Astrobiology and the Potential for Life on Europa

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The high likelihood that Europa harbors a contemporary, global, subsurface liquid water ocean makes it a top target in our search for life beyond Earth. Europa's chondritic composition, rocky seafloor, and radiolytically processed surface may also play an important role in the habitability of Europa. Here we review the availability and cycling of biologically essential elements, the availability of energy to power life, and the conditions on Europa that could be conducive to the origin of life. We also address the survivability and detection of possible biosignatures on the surface, and possible search strategies for future orbiting and landed missions.

1. ORIGINS AND HABITABILITY, PAST AND PRESENT

1.1. Introduction

Europa may be the premier place in the solar system to search for both extant life and a second origin of life. The discovery of extant life beyond Earth would provide critical information about the fundamental nature of life and the breadth of possible biological processes. The discovery of a second origin of life would provide a key benchmark to assess evolutionary processes and would lead to a better understanding of life in the broader context of the universe. Were we to discover a second origin, we would transform the universe from a barren expanse in which life is unique to Earth, to a biological universe in which life may arise wherever conditions are suitable. We could begin to separate the contingent from the necessary in our understanding of biological possibility (*Sagan,* 1974). These discoveries lie at the heart of some of humankind's most longstanding and profound questions, and the exploration of Europa has the potential to provide some answers.

Our understanding of life on Earth has led to three key requirements for habitability (Fig. 1). Liquid water, a suite of biologically essential elements, and a source of energy are all prerequisites for life as we know it. In the context of Europa, these three cornerstones may well be satisfied by the attributes listed in the periphery of Fig. 1. This chapter concerns the details of these attributes so that our physical, chemical, and geological understanding of Europa can guide our biological assessment of this world. We do not address in detail the case for liquid water on Europa, as that

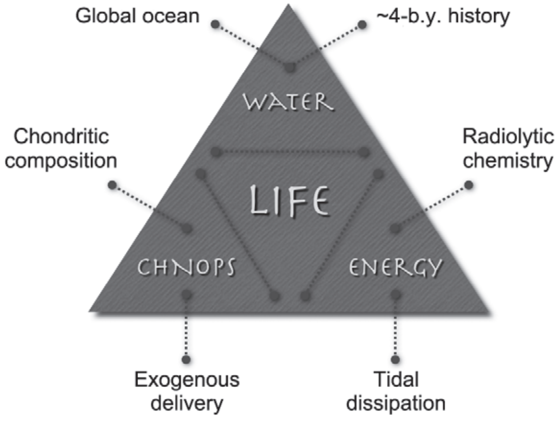

Fig. 1. Representation of the habitability of Europa. At present, our understanding of the conditions necessary for life can be distilled down to three broad requirements: (1) a sustained liquid water environment, (2) a suite of elements critical for building life (e.g., C, H, N, O, P, S, etc.), and (3) a source of energy that can be utilized by life. Here we suggest how these "keystones of habitability" intersect with our current understanding of the conditions on, and within, Europa.

subject is addressed in the chapters by McKinnon et al. and Khurana et al. We also refer the reader to the chapters by Carlson et al. and by Zolotov and Kargel for additional information regarding surface composition and ocean chemistry.

Significant to the issue of extant life on Europa is the fact that the subsurface liquid water reservoir has likely persisted for much of the moon's history. The ocean is predicted to be in contact with a rocky ocean floor, potentially creating a geochemically rich environment suitable for the origin and maintenance of life. Although the ocean lies beneath several to many kilometers of ice (*Pappalardo et al.,* 1998; *Greenberg et al.,* 1999; *Hand and Chyba,* 2007), the global nature of the ocean suggests that much of this world could be inhabited if the chemical conditions are suitable.

The contemporary, global ocean of Europa stands in contrast to several other potentially habitable worlds within our solar system. Mars, for instance, may have harbored a northern hemisphere surface ocean for the first ~2.0 G.y. of the planet's history (*Head et al.,* 1999). On present-day Mars, however, liquid water may be restricted to subsurface aquifers or transient pockets of near-surface meltwater (*Costard et al.,* 2002). The proximity of Mars to Earth could also raise interesting questions of contamination, were life on Mars discovered to be similar to life on Earth (e.g., DNA, RNA, and protein-based). The discovery of such life would certainly be profound, but contamination issues could confuse the discovery of a second origin.

Similarly, the question of contemporary subsurface liquid water on Enceladus remains open (*Spencer et al.,* 2006; *Kieffer et al.,* 2006), but the heat flux and high loss rate from the plumes implies that the observed events are likely transient. Stability of liquid water reservoirs over geological timescales (~10^5 yr or more) is an important consideration for habitability; Enceladus may have contemporary liquid water, but if it is a transient reservoir then the habitability of Enceladus could be compromised.

Titan may contain an ammonia-water mantle, part of which could be liquid (*Lorenz et al.,* 2008). The primary solvent available on Titan's surface and near-subsurface, however, is nonpolar hydrocarbons in liquid methane lakes (*Mitri et al.,* 2007). Such environments could be an interesting place to search for life *unlike* life as we know it (*Schulze-Makuch and Grinspoon,* 2005; *McKay and Smith,* 2005), but it is intellectually more tractable to focus our initial search on environments comparable to those in which we know biology could work. The contemporary ocean of Europa provides just such an environment. Our experience with life here on Earth has taught us that carbon-and-water-based life functions quite well throughout a considerable range of temperature, pressure, and chemical regimes. But given the right set of conditions, the question remains: Will life arise? If we were to discover carbon- and water-based life elsewhere in the solar system, would we see biochemistry comparable to the protein-, RNA-, and DNA-based life we have here on Earth?

Finally, we note that life on Europa could be vastly different in composition and biochemistry from life on Earth. Such "weird life" (*Benner et al.,* 2004) should of course not be excluded from consideration, or from our future search strategies (*NRC,* 2007). However, our working knowledge and scientific understanding is constrained to the carbon- and water-based life found here on Earth. Consequently, in the sections that follow, references to "life" should really be interpreted to mean "life as we know it."

1.2. Habitable Environments

The origin of life on Europa, and the habitability of Europa, are two distinct but related issues. Here we address both questions, starting first with habitability and then moving to the more difficult and less-well-constrained question of life's origin. We focus on conditions relevant to contemporary Europa, but it is important to distinguish Europa in the past from Europa in the present. For instance, *Hand and Chyba* (2007) have argued that the salinity of the contemporary ocean may be habitable by terrestrial standards, but not necessarily conducive to the origin of life. Considering a different aspect of ocean chemistry, however, models of the tidal evolution of Europa indicate that the heat flux may have been much greater in the past (*Hussmann and Spohn,* 2004), perhaps creating a liquid water environment with more hydrothermal activity and thus more chemistry of prebiological potential. We explore these possibilities below.

1.2.1. Inventory and cycling of biologically essential elements. Life requires a suite of biologically essential elements to serve as the basic building blocks and metabolic material for maintenance, growth, and reproduction (*Wackett et al.,* 2004). For life to persist such materials must be available, and their availability must be sustained through cycles within the geological and chemical processes of the ecosystem. On a planetary scale this means that the elemental cycles must be closed-loop systems; if large loss terms exist or the turnover rate is too slow, then life will be constrained by the limiting element.

Carbon-and-water-based life on Earth requires nitrogen, phosphorous, sulfur, and trace amounts of metals. At the orbital distance of Jupiter (5.2 AU), condensation and retention of the volatile elements would have been more efficient than for that of the terrestrial planets (*Lewis,* 1971). As discussed by *McKay* (1991) and *Chyba and Hand* (2006), based on abundance alone, the inner solar system is actually a poor locale for carbon chemistry and hence carbon-based life. High temperatures forced CO_2, NH_3, CO, H_2S, H_2O, and N_2 out beyond the asteroid belt where they could condense onto planetesimals. Although heating in the jovian subnebula also led to dehydration and loss of volatiles, the formation of Europa from chondritic material in the accretion disk of Jupiter likely led to a world rich in many of the biogenic elements (see chapters by Estrada et al. and by Canup and Ward). In Fig. 2 (inspired by *McKay,* 1991) we show the approximate bulk abundance for several biologically essential elements on worlds within our solar system. Values are given relative to solar abundance. To date, carbon, hydrogen, oxygen, and sulfur have all been observed

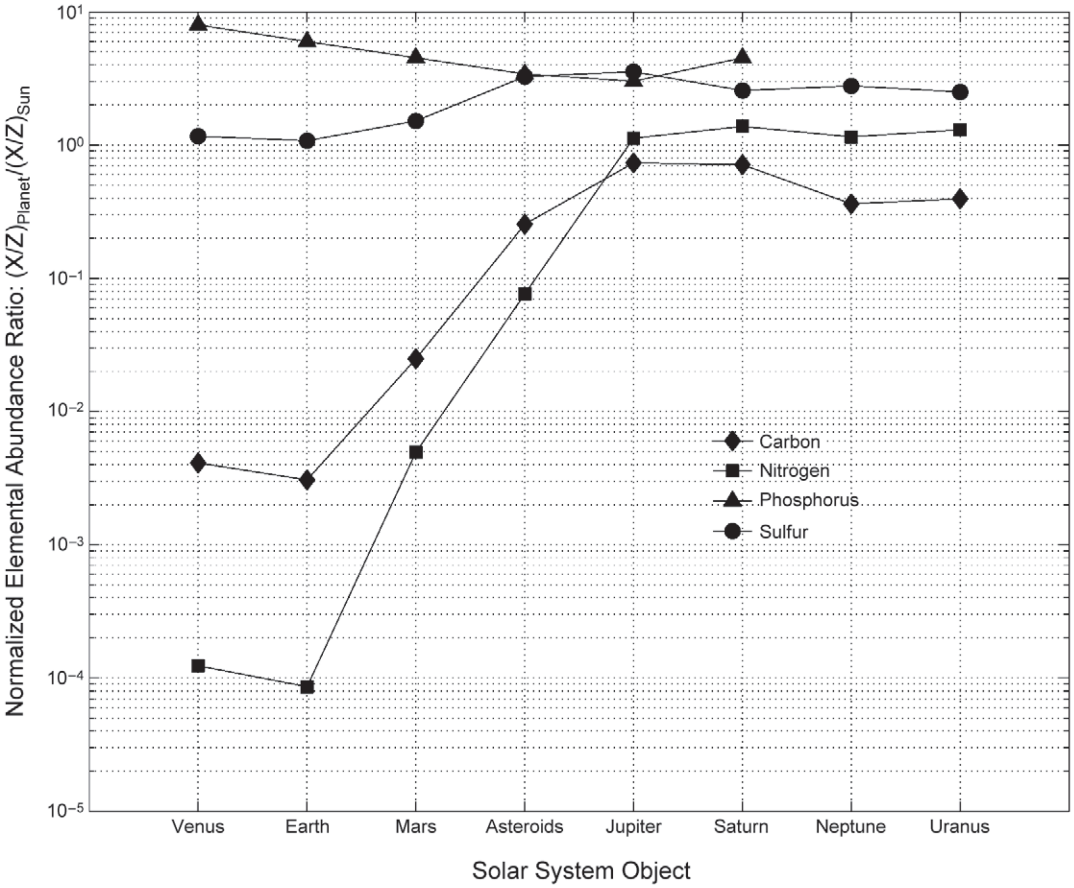

Fig. 2. Ratios of the biologically essential elements C, N, P, S to total heavy elements larger than helium, normalized to solar values (inspired by *McKay,* 1991). With increasing distance from the Sun the relative abundance of C, N, and S increases. This in part reflects the fact that C, N, and S were largely present in volatile species (e.g., CH_4, CO_2, N_2, and H_2S) that condensed into ices in the cold outer regions of the solar system (similar to H_2O). Values presented here were calculated from data and models provided in *Lodders and Fegley* (1998), *Lewis* (1971), *Lodders* (2003), *Taylor et al.* (2004), *Guillot* (2005), *Sotin et al.* (2007) and from references within *McKay* (1991). The values shown here are intended only to represent the broad, order of magnitude, trend in our solar system.

on the surface of Europa (see chapter by Carlson et al.). Here we examine observational and theoretical constraints for the abundance, phase, and cycling of each of these elements.

1.2.1.1. Carbon: The abundance of total carbon (organic + inorganic) incorporated into planets increases by over two orders of magnitude for worlds beyond the asteroid belt (*McKay,* 1991). Despite Earth's carbon-rich biosphere, our planet is depleted in carbon when compared to worlds of the outer solar system. The carbon abundance found in worlds beyond the asteroid belt is still a few orders of magnitude lower than the abundance of carbon found in life on Earth, but it gives us good reason to expect that the abundance of carbon on Europa will not be a limiting factor for autotrophic and heterotrophic biochemistry. Models for the formation of Europa from chondrites (*Crawford and Stevenson,* 1988; *Kargel et al.,* 2000; *Zolotov and Shock,* 2001) put the abundance of carbon at ~0.01–0.7 wt.%, most of which is in CO_2. The observation of 360 ppm CO_2 in the surface ice of Europa leads to 0.028 wt.% carbon on the surface, in good agreement with the models for Europa's bulk composition (*Fanale et al.,* 2001; *Hand et al.,* 2007; see chapter by Carlson et al.).

Other forms of carbon are expected on the surface, but have not yet been observed [e.g., CO and H_2CO_3 (*Hand et al.,* 2007)]. Spectral characterization of Europa by the Galileo spacecraft's Near-Infrared Mapping Spectrometer (NIMS) was limited by radiation noise, but observations of Ganymede and Callisto can serve as a guide to the molecules that might be produced on Europa from meteoritic impact and subsequent radiolysis. Ganymede and Callisto are less geologically active, with correspondingly greater surface ages, and they show more meteoritic debris accumulated on their surfaces. This is in part due to higher escape velocities and lower impact velocities for the large, outer satellites (*Pierazzo and Chyba,* 2002).

Two sets of spectral features of astrobiological interest are observed on Callisto, the first thought to be hydrocarbon C-H stretch features and the second assigned to the nitrile group (C≡N). The latter is also observed on Ganymede. *McCord et al.* (1997, 1998) identified possible CH_2 stretch bands on Callisto at 3.41 ± 0.02 μm and 3.58 ± 0.03 μm (in wavenumbers this corresponds to 2932 ± 20 cm^{-1} and 2793 ± 25 cm^{-1}, respectively). The positions of these bands are close to the 3.44- and 3.53-μm (2907-cm^{-1} and 2833-cm^{-1}) band positions observed on Enceladus (*Brown et al.,* 2006). Similar CH_2 symmetric and asymmetric stretch bands are found in residues produced in the laboratory by ultraviolet and proton-irradiation of ices containing ammonia and alkanes (*Allamandola et al.,* 1998; *Moore and Donn,* 1982). These features have been suggested to arise from aliphatic hydrocarbons dominated by methylene (CH_2) groups (*Allamandola et al.,* 1998). Polymethylene oxides and CH_2 groups associated with C≡N are also possible contributors (*Allamandola et al.,* 1998). These bands are not observed on Europa, although low-noise measurements in this wavelength region were very limited. Using such spectra (see chapter by Carlson et al.) and band strengths from *Moore and Hudson* (1998), we find an upper limit for the number of methylene groups per water molecule of $[CH_2]/[H_2O] < 1.5 \times 10^{-3}$. The number expected for meteoritic infall and burial by gardening, if all the C atoms are associated with methylene, is a factor of 3 less than this upper limit or ~500 ppm. If hydrocarbons are found locally on Europa with concentrations greatly exceeding tenth-of-a-percent levels, they could indicate an oceanic source of organic material, a point discussed in greater detail in section 3.

Cycling of carbon on an ice-covered ocean world, be it inhabited or uninhabited, may not differ considerably from the major attributes of the terrestrial carbon cycle. Most of the carbon on Earth is in geological reservoirs or dissolved in the ocean. Plate tectonics and volcanism are the critical ways to release geologically sequestered carbon back into the atmosphere, where CO_2 is a minor species present at levels comparable to the concentration found in Europa's surface ice. Life on our continents is largely dependent on carbon derived from atmospheric CO_2.

For an ice-covered ocean, however, dissolved inorganic carbon (HCO_3^-, CO_3^{2-}, H_2CO_3), precipitated carbonates, CO_2 clathrates, and organic carbon will be the primary phases of carbon. Tectonics would be required in order to completely recycle carbon, but even on a geologically dead Europa the balance between dissolved carbon and precipitated carbon could lead to a self-sustaining carbon cycle. The critical link for sustaining this cycle, and one key geochemical difference between the oceans of Europa and Earth, is the depth of Europa's ocean and the change in solubility of carbonates as a function of pressure and temperature. On Earth, where the average depth of the ocean is 4 km, the pressure is ~40.1 MPa and carbonates are stable for the bulk ocean pH of 8 and temperature of 4°C. At slightly greater depth (~4.2 km) the increase in pressure raises the solubility of carbon dioxide and precipitated carbonates are subject to dissolution in the unsaturated deep waters. This so-called carbonate compensation depth (CCD) explains why calcite and aragonite are rarely found in the deepest regions of our ocean. Coupled with this, the solubility of CO_2 increases with decreasing temperature. Thus, while carbonates precipitate easily in the warm surface waters of Earth's ocean, the cold deep waters of our ocean enhance dissolution (*Krauskopf and Bird,* 1995).

On Europa, the pressures and temperatures that are expected for the ocean will ensure that much of the inorganic carbon stays in dissolved form. Europa's ice-covered ocean must, on average, hover near the freezing point of water (otherwise the icy shell and ocean could not be stable), and therefore be conducive, at least in terms of temperature and pressure, for retaining inorganic carbon in dissolved forms. Clathrates of CO_2 may be the dominant phase of precipitated carbon (*Crawford and Stevenson,* 1988; *Kargel et al.,* 2000). One interesting consequence of the CO_2 solubility chemistry is that carbonate shells and other biological structures made of carbonate will be unstable at depths of ~30 km or more in Europa's ocean. Were the ice on Europa ~30 km in thickness, then no region of the ocean would be stable for carbonates, save for perhaps localized hot spots and pH extremes on the ocean floor. In other words, carbonate skeletal structures such as those found on Earth are unlikely to be stable throughout much of Europa's ocean. (The consequences for biosignatures on the surface of Europa are discussed later in this chapter.) A carbon cycle on Europa could then be completed by the complementary actions of precipitation of carbonate and organic carbon to depth, followed by dissolution. Ultimately this raises questions about the stability of organic carbon at the ocean floor: Would carbon-based life be stable against dissolution on Europa's seafloor? Evidence on Earth suggests it would. Soft-walled, single-celled foraminifera have been found in sediments at Challenger Deep in the Marianas Trench (*Todo et al.,* 2005), the greatest depth within our ocean (11 km, P = 110.1 MPa, comparable to a depth of roughly 80 km below the surface of Europa).

1.2.1.2. Nitrogen: Nitrogen is also expected to be in abundance at Europa and we have empirical evidence of nitrogen for Europa's outer neighbors. A feature at 4.57 μm in spectra of Ganymede and Callisto may be due to the stretching of a C≡N group, which shows strong, narrow absorption features between 4.4 and 4.9 ìm (*McCord et al.,* 1997, 1998). Most of the simple molecules containing C≡N have features that are inconsistent with the observed features, but polymeric HCN and some tholins possess absorption features in the 4.53- to 4.61-μm region that may be consistent with cyanogens (*Cruikshank et al.,* 1991; *Khare et al.,* 1994). With only one band evident it is impossible to provide unequivocal identifications, and other candidates are plausible, including CO trapped within zeolites and amorphous silica (*Cairon et al.,* 1998) as well as carbon suboxide, C_3O_2 (*Gerakines and Moore,* 2001; *Johnson et al.,* 2004). Spectra at Europa were too noisy to resolve the 4.57-μm feature; thus, while models predict nitrogen to be in abundance, we have at present no direct evidence for nitrogen on Europa.

Modeling estimates for the abundance of ammonia, and hence nitrogen, in primordial Europa are within the range of 5–10 wt.% NH_3 (*Lewis,* 1971; *Grasset and Sotin,* 1996).

Depending on formation mechanisms for the icy shell, ammonia may be excluded from the freezing ice, leading to a surface devoid of ammonia and an ammonia-rich subsurface ocean (*Grasset and Sotin,* 1996; *Spohn and Schubert,* 2003). Even if ammonia did reach the surface (e.g., cryovolcanism), the radiation environment of Europa would radiolyze much of the NH_3 to N_2 and H_2, which are easily lost to space (*Loeffler et al.,* 2006). Could Europa have lost a significant fraction of its nitrogen to space as a result of resurfacing and radiolysis over 4.5 G.y.? Such a loss is unlikely. Even if one assumes complete loss of nitrogen through a 1-m gardened surface layer that is replenished every 10 m.y., the amount of nitrogen lost is only about 0.5 wt.% relative to the starting concentration. Nitrogen should still be in abundance on contemporary Europa.

It has been argued that nitrogen, as opposed to water or carbon, should be used as the primary flag for habitable regions beyond Earth (*Capone et al.,* 2006). In this context, it is interesting to consider the various phases and possible pathways of nitrogen cycling in an ice-covered ocean. Unlike Earth, where atmospheric N_2 serves as the major reservoir for nitrogen, Europa's nitrogen will be in dissolved or solid forms in the ocean, frozen into the ice, or sedimented onto the seafloor. The initial phase of nitrogen during formation was primarily ammonia, but if Europa's waters became oxidized over time then nitrate would have become the dominant phase. Nitrate and the ammonium ion (NH_4^+) are very soluble in water and would be easily incorporated into the icy shell. Neither species was reported in the NIMS spectra, although radiation noise at longer wavelengths may have been a limiting factor for such detection.

The phase of nitrogen has important consequences for biochemistry, as illustrated by terrestrial ecosystems. On Earth, the advent of nitrogen fixation (N_2 → organic nitrogen, e.g., C-N-H) may have opened a bottleneck in biological productivity (*Capone et al.,* 2006; *Smil,* 2001). Prior to the evolution of N_2-fixation, life on Earth was limited to the nitrogen found in nitrates in the ocean. This amounts to approximately 0.4% of the available nitrogen ($\sim 5.5 \times 10^{21}$ g), of which ~73% resides in our atmosphere (*Capone et al.,* 2006). Our nitrogen-rich atmosphere resulted largely from the fact that N_2 is chemically inert. Nitrogen accumulated in the atmosphere from volcanic emissions and photolysis, but without biological fixation of N_2, there was no large sink term for biological or geological sequestration. Photolysis and fixation by lightning accounted for $\sim 10^{13}$ g N fixed per year, roughly two orders of magnitude lower than contemporary biological fixation (*Walker,* 1977). With the advent of biological N_2-fixation, however, nitrogen in biomass and sediments reduced the nitrogen content of the atmosphere by more than 20% over Earth's history (*Walker,* 1977). Maintaining our nitrogen-rich atmosphere, and our global nitrogen cycle, then became partially dependent on the complementary process of biological denitrification ($N_{Organic} \rightarrow NO_3^- \rightarrow N_2$).

