

## Research Paper

# Searching for Life on Mars: Selection of Molecular Targets for ESA's Aurora ExoMars Mission

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### ABSTRACT

The European Space Agency's ExoMars mission will seek evidence of organic compounds of biological and non-biological origin at the martian surface. One of the instruments in the Pasteur payload may be a Life Marker Chip that utilizes an immunoassay approach to detect specific organic molecules or classes of molecules. Therefore, it is necessary to define and prioritize specific molecular targets for antibody development. Target compounds have been selected to represent meteoritic input, fossil organic matter, extant (living, recently dead) organic matter, and contamination. Once organic molecules are detected on Mars, further information is likely to derive from the detailed distribution of compounds rather than from single molecular identification. This will include concentration gradients beneath the surface and gradients from generic to specific compounds. The choice of biomarkers is informed by terrestrial biology but is wide ranging, and nonterrestrial biology may be evident from unexpected molecular distributions. One of the most important requirements is to sample where irradiation and oxidation are minimized, either by drilling or by using naturally excavated exposures. Analyzing regolith samples will allow for the search of both extant and fossil biomarkers, but sequential extraction would be required to optimize the analysis of each of these in turn. **Key Words:** Biomarkers—Biomolecules—ExoMars (mission)—Irradiation, Mars—Sample extraction—Search for Life. *Astrobiology* 7, 578–604.

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## INTRODUCTION

ONE OF THE HIGHEST-PRIORITY scientific goals of the exploration of Mars is the detection of organic matter and, in particular, organic molecules that may be evidence of past or present life. Specific types of biosynthesized molecules (biomarkers) would constitute such evidence. Numerous publications list, in general terms, the compound classes that should be sought. These publications place emphasis on amino acids, carboxylic acids, fatty acids, sugars, pigments, and cell membrane constituents/derivatives as a whole (*e.g.*, Simoneit *et al.*, 1998; Westall *et al.*, 2000; Fox, 2002; Buch *et al.*, 2003; MEPAG, 2006). They do not, however, conclude a list of specific molecules or prioritize them. Here, we propose a list of over sixty targets, which consists of a mixture of specific molecules and generic structures. The list derives from a workshop held in support of development of a Life Marker Chip for the European Space Agency's (ESA's) ExoMars mission of the Aurora program, which is currently scheduled for launch no later than 2013 (Vago *et al.*, 2006). The Life Marker Chip is one of several potential instruments in the Pasteur payload to be accommodated on board the ExoMars Rover. Since the mission's main scientific objective is to detect past or present life, the model payload (pre-final mission configuration) also includes a gas chromatograph–mass spectrometer system and the Urey instrument suite, which focuses on aromatic hydrocarbons, amino acids and their chirality, and the identification of soil oxidants. The model payload also includes Raman spectroscopy. A number of other instruments will help to characterize the geological context of the samples. The Life Marker Chip will use immunoassay techniques (molecular receptors based on antibodies) to detect specific organic molecules or classes of molecules (Sims *et al.*, 2005). This instrument has very high specificity.

The molecular targets listed here partially reflect the specific method of analysis. Thus, we include generic structures (compound classes), to which general antibodies might be raised, in addition to specific molecules. Some targets do not yet have antibodies, and in some cases it may prove impossible to raise them. Accordingly, the list is extensive, which allows for the eventual omission of some targets. The list is further prioritized (A, B, C, in decreasing priority) to allow selectivity in targeting and antibody develop-

ment, but with flexibility in final choice. The extensive list has wider value because it identifies potential targets that can also be useful for other methods of analysis. We anticipate the list will evolve, both in general relevance to Mars exploration and in the particular application of the Life Marker Chip.

## THE SEARCH FOR BIOMARKERS ON MARS

In 1996, the ESA's Microgravity Directorate listed six criteria that could be applied to test for extant or fossil life (Westall *et al.*, 2000). These were: (i) the presence of water, (ii) derived inorganic minerals (that lack organic C-H bonds), *e.g.*, carbonate, (iii) carbonaceous debris, (iv) organic matter with complex structure, (v) chirality, and (vi) isotopic fractionation between reservoirs. Specific biomarkers proposed include minerals with structures that are biologically created (Friedmann *et al.*, 2001; Allen *et al.*, 2001); distinctive fossils, microfossils or biofilm deposits (Brack *et al.*, 1999; Westall, 1999; Westall *et al.*, 2000; Cady *et al.*, 2003), the presence of chiral molecules (Sparks *et al.*, 2005), isotopic fractionation (in particular carbon) (Schidlowski, 1997), and organic molecules associated with terrestrial life. We focus here on the organic molecules.

We are faced with the constraint that our understanding of what constitutes a biomarker is strongly influenced by terrestrial biology. Life may have a very wide definition (Cleland and Chyba, 2002; Ruiz-Mirazo *et al.*, 2004), and accordingly a wide range of chemistries could support life, including nonaqueous systems (Benner *et al.*, 2004; Bains, 2004). However, the strategy for searching for life on Mars remains one based on water, which is appropriate for our current state of knowledge. Leaving aside the possibility that any martian life may be related to terrestrial life by a panspermia model, in which meteorites from one planet seeded life on the other (Mileikowsky *et al.*, 2000), there is still reason to believe that molecular evolution would follow predictable pathways. Particular types of biomolecules, along with membrane components, energy production and storage mechanisms, and use of biomolecules such as RNA and DNA to store hereditary information, appear to be ubiquitously employed on Earth, though this must partly reflect a single common

ancestor (Davies and Lineweaver, 2005). Furthermore, the energy yielding reactions used by life are likely to be universal. For example, thermodynamically favorable oxidation-reduction (redox) couples are likely to be used by life, whatever the biochemical architecture. Hence, the use of terrestrial life as a proxy may be a reasonable strategy.

## DEGRADATION OF ORGANIC MATTER

### *Carbon budget*

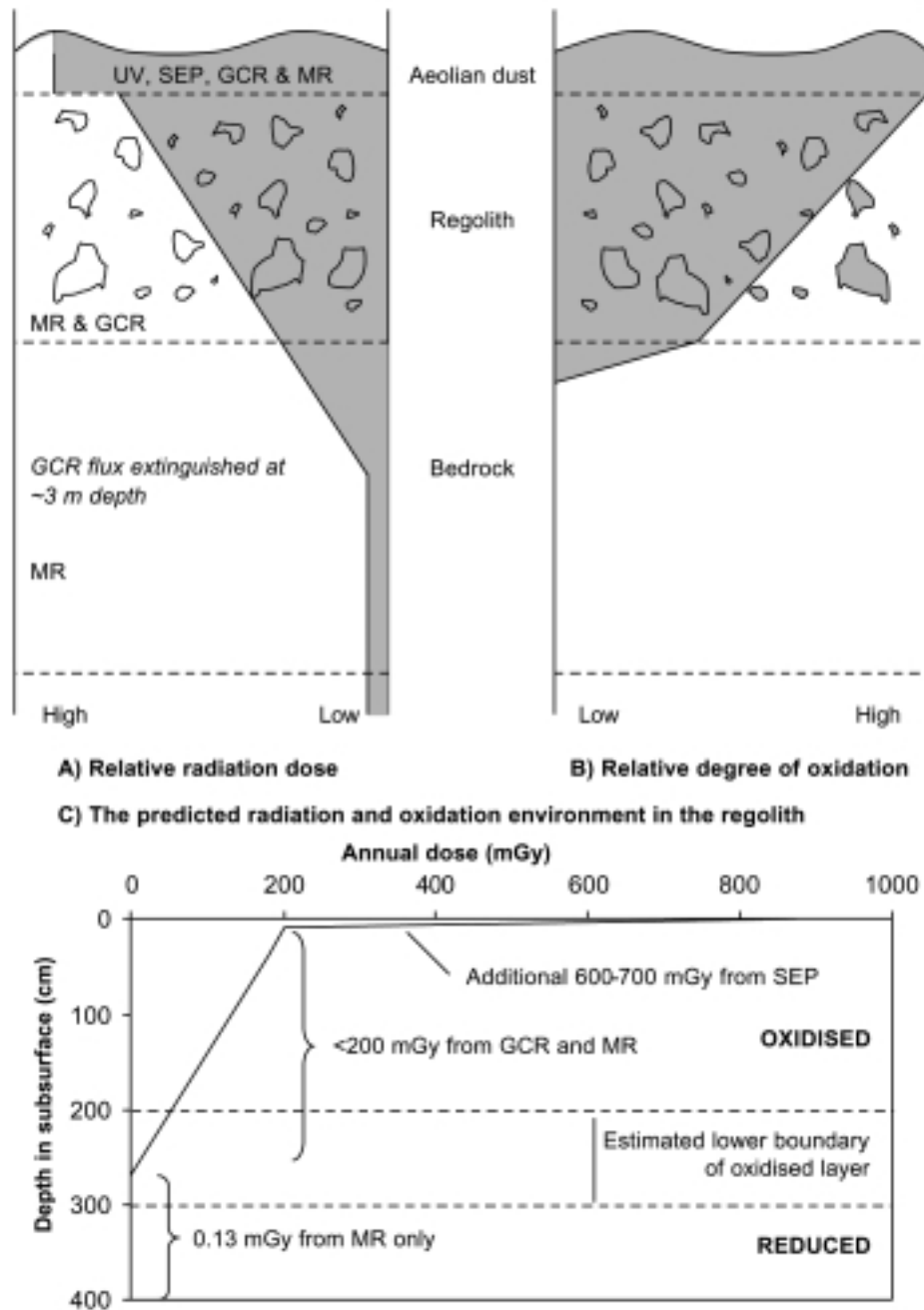
The martian surface, like the Earth, receives organic material from space, including meteorites, cometary matter, and interplanetary dust particles. The annual influx is estimated at approximately 240 tons per annum (Flynn, 1996). These materials are likely to include a range of organic molecules, from large aromatic structures to amino acids and carboxylic acids. The degradation rate due to ultraviolet irradiation (see below) probably means no net gain to the martian carbon budget (Stoker and Bullock, 1997). Evidence for carbon surviving on Mars comes from the carbonate alteration phases in martian meteorites (Bridges *et al.*, 2001). Data from meteorites also indicates an atmospheric component of carbon dioxide (Carr *et al.*, 1985) and associated carbon dioxide ice, and a mantle carbon reservoir (Grady *et al.*, 2004). The most interesting carbon-rich phase of astrobiological interest to date is methane in the atmosphere (Formisano *et al.*, 2004). Key aspects of this occurrence are as follows: (i) it is spatially variable, which indicates a localization of the source and (ii) the short residence time of methane in the atmosphere means that it is being replenished at a rate that makes meteorite impact or magmatic activity unlikely controls on its release (Oze and Sharma, 2005). A range of possible explanations for the methane include cometary impacts (Kress and McKay, 2004), abiotic water-rock interaction (Oze and Sharma, 2005; Lyons *et al.*, 2005), decomposition of buried organic matter (Oehler *et al.*, 2005), but also methanogenesis by extant microbial life (Krasnopolsky *et al.*, 2004; Krasnopolsky, 2006; Onstott *et al.*, 2006). After its initial formation, methane could have a long-term residence in gas hydrates (Pellenbarg *et al.*, 2003), fluid inclusions (Parnell *et al.*, 2006a), or fossil organic matter (Oehler *et al.*, 2005).

