Enceladus Astrobiology, Habitability, and the Origin of Life

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Analyses of the plume of icy material emanating from the south polar terrain of Enceladus by the Cassini spacecraft point to a habitable subsurface ocean. Icy particles in the plume, originating from an ocean of liquid water in contact with a rocky core, contain organic molecules, biologically available nitrogen, sodium, potassium, hydrogen, and carbon dioxide. The hydrogen is inferred to originate from alkaline hydrothermal activity and is present at a level adequate to support methanogenic microorganisms. The biogenic elements (C, H, N, O, P, S) have been detected or are expected to be present due to the rock–water interactions. The strong indication of habitable conditions contrasts with the question of the origin of life. If deep-ocean alkaline hydrothermal vents are suitable sites for the origin of life, then the most basic conditions are met for a second genesis (*McKay,* 2001) of life on Enceladus, and evidence of life could be found in the ocean. However, if life requires surface environments for its origin, and if panspermia has not been effective in the case of Enceladus, then this world could be habitable but uninhabited. Thus, near-term missions to Enceladus will try to shed light on these possible scenarios by searching for biomolecules in plume materials. Evidence for life might appear as a molecular pattern indicative of biological production; e.g., a set of several amino acids with common chirality that could represent a basis for protein assembly. Lacking analog environments on Earth that have all the features of the Enceladus ocean, a better understanding of possible ecosystems on Enceladus can still be constructed piecewise. Relevant features of analog environments on Earth include dark, anoxic water bodies virtually sealed by ice, anaerobic chemoautotrophic microbial ecosystems, and low-temperature alkaline hydrothermal vents.

1. INTRODUCTION

Enceladus is likely to be a world habitable for a range of Earth microorganisms. The plume emanating from the south polar region was discovered by Cassini in 2006 (*Hansen et al.,* 2006; *Porco et al.,* 2006; *Spencer et al.,* 2006; *Waite et al.,* 2006) and has been investigated extensively as the spacecraft flew through it repeatedly. The data from these flybys indicate that the plume originates from a global ocean below a surface layer of ice (*Iess et al.,* 2014; *Thomas et al.,* 2016). The Ion and Neutral Mass Spectrometer (INMS) on Cassini detected hydrogen, carbon dioxide, and organic compounds up to C_6 (*Waite et al.,* 2009, 2017). The hydrogen and carbon dioxide form a redox couple suitable for supporting methanogens (*McKay et al.,* 2008). Nitrogen is present in the

form of ammonia, and sulfur has been tentatively detected in the form of hydrogen sulfide (*Waite et al.,* 2009). If the preliminary analysis of *Khawaja et al.* (2015) is correct, then the Cosmic Dust Analyzer (CDA) instrument detected biologically available nitrogen in the form of amines. The CDA also detected organic compounds of high molecular weight in E-ring particles derived from the plume (*Khawaja et al.,* 2015; *Postberg et al.,* 2017). Sodium and other salts detected in the E ring and lower parts of the plume indicate that the ocean salinity is about 0.5% to 2% dominated by NaCl (*Postberg et al.,* 2009, 2011) — a water activity near unity that is suitable for life as we know it. The detection of nanometer-sized silica particles in the E ring, and inferred to be derived from the plume, suggest that the ocean hosts hydrothermal vents in which water is in contact with the

rocky core at temperatures of at least ~100°C and that the pH of the ocean is between 8.5 and 10.5 (*Hsu et al.,* 2015; *Sekine et al.,* 2015). All the major elements needed for life (C, H, N, O, P, S) have been detected or are expected to be present due to the rock–water interaction. Thus there is good indication that the ocean on Enceladus is habitable and that the icy particles in the plume are samples of that habitable water.

However, *habitable* does not necessarily mean *inhabited* (*Hand et al.,* 2009; *Cockell,* 2011). A search for signatures of life in the habitable ocean of Enceladus directly confronts different hypotheses for the origin of life (*Davis and McKay,* 1996). The astrobiology, habitability, and origin of life questions pertaining to Enceladus motivate future missions to sample icy grains from the plume and search for signatures of life, using comparisons with life on Earth. In this chapter we expand on these topics in more detail, reviewing our current knowledge of the astrobiology of Enceladus and emphasizing the big questions that lie ahead.

2. THE ORIGIN OF LIFE

A significant, if often overlooked, challenge in considering the astrobiological potential of Enceladus is the possibility of origin of life events. The difficulty arises from the lack of a consensus regarding how life originates. In the case of life on Earth, the only fact we have about the origin of life is that it happened more than 3.5 G.y. (e.g., *Djokic et al.,* 2017). We do not know where, when, or how life originated, or how long it took. The hypothesis that the one example of life we see on Earth originated on Earth and/or took a long time is not supported, nor excluded, by any geological evidence.

The scientific inquiry into the origin of life is further hampered by the lack of any necessary connection between environments that can support life and environments in which life can originate. As discussed above, there is compelling evidence that the ocean on Enceladus is an environment that can support life (it is likely habitable for many Earth microorganisms), but we have no criteria with which to judge if it is an environment that can promote the origin of life.

Similar to the case of life on Earth, there are two broad classes of hypotheses for an origin of life on Enceladus: (1) those that postulate a local origin of life event; and (2) those that postulate the transfer of life — panspermia — from other sources in the solar system, or from sources outside the solar system (i.e., presolar grains), even prior to its formation. The transfer of life to Enceladus from sources in the inner solar system (i.e., Earth or Mars) is unlikely due to its relative isolation from impact debris created in the inner solar system (*Worth et al.,* 2013). The transfer of life to Enceladus from sources in the outer solar system (i.e., other icy moons) might have been more likely, but has not yet been thoroughly addressed in the literature. The arrival of presolar grains carrying life forms that became seeds of life on Enceladus (and Earth) cannot be dismissed *a priori*,

and would have resulted in a common biochemistry across the solar system — a hypothesis that can only be tested when life is discovered outside the Earth.

While different forms of panspermia are worth considering, more attention is directed toward hypotheses that life originated on Earth itself. Here the debate of interest to Enceladus astrobiology is the role of submarine hydrothermal vents in the origin of life. *Russell* (2003) and *Russell et al.* (2014) have suggested that life originated on Earth in alkaline hydrothermal vents similar to the Lost City system; the evidence for hydrothermal activity on Enceladus is consistent with the origin of life there as well (see also *Barge and White,* 2017). In direct contrast with this hypothesis, *Deamer et al.* (2006), *Deamer and Georgiou* (2015), and *Hud et al.* (2013) have argued that the origin of life requires periodic drying to concentrate biologically relevant molecules in order to form biopolymers and enclosed vesicles. They suggest that the origin of life occurred on Earth in a land environment that experienced repeated cycles of wetting and drying. *Deamer and Damer* (2017) explicitly state that their work suggests that Enceladus may be habitable but would be uninhabited.

Even if the geochemical environment that could promote the origin of life was understood, the question of duration would still be relevant. The record of life on Earth provides very little information on how long it took for life to start on this planet; the first appearance of life occurred within 100 to 500 m.y. after the formation of Earth. Clearly this is an upper limit set by the resolution of the historical record, and the origin of life may have been much faster. Considering this question, *Lazcano and Miller* (1994) suggested that "in spite of the many uncertainties involved in the estimates of time for life to arise and evolve to cyanobacteria, we see no compelling reason to assume that this process, from the beginning of the primitive soup to cyanobacteria, took more than 10 million years." However, *Orgel* (1998) criticized this argument on the grounds that we do not understand the steps that lead to life and consequently we cannot predict the time required: "Attempts to circumvent this essential difficulty are based on misunderstandings of the nature of the problem." Thus the problem of the time required for the emergence of life, like the question of its location, remains unsolved with the current data.

The age of Enceladus is unknown, as is the persistence of the ocean over that age. Conventionally, it is assumed that Saturn's moons are nearly as old as the planet, and they formed near the end of the planet's formation (*Canup and Ward,* 2006). However, recent dynamical modeling may imply that the inner moons, including Enceladus, are only ~100 m.y. old (*Ćuk et al.,* 2016). If the origin of life does occur at low-temperature hydrothermal vents, would it have occurred in the ocean of Enceladus in 100 m.y.? We have no basis with which to answer this question, emphasizing again the importance of an empirical approach to the origin of life on other worlds.

