

# The Significance of Microbe-Mineral-Biomarker Interactions in the Detection of Life on Mars and Beyond

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## Abstract

The detection of biomarkers plays a central role in our effort to establish whether there is, or was, life beyond Earth. In this review, we address the importance of considering mineralogy in relation to the selection of locations and biomarker detection methodologies with characteristics most promising for exploration. We review relevant mineral-biomarker and mineral-microbe interactions. The local mineralogy on a particular planet reflects its past and current environmental conditions and allows a habitability assessment by comparison with life under extreme conditions on Earth. The type of mineral significantly influences the potential abundances and types of biomarkers and microorganisms containing these biomarkers. The strong adsorptive power of some minerals aids in the preservation of biomarkers and may have been important in the origin of life. On the other hand, this strong adsorption as well as oxidizing properties of minerals can interfere with efficient extraction and detection of biomarkers. Differences in mechanisms of adsorption and in properties of minerals and biomarkers suggest that it will be difficult to design a single extraction procedure for a wide range of biomarkers. While on Mars samples can be used for direct detection of biomarkers such as nucleic acids, amino acids, and lipids, on other planetary bodies remote spectrometric detection of biosignatures has to be relied upon. The interpretation of spectral signatures of photosynthesis can also be affected by local mineralogy. We identify current gaps in our knowledge and indicate how they may be filled to improve the chances of detecting biomarkers on Mars and beyond. Key Words: DNA—Lipids—Photosynthesis—Extremophiles—Mineralogy—Subsurface. *Astrobiology* 15, 492–507.

## 1. Introduction

CERTAIN QUESTIONS have always intrigued humankind: How did life evolve on Earth? Are we alone in the Universe, or is there life elsewhere? Does this life resemble life on Earth? These are also fundamental scientific questions. The discovery of organisms in extreme environments on Earth has fostered the search for these answers and provided further motivation for the exploration for life beyond Earth. Life, some of it surprisingly complex, has been and is being found in places on our own planet that were once considered to be uninhabitable. The cold dry valleys of Antarctica harbor diverse, specialized microbial communities (Pointing *et al.*, 2009), while multicellular eukaryotes have been encountered deep (>1 km) in the subsurface (Borgonie *et al.*, 2011). Life can thrive under conditions of

extreme radiation, oxidative stress, pressure, temperature, pH, aridity, and salinity and with scarce nutrient availability (Satyanarayana *et al.*, 2005). The potential for long-term (>250 million years) preservation of life-forms (Vreeland *et al.*, 2000) and their components (Brocks *et al.*, 1999) provides further motivation for the search for past and present life on other planets, or their moons, and exoplanets.

Limitations in technology and distances are critical constraints in the search for life in planetary exploration programs. The search for habitable planetary bodies will often be limited to the remote spectrometric detection of signatures of possible life (Hanslmeier, 2009). However, several planetary bodies are physically accessible. Mars is the most promising candidate for detecting (extinct) life (Irwin and Schulze-Makuch, 2001) and remains the major target for planetary exploration in the near future.

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A search for life requires consideration of how life may reveal itself elsewhere and how life or biomarkers indicative for life may be detected. A *biomarker* refers to a measurable indicator of some biological state or condition, such as cellular components or isotopic fractionation. Considerable effort is being spent in the development and optimization of high-quality scientific instruments to measure biomarkers. However, sensitive detection also requires the efficient extraction of biomarkers from various substrates and the selection of sampling locations with characteristics most promising for successful exploration. The aim of this review is to address the importance of considering mineralogy in relation to the selection of sampling locations and to the methodology of detection of biomarkers by providing a review of relevant mineral-biomarker and microbe-mineral interactions. We will focus on three types of organic biomarkers of high interest to *in situ* detection: amino acids, lipids, and nucleic acids. We will first describe how martian mineralogy informs on current and past environmental conditions and whether they are, or were, compatible with life as we know it on Earth. Next, we will review research that indicates that on Earth the abundances and identities of the organisms producing these biomarkers are influenced by the type of mineral present in their local growth environment. Chemical characteristics of certain minerals have also played an important role in the evolution of life and the preservation of biomarkers, which is related to strong adsorption and accumulation of biomarkers on these minerals. Subsequently, we will indicate that this adsorption interferes with efficient extraction of biomarkers for detection and that minerals may contribute to the destruction of organic biomarkers during attempts to detect them. As only a few planetary bodies are accessible for *in situ* detection, we will also address the remote detection of biomarkers and how mineralogy influences their detection. We will conclude with suggestions for future research.

## 2. Molecular Biomarkers

Ideally, we would prefer to directly observe extraterrestrial life-forms on other (exo)planets. However, with regard to Mars, it is obvious that this planet is not teeming with life. Even on Earth, most of the living biomass consists of microorganisms and is invisible to the human eye (Whitman *et al.*, 1998). Therefore, the detection of biomarkers is likely more rewarding and central to planetary life-detection missions (Hansmeier, 2009).

Life on Earth uses carbon-based molecules for structural and metabolic functions. In principle, life elsewhere could be based on a completely different chemistry. Silicon, an abundant element in many minerals, has often been proposed as an alternative for carbon (Pace, 2001; Zhang *et al.*, 2003). However, it cannot form the types of chemical bonds with many of the elements with which carbon does, which limits the flexibility needed for biological metabolism (Pace, 2001; Rizzotti, 2009). Furthermore, carbon chemistry is ubiquitous; a wide variety of complex carbon-based molecules have been identified in the interstellar medium and on comets and meteorites (Ehrenfreund and Charnley, 2000). Organic building blocks of the common factors of life are in general relatively easily formed and joined into polymers by chemical reactions (Ehrenfreund *et al.*, 2006). Organic

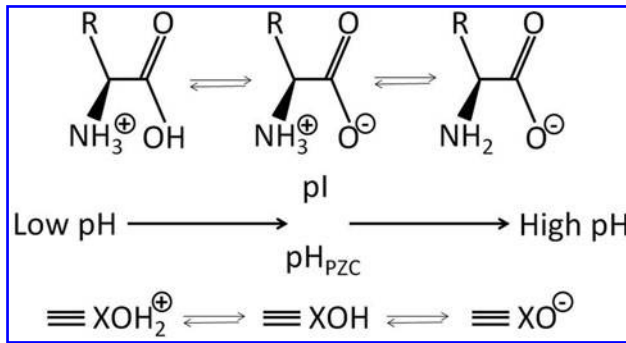
matter delivered by meteoritic impacts to planetary bodies also comprises a source of diverse amino acids and nucleobases (Sephton, 2002; Martins *et al.*, 2008). These factors may have contributed to the rapid establishment of life on Earth soon after cooling of its crust, 3.8 billion years ago (Derenne *et al.*, 2008; Westall, 2009), and warrant the extraterrestrial search for organic biomarkers.

Several classes of organic biomarkers have been proposed based on a number of biological features that are common to all known life-forms on Earth, such as a compartmentalized shape, energy-producing and storage mechanisms, and the use of biomolecules to store hereditary information (McKay, 1997; Parnell *et al.*, 2007; Summons *et al.*, 2008). These biomarkers comprise relatively simple monomers that constitute the building blocks of cells, such as L-amino acids, D-sugars, pigments, and lipids (*e.g.*, isoprenoids, hopanoids), and complex biopolymers such as nucleic acids.