### *The martian radiation environment*

Four types of ionizing radiation can be identified: ultraviolet radiation (UV), solar energetic particles (SEP), galactic cosmic rays (GCR), and mineral radiation (MR). The first three are exogenous to Mars, so their radiation will only affect the uppermost layers of the martian subsurface. In contrast, MR is ubiquitous. The intensity of solar UV radiation at the martian surface varies as a function of latitude, but the SEP and GCR fluxes are regarded as uniform because of the isotropic distribution of the GCR flux and the deflection of SEP by the interplanetary magnetic field (Badhwar, 2004), combined with the long-term chaotic tilt of Mars (Ward, 1979). The absence of a strong magnetic field and the thin atmosphere allows a SEP dose of 600–700 mGy yr<sup>-1</sup> to reach the surface and penetrate ~10 cm, while UV photons are limited to a penetration of just a few  $\mu\text{m}$ . More penetrating are GCR, which are typically capable of penetrating ~3 m into the subsurface, with a maximum dose rate of ~200 mGy yr<sup>-1</sup> at about 20 cm depth, a result of the formation of collision cascades. GCR have an isotropic distribution in the sky, and variations with latitude are not expected. MR, which consists of  $\alpha$ -,  $\beta$ -, and  $\gamma$ -rays from the decay of radioelements such as thorium, uranium, and potassium, is much weaker, with a current estimated average dose rate of 130  $\mu\text{Gy yr}^{-1}$ , having diminished from 350  $\mu\text{Gy yr}^{-1}$  at 3 Ga. However, the accumulated dose since 3 Ga is substantial, around 740 kGy (Mileikowsky *et al.*, 2000; Pavlov *et al.*, 2002; Kminek and Bada, 2006). This is the only radiation type capable of altering material in the deep subsurface. The radiation environment in the martian subsurface is shown schematically in Fig. 1.

### *Oxidants in the martian soil*

The Viking craft detected no organic matter in the martian soil, at the surface and at a depth of 10 cm, at ppb level for complex organic species and ppm level for simpler compounds (Biemann *et al.*, 1977; Biemann, 1979). Viking data were used to infer the presence of at least one oxidant in the soil. Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) is the oxidant normally used in modeling because of its ease of photochemical formation in the atmosphere (Hunten, 1979) and soil (Huguenin, 1982). However, H<sub>2</sub>O<sub>2</sub> has a rather short lifetime of 10<sup>4</sup> s (~1 day) against UV photolysis, and it cannot account



**FIG. 1. Schematic illustration (not to scale) of the radiation (A) and oxidation (B) environments expected in the martian subsurface.** All three components of the subsurface (dust, regolith, and bedrock) illustrated here may not be ubiquitous, and their relative thicknesses on Mars will vary greatly from the examples displayed here. Atmospheric processes distribute the UV and SEP doses and the oxidants that, where present, are uniformly distributed throughout the aeolian dust, as illustrated above. Where the aeolian dust layer that overlies the regolith is absent, the underlying regolith (bedrock) is exposed to the UV and SEP dose, resulting in a greater absorbed dose than illustrated in (A). The underlying regolith is exposed to, assuming a dust layer greater than ~10 cm thick, only the GCR and MR fluxes. A thin regolith, as shown here for the purposes of illustration, allows the GCR flux and oxidant to penetrate to the underlying bedrock. Diffusion of oxidant into the bedrock is hindered, relative to the regolith, because of the lower permeability and lack of impact redistribution, which would result in the steeper gradient, as illustrated above. In contrast, assuming similar densities of regolith and bedrock, the rate of change of the GCR flux would be unaltered. The GCR flux is effectively shielded beneath ~3 m depth, leaving MR as the only source of radiation. The depth of penetration of the oxidant is unknown, but modeling suggests a depth of 2–3 m. In (C), the predicted variation of radiation dose with depth in the regolith is marked, along with an estimate of the thickness of the oxidized layer. Data is derived from Bullock *et al.* (1994), Zent (1998), Kolb *et al.* (2002), Pavlov *et al.* (2002), Lammer *et al.* (2003), and Kminek and Bada (2006).

TABLE 1. ESTIMATES OF THE TIME REQUIRED FOR VARIOUS SPORE SURVIVAL FRACTIONS AT VARIOUS DEPTHS IN THE MARTIAN SUBSURFACE

Depth in subsurface (m)	Estimated time (kyr) for spore survival fraction of:		
	$10^{-8}$	$10^{-6}$	$10^{-12}$
	Pavlov et al. (2002)	Kminek et al. (2003)	
0.5	115	75	200
1.0	160	275	550
2.0	400	—	—
3.0	150,000	—	—

for the thermally stable oxidant suggested by the Viking data (Klein, 1979; Zent and McKay, 1994). Other proposed species are superoxide ( $\text{O}_2^-$ ), hydroxide ( $\text{OH}^-$ ), and perhydroxyl ( $\text{HO}_2$ ) radicals (Hunten, 1979; Yen *et al.*, 2000). Superoxides could be generated in the martian atmosphere via dust storm processes (Atreya *et al.*, 2006). Uptake of monolayers of water may also have an important role in destroying organic matter, as the water allows oxidizing acid reactions to occur (Quinn *et al.*, 2005).

The results of a number of models that simulate the effects of oxidation indicate that oxidants

do not penetrate more than 2–3 m into the subsurface (Kolb *et al.*, 2002), a penetration depth similar to that estimated for the GCR flux. Accounting for the effects of impact redistribution, if the accumulation of oxidants began during the Late Heavy Bombardment (LHB), modeling indicates that the oxidized layer may be 130–150 m thick. Conversely, if the accumulation of oxidants began after the LHB, modeling suggests a ~50% probability of finding reduced material at 1 m depth (Zent, 1998).

The failure of Viking to detect organic compounds in the martian soil is not proof of their nonexistence (Navarro-González *et al.*, 2006). Although oxidation would ultimately convert all organic species to  $\text{CO}_2$ , it is very likely that metastable intermediates, undetectable by the Viking experiments, have accumulated. Benner *et al.* (2000) proposed that organic acids and salts would form by oxidation and accumulate in the Mars regolith. The meteoritic organic infall would be dominated by complex macromolecular matter, whose oxidation rate and breakdown products are uncertain. It seems likely that organic compounds are capable of surviving the oxidizing environment on the surface of Mars in some form over geological time, though relating those compounds to their parent species may prove difficult.

