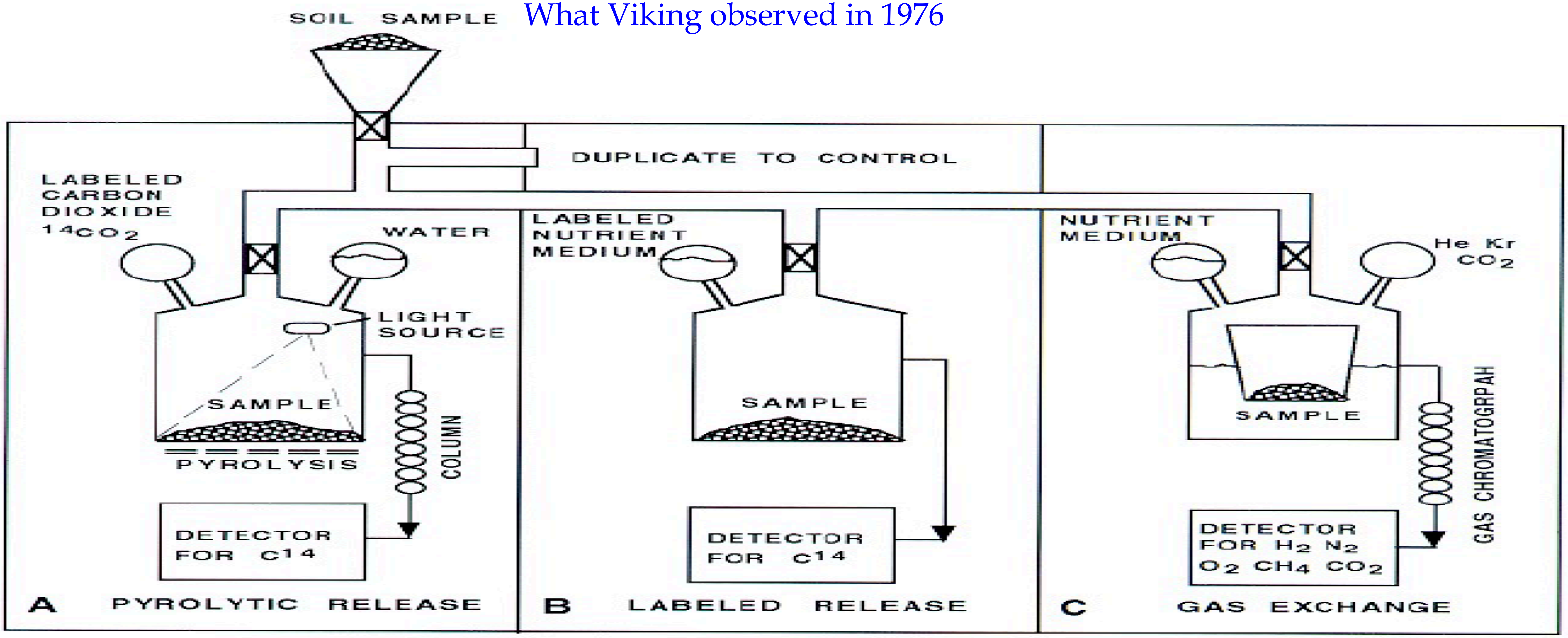
Searching for extant life on Mars before human arrival

Steven Benner, Chris Temby, Jan Špaček
Foundation for Applied Molecular Evolution (ffame.org)
Agnostic Life Finding Association (alfamars.org)

Cultural context from Viking 1976

What Viking observed in 1976



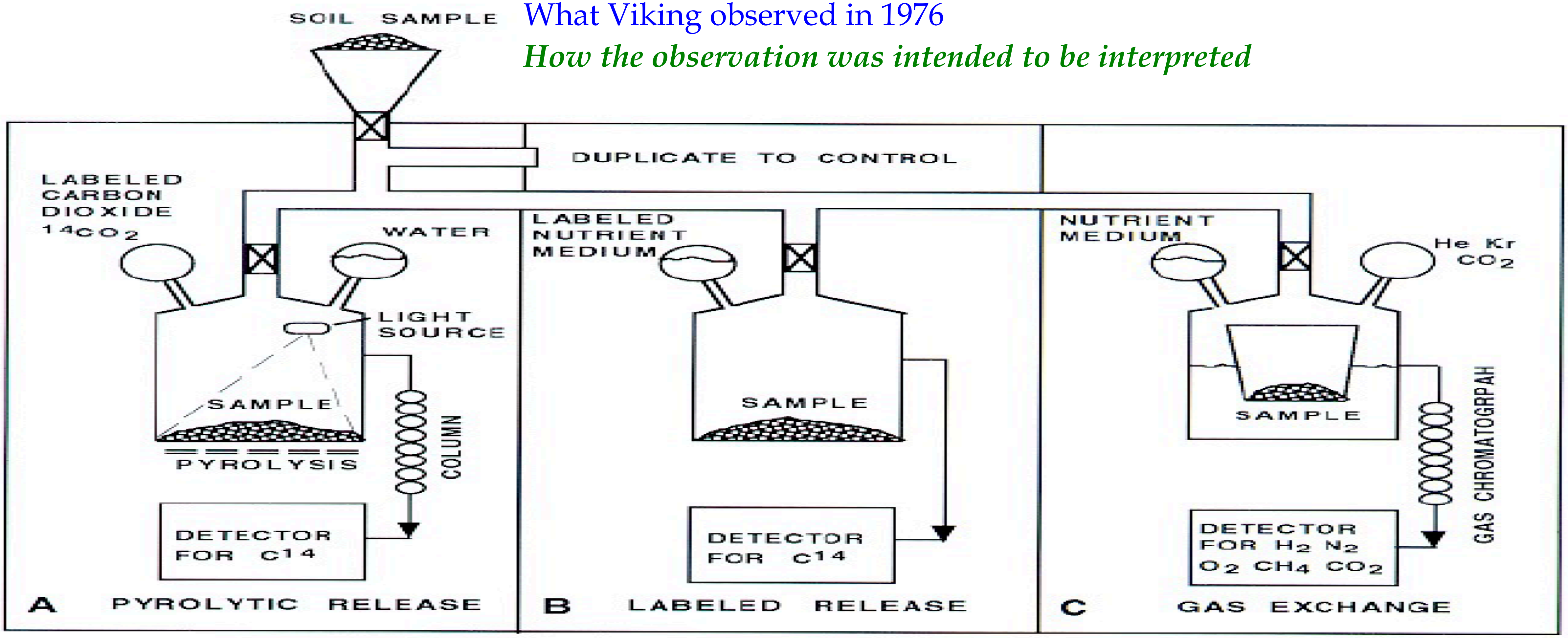
$^{14}CO_2$ fixed into organics

Radioactive food → $^{14}CO_2$ release

Moistening → O_2 rapid release

What Viking observed in 1976

How the observation was intended to be interpreted



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Photosynthetic autotrophy

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Other half of photosynthesis