The lack of scientific understanding of the origin of life even on Earth implies that we cannot state with confidence

that a search for life in the habitable ocean of Enceladus will be fruitful. But we can state that results of a search for life on Enceladus, whether positive or negative, will provide important constraints on our models for origins of life anywhere. The science that is currently limited to the single data point of the "habitable and inhabited Earth" would finally have a second data point, providing an enormous increase in available information and perhaps enabling the long-sought-after comprehensive theory for the emergence of life on Earth (*Lazcano and Hand,* 2012).

3. HABITABILITY: SUSTAINABILITY AND LIMITS FOR LIFE

In a comprehensive review of the requirements and limits for life in the context of exoplanets, *McKay* (2014a) provided three tables: one on the ecological requirements for life, a second on the elemental abundances by mass used in life, and a third on the ecological limits to life. These have been variously used and defined from previous studies (*McKay,* 2014a, and references therein). It is useful therefore to reiterate the entries in these tables and add a column for Enceladus. This is shown in Table 1.

At the basic level of the requirements for life — liquid water, energy, carbon, and other key elements — Enceladus meets all the requirements. The second set of requirements specifies the elements needed for life in detail. Here we find that Enceladus is *likely* to meet all the requirements, but the presence of P and Ca are inferred, not direct. Phosphorus is of particular importance and is expected based on the composition of meteoritic materials and especially its detection in comets (*Altwegg et al.,* 2016). However, the low solubility of phosphate minerals in alkaline water could limit the availability of P in the Enceladus ocean (*Zolotov,* 2012). The third set of requirements is that used to characterize the limits of life in extreme environments. Here, the ocean of Enceladus easily meets all requirements except for the level of sunlight needed for photosynthesis. Thus, the habitability of Enceladus is contingent on the presence of chemical redox couples or alternative sources of energy.

Although the habitability of Enceladus is formally established — pending the expected confirmation of the elements P and Ca, metals such as Fe, and a firmer detection of S — there are still gaps in our understanding of the physical and chemical characteristics of the Enceladus ocean that need to be addressed. In terms of the bulk ocean properties, the key values of pH and salinity are only crudely constrained. The pH in particular is uncertain by 4 orders of magnitude (in terms of H^+ activity) (*Glein et al.,* 2015). Direct measurements of pH to ±1 and salinity to ±10% are needed to refine the assessment of the habitability of Enceladus in terms of possible types of microbial ecosystems that could be present, and availability of nutrients and reaction pathways. The only potential biological energy source clearly indicated by the Cassini INMS results is the $CO_2 + H_2$ redox reaction (*Waite et al.,* 2017). It is interesting to note that this reaction is only used by Archaean methanogens, and if this is the only form

of biological energy available in the ocean of Enceladus, it points to a particular type of microbial ecosystem as the basis for all primary biological production. An interesting note is the high requirement for the element Ni observed in methanogens on Earth. This requirement has been suggested to have global ecological implications over Earth's history (*Konhauser et al.,* 2009). The concentration of Ni in the ocean of Enceladus is unknown, but may be restricted by the low solubility of Ni-bearing sulfide minerals in a reduced ocean (*Zolotov,* 2012).

To obtain a more quantitative understanding of the habitability of Enceladus' ocean, an initial step is to assess the amount of chemical (free) energy that could be harnessed if metabolic reactions were performed by organisms in the ocean. The Cassini INMS instrument detected CO_2, H_2, and CH_4 in the plume gas (*Waite et al.,* 2017), which enables an evaluation of the energy yield from the methanogenesis reaction

$$CO_2\,(aq) + 4H_2\,(aq) \rightarrow CH_4\,(aq) + 2H_2O\,(l) \qquad (1)$$

The amount of Gibbs free energy that would be released from the reaction is equivalent to the chemical affinity of the reaction (A), which can be calculated as

$$A = 2.3026RT\left(\log K - \log Q\right) \qquad (2)$$

where R denotes the gas constant (8.3145×10^{-3} kJ mol^{-1} K^{-1}), T ≈ 273 K for Enceladus' ocean, K designates the equilibrium constant, and Q the reaction quotient. The equilibrium constant can be computed from widely available standard-state thermodynamic data (i.e., Gibbs energies of formation). Here, we use log K = 37.44 (*Waite et al.,* 2017). The reaction quotient for the methanogenesis reaction can be approximated as

$$\log Q \approx \log\left(\frac{CH_4}{CO_2}\right) - 4\log\left[H_2\right] \qquad (3)$$

for dilute (ideal) aqueous solutions, where the CH_4/CO_2 ratio corresponds to the ocean and $[H_2]$ represents the molal concentration of H_2 in the ocean. *Waite et al.* (2017) assumed that the CH_4/CO_2 ratio in the ocean is similar to that in the plume (~0.4) for a scenario of rapid degassing of these volatiles from droplets of ocean water. They developed an approach for estimating the dissolved concentration of H_2 by assuming that the ratio of H_2/CO_2 is also conserved between the ocean and plume. This ratio can be expressed as $H_2/CO_2 = (H_2/H_2O)/(CO_2/H_2O)$, in which $CO_2/H_2O \approx 0.005$ (*Waite et al.,* 2017). Multiplying the H_2/CO_2 ratio times the concentration of CO_2 in the ocean gives the concentration of H_2. The concentration of CO_2 was constrained using a speciation model (*Glein et al.,* 2015) with input parameters of pH and total dissolved carbonate [nominally 0.03 molal HCO_3^- + CO_3^{-2} (*Postberg et al.,* 2009)]. This approach allows the affinity for methanogenesis to be calculated as a function of pH and the relative abundance of H_2 in the plume (Fig. 1).

TABLE 1. Habitability of Enceladus.

| Requirement	Limit (from *McKay,* 2014a)	Enceladus Ocean
Ecological Requirements for Life		
Energy		
Predominately light	Photosynthesis at 100 AU light levels	No
Chemical energy	e.g., $H_2 + CO_2 \rightarrow CH_4 + H_2O$	Only H_2, CO_2 confirmed
Carbon	Common as CO_2 and CH_4	CH_4, organics to C_6 + CO_2
Liquid water	Rare, certain on Earth	Firm detection of water of suitable salinity
N		Present as NH_3 and possibly amines
P, S, Na, and other elements	Likely to be common	P possible from water-rock interaction S tentatively as H_2S Na, K, Cl detected
Elemental Abundances by Number in E. coli		
O	68%	As H_2O
C	15	CO_2, organics to C_6 +
H	10.2	As H_2O, H_2
N	4.2	Present as NH_3 and possibly amines
P	0.83	Inferred from Na
K	0.45	Detected
Na	0.40	Detected
S	0.30	Tentatively detected as H_2S
Ca	0.25	Inferred from Na
Cl	0.12	Detected
Ecological Limits for Life		
Lower temperature	$\sim -15°C$	Above limit ($\sim 0°C$)
Upper temperature	122°C	Below limit in bulk ocean
Low light	$\sim 10^{-4}\ S_o$	Zero light
pH	0–11	5–10, within limits
Salinity	Saturated NaCl	3% salt, below limit
Water activity	0.6 (yeasts) 0.8 (bacteria)	0.97 above limit
UV	≥ 1000 J m^{-2}	Zero UV, below limit
Radiation	50 G.y./hr	Crustal radiation only, from U, Th, K on the order of ~ 0.2 rad/yr, below limit

For the present best constraints of pH ~9–11 and H_2 mixing ratio [~0.4–1.4% (see *Waite et al.,* 2017)], the affinity based on the preceding model lies between ~50 and ~120 kJ per mole of CH_4. This is larger than the free energy required for methanogens to grow, as determined experimentally by *Kral et al.* (1998) to be about 40 kJ mole^{-1}. It is much larger than the theoretical maintenance energy of ~10 kJ mole^{-1} (*Hoehler,* 2004; *Hoehler et al.,* 2007), which may be the minimum energy that is required to permit the persistence of organisms (at least on Earth). The excess of chemical energy at Enceladus provides a strong case that its ocean is energetically habitable.

