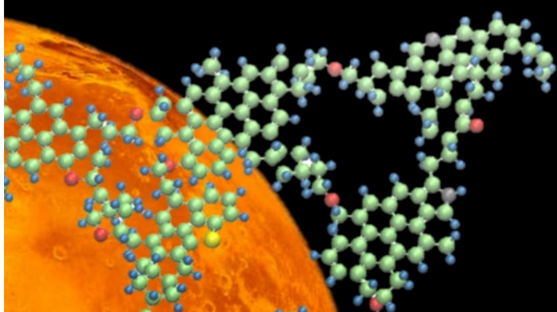
# Biosignatures & Life Detection Techniques

*(with a slight Mars tilt)*



September 19, 2022

Mary Beth Wilhelm
Space Science and Astrobiology Division
NASA Ames Research Center
Moffett Field, California

# Motivations for Life Detection Missions

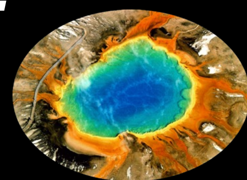
- **The search for life remains a key NASA science goal**
  - Extant & presently habitable
  - Ancient/preserved remains of life & previously habitable

- **Improve understanding of early prebiotic chemistry & link to early Earth**

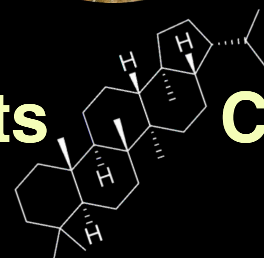
- **Understanding the carbon cycle**

# Searching for Indicators of Life

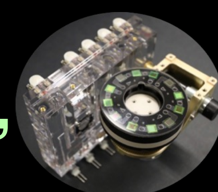

***Basic Understanding:***

Life's origins  Analog research

***Search Strategy:***

Diverse targets  Complementary measurements

***Tech Development:***

Collection, Processing,  Analysis instruments

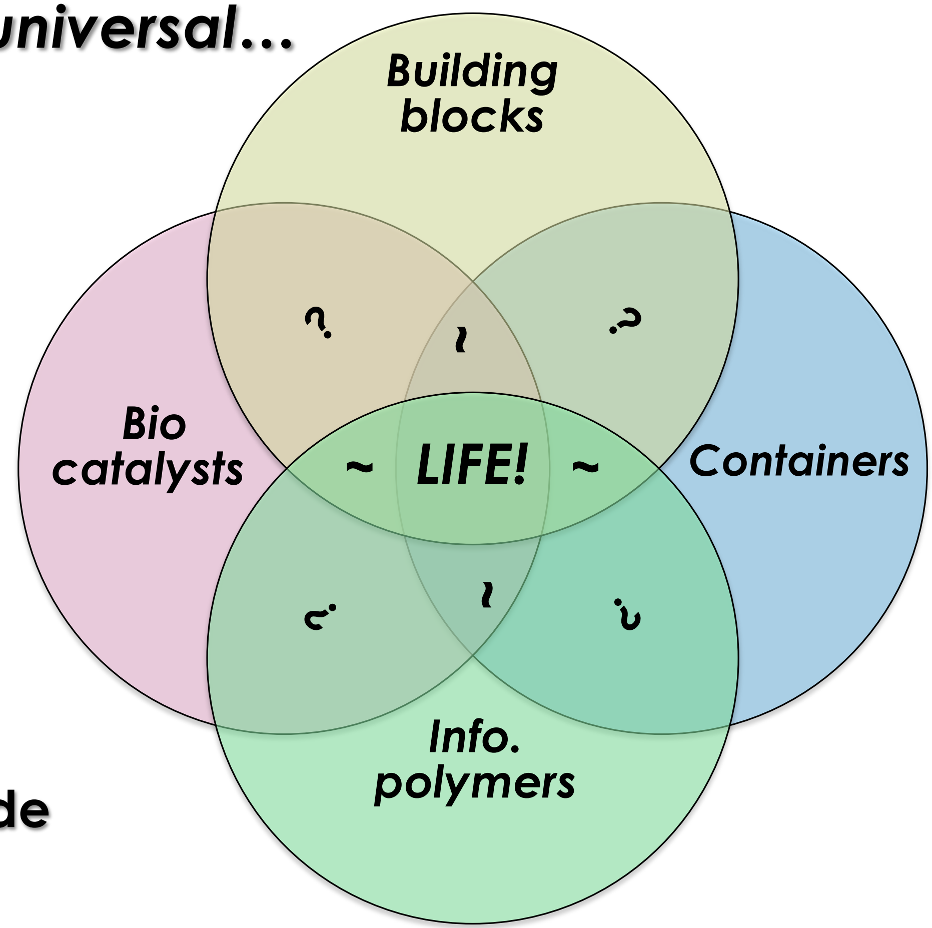
***Plan Missions:***

Choose destination  Develop concept 

# What should we look for?

***some aspects of life are likely to be universal…***

- Versatile chemical building blocks
- Complex multimeric biomolecules
- Containment structures
- Function-specific molecules




**Arguably, all are required for life**

- **Combined, these indicators could provide compelling evidence of life**

# Targets, Rationale, and Instruments for Life Search

| Measurement Target | Observed Parameter | Life Detection Rationale *examples* | Analytical Approach |
|---|---|---|---|
| **Molecular building blocks** | Chirality | **Enantiomeric excess** enables biochemistry<br>*amino acids, saccharides* | *Capillary Electrophoresis*<br>*Mass Spec* |
| **Functional molecules** | Catalysis | **Biochemical processes; electron transfer**<br>*kinases; quinones* | *Electrochemical BioSensors*<br>*Mass Spec* |
| **Biogenic organic polymers** | 'Structural' polymers | **Containers, energy, biochemistry**<br>*lipids* | *Mass spec*<br>*Capillary Electrophoresis* |
| | 'Information' polymers | **Information storage and transfer**<br>*poly nucleic acids* | *Sequencing*<br>*Mass Spec* |
| **Containers** | Morphology | **Containers, structures, barriers**<br>*cells, membranes* | *Fluorescence Microscopy with staining* |

# “Standards of Evidence” for Life Detection

From the 2021 “White Paper Report from the Biosignatures Standards of Evidence Community Workshop” produced by the NFOLD Network for Life Detection

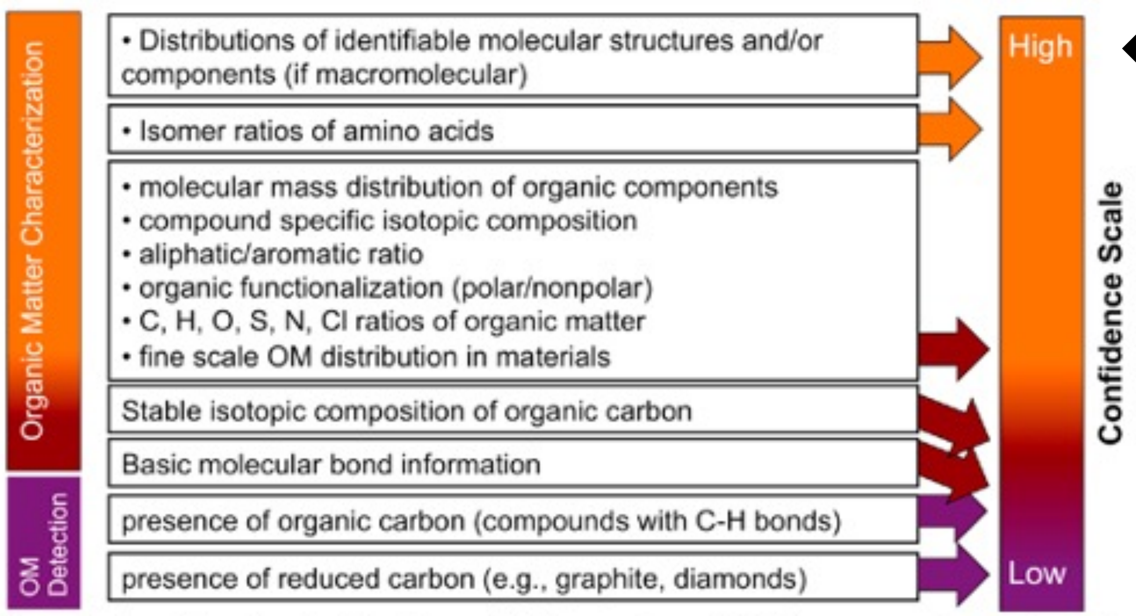
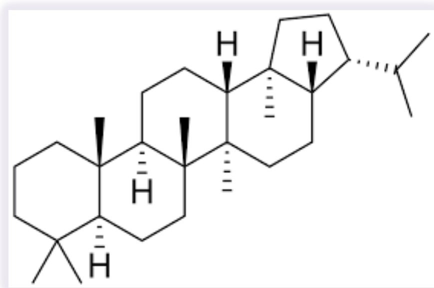



**Figure 5. Organic molecule detection methods are not as definitive as some types of organic matter characterization.** Confidence of detecting a definitive biosignature for various observation types for organic matter. Observation types (central column) in order of certainty that the observation could yield a definitive biosignature (right column). Left column denotes general type of observation: detection vs. characterization. (Adapted from Mustard et al., 2013).

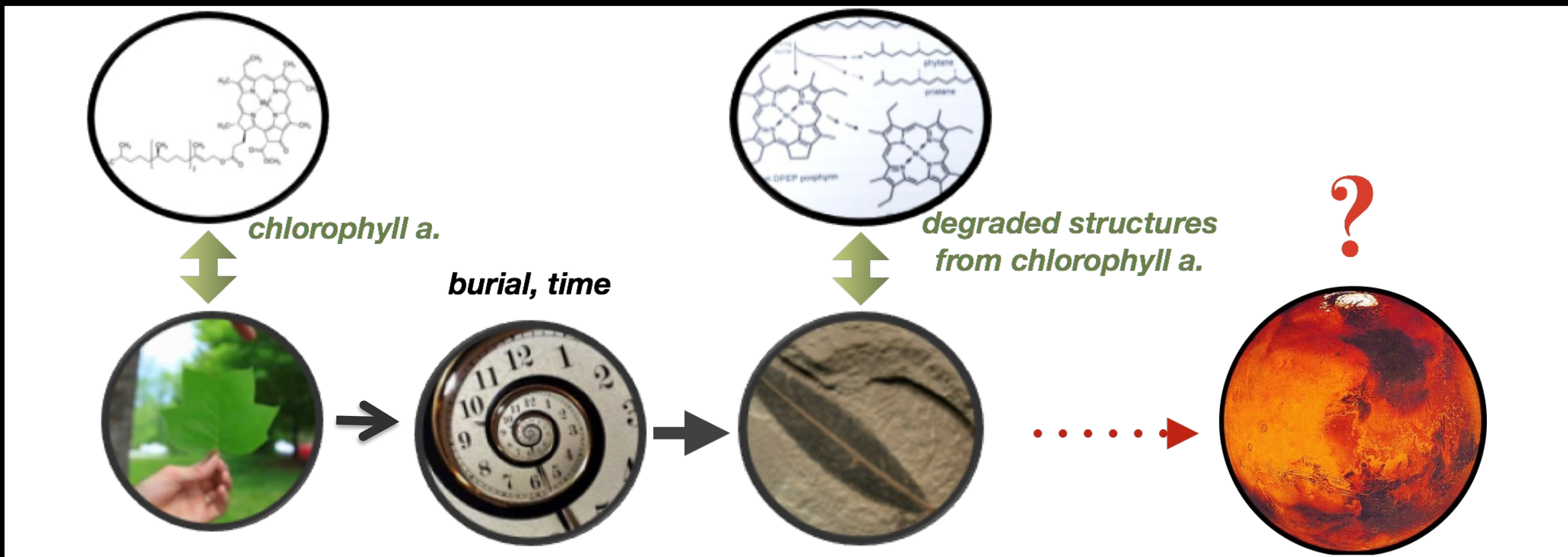
Ideally, we want to make measurements across this spectrum, prioritizing the highest-confidence measurements.

# Biomarkers

Biomarkers are organic compounds that retain **biological heritage** in their molecular structure through geologic time *(Meyers and Ishiwatari, 1993)*.



