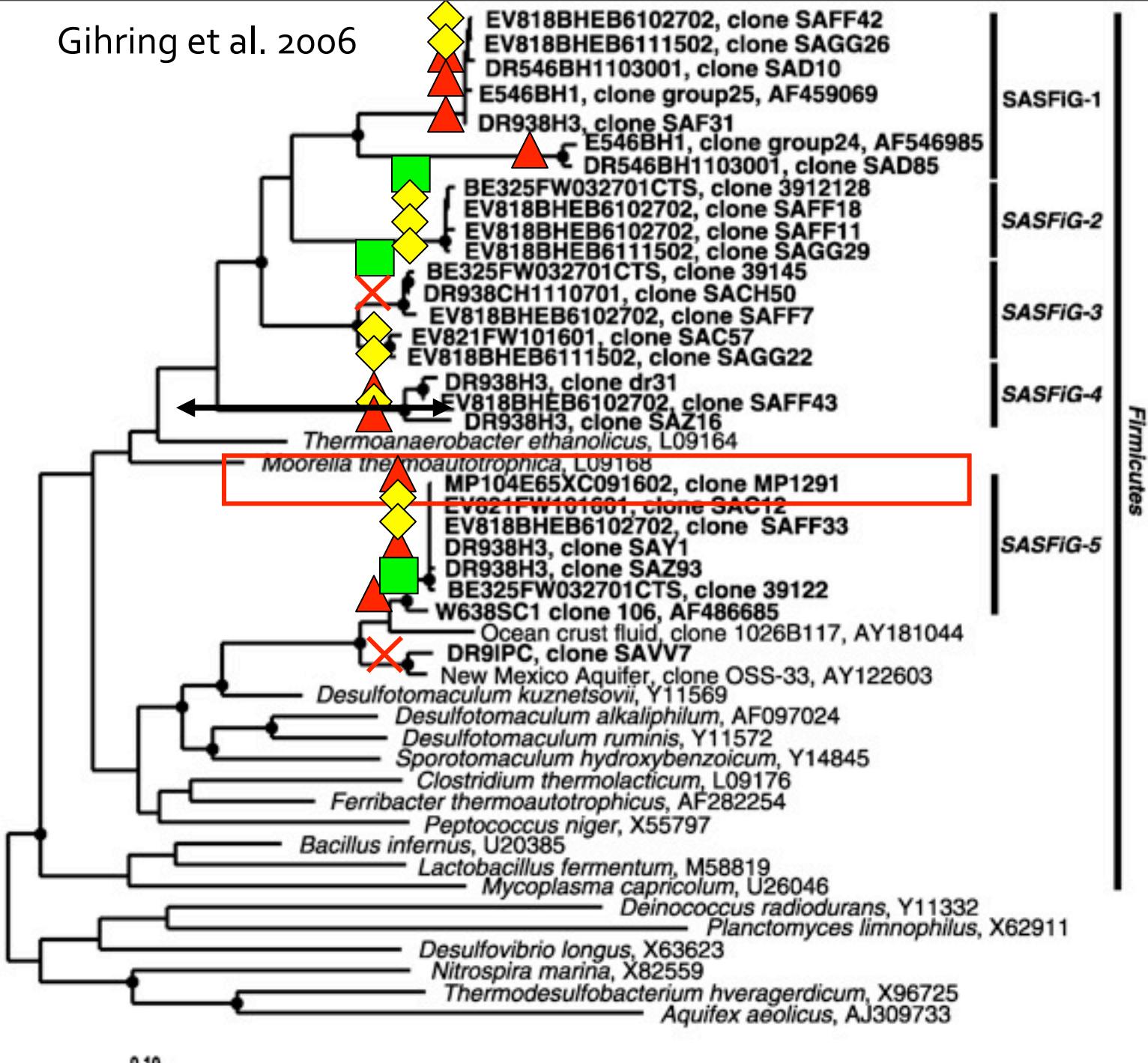
S-05	3 (13)	6 (18)	5
MP10455	MP10455X	MP10455X	MP10455X
01902	-0.07212	<130950	58-091652
rate	fracture	fracture	fracture
rate	water	water	water
rate	water	water	water
10.0 (55) 4.2(2.6/4)	2.3(2.7 0/4)	N/A	N/A
9.3	9.3	9.2	N/A
-10.0	-340	-263	N/A
52	52	52	20
N/A	7.1	8.9	1.4
N/A	23.5	35.7	5.8
N/A	71.9	84.9	0.42
N/A	177	218	3.8
N/A	908	1868	17.1
1100	N/A	1660	N/A
2100	2090	1725	N/A
31000	9120	16400	N/A
-dL	-61.	-61.	285
-44	-488	-695	N/A
-13.7	-32.8	-33.2	N/A
-0.47	-566	-393	N/A
0-10.2	15.8 ± 7.9	N/A	N/A
3-10.8	14.9 ± 8.4	N/A	N/A
0-3.8	21.0 ± 6.0	N/A	N/A
-3-3.8	23.8 ± 4.6	N/A	N/A
0-10.8	30 ± 35	30 ± 15	0 ± 1.5 ± 10
-dL	-5 × 10 <sup>-2</sup>	-5 × 10 <sup>-2</sup>	260 ± 100
N/A	N/A	1.1 ± 0.3 ±	

For sample 1, 27.6 mM for sample 2 and 60.0 mM for sample 3. (The 3 is corrected for dilution loss 18. Diffusion correction was not applied to the positive O<sub>2</sub> content for sample 1 as it may be derived from incomplete

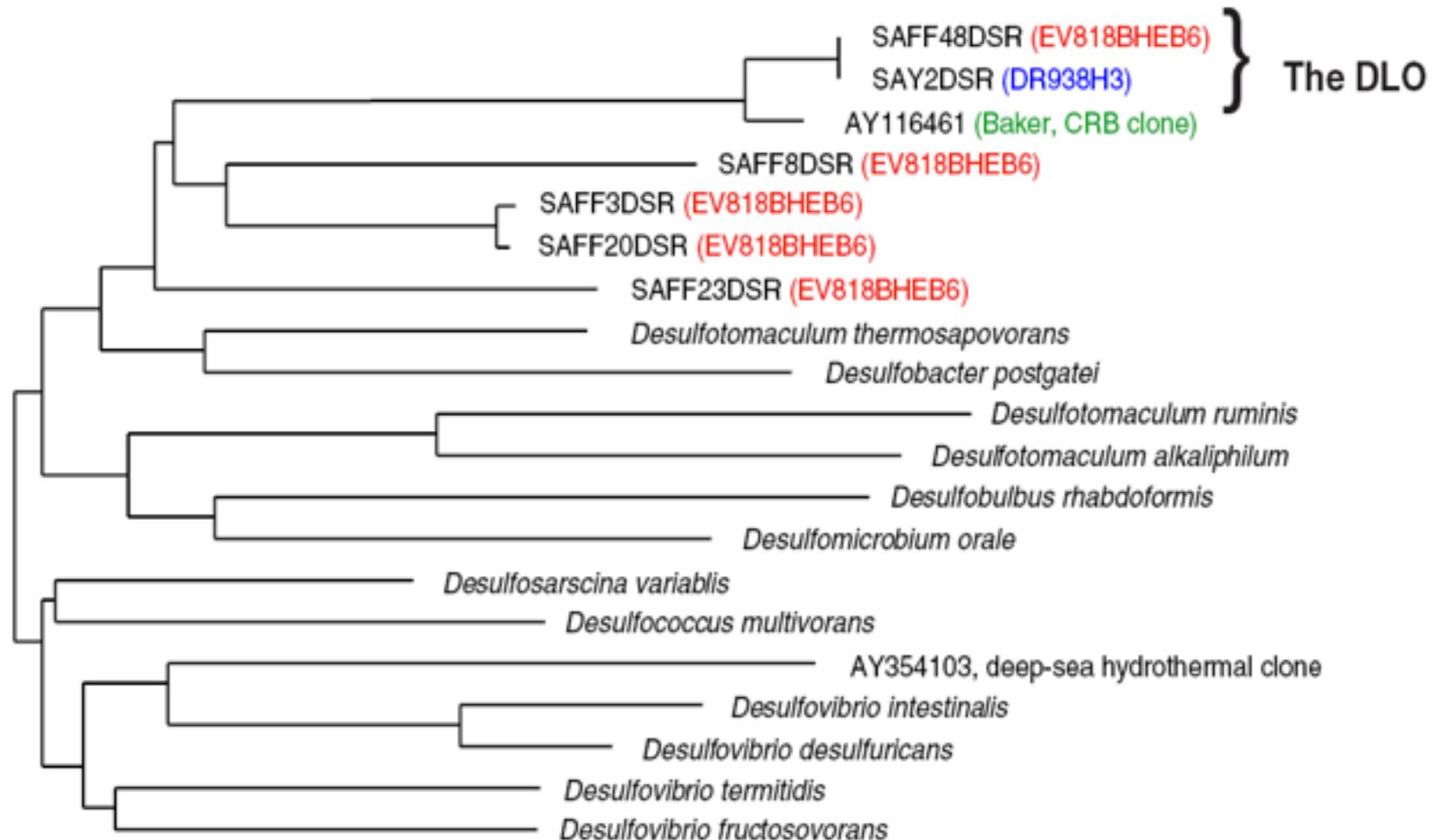
will be able to administer highly potent pressures until these sites. Jeffie Lafferty

The uncertainties for aqueous and gas chemicals are ±20% and ±10% for 50% desorption and 20% for 100% desorption.

