Anal Bioanal Chem (2013) 405:7931–7940
DOI 10.1007/s00216-013-6915-0

REVIEW

Enantioselective separation of amino acids as biomarkers indicating life in extraterrestrial environments

Maria Chiara Pietrogrande

Received: 15 January 2013 / Revised: 4 March 2013 / Accepted: 11 March 2013 / Published online: 29 March 2013

Abstract Traces of prebiotic amino acids, i.e., the building blocks of proteins, are excellent biomarkers that could provide evidence of extinct or extant life in extra-terrestrial environments. In particular, characterization of the enantiomeric excess of amino acids gives relevant information about the biotic or abiotic origin of molecules, because it is generally assumed that life elsewhere could be based on either L or D amino acids, but not both. The analytical procedures used in in-situ space missions for chiral discrimination of amino acids must meet severe requirements imposed by flight conditions: short analysis time, low energy consumption, robustness, storage for long periods under extreme conditions, high efficiency and sensitivity, automation, and remote-control operation. Such methods are based on gas chromatography, high-pressure liquid chromatography, and capillary electrophoresis, usually coupled with mass spectrometry; of these, gas chromatography–mass spectrometry (GC–MS) is the only such combination yet used in space missions. Preliminary in-situ sample derivatization is required before GC–MS analysis to convert amino acids into volatile and thermally stable compounds. The silylation reagent most commonly used, *N*-(*tert*-butyldimethylsilyl)-*N*-methyltrifluoroacetamide, is unsuitable for detection of homochirality, and alternative derivatization techniques have been developed that preserve the stereochemical configuration of the original compounds and are compatible with spaceflight conditions. These include the reagent *N*,*N*-dimethylformamide dimethylacetal, which has already been used in the Rosetta mission, a mixture of alkyl chloroformate, ethanol, and pyridine, a mixture of perfluorinated anhydrides and perfluoro alcohols, and hexafluoroacetone, the first gaseous derivatizing agent. In all the space instruments, solvent extraction of organic matter and chemical derivatization have been combined in a single automatic and remote-controlled procedure in a chemical reactor. Liquid-based separation systems have been used in space missions. In particular, microchip capillary electrophoresis, based on microfluidic lab-on-a-chip systems, enables high-performance chemical analysis of amino acids with low mass and volume equipment and low power and reagent consumption. Coupling with laser-induced fluorescence detectors results in ultra-low limits of detection. This critical review describes applications of the on-board instruments used in the Rosetta mission to comets and in the more recent Mars exploration program, i.e., the Mars Science Laboratory and ExoMars missions.

Keywords Enantioselective separation of amino acids · Extraterrestrial environments · Chemical biomarkers · Extinct or extant life

Introduction

Searching for traces of extinct and/or extant life in extraterrestrial environments is one of the major objectives of remote sensing and in-situ exploration in space missions [1–3]. Within this perspective, a strategy of "find the carbon" is followed, referring to the discovery of known terrestrial organic biomarkers. They are possible indicators of life because they are deemed to be exclusively produced by living organisms and most likely to survive under harsh planetary conditions [4]. Several classes of organic biomarker are known: amino acids, the monomer building blocks of proteins, biologically active nucleic acids and their associated

Published in the topical collection *Amino Acid Analysis* with guest editor Toshimasa Toyo'oka.

M. C. Pietrogrande (✉)
Department of Chemistry, University of Ferrara,
Via L. Borsari, 46,
44100 Ferrara, Italy
e-mail: mpc@unife.it

sugars, for example the monomers of DNA and RNA, and lipids or carboxylic acids as their degradation products. It is mandatory that these compounds have an unambiguously identifiable chemical structure and, thus, a unique spectroscopic or chromatographic "fingerprint". Among these compounds the amino acids are of particular interest, because they could have been utilized by any life originating in our planetary system. Amino acids have been detected in carbonaceous meteorites, for example Murchison, and in cometary dust particles [5–8].

Moreover, analysis of their optical activity and/or chirality should be a good complementary diagnostic tool from which to derive evidence of biotic or prebiotic activity. In fact, all living beings on Earth contain asymmetric compounds, with proteins and genes composed of L amino acids—19 of the 20 genetically encoded amino acids have a chiral centre, the exception being glycine—whereas abiotic systems contain racemic mixtures (equal amounts of the L and D forms). In addition, detection of an excess of D amino acids in an extraterrestrial environment could provide evidence that life evolved independently of Earth and could yield information about the origin of the homochiral life on Earth, i.e., endogenous (originated on the Earth itself) or exogenous (imported from space) [9–12]. In addition, integration with kinetics studies of racemization (conversion of one enantiomer to the other) may provide information on the time evolution of amino acids in an extant biosphere or in extinct biological species [10].

The search for indications of life in extraterrestrial environments is encouraged by the extraordinary discoveries made by recent space missions which proved the presence of ice deposits and methane on Mars, in addition to evidence of liquid water in the past [4, 8, 11]. For this reason, future space missions are being considered with the purpose of performing in-situ chemical analysis to seek any signs of enantiomeric excess [2, 4, 7, 8, 10–15].

The instruments useful for in-situ space analysis must fulfill several severe requirements imposed by light conditions: simplicity of construction, miniaturization (small size and mass), low energy (power) consumption, automation and/or remote control operation, and, most of all, tolerance of high mechanical shock and resistance to vibration. In addition, their detection limit must be as low as possible, because biomarker concentrations in extraterrestrial environments are not well known [15–17].

During the past few decades, many methods have been developed for amino acid analysis in extraterrestrial environments by use of conventional bench-top-scale instrumentation, for example infrared (IR), Raman, fluorescence, and nuclear magnetic resonance spectroscopy, gas and high-performance liquid chromatography (GC and HPLC), and capillary electrophoresis (CE) [16–24]. Among these, laser-induced fluorescence (LIF) spectroscopy has recently been suggested as a valuable method for detection of amino acids from any source, down to trace levels in Martian soil. Amino acids were labeled with fluorescamine to furnish fluorescent derivatives that are detected with high sensitivity and specificity; this is of fundamental importance for analytical procedures to be used on future space missions [20].

