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Astrobiological instrumentation for Mars – the only way is down

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Abstract: In this paper, in this edition of the Journal commemorating the life and work of David Wynn-Williams, we consider approaches to the astrobiological investigation of Mars. We provide a brief account of the scientific rationale behind the approach presented here. In particular, we outline the capabilities of the Raman spectrometer for the detection of biomarkers. David Wynn-Williams was an active champion of this instrument who was keen to field-qualify a version in Antarctica with a view to flying a Raman instrument onboard a Mars-bound space mission. We examine a scenario for the deployment of such an instrument in conjunction with other instrumentation and argue that subsurface deployment of scientific instruments is essential if we are to succeed in detecting any evidence that may exist for former life on Mars. We outline a mission scenario – Vanguard – which represents a novel but low-risk, low-cost approach to Mars exploration that was conceived and developed jointly by one of the authors (Ellery) and the late David Wynn-Williams.

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Introduction

The recent discovery of bacteria living in extreme environments on Earth – extremophiles – has opened up the possibility that Mars and/or Europa might harbour life under similar conditions where water is available, i.e. deep underground and/or at hydrothermal vents. Extremophiles (micro-organisms living in so-called extreme conditions of temperature, pH, salinity, pressure, radiation, etc.) are also considered to be excellent models for early evolutionary processes on Earth. On Mars, the spatial distribution of chemolithotrophs, if they exist, will probably be somewhat diffuse and heterogeneous, depending on redox couples, such as sulphides of iron, as energy sources where two molecules bond to share an electron, releasing energy during the reaction (Russell & Hall 1997, 1999). The energy availability from the Martian geothermal flux of $\sim$30–40 mW m^{-2} places constraints on the size of any microbial community to an average of 5 microbes cm^{-3} yr^{-1} over 4 Gyr, suggesting that life on Mars, if it currently exists, is sparse. Furthermore, given a Martian crust thermal conductivity of 2.0–3.25 W mK^{-1}, the requirement for liquid water suggests that such biota will be resident at great depths ($\sim$2.5–5.0 km) at the equator, increasing to 6.5–13.0 km at the poles. Such environments may harbour chemolithoautotrophic organisms as primary producers from resident CO_2 and H_2

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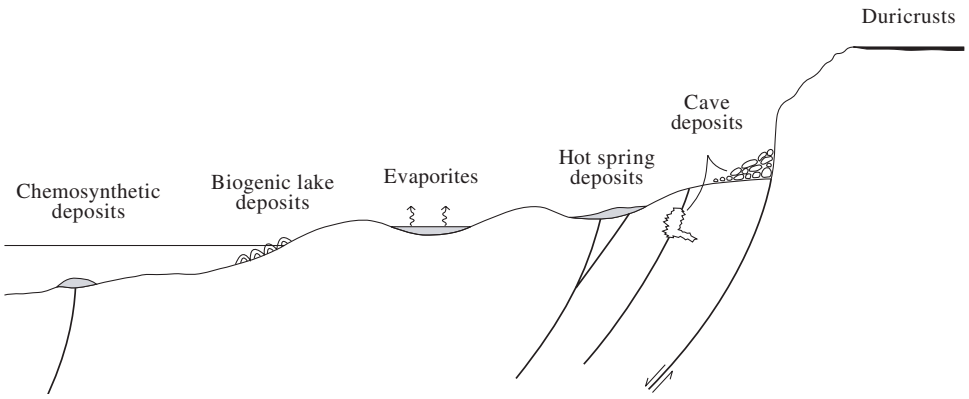

Fig. 1. Range of surface environments which astrobiologists have proposed should be targeted in the search for life on Mars. Model shows scenarios in vicinity of the fault-bound margin of a crater.

produced by the chemical reaction of water with Fe (II) minerals in basalt, but similar communities $\sim$1.5 km deep in the Columbia River Basalts are generally severely depleted in population, making them sparsely distributed. Furthermore, access to these deep subsurface habitats remains a technological challenge that is unlikely to be achieved in the near future. However, a more immediate step with a good chance of success in the quest for evidence of life on Mars, is the detection of biomolecules from former microbial biofilms, now buried and preserved in the near subsurface of former palaeolakes or other ancient water bodies. Numerous types of sedimentary deposit have been recommended by astrobiologists, representing a range of surface/near-surface environments (Fig. 1). These include chemosynthetic deposits around methane vents (Komatsu & Ori 2000), biogenic lake deposits including algal stromatolites (Westall *et al.* 2000), evaporites (Rothschild 1990), hot spring deposits (Farmer & Des Marais 1999), cave deposits (Grin *et al.* 1998) and soil zone duricrusts (Cid & Casanova 2001). Sedimentary deposits within palaeolakes indicative of aqueous origin are the primary targets – carbonates, phosphates, sulphates, halides, metallic oxides, sulphides and clays. It is such deposits that are of immediate importance for astrobiologists.

Life on Earth had evolved some 3.5 Gya in fully cellular form as evidenced by fossil stromatolite deposits, microbially precipitated $CaCO_3$ layers diagnostic of marine conditions (though the nature of the fossil deposits is currently under dispute, e.g. Schopf 1993). Evidence of $^{13}C/^{12}C$ isotopic ratios measured from deposits dating from $\sim$3.8 Gya suggests that metabolic activity was underway at this time (this is also disputed; see Schidlowski 1988). This is effectively geologically immediately following the last sterilizing impacts from the tail-off of the Earth's accretion process during solar system formation. Unfortunately, most of the evidence of the early epochs of Earth's geological history is denied to us due to Earth's active tectonics. However, the implication is clear – life emerged on Earth very rapidly once the conditions were amenable. If the conditions on early Mars were similarly amenable, and we assume that the emergence of life is a deterministic physico-chemical process, the corollary is that life could have emerged there also. The discovery of extremophilic bacteria on Earth living in habitats much more diverse than that associated with the 'continuous habitable zone' suggested that liquid water was a necessary (though not necessarily sufficient) condition for life (Madigan & Marrs 1997). Sufficiency requires an energy source, though not necessarily derived from the Sun. NASA's emphasis on 'follow the water' to find extraterrestrial life thus appears to be sound.

After the cessation of the heavy bombardment phase $\sim$3.8–3.9 Gya, Mars is believed to have had a much thicker atmosphere than at present, outgassed from its interior. This may have been >1 bar with a greenhouse effect resulting in surface temperatures high enough to permit the flow of relatively fresh liquid water to survive as evidenced by palaeolakes and water-cut channels (Carr 1986, 1987; Clifford 1993; Jakosky & Jones 1994). These could have been perpetuated for a long period by the flow of increasingly saline brines at temperatures as low as ~ -50 °C as in the Antarctic Dry Valleys. The nature of this greenhouse effect is disputed – the relative extent of the roles of sulphur dioxide and carbon dioxide in the Martian atmospheric greenhouse is currently unclear. The Martian meteorites recovered from terrestrial falls are all igneous in nature, but most show evidence of alteration by fluids such as the presence of carbonates, which are indicative of hydrous alteration on Mars and an atmosphere thick enough to support liquid water on the surface during early epochs.

Fox & Hàc (1997) investigated the $^{15}N/^{14}N$ isotope fractionation in dissociative recombination of N_2^+ and came to the conclusion that the Martian nitrogen isotope anomaly may indicate that Mars had in its past a much denser atmosphere of at least 114 mbar. Furthermore, atmospheric escape models suggest denser atmospheres with a pressure of up to about 1 bar 3.5 Gya (Lammer *et al.* 1996, 2001; Molina-Cuberos *et al.* 2001; Lammer & Bauer 2003). After strong atmospheric escape the pressure had dropped to low values with temperatures dropping below 0 °C (but still supporting saline liquid water), reaching its present state around 1.5 Gya. Lammer *et al.* (2002a) modelled the thermal and non-thermal

loss of water from Mars from the present to 3.5 Gya. The results of their study imply a total loss of water to space equivalent to a depth of about 17 m over the past 3.5 Gyr by considering a self-regulation mechanism between the loss of O and H as postulated by McElroy & Donahue (1972). They have shown that their modelled loss rates to space for O may keep up the self-regulation process for H, since flux values close to hydrodynamic escape for H may only be established before 3.5 Gya due to high solar extreme ultraviolet (EUV) fluxes and non-thermal O loss rates. The amount is lower than previous estimates by Kass & Yung (1995, 1996, 1999) of about 50–80 m, and could even be lower than 17 m if the H:O coupling proposed by McElroy & Donahue (1972) does not work perfectly. Measurements of D/H isotope ratios in the Martian atmosphere indicate an enrichment of D compared with H of about 5.5 times higher than that of the Earth's ocean value (Owen *et al*. 1988). The D and H can escape by atmospheric loss processes over the Martian past, where the atmosphere will be enriched in the heavier D isotope. Using the observed D/H isotope fractionation in the Martian atmosphere and the water loss value of about 17 m, one can estimate the water-ice reservoir that is exchangeable with the atmosphere on Mars using a Rayleigh law and the assumption that the Martian water had the terrestrial sea water ($D/H = 1.56 \times 10^{-4}$) or cometary ($D/H = 3.02 \pm 0.22 \times 10^{-4}$) D/H isotope ratio in the past (Eberhardt *et al*. 1995). Such studies indicate that Mars may at present have a comparable reservoir of a subsurface water-ice layer with a depth of about 10 m if the cometary D/H standard is used and about 3.5 m if one uses the terrestrial sea water D/H ratio about 3.8 Gya (Lammer *et al*. 2002b).