On Europa, N_2 is likely to be only a minor component of any abiotic global nitrogen cycle. Some of the primordial ammonia would have gone into ammonium (NH_4^+), nitrite (NO_2^-), and nitrate (NO_3^-), with speciation being largely a function of pH, temperature, and the availability of oxidants. With oxidants available, energetic niches for life exist in the pathways to nitrate, e.g., $NH_3 \rightarrow NO_2^- \rightarrow NO_3^-$. For terrestrial life to incorporate the nitrogen in nitrate into amino acids and macromolecules (e.g., proteins and nucleic acids), nitrate must be reduced to ammonium ions, $NO_3^- \rightarrow NO_2^- \rightarrow NH_4^+ \rightarrow N_{Organic}$. A complete cycle would then be fulfilled by (1) conversion of macromolecular nitrogen back into nitrate by nitrifying organisms, (2) conversion back into ammonia or the ammonium ion by abiotic or biological ammonification, and (3) radiolysis to oxidized species or nitriles on the surface of Europa. Additionally, anaerobic ammonium oxidation (anammox) — an important biological pathway in the nitrogen cycle of Earth's ocean — could serve to oxidize ammonium with nitrite in Europa's ocean, converting the nitrogen back to dissolved molecular nitrogen. Once nitrogen is in organic form it is advantageous for organisms to simply harvest the amino acids and nucleic acids rather than breaking these compounds down any further. This process, however, requires oxygen and would only be possible if radiolytically produced oxygen on the surface of Europa is delivered to the ocean (see section 1.2.2). Were that the case, a biologically active europan ocean could have much of its nitrogen sequestered and cycled in organic forms. Figure 3 shows possible nitrogen cycling and partitioning for three different scenarios in Europa's ocean. Each of these scenarios has important implications for the observation of nitrogen on the surface of Europa and the prospect of distinguishing abiotic organic chemistry from biologically mediated chemistry. These points are discussed in detail in sections 2 and 3.

1.2.1.3. Phosphorous: Phosphorous has not been observed on any of the Galilean satellites but it is seen in trace amounts in the jovian atmosphere (*Taylor et al.,* 2004). The bulk abundance of phosphorous, based on chondritic models for the formation of Europa (*Kargel et al.,* 2000; *Lodders and Fegley,* 1998), is ~0.1 wt.%. *Zolotov and Shock* (2001) estimated that full extraction of phosphorous from Europa's original chondritic material could yield 10–20 g of phosphorous per kilogram of ocean water. However, their more realistic partial extraction models yield concentrations of 0.34 to 4.2×10^{-6} g kg^{-1}, much closer to that found in terrestrial ocean water, 6×10^{-5} g kg^{-1}. The availability of phosphorous is a critical limiting element for life on Earth. The problem is not so much the bulk quantity, but rather the relative insolubility of phosphorous and the geological sequestration of phosphorous in minerals. Much of Earth's phosphorous is believed to reside in the lower mantle or core and only ~0.007 wt.% of the total phosphorous is dissolved in the ocean (*Macia et al.,* 1997). In contrast to nitrogen, phosphorous has no gaseous (atmospheric) reservoir. For the temperature and pressure conditions on Earth, and for those on Europa, phosphorous is found in solid phase as apatite or in relatively insoluble phosphoric acid and related salts. The readily available dissolved ions in water are

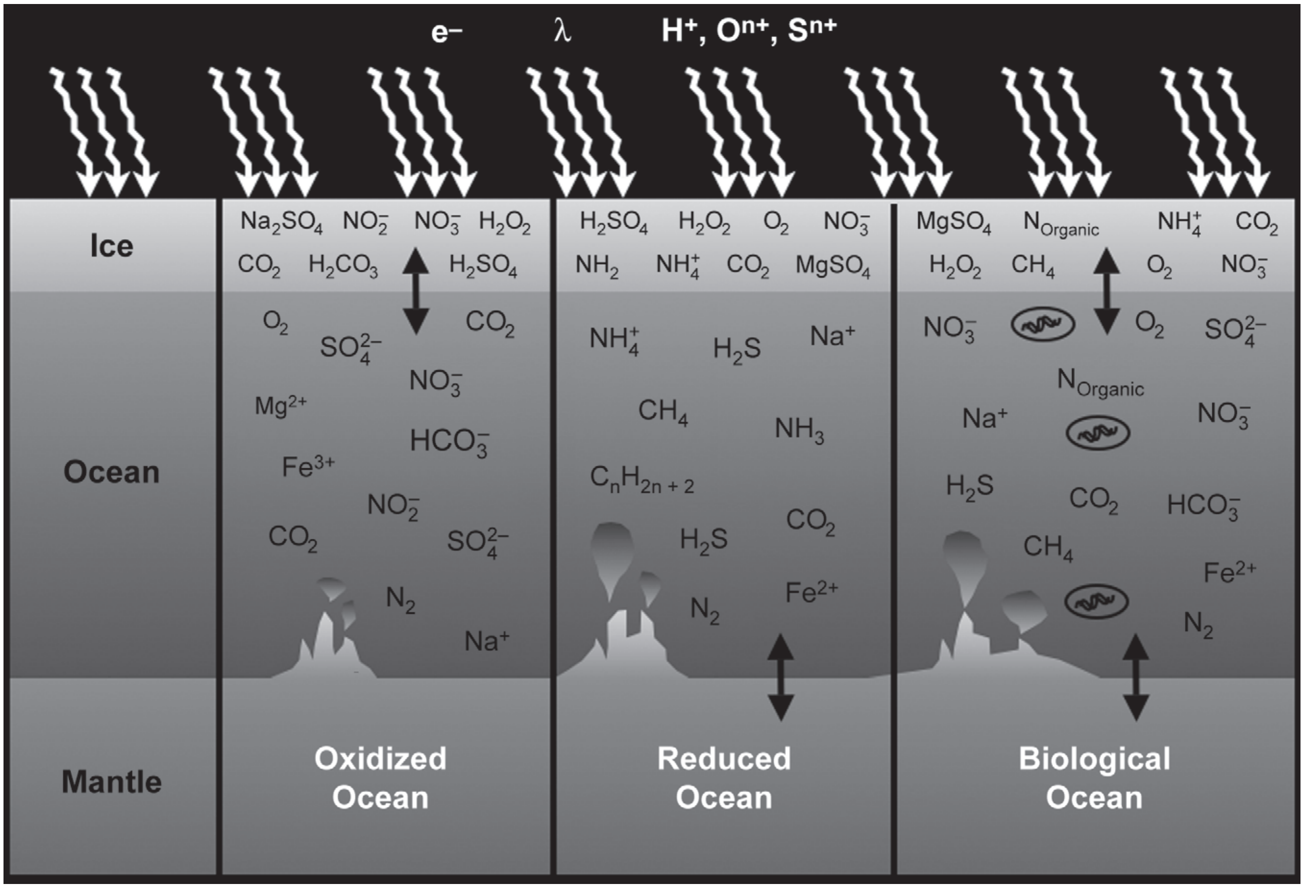

Fig. 3. Consideration of the sequestration of nitrogen on Europa. The sequestration and possible cycling of nitrogen on Europa will be considerably different than that of the Earth, since Europa lacks an N_2-rich atmosphere. Three possibilities are indicated in this diagram: (1) an oxidized ocean, resulting from cycling of the ocean with the radiolytically processed icy shell and dominated by nitrogen in the form of nitrate and nitrite; (2) a reduced high-pH ocean, resulting from cycling with the seafloor and dominated by ammonia and ammonium; and (3) a biological ocean (note the microbe symbols), in which cycling occurs with both the icy shell and seafloor and in which nitrogen is largely in organic form (e.g., amino acids, proteins, nucleic acids). Each scenario holds potentially different implications for nitrogen on the surface of Europa, an important consideration when searching for signs of life.

the phosphate ions PO_4^{3-}, HPO_3^{2-}, and $H_2PO_3^-$, where the former is most stable in basic solutions and the latter is more stable in acidic solutions.

The limiting nature of phosphorous raises the question of why terrestrial biochemistry is so intimately tied to this element. From the phosphodiester bonds of our genetic material to the energy-yielding bonds of ATP, all life on Earth is critically dependent on the availability of phosphorous. Surely there must be some biogeochemical alternative that would not suffer the geological limitations imposed by phosphates. *Westheimer* (1987) explored this question and argued that phosphate and phosphoric acid are well tuned to the needs of genetic biochemistry. Being trivalent, phosphate is able to provide two bonds for building polymers, while the remaining valency yields a negative charge for the molecule. This negative charge serves to keep the molecule within a lipid membrane, and it impedes hydrolytic destruction of the molecule. This latter property is critical for stability; if an information storage molecule is going to be useful for Darwinian selection, it must survive long enough for replication and reproduction. Ester bonds (R–COO–R'), such as those found in ethyl acetate, can survive on average for many months, but information storage requires thousands of such bonds, each of which is important to the integrity of the information stored. For example, if too many bonds are broken, the organism's ability to reproduce could be severely compromised (*Westheimer,* 1987). Here again phosphate is advantageous. Ester bonds with phosphate are typically a little more stable than the alternatives, but phosphate has the added advantage of the negative ionic charge, which protects the bonds from hydrolysis by the surrounding water.

Westheimer (1987) examined citric acid, arsenic acid, and silicic acid as alternatives, but none had the combined advantage of phosphoric acid and the associated phosphate. Sulfate — an anion observed over much of the surface of Europa — is insufficient in both its valency and stability. Sulfate is divalent and capable of making polymers, but the sulfate bonds hydrolyze much faster than comparable phosphate bonds. At the cold temperatures, high pressures, and neutral to alkaline pH [predicted from models for Europa's ocean (*Zolotov and Shock,* 2001)], stability increases and some of these other compounds may become viable alternatives, but phosphate will still be the best. As with our ocean, the ocean on Europa is likely at or near saturation with phosphoric acid and the available phosphorous provides a unique evolutionary advantage for any putative subsurface ecosystem. Finally, we note that for the case of lipids used in biological membranes, sulfolipids have been observed to replace phospholipids in cyanobacteria living in phosphorous deficient regions of the ocean (*Van Mooy et al.,* 2006).

1.2.1.4. Sulfur: Sulfur is unlikely to be a limiting factor for life on Europa. The surface chemistry, and potentially the subsurface chemistry, is dominated by sulfur and water. Formation models involving complete elemental extraction from a bulk silicate Europa calculate an upper limit for endogenous sulfur of 340 g $kg^{-1}_{H_2O}$ (*Zolotov and Shock,* 2001). However, the preferred partial extraction model of that work argues for only ~3 g sulfur $kg^{-1}_{H_2O}$. Coupled with this is the exogenous sulfur delivered from the Io torus (distinct from that of sulfur from micrometeorites, discussed below). Observations put the contemporary flux of iogenic sulfur ions and neutrals to the surface of Europa at 10^7–10^8 and ~10^6 atoms per cm^{-2} s^{-1}, respectively (*Johnson et al.,* 2004). The loss rate is ~10^7 atoms per cm^{-2} s^{-1}. Over ~4 G.y. this amounts to ~6.4×10^{10} kg of sulfur, or roughly 20 ng $kg^{-1}_{H_2O}$ added to Europa's water layer. Although perhaps a small net effect, it is important to emphasize the process taking place: volcanism on one world (Io) is having a measurable effect on the chemistry of another world (Europa). On the surface, spectra from the Galileo NIMS indicated regions where hydrated sulfate may constitute upwards of 90% by number of the molecular surface abundance. Considerable debate remains regarding the cation associated with the sulfate (see chapter by Carlson et al.), but radiolysis of the europan surface almost certainly drives a sulfur cycle, creating surface reservoirs of hydrated sulfate, sulfur dioxide, hydrogen sulfide, and various forms of elemental and polymerized sulfur (*Carlson et al.,* 1999a, 2002, 2005).

Biochemically, sulfur is in the amino acids cysteine and methionine, which are components of numerous enzymes and coenzymes that are critical to metabolism. Functionally, the thioester bond (CO–S) mediates electron transfer and group transfer in metabolism (*de Duve,* 2005). Specifically, the thiol within acetyl-coenzyme A plays a key role in the tricarboxylic acid cycle (a.k.a. the Krebs cycle). This cycle is responsible for generating reduced metabolic intermediates and adenosine triphosphate (ATP). ATP is the central molecule that fuels biosynthesis in all life on Earth. In modern metabolic pathways, the protonmotive force is used more than thoiester couplings to drive synthesis of ATP, but the thioester coupling is tied to some of the most primitive metabolic pathways (*de Duve,* 2005). Indeed, the role of sulfur in modern coenzymes and metabolic pathways has motivated some workers to examine whether primordial sulfur geochemistry may have served as the seed for the origin of life on Earth (*Wächtershäuser,* 1988; *Cody,* 2005).

The cycling of sulfur on Europa is potentially of great importance to the habitability of Europa. The oxidation states of sulfur, from H_2S to SO_2, offer a variety of useful biochemical pathways for metabolism. In a cold ocean with an active seafloor, H_2S or SO_4^{2-} will be the dominant form, with the balance being determined by the oxidation state of the bulk ocean. If Europa has an active seafloor but cycling with the icy shell is poor, then H_2S will dominate. Conversely, active cycling with the icy shell but not the seafloor will yield a sulfate-dominated ocean. A detailed discussion of sulfur in Europa's ocean can be found in the chapter by Zolotov and Kargel.

1.2.1.5. Exogenous delivery of biologically essential elements: Delivery of biologically essential elements from comets and micrometeorites over the age of the solar system would have also been an important source for Europa. *Pierazzo and Chyba* (2002) calculated this flux and found that over the past 4.4 G.y. Europa received 9×10^{11} to 1×10^{13} kg of carbon (the exact phase, e.g., organic or inorganic, depends heavily on the velocity of impact). Even had Europa somehow formed devoid of carbon, the accumulation of cometary carbon over solar system history would have still supplied this baseline reservoir. Similarly, the integrated flux would have led to a total of 2×10^{11} to 3×10^{12} kg of nitrogen, 2×10^{10} to 3×10^{11} kg of phosphorous, and 2×10^{11} to 2×10^{12} kg of sulfur delivered to Europa. These estimates account for much of the material being lost as ejecta upon impact, but they do not account for reaccretion. Therefore, these numbers serve as lower limits for the exogenous delivery of chondritic material. *Johnson et al.* (2004) calculated a micrometeorite flux of ~0.045 kg s^{-1} to Europa, resulting in an integrated flux for the above elements that is roughly an order of magnitude larger than *Pierazzo and Chyba* (2002). The *Johnson et al.* (2004) results do not account for ejecta loss and therefore serve as a good upper estimate.

Table 1 shows a comparison of elemental abundances for several different solar system objects. Also shown in Table 1 are the relative elemental abundances found in life on Earth.

1.2.2. The constraint of chemical energy. Life extracts energy from its environment and, at a minimum, exchanges it for heat energy and entropy. The energy from the environment is used to do the work of cellular maintenance, metabolism, and reproduction. The availability of energy within an environment can be a limiting factor for habitability (*Heijnen and van Dijken,* 1992; *Nealson,* 1997; *Hoehler et al.,* 2001). On Europa, it has been argued that while liquid water and biologically essential elements are available, the chemistry of the ocean may have reached thermodynamic equilibrium, resulting in an ocean devoid of the energy needed for life (*Gaidos et al.,* 1999).

The chemical potential, or molal Gibbs free energy (with units of kJ mol^{-1}), of a solution provides a metric by which we can assess the energetic constraint on habitability. This

TABLE 1. Elemental abundances for solar system objects and life.

C = 1	Life	Earth (bulk)	Earth (crust)	Earth (ocean)	CI chond	CV chond	LL chond	Comets	Jupiter (atm.)	Europa Ocean
H	5.67	10.1	51.5	47,290	7.03	6.34	NA	4.02	952	19.6
N	0.23	0.012	0.125	0.714	0.079	0.013	0.019	0.094	0.384	0.013
O	2.4	5400	1080	23,640	10.1	52.4	96.8	2.02	0.284	9.8
P	0.012	10.4	1.41	0.714	0.012	0.082	0.114	N/A	$2.9\text{x}10^{-4}$	0.082
S	0.012	76.1	0.589	12.1	0.588	1.56	2.54	0.071	0.039	1.54
Ref.	*Zubay* (2000)	*Kargel and Lewis* (1993)	*Zubay* (2000)	*Zubay* (2000)	*Lodders and Fegley* (1998)	*Lodders and Fegley* (1998)	*Lodders and Fegley* (1998)	*Lodders and Fegley* (1998) Dust + Ice, Comet P/Halley	*Taylor et al.* (2004)	*Zolotov and Shock* (2001)

Notes: Normalized, by number of atoms, relative to carbon. Numbers for Europa's ocean are based on the *Zolotov and Shock* (2001) 100-km bulk extraction ocean model with Fe-core. Nitrogen and phosphorous for Europa are scaled based on the abundance found in CV chondrites ("chond").

term is the chemical reaction equivalent of gravitational potential energy or electrical potential in circuits (*Krauskopf and Bird,* 1995). The change in Gibbs free energy per mole of a substance can largely be considered a change in energy level for a given phase relative to the initial state. The Gibbs free energy, G, is defined as

$$G \equiv H - TS \tag{1}$$

where H is the enthalpy, T is the temperature in Kelvin, and S is the entropy of the given state. Changes in enthalpy, where $H = E + PV$, account for changes in heat as a function of pressure P and volume V as well as internal energy. For exothermic reactions $\Delta H < 0$, while for endothermic reactions $\Delta H > 0$. In accord with the second law of thermodynamics, the entropy of the system plus its environment must always increase, until equilibrium is reached, at which point $dS = 0$ for the system considered. The change in Gibbs free energy is then (assuming constant temperature)

$$\Delta G = G_{\text{Products}} - G_{\text{Reactants}} = \Delta H - T\Delta S \tag{2}$$

where $\Delta G = 0$ at equilibrium. The value of ΔG (typically in kJ per mole reactant) provides a measure for predicting the tendency of compounds in a system to react. Negative ΔG for a given reaction indicates spontaneous progression of the reaction, while positive ΔG indicates that the reaction requires some input of energy into the system. It is not hard to show that a system with $\Delta G < 0$ and constant T corresponds to $\Delta S > 0$ for the system plus the environment, i.e., is entropically driven. Put another way, a negative ΔG indicates that the reaction can do work on the system (exergonic), while a positive ΔG requires an input of work (endergonic). This capacity for a reaction to do work, or yield free energy, is the difference between the total energy change and the waste heat. In terms of the first law of thermodynamics, this is $dW = dQ - dE$, where W, Q, and E refer to the work, heat, and energy respectively. It is important to note that while enthalpy is often referred to as "heat content" (indeed, this is consistent with the etymology), ΔH represents both waste heat and some energy to do work (e.g., the familiar $PdV = dW$ term in the differential of dH). Similarly, in a reversible process the $T\Delta S$ term of ΔG represents waste heat, ΔQ, but in practice almost all processes are irreversible, leading to $T\Delta S > \Delta Q$. All known biochemistry is critically dependent on this difference between waste heat and the energy capable of doing work in a given metabolic reaction. Variations of temperature, pressure, and concentrations of relevant chemical species can greatly alter this balance.

A related but distinctly different approach to Gibbs free energy illustrates this relationship. For a given reaction, with reactants X_n, products Y_n, and coefficients a_n and b_n,

$$a_1X_1 + a_2X_2 + \ldots a_nX_n \rightarrow b_1Y_1 + b_2Y_2 + \ldots b_nY_n \tag{3}$$

the equilibrium constant, K, can be found via

$$K = \prod_1^n [Y_m^{b_m}] \Big/ \prod_1^n [X_m^{a_m}] \tag{4}$$

Here brackets denote molal concentrations (mol kg^{-1} solution) of the reactants and products. The Gibbs free energy, can then be quantified by

$$\Delta G = \Delta G^\circ + RT\ln K \tag{5}$$

where R is the gas-law constant (8.314 J mol^{-1} K^{-1}), and ΔG° is the free energy as measured at a reference temperature and pressure (typically 25°C and 0.1 MPa, referred to as standard temperature and pressure, STP).

Two issues arise when addressing the limitations on life as imposed by the need for free energy. First, the availability of reactants, i.e., the left side of equation (3), is a hard constraint on ΔG. Without reactants, there is no reaction. Second, without the right set of reactants and the right set of conditions (e.g., temperature and pressure), the ΔG of the reaction may be insufficient for sustaining life. On Earth, the first limitation is largely solved by plate tectonics and geological cycling of elements important to life. Without this cycling, for instance, much of Earth's carbon would have been permanently sequestered into carbonates (*Kasting and Catling,* 2003). Also significant, especially in the context of icy moons with relatively low gravity, is that Earth does not lose much of its inventory of elements to space. Hydrogen escape is the only significant loss term. On Europa, loss of elements to space may be significant (see chapters by McGrath et al. and Johnson et al.).

The minimum ΔG needed for maintaining life has been a subject of considerable investigation, especially in recent years (*Heijnen and van Dijken,* 1992; *Hoelhler et al.,* 2001). This limit, sometimes referred to as the maintenance energy (ME), typically excludes the energy needed for growth and reproduction and focuses just on the energy needed for maintaining the organism. For the case of methanogenic *Archaea* utilizing the pathway

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \qquad (6)$$

measurements in anoxic sedimentary environments by *Hoehler et al.* (2001) put the limit for metabolizing H_2 at $\Delta G = -10.6 \pm 0.7$ kJ (mol CH_4)$^{-1}$ at 0.1 MPa and 22°C. Similarly they found that sulfate-reducing bacteria, utilizing the pathway

$$SO_4^{2-} + 4H_2 \rightarrow S^{2-} + 4H_2O \qquad (7)$$

could be supported by energy yields as low as $\Delta G = -19.1 \pm 1.7$ kJ (mol SO_4^{2-})$^{-1}$ at 0.1 MPa and 22°C. For aerobic heterotrophs, from nematodes to blue whales, the oxidation of hydrocarbons yields values of hundreds of kJ per mole for the net change in Gibbs free energy of the metabolic reaction.

The problem for Europa is that the ocean may be devoid of cycles that could maintain a chemical flux to drive equations (3), leading to what *Gaidos et al.* (1999) term "thermodynamics-driven extinction" and what *Chyba and Hand* (2001) have referred to as the "spectre of entropic death." Even if Europa were hydrothermally active, *Gaidos et al.* (1999) argued, the resulting chemistry would be dominated by reductants and little ΔG would be available from such reactions. Without a source of oxidants to Europa's ocean there will be no chemistry to drive equation (3) and the only free energy for life to harness would be through methanogenesis and sulfate reduction. Both of these metabolic pathways are limited by geological supply at the seafloor. Here we first review the case of chemical energy derived from low-temperature geochemistry in Europa's ocean; we then review the case for a hydrothermally active ocean; and finally we consider the energy available in an ocean that mixes with the icy shell and receives a flux of radiolytically produced oxidants from the surface. Figure 4, based on *Nealson* (1997) but adapted to the specific case of Europa, illustrates the limiting ΔG of oxidant and reductant (redox) pairs. Oxidants and reductants observed on the surface of Europa are shown in outlined boxes.

Zolotov and Shock (2003, 2004) considered the case of low-temperature geochemistry for Europa's ocean and found that biologically useful cycles of sulfur, carbon, and iron could persist provided new rock was periodically made available on the ocean floor. In the model of *Zolotov and Shock* (2003), quenched hydrothermal fluids rich in H_2, CH_4, and/or organics couple with dissolved sulfate to yield

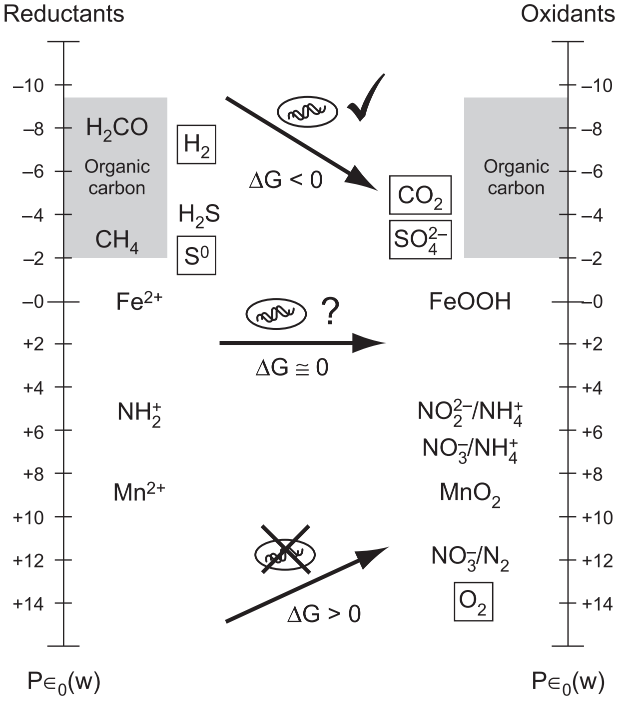

Fig. 4. Gibbs free energy and the energetic limits of life at Europa. Here we show several metabolic pathways that can, and cannot, support life. Life extracts energy from the environment in order to do the work of growth, reproduction, and maintenance. If a given reaction yields a sufficient negative change in Gibbs free energy, then life might be able to utilize that reaction (e.g., the microbe symbol marked with a check sign). The minimum negative change in Gibbs free energy required for life (microbe symbol with a question mark) is a topic of considerable study (see text). Pairing of reductants and oxidants that do not yield a negative change in Gibbs free energy are incapable of supporting life (e.g., the microbe symbol marked "X" for which the change in Gibbs free energy is positive). Reductants and oxidants in outlined boxes have been detected on Europa. Compounds are aligned along a relative redox scale. $P\epsilon_0$ is the negative logarithm of the electron activity. Highly electronegative compounds are plotted at the top of each scale, and the most electropositive compounds are at the bottom. Adapted from *Nealson* (1997).

