

Extreme life detection on Earth: Informing the search for life in our universe

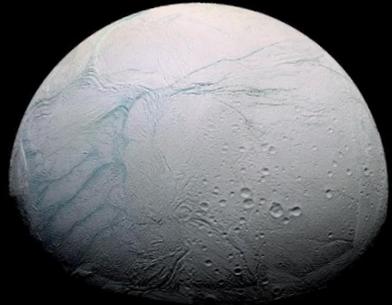
Dr. Jackie Goordial
Postdoctoral Research Fellow
Bigelow Laboratory for Ocean Sciences



What can we learn from analogs?

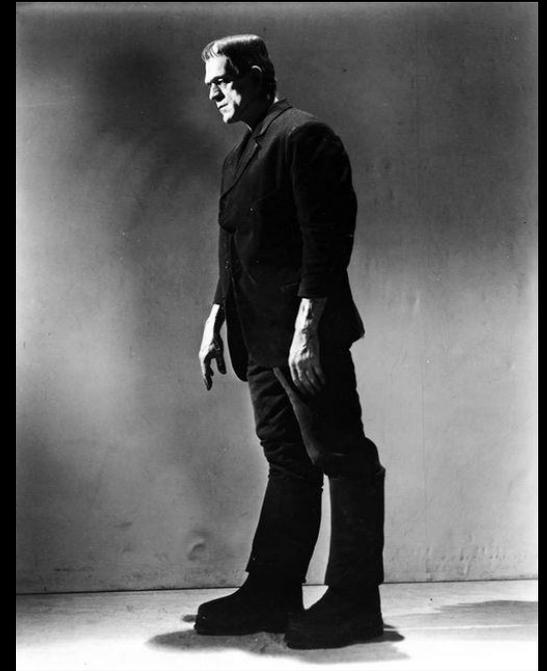


- The limits of life as we know it!
- Where can we find life?
- How can we identify and study life and its biomarkers?



Biomarker: traces (chemical, biological, morphological, isotopic etc.) of *past* or *present* life.

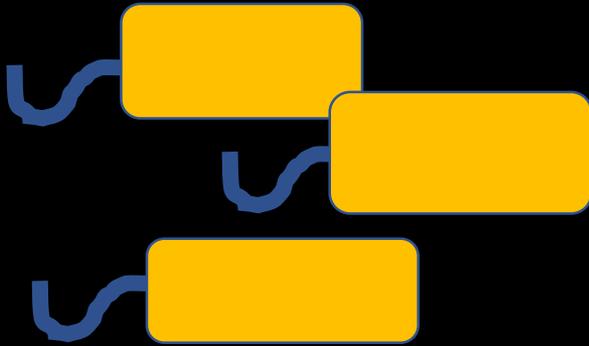
Extinct vs. Extant life



Central dogma:

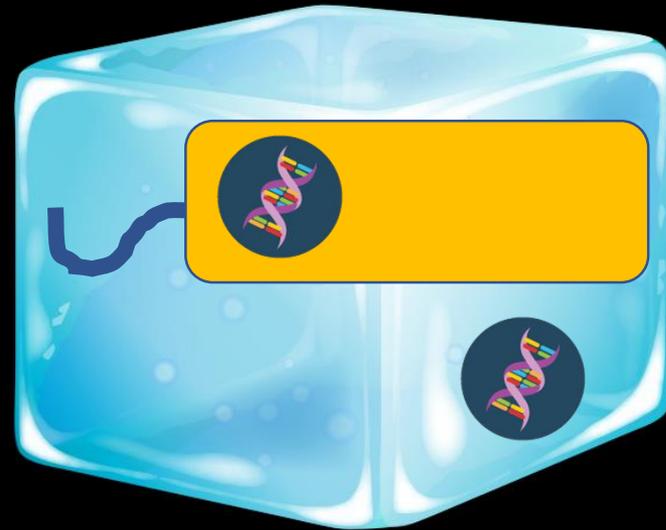
DNA → RNA → Protein

Clearly Alive:



- Cell division/ Growth
- Respiration
- RNA

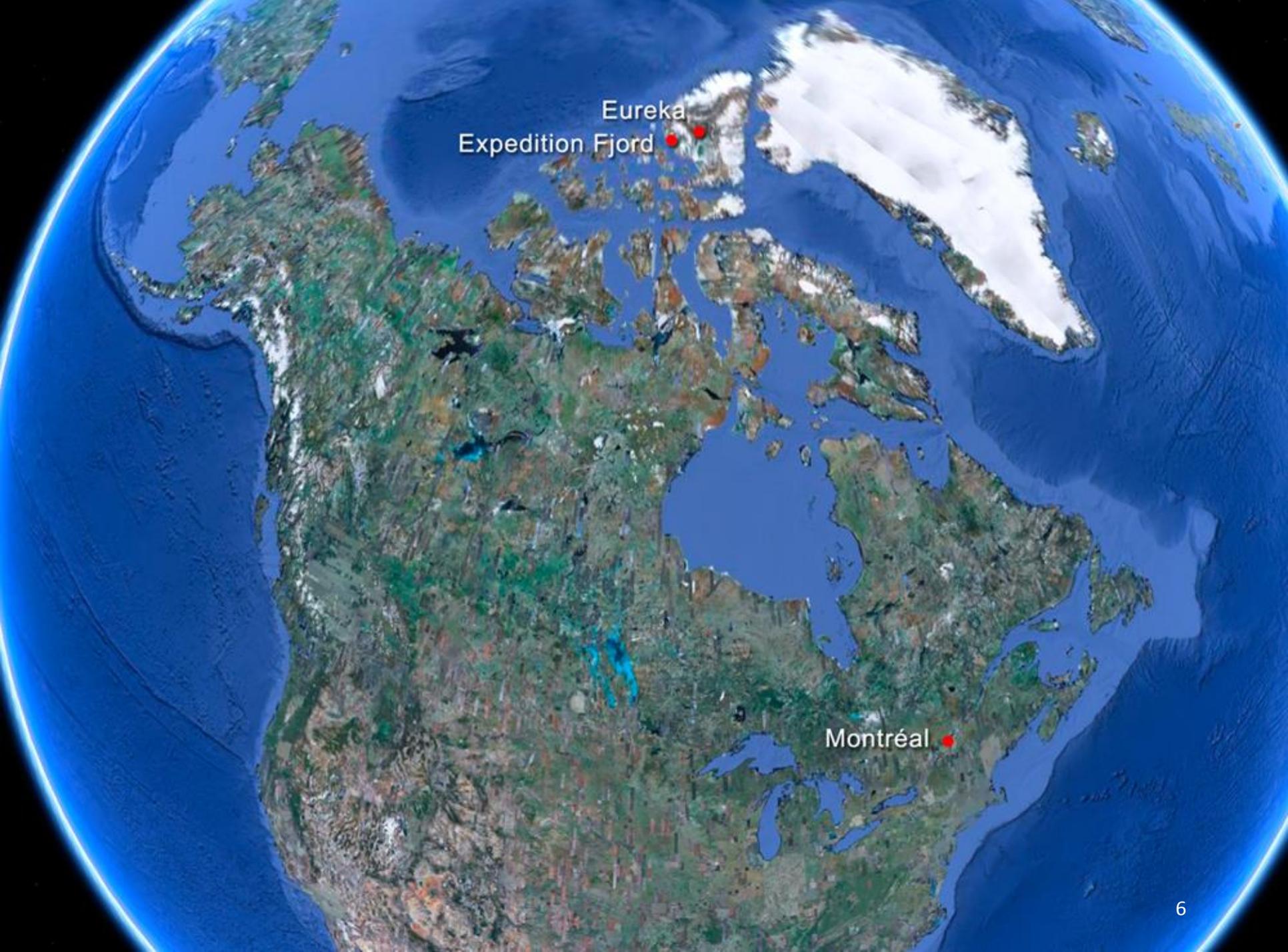
Not as clear:



- Preserved cells/ cell components
- DNA

In situ life and biomarker detection in Mars analogue
permafrost in the Canadian high Arctic





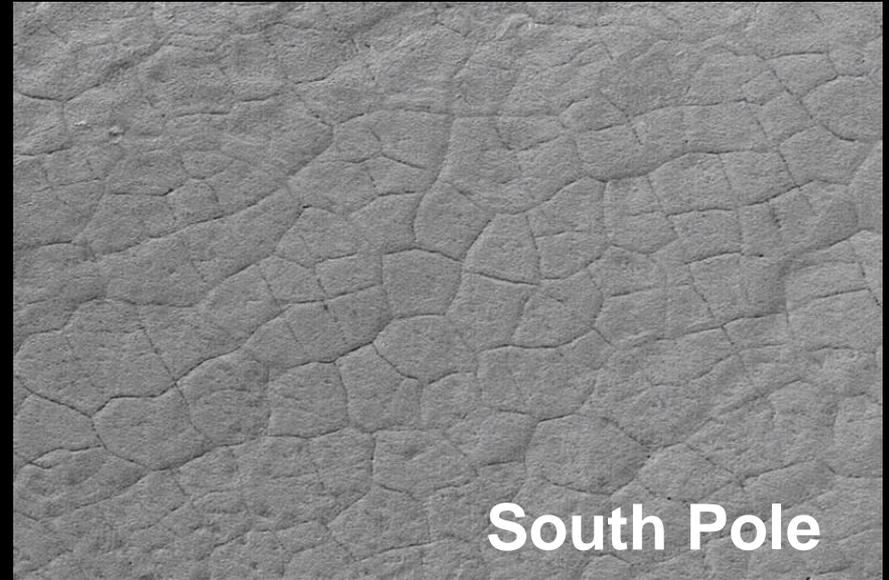
Eureka
Expedition Fjord

Montréal

Canadian High Arctic



Mars



South Pole



Phoenix Landing Site

Micro Life Detection Platform

Environmental samples and sample return

Samples from analog environments: ice wedge soil, subzero saline spring water, glacial ice, cryptoendoliths



Biosignature Detection

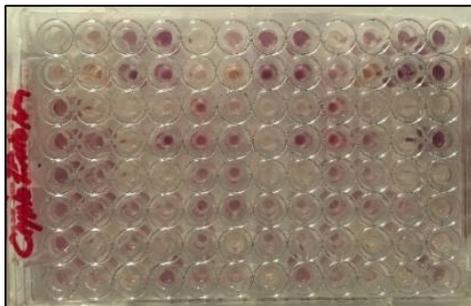
nucleic acid sequencing



Oxford Nanopore MinION

Viable extant life detection

Microbial Activity microassay (MAM)



Coloured wells indicate metabolizing microorganisms

CRYO-plate:



Culturing/ Isolation of novel microorganisms

Oxford Nanopore MinION



- Highly portable, lightweight, DNA sequencer with low energy requirements (usb powered).
- Successfully used in the Canadian high Arctic in Spring 2016 (79°26'N)



Pore-forming (nanosized) proteins



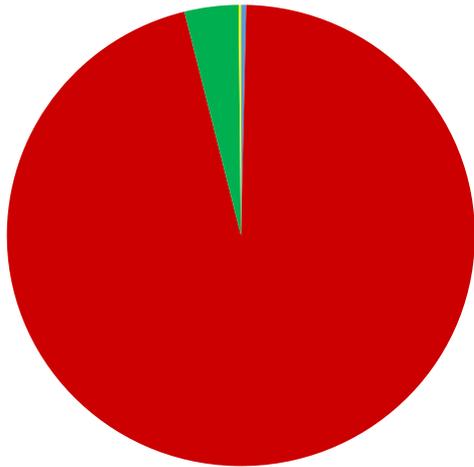
Credit: Oxford Nanopore

MinION Field sequencing

- **Manual** DNA extraction, quantification, and preparation for sequencing.
- Did local (offline) basecalling
- On select samples: ***uplinked data*** via satellite internet (sporadic and weather dependant) and ***downlinked*** basecalled data.
- We did not tax ID in the field in real time in spring 2016- ***but those capabilities exist now***

