CENTRO DE ASTROBIOLOGÍA

ASOCIADO AL NASA ASTROBIOLOGY INSTITUTE

Biomarkers in Planetary Exploration

Víctor Parro

Department of Molecular Evolution, Centro de Astrobiología (INTA-CSIC),
Carretera de Ajalvir km 4, Torrejón de Ardoz, 28850, Madrid, Spain;

UIMP, Santander, June 2011

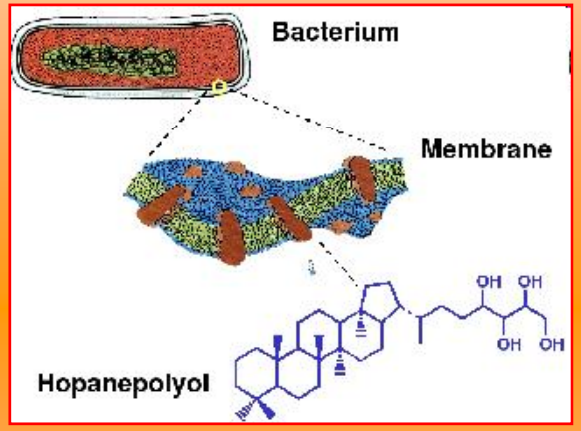
Topics

- **What is a Biomarker?**
- **Biomarkers for Astrobiology**
- **Prebiotic organics vs Biomarkers**
  - **Can aminoacids and monosaccharides be considered as biomarkers?**
  - **Chirality as a Biomarker?**
- **Preservation of biomolecules. Diagenesis**
  - **On Mars**
- **Geo-Biosignatures**
  - **Atmospheric biosignatures**
  - **Microfossils**
  - **Banded Iron Formations**
  - **Biominerals**
  - **Isotopic fractionation**
- **Target biomarkers for Mars**
  - **Extinct life**
  - **Extant life**
- **Detection methods of biomarkers in planetary exploration:**
  - **Volatiles by GC-MS and pyrolysis**
  - **Macromolecular complexes, colloids and organomineral particles**
  - **New approaches: Bio-affinity-based biosensors**
- **Biomarker profiling in Terrestrial analogs**

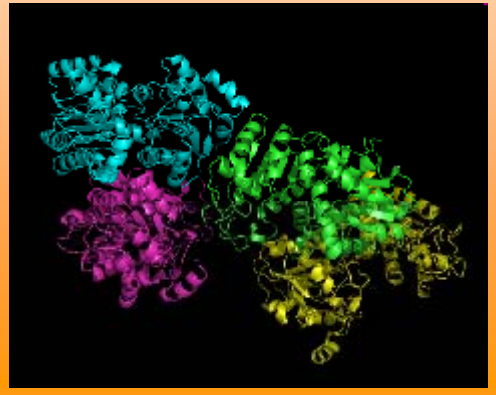
What is a biomarker?

- "A **biomarker**, or biological marker, is, in general, a substance used as an indicator of a biological state. It is a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention".

- In Geology and astrobiology "A (*molecular*) biomarker can be **any kind of molecule** indicating the existence, past or present, of living organisms. In the fields of geology and astrobiology, biomarkers, versus geomarkers, are also known as biosignatures."

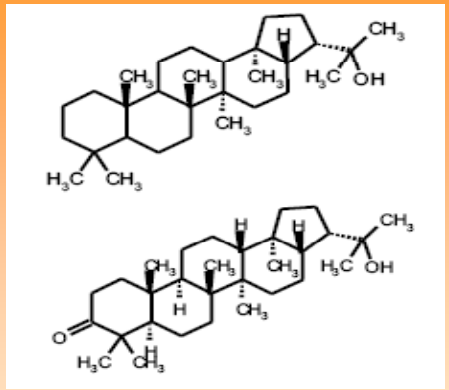


Examples of biomarkers



A serum protein as marker of any disease

Termite mound

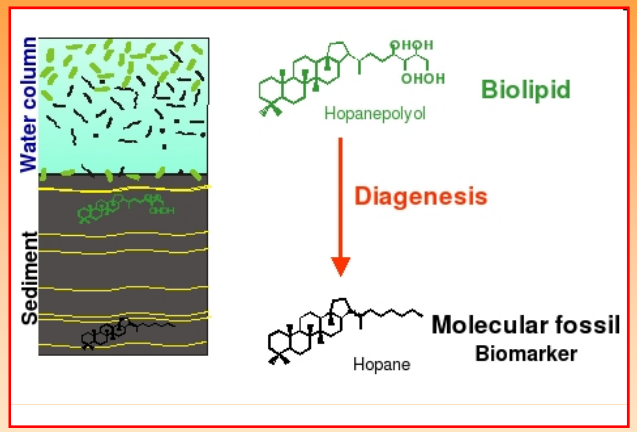


Hydroxyhopanes

We know they are biomarkers because we know them very well.

Biomarkers for Astrobiology

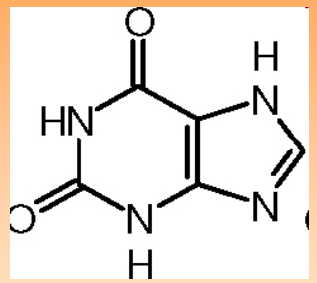
- Atmospheric biosignatures
- Geological biosignatures or fingerprints
  - Macrostructures
  - Biominerals
  - Isotopic fractionation
- Molecular fossils
  - Diagenetic products of biological compounds
- Current Biomolecules
  - Simple molecules to polymers
- Cells and organelles



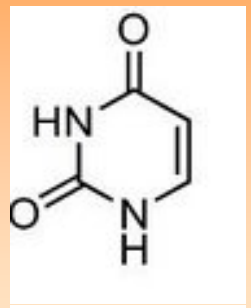
Prebiotic organics vs Biomarkers

- In Astrobiology we are moving in the boundaries of the biochemistry and prebiotic chemistry.
- Aminoacids, some monosaccharides and some nucleobases are also obtained from chemicals sources. They have been found in carbonaceous chondrites meteorites.

Extraterrestrial nucleobases in the Murchison meteorite



xanthine



uracil

Martins et al., Earth Planet. Sci. Lett. *270: 130-136*

Can amino acids and monosaccharides be considered as biomarkers?

- A relative long list of them have been found in meteorites.
- Their presence alone is not a biomarker.

TABLE 3. TYPES AND ABUNDANCES OF COMPOUNDS IN THE MURCHISON METEORITE (SEPHTON, 2002; SEPHTON AND BOTTA, 2005)

	Abundances	
Compounds	*%*	*$\mu g\ g^{-1}$ (ppm)*
Macromolecular material	1.45	
Carbon dioxide		106
Carbon monoxide		0.06
Methane		0.14
Hydrocarbons: aliphatic		12–35
aromatic		15–28
Acids: monocarboxylic		332
dicarboxylic		25.7
α-hydroxycarboxylic		14.6
Amino acids		60
Alcohols		11
Aldehydes		11
Ketones		16
Sugar-related compounds (polyols)		~24
Ammonia		19
Amines		8
Urea		25
Basic N-heterocycles (pyridines, quinolines)		0.05–0.5
Pyrimidines (uracil and thymine)		0.06
Purines		1.2
Benzothiophenes		0.3
Sulphonic acids		67
Phosphonic acids		1.5

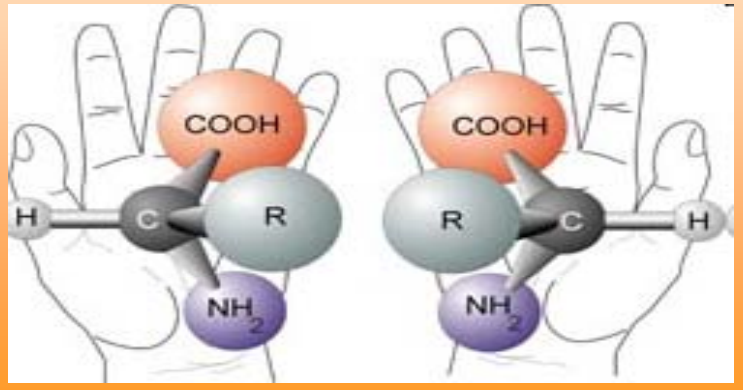
Chyrality as a Biomarker?

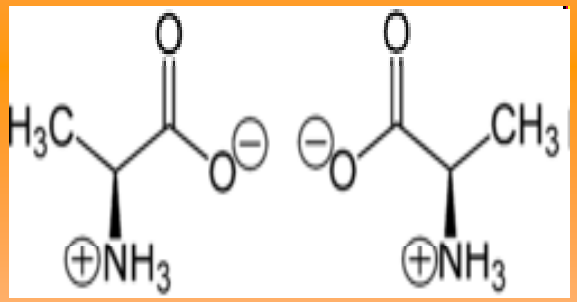
- Life:

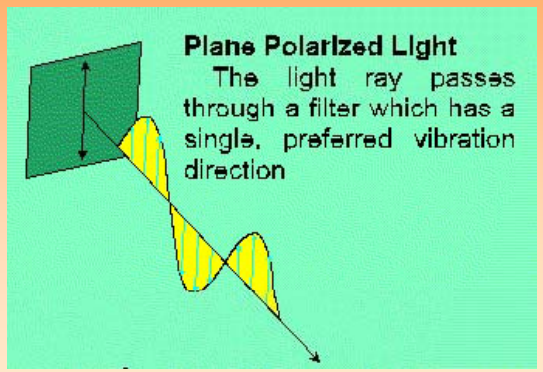
  D-monosaccharides

  L-amino-acids
- Carbonaceous condrites: Mixture of both.
- Polarized light may render D or L isoforms

