Forum Article

Lipids as Universal Biomarkers of Extraterrestrial Life

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Abstract

In 1965, James Lovelock published a general statement, based on thermodynamic chemical equilibrium principles, about how to detect extant or extinct life on a planet other than Earth. Nearly 50 years later, it is possible to make such measurements with robotic missions such as current and future Mars rovers, and probes to sample icy plumes of Enceladus or Europa. We make a specific recommendation that certain characteristic patterns in the composition of lipid hydrocarbons can only result from a biological process, because the signal arises from a universal requirement related to lipid bilayer fluidity and membrane stability. Furthermore, the pattern can be preserved over millions of years, and instrumentation is already available to be incorporated into flight missions. Key Words: Biomarkers—Lipids—Biomembranes—Hydrocarbons—Extraterrestrial life. Astrobiology 14, 541–549.

1. Introduction

JAMES LOVELOCK first proposed that biotic processes generate patterns in chemical structures and compositions that are utterly improbable in terms of thermodynamic principles:

> a planet bearing life is distinguishable from a sterile one as follows: (1) The omnipresence of intense orderliness and of structures and of events [are] utterly improbable on a basis of thermodynamic equilibrium. (2) Extreme departures from an inorganic steady-state equilibrium of chemical potential. This orderliness and chemical disequilibrium would to a diminished but still recognizable extent be expected to penetrate into the planetary surface and its past history as fossils and as rocks of biological origin. (Lovelock, 1965)

To our knowledge, Lovelock was among the first to describe what we would now call a biomarker. Because the term has come into general use in multiple ways in different contexts, for our purposes we will define a biomarker simply as a chemical pattern that preserves a recognizable signature of life over long time intervals and can be detected by one or more instruments. Examples of biomarkers in this general sense have been considered to include homochirality, stable isotope distribution, nonrandom assortments of organic compounds associated with metabolic processes (McKay, 2004), and specific organic molecules that have no known abiotic source.

Biomarker patterns are created by an evolutionarily conserved repetition of biosynthetic steps such as the enzyme-catalyzed metabolism that synthesizes amino acids, monosaccharides, and nucleobases. However, biomarkers that rely on the properties of polymers associated with life—proteins, polysaccharides, and nucleic acids—present significant difficulty in providing convincing evidence for extinct life that may have originated and thrived millions to billions of years ago. The reason is that biologically relevant properties rapidly disappear over time intervals of thousands to millions of years due to hydrolysis, racemization, and other forms of chemical degradation.

Furthermore, many of life's monomers can be synthesized by nonbiological reactions, a prime example being the array of amino acids and nucleobases. Their presence in carbonaceous meteorites has been established by numerous studies (Kvenvolden *et al.*, 1970, 1971; Cronin *et al.*, 1981, 1985; Engel and Nagy, 1982; Cronin and Pizzarello, 1983, 1986; Bhatia and Hajdu, 1988; Naraoka *et al.*, 1999; Cooper *et al.*, 2001; Engel and Macko, 2001; Pizzarello *et al.*, 2006; Yabuta *et al.*, 2007; Pizzarello and Shock, 2010; Callahan *et al.*, 2011; Glavin *et al.*, 2011). In particular, abiotic amino acids and related products can be the outcome of the Strecker synthesis (Strecker, 1850, 1854), as also demonstrated by the Miller and Urey experiment (Miller and Urey, 1959). Moreover, although hydrocarbon derivatives are universal components of terrestrial life, similar suites of shorter-length (in $\geq C_{15}$ decreasing abundance) hydrocarbon derivatives can be synthesized abiotically by Fischer-Tropsch-type reactions that simulate high-pressure/temperature geochemical conditions (McCollom *et al.*, 1999; Simoneit, 2004). In addition, the use of enantiomeric excess of L amino acids and D sugars as a biomarker (Tegelaar *et al.*,

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1989; Engel and Macko, 1993; Fontaine *et al.*, 2007; Summons *et al.*, 2008) has been challenged by the observation of enantiomeric enrichments for some L-amino acids in the Murchison, Orgueil, and SCO 06043 meteorites (Engel and Nagy, 1982; Cronin and Pizzarello, 1997; Engel and Macko, 2001; Glavin *et al.*, 2011). Another commonly used biomarker is the $^{13}C/^{12}C$ isotope ratio related to metabolic uptake of the lighter carbon isotope, but similar ratios have been produced by exposing Murchison material to UV light (Keppler *et al.*, 2012). All these observations further blur the distinction between biological and nonbiological patterns.

To overcome these difficulties, we need to look for chemical and physical properties of extremely stable organic molecules, which were acquired from their interaction with living organisms. Such biomarkers should conform to the following principles:

- They must be strictly biogenic (not found in the abiotic organics in nature) and possess universal chemical and physical properties required for any form of life, terrestrial or extraterrestrial.
- They should be integral components of any water-based life-form.
- They must be chemically stable compared to the other organics of life to serve as molecular biomarkers of extinct life.
- They should preferably constitute a substantial portion of life's organics and should be easily isolated for increased sensitivity and minimal interference in their detection.

Are there universal biomarkers in terrestrial life that might be expected to occur in extraterrestrial life? Here, we propose a specific biomarker related to Lovelock's challenge: compositional patterns of hydrocarbon derivatives associated with biogenic lipids.

2. Patterns in Lipid Hydrocarbons

Living organisms from bacteria to multicellular animals and plants have had over 3 billion years to explore a variety of conditions and morphologies, yet there are no exceptions to the rule that cells are the fundamental unit of life. We assume that this rule also holds for extraterrestrial organisms. Given that all life is cellular, there must also be boundary structures that prevent free diffusion of solutes and maintain systems of functioning polymers in compartments. A second rule without exception is that the permeability barriers are composed of lipid bilayer membranes. Because the lipids must have properties that fit within well-defined parameters related to stability, fluidity, and permeability imposed by the aqueous substrate of life, there are essential features of composition that can fulfill the requirements for a universal biomarker. Third, most forms of terrestrial life also use lipids such as triglycerides to store energy or as a source of fatty acids required for boundary membranes. The storage lipids have fatty acid compositions that maintain them in the fluid state at the ambient temperature in which the organism goes through its life cycle. We will focus the following discussion on biomembranes because the requirement of lipid fluidity is most apparent in these structures, keeping in mind that the same arguments apply more generally to storage lipids in whichever form they may exist in extraterrestrial life.