The implicit assumption in proposing particular biomarkers is an expected commonality of chemistry in life's processes (Pace, 2001). However, although the core classes of molecules in extraterrestrial life-forms might be similar to those of life on Earth, their overall structural makeup could be different in some aspects. Therefore, planetary missions aim to address a wide range of biomarkers in order to provide the strongest evidence for extraterrestrial life (Parnell *et al.*, 2007). The search should be broad enough to include detection of biomarkers that are not necessarily completely identical to those used in Earth's biology. In this review, we will focus on three different types of biomarkers that, taken together, cover these aspects: amino acids, membrane lipids, and nucleic acids.

Amino acids and lipids are building blocks of life, which are considered high-priority molecular targets in the search for life on Mars because of their great chemical stability and potential for persisting for billions of years in the geological record (Brocks *et al.*, 1999; Aubrey *et al.*, 2006; Kminek and Bada, 2006). Amino acids consist of an amine group, a carboxylic acid, and a structural group, which is different in each amino acid and partially determines its characteristics. More than 500 different amino acids have been detected (Wagner and Musso, 1983). Of these, the  $\alpha$ -amino acids play an important role in life on Earth. These amino acids have an amine and carboxylic acid group attached to the first carbon ( $\alpha$ ) atom and have an organic substituent as the functional side chain (Fig. 1). The  $\alpha$ -amino acids include the 23 proteinogenic amino acids, which are linked together by the protein synthesis machinery into peptides resulting in the formation of proteins in all domains of life. Amino acids are relatively easily formed abiotically and have been detected in extraterrestrial meteorites (Sephton, 2002; Martins *et al.*, 2007), which can be used as an argument for their potential universal use in life. Amino acids are rapidly degraded when exposed to ionizing radiation and other oxidizing conditions at the surface (ten Kate *et al.*, 2006; Dartnell, 2011; Pavlov *et al.*, 2012), but when buried at a depth of 2 m, and in the absence of enzymatic degradation, they can persist up to 3.5 billion years (Aubrey *et al.*, 2006; Kminek and Bada, 2006).

The abiotic formation of amino acids appears to undermine their usefulness as unambiguous biomarkers. However, most amino acids display isomerism, appearing as levorotatory (L-) and dextrorotatory (D-) enantiomers, which are each other's non-superimposable mirror image. Life on Earth



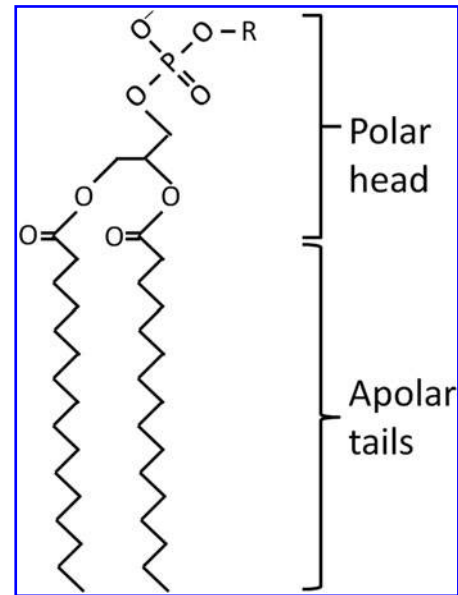
**FIG. 1.** General molecular structure of L- $\alpha$ -amino acids, revealing their zwitterionic characteristics and the dependence of the charge of their carboxylic acid and  $\alpha$ -amino groups on pH. At a pH equal to the isoelectric point (pI), the net charge is zero. R is the side chain specific to each amino acid. The bottom part shows a hydroxide mineral, which reveals a similar impact of pH on charge, with  $\text{pH}_{\text{pzc}}$  indicating the pH corresponding to the point of zero charge (pzc).

primarily uses amino acids in the L-configuration (Fig. 1). Amino acid mixtures become racemic (*i.e.*, the two different enantiomers are present in equal amounts) over time due to natural processes. Deviations from racemic mixture hints at a biological origin, which provides additional motivation to target amino acids as indicators for life (Meierhenrich, 2008). However, it is becoming increasingly clear that abiotic processes can also introduce enantiomer excesses, so care must be taken when using these excesses as an exclusive indication of biotic origin (Jorissen and Cerf, 2002; Glavin *et al.*, 2013). Carbon isotope composition can also reveal the biological or abiotic origin of individual amino acids (Ehrenfreund *et al.*, 2001).

Lipids are the major component of cell membranes in life-forms on Earth and play an essential role in membrane permeability, as protein anchor, and in energy conservation (Madigan *et al.*, 2012). Eight classes of lipids are distinguished. An example of phospholipids is shown in Fig. 2. These lipids contain a polar head that interacts with water and hydrophobic tails. Lipids can self-assemble to form double-layered membranes with heads facing an aqueous phase and tails facing each other. The ability for self-assembly into double-layered membranes has also been observed for abiotic molecules identified in meteorites; however, these molecules were much shorter than those observed in organisms (Deamer, 1985; Naraoka *et al.*, 1999).

Membranes in living organisms are generally characterized by a specific distribution profile of lipids, with a strong dominance of lipids with an even number of 14–20 carbons (Georgiou and Deamer, 2014). Many lipids, such as isoprenoids, have no known abiotic source and are derived from unique structural building blocks (*e.g.*, isoprene units) specific to life (Summons *et al.*, 2008). Biological lipids and their derivatives can survive for billions of years in the geological record, such as in the 2.7 billion-year-old shales from the Pilbara Craton, Australia (Brocks *et al.*, 1999). Altogether, this makes lipids an obvious target for life detection (Summons *et al.*, 2008).

Compared to amino acids and lipids, the detection of biopolymers, such as molecules storing hereditary information, would be far more compelling evidence for life due to



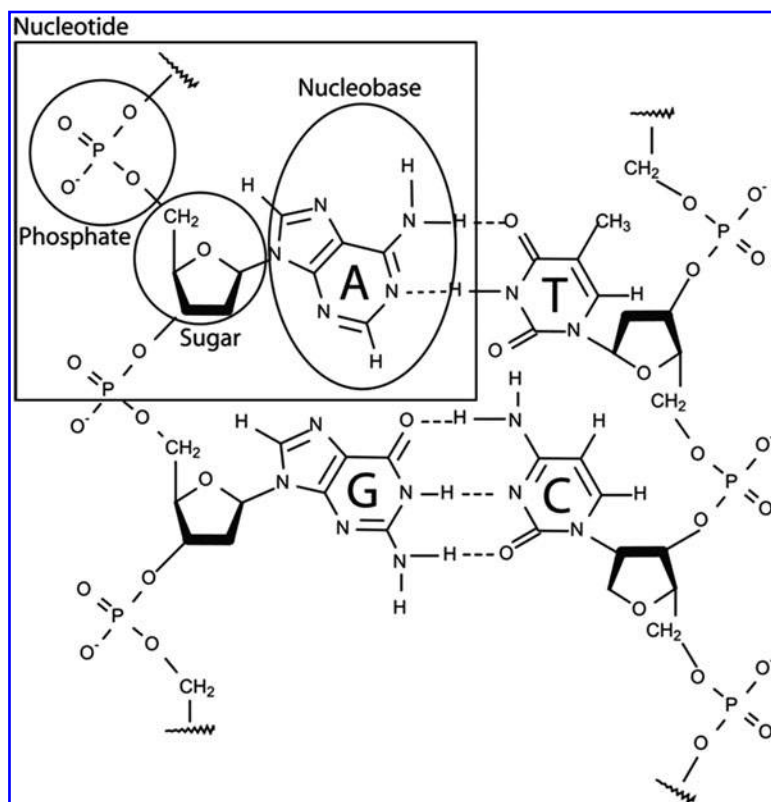
**FIG. 2.** Example of a lipid: structure of a membrane phospholipid, containing a diglyceride, a phosphate group, and an organic molecule R. The diglyceride contains two saturated C16 fatty acid chains, which form a hydrophobic tail. The fatty acids are covalently bound to glycerol through ester linkages. The glycerol backbone and phosphate group provide a hydrophilic, charged polar head.