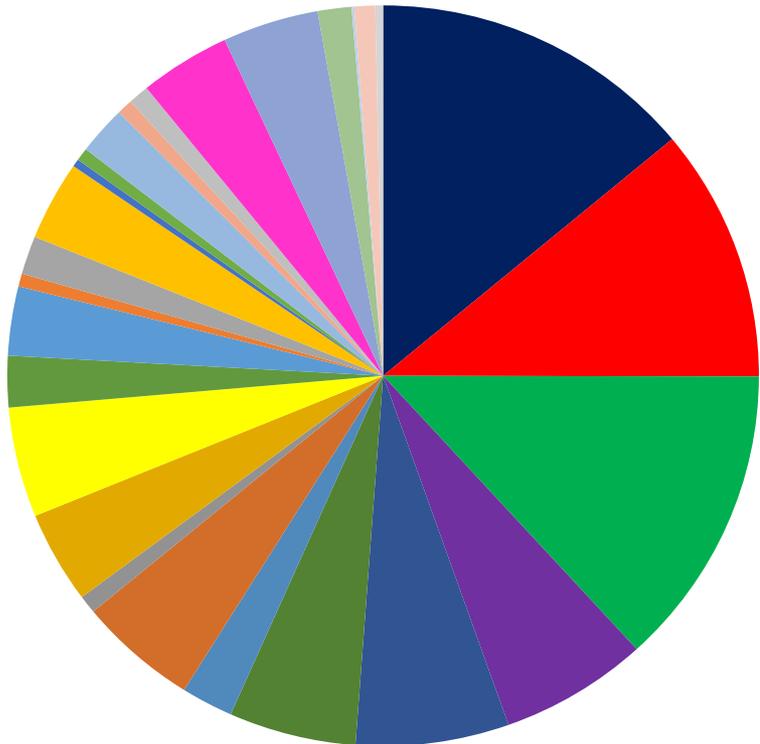
Identified microorganisms across multiple domains of life!

- Archaea
- Bacteria
- Eukaryota
- Viruses

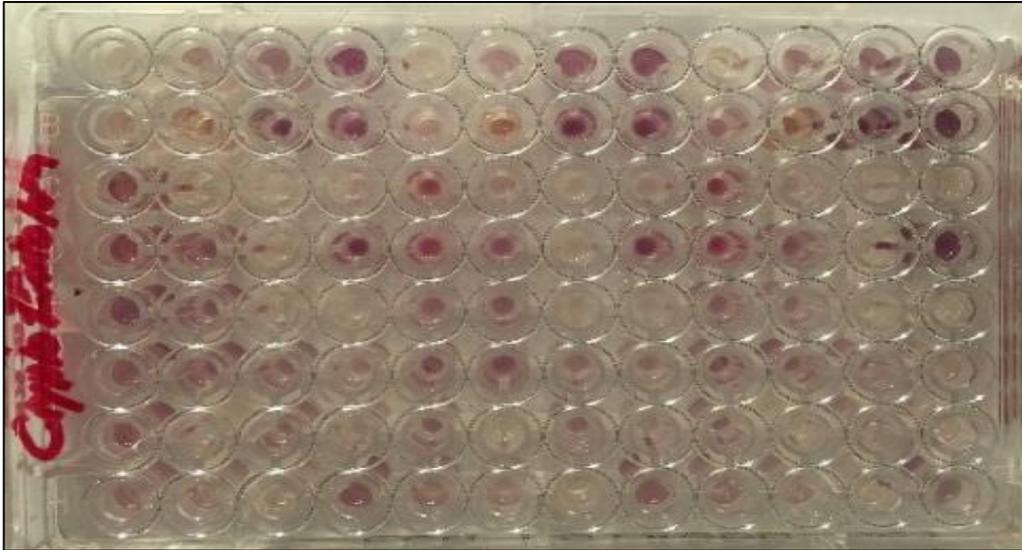


Can also identify genes and function in the community

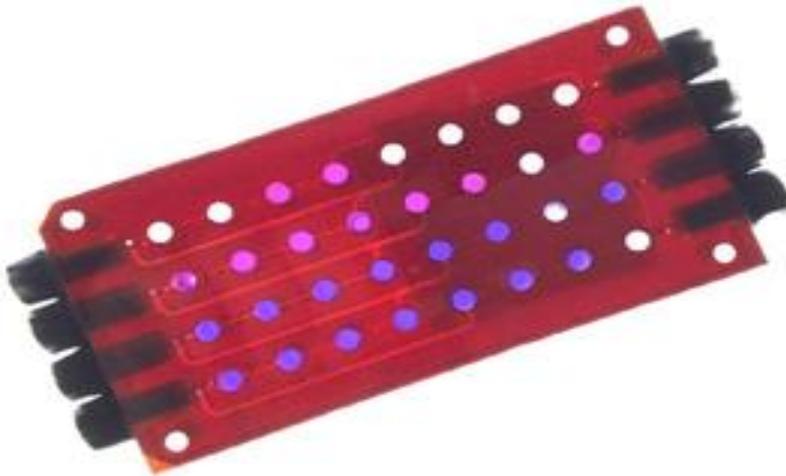
- Carbohydrates
- Amino Acids and Derivatives
- Clustering-based subsystems
- Miscellaneous
- Protein Metabolism
- Cell Wall and Capsule
- Membrane Transport
- DNA Metabolism
- Phosphorus Metabolism
- Respiration
- Cofactors, Vitamins, Prosthetic Groups, Pigments
- Fatty Acids, Lipids, and Isoprenoids
- Stress Response
- Motility and Chemotaxis
- Sulfur Metabolism
- Virulence, Disease and Defense
- Iron acquisition and metabolism
- Nitrogen Metabolism
- RNA Metabolism
- Regulation and Cell signaling
- Metabolism of Aromatic Compounds
- Nucleosides and Nucleotides
- Phages, Prophages, Transposable elements, Plasmids
- Cell Division and Cell Cycle
- Dormancy and Sporulation
- Potassium metabolism
- Secondary Metabolism



Microbial activity microassay (MAM) (Biolog Ecoplate)