The amphiphilic molecules that compose cell membranes have polar heads that interact strongly with water and hydrophobic tails composed of hydrocarbon chains. In water, certain amphiphiles such as phospholipids self-assemble to form double-layered membranes with heads out and tails in. Lipid bilayers form a barrier that sequesters the contents of the cell because ionized and polar solutes like KCl, glucose, and amino acids are relatively insoluble in the low dielectric oily interior of the bilayer (Parsegian, 1969). The hydrocarbon-based acids and alcohols that contribute the amphiphilic property of contemporary membrane lipids in most bacteria (eubacteria) and in eukaryotes have typical chain lengths between 14 and 18 carbons. Such chain lengths, if present as saturated hydrocarbons, would be in a gel state at ordinary temperature ranges (Mansy, 2009), but membrane function requires lipids to be in a fluid state. To produce fluidity at ordinary temperature ranges, the hydrocarbon chains of eukaryotes have unsaturated *cis*-double bonds, while many prokaryotes have branched chains with methyl groups along the chain to introduce fluidity (Kaneda, 1991). The complexity of such membrane lipid components and final assembly construction process has been perfected by terrestrial life through 3 billion years of evolution.

It is important to note that amphiphilic organic compounds with the capacity to assemble into bilayer structures are not strictly biogenic. For instance, abiotic hydrocarbon derivatives were identified in the Murchison meteorite as single-chain aliphatic saturated carboxylic acids through C_{12} (Naraoka *et al.*, 1999). Together with other nonpolar extracts from this meteorite, they can spontaneously self-assemble into membranous bilayer vesicles in aqueous phases (Deamer, 1985; Deamer and Pashley, 1989). Although membranes can assemble spontaneously in the absence of life and therefore cannot be considered to be a reliable biomarker, the membranes of living cells have distinctive patterns within their components that result from biosynthetic processes, and it is these patterns that can be used as biomarkers.

The characteristic properties become clear by inspection of Fig. 1, which schematically compares the composition of hydrocarbons and their derivatives isolated from abiotic and biotic sources. The alkanes, alcohols and monocarboxylic acids from Fischer-Tropsch synthesis (Fig. 1A), are produced by the catalyzed addition of single carbons, so no obvious patterns emerge in chain lengths and carbon number (McCollom and Seewald, 2007). Similarly, as illustrated in Fig. 1B, there is no apparent pattern in the monocarboxylic acids extracted from a carbonaceous meteorite (Huang *et al.*, 2005). The chain lengths are too short to assemble into a bilayer membrane, and there are also various branched-chain acids that would not be present in a biological sample. In contrast, the monocarboxylic acids from a bacterial source (Hartgers *et al.*, 2000) show an obvious pattern in which chains with an even number of carbons dominate. We will refer to this as the odd-even pattern, which occurs because carbon atoms are added two at a time during biosynthesis. Furthermore, the majority of the chains have lengths of 14–20 carbons and are well able to assemble into stable bilayer membranes.

It is significant that the odd-even pattern and chain lengths can be preserved indefinitely, as illustrated by the
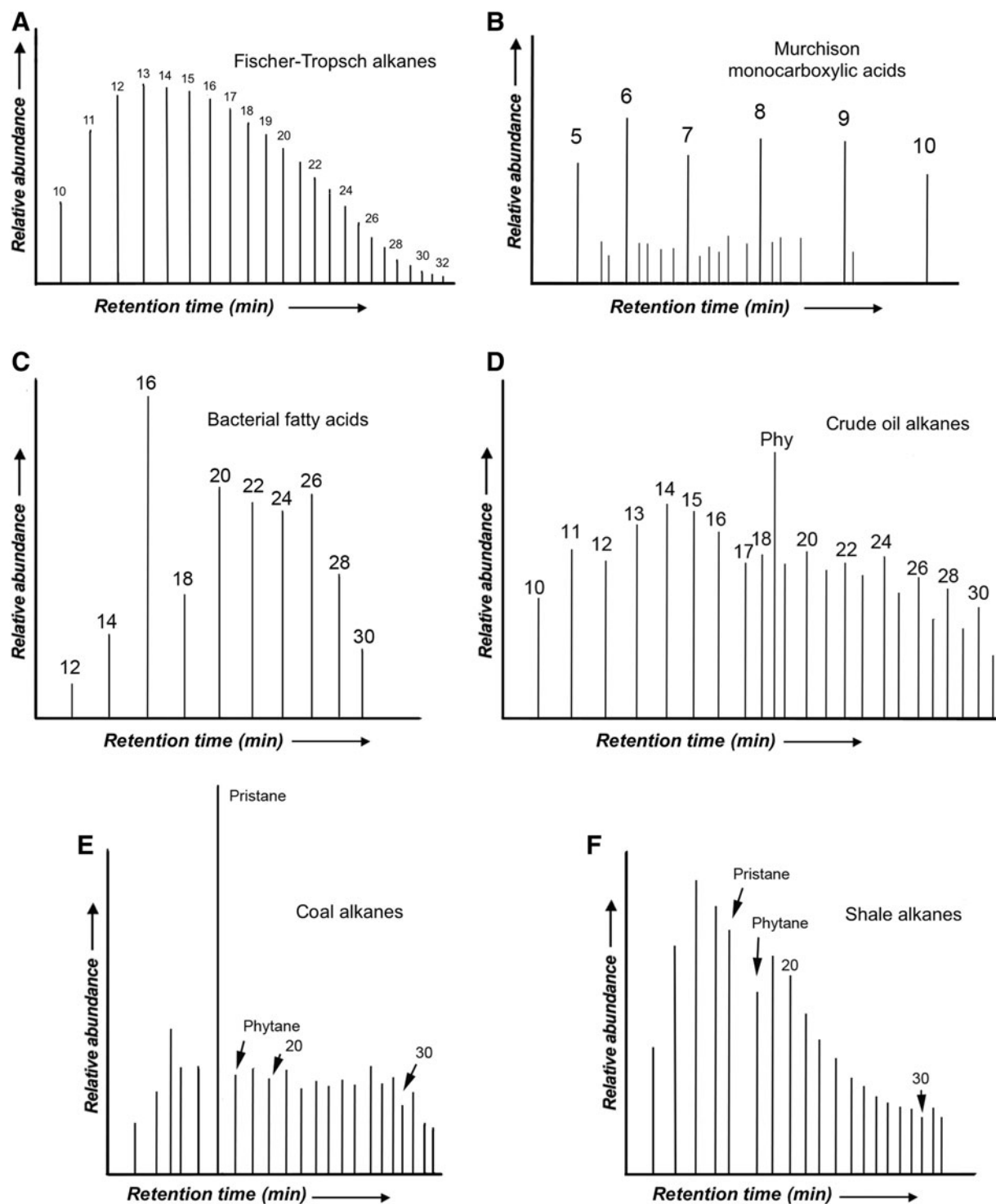
FIG. 1. Compositional patterns in hydrocarbon derivatives from abiotic and biological sources. The chromatograms are shown schematically for illustrative purposes. Chain lengths are indicated numerically, and line heights and relative retention times correspond precisely to peaks on published gas chromatograms. (**A**) Typical reaction products of Fischer-Tropsch synthesis. The major peaks correspond to a homologous series of abiogenic *n*-alkanes (adapted from Fig. 3 in McCollom and Seewald, 2007). (**B**) Abiogenic monocarboxylic acids found in the Murchison meteorite (adapted from Fig. 1a in Huang *et al.*, 2005). The primary peaks are *n*-carboxylic acids from 5 to 10 carbons long, and smaller peaks are branched-chain acids having one or two methyl groups. (**C**) Gas chromatogram of the fatty acid fraction isolated from the bacterial extract of Lake Cisó (adapted from Fig. 2 in Hartgers *et al.*, 2000). The fatty acids are virtually all even-numbered. (**D**) Whole crude oil gas chromatogram showing the distribution of biogenic *n*-alkanes (''Phy'' represents phytane; adapted from Fig. 9a in Cai *et al.*, 2005). (**E** and **F**) Hydrocarbon fractions from coal (E) and shale (F) showing the disappearance of the odd-even preference in the shale sample (adapted from Fig. 3B in Hatch *et al.*, 1986).