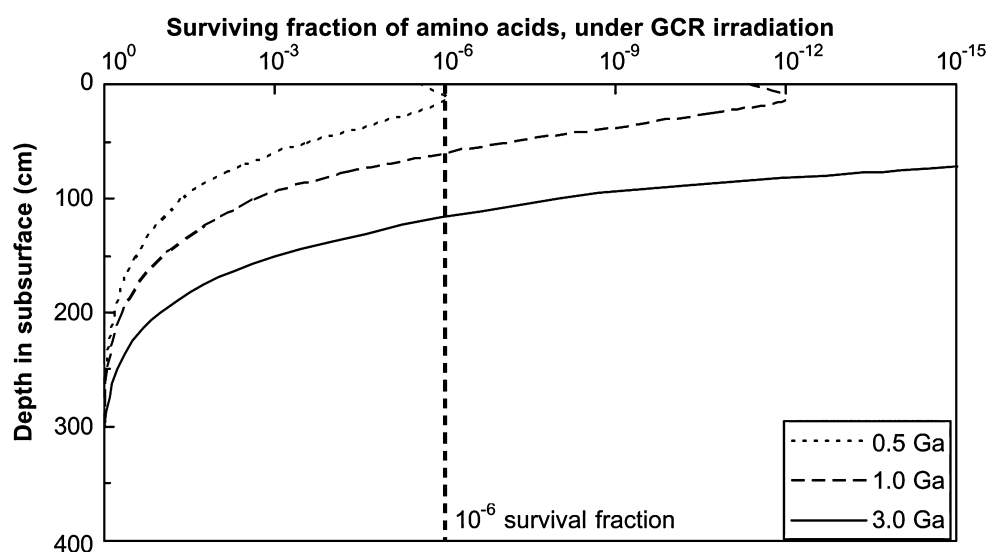


FIG. 2. The relationship between the survival of amino acids and depth in the subsurface upon exposure to the GCR flux since 0.5, 1.0, and 3.0 Ga. Given an initial abundance of 10 ppm and a detection limit of 10 ppt, a decrease in abundance of 6 orders of magnitude is possible, which could allow amino acids from 0.5, 1, and 3 Ga to survive the GCR flux at any depth below ~50 cm and below ~120 cm, respectively. However, this does not account for the destruction of compounds by oxidation. Based on Kminek and Bada (2006).

*Alteration of organics in martian environments*

The estimated annual dose from SEP and GCR, of 800–900 mGy, is not deadly to active life. Of more importance is the UV dose. Although 1 mm of Mars dust analogue provides complete protection (Mancinelli and Klovstad, 2000) from UV radiation, exposed bacteria and spores will be killed in minutes (Schuerger *et al.*, 2006), and amino acids will be degraded in hours (ten Kate *et al.*, 2005; Garry *et al.*, 2006). Consequently, detectable simple organic compounds, such as amino acids, and active life or bacterial spores are not expected to occur in aeolian dust. Larger compounds are less vulnerable to UV photolysis. Polycyclic aromatic hydrocarbons (PAH) should not be affected by UV on the martian surface, as the more energetic photons are absorbed by the atmosphere (ten Kate *et al.*, 2003). However, such UV-resistant species will still be vulnerable to radiolysis by the GCR and SEP fluxes. The effects of irradiation are difficult to predict, but irradiation will induce cleavage of bonds and the formation of free radicals, and accelerate ongoing reactions, such as those with the oxidants in the soil (Baumstark-Khan and Facius, 2001).

If aeolian dust is absent, the SEP and UV fluxes will irradiate the uppermost regolith or bedrock, with UV penetrating less than a millimeter and the 600–700 mGy yr<sup>-1</sup> SEP flux no more than 10 cm. The effects of irradiation on spores and amino acids have been estimated (Pavlov *et al.*, 2002; Kminek *et al.*, 2003; Kminek and Bada, 2006). Modeling suggests an annual GCR dose of 0.5 mGy at a shielding depth of 700 g cm<sup>-2</sup>, which represents 700 cm of material of density 1 g cm<sup>-3</sup> (Pavlov *et al.*, 2002) and corresponds to a depth of ~270 cm, assuming a regolith density of 2.6 g cm<sup>-3</sup> (Moore and Jakosky, 1989). These doses are insufficient to kill active life, but can, over geological time, deactivate spores and degrade organic species (Dartnell *et al.*, 2007). The survivability of organic species and spores increases with increasing depth as a result of decreasing GCR flux. Active bacterial life, which possesses mechanisms capable of repairing radiation damage, will be unaffected by the GCR flux. However, spores rely on repairing genetic damage upon germination. Hence, it is not the dose rate that kills spores, but the total dose absorbed. Experiments suggest that the fraction of viable spores in the deep subsurface will be 10<sup>-14</sup> after 100–160 Myr, given the current estimated annual

MR flux of 0.13 mGy (Mileikowsky *et al.*, 2000; Kminek *et al.*, 2003). Closer to the surface, the GCR flux accelerates the deactivation of spores, with a survival fraction of 10<sup>-12</sup> occurring after 200 kyr at 0.5 m depth and ~550 kyr at 1 m depth. Modeling by Pavlov *et al.* (2002) gave similar results, which suggests that a dose of 20 kGy would result in a spore survival fraction of 10<sup>-8</sup>. The radiation profile outlined in Fig. 1c gives the spore lifetimes in the martian subsurface detailed in Table 1. There is general agreement that spores exposed to the GCR and MR fluxes will be deactivated on a timescale of hundreds of kyr, rising to ~150 Myr when only the MR flux is considered.

Although spores may be deactivated by radiation, their organic remains, including amino acids (Neidhardt *et al.*, 1990), may still be detectable. Given plausible values for the initial abundances and detection limits, the GCR flux will not prevent the detection of amino acids from 0.5 Ga at any depth in the martian subsurface (Kminek and Bada, 2006; Fig. 2). Increasing the simulated amino acid exposure to 1 and 3 Ga results in upper depth limits of ~50 and ~120 cm, respectively. Given that the more intense UV and SEP fluxes cannot influence deeper than a mixing boundary, or penetrate deeper than 1 mm or ~10 cm, respectively, ancient amino acids should be detectable, if present, at around 150 cm depth. This result strongly suggests that, to be able to search for traces of fossil life, a mission like ExoMars should have the ability to collect subsurface samples from a depth of at least 150 cm in bedrock.

## CHOICE OF BIOMARKERS

Target compounds have been selected to represent meteoritic input, fossil organic matter, extant (living, recently dead) organic matter, and contamination (Appendix 1, Table 2). Fossil organic matter in the martian context is assumed to represent life that colonized early Mars (3–4 Ga ago). The tabulated compounds given in Table 2 provide a short list of feasible targets, which has been further prioritized to 3 ranks (A, B, C) of decreasing importance. The targets are a mix of specific molecules and generic compound classes, in some cases with overlap: this should allow flexibility in antibody preparation.

TABLE 2. SELECTION OF TARGET BIOMARKERS, THEIR LEVEL OF PRIORITY, AND BIOLOGICAL SIGNIFICANCE

<i>Target category no.</i>	<i>Priority</i>	<i>Biomarker</i>	<i>Molecular type</i>	<i>Polar molecule</i>	<i>Biological significance</i>
<i>Extant</i>					
1	A	ATP	Phosphate	Yes	Energy
2	A	Phosphoenolpyruvate	Phosphate	Yes	Energy
3	B	Acetyl phosphate	Phosphate	Yes	Energy
4	C	cyclic AMP	Phosphate	Yes	Signalling
5	A	Generic pyrimidine base	Nucleobase	Yes	Information
6	A	Generic purine base	Nucleobase	Yes	Information
7	A	DNA	Nucleobase	Yes	Information
8	A	Nicotinamide (generic NAD, NADP)	Vitamin	Yes	Electron transfer
9	C	Flavin (isoalloxazine ring)	Vitamin	Yes	Electron transfer
10	C	Fe-S centers	Redox center	Yes	Electron transfer
11	C	Quinones	Electron transport	No	Electron transfer
12	B	Generic carotenoid	Pigment	No	Light harvest
13	C	Phycocyanin	Pigment	No	Light harvest
14	C	Thioesters	Ester	Yes	Energy
15	A	Generic extant porphyrin	Porphyrin	No	Electron transfer
16	B	Chaperones	Protein	Yes	Stress protein
17	A	ATP Synthase	Protein	Yes	Enzyme
18	A	Phytane	Hydrocarbon	No	Archeal lipid
19	A	Fatty acids (1 or 2)	Carboxylic acid	Weakly	Lipid (18 bio, 19 not)
20	A	Teichoic acid	Amino acid + phosphate polymer	Yes	Gram-positive wall
21	A	LPS	Macromolecule	Yes	Gram-negative wall
22	B	Ectoine	Compatible solute	Yes	Osmotic protectant
23	C	Trehalose	Compatible solute	Yes	Osmotic protectant
24	B	Squalene	Hydrocarbon	No	Lipid biosynthesis (isoprenoid precursor)
25	C	Diploptene	Hopanoid	No	Bacterial membrane
26	B	Melanoidins	Macromolecule	No	Sugar degradation
<i>Sediment/cell extracts:</i>					
27	C	1. Acid mine drainage	Multiple	Some	Whole cells
28	C	2. Methanogens	Multiple	Some	Whole cells
29	C	3. Cyanobacteria	Multiple	Some	Whole cells
30	C	4. Mars energy users	Multiple	Some	Whole cells
31	C	5. Extract/abiotic mix	Multiple	Some	Whole cells
<i>Fossil</i>					
32	A	Generic isoprenoid	Hydrocarbon	No	Chlorophyll, quinones, archeal membranes
33	A	Pristane	Hydrocarbon	No	Chlorophyll, quinones, archeal membranes
18	A	Phytane	Hydrocarbon	No	Chlorophyll, quinones, archeal membranes
34	A	$\beta,\beta$ -carotane	Hydrocarbon	No	Fossil carotenoids
35	C	Tetramethyl benzenes	Hydrocarbon	No	Fossil carotenoids
36	C	Tetramethyl cyclohexanes	Hydrocarbon	No	Fossil carotenoids
37	C	Squalane	Hydrocarbon	No	Membranes (prokaryotes)
38	A	Generic ABC terpane	Hydrocarbon	No	Membranes (prokaryotes)
39	A	Generic hopane	Hydrocarbon	No	Membranes (prokaryotes)
40	C	Gammacerane	Hydrocarbon	No	Membranes (prokaryotes)
41	B	Generic diasterane	Hydrocarbon	No	Membranes (eukaryotes and prokaryotes)