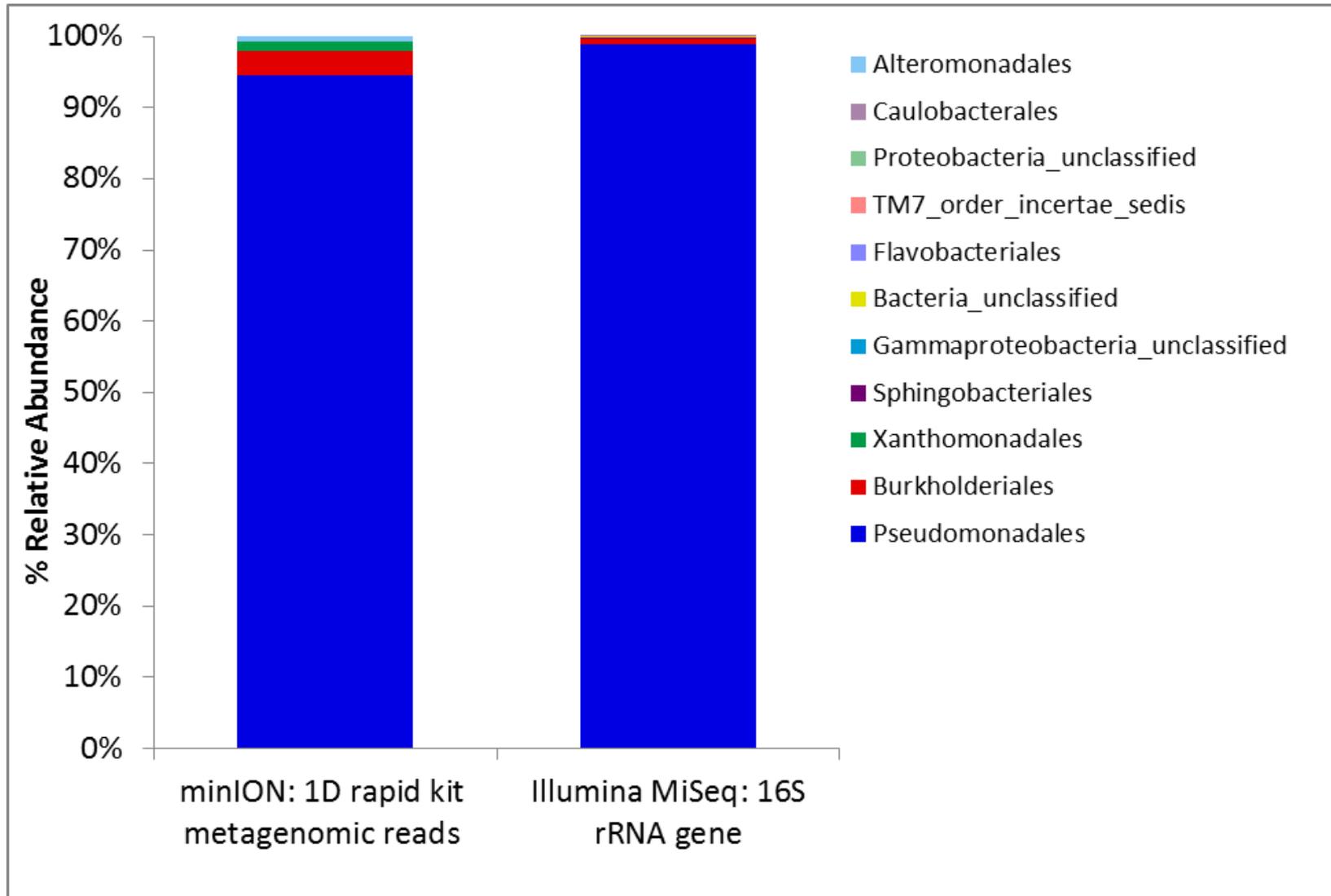
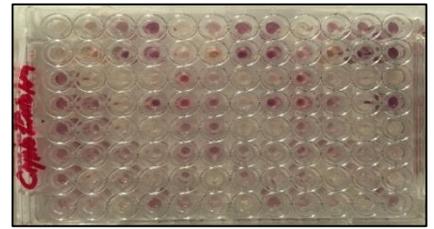


Coloured wells indicate substrates which have been metabolized by viable microorganisms



The Biosentinel microfluidic card prototype (right) displays metabolically active cells in a colourimetric assay (pink wells contain active cells).