An alternative energy source motivated by the possible presence of complex organics detected in the plume is fermentation. Fermentation is a general term that refers to the acquisition of biological energy that does not require an external oxidizing or reducing agent, but instead the organic compound undergoes self-disproportionation (fragmentation to more oxidized and more reduced species). The familiar example of this is the process that converts glucose to smaller organic molecules. Typically fermentation does not proceed to completion and, for example, one glucose molecule can be converted into two ethanol molecules and two carbon dioxide molecules

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 \tag{4}$$
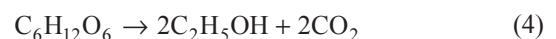
in a reaction, well known and often banned. As another example to note, bacteria such as *Clostridium pasteurianum* ferment glucose, producing carbon dioxide and hydrogen gas plus other organics via reactions such as (see review by *Thauer et al.,* 1977)

$$C_6H_{12}O_6 + 4H_2O \rightarrow$$
$$2CH_3COO^- + 2HCO_3^- + 4H^+ + 4H_2 \tag{5}$$
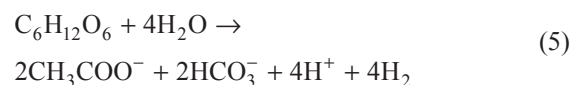
There is no evidence of glucose in the plume of Enceladus, but even if present, it would not have been detectable by

the instrumentation on Cassini because of its relatively high mass and its many isomers.

Schink (1985) showed that C_2H_2 can be the basis of a fermentation metabolism by the organism *Pelobacter acetylenicus*. This is perhaps not surprising given the chemical energy stored in the carbon-carbon triple bond. The reaction generates acetaldehyde by the reaction

$$C_2H_2 + H_2O \rightarrow CH_3CHO \qquad (6)$$

Oremland and Voytek (2008) have suggested C_2H_2 fermentation as a possible metabolism for life in Enceladus and CH_3CHO as a potential biomarker. However, *Waite et al.* (2009) reported an upper limit of 7×10^{-4} on the mixing ratio of C_2H_4O in the plume gas. Detection of C_2H_2 in the plume of Enceladus remains ambiguous partly because C_2H_2 (and other "high-energy" organics) could be produced by impact chemistry of complex organic materials in the titanium antechamber of the Cassini INMS (*Waite et al.,* 2009). *McKay et al.* (2012) suggested that if C_2H_2 is present in Enceladus, it would reflect an accreted source of this compound because thermal, hydrothermal, or biological processes would not be expected to produce it. C_2H_2 and other unsaturated hydrocarbons such as C_2H_6 can release energy by reaction with H_2, as has been suggested for Titan (*McKay and Smith,* 2005).

A further energy source could be the direct use of geophysically released electrons. Recent studies have shown that some microorganisms can directly use electrons for their metabolism and biomass production (*Nielsen et al.,* 2010; *Rosenbaum et al.,* 2011; *Bose et al.,* 2014). Electrons can be

made available within seafloor sediments and hydrothermal fields (*Nielsen et al.,* 2010; *Yamamoto et al.,* 2017).

The persistence of H_2 in the ocean is key to available energy for life. As discussed above, the H_2 and CO_2 present in the ocean provide a basis for methanogens that form CH_4 and biomass, and are the primary producers in the microbial community that potentially form biofilms at the core-ocean interface (or in fractures inside the core). Clearly, with no way to recycle this CH_4 and biomass, the system would eventually die down.

In addition to being produced by rock-water interactions, H_2 on Enceladus can be regenerated from CH_4 at high temperature — effectively cycling biological carbon. Figure 2 shows a schematic of how H_2 could be recycled in the hot core of Enceladus, providing a continuing fuel for a methanogenic ecosystem in the ocean. Water flowing through the subsurface could reform H_2 and CO_2 from CH_4 and H_2O. For simple cometary ratios of C, H, and O (e.g., *McKay and Borucki,* 1997; *Kress and McKay,* 2004), the shift in thermodynamic stability with temperature favors CO_2 above about 500°C. It is not clear that the core of Enceladus could be this hot at present. Thermodynamic calculations (*Glein et al.,* 2008) imply lower temperatures in the presence of mineral redox buffers that set the equilibrium oxidation state. For magnetite and hematite (i.e., the MH buffer) the minimum temperature for recycling CH_4 to CO_2 is as low as 25°C if the abiotic reaction kinetics are suitable (*Glein et al.,* 2008, Fig. 1). However, the formation of hematite usually requires highly oxidizing environments (e.g., for Earth, an O_2-rich atmosphere). In contrast, pyrrhotite-pyrite-magnetite (PPM) or fayalite-magnetite-quartz (FMQ) buffers are more representative of submarine hydrothermal systems on Earth. For these buffers, the required temperatures for a significant amount of CO_2 reformation exceed ~250°C (*Glein et al.,* 2008, Fig. 1). A key question then is the maximum temperature of ocean water as it circulates through the core of Enceladus and how this relates to the observed (high) levels of H_2 in the plume (*Waite et al.,* 2017) and the inferred conditions of the hydrothermal system (*Hsu et al.,* 2015; *Sekine et al.,* 2015).

McCollom (1999) developed a quantitative model of a methanogen-based biota for Europa and a similar analysis has been applied to Enceladus (*Steel et al.,* 2017). Steel
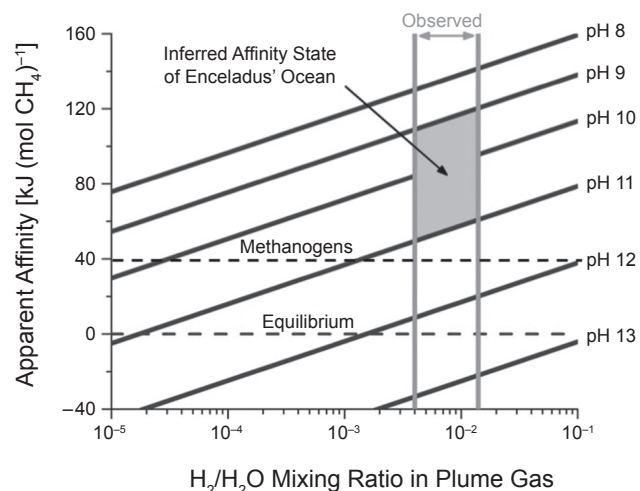

Fig. 1. Reaction energy for the reaction of hydrogen and carbon dioxide in the ocean of Enceladus as a function of the H_2/H_2O mixing ratio in the plume for various values of pH. The dotted lines show energy of 0 and energy of 40 kJ/mole — the minimum for methanogens based on experiments by *Kral et al.* (1998). The inferred state of Enceladus' ocean is shown by the shaded regions and indicate sufficient energy to support methanogens. Modified from *Waite et al.* (2017).
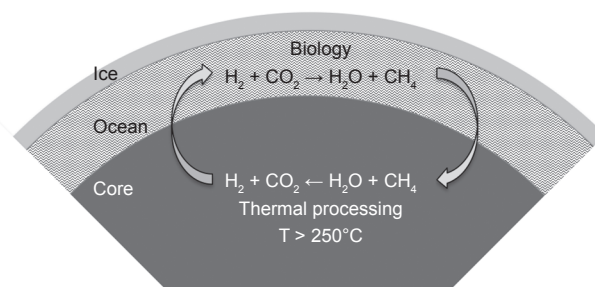

Fig. 2. Possible carbon cycle on Enceladus based on methane recycling by thermal reactions in the core.

et al. estimated, based on the energy flux observed at the south pole and the inferred internal hydrothermal activity, that H_2 production is 0.6–34 mol s^{-1} from serpentinization, sufficient to sustain abiotic and biotic amino acid synthesis of 0.005–0.25 g s^{-1} and 1–52 g s^{-1}, respectively. Assuming methanogens consume virtually all the H_2 implies up to 90 µM concentrations of amino acids and cell concentrations of 80–4250 cells cm^{-3} in the plume and the ocean (*Steel et al.,* 2017). Abiotic processes alone imply glycine, alanine, α-aminoisobutyric acid (AIB), and glutamic acid in the plume and in the ambient ocean would all be above 0.01 µM. No enantiomeric excess is expected in the ocean in either case, because racemization timescales are short compared to production timescales. Clearly the H_2 concentrations reported by *Waite et al.* (2017) of 0.4% to 1.4% in the plume are not consistent with the assumption that biology consumes all the H_2 produced, suggesting either the lack of biology or greatly reduced efficiency of H_2 consumption, due perhaps to a limited spatial extent of the methanogen biofilms. For example, *Kral et al.* (1998) showed a variety of methanogens will consume H_2 to low levels, to partial pressures as low as ~4 Pa. Given the utility of H_2 in all energy schemes discussed above, its abundance in the ocean at levels well above 4 Pa [~20–~10^4 Pa (*Waite et al.,* 2017)] would seem inconsistent with biological consumption — high levels of H_2 may be an anti-biomarker.