chromatogram of crude oil (Fig. 1D). The petroleum is derived from microorganisms that lived 50–200 million years ago, yet starting with the 16-carbon alkane the odd-even pattern can be clearly distinguished. It should be noted that the pattern is lost if the biological material is exposed to temperature and pressure regimes that cause further extensive degradation of the organic compounds. For instance, Figs. 1E and 1F compare hydrocarbons present in coal and shale from neighboring sites in Oklahoma (Hatch *et al.*, 1986). The coal hydrocarbon extract has maintained the odd-even pattern in the alkanes between 20 and 30 carbons in length, but the pattern has been obliterated in the shale source, presumably because it is the product of extensive cracking processes occurring at higher temperatures and pressures.

Other potential biomarkers include biological hydrocarbons that are synthesized by addition of 5-carbon isoprene units rather than 2-carbon acetyl groups. The resulting branching of methyl groups along the chain is an alternative to unsaturation for modulating membrane fluidity. Isoprenoids are present in petroleum samples in the form of terpenoids and hopanoids, and two of these, phytane and pristane, are obvious in the chromatograms shown in Figs. 1D–1F. A similar comparison of hydrocarbons in the Murchison meteorite shows a large variety of aliphatic and aromatic hydrocarbons with no unexpected dominant compounds (Cronin and Pizzarello, 1990). The presence of isoprenoids in extensively processed petroleum illustrates the stability of hydrocarbons; no other biomarkers such as amino acids or nucleobases have survived. Further discussion of petroleum hydrocarbon patterns and mechanisms of formation can be found elsewhere (Emmerton *et al.*, 2013; Sephton and Hazen, 2013).

The enzyme-catalyzed addition of acetyl or isoprene groups during fatty acid synthesis is presented as an example, not as a universal pattern. Our main point is that a search for lipid biomarkers should include tests for deviation from abiotic products such as those present in carbonaceous meteorites or synthesized by the Fischer-Tropsch reaction. The relatively simple metabolic processing of lipids suggests that similar evolutionary paths will be followed by any life-form irrespective of planetary origin. These lipid modifications are discussed in the following sections because their patterns are likely to represent a universal biomarker for extraterrestrial life.

3. Membrane Function Requires Specific Chemical and Physical Properties of Component Lipids

To adapt to various environmental challenges, biological membranes must have a certain degree of fluidity that is related to lipid properties. Within membranes, the number and position of double bonds and acyl chain length markedly influence fluidity, stability, and permeability (Cook and McMaster, 2002). Fatty acyl chain length and positions of double bonds within the chain can be altered by life to confer widely differing properties that are necessary for optimum membrane function in different environments (Hazel and Williams, 1990). Biomembranes require acyl chain length in the range of 14–18 carbons to be stable at ordinary temperature ranges, defined here as 0–40°C. Shorter chain lengths cannot form bilayers that have suffi-

cient stability to act as barriers to the free diffusion of solutes, and longer chain lengths tend to exist in the gel state (Mansy, 2009).

Adaptive response to varying temperatures is also related to chain length because the phase transition temperature between gel and fluid states increases markedly as chains grow longer (Falcone *et al.*, 2004). For instance, the transition temperature of dimyristoylphosphatidylchloline is 24°C, but lengthening the fatty acids from C_{14} to C_{16} increases the transition temperature to 41°C. Thermophilic microorganisms (archaebacteria and eubacteria) achieve membrane stability by incorporating ether lipids with much longer chains, some even spanning the membrane. The ether bonds are not readily hydrolyzed, and the membrane-spanning lipids presumably are adaptations to temperature ranges up to near-boiling niches in geothermal hot springs (Pond and Langworthy, 1987; Koga, 2012).

In contrast, in low–temperature environments membrane fluidity is maintained by introduction of double bonds (Cook and McMaster, 2002). These serve to increase the fluidity of membranes and stabilize them in the cold by decreasing the phase transition temperature (Nichols *et al.*, 1993, 2004). A saturated phosphatidycholine with a C_{16} and a C_{18} fatty acid has a transition temperature of 49°C, but adding a single *cis* double bond decreases this to $-2°C$. Archaebacteria do not contain polyunsaturated fatty acids but instead adapt to cold temperatures by increasing the degree of unsaturation within the phytenyl chains of their ether phospholipids (Nichols *et al.*, 2004). Unsaturation is a strictly biogenic process that takes place in bacteria in the absence or presence of oxygen, or both (Marrakchi *et al.*, 2002; Feng and Cronan, 2011), thus making the presence of double bonds a reliable biomarker. Addition of methyl groups to hydrocarbon chains has the same effect, and branched-chain fatty acids with methyl groups are common constituents of bacterial lipids (Kaneda, 1991). The most common branched-chain fatty acids are mono-methyl-branched, but di- and poly-methyl-branched fatty acids are also known. Both chain branching and unsaturation increase fluidity by reducing the ability of hydrocarbon chains to pack tightly, as illustrated in Fig. 2. Branching and unsaturation increase the spacing between hydrocarbon chains, which reduces the van der Waals forces that stabilize chain-chain interactions.