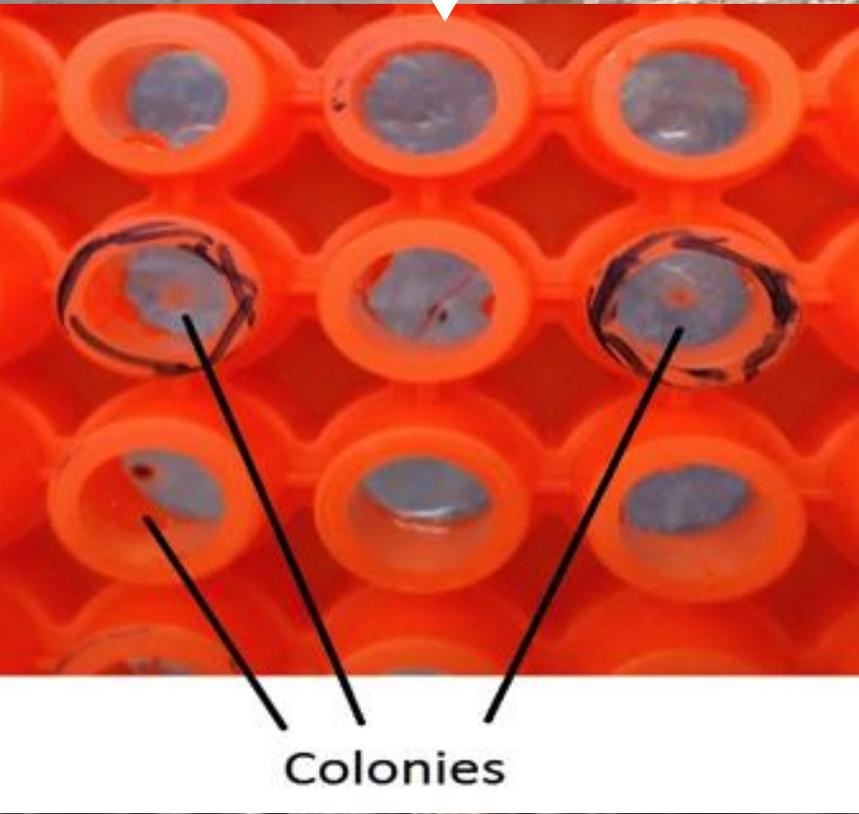
L-serine enriched communities



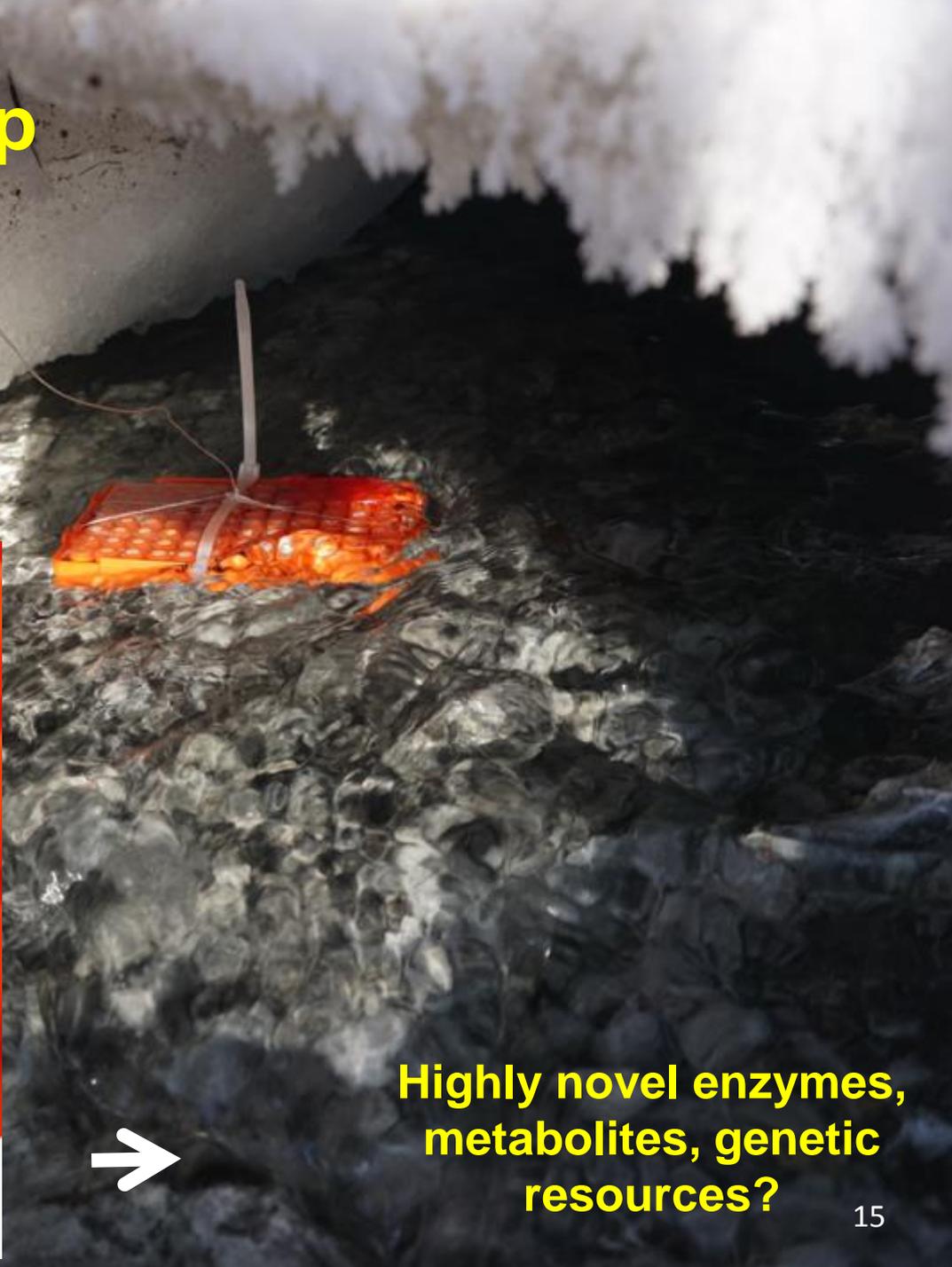
Culturing the unculturable via ichip methods



Highly novel strains?