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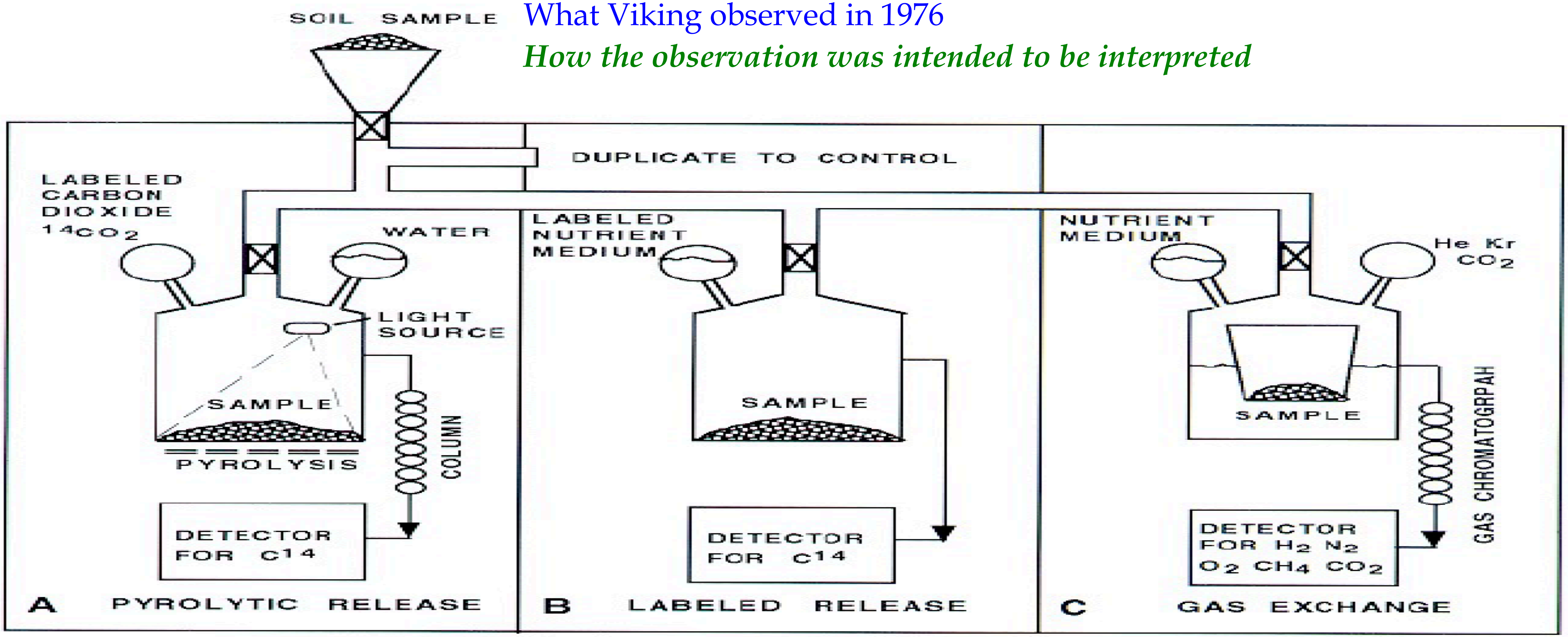

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Photosynthetic autotrophy | *Respiration* | *Other half of photosynthesis*

And then gas chromatography-mass spectrometry data were said to show **an absence of organics in soil**

Gerald Soffen: "That's the ball game. No organics. No life."

The evolution of the interpretation

Biemann et al. (1977) *J. Geophys. Res.* reported the results in detail

- Freon and methyl chloride were detected by the GC-MS on Mars
- Freon was detected by the GC-MS during the flight to Mars
- Methyl chloride was *not* detected in flight to Mars
- *"Methyl chloride could conceivably be indigenous to Mars."*

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II. Organic	None detected (see Table 3 for detection limits)
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And people then raced to generate non-biological explanations for the three positive life detection results.

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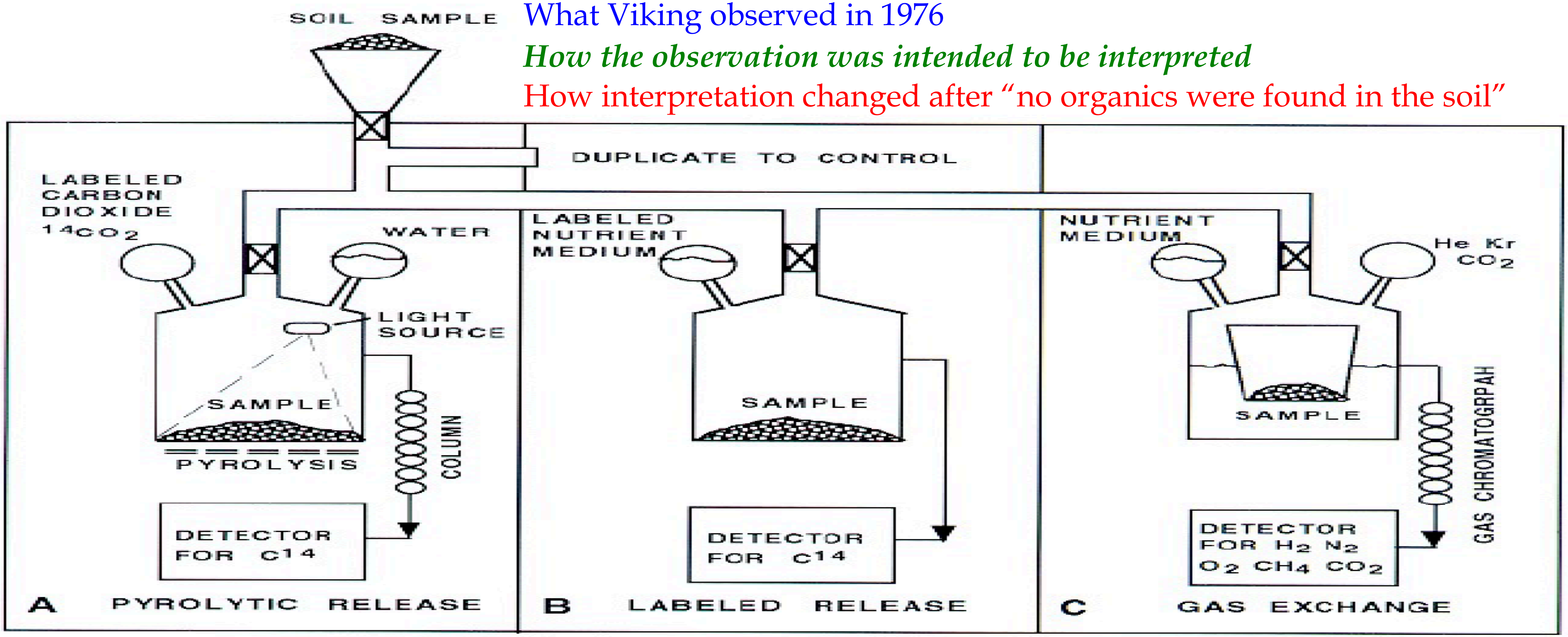
How interpretation changed after "no organics were found in the soil"