# How might these processes work on other bodies?

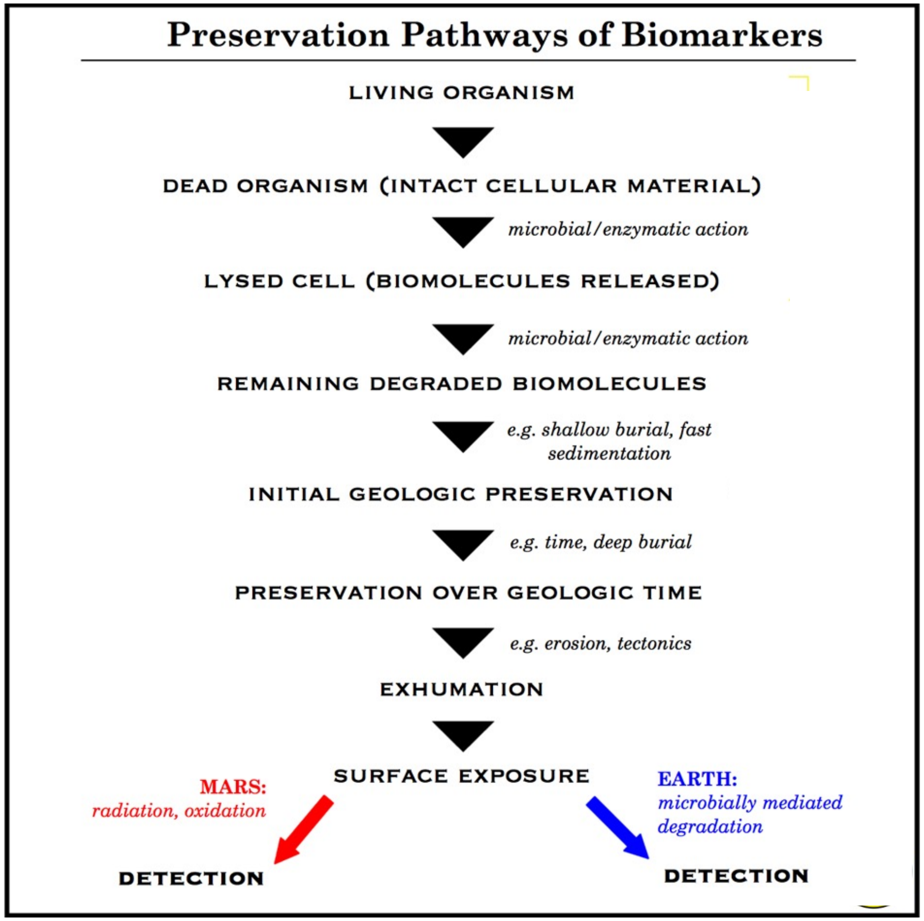


# Taphonomic Processes on Earth

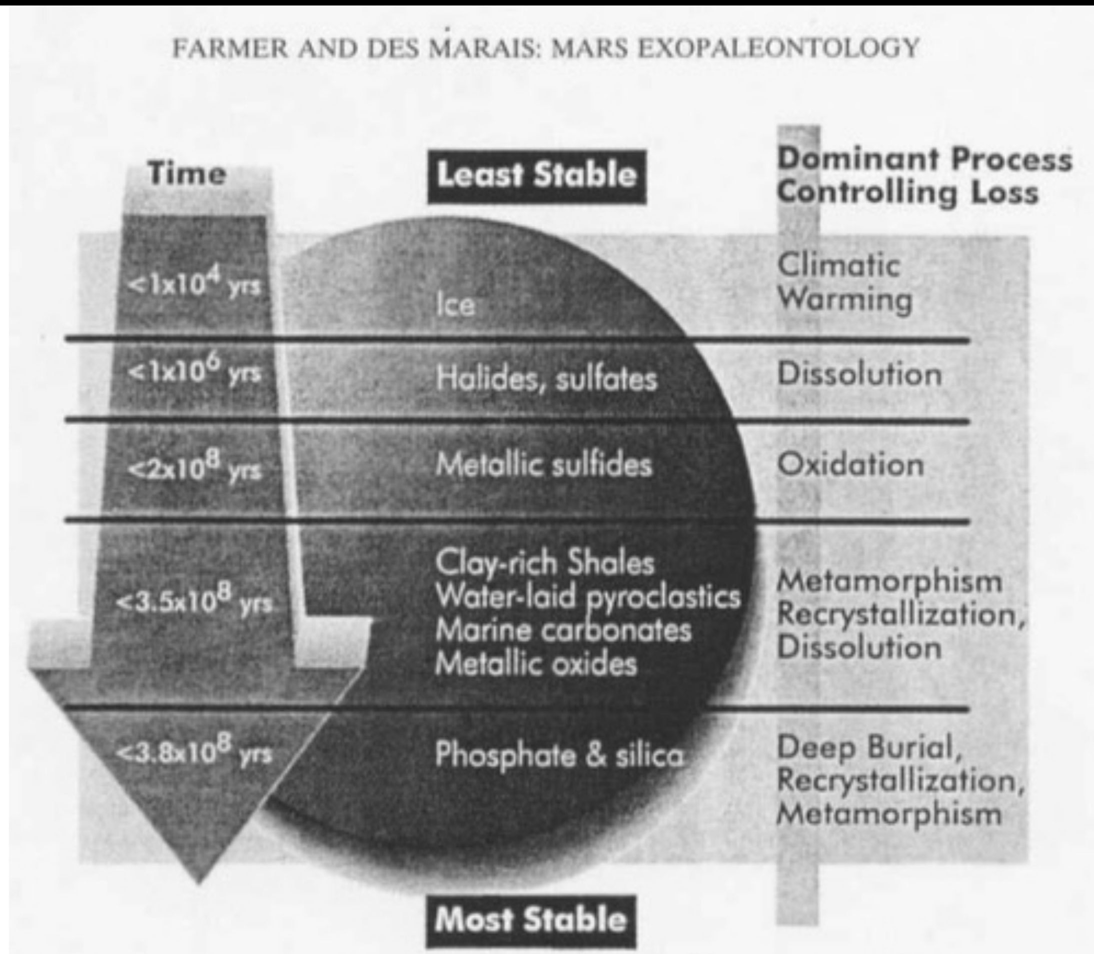
- Microbial processes mediate the majority of organic decomposition on Earth (>99.9% recycled;
  *Des Marais, 2001*).

- Initial preservation of biomarkers dictates long-term survival

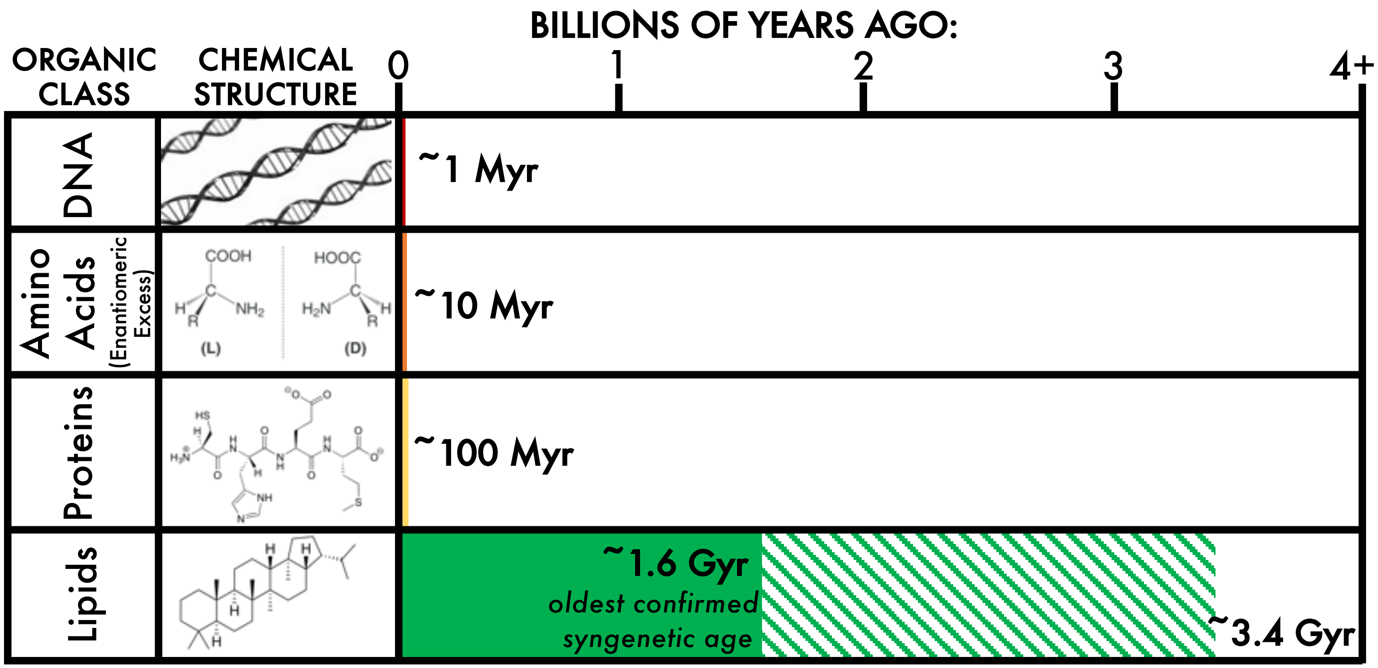
- Most of what we know about biomarker preservation is from aquatic environments.



# Stability of Geologic Deposits

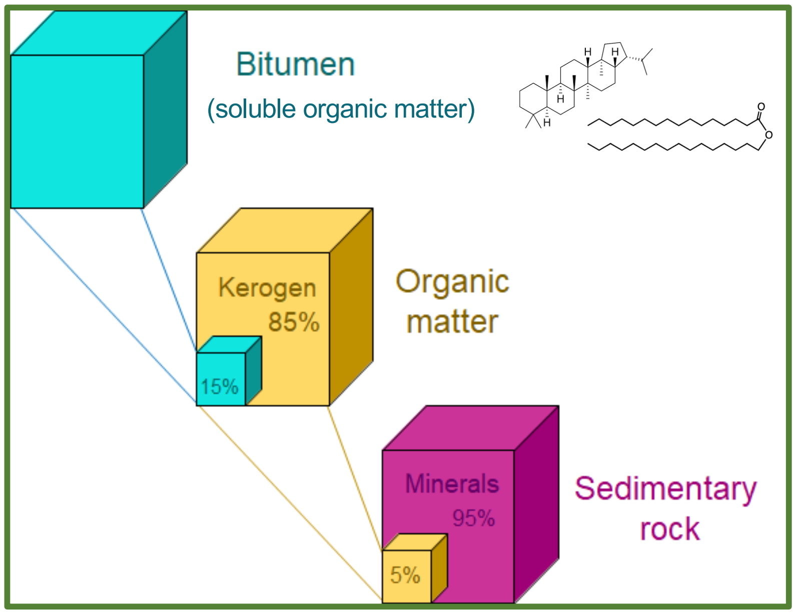


# Geological Longevity of Biomarkers



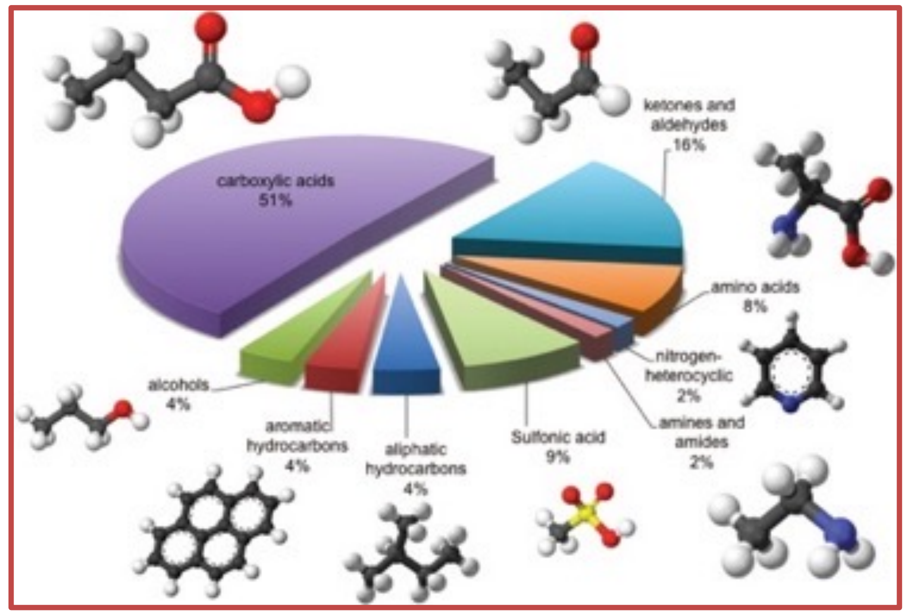
# Organic Content in Biotic & Abiotic Settings