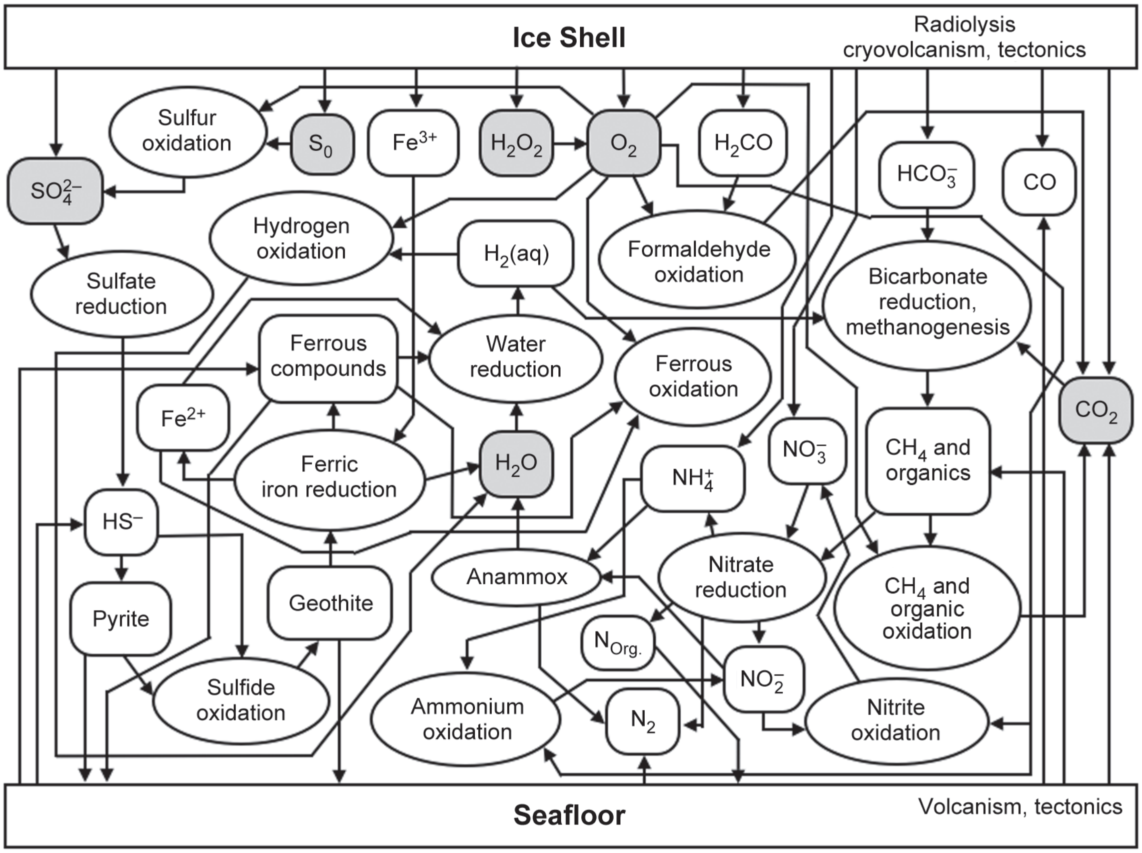

Fig. 5. Possible biogeochemical cycles and energetic niches on Europa. Molecular species are shown in rectangles, and reactions — either abiotic or metabolically mediated — are shown in ovals. Most of the reaction niches indicated are known metabolic pathways for terrestrial microbes. However, direct oxidation of ferrous iron by water, sulfate, or bicarbonate, and oxidation of bisulfide (HS^-) by water, have no known microbial analog. Cycling of surface material and seafloor material would help to maintain oceanic biogeochemical pathways. Compounds in gray have been observed on the europan surface. The diagram is based on Fig. 9 of *Zolotov and Shock* (2004), but here surface oxidants are added.

biologically useful energy. This is comparable to known sulfate-reducing microbial ecosystems in anoxic environments on Earth (*D'Hondt et al.,* 2002; *Amend and Shock,* 2001). Example pathways are

$$SO_4^{2-} + H^+ + 4H_2(aq) \Rightarrow HS^- + 4H_2O(l) \qquad (8)$$

$$SO_4^{2-} + 2H^+ + 4H_2(aq) \Rightarrow H_2S(aq) + 4H_2O(l) \qquad (9)$$

The equilibrium constant at estimated values for T and P of the europan seafloor (T = 273 K, P = 137.5 MPa) are $\log_{10}K = 13.22 – 8720T^{-1} + 6.54 \times 10^6T^{-2} – 6.77 \times 10^8T^{-3}$, and $\log_{10}K = 34.36 – 24174T^{-1} + 1.17 \times 10^7T^{-2} – 1.21 \times 10^9T^{-3}$. Under these conditions, the change in Gibbs free energy for the reaction in equation (9) is –263 kJ mol^{-1} (*Zolotov and Shock,* 2003). The low temperature serves to inhibit the abiotic reduction of sulfate, allowing for biological mediation of the reaction. Based solely on estimates for sulfate and hydrogen available in the original hydrothermal fluid after the formation of Europa, *Zolotov and Shock* (2003) calculate that sulfate reduction in a 100-km-deep ocean could yield a total of ~10^{24} J. This corresponds to ~10^{16} kg of total biomass throughout the ocean and over the history of the ocean (*Heijnen and Dijken,* 1992; *McCollom,* 1999). By comparison, terrestrial primary productivity, which is dominated by photosynthesis, yields ~10^{14} kg yr^{-1} (*Field et al.,* 1998; *McCollom,* 1999). Ultimately the above pathways are limited by geological activity at the seafloor supplying H_2, CH_4 and organics to the ocean. A sulfate and carbon flux from the icy shell would serve to maintain a cycle of sulfate reduction on a hydrothermally active Europa.

Considering the combined geochemistry of sulfur, iron, and carbon, in a cold, mildly alkaline europan ocean, *Zolotov and Shock* (2004) examined a host of possible metabolic pathways and biogeochemical cycles that could provide chemical energy for a subsurface biosphere. Figure 5 provides a summary of some of these cycles and possible energetic niches for life. Almost all the niches identified map to metabolic pathways for known terrestrial microbes, but several reactions — including direct oxidation of ferrous iron (dissolved or mineralized) by water, sulfate, or bicarbonate, and oxidation of bisulfide (HS^-) by water — have no known microbial analog. Maintaining the cycles identified by *Zolotov and Shock* (2004) into the present requires periodic delivery of new mantle rock or aqueous fluids from the seafloor.

McCollom (1999) considered a hydrothermally active Europa and used methanogenesis (equation (6)) as a model pathway for assessing biomass production. Both a reduced and oxidized ocean were considered. In the reduced model, CH_4 and H_2S dominate, whereas in the oxidized ocean model SO_4^{2-} and HCO_3^- are in abundance. Carbon dioxide and hydrogen released from fluid-rock interactions were taken to be the sources of oxidants and reductants, and McCollom notes that CO_2 in terrestrial vent fluids is largely derived from leaching of magmatic gases, or from interaction with gases exsolved as the underlying magma chamber solidifies, i.e., it is new carbon, not recycled HCO_3^-. Important to the thermodynamics of both McCollom and *Zolotov and Shock* (2004), abiotic reduction of CO_2 is very slow at the low temperatures expected for Europa's ocean (<300 K). The range of energy available per kilogram of vent fluid in the oxidized ocean and reduced ocean was found to be 250–500 J, capable of yielding ~6–13 mg of dry weight biomass per kg of fluid, based on terrestrial microbial analogs (*McCollom,* 1999; *Heijnen and Dijken,* 1992). Taking Europa's contemporary hydrothermal flux to be 10^{-3} that of Earth's annual 3×10^{13} kg of hydrothermal fluid, *McCollom* (1999) estimates an annual europan biomass productivity of 10^5–10^6 kg yr^{-1}, or about 10^{-5} that of terrestrial hydrothermal biomass productivity. Integrated over 4 G.y., this is comparable to the results of *Zolotov and Shock* (2003) for sulfate reduction.

Cycling of ocean water with the seafloor crust is an important consideration for the supply of reductants on Europa, but the total energy available is greatly limited by the availability of oxidants. Cycling of the ocean with the icy shell may, however, introduce oxidants such as sulfate, carbonic acid, O_2, and H_2O_2 into the ocean, yielding a solution with high chemical potential. The production of oxidants at the surface results from the radiolytic processing of the surface ice by the 7×10^{15} eV cm^{-2} flux of energetic particles, the majority of which are high-energy electrons (*Cooper et al.,* 2001; see chapter by Paranicas et al.).

Using the hydrogen peroxide surface abundance of 0.13% by number relative to water, as constrained by observations from Galileo NIMS (*Carlson et al.,* 1999a), *Chyba* (2000) and *Chyba and Phillips* (2001) examined the possible delivery of hydrogen peroxide to the ocean in the context of habitability. If the surface is gardened (i.e., mixed by meteorites and micrometeorites) to an average depth of 1.3 m over 10^7 yr (*Cooper et al.,* 2001), and the gardened layer contains the above peroxide concentration throughout, then ~4×10^{16} moles of H_2O_2 will be available in the surface reservoir. *Chyba and Phillips* (2001) argue that the resurfacing of Europa could deliver this reservoir to the subsurface ocean, where it would rapidly (~10 yr) decay to O_2 and serve as a useful oxidant for metabolism. Gardening depths may be one to two orders of magnitude lower (see chapter by Moore et al.) and resurfacing rates an order of magnitude higher (*Zahnle et al.,* 2008), leading to delivery of ~10^6–10^7 moles per year of H_2O_2. *Chyba and Phillips* (2001) estimate that radiolysis of CO_2 and CO in ice would yield a flux of ~3×10^5 moles per year of the biologically useful reductant formaldehyde (H_2CO), although that compound was not observed by NIMS. Again, this estimate is decreased by two orders of magnitude if one assumes both shallow gardening and longer resurfacing periods. The calculated abundance of H_2CO is consistent with laboratory measurements of electron radiolysis of CO_2-enriched ice (*Hand et al.,* 2007).

Based on the high-end fluxes, and using the metabolism of the soil bacterium *Hyphomicrobium* as an example,

$$H_2CO + O_2 \rightarrow H_2O + CO_2 \tag{10}$$

Chyba and Phillips (2001) estimated that delivery of radiolytically produced oxidants (H_2O_2) and reductants (H_2CO) from the surface could alone support an ecosystem of ~10^{23}–10^{24} microbial cells, or ~10^6–10^7 kg of steady-state biomass. If the above reaction were not limited by the availability of H_2CO, then steady-state biomass could reach ~4×10^{11} kg. Considering the gardening and resurfacing uncertainty, steady-state biomass utilizing H_2O_2 as an oxidant ranges from ~10^9 to 10^{11} kg.

Considering only the observed peroxide concentration underestimates the total oxidant abundance on the surface of Europa, and consequently, the possible flux to the ocean. *Cooper et al.* (2001) and *Chyba and Hand* (2001) calculated that radiolytically produced O_2 on the europan surface could lead to combined oxidant fluxes ($H_2O_2 + O_2$) as high as 10^{12} moles per year. Coupled with this is the decay of ^{40}K (half-life = 1.25 G.y.) in both the icy shell and ocean. Scaling potassium concentrations from estimates for salt concentrations given in *Kargel et al.* (2000), *McCord et al.* (1999), and *Fredriksson and Kerridge* (1988), *Chyba and Hand* (2001) find that O_2 produced via ^{40}K decay in a 10-km icy shell could yield 10^7–10^8 mol O_2 yr^{-1}, while that in a 100-km-deep ocean would yield 10^{10} mol O_2 yr^{-1} in the contemporary ocean, and 10 times as much over 4.2 G.y.

Groundbased observations by *Spencer and Calvin* (2002) of absorption at 577.1 nm on Europa indicate solid-phase O_2 trapped in the ice. The 577.1-nm absorption results from interacting pairs of O_2 molecules. Using this quadratic dependence, *Hand et al.* (2006) modeled the radiative transfer of the absorption to calculate O_2 abundances as high as 1.3–4.6% by number relative to water. The lower limit is derived from considering water ice with O_2 as the only non-ice component, while the upper estimate allows for absorption by other compounds in the ice. In either case, the O_2 abundance in the surface ice is more than an order of magnitude greater than the observed hydrogen peroxide (see chapter by Carlson et al.).

Delivery of this much oxygen to the ocean, even at periods of hundred of millions of years, will serve to oxidize a geologically inactive ocean (*Hand et al.,* 2007). If the seafloor is active, then the redox coupling of O_2 and SO_4^{2-}

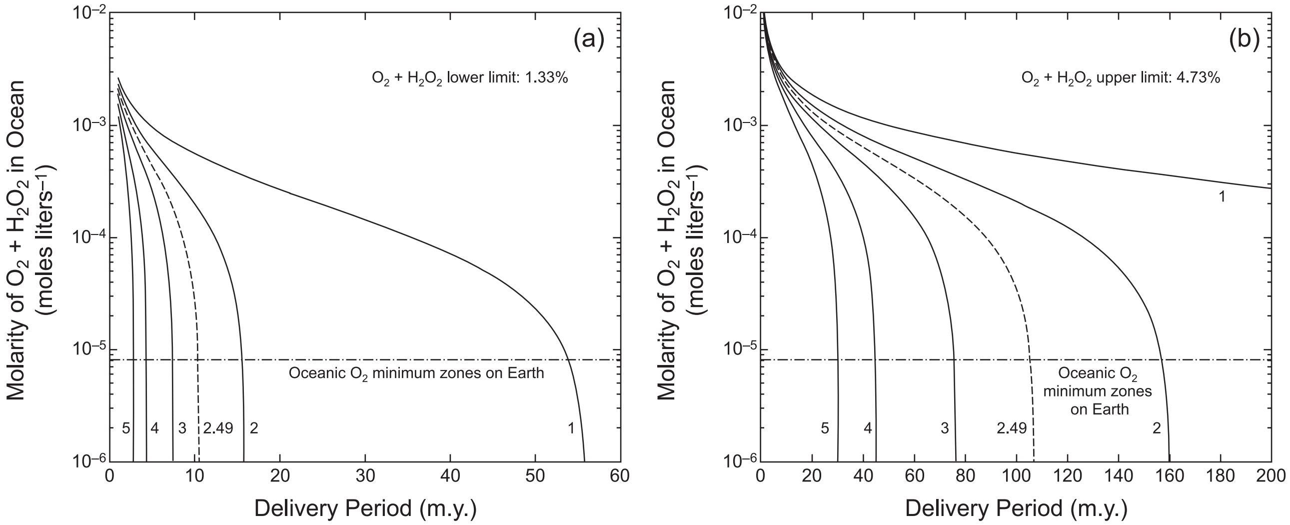

Fig. 6. Molarity of O_2 and H_2O_2 (which rapidly becomes O_2) in Europa's ocean as a function of the time it takes for radiolytically produced surface material to reach the ocean (delivery period). The quantity of O_2 and H_2O_2 delivered accounts for surface gardening (see text) **(a)** for a radiolytically produced surface abundance of 1.33% by number relative to water, and **(b)** for 4.73% by number abundance. In each case, a flux of seafloor reductants is assumed, and the net molarity of O_2 and H_2O_2 in the ocean (assumed 100 km deep) is what is left after reaction with the seafloor reductants. Solid contours, from left to right are for 5, 4, 3, 2, and 1 × 10^9 moles per year of reductants. Dashed line is for 2.49 × 10^9 moles per year, corresponding to the seafloor activity estimate of *McCollom* (1999). The molarity of Earth's oceanic O_2 minimum zones (dash-dotted line) is shown for reference. For a lower O_2 and H_2O_2 concentration **(a)**, a shorter delivery period or a lower reductant flux are required in order for the molarity to exceed that of Earth's O_2 minimum zones. For higher O_2 and H_2O_2 concentration **(b)**, the molarity exceeds that of Earth's minimum zones even for long delivery periods and high reductants fluxes. Sulfate concentrations exceed the O_2 and H_2O_2 by at least an order of magnitude; thus, achieving an oxidized ocean with sulfate is considerably easier than with O_2 and H_2O_2.

from the icy shell and H_2, H_2S, Fe^{2+}, and CH_4 from the seafloor would be a useful energetic resource in the context of habitability. Abiotic oxidation would precipitate magnetite and pyrite, but even for terrestrial banded iron formations biological mediation of these products is believed to be significant (*Canfield,* 1998).

In Fig. 6, adapted from *Hand et al.* (2007), the molarity of O_2 in Europa's ocean is plotted as a function of delivery period, where the delivery period, τ_d, is defined as the average time it takes for material on the surface of Europa to reach the subsurface ocean. In the work of *Chyba* (2000), *Chyba and Phillips* (2001), and *Chyba and Hand* (2001) the delivery period was taken to be roughly equivalent to the observed surface age, t_{ice}, of tens of millions of years (*Zahnle et al.,* 2003, 2008; see chapter by Bierhaus et al.). This was a reasonable assumption, but other alternatives are possible. The molarity of oceanic O_2 was determined by calculating the number of moles delivered to the ocean per year, η_i, for a given delivery period

$$\eta_i = \frac{A d_g \varepsilon_i}{\tau_d} \qquad (11)$$

This flux is a function of A, the surface area of Europa, the gardening depth, d_g, to which surface materials are buried, and ε_i, the concentration of each compound throughout the gardened layer (in units of moles per cubic meter). Here the gardening depth has been calculated based on *Cooper et al.* (2001), but recent work summarized in the chapter by Moore et al. suggests that the gardening depth could be reduced by one to two orders of magnitude. From this flux we have then subtracted a sink term based on the hydrothermal flux of ~10^9 moles per year of reductants as calculated by *McCollom* (1999). By subtracting this sink term we account for a rough estimate of abiotic sinks for oxidants in the ocean. The lines shown in Fig. 6 correspond to sink terms of 1, 2, 3, 4, and 5 × 10^9 mol yr^{-1} reductants. The dashed line for 2.49 × 10^9 mol yr^{-1} is the oxidant consumption based on the reductant flux estimated by *McCollom* (1999). The resulting oceanic molarity is then the net accumulation of O_2 after accounting for hydrothermal sinks. Figure 6a shows the results based on a percent by number abundance of O_2 and H_2O_2 of 1.33%, and Fig. 6b shows the same calculations based on a 4.73% by number abundance. The dotted horizontal line shows the molarity of O_2 in the most oxygen-depleted regions of Earth's ocean. Even in these energetically challenging environments on Earth, multicellular creatures are found, such as the 3.5–12.5-cm-long crustacean *Gnathophausia ingens* (*Childress,* 1968). For the lower limit oxygen concentration in Europa's ice (Fig. 6a), the molarity of O_2 in the ocean exceeds terrestrial O_2 minima levels if the delivery period is <54 m.y. and the sink term is 1 × 10^9 moles per year. If the sink term is doubled, then the delivery period must be <16 m.y. if O_2 molarity is to surpass the minima level. These values for the delivery period are consistent with published values for the surface age (*Zahnle et al.,* 2003). For a sink term of 5 ×

10^9 mol yr^{-1} the delivery period would have to be less than 2.8 m.y. in order to surpass the O_2 minima level. This is clearly much younger than the surface age and thus unlikely.

For the case of $O_2 + H_2O_2$ concentrations of 4.73% by number in the ice, the delivery period can be significantly longer while still achieving an oceanic O_2 molarity suitable for terrestrial macrofauna. With the 5×10^9 mol yr^{-1} sink term subtracted from the oxidant flux, the delivery period need only be <30 m.y. in order to maintain an ocean above terrestrial O_2 minima zone levels. If the reductant sink is decreased, e.g., to 2×10^9 moles per year, then delivery periods can be as long as 160 m.y. and still achieve minima levels. Decreasing the sink term even more allows an O_2-rich ocean to be maintained even with delivery periods of several hundred million years. The delivery periods in the case of a 4.73% by number $O_2 + H_2O_2$ concentrations can greatly exceed the surface age of Europa and still achieve an ocean with high levels of oxygen. We note here that much of our terrestrial ocean is supersaturated with O_2, reaching levels of ~250 μM or roughly 3% supersaturation (*Millero,* 2005). The temperature and pressure environment of Europa's ocean will permit levels of dissolved O_2 in the range of 50 mM (*Lipenkov and Istomin,* 2001). Depending on the delivery period and sink term for oxidants, it is possible that oxygen levels in Europa's ocean could greatly exceed those found in Earth's ocean. While *Gaidos et al.* (1999) argued that habitability may be hindered by limited availability of oxidants, it is possible that the ocean could be highly oxidized with sulfate, oxygen, and hydrogen peroxide if there is even moderate mixing of the ocean with surface material. If this is the case, habitability could actually be inhibited by an overabundance of oxidants. While terrestrial macrofauna have evolved mechanisms to accommodate our oxygen-rich atmosphere, the origin of life on Europa may have been severely compromised if such an oxidant-rich ocean persisted since the formation of Europa's ocean.

1.2.3. Photosynthesis as a possibility. Photosynthesis cannot strictly be ruled out for Europa (*Reynolds et al.,* 1983; *Greenberg et al.,* 2000). Nevertheless, it is considered an unlikely metabolic pathway because the average thickness of the icy shell is generally thought to be at least several hundred meters in thickness (*Carr et al.,* 1998; *Pappalardo et al.,* 1998; *Greenberg et al.,* 2000), and light cannot penetrate this deeply in ice.

The equatorial daytime flux of photosynthetically active radiation (PAR, 375–725 nm) is ~21.87 W m^{-2} (~100 μmol photons m^{-2} s^{-1}) at Europa. For light of wavelength λ and incident intensity I_0, the intensity at depth x is

$$I(x) = AI_0e^{-ax} \qquad (12)$$

where A is the local albedo of Europa and a is the absorption coefficient at wavelength λ (*Hobbs,* 1974). With A = 0.67, I_0 = 21.87 W m^{-2}, and a = 8×10^{-4} cm^{-1} for visible light through clear ice with no bubbles (*Hobbs,* 1974), the maximum penetration depth is ~100 m. Adding bubbles and frost to the ice increases a to ~2.8×10^{-4} m^{-1} and reduces the penetration depth to a few meters. Unless regions of the icy shell are thinner than these values, photosynthesis will not be possible.