their high complexity. On Earth, life-forms store their hereditary information in nucleic acids—in DNA or RNA (Madigan *et al.*, 2012). These nucleic acids are composed of nucleotide monomers containing a sugar, a phosphate, and a nucleobase (Fig. 3). The most common nucleobases found in nucleic acids of organisms on Earth are adenine, thymine, cytosine, uracil, and guanine. DNA has deoxyribose as the sugar group, and RNA has ribose. The presence of DNA- and RNA-like molecules in extraterrestrial life is plausible since building blocks of nucleic acids also have been delivered from space to Earth. Nucleobases encountered in carbonaceous chondritic meteorites include guanine, hypoxanthine, xanthine, adenine, and uracil (Martins *et al.*, 2008; Callahan *et al.*, 2011). Riboses have been identified in the gaseous phases of interstellar medium (Cocinero *et al.*, 2012).

A drawback to nucleic acids as biomarkers is their relative instability in comparison to amino acids and lipids; the theoretical life span of nucleic acids is 50,000–100,000 years (Lindahl, 1997). Still, there are reports of ancient DNA and even living microorganisms (thus containing intact DNA) being recovered from samples that are millions of years old (Vreeland *et al.*, 2000; Panieri *et al.*, 2010). Nucleic acids rapidly degrade under the strong UV radiation and oxidizing conditions of Mars' surface (Martins, 2011). However, in the subsurface they are more protected from these destructive forces, and martian low temperatures and dryness may help preserve remnants of life even better than on Earth (Sephton, 2010).

### 3. Mineralogy of Mars in Relation to Conditions for Life

Mars is the major destination of current astrobiological missions. Studies of martian meteorites (*e.g.*, Bouvier *et al.*,



**FIG. 3.** The double-stranded DNA structure and its four nucleotides. DNA is composed of nucleotide monomers containing a phosphate, a sugar (deoxyribose), and a nucleobase. In DNA, the nucleobase adenine (A) pairs with thymine (T), and guanine (G) pairs with cytosine (C). In RNA, thymine (T) is replaced by uracil (U), and the sugar is ribose.

2005) and orbiter, lander, and rover missions have provided detailed insight on its mineralogy and therewith its evolution and habitability (e.g., Hanslmeier, 2009; Arvidson *et al.*, 2014; Grotzinger *et al.*, 2014). While current conditions on the surface of Mars are hostile to life and the persistence of biomarkers, conditions in the past were more compatible with life. Three major climatic stages have been proposed for the geological history of Mars (Bibring *et al.*, 2005). During its first 0.7–0.9 billion years, Mars was likely water-rich and possessed a magnetic field and thicker atmosphere (Walter and Des Marais, 1993; Kasting, 1997). Physicochemical conditions of the surface of Mars were mainly controlled by a global hydrological cycle on its surface, and in its subsurface, by volcanic activity (Walter and Des Marais, 1993), and by meteoritic impacts delivering organics (Flynn, 1996; Grady, 2008). These conditions were rather similar to those on early Earth where life evolved in this period, soon after cooling of the Earth's crust, 3.8 billion years ago (Poulet *et al.*, 2005; Bénilan and Cottin, 2007). The presence of evaporite minerals and hydrated silicate minerals is indicative of past aqueous activity (Meslin *et al.*, 2013).

Martian mineralogy is diverse and spatially variable, with iron oxides (magnetite and hematite), iron oxyhydroxides (goethite and ferrihydrite), iron sulfides, silicates (feldspar, olivine, pyroxene, and plagioclase), evaporites (sulfates, e.g., jarosite and gypsum), carbonates, clay minerals (phyllosilicates; e.g., montmorillonite and kaolinite), and quartz (e.g., Squyres *et al.*, 2004a, 2004b; Poulet *et al.*, 2005; Grady, 2008; Mustard *et al.*, 2008; Bish *et al.*, 2013; Arvidson *et al.*, 2014; Grotzinger *et al.*, 2014; Vaniman *et al.*, 2014). Mineral assemblages such as jarosite suggest that acidic, oxidizing, and saline conditions once prevailed at Gusev Crater and

Meridiani Planum (Hurowitz and McLennan, 2007). Smectite signatures associated with fine-grained, layered rocks containing spherules indicate aqueous environments of slightly acidic to circumneutral pH in the Endeavour Crater, which would have been more favorable for prebiotic chemistry and microorganisms than those recorded by younger sulfate-rich rocks at Meridiani Planum (Arvidson *et al.*, 2014). The mineralogy at Yellowknife Bay, Gale Crater, indicates a habitable fluviolacustrine environment characterized by neutral pH and low salinity and variable redox states of both sulfur and iron species (Grotzinger *et al.*, 2014).

A climate shift occurred on Mars about 3.6–3.8 billion years ago, which led to a drastic decline in global temperature and water availability (Fairén *et al.*, 2010). During this second stage, a snowball Mars arose, with greatly diminished surface runoff and large areas becoming ice-covered, as has also been hypothesized to have occurred on Earth at least once (Fairén *et al.*, 2010). The third and current stage of Mars is characterized by extremely arid and cold conditions (average surface temperature is about  $-60^{\circ}\text{C}$ , varying from  $-130^{\circ}\text{C}$  at the poles in winter to  $+30^{\circ}\text{C}$  during daytime at the equator in summer), with a thin atmosphere ( $\sim 6$  mbar) composed of  $\sim 95\%$   $\text{CO}_2$ . Surface exposure to galactic cosmic radiation and solar energetic particles is high (Pavlov *et al.*, 2002; Dartnell *et al.*, 2007; Pavlov *et al.*, 2012; Hassler *et al.*, 2014), and it has been speculated that radiation generates strong oxidants on the martian soil surface (Yen *et al.*, 2000). Indeed, perchlorates, salts with strong oxidizing properties, especially when heated, appear to be globally distributed on Mars (Hecht *et al.*, 2009; Glavin *et al.*, 2013; Leshin *et al.*, 2013).

Analogues that emulate several of the physicochemical conditions during these three geological stages of Mars can



be found on Earth, such as phyllosilicate-rich field locations, acidic aqueous environments, permafrost, and hyperarid deserts (Fairén *et al.*, 2010). Investigation of these Mars analogues has revealed their habitability. However, the combination of all surface conditions on Mars is not found anywhere on current Earth. On present-day Mars, the continuously high radiation is expected to destroy life and its biomarkers on, and close to, the surface (Pavlov *et al.*, 2002; Dartnell *et al.*, 2007; Pavlov *et al.*, 2012; Hassler *et al.*, 2014). It has been suggested that after surface conditions on Mars became hostile for life, life may have found a refuge in the martian subsurface (Boston *et al.*, 1992). Organics may also have been preserved buried underneath the oxidizing surface of Mars in, for example, evaporites that filter radiation effectively (Kanavarioti and Mancinelli, 1990; Vreeland *et al.*, 2000). Even on Earth, the majority of microorganisms are found in the subsurface, and the microbial biomass in the subsurface has been estimated to roughly equal or even outweigh the plant biomass on the surface of Earth (Whitman *et al.*, 1998). Evidence for long-term (>1 million years) sustained life in Earth's subsurface that does not depend on solar energy and products of photosynthesis is accumulating (Lin *et al.*, 2006). Radiolysis and hydrogen-generating reactions between iron-containing minerals and water may fuel this subsurface life (Pedersen, 2000).