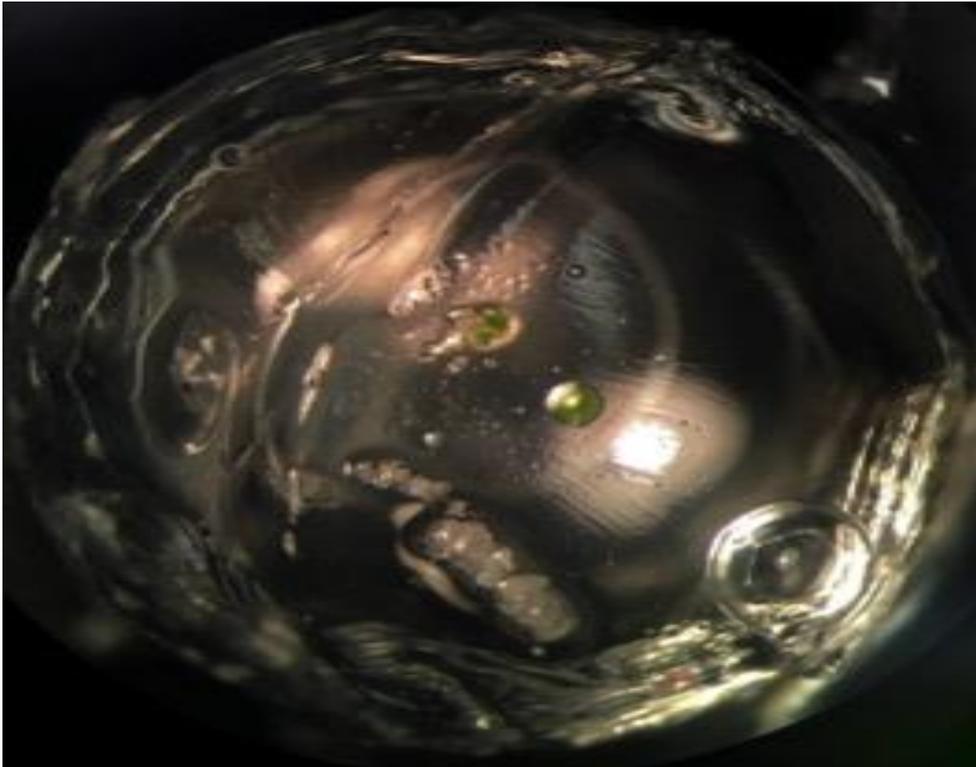
Colonies



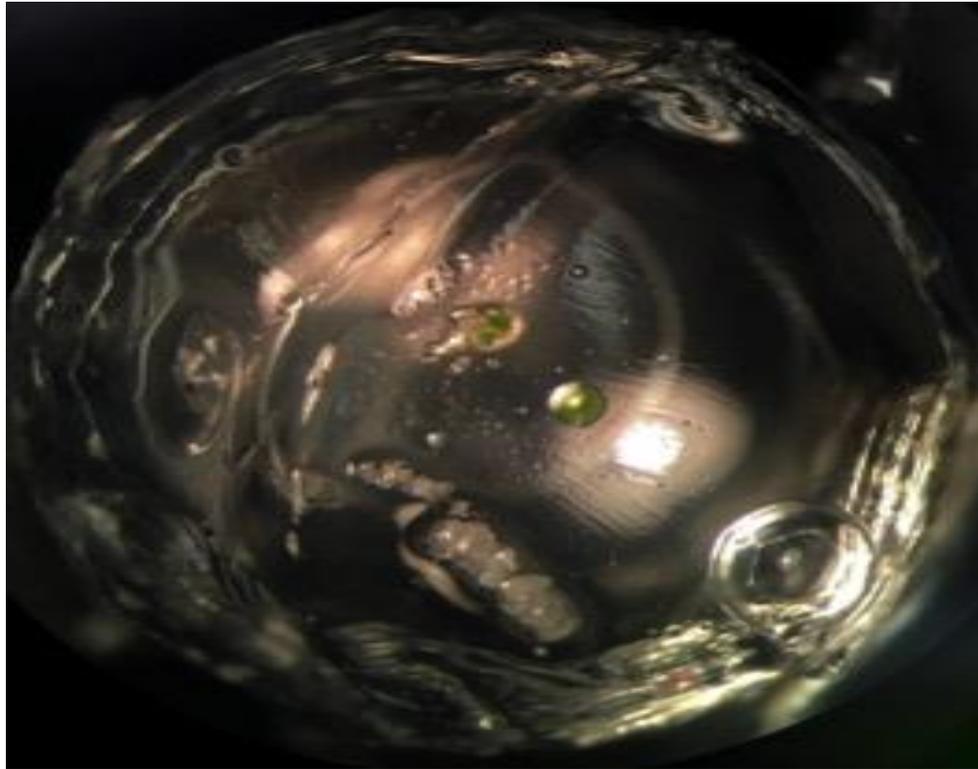
Highly novel enzymes, metabolites, genetic resources?

Cryo-iPlate 3.0

- Incubated *in situ*
- Extracted 106 isolates from 5 cryo-iPlates
- 72 (68%) were sub-culturable



Partial *Pedobacter* sp. genome sequence with the minION.



2 Million base pairs of high quality reads (closest *Pedobacter* genome size is ~5 Mbp)

~800 identified proteins, 6250 hypothetical proteins

What about the microorganisms we can't culture?

How can we tell if they are active?