On Earth, photosynthesis by phytoplankton has been measured beneath up to 7 m of ice at photon flux levels near 0.1% of the incident surface irradiance (~1 μmol photon m^{-2} s^{-1}) in lakes of the McMurdo Dry Valleys, Antarctica (*Priscu,* 1995). Similarly, adequate light penetrates the 3–4-m-thick ice cover of Lake Vanda (McMurdo Dry Valleys) and penetrates the highly transparent waters (extinction coefficient ~0.033 m^{-1}) permitting photosynthesis to depths of ~65 m beneath the ice surface (*Howard-Williams et al.,* 1998). Clearly, viable terrestrial ecosystems demonstrate that photosynthesis can occur at considerable depths beneath ice. In addition to relatively high penetration of PAR, UV radiation can also be transmitted through the ice covers on Antarctic lakes. *Vincent et al.* (1998) found that UV penetration through the ice cover of Lake Vanda can be an impediment to photosynthetic productivity in the upper regions of the liquid water column. The ice covers of Antarctic lakes can themselves harbor an active ecosystem fueled by photosynthesis, providing an oasis for life in what would otherwise seem to be an inhospitable environment (*Priscu et al.,* 1998; *Paerl and Priscu,* 1998; *Priscu et al.,* 2005a).

Reynolds et al. (1983) approached the problem of access to sunlight on Europa by considering crack formation and refreezing. *Buck et al.* (2002) reconsidered this problem and incorporated models for tidal heating of the ice. While formation of the initial surface layer of ice occurs rapidly, the ice growth rate slows as the ice becomes thicker. This allows for periods of days to years during which light may reach the liquid water interface. Using an annual resurfacing area of 5 km^2 based on frost deposition rates from *Squyres et al.* (1983), *Reynolds et al.* (1983) found that 2×10^{15} J yr^{-1} would be available for photosynthesis in the 375–725-nm range. This leads to a total equilibrium biomass (for photosynthesis only) throughout Europa of ~10^4–10^5 kg, or roughly one-billionth of Earth's annual photosynthetic productivity (*Field et al.,* 1998). We note that in the *Reynolds et al.* (1983) model the influence of the crack sidewalls was neglected in the refreezing calculations. This effect would decrease the total power available for photosynthesis by a factor of ~2, depending on the ratio of crack width to depth.

Despite the thickness of the icy shell being an impediment to the emergence of photosynthesis as a metabolic pathway, several aspects of such an ecosystem warrant closer inspection in the context of future robotic exploration. First, the selection effects for life detection on orbiter or lander missions would be biased toward detecting near-surface life utilizing pigments easily detectable with spectroscopy. Photosynthetic organisms satisfy both these conditions. Their need for sunlight would lead them to concentrate in any regions where the ice is thin and thus likely young in age. Second, the molecular pigments used for photosynthesis (e.g., chlorophyll-a, b, c, and various carotenoids and xan-

TABLE 2. The limits for growth and reproduction of life on Earth.

Parameter	Limits	Description
Temperature	–20°C to 121°C	Limits correspond to life in ice veins to hydrothermal systems.
Pressure	0 to 1680 MPa	Spore formation permits survival at low pressures. *Escherichia coli* and *Shewanella oneidensis* can remain metabolically active for ~1 day at the high end limit.
pH	–0.6 to >11	Possible metabolic activity at levels as high as 12.5–13 have been reported.
Salinity	0 to near salt saturation	Upper limit is ~260 g of $MgSO_4$ per kg of water, and to nearly 300 g NaCl per kg of water.
Water activity	>0.6	Survival at activities of zero are possible by forming spores. Unpublished results suggest that metabolically active populations may be possible for activities lower than 0.6.
Ionizing radiation	0 to 6000 Gy	Upper limit corresponds to *D. radiodurans*, which needs to actively repair its DNA. Limits of several tens of Gy (J kg^{-1}) are more typical of bacteria like *E. coli* and *B. subtilis*.

Notes: These limits apply to various types of *Bacteria* and *Archaea*; *Eukaryotes* can only tolerate a subset of the conditions listed. Based on *Nealson* (1997), *Rothschild and Mancinelli* (2001), and *Marion et al.* (2003).

thophylls, in the case of Earth organisms) are evolution's gift to spectroscopists. They are molecules tuned for optimal absorption over a set bandpass. Chlorophyll-a, for example, absorbs sharply at ~400 and 660 nm and is easily detectable from Earth orbit. Chlorophyll-a also has unique fluorescence properties and fluoresces at 683 nm when excited with light near 400 nm. These selection effects for detection of life on Europa imply that while photosynthesis may occupy an unlikely or small niche on Europa, such an ecosystem may satisfy a disproportionately large solution space for our detection techniques. We discuss these issues in greater detail in section 3.

1.2.4. Earth ecosystems as an analog for Europa? Is Europa's ocean an "extreme" environment relative to Earth's ocean? In this section, we begin by considering temperature, pressure, and salinity, finding that Europa's ocean is probably well within the known limits of life. Then we consider some possible extreme terrestrial environmental analogs to Europa.

Europa's ocean is not a particularly extreme environment, in terms of temperature and pressure, at least. The temperature of the water may be suppressed to ~260 K (–13°C) by the dissolution of salts, but it cannot go much lower and still remain liquid (*Kargel et al.,* 2000; *Marion et al.,* 2003; *Melosh et al.,* 2004). In the eutectic limit with 213 g of chlorine per kg H_2O, the freezing point could get considerably lower [238.65 K at 0.1 MPa (*Marion et al.,* 2003)], but this would require ~6 × 10^{20} kg of chlorine globally, a value hard to reconcile with a chondritic origin for Europa (Cl is present at 700 ppm in CI chondrites) (*Kargel et al.,* 2000; *Lodders and Fegley,* 1998).

The pressure environment within Europa is also not that extreme compared to terrestrial ecosystems. Despite possibly harboring a deeper ocean than that of the Earth, ~100 km on Europa vs. a 4-km average and 11-km maximum depth on the Earth, the pressures are not that great because the gravitational acceleration, g, on Europa is less than one-seventh that of our considerably more massive Earth. For a given depth, d, in kilometers below the surface of Europa, the pressure in MPa is ~1.3 d, whereas on Earth it is ~10 d; therefore, the 110 MPa pressures in the 11-km Marinas Trench on Earth are comparable to the seafloor of a ~100-km icy shell plus ocean on Europa. Many large and complex life forms are found on Earth at the average seafloor depth of 4 km (*Van Dover,* 2000), corresponding to a comparable pressure at a depth of roughly 30 km on Europa. In the sediments of the Marinas Trench and other deep regions of our ocean, soft-walled foraminiferans have been found to flourish (*Gooday et al.,* 2004; *Todo et al.,* 2005). The pressures expected throughout the ocean on Europa are not a challenge for life as we know it.

Aside from a supply of chemical energy, life in the ocean of Europa could be most challenged by the salinity. Table 2 provides a summary of the limits for growth and reproduction of life on Earth based on *Nealson* (1997), *Rothchild and Mancinelli* (2001), and *Marion et al.* (2003). Although still highly uncertain, estimates for Europa ocean salinity range from a few tens of grams of salt per kilogram H_2O to saturation with magnesium sulfate or sodium chloride (*Kargel et al.,* 2000; *Zolotov and Shock,* 2001; *Hand and Chyba,* 2007). Even so, terrestrial halophiles, such as the archaean *Haloferax volcanii* can survive in near-saturated solutions of ~240 g $MgSO_4$ per kg H_2O, when no other ions are present (*Oren,* 1994). Methanogens are capable of surviving in solutions of up to 100–150 g NaCl per kg H_2O (*Oren,* 2001). Water activity is a measure of the availability of water, which, when considering surface water, is simply the water fugacity, and is often invoked as a limitation for life. While brine pockets and cracks in the icy shell are likely to have low water activities, a global ocean saturated with salt will still have an activity of 0.72 or higher and would be suitable for several terrestrial microbes (*Siegel,* 1979; *Nealson,* 1997; *Marion et al.,* 2003).

There are no perfect terrestrial ecosystem analogs for what we might expect to find on Europa. Hydrothermal vent ecosystems are often invoked for comparison. This is certainly a legitimate analogy in that physical conditions (temperature, pressure, lack of light) and chemical conditions (sulfur, iron, methane) could be similar, but the chemistry of the surrounding ambient water remains unknown. Whether deep ocean material mixes with shallower waters on Europa, thereby possibly bringing hydrothermal materials upward toward the ice, is also highly uncertain. In the context of exploration, the bottom of Europa's ocean is arguably the most inaccessible part of Europa's potentially habitable ocean. Spacecraft landers many decades from now may carry submersibles capable of interrogating the seafloor, but for the near-term exploration of Europa our search will be largely restricted to the surface and the ice-shell/ocean interface. The habitable volume of the ocean accessible to these searches will, at best, be the upper few kilometers of water. Considering the habitability of this region of the ocean, the liquid water environments of the recently discovered subglacial lakes in Antarctica provide some intriguing examples of how life on Earth has evolved and adapted to comparable cold and dark environments (*Priscu et al.,* 2005b, 2008).

Direct sampling of the liquid water from Antarctica's Lake Vostok has not yet occurred, but data from the region of the ice core just a few tens of meters above the lake indicate that the lake likely harbors life (*Priscu et al.,* 1999; *Karl et al.,* 1999). This region of the ice, approximately 240 m in thickness, which lies below a depth of 3590 m beneath the ice sheet surface, is accreted ice, so called because it is ice that forms from the lake water and accretes on the bottom of the East Antarctic ice sheet. Cell counts from this ice reveal microbial concentrations of 83–260 cells ml^{-1} (*Priscu et al.,* 1999; *Christner et al.,* 2006), which, based on estimated partitioning coefficients, equates to cell concentrations in the surface waters of Lake Vostok ranging from 150 to 460 cell ml^{-1} using epifluorescence microscopy of DNA stained samples (*Christner et al.,* 2006), and between 10^5 and 10^6 cell ml^{-1} using scanning electron microscopy (*Priscu et al.,* 1999).

Phylogenetic analysis of the 16S rRNA gene sequences of these cells places them among the δ-, γ-, and β-proteobacteria (*Christner et al.,* 2006), leading to the conclusion that the surface waters of Lake Vostok harbor a microbial consortium consisting of chemotrophs and heterotrophs. The Vostok γ-proteobacteria are likely chemolithotrophs harnessing energy from Fe^{2+} oxidation, or reduction of Fe^{3+} and elemental sulfur (S°) with hydrogen. Coupling of S° with Fe^{3+} may also be an energetically useful pathway for these microbes. The Vostok δ-proteobacteria are likely chemotrophic anaerobes yielding energy from reduction of sulfate, S°, Fe^{3+}, and Mn^{4+}. Finally, the Vostok β-proteobacteria appear to be heterotrophs utilizing single carbon compounds such as methanol, formate, and carbon monoxide. The Vostok ecosystem therefore derives much of its energy from redox couples associated with iron and sulfur within the subglacial environment (*Christner et al.,* 2006; *Priscu et al.,* 2006). Dissolved organic carbon, CO_2, N_2, O_2, and other minerals are also advected into the system from the overlying ice sheet as it melts.

Our understanding of the Vostok ecosystem is limited to the accretion ice, and by inference the surface waters. Very little is known about deep-water column processes and the degree to which the deep waters of Vostok mix with the surface waters. Based on comparison with lakes in the McMurdo Dry Valleys, the deep waters of Vostok (below perhaps 400 m) may be a bit saltier than the surface waters and may even be warmer owing to geothermal heating. These waters may also be anaerobic, depending on the gas flux from the melting ice and geothermal sources as well as pos-sible *in situ* respiration. The sediments would be an additional source of minerals and carbon to drive life in the deeper waters. The fact that the accreted ice serves as our only window into the ecosystem of Vostok is perhaps useful for the comparison to Europa. Over the next few decades, our search for life on and within Europa will be limited to the surface ice and, in an optimistic scenario, the water near the ice-water interface. Thus, while sedimentary and hydrothermal ecosystems are interesting in the context of Europa, it is really the near-surface water environments of subglacial lakes that provide the best comparison for what waters we might expect to encounter first with robotic spacecraft. Information on deep convective mixing of this surface layer would also provide information on processes that occur deeper within the water column.

Microbes are also found in veins and brine pockets within the Antarctic ice sheet. Although small — a typical vein might only be 10 µm in diameter — such environments are plenty spacious for a 1-µm-diameter bacterium. Additionally, even in the coldest regions of the ice sheet, thin films of water can exist at mineral or ice surfaces and only approximately three monolayers of water are needed to support metabolism. Given the presence of liquid water and biogenic elements in subglacial lakes and brine pocket veins of the ice sheet, what can be said about the energetic limitations of life in these environments? Based on models by *Jepsen et al.* (2007), most of the available metabolic energy is used for cellular maintenance. This may also be the case for organisms in the upper portion of the ice sheet where temperatures are <–20°C. However, in the deeper portion of the ice sheet, veins are larger and temperatures warm to ~–3°C, which is arguably a clement environment for life. Based on the increase in bacterial density observed in accretion ice, relative to the overlying glacial ice and in concert with the stable and relatively warm liquid water body thought to exist in Vostok, bacteria should be doubling once every 10 to 60 days in these veins.

Metazoan populations (multicellular organisms) may not be present in the Antarctic ice ecosystems. This is consistent with the following ecosystem constraints: (1) not enough

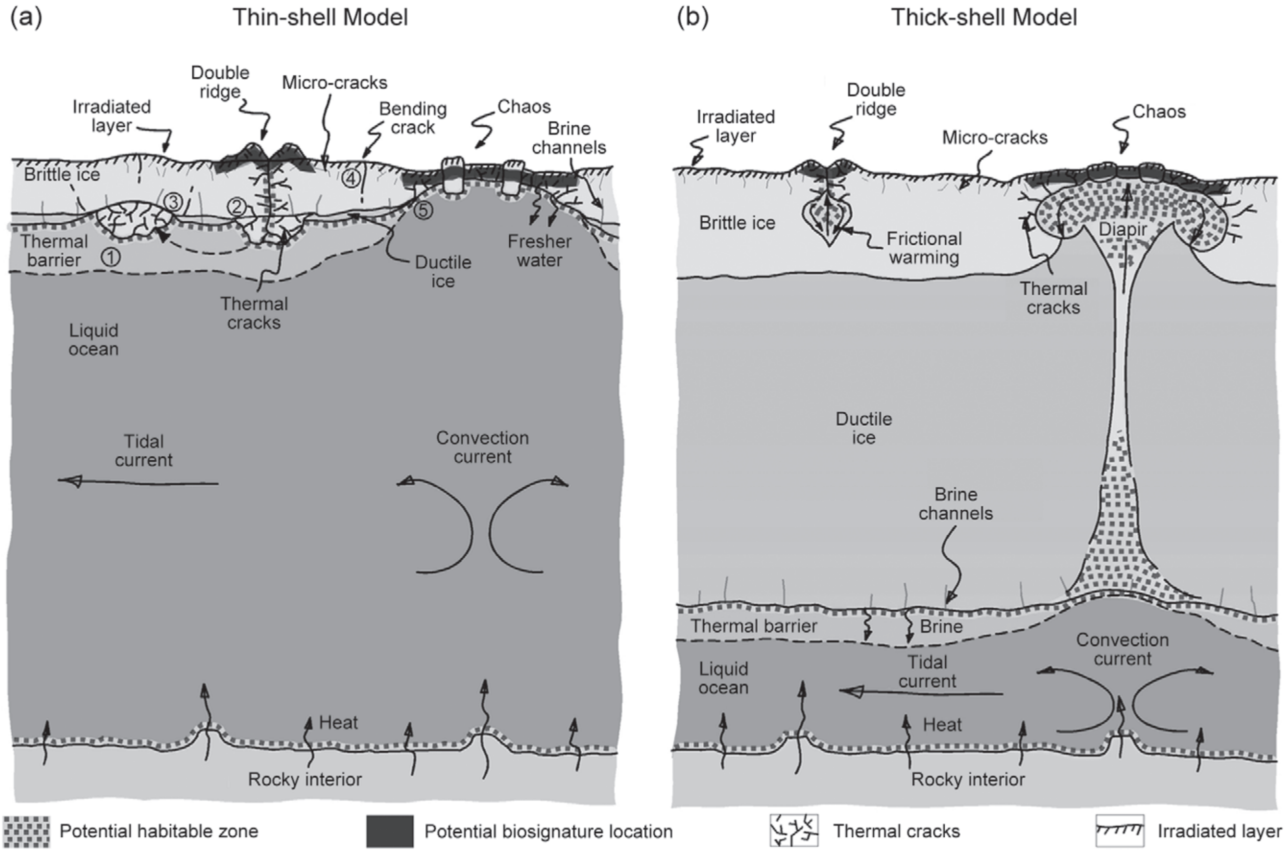

Fig. 7. Schematic cross-sections of Europa's icy shell, ocean, and seafloor showing possible habitats and biosignatures (from *Figueredo et al.,* 2003). **(a)** Thin ice-shell and **(b)** thick ice-shell models are shown for comparison, but in both cases, the regions expected to be most conducive to life are the chemically rich interfaces at the seafloor-ocean boundary and the ice-ocean boundary. Regions within the icy shell may also be habitable. The numbers given in the thin-shell model indicate the following specific features: (1) a sub-ice mixing layer, in which materials are exchanged between the ice and ocean; (2) a crushed-ice "keel," corresponding with isostatic support for surface ridges; (3) migration of subsurface ice material to other regions, causing subsequent upwarping; (4) cracks produced by bending of the icy shell; and (5) cracks resulting from thermal effects at the ice-water interface. In the thick-shell model, convection and the resulting diapirs and brine channels could provide habitable niches. Surface regions where biosignatures may be expressed are also shown.

energy in the primary food base to support higher trophic levels (energy transfer up food webs is not very efficient) and (2) metazoans (eukaryotes for that matter) contain a complex membrane network associated with organelles as well as the plasma membrane. These membranes are made of a mosaic of phospholipid bilayers interspersed with functional proteins (there is often a complex outer membrane matrix as well). Cold temperatures can change the fluidity of the membranes, rendering them metabolically useless (e.g., protonmotive forces like that used to produce ATP can come to a halt at cold temperatures). Finally, ice crystals can form in the membranes, ruining their integrity. Prokaryotic organisms are more capable of surviving freeze/thaw processes and typically dominate icy ecosystems on Earth owing to their paucity of membranes and high metabolic diversity relative to eukaryotic organisms, which can be considered structurally diverse (by virtue of their membrane bound organelles) and metabolically conservative.

The habitability of Europa's ocean will depend on the degree to which, and frequency with which, the ocean water cycles through the mantle rock of the ocean floor and with ice in the icy shell. Cycling through the ocean floor yields a solution rich in reductants while cycling with the icy shell introduces oxidants, both of which are needed to power life. Based on the need for energy, liquid water, and a suite of biologically essential elements, the prime habitats for life on Europa (were life to exist) are likely to be at the seafloor-ocean interface and at the ice-ocean interface. Here chemical energy and useful compounds and elements may combine with liquid water to provide the conditions needed for life. Figure 7, adapted from *Figueredo et al.* (2003), shows a cross section of possible habitats, and their surface expression, on Europa. *Lipps and Rieboldt* (2005) provide a detailed classification and taphonomic analysis of such possible habitats. Ongoing work in the polar regions of the Earth, and in particular in subglacial sulfur-rich springs (*Grasby et al.,* 2003), will help guide our understanding of life in ice and the chemistry and conditions needed to sustain life in such regions.

The availability of chemical energy, as discussed above, addressed the capacity of Europa's ocean to support life, but had little bearing on the issue of life's origin. In that context, a reduced ocean may be highly preferable. We explore our current understanding of life's origin, and the implications for Europa, in the next section.

1.3. Constraints on Origins

The origin of life on Earth remains one of the great, unanswered scientific questions. Lacking a complete understanding of how our own tree of life came to be, we cannot read too much into the chemistry of Europa and possible origins on that world. Nevertheless, the question of life's origin is precisely why Europa is such a prime astrobiological target — it poses a testing ground for a second, independent origin of life in our solar system. Our understanding of terrestrial prebiotic chemistry indicates that life may have also arisen on Europa. There is, as yet, no equation for such a prediction, but informally, the conventional scientific wisdom is that

$$\begin{aligned}&\text{Liquid water} + \text{Biologically essential elements} +\\&\quad\text{Energy} + \text{Catalytic Surfaces} + \text{Time} \Rightarrow \text{Life}\end{aligned} \tag{13}$$

Were we to discover that Europa is not inhabited, but that the conditions on Europa fully satisfied the lefthand side of the above expression — and many of the other conditions thought to be necessary for origin of life (see, e.g., *Chyba and McDonald,* 1995) — we would gain new insight into the unique conditions that lead to the origin of life on Earth. Similarly, were life discovered on Europa, we could then begin a rigorous cross-comparison to investigate the conditions leading to the emergence of life on both worlds.

Laboratory experiments (*Miller,* 1953; *Palm and Calvin,* 1962; *Ponnamperuma et al.,* 1963; see *Wills and Bada,* 2001, for a review) have shown how the lefthand side of equation (13) can readily lead to the production of amino acids and nucleobases, and to some extent, short polymers. Similarly, biologists have sufficiently deconstructed the righthand side of this equation to understand that once the innovation of ribonucleic acids (RNA) occurred, chemistry was well on its way toward mutation, replication, and Darwinian selection — i.e., well on its way toward life itself. While many gaps remain in our understanding of life's origin, the largest gap is arguably that between the formation of monomeric building-blocks [e.g., amino acids and nucleobases as in *Miller and Urey* (1959)] and the emergence of an RNA-world, in which RNA serves the critical dual functions of an information storage molecule and a protein synthesizer (*Joyce,* 1989).

While Europa lacks the tidal pools, warm springs, and the atmospheric chemistry often invoked for terrestrial prebiotic chemistry, several attributes of the europan system make it an attractive host for life's origin. Foremost among these attributes are a rocky seafloor in contact with the ocean water, and a radiolytically processed icy shell that may harbor cracks and pockets of water in which eutectic freezing can concentrate solutes. In this section, we analyze these two attributes in the context of prebiotic chemistry. Specifically, we examine the icy shell and seafloor in the context of (1) the production, stability, and polymerization of amino acids and nucleobases; (2) chemical pathways and protometabolisms for self-organizing biochemical cycles; and (3) formation of membranes and vesicles that could be important for compartmentalization and differentiation of early life. These are three of the most critical aspects of life's origin on Earth and they serve as good starting points for assessing origins elsewhere.

1.3.1. The rock-water interface. An ocean in contact with a rocky seafloor may be an attribute rare among our solar system's four to seven known or probable contemporary liquid water oceans (Earth, Europa, Ganymede, and Callisto, and possibly Titan, Enceladus, and Triton). Ganymede and Callisto, both with oceans of ~10 times the volume of Earth's, likely have seafloors of ice III (*Spohn and Schubert,* 2003). Titan may have ammonia-rich seas trapped between an ice and hydrocarbon-rich crust and an ammonia-hydrate mantle (*Sagan and Dermott,* 1982; *Lorenz et al.,* 2008). Triton too may have a subsurface ocean, but again its liquid water-nitrogen mixture would likely be confined to a region between high-pressure phases of ice or ammonia-hydrate (*Cruickshank et al.,* 1984). Although the south polar plumes of Enceladus are intriguing evidence consistent with a subsurface sea (*Collins and Goodman,* 2007), our knowledge of this world is still too immature to know whether or not a substantial body of liquid is in contact with rock. Europa, the Earth, and potentially an ancient Mars are the only worlds likely to have had vast quantities of liquid water in contact with mantle rocks over geologically-long periods of time. Chemical weathering of these rocks can supply reduced compounds and metals that may be important for the origin of life (*Nisbet and Sleep,* 2001).