The occurrence of methane on Mars was for a long time debated (Webster *et al.*, 2013), but the Sample Analysis at Mars (SAM) instrument on the Curiosity rover recently detected methane (Webster *et al.*, 2015). Assuming a biotic origin for previously reported methane concentrations on Mars (Formisano *et al.*, 2004), Tung *et al.* (2005) estimated that Earth-like methanogens may be present at just around one cell per milliliter, if distributed uniformly over a 10 m thick layer at a temperature of about 0°C. This temperature occurs between 150 and 8 km depth on Mars. This low abundance and the remote location provide challenges for detecting life on Mars. As already briefly alluded to above, when we described reactions between minerals and water fueling subsurface life, the relationships between mineralogy and microorganisms and their associated biomarkers need to be taken into account in order for the odds of detecting extinct or extant life on Mars to be as high as possible.

#### 4. Microbe-Mineral Interactions

The numbers of microorganisms associated with solid surfaces in subsurface environments on Earth are typically more than one order of magnitude higher than those of the free-living microorganisms in pore water, per volume unit (Albrechtsen and Winding, 1992; Holm *et al.*, 1992). The identities and metabolic potentials of attached microorganisms differ from those in the nearby liquid phase (Albrechtsen *et al.*, 1996; Crump *et al.*, 1999; Röling *et al.*, 2001).

Attachment to minerals can help microorganisms protect themselves against extreme environmental conditions and physicochemical stresses (Decho, 2000) or against predators (Wey *et al.*, 2008). Nutrient availability in minerals is also an important reason for attachment (Roberts, 2004; Carson *et al.*, 2009). Electrostatic interactions between charged minerals and cell surface components play an important role in attachment (Roberts, 2004; Walker *et al.*, 2004). Microorganisms may also become associated with minerals due to

entrapment as the result of mineral precipitation (Fortin *et al.*, 1997; Southam and Saunders, 2005).

The distribution and abundances of mineral-associated microorganisms are affected by the type of mineral (Boyd *et al.*, 2007; Mauck and Roberts, 2007) and their particle sizes (Albrechtsen, 1994; Sessitsch *et al.*, 2001). These factors, and associated differences in pore sizes (Ruamps *et al.*, 2011), pore connectivity (Carson *et al.*, 2010), and other physicochemical properties (Killham *et al.*, 1993), may contribute to heterogeneous and complex microbial communities over short distances, on a centimeter scale (Franklin *et al.*, 2002), and thus are important to consider in relation to astrobiological missions and sampling extraterrestrial soils for biomarker detection.

Elemental mineral composition plays a major role in attachment and the abundances of microorganisms (Banfield *et al.*, 1999; Roberts, 2004; Mauck and Roberts, 2007). Essential elements for growth (such as P and K) are often scarce in groundwater (Banfield *et al.*, 1999). Silicate weathering by microorganisms is determined by nutrient requirements. Microorganisms can display a preference for attachment to, and dissolution of, minerals containing growth-limiting nutrients (Rogers *et al.*, 1998; Bennett *et al.*, 2001). Weathering of minerals like apatite provides phosphate required for microbial activity and growth (Bennett *et al.*, 2001). Dissolution of sulfide minerals and iron-magnesium silicates supplies enzyme cofactors like Cr, Cu, Fe, Mg, Mo, Ni, and Zn (Banfield *et al.*, 1999; Rogers and Bennett, 2004).

Microorganisms can also use redox-reactive elements in minerals for their energy metabolism (Edwards *et al.*, 2003; Lovley *et al.*, 2004). Generally, large quantities of these elements are required for a chemotrophic lifestyle. In particular, Fe- and S-bearing minerals are utilized as electron donors, such as Fe(II)- or sulfide-containing minerals (Edwards *et al.*, 2003), or as electron acceptors, such as sulfur and Fe(III) (Lovley *et al.*, 2004). A wide range of iron (oxyhydr)oxides and clays can be utilized for Fe(III) reduction or Fe(II) oxidation (Shelobolina *et al.*, 2004; Dong *et al.*, 2009). Anaerobic microbial iron oxidation and reduction has been hypothesized to be one of the most primordial microbial metabolisms on Earth and is proposed as a possible metabolism for other iron-mineral-rich planets like Mars (Weber *et al.*, 2006). Geochemical, phylogenetic, and physiological data suggest that anaerobic Fe(III) reduction by thermophilic microorganisms may have been the first electron-accepting process on Earth (Vargas *et al.*, 1998). The postulated variable redox states of both sulfur and iron species, in association with neutral pH and low salinity, may have once supported a chemolithoautotrophy-fueled biosphere at Yellowknife Bay in the Gale Crater of Mars (Grotzinger *et al.*, 2014).

On the other hand, minerals can also have antimicrobial properties. Natural antibacterial clays containing nanoscale illite-smectite and reduced iron phases buffer conditions that promote iron solubility, leading to subsequent intracellular iron accumulation, oxidation, and precipitation, and the formation of iron-associated deadly radicals (Williams *et al.*, 2011). Aluminum, such as that found in the phyllosilicate kaolinite ( $\text{Al}_2\text{Si}_2\text{O}_5(\text{OH})_4$ ), is potentially toxic to some bacteria (Roberts, 2004).

Thus, mineralogy affects the abundances of microorganisms and thereby the abundances of their biomarkers. It also

impacts the type and diversity of biomarkers, as mineralogy also influences the structure and diversity of bacterial communities associated with geological substrates incubated *in situ*. Most of these observations are based on studies that involved only a limited number (~3–6) of minerals or other growth supports (Reardon *et al.*, 2004; Boyd *et al.*, 2007; Mauck and Roberts 2007; Carson *et al.*, 2009; Direito *et al.*, 2012; Mitchell *et al.*, 2013) exposed to a variety of incubation procedures, ranging from direct incubation of minerals in soils (Carson *et al.*, 2009) to the use of different solid supports in aquifers (Mauck and Roberts, 2007). These studies included anaerobic subsurface environments (Mauck and Roberts, 2007; Direito *et al.*, 2012), which are of relevance with respect to detecting life on Mars. In an iron-reducing aquifer, a positive correlation between Fe content of minerals and attached biomass was observed (Mauck and Roberts, 2007). Recently, the effect of bedrock mineral composition on microbial abundances and diversity in a subglacial environment was determined. This study demonstrated that Fe- and S-containing minerals, especially pyrite, can have a large influence on bacterial community structure and biomass density (Mitchell *et al.*, 2013). Reported biomass per gram mineral was about tenfold higher on Fe- and S-containing minerals compared to quartz. Fe(III) is present in a wide range of martian minerals. When Fe(III)-containing minerals are the sole source of electron acceptor in anaerobic settings, different forms of these minerals may support different communities, as has been shown experimentally (Lin *et al.*, 2007).