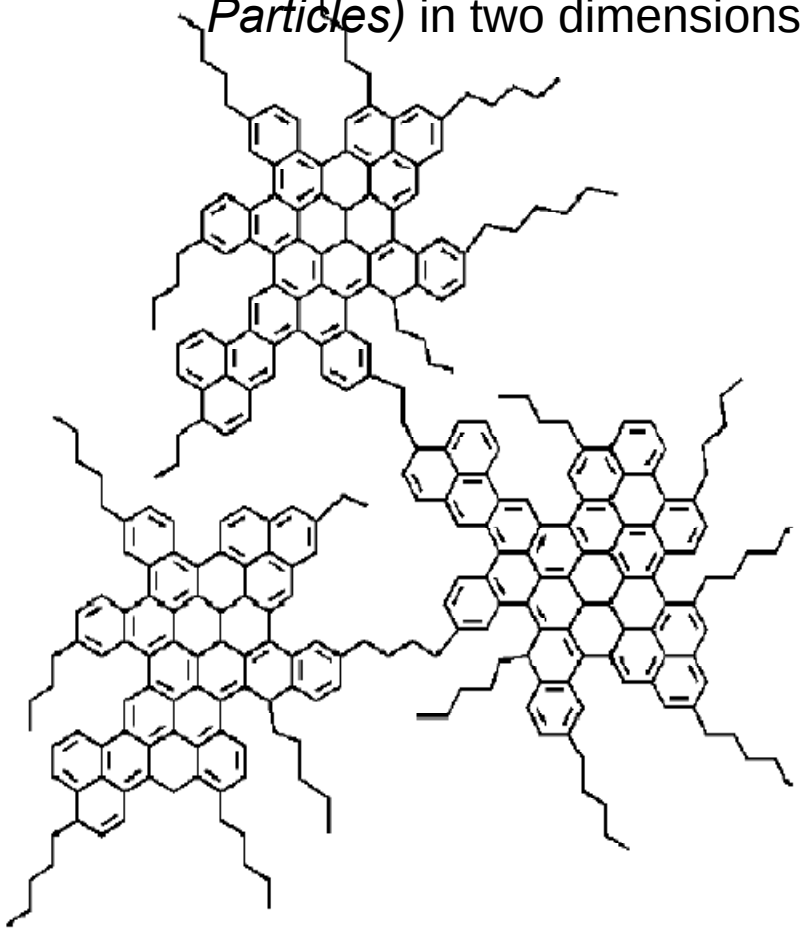
- Not a biomarker

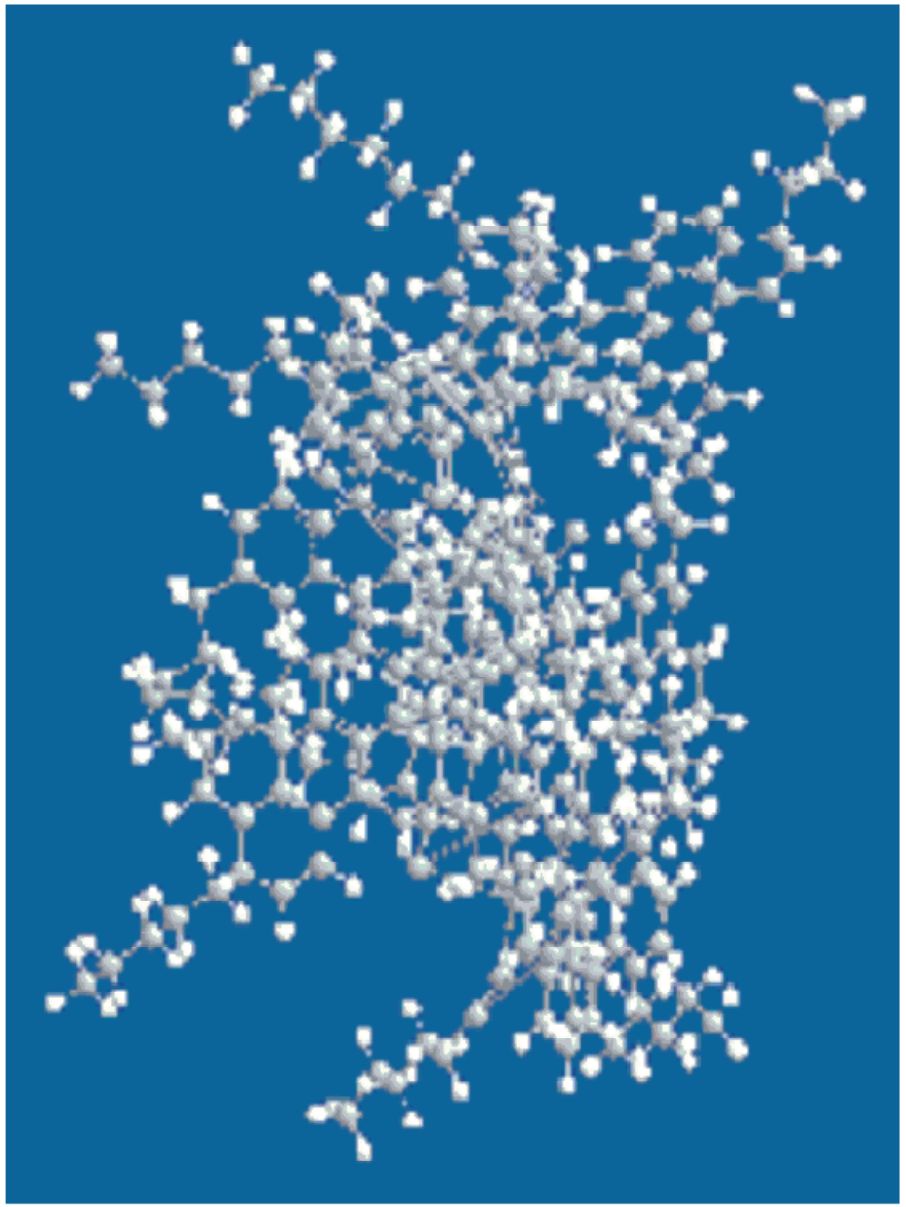






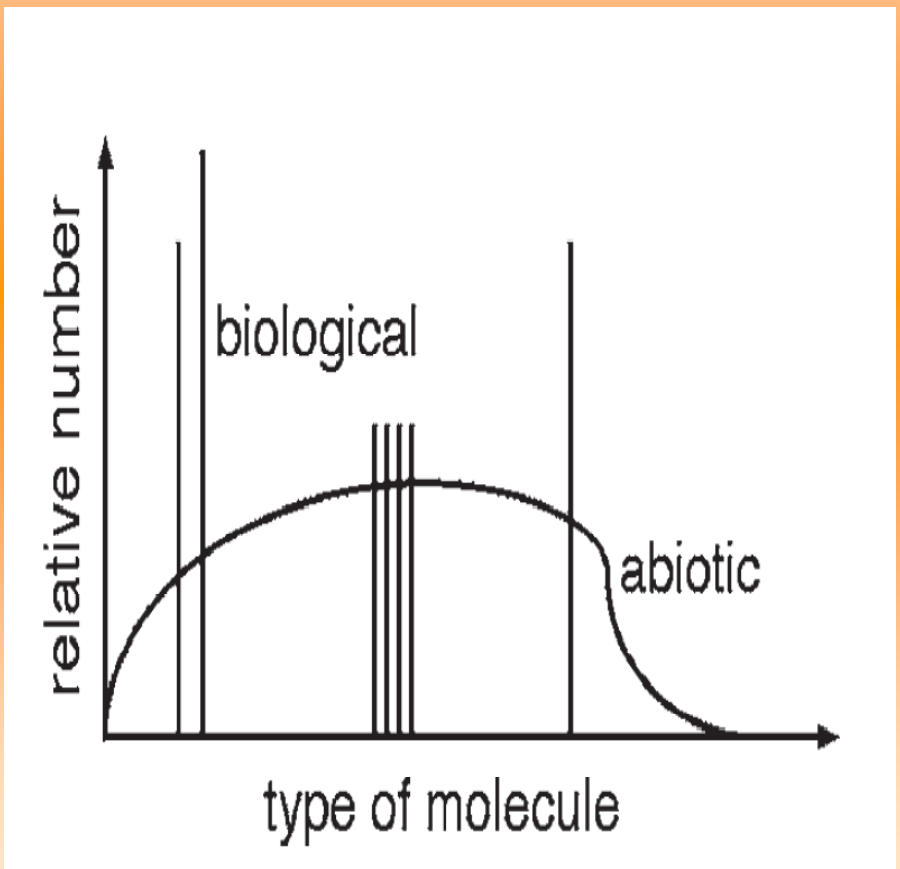
Expected substructure unit for the a-C:H observed in many IDPs (*Interplanetary Dust Particles)* in two dimensions





Biogenic vs Nonbiogenic organic matter

- Nonbiological processes produce smooth distributions of organic material (curve).
- Biology, in contrast selects and uses only a few distinct molecules (spikes), e.g., the 20 L-amino acids on Earth).
- Analysis of a sample of organic material from Mars or Europa may indicate a biological origin if it shows such selectivity.



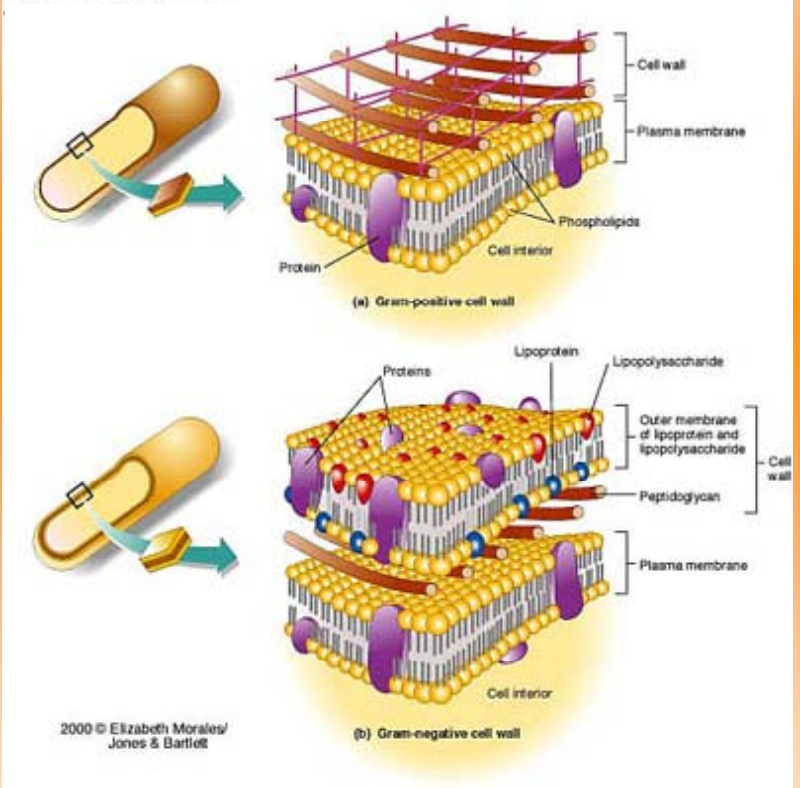
Chris McKay

Molecular Biomarkers

Compound class[a]	Common biotic source[b]	Analytical detection method
Labile compounds to assess contemporary or recent life processes		
L-Amino acids/peptides	All life	GC, Py-GC
Sugars/polysaccharides	Flora (some fauna)	GC, GC-MS, GC-MS/MS
Nucleotides, bases, etc.	All life	Specific methods
Unsaturated lipid compounds	All life	GC, GC-MS
Biopolymers	All life	Py-GC-MS, specific methods
Lipid/bitumen compounds to assess biosynthesis in prior geological times		
Aliphatic hydrocarboms	Ubiquitous/not specific	GC, GC-MS, GC-IRMS
Aliphatic acids, alcohols	Ubiquitous/some specific	GC, GC-MS, GC-IRMS
Isoprenoids	Biogenic	GC, GC-MS, GC-IRMS
Steroids	Flora/fauna	GC-MS, GC-IRMS
Triterpenoids	Flora/microbes	GC-MS, GC-IRMS
Diterpenoids	Flora/microbes	GC-MS, GC-IRMS
Pigments	Flora/microbes	HPLC-MS, HPLC-IRMS
Biopolymers	Flora/microbes	Py-GC-MS
Novel and unknown biomarkers	–	GC-MS, Py-GC-MS, GC-IRMS
Abiogenic organic compounds		
D/L-Amino acids	Abiotic	GC, Py-GC
Aliphatic alcohols, acids, hydrocarbons	Abiotic	GC, GC-MS, GC-IRMS
PAH	Abiotic	GC, GC-MS, GC-IRMS

Preservation of biomolecules.

- **Factors that favor biomolecules degradation**:
  - enzymatic and microbial activities
  - radiation (UV and others)
  - oxidation
  - metal attacks
  - Maillard reactions
  - high temperatures and extreme pHs
- **Factors that estabilize biomolecules**
  - low temperatures
  - inclusions in hypesaline solutions
  - a rapid burial
  - precipitation or binding on the surface of colloids, mineral particles, or organic macromolecular aggregates
  - rapid dehydration
  - mild pH values
  - absent of reactive and degradative metal ions
  - Protective effect of clay minerals



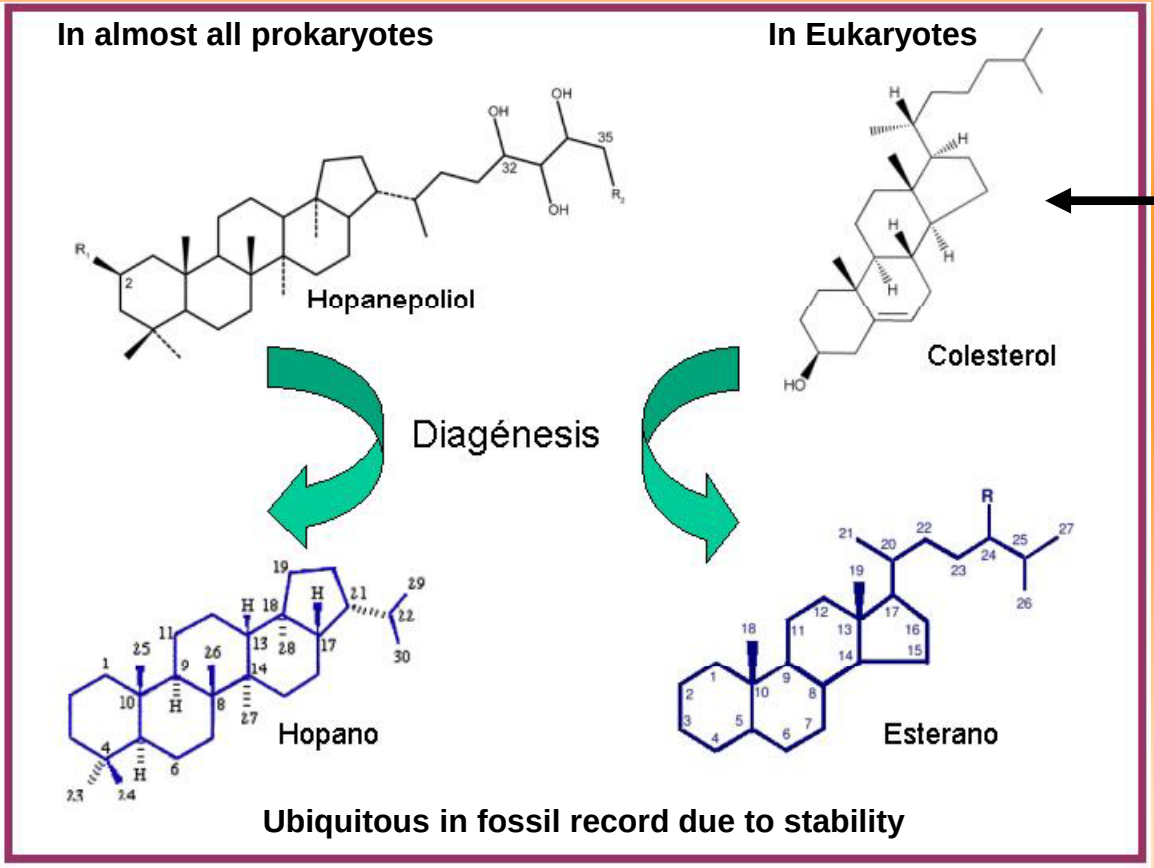
BACTERIA IN THE ENVIRONMENT

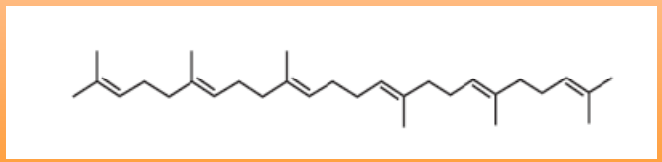
Number of bacteria in natural environments

Environment	Number of bacteria		References
	Total counts (cells/g dw)	Viable counts (cfu/g dw)	
Soils	10^8–10^{10}	10^5–10^8	[4,5]
Sediments			
up to 10 m	10^7–10^{10}	10^2–10^6	[6]
up to 550	$< 10^6$–10^8	$< 10^2$–10^8	[2]
Permafrost sediments			
Arctic (> 300 m)	10^7–10^9	10^2–10^8	[3,7,8]
Antarctic (20 m)	10^7–10^8	10–10^4	[9]
Permafrost buried soils	10^9	10^4–10^6	[7]
Bottom sediments of lakes			
upper layers	10^8–10^9	10^4–10^5	[10]
layers at 3–8 m	10^8	10^2–10^3	
Central part of the ocean	cells/ml	cells/ml	[11]
water	10^2–10^6	0–10	
upper layer of sediments	10^7	10^2–10^6	
Groundwater (up to 2000 m)	10^3–10^6	10–10^5	[2]
Antarctic glaciers (up to 1800 m)		single cells/l	[12]

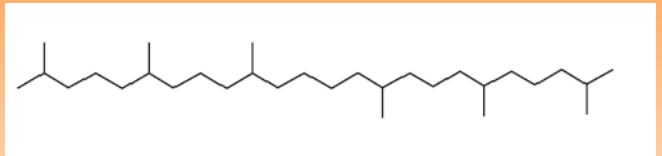
From Vorobyova et al. FEMS microbiology reviews 20 (1997) 277-290

Transformation of biomolecules by digenetic processes



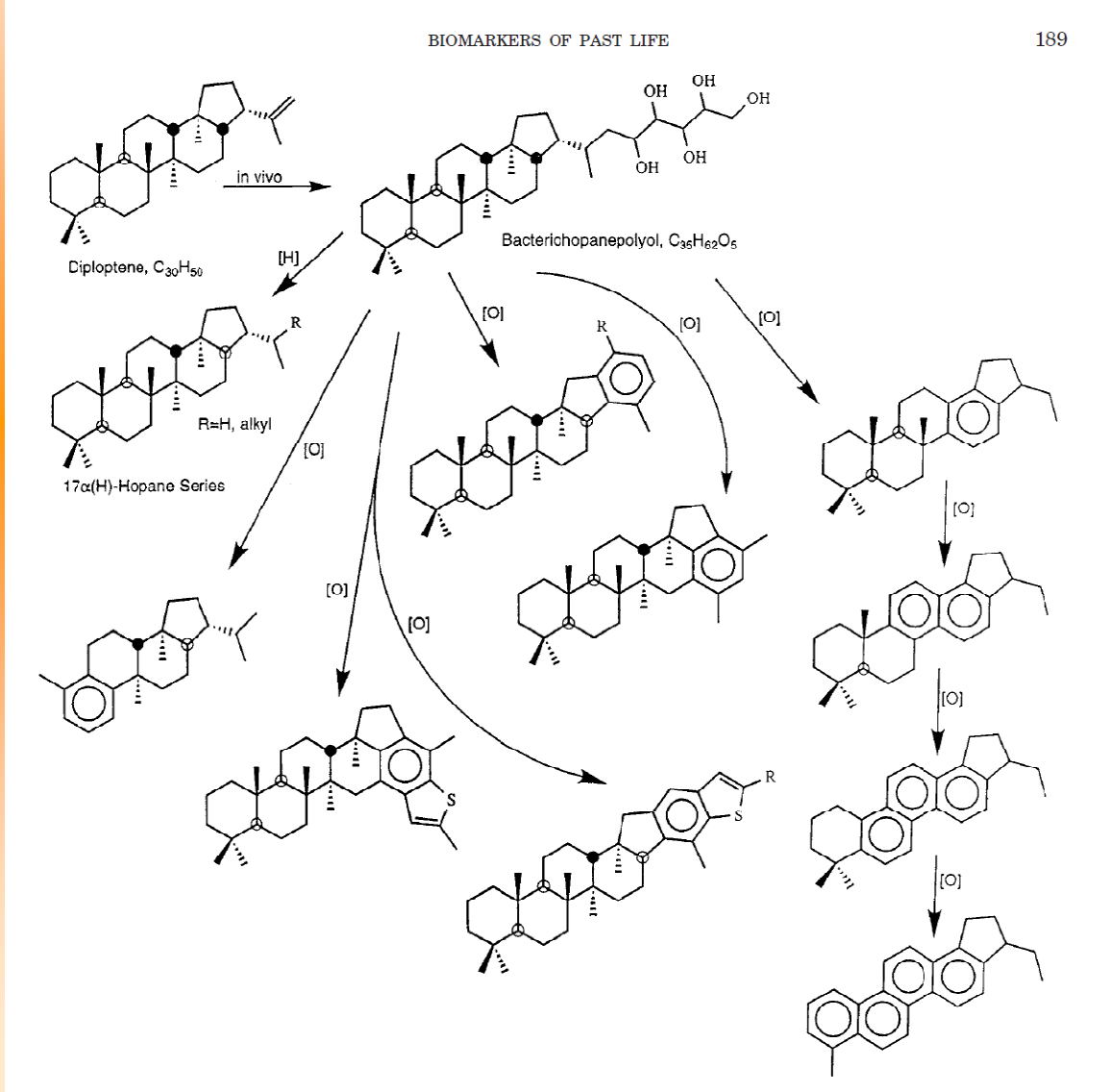


Squalene
Common in all domains of life. Biological precursor of larger terpenoinds