However, Mars gradually lost the majority of this atmosphere so that by 3 Gya, the pressure had dropped to 100 mbar with temperatures dropping below 0 °C (but still supporting saline liquid water), reaching its present state around 1.5 Gya. Hydrological models suggest four epochs of hydration on Mars with equivalent habitats in Antarctica:

(1) the first hydrological cycle ~4.2–3.8 Gya with abundant surface and subsurface water and characterized by life originating and evolving to photosynthetic cyanobacteria in surface waters and riverbeds;
(2) the second hydrological cycle ~3.8–3.1 Gya with surface water restricted to ice-covered hypersaline lakes with benthic cyanobacterial stromatolitic communities beneath the ice similar to those in Lake Hoare, Antarctica;
(3) the third hydrological cycle ~3.1–1.5 Gya with water restricted to moisture in porous rocks as in the translucent Beacon sandstone of Victoria Land, Antarctica forcing desiccation-tolerant cyanobacterial communities to near-starvation conditions;
(4) the fourth hydrological cycle ⩽1.5 Gya to the present, characterized by desertified of the surface of Mars, possibly underlain by permafrost with possible anhydrobiotic microbes and deep chemosynthetic life.

Any indigenous surface life that may have existed on early Mars would have retreated below ground into porous rock as endoliths and deep subsurface sediments as chemolithotrophs during surface desiccation and freezing. Some populations may still be preserved freeze-dried in a dormant state in permafrost. Alternatively, their fossils buried in stromatolitic sediments may have been flushed on to or near the surface by relatively recent transient hypersaline flows.

We start from the assumption that life originated on terrestrial planets such as Earth and Mars in hot, dark geothermal habitats using redox couples as energy sources (Corliss *et al*. 1979). For instance, chemoautotrophic bacteria in hydrothermal vents along the Galapagos Rift ~2.5 km deep in the Pacific Ocean use sulphides and other materials vented from the subsurface as their primary source of energy. We hypothesize that life expanded into illuminated surface habitats, and that the selective pressure of solar radiation on evolution would have driven the original microbiota to develop photosynthetic and UV-protective pigments to harness the ubiquitous and energy-efficient photosynthetically active radiation (PAR). This convergent evolution scenario between Earth and Mars is rooted in the nature of physico-chemical processes of biological energy transduction (unlike appeals to convergent evolution for the emergence of extraterrestrial intelligence, which are misapplied to behavioural evolution rather than morphological evolution). Furthermore, estimation of the time required for the evolution of cyanobacteria from a prebiotic condition is just $\sim 7 \times 10^6$ yr, including bottlenecks (the origin of self-replicating systems and the emergence of protein biosynthesis straddling the RNA world) constrained to the period for the complete recycling of the ocean through the prebiotically destructive deep-sea vents every 10^7 yr (Lazcano & Miller 1994). Microbial communities that migrate towards the surface to exploit light will be restricted to the focused zones penetrated by PAR and UV radiation. The result of this constraint is a laterally homogeneous stratified biofilm, influenced by the seasons. They would have required protective pigments against UV radiation damage to vital molecules such as nucleic acids and proteins. Such biofilms typically contain high concentrations of photosynthetic and UV-protective pigments with distinctive functional molecular structures produced as a response to environmental stress. If this is correct, the occurrence and spatial distribution of preserved pigments or their derivatives in the near-subsurface profile beneath the oxidized zone should be detectable *in situ* by non-destructive laser Raman spectroscopy, as on Earth. Both photosynthetic and UV-protective pigments generate distinctive Raman spectra, independent of their hydration state. Microbial communities in extreme Antarctic desert habitats analogous to those of early Mars indicate that these key biomolecules can be detected *in situ* within the biochemical pool of mixed populations. Photosynthetic cyanobacteria that are dominant in extreme Antarctic desert habitats near the limits of life on Earth, are good sources of pigments and other biomolecules that could act as biomarkers for former life on Mars. The overall Raman spectrum not only reveals organic matter and the composition of its mineral substratum, but also reveals the nature of biomolecules present and their potential function (such as UV absorption).

Scientific rationale for Mars astrobiology

We assume that the gradual loss of water from Mars would have led to ever-shrinking bodies of water of increasing salinity as in the lakes of the Antarctic Dry Valleys (such as Don Juan Pond), the coldest, driest places on Earth with <10 mm of precipitation per year. The lowest limit for metabolic activity detected in the natural environment is microbial enzyme activity (acetate incorporation into lipids) in Siberian permafrost at $-20\,^{\circ}$C (Rivkina *et al.* 2000). In saline Antarctic soils, water does not freeze at a unique temperature but undergoes a phase change so that some liquid can exist at $-70\,^{\circ}$C (Anderson & Tice 1989; Wynn-Williams *et al.* 2002b). Viable (but inactive) bacteria are found in the hypersaline Don Juan Pond (saturated with CaCl solution), Antarctica at $-53\,^{\circ}$C. Freezing point does not imply the absence of liquid water – NaCl lowers the melting point of water to $-24\,^{\circ}$C, MgCl to $-33\,^{\circ}$C, CaCl to $-53\,^{\circ}$C and a mixture of MgCl/CaCl would lower it to $-63\,^{\circ}$C. This is about the limit of freezing point depression, as supersaturation beyond this would increase the viscosity of the solution giving a density of 1.4 g cm^{-3}. Antarctic hypersaline solutions with 270 g l^{-1} of salt with a freezing point of $-28\,^{\circ}$C have yielded the halophilic bacterium *Halobacterium lacusprofundi*. Cyanobacteria, which are common under arid Antarctic conditions, use glycine betaine as an anti-freeze though it is costly to manufacture. During desiccation at extremely low temperatures, the sugar trehalose can act as a water replacement molecule to maintain the structure of membrane proteins and enzymes against denaturation (Potts 1999). Cyanobacterial mat communities, both exposed and shaded, are ubiquitous in the Antarctic. Such communities use a number of techniques for survival in extreme environments such as the use of intracellular mycosporine-like amino acids but it is rapidly degraded. The UV-sunscreen pigment scytonemin is widely secreted as part of the outer protective sheath of many cyanobacterial species, suggesting its early evolution on Earth (Wynn-Williams & Edwards 2002). Sunscreen pigments allow cyanobacteria to trap light and direct it to chlorophyll, giving them the capability to survive with very faint illumination levels beneath the ice-covered lakes and translucent rock. Communities under extreme stress, as in hot and cold deserts, adopt a stratified community structure and avoidance strategies for survival. Cyanobacteria requiring photosynthetically active radiation must be concurrently protected from damaging UVB radiation or must be shade-adapted to grow at low light levels where the UVB stress is minimal – translucent sandstone and the mutual shading of a microbial mat are both strategies that have been adopted in Antarctic cyanobacteria. Living within ice inclusions a few millimetres below the surface of porous translucent rock such as sandstone allows sunlight to penetrate to melt the inclusions, though at a much reduced intensity of ~ 0.1 μMphoton m^{-2} s^{-1} (Thomas & Schimel 1991). The temperature within the rock can be $\sim 10\,^{\circ}$C higher than ambient conditions and the endolithic habitat protects organisms from desiccation by ambient winds (Cabrol & Grin 1995). Endolithic cyanobacteria, however, have reduced metabolic rates with $\sim 10^5$ yr for a metabolic cycle due to the paucity of water. Mars, however, is drier than Antarctica with little possibility of liquid water fluid inclusions; furthermore, Martian rock is generally opaque as they are generally covered in a layer of oxidized dust.

Table 1. *Timescales for microbe and biomolecular integrity (from Wynn-Williams D. 2002 private communication)*

Microbe/ biomolecule	Location	Period	Timescale (Ma)
Fossil cyanobacteria?	Apex chert, Pilbara, Australia	Precambrian	3500
Cyanobacterial hopanoids	Apex chert, Pilbara, Australia	Precambrian	2500
Porphyrins	Siberian shales	Cambrian	~550
Chlorobiaceae carotenoids	Vena-del-Gesso, Italy	Ordovician	~450
Halobacteria	Cleveland potash, UK	Permian	270
Isoprenoids	Lower Albial shale, France	Cretaceous	100
Viable bacteria	Mt Feather permafrost, Antarctica	Holocene	~3

Cyanobacteria are very hardy and have been tentatively identified in stromatolite fossils on Earth dating back to 3.5 Gya (Schopf 1993). Terrestrial hopanoid derivatives from cell membranes of photosynthetic cyanobacteria have been identified from relict cyanobacteria in 2.5 Gya Precambrian chert stromatolites (Summons *et al.* 1999) when there would still have been liquid water on Mars (see Table 1).