Mechanistic understanding on the distribution and abundances of microbial species in relation to mineralogy is rudimentary. Next to physiological drivers, microbial attachment to mineral surfaces is frequently governed by differences in the surface charge of cell membranes and minerals. Electrostatic interactions were found to dominate microbial colonization on positively charged oxide surfaces irrespective of mineral composition, while on negatively charged silicate minerals, the solid phase composition determined the extent of microbial colonization and diversity (Roberts, 2004).

### 5. Adsorption of Organics to Minerals: The Origin of Life and Preservation

Mineral type not only affects the abundances and diversity of microorganisms but also the abundances and diversity of organic biomarkers attached to minerals. Crystalline surfaces of minerals are postulated to have contributed to the origin of life on Earth by participating in the synthesis, selection, concentration, and organization of organic molecules in the transition from geochemistry to biochemistry (Bernal, 1951; Hazen and Sverjensky, 2010). Transition metal sulfides and oxides can promote a variety of organic reactions, such as nitrogen reduction, hydroformylation, and amination. Specific amino acids, sugars, and other molecules are concentrated on the surfaces of oxides, silicates, and carbonates, which can also enhance their stability. Clay minerals and hydroxides have the capacity to facilitate self-organization and condensation polymerization. Clays tend to adsorb organic molecules strongly (*e.g.*, Saeki and Sakai, 2009), and this contributes to their ability to catalyze a diversity of organic reactions, such as RNA formation and elongation on montmorillonite clay (Ferris *et al.*, 1996).

Illite and hydroxylapatite can act as scaffolds in the oligomerization of amino acids (Ferris *et al.*, 1996; Lambert, 2008). Minerals may even have contributed to homochirality, because chiral surfaces of a range of minerals, including quartz, alkali feldspars, and clinopyroxene, can separate L- and R-configured molecules, such as L- and D-amino acids (Hazen and Sverjensky, 2010).

Minerals implicated in the origin of life on Earth are also of interest to astrobiological missions because biomarkers may be protected by adsorption to them. For instance, DNA binding to clay minerals protects against enzymatic digestion (Aardema *et al.*, 1983; Scappini *et al.*, 2004), UV radiation (Scappini *et al.*, 2004), and X-rays (Ciaravella *et al.*, 2004).

The strength of the adsorption of biomarkers to minerals depends on properties of both. Minerals that adsorb one type of biomarker strongly may adsorb other biomarkers weakly. Hematite adsorbs a range of amino acids (Bentaleb *et al.*, 1994), but DNA hardly at all (Direito *et al.*, 2012). Minerals adsorb amino acids with charged side groups much more than those with uncharged side groups (Zaia, 2004). Also the structure of biomolecules affects their adsorption; linear DNA binds more strongly than supercoiled DNA, likely by having a continuous line of contact with the surface (Melzak *et al.*, 1996).

The mechanisms of adsorption of biomarkers to minerals are diverse, again relating to properties of both minerals and biomarkers and to local physicochemical conditions, such as solution chemistry and pH. Adsorption of organic molecules on oxide surfaces is affected by complex surface speciation in relation to mineral structure, with inner and outer sphere species varying in relative importance over a range of pH, ionic strength, and surface coverage (Hazen and Sverjensky, 2010). The substitution of the inorganic interlayer cations that occur naturally in smectite clays by small organic cations alters the adsorption characteristics of clays for amino acids (Fraser *et al.*, 2011). The differences in side chains between amino acids have an effect on their charge and polarity, although most amino acids are readily soluble in water. Electrostatic interactions generally play a dominant role in the adsorption of amino acids to minerals (Churchill *et al.*, 2004). The net charge of mineral surfaces is a function of pH; the net charge is zero when the pH of the solution equals the so-called “point of zero charge,”  $pH_{pzc}$ . When the pH of the solution is below this  $pH_{pzc}$ , the net surface charge of the mineral is positive, and it is negative when the pH of the solution is above  $pH_{pzc}$  (Fig. 1). The charge of an amino acid is similarly affected by solution pH. Amino acids are zwitterions; they contain both acid and base groups (Fig. 1). The charge of an amino acid is zero when solution pH equals the “isoelectric point,”  $pI$ , which is determined by the side group of an amino acid (Fig. 1). Both  $pI$  and  $pH_{pzc}$  differ strongly among amino acids and among minerals, respectively, ranging from acidic to alkaline. Adsorption between minerals and amino acids is stronger when their respective  $pH_{pzc}$  and  $pI$  values differ more from each other (Churchill *et al.*, 2004). However, other factors besides net electrostatic interactions play a significant role in selective adsorption and its strength (Bentaleb *et al.*, 1994; Churchill *et al.*, 2004), such as the geometrical distribution of positive and negative charge centers associated with specific mineral surfaces and individual amino acids (Churchill *et al.*, 2004).

Clays are of great interest to astrobiology because of their surface electrostatic charges and their fine-grained particles

with large reactive surface areas, which enable them to adsorb strongly polar biomarkers such as DNA and RNA (Novinscak and Filion, 2011; Direito *et al.*, 2012). Saeki and Kunito (2010) suggested that DNA adsorption is more likely to depend on mineral surface charges than on mineral surface area. Adsorption is proposed to occur at the edges of sheets containing aluminum(III) groups that coordinate with water molecules ( $\text{Al-OH}_2^+$ ), promoting acidity and causing negatively charged substances, such as the DNA phosphate groups, to bind (Ferris, 2005; Saeki and Sakai, 2009). DNA molecules have low affinity for silica ( $\text{SiO}_x$ ); however, DNA adsorption is enhanced by the presence of cations (Saeki and Kunito, 2010). Monovalent cations, like  $\text{Na}^+$ , reduce the electrostatic barrier between DNA and silica, while divalent cations, like  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ , neutralize charges by binding to the DNA phosphate backbone and binding to Si-OH groups of the silica, reducing electrostatic repulsion and forming bridges between mineral and DNA (Nguyen and Elimelech, 2007). Preferential adsorption of small DNA fragments to soils and clays may be due to size exclusion or mechanisms related to the kinetics of diffusion (Ogram *et al.*, 1994).

The distribution of a biomarker on a mineral phase and in a liquid phase is related to its concentration. Relatively more biomarker will adsorb to minerals when the overall biomarker concentration decreases. These adsorption characteristics can be described by Freundlich or Langmuir isotherms (Benaziz *et al.*, 2001), but they have been little studied in the context of life detection. Up to now, adsorption has been studied at most with a combination of a few molecules and a few minerals. Recently, a systematic study on the adsorption of DNA to a wide range of pure minerals, of significance to Mars, was conducted (Direito *et al.*, 2012). Not unexpectedly, clays were found to adsorb DNA strongly; more than 99% of added DNA was adsorbed and could not be recovered with conventional methods for DNA isolation. However, other minerals, such as jarosite and the silica-bearing minerals olivine, diopside, labradorite, and apatite also strongly adsorbed DNA. In contrast, quartz and iron oxides show relatively limited adsorption (<50%). The low adsorption by iron (oxyhydr)oxides is a promising result considering that these minerals are of interest in the search for life on Mars. Besides DNA, RNA can also adsorb strongly to clays (Novinscak and Filion, 2011).