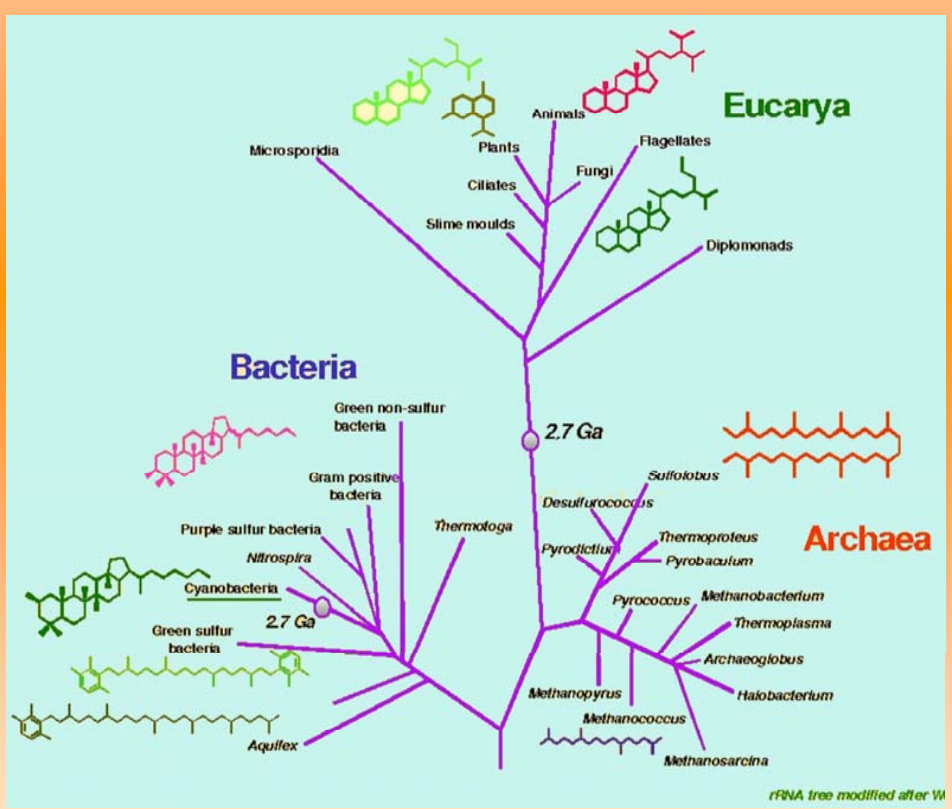
Squalane
Variable abundance in fossil record due to instability of its precursor

Alteration scheme for hopanoids by reduction to the geo-hopanes, by oxidation to aromatic hydrocarbon derivatives, and by reaction with sulfur to thio-aromatic products.



Taphonomy and paleo-ecology

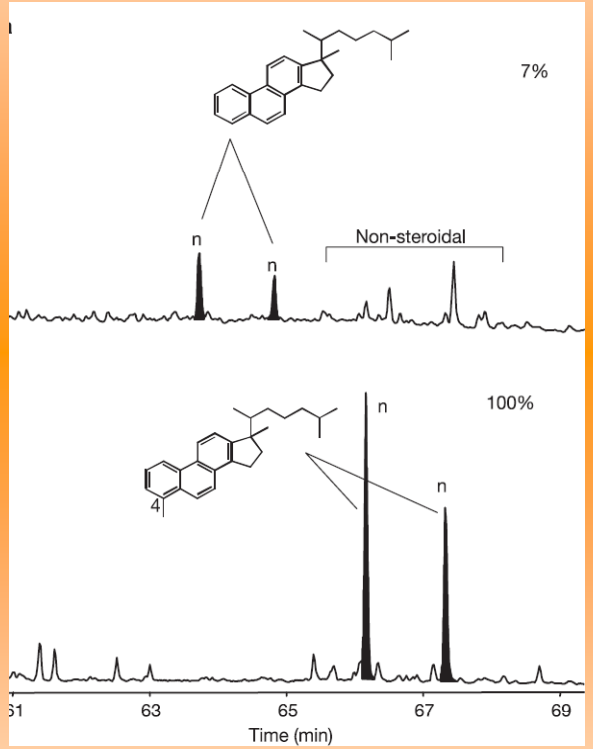
- **Taphonomy** (from the Greek *taphos - τάφος* meaning burial, and *nomos - νόμος* meaning law), is the study of the transition of remains, parts, or products of organisms, from the biosphere, to the lithosphere, *i.e.* the creation of fossil assemblages. Taphonomy tries to understand the geo-ecological conditions operating at the time where the fossils were found.



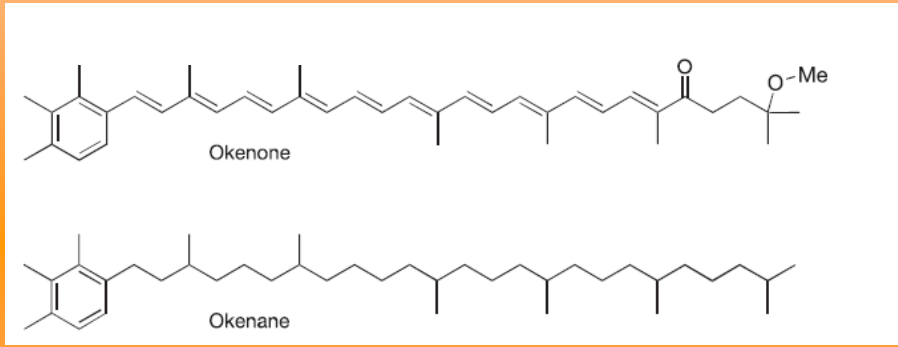
JJ Brocks and R. Summons

Molecular Taphonomy

Biomarker evidence for green and purple sulfur bacteria in a stratified Palaeoproterozoic sea



Selected-ion-recording chromatograms of triaromatic steroids from a representative sample of the **1.64-Gyr-old Barney Creek** Formation (BCF) of the McArthur Group, northern Australia

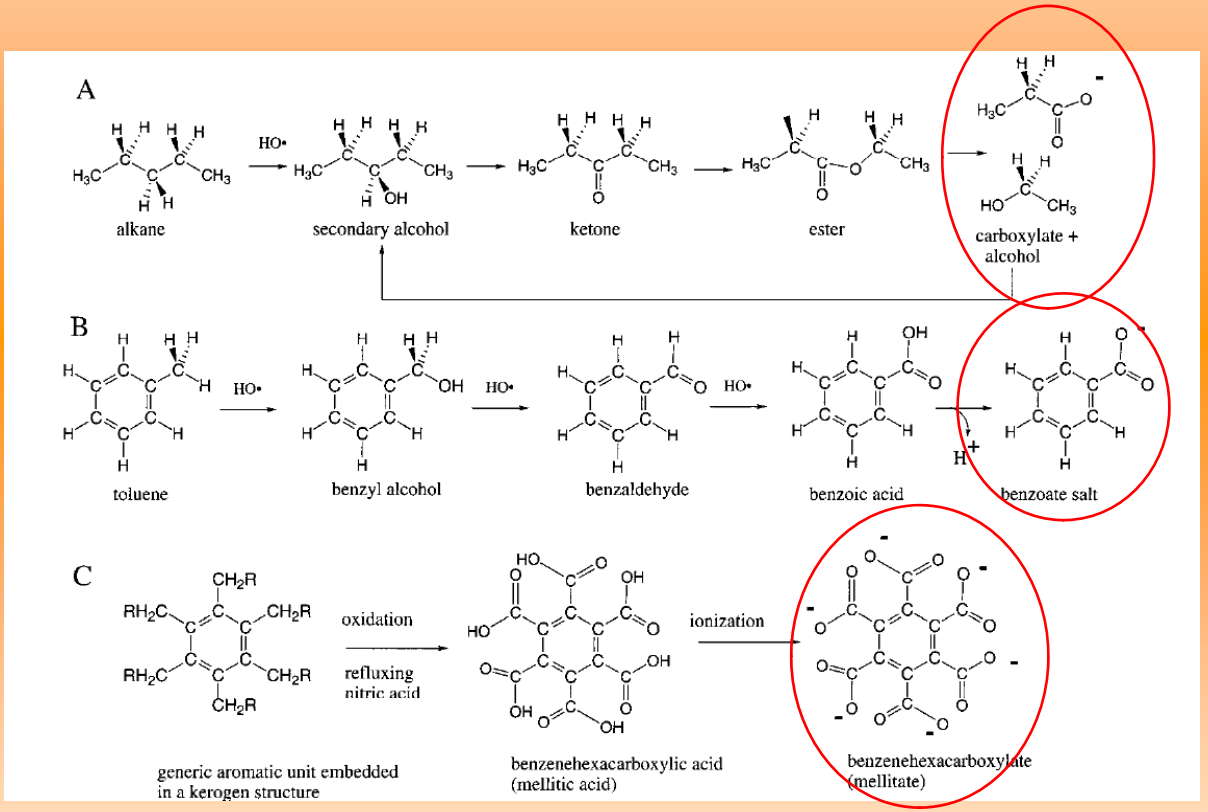


Chemical structures of the carotenoid pigment okenone and its hydrocarbon fossil equivalent okenane

Brocks JJ, et al., 2005. NATURE|Vol 437:866-870.

Stability of organic matter in planetary bodies, the case of Mars

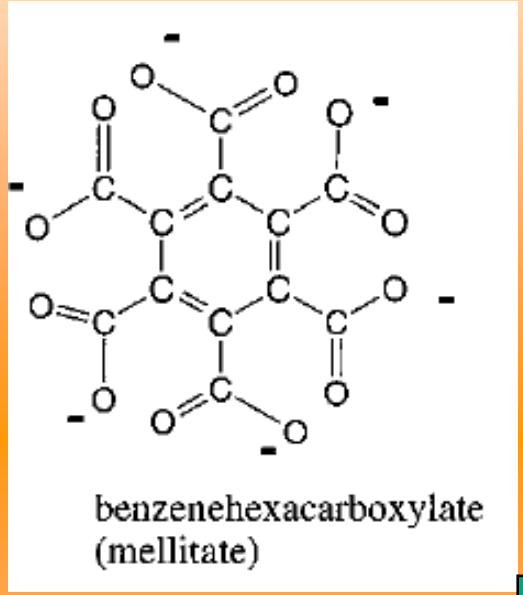
- High amounts of reduced carbon estimated to reach the Mars surface each year in meteors (up to 2.4×10^8 g)
  - UV radiation
  - Powerful oxidant on martian surface

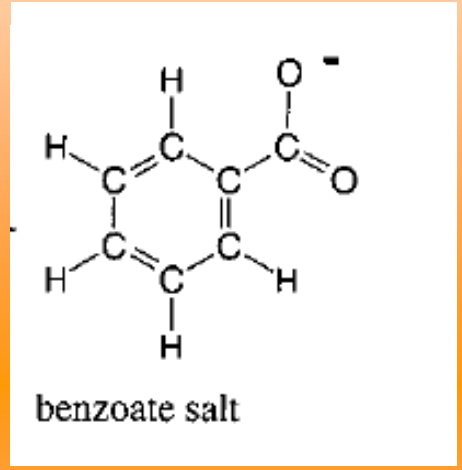


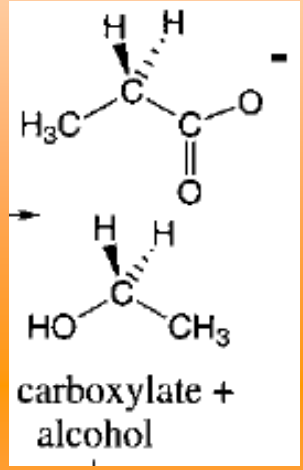
G. J. Flynn, Earth, Moon and Planets 72, 469 (1996)

Benner et al., 2000. The missing organic molecules on Mars. Proc Natl Acad Sci U S A. 97:2425-30.

Photocatalytic Decomposition







Radiation

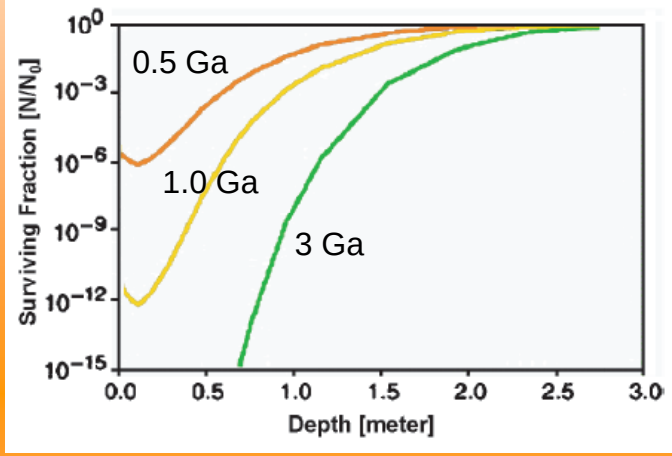
CO_2 CH_4

- Photocatalytic Decomposition of Carboxylated Molecules on Light-Exposed Martian Regolith in the presence of Fe(III) minerals would produce CO_2 and CH_4 as end products

Shkrob et al., 2010. Astrobiology 10:425-436

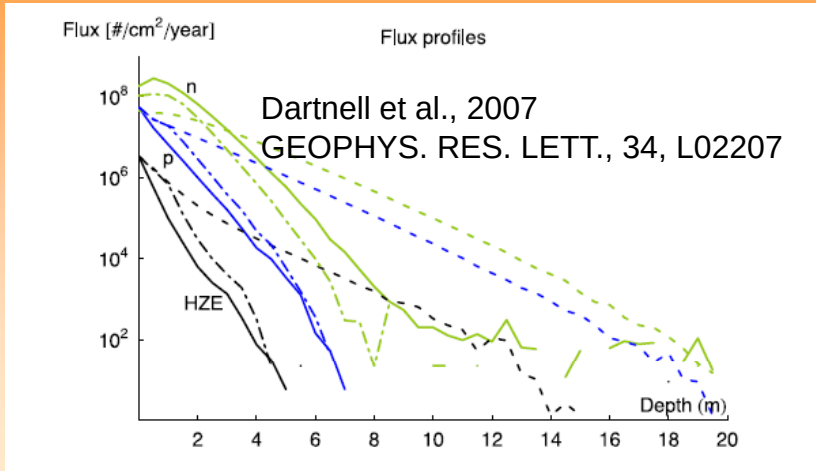
Modeling the surface and subsurface Martian radiation environment

- Some estimations indicate that some biomolecules can be preserved at 2 m deep



G. Kminek and J. L. Bada,
Earth Planet. Sci. Lett. 245, 1 (2006)

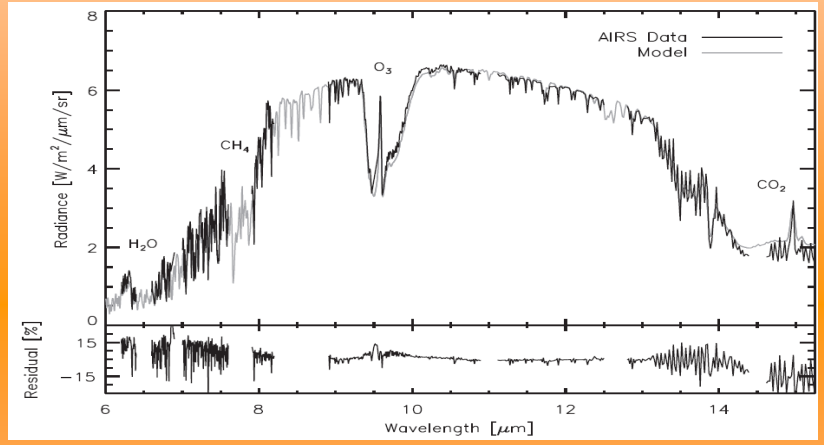
- Others suggest that the recovery of viable cells cryopreserved within the putative Cerberus pack-ice requires a drill depth of at least 7.5 m.