www.nationalbank.ae - National Bank of the United Arab Emirates

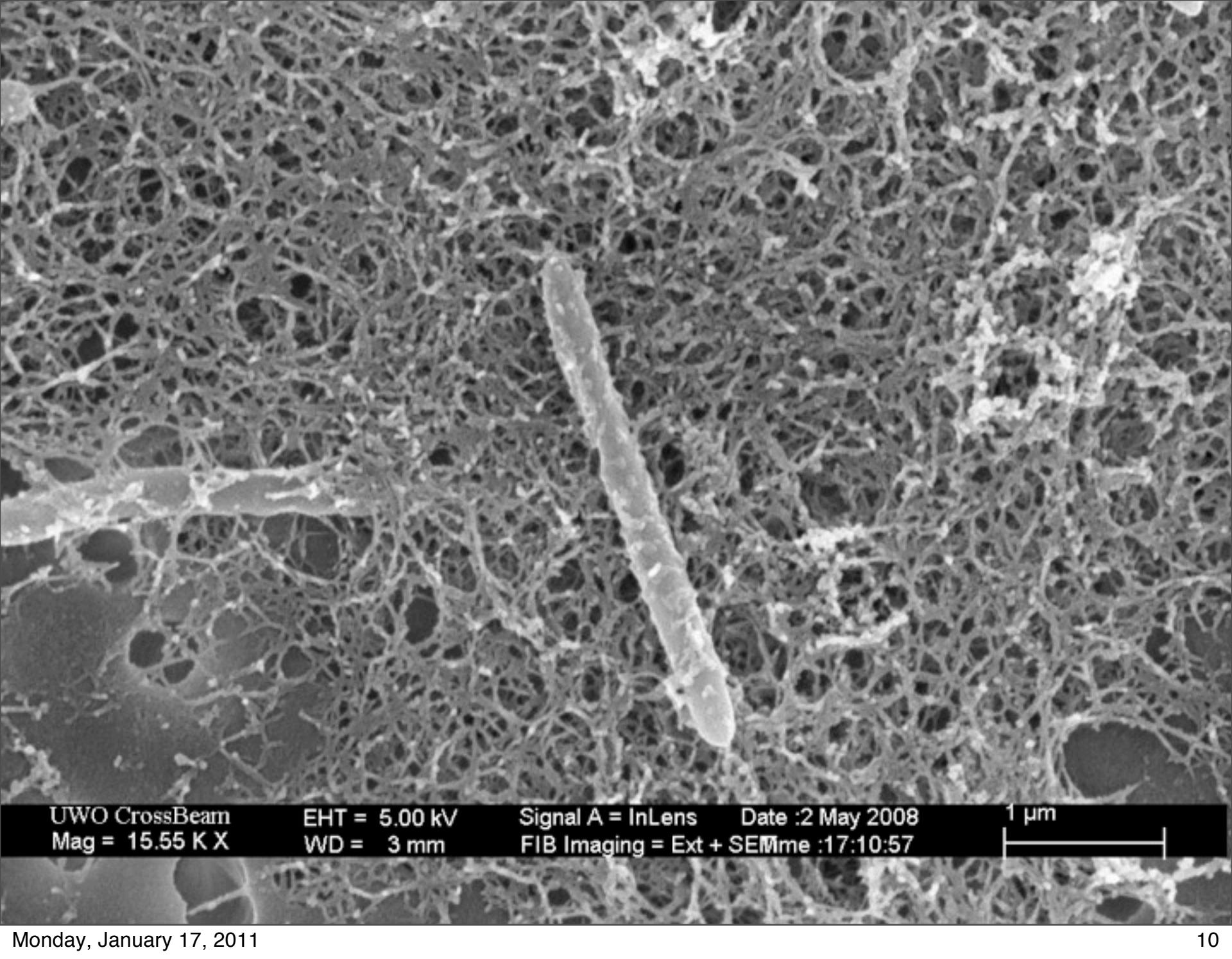


# dsrB gene



0.10

Gihring pers. comm. 2004



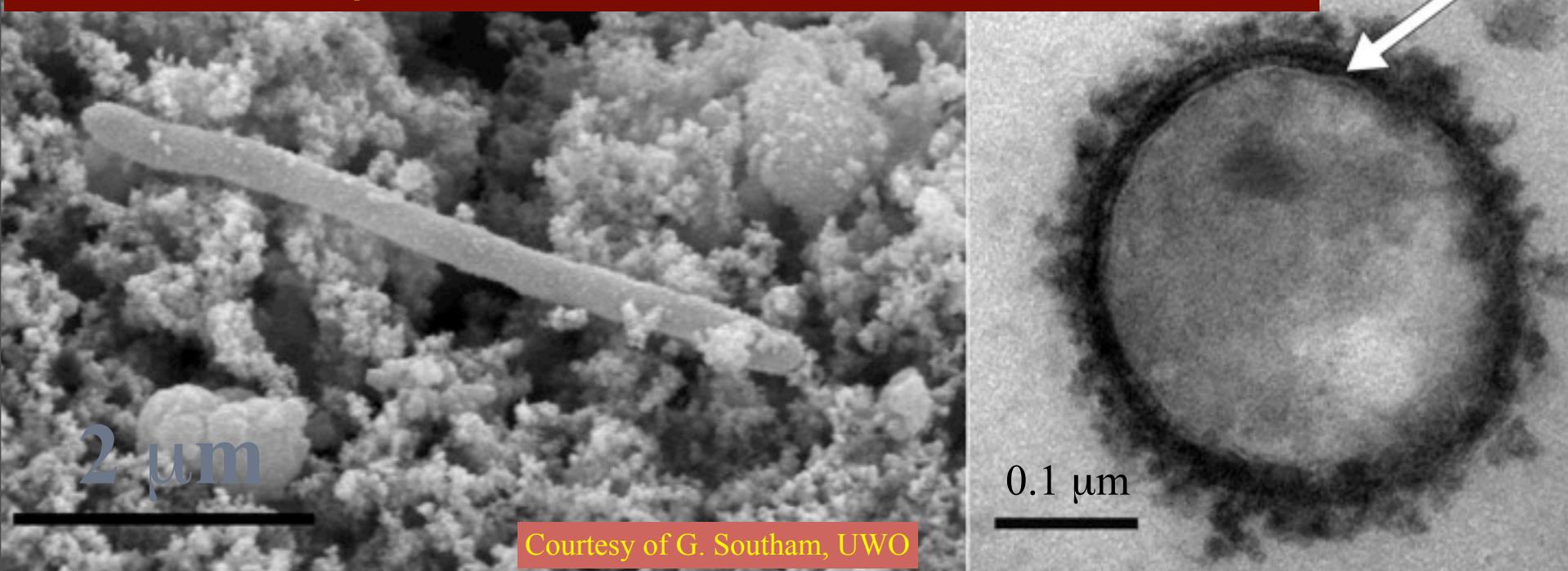
UWO CrossBeam  
Mag = 15.55 KX

EHT = 5.00 kV  
WD = 3 mm

Signal A = InLens  
FIB Imaging = Ext + SEM  
Date : 2 May 2008  
Time : 17:10:57

1 μm

# Candidate species - Desulfurudis Audaxviator

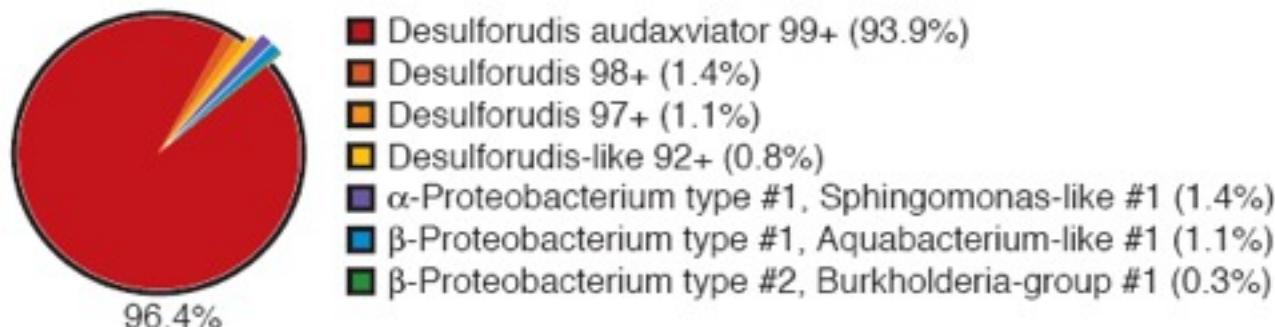


*"In Snejfels Joculis craterem quem delibat Umbra Scartaris Julii intra calendas descende, Audax viator, et terrestre centrum attinges."*

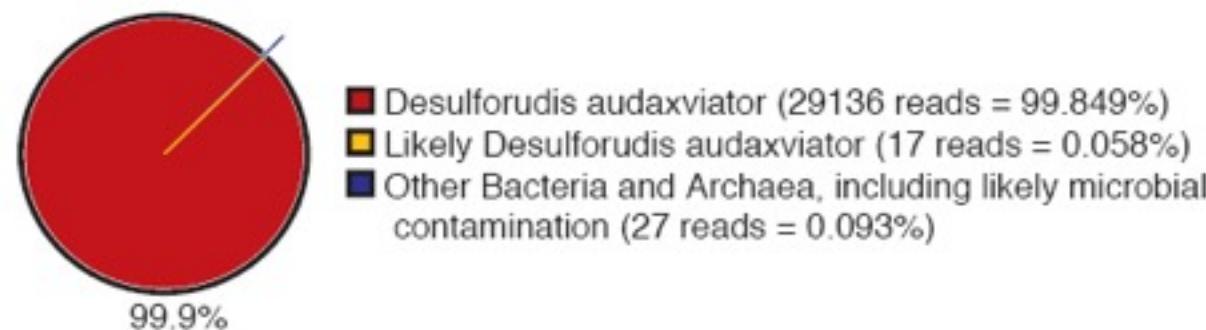
(*"Descend, bold traveller, into the crater of the Jokul of Snejfels, which the shadow of Scartaris touches before the kalends of July, and you will attain the center of the earth."*)