On Earth tectonic activity results in convective cooling of magma by flow of ocean water through the seafloor. The deep-sea hydrothermal vents powered by this activity have been repeatedly invoked as a crucible for life's origin on Earth (*Corliss et al.,* 1979, 1981; *Wächtershäuser,* 1988; *Cody,* 2005; *Martin et al.,* 2008). The reasons are manifold. The reductant-rich and warm environments would have been largely sheltered from impact sterilization (*Nisbet and Sleep,* 2001). Additionally, although debated, our top-down approach to understanding the root of Earth's tree of life suggests that hyperthermophiles — i.e., heat-loving microbes — may have been among the earliest life forms on Earth (*Pace,* 1997; *Madigan et al.,* 2003). The chemistry within the vent fluids and chimneys are conducive to early biochemistry, and the chimneys themselves create natural flow-through reactors with mineral-rich pores that could serve to prevent reaction products from diffusing into the ocean (*Russell et al.,* 1988; 1989). We have no knowledge of seafloor activity on Europa, but tidal energy must be dissipated, and the extent to which that energy is dissipated in the mantle will determine seafloor activity. Models for tidal dissipation at the europan seafloor range from 8 mW m^{-2} to more than 100 mW m^{-2} (*Squyres et al.,* 1983; *Thomson and Delaney,* 2001). By comparison, the seafloor flux on Earth is ~80 mW m^{-2} on average and ~200 mW m^{-2} in active regions (*Stein and Stein,* 1994). Although poorly constrained, the existence of hydrothermal systems on Europa seems plausible. Coupled with this, models of the thermal-orbital evolution

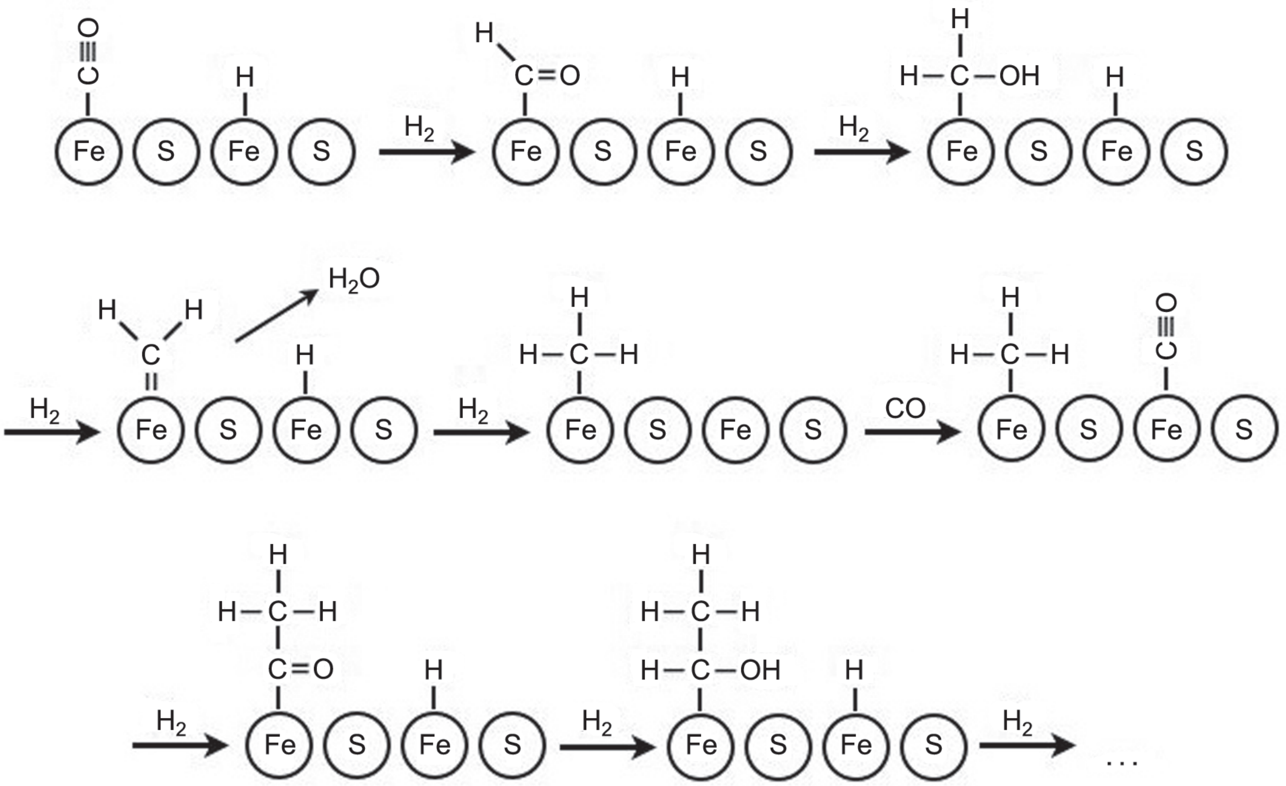

Fig. 8. Fischer-Tropsch-type surface catalysis on the surface of an iron sulfide cluster can yield organics of possible importance to the emergence of life. The positive charge of the mineral surface promotes ionic bonding of electronegative reduced compounds to the surface. Sequential reduction of compounds such as CO_2, CO, and COS leads to organic molecules that could be important for prebiotic chemistry. Adapted from *Cody* (2005).

of Europa make a strong case for a higher heat fluxes billions of years ago (*Hussmann and Spohn,* 2004). Certainly on a young Europa the increased radiogenic flux alone would have contributed to seafloor activity. The contemporary radiogenic flux is between 6 and 8 mW m^{-2}, whereas 4 G.y. ago this would have been roughly an order of magnitude larger. Additional considerations on seafloor activity can be found in the chapter by Vance and Goodman.

But what makes the chemistry of hydrothermal vents amenable to the origin of life? At the most basic level, it is the capacity for the reduced fluids and minerals to synthesize organic compounds from inorganic carbon (*Wächtershäuser,* 1988), although high temperatures at some vent environments may limit the stability of such compounds (*Bada and Lazcano,* 2002). The sulfides H_2S, HS^-, and FeS react with CO and CO_2 to yield hydrocarbons and other organic molecules. Such pathways toward life are referred to as autotrophic origins (*Bada and Lazcano,* 2002), as opposed to heterotrophic pathways that depend on abiotic organic compounds such as carbohydrates, hydrocarbons, and amino acids [e.g., most "prebiotic soup" models (*Miller,* 1953)]. Autotrophic models are attractive for prebiotic chemistry on early Earth, in part because the atmosphere may have been less reducing than initial estimates assumed (*Walker,* 1977; *Holland,* 1984). For Europa, where the ocean may be rich in oxidized forms of carbon, hydrothermal chemistry may also play a critical role in organic synthesis.

The sequential elongation of a hydrocarbon chain by reduction and addition of CO amounts to Fischer-Tropsch-type (FTT) synthesis, which has been well-studied in the laboratory (*Berndt et al.,* 1996; *Heinen and Lauwers,* 1996; *McCollom et al.,* 1999) and in the environment (*Lollar et al.,* 2002). The abiotic methane associated with deep-sea serpentinization sites, e.g., Lost City (*Kelley et al.,* 2001), is derived from a similar pathway. Figure 8, adapted from *Cody* (2005) and described in more detail below, shows a model of FTT surface catalysis on an iron sulfide cluster.

The autotrophic capacity of vent and seafloor chemistry has also been employed to argue for an alternative way of thinking about life's origin. This idea has roots in the work of *Ycas* (1955) and *Corliss et al.* (1979; 1981), but it was the work of *Wächtershäuser* (1988) that was largely responsible for linking the concept, chemistry, and hydrothermal locale in considerable detail. Central to this work is the idea that metabolism would have been the first step toward origins (as opposed to membrane formation or information storage). In its earliest form, life would have consisted of self-sustaining auto-catalytic reaction pathways that served as a protometabolism (*Orgel,* 1998). In the iron-sulfur model championed by *Wächtershäuser* (1988), the metabolic pathway is organic catalysis on a mineral surface in low pH hydrothermal vents, with genetic control and cellular organization arising as a result of a metabolic reaction network evolving toward higher complexity (*Wächter-*

shäuser, 1998). *Goldschmidt* (1952) and *Russell et al.* (1989) contend that hydrogen is the ultimate primordial fuel for life, leading to a preference for low-temperature serpentinization-driven vents as the cauldron for the emergence of life. Both of these autotrophic models may be applicable to the seafloor of Europa, where hot sulfide-rich acidic vents or alkaline low-temperature serpentinization-driven vents are plausible.

Central to the iron-sulfur model is the pyrite-forming reaction

$$FeS + H_2S \rightarrow FeS_2 + 2H^+ + 2e^- \qquad (14)$$

Central to the hydrogen model are reactions such as

$$\begin{array}{c} 3MgFeSiO_3 + 7H_2O \rightarrow 3SiO_2 + \\ Fe_3O_4 + 3Mg^{2+} + 6OH^- + 4H_2 \end{array} \qquad (15)$$

In the iron-sulfur model for the origin of life, the electrons made available during pyrite formation in equation (14) are used to reduce CO_2, CO, and COS, and in the process organic molecules are created and replicated on the mineral surface in a fashion similar to FTT. Because of the positive charge of the mineral surface, the electronegative reduced compounds become ionically bonded to the surface, allowing for continued synthesis of larger molecules. Pyrite is an attractive host for anionically bound surface reactions because, like many heavy metal sulfides, it has positive surface charges and is capable of forming strong bonds with anionic carboxylate and mercapto groups (*Wächtershäuser,* 1990). Complementing this, fixation of CO_2 results in both carboxylate ($-COO^-$) and mercapto ($-SH$) groups. As a result, the oxidative pyrite reaction leads directly to products that are capable of ionic bonding. Combining the pyrite forming reaction with CO_2 reduction at standard conditions yields formate

$$\begin{array}{c} FeS + H_2S(aq) + CO_2(aq) \rightarrow \\ FeS_2 + H_2O + HCOOH; \\ \Delta G^\circ = -8.2 \text{ kJ/mol} \end{array} \qquad (16)$$

A host of other reactions have been proposed, some more thermodynamically favorable than others. Below is Wächtershäuser's surface metabolic pathway for the theoretical formation of succinic acid formation, a highly exergonic reaction and an important step toward more complex prebiotic chemistry (*Wächtershäuser,* 1990)

$$\begin{array}{c} 4CO_2 + 7FeS + 7H_2S \rightarrow \\ (CH_2COOH)_2 + 7FeS_2 + 4H_2O; \\ \Delta G^\circ = -420 \text{ kJ/mol} \end{array} \qquad (17)$$

Several aspects of the iron-sulfur world chemistry have been demonstrated experimentally, although some of these results are debated (*Schoonen et al.,* 1999). *Heinen and Lauwers* (1996) produced methane thiol (CH_3SH) upon reaction of FeS, H_2S, and water with dissolved CO_2. *Blochl et al.* (1992) used the pyrite reaction to pull nitrate to ammonia, alkynes to alkanes, thioglycolic acid to acetic acid, and phenylpyruvate to cinnamate and phenylproprionate. More recently, *Huber and Wächtershäuser* (1997) have demonstrated conversion of the methane thiol directly to acetic acid by CO insertion using a mixed iron-nickel sulfide, (Ni,Fe)S, or nickel sulfate, $NiSO_4$.

In an effort to further elucidate the role of iron sulfide in the evolution of early metabolic pathways, *Cody et al.* (2000) demonstrated synthesis of pyruvic acid (CH_3–CO–COOH) from formic acid at 250°C and 200 MPa, conditions comparable to what might be expected at hydrothermal sites in the depths of both Earth's ocean and Europa's ocean. Metabolically, pyruvic acid plays a key role in the reductive citric acid cycle and in the production of amino acids and sugars. Synthesis of pyruvic acid via pyrite-pulled reactions has helped build a simplified, but experimentally supported, picture of how life might have originated in an iron-sulfur hydrothermal environment (*Wächtershäuser,* 2000).

In the hydrogen-rich, alkaline model for life's origin, serpentinization reduces CO_2 and yields H_2 (*Russell et al.,* 1989; *Schulte et al.,* 2006; *Bach et al.,* 2006). The hydrogen/carbon dioxide potential, augmented by the natural proton-motive force (pmf) created as a consequence of the acidic (carbonic) ocean water interfacing with the alkaline interiors of hydrothermal compartments through FeS-bearing inorganic membranes, then drives metabolism. The proton gradient across inorganic precipitate membranes aids condensation and polymerization of simple organic molecules. Based on this possibility, *Russell and Hall* (2006) and *Martin and Russell* (2007) have argued that low-temperature (~100°C) alkaline hydrothermal systems on the early Earth would have been conducive to the emergence of an H_2-driven metabolism, production of organic polymers, and the formation of early membranes. In this model acetogenesis and methanogenesis are catalyzed by iron and nickel mineral surfaces (e.g., mackinawite, FeS, and greigite, Fe_5NiS_8). Along with enhanced macromolecular stability in alkaline low-temperature fluids, such systems have the added advantage of favoring phosphate and amine chemistry (*Russell,* 2003). Such models could be applicable to low-temperature serpentinization-driven hydrothermal systems on Europa (*Vance et al.,* 2007). In other words, even if Europa lacked an active seafloor with spreading ridges and hot vents, the exothermic reaction of Europa's ocean water with mantle rock may have provided an environment conducive to the origin of life.

Interestingly, the global ocean of Europa may actually pose problems for the formation of complex molecules important to prebiotic chemistry. The synthesis of peptides and other polymers is impeded in environments rich with liquid water. This is a long-standing problem in origin of life chemistry (*Miller and Bada,* 1988), and is particularly germane to origins in the ocean of Europa. The reaction can be summarized by

$$n \text{ amino acids} \Leftrightarrow \text{peptide} + nH_2O \qquad (18)$$

where synthesis of the peptide results in production of water, an unfavorable product in a liquid water solution. With abundant water, the hydrolysis of the peptide — i.e., pushing equation (18) from right to left — is favorable. Consequently, formation of peptides and strands of nucleic acids is impeded in liquid water environments. On the Earth, tidal pools and evaporitic environments provide desiccating conditions that may have allowed equation (18) to proceed from left to right. Comparable environments do not exist on Europa. Instead, mineral surface interactions and hydrothermal vent chemistry may provide a mechanism for circumventing this problem. Formation of peptide bonds linking amino acids under simulated, although alkaline, hydrothermal conditions has been demonstrated (*Huber and Wächtershäuser,* 1998) and may be linked to carbonyl sulfide (COS) within the solution (*Leman et al.,* 2004; *Cody,* 2005). Along with the (Ni,Fe)S results of *Huber and Wächtershäuser* (1998), adsorption of monomers onto mineral surfaces has been shown to promote polymerization (*Ferris et al.,* 1998). Flow-through reactor experiments replicating vent chemistry show that the metal ions and quenching processes in vent fluids can yield oligopeptides (short peptides with fewer than ~10 amino acids) (*Imai et al.,* 1999). Thus, while the vast liquid water ocean of Europa may dilute and inhibit the formation of biopolymers, a hydrothermally active seafloor may provide microenvironments in which such reactions could occur.

Finally, we note that the presence of significant concentrations of salt in Europa's ocean may be an impediment to the origin of life. The induced magnetic field signature observed by Galileo requires salt concentrations of several grams to hundreds of grams (saturation) of salt per kg of water in order to achieve the necessary conductivity (*Hand and Chyba,* 2007). The ionic inorganic solutes influence self-assembly of monocarboxylic acid vesicles, and the nonenzymatic, nontemplated polymerization of activated RNA monomers (*Monnard et al.,* 2002). Sodium chloride or sea salt concentrations as low as 25 mM NaCl (1.46 g per kg of H_2O) reduce oligomerization, and higher concentrations had correspondingly worse effects. If the ratio of the cation to amphiphile molecules falls below ~1, then the excess amphiphile cannot form membranes. This ratio needs to be above ~2 in order for amphiphile to precipitate and form membranes. We note that these experiments have not yet been performed with $MgSO_4$, the "preferred" salt for many Europa models (*Kargel et al.,* 2000; *Zolotov and Shock,* 2001). The *Monnard et al.* (2002) results therefore support the contention that life originated in a freshwater solution, and suggest that the upper range of salinities implied by the Galileo magnetometer results could pose a serious challenge to the origin of life in Europa's bulk contemporary ocean, were abiotic RNA oligomerization or amphiphile membrane formation critical steps toward the origin of life.

If life were to be discovered on Europa, theories for a hydrothermal origin of life would certainly be bolstered, because such environments provide one of the only common geochemical threads between liquid water environments on the Earth and Europa. The other large mineralogical interface on Europa that may provide useful chemistry for origins is the ice-liquid water boundary and melt-water pockets within the icy shell. Similar environments may have existed on the early Earth, particularly given the likelihood of a faint young Sun (*Sagan and Mullen,* 1972; *Newman and Rood,* 1977). Such pathways have not been examined in great detail, but we review this work in the context of Europa in the following section.

1.3.2. The ice-water interface. The origin of life in a cold environment has several advantages over the canonical "warm little pond" of Darwinian fame (*Darwin,* 1871; *Dyson,* 1999; *Nisbet and Sleep,* 2001). Foremost is the ability to preserve large compounds once they are synthesized. Of the amino acids, the aliphatics are the most stable. Even so, temperature variations show a dramatic decrease in stability with increasing temperature. Studies of alanine decomposition via decarboxylation, i.e., conversion of –COOH to CO_2, show a half-life of billions of years for temperatures <25°C but a half-life of only 10 yr at 150°C (*Miller and Orgel,* 1974). Many of the amino acids are known or expected to have a half-life of just a few thousand years at 25°C, but millions to billions of years at temperatures approaching 0°C. Similarly, many purines, pyrimidines, and nucleosides show a decrease in half-life of an order of magnitude to several orders of magnitude as temperatures drop from 25°C to 0°C. Peptides, DNA, and RNA all show a similar trend in stability. At 25°C the phosphodiester bonds in RNA have a hydrolysis half-life of just 30 yr, vs. 900 yr at 0°C (*Miller and Orgel,* 1974). The counterargument to the benefit of enhanced stability of low temperatures is that production rates go up with increasing temperature. This, however, is not necessarily true, especially for the case of reactions with abundant liquid water. Studies of glacial ice (*Price,* 2007) and simulated sea ice (*Trinks et al.,* 2005) have shown that prebiotic chemistry, and even polymerization of substantial nucleotide chains, can be advanced in cold, icy environments.

Ice offers a means through which to concentrate and dehydrate solutes. As liquid water pockets within ice freeze, most nonwater constituents are excluded from the ice matrix, leaving a more concentrated form of the original solution. *Miyakawa et al.* (2002) explored the possibility of ice and eutectic freezing as a means for concentrating HCN and formamide to promote synthesis of prebiotic compounds. Hydrogen cyanide has long been a keystone compound for prebiotic synthesis, dating back to the experiments of *Oró* (1960, 1961) and *Oró and Kamat* (1961), who showed that under Earth-like conditions they could advance reactions such as

$$5\ \mathrm{HCN} \rightarrow \mathrm{Adenine} \qquad (19)$$

Concentration and stability of HCN, however, proves to be a critical limiting factor when moving from the controlled laboratory settings to the natural environment. In dilute aqueous solutions, hydrolysis of HCN leads first to formamide and then to formic acid. Only at high concentrations will HCN lead to the synthesis of nucleobases and amino acids. Formamide is also directly useful for synthesis of prebiotic compounds (*Ochiai et al.,* 1968; *Philipp and Seliger,* 1977), and it has the added benefit of being a good solvent in which to synthesize nucleosides, peptides, and even adenosine-triphosphate (ATP) (*Benner et al.,* 2004). In water these compounds would hydrolyze to ammonia and acids, but in formamide they are stable. *Miyakawa et al.* (2002) found that the low concentrations expected in the ocean of the early Earth would have been insufficient to overcome hydrolysis relative to adenine synthesis, but eutectic freezing in cold-water environments could have provided important niches for enhancing HCN concentrations and promoting polymerization. Miller had the great foresight to prepare, and then freeze (at –20°C and –78°C), solutions of ammonium cyanide (NH_4CN) in 1972. Although at the time the relevance to Europa surely would have been uncertain, the analysis, performed 25 years later (*Levy et al.,* 2000), revealed production of adenine, guanine, and several amino acids, including glycine and alanine.

But achieving HCN polymerization through eutectic concentration still necessitates a nonzero starting concentration for HCN and other prebiotic compounds. The experiments of Oró and colleagues typically involved HCN solutions of >1 M (mole per liter of solution). By comparison, *Sanchez et al.* (1966) froze 0.1-M HCN solutions and did not find adenine, but instead found diamino-maleonitrile (DAMN), an important intermediary in the HCN to adenine pathway. In the NH_4CN experiments of *Levy et al.* (2000), the initial 0.1-M NH_4CN solution yielded adenine at 0.035–0.040% the concentration of NH_4CN, and guanine at about an order of magnitude lower concentration. In other words, even for relatively high initial concentrations, yields are low. By comparison, a warm ocean on the early Earth is estimated to have HCN concentrations of 10^{-10}–10^{-15} M (*Stribling and Miller,* 1987; *Miyakawa et al.,* 2002). This range is based in part on Stribling and Miller's production rate of 100 nmol cm^{-2} yr^{-1} for HCN in spark discharge experiments. *Chyba and Sagan* (1992) calculated HCN production rates an order of magnitude higher. *Miyakawa et al.* (2002) experimentally determined hydrolysis of HCN at different temperatures and found greatly improved stability at low temperatures. Using the Stribling and Miller production rate, those workers argue that a 0°C ocean on Earth could maintain HCN concentrations of 10^{-5}–10^{-6} M, which if concentrated via freezing in an ice pocket, could lead to concentrations high enough to support polymerization. Similar eutectic concentration mechanisms could apply to the icy shell of Europa, but what might we expect for the availability of initial monomers such as HCN?

Pierazzo and Chyba (2002) modeled cometary delivery of amino acids and organics to the surface of Europa, but concluded that most of the inventory is destroyed upon impact or ejected to space. Even so, *Bada et al.* (1994) have pointed out that impacts into frozen oceans on a young Earth could have enhanced prebiotic synthesis as a result of eutectic synthesis and enhanced preservation in ice. Similar arguments can be applied to impact melts on Europa. *Zolotov et al.* (2006) modeled the primordial ocean of Europa and argued that N-bearing organic compounds would have been released into the ocean as a result of differentiation and leaching from an initial composition comparable to CI/CM carbonaceous chondrites. If, as described in section 1, Europa's ocean were to have significantly more ammonia and carbon dissolved in it than Earth's early ocean, then HCN may have been available on primordial Europa. Eutectic freezing in the icy shell could then have provided a pathway toward enhancing concentrations and polymerizing HCN to yield nucleobases and amino acids.

On the early Earth, lightening and UV-photolysis were available to drive prebiotic chemistry in the atmosphere. Europa lacks a thick atmosphere today, but it does have an incessant flux of UV, ions, and electrons bombarding its icy surface. It is a cold, yet highly energetic environment. For pure water ice, the resulting radiolytic chemistry, as detailed in section 1 and in the chapter by Carlson et al., produces the oxidants H_2O_2 and O_2. If other compounds are mixed with the ice, e.g., hydrocarbons and ammonia, then the resulting chemistry can lead to larger compounds of prebiotic interest. Considerable laboratory work has been done on astrophysical (<50 K) and planetary (>50 K) ices (e.g., see *Khare et al.,* 1989; *Bernstein et al.,* 1995; *Hudson and Moore,* 1999; and references therein). Here we highlight select results particularly germane to prebiotic chemistry and to the conditions on Europa. We note, however, that in most laboratory studies, the concentration of nonwater compounds typically exceeds our current understanding of the surface chemistry of Europa.