As an alternative, a use of a miniature Raman spectrometer (low mass and compact dimensions) has been proposed for use in the forthcoming ExoMars mission as an analytical first-pass screening device to identify specific biomarkers from their characteristic spectra [21, 22]. Raman spectroscopy is a vibrational spectroscopic technique which enables unambiguous identification of molecular species on the basis of their unique chemical functional groups, providing information on the nature of the molecules, their spatial arrangement, and their relative concentrations. This technique has several advantages in this research, for example its ability to record spectra of micrometer-sized grains of inorganic (geological minerals), organic, and biological materials yet requiring minimum (if any) sample preparation [22].

However, despite these capabilities, spectroscopic techniques cannot distinguish D or L optical isomers.

Discrimination between the enantiomers of individual amino acids requires chiral separation techniques, including GC, HPLC, and CE, based on stereoselective interactions between the analyte and chiral stationary or mobile phases [17, 23–38]. GC, usually hyphenated with spectrometric devices and with multicolumn operation, has been the analytical technique most used for in-situ space missions [14–17, 31–38]. Because of significant developments in microfluidic lab-on-a-chip systems, LC and CE devices using microfluidic systems have also been suggested as suitable alternatives to GC for in-situ space analysis of organic compounds [23–25].

This paper reviews the relevant analytical methods developed for chiral separation of amino acids during in-situ space missions. This search has so far concentrated on priority targets in our solar system, for example comets, that retain traces of Earth's early evolution [32–36], and Mars, the planet which most closely resembles Earth [4–8, 11–15, 21, 25, 31, 37–45].

GC–MS analysis in exploration of comets and Mars

In-situ experiments in the search for chirality in space started with the 1976 Viking mission to Mars [17, 31], and continued with the cometary sampling and composition (COSAC) experiment in the Rosetta mission to a comet [32–36] up to the robotic spacecraft developed for Mars exploration [6, 8, 11–15, 37–45]. In all these missions, GC instruments coupled with mass spectrometry have been

installed on probes: an overview of the GC instrumentation is given in Table 1. Advantages of this technique include the ability to analyze small samples, to separate complex mixtures of organic compounds, and to identify small concentrations of biomolecules (frequently in the ng or pg range) [26, 27].

Unfortunately, amino acids require preliminary chemical conversion into volatile and thermally stable derivatives suitable for GC analysis. So all GC experiments designed for biomarker detection include a pre-analysis chemical derivatization procedure [14, 17, 37]. To date, the most popular derivatizing agent used in in-situ space GC analysis is *N*-(*tert*-butyldimethylsilyl)-*N*-methyltrifluoroacetamide (MTBSTFA), which furnishes silyl derivatives [14, 35, 37, 40, 41, 44, 45].

Cometary mission Rosetta

The ESA-directed Rosetta Cometary Mission, launched in 2004, intends to determine whether cometary nuclei do contain asymmetric prebiotic molecules. The Rosetta spacecraft is currently expected to rendezvous with the comet 67P/Churyumov–Gerasimenko in November 2014, when it is planned the lander Philae will soft-land on the surface of the comet nucleus. It is equipped for in-situ enantioselective analysis of amino acids as a means toward further understanding of the origin of asymmetric biological molecules [9, 32–36].

Among the eight instruments aboard the Rosetta lander, the COSAC experiment is dedicated to the in situ GC–MS analysis of organic compounds obtained by thermal volatilization of the material in the comet's nucleus by use of a pyrolyzer (maximum heating temperature: 800 °C) and chemical derivatization by use of MTBSTFA [32, 35]. The GC system contains eight capillary columns, mounted in parallel, equipped with different stationary phases, to enable unambiguous identification of the broad range of compounds expected in comets. Three of the stationary phases are chiral, enabling enantioselective separation of chiral aliphatic hydrocarbons and cometary amino acids. GC detection will be achieved by use of a miniaturized thermal conductivity detector (TCD) and a miniaturized (<1 kg) time-of-flight (TOF) high-resolution mass spectrometer with a mass range of 12–1500 amu (Table 1, second row) [36].

Mars exploration

Mars is a key target of astrobiologists because its past environmental conditions are believed to have been more favorable for existence of the prebiotic organics that led to life [4, 8, 11]. Several missions to Mars in the next two decades, from NASA and European Space Agency (ESA), will concentrate on identification of "biosignatures" in the Martian surface and subsurface in preparation for a manned mission to Mars [4, 6, 7, 11, 15, 39–43].

In the NASA Viking mission, for the first time ever, a pyrolysis-based GC–MS instrument was installed on the Viking space probe (Table 1, first row). However, at that time, polar organic compounds, for example amino acids and carboxylic acids, were not detected because of lack of detection sensitivity of the GC–MS instrumentation (above the parts per billion level) and because the experimental conditions of Viking's pyrolysis were not compatible with their extraction from the Martian soil [12, 31]. Later, Mars exploration continued with several space missions—including the Mars Exploration Rovers Spirit and Opportunity, the Mars Express probe, the Mars Reconnaissance Orbiter, and the Phoenix lander—and provided novel miniaturized instrumentation for sample handling and treatment to be installed on the rover vehicles [4, 11, 14, 39, 41–43].

The largest, most advanced suite of instruments yet sent to the Martian surface is the Sample Analysis at Mars (SAM) system included in the Mars Science Laboratory (MSL) mission launched on November 26, 2011, which landed on Mars on August 5, 2012 [14, 38, 40–43]. To detect a wide range of chemical indicators of life, the SAM analytical components include a quadrupole mass spectrometer (QMS), two pyrolysis ovens, six gas chromatographic columns, and a tunable laser spectrometer (TLS). Volatile compounds may be introduced into the QMS and TLS either directly from the atmosphere or by heating solid samples in the pyrolysis ovens. Nine pyrolysis cups on board SAM are devoted to wet chemistry, and combine single-step solvent extraction with chemical derivatization by use of a mixture of MTBSTFA and dimethylformamide (DMF) [14, 37, 38, 40, 41]. To detect polar organics that are bound to more complex organic molecules (e.g. amino acids in proteins) a thermochemolysis process (i.e., thermally assisted hydrolysis and methylation at temperatures >340 °C) is also implemented in SAM [41].

The GC assembly contains six complementary chromatographic columns with different stationary phases selected to provide good resolution capability for volatile organic compounds with a broad range of molecular weight and polarity, taking advantage of the significant advances in throughput and sensitivity provided by new columns in comparison with the instruments on the Viking. The SAM system also includes a ChiralDex column for discrimination of the enantiomers of chiral molecules (Table 1, fourth row). Such an instrument designed for SAM has been successfully tested with soil from Atacama Desert, the driest and oldest desert on Earth believed to be one of the best analogues of the Mars surface: traces of organic material (benzoic acid and amino acids) up to 10 pmol were detected in 100 mg soil [37]. As this article goes to press, this instrument is on Mars as part of the MSL rover.