-- 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"

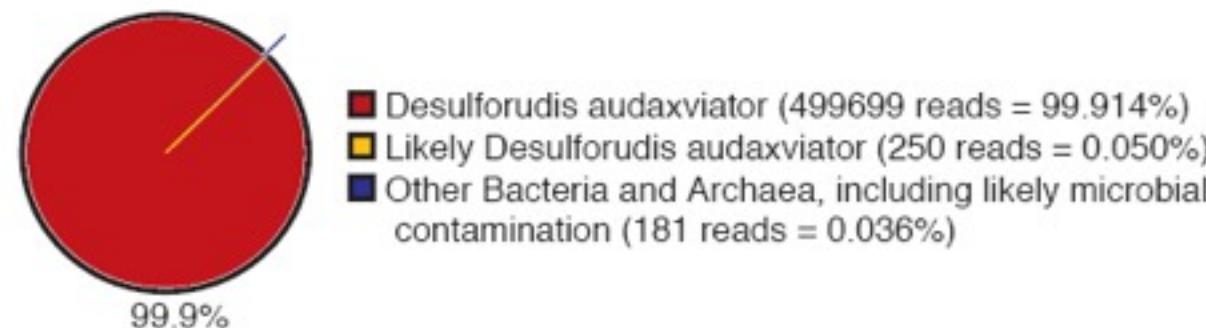
(b) SSU rRNA clone library (361 clones)

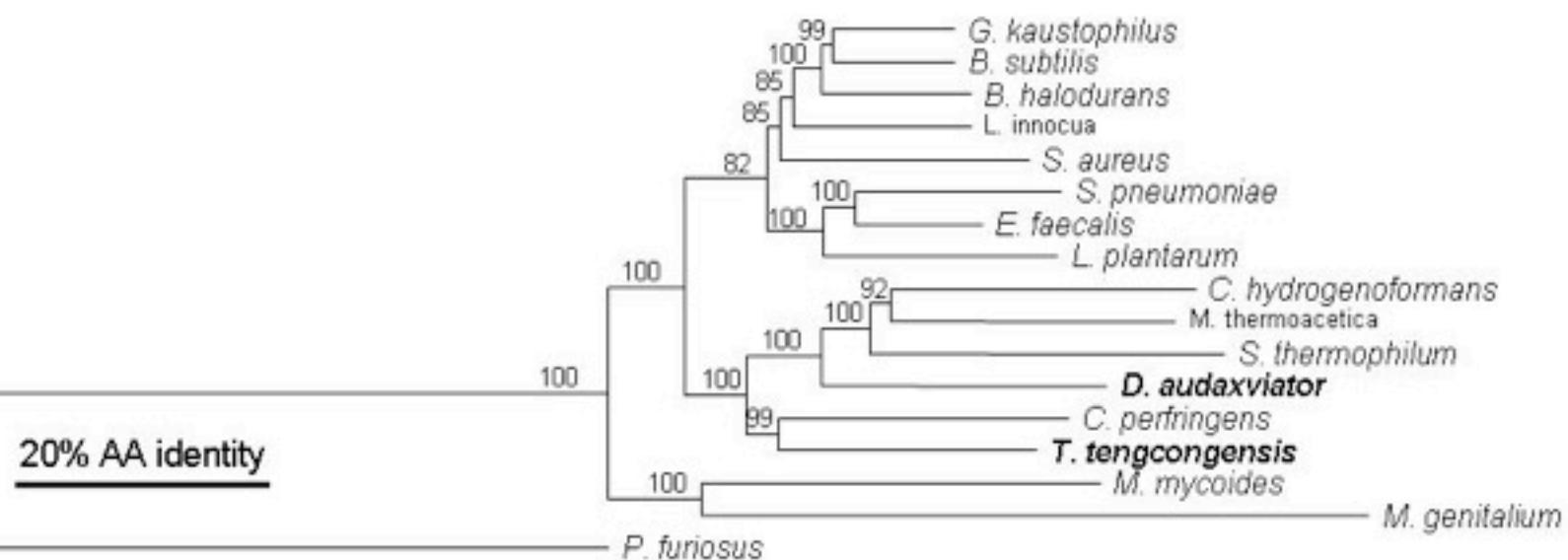


(c) Sanger metagenomic sequence (29180 microbial reads)



(d) 454 metagenomic sequence (500130 microbial reads)





A thousand copies of Hamlet.

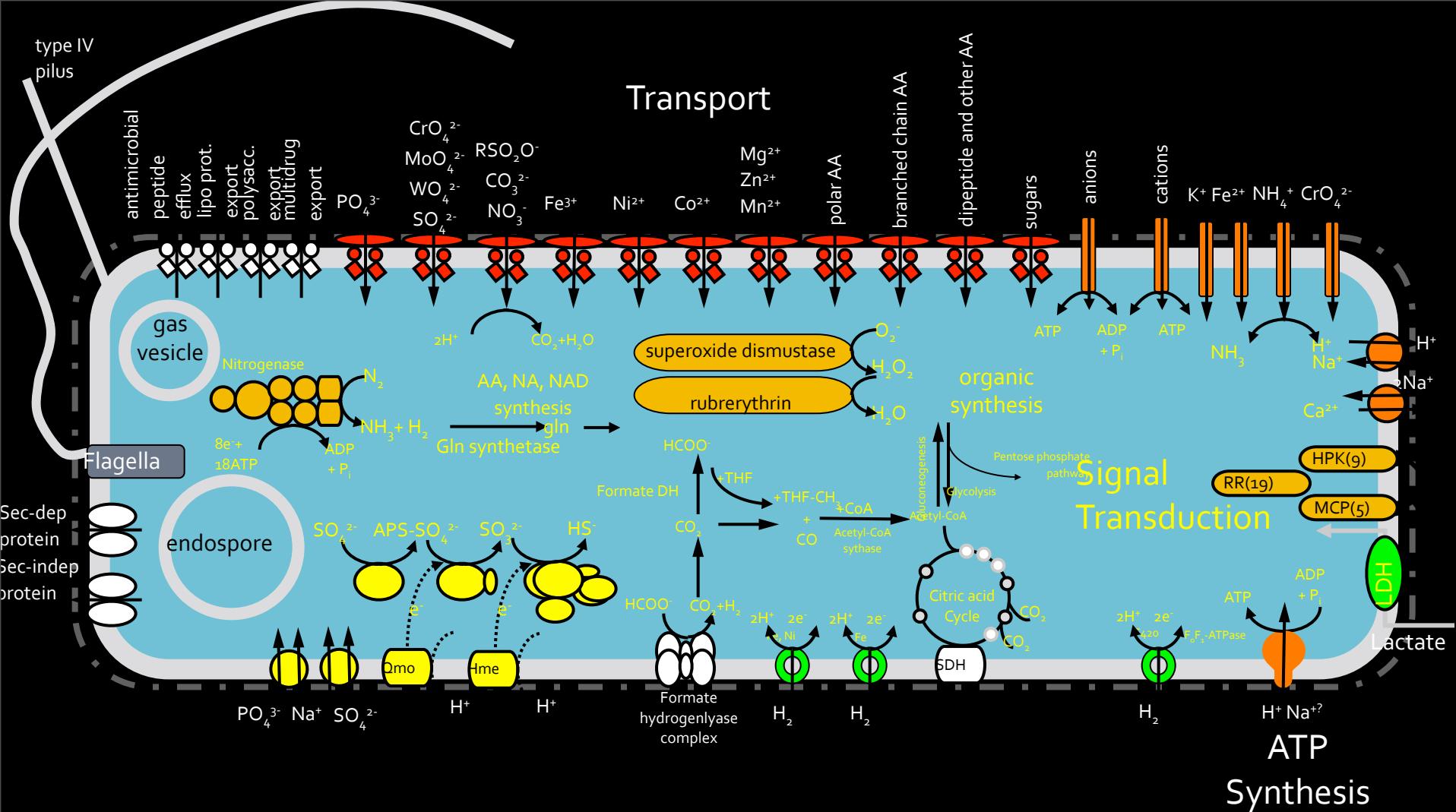
But not the library of congress.