## BIOTIC

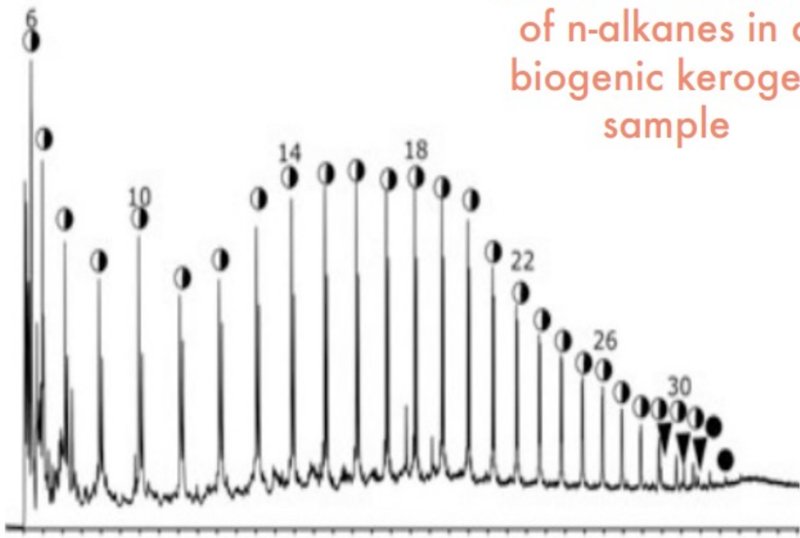



Courtesy of Carina Lee

## ABIOTIC

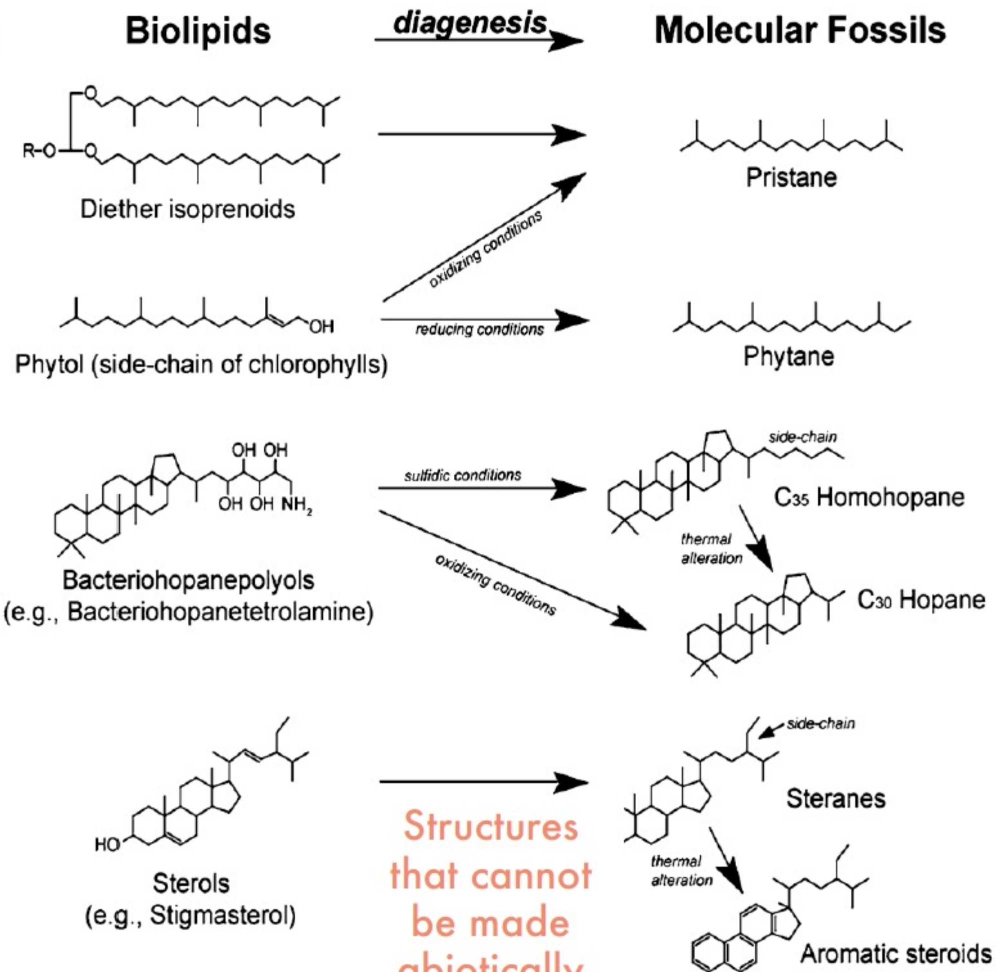



Remusat (2014)




Normal distribution of n-alkanes in a biogenic kerogen sample

Distributions of key molecular features or specific molecular structures are unique to biolipids. These features are maintained through diagenesis and preserved in the geologic record.




Structures that cannot be made abiotically

*Courtesy Allie Fox*

# Early Thinking in Biosignature Detection

## A PHYSICAL BASIS FOR LIFE DETECTION EXPERIMENTS

By Dr. J. E. LOVELOCK
Bowerchalke, Nr. Salisbury, Wiltshire

THE design of an efficient and unequivocal experiment in extra-terrestrial life detection should take into account: (1) A definition of life stated in terms favourable for its recognition. (2) A description of the past and present environment of the planet to be sampled.

As yet, there is no formal physical statement to describe life from which an exclusive definition for experimental purposes could be drawn. Moreover no comprehensive description is available of the atmospheric as well as the surface physical and chemical environment of any of the planetary bodies.

It is not surprising, in view of the vast expense of space-probe experiments and of the formidable uncertainties already stated here, that the proposed experiments in life detection all ask the cautious geocentric question: "Is there life as we know it?" Most certainly it is difficult to envisage in detail an alien biochemistry; it would seem pointless and very uneconomic to send a space probe to detect a speculative life-form.

It is the object of this article to show that we are not necessarily limited to experiments based on the recognition of a specific life-form, either Earth-like or alien. Also, that it is possible, by accepting a limited phenomenological definition of life, to design simple experiments from the general recognition of life phenomena, including that with which we are familiar. The application of this approach to experiments in life detection is the basis of the discussion which follows.

differs from the other phenomena so classified in its singularity, persistence, and in the size of the entropy decrease associated with it. Vortices appear spontaneously but soon vanish; the entropy decrease associated with the formation of a vortex is small compared with energy flux. Life does not easily form, but persists indefinitely and vastly modifies its environment. The spontaneous generation of life, according to recent calculations from quantum mechanics[4,5], is extremely improbable. This is relevant to the present discussion through the implication that wherever life exists its biochemical form will be strongly determined by the initiating event. This in turn could vary with the planetary environment at the time of initiation.

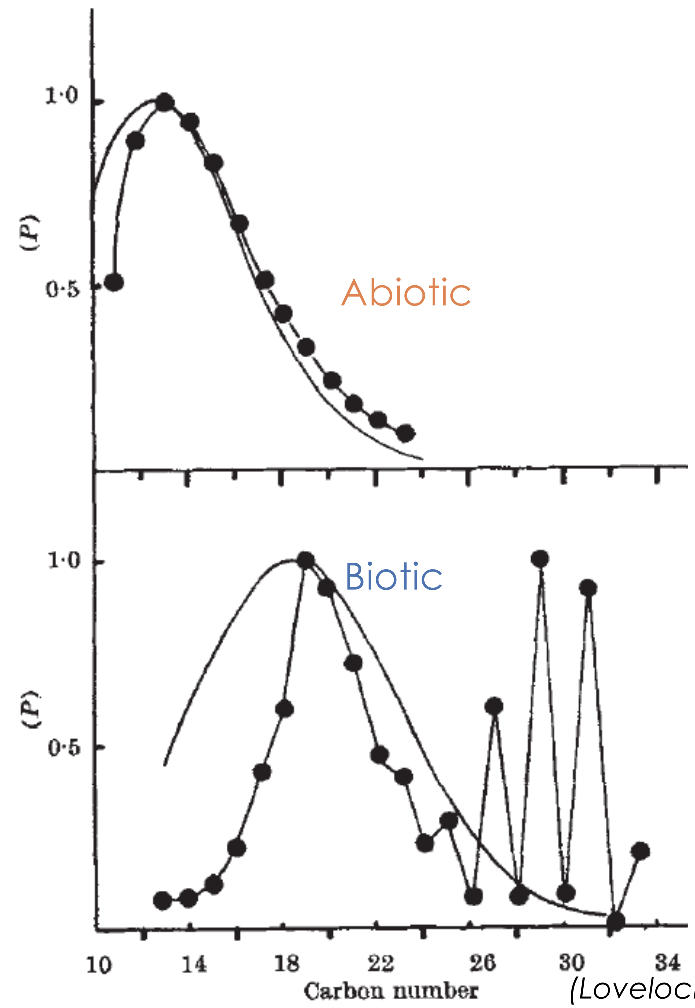
On the basis of the physical phenomenology already mentioned, a planet bearing life is distinguishable from a sterile one as follows: (1) The omnipresence of intense orderliness and of structures and of events utterly improbable on a basis of thermodynamic equilibrium. (2) Extreme departures from an inorganic steady-state equilibrium of chemical potential.

This orderliness and chemical disequilibrium would to a diminished but still recognizable extent be expected to penetrate into the planetary surface and its past history as fossils and as rocks of biological origin.

### Experiments for Detection of Life

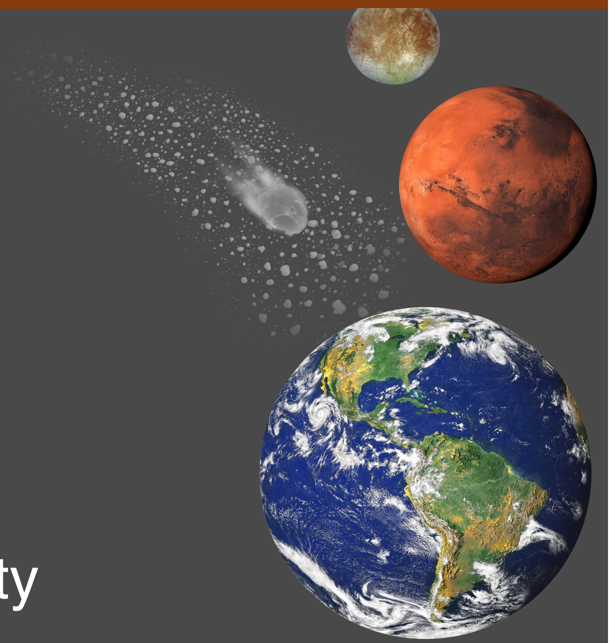
The distinguishing features of a life-bearing planet,

1919-2022




*(Lovelock, 1965)*

# “Origin-Diagnostic” Features and Patterns



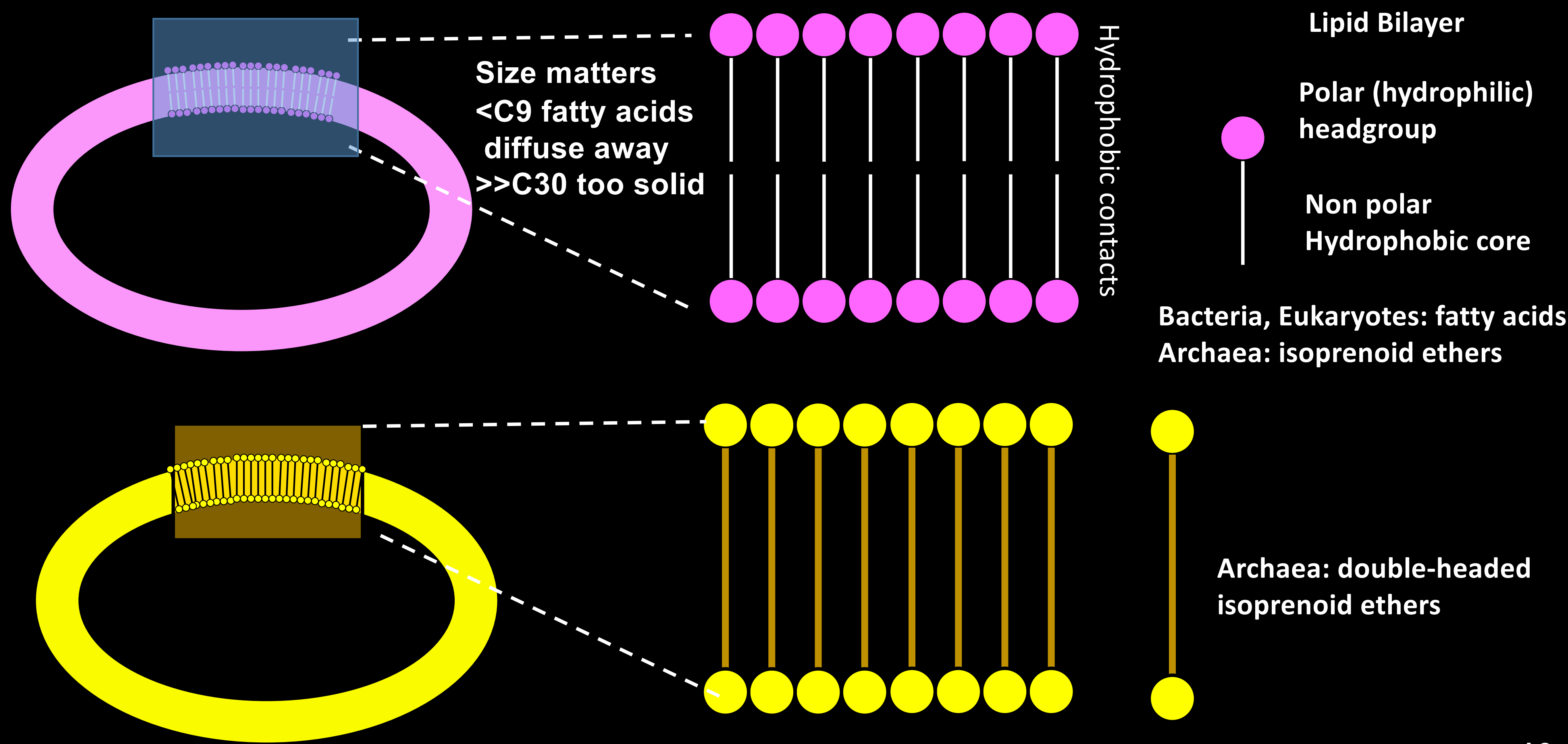
**Molecular form begets biological function:**

features reflect and enable membrane geometry

- Fatty acid chain lengths
- Polyunsaturations regulate membrane fluidity
- Polycyclic isoprenoids enhance structural stability