Dartnell et al., 2007
GEOPHYS. RES. LETT., 34, L02207

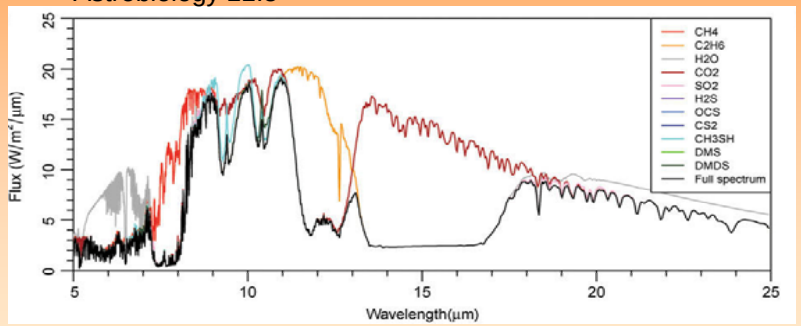
Molecular Biomarkers vs Biosignatures:
Atmospheric Biosignatures

- Earth atmosphere from space as a biosignature. Earth as an Extrasolar Planet



Mid-infrared, 24-hour average spectra of Earth from a March model (gray) and as generated from AIRS observations (black) (Hearty et al., 2009). Robinson et al., 2011: Astrobiology 11:5

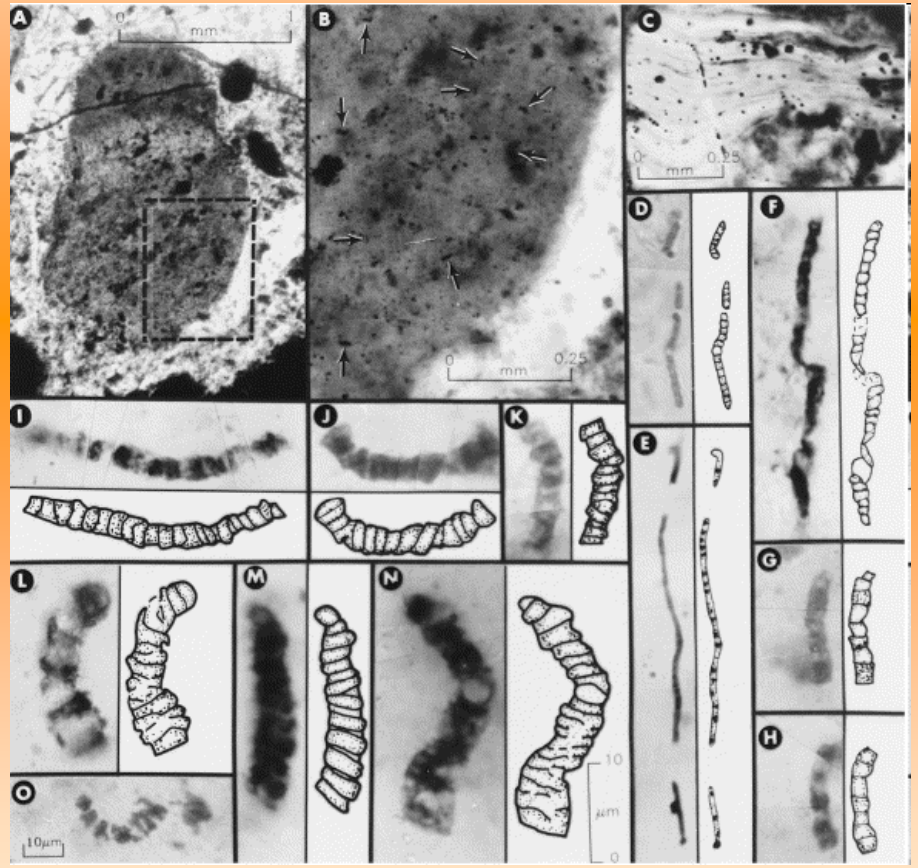
- Simulated absorption spectra for biogenic sulfur gases in anoxic planets CS_2, OCS, CH_3SH, CH_3SCH_3, and $CH_3S_2CH_3$)



Domagal-Goldman et al., 2011. Astrobiology 11:5 p419-441

Microfossils

- Stromatolite-like clasts and filamentous microfossils reveal prokaryotic assemblage indicating that cyanobacterium-like microorganisms were extant and morphologically diverse at least as early as approximately **3.4 Ga** ago and suggests that oxygen-producing photoautotrophy may have already evolved by this early stage in biotic history.
- Some may be direct proof of life.



Microfossils from the early Archean Apex Chert of Australia (from Schopf, 1993. Science 260:640-6.)

Geo-mineralogical Biosignatures

Banded Iron Formation (BIF) a an indirect biosignature from oxygenic photosynthesis (From 3.8 to 1.8 Ga)

Oxygen from photosynthetic microorganisms was firstly accumulated in rocks and iron minerals. It was not until probably only 1 billion years ago that the reservoirs of oxidizable rock became saturated and the free oxygen stayed in the air

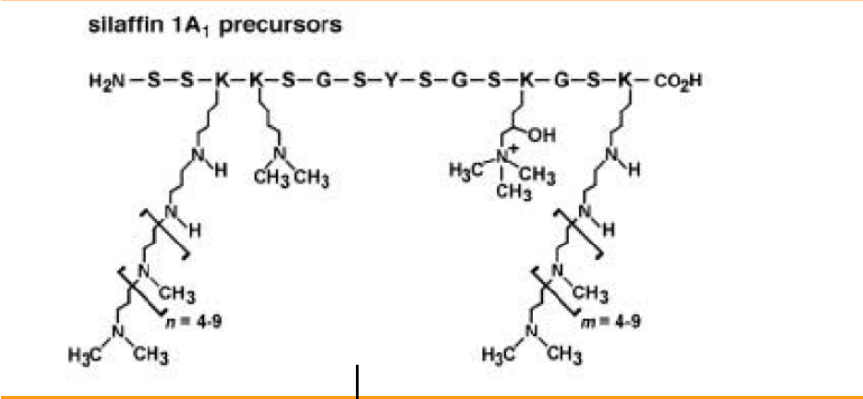
Banded iron formations, such as these found in Ontario, Canada, show the chemical features of ancient seawater when they formed in iron-rich oceans billions of years ago.

Stromatolites

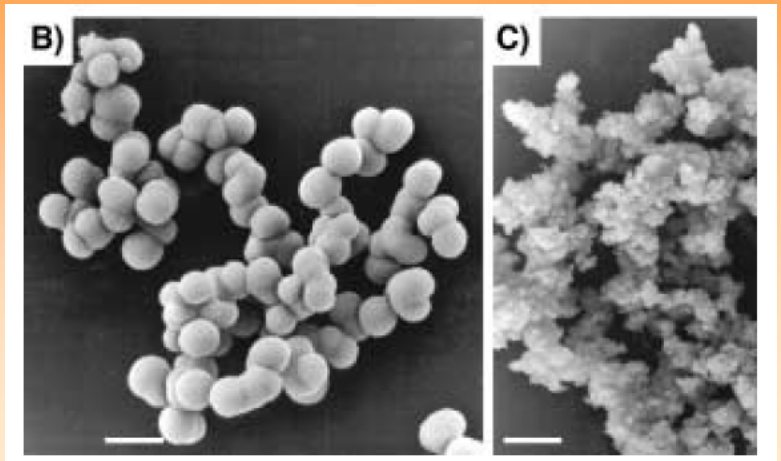
Layered accretionary structures formed in shallow water by the trapping, binding and cementation of sedimentary grains by biofilms of microorganisms, especially cyanobacteria.

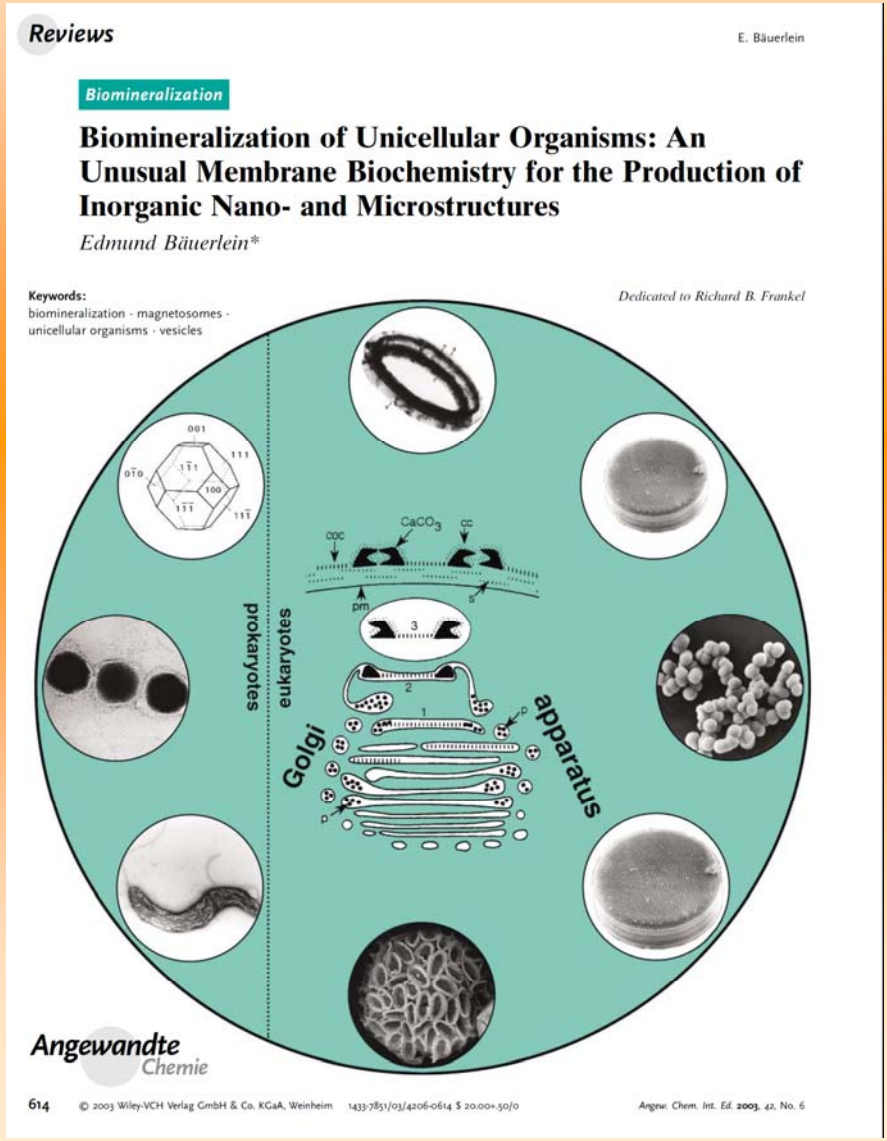
They include some of the most ancient records of life on Earth.

Mineralization mediated by microorganisms



Silica precipitation induced by microbial peptides





Reviews

E. Bäuerlein

Biomineralization

Biomineralization of Unicellular Organisms: An Unusual Membrane Biochemistry for the Production of Inorganic Nano- and Microstructures

*Edmund Bäuerlein**

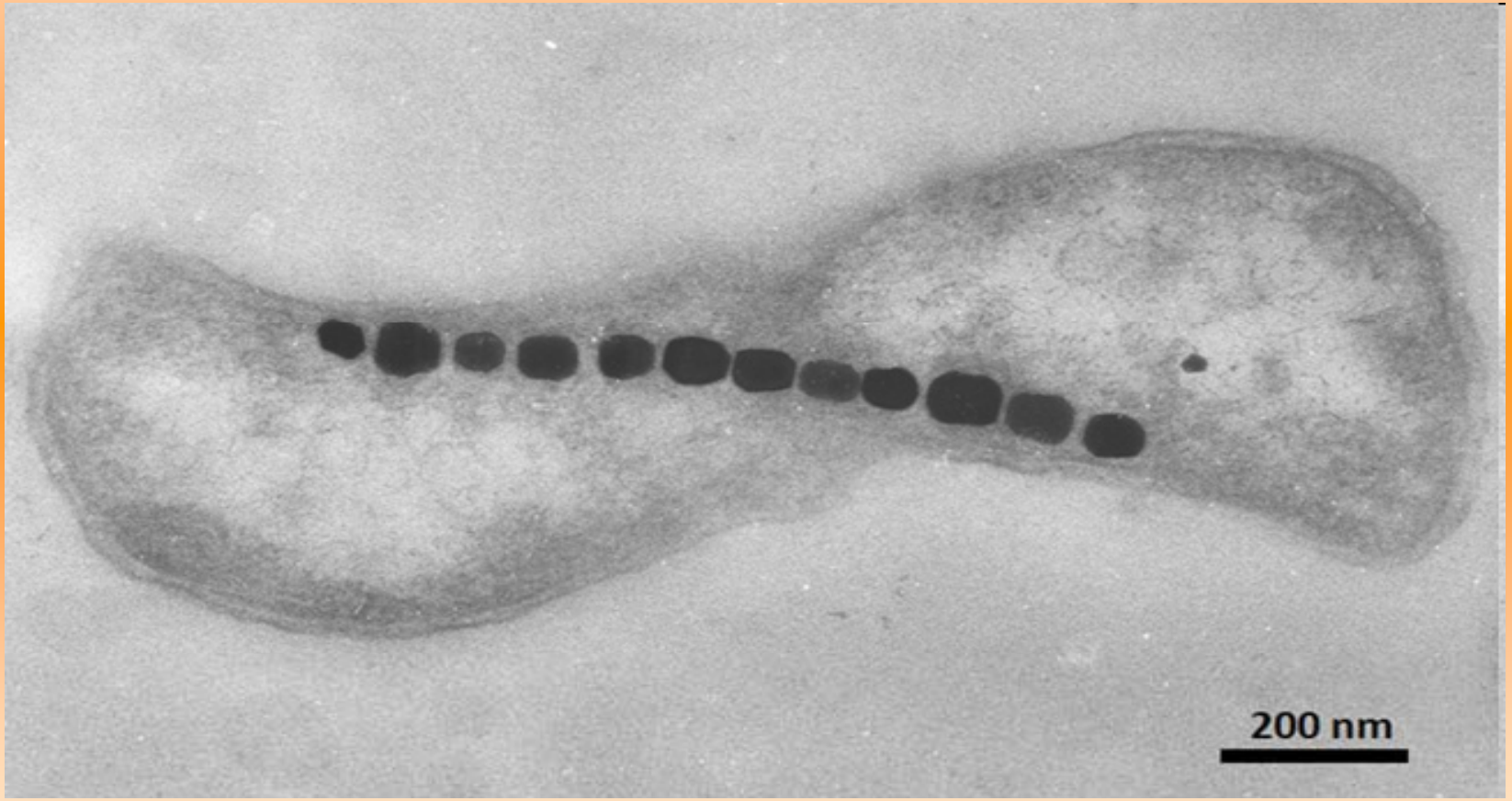
Keywords: biomineralization · magnetosomes · unicellular organisms · vesicles