Phosphate is a universal component of the membrane lipids in terrestrial organisms, providing the essential ionized hydrophilic head group required for membrane self-assembly. Unlike C, N, O, and H, phosphorus in the form of phosphate does not exist in a common gaseous form that would be available to those niches where life emerged. Moreover, the low solubility of phosphate minerals in seawater makes it a rate-limiting growth factor even for terrestrial life today (Tawfik and Viola, 2011). However, the conditions early in planetary history may have markedly increased phosphate availability. For instance, when liquid water was abundant on Mars over 3 billion years ago, phosphate release rates from phosphate minerals are predicted to have been as much as 45 times faster than those on Earth, so that phosphate concentrations would have been more than twice those of Earth at that time. If so, the increased available phosphate may have mitigated one of the hurdles to abiogenesis on Mars (Adcock *et al.*, 2013).
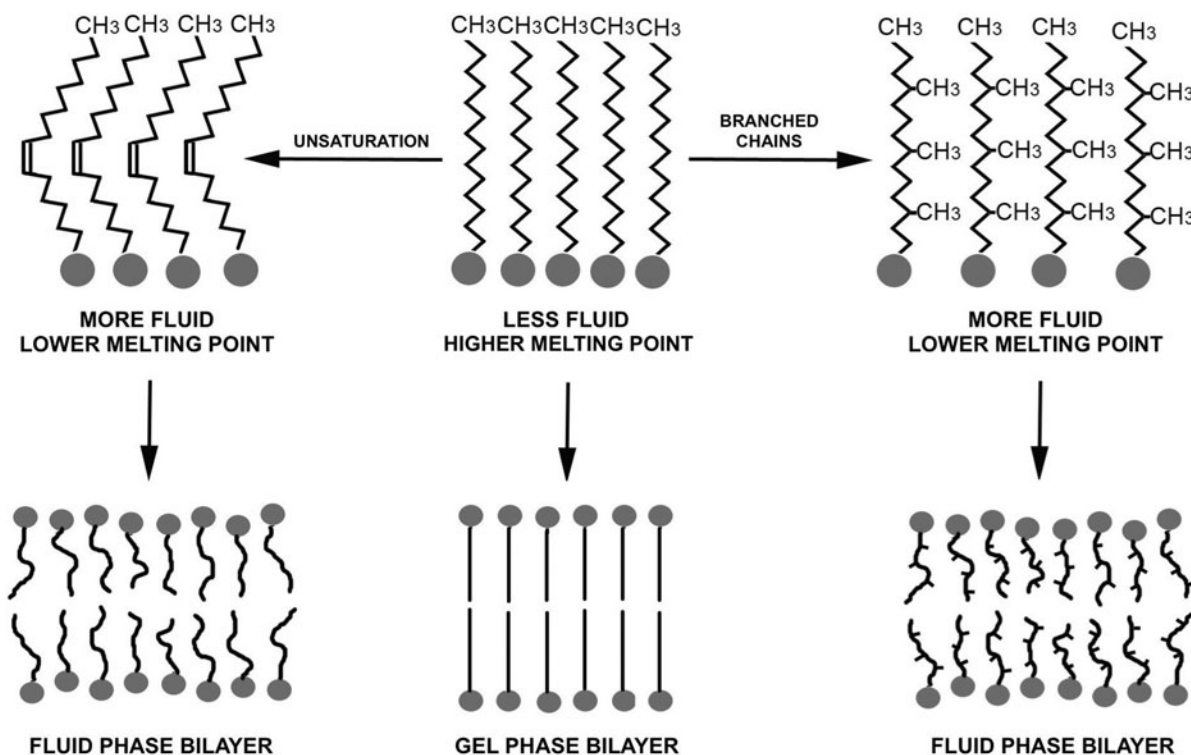
FIG. 2. Effect of unsaturation and branched chains on lipid fluidity. The top row shows unsaturated, saturated, and branched chains attached to hydrophilic head groups, and the bottom row shows the effect of each property on the fluidity of a bilayer membrane. See text for details.

The incorporation of phosphate in membrane lipids raises the possibility that it would represent a universal contribution to the polarity of membranes in extraterrestrial life as well. However, phosphate-based lipids are rapidly degraded by hydrolysis after cell death (Petsch *et al.*, 2001), and their component α-glycero-phosphoric acid is short-lived in the environment (Simoneit, 2004). This would also be true for the polyprenyl phosphates that have been proposed as possible lipid ancestors (Ourisson and Nakatani, 1999). Thus, phosphate alone cannot serve as a geochemical biomarker, especially since the discovery of nonbiogenic alkyl phosphonic acids in the Murchison meteorite (Cooper *et al.*, 1992). Although lipid phosphate esters are unlikely to survive long enough to serve as reliable biomarkers, phosphate esters identified in lipidlike molecules could be used as a biomarker of extant life.

In summary, the characteristic distribution profile of hydrocarbons and their derivatives from biological sources allows them to be distinguished from abiotic sources. In fact, a wide range of lipid types have been used as molecular fossils of ancient terrestrial life (Brocks *et al.*, 1999; Xie *et al.*, 2003; Brocks and Pearson, 2005; Eigenbrode, 2008). For instance, polyunsaturated fatty acids were detected in 5–10 million-year-old sediments in Rossano Basin, northern Calabria, Italy (Guido *et al.*, 2007), and linoleic acid with two double bonds was found in 230-million-year-old fossil teeth (Das and Harris, 1970). Hydrocarbons were preserved in oil trapped inside fluid inclusions for more than 2 billion years (George *et al.*, 2008), as well as in a 2.5–2.7 billion-year-old location in Canada and Australia (Brocks *et al.*, 1999, 2003a, 2003b; Ventura *et al.*, 2008; Waldbauer *et al.*,

2011). The archaebacterial lipid biphytane and its acyclic derivatives were extracted from 2.5-billion-year-old (late Archean) argillites and greywackes of the southern Abitibi Province of Ontario, Canada (Brocks *et al.*, 2003b; Ventura *et al.*, 2008). *n*-Alkanes are by far the most abundant hydrocarbons in all late Archean bitumens and have been extracted from 2.45–2.78 billion-year-old sedimentary rocks from the Pilbara region of Western Australia; alkanes, in particular, were also detected with chain lengths from C_{9-23} (and one case with C_{28}) (Brocks *et al.*, 2003a, 2003b). Molecular fossils of biological lipids were preserved in 2.7-billion-year-old shales from the Pilbara Craton, Australia, specifically 2a-methylhopanes and steranes, which are characteristic of cyanobacteria and eukaryotes, respectively (Brocks *et al.*, 1999; Eigenbrode *et al.*, 2008; Waldbauer *et al.*, 2011). These studies illustrate the remarkable chemical stability of lipid hydrocarbons. Their physical and chemical properties related to metabolism and maintaining fluidity in different environments suggest that they may serve as biomarkers of extraterrestrial life.