Or the Library of Babel

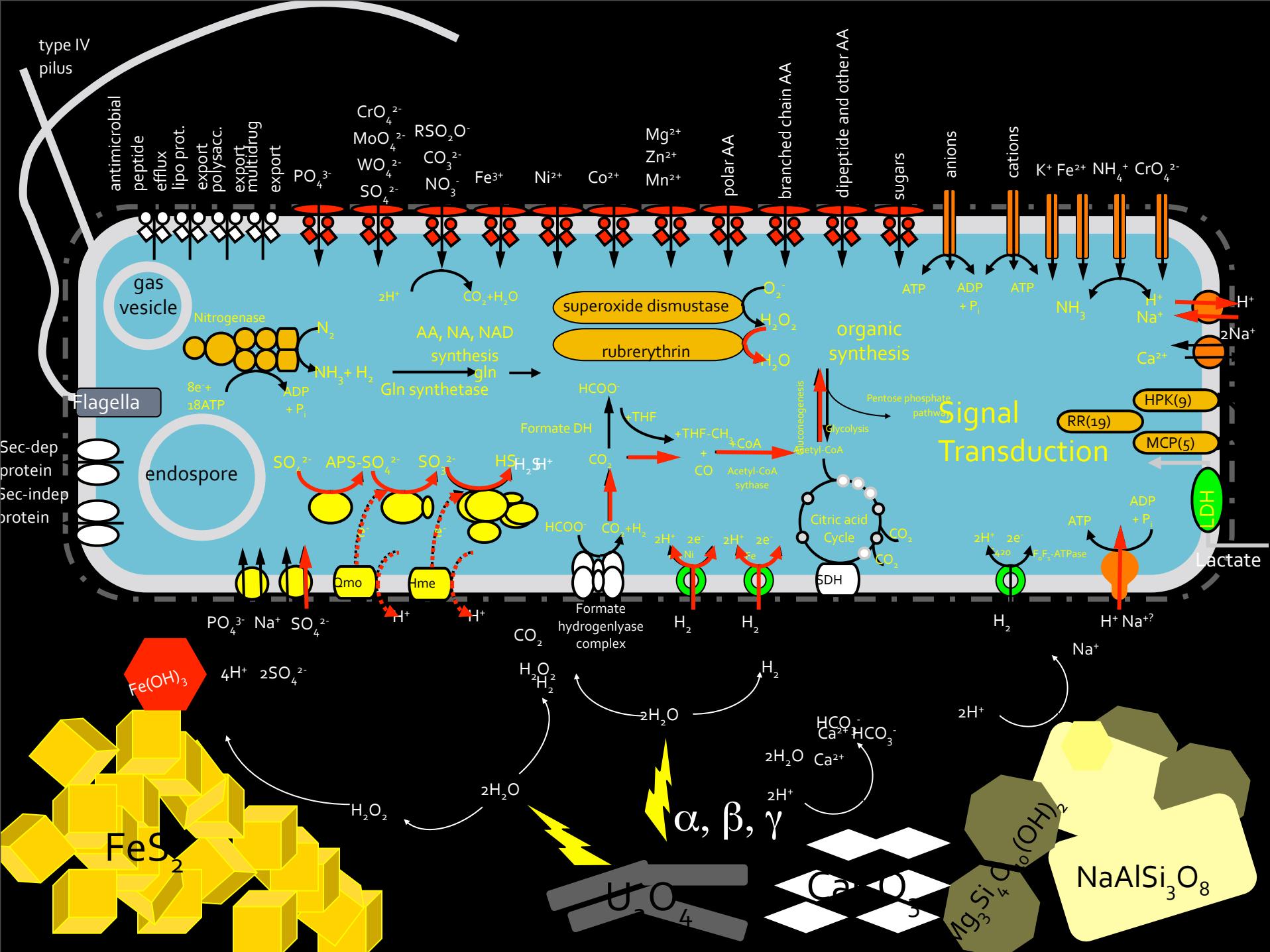
KEY	
NF: Nitrogen Fixation	* region drawn at 1/2 scale
CF: Carbon Fixation	No recent extremophile HGT
HGT: extremophile only	
HGT: extremophile top hit	
HGT: extremophile with clade	

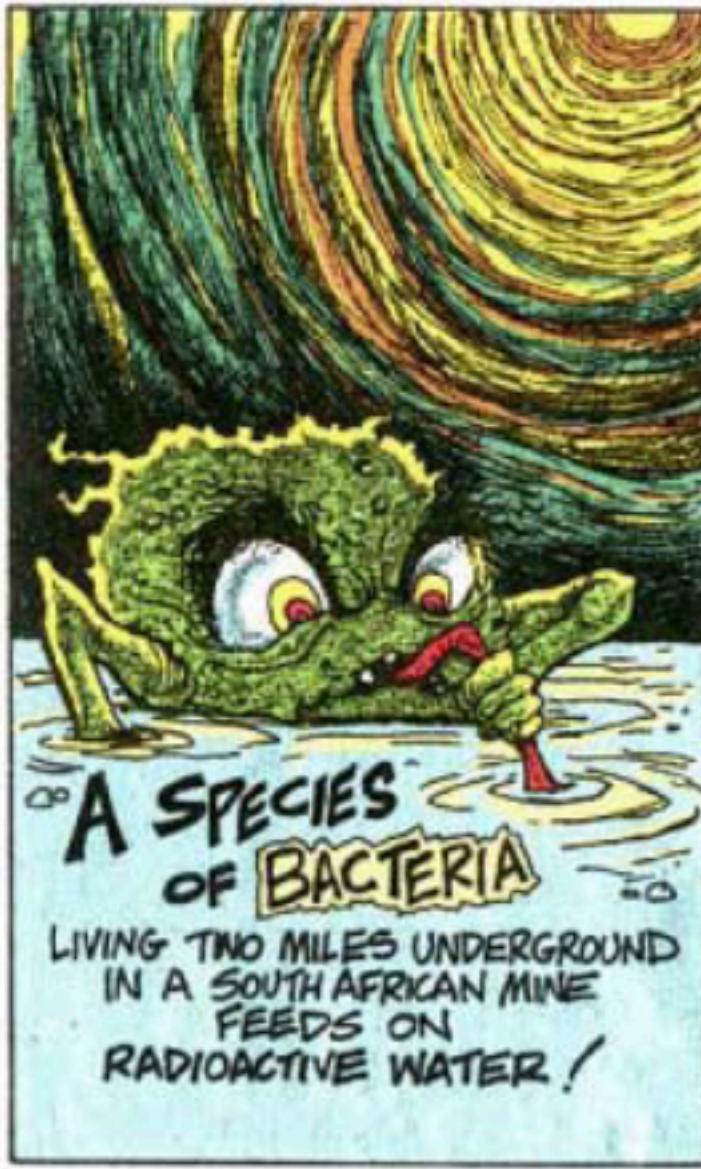
# Physiological Features

Feature	Notes
Endospores	Yes
Flagella	Yes
Sulfate reduction	SRB, $\text{Na}^+/\text{SO}_4^{2-}$ symporter
Carbon fixation	
TCA - Reverse TCA	Incomplete
CODH - Wood Ljungdahl -	2 types, one archaeal
N fixation	Nitrogenase, archaeal
“hyperthermophile” genes	~50
Archaeal genes	85
Catalase, peroxidase, SOR	No



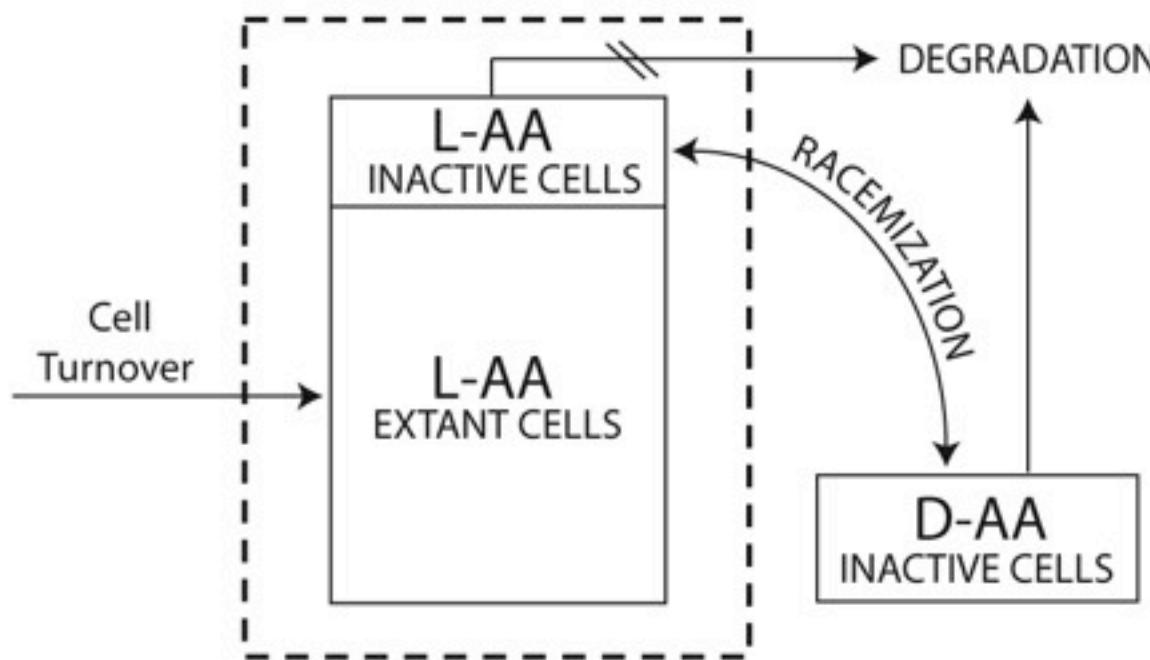
2.3 Mb with 2241 ORF's





• A SPECIES  
OF BACTERIA •

LIVING TWO MILES UNDERGROUND  
IN A SOUTH AFRICAN MINE  
FEEDS ON  
RADIOACTIVE WATER!