Viking's lack of detection of organic molecules on the Martian surface by its gas chromatograph/mass spectrometer (GCMS) (expected by delivery of $\sim 2.4 \times 10^8$ kg of carbon per year from comet and asteroid impacts) has been attributed to the highly oxidizing environment at the surface due to the peroxides generated by the solar ultraviolet flux, particularly the high-energy UVC component (though this hypothesis is not universally accepted). Inorganic surface soil oxidant is currently the favoured explanation for the evolution of oxygen upon humidification and exposure to carbon compounds of the Martian soil during the Viking biological experiments (Zent & McKay 1994). Thus, the Martian surface is percolated by peroxides and superoxides generated by the action of solar UV radiation, which produces oxygen ions. These rapidly oxidize surface biomolecules to carbon dioxide and unrecognizable residues (Stoker & Bullock 1997). The OH radical reacts with most organic molecules to yield benzenecarboxylic acid derivatives from kerogen (the most abundant organic material in meteorites), metastable oxalic acid derivatives of amino acids, phthalic acid and other carboxylic acid derivatives of PAHs, and perhaps the carboxylic acid derivatives of alkanes (Benner *et al.* 2000). These derivatives, particularly carboxylic acids such as acetic acid may be stable to further degradation and such derivatives would not have been detectable by the Viking GCMS instrument due to the pyrolyic degradation process involved in its operation. However, unless organics were resupplied, these molecules would

be further degraded through Fenton's reaction catalysed by iron oxide given sufficient time. The intense solar UV flux is also destructive to biota as it causes mutations in nucleic acids and disrupts proteins and cell membrane lipoproteins. These two factors eliminate the possibility of life on the surface itself, although radiation-tolerant bacterial species such as *Deinococcus radiodurans* do survive extreme UV flux $\sim$1–3 Mrad (compared with endospore tolerance to 0.3–0.4 Mrad doses) on Earth (this is generally attributed to survival capacity under arid conditions which causes oxygen damage). The diffusion of oxidants into the Martian regolith and their chemical concentration decline in the subsurface environment results in a certain oxidant extinction depth, which is of importance for astrobiology on Mars. The degree of oxidation of Martian soil components decreases with depth and was shown to depend on oxidant extinction depth and meteoritic gardening scenarios (Zent 1998). This extinction depth can be estimated by applying photochemical/geochemical models of oxidation and meteoritic gardening effects using obtained mean regolith depths as monitor parameters (Kolb *et al*. 2002a). Based on this work the range of global mean values for oxidant extinction depths should exceed 2 m, but should certainly be smaller than 5 m. An onset of oxidation of about 1.5 Gya was considered in this study, estimated from atmosphere evolution modelling (Lammer *et al*. 2002a). This is consistent with regolith mixing due to later cratering by post-heavy bombardment modelled by the Carr meteoritic production function, which suggests that oxidant stirring yields a 1/e oxidant depth of 0.5–0.85 m, i.e. an effective extinction depth of 2–3 m. A similar result arose from the calculation of an upper limit for the extinction depth of about 2.7 m for the oxidant in the Viking Labelled Release (LR) experiment (Bullock *et al*. 1994). Oxidant comprising H_2O_2, O_3 and HO_2 originates from water photochemistry at the base of the Martian atmosphere $\sim 2 \times 10^9\ cm^{-2}\ s^{-1}$ with a residency of 10^5 yr, impregnating the soil at $\sim$1–10 ppm prior to incorporation into Ti and Fe oxides. Reinvestigation of the oxygen release in VIKING GEx experiments revealed an amount of 34 ppm (g) or 4×10^{12} N cm^{-2} adsorbed superoxide to be present in the Mars soil (Yen *et al*. 2000). The UV surface flux may be modelled from the incident flux at the top of the Martian atmosphere undergoing a Beer's law exponential decay from atmospheric dust in the lower 20 km of the Martian atmosphere (atmospheric dust distribution modelled by the Delta-Eddington approximation). The diffusion of peroxide was modelled by Fick's law and extinction depth was defined to be the point at which peroxide density dropped to 10^{-6} of its surface value, giving an oxidant depth of 30 cm by diffusion alone. The model assumed that the onset of oxidizing conditions occurred after the end of the heavy bombardment phase $\sim$3.8 Gya (when the cratering rate was 10^4 times as great as subsequently) as oxidant cannot survive in aqueous environments. Given the uncertainties in this model, determining the thickness of this layer of superoxides is one of the most important questions in Martian geochemistry – indeed, such a measurement would provide an insight into the early processes, including the relationship between the end of heavy bombardment and the loss of volatiles. The oxidized layer thickness may be modulated by local UV-irradiation conditions and water adsorption (Patel *et al*. 2002). Palaeolake depressions at mid latitudes may exhibit lower oxidant production rates and moreover, due to enhanced water adsorption, less effective oxidant diffusion into the soil. It was shown that some depression zones on Mars go hand in hand with putative subsurface water-ice deposits (Boynton *et al*. 2002; Mitrofanov *et al*. 2002), which should be candidates for regions with higher water adsorption. Thus, the search for appropriate landing sites depends on the knowledge of oxidant chemistry and its thermodynamic/kinetic behaviour. Experiments to determine the adsorbate chemistry under Martian conditions are therefore indispensable (Kolb *et al*. 2002b).

The regolith itself is a mixture of loose soil, dust and rock on the surface, which has probably become compacted to a greater or lesser degree with depth. Drilling to depth can be difficult to accomplish – the Apollo astronauts had great difficulty in drilling and coring into the lunar regolith that they penetrated to a depth of only 3 m due to the high compaction from meteoritic bombardment, though this is unlikely to be the case on Mars. However, the nature of the degree of compaction on Mars is not known and this will be critical in determining the reachability of depths. This suggests that any search for extant or relict microbial life may have to be conducted below a depth of >3 m. For these reasons, we favour shallow searches below 3 m depths for the remains of photosynthetic bacterial and cyanobacterial communities rather than deep drilling for chemolithotrophic bacteria.

Definition of biomarkers

The next question concerns biomarkers that may be exploited in the search for life. One of the most basic assumptions is that life will be based on CHONPS elements. The identification of biomarkers may be achieved by direct or indirect methods. Direct detection of molecular entities includes metabolic products such as methane or carbon dioxide, and metabolic components such as ATP, lipids or nucleic acids. Indirect detection may be achieved by observing a biological process such as enzyme function, CO_2 fixation or CO_2 release – this was the approach adopted on Viking (1976). Three different life-detection experiments plus a gas chromatograph/mass spectrometer provided the means for 26 tests for tests for living micro-organisms in the Martian soil. The life detection experiments gave positive results but were attributed to geochemical reactions in the light of the lack of organics detected by the GCMS (though this is disputed (Levin 1997; Levin & Straat 1981)). Such methods are no longer regarded as being a diagnostic of biotic activity.

Fossilization and preservation of organic matter is enhanced under anoxic conditions, rapid burial rates, rapid desiccation, hypersaline conditions and subzero temperatures. Bacterial morphological fossils are unlikely, as they do not fossilize readily and are often indistinguishable from artefacts in the imaging process, but fossil evidence of their existence

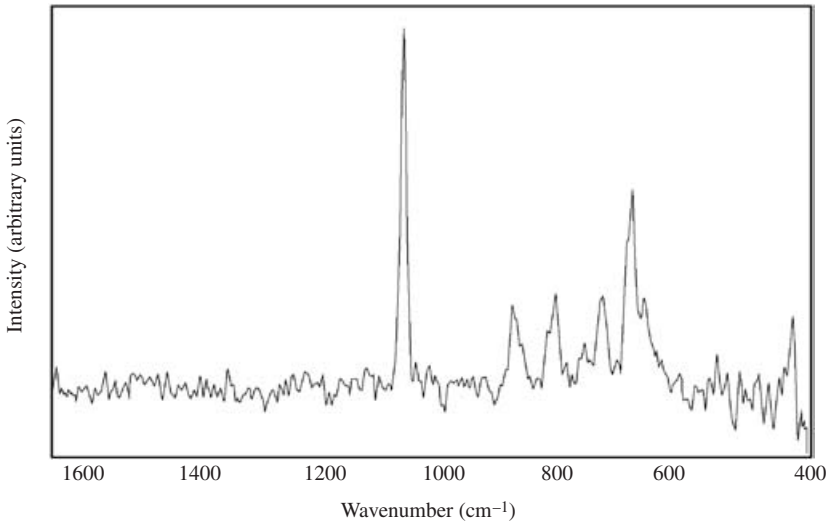

Fig. 2. FT Raman spectrum of hopanoid biomolecule from cell membrane of a fossil cyanobacterium $\sim$2.5 Gya (from Wynn-Williams D. 2002 *private communication*).