# Terrestrial cell membranes: bacteria and archaea

**Bilayers and monolayers**

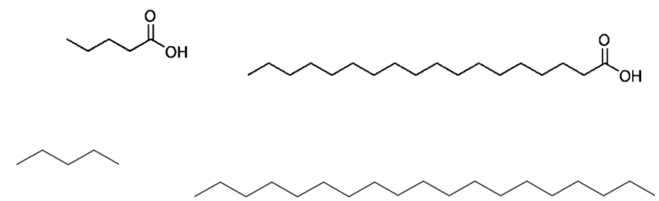



*Courtesy Mike Malaska*

# Origin Diagnostic Structural Features & Distributions

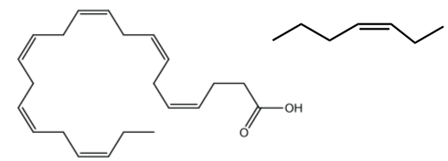
Review of ~1500 terrestrial & ~90 meteorite samples containing fatty acids

## 1. Chain length:



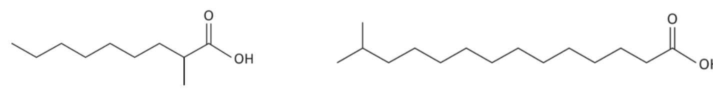

- Min-max (shortest-longest)
- Even vs odd preference
- Most abundant molecule
- 2nd most abundant molecule

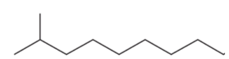
## 2. Unsaturations:

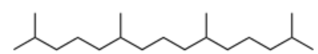


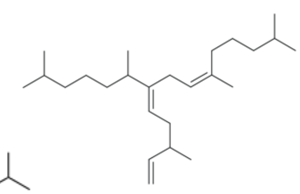

- Presence y/n
- Max # unsaturations in a molecule
- Most abundant unsaturated molecule

## 3. Branching:







- Presence y/n
- Min-max chain length containing a branch
- First-last branch position
- Max # branches in a molecule
- Max # carbons in a branch
- Most abundant branched molecule & conformation

(Buckner et al., in prep)

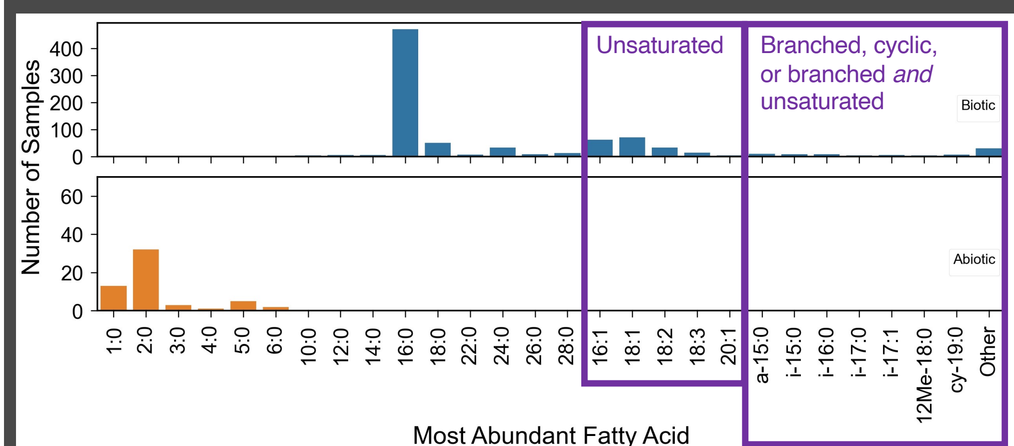
# Fatty Acid chain length distributions

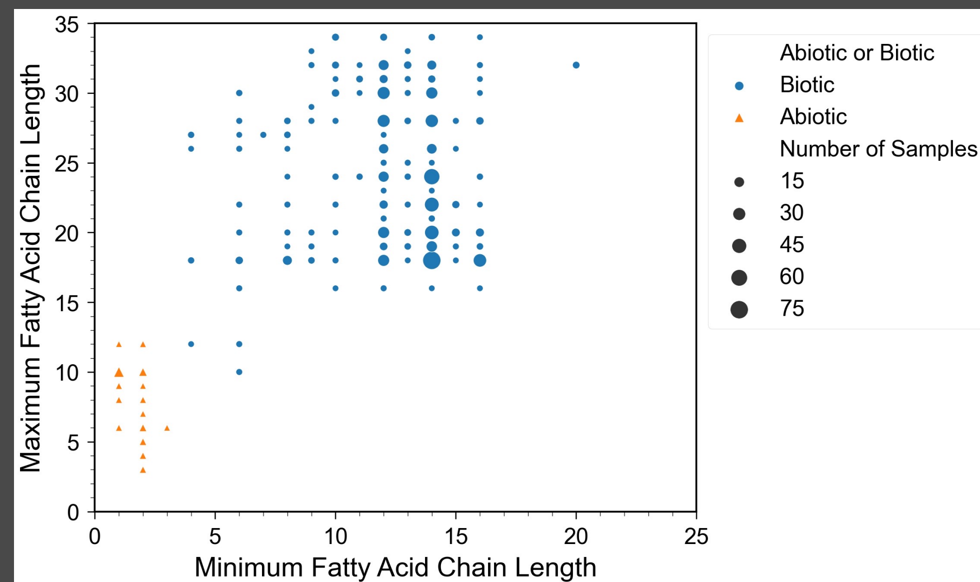
## Terrestrial fatty acids

- Longer chains (4-34 carbons)
- $C_{16:0}$ usually the most abundant molecule

## Meteoritic fatty acids

- Shorter chains (1-12 carbons)
- $C_{2:0}$ usually the most abundant molecule

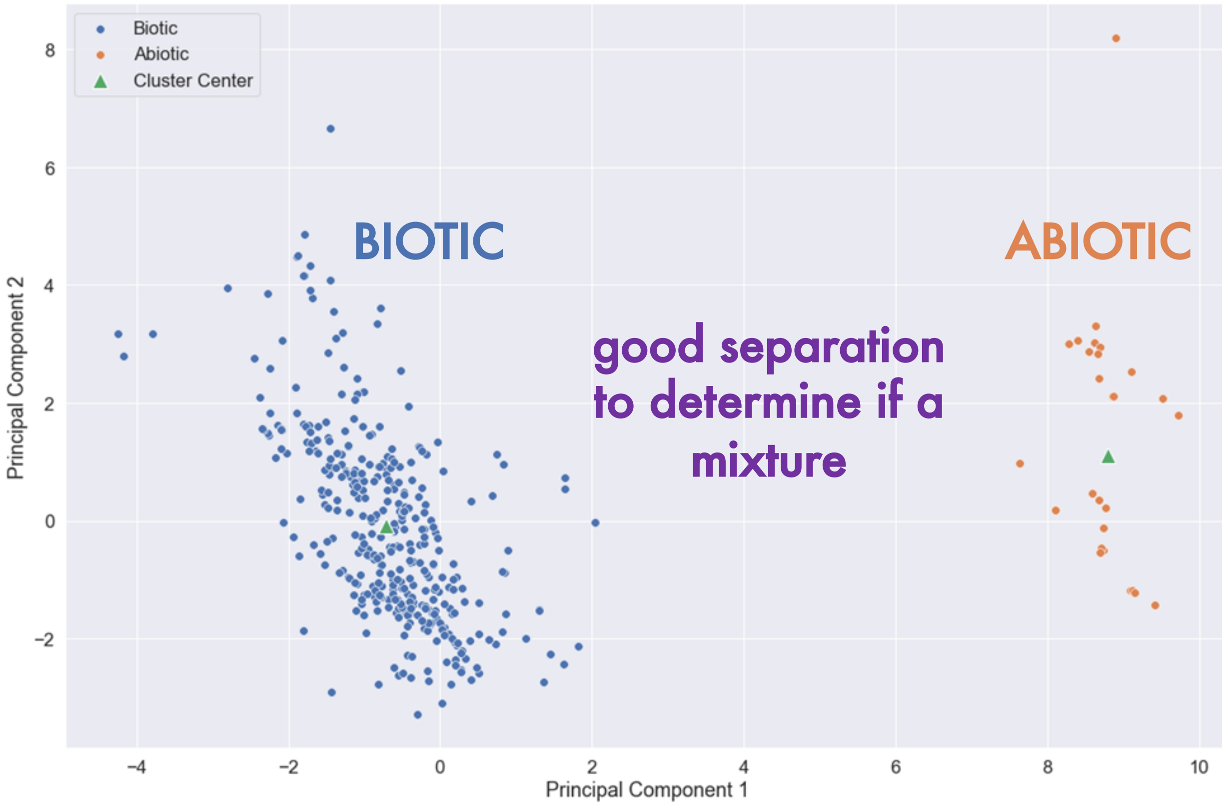



(Buckner et al., in prep)

# Molecular Measurements are Additive!

**Lipid features & patterns are <u>additive</u> in determining biotic vs. abiotic origin:**



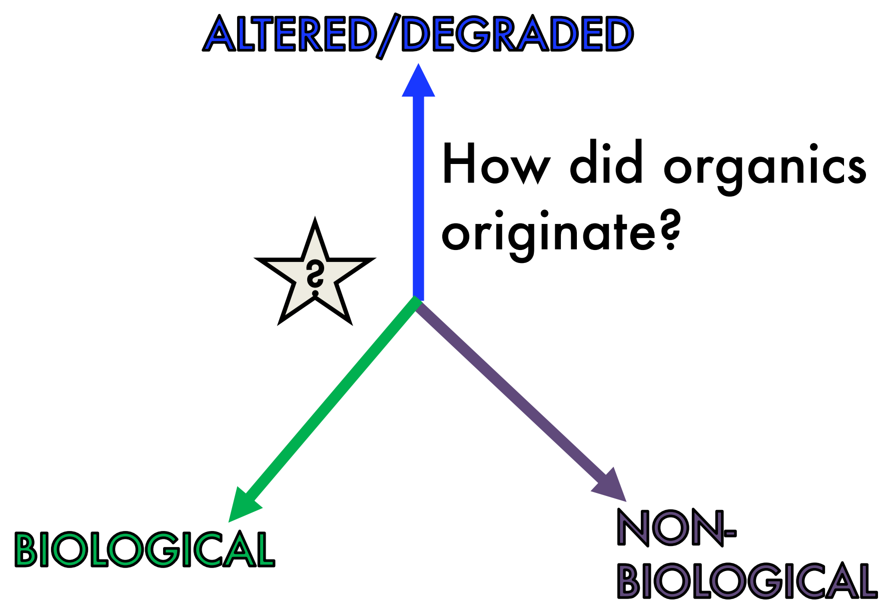

*14 diagnostic molecular patterns & features are represented in this principle component analysis*

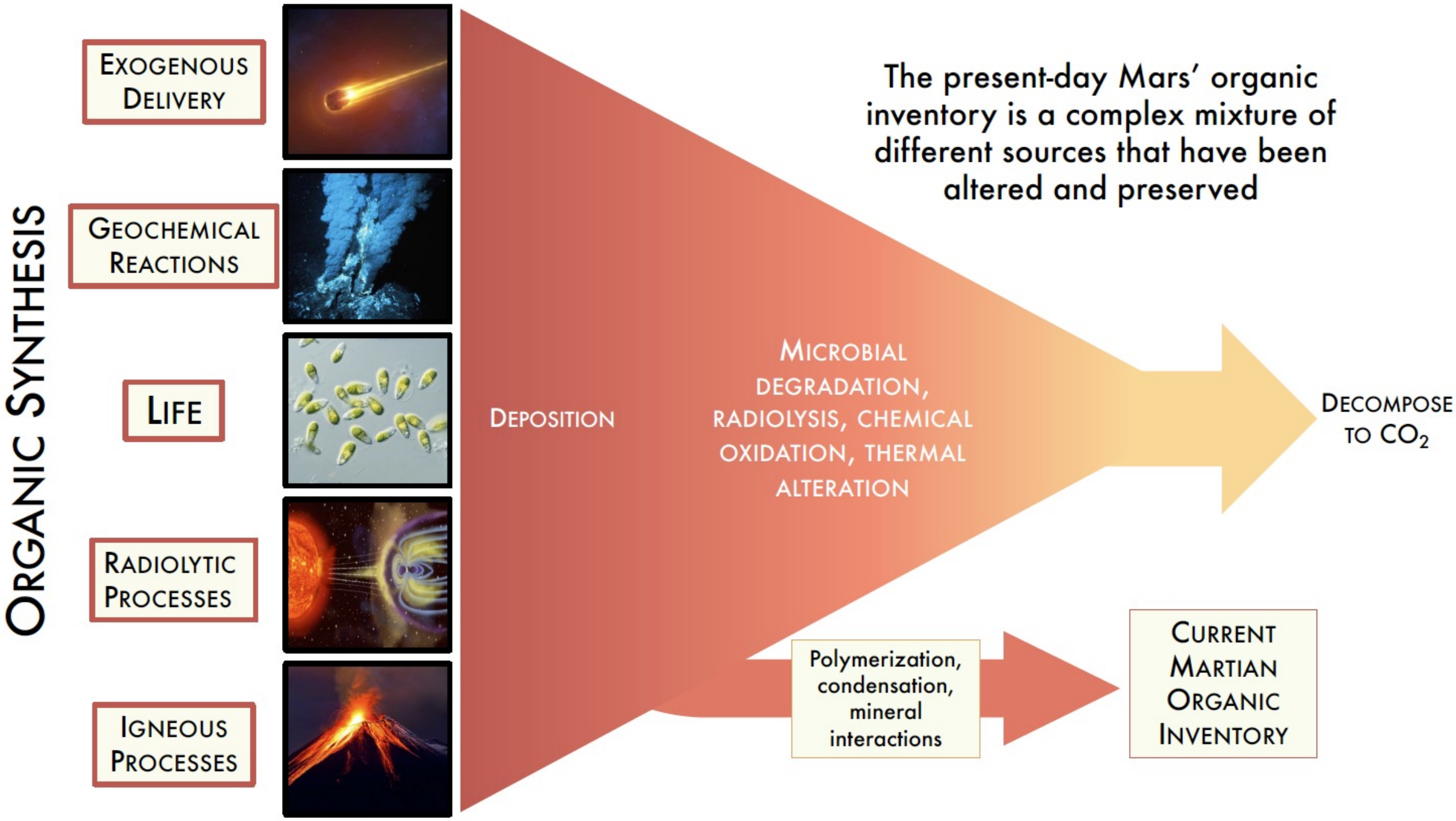
(Buckner et al., in prep)