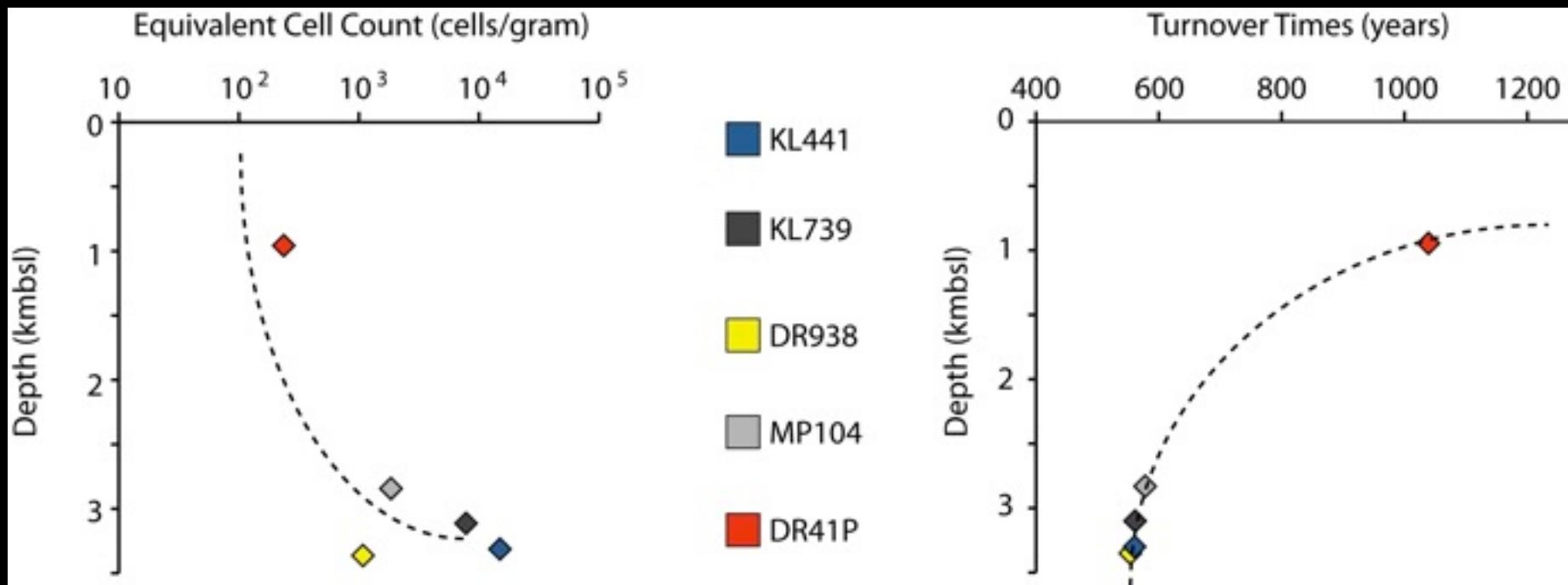
	Rate (yr <sup>-1</sup> )	L-Amino Acid Source	Sink
Cell Turnover		●	
Racemization <sup>1</sup>	1.25 x 10 <sup>-3</sup>	●	●
Degradation <sup>2</sup>	~1 x 10 <sup>-5</sup>		●

<sup>1</sup>in vivo racemization rate (Masters et al., 1977)

<sup>2</sup>rates of degradation estimated ~10<sup>-5</sup> yr<sup>-1</sup>, approximately 100 times slower than racemization, and can be assumed insignificant. Recycling may significantly affect the fate of the amino acids from inactivated bacteria and is an unknown.

$$\left( k_{TO} \cdot \frac{[L-AA]}{cell} \right) + (k_{RAC} \cdot [D-AA]) = (k_{RAC} \cdot [L-AA]_{DA}) + (k_{DEG} \cdot [L-AA]_{DA})$$

Assuming a steady-state model of growth and decay the turn over times can be derived.



The data suggest that turnover times must decrease with increasing depth and temperature probably due to the harsher environment. This also means that the average age of *D. Audaxviator* in MP104 is ~580 years.

# Single Nucleotide Polymorphism's

Category	Phred $\geq 15$ +duplicates	Phred $\geq 25$ +skip first 50	Phred $\geq 25$	Phred $\geq 20$ +skip first 50	Phred $\geq 20$
Intergenic	7	25	31	87	98
Pseudogenes	0	5	5	10	10
RNA	0	2	2	8	8
Synonymous in protein-coding genes	11	59	68	201	214
Non-synonymous in protein-coding genes	14	91	102	316	339
Total SNPs	32	182	208	622	669
Average rate of SNPs	0.0014%	0.0077%	0.0089%	0.026%	0.028%
Average depth of coverage	9.567 X	8.033 X	8.106 X	8.800 X	8.895 X

# Single Nucleotide Polymorphism's

- $2 \times 10^{11}$  cells => 38 generations
- mutations per base pair =  $1.4 \times 10^{-5}$  which is 60 times lower than previously reported environmental metagenomes and many of the SNP's are concentrated in a single ORFAN gene
- mutations per base pair per generation for *E. Coli* =  $5 \times 10^{-9-10}$  and 50 to 100 generations required for fitter individual to change community composition (Chao & Cox, 1998)
- at this mutation rate 24 to 27 generations would be required to produce observed SNP's.
- if turn over times per cell are  $10^3$  years, then lag times for sweeps of a higher fitness mutant would be ~50 to 100 kyr.
- subsurface residence time for this fracture water is > 3 to 5 myr.

# Self-sufficient life below the planet surface

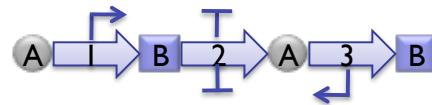
- Sulfate brine solutions can remain stable in martian conditions and effectively lower the freezing point of water.
- Sulfates have been shown to be widespread on the surface of Mars and sulfate-brine solutions have been correlated with recent water activity.
- Subsurface would be the likely first target for environmental deployment of microbes to protect from UV damage.
- However, martian subsurface much cooler than our South African Gold Mine. Perhaps there is not enough hydrogen for the key energetic reactions for survival to execute.

# Self-sufficient life below the planet surface

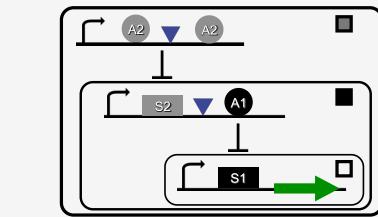
Sulfate reducing microbes: *Audax viators*!

- Ultra low growth rates with “persistent” activity.
- Resultant low mutation rates
- Chemoautrophic to allow
- Genomics systems are not “minimal” and contain numerous stress response and alternative metabolic elements
- Subsurface sulfate reducers are a possible chassis for terraforming, biotransformation of environment to chemicals/fuels/materials for human use on other planets
  - But they are also biocorrosion and souring agents

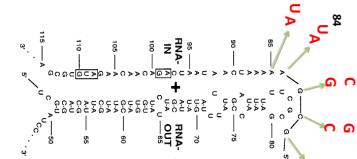
# Programmable DNA



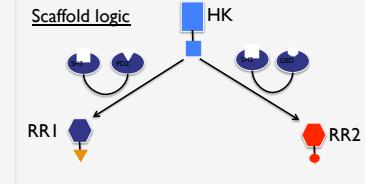
## Programmable transcription



## Programmable translation



## Programmable sensing

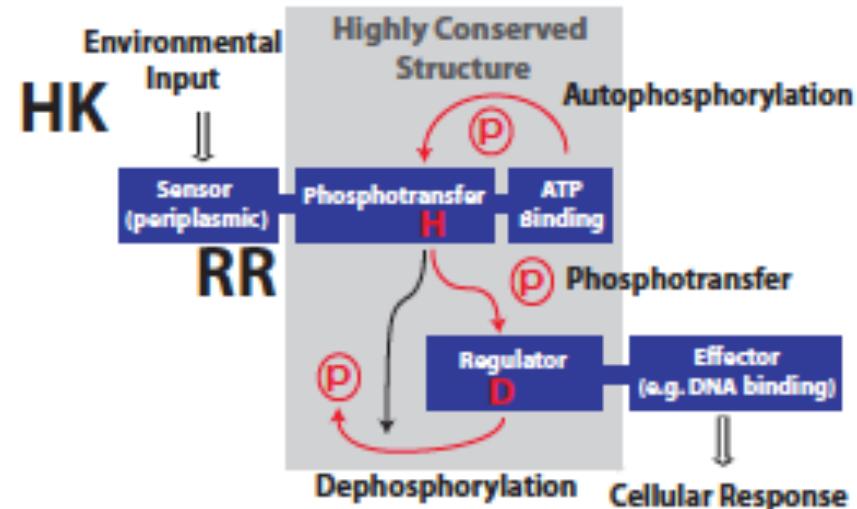
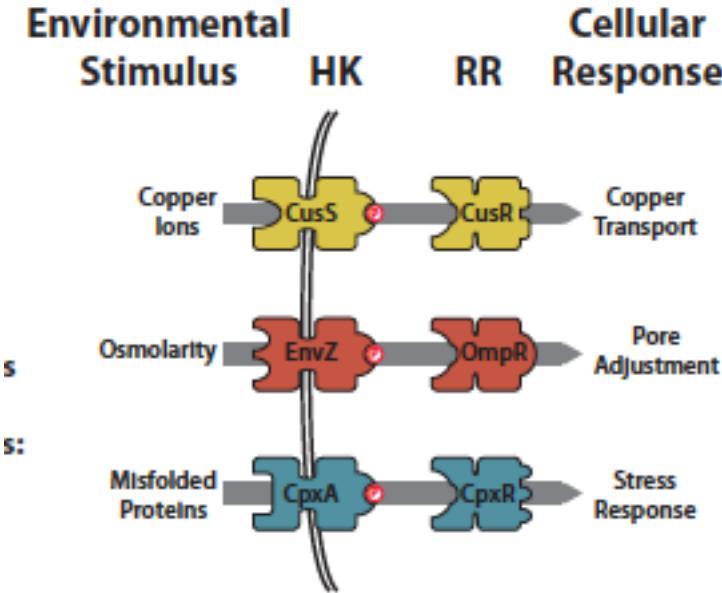


Towards a basis set of homogeneous parts for regulatory engineering of cells.