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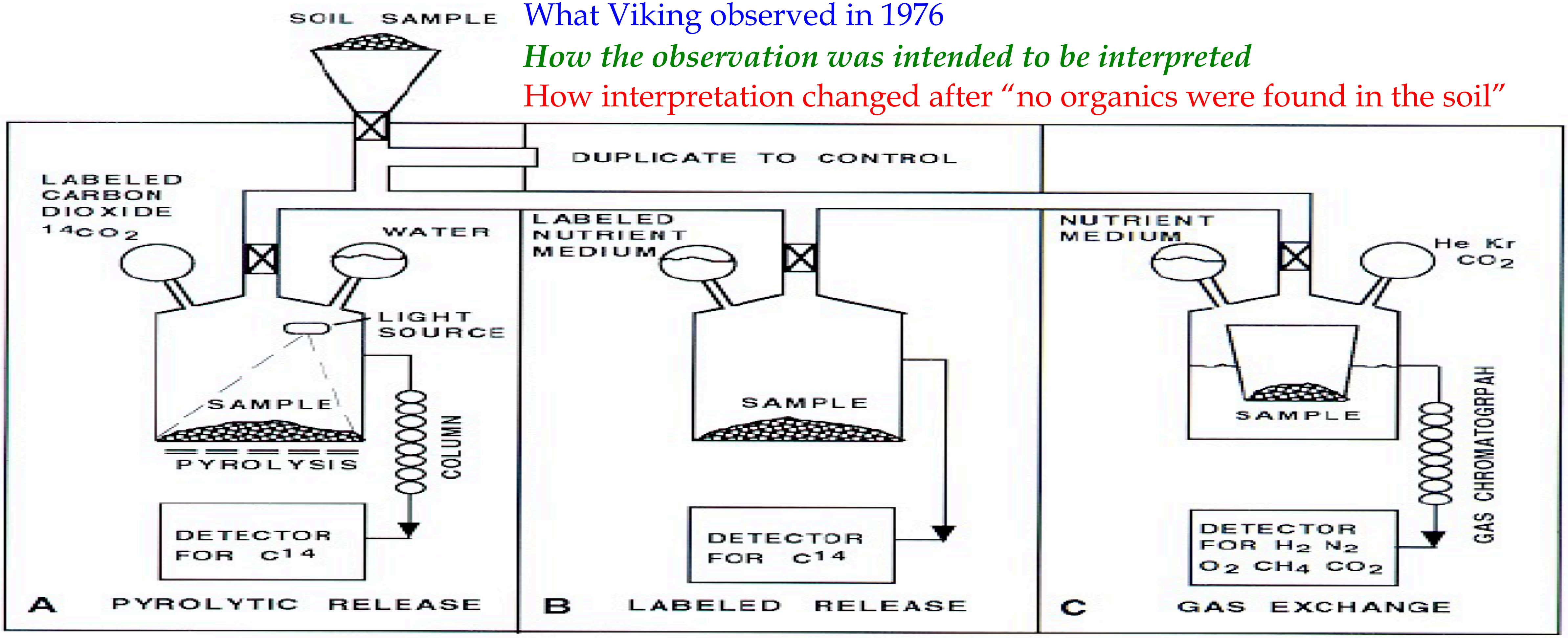

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But there is a **paradox**, recognized at the time by Horowitz. ""[I]t was surprising that in such a strongly oxidizing environment … organic material could be fixed in the soil." **But not to worry, Horowitz wrote:** "Investigations into the problem are now under way [that will find a] solution to the puzzle."

We now understand what Viking saw

- GC-MS would not detect principal meteoritic organics even sitting on a pile of them.
- GC-MS was too insensitive to detect bio-organics at the level of Atacama desert life.
- Soil perchlorate destroyed organics present upon heating to 400 °C.
- Methyl chloride is a byproduct of perchlorate + heat + organics.

Benner et al. (2000). The missing organic molecules on Mars. *Proc. Natl Acad Sciences 97*(6), 2425-2430.
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Viking's GCMS LOD ~10^6 cells/ml; Atacama life: ~10^2 cells/ml. LR LOD ~10 cells/ml.

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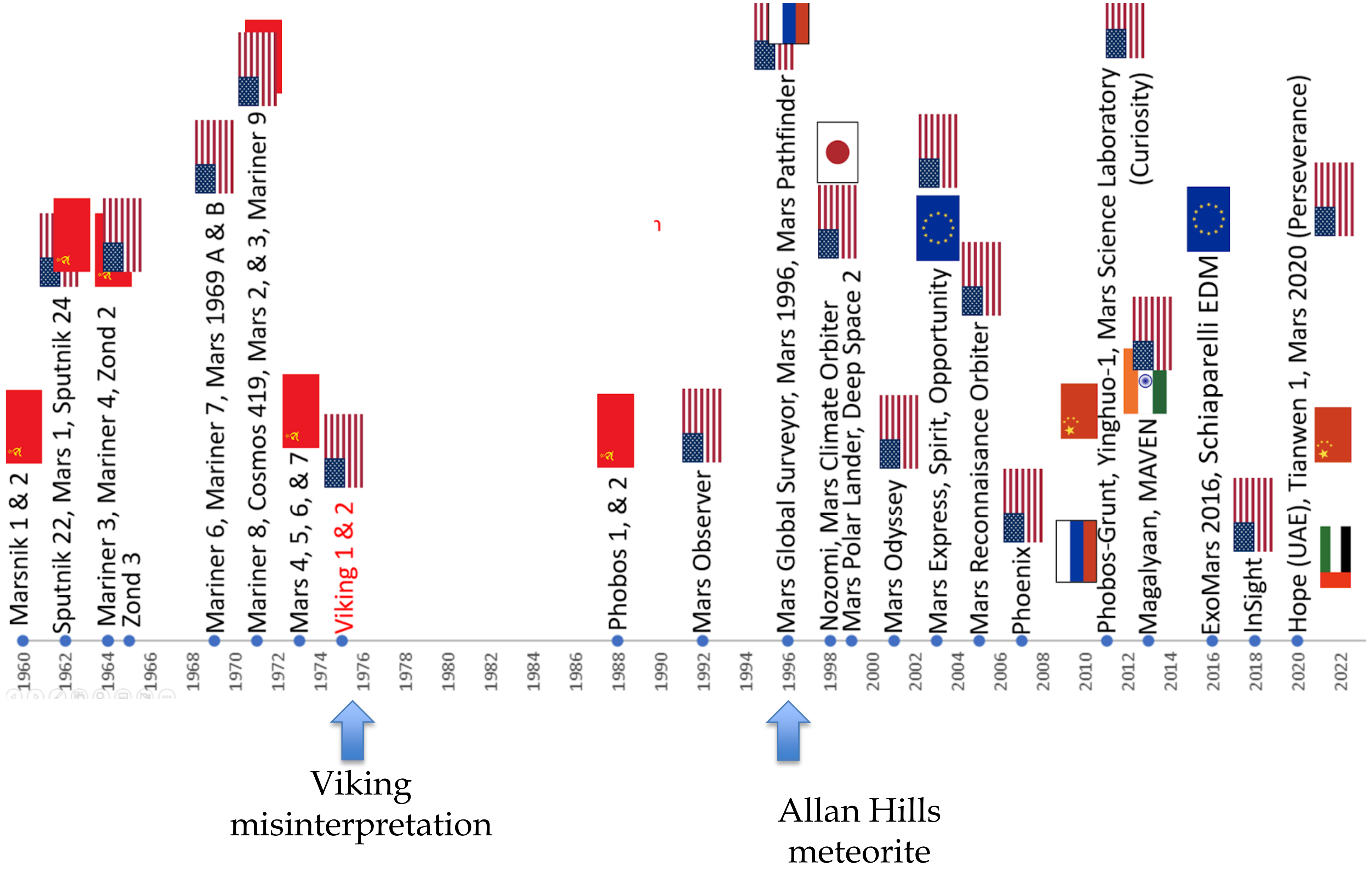
Meadows, Graham et al. NASA 2022 "Community Standards" report. "[S]ince Viking it's been recognized that there are numerous possible non-biological explanations for the [Viking] results. The results of the Viking Gas Exchange Experiment, which demonstrated that the regolith was chemically reactive, and the search for organics using the Viking GC-MS further support a non-biological interpretation of the [Viking] results. Additionally, detections of oxychlorine compounds on Mars (perchlorate and chlorate) during the Phoenix and Mars Science Laboratory missions indicate the occurrence of processes that can explain both the Viking LR and GCMS results."