4. Sampling Lipid Biomarkers in Future Life-Detection Missions

Lovelock (1965) proposed that hydrocarbons could be used to detect extraterrestrial life based on chemical equilibrium considerations. He argued that abiotic processes such as Fischer-Tropsch-type synthesis produce hydrocarbon distribution curves that reflect an equilibrium state, while those derived from biogenic aliphatic hydrocarbons are expected to show large departures from this equilibrium

state. The approach we describe here differs from Lovelock in that it is based on metabolic processing that endows hydrocarbons with an essential property related to fluid lipid phases and membrane stability. To conclude this discussion of lipid biomarkers, it will be useful to compare some of the instruments that have already been designed for extraterrestrial biomarker analysis and then show how a lipid analysis instrument could be incorporated into future flight missions and landers.

The instrument should be designed to detect a suite of chemical properties that we have described here:

(1) A distinctive pattern of chain lengths such as the odd-even pattern of terrestrial life.
(2) Modifications such as chain branching or unsaturation required to maintain fluidity.
(3) A dominant chain length that does not fit the profile of alkanes, fatty acids, or alcohols produced by Fischer-Tropsch synthesis or observed in carbonaceous meteorites.
(4) Presence of specific biomarkers such as isoprenoid derivatives and phosphorus that have no known abiotic source.

There have been previous proposals for detecting extraterrestrial life that recognize biological precursors of lipids (fatty acids, phytane, and pristine) as high-priority biomarkers for searching for life on Mars because of their stability (Parnell *et al.*, 2007; Summons *et al.*, 2008). However, these propositions are based on detection of specific compounds rather than the physicochemical properties of lipids related to lipid bilayer fluidity and stability. From the discussion above, it is clear that the pattern of hydrocarbon chain properties of membrane lipids can distinguish between the sources of a suite of other organic compounds in terms of a biotic or abiotic origin. Because collection, extraction, and analysis of lipids are already automated in commercial instruments, we foresee no insurmountable problems in adapting a similar technology for a flight mission.

An important question related to the use of lipid biomarkers for detecting extraterrestrial life concerns the nature of the sample and the method of its extraction and concentration for analysis. We will consider two potential sources of samples: Mars and the recently discovered plumes of Enceladus and Europa. Mars is a good example because there is likely to be a lander dedicated to life detection in the next decade. What samples might be feasible? We will not consider the simplest to obtain, which is to sample the first few centimeters of loose soil. This material has been exposed for millions of years to oxidizing perchlorate salts and solar UV while being stirred by wind, so it is unlikely that any lipid derivatives remain.

A preferred sample type would be ice preserved under an ancient lake bed, and several such sites were proposed by Heldmann *et al.* (2014). A drilling technology named Icebreaker has already been developed to sample ice-cemented ground in the north polar plains of Mars. A primary goal of Icebreaker is to search for specific biomolecules that would be conclusive evidence of life (McKay *et al.*, 2013). An alternative sample is the powder obtained by grinding what appears to be a layered sedimentary rock that may contain preserved organic material. The Curiosity rover has already demonstrated this capability.

After collecting a sample, the next step is extraction and concentration of soluble components. We assume that after millions of years no intact lipids associated with extinct life will remain in their original state, so an extraction procedure should be designed to dissolve small amounts of degradation products, primarily fatty acids and alcohols, or hydrocarbons that resemble terrestrial petroleums if the sediments have been exposed to similar temperature and pressure regimes. Such compounds can be selectively isolated in nonpolar solvents from other hydrophilic organic/inorganic matter because of their hydrophobicity. Water-soluble alkyl phosphonic acids such as those found in the Murchison meteorite (Cooper *et al.*, 1992) will not co-fractionate with phosphorus-containing lipids in nonpolar solvents, thus allowing them to be identified by instruments designed to detect phosphorus. Standard methods for the anhydrous extraction/isolation of lipids are based on typical solvents such as chloroform/methanol, hexane/ethanol, methanol, ethanol, isopropanol, and butanol, sometimes combined with sonication (Carrapiso and García, 2000; Lee *et al.*, 2010; Sheng *et al.*, 2011). An alternative and preferred solvent is supercritical CO_2 that dissolves and extracts lipids from any source (soil, water, etc.) by flashing them with liquid CO_2 and concentrates them by subsequent CO_2 evaporation at low pressure (Montanari *et al.*, 1996; Bautista *et al.*, 1999; Boselli and Caboni, 2000; Hanif *et al.*, 2012). Such supercritical fluid lipid extraction technology is already commercially available (Applied Separations, Allentown, PA). A miniaturized version can be employed in Mars missions in conjunction with a soil sampler such as Icebreaker. Analysis would be performed by a miniaturized gas chromatograph–mass spectrometer (GC-MS) instrument such as the TRIDION-9 GC-TMS and GUARDION-7 GC-TMS by Torion Technologies, Inc. (Contreras *et al.*, 2008).

There is increasing evidence that liquid water exists on Enceladus (Hansen *et al.*, 2006) and more recently Europa (Roth *et al.*, 2013), and it is interesting to speculate that primitive forms of life could emerge in such environments and may still exist. Because of their distance from Earth and the nature of the surfaces of these moons, designing a lander to search for life is much more challenging than what has proven to be feasible on Mars. However, it may be possible to obtain samples from the plumes emerging from the surfaces. These appear to result from a geyserlike activity in which liquid water is ejected into space as vapor and ice, presumably carrying with it any material present in the subsurface water. A feasibility study of a proposed flight mission to sample an Enceladus plume has already been completed (Spencer, 2010). Sample collection from the Enceladus and Europa ice plumes can be performed by the use of a National Center for Atmospheric Research (NCAR) counterflow virtual impactor (CVI), which has already been tested by NASA (Twohy *et al.*, 2003). The NCAR-CVI (Noone *et al.*, 1988; Twohy *et al.*, 1997) is an airborne instrument with an inlet tip that collects cloud droplets or ice crystals larger than about 8 μm aerodynamic diameter. These are separated from the interstitial aerosol by a counterflow stream of dry nitrogen out the CVI tip, which assures that only larger particles (cloud droplets or ice crystals) are sampled and then concentrated. The water vapor and nonvolatile residual nuclei remaining after droplet evaporation are analyzed downstream of the inlet with selected

instruments. These may include a Lyman-alpha or similar hygrometer, a condensation nucleus counter, an optical particle counter, filters for chemical analyses. The supercritical fluid system for lipid extraction, we propose here, would be connected on line with a GC-MS lipid analysis instrument.