# Extensible Engineering of Microbes

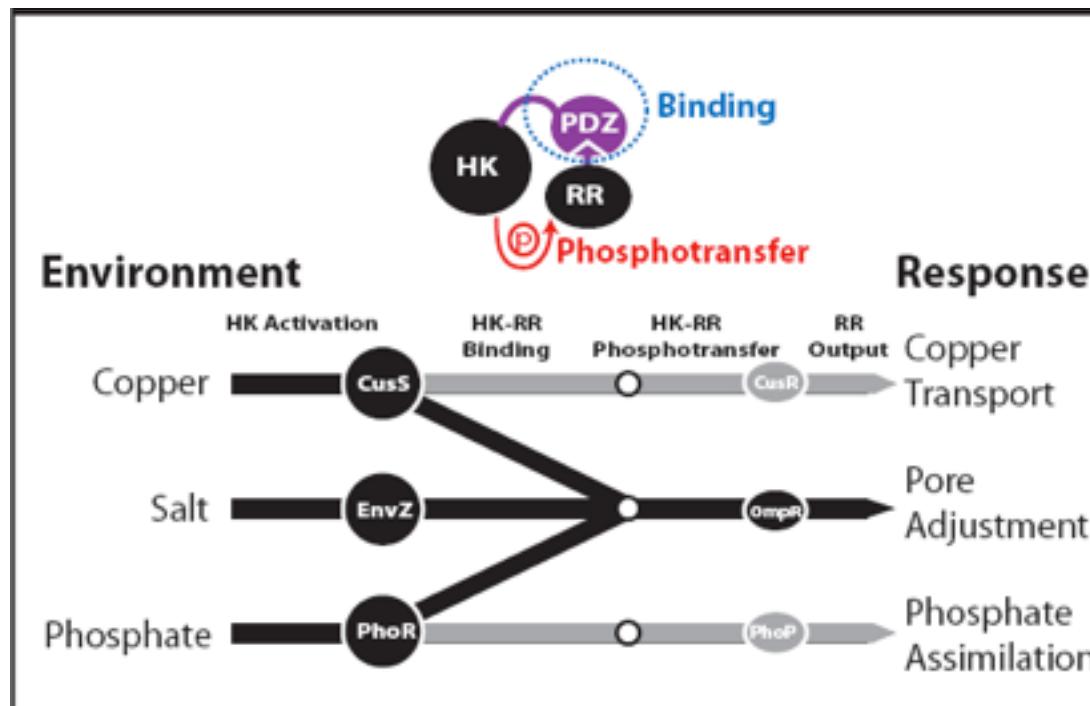
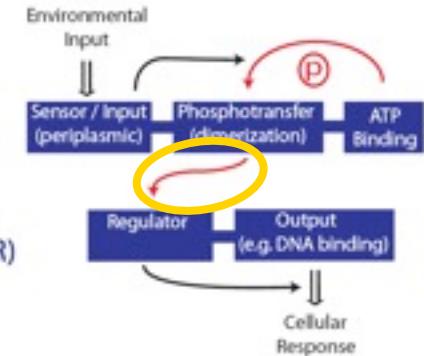
# Signal Engineering

## Common Blueprint



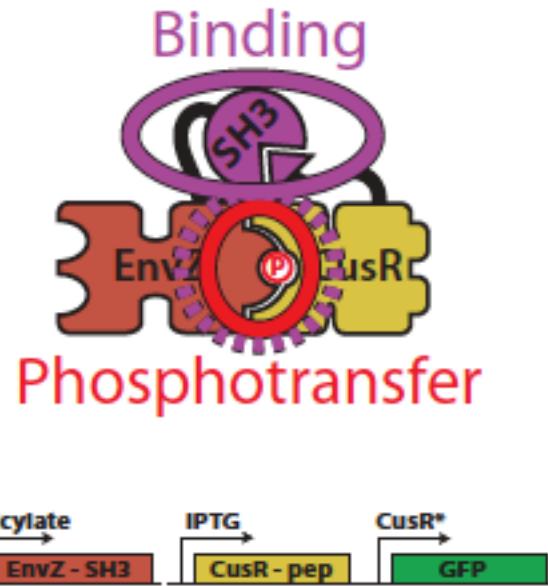
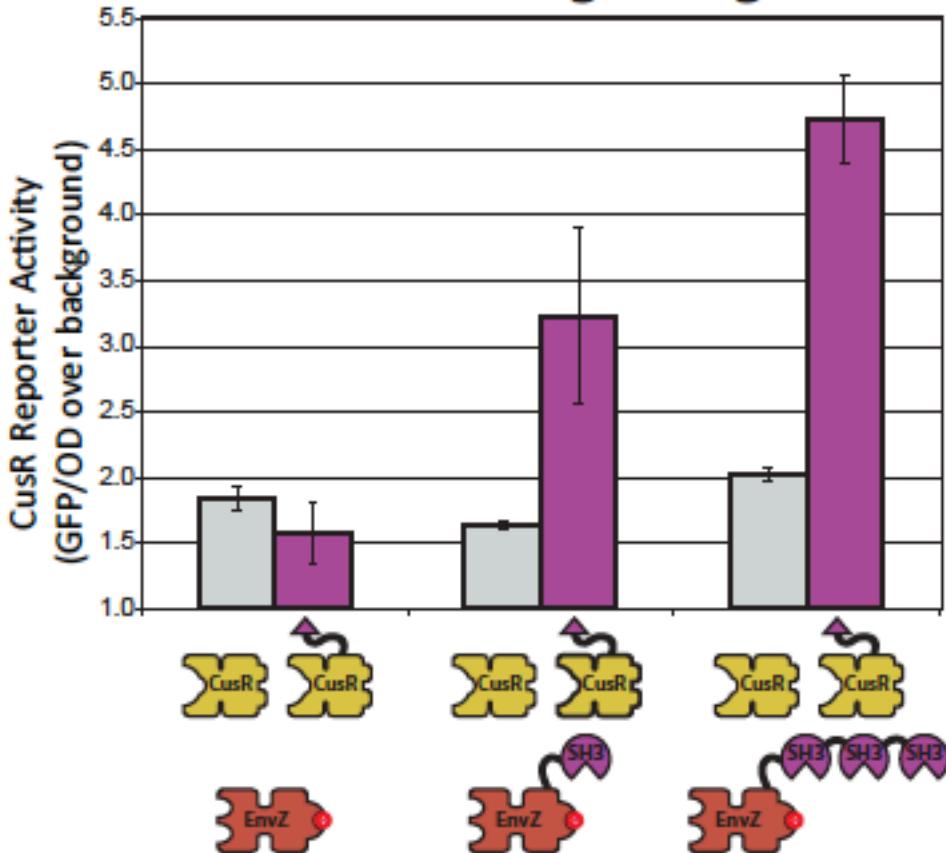
# Controlling signalling

Separating HK-RR binding from phosphotransfer introduces an additional node of control.