GC-MS misinterpretation blocked Mars exploration



Courtesy
Jan Spacek
Chris Temby

GC-MS misinterpretation blocked development of healthy pro/anti life dialectic, needed for science

Results at face value *define* the kind of life Viking found

- Horowitz observed fixation of carbon, photosynthetic autotrophy

The 6th grader question asks: What do autotrophs do at night: They do respiring metabolism.

- Levin and Straat observed respiration

The 6th grader asks:

What do autotrophs at night respire with? O_2 from the atmosphere (20% on Earth)

What if atmosphere has little O_2? Store O_2 created in the day for respiration for overnight

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The point: If life was extant at Viking sites, it is everywhere on Mars

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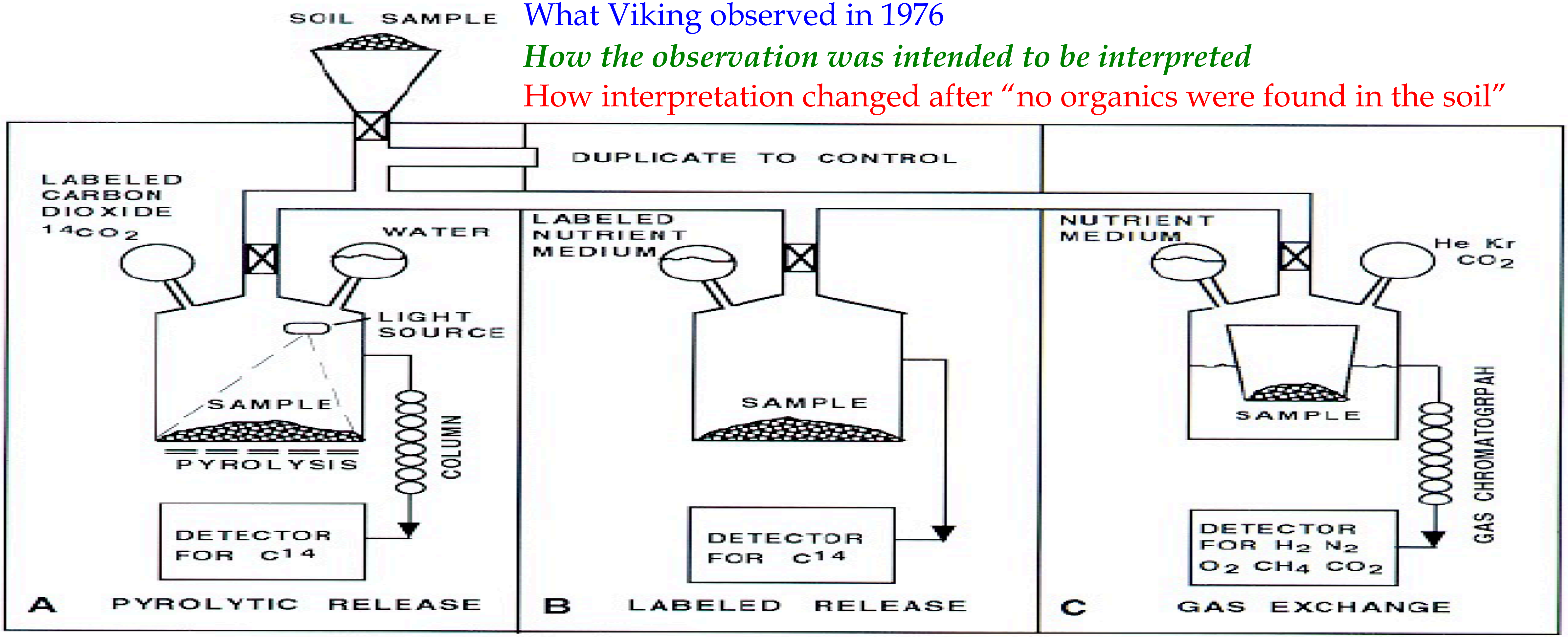

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So now that we know the case for an extant biosphere on/near the Martian Surface, how do we go look for it?

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Search for extant life on Mars is the only space endeavor that cannot wait.

Human missions to Mars are imminent, from many actors worldwide.

Extant Mars life is the only scientific object above our atmosphere that might be negatively affected by human activities in the next decade or two. Not the Moon, not Europa, not Venus.

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Need more than a "yes-no" answer. We must *study* Martian biochemistry.

The essence to planetary protection is **orthogonality.**

If Martian biochemistry is orthogonal to terran biochemistry, little planetary protection hazard.

It cannot infect us, we cannot infect it.

The dreaded "biosignature question"

We agree that Darwinian evolution is the only mechanism that allows matter to self-organize to give properties that we value in life.

We can look for molecules/molecular aggregates/structures that are the products of Darwinian evolution

Amino acids, nucleobases, complexity in molecular structures, features of molecular aggregates …
False positives, false negatives, analytical chemistry challenges, endless debates (e.g. Allan Hills) …

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Or, we can look for molecules/molecular aggregates/structures that are required to enable Darwinian evolution, in particular, its informational needs

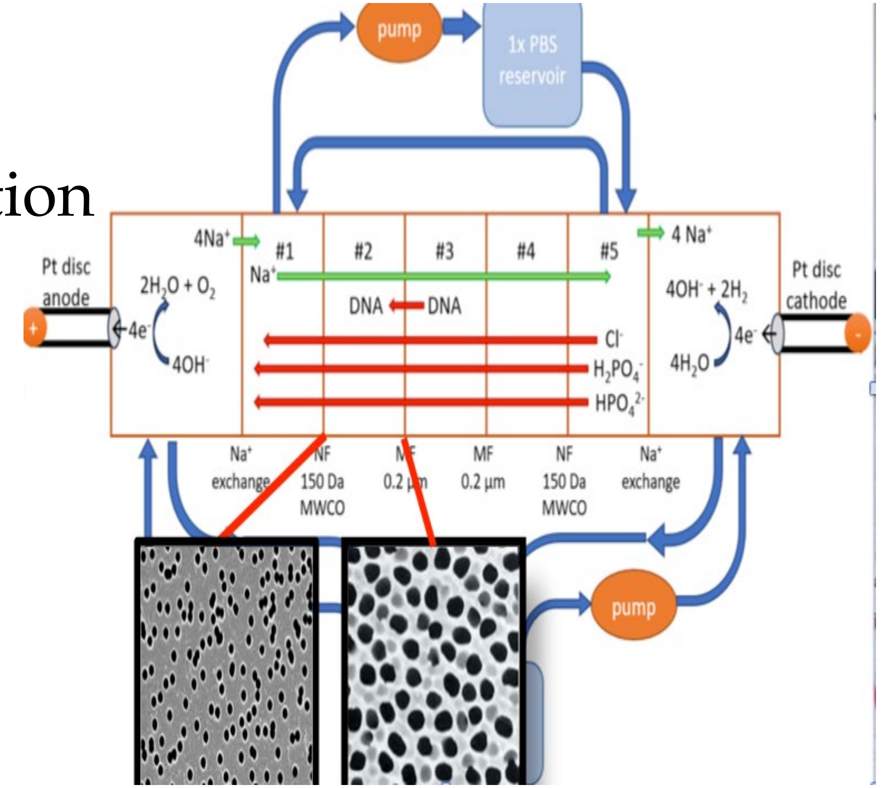
Polyelectrolyte Theory of the Gene:
For life in H_2O, informational needs of Darwinian evolution can be met only by a biopolymer with a repeating backbone charge built from a small vocabulary of size/shape regular units.
https://en.wikipedia.org/wiki/Polyelectrolyte_theory_of_the_gene