TABLE 2. SELECTION OF TARGET BIOMARKERS, THEIR LEVEL OF PRIORITY, AND BIOLOGICAL SIGNIFICANCE (CONT'D)

Target category no.	Priority	Biomarker	Molecular type	Polar molecule	Biological significance
42	C	Generic sterane	Hydrocarbon	No	Membranes (eukaryotes and prokaryotes)
43	A	Generic fossil porphyrin	Porphyrin	No	
44	A	Generic straight-chain fatty acid	Carboxylic acid	Weakly	Membrane
19 a, b	A	2 individual fatty acids	Carboxylic acid	Weakly	18 bio, 19 not
45	A	Generic amino acid	Amino acid	Yes	
46	B	Quaternary carbon alkane	Hydrocarbon	No	Origin unclear
<i>Meteoritic</i>					
47	A	Napthalene	Hydrocarbon	No	N/A
48	A	Coronene	Hydrocarbon	No	N/A
49	B	Pyrene	Hydrocarbon	No	N/A
50	A	1,3 Dimethylbenzene	Hydrocarbon	No	N/A
51	A	1,4 Dimethylbenzene	Hydrocarbon	No	N/A
45, OR	A	Generic amino acid	Amino acid	Yes	N/A
52 AND	A	Isovaline	Amino acid	Yes	N/A
53	A	$\alpha$ -aminoisobutyric acid	Amino acid	Yes	N/A
54	B	Generic aromatic carboxylic acid	Carboxylic acid	Yes	N/A
55	A	Experimental abiotic	Complex		N/A
<i>Contaminants</i>					
56	A	Generic fungal			Fungal
20	A	Teichoic Acid	Amino acid + phosphate polymer	Yes	Cell wall Gram-positive
21	A	LPS	Macromolecule	Yes	Cell wall Gram-negative
57	A	<i>Staphylococcus</i>	Bacterium genus		Whole bacterium
58	A	<i>Streptococcus</i>	Bacterium genus		Whole bacterium
59	A	<i>Bacillus</i>	Bacterium genus		Whole bacterium
60	A	<i>Micrococcus</i>	Bacterium genus		Whole bacterium
61	A	<i>Pseudomonas</i>	Bacterium genus		Whole bacterium
62	A	Dipicolinic acid	Carboxylic acid	Weakly	Spores
63	A	Hydrazine (or equivalent)	Base	Yes	(Fuel)

N/A, not applicable.

### Extant biomarkers

Extant terrestrial biology is characterised by (i) energy conservation that involves redox reactions that result in the production of energy-rich storage compounds, (ii) informational macromolecules, and (iii) compartmentalization via lipid-rich membrane systems.

Central to energy conservation are virtually universal electron carriers and energy-rich, short-term storage compounds. The most ubiquitous and important of the energy storage compounds is the anhydride phosphate, ATP (see Table 2, No. 1, Priority A). There are, however, other simpler phosphate compounds, such as phospho-

enolpyruvate (2, A) and acetyl phosphate (3, B), that have an analogous function. Another phosphate compound, cyclic AMP (4, C), has a widespread signaling function (Robison *et al.*, 1968). In addition to energy-rich phosphate bonds, thioester bonds (14, C), such as in Coenzyme A, fulfill similar functions, particularly under anaerobic conditions (Weber, 2006). All of these energy-storage compounds are dynamic entities in living cells. These compounds are continuously broken down to drive biosynthetic reactions, resynthesized by catabolism, and do not persist in the environment outside cells due to the intrinsic lability of their high-energy bonds. As such, ATP in particular has been widely used as



an extant biomass marker, largely because a sensitive assay has been widely available for more than 30 years (Stanley, 1989). In principle, however, other compounds could equally be used if simple sensitive specific assays were available. A related target molecule is ATP synthase (17, A), a highly conserved enzyme complex that is universal in producing ATP (Murray *et al.*, 2004). Several target molecules have a role in the electron transport chain. These include flavin mononucleotide (Kaplan, 1955) of which the immunoassay technique could target the isoalloxane ring (9, C). Another widespread group of redox compounds have centers of coordinated Fe and S atoms, which could be targeted as a generic structure (10, C). However, the Fe-S center does not exist outside its protein, and since these proteins are quite sensitive to oxidants and rapidly decompose when outside a cell, their survival will be limited; hence, they are considered a low-priority target. These structures may have an ancient heritage and represent an autotrophic alternative to conventional ATP-driven reactions in metabolism (Maden, 1995). Another interesting group are the quinones, the analysis of which has been used to study microbial community structure (Hu *et al.*, 2001) (11, C), though they may also be formed abiotically as an oxidation product of polyaromatic hydrocarbons (Anderson and Johns, 1986). Nicotinamide (8, A) is selected as a generic structure within NAD and NADP, which are labile crucial and ubiquitous electron carriers, particularly in fermentation (Markham *et al.*, 2003). Another generic target is the porphyrin structure with associated side chains bearing carboxyl groups (15, A) at the center of the cytochrome structure used for electron transfer in cell membranes. The tetrapyrrole-based porphyrin structure also occurs within chlorophyll molecules that are used as the primary light-harvesting pigment in oxygenic and anoxygenic phototrophy, and, additionally, at the active site of one of the cofactors in methanogenesis. Tetrapyrrole components are also well-preserved in ancient sediments (Nagy, 1982; Eckhardt *et al.*, 1991; Xiong, 2006). Other targets involved in phototrophy are the secondary light-harvesting pigments, including a generic carotenoid structure (12, B) used by anoxygenic phototrophs that are well-preserved in sediments (Hebting *et al.*, 2006), and phycocyanin (13, C), one of the analogous pigments used by cyanobacteria (Izydorczyk *et al.*, 2005).

Deoxyribonucleic acid, or DNA (7, A), is by far the most stable of the informational polymers and constitutes the ultimate repository of information in extant life. Detection of DNA would be unequivocal proof of extant biology or, at least, recently dead biology. There is debate about how long DNA persists in the fossil record. Little disagreement exists with regard to time scales that span tens of thousands of years, but there are also controversial claims that DNA has persisted over hundreds of millions of years in particular sites such as evaporites (Pääbo *et al.*, 2004). The general consensus is that DNA does not have long-term stability and claims of million-year-level survival are misleading (Sykes, 1997; Grant *et al.*, 1998; Wayne *et al.*, 1999). The relative lability of ribonucleic acid, or RNA, and polypeptides makes these unlikely to persist outside cells over significant geological periods, though there are reports of identifiable polypeptides in material some tens of thousands of years old (Ambler and Daniel, 1991). The nucleobases within DNA and RNA are divided into a generic pyrimidine base (5, A) and a generic purine base (6, A). As far as specific polypeptide targets are concerned, molecular chaperones (16, B), proteins that cope with stress response such as heat shock, are present in relatively large concentrations in cells. For this reason, they are regarded as reasonable targets compared with other polypeptides that may be present as only a few molecules (Saibil and Ranson, 2002).

Lipid-based membranes in extant biology on Earth comprise two main types: those based on hydrophobic fatty acid chains and those based on hydrophobic-branched isoprenoid chains. Both of these classes of compound persist in the fossil record and are good biomarkers for extinct and extant life (Ourisson *et al.*, 1982). Straight-chain fatty acids (19, A) can also have an abiotic origin, but distinction of the C16 or C18 acid, which can have a biological origin, and the C19 acid, which has an almost exclusively abiotic origin, would be very helpful. Phytane (18, A), the isoprenoid core component of archaeal membranes, including those of methanogens (Woese *et al.*, 1990), is a particularly attractive biomarker in view of the evidence for methane in the Mars atmosphere (Formisano *et al.*, 2004). Squalene (24, B) is a precursor of the isoprenoids and is widely present in living organisms and, to a limited extent, in extracts of sediments (Peters *et al.*, 2005). Enzymatic cyclization of squalene produces the hopanoid

diploptene (25, C), which also is widely present in prokaryotes (Prahl *et al.*, 1992). Diagenesis of diploptene converts it to hopane (see below). Specific marker compounds of the outer structures of cell walls in Gram-positive and Gram-negative bacteria are teichoic acids (20, A) and lipopolysaccharides (21, A), respectively (Caroff and Karibian, 2003; Schär-Zammaretti and Ubbink, 2003).

Two other proposed compounds are ectoine (22, B) and trehalose (23, C), which are compatible solutes used by bacteria to cope with osmotic potential in hypersaline conditions (Grant, 2004). Melanoidins (26, B), polymerization products of sugars formed during early microbial degradation, have been proposed as well (Collins *et al.*, 1992).