Table 1 Overview of the GC instruments installed on probes of in-situ space missions for chiral discrimination

Mission; launch, arrival year	Experiment; sample type	GC columns	Detectors	Ref.
NASA Viking to Mars; 1975, 1976	GEX: gas GC–MS: soil	Pairs of packed Porapack Q Tenax coated with polymetaphenoxylene	Thermistore TCD MS	[31]
ESA Rosetta mission to comet; 2004, 2014	GC–MS; comet nucleus	Six WCOTs and two PLOTs in parallel; three chiral columns	One TOF MS and eight nanoTCDs in parallel	[32–36]
Phoenix mission; 2007, 2008	TEGA; Mars soil	Six GC capillary columns	MS	[7, 13]
NASA Mars Science Laboratory mission; 2011, 2012	Sample analysis at Mars (SAM); Mars soil	Six GC capillary columns for hydrocarbons, including a ChirasilDex for separation of chiral compounds	TCD, QMS	[14, 38, 40, 41, 43]
ESA ExoMars Mission to Mars biological environment; 2013	Mars organic molecule analyzer (MOMA); Mars soil	Four GC capillary columns, including a ChirasilDex for separation of chiral compounds; a pulsed ultraviolet laser for solid sample desorption and ionization	TCD, QMS	[42]
		MOA, a portable microfabricated capillary electrophoresis instrument	Fluorescence detector	[24]

A similar set of instruments is currently under development for installation in the Mars Organic Molecule Analyzer (MOMA) on board the forthcoming ExoMars mission (2016–2018) [43]. It includes pyrolysis ovens for single use, a GC with four different column types, a pulsed ultraviolet laser for solid sample desorption and ionization, and a mass spectrometer. In addition to the pyrolysis-GC–MS mode of operation common to SAM, MOMA can also use laser desorption and ionization procedures for direct MS analysis of molecular (organic and/or inorganic) fragments.

A "one-pot" extraction and chemical derivatization procedure has recently been developed for the SAM and MOMA experiments: the temperature and duration of the derivatization reaction, the procedure for pre-concentration of the chemical derivatives, and the GC–MS conditions were optimized under SAM instrument design constraints [41]. The samples were extracted and derivatized with a mixture of MTBSTFA and DMF before GC–MS analysis. The method has been validated on a fragment of the CM2 carbonaceous meteorite Murchison and a variety of terrestrial Mars analog materials, including surface soil samples collected from the Atacama Desert and a carbonate-rich mineral representative of those recently detected on Mars by the Phoenix lander and the Mars Reconnaissance Orbiter. The most promising results were obtained from the carbonate-rich mineral: leucine (0.02 nmol g^{-1}) and proline (0.01 nmol g^{-1}) and several unidentified compounds were successfully extracted and detected.

Search for chirality in space

The extremely popular MTBSTFA derivatization technique is unsuitable for separation of the enantiomers of amino acids, because it does not preserve the stereochemical configuration of the original compounds. Alternative esterification and acetylation procedures have therefore been developed to avoid racemization phenomena and enable correct determination of enantiomeric excess under flight-compatible conditions [26, 27].

The *N*,*N*-dimethylformamide dimethylacetal (DMF-DMA) reagent has been used in a single step derivatization procedure incorporated in the Rosetta mission's COSAC experiment [32–35]. This procedure has been further developed and optimized for inclusion in the SAM and MOMA experiments coupled with miniaturized GC–MS formats [46]. It is a rapid, one-step reaction (without any cofactors) that can occur at relatively low temperatures (140 °C); it can be easily automated and it yields low-molecular-weight products compatible with space MS performance (limited mass range for detection). This derivatization procedure enabled separation and characterization of the enantiomers of 20 protein amino acids on the chiral capillary column (Chirasil-Dex) that will be used in the SAM and MOMA experiments: resolution, R_S, of the pairs of enantiomers are reported in Table 2 (first column). In addition, low detection limits (LOD values ~1 pmol) facilitate its implementation in the in-situ Mars experiment.

A derivatization reaction that uses an alkyl chloroformate–alcohol–pyridine mixture to obtain the *N*(*O*,*S*)-alkyl alkoxy carbonyl esters of amino acids may be a suitable candidate for in-situ discrimination of enantiomers [47, 48]. Among the different reagents studied—methyl (MCF), ethyl (ECF), and isobutyl chloroformates (IBCF) in combination with alcohols with an alkyl chain identical with or different from that of the chloroformate—the combination of HFB (2,2,3,3,4,4,4-heptafluoro-1-butanol) with MCF provided the best separation, because five pairs of enantiomers of the 20 proteinogenic amino acids could be separated with a good resolution close to 2 (R_S values for pairs of enantiomers reported in Table 2, second column).

Another one-step derivatization procedure uses a mixture of perfluorinated anhydride and perfluoro alcohols to obtain the *N*(*O*,*S*)-perfluoroacyl perfluoroalkyl derivatives by simultaneous esterification and acylation [49, 50]. Different combinations of the derivatization reagents, i.e., trifluoroacetic (TFAA) and heptafluorobutyric (HFBA) anhydrides, 2,2,2-trifluoro-1-ethanol (TFE), and 2,2,3,3,4,4,4-heptafluoro-1-butanol (HFB) alcohols, have been investigated. All the reagent combinations yield derivatives with similar enantiomeric selectivity on a Chirasil-L-Val column (R_S values in Table 2, third, fourth, and fifth columns). Eight pairs of enantiomers of the 20 proteinogenic amino acids can be completely separated with $R_S \approx 2$ at a temperature lower than the column bleed limit (200 °C). The derivatives obtained were also separated on a γ-cyclodextrin (Rt-γ-DEXsa) chiral column, the enantiomeric selectivity of which is different from and complementary to that of Chirasil-L-Val. As an example, the GC–MS signal obtained from TFAA-TFE derivatives separated on the Chirasil-L-Val column is reported in Fig. 1.

Comparison of the chloroformate and anhydride procedures revealed that both enabled good chiral discrimination of several amino acids on the Chirasil-L-Val column, the same stationary phase as is used in the COSAC experiment [50].