The high levels of H_2 in the plume (*Waite et al.,* 2017) have been attributed to production by hydrothermal reactions (e.g., through serpentinization) within the core or, as discussed above, by thermal processing in the core. However, models of these processes do not predict ongoing production (e.g., *Malamud and Prialnik,* 2013). Furthermore, the mineralogy of the core and, in particular, indications that it has not undergone igneous differentiation (*Sekine et al.,* 2015) may further suggest a short timescale for H_2 production by consumption of unaltered, primary minerals by hydration within Enceladus. These explanatory issues notwithstanding, the direct measurement of H_2 in the plume motivates the study of this microbial energy source.

The movement of ocean water through the core of Enceladus could also recycle nitrogen. Organic matter from carbonaceous chondrites releases NH_3 when heated to 300°C (*Pizzarello and Williams,* 2012). For this moderate level of heating, NH_3 dissociation into N_2 and H_2 is kinetically inhibited (*Sekine et al.,* 2015). For much higher temperatures (e.g., ~500°C), where CH_4 and H_2O are effectively converted into CO_2 and H_2, NH_3 also could dissociate into N_2 and H_2 (*Matson et al.,* 2007). However, such high-temperature processing is not expected and would be inconsistent with the observations of low N_2 in the plume (*Hansen et al.,* 2011; *Waite et al.,* 2017).

4. ANALOGS ON EARTH

An astrobiology analog on Earth of the ocean on Enceladus would be (1) a dark, anoxic water body virtually sealed by ice; (2) an environment containing an anaerobic chemo-autotrophic microbial ecosystem; and (3) a recirculating, low-temperature, alkaline, hydrothermal vent. No known environment on Earth has all three of these features, but there are systems that have one of the three characteristics, and these systems can help inform our understanding of the astrobiological potential of Enceladus.

Physical analogs for Enceladus are ecosystems sealed under ice, but such systems are not common. Most of the lakes in the Dry Valleys (Lakes Vanda, Hoare, Fryxell, Bonney, and Joyce) are ice-covered, not ice-sealed. The ice floats, and each summer a moat forms. Indeed, the energy balance that sets the thickness of the ice cover and the area of these lakes depends on summer melt water flowing into the lake (e.g., *McKay et al.,* 1985; *Chinn,* 1993). The lakes found beneath kilometers of ice on the polar plateau (e.g., Lake Whillans and probably Lake Vostok) are also not sealed — they are part of an extensive network of flow underneath the plateau (see, e.g., *Fricker and Scambos,* 2009; *Vincent and Laybourn-Parry,* 2008).

The best known example of an ice-sealed ecosystem is Lake Vida in the Dry Valleys of Antarctica (*Murray et al.,* 2012). This system receives no material flow and no sunlight. Early observers and models of Lake Vida assumed it was frozen to its base (*Calkin and Bull,* 1967; *McKay et al.,* 1985). However, radar revealed a constant highly reflecting interface about 19 m below the surface of the ice in the interior of the lake. Drilling through the ice revealed the presence of a brine layer (~245‰ NaCl) in the lake extending from about 16 m downward. Radiocarbon dating of organic matter sampled at 12 m in the lake ice cover suggests that the brine has been isolated for more than 2800 years (*Doran et al.,* 2003). Analysis of microbial assemblages within the perennial ice cover of the lake revealed a diverse array of bacteria in this sealed brine ecosystem at –13°C (*Murray et al.,* 2012).

A surprising result of the analysis of the material in the brine was the mix of oxidized and reduced species. For example, nitrate, nitrite, and ammonium are present at concentrations of about 900, 23, and 4000 µM/L, respectively. Iron (Fe) is present at high levels (>300 µM L^{-1}) and there are also high levels of dissolved organic carbon (580 mg L^{-1}). Most surprisingly, perchlorate is present at 50 µg L^{-1}, the highest concentration of any Dry Valley lake. Due to this large perchlorate level, the ratio of nitrate to perchlorate (~10^3) is lower than anywhere else on Earth, other than the Atacama Desert — typical values of nitrate to perchlorate ratio on Earth range from 10^4 to 10^5 (*Jackson et al.,* 2015). Even allowing for the slowdown of metabolism with temperature, the expectation from other closed systems is that this system would have long ago run down, depleting energy reserves and going down the redox couple ladder toward sulfate reduction and methanogenesis (see, e.g., *Nealson,* 1997). Perchlorate and nitrate would have long ago been used to oxidize organic material, making their presence alongside relatively high levels of organic carbon interesting, but challenging to explain. *Murray et al.* (2012) suggested that the high levels of dissolved and gaseous nitrogen compounds,

Fe and H_2, suggest that rock-water reactions may be occurring at the base of the brine, producing H_2 and maintaining redox disequilibrium. How this works is not understood, and the rate of such reactions at the low temperatures involved is also unknown, but if such a source of H_2 is confirmed and is playing a role in maintaining the redox mix in the Lake Vida brine, this may have interesting implications for the chemistry, and habitability, of the ocean on Enceladus.

The Lake Vida brine is rich with evidence of microbial life, dominated by bacteria (~10^7 cells ml^{-1}) (*Murray et al.,* 2012). Eight bacterial phyla were identified from a 16S rRNA gene clone library from brine collected by filtration on 0.2-μm pore-sized filters: *Proteobacteria* (classes γ, δ, and ε), *Lentisphaerae, Firmicutes, Spirochaeta, Bacteroidetes, Actinobacteria, Verrucomicrobia,* and Candidate Division TM7. This is a rather large diversity for a low-temperature brine environment, but may not be a good biological analog for Enceladus. Methanogenic archaea, expected to be a useful analog organism for the base of any microbial ecosystem present on Enceladus (as discussed above), comprise a negligible fraction of the organisms detected in the Lake Vida brine and methane is only found at trace levels.

Lake Untersee, also in Antarctica, is an ultra-oligotrophic lake located at –71.342°, 13.473° in Dronning Maud Land, in the region due south of Africa. Lake Untersee occupies a basin dammed by the terminus of the Anuchin Glacier. The lake is 563 m above sea level, with an area of 11.4 km^2, and is among the largest surface lakes in East Antarctica. This lake is also effectively sealed by ice cover from the atmosphere, which transmits ~5% of the visible wavelengths of sunlight (*Andersen et al.,* 2011). The lake has two sub-basins; the largest, 169 m deep, lies adjacent to the glacier face and is separated by a sill at 50 m depth from a smaller, 100-m-deep basin in the southwest corner. The deep basin is well mixed and oxygenated to the bottom. In contrast, the shallow basin is density-stratified below the sill depth and is anoxic at its base (*Wand et al.,* 2006). The deep basin and upper part of the anoxic basin have similar water chemistry and are well-mixed. Two unusual features of the lake are a high concentration of methane (>20 mmol l^{-1}) in the deep part of the anoxic basin, and a pH of ≥10.4 in the mixed layer of the lake.