Studies on the interactions of minerals with lipids in relation to the origin of life are relatively limited, in spite of their high utility as unique biomarkers with high conservation potential. Possibly this relates to their largely hydrophobic, uncharged molecular characteristics, on the basis of which one would expect low adsorption to minerals. Adsorption studies with lipids appear to have mainly focused on the interaction between anionic fatty acids and cationic mineral surface sites. Surprisingly, an increasing affinity for carbonate surfaces was found with increasing alkyl chain length for these fatty acids (Zullig and Morse, 1988; Thomas *et al.*, 1993). Adsorption is largely irreversible in aqueous systems (Thomas *et al.*, 1993). Adsorption isotherms that describe the amount of surface-bound lipid as a function of the lipid present in solution are S-shaped. Roughly three regions can be distinguished: in the first and third regions, a relatively slow increase in bound lipid is observed with increasing lipid in solution, compared to the second region. In the first region, chemisorption between the charged groups of

lipids and minerals is important, but in the second phase hydrophobic bonding between alkyl chains dominates adsorption behavior, while in the third phase the mineral is covered by a monolayer that prevents further adsorption of lipids (Zullig and Morse, 1988).

## 6. Impact of Mineralogy on the Extraction of Biomarkers

On the one hand, certain minerals favor the attachment of microorganisms and the adsorption and preservation of organic biomarkers, while on the other hand, the strong adsorption potential and other properties of minerals may interfere with the efficient extraction and detection of biomarkers. The degree of interference is influenced by the method of biomarker extraction, for which a large variety of approaches exist.

Conventional methods to characterize biomarkers in samples on Earth generally employ solvents to extract amino acids, nucleic acids, and lipids. These liquid extracts are subsequently analyzed. Use of equipment on Mars that requires solvents for the detection of biomarkers is avoided because it poses a risk of contamination. Viking 1 and 2, the first robotic landers on Mars, were equipped with instrumentation employing thermovolatilization, or pyrolysis, to search for life: soil samples were directly heated, and the gaseous phase was analyzed by gas chromatography (GC) followed by mass spectrometry (MS) (Klein, 1978). Since August 2012, NASA's Curiosity rover has been investigating the habitability of the martian surface, employing the SAM instrument, which conducts thermal volatilization GC-MS and can chemically derivatize organics to make them more volatile. The 2018 ExoMars mission will include the Mars Organic Molecule Analyzer (MOMA), which is able to detect and identify organic molecules at low concentrations (ppb to ppt) with an ion-trap mass spectrometer. Two major ion sources are employed. The first is classical GC in which solid soil samples are pyrolyzed and GC separates the volatile fraction, including separation based on chirality. The second is laser desorption/ionization (LDI) where larger and likely less volatile molecules are ionized by short laser pulses. LDI has an additional advantage in that it can also provide mineral signatures (Ehrenfreund *et al.*, 2011).

We will deal first, and especially, with solvent-based extraction, as most research on the relation between minerals and biomarker extraction efficiency has been obtained by using this approach. Furthermore, solvent-based extraction is essential when one wants to target more specific and complex biomarkers as indicators of past or present life on Mars. The Life Marker Chip (LMC) instrument, which in the end was not selected for the payload of the ExoMars 2018 mission, has this potential. It relies on antibody microarray technology, in which immobilized antibodies are used as receptors to capture specific biomarkers (Parnell *et al.*, 2007).

The three types of biomarkers central to this review are extractable by solvents, but while aqueous solutions are generally used for amino and nucleic acids, organic solvents are typically employed for lipids. Subsequently GC-MS allows detection and quantification of chemically derivatized amino acids and their chirality, and lipids. GC-MS is not possible for complex nucleic acids, but detection of even one single nucleic acid molecule can be achieved by *in vitro* amplification (Mullis *et al.*, 1986). Novel nonselective



amplification methods such as multiple displacement amplification offer advantages in astrobiology over the conventional polymerase chain reaction, which requires *a priori* sequence information (Direito *et al.*, 2014). These techniques could even be adapted to detect heritable information that deviates from the nucleic acids utilized in life on Earth (Direito *et al.*, 2014).

Solvent extraction methods currently considered in relation to space missions (subcritical water extraction, solvent extraction using ultrasonication) are predicted to be unable to fully recover biomarkers because recovery efficiencies among biomarkers differ and because destruction of biomarkers may occur (Skelley *et al.*, 2005; Court *et al.*, 2010). Specific combinations of biomarkers and minerals may present methodological problems, as observed for the SAM (Stalport *et al.*, 2012) and LMC instruments (Fornaro *et al.*, 2013). For instance, the extraction procedure for the LMC instrument is optimized for aliphatic biomarkers, employing sonication in a solvent system of 20:80 methanol:water, with 1.5 mg/mL Tween, a polysorbate detergent. However, this procedure results in low extraction of nucleobases adsorbed to magnesium oxide (Fornaro *et al.*, 2013). The SubCritical Water Extractor (SCWE) is one of the components of the Urey instrument, now de-scoped from the ExoMars mission. It has been applied to extract amino acids from mineral matrices but might be employed to extract other organic compounds (Aubrey *et al.*, 2008). The use of water as a solvent to extract DNA would be ideal for planetary missions instead of a more hazardous organic solvent. However, a temperature of 100°C damages the DNA and negatively influences its detection (Direito *et al.*, 2012).

Solvent extraction methods have been intensively investigated in the field of microbial ecology, with respect to representative extraction of biomarkers indicative of microbial community composition. The choice of extraction method has a strong influence on the final outcome, irrespective of whether one addresses polar lipid fatty acids (Nielsen and Petersen, 2000) or DNA (Frostegård *et al.*, 1999) to profile communities. DNA extraction procedures include a cell lysing step that can be performed in several ways: by bead beating, heating or detergent treatment, grinding-freezing-thawing, or sonicating a sample directly or indirectly after separating cells from their environmental matrix (Robe *et al.*, 2003). Indirect cell extraction and the use of either differential centrifugation approaches or chemicals to dissolve the mineral matrix generally lead to severe loss of cells or damage their biomarkers (Robe *et al.*, 2003; Direito *et al.*, 2012). On the other hand, during direct DNA extraction, DNA is released from cells and can adhere to minerals. This adsorption also has a detrimental impact on microbial community studies. Samples from the Mars Desert Research Station (MDRS) in the Utah desert revealed a wide variety of putative extremophiles and all three domains of life (Archaea, Bacteria, and Eukarya), but these were not detected in all samples (Direito *et al.*, 2011). Large differences over short distances, even on the centimeter scale, were uncovered in the occurrence and diversity of microorganisms. Spiking with defined quantities of a gene fragment showed that this heterogeneity may relate to low DNA recovery from some samples, due to strong adsorption or degradation (Direito *et al.*, 2011).

Understanding the strength of adsorption and mechanisms contributing to adsorption enables a more rational design of

methods for the efficient extraction of biomarkers and their subsequent detection. We have been able to improve DNA recoveries by up to a hundredfold by modifying the chemical composition of extraction solutions (Direito *et al.*, 2012). A high concentration of phosphate was applied to compete with the phosphate in DNA (Fig. 3) for adsorption to minerals. A high temperature (85°C) was also applied. The phosphate was dissolved in a solution of 15% ethanol. Ethanol helps to disrupt cell membranes, while it makes DNA change conformation at concentrations of 15% or higher, promoting DNA desorption from mineral surfaces (Fang *et al.*, 1999). A relatively low concentration of 15% avoids precipitation of DNA. The introduction of this step in extraction resulted in a much higher DNA yield for several mineral samples, both clay and non-clays, while maintaining reproducible microbial community profiles (Direito *et al.*, 2012). The latter was in line with expectation, as nucleobases are not involved in the adsorption to clays (Pietramellara *et al.*, 2001).