In addition, both methods can be coupled with an automatic processing procedure to handle the complex GC–MS signals and extract information about the enantiomeric excess of the amino acids. As an example, the arrows in Fig. 1 indicate the constant distance between the chromatographic peaks of each pair of enantiomers that can be used to automatically estimate the presence and number of isomers in the sample [50]. Automatic data handling is especially helpful for saving labor and time and enabling rapid delivery of the results from in-situ space experiments, in which severe restrictions on data storage (analysis time generally limited to 10–20 min) and transmission are imposed by on-board conditions [17].

Recently, a new derivatization procedure using hexafluoroacetone, the first gaseous derivatizing agent, has been proposed to satisfy space constraints, in particular low energy consumption. The reaction introduces halogen atoms leading to high-mass ions that are more specific than low-mass ions and result in enhanced derivative detectability [51]. The automated procedure performs derivatization in an hour, at low

Table 2 Resolution (R_S) of amino acid enantiomer pairs after derivatization with different reagents

Amino acid	DMF–DMA[a]	HFB–MCF[b]	TFAA–TFE[b]	TFAA–HFB[b]	HFBA–HFB[b]	HFA[a]	HFA[c]
Alanine	6.59	2.11	1.79	1.84	1.71	1.05	0.50
Valine	0.83	2.39	1.49	2.26	1.20	1.11	0.67
Isoleucine		6.24	1.56	1.93	1.44	1.85	1.90
Leucine		3.88	3.22	3.97	4.13	0	2.00
Methionine		2.43	2.25	2.93	2.31	0	1.92
Glycine		1.38	3.43	2.31	2.07		
Phenylalanine	0.84	0.93	2.23	2.27	2.05		
Tyrosine	0.70	0.85	1.61	1.60	1.46	14.9	0
Proline	1.67					0.70	0
Norvaline						1.83	2.12
Threonine	4.73					2.23	1.91
Serine	6.36					1.42	2.20
Tryptophan						nd	1.52

Reagents: dimethylformamide (first column), chloroformate (second column), perfluoro anhydrides (third to fifth columns) and hexafluoroacetone (sixth and seventh columns)

DMF, *N*,*N*-dimethylformamide; DMA, dimethylacetal [46]; HFB, 2,2,3,3,4,4,4-heptafluoro-1-butanol [50]; MCF, methyl chloroformate; TFAA, trifluoroacetic anhydride; HFBA, heptafluorobutyric anhydride; TFE, 2,2,2-trifluoroethanol; HFA, hexafluoroacetone [51]

[a] Separation on β-cyclodextrin column

[b] Separation on Chirasil-L-Val column

[c] Separation on γ-cyclodextrin column

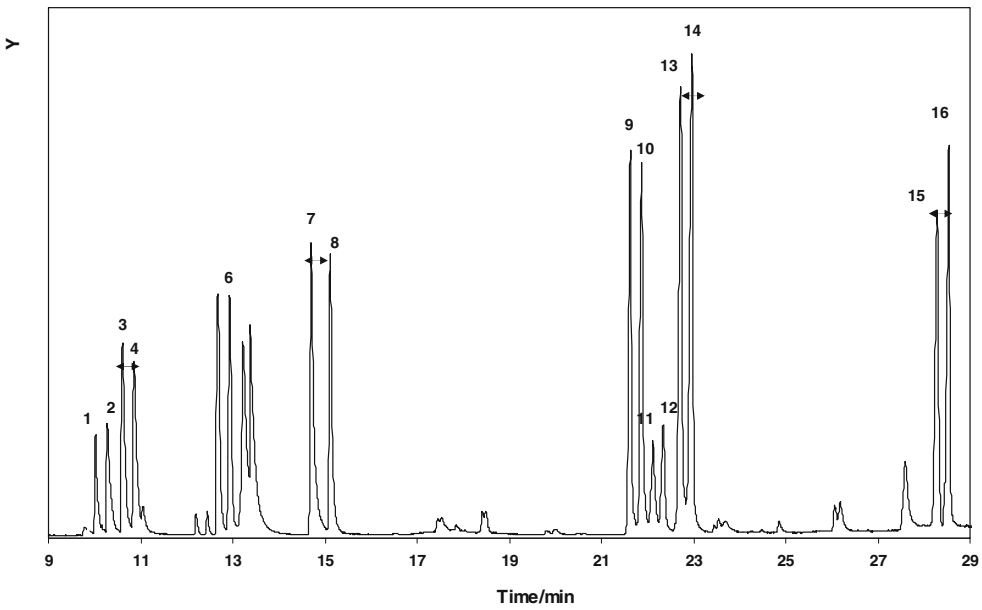

Fig. 1 GC–MS separation of TFAA–TFE derivatives. *Arrows*: constant interdistance Δt=0.25 min between the pairs of enantiomers. *1*, *2*, D,L-Val; *3*, *4*, D,L-Ala; *5*, *6*, D,L-Ile; *7*, *8*, D, L-Leu; *9*, *10*, D,L-Met; *11*, *12*, D, L-Glu; *13*, *14*, D,L-Phe; *15*, *16*, D,L-Tyr. GC column temperature program: from 40 to 200 °C at 4.4 ° min^{-1}, followed by isothermal conditions. Reprinted from Ref. [50]; copyright (2010), Elsevier, reproduced with permission

temperature, without stirring and solvent. The best chiral separation was achieved on a γ-cyclodextrin capillary column, from which all derivatives eluted in less than 30 min with good precision and low detection limit (~2.5–20 pmol injected for each amino acid) compatible with in-situ analysis. Among the 11 chiral amino acids analyzed, eight pairs of enantiomers could be separated with a constant order of elution, because the levorotatory form always eluted first (GC–MS signal reported in Fig. 2). Resolution, R_S >1.5 was obtained for six pairs of enantiomers, i.e., those of norvaline, leucine, isoleucine, threonine, methionine, and tryptophan; resolution was lower ($0.5 \geq R_S \geq 1.5$) for two other pairs (Table 2, sixth and seventh columns).

In general, all the alternative or complementary derivatization methods described are suitable for inclusion in the Mars missions, because they all enable good chiral discrimination of several amino acids with a wide linear response at trace levels (nmol detection limits).