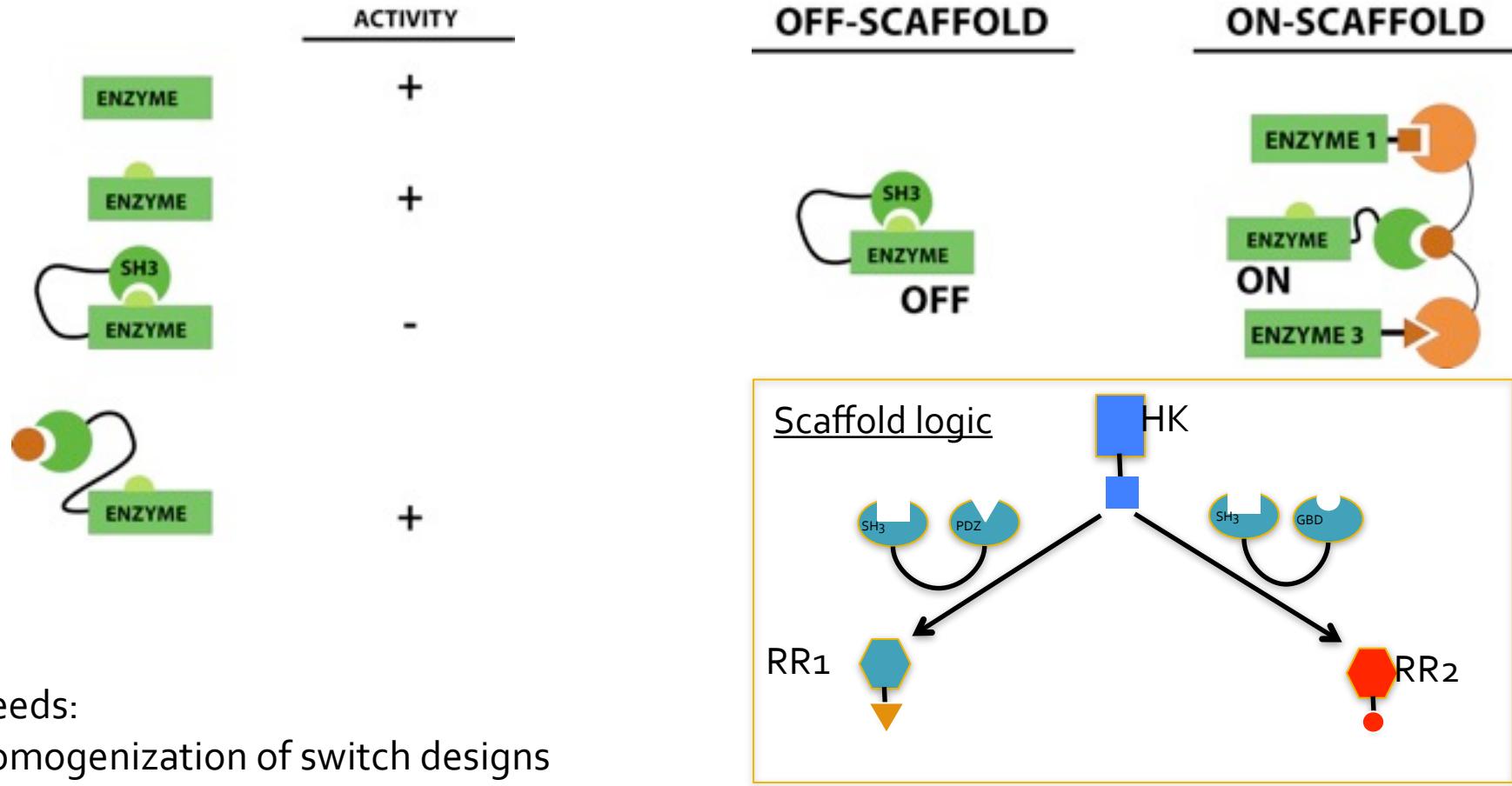
# Specificity through Colocalization

## Modular Binding Domains Used to Control Signaling

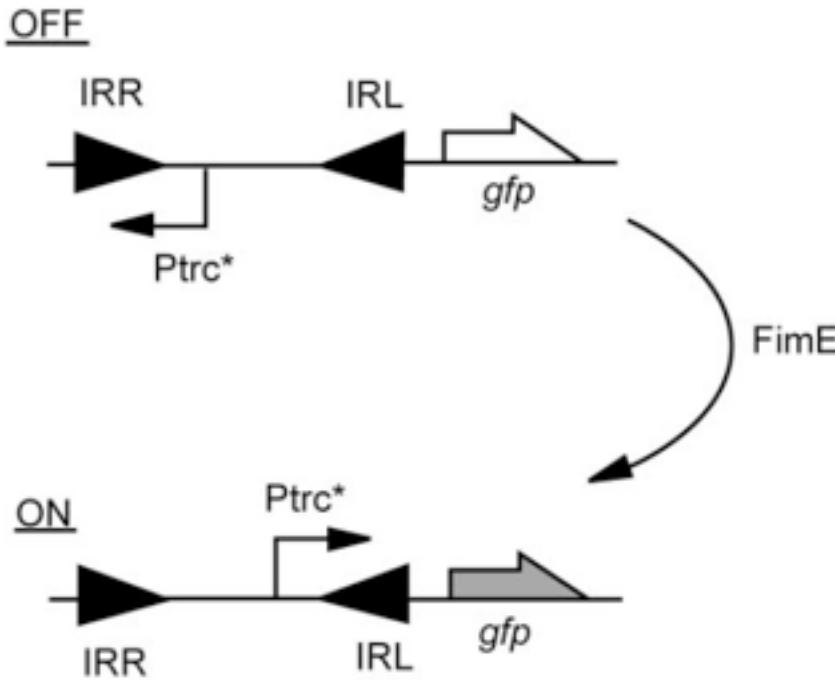


An *in vivo* signaling assay with phosphorylated CusR driving GFP expression shows colocalization controls non-cognate signaling.

# Use of Modular Parts to Spatially Regulate Enzyme Activities: Modular allostery and



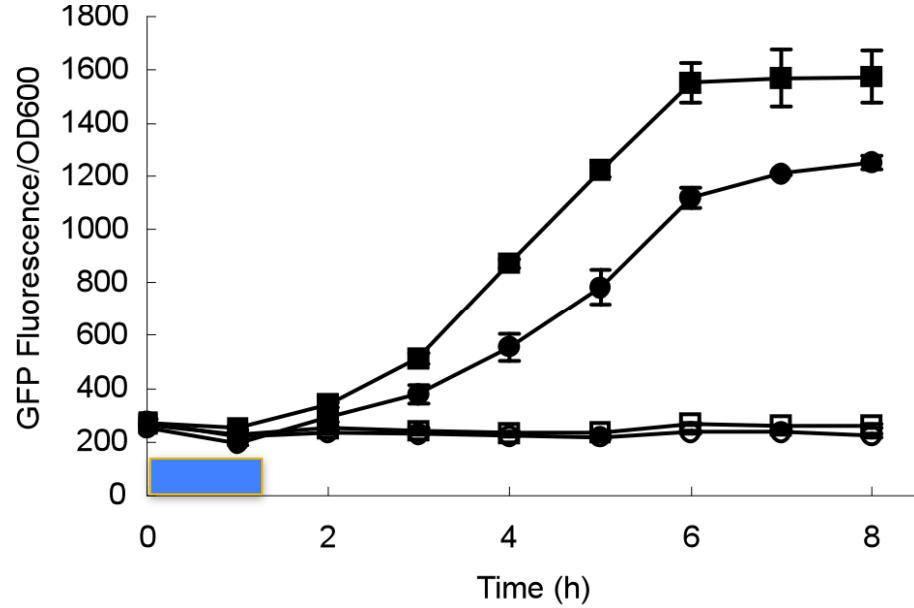
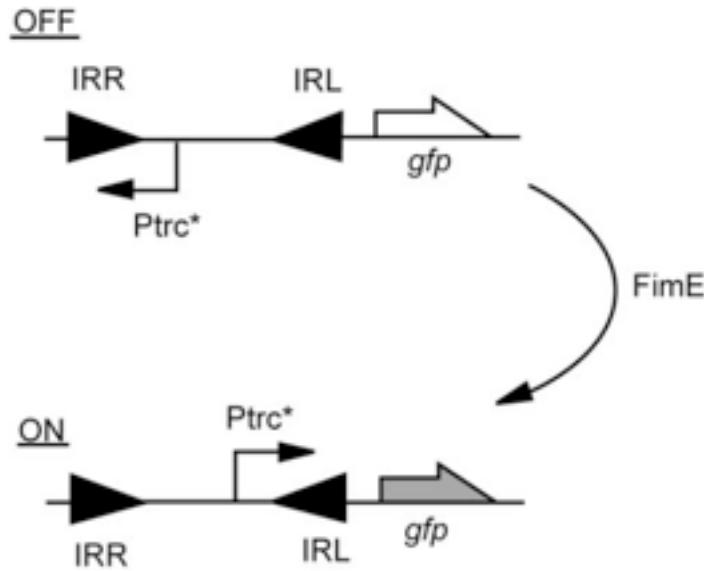
# Recombinases



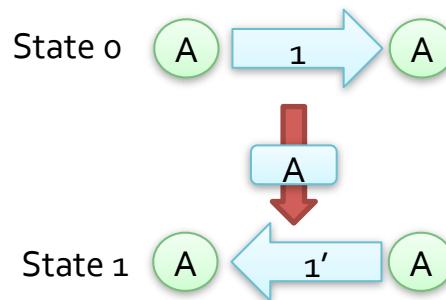
- Interesting operations
  - Inversion
  - Insertion
  - Excision
- Nearly Boolean
- State accessible after death and transmissible between cells
- Geometrically programmable?
- Possible huge state space for logical machines encodable in relatively little DNA
- Flexible acceptance of active elements in internal regions.