Graphic credit: Walt Alvarado

# Life Detection is not a Binary

- Not a binary of biotic or abiotic
  1. Abiotic synthesis—multiple types
  2. Known or unknown biotic synthesis
  3. Altered/degraded signal (e.g., radiation, geological processes, oxidation)
- Measurement requirements should cover potential pools
- **Abiotic "baseline" needs to be defined for a given body**






*Courtesy Allie Fox*

# Additional Considerations in Analysis of Organics

1. **Inorganic Interference**
   - Oxidants, salts, sulfurous minerals

2. **Geologic Heterogeneity of Biomarkers**
   - Data from deserts on Earth indicate heterogeneity of biomarker distribution is on the cm-scale

3. **Organic Material is Complex**
   - Organics can be bound inside minerals
   - Keil and Mayer, 2014

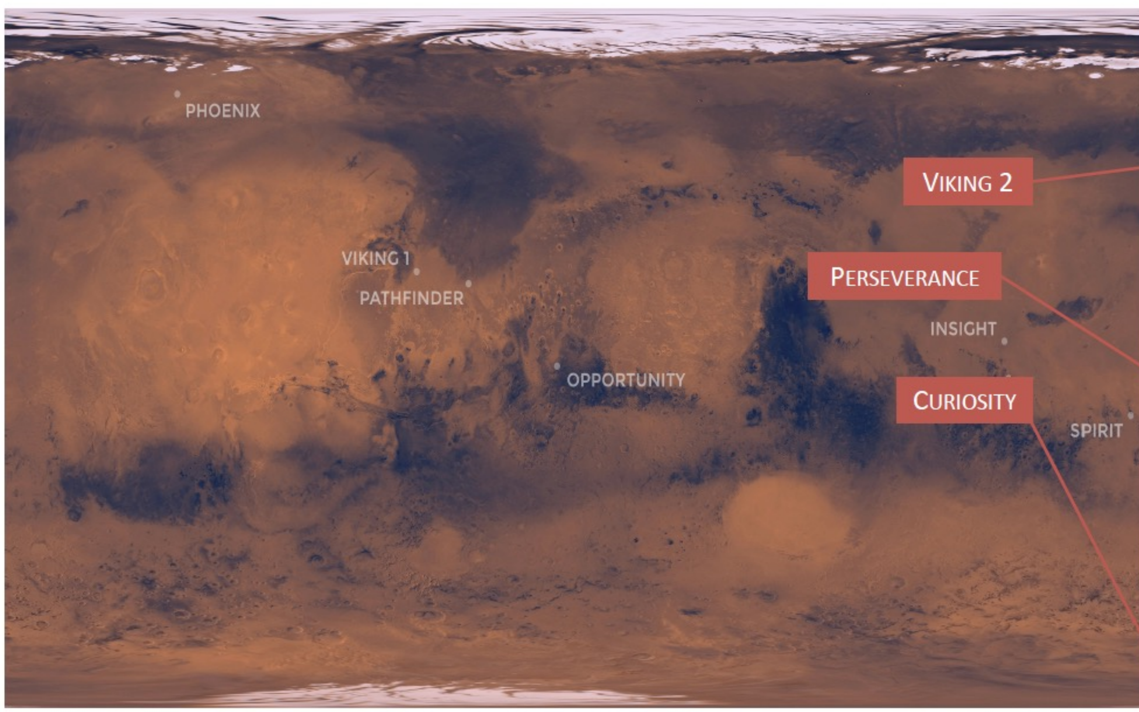
# Low Limit of Detection is Key

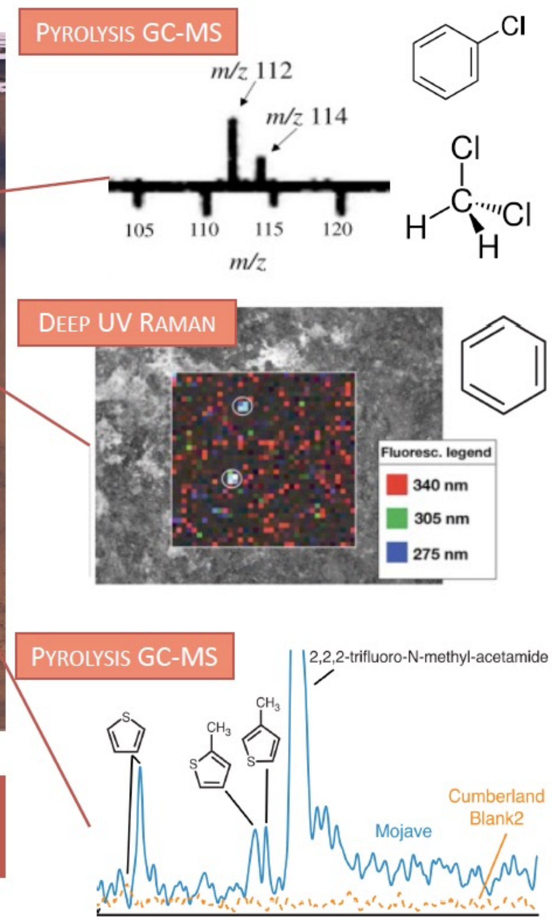
- **Detection of overall organics present not enough —**

**<u>Need to see molecules low in abundance</u>**

| | Viking | MSL (SAM) | MSR |
|---|---|---|---|
| **Year** | 1976 | 2012 | 2036 |
| **Depth** | 0 cm | ≤5 cm | ≤7 cm |
| **Sample Volume** | 0.1 cc | 0.08 cc | 8 cc /tube |
| **Analytical Instrument LoD** | $10^{-8}$ mol | $10^{-12}$ mol | TBD |
| | $10^{-7}$ mol/cc | $10^{-11}$ mol/cc | TBD |

# Quality of Molecular Information is Critical



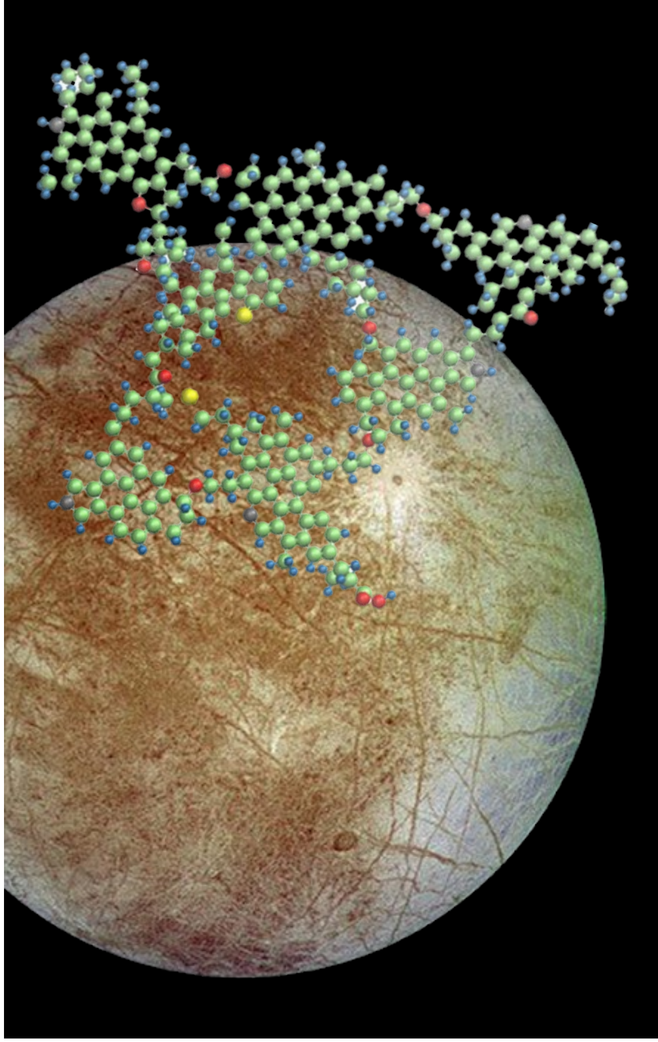

Previous missions to Mars have only detected small, simple organic material that are not diagnostic of their origins.

*Courtesy Allie Fox*

# Lab Approach to Biomarker Analysis

- High extraction efficiency, maintains structural integrity of molecules
- Overcomes analytical challenges associated with low-biomass, mineral interference, organic complexity
- Demonstrated on >>100,000 terrestrial samples
- Many steps
- employ large volumes of solvents, samples, consumables

# Organic Extraction from Icy Moon Samples will be Challenging



An ideal instrument design would be capable of extracting and purifying organics from an ice sample:

- Without modifying origin-diagnostic molecular characteristics
- Removing inorganics that would potentially interfere with analytisis(sulfur, salts, etc).
- Increasing the signal of the organics to increase the likelihood of detection

**Technology focused on *Liquid Processing Systems* integrated with:**

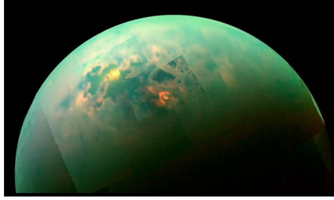
1) Chemical and Environmental Sensors
2) Optical Spectroscopy and Microscopy
3) Microfabricated Devices for Chemical Analysis

**Technology Lineage and Heritage:**

1) CubeSat Astrobiology and Space Biology Missions
2) Mars Exploration
3) Human Health, Performance and Safety Instrumentation

**Current and Emerging Applications:**

1) Astrobiology Life Detection Missions
2) Gateway Science Payloads and Environmental Monitoring
3) Robotic and Human Lunar Exploration



# Tradespace Brainstorm

### ASTROBIO RELEVANT MOTIVATIONS
- Modern habitability assessment
- Search for ancient preserved remains

### POTENTIAL MEASUREMENTS
- Variability of astrobio-relevant gases
- Organics in regolith
- Liquid water variability, salts, redox energetics

### ARCHITECTURES
- Impactor
- Drones
- Single lander, small payload
- Many landers, even smaller payloads
- Impact craters
- Buildable missions
- Scout mission for bigger, more expensive life detection mission
- Mole with sensors
- Pyrotechnics

### SMALL INSTRUMENTS
- Sample handling
- Fluorescence (label addition)
- SERS
- LD-MS
- GC-MS
- CE-MS
- CE-LIF
- Micro-spot XPS/Auger
- nSIMS
- Atomic absorption microscopy
- CNT gas sensors other Chemical Gas Sensors
- Pyrolysis augmented CNTs
- Electrochemical sensors for ions
- Electrochemical sensors for TOC, other organics
- Raman
- Micro LIBS TLS