Capillary electrophoresis in Mars exploration

Because of the high polarity of amino acids, liquid-based procedures have been the instrumental techniques of choice, because they have the great advantage of achieving direct

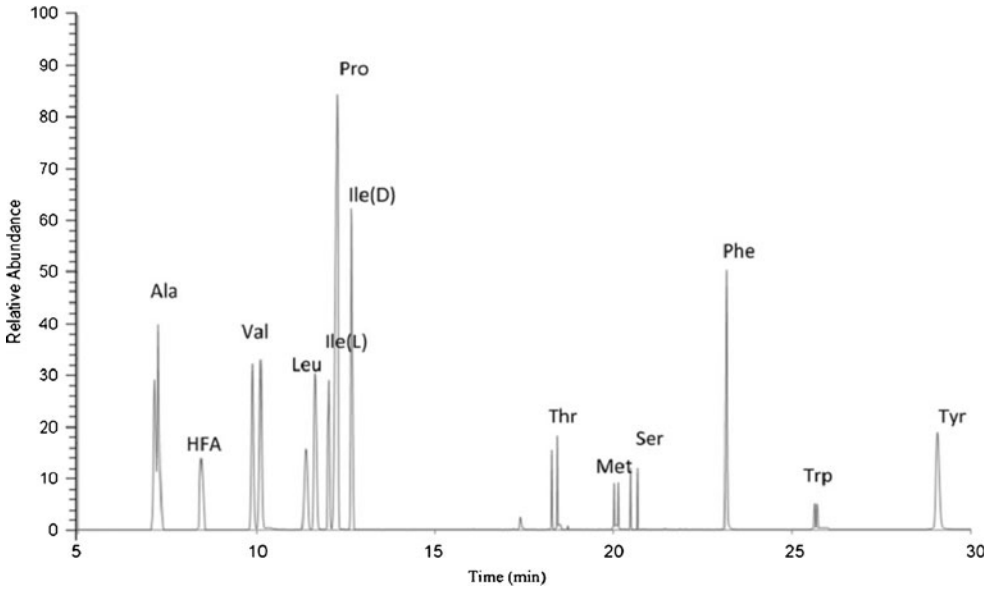

Fig. 2 GC–MS analysis of 11 HFA (L,D) derivatives on a γ-cyclodextrin column. L forms elute first. Split injection ratio 50:1. Column temperature program: from 60 (5 min) to 200 °C at 5 ° min^{-1}. Reprinted from Ref. [51]; copyright (2012) Elsevier, reproduced with permission

separation of amino acid enantiomers in solution, in comparison with the GC approach that requires preliminary sample pretreatment. Amino acid analysis is traditionally performed by use of chiral HPLC columns or, more recently, chiral capillary electrophoresis, because of its simplicity, rapidity, and high efficiency and resolution; furthermore, CE is highly flexible and versatile, with minimum use of expensive chiral reagents [22–24].

However, these methods do not have the low instrument mass and analysis volume required for in-situ space analysis. The advent of microfluidic systems has revolutionized this methodology, because existing separation systems can be miniaturized, enabling development of the ultra portable, fully automated, highly sensitive instrumentation that is essential for space research. In particular, microchip capillary electrophoresis (μCE), based on microfluidic lab-on-a-chip systems, enables high-performance chemical analysis of a broad range of compound classes with low mass and volume equipment and low power and reagent consumption. The coupling to laser-induced fluorescence (LIF) detection is extremely attractive because it results in ultra-low limits of detection of approximately 70 pmol L^{-1} for amino acids [23–25, 52–57].

μCE is well-suited to integration with other microfabricated components, including heaters, resistive temperature detectors, and pH-sensitive electrodes, enabling the automation required for spaceflight-ready liquid-based analysis of organic compounds [25, 52, 56, 57].

A microfabricated CE device with cyclodextrin as chiral selector in the running buffer was first proposed for chiral resolution of amino acids in space. Amino acids were labeled with fluorescamine and the chiral dye derivatives were analyzed in less than 200 s with high-quality chiral resolution, low background, and limits of detection of ~50 nmol L^{-1} [23].

In addition to conventional μCE, an electrophoretic separation using gradient elution isotachophoresis (GEITP) has been developed for chiral separation of amino acids labeled with different fluorescent agents [24]. The primary advantage of this technique is its simplicity, which ensures its suitability for in-situ space analysis of amino acid chirality. It uses the simplest possible microfluidic device—a short, straight microchannel connecting two reservoirs. Furthermore, there is no voltage switching to define an injection and no requirement for a temperature gradient.

A portable μCE instrument is a crucial part of the Mars organic analyzer (MOA) developed for the forthcoming ExoMars mission to detect primary amines and amino acids and amino acid chirality in the Martian environment [25] (Table 1, fifth row). The MOA was originally developed to analyze a wide variety of fluorescamine-labeled biomarkers containing the amine group, including amino acids: they were effectively labeled, separated, and detected at concentrations <500 nmol L^{-1} [58]. Labeling with a highly fluorescent amine reactive probe (Pacific Blue succinimidyl ester, PB) enhanced optical and chemical properties for biomarker detection, yielding a 200-fold increase in sensitivity, compared with fluorescamine, at concentrations as low as 75 pmol L^{-1} (sub-parts-per-trillion). This future Mars in-situ instrumentation was tested on Mars analogues: Fig. 3 reports the electrophoretic separation obtained from an Atacama Desert soil sample. The good resolution and efficiency of the capillary zone electrophoresis (CZE) separation (Fig. 3a) can be further enhanced by use of micellar electrokinetic chromatography (MEKC) with a zwitterionic surfactant (45 mmol L^{-1} CHAPSO, pH 6 at 5 °C) (Fig. 3b). With this procedure, most amino acids are resolved to baseline, and the different interactions between the L and D forms of the amino acids and the chiral center on the CHAPSO surfactant enables separation of the enantiomers of alanine and serine [25]. Another advantage of MEKC is that it is an orthogonal retention method for enhanced identification of