Dedicated to Richard B. Frankel

Angewandte Chemie

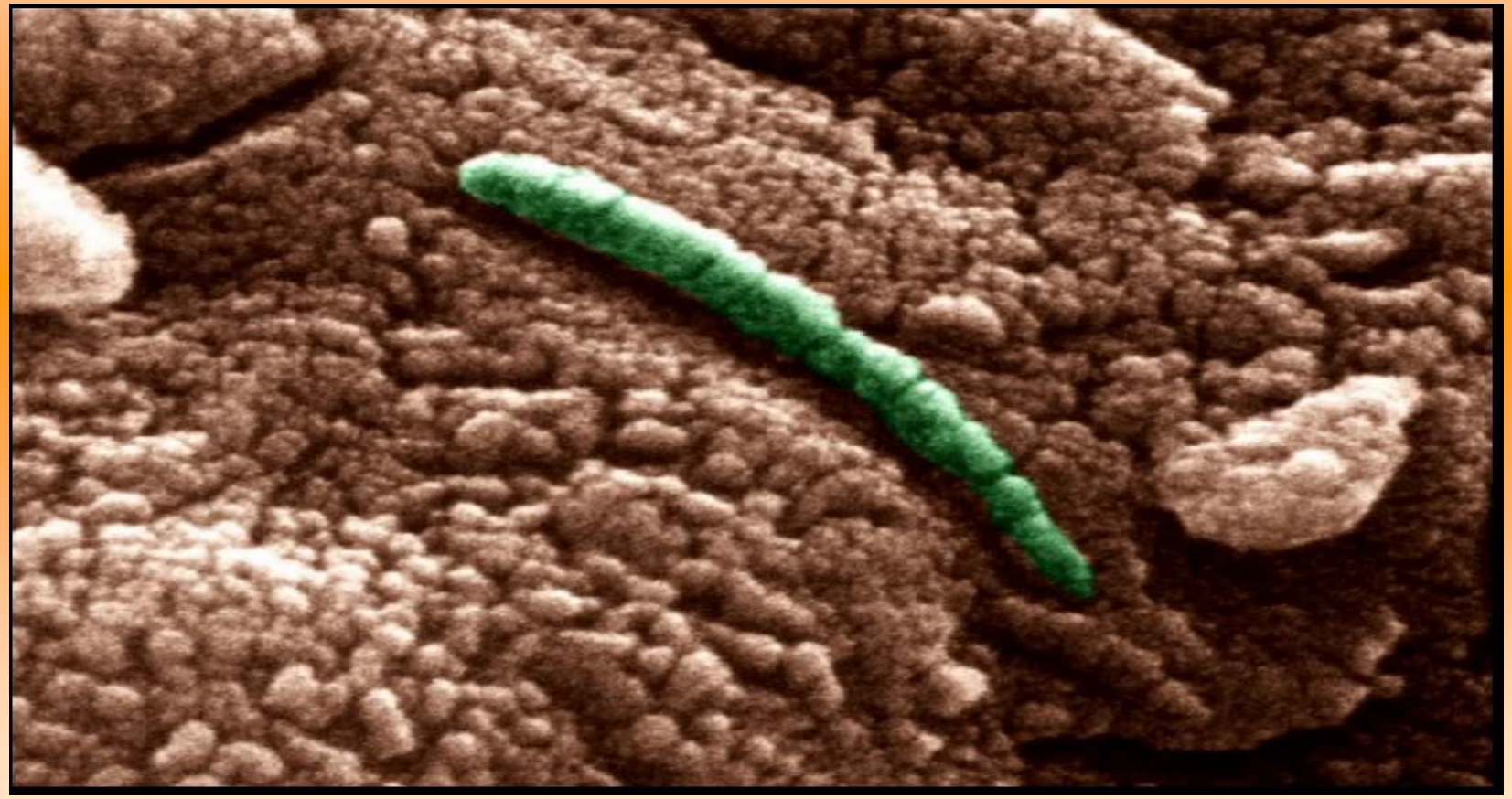
614 © 2003 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim 1433-7851/03/4206-0614 $ 20.00+.50/0 Angew. Chem. Int. Ed. 2003, 42, No. 6

Magnetite crystals characteristic of magnetotactic bacteria



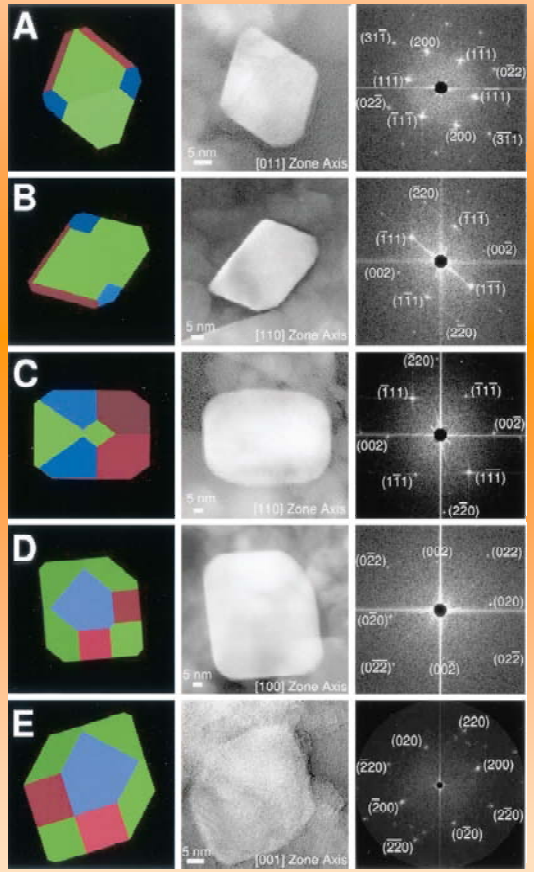
TEM image of a magnetotactic bacterium
Note the chain of twelve magnetite (Fe_3O_4) nanoparticles that are arranged along the long axis of the cell. The magnetic magnetite chain allows the organism to behave as a tiny motile compass needle. Scale bar is provided in the lower right corner. © 2010 Nature Education All rights reserved.

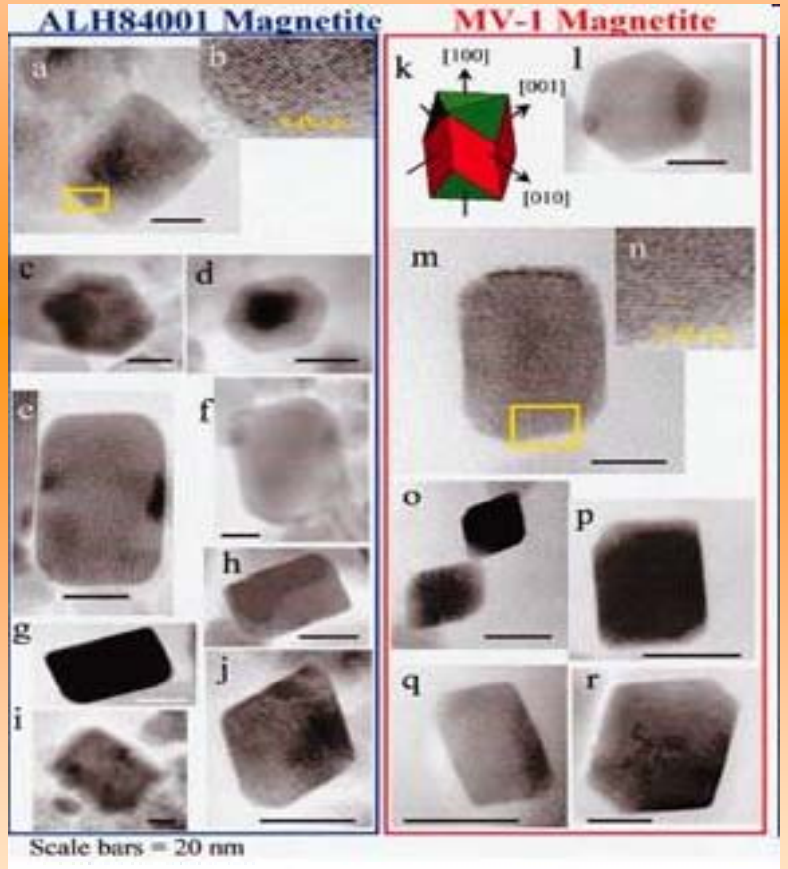
ALH84001 Martian meteorite: Morphology by itself is not enough



McKay et al., 1996. Science

Magnetite crystals similar to that of magnetotactic bacteria.





Thomas-Keprta et al., APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Aug. 2002, p. 3663–3672

Isotopic fractionations as biosignatures

- Life always prefers light versus heavier isotopes
- High ratio $^{12}C/^{13}C$ indicated carbon fixation via photosynthesis **3,8 Ga** ago (Schidlowski, 1988).
- Fe and C isotope fractionation supports ecosystem and environmental diversity **2.7—2.5 Ga** in Hamersley Province, Australia (Czaja et al., 2010)

Simplified metabolic reactions of Neoarchean sedimentary environments.

Metabolism	Reaction
Oxygenic photosynthesis	$CO_2 + H_2O \rightarrow CH_2O + O_2$
Anoxygenic photosynthetic iron oxidation (APIO)	$4Fe^{2+} + CO_2 + 4H^+ \rightarrow CH_2O + 4Fe^{3+} + H_2O$
Dissimilatory iron reduction (DIR)	$4Fe(OH)_3 + CH_2O \rightarrow 4Fe^{2+} + HCO_3^- + 7OH^- + 3H_2O$
Bacterial sulfate reduction (BSR)	$SO_4^{2-} + CH_2O \rightarrow 2HCO_3^- + H_2S$
Methanogenesis	$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$ or $CH_3COOH \rightarrow CH_4 + CO_2$
Aerobic methanotrophy (AMT)	$7CH_4 + 8O_2 \rightarrow 6CH_2O + CO_2 + 8H_2O$
Anaerobic oxidation of methane	
Coupled to sulfate reduction (AOM-SR)	$CH_4 + SO_4^{2-} \rightarrow HCO_3^- + HS^- + H_2O$
Coupled to iron reduction (AOM-IR)	$CH_4 + 8Fe(OH)_3 + 15H^+ \rightarrow HCO_3 + 8Fe^{2+} + 21H_2O$

Czaja et al., 2010. Earth and Planet. Sci. Lett. 292: 170-180.

Mars Taphonomy from MSL

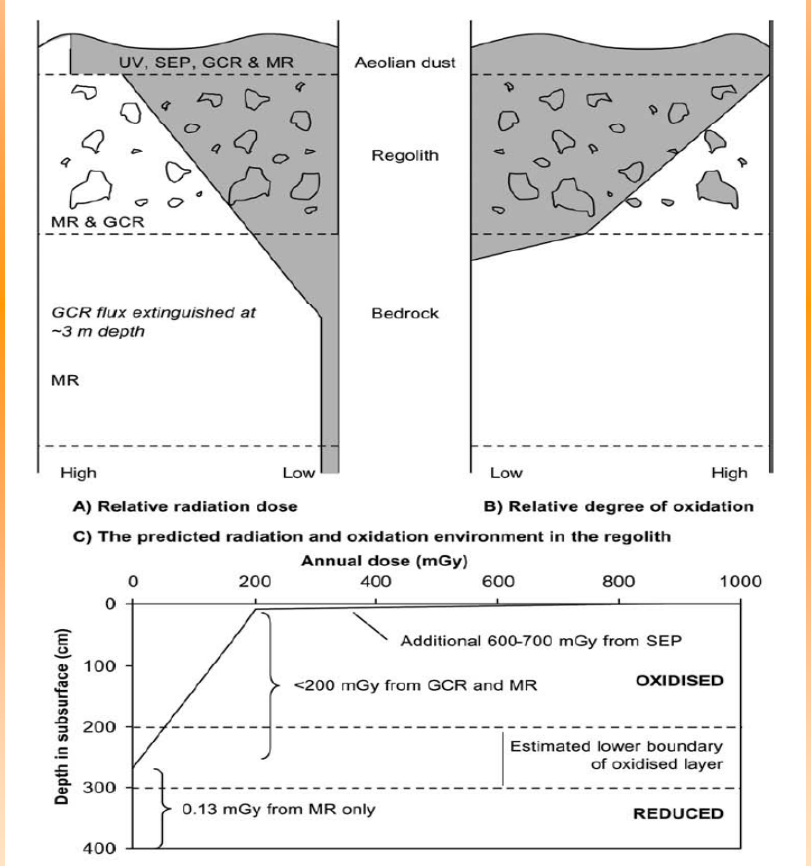
Table 12. Summary of Mineralogical Features Relevant to Biosignature Preservation in Sedimentary Environments on Early Mars

Important factors	*Factor-related feature*	*Preservation window*	*Payload elements*
Sediment source	Detrital mineral assemblages	Early lithification is favorable. Avoid the following: sediments altered by recrystallization and dissolution (especially evaporites), redox changes toward oxidizing conditions, and strong structural alteration and strong thermal metamorphism	Mastcam, MAHLI, APXS, ChemCam, CheMin, SAM-EGA
H_2O solute chemistry and flux	Evaporite assemblages and trace element distribution		
Redox and pH	Redox-sensitive (Fe, Mn, S, U, etc.) and pH-sensitive (jarosite, alunite, kaolinite, etc. vs. carbonates) mineral phase and abundance		
Biomineral interactions	Biomagnetite, biocarbonate, biosulfides, oxides, phosphate precipitation; bioleaching and dissolution products		