An alternative approach is to raise antibodies to extracts from whole sediment samples and microbial communities (Parro *et al.*, 2005). This approach has the advantage that it maximizes the chance of detecting something, and it can be tailored toward particular energy sources that might be used by a microbial community. Hence, we have identified four possibilities, but at low priority: acid mine drainage samples (27, C), a methanogen-based community (28, C), a cyanobacterial community (29, C), and a community selected to use energy sources feasible on Mars (30, C). In addition, there is the possibility of designing mixtures of microbial extracts and experimental abiotic assemblages (31, C) to calibrate the response from biotic-abiotic mixtures.

#### *Fossil biomarkers*

The chemical fossils found within the geological record on Earth are typically the most recalcitrant biomolecules or the stable breakdown and alteration products of these biomolecules. This creates a bias within the list of fossil biomarkers for compounds that were originally present within cell membranes. These types of compounds are stable when exposed to the chemically hostile environment exterior to the cell.

As the chemical, physical, and biological conditions on the surface of Mars are different from those on Earth, it is possible that many biomolecules that have not been demonstrated to survive for long periods on Earth may persist for longer periods on Mars. Amino acids (45, A) and proteins are compound types that typically do not persist for long within the fossil record on Earth,

as they are very susceptible to microbial degradation (Tegelaar *et al.*, 1989), but they could be well-preserved within the geological record on Mars.

Phytane, pristane, and other isoprenoids (18, A; 32, A; 33, A) can derive from biomolecules with a diterpanoid structure, such as phytol. Changes to the original biological structure include the loss of the alcohol group and the saturation of any conjugated bonds (see Hebling *et al.*, 2006 for recent review). Chemical conditions during early burial can also lead to the shortening in chain length of isoprenoids, *e.g.*, pristane can degrade to phytane (Hughes *et al.*, 1995). Pristane and phytane are very stable. They are present in ancient sedimentary deposits (Brocks *et al.*, 1999) that contain fossil organic matter, and they persist in deposits that have been exposed to high temperatures (>200°C; Price, 1993). Furthermore, many precursor biomolecules can be modified to form pristane and phytane. For example, both chlorophyll and the tetraether lipids that comprise the cell membranes of archaea can yield phytol on the breakdown of the parent biomolecule (Didyk *et al.*, 1978). Thus, there is a plethora of biological precursors for phytane and pristane, and their stability makes these compounds high-priority targets.

Carotenoids (12, B) are relatively large (>C<sub>40</sub>) isoprenoids, though many of the fossil derivatives of these compounds are smaller. This is because the precursor molecules contain highly reactive conjugate bond systems that are not chemically stable over long periods. Terminal ring groups and part of the isoprenoid chain are lost or altered during early diagenesis, and the fossil derivatives such as  $\beta,\beta$ -carotane contain a saturated isoprenoid chain (34, A). The alkyl trimethylcyclohexene ring structure present in  $\beta,\beta$ - and  $\gamma$ -carotenes also becomes saturated to form the alkyl trimethylcyclohexane (Hall and Douglas, 1981; 36, C). Alternatively, it may aromatize (Koopmans *et al.*, 1996) to form alkyl trimethylbenzenes (35, C). Alkyl trimethylbenzenes also derive from the carotenoid isorenieratene (Summons and Powell, 1986). Within the oldest sedimentary deposits that contain fossil carotenoid derivatives on Earth, the saturated shorter-chain, single-ring compounds are far more abundant than the saturated C<sub>40</sub> fossil compounds. While certain fossil carotenoids are limited to specific kingdoms,  $\beta,\beta$ -carotene is produced by many microorganisms, and the shorter

chain compounds (35, C; 36, C) can derive from many carotenoid precursors.

Squalene (24, B) is an isoprenoid from which many other triterpenoids are biosynthesized by the cyclization of the squalene structure (Harwood and Russell, 1984; Ourisson and Rohmer, 1992; Volkman, 2005). Therefore, it may be reasonable to assume that squalane (37, C), its fossil counterpart, would be prominent within the fossil record. This is not the case, as squalene is very unstable and has been found to decay rapidly under ambient light (Archer *et al.*, 2005). Thus, even though squalene is widely found in living organisms and its fossil counterpart has been detected in the fossil record back to the Precambrian (Brocks *et al.*, 2005), squalane is not a high-priority target because of its generally low preservation potential.

Four of the target molecules share a similar polycyclic structure. Variations of the structure are present in the generic ABC tricyclic terpane (38, A) found in the fossil biomarkers, hopanes, gammacerane, and steranes. These polycyclic structures appear to be highly stable, and hopanes (39, A) are among the most recalcitrant biomarkers present in fossil organic matter (Peters and Moldowan 1991; Tritz *et al.*, 1999). Hopanes with carbon numbers fewer than C<sub>30</sub> can derive from diploptene (25, C) or diplopterol, or from other C<sub>35</sub> hopanoids and hopane degradation products. Hopanoids (though initially discovered within a tree resin) are greatly conserved within the prokaryote domain (Ourisson and Rohmer, 1992). Because of their great chemical stability and persistence within the geological record, hopanoids are high-priority astrobiological targets. Given the wide variation observed in both bio- and geo-hopanoids, a generic hopane antibody is most desirable. Gammacerane (40, C) is another highly stable terpane biomarker of microbial origin but typically not as abundant as the geohopanoids (Peters *et al.*, 2005); thus, it has been given a lower priority.

Steranes and diasteranes are chemical fossils derived from sterols. In a similar way to hopanes, these compounds are geologically stable. Diasteranes (41, B) are slightly more stable than steranes (42, C). Many sterols and fossil sterol degradation products have been reported (Killops and Killops, 2005); thus, a generic antibody test is preferable. Individual sterols can be kingdom-specific, and sterol synthesis is mostly confined to the eukaryote domain, though a very lim-

ited number of bacteria also synthesize sterols (Volkman, 2003). Unlike hopanoids, they require oxygen for their formation, which may not have been present on early Mars. Sterols are also less stable than hopanes (Tritz *et al.*, 1999), so steranes have received a lower priority than hopanes.

Long-chain fatty acids are the main constituents of the phospholipids that form the cell membranes of eukaryotes and true bacteria (Harwood and Russell, 1984). Although fatty acids may be synthesized abiotically by Fischer–Tropsch-type processes, the probability of such a process producing large straight-chain acids (up to C<sub>18</sub>) in length is small. Because these compounds are very stable relative to other compounds (Tegelaar *et al.*, 1989) and are synthesized by two of the three domains of life, they are high-priority biomarkers. Two tests are proposed: one for a generic straight-chain compound (44 A) and one to address biogenicity by comparing the quantities of stearic acid to nonadecanoic acid (19, A).

Porphyryns derive from several different biological precursors, most notably the chlorophylls, and comprise four pyrrole units in a closed ring (Killops and Killops, 2005). Metal chelation by the porphyrin structure increases stability; these compounds are geologically persistent (Baker and Louda, 1986) and occur in both coal (Bonnett, 1996) and oils (Sundararaman *et al.*, 1988). Given the wide range of sources and the geological survivability of fossil porphyryns, they have been given a high priority (43, A).

Another widespread group of structures with a record back to the early Proterozoic is a series of branched aliphatic alkanes with a quaternary substituted carbon atom (Kenig *et al.*, 2003). Their origin is unclear, and they are very rare in modern biological material. They may, however, have an association with sulphidic environments. Their wide distribution and longevity make them a good target (46, B).

#### *Markers of meteoritic organic matter*

The influx of meteoritic organic matter to a planetary surface includes contributions from interplanetary dust particles, comets, and meteorite fragments derived from asteroids (Chyba and Sagan, 1992; Sephton and Botta, 2005). The accumulation rate on Mars is higher than on Earth (Bland and Smith, 2000), and the ratio of meteoritic organic matter to any indigenous organic

matter on Mars should, therefore, be very high. Meteoritic organic matter comprises a sample of unequivocal 4.5 billion-year-old abiogenic organic compounds. Its constitution provides firm indications of what abiogenic organic molecules should look like on Mars. Table 3 indicates the types and abundances of molecules in the Murchison meteorite.

Abiogenic molecules show some clear characteristics that distinguish them from their biogenic counterparts (Sephton and Botta, 2005):

- i) Complete structural diversity. Abiogenic molecules are not the product of directed synthesis enabled by enzymes and, consequently, exhibit a wide range of structural isomers. In fact, most meteoritic compound classes show complete structural diversity.
- ii) Branched chains dominate. A logical consequence of structural diversity is that branched chains will dominate any abiogenic organic assemblage.
- iii) Decrease in abundance with increase in carbon number. For small (solvent-insoluble) molecules, the smaller molecules are more abundant.