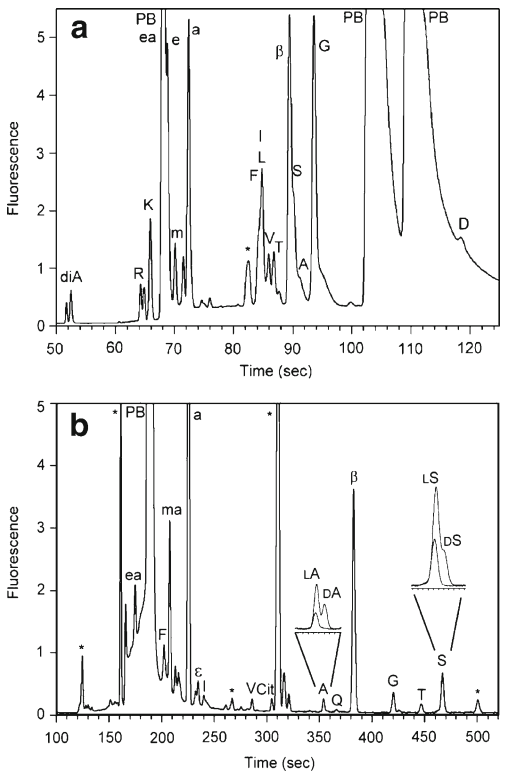

Fig. 3 Microchip electrophoretic analysis of the subcritical water extract from duracrust from the Yungay Hills (Atacama Desert, Chile). (**a**) capillary zone electrophoresis (CZE); (**b**) micellar electrokinetic chromatography (MEKC). The reaction product was diluted sevenfold for analysis. Peaks were identified on the basis of their mobility relative to the Pacific blue peaks (*PB*) and by spiking. Peaks: *a*, ammonia; *m*, methylamine; *e*, ethylamine, *ea*, ethanolamine. *Asterisks* indicate peaks having signals equivalent to those from the blank. Standard amino acids are designated by their one-letter designations. The *insets* of (**b**) show magnified views of the sample and the sample spiked with a 1:1 ratio of L and D-alanine and L and D-serine. Reprinted from Ref. [25]; copyright (2009), ACS Publications, reproduced with permission

amino acids, because it separates amino acids on the basis of different hydrophobicity.

Concluding remarks and future perspectives

Discovery of an enantiomeric excess of amino acids, as an indicator of biotic or prebiotic activity, will be of crucial importance in the search for extra-terrestrial life and in explanation of the emergence of the enantiomers present in the universe: both laboratory models and extraterrestrial investigations, for example the Rosetta, ExoMars, and MOMA missions, will be necessary to obtain more definitive answers. Therefore, starting from conventional benchtop scale instrumentation, dramatic improvements have led to sophisticated instruments suitable for chiral separation, mainly chiral CE, in in-situ space missions, in addition to the traditional GC approach.

It is, however, true that new breakthroughs are required to further enhance space techniques. The most pressing demand is miniaturization of equipment and methods. Microfluidic chips will certainly become high-performance analytical tools addressing such issues as energy saving, speed, throughput, and automation. In addition, microchips will enable growing opportunities which may lead to the development of new pretreatment tools suitable for integration of sampling procedures, including liquid extraction techniques, trapping and thermal desorption, preconcentration, and chemical derivatization. In this context, by using the novel ability of microfluidic systems to move in two dimensions, lab-on-a-chip systems can also be implemented to create two-dimensional apparatus combining separations in two independent achiral and chiral systems.

Other new breakthroughs in space applications concern detector performance: fluorimetric detectors and mass spectrometers can be miniaturized to provide high sensitivity and specificity in the detection of a wide variety of organic compounds. Simultaneously, new methods of ionization can be developed and adapted to direct MS analysis of condensed-phase extraterrestrial samples.

Recently, a new generation of analytical devices has been developed for the search for extra-terrestrial life in forthcoming space missions [58–61]. These use antibody microarray-based biosensors and are based on the capacity of antibodies or other antibody-like molecules for biomolecular interaction and specific recognition. One is the signs of life detector (SOLID) that uses a sandwich-type immunoassay to detect large-molecular-weight compounds. In addition, the life marker chip (LMC), one of the Pasteur payload instruments selected for the ExoMars mission, uses an inhibition microarray immunoassay to detect small size biomarkers. The next logical step of this approach is the production of new antibody microarrays to extend immunoassay detection to other highly relevant compounds, including stereospecific enantiomers, which might be essential components of the organic compounds present on Mars.

Finally, a new biological oxidant and life detection mission (BOLD) has been proposed for detecting life on Mars [62]. It uses new and sophisticated single-molecule biosensors that utilize the fully planar optofluidic lab-on-a-chip systems provided by the recent developments in liquid-core optical waveguides. The BOLD mission will also include a new generation of chiral detection experiments, able to detect life forms that are based on enantiomers. Labile organic compounds will be added to soil as pure enantiomers and the degradation products will be detected. Selective consumption of either D or L organic compounds is assumed to be evidence for biological activity, because microorganisms are expected to be selective and consume only one enantiomer whereas chemical oxidants would destroy both D and L isomers. It is desirable that the next step of the search for extraterrestrial life includes the integration of chemical and biochemical analytical procedures to be implemented in micro devices especially designed for comprehensive detection of a variety of target biomarkers.

References

1. Benner SA (2010) Defining Life. Astrobiol 10:1021–1030
2. Blumberg BS (2011) Astrobiology, space and the future age of discovery. Philos Transact A Math Phys Eng Sci 369:508–815
3. Gibson CH, Schild RE, Wickramasinghe NC (2011) The origin of life from primordial planets. Int J of Astrobiology 10:83–98
4. An Astrobiology Strategy for the Exploration of Mars (Free Executive Summary) (2007) National Academies Press, Washington, DC, http://www.nap.edu/catalog/11937.html
5. Pizzarello S, Huang Y, Fuller M (2004) The carbon isotopic distribution of Murchison amino acids. Geochim et Cosmochim Acta 68:4963–4969
6. Heinrich MN, Khare BN, McKay CP (2007) Prebiotic organic synthesis in early Earth and Mars atmospheres: Laboratory experiments with quantitative determination of products formed in a cold plasma flow reactor. Icarus 191:765–778
7. Navarro-Gonzalez R, Iniguez E, de la Rosa J, McKay CP (2009) Characterization of organics, microorganisms, desert soils, and Mars—like soils by thermal volatilization coupled to mass spectrometry and their implications for the search for organics on Mars by Phoenix and future space missions. Astrobiol 9:703–715
8. Summons RE, Amend JP, Bish D, Buick R, Cody GD, Des Marais DJ, Sumner DY (2011) Preservation of Martian organic and environmental records: Final report of the Mars Biosignature Working Group. Astrobiol 11:157–181
9. Cagliotti L, Holczknecht O, Fujii N, Zucchi C, Palyi G (2006) Astrobiology and biological chirality. Orig Life Evol Biosph 36:459–466
10. Li F, Fitz D, Rode BM (2013) Isoleucine as a possible bridge between exogenous delivery and terrestrial enhancement of homochirality. Amino Acids 44:725–732
11. Pratt LM, Allen C, Allwood A, Anbar A, Atreya S, Carr M, Des Marais D, Grant J, Glavin D et al (2010) The Mars Astrobiology