The primary source of methane in the anoxic basin of Lake Untersee is microbial and provides a plausible ecological model of a methanogen-based ecosystem on Enceladus. The microbial reactions occur at the bottom, ~100 m depth, in the anoxic zone in Lake Untersee, resulting in the production of CH_4 from H_2 and CO_2 (*Wand et al.,* 2006). The CH_4, and other biogenic products such as NH_3 produced at the bottom, diffuse upward through the stagnant water column in the anoxic trough. There are no sources or sinks for these biogenic gases until they reach the oxygen-rich layer and are consumed by microbial oxidation. This oxidation begins at about 80 m depth and is complete by ~75 m depth (*Wand et al.,* 2006). With the exception of the oxidation layer, this is a model in miniature of the situation hypothesized for

Enceladus based on the detection of H_2 and CO_2 (as well as CH_4) in the plume. It is presumed that a putative methanogenic ecosystem on Enceladus could be operating at the interface between a core and the ocean. Hydrogen released from reactions in the core is carried outward and would be consumed by the methanogens using CO_2 from the water column — analogous to the bottom of the anoxic zone in Lake Untersee. The CH_4 produced, as well as other biogenic products, would be carried upward from the source region into the plume without much further loss other than by dilution (*Steel et al.,* 2017) because unlike Lake Untersee, Enceladus does not, to the best of our knowledge, have an upper O_2-rich layer. Thus, the ocean and plume of Enceladus corresponds to the zone between 80 m and 100 m depth in Lake Untersee. It is interesting to note that, as on Enceladus, NH_3 as well as CH_4 are present. Direct sampling of the water in the CH_4 flow region (80–100 m depth) of Lake Untersee for biogenic gases and microbially produced biomarkers could provide a detailed basis for defining target biosignatures, and expected concentrations, in the search for life in the plume of Enceladus.

The high pH in the oxic waters of Lake Untersee is not fully explained, but an important clue to its cause is the fact that carbon entering the lake does not accumulate as carbonate, but rather as biologically produced organic carbon on the lake bottom (*Andersen et al.,* 2011). The photosynthetic microbial mats are carbon-starved and draw down CO_2. It has been shown that consumption of CO_2 by phototrophs can cause the pH to be as high as 10 when there is limited gas exchange with the atmosphere (see, e.g., *Talling,* 1976). Thus, the high pH in Lake Untersee appears to be a biological effect. While photosynthesis is not likely a viable niche on Enceladus, it is important to consider how various biological processes can mediate environmental parameters, such as pH.

Considering the available evidence, relevant analog systems for microbiology on Enceladus are, for the most part, anaerobic chemoautotrophic closed systems. As discussed above, H_2 is a likely "fuel" molecule on Enceladus and methanogens could be the primary producers. Most of the surface and subsurface biosphere on Earth is based, ultimately, on photosynthesis directly, or on heterotrophic decomposition of organic material produced at the surface, usually reacting with oxygen produced from the surface. This is not relevant to the subsurface of worlds that have no surface biosphere or other source of oxidants. There are three isolated ecosystems reported in the literature that are anaerobic chemoautotrophic, two of which are based on methanogens that use H_2 derived from rock-water reactions (*Stevens and McKinley,* 1995; *Chapelle et al.,* 2002), and a third based on sulfur-reducing bacteria that use redox couples produced ultimately by radioactive decay (*Lin et al.,* 2006).

The first example of a microbial community completely independent of surface photosynthesis and O_2 was reported by *Stevens and McKinley* (1995). The system is deep below the surface of the Columbia River basalts and H_2 is produced by the serpentinization of olivine in the rock. *Chapelle et al.*

(2002) found a similar system in the massive basalts in the Twin Falls area of Idaho. These two systems are useful examples of strongly metabolically constrained but biologically active ecosystems within rock-hosted environments on Earth.

There is one clear analog on Earth for hydrothermal vents in Enceladus' ocean: the Lost City field along the Atlantic Massif to the west of the mid-Atlantic ridge (Fig. 3) (*Kelley et al.,* 2001). The measurements made by the Cassini spacecraft support the conclusion that the subsurface liquid water of Enceladus is cycling through warm, alkaline hydrothermal systems at the bottom of the ocean. On Earth the discovery of such systems within our own ocean occurred only relatively recently, although their existence had been predicted based on geological observations (*Kelley et al.,* 2001). Even before their discovery it had been argued that alkaline hydrothermal systems could have been important locales for the origin of life (*Russell et al.,* 1988, 1989; *Russell,* 2003; *Barge and White,* 2017).

Russell (2003) and *Hanczyc et al.* (2003) showed that alkaline environments such as Lost City could provide a geochemical interface that supports lipid vesicle formation. Importantly, however, a moderate to high pH is problematic for the stability of RNA and to a lesser extent for DNA, but the functionality of RNA may have been of much greater importance to the origin of life as we know it (e.g., *Joyce,* 1989). High salinity, and in particular the prevalence of divalent cations such as magnesium, also poses a problem for the formation and stability of RNA and other polymers

(*Monnard et al.,* 2002; *Hand and Chyba,* 2007). *Kelley et al.* (2001) measured magnesium at Lost City to be in the range of 9–19 mmol kg^{-1}, which is considerably lower than seawater (54 mmol kg^{-1}) but significantly higher than high-temperature, low-pH, axial hydrothermal systems (~0 mmol kg^{-1}).

The Lost City site has become the canonical example of an active, off-axis, serpentinizing hydrothermal system, generating low-temperature (~70°C) alkaline fluids. Several additional sites have been discovered over a range of seafloor depths and host-rock conditions (see, e.g., *German et al.,* 2010; *Schrenk et al.,* 2013). *Brazelton et al.* (2006) measured the hydrogen and methane concentrations at active venting sites within the Lost City field to be 1 to 15 mmol kg^{-1} and 1.28 to 1.98 mmol kg^{-1}, respectively. The corresponding pH range was measured to be between 9 and 11, with temperature variations between approximately 40°C and 90°C. Carbon dioxide has been found to be largely absent (*Kelley et al.,* 2005), which restricts the availability of inorganic carbon in these systems.

The exothermic (heat-releasing) serpentinization reactions produce Ca-OH fluids that mix with seawater, leading to the formation of carbonate chimneys, several of which have grown to ~50 m in height over a lifetime of approximately 30,000 years (*Fruh-Green et al.,* 2003). As measured by *Brazelton et al.* (2006, 2011), within the carbonates are microbial populations feeding off the vent fluid, generating a biomass of 3.4×10^6 to 1.4×10^9 cells g^{-1} of carbonate. Considering fluids sampled from active vents, these numbers
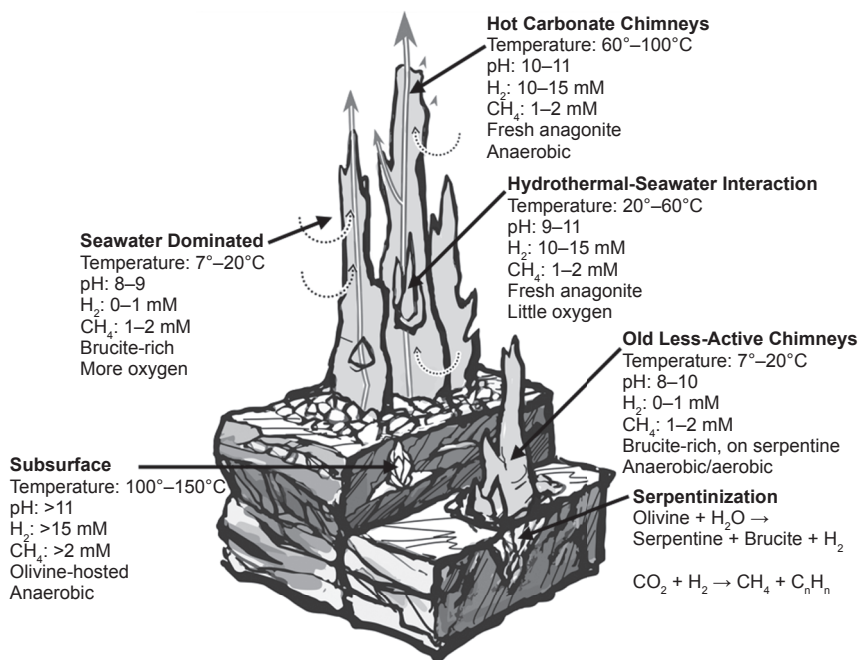