such as stromatolite deposits would provide a bio-marker. Organic molecule chirality has often been suggested as the *de facto* characteristic of biotic processes – indeed, the Rosetta Lander (RoLand) carries a chirality-sensitive GCMS instrument to determine the enantiomeric excess of any organic materials in the target comet samples. All life on Earth is characterized by chirally pure mixtures of L-amino acids in proteins, D-ribose in nucleic acids and D-glucose in sugars – the construction of regular, asymmetric biomolecular geometries such as α-helices and β-sheets essential for catalytic activity would not be possible with both L and D enantiomers. However, an enantiomeric excess by itself can be generated abiotically – perhaps through circularly polarized synchrotron radiation generated by Mie scattering in dust grains within interstellar clouds. Other possibilities include catalysis on chiral substrates, and the influence of weak nuclear force β-decay, which exhibits CP asymmetry – indeed, each of these mechanisms has been postulated as the causative agent in the origin of chiral selection during early biogenic evolution. Such mechanisms may be responsible for the small enantiomeric excess in the Murchison meteorite of α-methyl α-amino acids and the 9% L-enantiomer excess of non-biological organics. Furthermore, given the rapid degradation of enantiomer excess into racemic mixtures due to natural diffusion with the environment, this approach requires the existence of an extant community of organisms. It has been suggested that under the cold, dry Martian conditions, amino acid racemization would not go to completion over the Mars lifetime $\geqslant 10^{10}$ yr (Bada & McDonald 1995) – the most slowly racemizing amino acids, isoleucine and valine, have a racemization rate one-third that of adenine and one-seventh that of aspartic acid (aspartic acid has the fastest racemization rate similar to the depurination rate of DNA under such conditions) and may survive under favourable conditions. However, any persistent or episodic liquid water in the environment would cause rapid racemization within $\leqslant 5 \times 10^6$ yr similar to that on Earth. Following racemization, it is possible that below the oxidizing layer, organic matter such as amino acids *per se* (using phenylalanine as a typical amino acid with a typical Arrhenius decay rate constant of $\sim 4 \times 10^{-17}\,\mathrm{s}^{-1}$) may be preserved beneath the oxidizing layer recognizable from being part of an assemblage ($\sim$1.6% of the original amino acid concentration) including amines and hydrocarbons (the first and second decay products of amino acids by slow decarboxylation and even slower deamination, respectively) – more stable amino acids such as alanine with a lower decay rate constant $\sim 2 \times 10^{-22}\,\mathrm{s}^{-1}$ will survive virtually undegraded, while less stable amino acids such as serine, with a decay rate constant of $\sim 1 \times 10^{-14}\,\mathrm{s}^{-1}$, may have been almost totally oxidized to kerogen and perhaps graphite (Kanavarioti & Mancinelli 1990).

We therefore suggest that the search should be for macro-biomolecular species with molecular structures that define their functionality, such functionality being fundamental to all organisms. Derivatives of photosynthetic and UV-protective pigments which are well defined and support fundamental requirements of surface biota, may remain preserved in stratified sedimentary layers beneath the oxidized zone of the near-subsurface Martian regolith. Chlorophyll degrades to porphyrins, carotenoids degrade into isoprenoids (derived from ether lipids), and cell membranes degrade into hopanoids (derived from bacterio-hopanetretol), all of which can act as long-lived fossil biomarkers stable to degradation, which are also recognizable by the Raman spectra of their molecular characteristics (Fig. 2). The cyanobacterium *Nostoc* is evolutionarily ancient and exhibits a Raman spectrum including the signatures of carotenoids, hopanoids and isoprenoids when exposed to UV radiation. None of these molecular species is known to form abiotically. Macromolecules such as kerogen, oligonucleotides or hopanoids would offer evidence of biogenic processes. Scytonemin resists degradation and would be an excellent biomarker for cyanobacteria. For the search for fossilized biota, the Raman spectrometer is currently one of the instruments of choice

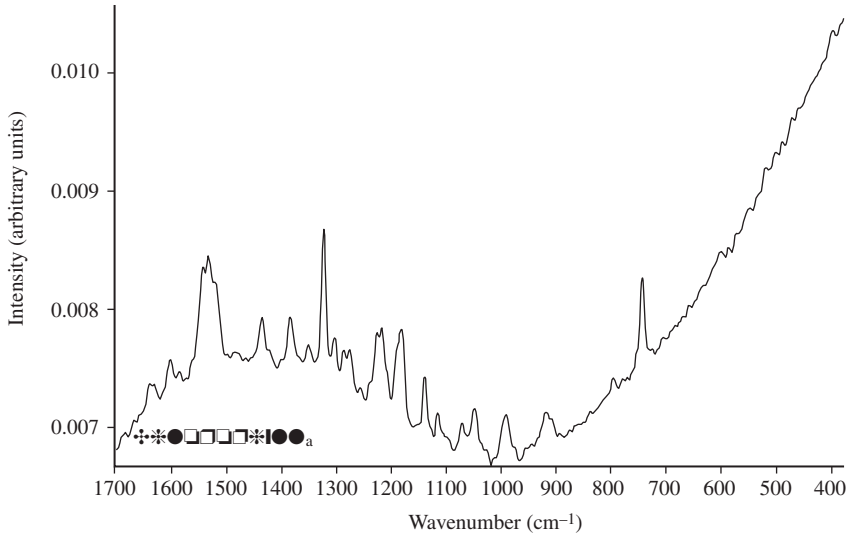

Fig. 3. Raman spectrum of chlorophyll (from Wynn-Williams D. 2002 *private communication*).

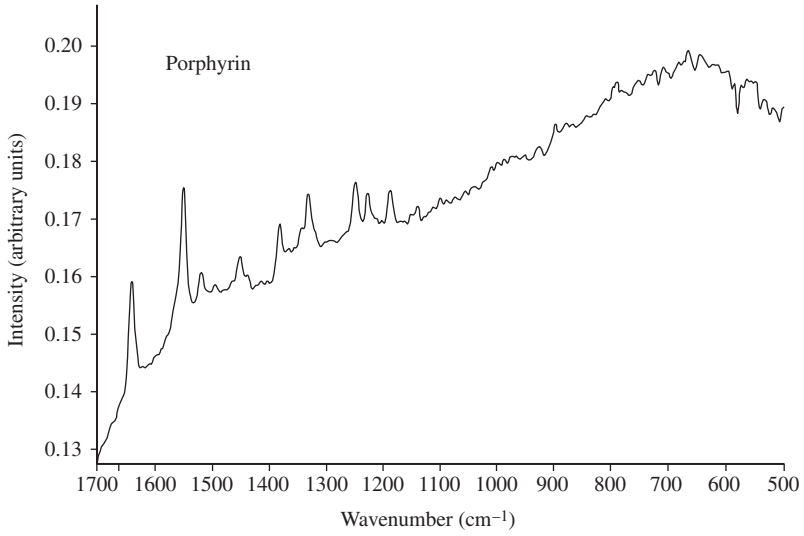

Fig. 4. Raman spectrum of porphyrin (from Wynn-Williams D. 2002 *private communication*).

in palaeobiology (Schopf 2002). Whilst being inclusive for our quest for any biomolecules and inorganic patterns left by former life (Friedmann & Weed 1987), we are focusing our attention on pigments because they are vital to the survival of any surface microbiota on a terrestrial planet in a solar system and they would have evolved in some form if life itself arose at all on the surface. By definition, their near-surface location also makes analysing them by a non-intrusive system such as Raman spectroscopy technologically feasible. Their distinctive Raman spectra due to each molecular specie giving a unique spectral fingerprint derived from their composition and chemical bonding adds to their suitability as biomarkers. A laser-Raman spectral database is being accumulated at the University of Bradford for recalcitrant derivatives (such as fossil porphyrins and isoprenoids) of key pigments such as chlorophyll and carotenoids (Edwards H. 2002 *private communication*) (Figs 3, 4). A miniature infrared (IR) laser Raman spectrometer has the concurrent potential for confocal microscopy of the strata and biomolecular debris, together with IR spectroscopy for water detection, with the same optics. Combination of these corroborative analyses will help to provide unequivocal evidence of former life.