5. Summary

For cellular life to exist, certain physical properties related to the essential fluid state of lipid components must be fulfilled. These properties are modulated by metabolic reactions that adapt the organism to the ambient temperature at which it goes through a life cycle. The resulting patterns of chain length, branching, and unsaturation can be used to distinguish between biotic and abiotic sources of hydrocarbon chains. Because hydrocarbons are easily analyzed and are stable over time intervals measured in millions to billions of years, the patterns represent a preferred biomarker for future life-detection missions.

Acknowledgments

Author Disclosure Statement

The authors declare that no competing financial interests exist.

Abbreviations

CVI, counterflow virtual impactor; GC-MS, gas chromatograph–mass spectrometer; NCAR, National Center for Atmospheric Research.

References

Adcock, C.T., Hausrath, E.M., and Forster, P.M. (2013) Readily available phosphate from minerals in early aqueous environments on Mars. *Nat Geosci* 6:824–827.

Bautista, J.M., González-Vilaa, F.J., Martín, F., del Rio, J.C., Gutierrez, A., Verdejo, T., and González, A.G. (1999) Supercritical-carbon-dioxide extraction of lipids from a contaminated soil. *J Chromatogr A* 845:365–371.

Bhatia, S.K. and Hajdu, J. (1988) Stereospecific synthesis of ether and thioether phospholipids. The use of L-glyceric acid as a chiral phospholipid precursor. *J Org Chem* 53:5034–5039.

Boselli, E. and Caboni, M.F. (2000) Supercritical carbon dioxide extraction of phospholipids from dried egg yolk without organic modifier. *J Supercrit Fluids* 19:45–50.

Brocks, J.J. and Pearson, A. (2005) Building the biomarker tree of life. *Reviews in Mineralogy and Geochemistry* 59:233–258.

Brocks, J.J., Logan, G.A., Buick, R., and Summons, R.E. (1999) Archean molecular fossils and the early rise of eukaryotes. *Science* 285:1033–1036.

Brocks, J.J., Buick, R., Logan, G.A., and Summons, R.E. (2003a) Composition and syngeneity of molecular fossils from the 2.78 to 2.45 billion-year-old Mount Bruce Super-

group, Pilbara Craton, Western Australia. *Geochim Cosmochim Acta* 67:4289–4319.

Brocks, J.J., Buick, R., Summons, R.E., and Logan, G.A. (2003b) A reconstruction of Archean biological diversity based on molecular fossils from the 2.78 to 2.45 billion-year-old Mount Bruce Supergroup, Hamersley Basin, Western Australia. *Geochim Cosmochim Acta* 67:4321–4335.

Cai, C., Worden, R.H., Wolff, G.A., Bottrell, S., Wang, D., and Li, X. (2005) Origin of sulfur rich oils and H_2S in Tertiary lacustrine sections of the Jinxian Sag, Bohai Bay Basin, China. *Appl Geochem* 20:1427–1444.

Callahan, M.P., Smith, K.E., Cleaves, H.J., II, Ruzicka, J., Stern, J.C., Glavin, D.P., House, C.H., and Dworkin, J.P. (2011) Carbonaceous meteorites contain a wide range of extraterrestrial nucleobases. *Proc Natl Acad Sci USA* 108:13995–13998.

Carrapiso, A.I. and García, C. (2000) Development in lipid analysis: some new extraction techniques and *in situ* transesterification. *Lipids* 35:1167–1177.

Contreras, J.A., Murray, J.A., Tolley, S.E., Oliphant, J.L., Tolley, H.D., Lammert, S.A., Lee, E.D., Later, D.W., and Lee, M.L. (2008) Hand-portable gas chromatograph-toroidal ion trap mass spectrometer (GC-TMS) for detection of hazardous compounds. *J Am Soc Mass Spectrom* 19:1425–1434.

Cook, H.W. and McMaster, C.R. (2002) Fatty acid desaturation and chain elongation in eukaryotes. In *Biochemistry of Lipids, Lipoproteins and Membranes,* edited by D.E. Vance and J.E. Vances, Elsevier, New York, pp 181–204.

Cooper, G.W., Onwo, W.M., and Cronin, J.R. (1992) Alkyl phosphonic acids and sulfonic acids in the Murchison meteorite. *Geochim Cosmochim Acta* 56:4109–4115.

Cooper, G.W., Kimmich, N., Belisle, W., Sarinana, J., Brabham, K., and Garrel, L. (2001) Carbonaceous meteorites as a source of sugar-related organic compounds for the early Earth. *Nature* 414:879–883.

Cronin, J.R. and Pizzarello, S. (1983) Amino acids in meteorites. *Adv Space Res* 3:5–18.

Cronin, J.R. and Pizzarello, S. (1986) Amino acids of the Murchison meteorite. III. Seven carbon acyclic primary alpha-amino alkanoic acids. *Geochim Cosmochim Acta* 50:2419–2427.

Cronin, J.R. and Pizzarello, S. (1990) Aliphatic hydrocarbons of the Murchison meteorite. *Geochim Cosmochim Acta* 54:2859–2868.

Cronin, J.R. and Pizzarello, S. (1997) Enantiomeric excesses in meteoritic amino acids. *Science* 275:951–955.

Cronin, J.R., Gandy, W.E., and Pizzarello, S. (1981) Amino acids of the Murchison meteorite: I. Six carbon acyclic primary alpha-amino alkanoic acids. *J Mol Evol* 17:265–272.

Cronin, J.R., Pizzarello, S., and Yuen, G.U. (1985) Amino acids of the Murchison meteorite: II. Five carbon acyclic primary beta-, gamma-, and delta-amino alkanoic acids. *Geochim Cosmochim Acta.* 49:2259–2265.

Das, S.K. and Harris, R.S. (1970) Lipids and fatty acids in fossil teeth. *J Dent Res* 49:126–130.

Deamer, D.W. (1985) Boundary structures are formed by organic components of the Murchison carbonaceous chondrite. *Nature* 317:792–794.

Deamer, D.W. and Pashley, R.M. (1989) Amphiphilic components of the Murchison carbonaceous chondrite: surface properties and membrane formation. *Orig Life Evol Biosph* 19:21–38.

Eigenbrode, J.L. (2008) Fossil lipids for life-detection: a case study from the early Earth record. *Space Sci Rev* 135:161–185.

Eigenbrode, J.L., Freeman, K.H., and Summons, R.E. (2008) Methylhopane biomarker hydrocarbons in Hamersley Province sediments provide evidence for Neoarchean aerobiosis. *Earth Planet Sci Lett* 273:323–331.

Emmerton, S., Muxworthy, A.R., Sephton, M.A., Aldana, M., Costanzo-Alvarez, V., Bayona, G., and Williams, W. (2013) Correlating biodegradation to magnetization in oil bearing sedimentary rocks. *Geochim Cosmochim Acta* 112:146–165.