Khare et al. (1989) used low-energy electrons (mean energy of a few eV) to irradiate methane clathrate at 77 K with a flux of roughly 3×10^{16} eV s^{-1} cm^{-2} for upwards of a week. Subsequent to irradiation, samples were warmed to room temperature and a residue rich in alkanes, aldehydes, and alcohols was found to persist, with perhaps some contribution from alkenes and aromatics. *Hudson and Moore* (1999) found that even with just CO in ice, irradiation with 0.8-MeV protons yielded methanol, formaldehyde, and formic acid. Although their work was conducted at 16 K, a temperature considerably lower than temperatures on Europa, experiments with high-energy electrons and CO_2-rich ice at 100 K show comparable results (*Hand,* 2007). Working with polycyclic aromatic hydrocarbons in 10 K ice, *Bernstein et al.* (1999) produced aromatic alcohols, ketones, and ethers upon irradiation with UV photons at Lyman-α (121.6 nm) and 160-nm wavelengths. Upon warming to 300 K, aliphatic hydrocarbon features were also observed. In summary, despite the simultaneous production of oxidants that might be expected to "burn" any reduced compounds, irradiation of CO_2 or short-chain hydrocarbons in ice under europan conditions appears to consistently lead to complex organics (*Hand,* 2007).

Adding nitrogen to their UV experiments, *Bernstein et al.* (1995) observed that photolysis of methanol and ammonia-rich ices (e.g., $H_2O:CH_3OH:CO:NH_3$ at a ratio of 100:50:10:10) leads to various nitriles (–C≡N) and specifically to vinyl cyanide ($R_2C{=}CH{-}C{\equiv}N$). When warmed slowly from 12 to 200 K, products were found to include ethanol, formamide, acetamide, and additional nitriles. Those workers found that of the initial carbon and nitrogen in the ice, approximately 20% and 50%, respectively, was retained in the final residue that remained at 300 K. The nitrile band observed by *Bernstein et al.* (1995) and many previous workers at 2165 cm^{-1} (4.62 μm) has long been observed on outer-solar-system bodies (*Cruickshank et al.,* 1991) and may help explain the 4.57-μm feature on Ganymede and Callisto (*McCord et al.,* 1999). Only somewhat recently, however, has the feature observed in the laboratory been confirmed as resulting from the cyanate ion (OCN^-) in ice (*Hudson et al.,* 2001). The proposed formation pathway for OCN^- utilizes CO as a precursor and leaves the ammonium ion in ice

$$\begin{gathered} NH_3 \rightarrow NH_2 \text{ and/or } NH \\ NH_2 + CO \rightarrow H + HNCO \\ NH + CO \rightarrow HNCO \\ HNCO + NH_3 \rightarrow NH_4^+ + OCN^- \end{gathered} \tag{20}$$

While the importance of HCN to prebiotic chemistry is well established, little work on OCN^- has been done in this context. This is an important area for future work since the cyanate ion may be the dominant nitrile form in icy solar system bodies. Upon irradiation of HCN in ice with NH_3, *Gerakines et al.* (2004) produced the cyanate ion along with the ammonium ion, cyanide ion (CN^-), isocyanic acid (HCNO), formamide ($HCONH_2$), and likely HCN-polymers similar to those measured by *Khare et al.* (1994). The connection between OCN^- production in simple, carbon-poor ices (*Hudson et al.,* 2001) and the products produced by irradiation of HCN (*Gerakines et al.,* 2004) must be further examined if we are to advance our understanding of how radiolytic chemistry on icy surfaces may drive prebiotic chemistry.

To examine production of amphiphiles and self-assembly of vesicular structures relevant to the origin of life, *Dworkin et al.* (2001) photolyzed a variety of ice mixtures at 15 K with UV photons. They discovered that with methanol and ammonia as part of their initial mixture (e.g., $H_2O:CH_3OH:CO:NH_3$ at a ratio of 100:50:1:1), a residue containing amphiphilic compounds remained upon warming to 300 K. The amphiphilic fraction was then rehydrated and shown to form membranous vesicles capable of hosting a polar dye. Vesicle sizes ranged from a few to a few tens of micrometers. Similar experiments were conducted by *Dworkin et al.* (2001) with organics extracted from the Murchison meteorite, showing that both in the laboratory and in the solar system environment, formation of molecules needed to achieve compartmentalization in the early stages of biochemistry is possible.

Amino acid production via irradiation of a Titan-analog gas mixture was shown by *Khare et al.* (1986), and *Ponnamperuma et al.* (1963) produced the nucleic acid base adenine with pulsed, 4.5-MeV electrons in a simple mixture of $H_2O + CH_4 + NH_3$ under conditions relevant to the early Earth. The first definitive results of amino acid synthesis in the solid-phase under astrophysical conditions come from *Bernstein et al.* (2002). In that work 15-K ice with methanol, HCN, and NH_3 was irradiated with UV and found to yield glycine and racemic mixtures of alanine and serine. Racemic alanine was also observed after 10-keV electron irradiation of 80-K ice with ammonia and propane ($H_2O:NH_3:C_3H_8$) in a ratio of approximately 5:1:1 (*Hand,* 2007).

Although direct compositional observations of Europa's surface have been very limited, there have been repeated, relatively facile, laboratory production of a host of complex organic compounds of prebiotic interest under conditions ranging from that of the early Earth to interstellar ice grains. This supports the conclusion that the cold but heavily irradiated surface of Europa may play a significant role in providing the subsurface ocean with the building blocks for life, if the ice does mix with the ocean. The largest uncertainty is in the initial composition and concentration of compounds in the ice; if simple compounds (e.g., CH_4 and NH_3) can be concentrated on the surface, then irradiation will likely yield complex organics similar to what is observed in the laboratory. Sputtering effects from heavy ions (S^{n+} and O^{n+}) will likely destroy material in the upper few micrometers, but materials synthesized by the electrons and UV photons that penetrate beneath the sputtering region will be protected. *Miller and Orgel* (1974) long argued that a "cold, concentrated soup would have provided a better environment for the origins of life." Radiolytic products, produced on the surface of Europa but then buried in the icy shell, may have been concentrated in tectonic cracks or fractures where synthesis of peptides and other large molecules could have taken place before mixing into the vast body of liquid water below. Finally, it is interesting to note that formamide as a solution has a density of 1.13 g cm^{-3} and therefore may be buoyant relative to a salty ocean. Many hydrocarbons will also be buoyant in Europa's ocean. Depending on mechanisms for the delivery of compounds from the surface, the ice-water interface on Europa could have been, and may still be, a very interesting and rich environment for prebiotic chemistry.

2. THE SURFACE EXPRESSION OF SUBSURFACE LIFE: POTENTIAL BIOMARKERS ON THE SURFACE OF EUROPA

An inhabited world is of little scientific utility if we have no way of detecting its inhabitants. The upper few meters of ice on Europa are, by any terrestrial metric, certainly uninhabitable. Yet it is this region to which our near-future reconnaissance of Europa will be constrained. The detection of life — were the ocean to be inhabited — therefore depends on the degree to which the surface ice mixes with, and serves as a proxy for, the ocean water. This is essentially an inversion of the resurfacing problem discussed in section 1.2; rather than delivering surface oxidants to the ocean, we are now concerned with delivering potential oceanic life forms to the surface.

The argument can be made that the ocean of Europa never mixes with the surface, and therefore no signs of life will be present. For many of the same reasons discussed in section 1.2, however, we find this hypothesis unlikely. The young surface age (see chapter by Bierhaus et al.) combined with the known sodium escape (*Brown and Hill,* 1996; *Leblanc et al.,* 2002) make a strong case for communication of ocean material with the surface on timescales of less than a few hundred millions years. If life were brought to the surface, its signature on the surface, i.e., its biosignature, would need to survive the surface environment for this period of time in order to permit detection by a spacecraft. Of course, one of the primary goals of a future mission would be to identify young surfaces, thereby reducing the exposure time of any entrained biological material. Here we address terrestrial biosignatures, and their survival through time and under a variety of conditions, in order to better understand possible surface biosignatures on Europa. The term "biosignature" is used here to refer to any sign of life in the environment, whereas the term "biomarker" is used here as a subset of biosignatures and refers to specific compounds that can be connected to life. Often these terms are used interchangeably (*Summons et al.,* 2007), but because we are concerned with detection from orbit, it is useful to differentiate between biomarker compounds that have spectroscopic signatures and those that do not.

Biosignatures on Earth include morphological, molecular, and chemical indicators of past or present life. Morphological biosignatures range from macroscopic fossils to trace fossils and structural indicators of past life, such as stromatalites and microbiolites. With centimeter-scale imagery, such biosignatures might be observable from orbit around Europa. As discussed in section 1.2, large oxygen-utilizing organisms might be possible on Europa if cycling of surface material into the ocean occurs and if heterotrophic food chains exist. Fossil remnants of such organisms on the surface of Europa are perhaps an unlikely, but nevertheless distinct, possibility. The icy shell itself may be modified by biological processes, comparable in concept to microbially mediated reefs structures on Earth. If the ice is chemically rich with radiolytic products, the ice surface at the ice-water interface could be a biologically active region. Overturned blocks of ice in a thin europan shell, one interpretation of chaos features (*Carr et al.,* 1998; *Greenberg et al.,* 1999), would then bring such morphological biosignatures to the surface where they could be detected remotely.

Molecular biomarkers refer to those compounds unique to life and biological processes that, when found in the geological record, can be used to infer the presence of life in the past. DNA is an obvious example, but the survival of DNA is short on geological timescales [~10^3–10^5 yr over the range of surface temperature conditions found on Earth (*Bada and Lazcano,* 2002)]. The efficacy of molecular biomarkers is rooted in the simple fact that in life "an enormous diversity of large molecules are built from a relatively small subset of universal precursors" (*Summons et al.,* 2007). Namely, the nucleobases of DNA and RNA (uracil, thymine, adenine, guanine, and cytosine), 20 amino acids, and two lipid building blocks (acetyl and isopentenyldiphosphate precursors). Alone, such compounds are ambiguous, but in polymer form the structure and complexity helps to build the case for a biogenic origin.

Coupled with the structural subunits is the selectivity and order in which they are polymerized. On the issue of selectivity, this largely refers to chiral, or enantiomeric, preference for subunits (e.g., proteins are constructed from only L-amino-acids). The construction of complex molecules from subunits brings with it the capacity for a large number of permutations for various branches and functional group arrangements of the same molecule. This amounts to a compounded chirality since with each addition new chiral centers are created, making it possible for a larger pool of possible variations on the same molecule. This is referred to as isomerism and a given spatial arrangement for a specific compound is referred to as a diastereomer (*Peters et al.,* 2005; *Summons et al.,* 2007). Biological processes not only preferentially select subunits of a specific chirality, but they also synthesize compounds with a diastereomeric preference.

Lipid biomarkers have arguably been the most robust window into the early history of life on Earth. The acetogenic lipids (e.g., fatty acids) occur in units of methylene ($–CH_2–CH_2–$) and consequently yield gas chromatograph mass spectrometer (GCMS) results patterned in a series of even-numbered carbon compounds (e.g., C_{14}, $C_{16} \ldots C_{28}$, C_{30}, C_{32}). The lipids constructed from isopentenyldiphosphate result in lipids of linked isoprene (C_5H_{10}) units. These are commonly referred to as terpenoids and this family of lipids consists of complex arrangements of linear and ring structures built from the isoprene subunit. It is largely the survival of terpenoid compounds (specifically hopanes and steranes) that have permitted exploration of the history of life in the terrestrial Archaean rock record. These are derivatives of lipids found in the cellular membranes of *Bacteria, Eukaryotes,* and *Archaea*. Through time, heat, and pressure, the starting compounds are reduced to more stable forms that typically have fewer methyl and hydroxyl groups. *Brocks et al.* (2003) identified many of these molecules in 2.5–2.7-G.y. shales from Australia, proving their long-term stability. But the preservation of such molecules typically depends on deposition of the initial biological material into anoxic sediments. The subsequent diagenesis then does little to oxidize the molecule. Conditions on Europa are likely to pose serious challenges for comparable anoxic preservation.

Chemical biosignatures are typically the result of metabolic differentiation or selective production. Common examples include the isotopic fractionation of carbon (biological material on Earth is depleted in ^{13}C relative to the environment), and the use in biological systems of only laevorotatory amino acids and dextrorotatory sugars. Although heavily debated, the relative depletion of ^{13}C ($\delta^{13}C \approx -28‰$) in graphite from 3.8-b.y.-old metasediments of the Isua supracrustal belt in Greenland has been used to argue for signs of life on Earth not long after the late heavy bombardment (*Mojzsis et al.,* 1996; *Rosing,* 1999). The depletion results from kinetic and thermodynamic effects that are largely a function of differences in the mass and quantum characteristics of the element and its isotopes (*Schidlowski et al.,* 1983). As an example, during photosynthesis the Calvin cycle utilizes the carboxylase ribulose–1,5–bisphosphate (RuBP) to fix CO_2. This reaction favors ^{12}C over ^{13}C leading to a ^{13}C depletion of –20‰ to –35‰. Several metabolic pathways and enzymes associated with chemosynthesis (as opposed to photosynthesis) also yield measurable carbon-isotopic fractionations. In particular, methane producing and consuming metabolisms can result in a carbon signature with very strong ^{13}C depletion. Coupling of methanogens feeding methanotrophs, which in turn feed CO_2 to the methanogens, has been invoked to explain an excursion of $\delta^{13}C \approx -60‰$ (*Hayes,* 1994). The chemistry to drive a comparable ecosystem on Europa was explored by *McCollom* (1999). Were such an ecosystem to exist, the carbon isotopic signature of carbon in the surface ice could provide clues to the subsurface life.

Sulfur and sulfur isotopes also play important roles as biosignatures for terrestrial life. Organic sulfur compounds are better preserved, and they are less likely to be degraded by other organisms (*Peters et al.,* 2005; *Summons et al.,* 2007). Additionally, anoxic marine environments are critical for long-term preservation, and such conditions are often rich in sulfide. Sulfur has four stable isotopes (^{32}S, ^{33}S, ^{34}S, and ^{36}S) which makes for a highly complicated isotopic fractionation story, especially in the context of biology. Over a range of temperatures, reduction of sulfate to sulfides by bacteria results in sulfide depleted in ^{34}S by 13–40‰ relative to seawater sulfate (*Canfield et al.,* 2000). These sulfides then form sediments on the ocean floor. Sulfur or sulfide oxidation to sulfate, which could remain in suspension in seawater, leads to enhanced ^{34}S concentrations in the sulfate relative to the initial sulfur; however, progressive oxidation of the sulfide can ultimately lead to ^{34}S depletion (*Philippot et al.,* 2007). Without mineral samples to couple to aqueous sulfate, it is difficult to establish any biogenic fractionation. Given that sulfur dominates much of the observed surface chemistry of Europa, and that it is likely an important constituent in ocean chemistry, sulfur and sulfur isotope biosignatures are important to consider [see *Pilcher* (2003) for an interesting analysis of possible organosulfur biomarkers]. Interpretation of surface samples, however, will be greatly complicated by the radiation environment and the lack of access to sedimentary sulfides.

It is reasonable to expect that isotopic fractionation effects would carry over to alien biochemistry, and thus that isotopic ratios could be a useful biosignature beyond Earth (*Schidlowski,* 1992). However, many terrestrial isotopic biosignatures are heavily mediated by chemical and geological processes and only make sense as biosignatures when taken in the context of the environment. In other words, context is critical for understanding the partitioning of various isotopes. As a tool for astrobiology and life detection, isotopic signatures will be only as useful as our knowledge of the cycles and processes that govern the planetary environment.

On Europa, the morphological, molecular, and chemical biosignatures used here on Earth serve as a valuable but limited guide. Ultimately, the radiation environment of Europa is likely to be the major process controlling preservation of surface biosignatures. Considering irradiation of biomarkers, naturally occurring uranium-enriched shales on Earth offer a unique opportunity for studying the effects of radiation on biomarker survivability. Unlike laboratory experiments that must trade irradiation time for intensity, the so-called “hot shales” (*Dahl et al.,* 1988a) provide the experiment *in situ* on geologic timescales. Several regions are known to have such sediments: the Witwatersrand sequence of South Africa, the Grants Uranium District of New Mexico, Cluff Lake in Saskatchewan, the Arlit formation in Nigeria, and the Oklo formation in Gabon are just a few examples of the best known regions. Here we examine one particularly well-studied region, the Alum Shales of Sweden (*Dahl et al.,* 1988a), in order to gain a better understanding of exactly what is expected from the irradiation of organic matter through time. Uranium concentrations in the Alum Shales have been found to vary between 7 ppm and 250 ppm. For samples with present-day concentrations of 190 ppm, *Dahl et al.* (1988a) calculated that uranium decay has yielded a total dose of 10^7 Gy over the past 500 m.y.

Sundararaman and Dahl (1993) summarize some of the effects of ~10^7 Gy (1 Gy = 1 J kg^{-1}) on organic matter as follows: (1) A decrease in the amount of total extractable organic matter. While uranium decay contributes directly to the destruction of organic compounds, it also dissociates water and thus facilitates oxidation. The radiolytically produced oxidants on Europa’s surface may also play a role in the destruction of organic compounds. (2) A decrease in the H/C ratio. Irradiation experiments have been shown to release hydrogen, methane, ethane, and other light hydrocarbons from organic matter while also yielding aldehydes in the remaining material, thus reducing the H/C ratio (*Swallow,* 1963). (3) The concentration of the lipid membrane biomarkers, such as hopanes and steranes, decreases. (4) The ratio of aromatics to saturated hydrocarbons increases. Aromatics are known to be more stable under irradiation than are aliphatics (*Dahl et al.,* 1988b). On Europa, this could complicate the challenge of distinguishing biogenic aromatics from the background flux of exogenously delivered abiotic polyaromatic hydrocarbons. (5) An increase in the $^{13}C/^{12}C$ ratio, i.e., $\delta^{13}C$ trends toward a nonbiological isotopic signature, possibly masking a biogenic origin. The mechanism for this change is likely the liberation of lighter isotopes via irradiation.

The parameters above are just a few of the measured effects of irradiation on terrestrial biosignatures. *Court et al.*

(2006) performed a comparison across several uranium-rich shales and found results consistent with those of *Sundararaman and Dahl* (1993) and *Dahl et al.* (1988a,b). Importantly, for shale samples with ~250 ppm ^{238}U, *Dahl et al.* (1988b) found no measurable hydrocarbons, saturates, or aromatics. Comparing the Alum shale to the surface of Europa is obviously imperfect. The environments of deposition and preservation are completely different. Nevertheless, the results above included a cross-comparison with organics in other terrestrial shales and thus help isolate the effects of irradiation. When comparing terrestrial irradiation in shales via the ^{238}U > ^{206}Pb decay series to that of the ion and electron impact environment of Europa, it is important to consider differences that may exist in the radiation particles. For the case of uranium, the radiation is a series of α- and β-decays. Each α-decay corresponds to the ejection of a helium nucleus with approximately 4.7 MeV of energy. The β-decay stages each result in the emission of a ~0.3-MeV electron. The electrons impacting Europa are similar in energy to those of β-decay (keV to MeV), and the H^+ ions are at least comparable in atomic cross-section, if not in total kinetic energy, to the He nuclei from α-decay. The O^{n+}, and S^{n+} ions, however, are much larger — both in mass and in cross-section — than what is seen in the decay of uranium.

For a surface age on Europa of ~10^7 yr, the total radiation dose at 1 cm below the surface would be ~3.6×10^{11} Gy, or ~3×10^3 Gy per month. This is over 100 times the highest dose experienced by the Alum Shales. Consequently, the remnants of any subsurface ocean biota are not likely to be well-preserved for this combination of depth and surface age. For surface ages of just a few thousand to tens of thousands of years, however, the total dose at 1 cm below the surface on Europa is then comparable to, or less than, that of Alum Shale samples that did reveal organics and possible biomarkers. Clearly, identifying young surfaces on Europa is critical from the standpoint of biosignature preservation on the surface.

Trading time for depth, at 1 m below the surface of Europa, the direct radiation dose is reduced to just 1 Gy per month. Using the same resurfacing timescale, biomarkers would be subject to one-tenth the dose experienced by the biomarkers in the Alum Shale with the highest uranium concentrations. Comparable Alum Shale samples are those with roughly 10 ppm uranium. Such samples have saturates and aromatics in the range of ~ 0.5–1.0 mg g^{-1}, well within the limits of detectability for a landed spacecraft with a GCMS. To provide some comparison to the survivability of spores exposed to radiation, we note that *Rivkina et al.* (2005) discovered viable microorganisms in the 3-m.y.-old permafrost of the Kolyma tundra in Siberia, where the estimated radiation dose would have been ~600 Gy. Such organisms could potentially survive for 50 yr on Europa, were they buried 1 m below the surface.

Positive detection of terrestrial biosignatures is largely dependent on geological context. The complex mineralogy of Earth's lithosphere is in part responsible for the challenge faced when attempting to establish biosignatures. On Europa, the radiation environment and ocean chemistry may obscure biosignatures, but the mineralogy is largely limited to ice, clathrate hydrates, and hydrated sulfate phases (see chapters by Carlson et al. and by Zolotov and Kargel; see also *Kargel et al.,* 2000; *Hand et al.,* 2006). The ~100-K surface temperatures would facilitate long-term survival of biomarkers, but the oxidant-rich surface, and gardening and sputtering of that surface by micrometeorites and ions (see chapter by Moore et al.), would serve to degrade biological material. Assessing the remnants of such material with an *in situ* lander would permit the application of a variety of tools and techniques. With solely an orbiting spacecraft, however, remote detection of such degraded material, e.g., through spectroscopy of the surface or mass spectrometry of Europa's sputtered atmosphere, would be our only window to life in the subsurface.

Application of spectroscopic biosignatures to the case of Europa is largely limited to the study of *Dalton et al.* (2003), and more recently to the work of Hand and Carlson (*Hand,* 2007). Noninvasive spectroscopic techniques for characterization and identification of microbes are underutilized and little explored. Much of the existing work stems from industrial applications for food security and sterilization (*Naumann et al.,* 1991; *Kummerle et al.,* 1998). *Dalton et al.* (2003) characterized near-infrared features of DNA, proteins, lipids, and carbohydrates at room temperatures (Fig. 9a). Lipids and proteins display C–H absorption features near 2.3 μm, as well as C–N features at 2.05 and 2.17 μm arising from amide bonds and polypeptide chains. Nucleic acids and carbohydrates have simpler spectra, but all four types of cellular components have strong features associated with water molecules near 1.2, 1.5 and 2.0 μm. To further explore the question of biosignatures on icy worlds, *Dalton et al.* (2003) froze *Sulfolobus shibatae, Deinococcus radiodurans,* and *Escherichia coli* and characterized their spectral signature under europan conditions of high vacuum and low temperature (100 K). In the complex, multiply-scattering and water-dominated cellular environment, the water (much of it bound) dominates the other cellular components and produces spectral features highly similar to those observed by the Galileo spacecraft at Europa. The near-infrared amide features of the microbes were distinguishable in the laboratory spectra, prompting those workers to suggest the possibility of using the 2.05- and 2.17-μm C–N bands as candidate biosignatures (Fig. 9b).