Fig. 3. Cross-section diagram of geological, geochemical, and biological zonation within the Lost City hydrothermal vents. The towers are several tens of meters in height and the system is powered by the exothermic serpentinization reaction that occurs between the ultramafic peridotite and gabbro of the Atlantis Massif and the surrounding seawater. The combination of reductants such as H$_2$ and CH$_4$ with oxidants in seawater helps to support a diverse array of microbial populations within Lost City. LCMS refers to the methanotrophic archaea Methanosarcinales. Adapted from *Brazelton et al.* (2006).

drop to 4.7×10^4 to 3.9×10^5 cells cm^{-3} of vent fluid. Within and beneath the chimneys, H_2 and CH_4 directly derived from serpentinization and fluid circulation drive microbial activity, predominantly in the form of methane-oxidizing archaea. Given the observation that some methane-oxidizing microbes also carry genes for methanogenesis (*Hallam et al.,* 2004), there is some debate as to the magnitude of biological processes as a source and sink for methane at Lost City. Further up the chimneys, and closer to the exterior, seawater cycling brings oxidants such as oxygen and sulfate into the fluids, permitting additional metabolic pathways for microbial populations.

The putative hydrothermal systems of Enceladus are clearly not as well characterized as the Lost City vents, but there appears to be a basis for the Lost City analogy. Alkaline systems on Enceladus may have lower concentrations of Mg than Lost City fluids, because submarine fluids on Earth are mixed with Mg-rich seawater. Based on the 0.4% to 1.4% H_2 in the plumes and the assumption that the H_2/CO_2 ratio is conserved between the ocean and plume, *Waite et al.* (2017) estimated that the dissolved H_2 in the ocean on Enceladus ranges from 1×10^{-4} to 2×10^{-7} mol kg^{-1}, for pH from 9 to 11, respectively. This is considerably lower than the Lost City values of 1 to 1.5×10^{-2} mol kg^{-1}, but the Lost City values were made from fluids collected at the vent field and do not reflect significant dilution, as could be expected for the case of Enceladus' ocean.

A fundamental difference between the Lost City vents and the possible vents on Enceladus is the energy available for life through various metabolic pathways. The Enceladus plume chemistry indicates that only H_2 and CO_2 are definitely available as a redox energy source. Sulfate and O_2 are not observed, and are perhaps unlikely to be present. Thus, given our current understanding of Enceladus' chemistry, oxidation reactions using these species, while important on Earth, may not be relevant for Enceladus.

If the hydrothermal outflow on Enceladus has a concentration of cells similar to that seen in the Lost City fluids, $\sim$10^5 cells cm^{-3}, biological material could be detectable in a sample collected by a spacecraft flying through the plume, even if the hydrothermal outflow is diluted by 10 to 1 with ambient ocean water, as determined by two-dimensional fluid flow calculations of *Steel et al.* (2017). In a related study, *Porco et al.* (2017) estimated that microbial concentrations at hydrothermal vents on Enceladus could be comparable to those on Earth, $\sim$10^5 cells cm^{-3}, by scaling the average geothermal flux into the sea beneath Enceladus' south polar terrain to that of the average Atlantic ocean, and assuming energy and metabolic partitioning is the same on both worlds.

The analogs discussed above — two physical analogs based on ice-sealed Antarctic lakes, two biogeochemical analogs based on methanogens, and the Lost City hydrothermal system — each have features that may resemble the ocean on Enceladus. These analogs have not yet been adequately studied in relation to how they might inform ecological models of Enceladus, but such work would be useful as missions to Enceladus are developed.

5. LIFE DETECTION INSTRUMENTATION APPROACHES

The Viking Missions to Mars in 1976 were the first, and to date only, direct search for life on another world. Each of the two landers carried three biology experiments designed to detect metabolic activity in samples from the top few centimeters of the martian soil. The pyrolytic release experiment (*Horowitz and Hobby,* 1977) detected the capability to incorporate radioactively labeled carbon dioxide in the presence of sunlight (i.e., photosynthesis). The labeled release (LR) experiment (*Levin and Straat,* 1977) attempted to detect life by the release of radioactively labeled carbon initially incorporated into organic compounds in a nutrient solution. The gas exchange experiment (GEx) (*Oyama and Berdahl,* 1977) was designed to determine if martian life could metabolize and exchange gaseous products in the presence of water vapor and in a nutrient solution. In all three cases the Viking Biology Experiments required that living organisms were present in the sample and that these organisms would respond to the conditions and nutrients provided. The results were considered negative (*Klein,* 1978, 1979, 1999) but controversy remains over the LR results (*Levin and Straat,* 2016).

Another key instrument on the Viking landers was the gas chromatograph/mass spectrometer (GCMS), which searched for organics in the soil. The most surprising result of the Viking mission was the apparent inability to detect organics in surface samples, and from samples below the surface (*Biemann et al.,* 1977). The explanation has emerged that the lack of detection was due to perchlorate in the soil (*Hecht et al.,* 2009; *Quinn et al.,* 2013) and the reactivity of perchlorates when heated (*Navarro-González et al.,* 2010; *Glavin et al.,* 2013).

Life detection on future missions will not follow the approach of the Viking Biology Experiments and expose samples to nutrient solutions (see, e.g., *Hand et al.,* 2017). Since Viking, the microbiology community has discovered that the ability of soil microorganisms to metabolize or grow in an experimental nutrient solution (e.g., culturing) is severely limited (*Lok,* 2015). On Earth the methods for detecting and characterizing microorganisms is based on non-culture methods. Such methods include the detection of key biomolecules such as phospholipids, Adenosine triphosphate (ATP), and DNA, and upon the direct sequencing of DNA and RNA to infer phylogeny and gene expression.

Analysis of specific biological molecules (e.g., ATP, chlorophyll) and genetic and protein sequencing are powerful methods but they are quite specific to Earth biology. Indeed, they would be essentially blind to an alternative type of life here on Earth — the proposed shadow biosphere (*Davies et al.,* 2009). They do not provide general tools for the search for life that may differ even slightly from life on Earth.

Instruments have been designed to detect signs of life on Enceladus and other solar system targets based on multiple biosignatures (Fig. 4), including cellular morphology and motility (*Lindensmith et al.,* 2016), and large biopolymers
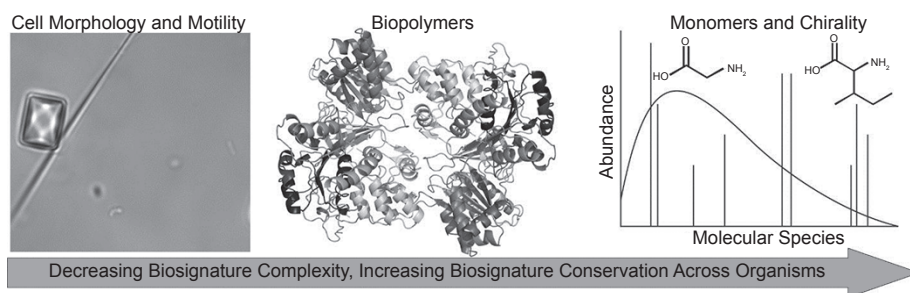
Fig. 4. Biosignatures, from highly complex but not well-conserved cell morphology and motility, to simple and well-conserved patterns of small organic molecules.