Engel, M.H. and Macko, S.A. (1993) *Organic Geochemistry: Principles and Applications*, Plenum Press, New York.

Engel, M.H. and Macko, S.A. (2001) The stereochemistry of amino acids in the Murchison meteorite. *Precambrian Res* 106:35–45.

Engel, M.H. and Nagy, B. (1982) Distribution and enantiomeric composition of amino acids in the Murchison meteorite. *Nature* 296:837–840.

Falcone, D.L., Ogas, J.P., and Somerville, C.R. (2004) Regulation of membrane fatty acid composition by temperature in mutants of *Arabidopsis* with alterations in membrane lipid composition. *BMC Plant Biol* 4:1–15.

Feng, Y. and Cronan, J.E. (2011) Complex binding of the FabR repressor of bacterial unsaturated fatty acid biosynthesis to its cognate promoters. *Mol Microbiol* 80:195–218.

Fontaine, S., Barot, S., Barre, P., Bdioui, N., Mary, B., and Rumpel, C. (2007) Stability of organic carbon in deep soil layers controlled by fresh carbon supply. *Nature* 450:277–280.

George, S.C., Volk, H., Dutkiewicz, A., Ridley, J., and Buick, R. (2008) Preservation of hydrocarbons and biomarkers in oil trapped inside fluid inclusions for >2 billion years. *Geochim Cosmochim Acta* 72:844–870.

Glavin, D.P., Callahan, M.P., Dworkin, J.P., and Elsila, J.E. (2011) The effects of parent body processes on amino acids in carbonaceous chondrites. *Meteorit Planet Sci* 45:1948–1972.

Guido, A., Jacob, J., Gautret, P., Laggoun-Défarge, F., Mastandrea, A., and Russo, F. (2007) Molecular fossils and other organic markers as palaeoenvironmental indicators of the Messinian Calcaredi Base Formation: normal versus stressed marine deposition (Rossano Basin, northern Calabria, Italy). *Palaeogeogr Palaeoclimatol Palaeoecol* 255:265–283.

Hanif, M., Atsuta, Y., Fujie, K., and Daimon, H. (2012) Supercritical fluid extraction of bacterial and archaeal lipid biomarkers from anaerobically digested sludge. *Int J Mol Sci* 13:3022–3037.

Hansen, C.J., Esposito, L., Stewart, A.I.F., Colwell, J., Hendrix, A., Pryor, W., Shemansky, D., and West, R. (2006) Enceladus' water vapor plume. *Science* 311:1422–1425.

Hartgers, W.A., Schouten, S., Lopez, J.F., Sinninghe Damsté, J.S., and Grimalt, J.O. (2000) ^{13}C-contents of sedimentary bacterial lipids in a shallow sulfidic monomictic lake (Lake Cisó, Spain). *Org Geochem* 31:777–786.

Hatch, J.R., King, J.D., and Daws, T.A. (1986) *Geochemistry of Cherokee Group Oils of Southeastern Kansas and Northeastern Oklahoma,* Subsurface Geology Series 11, Kansas Geological Survey, Lawrence, KS.

Hazel, J.R. and Williams, E.E. (1990) The role of alterations in membrane lipid composition in enabling physiological adaptation of organisms to their physical environment. *Prog Lipid Res* 29:167–227.

Heldmann, J.L., Schurmeier, L., McKay, C., Davila, A., Stoker, C., Marinova, M., and Wilhelm, M.B. (2014) Midlatitude ice-rich ground on Mars as a target in the search for evidence of life and for *in situ* resource utilization on human missions. *Astrobiology* 14:102–118.

Huang, Y., Wang, Y., Alexandre, M.R., Lee, T., Rose-Petruck, C., Fuller, M., and Pizzarello, S. (2005) Molecular and compound-specific isotopic characterization of monocarboxylic acids in carbonaceous meteorites. *Geochim Cosmochim Acta* 69:1073–1084.

Kaneda, T. (1991) Iso- and anteiso-fatty acids in bacteria: biosynthesis, function, and taxonomic significance. *Microbiol Rev* 55:288–302.

Keppler, F., Vigano, I., McLeod, A., Ott, U., Früchtl, M., and Röckmann, T. (2012) Ultraviolet-radiation-induced methane emissions from meteorites and the martian atmosphere. *Nature* 486:93–96.

Koga, Y. (2012) Thermal adaptation of the archaeal and bacterial lipid membranes. *Archaea* 2012:1–6.

Kvenvolden, K., Lawless, J., Pering, K., Peterson, E., Flores, J., Ponnamperuma, C., Kaplan, I.R., and Moore, C. (1970) Evidence for extraterrestrial amino-acids and hydrocarbons in the Murchison meteorite. *Nature* 228:923–926.

Kvenvolden, K.A., Lawless, J.G., and Ponnamperuma, C. (1971) Nonprotein amino acids in the Murchison meteorite. *Proc Natl Acad Sci USA* 68:486–490.

Lee, J.-Y., Yoo, C., Jun, S.-Y., Ahn, C.-Y., and Oh, H.-M. (2010) Comparison of several methods for effective lipid extraction from microalgae. *Bioresour Technol* 101:S75–S77.

Lovelock, J.E. (1965) A physical basis for life detection experiments. *Nature* 207:568–570.

Mansy, S.S. (2009) Model protocells from single-chain lipids. *Int J Mol Sci* 10:835–843.

Marrakchi, H., Choi, K.-H., and Rock, C.O. (2002) A new mechanism for anaerobic unsaturated fatty acid formation in *Streptococcus pneumoniae. J Biol Chem* 277:44809–44816.

McCollom, T.M. and Seewald, J.S. (2007) Abiotic synthesis of organic compounds in deep-sea hydrothermal environments. *Chem Rev* 107:382–401.

McCollom, T.M., Ritter, G., and Simoneit, B.R.T. (1999) Lipid synthesis under hydrothermal conditions by Fischer-Tropsch-type reactions. *Orig Life Evol Biosph* 29:153–166.

McKay, C.P. (2004) What is life—and how do we search for it on other worlds? *PLoS Biol* 2:1260–1263.

McKay, C.P., Stoker, C.R., Glass, B.J., Davé, A.I., Davila, A.F., Heldmann, J.L., Marinova, M.M., Fairen, A.G., Quinn, R.C., Zacny, K.A., Paulsen, G., Smith, P.H., Parro, V., Andersen, D.T., Hecht, M.H., Lacelle, D., and Pollard, W.H. (2013) The Icebreaker Life mission to Mars: a search for biomolecular evidence for life. *Astrobiology* 13:334–353.