In an effort to establish possible spectroscopic biosignatures that persist after irradiation, Hand and Carlson irradiated spores of the common soil bacterium *Bacillus pumilus,* with 20-keV electrons under Europa-like conditions (*Hand,* 2007). This bacterium was selected because of its relevance to spacecraft sterility and planetary protection. *LaDuc et al.* (2003) studied the Mars Odyssey spacecraft and found *B. pumilus* to be one of the most abundant viable organisms on the spacecraft after the craft was cleaned with H_2O_2, exposed to intense UV, and baked to high temperatures. The fact that *B. pumilus* can tolerate H_2O_2 cleaning and UV irra-

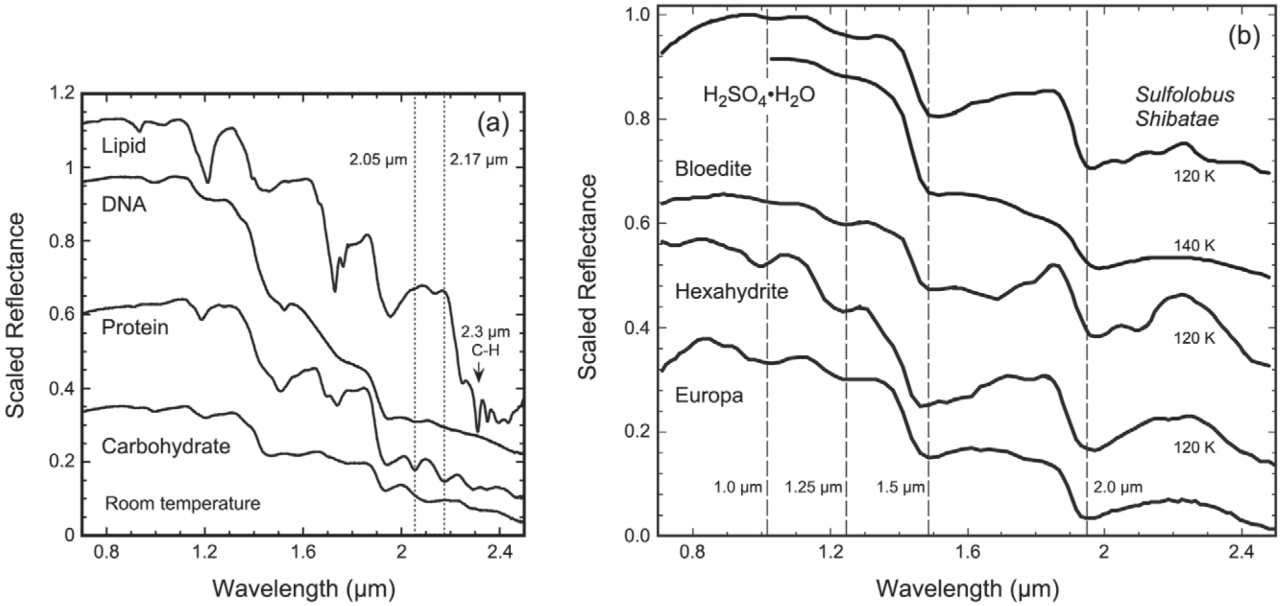

Fig. 9. **(a)** Infrared spectra of infrared-active cellular components, obtained at room temperature by *Dalton et al.* (2003). Vertical dashed lines at 2.05 and 2.17 μm indicate positions of absorption features arising from amide bonds. **(b)** Infrared spectra of Europa's non-ice material and proposed surface constituents, obtained at 120 K by *Dalton et al.* (2003), except for the sulfuric acid spectrum, obtained at 140 K by *Carlson et al.* (1999b). Vertical dashed lines near 1.0, 1.25, 1.5, and 2.0 μm denote positions of asymmetric and distorted spectral features in Europa's dark and disrupted terrains. Features in the spectrum of frozen microbes, *S. shibatae*, show intriguing similarity to the hydration features seen in spectra of Europa's dark surface material.

diation makes it a good candidate for assessing possible chemical or molecular biosignatures under europan surface conditions. Organisms such as *Deinococcus radiodurans* are often touted for their ability to withstand harsh radiation, but in order to do so *D. radiodurans* must actively repair its DNA, something it would not be able to do in the cold, desiccating surface environment of Europa. Radiation tolerant organisms are not particularly relevant to the habitable region of Europa (the ocean and water-filled regions of the icy shell), although spore-forming organisms that can shut down metabolically and survive cold, dry, radiation-intense environments (*Rivkina et al.,* 2005) are interesting in the context of exposure to the surface, followed by reintroduction to the ocean.

Figure 10 shows spectra of *B. pumilus* spores in ice at 100 K before irradiation (lower spectrum) and after irradiation with 20 keV electrons at a current of 1 μA for approximately 24 hr (~10^9 Gy) (middle spectrum). Also shown is a spectrum of abiotic organic chemistry resulting from electron radiolysis (upper spectrum). The asymmetric stretch of $-CH_3$ at 2965 cm^{-1}, the symmetric stretch of $-CH_3$ at 2874 cm^{-1}, and the asymmetric stretch of $-CH_2$ at 2936 cm^{-1} are all indistinguishable from one spectrum to the next. The hydrocarbon chemistry of biology offers little in the way of clues toward a biogenic origin. This is unfortunate as this is the easiest band to observe, and it has been reported for Enceladus at 3.44 μm (2870–2970 cm^{-1}), as well as for numerous other solar system objects (*Hansen et al.,* 2006).

Several other spectral features related to carbon or organic chemistry are also indistinguishable across spectra. The CO_2 (2340 cm^{-1}), CO (2130 cm^{-1}), nitrile band (2164 cm^{-1}), and methane bands (1304 cm^{-1}) are all very similar. The region of the spectrum where life begins to distinguish itself from nonlife is in the region <1700 cm^{-1} (i.e., wavelengths longer than 5.88 μm). In the *B. pumilus* spectrum taken prior to irradiation strong peaks are centered at 1650 cm^{-1} and 1514 cm^{-1}. These peaks correspond to the amide I and amide II bands respectively. The amide bond, and the associated vibrational excitations, specifically refers to the C–N linkage between amino acids and the interaction with the C=O and N–H on either side of that bond. The 1514 cm^{-1} band is sometimes denoted the tyrosine band, as it is a hallmark of the presence of that amino acid in proteins (*Naumann et al.,* 1996; *Maquelin et al.,* 2002). The band sits next to, and in conjunction with, the amide II band at 1520–1550 cm^{-1}, characteristic of the C–N stretch and C–N–H bend in the peptide structures of proteins. The observed decrease in band strength in these experiments indicates destruction and loss of proteins.

Amide bands persist after irradiation and are distinguishable from the abiotic radiolytic products. The critical difference between the abiotic and biogenic is that amino acids in proteins are arranged in ordered sheets and helical structures, whereas amino acids produced radiolytically do not share the peptide structure and associated infrared band shift (*Hand,* 2007). The amide I band of α-helical structures in proteins, resulting from the carbonyl bond in the right-handed spiral of the helical structure, is seen at 1655 cm^{-1}. The NH_3 residue likely consists of carbonyls with a greater diversity of secondary bonds, thereby shifting the peak to-

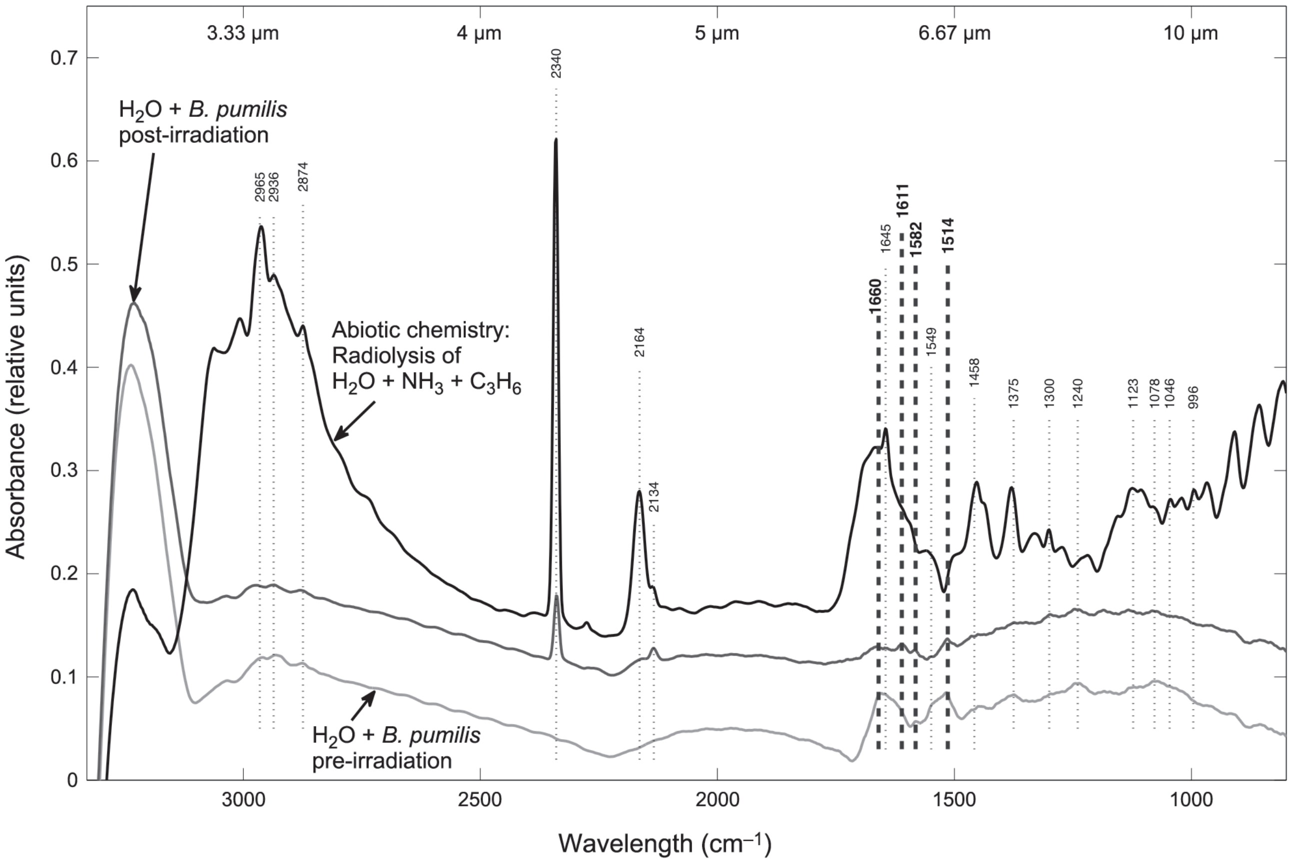

Fig. 10. Possible spectroscopic biosignatures in the amide region of the midinfrared. The bottom spectrum shows spores in 100 K ice before irradiation, the middle spectrum shows similar spores after irradiation with 20-keV electrons, and the top spectrum shows results from irradiation of an abiotic mixture of H_2O, NH_3, and C_3H_6. The spectrum of irradiated microbes is distinguishable from the abiotic radiolysis spectrum primarily due to persistent amide bands (O=C-N-H) at ~6 μm (~1600 cm^{-1}) associated with protein structures (bold dashed lines). From Hand and Carlson (in preparation).

ward the C–C=O carbonyl at 1710 cm^{-1}, as opposed to that of the amide bond (N–C=O) at 1655 cm^{-1}. Also contributing to this difference is the amide I band of the β-pleated sheet structures in proteins. This band, at 1637 cm^{-1} (*Naumann et al.,* 1996), is also due to the carbonyl stretch interacting with the amide N–H, but in this case the strands of linked amino acids form a sheet by hydrogen bonding with neighboring strands. The change in molecular geometry shifts the amide I in β-sheets to the longer wavelength. This band is seen on the shoulder of the α-helix amide I band.

At even longer wavelengths, centered at 1240 cm^{-1}, is a band corresponding to the asymmetric stretch of the P=O bond in a phosphodiester ($>PO_2^-$) linkage (*Maquelin et al.,* 2002). The phosphodiester bond is responsible for connecting the nucleotides of RNA and DNA. The diesters of ATP occur at slightly shorter wavelengths, ~1260 cm^{-1} (*Liu et al.,* 2005). The second broad band, centered at 1078 cm^{-1}, also corresponds to the phosphodiester bond, but in this case the band results from the symmetric stretch of P=O (*Choo-Smith et al.,* 2001; *Maquelin et al.,* 2002).

The host of biosignatures described above provides some context for the manifestation of preserved biological products through time and under irradiation. The described suite of biosignatures is, however, severely limited by our lack of a comprehensive understanding for how life on Earth is modified and preserved through time. Earth is awash with life, and deconvolving signatures of life in modern environments from those preserved in ancient environments is an ongoing challenge to understanding the history of life on this planet (for extensive reviews, see *Knoll,* 2003; *Schopf,* 1999). In some respects, the surface environment of Europa offers a more homogenous preservational environment than either Earth or Mars. The frozen, sulfate-rich ice covers the surface, and while there is a leading/trailing hemisphere dichotomy for the electron irradiation, the majority of the surface is heavily bombarded by energetic particles (*Cooper et al.,* 2001; see chapter by Paranicas et al.). The traditional rock cycle of igneous, sedimentary, and metamorphic rocks complicates the signature of ancient life on Earth. On Europa, this cycle still applies, but it is likely to be greatly simplified by the fact that one mineral, ice and its polymorphs, dominate all processes. We anticipate that more complex chemical heterogeneity would be revealed with future missions, adding new dimensions to the europan rock cycle, but we also anticipate that such heterogeneity will provide clues to a potentially habitable, or even inhabited, subsurface. We discuss some of these possibilities in the following section.

3. DETECTING LIFE FROM AN ORBITER AND/OR LANDER

The challenge of searching for life on Europa is exacerbated by the fact that we still lack a broadly accepted definition for life (*Cleland and Chyba,* 2002). Without such a definition, establishing a succinct set of search criteria is difficult. Proposed definitions have employed metabolic, biochemical, physiological, genetic, and thermodynamic parameters, but all such definitions face considerable drawbacks (*Sagan,* 1970; *Chyba and McDonald,* 1995; *Chyba and Phillips,* 2002). A popular working definition is that life is "a self-sustained chemical system capable of undergoing Darwinian evolution" (*Joyce,* 1994). While potentially useful on Earth, this definition is difficult to utilize when developing a search strategy. As pointed out by *Chyba and Phillips* (2002), "How long do we wait to determine if a candidate entity is 'capable of undergoing Darwinian evolution'?"

Only once before has a rigorous chemical search for life been conducted *in situ* with robotic spacecraft on another planet. The Viking Landers had three experiments as part of the biology payload (*Klein et al.,* 1972; *Klein,* 1978). The gas exchange experiment involved incubating soil samples with nutrients to prompt growth and gas exchange; the labeled release experiment used ^{14}C-labeled nutrients to monitor production of ^{14}C-gases evolved from the inoculated soil; and finally, the pyrolitic release experiment examined incorporation of ^{14}C into solid biomass within the soil. All three of the experiments implicitly utilized a metabolic definition of life, i.e., it was a search for the effects that living organisms have on the chemistry of their environment. The results of the biology experiments may have been ambiguous had it not been for the gas chromatograph mass spectrometer results showing that no organics were present to the limit of parts-per-billion (*Biemann et al.,* 1977). In this case, a biochemical definition (i.e., organics are needed for carbon-based life) trumped a metabolic definition (*Chyba and Phillips,* 2002).

Based on the lessons learned from the Viking Landers, *Chyba and Phillips* (2001) proposed the following approach for future robotic searches for life: (1) If the payload permits, conduct experiments that assume contrasting definitions for life. (2) Given limited payload, the biochemical definition deserves priority. (3) Establishing the geological and chemical context of the environment is critical. (4) Life-detection experiments should provide valuable information regardless of the biology results. (5) Exploration need not, and often cannot, be hypothesis testing. Planetary missions are often missions of exploration, and therefore the above guidelines must be put in the context of exploration- and discovery-driven science.

On the surface of Europa, our search will largely take the form of a biochemical search. Given the harsh surface conditions, we do not anticipate finding viable life forms on the surface, and thus metabolic or even genetic definitions would prove challenging. Such definitions could be useful with advanced landers capable of excavating to several tens of centimeters in depth, or if freshly exposed subsurface material from the ocean was accessible. For the case of point (3) in the above list, *Figueredo et al.* (2003) have established several geological criteria, specific to the surface of Europa, that can be used to guide the search for biosignatures. A variety of surface units (specifically chaos regions, bands, ridges, craters, and plains) were assessed in the context of the following criteria: (1) evidence for high material mobility; (2) concentration of non-ice components; (3) relative youth; (4) textural roughness (providing a possible shield from the degrading effects of radiation — although obviously complicating engineering considerations for a lander); and (5) evidence for stable or gradually changing environments.

Figueredo et al. (2003) argue that recent, low-albedo bands that fill interplate gaps present very high potential for biosignatures, based on the above criteria (see also Fig. 7). Similarly, smooth, salt-rich plains also rank very high. The evidence for melting and/or material exchange seen in chaos regions, along with their potential for being young in age and salt-rich, merits those surface units a high potential for biosignatures. Large craters and ridges both rank as moderate locales for hosting biosignatures, although we note that impact events could yield abiotic chemistry that complicates detection.

Using the limited high-resolution imagery from Galileo spacecraft, *Figueredo et al.* (2003) present several candidate features that earned the "very high" ranking. In Fig. 11 we show two smooth plain sites and one chaos site. The smooth plains have the added advantage of likely being a more welcoming site for future landed missions.

Ultimately the search for life on Europa is an exercise in geography, with the goal of connecting surface chemistry with the landscape on a local and global scale in order to understand the habitability of the subsurface and the expression of any subsurface life on the surface. A detailed map of surface age, registered with surface chemistry and radiolytic processing, will help determine endogenous vs. exogenous origins and potentially reveal clues about life in the subsurface. Chemical differences between surface features of similar age and type, but spread geographically across the surface of Europa, will serve as one mechanism for assessing radiolytic processing and modification. For example, were we to discover two geologically similar sites both very young in age — one on the leading hemisphere and one on the trailing hemisphere — but find that the trailing hemisphere site was rich with C–H, CO_2, CO, and C≡N, while the leading hemisphere site was rich in O=C–N and N–H, we might infer that the leading hemisphere material experienced less radiolytic processing and thus is more in-

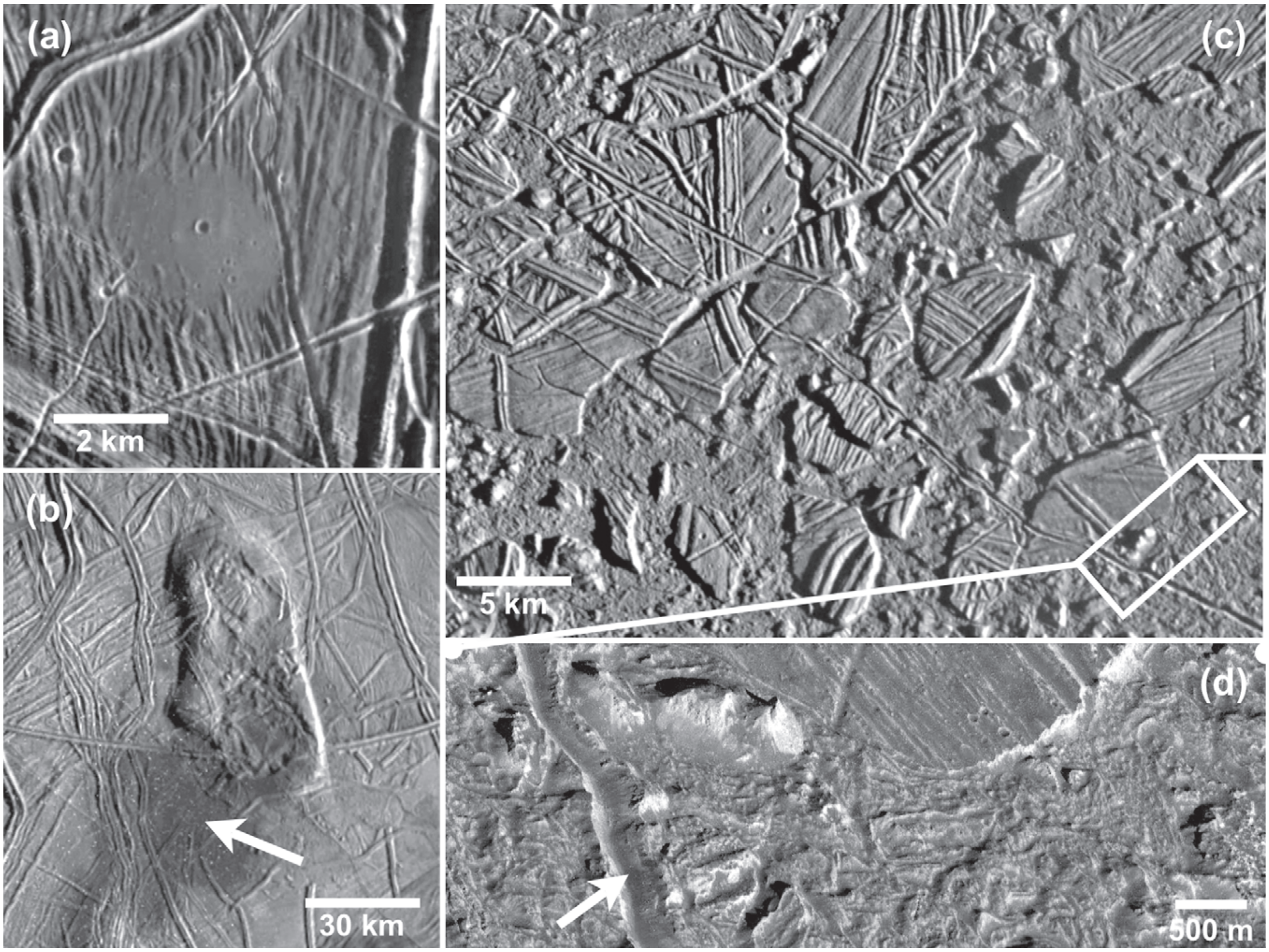

Fig. 11. Surface units on Europa with a "very high" biosignature potential, as determined by the criteria of *Figueredo et al.* (2003). **(a)** A smooth plains "puddle" feature; **(b)** the low-albedo, smooth terrain of Castalia Macula (arrow); **(c)** a portion of Conamara Chaos; and **(d)** a high-resolution version of the boxed inset (arrow indicates possible concentration of non-ice components along a fracture). Smooth plains features [e.g., **(a)** and **(b)**] have the added benefit of possibly offering a relatively benign landing site for future missions.

dicative of the parent endogenous material, possibly of biological origin. Complete radiolytic processing of the surface observable with orbital techniques (the upper ~100 μm of ice) is rapid compared to geological timescales, taking perhaps days to a few years depending on the composition and longitudinal position of the initial material. For a lander on the leading hemisphere, where sputtering erosion rates will also be low (*Paranicas et al.,* 2001; *Cooper et al.,* 2001), surface ages of 10^4–10^5 yr will be gardened to at most 1–10 cm (*Chyba and Phillips,* 2001; see chapter by Moore et al.), thus perhaps eliminating the need to employ drills to reach unprocessed material (melting, scooping, or a soft impact could provide the necessary excavation). As a result, for both orbital and lander measurements, identification of young or active regions is critical to assessing the endogenous chemistry and possible biomarkers on Europa.

Sublimation driven resurfacing may offer one way to access fresh, relatively unprocessed material on the surface of Europa. Regions with surface temperatures of 130–150 K will experience sublimation loss of H_2O at a rate of 1–1000 μm yr^{-1} (*Fagents et al.,* 2000). Non-ice material will be left behind and possibly concentrated as a result of water loss. Shear heating along ridge structures could be one place where thermal upwarping (*Nimmo and Gaidos,* 2002) leads to sublimation and exposure of fresh non-ice compounds. *Carlson et al.* (2005) used Galileo NIMS spectra to argue that such a mechanism, coupled with radiolysis and mass wasting, could explain the strong hydrated sulfate feature seen in the talus and on the flanks of ridges.

Detecting any biosignature on the surface — with a lander or orbiter — could be strongly limited by the concentration of biomass in the surface ice. Table 3 shows cell concentrations for several different environments on Earth. Concentrations in the surface waters of Earth's ocean are in the range of 5×10^3–5×10^5 cells ml^{-1}, or 10^{-6}–10^{-4} g of dry cell mass per kg of water [using 2.3×10^{-13} g cell^{-1} (*Madigan et al.,* 2003)]. The majority of this biomass is photosynthetic, not necessarily appropriate to Europa. The lowest biomass concentrations in the ocean are found in deep-ocean basins devoid of hydrothermal activity. In these

TABLE 3. Bacterial abundance of terrestrial ecosystems, in cells per milliliter.