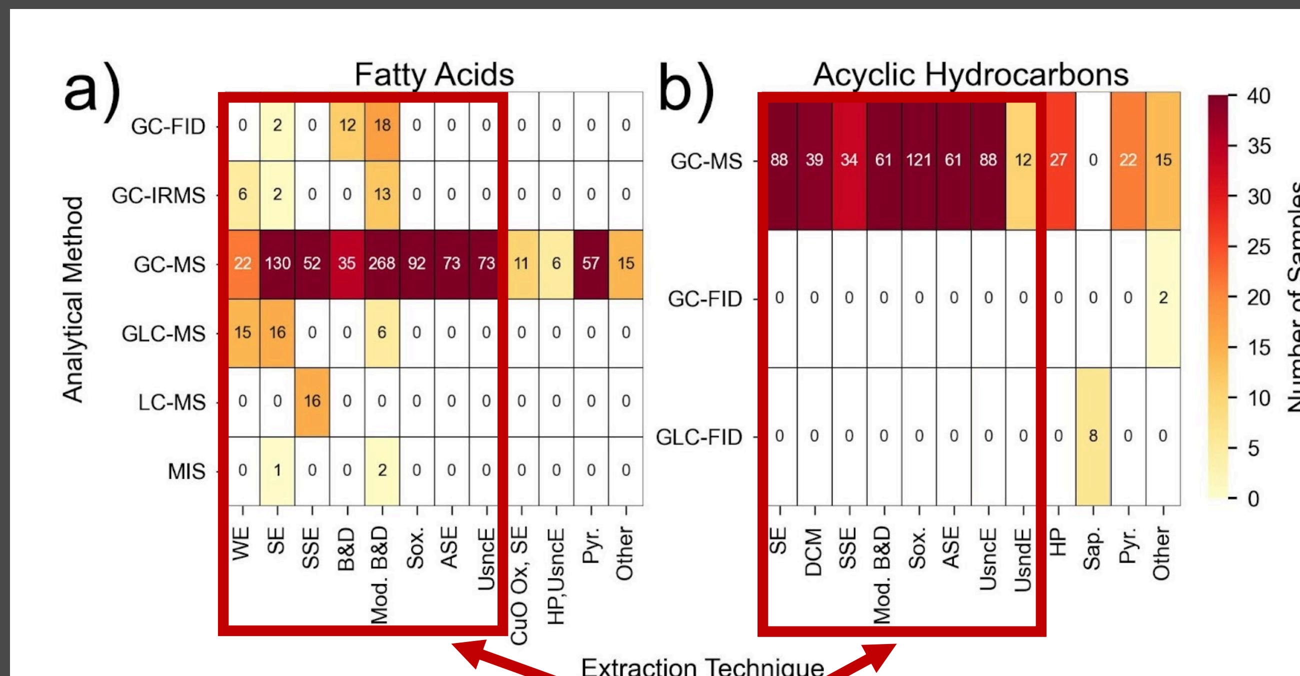
### OTHER CONSIDERATIONS
- Planetary Protection

# BACKUP

# Trends in Lipid Detection Techniques

Solvent extraction preserves molecular features that can indicate origin

MS can characterize structures that indicate origin



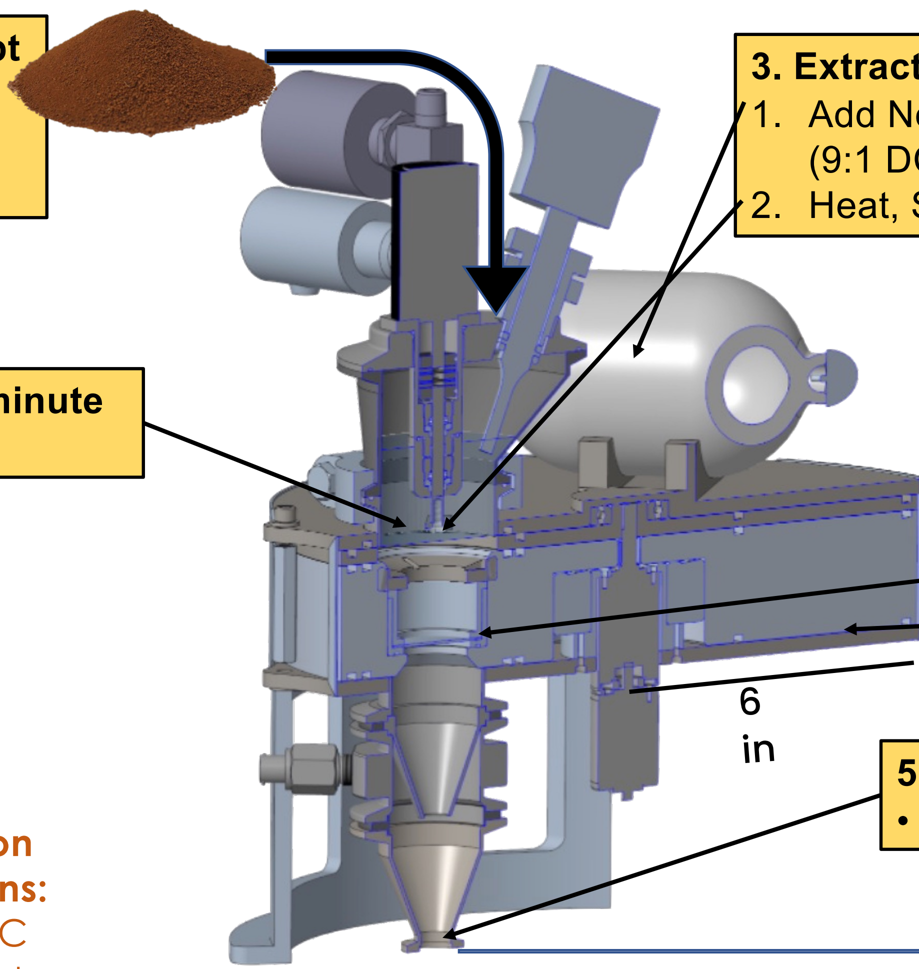

Buckner et al., in prep

*Solvent-based techniques*

# ExCALiBR ConOps Enabling Analysis with Preservation of Origin-Diagnostic Features for Martian Samples

Ames Research Center

**1. Accept Sample**
25 cc regolith




**2. Comminute Sample**

**3. Extract Lipids:**
1. Add Non-Aqueous Solvents (9:1 DCM:MeOH)
2. Heat, Sonicate, Mix

**4. Filter Out Minerals**
- Filter ~ 0.2 µm
- Carousel (5 filters)

**5. Concentrate Extract**
- To dryness

**Working extraction conditions:**
0 – 110 °C
0.01 – 10 bar

**Analytical Measurements**

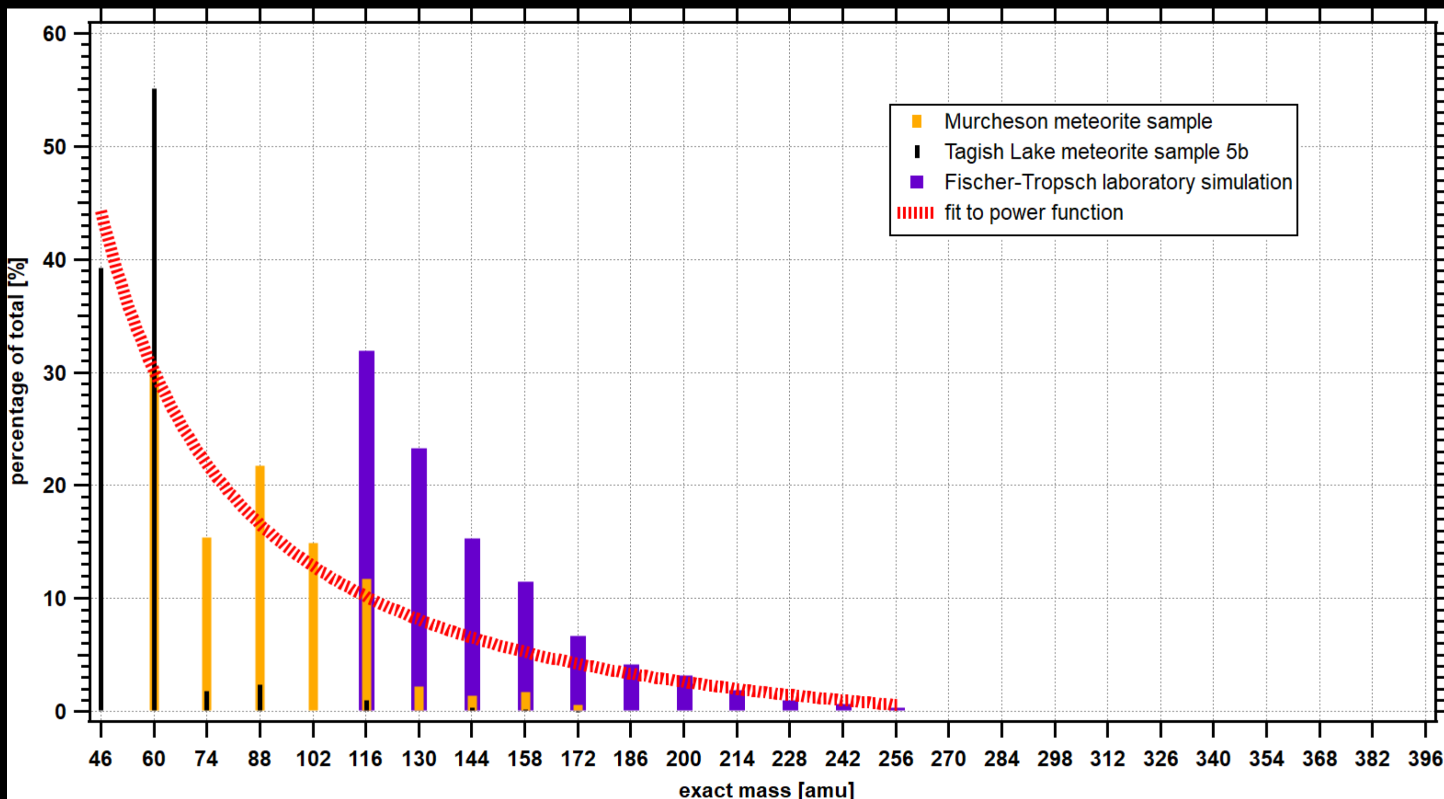
**SERS**
**Presence & abundance of lipid classes and specific chemical functionalities**

**MS**
**Molecular mass & relative amounts of lipids & fragments; fragmentation patterns**

**Analyze "Total Lipid Extract"**

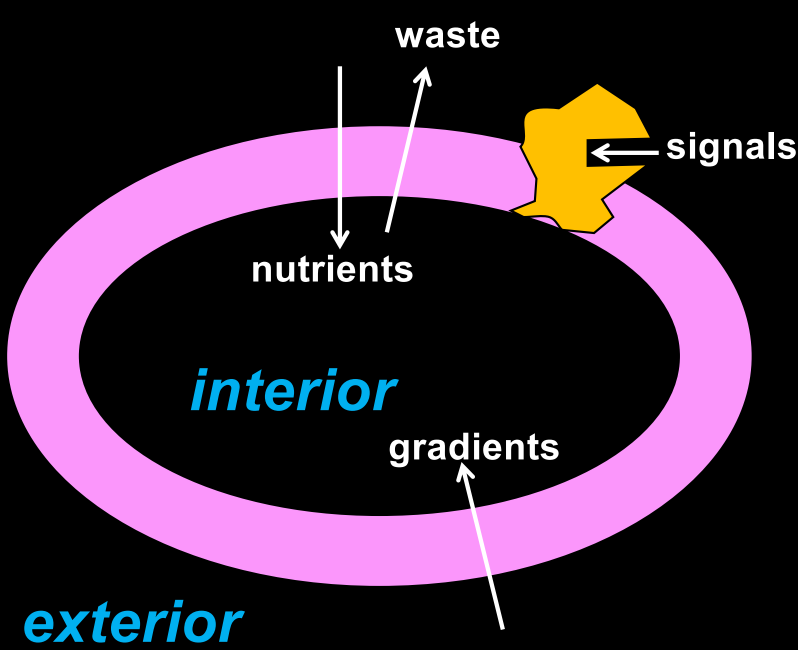
# Exponential decay in abiotic fatty acid synthesis