like DNA and proteins (*Parro et al.,* 2011; *Sims et al.,* 2005; *Benner,* 2017). However, searching for cells or large biopolymers carries important Earth-centric assumptions and suffers from a low probability of conservation across independent origins. An alternative approach to search for life on other worlds is to search for the building blocks of these large molecules. The key observation is that biology uses a small set of monomers for the construction of the large polymers that are used for structure, function, and information content. Thus, polymers such as DNA, RNA, polysaccharides, lipids, and proteins are constructed of specific monomers without the use of chemically similar compounds. *McKay* (2004) referred to this as the "Lego Principle" and suggested that the search for life on other worlds in the solar system could be based on the identification of this biological selectivity (Fig. 5). Perhaps the best example of this is the proteinogenic amino acids. Life on Earth uses 20 amino acids to make proteins, with small variations. Ten of the proteinogenic amino acids (the structurally simpler ones) are commonly found in abiotic chemistry and were likely present in the
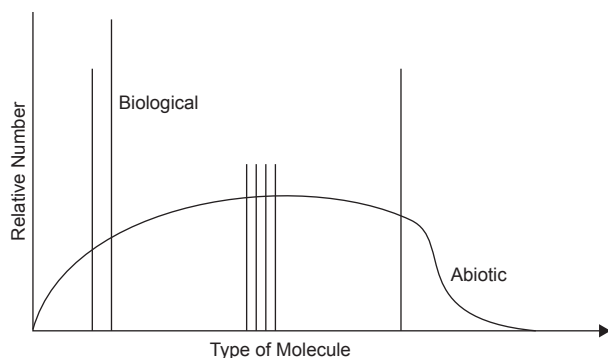

Fig. 5. Comparison of biogenic with abiogenic distributions of organic material. Nonbiological processes produce smooth distributions of organic compounds, illustrated here by the curve. Biology, in contrast, selects and uses only a few distinct molecules, shown here as spikes (e.g., the 20 L-amino acids on Earth) and builds up complex biomolecules from the combination of these few building blocks. From *McKay* (2004).

prebiotic world. The other 10 amino acids (structurally more complex) are only known as byproducts of biochemical synthesis. Life elsewhere arising in liquid water environments such as the Enceladus ocean would likely use some of the same 10 simple amino acids, but the chances of alien life making the exact same choices for the large amino acids are small (*Davila and McKay,* 2014). Indeed, while selective pressures clearly shape the set of amino acids life will use, the set of 20 in Earth life is not unique. *Philip and Freeland* (2011), and more recently *Ilardo et al.* (2015), show through computational analysis that the set of 20 amino acids found within the standard genetic code is the result of considerable natural selection, but that alternative choices for the large amino acids are possible. In addition, to optimally form proteins, all the amino acids used must have the same handedness (chirality). Life on Earth uses only L-amino acids to form proteins; life on Enceladus could have made the same choice, or could have chosen the mirror image of the entire suite of compounds. This selectivity of biology with respect to the possible amino acids is shown in Fig. 6.

When considering a search for signs of life on other worlds there is often an implicit assumption that the choices will be binary: life or no life. However, it may be possible that we discover chemistry on its way to life that is arguably neither abiotic chemistry nor life undergoing Darwinian evolution, such as a protometabolic chemical system. The existence of such a "missing link" between chemical evolution and Darwinian evolution is logically required, but the nature of this link is unclear. Fundamental aspects of living systems, such as homochirality, may have been present in this intermediate stage even before Darwinian evolution, and hence life, became operative (e.g., *Benner et al.,* 2017). Future investigations of Enceladus or other habitable worlds should be prepared to investigate this question — a question in many ways just as interesting as the discovery of a second genesis of life.

6. EXPLORATION TECHNOLOGIES

To further investigate Enceladus' habitability and potential inhabitants, robotic exploration will someday lead to additional sampling of the plume material, landing on
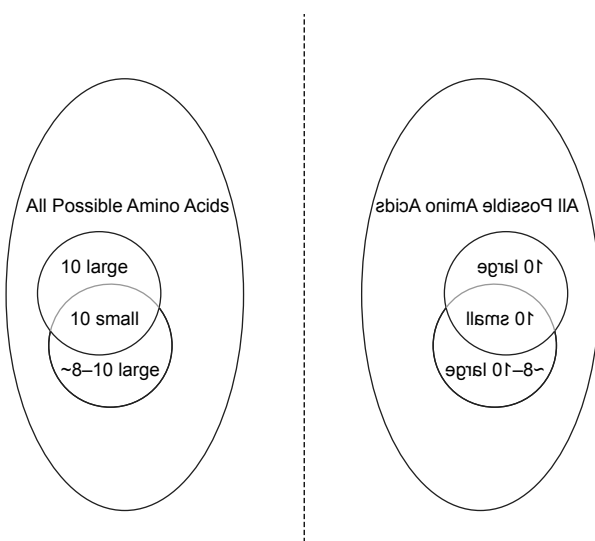
trace (parts-per-million or lower) organic molecules in the plume requires a highly sensitive detection system and a separation method to resolve different species and enantiomers. Highly sensitive detection systems include mass spectrometry (MS), which is currently the predominant organic detection system for space flight, and laser-induced fluorescence (LIF), which has been in development for space flight for a decade and is capable of very low limits of detection [sub-parts-per-trillion (*Chiesl et al.,* 2009; *Creamer et al.,* 2016; *Mathies et al.,* 2017)]. However, for compositional and chiral resolution, a front-end separation method is typically needed. While gas chromatography has been used with success on Mars missions, capillary electrophoresis (CE) is showing new promise. The Enceladus Organic Analyzer is an instrument concept from the University of California (UC) Berkeley and the Berkeley Space Science Laboratory (*Butterworth et al.,* 2016; *Mathies et al.,* 2017) that uses microcapillary CE (μCE-LIF) for quantitative compositional and chiral analysis of amino acids and would be suitable for a flyby mission. The Mathies group at UC Berkeley has already demonstrated μCE-LIF for quantitative compositional analysis of amines, amino acids, dipeptides, carboxylic acids, aldehydes, ketones, and polycyclic aromatic hydrocarbons in astrobiologically relevant samples including the Murchison meteorite (*Chiesl et al.,* 2009), the Rio Tinto (*Stockton et al.,* 2009a), the Atacama Desert (*Chiesl et al.,* 2009), hydrothermal surface pools (*Stockton et al.,* 2010), submarine hydrothermal vents (*Stockton et al.,* 2009b), etc., and have proposed this instrument for Enceladus (*Mathies et al.,* 2017). They additionally successfully dated the age of the Atacama Desert using chiral analysis of amino acids with a field-portable μCE-LIF instrument (*Skelley et al.,* 2007). *Fujishima et al.* (2016) demonstrated CE with MS to resolve biopolymers and chiral amino acids, and *Creamer et al.* (2016) reported on the use of microcapillary CE to detect biological amino acids in the brine of Mono Lake.

Benner (2017) has proposed a concept instrument that could detect any linear biopolymer (in water) that would be able to encode information and thus support Darwinism. The detection method is based on the conclusion that any such linear molecule would be a polyelectrolyte. The essential feature of this approach is the view that linear biomolecules
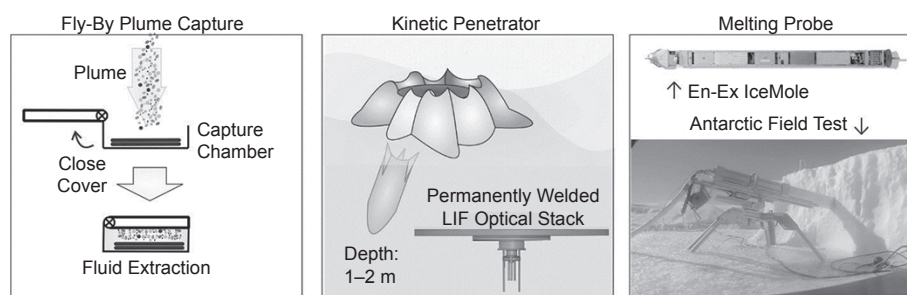

Fig. 6. The "Lego Principle" applied as the basis for a search for alien biochemistry using amino acids. Life on Earth appears to use 10 simple amino acids inherited from prebiotic processes and 10 large amino acids that were selected by evolution and biosynthesized from precursors not incorporated from prebiotic production directly (lefthand panel). Life elsewhere arising in liquid water environments would likely use the same 10 simple amino acids, but the chances of alien life making the same choices for the large amino acids are small. In addition to this choice, life can choose the mirror image of the entire site — as represented by the righthand panel. Amino acids are found naturally in meteorites and comets, but the distribution and chirality are distinct from a proteinogenic set. From *McKay* (2014b).