Explorer-Cacher (MAX-C): A potential rover mission for 2018. Final report of the Mars Mid-Range Rover Science Analysis Group (MRR-SAG) October 14, 2009. Astrobiol 10:127–63
12. DiGregorio B (2005) The search for organic molecules on Mars. Anal Chem 77:348A–353A
13. Navarro-Gonzalez R, Iniguez E, de la Rosa J, McKay CP (2009) Characterization of organics, microorganisms, desert soils, and Mars-like soils by thermal volatilization coupled to mass spectrometry and their implications for the search for organics on Mars by Phoenix and future space missions. Astrobiol 8:703–15
14. Buch A, Sternberg R, Szopa C, Freissinet C, Garnier C, Bekr EJ, Rodier C, Navarro-Gonzalez R, Raulin F, Cabane M, Stambouli M, Glavin DP, Mahaffy PR (2009) Development of a gas chromatography compatible Sample Processing System (SPS) for the in-situ analysis of refractory organic matter in Martian soil: preliminary results. Adv Space Res 43:143–151
15. Mahaffy PR (2009) Sample analysis at Mars: Developing analytical tools to search for a habitable environment on the Red Planet. Geochem News 141 http://www.geochemsoc.org/publications/geochemicalnews/gn141oct09/sampleanalysisatmars
16. Kaal E, Jansse HG (2008) Extending the molecular application range of gas chromatography. J Chromatogr A 1184:43–60
17. Pietrogrande MC (2012) Gas chromatography in space exploration. In: Poole CF (ed) Gas chromatography. Elsevier, Waltham, MA, pp 711–720
18. Geffroy-Rodier C, Grasset L, Sternberg R, Buch A, Ambles A (2009) Thermochemolysis in search for organics in extraterrestrial environments. J Anal Appl Pyrolysis 85:454–459
19. Gerakines PA, Hudson RL, Moore MH, Bell JL (2012) In situ measurements of the radiation stability of amino acids at 15–140 K. Icarus 220:647–659
20. Chan HS, Martins Z, Sephton MA (2012) Spectrofluorimetric analysis of amino acid mixtures: Implications for future space missions. Plan Space Sci 60:336–341
21. Jorge-Villar SE, Edwards HGM, Benning LG, AMASE 2004 team (2011) Raman spectroscopic analysis of arctic nodules: Relevance to the astrobiological exploration of Mars. Anal Bioanal Chem 401:2927–2933
22. Vandenabeele P, Jehlicka J, Vıtek P, Edwards HGM (2012) On the definition of Raman spectroscopic detection limits for the analysis of biomarkers in solid matrices. Plan Space Sci 62:48–54
23. Skelley AM, Scherer JR, Aubrey AD, Grover WH, Ivester RHC, Ehrenfreund P, Grunthaner FJ, Bada JL, Mathies RA (2005) Development and evaluation of a microdevice for amino acid biomarker detection and analysis on Mars. Proc Nat Acad Sci USA 102:1041–1046
24. Danger G, Ross D (2008) Chiral separation with gradient elution isotachophoresis for future in situ extraterrestrial analysis. Electrophoresis 29:4036–4044
25. Chiesl TN, Chu WK, Stockton AM, Amashukeli X, Grunthaner F, Mathies RA (2009) Enhanced amine and amino acid analysis using pacific blue and the mars organic analyzer microchip capillary electrophoresis systems. Anal Chem 81:2537–2544
26. Schurig V (2002) Chiral separations using gas chromatography. Trends Anal Chem 21:647–661
27. Schurig V (2011) Gas chromatographic enantioseparation of derivatized α-amino acids on chiral stationary phases e past and present. J Chromatogr B 897:3122–3140
28. Ilisz I, Berkecz R, Peter A (2008) Application of chiral derivatizing agents in the high-performance liquid chromatographic separation of amino acid enantiomers: A review. J Pharm Biomed Anal 47:1–15
29. Kirschner DL, Green TK (2009) Separation and sensitive detection of D-amino acids in biological matrices. J Sep Sci 32:2305–2318
30. Waldhier MC, Gruber MA, Dettmer K, Oefner PJ (2009) Capillary electrophoresis and column chromatography in biomedical chiral amino acid analysis. Anal Bioanal Chem 394:695–706
31. Mukhopadhyay R (2007) The Viking GC–MS and the search for organics on Mars. Anal Chem 79:7249–7256
32. Sternberg R, Szopa C, Rodier C (2002) Analyzing a comet nucleus by capillary GC. Anal Chem 74:481A–487A
33. Szopa C, Sternberg R, Raulin F, Rosenbauer H (2003) What can we expect from the in situ chemical investigation of a cometary nucleus by gas chromatography: first results from laboratory studies. Planet Space Sci 51:863–877
34. Szopa C, De Pra M, Tellini I, Sternberg R, Pietrogrande MC, Vidal-Madjar C, Raulin F (2004) Dual column capillary gas chromatographic system for the in situ analysis of volatile organic compounds on a cometary nucleus. J Sep Sci 27:495–503
35. Goesmann F, Rosenbauer H, Roll R, Szopa C, Raulin F, Sternber R, Munoz-Caro G (2007) Cosac, the cometary sampling and composition experiment on philae. Space Sci Rev 128:257–280
36. Todd JFJ, Barber SJ, Wright IP, Morgan GH, Morse AD, Sheridan S, Leese MR, Mark R et al (2007) Ion trap mass spectrometry on a comet nucleus: The Ptolemy instrument and the Rosetta space mission. J Mass Spectrom 42:1–10
37. Meunier D, Sternberg R, Mettetal BFA, Coscia D, Szopa C, Raulin F (2007) A laboratory pilot for in situ analysis of refractory organic matter in Martian soil by gas chromatography–mass spectrometry. Adv Space Res 39(3):337–344
38. Cabane M, Coll P, Israël G, Raulin F, Niemann H, Mahaffy P, Brinckerhof W (2002) Organic and inorganic signatures in Mars ground and underground, one of the goals for "SAM" (Sample Analysis at Mars). In: Lacoste H (ed) Exo-Astrobiology, vol. 518, 323–326
39. Getty SA, Inge LF, Feng L, Brinckerhoff WB, Cardiff EH, Holmes A, King VE, Li TT, Mumm E, Mahaffy PR, Glavin DP (2010) Development of an evolved gas-time-of-flight mass spectrometer for the Volatile Analysis by Pyrolysis of Regolith (VAPoR) instrument. Int J Mass Spectrom 95:124–132
40. Mahaffy PR, Webster CR, Cabane M, Conrad PG, Coll P (2012) The sample analysis at mars investigation and instrument suite space. Sci Rev 170:401–478
41. Stalport F, Glavin DP, Eigenbrode J, Bish D, Blake D, Coll P, Szopa C, Buch A, McAdam A, Dworkin JP, Mahaffy PR (2012) The influence of mineralogy on recovering organic acids from Mars analogue materials using the "one-pot" derivatization experiment on the Sample Analysis at Mars (SAM) instrument suite. Plan Space Sci 67(1):1–13
42. Bramall NE, Quinn R, Mattioda A, Bryson K et al (2012) The development of the Space Environment Viability of Organics (SEVO) experiment aboard the Organism/Organic Exposure to Orbital Stresses (O/OREOS) satellite. Plan Space Sci 60:121–130
43. Bishop JL, Franz HB, Goetz W, Blake DF, Freissinet C, Steininger H, Goesmann F, Brinckerhoff WB, Getty S, Pinnick VT, Mahaffy PR, Dyar MD (2012) Coordinated analyses of Antarctic sediments as Mars analog materials using reflectance spectroscopy and current flight-like instruments for CheMin, SAM and MOMA Icarus. doi:10.1016/j.icarus.2012.05.014
44. Rodier C, Sternberg R, Raulin F, Vidal-Madjar C (2001) Chemical derivatization of amino acids for in situ analysis of Martian samples by gas chromatography. J of Chromatogr A 915:199–207
45. Buch A, Sternberg R, Meunie D, Rodier C, Laurent C, Raulin F, Vidal-Madjar C (2003) Solvent extraction of organic molecules of exobiological interest for in situ analysis of the Martian soil. J Chromatogr A 999:165–174
46. Freissinet C, Buch A, Sternberg R, Szopa C, Geffroy-Rodier C, Jelinek C, Stambouli M (2010) Search for evidence of life in space: Analysis of enantiomeric organic molecules by N, N-dimethylformamide dimethylacetal derivative dependant Gas Chromatography–Mass Spectrometry. J Chromatogr A 1217:731–740
47. Zampolli MG, Basaglia G, Dondi F, Sternberg R, Szopa C, Pietrogrande MC (2007) Gas chromatography–mass spectrometry