Polyelectrolyte Theory of the Gene comes from hundreds of molecules made by synthetic biologists seeking to find molecules other than DNA/RNA able to support evolution

For Terran DNA and RNA, the repeating backbone charge is negative.
Synthetic biologists have made alternatives with a repeating positive backbone charge.
Polyelectrolyte allows information content of genes to change without large changes in properties
Size-shape regular building blocks allows high fidelity of replication ("aperiodic crystal")

Benner (2023) Rethinking nucleic acids from origins to applications. *Phil. Trans. Roy. Soc. B: Biol. Sci.* **378**, 1871.

Polyelectrolytes are easy to concentrate from dilute solution
Once concentrated, easy to study molecular structure
If size-shape regular, indisputable biosignature
No false positives; Darwinism needed to sustain structure
No false negatives; polyelectrolytes required for Darwiniam



Courtesy. Jan Spacek, Chris Temby,
Agnostic Life Finding Association, ALFA-Mars

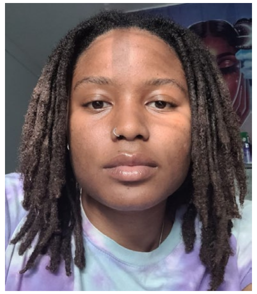
Particulates

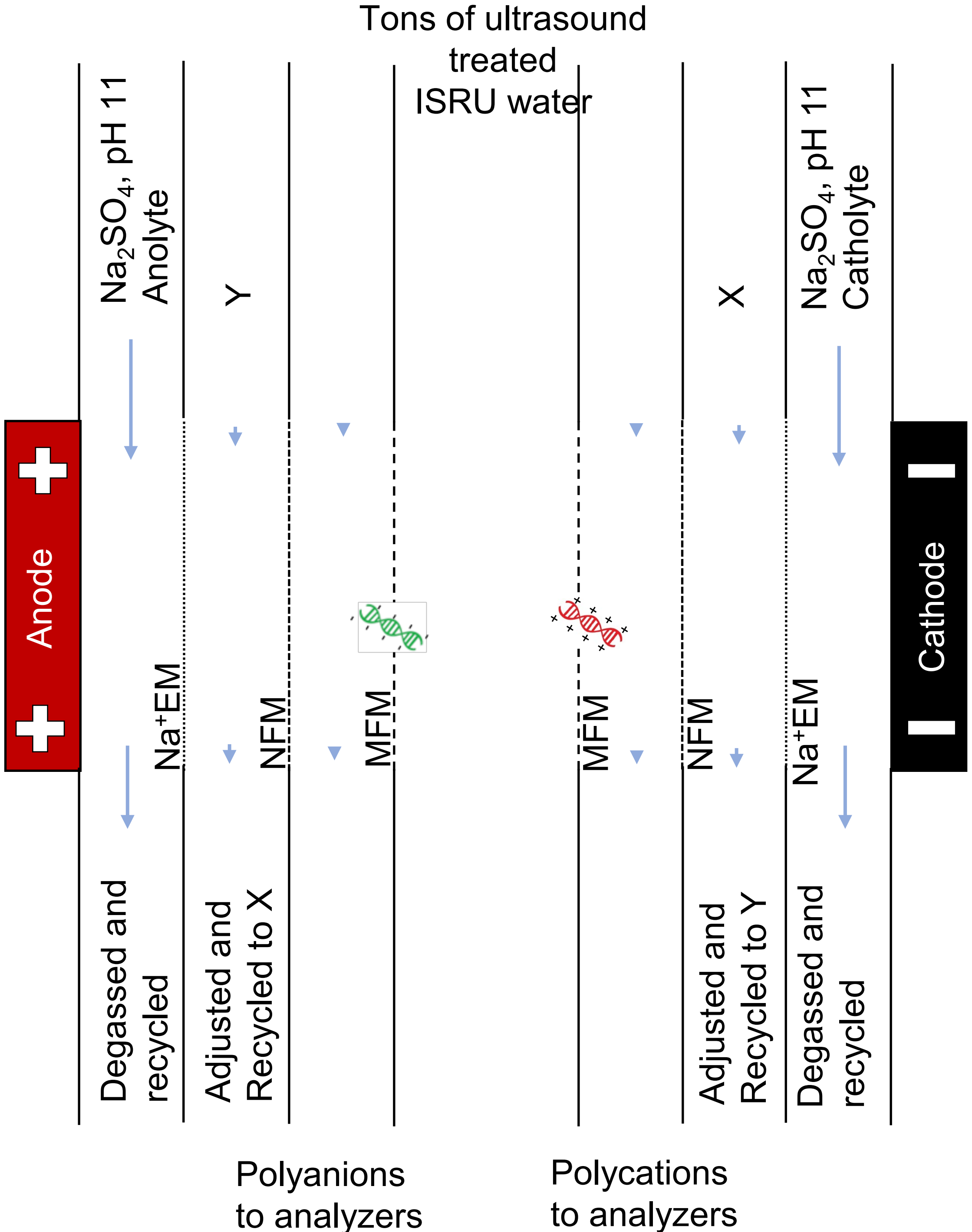
Polyanions (e.g. DNA)

Polycations (e.g. alien genetic polymer)

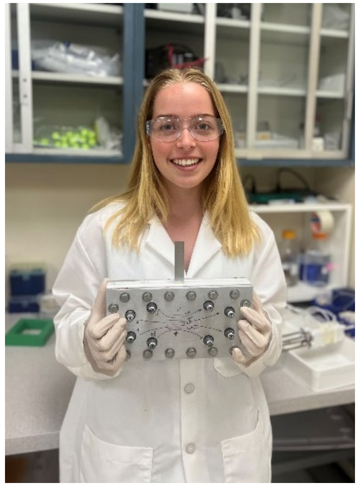
2024





Ultrasound releases polyelectrolytes from cells, promotes desorption from minerals.