**Abiotic synthesis decreases with length**
**Short molecules can’t form membranes**




*Courtesy Mike Malaska*

# Membranes define cells

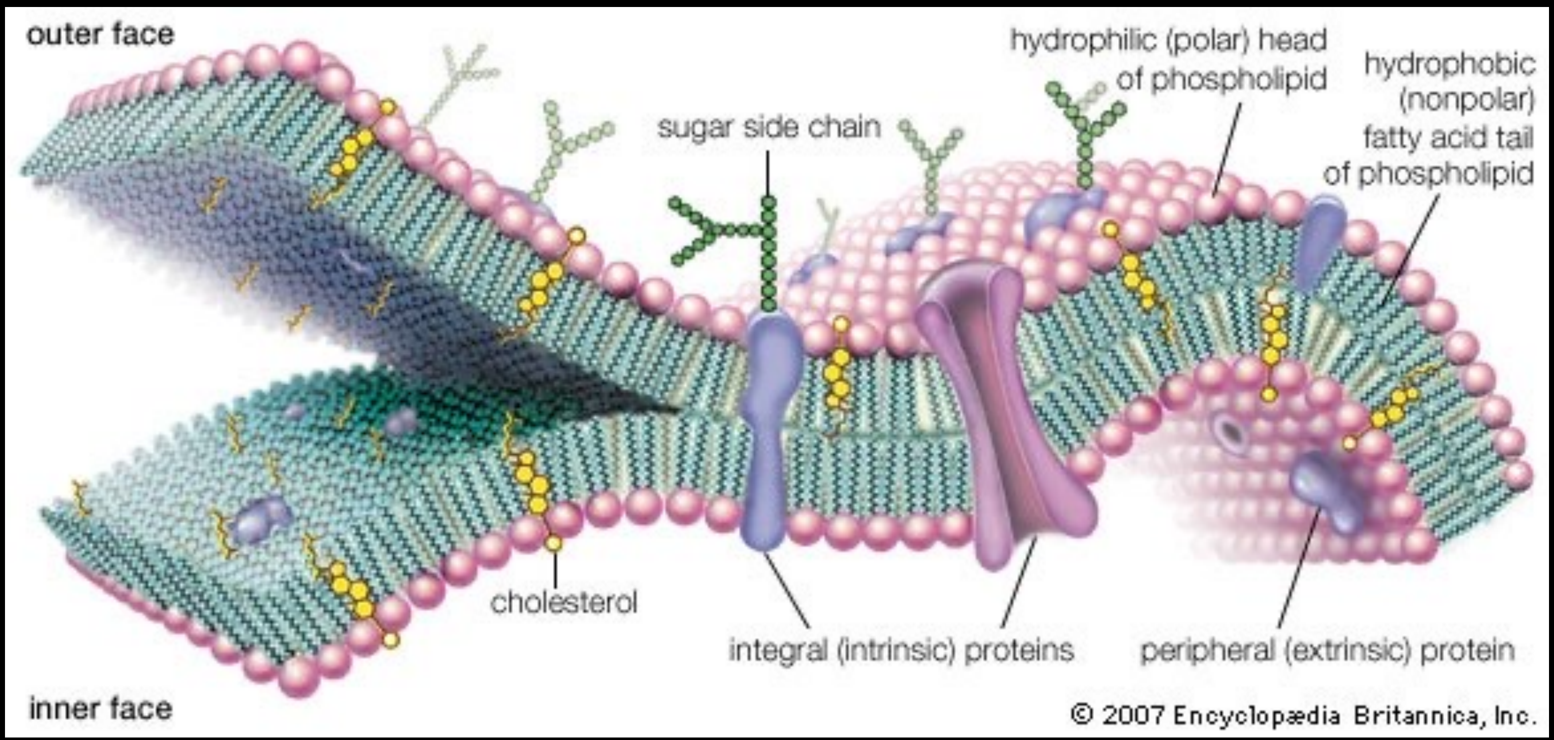



**Bulk average membrane requirements:**

- Be surrounded by outer aqueous environment
- Encapsulate inner aqueous environment
- Be sufficiently fluid on average
- Allow some molecules to diffuse across (gases, limited water)
- Impermeable to key ions (first $Na^+$, later $H^+$)
- Not allow molecules to diffuse or wander away from membrane construct
- Adjustable for different temperatures and ionic conditions
- Support transmembrane constructs (porins, transporters, receptors, etc.)

*Courtesy Mike Malaska*

# Bilayer plasma membrane detail




**archaeal membranes have a monolayer; use GDGTs not fatty acids**

**Image source: https://www.britannica.com/science/cell-membrane#/media/1/463558/4555**

*Courtesy Mike Malaska*

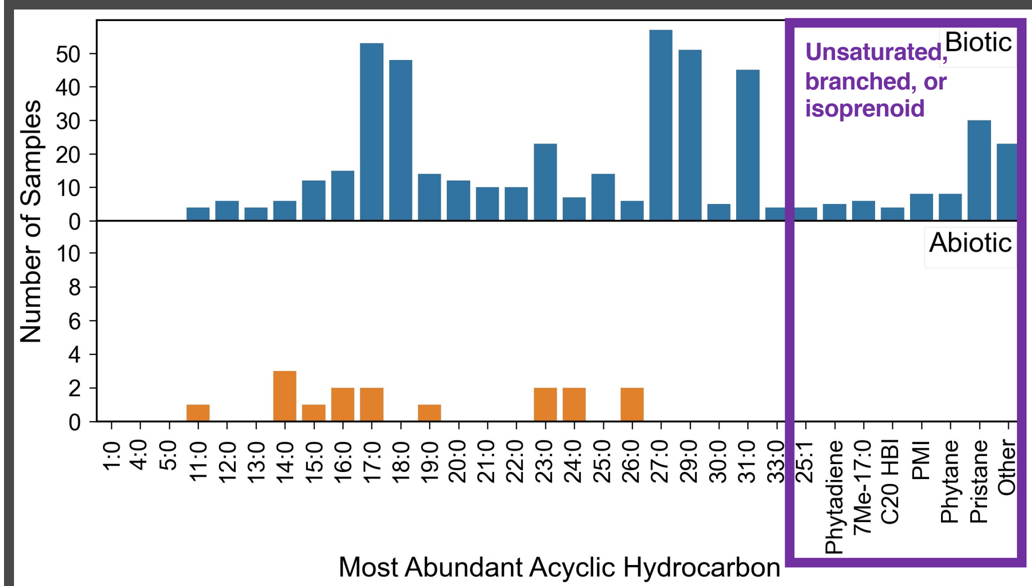
# Acyclic hydrocarbon chain length distributions

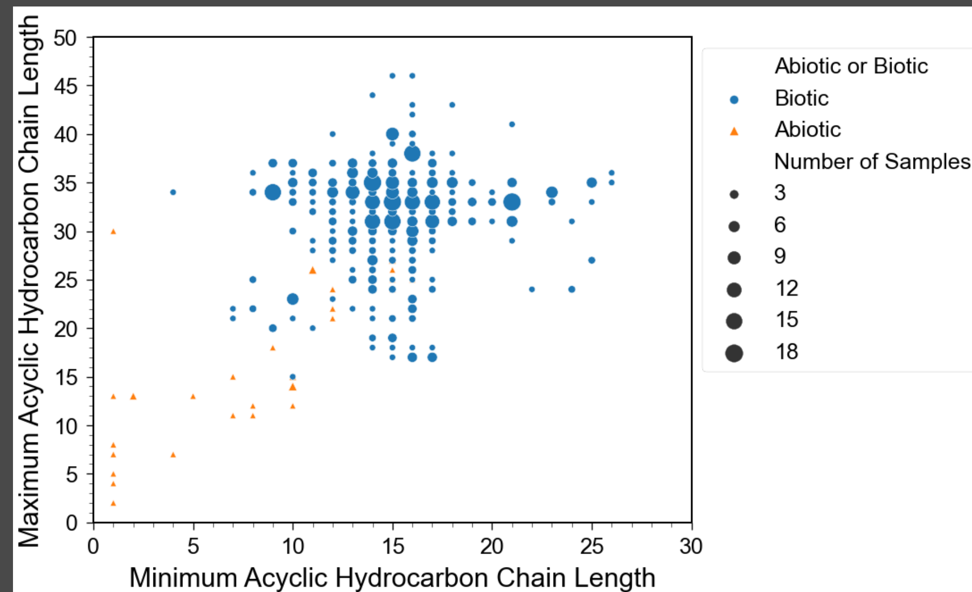
**Terrestrial acyclic hydrocarbons**

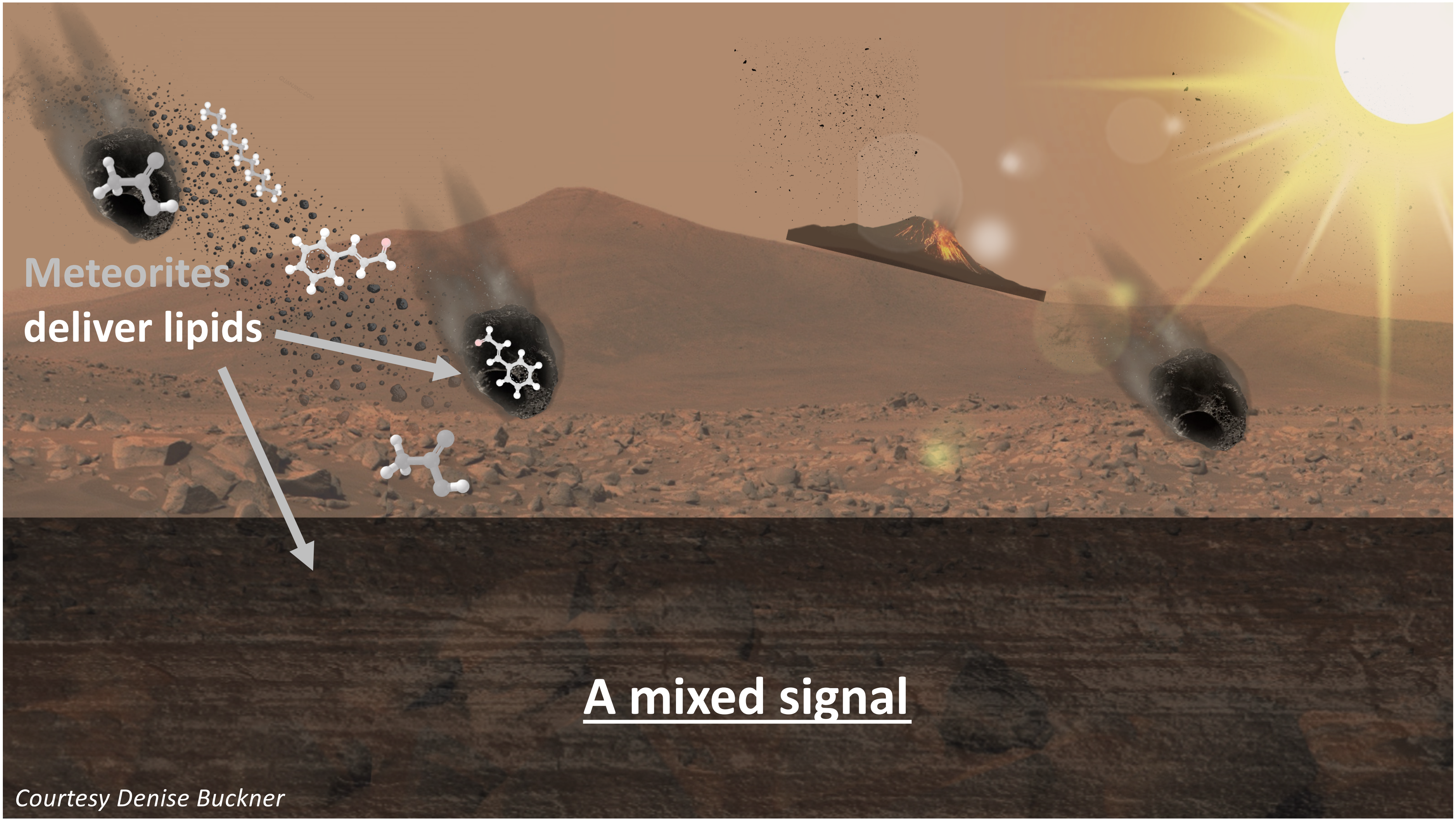
- Longer chains (4-46 carbons)
- $C_{27:0}$ or $C_{17:0}$ usually the most abundant molecule