Modification of extraction solutions for other biomarkers may also help enhance their recovery. The use of a mixed organic-aqueous solvent to provide both ionic replacements, in the form of orthophosphate, and solubility for lipids, helped increase the yield of lipids (Zullig and Morse, 1988). The amount of lipid biomarkers was not strongly affected when vegetative cells were extracted with a variety of methods; however, pressurized hot solvent extraction yielded significantly higher amounts of lipids from spores than other methods (Macnaughton *et al.*, 1997).

DNA is very sensitive to chemical and physical conditions; however, other biomarkers are much less sensitive. Hydrofluoric acid treatment can dissolve the mineral matrix while leaving cell morphology intact. For example, proteins were shown to be recovered from inorganic material by dissolving mineral matrices when using 10% hydrofluoric acid (Schulze *et al.*, 2005). The large differences in mechanisms of adsorption and in properties of minerals and biomarkers suggest that it will be difficult to design a single solvent-based extraction procedure for a wide range of biomarkers.

Thermovolatilization of biomarkers in soil samples avoids the use of liquid solvents. Research on the impact of adsorption to minerals on thermovolatilization-based detection of biomarkers appears to be absent from the scientific literature. The presence of oxidizing minerals such as sulfates and perchlorate may compromise the detection of organic biomarkers (Navarro-González *et al.*, 2010; Lewis *et al.*, 2015). The failure of the Viking gas chromatograph-mass spectrometer to detect organics at the part-per-billion level in the top few centimeters of the martian soil (Biemann *et al.*, 1976) is now assumed to have been due to chemical oxidation of organics by perchlorate at high temperatures in the oven of the gas chromatograph instrument (Navarro-González *et al.*, 2010). Perchlorates likely also influenced analyses by SAM (Leshin *et al.*, 2013; Ming *et al.*, 2014).

Laser desorption/ionization avoids the problem of oxidation of organic compounds by minerals at high temperature (Getty *et al.*, 2012). However, a systematic evaluation of LDI efficiency in extracting biomarkers appears to be lacking. A few studies have addressed how minerals may act as a geological matrix that aids desorption and ionization of amino acids, fatty acids, and small proteins by a laser, comparable to matrix-assisted laser desorption/ionization (MALDI) (Yan *et al.*, 2007; Kotler *et al.*, 2008; Richardson *et al.*, 2008). The



minerals impact detection; desorption and ionization of the biomarker is dependent on the energy of the laser absorbed by the mineral matrix and converted to heat. Evaporates like halite and jarosite yielded a signal, but hematite did not (Yan *et al.*, 2007; Kotler *et al.*, 2008; Richardson *et al.*, 2008). Amino acids incorporated within halite yielded a better signal-to-noise ratio than those applied to its surface (Yan *et al.*, 2007). The properties of biomarkers affect detection and fragmentation and may complicate their identification (Yan *et al.*, 2007; Richardson *et al.*, 2008). MALDI itself is not effective when biomarkers are strongly adsorbed to minerals, as dissolution of biomarkers in MALDI solvents is required for detection (Yan *et al.*, 2007). Biomarkers were also not detectable when directly exposed to LDI, without a matrix (Yan *et al.*, 2007; Richardson *et al.*, 2008).

### 7. Remote Detection of Indicators for Life on Planets and Exoplanets

Mars is one of only a few planetary bodies that are accessible for direct assessment of biomarkers in the near future. However, for the very large majority of planets and exoplanets, we will have to resort to remote detection in which spectrometry will play a key role. Spectrometric biosignatures are spectral characteristics that can be remotely detected and inform on the habitability of a planetary environment or are indicative of life itself. Most attention has so far been given to atmospheric biosignatures (*e.g.*, detection of molecular oxygen) (Kaltenegger *et al.*, 2012), even though surface biosignatures are a more specific indicator of life. In particular, two surface biosignatures relating to photosynthesis are of high interest: the “red edge” and circular polarization indicating homochirality.

Photosynthesis, employing (bacterio)chlorophylls, is the major driving factor of life on Earth since at least 2.5 Ga (Madigan *et al.*, 2012). Bacterial photosynthesis likely developed soon after the emergence of life on Earth (Xiong and Bauer, 2002; Blankenship, 2010). For other (exo)planets, we may also expect that photosynthesis is essential in sustaining life over long periods of time (Seager *et al.*, 2005; Kiang *et al.*, 2007). The selective use of light energy produces spectral biosignatures in land plants: the “green bump” caused by absorbance of blue and red light by chlorophyll and the “red edge” caused by the contrast between absorbance in the red by chlorophyll and its reflectance in the near IR. The red edge is a very strong, specific signal that is employed for remote spectrometric sensing of vegetation cover on Earth (Joint and Groom, 2000). The red edge has not only been observed for plants but also for several photosynthetic bacteria and algae (Kiang *et al.*, 2007). A second important and likely universal characteristic of life is that almost all biological molecules display homochirality. Scattering of light by biological molecules of photosynthetic organisms can result in circular polarization by circular dichroism, that is, the differential absorption of left and right circular polarized light. This circular polarization is also detectable remotely (Sparks *et al.*, 2009a).

Photosynthetic organisms are highly diverse and widespread on present-day Earth (Kiang *et al.*, 2007; Madigan *et al.*, 2012). Two major photosynthetic processes occur: anoxygenic and oxygenic photosynthesis. Anoxygenic photosynthetic bacteria are considered to have been the ancestors of

oxygenic photosynthetic organisms (Xiong and Bauer, 2002; Blankenship 2010). Anoxygenic photosynthetic bacteria are phylogenetically and physiologically diverse, using organic compounds, hydrogen, ferrous iron, sulfur, or sulfide as reductant via a variety of bacteriochlorophylls (Konhauser, 2007; Madigan *et al.*, 2012). The type of anoxygenic photosynthetic microorganism that evolved first is still a subject of debate (Xiong and Bauer, 2002; Kiang *et al.*, 2007; Blankenship, 2010). Cyanobacteria are considered to have been the first oxygenic photosynthetic organisms, oxygenating Earth’s atmosphere by using light to energize the oxidation of water to oxygen gas. There are a large variety of chlorophylls and bacteriochlorophylls that mediate photosynthesis, each with its unique absorption spectrum and light reflection characteristics. Different biological species produce different (bacterio)chlorophyll-binding proteins and accessory pigments (*e.g.*, carotenoids, phycobilins) that affect light absorption properties (Madigan *et al.*, 2012). Photosynthesis on Earth occurs under a wide range of environmental conditions, such as at extreme low and high temperatures, low and high pH, and in arid and saline settings (Zettler *et al.*, 2002; Satyanarayana *et al.*, 2005; Konhauser, 2007; Pointing *et al.*, 2009).

Research on remote detection of the red edge and, in particular, homochirality, has so far been limited to a small set of organisms and conditions, in particular plants and algae under “normal” environmental conditions (aerobic, circumneutral pH, ambient temperature, etc.) (Kiang *et al.*, 2007; Sparks *et al.*, 2009a, 2009b; Martin *et al.*, 2010). This hardly covers the wide range of conditions under which photosynthesis occurs on Earth. If the organisms that thrive in extreme environments and the organisms that represent the evolution of photosynthesis on Earth also consistently reveal a specific reflectance signal and can be detected on the basis of homochirality, this would provide larger possibilities to indicate possible life on other (exo)planets, that is, planets with general conditions that are at the limits for life on Earth or planets that are in an earlier evolutionary phase.