Summer 2023: Kate Sheldon, Princeton

Summer 2022: Chris Temby, UCSB

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AGNOSTIC LIFE FINDING ASSOCIATION
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ABOUT US

AGNOSTIC LIFE FINDING ASSOCIATION – MARS

ALFA Mars consists of a group of scientists, engineers, science communicators and volunteers aiming to determine whether indigenous life is present today on Mars before humans walk on its surface.

ALFA Mars is administered by Agnostic Life Finding Association Inc., 501(c)(3) registered in Florida.

JAN ŠPAČEK

Dr. Jan Spacek, founder of the ALFA Mars project. Inventor of ALF (Agnostic Life Finder) and IMPRESS mission. Senior research scientist at Foundation for Applied Molecular Evolution, molecular biologist, and analytical (electro)chemist.

– Read Full Bio

CHRIS TEMBY

Chris Temby received his Bachelors of Science degree in Physics, with minors in Astronomy and Planetary Sciences and History, from the University of California, Santa Barbara. He is an aspiring astrobiologist and astronaut, and currently a private pilot and scuba divemaster.

– Read Full Bio

GABBY RIZZO

Gabby Rizzo is a first year Ph.D. student in the Genetics, Cellular & Molecular Biology program at the University of Nebraska-Lincoln. As an aspiring astrobiologist, she is interested in the search for evidence of past or present life beyond Earth by studying various extremophilic microorganisms and their environments.

– Read Full Bio

COURTNEY PASTERNAK

Courtney is a Junior at Embry-Riddle Aeronautical University studying Aerospace Engineering with a concentration in Astronautics, while minoring in computer-aided design (CAD) and computer-aided manufacturing (CAM).

– Read Full Bio

STEVEN BENNER

Dr. Steven Benner, founder of the Foundation for Applied Molecular Evolution and Firebird Biomolecular Sciences. Expert in astrobiology, synthetic biology, paleogenetics, and evolutionary bioinformatics.

– Read Full Bio

LYLE WHYTE

Prof. Lyle Whyte, McGill University; Member, McGill Space Institute. Lyle studies the ecology and activity of microbial communities at subzero temperatures in high arctic analogues to Martian environments.

– Read Full Bio

VICTOR PARRO

Prof. Victor Parro, PhD, Senior Scientist at Spanish Astrobiology Center (Centro de Astrobiología), studies the environmental microbiology of extremophiles. Victor searches for (alien) biomolecules using antibody microarrays in Antarctica.

– Read Full Bio

SHANNON BOETTCHER

Prof. Shannon Boettcher is a Director of the Oregon Center for Electrochemistry and an expert in inorganic materials science and fundamental electrochemistry, including membrane electrodialysis.

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SUKRIT RANJAN

Sukrit Ranjan is Professor of Planetary Science at the University of Arizona. His research interests lie in modeling the surface-atmosphere system of rocky planets with an emphasis on early Earth and terrestrial exoplanets, in service of (1) efforts to understand the emergence of life on Earth and (2) to search for life on exoplanets.

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MIGUEL ÁNGEL FERNÁNDEZ-MARTÍNEZ

Dr. Miguel Ángel Fernández-Martínez is a 'María Zambrano' excellence program postdoctoral researcher at Universidad Autónoma de Madrid (Spain), specializing in microbial ecology of Mars terrestrial analogs and extreme environments.

– Read Full Bio

ERIK BETHKE

Erik Bethke, CEO of Million on Mars, online game developer focused on settling the solar system. Created Starfleet Command and GoPets. Exited to Zynga, leadership on MafiaWars and FarmVille. Wrote Game Design and Production, and Settlers of the New Virtual Worlds.

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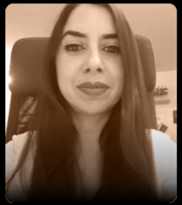


XIMENA C. ABREVAYA

Dr. Ximena Abrevaya is an Astrobiologist, Senior Research Scientist at Instituto de Astronomía y Física del Espacio, Argentina, and Founding Director of the Argentinian Research Unit in Astrobiology (Astrobio.ar). Her expertise in Astrobiology involves interdisciplinary studies, combining astrophysics, biology, and geology.

– Read Full Bio

CHARLES COCKELL

Charles Cockell is professor of Astrobiology at the University of Edinburgh. His scientific interests are on life in extremes, habitability and the human exploration of space.

– Read Full Bio

JANUSZ PĘTKOWSKI

Dr. Janusz Pętkowski is an astrobiologist, Research Affiliate in the Department of Earth, Atmospheric and Planetary Sciences (EAPS) at MIT. Janusz is a Deputy PI on the MIT-Breakthrough Venus Life Finder Mission Concept Study.

– Read Full Bio

GAGE OWENS

Gage Owens is a Ph.D. candidate in Chemistry at the University of Washington. He has worked in synthetic biology and organic chemistry at Foundation for Applied Molecular Evolution.

– Read Full Bio

KATE SHELDON

Kate Sheldon is a freshman at Princeton University studying astrophysics and chemistry. Kate intends to work in astrobiology and astrochemistry and hopes to study life on Mars – while on Mars.

– Read Full Bio

HOLDEN ALPERN

Holden Alpern is a sophomore at the University of Florida studying Geology, looking to double major in Microbiology. After college, he aims to conduct graduate research in Geobiology, focused on finding life on other worlds. He strives to turn childhood aspirations of space exploration to reality, helping build ALF at the FfAME lab, and hopes to one day see the Red Planet for himself.

– Read Full Bio

SEAN BROWN

Sean Brown, a Biological Sciences PhD candidate at the University of Maryland, Baltimore County, explores astrobiology, synthetic biology, and theoretical biochemistry. His research delves into the question of how an alien or alternative protein biochemistry might differ from what we know here on Earth.

– Read Full Bio

IAN FERNANDEZ

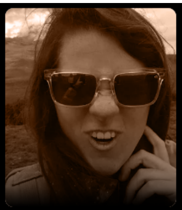
Ian Fernandez is a graduate student in the Microbiology and Cell Science program at the University of Florida. After graduate school, Ian intends to work in the biotechnology sector, while continuing his involvement in

LIGIA FONSECA COELHO

Dr. Lígia Fonseca Coelho is a Fulbright Researcher at the Department of Astronomy, Cornell University. Lígia is interested in biosignatures (pigments), extreme environments, and planetary field analogs

ZACK COHEN

Zack Cohen is a Ph.D. candidate in the Chemistry department and Astrobiology program at the University of Washington in Seattle. Zack investigates the behavior of fatty acid membranes



OLIVIA TRAENKLE

Olivia Traenkle is a chemist and marine scientist, a PhD candidate working in Electrochemistry and Material Science Laboratory (University of Oregon) on bipolar membrane water electrolysis

HANNAH DROMIACK

Hannah Dromiack is a Ph.D. candidate (Physics) at Arizona State University studying new methods for agnostic life detection using macroscale behaviors displayed by living organisms. She also is a contractor for NASA

TOMÁŠ PETRÁSEK

Tomáš Petrásek is a Czech neuroscientist, astronomy popularizer, astrobiology teacher and science fiction writer, a member of Czech space enthusiast society Kosmo Klub. He leads an astrobiology course at the Charles

CADEN HOWLETT

Caden Howlett is a PhD candidate in Geosciences at the University of Arizona, broadly interested in the processes that create and destroy mountain belts. He is a field-based structural geologist who integrates