analysis of amino acid enantiomers as methyl chloroformate derivatives: Application to space analysis. J Chromatogr A 1150:162–172
48. Zahradnickova H, Husek P, Simek P (2009) GC separation of amino acid enantiomers via derivatization with heptafluorobutyl chloroformate and Chirasil-L-Val column. J Sep Sci 32:3919–3924
49. Zampolli MG, Meunier D, Sternberg R, Raulin F, Szopa C, Pietrogrande MC, Dondi F (2006) GC–MS Analysis of Amino Acid Enantiomers as Their N(O, S)-Perfluoroacyl Perfluoroalkyl Esters: Application to Space Analysis. Chirality 18(4):279–295
50. Pietrogrande MC, Basaglia G (2010) Enantiomeric resolution of biomarkers in space analysis: Chemical derivatization and signal processing for gas chromatography–mass spectrometry analysis of chiral amino acids. J Chromatogr A 1217:1126–1133
51. Geffroy-Rodier C, Buch A, Sternberg R, Papot S (2012) Gas chromatography–mass spectrometry of hexafluoroacetone derivatives: first time utilization of a gaseous phase derivatizing agent for analysis of extraterrestrial amino acids. J of Chromatogr A 1245:158–166
52. Skelley AM, Scherer JR, Aubrey AD, Grover WH, Ivester RHC, Ehrenfreund P et al (2005) Development and evaluation of a microdevice for amino acid biomarker detection and analysis on Mars. Proc Natl Acad Sci U S A 102:1041–1046
53. Benhabib M, Chiesl TN, Stockton AM, Scherer JR, Mathies RA (2010) Multichannel capillary electrophoresis microdevice and instrumentation for in situ planetary analysis of organic molecules and biomarkers. Anal Chem 82(6):2372–2379
54. Stockton AM, Tjin CC, Huang GL, Benhabib M, Chiesl TN, Mathies RA (2010) Analysis of carbonaceous biomarkers with the Mars Organic Analyzer microchip capillary electrophoresis system: aldehydes and ketones. Electrophoresis 31:3642–3649
55. Stockton AM, Tjin CC, Chiesl T, Mathies R (2011) Analysis of carbonaceous biomarkers with the Mars Organic Analyzer microchip capillary electrophoresis system: carboxylic acids. Astrobiol 11:519–528
56. Cable ML, Stockton AM, Mora MF, Willis PA (2013) Low-temperature microchip nonaqueous capillary electrophoresis of aliphatic primary amines: Applications to titan chemistry. Anal Chem 85:1124–1131
57. Stockton AM, Mora MF, Cable ML, Willis PA (2013) Design rules and operational optimization for rapid, contamination-free microfluidic transfer using monolithic membrane valves. Sensors and Actuators B 17:668–675
58. Skelley AM, Cleaves HJ, Jayarajah CN, Bada JL, Mathies RA (2006) Application of the mars organic analyzer to nucleobase and amine biomarker detection. Astrobiol 6:824–837
59. Sims MR, Cullen DC, Rix CS, Buckley A, Derveni M et al (2012) Development status of the life marker chip instrument for ExoMars. Plan Space Sci 72:129–137
60. Blanco Y, Rivas LA, Ruiz-Bermejo M, Parro V (2012) Immunological detection of mellitic acid in the Atacama Desert: Implication for organics detection on Mars. Icarus. doi:10.1016/j.icarus.2012.06.006
61. Azua-Bustos A, Urrejola C, Vicuña R (2012) Life at the dry edge: Microorganisms of the Atacama Desert. FEBS Lett 586:2939–2945
62. Schulze-Makuch D, Head JN, Houtkooper JM, Knoblauch M, Furfaro R, Fink W et al (2012) The biological oxidant and life detection mission: A proposal for a mission to Mars. Plan Space Sci 67:57–69