Compound classes of interest include amino acids, carboxylic acids, sugar-related compounds, nucleobases, and polyaromatic hydrocarbons. To date, over seventy-five amino acids have been detected in the Murchison meteorite, only eight of which are found in terrestrial proteins, and eleven more have a restricted occurrence on Earth. The remaining amino acids appear unique to carbonaceous chondrites (Kvenholden *et al.*, 1970, 1971; Cronin *et al.*, 1995; Ehrenfreund *et al.*, 2001). In addition to the generic amino acid (45, A) selected as a fossil life marker, we propose the relatively simple and abundant isovaline (52, A) and  $\alpha$ -aminoisobutyric acid (53, A) as representatives of amino acids resistant to secondary racemization and non-terrestrial amino acids, respectively. Carboxylic acids are the most abundant solvent-soluble organic compounds in the Murchison meteorite, but in contrast to biological systems, small molecules are the most common (Sephton, 2002). Aromatic carboxylic acids are a predicted and observed degradation product of the most abundant meteoritic organic component (Sephton *et al.*, 2004) and are likely to be encountered on the surface of Mars (Benner, 2000). Consequently, we propose a generic aromatic carboxylic acid (54, B).

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

Compounds	Abundances	
	%	$\mu\text{g g}^{-1}$ (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
$\alpha$ -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

The most dominant molecules in carbonaceous chondrites, when all solubility classes are considered, are the aromatic or polycyclic aromatic hydrocarbons (PAH). PAH constitute over 60% of the organic matter in the Murchison meteorite; the vast majority of the organic matter is present as an intractable macromolecular component. PAH have no direct role in terrestrial biochemistry. They can be produced from biological organic matter only through partial combustion or maturation, following burial in the subsurface. PAH, therefore, are good indicators of abiogenic matter, though Ehrenfreund *et al.* (2006) hypothesized that they could be utilized in an alternative (non-terrestrial) pathway for the evolution of life. The choice of PAH targets is naphthalene (47, A), pyrene (48, B), and coronene (49, A). PAH of different sizes have variable susceptibilities to degradation by UV. The relative abundances of the three PAH can provide an insight into any record of organic destruction by UV.

The meta-form of PAH is the final form reached by meteoritic organic matter under thermodynamic equilibrium conditions (Sephton *et al.*, 1998; 2000). Meta-/para- ratios for dimethylated aromatics can be used as indicators of indigeneity (Hayatsu *et al.*, 1977). Thus, two forms of the aromatic structure dimethylbenzene can help to distinguish a meteoritic signature, which includes the meta-form 1,3 dimethylbenzene (50, A) from a more general abiotic or even biotic signature that could additionally include the para-form 1,4 dimethylbenzene (51, A).

We also propose an experimental abiotic assemblage (55, A), which will also be used in mixtures with microbial extracts (31, C, see above).

#### Contaminant biomarkers

In identifying potential contaminants, we have made the assumptions that a rigorous cleanup procedure will remove traces of fingerprints, varnishes, etc. from the spacecraft and the major source of contamination will be from particulate matter after the clean-up procedure (Mahaffy *et al.*, 2004). Microbiological studies of previous spacecraft show that, without rigorous treatment, the spacecraft will carry a high level of contamination (Puleo *et al.*, 1977). Five of the most widely distributed bacterial genera are targeted: *Staphylococcus* (57, A), *Streptococcus* (58, A), *Bacillus* (59, A), *Micrococcus* (60, A), and *Pseudomonas* (61, A). *Staphylococcus epidermidis* is shed in vast numbers

from human skin (La Duc *et al.*, 2003), and *Streptococcus* occurs in the human respiratory system. *Bacillus*, *Micrococcus*, and *Pseudomonas* occur in soil and are relatively resistant to ultraviolet irradiation and desiccation, so they are well-suited to survive in a clean integration room (Pierson, 2001; La Duc *et al.*, 2003). In addition to these specific genera, contamination detection would also incorporate monitoring of anomalous levels of teichoic acid (20, A) and lipopolysaccharides (21, A) as indications of gram-positive and gram-negative bacteria, respectively. Lipopolysaccharides represent an extremely sensitive test for contamination and were used on the Mars Exploration Rovers program. We propose a generic fungal target (56, A); additionally, bacterial spores can be recognized by dipicolinic acid (62, A), which occurs in the spore cores (Goodacre *et al.*, 1999; Farquharson *et al.*, 2004).

A spacecraft fuel marker will also be targeted. Currently, this is identified as hydrazine (63, A) (Ritz *et al.*, 1999), but could be changed according to the fuel used during the mission. Note that hydrazine also has a role as an intermediate in anaerobic ammonia oxidation (Strous *et al.*, 2006).

## DISCUSSION

### *Sampling fossil organic molecules on Mars*

It is widely accepted that Mars is more likely to have a fossil record of life, dating from a warmer, wetter time in early martian history, than an extant biota (Bibring *et al.*, 2006a; Knoll and Grotzinger 2006). If any extant anaerobic microorganisms inhabit the martian subsurface (Fisk and Giovannoni, 1999), their biomass is probably very low and difficult to detect (Westall and Southam, 2006). Accordingly, ESA's strategy is to focus on the detection of fossil life (Brack *et al.*, 1999; Vago *et al.*, 2006). There has been an emphasis on examining terrestrial analogues from our own early history, such as the Pilbara Craton in Western Australia (Westall, 1999; Brown *et al.*, 2004; Westall *et al.*, 2006a) and the Barberton Formation in South Africa (Westall and Southam, 2006; Westall *et al.*, 2006b). However, although we point to the preservation of microfossils in rocks of great age (Archean, >2.5 Ga, with convincing records back to 3.5 Ga) or in potential Mars analogues such as volcanic-hosted mineral veins and hydrothermal sinters (*e.g.*,

Westall, 1999; Hofmann and Farmer, 2000; Farmer, 2000), the likelihood of detecting organic molecules in samples of these materials from the terrestrial geological record is low, even when very carefully selected. On Earth, we progress through a series of stages that include: regional fieldwork; site selection, including access to localities that would be inaccessible autonomously; sample selection aided by extensive experience and real-time prioritization; extensive preparation to present the sample at its optimum; and analysis by high-resolution techniques that permit iteration in methodology. Despite this, many terrestrial samples may yield no useful results. Most martian samples have been spared the regional metamorphism that affects very old terrestrial samples, but even so, the expectation that we may detect molecular signatures in them is highly optimistic. We generally regard terrestrial molecular signatures >1.0 Ga as exceptional, even though they are only one third the age of the early Mars samples and are much more specifically sampled. Even then we require organic-rich samples; obtaining a signature from a veined volcanic rock or a hydrothermal deposit of just 100 Ma age and unaffected by metamorphism would be a significant achievement. If we could achieve on our planet what we hope to do on Mars, our molecular record of life on Earth would be much better than it is. So how can we optimize our chances on Mars? In the search for fossil life, by far the most realistic option is to target bedded sediment from the early geological record. Samples could be taken either *in situ* or where they have been excavated by fluid movement or impact ejection. As discussed above, one of the overriding factors for the preservation of organic material on Mars will be protection from irradiation and oxidation at the surface (Pavlov *et al.*, 2002; Kminek and Bada, 2006; Dartnell *et al.*, 2007). Hence, material excavated relatively recently would be required. Targeting bedded sediments that are widely distributed could improve the mission's chances of acquiring samples for analysis from recently exhumed locations. The arid, oxidizing environment encountered by the Opportunity Rover at Meridiani Planum has been suggested to imply poor preservation potential for fossil organic matter in sulphate-rich sediments (Sumner, 2004). However, sulphates, and evaporite minerals more generally (*i.e.*, including halite), have been widely advocated as a target for astrobiological investigation (*e.g.*, Cid and

Casanova, 2001; Mancinelli *et al.*, 2004; Stivaletta *et al.*, 2005), and microbial sulphate reduction is implicated in rocks that contain the oldest known evidence of life at ~3.5 Ga (Shen *et al.*, 2001). The organic content of evaporite minerals is usually very low, and biomolecules have only been extracted from samples dating to tens of millions of years (*e.g.*, Grice *et al.*, 1998). However, despite the likelihood that terrestrial evaporites experience recrystallization and dissolution due to interaction with water, thick-bedded evaporites retain primary fluid inclusion signatures over hundreds of millions of years (Roberts and Spencer, 1995; Kovalevich *et al.*, 1998), which offers encouragement that biomolecular signatures can also survive over this period. As water-rock interaction on Mars should have been less frequent than on Earth, there is similarly reason to believe that primary geochemical signatures may be preserved in martian evaporites. Accordingly, despite the acid, oxidizing conditions, the sulphate-rich sediments at Meridiani Planum are suggested as a habitable environment worthy of astrobiological investigation (Knoll *et al.*, 2005; Squyres and Knoll, 2005; Aubrey *et al.*, 2006). The evaporative salts may actually help to inhibit the decay of organic molecules (Tehei *et al.*, 2002).

#### *Optimization of sampling fossil and extant biomarkers*

A specific Sample Preparation and Distribution System is being developed for inclusion in the suite of analytical instruments in the Pasteur payload (Vago *et al.*, 2006). Samples would be milled to increase volatilization of organic material and increase the likelihood of extraction (Beatty *et al.*, 2004). However, there are more general issues relating to how extraction is tailored to our objective of seeking evidence for both fossil and extant molecules.