AGNOSTIC LIFE FINDING ASSOCIATION
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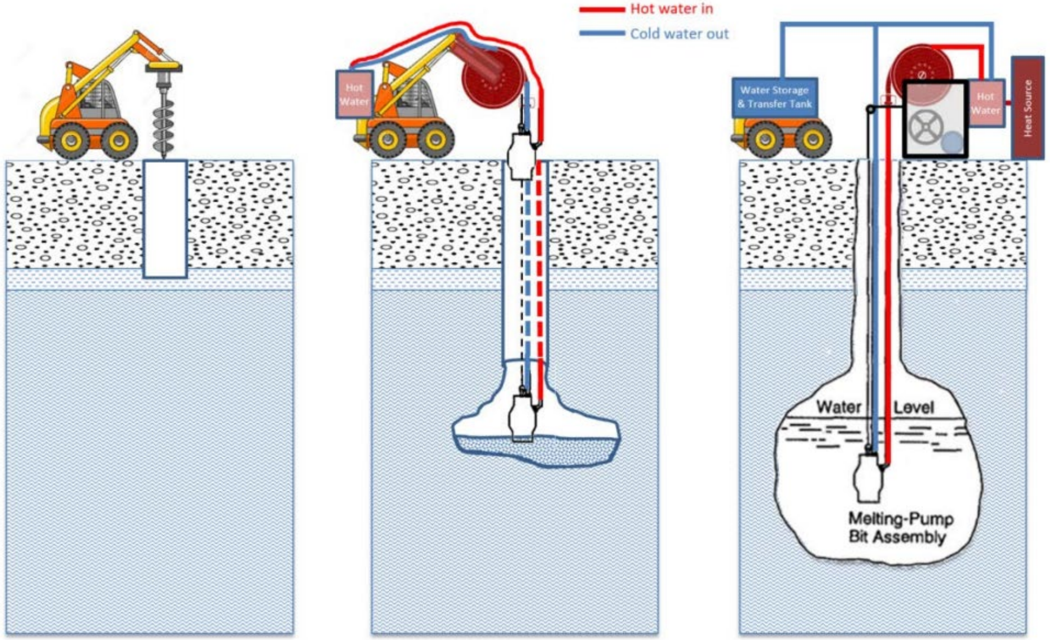
ALF can be a low cost addition to a water mining operation, standing astride water flow of any size, pulling polyelectrolytes out and concentrating them for study.

- Water mining for propellant
- Mid-latitude subsurface ice-dust layered “lasagna”
- On Mars likely long before humans leave Earth
- Make water mining into extant life prospecting missions
- Very large scale astrobiology experiment with little added cost

Spacek, J., Benner, S. A. (2022). Agnostic life finder (ALF) for large-scale screening of Martian life during in situ refueling. *Astrobiology*, *22*(10), 1255-1263.

Honeybee Robotics' Red Water Rod Well. Heldmann, J. L., *et al* (2022) *New Space 10*, 259-273.



Phase 1: Drill through overburden into top of ice.

Phase 2: Melt into ice. Begin forming water pool.

Phase 3: Steady state operation.

Fig. 7 Steps used to establish a Rodwell on Mars

Alternative timetables and mission architectures

Spry et al. (2024)

- **Multiple Mars pre-landers, with the first lander(s) pre-deploying cargo to prepare for a later crew landing; this would include propellant for the return trip.**

- **Modest initial surface infrastructure: a ~10 kWe fission surface power system and communications infrastructure, but no fixed surface and no requirement for return mission-critical *in situ* resource utilization (ISRU) propellant production.**

To meet the planetary protection goals, we must insert here a RodWell with a with an 100+ liter capacity/ for one Sol and the capability to detect life in it. Compare Starship refueling ~600,000 liters of water.

- **An "all-up mission" approach, with crew departing Earth with all the transit propellant they need for the round-trip journey, a consequence if there is no ISRU propellant for the first mission.**

- **A light initial exploration footprint, sending four crew members to Mars orbit, with two of those crew members descending and living on the surface for a 30-sol surface stay**

Alternative Mission Architectures: IMPRESS International Mars Prospecting Ride-Share System

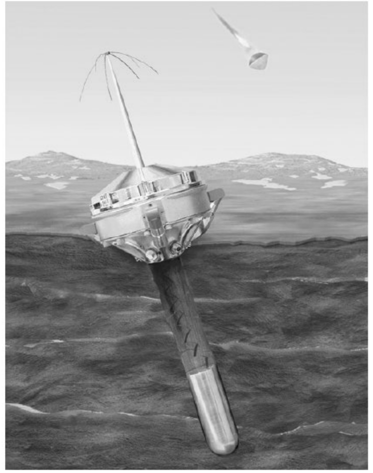


Fig. 26. Artist's impression of the DS-2 penetrator deployed on the surface. The forebody and aftbody are connected with a flexible tape umbilical.

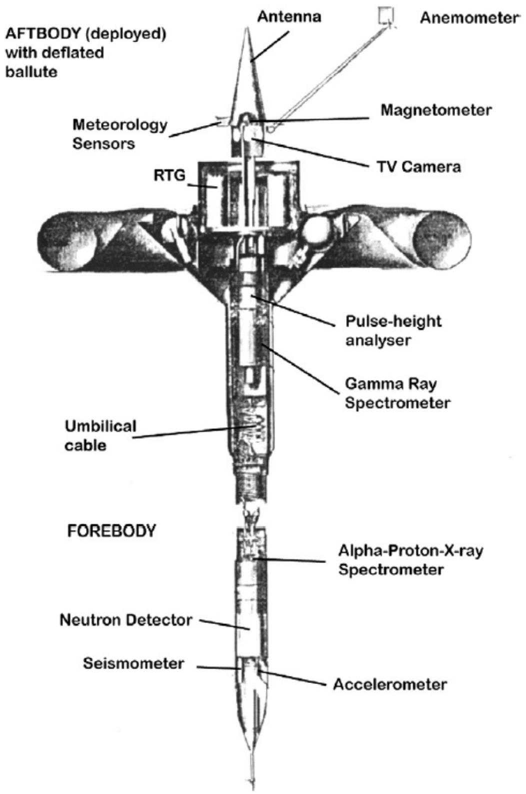
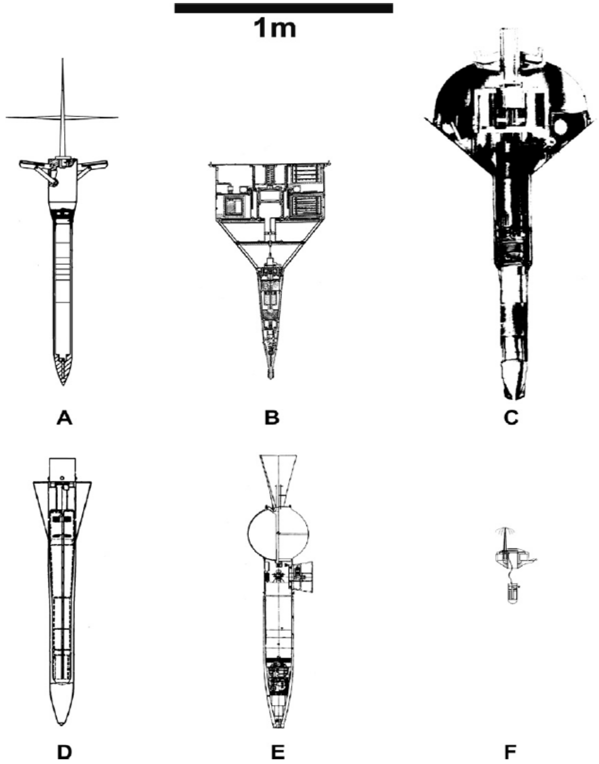