Summons et al., 2011. Astrobiology 11:157-180

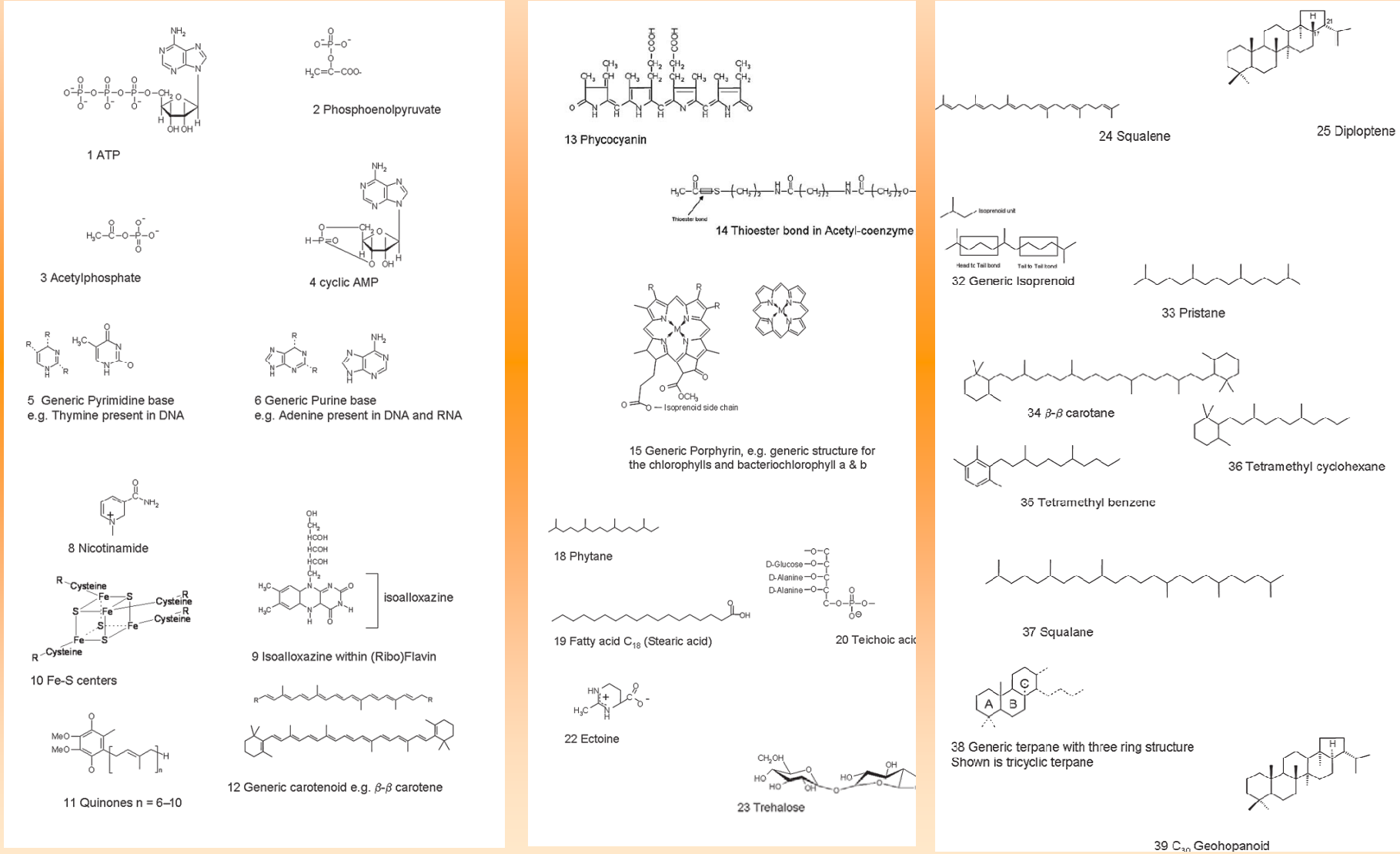
Target biomarkers for ExoMars

- Radiation and oxidation are the main factors affecting the stability of organic matter.
- In the best scenario, up to two meters are necessary to drill to obtain intact organic material

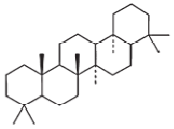


Parnell et al., 2007. Astrobiology 7:578-604

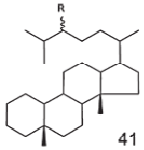
Target bimarkers for Mars



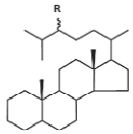
Molecular Targets for Mars



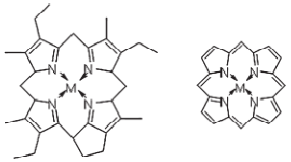
40 Gammacerane



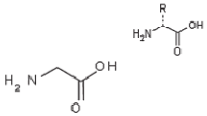
41 Diasteranes



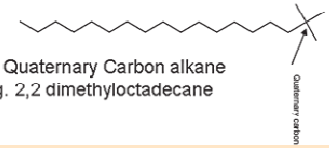
42 Steranes



43 Generic Porphyrin e.g. DPEP (deoxophylloerythroetio)



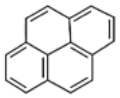
45 Generic Amino acids e.g. structure of glycine



46 Quaternary Carbon alkane e.g. 2,2 dimethyloctadecane

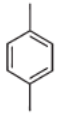
47 Napthalene

48 Coronene

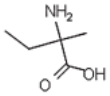


49 Pyrene

50 1,3-dimethylbenzene



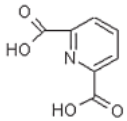
51 1,4-dimethylbenzene



52 Isovaline

53 α-aminoisobutyric acid

54 Benzocarboxylic acid



62 Dipicolinic acid

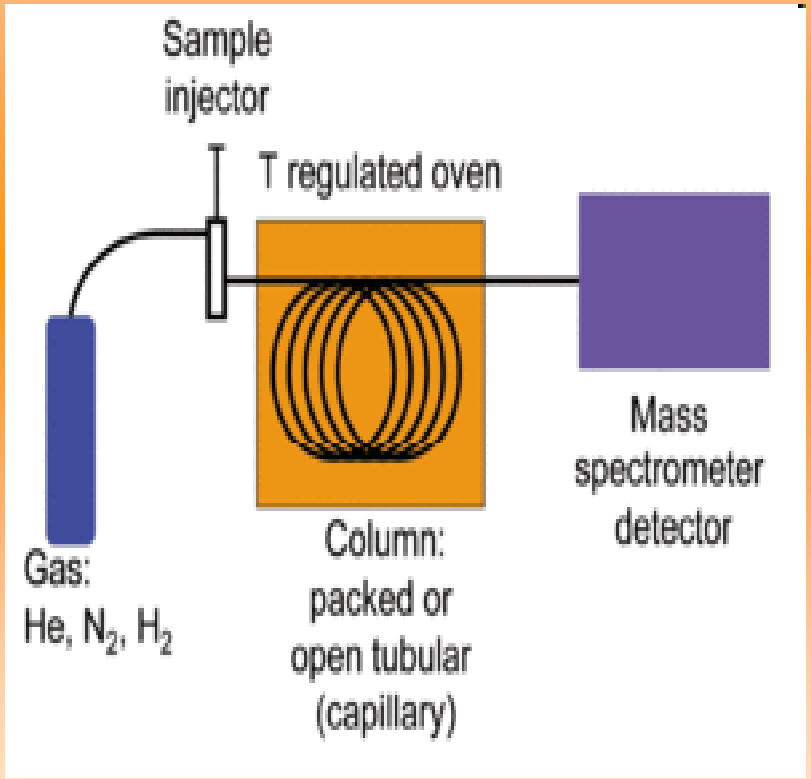
63 Hydrazine

Detection methods of biomarkers in planetary exploration

Technique Instrument	Presentation of sample	Target Type	Target Size
Pyrolisis/MS	Solids	Total Carbon	Elemental
IR	Solids	Chemical bonds	From small mol.
Urey-MOD	Solids	Volatiles aa, nucleobases, PAHs	100-500
GC-MS	Solids	Volatiles fatty acids, PAHs..	300-600 Da
Laser desoption TOF-MS	Solids	Small oligo and polymers	600-10.000 Da
SOLID (Immunoassays)	**Liquid suspension**	**from aa to cells**	**?100Da to μm particles organo-minerals, aggregates, etc.**
Microscope	Solid surface	Micro-structures	cells-colonies

Gas Chromatography Mass Spectrometry (GCMS): The preferred method for organic detection in planetary exploration

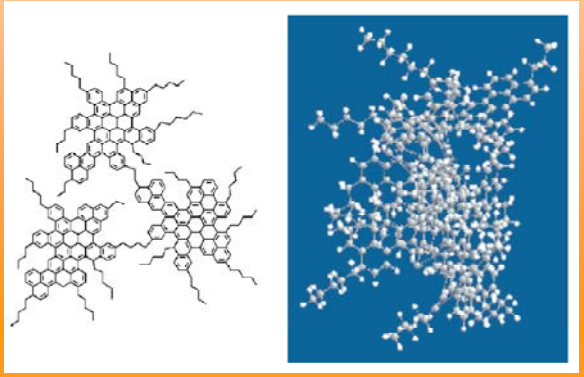
- Easy sample preparation:
- Sample is heated or pyrolysed and the volatile compounds enter in a GCMS instrument
- Accuracy and sensitivity
- Standards are required
- Only volatiles



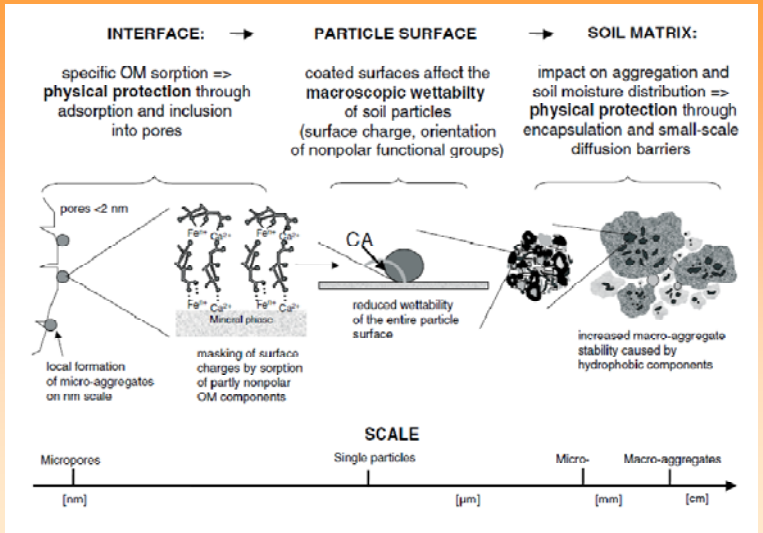
How is organic matter in nature?:
Extractable and Refractory matter

- Up to 90 % of the organic matter in a meteorite consists of an insoluble macromolecular material superficially resembling terrestrial kerogen. Only about 10% exists in the form of soluble characterizable molecules.

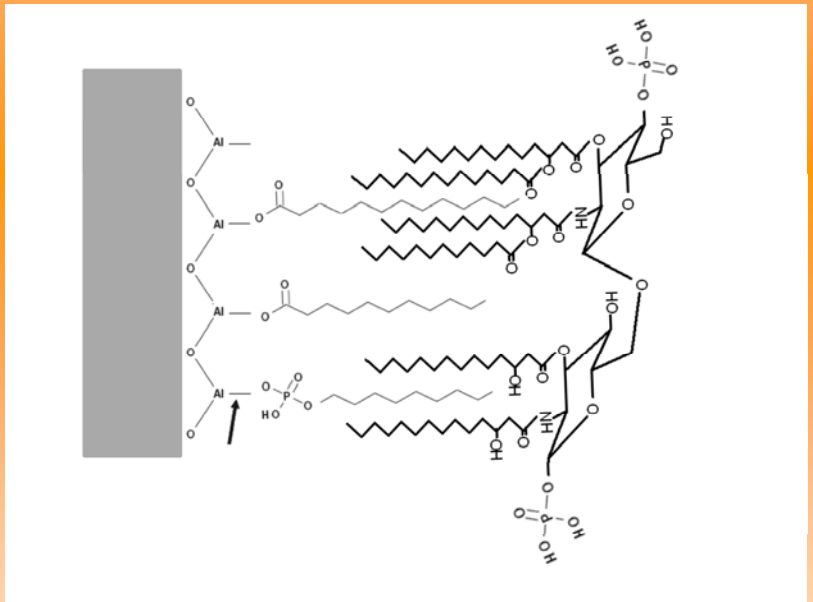
- On Earth, the organic matter interacts and modifies the mineral surfaces

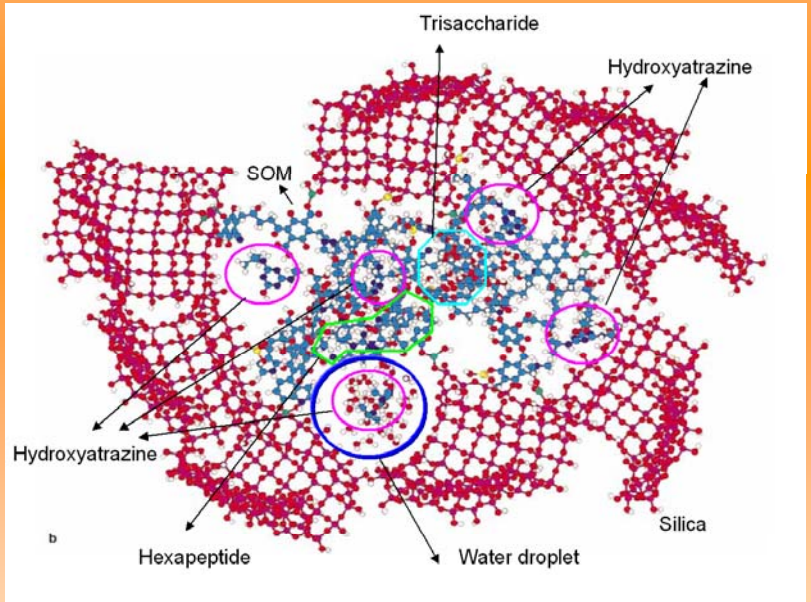


Proposed structure of the Solid carbonaceous and organic matter in Space



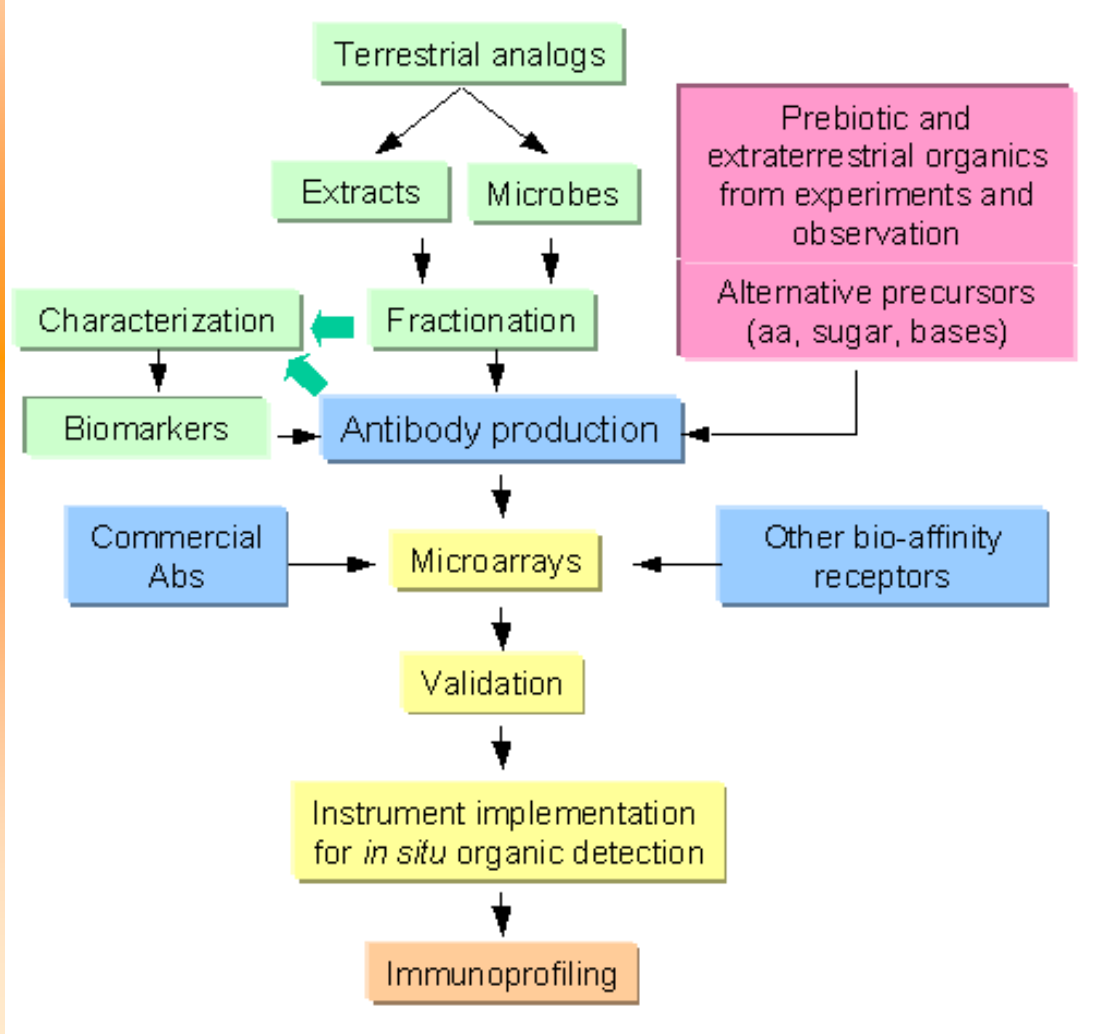
Organics and biomolecules bind to mineral particles





Bioaffinity-based microarray sensor for a "Signs Of LIfe Detector" instrument.