Laser Raman spectroscopy

There is a need for a scientific instrument to characterize *in situ* the micro-structure and inorganic composition of potential microbial habitats on Mars with concurrent analysis of molecular components of organic material and biomolecules in particular, without any preparation or prior identification of compounds. Specific requirements include:

- non-intrusive *in situ* analysis of the micro-structure and inorganic composition of potential micro-habitats for microbial life;

Table 2. *Raman spectral bands and absorption maxima for microbial photo-pigments (from Wynn-Williams D. 2002 private communication)*

Pigment	Pigment type	Raman vibrational bands (wavenumber cm^{-1})					Absorption peaks (nm)				
							UVC	UVB	UVA	Visible	
							<280	280–320	320–400	>400	
Usnic acid	Cortical acid	2930	1607	1322	1289		>220	290	325	>400	
Pulvinic dilactone	Pulvinic derivative	1672		1405			>246	290	367	>400	
Parietin	Anthraquinone	1675		1099	551		>257	288		431	422
Calycin	Pulvinic derivative		1611		1379		>269			>400	
Atranorin	*para*-depside	2942	1666	1303	1294	1266	>274			>400	
Gyrophoric acid	Tri-depside	1661		1290			275	304		>400	
Fumarprotocetaric acid	Depsidone	1642	1630	1290	1280		273	315		>400	
Emodin	Quinone	1659						291		440	
MAA (*Nostoc*) 7437	Mycosporine Am. acid							(>310)	330	>400	
Scytonemin	8-ring dimer	1590	1549	1323	1172		252	300	370	>400	
ß-carotene	Carotenoid		1524	1155			>246	283	384	429	451
Rhizocarpic acid	Isoprenoid	1665	1620	1596			na	na	na	>400	
Porphyrin	Tetrapyrrole ring		1453							>650	
Chlorophyll$_a$ (cyano)	Tetrapyrrole ring		1360	1320						680	700
Bchla (Rh. spheroides)	Tetrapyrrole									850	870
Chlorobium Chl.	Tetrapyrrole									650	660
Phycocyanin	Phycobilin	1630	1351							560	620
Phycoerythrin	Phycobilin	na								544	

- non-selective analysis of organic compounds in near-surface substrata below the oxidized regolith zone without any extraction or preparation;
- diagnosis of biomolecules from their functional components (e.g. rings and isoprenoid units) without prior knowledge of the identity of compounds;
- diagnosis of focused strata of biomolecules (e.g. light-constrained pigments in the profile of palaeolake sediments) in spatially dispersed samples to minimize the chance factor of heterogeneity;
- emphasis on the ecological function of component parts of molecules (e.g. UV-absorbing rings for former surface microbial survival) in environmentally challenging conditions;
- a database of terrestrial microbial biomolecules (especially photosynthetic and photoprotective pigments from potentially analogous primitive microbes on Earth), e.g. photosynthetic bacteria and cyanobacteria;
- simultaneous confocal microscopic imagery of the habitat structure and any fossil or preserved microbes;
- proven suitability for the detection of similar analogues in Antarctic samples;
- capacity for miniaturization of the instrument with a low power requirement for a Mars lander or rover mission.

Raman spectroscopy fulfils these requirements. Laser Raman spectroscopy is a favoured technique for the analysis of minerals in Mars meteorites (Edwards *et al.* 1999) and potentially for analysis of Martian regolith *in situ* (Edwards & Newton 1999; Popp *et al.* 2001) and concurrently characterizes the mineral environment of any biomolecules. The emphasis for Raman research on Mars has been placed either on rock crusts, such as the varnish on rocks of Martian geology (Israel *et al.* 1997) or using point-count methods on the surface of lithosols (Haskin *et al.* 1997). The JPL Raman spectrometer that was originally proposed for the original Athena payload for the Mars Exploration Rovers (MER) has been dropped from the MER payload (Wang *et al.* 1998). Although the miniature Raman system developed by Haskin's group has value for surface mineralogy (Wang *et al.* 1998), its restriction to the oxidized, irradiated surface precludes detection of biomolecules. However, biomolecules, especially pigment derivatives, may still be detectable beneath the oxidized zone in the near-subsurface profile. These biomolecules have been detected in stromatolitic and endolithic microbial communities that may similarly have developed on the surface of Mars (McKay 1997; Wynn-Williams & Edwards 2000). Doran *et al.* (1998) have shown recognizable stromatolitic layers in the beds of Antarctic palaeolakes, and it is these types of profiles that would make good targets for the detection of biomolecules on Mars. Such promising targets may reside in places such as the delta within the Gusev Crater (Grin *et al.* 1997; Wynn-Williams *et al.* 2001).

Raman spectroscopy does not require sample preparation and offers fibre optic delivery and cleaner spectra than are obtainable from infrared spectrometry and a range below 400 cm^{-1}, which provides for organic and inorganic species identification and differentiation. Raman spectroscopy is a laser-based technique that can be used to detect organic and inorganic compounds *in situ* by the detection of scattered light (Table 2). The wavelength of monochromatic laser light is scattered from the surface of inorganic and organic compounds (especially biomolecules which characterize molecular structures) and is shifted by the vibrations and rotations of the chemical bonds of the molecular components of the sample. The vibrational signature of the molecules arises from inelastic collisions between the photons of the exciting laser source and the component parts of the target molecule. A small proportion ($\sim 10^{-5}$%) of the incident photons are

scattered with different wavelengths and intensities due to inelastic Raman scattering, which are collected by the excitation optics for subsequent analysis. The vast majority of the scattered light is unchanged in frequency due to elastic Rayleigh scattering, which can be filtered out – the Rayleigh scatter line at the excitation wavelength is normalized to a wavenumber of zero. Spectral lines which are shifted to energies lower than that of the laser source (Stokes lines) are produced by ground-state molecules, whilst lines at higher frequencies (anti-Stokes lines) are due to molecules in higher excited vibrational states. For Raman spectra, wavelengths (λ) are traditionally expressed as the equivalent wavenumber (cm^{-1}), which numerically is $1/\lambda$. The spectrum of a given compound consists of a unique fingerprint of all its atoms, groups and bonds and their interactive effect (stretching, deformation, rotation) on each other. This produces for any compound a spectrum that is a unique fingerprint for any molecule. The Raman spectrum represents a set of corroborative bands that describe the sum of the components in the spectrum resulting from a mixed biomolecular pool of a cell or natural community so that we can predict the function of the target molecules, such as the absorption of UV light. Through the examination of biomolecules from organisms that we know can survive in extreme conditions on Earth, we can extrapolate this knowledge to similar habitats on Mars. Pigment biomolecules of cyanobacteria in microbial mats and of endolithic cyanobacteria within translucent sandstone in Antarctica have been studied using this technique. Inorganic compounds of ecological significance such as UV screening haematite and bio-weathering calcium oxalate have similarly been studied using laser Raman spectroscopy. Calcium oxalate can be detected as evidence of rock bioweathering (Russell *et al.* 1998). The Raman water signature is small so that it is applicable to untreated field-fresh material.

A UV laser Raman instrument operating at 220–250 nm can detect fragments of RNA and DNA but such short wavelengths can excite autofluoresecence so longer wavelengths in the infrared spectrum are preferred. Photosynthetic pigments (chlorophyll and accessory pigments such as phycocyanin) and certain photo-protective UV screening and quenching pigments (such as carotenoids) autofluoresce under blue/green illumination. Their wavelength response is typically 200–4000 cm^{-1}. Raman spectroscopy can detect organic compounds, including C–H bonds of organic molecules near 3000 cm^{-1} and measure symmetric bonds such as S–S, which are weak in the infrared absorption spectrum. At wavenumbers between 500 and 100 cm^{-1}, there are frequently inorganic bands that are valuable for describing the mineral composition of the habitat (e.g. quartz, silica and iron oxides at 460 cm^{-1}). Organic components give signatures at 2750–3000 and 1000–1700 cm^{-1} ranges such as carotenoids and chlorophyll. Near-IR (1064 nm) excitation is optimal for minimal interference by autofluorescence of cyanobacterial pigments, but typically requires an interferometer to analyse the spectra (Edwards & Newton 1999). A compromise with an 852 nm laser (giving some autofluorescence) permits miniaturization with a charge-coupled device (CCD) detector (Dickensheets *et al.* 2000). The micro-objective can be used to magnify the target so that the laser beam illuminates a selected spot which can be as small as 5 μm diameter. Using the same fibre-optic path as the Raman laser, confocal microscopy can be used to image the target prior to Raman analysis (Dickensheets & Kino 1998). However, the development of IR-sensitive InGaAs detectors now permits the miniaturization of a Raman spectrometer for fieldwork and planetary missions whilst retaining the optimal near-infrared 1064 nm laser wavelength (Wynn-Williams *et al.* 2001).

For studies of Antarctic samples of cryptoendolithic microorganisms, spectra were recorded using a Fourier transform (FT) Bruker IFS66 (Bruker IR Analytische GmbH, Karlsruhe) laboratory instrument and FRA 106 Raman module attachment with 350 mW Nd/YAG laser excitation at 1064 nm and a liquid nitrogen-cooled germanium detector (Russell *et al.* 1998; Newton *et al.* 2000). For spectroscopy of rock profiles and surface crusts, this was coupled via a TV camera to a Raman microscope with a $\times 40$ objective giving a spot diameter of ~ 40 μm at the sample. With suitable objectives, the target spot size can be as small as 5 μm. The power level was set as low as possible to minimize sample degradation whilst optimizing signal quality, and was typically 20 mW. Between 4000 and 10 000 scans (accumulated at ~ 2 scans per second) were needed to obtain good spectra at 4 cm^{-1} resolution with wavenumbers accurate to ± 1 cm^{-1} or better. The total energy input for 4000 scans was therefore ~ 40 J and exhaustive trials with a variety of endolithic samples showed degradation during scanning to be negligible. In contrast to many spectrometers, the Raman spectrometer can perform its analysis over periods of minutes to a small number of hours per sample.