	Abundance (cells ml^{-1})	References
Ocean, surface	5×10^3–5×10^5	[1,2]
Ocean, deep basins	10^3–10^4	[3]
Hydrothermal vents	10^5–10^9 (in suspension)	[3]
Hot Springs	~10^6	[4]
Microbial mats	10^7–10^9	[6]
Sierra Snowpack	10^3–10^4	[5]
Glacial ice	120	[1]
Vostok accretion ice	83–260	[1]
Vostok water (predicted)	150	[1]

References: [1] *Christner et al.* (2006); [2] *Whitman et al.* (1998); [3] *Van Dover* (2000); [4] *Ellis et al.* (2005); [5] *Painter et al.* (2001); [6] *Sorenson et al.* (2005).

regions, measured values are one to two orders of magnitude lower than surface waters. Cell concentrations in the water around hydrothermal vents can reach 10^9 cells ml^{-1} or roughly 0.2 g kg^{-1} (*Van Dover,* 2000). Of course on Europa, the hydrothermal population would need to somehow translate to a surface concentration, were we to be able to detect it with an orbiter or lander. *Winn et al.* (1986) studied the biological material in plumes above the Juan de Fuca Ridge and found detectable populations of bacteria and ATP for several hundred meters above the plume (Fig. 12). Plume dynamics on Europa could help transport such organisms to the ice-water interface (*Goodman et al.,* 2004; see chapter by Vance and Goodman), but populations would be severely diluted. Subglacial lakes, Vostok accreted ice, and glacial ice are all found to have a few tens to a few hundred cells per milliliter, or ~10^{-8}–10^{-7} g kg^{-1} of water (*Christner et al.,* 2006). Estimates of the total prokaryotic biomass within all Antarctic subglacial lakes, the ice sheet itself, and the suspected subglacial aquifer are 10^{24}, 10^{21}, and 10^{24} cells, which equates to 4.3×10^{-8}, 1.1×10^{-4}, and 1.15 Pg (petagram) of bacterial carbon (1 Pg = 10^{15} g) (*Priscu et al.,* 2008). The majority of this prokaryotic biomass is suspected to be present in the Antarctic subglacial aquifer. This south polar sub-ice biomass rivals many of the surface bacterial carbon pools on Earth (*Priscu and Christner,* 2004; *Priscu et al.,* 2008). Algal blooms in springtime snow can reach several thousands of cells per milliliter (10^{-7}–10^{-6} g kg$_{H_2O}^{-1}$) (*Painter et al.,* 2001). Microbial mats reach cell densities of 10^7–10^9 cells ml^{-1} (*Sorensen et al.,* 2005). In an ideal case for detection on Europa, similar densities would populate niches within and directly beneath the ice layer. Using radiolytic oxidants and carbon availability in the icy shell as limiting factors, *Chyba* (2000) estimated that cell densities could reach ~10^7–10^{10} cells ml^{-1} for ~1 mm of ice melt. Spread globally through a 100-km-thick subsurface ocean, however, this amounts to just 1 cell ml^{-1}.

Does the above range of concentrations permit detection from orbit? As mentioned in section 1.2, photosynthetic pigments such as chlorophyll, carotenoids, and xanthophylls are, through eons of selective pressure, uniquely suited to spectroscopic detection — they are tuned for light absorption and reflection and thus they are particularly sensitive

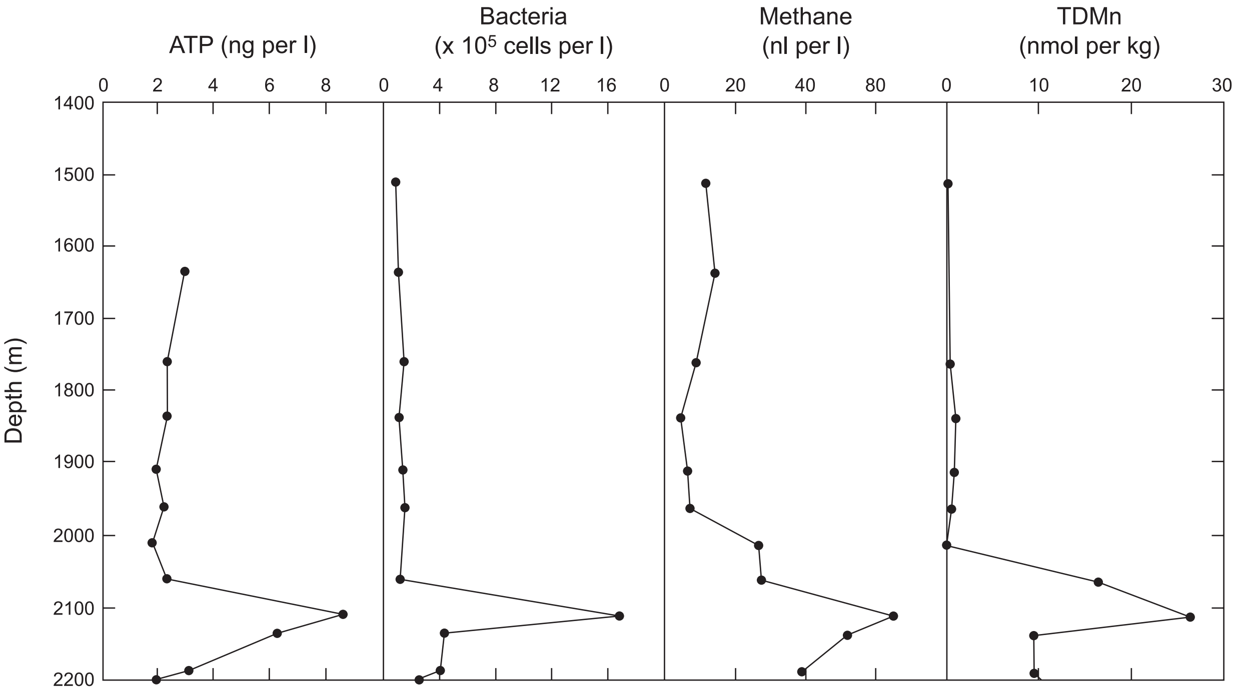

Fig. 12. Distribution of ATP concentration, bacterial cell number, methane, and total dissolved manganese (TDMn) at and above a hydrothermal vent on the Juan de Fuca Ridge, off the coast of the northwestern United States. Reproduced from *Winn and Karl* (1986), courtesy of Nature Publishing Group.

to detection via light spectroscopy. Sea surface phytoplankton populations are easily observable from orbit by monitoring chlorophyll-a absorption features in the 0.4–0.7-μm range (*Montes-Hugo et al.,* 2005). Similarly, using airborne spectroscopy, *Painter et al.* (2001) were able to detect the snow algae *Chlamydomonas nivalis* down to levels of ~2300 cells ml^{-1} by observing carotenoid absorption between 0.4 and 0.64 μm and chlorophyll-a and -b absorption at ~0.68 μm.

But photosynthesis is not a great metabolic model for a world covered in ice. Other colorful biological compounds associated with sulfur-, iron-, or oxygen-metabolizing microbes may serve as better biosignatures. Setting aside such compounds, however, what spectroscopic strategy might be useful based on just the organic chemistry of cell biomass? Consistent with the laboratory results discussed in section 2 (Fig. 10), the first spectroscopic line to look for should be CO_2, as radiolysis of carbon-based life on the surface of Europa will release this product, and it provides a strong absorption line. Nitriles and C–H features are also important, but would be hard to distinguish from abiotic radiolytic chemistry. The amide bands at 2.05, 2.17, 6.11, and 6.6 μm would help build the case for biochemistry. Table 4 shows the various frequencies and wavelengths for detection of biologically relevant molecules and functional groups. Approximately 55% of the dry cell mass of a microbe is attributable to proteins, 9% to lipids, and 20.5% to RNA (DNA is 3.1%) (*Madigan et al.,* 2003; *Loferer-Krössbacher et al.,* 1998). Given the above cell densities and mass fractions, this means that spectroscopic techniques must be sensitive to the ~1 ppb–200 ppm level to detect biochemistry at levels comparable to those found throughout our ocean and in hot springs, microbial mats, and snowpack. For glacial ice and Lake Vostok cell concentrations, sensitivities greater than 1 ppb would be required (*Priscu et al.,* 2006; *Christner et al.,* 2006). On Europa, geological processes such as the sublimation and mass wasting discussed above could help overcome low concentrations in the initial ice.

While infrared spectroscopy from orbit around Europa is hindered by water absorption and is limited to detecting only major species on the surface, naturally occurring ion bombardment (sputtering) constantly ejects molecules from the surface, potentially delivering them to orbital altitude where they could then be measured by an orbiting mass spectrometer or microwave spectrometer (*Johnson et al.,* 1998; *Allen et al.,* 2008). Europa's sputtered atmosphere is produced by ejection of molecules from the surface at velocities of up to ~1 km s^{-1} and these molecules can reach ~300 km in altitude. Both the parent molecule and decomposition products are ejected. For example, in H_2O sputtering, the products are mostly H_2O with a few percent as O_2 and H_2. Large organic molecules can be ejected intact or as decomposition products. If the amino acid glycine were sputtered off the surface, the glycine molecule plus the decomposition products NH_3, CO_2, CH_4, H_2O, and H_2CO, would be in the atmosphere. For a surface concentration of 100 ppm glycine, there could be a total glycine-related column density of 5×10^9 cm^{-2} and a volume density at orbital altitudes of a few hundred per cubic centimeter. This volume density is within the anticipated detection capability of modern, improved flight mass spectrometers (*Waite et al.,* 2006). Highly sensitive rotational-line millimeter-wave spectroscopic measurements of the sputtered atmosphere could make comparable detections (*Allen et al.,* 2008).

Unless the signs of life are readily apparent, e.g., fossils, strong pigment signatures, and other biosynthetic biomarkers, it will be difficult to make a *convincing* case for life on Europa from orbit. A compelling case is possible with the combination of orbital techniques and geography, but ultimately it will take a lander to resolve uncertainties. With a landed gas chromatograph mass spectrometer (GCMS) and microscope a much more certain case could be made for a definitive detection of life. *Chyba and Phillips* (2001) calculated that with onboard fluorescent high performance liquid chromatography roughly 10^2–10^3 kg of ice would need to be melted and filtered to achieve detection at levels of 0.1–1 cell ml^{-1}. For concentrations of 10^2–10^3 cells ml^{-1} the filtering requirement drops to 0.1–1 kg of ice. For ~50–100-g samples of Vostok ice, *Priscu et al.* (2007) were able to detect cell counts down to 10 cell ml^{-1} using the combination of epifluorescence microscopy and DNA stained cells. Because melting, evaporating, and drilling ice all require energy from the spacecraft, these are important engineering considerations (*Chyba and Phillips,* 2002). Additional decontamination protocols would also have to be implemented to ensure sample integrity (e.g., *Christner et al.,* 2005).

Finally, we note that at least in one instance, a spacecraft designed for the study of the Jupiter and the Galilean satellites was capable of discovering life on an inhabited world. The Galileo team famously detected life on Earth during Galileo's Earth flyby (*Sagan et al.,* 1993). Those results were, of course, largely biased toward the photosynthetic and technological signatures of life on Earth and would probably have been ineffective if focused on Earth's polar ice sheets. Nevertheless, the analyses revealed some of the challenges and ambiguities associated with biosignatures known to be derived from life here on Earth. Even on a world ripe with life, detection and confirmation of that life from a spacecraft is not entirely straightforward. Indeed, as we move forward with the challenge of searching for life beyond Earth, and as we work to define convincing signs of life on other worlds, we would do well to keep in mind advice from *Sagan et al.* (1993): "Life is the hypothesis of last resort."

4. PLANETARY PROTECTION

The potential habitability of Europa bestows a great responsibility on our future exploration of the europan surface and subsurface. Planetary protection refers to those policies and practices intended to (1) prevent the contamination of a celestial body by terrestrial microorganisms that could be present on a spacecraft at launch, survive the space

TABLE 4. Biologically important functional groups and molecular assignments for infrared spectroscopy at STP.

Frequency, cm^{-1} (Wavelength, μm)	Functional Groups and Molecular Assignments
25,000 (0.4–0.58)	Carotenoid pigment
16,667 (0.6–0.7)	Chlorophyll-a, -b pigments
10,000 (1.0)	O–H of water of hydration of parent compound ($\bullet H_2O$)
8000 (1.25)	O–H of water of hydration of parent compound ($\bullet H_2O$)
6667 (1.5)	O–H of water of hydration of parent compound ($\bullet H_2O$)
5000 (2.0)	O–H of water of hydration of parent compound ($\bullet H_2O$)
4878 (2.05)	Amide in proteins, N–H vibration with C–N–H bend
4608 (2.17)	Amide in proteins, N–H fundamental with C–N stretch
4348 (2.3)	C–H and methane
~3500 (2.86)	O–H stretch of hydroxyl groups
~3200 (3.1)	N–H stretch (amide A) of proteins
~2955 (3.38)	C–H stretch (a) $-CH_3$ in fatty acids
~2930 (3.4)	C–H stretch (a) $>CH_2$
~2918 (3.43)	C–H stretch (a) of $>CH_2$ in fatty acids
~2898 (3.45)	C–H stretch, CH in methine group
~2870 (3.48)	C–H stretch (s) of $-CH_3$
~2850 (3.51)	C–H stretch (s) of $>CH_2$ in fatty acids
2590–2560 (3.88)	–S–H of thiols
~1740 (5.75)	>C=O stretch of esters
~1715 (5.83)	>C=O stretch of carbonic acid
~1680–1715	>C=O stretch of nucleic acid
~1695 (6.0)	Amide I band components
~1685 (5.93)	Resulting from antiparallel pleated sheets
~1675 (5.97)	Amide I β-turns of proteins
~1655 (6.04)	Amide I of α-helical structures
~1637 (6.11)	Amide I of β-pleated structures
~1550–1520 (6.52)	Amide II
~ 1515 (6.6)	"Tyrosine" specific band
~1468 (6.81)	C–H deformation of $>CH_2$
~1400 (7.14)	C=O stretch (s) of COO^-
~1310–1240 (7.8)	Amide III of proteins
1304 (7.67)	CH_4, methane
~1250–1220 (8.0)	P=O str $>PO_3^-$, phosphodeiesters
1240–180 (8.26)	O–S=O stretch of sulfites
~1200–900 (8–11)	C–O, C–C, str of carbohydrates
~1200–900 (8–11)	C–O–H, C–O–C def. of carbohydrates
~1100–1000 (9.52)	P–O, PO_4^{-3} stretch
~1090 (9.17)	P=O stretch (s) $>PO_2$
~1085 (9.2)	C–O stretch
~1061 (9.4)	C–N and C–C stretch
1140–1080 (9.0)	S–O– stretch of inorganic sulfates
1070–1030 (9.52)	C–S=O of sulfoxides
~1004 (9.96)	Phenylalanine
~852 (11.7)	Tyrosine
~829 (12)	Tyrosine
~785 (12.7)	Cytosine, uracil (ring stretch)

Compiled from *Naumann et al.* (1991), *Naumann et al.* (1996), *Kummerle et al.* (1998), *Smith* (1999), *Choo-Smith et al.* (2001), *Painter et al.* (2001), *Maquelin et al.* (2002), *Dalton et al.* (2003), *Liu et al.* (2005), and *Hand* (2007).

voyage, and grow and multiply on that body ("forward contamination"); and (2) prevent any "back-contamination" of Earth by possible extraterrestrial biota that might be brought to Earth on a sample return mission. The National Research Council (*NRC,* 2006) has critically reviewed the history and current basis of planetary protection policy and practice in the Mars context; this section draws on that discussion, together with an earlier *NRC* (2000) report specific to Europa, and considers implications for preventing the forward contamination of Europa.

The idea of planetary protection dates to the beginning of the space age, with a letter from Nobel laureate Joshua Lederberg in 1957 (*Lederberg,* 1957) and a 1958 resolution by the U.S. National Academy of Sciences (*NAS,* 1958) stating that the Academy "urges that scientists plan lunar and planetary studies with great care and deep concern so that initial operations do not compromise and make impossible forever after critical scientific experiments." In 1964, the international Committee on Space Research (*COSPAR,* 1964) declared that since the forward biological contamination of Mars would make a search for life there far more difficult, "all practical steps should be taken to ensure that Mars be not biologically contaminated until such time as this search can have been satisfactorily carried out." From the beginning, then, planetary protection was concerned with what is sometimes called "protection of the science" rather than "protection of the planet" or protection of a possible alien biosphere *per se*.

The 1967 Outer Space Treaty provides the current legal requirement to conduct planetary protection (*UNOOSA,* 2007). Article IX of the treaty asserts that "States Parties to the Treaty shall pursue studies of outer space, including the moon and other celestial bodies, and conduct exploration of them so as to avoid their harmful contamination . . . and, where necessary, shall adopt appropriate measures for this purpose." The treaty does not define "harmful contamination," and one unofficial legal review of the treaty (*Cypser,* 1993) argues that "harmful contamination" is appropriately interpreted as "harmful to the interests of other states"; therefore, the concern must be with protecting the science that states might wish to pursue. Current NASA policy affirms that the purpose of planetary protection is to protect scientific investigations (e.g., *Rummel and Billings,* 2004), and this has been the approach taken by COSPAR for the past four decades. Notably, however, the National Research Council (*NRC,* 2000) report on *Preventing the Forward Contamination of Europa* explicitly stated that limiting the forward contamination of Europa "is necessary to preserve the scientific integrity of future biological studies and to protect any indigenous life forms." Similarly, the *NRC* (2006) panel on *Preventing the Forward Contamination of Mars* called for an international workshop to discuss whether planetary protection should be extended beyond protecting the science to protecting the destination planet's possible biosphere. As a practical matter, especially in harsh and cold conditions where biological growth and reproduction might be expected to be quite slow, such a commitment might lead to substantially more rigorous planetary protection requirements than would otherwise be the case.

The threat of biological contamination of Europa via spacecraft cannot be easily dismissed. Experiments on NASA's Long Duration Exposure Facility (LDEF) demonstrate that spores of *Bacillus subtilis* survive six years in space at the 1% level, provided that they are shielded from solar ultraviolet light, as any organism within a spacecraft would be (*Horneck et al.,* 1994, 1995). The timescale of the LDEF experiment approximates that required for a spacecraft to reach Jupiter from Earth. Spacecraft assembled within standard NASA class-100,000 clean rooms (meaning a clean room with 100,000 0.5-μm-diameter particles per cubic foot of air) have bacterial spore surface densities of ~10^3 spores m^{-2} (*Barengoltz,* 2004); total spacecraft surface bioburden is at least two orders of magnitude greater (*NRC,* 2006). Therefore, additional measures must be taken or other factors must come into play to prevent a spacecraft landing or crashing on the europan surface from delivering viable microbes.

Whether such organisms could then reach the ocean in a still-viable state, and grow and multiply within that ocean, is a question whose answer is very difficult to quantify. Such organisms would have had to survive the jovian radiation environment while in orbit around Jupiter and/or Europa, and be buried in Europa's ice quickly enough to survive the surface radiation environment. They would then have to be delivered into Europa's ocean, and be able to survive and reproduce in that environment. Clearly, this adds up to a formidable set of challenges. However, without intervention, a potentially large number of microorganisms of currently poorly known characteristics might be present at the time of launch, so the possibility, while likely remote, cannot currently be excluded.

The Task Group on the Forward Contamination of Europa (*NRC,* 2000) attempted to quantify the probability of forward contamination by considering four classes of microorganisms: common microorganisms, general spore-formers, radiation-resistant spore-formers, and non-spore-forming highly radiation-resistant microbes. Majority opinion in the Task Group argued that NASA should adopt a requirement that the probability P_c of forward contamination of Europa be below 1 in 10,000. They argued that this criterion could be met with minimal groundbased spacecraft bioburden reduction, largely because of postlaunch sterilization effects in Europa's radiation environment.

Greenberg and Tufts (2001) criticized the Task Group's approach, arguing that P_c should be based on a transparent criterion, and recommended that the criterion chosen be that the probability that the space program contaminate Europa be "substantially smaller than the probability that such contamination happens naturally." Certainly P_c should be set low enough so that, integrated over the total number of future missions to Europa (perhaps 10, but maybe 100?), the probability of contamination is small. How small is acceptably small may depend in part on whether one's concern is with "protecting the science" or protecting a possible alien biosphere. The "natural background" criterion of *Greenberg and Tufts* (2001) may prove to set an extremely stringent requirement for Europa, because viable microbial transfer in terrestrial meteorites to Europa is likely very difficult (summarized by *Chyba and Phillips,* 2007). Understanding this problem in detail (*Gladman et al.,* 2006) may be important for providing context for setting planetary protection requirements.

5. CONCLUSIONS

Europa is a prime target for astrobiology. Our current understanding of the geology, geophysics, and chemistry of Europa provide good reason to expect that Europa may be habitable at present, and may have been habitable throughout much of its past. Foremost among the attributes that could make Europa habitable is the likely presence of a global subsurface liquid water ocean with some 2–3 times the volume of all the liquid water on Earth. But while liquid water is a necessary condition for life as we know it, it is not a sufficient condition. Also required is a suite of elements essential to life and a source of energy that can sustain the growth, reproduction, and maintenance of life. In the context of elemental abundance, the formation of Europa in the jovian subnebula likely led to a bulk composition comparable to that of chondritic material, which is rich in carbon, nitrogen, phosphorous, sulfur, and other key elements needed for life.

Energy for life on Europa may be a critical limiting factor. The surface temperature and pressure and radiation environment of Europa make it an inhospitable environment for life, thus photosynthesis on the surface will not be possible. Penetration of sunlight through meters to tens of meters of ice could power photosynthetic life beneath the surface; however, such an ecosystem necessitates very thin ice, a requirement inconsistent with many models. Therefore, harnessing the energy passively received from the Sun is likely a limited niche. Alternatively, chemosynthetic metabolic pathways within the ocean and at the seafloor could present a much larger energetic niche for life in the europan subsurface. Important to maintaining such pathways through time is the active geological cycling of seawater through reducing mantle material on the europan seafloor, and with oxidizing material produced radiolytically on Europa's surface. Reductant delivery via seafloor hydrothermal activity is a well-studied process that helps drive ecosystems here on Earth. Radiolytic production of oxidants in ice, however, has no direct terrestrial analog other than atmospheric photolysis of water. Europa's radiation environment may play a critical role in maintaining the reductant-oxidant (redox) chemical pairing needed to power life.

Life on Europa would likely represent a second, unique origin of life within our solar system. The salinity and oxidation state of the ocean could, however, be an impediment to prebiotic chemistry. Hydrothermal seafloor systems or eutectic melts within the icy shell would provide useful microenvironments for promoting chemistry important to life's origin. Additionally, laboratory work indicates that radiolytic surface chemistry on Europa could result in the synthesis of prebiotic compounds such as amino acids and nucleobases.

A key question, and common theme for understanding the habitability and detectability of life on Europa, persists: Is Europa geologically active? If so, where is the activity and what is the timescale for cycling of material? Tidal dissipation may create an active seafloor and icy shell, driving the chemical cycling needed to maintain life on Europa and needed to bring such life to the surface, where it can be discovered by future missions.

Detection of life on Europa in the coming decades will be limited by the extent to which the surface ice provides a window to the subsurface ocean chemistry. Orbiting spacecraft and surface landers could target young and fresh surfaces where radiolytic processing has not yet destroyed compounds and structures indigenous to the ocean. Chemical bonds indicative of structural complexity and organization, such as those associated with the amide bands and phosphodiester bands of proteins and nucleic acids, could serve as spectroscopic biosignatures. Mass spectrometry of material showing possible biosignatures could be critical for confirming a biochemical detection of life, and microscopic imagery would be essential for confirming morphological and structural biosignatures.

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