A shotgun strategy for antibody production for life detection



Natural extracts:
- Cells
- Exopolymers
- Proteins
- DNA
- Humic macromolecules

Cultures:
- Cells
- Exopolymers
- Proteins
- DNA
- Metabolites

Purified compounds:
- Known Proteins
- EPS, LPS
- Biosurfactants
- DNA
- Metabolites

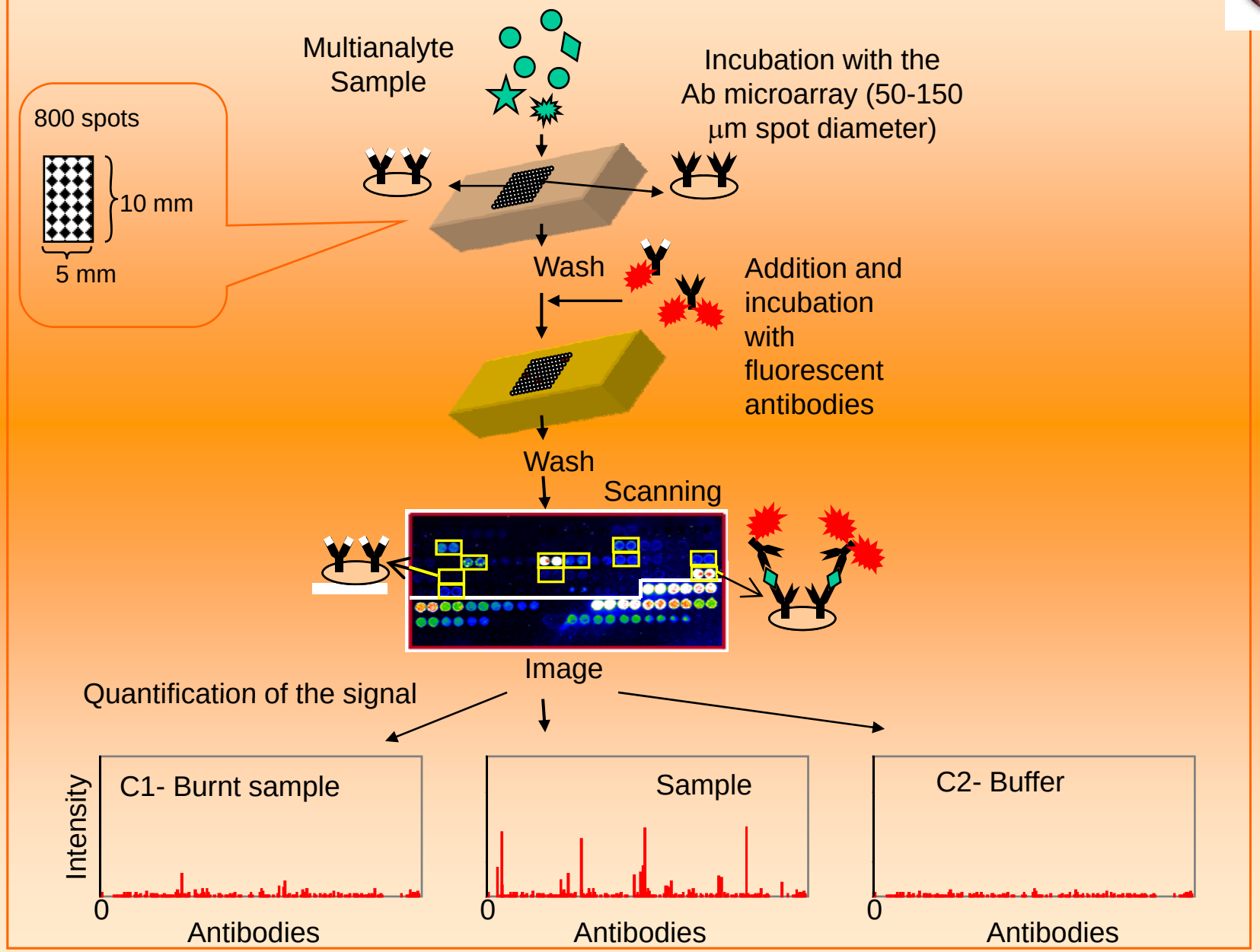
Commercial Abs:
- Aa
- Cells
- Proteins, etc.

Rivas et al., 2008; Parro et al., 2011

LDChip300 Target biomarkers

Target Type	Nº of Abs	Examples of Targets
Environmental Biomarkers	55	Polymeric substances from environmental samples
Microbial strains	110	Vegetative cells, spores and extracellular components (from 50 prokaryotic species)
Proteins and peptides	81	Universal proteins and specific metabolic enzymes
Nucleic Acids	4	ssDNA, dsDNA, thymine dimer, 5.8S rRNA (Euk)
Cell wall components	11	Peptidoglycan, NAG, NAM, LTA, LipidA, etc
Small metabolites	21	Universal metabolites: NAD+, cAMP, cGMP, etc.
Amino acids	16	Asp, Glu, Trp, Tyr, Phe, etc
Extinct biomarkers	3	Triterpens derivatives, carotane derivatives
Xenobiotics	3	Triazines
PAHs	2	Naphthalene, diphenylphenol
Carboxilic acids	2	Mellitic acid, benzoic acid
Polymeric compounds	2	Humic acids, polyglutamic
Total	307	

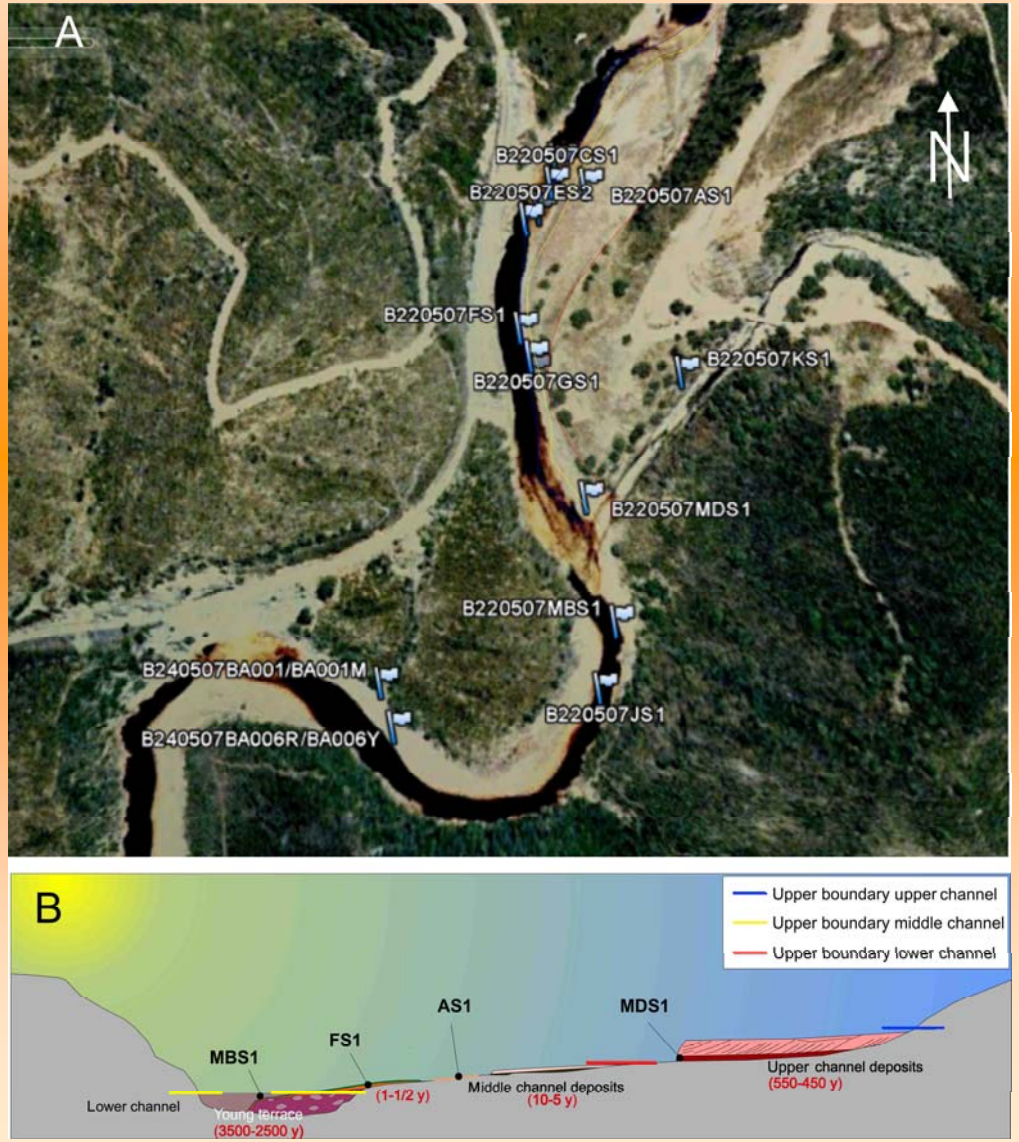
Sandwich Microarray Immunoassay (SMI) with LDCHIP300



Performance of LDCHIP300: a 300 antibodies containing microarray detecting polymeric biomarkers in rock samples

Blank control. Only positive control spots are detected

Terrestrial analogs: Biomarker profiling with old and recent sediments from Río Tinto

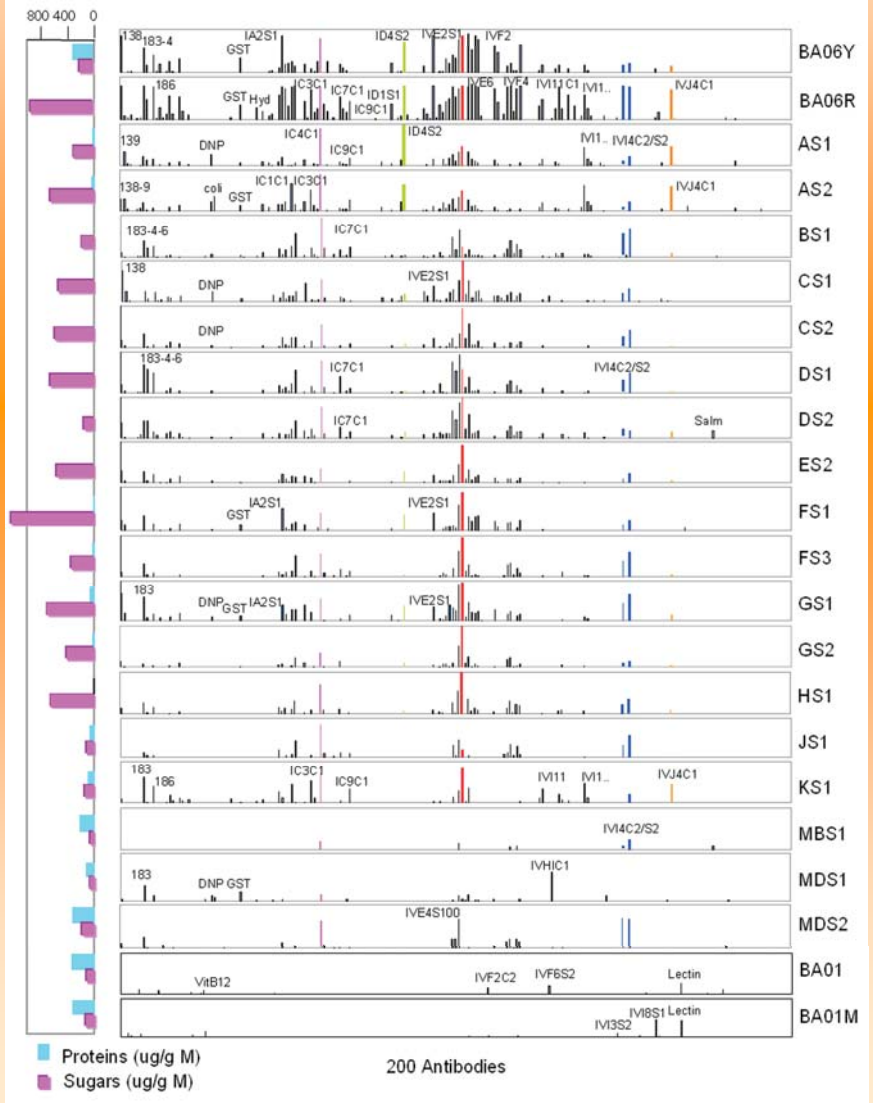


Old and recent sediments from Río Tinto

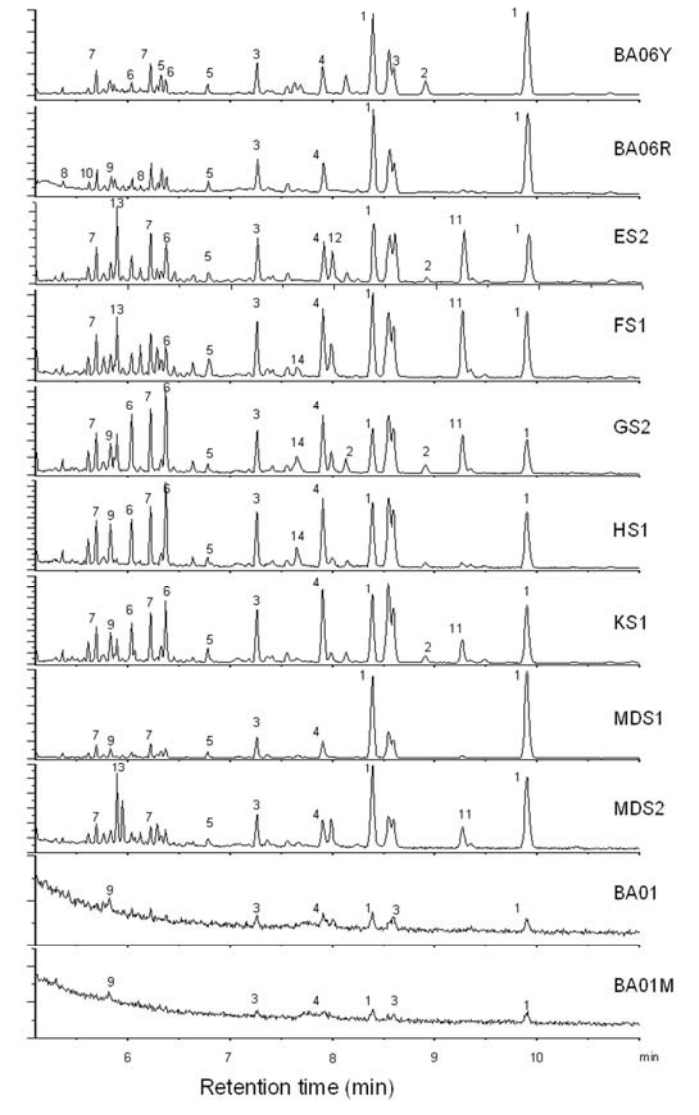
Parro et al., 2011, Astrobiology 11:29-44