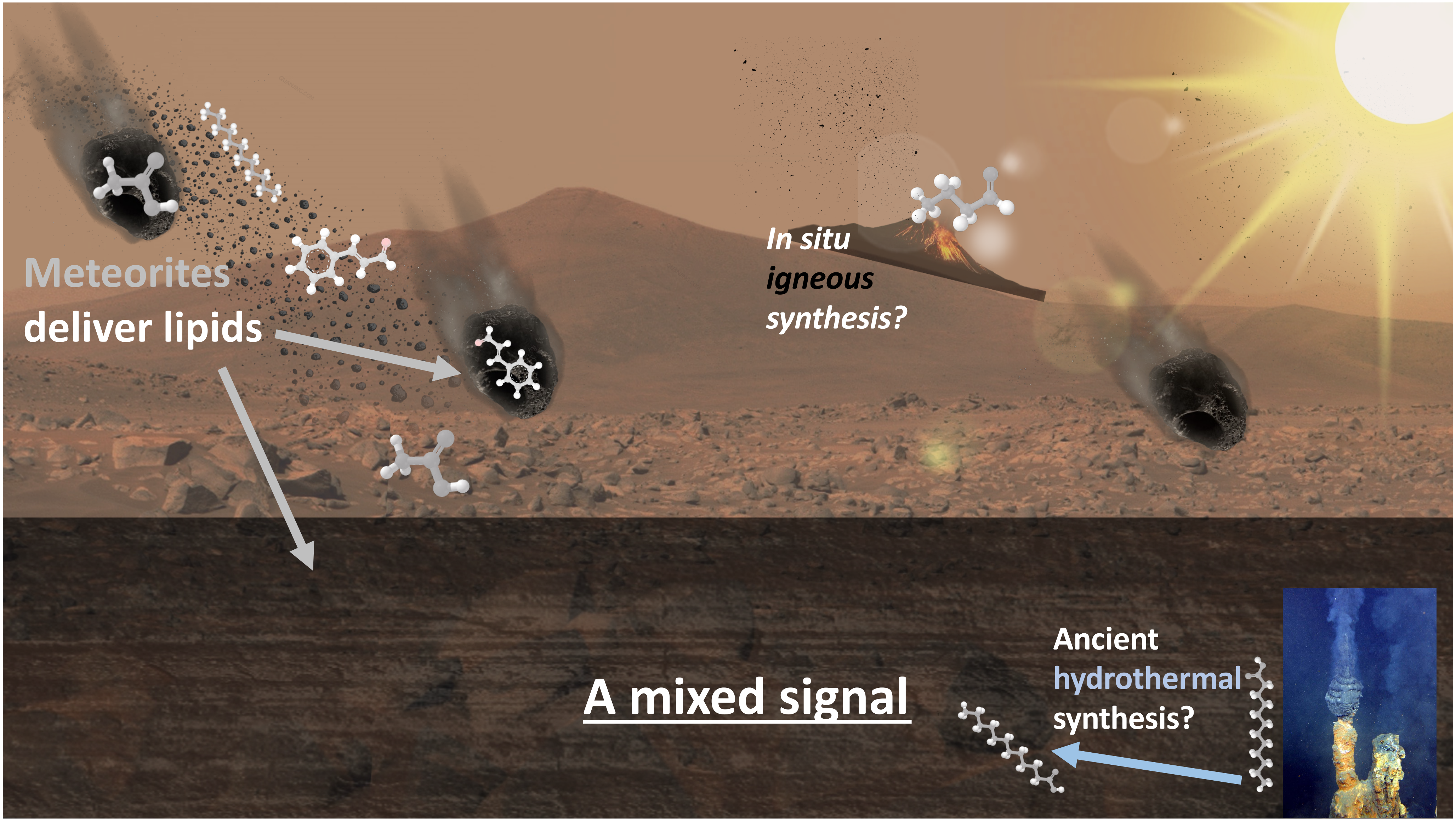
**Meteoritic acyclic hydrocarbons**

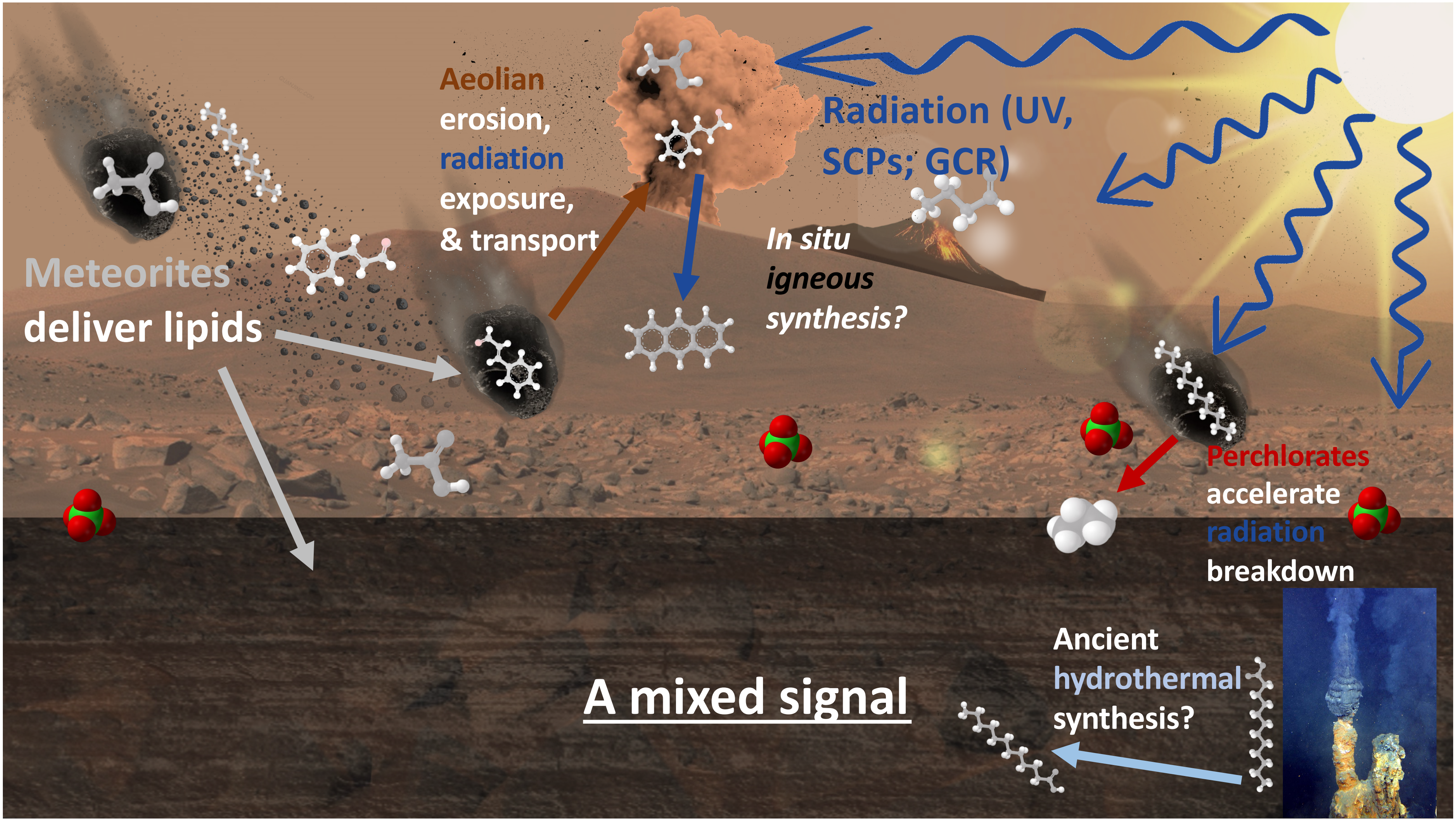
- Shorter chains (1-31 carbons, usually max at 13, 14, or 15)
- No trends in the most abundant molecule

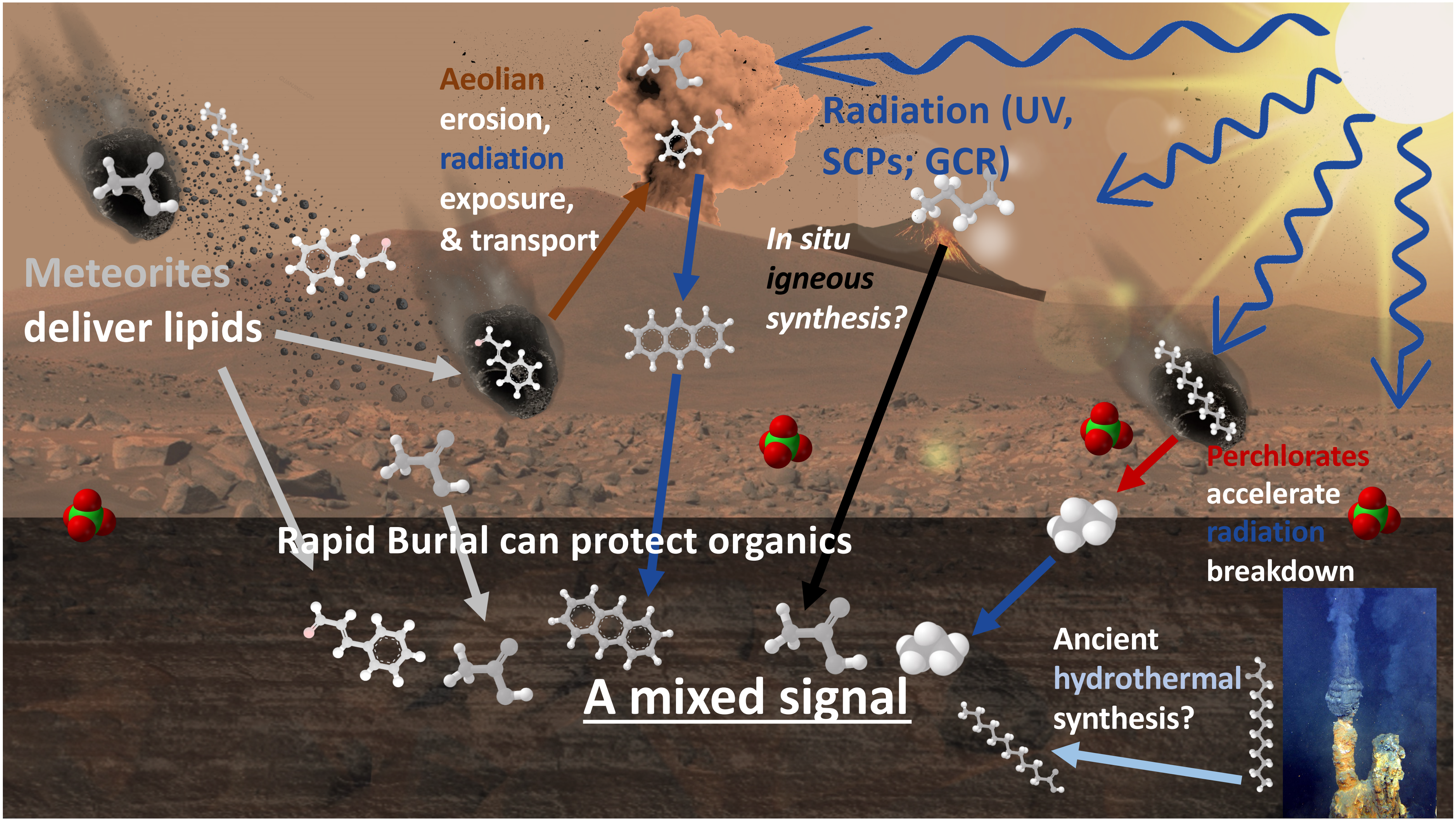


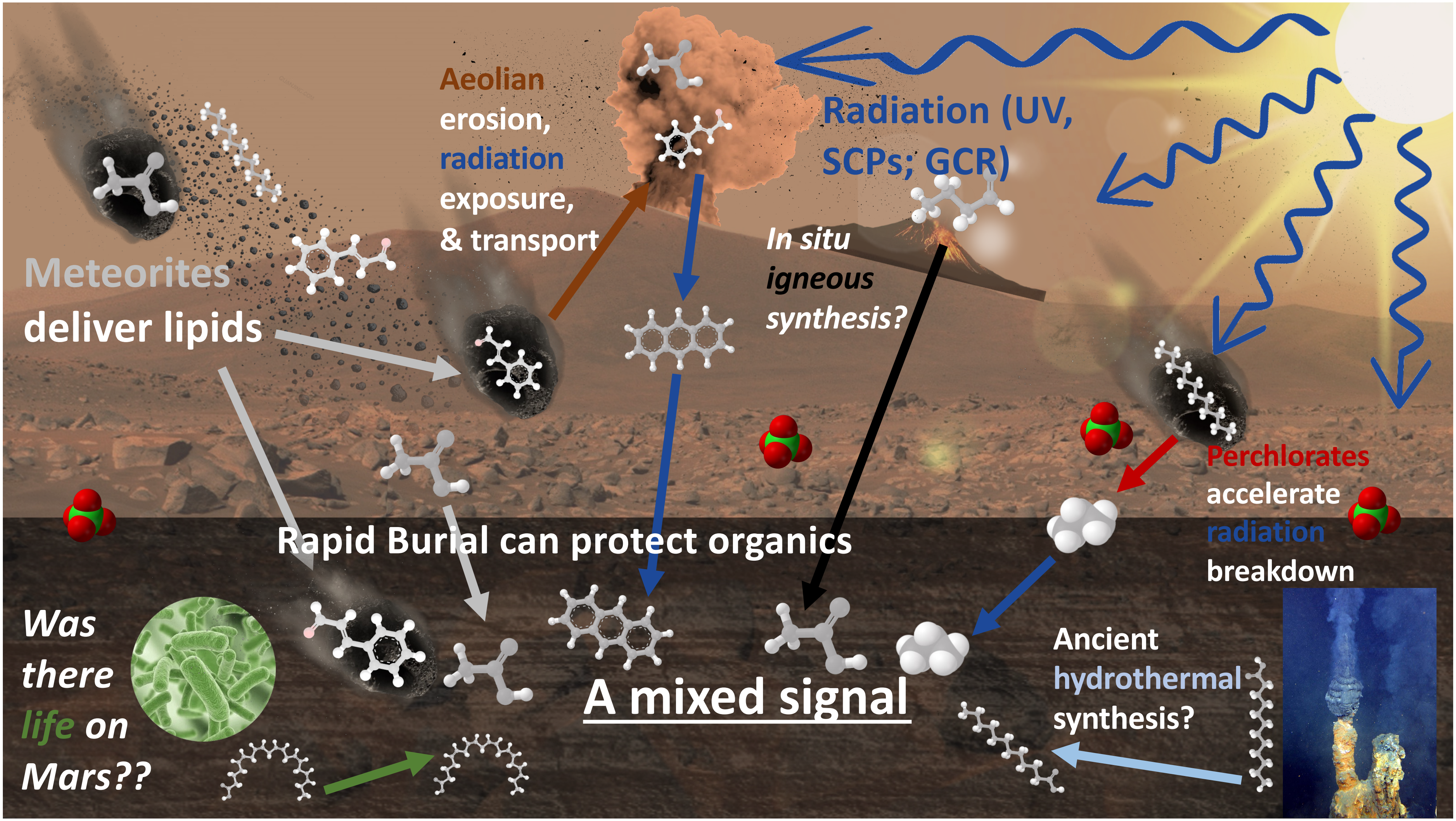

Meteorites
deliver lipids
A mixed signal
Courtesy Denise Buckner


Meteorites
deliver lipids
In situ
igneous
synthesis?
Ancient
hydrothermal
synthesis?
A mixed signal


Aeolian
erosion,
radiation
exposure,
& transport
Radiation (UV,
SCPs; GCR)
In situ
igneous
synthesis?
Meteorites
deliver lipids
Perchlorates
accelerate
radiation
breakdown
Ancient
hydrothermal
synthesis?
A mixed signal



Aeolian erosion, radiation exposure, & transport
Radiation (UV, SCPs; GCR)
Meteorites deliver lipids
In situ igneous synthesis?
Perchlorates accelerate radiation breakdown
Rapid Burial can protect organics
A mixed signal
Ancient hydrothermal synthesis?



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Was
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Mars??
A mixed signal
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