Drilling Into the Atlantis Massif

Hunt for active life



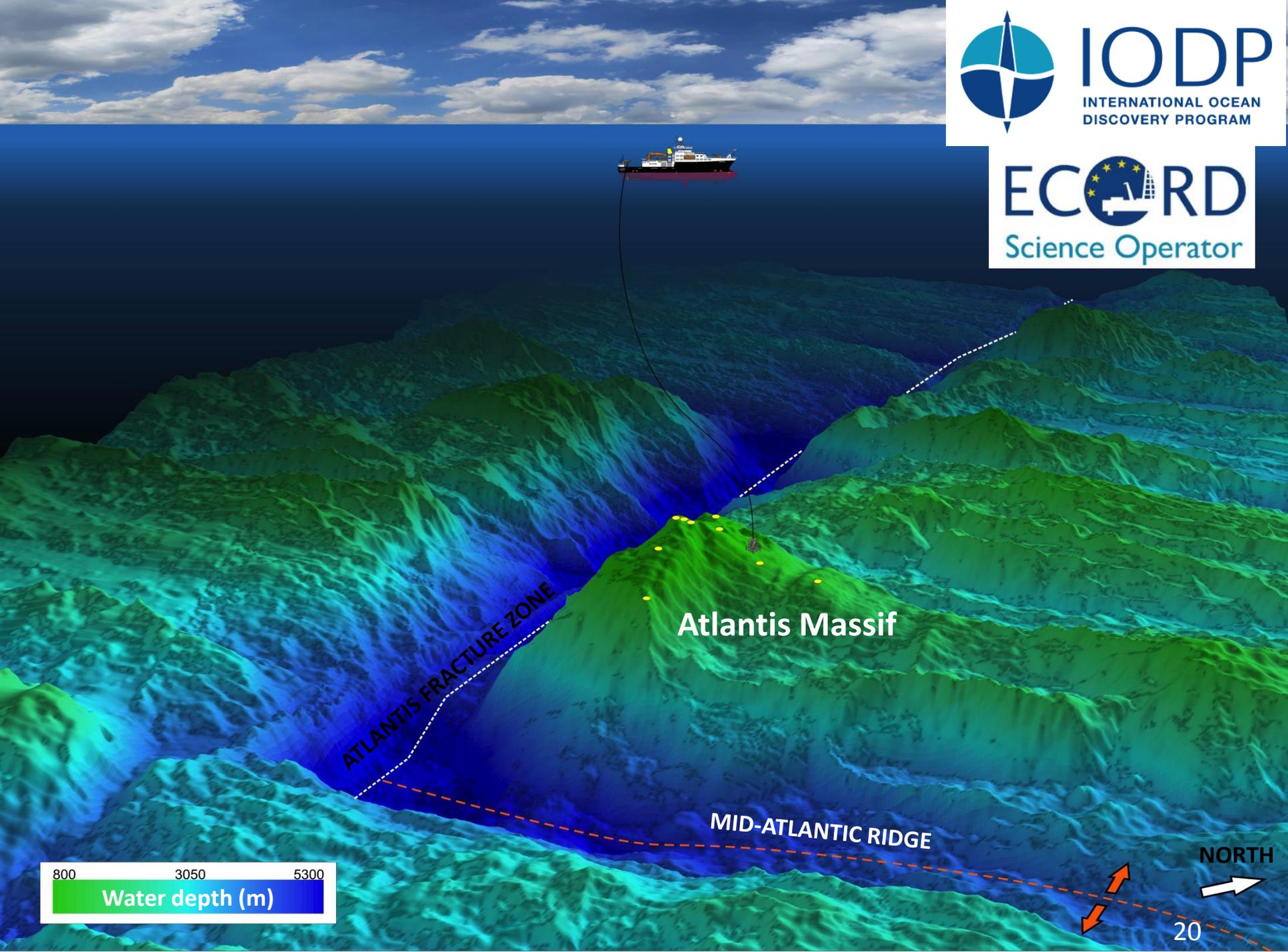
IODP Expedition 357
Oct-Dec 2015



Bigelow Lab

Atlantis
Massif
& Lost City





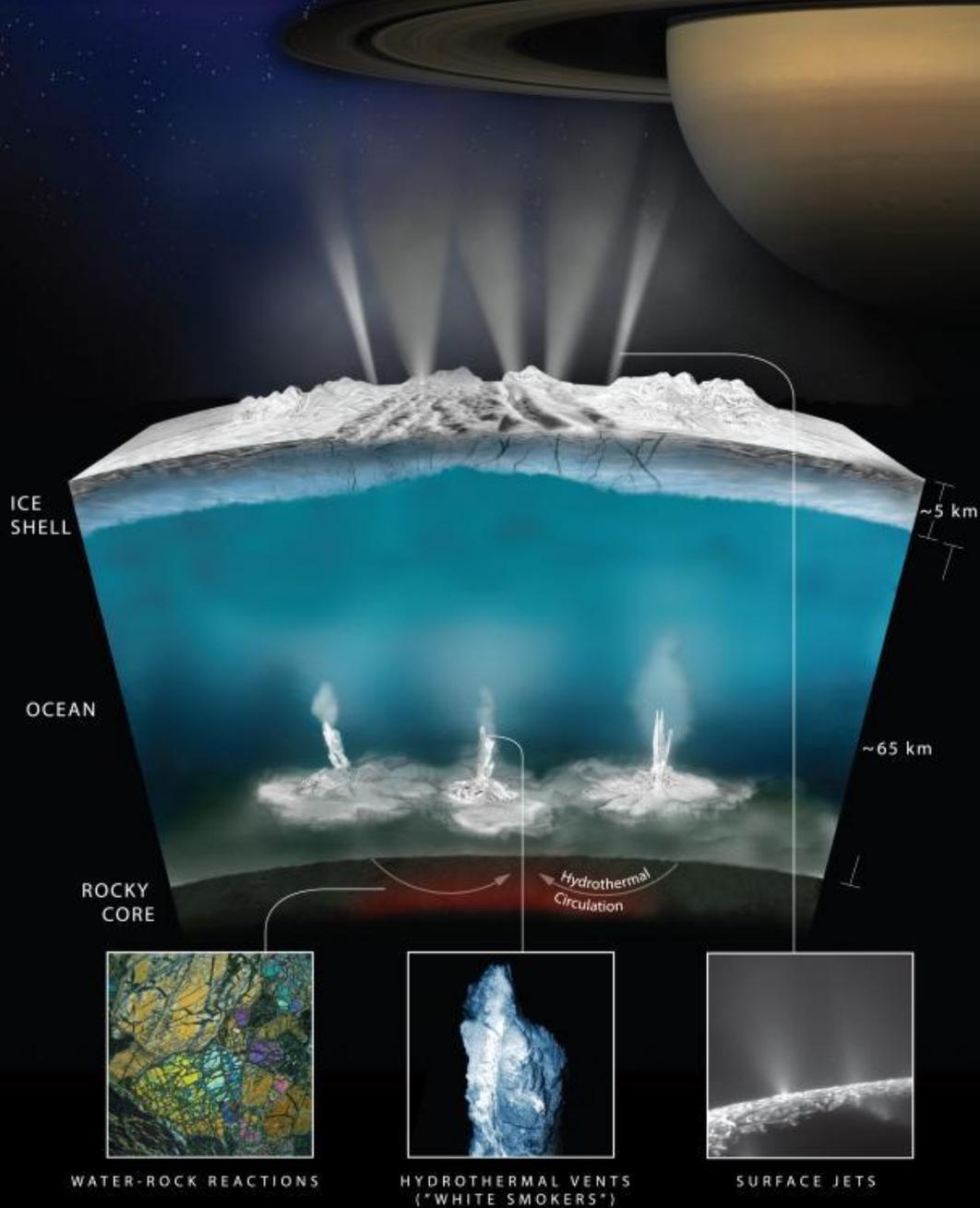
Serpentinization can support microbial life

Olivine + water + carbonic acid → serpentine ± brucite + magnetite + methane + hydrogen



* also generates heat (from hydration of olivine) and lowers density of rock





Credit: NASA/JPL-Caltech/Southwest
Research Institute

ENCELADUS

Getting down to the single cell

DNA → RNA → Protein

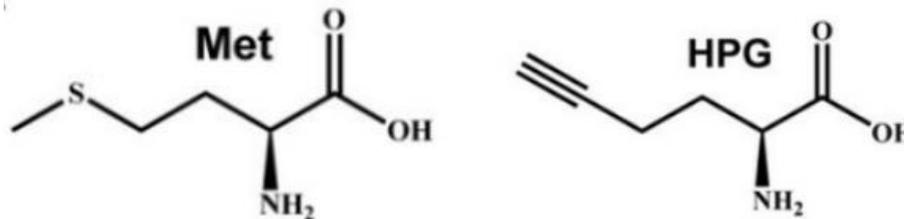
New tools are allowing us to separate active microorganisms from the rest of the community