It will again be important to consider the influence of minerals, soils, and rocks on the spectrometric signals. Firstly, minerals also reveal spectrometric signals, mainly caused by crystal field effects (Hunt, 1977). Minerals absorb and reflect light, and while most minerals investigated so far have shown reflection spectra clearly different from microorganisms, some minerals reveal edges at visible and near-IR wavelengths that might prove difficult to distinguish from biological signatures (Seager *et al.*, 2005). Similarly, the few minerals investigated in relation to remote detection of homochirality did reveal circular dichroism, but at low amplitude and different from organisms (Sparks *et al.*, 2009a). Secondly, microorganisms often grow in biofilms on mineral surfaces, or are encrusted by minerals under some extreme conditions, such as photosynthetic endoliths in arid environments (Wynn-Williams, 2000). Finally, anoxygenic photosynthetic microorganisms using Fe(II) produce solid iron minerals (Ehrenreich and Widdel, 1994). Shielding by minerals may interfere with remote detection of photosynthetic microorganisms.

### 8. Conclusions and Perspective

Studies of mineral-molecule and microbe-mineral interactions are still in their infancy when compared to the wide range of extreme environments, minerals, and biomarkers on Earth

that are of relevance to astrobiology. Yet the importance of mineralogy with respect to detecting extraterrestrial life is evident. The type of mineral has a big impact on the potential abundances and types of biomarkers and microbes associated with these minerals and influences extractability and thus detection. The impact of mineral type on microbial abundances informs in particular on current life, but one may postulate that a soil that once supported a large living biomass may have a higher chance of retaining biomarkers over long periods of time than a mineral soil that supported less biomass, in particular if the soil has a high capacity to adsorb released biomarkers. Other direct and indirect interactions between minerals and microorganisms or their biomarkers are also of importance to biomarker detection: secondary minerals produced by microorganisms may also be good biomarkers (Weber *et al.*, 2006), and compounds released as the result of microbial activity on minerals may help to stabilize biomarkers; for example, sulfurization can protect biomarkers against biodegradation (Summons *et al.*, 2008). Hence, local mineralogy is a factor that should be taken into account when selecting sampling locations for the detection of life and designing biomarker-detection protocols.

More systematic studies on mineral-biomarker interactions and understanding the mechanisms that contribute to adsorption are needed. This will aid in a more rational design of methods for high extraction efficiency of a wide range of biomarkers. We advocate the approach that we recently applied in the case of nucleic acid biomarkers (Direito *et al.*, 2012): spike “clean” minerals (*i.e.*, minerals free of biomarkers) with known quantities of biomarkers and then quantify their recovery by using extraction protocols optimized on the basis of mechanistic insight on the adsorption of biomarkers to minerals.

Despite the significant improvements we made for nucleic acid extraction, extraction from some minerals, clays in particular, remained suboptimal, as “only” 1% of added DNA could be extracted (Direito *et al.*, 2012). Therefore, it is also important to include internal controls in life-detection strategies, that is, spike samples retrieved from extreme environments on Earth or from planetary bodies with known quantities of distinctive markers prior to extraction in order to quantify their recovery (Direito *et al.*, 2011, 2012). This information will be essential to better interpret negative results: Are these due to the absence of biomarkers (that is, biomarkers are present below the detection limit) in the investigated sample or due to strong loss of biomarkers due to adsorption to minerals or destruction during the extraction procedure? This is an especially important issue when investigating low-biomass environments such as are expected on Mars. On the other hand, it is important to avoid false positives due to contamination of equipment with the biomarkers used as spikes.

Life-detection strategies should be robust and recover biomarkers of high quality with great efficiency from samples. For this, it is important also to retrieve biomarkers that are present in cells, spores, and fossils. The astrobiology community pays relatively little attention to these intracellular biomarkers, which are considered in microbial ecology as we indicated in relation to the use of nucleic acids to study microbial communities in extreme environments.

Differences in mineral composition over short distances can lead to large differences in microbial community and biomarker composition, and indicate that future sampling mis-

sions should employ high-density sampling at any location of interest to maximize the chance of finding life (Direito *et al.*, 2011). Similarly, mineralogy is of high importance in relation to the remote detection of spectral features of photosynthesis. More knowledge is required on the *in situ* interaction between minerals and microorganisms and the implication for biomarker detection. The unavailability of extraterrestrial samples, with the exception of a few available meteorites (with possible contamination risks), makes it essential to further study the interaction between minerals and microbes and their biomarkers in extreme environments on Earth.

As Mars is a prime target in the near future, the environments to study should include terrestrial representatives of the three geological stages of Mars and ideally be linked to available data on biota and their abiotic environment, as such information can benefit experimental design and data interpretation. Current extant martian life, if it exists, would face challenges from low temperature, aridity, and high radiation. The McMurdo Dry Valleys and MDRS are analogues to this situation. The McMurdo Dry Valleys, in Antarctica, constitute a cold hyperarid polar desert. Despite their very extreme and stressful conditions, they contain a highly specialized microbial diversity, revealing radiation- and desiccation-tolerant microorganisms, including photosynthetic endoliths (Pointing *et al.*, 2009). MDRS, in Utah, is an arid, oxidizing environment with high temperatures during the day and low temperatures at night. MDRS has a mineralogy comparable to that found on Mars and a patchy distribution of biomarkers and microorganisms (Clarke and Stoker, 2011; Direito *et al.*, 2011; Ehrenfreund *et al.*, 2011; Martins *et al.*, 2011). Parts of Mars may have been highly acidic and saline in the past, resembling conditions currently found at Río Tinto, Spain. This acidic (pH 2–3), iron-rich river system is produced from rock-water-biology interactions. Pyrite effectively provides the energy for the development of the Río Tinto ecosystem, which is surprisingly rich (Fernández-Remolar *et al.*, 2005). Mars’ early history resembled that of early Earth: moist and warm. Hydrothermal systems have been suggested as suitable environments for the appearance of life on early Earth (Russell and Hall, 1997) and were likely active in the crust of Mars.

Studying natural environments where life had time to evolve and adapt to multiple stressors will contribute to the optimization of sampling strategies and life-detection methodologies and will also indicate the type of organisms and biomarkers to expect in such environments (*e.g.*, Cockell *et al.*, 2005). We will obtain more insightful information on mineral-microbe-biomarker interactions from these natural environments than from relative short-term incubations in Mars simulation chambers (*e.g.*, Osman *et al.*, 2008), for which survival and growth of microorganisms might be insufficient to establish meaningful relationships between minerals, microbes, and biomarkers. Nevertheless, simulation chambers could be useful to determine protective properties of minerals on the conservation of biomarkers under simulated martian conditions.

Studying the interactions between minerals, biomarkers, and microbes is not only relevant for astrobiology but also for other research fields such as microbial ecology, evolutionary sciences, forensic sciences, and bioarchaeology. These research areas can, and should, influence each other mutually to contribute new methodologies and mechanistic insight on microbe-mineral-biomarker relationships.

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#### Abbreviations Used

GC = gas chromatography  
LDI = laser desorption/ionization  
LMC = Life Marker Chip  
MALDI = matrix-assisted laser desorption/ionization  
MDRS = Mars Desert Research Station  
MS = mass spectrometry  
SAM = Sample Analysis at Mars