Invertases: the Fim system

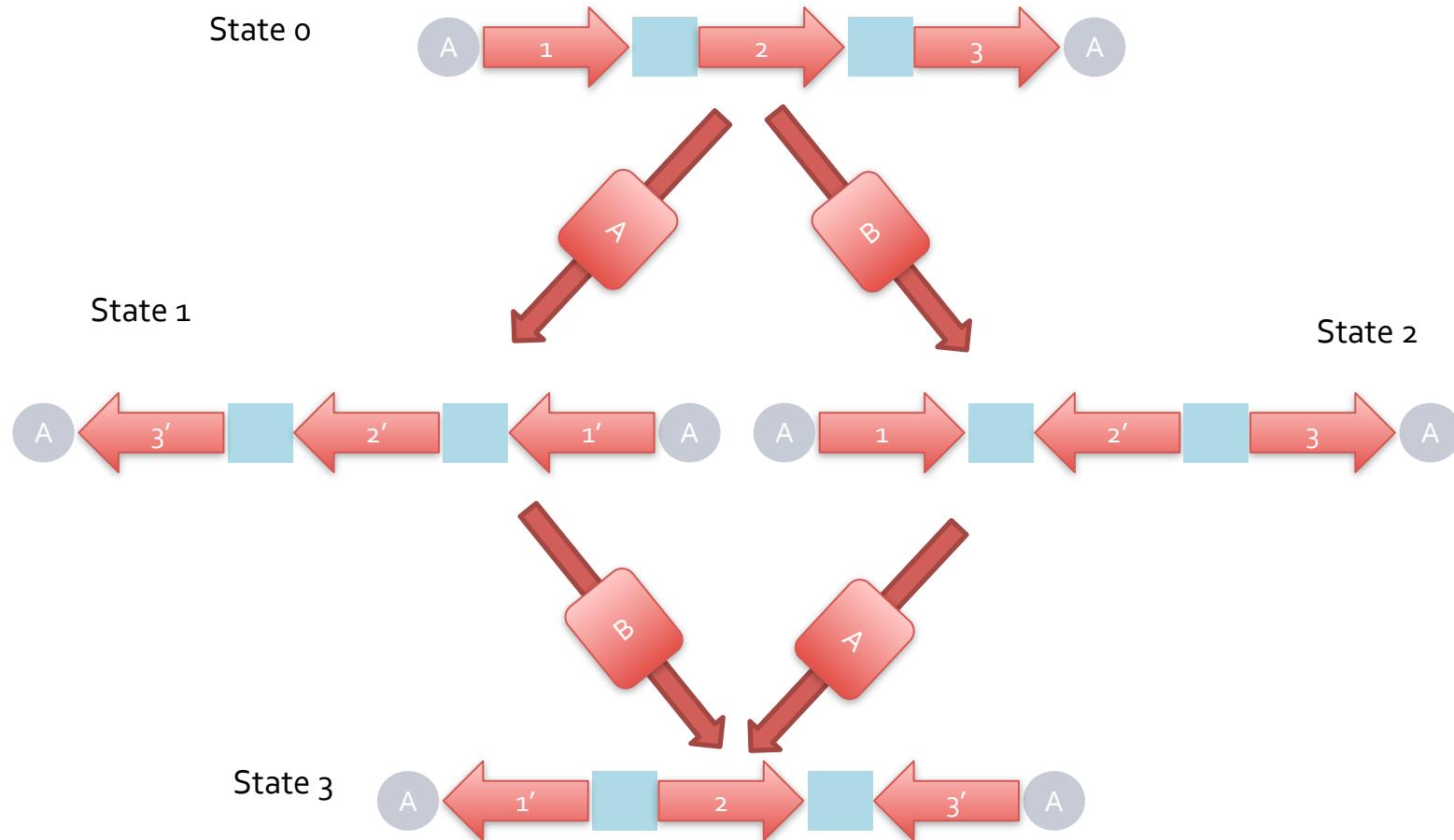
# Invertase dynamics



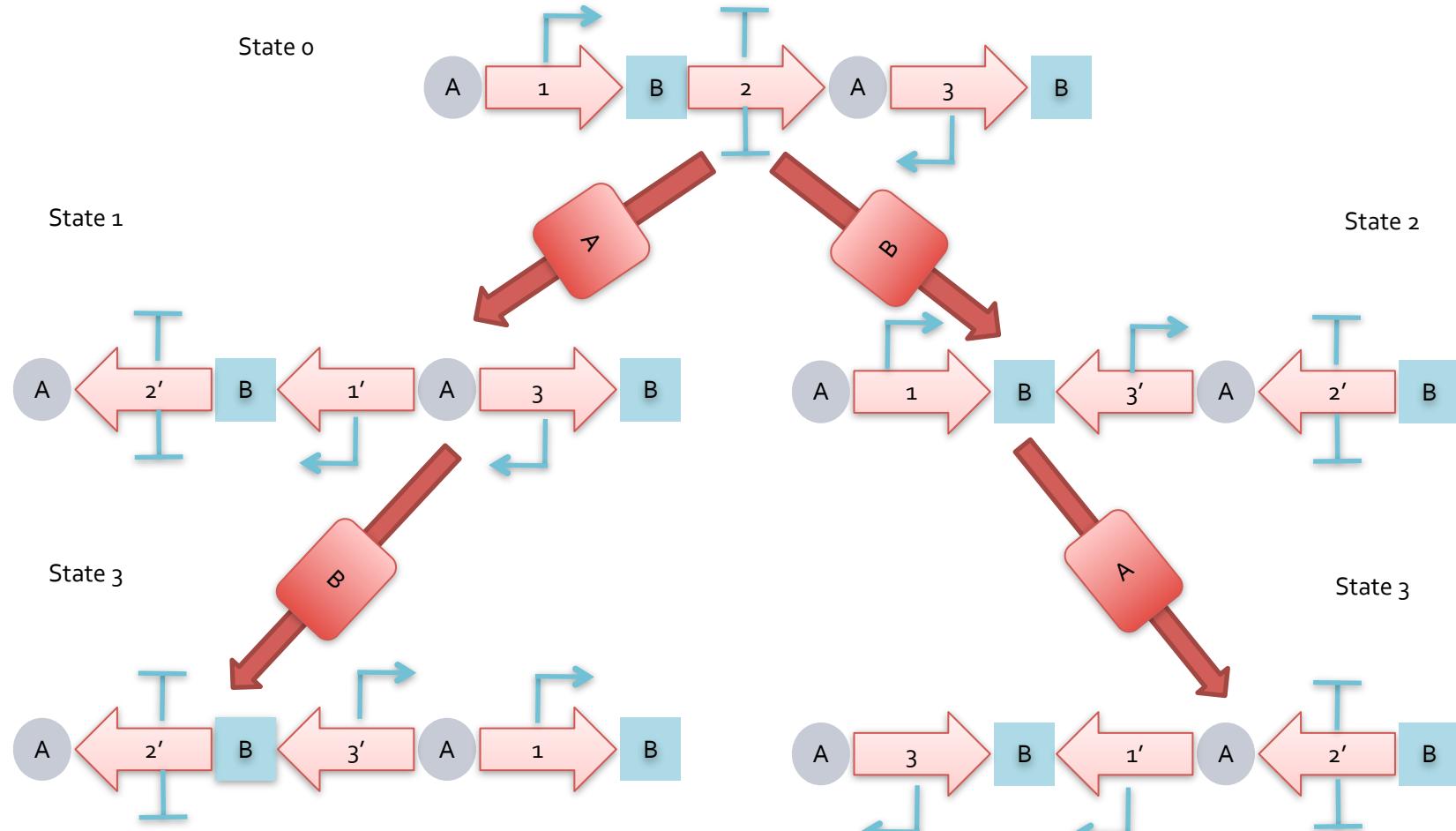
Leakless  
System can hold “state” after pulse



# Placement of two invertase sites.

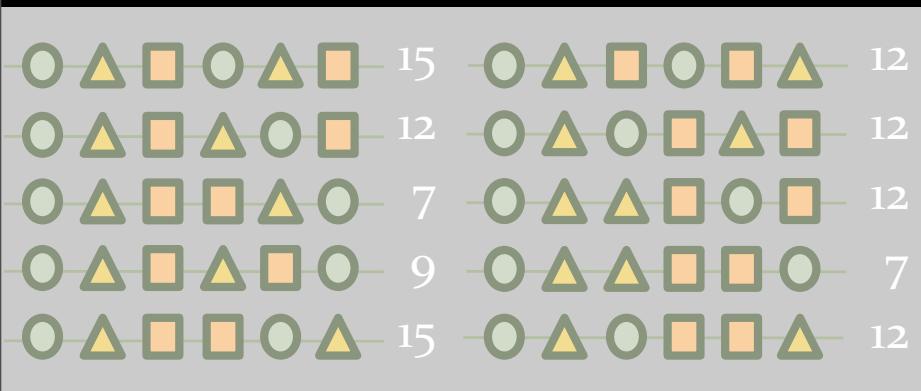


# Placement of two invertase sites.



$$S(N) = \sum_{k=1}^N k! C(N, k)$$

# Number of devices and state space grows rapidly with N.



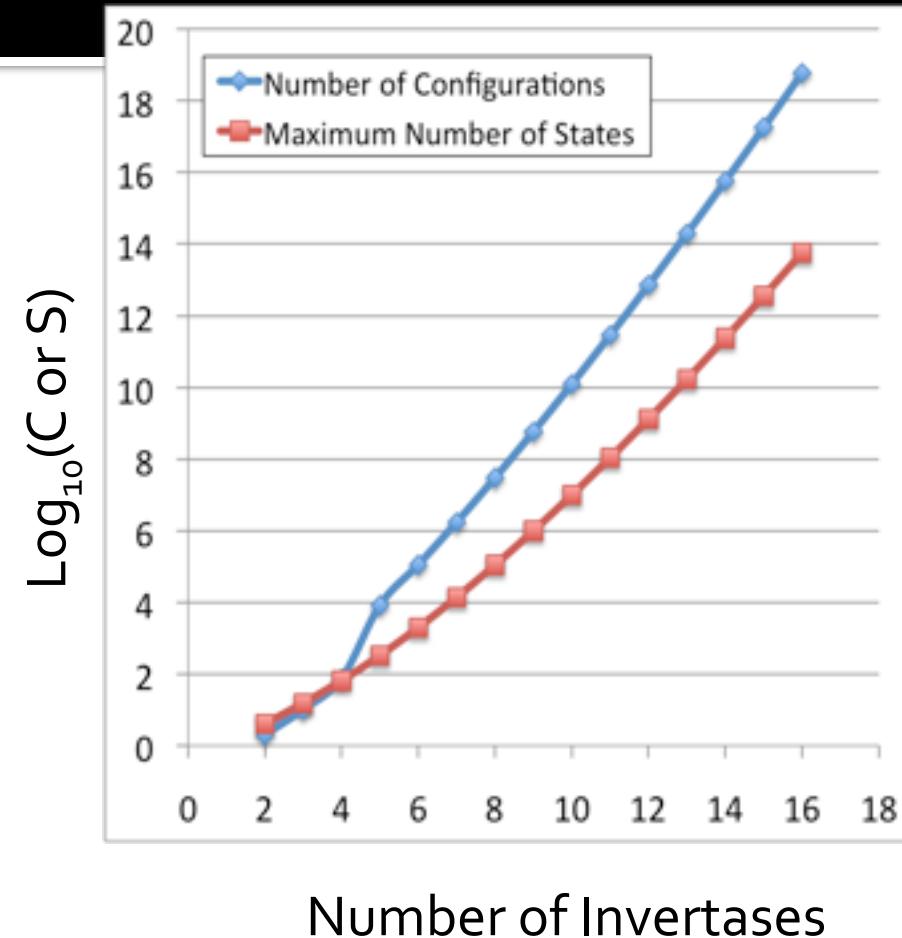
For  $n=N-1$  there are:

$$a(0) = 1; \text{ for } n > 0, a(n) = (2n-1)!! - \sum_{k=1}^{n-1} (2k-1)!! a(n-k)$$

configurations.

If the minimum length of a flippable region is 500 bp and inversion sites are 30bp then the device is less than  $2*30*N+500\text{bp}$  long at minimum.

So for  $N=10$ :  $S \sim 10^{11}$  and  $L \sim 1.1 \text{ kb}$



# Some numbers to contemplate

- $10^6$  microorganisms per gram of soil ( $10^{33}$  on earth)
- $10^9$  in a ml of rich media
- Divide every 20 minutes
- Flip-operations-per-molecule-per-second-per cell ~ 0.01
- 100-200 plasmids per cell.
- 5-6 “machines” per plasmid
- 100-1000 “machines” per genome
- Plasmids passed per cell per generation ~ 0.01
- What is the computational capacity?

# Summary

- There are promising chassis that might provide a robust, flexible starting point for synthetic biological engineering of extraterrestrial microbes
  - Complex materials from simple building blocks
  - Soil Transformation
- Modular synthetic biology is allowing the reprogramming of regulation, and signaling for human purpose.

# Enablers and Co-dependents

- SRB
  - Dylan Chivian
  - Tullis Onstott
  - Dwayne Moser
  - Terry Hazen
  - A large number of others
- Synthetic Biology
  - Stefano Cardinale
  - John Dueber
  - Tim Ham
  - Julius Lucks
  - Vivek Mutalik
  - Stanley Qi
  - Weston Whitaker
  - Jay Keasling

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