The probe can be mounted within a ground-penetrating mole (such as the PLUTO – Planetary Underground Tool – mole developed by Lutz Richter at DLR, Germany for Beagle 2 (Richter *et al.* 2001a, b)). This provides the basis for *in situ* subsurface analysis of the geology, mineralogy and organic chemistry of the borehole environment. Rotary drilling is a commonly proposed technique for subsurface penetration, but drilling introduces a number of potential problems (Mellor 1989; Ellery *et al.* 2002a). First, integrating sensory instruments such as a Raman sensor head into the drill collar is not a trivial task. Secondly, single-segment drills are limited to ~ 1 m depth capabilities due to the packaging requirements imposed by the lander volume. Thirdly, penetration to depths over ~ 1 m imposes the requirement of autonomous robotic assembly of the drill string from the drill segments. These considerations suggest the use of self-contained moles for subsurface penetration (Gromov *et al.* 1997; Kochan *et al.* 2001). The CMaRS instrument can perform spot chemical analysis at any selected point within the borehole (high-resolution spot diameter < 5 μm), while the scanning confocal microscope (based on MEMS-manufactured laser scanning mirrors at the end of the fibre optic probe) can provide images of soil/rock morphology (e.g. sedimentary strata), crystal structure, and microscopic

images of suspected lifeforms (organics to be detected by the spectrometer).

The electronics of the CMaRS is based on CCD technology, limited to wavelengths <980 nm. Upgrading the 852 nm Raman system to use a laser of wavelength 1064 nm has required a dispersive spectrometer with a novel InGaAs linear diode array detector sensitive to near-infrared wavelengths. This has produced Raman spectra greatly superior in breadth and resolution than the 852 nm system with a further reduction in fluorescence of pigment molecules, of similar quality to the laboratory FT–Raman 1064 nm system. The current miniature prototype developed by Micron Optical Systems Inc. of Norfolk, Virginia, has a wavenumber range from 500 to 3500 cm^{-1} and a resolution of 8 cm^{-1}. It has a fixed grating (i.e. no moving parts), incorporating a linear axis. This linear axis provides insensitivity to temperature fluctuations as well being rugged and compact in design. The combination of the diode-pumped solid-state laser with the InGaAs linear diode array is patent pending under Micron Optical Systems Inc. The complete instrument is projected to have a mass of <1 kg with a power consumption of <4 W.

Site selection

There are several possible locations for former or even extant life on Mars, each of which is not necessarily exclusive:

(1) regions where water existed for significant periods of time such as palaeolakes and water-cut channels (Doran *et al.* 1998);
(2) hypersaline brines (Wynn-Williams *et al.* 2001) as found in Antarctic Dry Valley lakes, or evaporite deposits indicative of salt mineral deposition in water – ice-covered lakes during Mars' second hydrological phase would have remained liquid for $\sim$100 Myr;
(3) deposits associated with hydrothermal vents powered by volcanic activity offer a potential site for evidence of biota;
(4) the permafrost–water interface may potentially harbour life (Vorobyova *et al.* 1997);
(5) localized volcanic activity may have created localized hydrothermal regions at shallower depths, perhaps as shallow as 500 m – such localized water deposits could persist as liquid for as long as 10–100 Myr;
(6) impact craters are another possible source of hydrothermal heating (Cabrol *et al.* 2002), particularly as sites of lakes from catastrophic outflows, which could have persisted for 10 000–100 000 yr;
(7) hydrothermal systems supported by volcanic activity appear to offer the best candidates for supporting a limited extant ecology (Walter 1996);
(8) dry beds of one of the ancient lakes of the northern hemisphere such as the recently discovered regions of sedimentary layering.

Recent evidence of liquid water on Mars has been found by the Mars Global Surveyor in gulleys on the cold, shaded sides beginning 100 m from the top of the slope (Malin & Edgett 2000). At the equator, the current equatorial temperature ranges from -110 to $+7$ °C, so melting water ice is a possibility. During the gradual loss of water from early Mars, salts accumulated in ever-shrinking water bodies until they became hypersaline, as in lakes of the Antarctic Dry Valleys, including the Don Juan Pond which consists of saturated calcium chloride solution and does not freeze until -53 °C. On Mars, the concurrent effects of the elevated melting point of hypersaline brines and the potential 'water greenhouse' effect of high relative humidity localized in the channels (Pathare & Paige 1996) indicate that emerging brine water, even if temporary, could flow over the surface. This is consistent with the hypersaline brines postulated from MOLA (Mars Orbiter Laser Altimetry) data by Lobitz *et al.* (2001). Clifford (1993) described how groundwater that resides in the Earth's crust for $\sim$Myr generally evolves into highly mineralized brine (a saturated mixture including chlorides, carbonates and sulphates) and suggested that this process would probably be accelerated on Mars by the influx of minerals leached from the crust by low-temperature hydrothermal circulation. Eventually, brine emerging on to the surface would evaporate after accumulating in an accompanying alluvial fan, as suggested by the Noachis Crater seepage feature shown by Malin & Edgett (2001). However, such sites are generally in difficult-to-access locations and thus represent an impractical proposition for a near-term robotic mission.

The dry beds of ancient lakes represent a potentially highly productive region for preserved biomolecules. The lacustrine plains offer the most promising sites for astrobiological prospecting, particularly lacustrine environments at the outlet of fluvial features (Cabrol & Grin 1995) – the Gusev crater of age $\sim$3.4–3.6 Gya is one such site at the outlet of the Ma'adim Vallis system with indications of deltaic features and extensive sequences of fluvial deposition over $\sim$200 Myr (an estimated sedimentary deposition volume of 14 000 km^3 for an 800 m thick sequence) (Cabrol *et al.* 1998). Evaporite deposits represent a promising location for sampling sites – terrestrial alkaline lakes concentrated by evaporation have high pH $\sim$10–12 within which bacteria survive by forming spores. Such sites may harbour extant but dormant lifeforms (e.g. 'White Rock'). Halophilic bacteria in the Dead Sea and Great Salt Lake can survive high salt concentrations due to their proteins having high proportions of charged amino acids to bind water tightly. Such halobacteria, such as *Halococcus salifodinae*, which can survive up to 25–30% NaCl solution, can exhibit longevity over geological timescales (Stan-Lotter *et al.* 2001). They are found in evaporite salt lakes and can be trapped in salt crystals during evaporation. They are indicated by the presence of carotenoids and bacteriorhodopsin pigments in their membranes. During the Permian–Triassic periods $\sim$245–280 Mya on Earth, around 1.3 Mkm^3 of salt sediments were deposited in the large basins of the supercontinent Pangaea and subsequently dried up due to the warm, arid climate. The revival of halophilic bacteria from such ancient deposits indicates high survival capacity under starvation conditions. Evaporite deposits on Mars

(probably of Mg and Ca sulphates) offer potential sources of fossil life.

Gestation and birth of a robotic astrobiology mission proposal – Vanguard

Mars recently hit the headlines when NASA scientists led by David McKay announced in 1996 that they had found fossil evidence of life on Mars within a Martian meteorite ALH84001 recovered from the Antarctic ice field (McKay *et al.* 1996). Although these findings are hotly debated, they have nevertheless intensified research into the embryonic field of astrobiology, both in the US and Europe. The UK research community have embraced astrobiology as a major discipline of research supported by the UK Science Minister, Lord Sainsbury. The US ramped up its Mars exploration programme following the ALH84001 press release. The US Pathfinder mission with the Sojourner rover centrepiece in 1997 indicates the popular support for the exploration of Mars (Shirley & Matijevic 1995; Matijevic 1997, 1998) – the Pathfinder website attracted millions of hits when the internet community was a third of its present size (556M in the first month of operation). However, the recent catastrophic losses of the US Mars Climate Orbiter and the Mars Polar Lander has placed Europe, and the UK in particular, in a unique position by placing Beagle 2 centre-stage in Mars astrobiology exploration (Sims *et al.* 1999, 2000; Clemmet 2001). The next US Mars missions are the landing of two large Athena-class Mars Exploration Rovers on to the Martian surface to conduct primarily geochemistry experiments in 2003/4 soon after Beagle 2's landing. Unlike the US MER programme, Beagle 2 is focused specifically on astrobiology and will analyse the atmosphere for signs of methane and analyse rock and soil samples for the presence of carbonates and organic material. Given the imminent flight of Beagle 2 on Mars Express in 2003, it is timely to consider post-Beagle 2 European robotic missions with astrobiology as the prime focus.