We assume that extant and fossil organic matter will be sought at a single site. However, while low-permeability samples are optimal for preserving fossil organic compounds, organic compounds incorporated after rock formation, from younger (including extant) life or meteoritic origin, are more likely to penetrate high-permeability samples. In a regolithic profile on solid bedrock, the downward decrease in permeability and porosity engenders an increasing potential for fossil organic matter. Regolith samples with well-preserved fragments of bedrock would have

potential for adhering organic matter of extant/meteoritic origin and encapsulated fossil organic matter. Thus, the relative importance of any extinct organic matter will increase with penetration depth into solid rock, while extant organic matter will be proportionally more important with increasing particle surface area. Depth and particle size (particle surface area) are both factors that can be considered in a sampling program. Up to 2 m depth may be accessed by the subsurface drill designed for the ExoMars mission (Magnani *et al.*, 2004; Vago *et al.*, 2006).

For the Life Marker Chip, a wet extraction process could be optimized for either fossil or extant organic matter. Most fossil biomarkers are apolar, whereas many extant biomarkers are polar, so the choice of extraction solvent can enhance what is measured. However, this implies the need for alternative extraction systems, which adds to the complexity of the procedure.

Sequential extractions from a single sample would enhance fossil or extant biomarkers in different extracts. Three options for this are:

- (i) Extraction prior to and after fine milling, which enhances the potential to detect extant and fossil biomarkers, respectively. This has been demonstrated in impact crater samples (Parnell *et al.*, 2007).
- (ii) Extraction from untreated particulate matter, followed by extraction after acid digestion to release components entrained in a mineral matrix. In terrestrial samples, this would be valuable for analyses on carbonates. On Mars, it may be a useful strategy to analyze sulphates, as demonstrated by Bowden *et al.* (2007).
- (iii) Extraction using an aqueous solvent (water) followed by an organic solvent, to extract polar biomarkers first and enhance recovery of the extant biomarker suite.

The main purpose of sampling with the aid of a drill is to penetrate through the surface zone where biomarkers are likely to be destroyed or altered by irradiation or oxidation. The effects of these alteration processes could also be reduced by sampling at sites where rocks from depth have been recently exposed by natural processes, which include impact cratering (Grier and Hartmann, 2000) and channel erosion (Baker, 2001, 2006; Burr *et al.*, 2002). Ejecta blankets can be mapped around many martian craters (Barlow *et al.*, 2000), and phyllosilicate-rich Noachian rocks

have survived without dehydration (Poulet *et al.*, 2005; Bibring *et al.*, 2006a, b). A crude estimate for small craters, assuming that ejecta are derived mostly from the top third of the crater, suggests that there is a high level of confidence that a single ejecta block derives from below a 2 m alteration depth for a crater of 300 m+ diameter (Parnell and Lindgren, 2006b). Although this strategy is designed to optimize the chance of detecting fossil biomarkers, large craters provide an additional means by which to search for possible deep subsurface biota from depths where liquid water is stable (Cockell and Barlow, 2002).

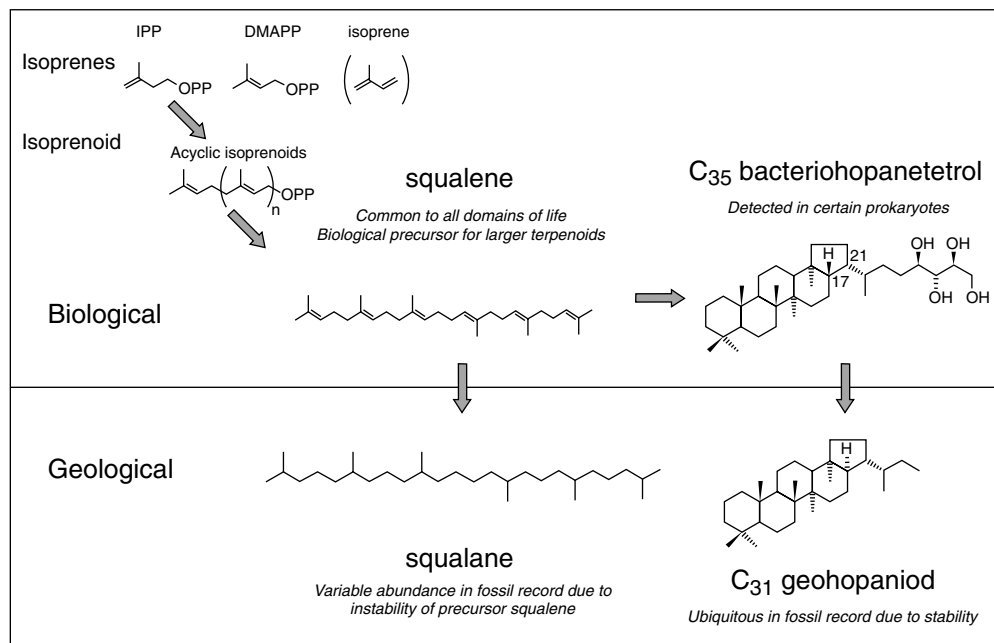
#### *Data interpretation*

The target compounds represent a range of levels of information, progressing from recognition of (i) organic matter of nonspecific origin, to (ii) organic matter of biological origin, to (iii) extant biological organic matter, (iv) organic matter of meteoritic origin, and (v) fossil organic biological matter. The choice of target molecules will evolve as progress is made with extraction protocols and assay development, but revisions can be incorporated into this information framework. The level of information obtained will stem not from detection of a single compound, but from the detailed distribution of compounds detected and undetected.

Three other trends can aid data interpretation: (i) Vertical concentration gradients at sites chosen for sampling at multiple depths can provide basic information on survival against alteration, but differential survival of different compounds will also support interpretation of organic matter composition; (ii) If any contamination is detected, this should become proportionally less important with successive analyses. Clearly, contamination should be avoided if at all possible, but being aware of it would still allow significant interpretation to be made from indigenous organic matter, especially if repeat analyses were made; (iii) Concentration gradients may be expected from analyses of generic structures to analyses of increasingly specific structures (Fig. 3).

## CONCLUSIONS

The prime purpose of this work has been to identify a range of biomarkers that may be targeted by the ExoMars mission and, in particular,



**FIG. 3.** Schematic pathway that shows the progressive development of terpenoid structures from isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), the biological isoprene equivalents. Terpenoids fulfill a wide range of biological functions, but all are constructed from two or more isoprenoid units (*e.g.*, acyclic regular isoprenoids). The long-term survival of terpenoids within the fossil record appears to be dependant on the stability of the molecule rather than biological abundance. Squalene, the biological precursor for many steroids and terpenoids, does not have the long term stability of 17 $\alpha$ ,21 $\beta$ (H) hopanoids. OPP, oxygen bond to diphosphate.

by the Life Marker Chip instrument. This list will inevitably change through developments in theory and instrumentation and, possibly, future observations on Mars. Nevertheless, such a list is very useful as a starting point for detailed preparations of the Life Marker Chip and will be of value to other life-detection programs.

The strategy for seeking biomarkers is underpinned by several conclusions about optimizing the chances for success:

- (i) With our current state of knowledge, our thinking is inevitably informed by terrestrial biology. Given the results of the Viking mission, proving the presence of *any* organic molecules on Mars, even of meteoritic origin, will constitute a fundamental step forward. If organic molecules are found, establishing their possible biological origin may be challenging. For this reason, the use of several instruments (certainly more than one) that can complement and reinforce each other's findings is very important for the credibility of the ExoMars results.
- (ii) Even if the mission were to encounter biology that is not Earth-like, we may derive im-

portant clues if the relative proportions of molecules that are recorded deviate from what is expected, *i.e.*, the distribution of molecules appears to be in disequilibrium.

- (iii) Seeking concentration gradients will also provide valuable information on contamination and surface alteration processes.
- (iv) In a mission whose prime objective is the detection of organic molecules, as opposed to exploration of new ground, targeting a known terrain is an attractive strategy that would allow optimization of the analytical procedure.
- (v) The analytical strategy needs to be developed in such a way as to ensure that both extant and fossil biomarkers can be detected.
- (vi) Both oxidation and galactic cosmic irradiation may cause alteration of fossil organic matter down to 2–3 m depth. This confirms the need to access material that has been protected by drilling and, preferably, in settings that have been excavated recently by natural processes.
- (vii) Finally, it is critically important that an instrument or instruments flying on the ExoMars mission be capable of characterizing the presence and reactivity of oxidants in the soil.



In the case where no organic molecules are detected, we need to understand why.

## ABBREVIATIONS

ESA, European Space Agency; GCR, galactic cosmic rays; MR, mineral radiation; PAH, polycyclic aromatic hydrocarbons; SEP, solar energy particles; UV, ultraviolet radiation.

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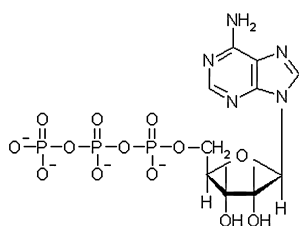
John Parnell

Department of Geology and Petroleum Geology  
University of Aberdeen  
Aberdeen AB24 3UE, UK

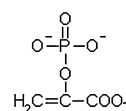
E-mail: J.Parnell@abdn.ac.uk

## APPENDIX

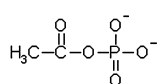
Structures of target molecules (as in Table 2). Macromolecules and complex materials are omitted.