Enceladus' active south polar terrain, and perhaps eventually navigating into plume fractures, enabling exploration through the ice shell and into the ocean (Fig. 7).

Enceladus has a plume that launches samples into space, which makes flyby *in situ* missions possible in the near term. Flyby systems are relatively low cost and benefit from the heritage of particulate sample collection enabled by sample return missions like Stardust and Hayabusa. Instrumentation to conduct quantitative compositional and chiral analyses of

Fig. 7. Instrument concepts for accessing subsurface samples at Enceladus. A flyby mission can access samples from the interior via the plume, while kinetic penetrators and melt probes are landed missions to the icy surface itself. Melt probes may be able to access the subsurface liquid water ocean.

would necessarily precede the development of chirality or other aspects of molecular selectivity (amino acids and lipids) — the hypothetical RNA world is an example (e.g., *Joyce,* 1989).

In the longer timeframe, surface missions might be possible on Enceladus. Relatively fresh biomarkers could be accessible in the "snow" falling out of the plume onto the surface in regions near the base of the jets, in the south polar terrain. Drilling or melting into the subsurface is an obvious route to access near-surface samples, and recently kinetic impactors have provided an innovative, and potentially lower-cost, avenue to these sought-after samples. Kinetic impactors must survive up to a 5 km s^{-1} impact with cryo-ice (roughly the hardness of granite) or about 50,000 g. *Stockton et al.* (2016) proposed a microfluidic processor with a LIF detector for this application. Currently the results of models indicate that the systems can penetrate to 1–2 m depth, and testing indicates that the monolithic optical stack for LIF will survive these extreme impact conditions. Planned and ongoing testing at high impact forces will help inform and constrain designs for this ambitious scheme for low-cost subsurface access.

However, direct investigations of the ocean would require deep-subsurface access. Considering only the pressure conditions on Enceladus, numerous existing technologies and submersible systems could be used, were they somehow transported there and delivered through at least a few kilometers of ice. The pressure beneath the ice of Enceladus is only a few megapascals (for the global average crust thickness) or less (for the crust thickness at the south pole regions) and at the seafloor (assuming a total ice + ocean depth of ~100 km), the pressure is only ~10 MPa. Within Earth's ocean this range corresponds to a depth of 1–2 km, or roughly twice the depth of Lost City [~700–900 m depth (*Kelley et al.,* 2005)]. The German Enceladus Explorer (EnEx) initiative uses a miniaturized probe to access subsurface liquid pockets near the surface via melting (*Kowalski et al.,* 2016). The probe uses an ice screw at the head and differential heating along its surfaces to enable steerable subsurface operation, and has been demonstrated under laboratory tests. Two prototypes were successfully tested on glaciers in Switzerland and Iceland, demonstrating directional melting in all degrees of freedom, as well as curve driving and dirt-layer penetration. The EnEx-IceMole probe enables obstacle avoidance and was tested on Canada Glacier and Taylor Glacier in Antarctica. During the Taylor Glacier deployment, the probe successfully returned samples from the source of Blood Falls, demonstrating the capability for clean access, sampling, and return from highly protected areas. The redesign and modeling of a lighter-weight probe is underway.

As a first site for investigation, the ice-water interface may supply sufficient chemical disequilibrium so as to sustain an ecosystem within, or just beneath, the ice shell. Robotic vehicles that provide mobility and sampling at this interface would be desirable. One such solution is a small, low-mass, low-power system that operates as a roving vehicle buoyantly supported against the ice-water interface (*Leichty et al.,* 2013). Getting through the ice and autonomously navigating to scientifically compelling sites within the subsurface ocean will require a host of additional technologies. *Stone et al.* (2010, 2014) and *Richmond et al.* (2011) describe the development and successful deployment of robotic vehicles in Antarctic subice environments with some of the capabilities that would be needed for an eventual Enceladus vehicle. Additional work by those teams continue to advance both the power and communication relays' need for penetrating through kilometers of ice and communicating data back to the surface.

Spectral analysis to detect organics, and even biomarkers, in the plume may be an important contribution by spacecraft flying through the plume or telescopes at Earth. On missions that ultimately sample the plume, a pre-encounter phase may utilize plume characterization by spectroscopy. Low-cost missions are also under consideration that consist only of high-speed flybys of the plume. Large telescopes at Earth deployed for exoplanetary research, and the associated methods of transit analysis, could also detect organics in the plume.

Using data from the Visual and Infrared Mapping Spectrometer (VIMS) on the Cassini spacecraft, *Clark et al.* (2005) mapped organic signatures attributed to aromatic or cycloalkane hydrocarbons on Saturn's satellite Phoebe. *Cruikshank et al.* (2008) demonstrated the detection of organics on the surface of Iapetus and refined the *Clark et al.* (2005) interpretation of organics on Phoebe, reporting aromatic and aliphatic units in complex macromolecular carbonaceous material with a kerogen- or coal-like structure, similar to that in carbonaceous meteorites on both satellites. *Dhingra et al.* (2017) reported on VIMS observations of the plume of Enceladus. Their results suggest that organic features in the 3-μm range could be detected in the plume if the signal to noise could be increased by about a factor of 10.

Drabek-Maunder et al. (2017) report a methanol (CH_3OH) detection in the vicinity of the plume. The abundance (>0.5% with regard to H_2O) exceeds the observed abundance in the direct vicinity of the vents (~0.01%), suggesting CH_3OH is produced chemically in the plume, probably from the photolysis products of CH_4 and H_2O. Radiolysis of more complex organics mixed with ice might provide an additional source of CH_3OH.

Judge (2017) presents a detailed analysis of the possibilities of organic and biomarker detection by next generation groundbased and orbital telescopes. An important opportunity in this regard is that Enceladus will transit the face of Saturn in spring 2022. The sophisticated methods of transit analysis developed for exoplanet research may be fruitful when applied to this transit of Enceladus.

Beyond organic detection it is possible that spectral methods could detect biomarkers. For example, identification of specific amino acids may be possible with reflectance spectroscopy and possibly even detection of chirality [see the U.S. Geological Survey dataset (*Kokaly et al.,* 2017)]. In another example, current studies of flyby missions suggest

that UV fluorescence can be used to detect aromatic amino acids and to distinguish them from polycyclic aromatic hydrocarbons. The spacecraft would fly by the plume and shine a pulsed UV laser on the plume at close range. Potential target molecules include tyrosine, tryptophan, and phenylalanine (*Johnson et al.,* 2011; *Smith et al.,* 2014; *Abbey et al.,* 2017). A challenge for remote sensing is the unambiguous identification of biomarker targets if they are present as complex mixtures with other organic spectral signatures.

7. CONCLUSIONS

Measurements of the plume of Enceladus by the Cassini spacecraft have clearly indicated that there is an ocean of liquid water below the ice and that this ocean is likely to be habitable for a range of terrestrial microorganisms. The samples of this habitable ocean coming out in the plume provide a unique opportunity for astrobiology. Future missions can refine our understanding of the habitability of the ocean perhaps to the level that we can specify, in detail, the type of ecosystem that can be expected. If the ocean is rich in biomarkers, and these biomarkers are similar to those from Earth biology, then *in situ* studies of the plume may reveal the presence and biochemical nature of life on Enceladus. If the signs of life are at a low level, swamped by non-biological sources of the same molecules, or if the molecules produced by life on Enceladus are profoundly different from those associated with life on Earth, then *in situ* investigations are likely to be inadequate and a sample return will be required. Whatever the outcome, the investigation of the habitable ocean on Enceladus will inform us not just about life on that world, but also about the nature of the origin of life in general and the prevalence of habitable environments in the universe.

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