In early 2001, David Wynn-Williams and one of the authors (Ellery) were concerned that there were no European astrobiology-focused Mars mission proposals following Beagle 2. It was our belief that Europe should be thinking ahead beyond Beagle 2 to follow up on its Mars astrobiology mission. However, ESA (European Space Agency) has recently initiated its Aurora programme – a technology-driven programme for planetary exploration leading ultimately to a human mission to Mars. The linchpin in this programme will be technology demonstration through robotic Mars missions. Given this constraint, it would make sense to combine some of the technology-demonstration requirements of Aurora with a strong astrobiology science focus in a single mission – a mission which is comparatively inexpensive, feasible and politically supportable. Vanguard is one such mission proposal – the product of discussions between David Wynn-Williams as a scientist and Ellery as an engineer. This negotiation approach ensured that at the outset the scientific returns would be maximized while conforming to the severe engineering constraints. The primary objective for Vanguard was to attempt to find *unequivocal* evidence of extinct life on Mars. The primary science requirement was the need for replicability – multiple data sets – to avoid the problems that plagued the ALH84001 data. The resulting engineering solution was that a surface package was required with two capabilities:

(1) subsurface capability to penetrate below the surface oxidized layer which rapidly degrades organic material;
(2) surface mobility capability for the selection of multiple target sites for the replication of data sets.

Note that these two engineering requirements are essentially robotic requirements, illustrating the intimacy between robotics and science in space-based experimentation including that applied to astrobiology (Chicarro *et al.* 1998). Gardening models suggest that the subsurface layer is estimated to be 2–3 m thick (Bullock *et al.* 1994; Zent 1998; Kolb *et al.* 2002a; Lammer *et al.* 2002a) – we set our target penetration depth at 5 m. NASA has delineated somewhat arbitrarily six milestone depths which are more indicative of technology requirements than scientific value: 1, 2, 10, 100 m, 1 km and 1 km+. Our 5 m target encompasses the first two targets but falls short of the third, but is selected on the basis of scientific value. In comparison, the proposed European Exo-Mars Aurora flagship mission is a large astrobiology mission proposal, which will land a 190 kg rover on to the surface of Mars carrying the 70 kg PASTEUR exobiology multi-user facility (Brack *et al.* 1999; Clancey *et al.* 2000; Westall *et al.* 2000; Battistelli *et al.* 2001; Vago 2002). At the cost of a 600 kg Mars entry probe that will require a new design of Mars spacecraft for delivery, the PASTEUR package is equipped with a small drill to penetrate 1 m into the Martian soil to return samples to the lander for analysis. Our analysis suggests that ExoMars is likely to be unsuccessful in its quest to find evidence of biotic activity – the penetration depth is too shallow, though Exo Mars will provide highly valuable data from its broad range of scientific instrumentation. Although penetration to depths of 5 m does not guarantee success in detecting evidence of biotic activity, penetration to such depths would maximize the probability of such and provide a sufficient depth profile to extrapolate the distribution and depth gradient of the oxidant material if the current models are incorrect (Kolb *et al.* 2002c) – this will be critical for furthering both Martian astrobiology and geochemistry data. Peroxides and superoxide ions are Raman and/or IR active. This fact has been used for a long time in technical catalysis studies (Li *et al.* 1989; Long *et al.* 1997). Spectroscopic packages, send to Mars, could be capable of investigating the Martian oxidant environment if they are designed to reach this environment *in situ* – as is the case for Vanguard. In contrast to spectroscopy, GCMS does not provide sufficient selectivity concerning different oxidants. Furthermore, GCMS fails – as even-laboratory grade systems – in the determination of low water contents and in the investigation of water-related catalytic exchange reactions. It is not necessary to emphasize that the variability of water is one of the most important scientific targets in Mars

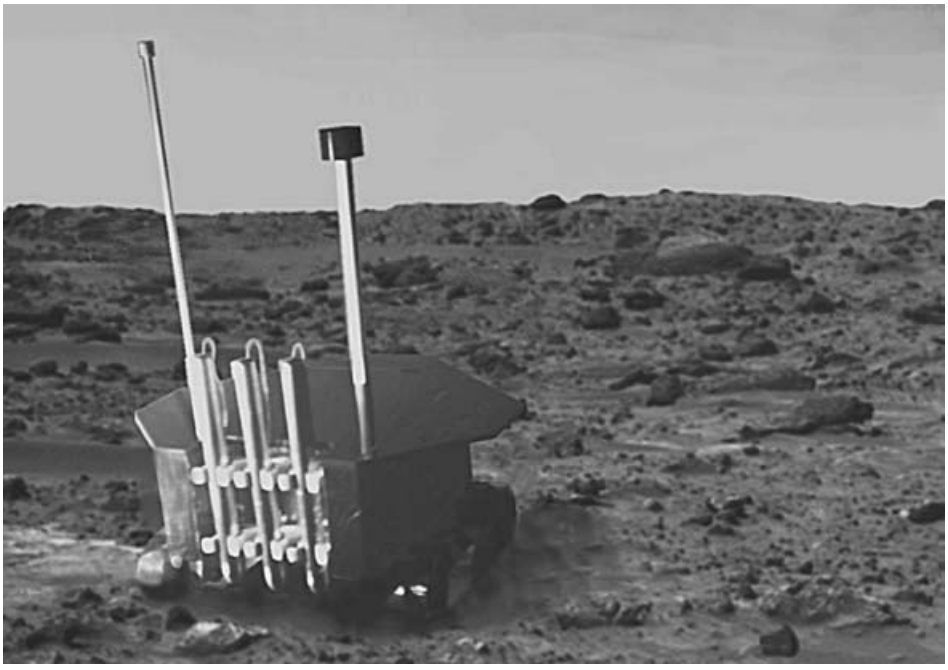

Fig. 5. Vanguard rover (Endurance) with its three vertically mounted moles (courtesy of Ashley Green).

research. IR spectroscopy is the most potent method for the determination of traces of water.

Wynn-Williams suggested that mobility was essential over at least $\sim$1 km to provide a wide selectivity of target sites for subsurface penetration and to accommodate variances in regolith depth. In terms of scientific instrumentation, Wynn-Williams suggested that for unequivocal evidence of life, we should focus on detecting hopanoids (and other biomolecules) that yield unique Raman spectra and which, more importantly are long-lived over geological timescales $\sim$2.5 Gyr. Wynn-Williams was particularly keen to focus on a lander mission to the Gusev palaeolake crater (14° S, 184° W) as a potential site for subsurface fossilized Martian biota. Our discussions led to Vanguard, a robotic astrobiology-focused Mars mission proposal.

The basic concept of the Vanguard mission proposal is to deliver to the surface of Mars a tri-utility surface package – a small lander, a mobile rover and three subsurface moles to be transported to Mars by a Mars Express-class carrier (Ellery & Wynn-Williams 2002; Ellery *et al*. 2002b). In many respects, it resembles the original Beagle 2 concept but there are significant differences (Sims *et al*. 1998). The rover (Fig. 5) offers great utility as a scientific instrument platform whose mobility provides great flexibility in sample and site selection during the mission (Wilcox *et al*. 1990). Its primary mission focus will be astrobiology unlike the currently scheduled US Mars missions, which are primarily geochemical and concentrated on the Martian surface or Martian atmosphere. This proposed mission would be the first Mars mission that specifically searches for bio-markers below the near-surface oxidized zone. Wynn-Williams suggested the name Endurance after the polar explorer Shackleton's ship for the rover and the name Orpheus has been provisionally selected as the name for the moles. The Endurance rover is the centrepiece of the Vanguard mission in providing the mobility component from the lander to the mole penetration sites. The laser source of the Raman spectrometer is implemented onboard the rover, while a compact detector head on a fibre-optic cable can be integrated in each mole with side-scanning sapphire windows (Richter *et al*. 2001b) for underground deployment to search for strata of biogenic materials whilst concurrently characterizing their mineral habitat. Vertical rearward mounting of the moles on the micro-rover eliminates the need to orient the moles for delivery into the surface and permits jettisoning of the mole(s) in the event of failure to penetrate into the subsurface. As the moles descend, soil physical parameters such as soil cohesion strength, friction angle, density and porosity may be obtained.