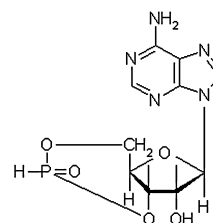
1 ATP



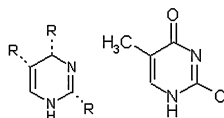
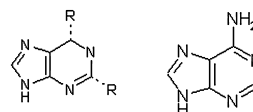
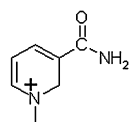
2 Phosphoenolpyruvate



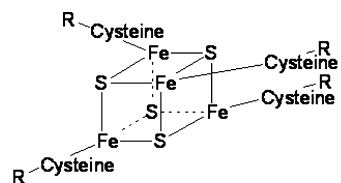
3 Acetylphosphate



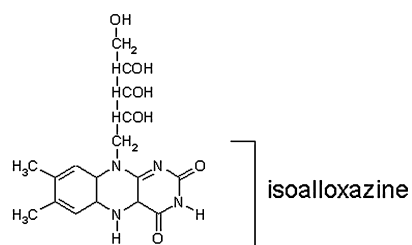
4 cyclic AMP

5 Generic Pyrimidine base  
e.g. Thymine present in DNA6 Generic Purine base  
e.g. Adenine present in DNA and RNA

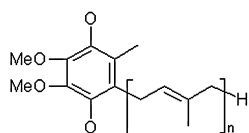
8 Nicotinamide



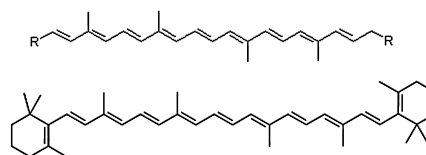
10 Fe-S centers



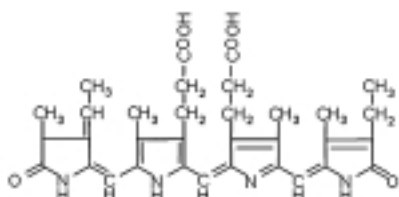
9 Isoalloxazine within (Ribo)Flavin



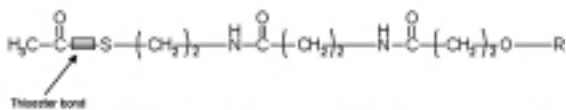
11 Quinones n = 6-10

12 Generic carotenoid e.g.  $\beta$ - $\beta$  carotene

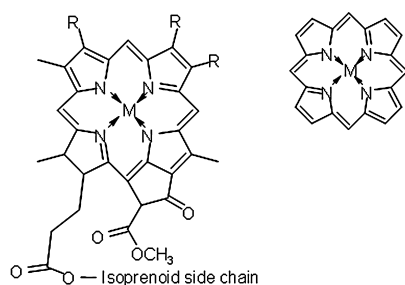
APPENDIX (continued)



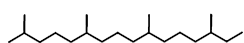
13 Phycocyanin



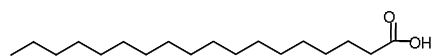
14 Thioester bond in Acetyl-coenzyme A



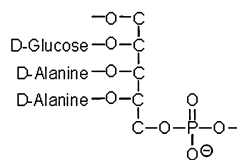
15 Generic Porphyrin, e.g. generic structure for the chlorophylls and bacteriochlorophyll a & b



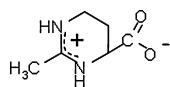
18 Phytane



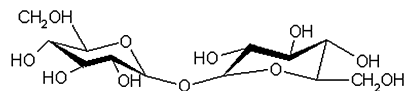
19 Fatty acid C<sub>18</sub> (Stearic acid)



20 Teichoic acid



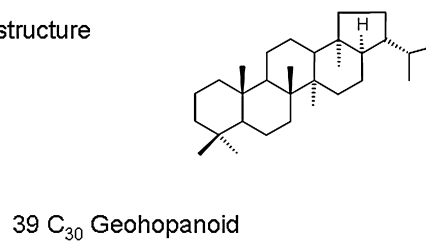
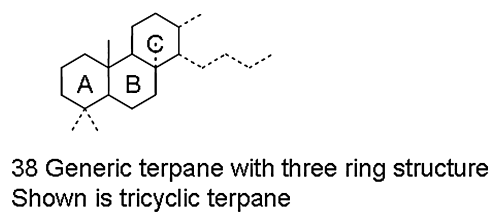
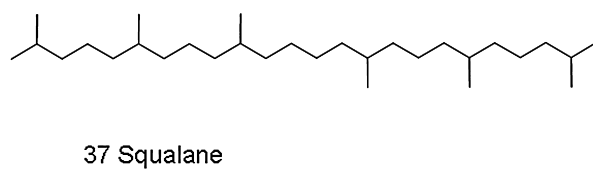
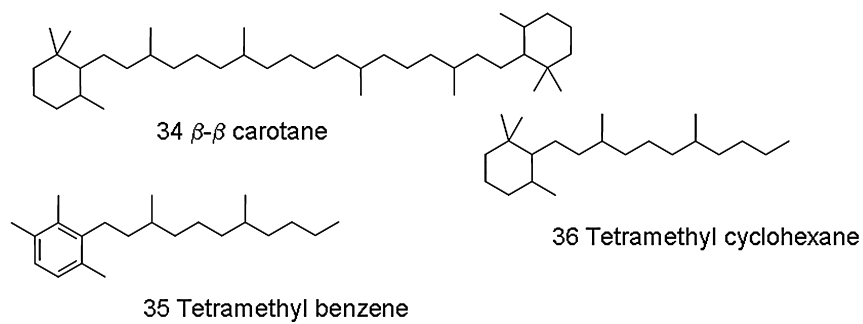
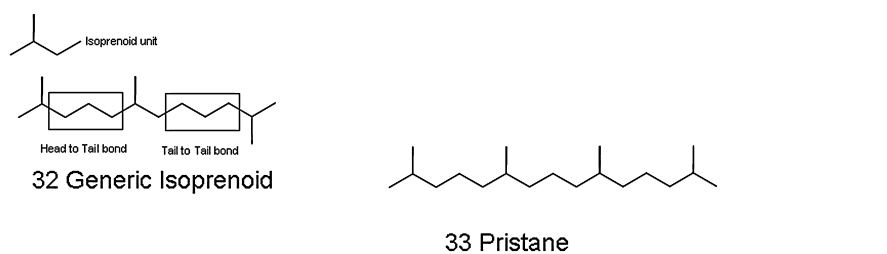
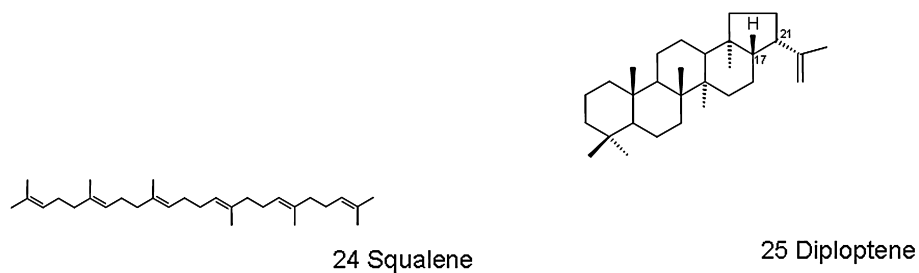
22 Ectoine



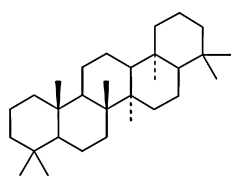
23 Trehalose



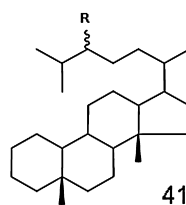
## APPENDIX (continued)



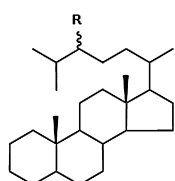
## APPENDIX (continued)



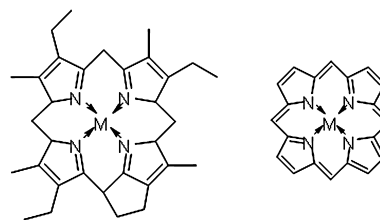
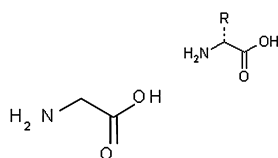
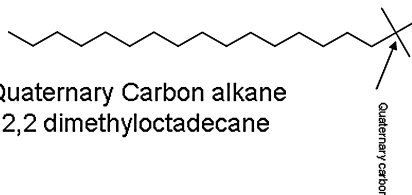
40 Gammacerane



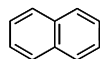
41 Diasteranes



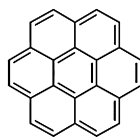
42 Steranes

43 Generic Porphyrin e.g. DPEP  
(deoxophylloerythroeti)45 Generic Amino acids  
e.g. structure of glycine46 Quaternary Carbon alkane  
e.g. 2,2 dimethyloctadecane

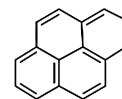
## APPENDIX (continued)



47 Naphthalene



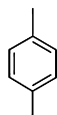
48 Coronene



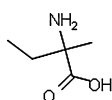
49 Pyrene



50 1,3-dimethylbenzene



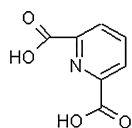
51 1,4-dimethylbenzene



52 Isovaline

53  $\alpha$ -aminoisobutyric acid

54 Benzoic acid



62 Dicolinic acid



63 Hydrazine