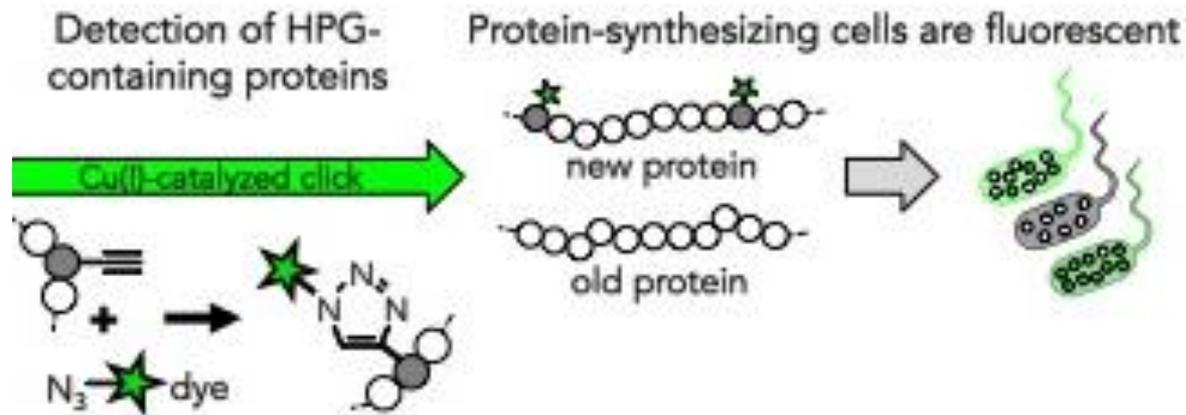
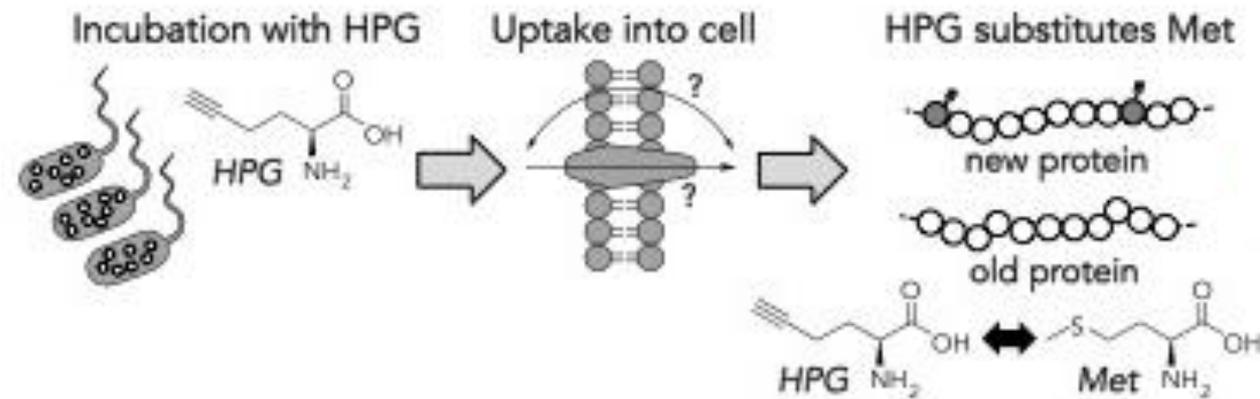
Rock samples

Bioorthogonal Noncanonical amino acid tagging (BONCAT)

Synthetic amino acids get incorporated in active microbes making new proteins

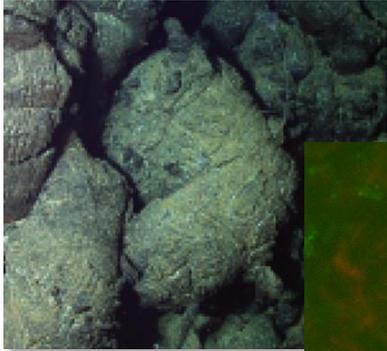


Bioorthogonal Noncanonical amino acid tagging (BONCAT)

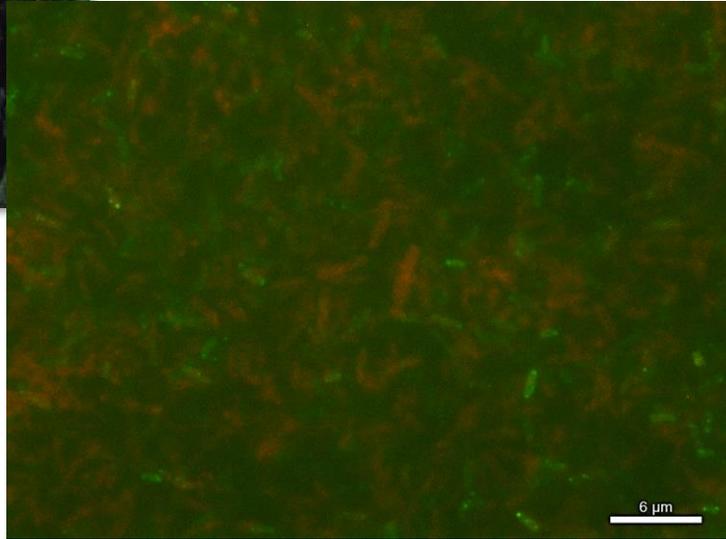


Sorting active cells

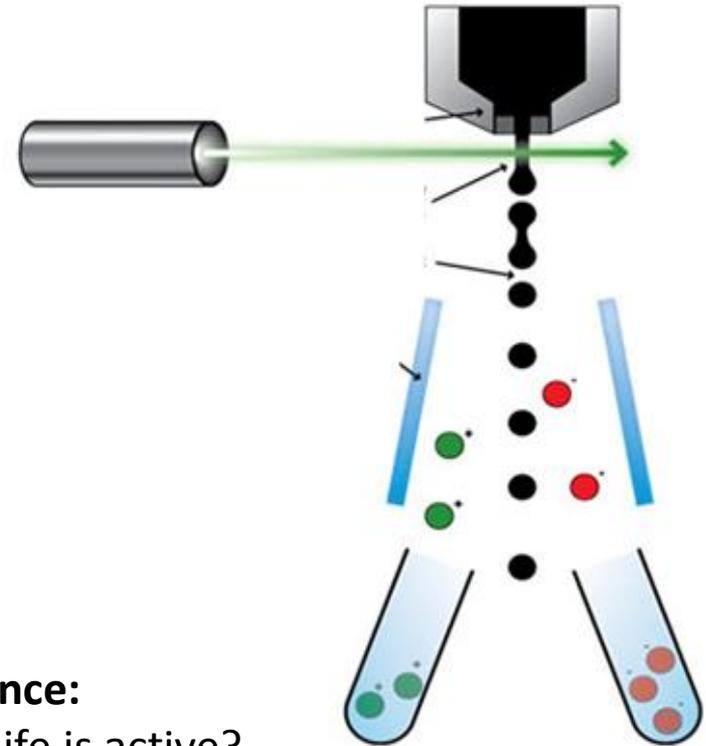
1. Cell Extraction
(shake cells off rocks)



2. BONCAT



3. Fluorescence
activated cell sorting
(flow cytometer)



Early results:

There is a low diversity, low biomass, **active**, microbial community in the ocean crust at Atlantis Massif!

Sequence:

What life is active?
What genetic adaptations do they have?

Conclusions:

- Most of methodology presented here is pre-existing, light weight, with low energy requirements.
- The nanopore minION is a promising tool for *in situ* nucleic acid sequencing, with clear application for life detection missions as well as environmental microbiology.
- This tool can be integrated with other microbiology based assays for life detection
- There is a need for automated systems. This is no small feat!
Low biomass will be an obstacle
- Future missions will likely (should) look for extant microbial life, there is a need for direct and unambiguous life detection instrumentation.

Thanks!

Many people in large teams make these projects happen!
I would especially like to acknowledge:

Dr. Lyle Whyte, McGill University

Dr. Beth Orcutt, Bigelow Laboratory for Ocean Sciences