Miller, S.L. and Urey, H.C. (1959) Organic compound synthesis on the primitive Earth. *Science* 130:245–251.

Montanari, L., King, J.W., List, G.R., and Rennick, K.A. (1996) Selective extraction of phospholipid mixtures by supercritical carbon dioxide and cosolvents. *J Food Sci* 61:1230–1234.

Naraoka, H., Shimoyama, A., and Harada, K. (1999) Molecular distribution of monocarboxylic acids in Asuka carbonaceous chondrites from Antarctica. *Orig Life Evol Biosph* 29:187–201.

Nichols, D.S., Nichols, P.D., and McMeekin, T.A. (1993) Polyunsaturated fatty acids in Antarctic bacteria. *Antarct Sci* 5:149–160.

Nichols, D.S., Miller, M.R., Davies, N.W., Goodchild, A., Raftery, M., and Cavicchioli, R. (2004) Cold adaptation in the Antarctic archaeon *Methanococcoides burtonii* involves membrane lipid unsaturation. *J Bacteriol* 186:8508–8515.

Noone, K.J., Ogren, J.A., Heintzenberg, J., Charlson, R.J., and Covert, D.S. (1988) Design and calibration of a counterflow virtual impactor for sampling of atmospheric fog and cloud droplets. *Aerosol Science and Technology* 8:235–244.

Ourisson, G. and Nakatani, Y. (1999) Origins of cellular life: molecular foundations and new approaches. *Tetrahedron Lett* 55:3183–3190.

Parnell, J., Cullen, D., Sims, M.R., Bowden, S., Cockell, C.S., Court, R., Ehrenfreund, P., Gaubert, F., Grant, W., Parro, V., Rohmer, M., Sephton, M., Stan-Lotter, H., Steele, A., Toporski, J., and Vago, J. (2007) Searching for life on Mars: selection of molecular targets for ESA's Aurora ExoMars mission. *Astrobiology* 7:578–604.

Parsegian, A. (1969) Energy of an ion crossing a low dielectric membrane: solutions to four relevant electrostatic problems. *Nature* 221:844–846.

Petsch, S.T., Eglinton, T.I., and Edwards, K.J. (2001) ^{14}C-Dead living biomass: evidence for microbial assimilation of ancient organic carbon during shale weathering. *Science* 292:1127–1131.

Pizzarello, S. and Shock, E. (2010) The organic composition of carbonaceous meteorites: the evolutionary story ahead of biochemistry. *Cold Spring Harb Perspect Biol* 2, doi:10.1101/cshperspect.a002105.

Pizzarello, S., Cooper, G.W., and Flynn, G.J. (2006) The nature and distribution of the organic material in carbonaceous chondrites and interplanetary dust particles. In *Meteorites and the Early Solar System II,* edited by D.S. Lauretta and H.Y. McSween Jr., University of Arizona Press, Tucson, pp 625–651.

Pond, J.L. and Langworthy, T.A. (1987) Effect of growth temperature on the long-chain diols and fatty acids of *Thermomicrobium roseum. J Bacteriol* 169:1328–1330.

Roth, L., Saur, J., Retherford, K.D., Strobel, D.F., Feldman, P.D., McGrath, M.A., and Nimmo, F. (2013) Transient water vapor at Europa's south pole. *Science* 343:171–174.

Sephton, M.A. and Hazen, R.M. (2013) On the origins of deep hydrocarbons. *Reviews in Mineralogy and Geochemistry* 75:449–465.

Sheng, J., Vannela, R., and Rittmann, B.E. (2011) Evaluation of methods to extract and quantify lipids from *Synechocystis* PCC 6803. *Bioresour Technol* 102:1697–1703.

Simoneit, B.R.T. (2004) Biomarkers (molecular fossils) as geochemical indicators of life. *Adv Space Res* 33:1255–1261.

Spencer, J. (2010) *JPL Rapid Mission Architecture (RMA) Enceladus Study Final Report: Planetary Science Decadal Survey*, NASA Technical Document, NASA, Washington, DC. Available online at http://www.archive.org/details/EnceladusFlybySampleReturnConceptStudies.

Strecker, A. (1850) Ueber die künstliche bildung der milchsäure und einen neuen, dem glycocoll homologen Körper. *Annalen der Chemie und Pharmazie* 75:27–45.

Strecker, A. (1854) Ueber einen neuen aus aldehyd—Ammoniak und blausäure entstehenden Körper (p). *Annalen der Chemie und Pharmazie* 91:349–351.

Summons, R.E., Albrecht, P., McDonald, G., and Moldowan, J.M. (2008) Molecular biosignatures. *Space Sci Rev* 135: 133–159.

Tawfik, D.S. and Viola, R.E. (2011) Arsenate replacing phosphate: alternative life chemistries and ion promiscuity. *Biochemistry* 50:1128–1134.

Tegelaar, E.W., de Leeuw, J.W., Derenne, S., and Largeau, C. (1989) A reappraisal of kerogen formation. *Geochim Cosmochim Acta* 53:3103–3106.

Twohy, C.H., Schanot, A.J., and Cooper, W.A. (1997) Measurement of condensed water content in liquid and ice clouds using an airborne counterflow virtual impactor. *Journal of Atmospheric and Oceanic Technology* 14:197–202.

Twohy, C.H., Strapp, J.W., and Wendisch, M. (2003) Performance of a counterflow virtual impactor in the NASA Icing Research Tunnel. *Journal of Atmospheric and Oceanic Technology* 20:781–790.

Ventura, G.T., Kenig, F., Reddy, C.M., Frysinger, G.S., Nelson, R.K., Mooy, B.V., and Gaines, R.B. (2008) Analysis of unresolved complex mixtures of hydrocarbons extracted from Late Archean sediments by comprehensive two-dimensional gas chromatography (GCGC). *Org Geochem* 39:846–867.

Waldbauer, J.R., Newman, D.K., and Summons, R.E. (2011) Microaerobic steroid biosynthesis and the molecular fossil record of Archean life. *Proc Natl Acad Sci USA* 108:13409–13414.

Xie, S., Lai, X., Yi, Y., Gu, Y., Liu, Y., Wang, X., Liu, G., and Liang, B. (2003) Molecular fossils in a Pleistocene river terrace in southern China related to paleoclimate variation. *Org Geochem* 34:789–797.

Yabuta, H., Williams, L.B., Cody, G.D., O'D Alexander, C.M., and Pizzarello, S. (2007) The insoluble carbonaceous material of CM chondrites: a possible source of discrete organic compounds under hydrothermal conditions. *Meteorit Planet Sci* 42:37–48.

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