The Vanguard lander is to be delivered to the Martian surface by a Mars Express-type spacecraft, and an entry, descent and landing system (EDLS) similar to that adopted for Beagle 2 (ablation shield, parachute and airbag). We present our first Vanguard mass budget analysis (Ellery *et al*. 2002c) – these figures are highly provisional and err on the side of conservatism rather than undue optimism (we thus anticipate further revisions to reduce allocated masses and increase the mass margin). The Mars Express has a scientific payload capacity of 176 kg including orbiter instruments. The surface probe (including EDLS) is limited to $\sim$126 kg in mass, of which $\sim$65 kg comprises the surface segment, leaving $\sim$50 kg for orbiter instruments. The surface segment comprises the small tetrahedral Vanguard lander of 34 kg mass to provide a communications relay to the orbiter (during its one overhead perigee pass per day), the Endurance micro-rover of 26.5 kg mass, and three ground-penetrating PLUTO moles of 1.6 kg each. The design lifetime of the

Table 3. *Vanguard scientific instrument mass and power budgets* (*Ellery et al. 2002c*)

Scientific instruments	Mass (kg)	Power (W)
Mars Lander		
Meteorology + Envt pack (sim to Beagle 2)	0.2	2.0
Seismometer	0.3	0.02
Subtotal	0.5	2.02
Mars Micro-rover		
Microscope	0.5	5.0
Raman/IR/LPS pack	3.0	5.0
Ground-penetrating radar	3.3	22.0
Subtotal	6.8	32.0
Mars mole $\times 3$		
3-axis accelerometer $\times 3$	0.025	0.1
Thermocouple $\times 3$	0.3	4.0
Electrochemical sensor $\times 3$	0.1	0.2
Subtotal	0.425	4.3
Total	8.575	38.32

Table 4. *Preliminary Vanguard mass budgets* (*Ellery et al. 2002c*)

Surface element	Subsystem	Mass (kg)
Mars lander	Instruments	0.5
	Structure	6.63
	Computer/electronics (ERC32)	1.8
	Telecommunications	0.83
	Battery	2.1
	Solar panels	10.5
	Power dist/conv	1.3
	Thermal control	6.0
	Rover support structure	3.0
	Miscellaneous (e.g. wh, etc.)	1.5
	Subtotal	34.2
Mars rover	Structure	2.0
	Solar panels	7.4
	Power dist/conv	0.8
	Thermal control	2.0
	Nav camera stereo-pair	0.5
	Panoramic stereo-camera pair (1.8 m)	0.5
	Autogyro	0.25
	SEO LRF-200 laser rangefinder	0.5
	Proximity/contact sensor	0.1
	Mobility/chassis system	2.5
	Computer/electronics (ERC32 + 2 × T865)	1.8
	Telecommunications	0.83
	Miscellaneous (wh, etc.)	0.5
	Instruments	6.8
	Subtotal	26.5
Moles $\times 3$	Structural mass	0.7
	Tether	0.5
	Instruments	0.425
	Subtotal	1.6
Surface segment total		65.5
EDLS		60
Total		126

lander is 90 days, limited by the accumulation of dust on the solar panels. The moles are mounted vertically to the rear of the micro-rover. Each mole is deployed independently to penetrate into the Martian subsurface to a nominal depth of 5 m, significantly below the estimated 2–3 m depth of the oxidized layer. Each mole is tethered to the micro-rover with mole-spooled cabling that carries optical fibres and power transmission wiring. The micro-rover provides the mounting platform for the scientific instrument package (remote sensing instruments) while each mole carries a sensor head to the laser-based instruments. The Endurance micro-rover is a free-ranging rover with free-space communications to the lander and independent onboard power generation. The micro-rover provides the basis for surface mobility across a $\sim$1.0 km transect which provides the capacity for surface site replicability of depth profile data. It has a design life of 45 days, limited by the capacity of its primary batteries.

There is a mass budget of 8.6 kg for scientific instruments (see Table 3). We focused on remote sensing instrumentation for reasons that will become apparent. We wished to eliminate from the payload instruments such as the gas chromatograph/mass spectrometer as such instruments require sophisticated robotic sampling capabilities, a minimum volume for pyrolytic ovens, and high power and mass overheads. Most importantly, remote sensing methods reduce the need for complex robotic sampling, which represents potential single-point failure modes. The combination of the Raman spectrometer and confocal imager offers a remote sensing method for the detection of organic material (Dickensheets & Kino 1998). The Raman spectrometer cannot detect water readily but the integration of an infrared spectrometer with the same optics provides this capability (Keraven *et al.* 1999). The CMaRS can be switched to a role as an infrared spectrometer to find water and may be readily incorporated into the instrument assembly and the same optical chain (Dickensheets D. 2001 *private communication*). Both the Raman and infrared spectrometers can provide complementary mineralogical data – data that has yet to be obtained from any previous Mars mission. Furthermore, a laser plasma spectrometer (LPS) can also be integrated into the same optical chain to provide rapid elementary composition data on samples (much more rapidly $\sim$seconds to minutes compared with the 8–10 h required for the alpha–proton–X-ray spectrometer (APXS) which is being adopted for the Mars Exploration Rovers) (Reider *et al.* 1997; Castle *et al.* 1998; Bertrand *et al.* 2002; Bertrand R. 2002 *private communication*). The LPS/Raman has a passive front-end linking the optical outlet/inlet for both laser-based plasma generation and collection of spectral light with the actual instrument and laser pump electronics by an optical fibre. The LPS/Raman laser may be transmitted through the same optics and sapphire window. The distance between the laser outlet and the actual target is not an optical issue. The LPS plasma spot may be focused beyond the sapphire window, particularly if it recessed – this would eliminate the possibility of degrading the sapphire window. These three instruments provide a powerful integrated scientific package with a rapid availability of results unachievable by an APXS or Mossbauer spectrometer. This integrated package is supplemented

by a rover-mounted ground-penetrating radar to provide subsurface investigation prior to mole deployment to maximize the probability of successful penetration to full depth. Smaller instrument packages to be included are a thermal probe and a magnetometer on each mole, and seismometer, environment and meteorological packages on the lander (Table 4).

The advantage of using remote-sensing laser-based instruments is that the moles are deployed on a descent-only profile – no provision is made for their recovery. Once each mole reaches its maximum depth, the tether is severed, and the micro-rover traverses to another site. Once all the moles are deployed, the Vanguard secondary mission involves using the Raman/IR/LP spectrometer package on surface targets (the LPS has a range of $\sim$20 m through air). The primary mission yields three separate depth profiles. An important corollary of the one-way mole trajectory is that as there is no requirement for reversal of the mole hammering mechanism, a rover-mounted expert system is required to perform real-time quick-look analysis during the mole descent to perform autonomous decision making. Such decision making will determine, as part of a closed-loop control system, the speed of descent of the mole and the need for further spectral integrations. Vanguard thus represents an autonomous astrobiology expert system.

The subsurface of Mars remains unknown (e.g. the depth of bedrock and the nature of compaction with depth) and the technology required to penetrate the surface at any depth and return samples to the surface for biochemical analysis is likely to be highly complex technologically. The greatest complexity concerns the return of samples to the surface which may impose the requirement for casing of the borehole, strengthening the tether for tension loads, back-driving the mole, respooling of the tether and the problem of re-mating the mole into its launch tube. The primary goal is to develop the technology required to borehole into the Martian surface to depth in order to ascertain the nature of the geological environment with depth as well as searching for biological material. Rather than attempting to retrieve samples, the moles undergo a one-way trip generating data as they descend. In this way, the rover can deliver a number (nominally three) of penetrating moles to different sites, each analysed remotely in turn by the rover's onboard Raman spectrometer and other instruments. Furthermore, such *in situ* characterization analyses biomolecules in their natural state and context. Replicated datasets taken from a palaeolake bed such as the Gusev crater or a similar former aquatic environment would be invaluable for astrobiology. The Raman spectrometer can yield unequivocal evidence of life from organic material below the surface if it exists and is readily found. What is learned from this mission will prove invaluable for any future mission that attempts to retrieve core samples to the surface from depth.

The Vanguard approach to astrobiological investigation is a robust and reliable one. The lander and micro-rover concepts are flight-proven. The mole concept is to fly on Beagle 2 though it will penetrate to a depth only 1.5 m below the surface and will be required to surface with its recovered sample for analysis by lander onboard gas analysis package (GAP). The Vanguard approach minimizes robotic complexity by:

(1) the use of moles for modest depth capability over drills which require autonomous assembly of the drill string for depths >1 m (Ellery *et al.* 2002c);
(2) the descent-only trajectory of each mole eliminates the requirement for mole recovery, for tether tension strengthening and for mole-launch tube mating on recovery;
(3) there is no need to ensure hole integrity through the reinforcement of the borehole by casing;
(4) the lack of physical sampling eliminates the need for direct sample analysis instruments such as GCMS.

By minimizing the complexity of the robotic infrastructure, we enhance reliability.

Conclusions

We have outlined an astrobiology theory on how putative early life on Mars may have evolved. Based on this, we have provided an astrobiological rationale for the need to penetrate into the Martian subsurface to maximize the probability of finding evidence of this early life that may have existed in early epochs. We have highlighted the laser Raman spectrometer as offering the best space-portable instrument technology to detect such evidence. We have completed the package by introducing a potential robotic Mars mission concept to deliver this instrument – and others – to the Martian environment. This mission proposal (Vanguard) represents a relatively inexpensive approach to astrobiological Mars exploration within conservative engineering constraints, i.e. maximizing reliability. Vanguard represents the next logical step for Mars astrobiology, and indeed, astrobiology as a discipline.

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