Biomarker profiling of Río Tinto Sediments

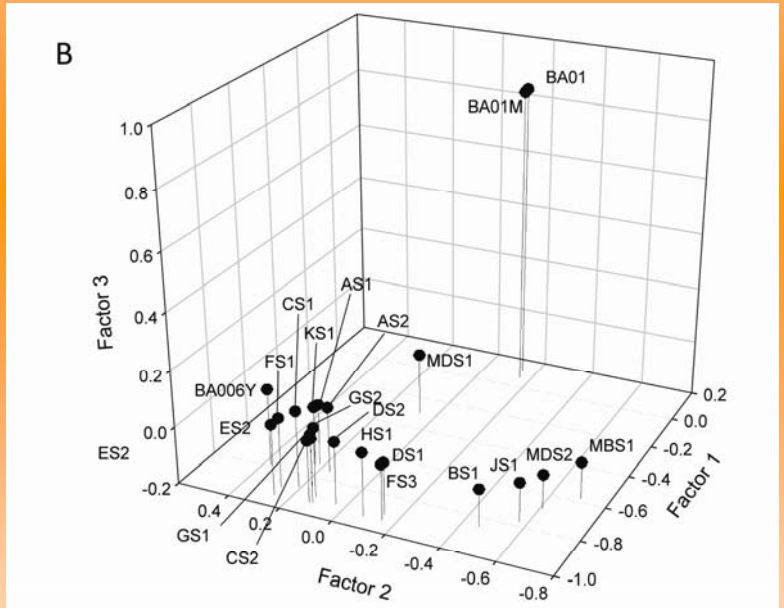
Immunoprofiling with LDChip200

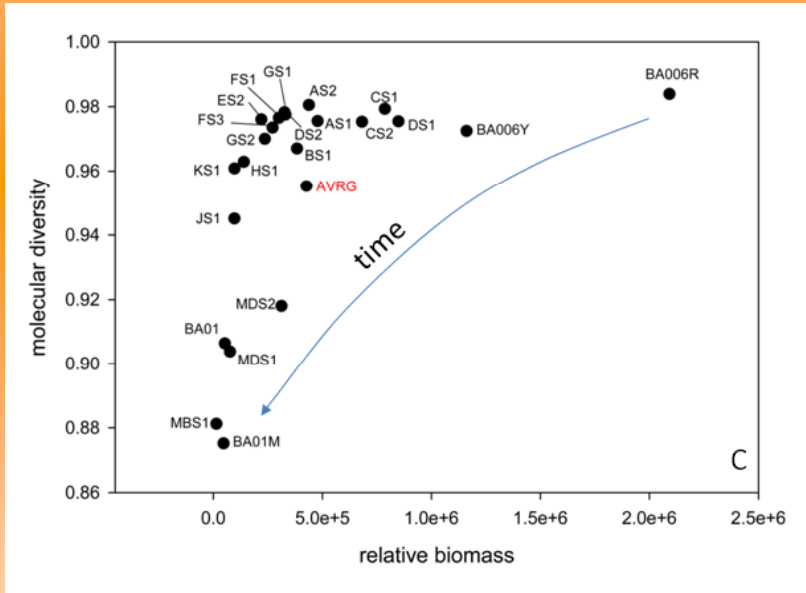


Monosaccharides from the EPS extract (GCMS)



Multifactorial analysis allows sample classification as a function of time and biomarker content

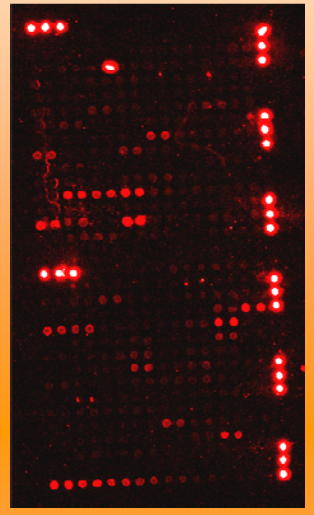




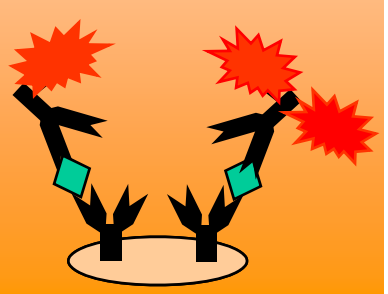
Parro et al., 2011, Astrobiology 11:29-44

Terrestrial Analogs: Mars on Earth

SOLID V2.0 instrument: a field prototype showing its 18 analysis modules, each of them loaded with an LDCHIP200



An example of the LDCHIP200 processed image from SOLID2

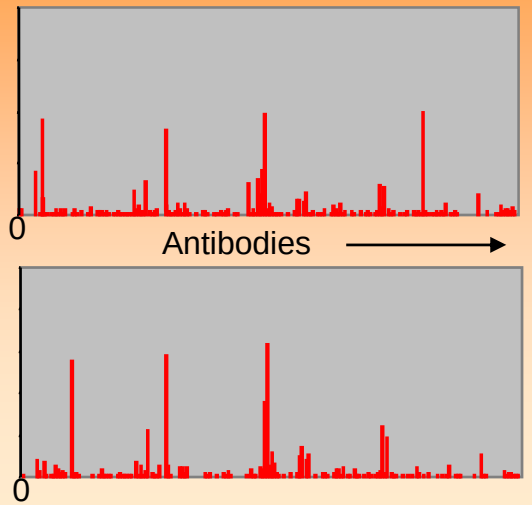


Sample analysis module

b

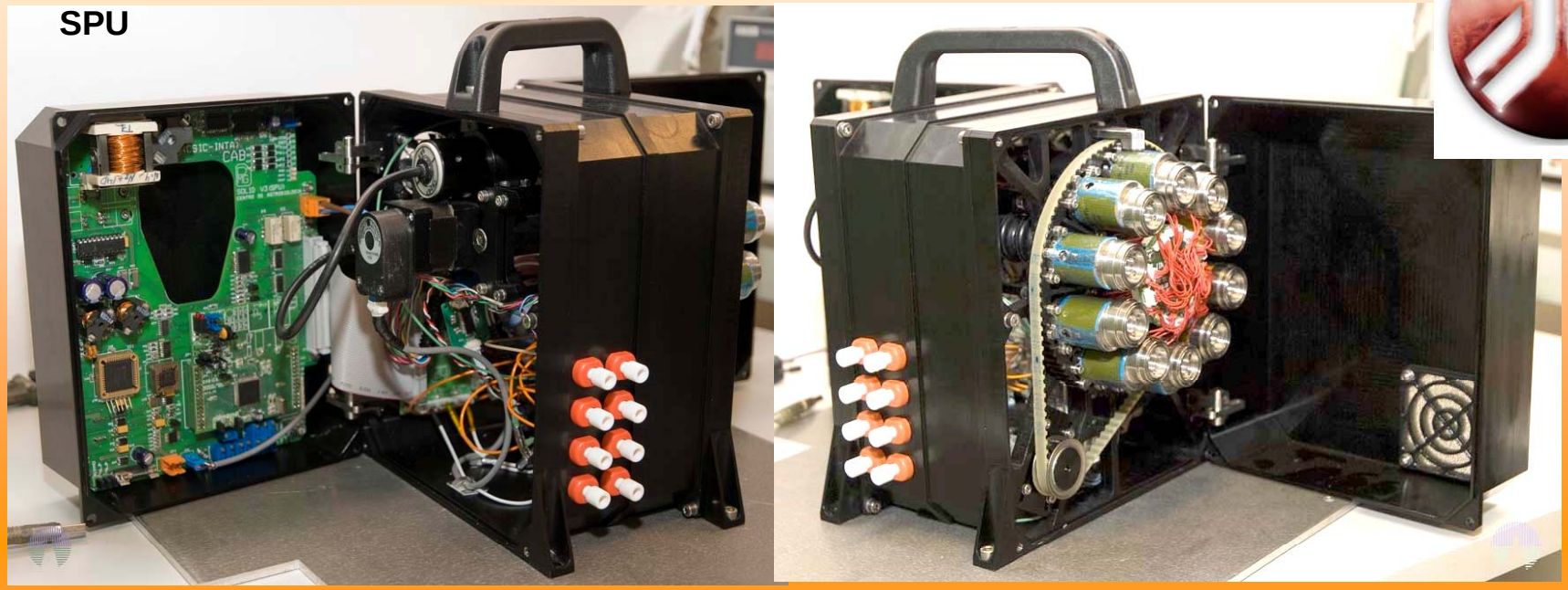
1. Homogenization chamber
2. Hybridization chamber
3. LDCHIP
4. Waste deposit
5. Additional chambers for reactants and antibodies

Quantification of fluorescent signal and plotting

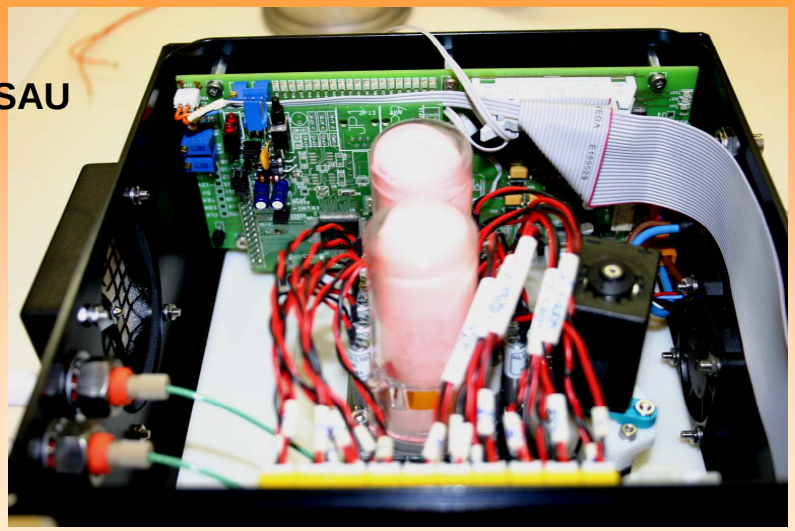


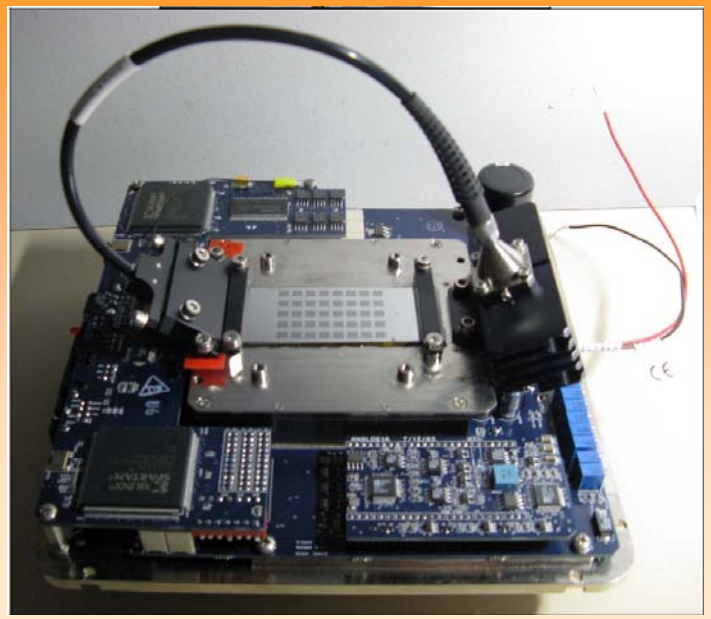
SOLID3.0: A Signs Of Life Detector

SPU



SAU





Parro et al., 2011, Astrobiology 11:15-28

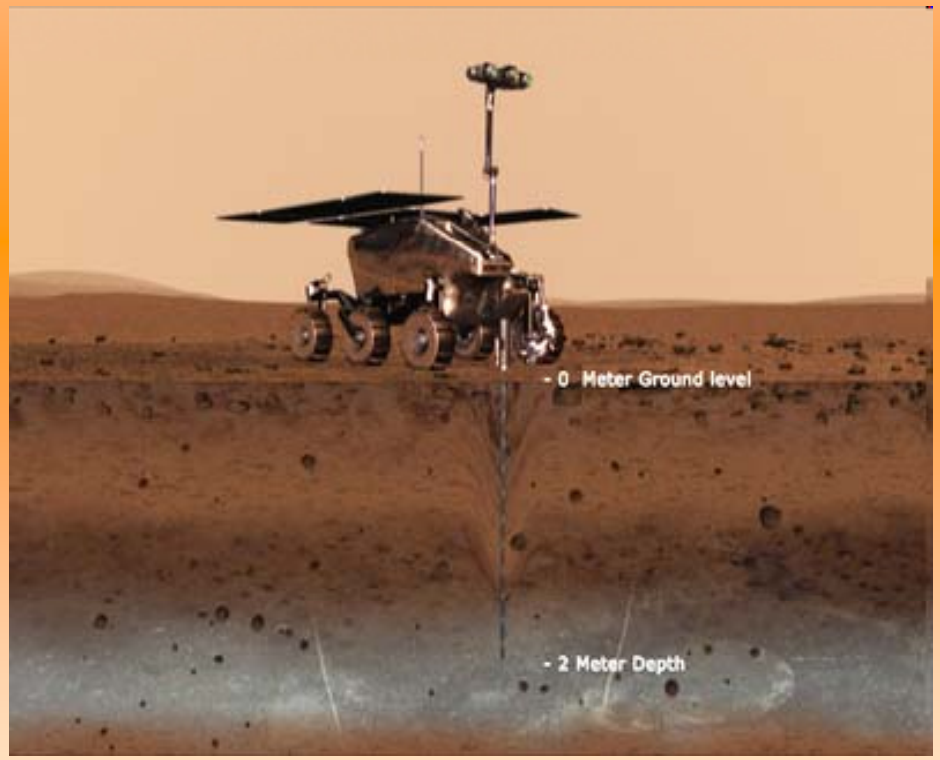
Next Challenge:
The ESA-NASA ExoMars programme 2016-2018

- **Investigate the Martian environment and to demonstrate new technologies paving the way for a future Mars sample return mission in the 2020's**
- **Search for signs of past and present life on Mars;**
- **Investigate how the water and geochemical environment varies**
- **Investigate Martian atmospheric trace gases and their sources.**

The main focus for the ExoMars Rover is the subsurface; it will penetrate the Martian soil, collect samples and analyse molecules in order to study the Martian geology and mineralogy, and search for biosignatures. The ExoMars Drill will extract soil samples down to a **maximum depth of 2 metres**, in a variety of soil types.

ExoMars payload instrumentation

- **PanCam** - The **Pan**oramic **Cam**era
- **MOMA** – **M**ars **O**rganic **M**olecule **A**nalyser (GC-MS and Laser Desorption-Mass Spectrometry LD-MS)
- **MicrOmega** A micro-imaging system
- **MARS-XRD** - **MARS X**-**R**ay **D**iffractometer
- **Raman Spectrometer**
- **WISDOM** - **W**ater **I**ce and **S**ubsurface **D**eposit **O**bservation **O**n **M**ars
- **Ma_MISS** - **M**ars **M**ultispectral **I**mager for **S**ubsurface **S**tudies
- **CLUPI** - **Cl**ose - **UP** **I**mager
- **LMC** - **L**ife **M**arker **C**hip



People

CAB

- **Biology**
  - Luis A Rivas
  - Mercedes Moreno
  - Miriam García-Villadangos
  - Yolanda Blanco
  - Patricia Cruz-Gil
  - Graciela de Diego
  - Manuel J. Gómez
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