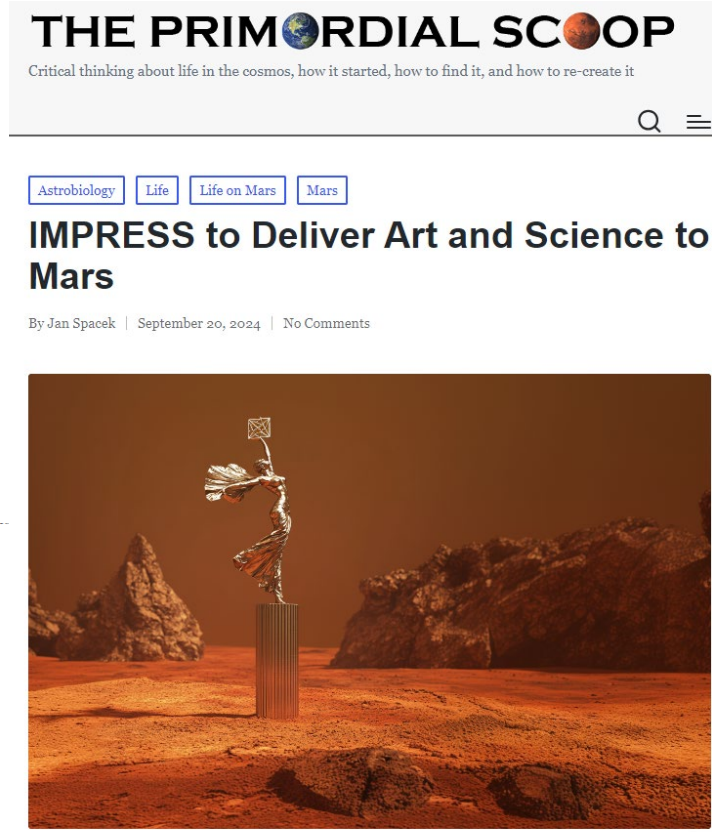
Fig. 17. Cross-section of the deployed Mars-96 penetrator, showing the extensive instrumentation and the split into fore- and aft-bodies connected by umbilical. Note the RTG is in the aftbody where it can effectively reject heat.



Advances in Space Research 48 (2011) 403–431

Planetary penetrators: Their origins, history and future

Ralph D. Lorenz *



Jan Spacek

A final word about orthogonality and planetary protection

This question has been addressed the "anthropogenic biology" community

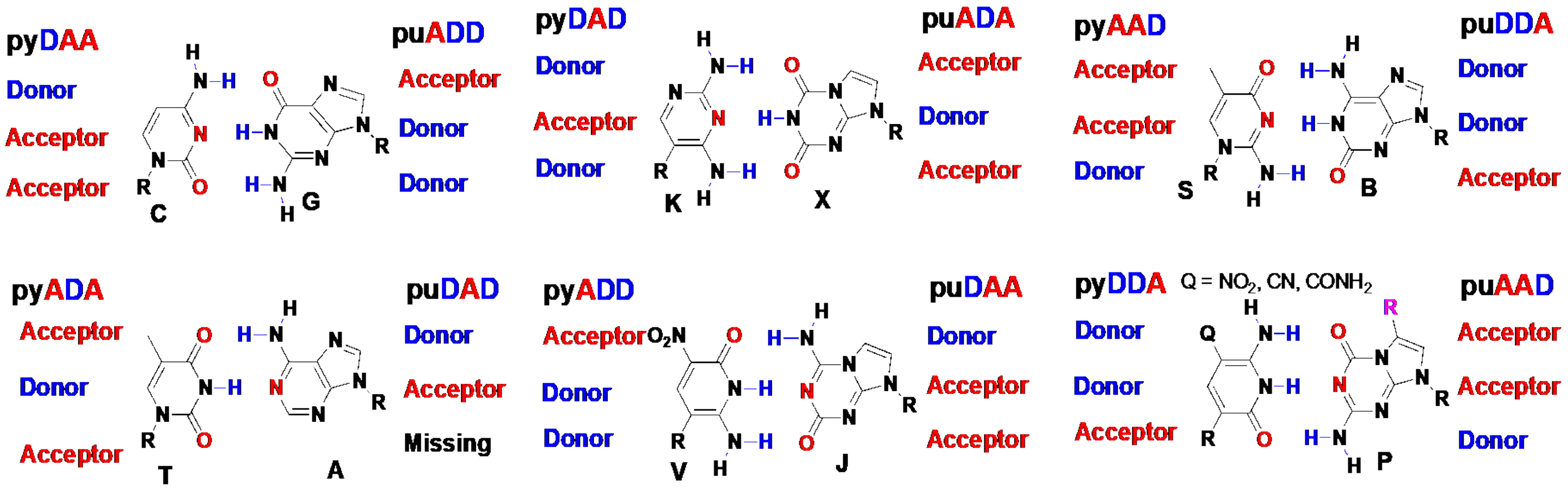
For example, Romesberg and Benner groups have made cells that replicate "alien DNA"

Zhang et al. (2017) A semi-synthetic organism that stores & retrieves increased genetic information. *Nature* **551**, 644.

The more distant the organism's molecular biology, the less likely it is to be a pathogen, predator, or prey.

Schmidt, M. (2010) Xenobiology: A new form of life as the ultimate biosafety tool. *Bioessays* 32, 322-331
Marliere, P. (2009). The farther, the safer: A manifesto for securely navigating synthetic species away from the old living world. *Systems & Synthetic Biology* **3**, 77-84.

- BARSOOM life adapted to the Viking site has no chance of infecting a human, or return infecting the Earth, or being extinguished by Earthlings.
- Analysis of ALF-concentrated polyelectrolytes will show how divergent Martians are.
- From this, risk is assessed.
- ALFs distributed around landing site assess spread of contaminants (compare Antarctica).



There is a dynamic range problem, finding indigenous biology in Matt Damon's poop.

Summary

The community must do a better job managing its culture

At the very least, people need to read the original papers

Misinterpretation of Viking, propagated for a half century, is an object lesson

Given the likelihood that extant life is everywhere, cannot wait

And we need more than yes/no. Need some molecular biology informaton

Polyelectrolyte Theory of the Gene gives a universal biosignature

In water, a handle to recover using electrodialysis

An Agnostic Life Finder (ALF) presently being developed with NIAC support

Low cost add on to ISRU water mining. Continuous presence to monitor spread

Given different architectures, different timing

If no ISRU in an all-up mission, must go with pre-arrivals to cache supplies

International Mars Prospecting Ride-Share System (IMPRESS) = inexpensive

Anthropogenic biologists have explored orthogonality for biosafety

Essentially no risk for predator/prey, extinction for surface Mars life

We must, however, manage signal/noise problem

…for further information

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