
















# The Science Case for a Return to Enceladus

Morgan L. Cable<sup>1,15</sup> , Carolyn Porco<sup>2</sup>, Christopher R. Glein<sup>3</sup>, Christopher R. German<sup>4</sup> , Shannon M. MacKenzie<sup>5</sup> , Marc Neveu<sup>6</sup> , Tori M. Hoehler<sup>7</sup>, Amy E. Hofmann<sup>1</sup> , Amanda R. Hendrix<sup>8</sup> , Jennifer Eigenbrode<sup>6</sup>, Frank Postberg<sup>9</sup> , Linda J. Spilker<sup>1</sup> , Alfred McEwen<sup>10</sup>, Nozair Khawaja<sup>11</sup> , J. Hunter Waite<sup>3</sup> , Peter Wurz<sup>12</sup> , Jörn Helbert<sup>13</sup>, Ariel Anbar<sup>14</sup> , Jean-Pierre de Vera<sup>13</sup> , and Jorge Núñez<sup>5</sup>

<sup>1</sup>NASA Jet Propulsion Laboratory, California Institute of Technology, Pasadena, CA 91109, USA

<sup>2</sup>Space Sciences Lab, University of California, Berkeley, CA 94704, USA

<sup>3</sup>Southwest Research Institute, San Antonio, TX 78238, USA

<sup>4</sup>Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA

<sup>5</sup>Johns Hopkins University/Applied Physics Laboratory, Laurel, MD 20723, USA

<sup>6</sup>NASA Goddard Space Flight Center/University of Maryland, Greenbelt, MD 20771, USA

<sup>7</sup>NASA Ames Research Center, Moffett Field, CA 94035, USA

<sup>8</sup>Planetary Science Institute, Tucson, AZ 85719, USA

<sup>9</sup>Freie Universität Berlin, 14195 Berlin, Germany

<sup>10</sup>Lunar and Planetary Laboratory, University of Arizona, Tucson, AZ 85721, USA

<sup>11</sup>Freie Universität Berlin, 14195 Berlin, Germany

<sup>12</sup>University of Bern, 3012 Bern, Switzerland

<sup>13</sup>German Aerospace Center (DLR), Cologne, Germany

<sup>14</sup>Arizona State University, Phoenix, AZ 85004, USA

Received 2021 February 15; revised 2021 April 16; accepted 2021 April 20; published 2021 July 21

## Abstract

The plume of Enceladus is unique in the solar system in providing direct access to fresh material from an extraterrestrial subsurface ocean. The Cassini Mission, though not specifically designed for it, was able to take advantage of the plume to conduct the best characterization to date of an extraterrestrial ocean. Evidence gathered from multiple instruments points to a global, subsurface liquid water ocean rich in salts and organic compounds, with water-rock interactions occurring presumably in hydrothermal systems at or below the moon's sea floor. Meeting the criteria of “extended regions of liquid water, conditions favorable for the assembly of complex organic molecules, and energy source(s) to sustain metabolism,” the ocean of Enceladus can therefore be considered habitable. It is also the only confirmed place beyond the Earth where we can easily sample fresh material from a demonstrably habitable environment without the complications of digging or drilling. The next step is to investigate whether Enceladus' ocean is actually inhabited. Here, we summarize the evidence for Enceladus' ocean and its habitability, identify constraints and outstanding questions on the detectability of life within its ocean, and recommend a return to Enceladus with a dedicated search-for-life mission (or missions).

*Unified Astronomy Thesaurus concepts:* [Saturnian satellites \(1427\)](#); [Astrobiology \(74\)](#); [Biosignatures \(2018\)](#); [Ocean planets \(1151\)](#)

## 1. An Accessible, Global Ocean

Enceladus is a relatively small (505 km mean diameter) moon of Saturn, and one of the brightest objects in the solar system, with a geologically young surface (Smith et al. 1982; Patterson et al. 2018) that is coated by fresh material (Pang et al. 1984). It is located within Saturn's E ring, which we now understand to be fed by ice grains emanating from Enceladus' plume (Hillier et al. 2007; Schmidt et al. 2008; Mitchell et al. 2015; Kempf et al. 2018). While Voyager data suggested a link between Enceladus and the E ring (Haff et al. 1983), the Cassini Mission provided the first direct evidence for the existence of the plume (Figure 1) and is therefore credited with its discovery (Porco et al. 2006; Dougherty et al. 2006; Hansen et al. 2006; Spahn et al. 2006; Tokar et al. 2006; Waite et al. 2006). The plume is formed, in part, by about 100 jets erupting

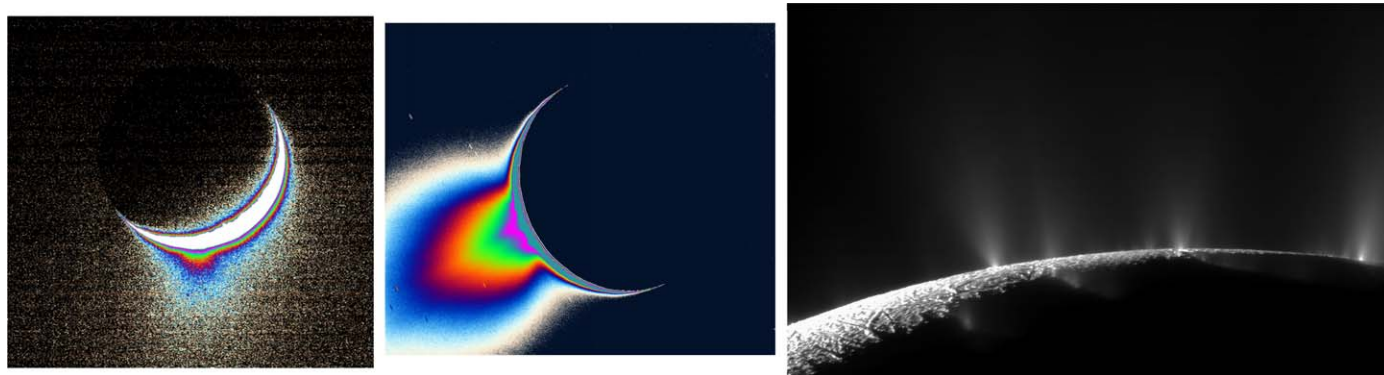
from four main surface fissures, the “Tiger Stripes,” in the South Polar Terrain (Porco et al. 2014); material may also be erupting from these fissures in the form of sheets (Porco et al. 2014; Spitale et al. 2015). The detection of sodium salts (mainly NaCl) in the ice grains ejected in the plume indicates that this material originates from an ocean in contact with Enceladus' rocky core (Postberg et al. 2009, 2011). Modeling of Cassini data suggests that the diurnal variation in the plume's brightness (and hence mass) is controlled by the cyclical variation of tensional stresses across the South Polar Terrain (Patthoff & Kattenhorn 2011; Nimmo et al. 2014; Běhounková et al. 2015), meaning the plume is likely a long-lived phenomenon that could be sustained for tens of millions to billions of years (Choblet et al. 2017; Hemingway et al. 2020; Liao et al. 2020). While other ocean worlds also harbor plumes, such as Triton (Hansen et al. 1990; Soderblom et al. 1990; Kirk et al. 1995) and possibly Europa (Roth et al. 2014; Sparks et al. 2016, 2017; Jia et al. 2018), at present Enceladus is the only body where material from a subsurface liquid water ocean laced with organic material is confirmed to be actively and continuously venting into space.

Two independent lines of evidence show that the plume's subsurface water reservoir is not a regional sea but a global

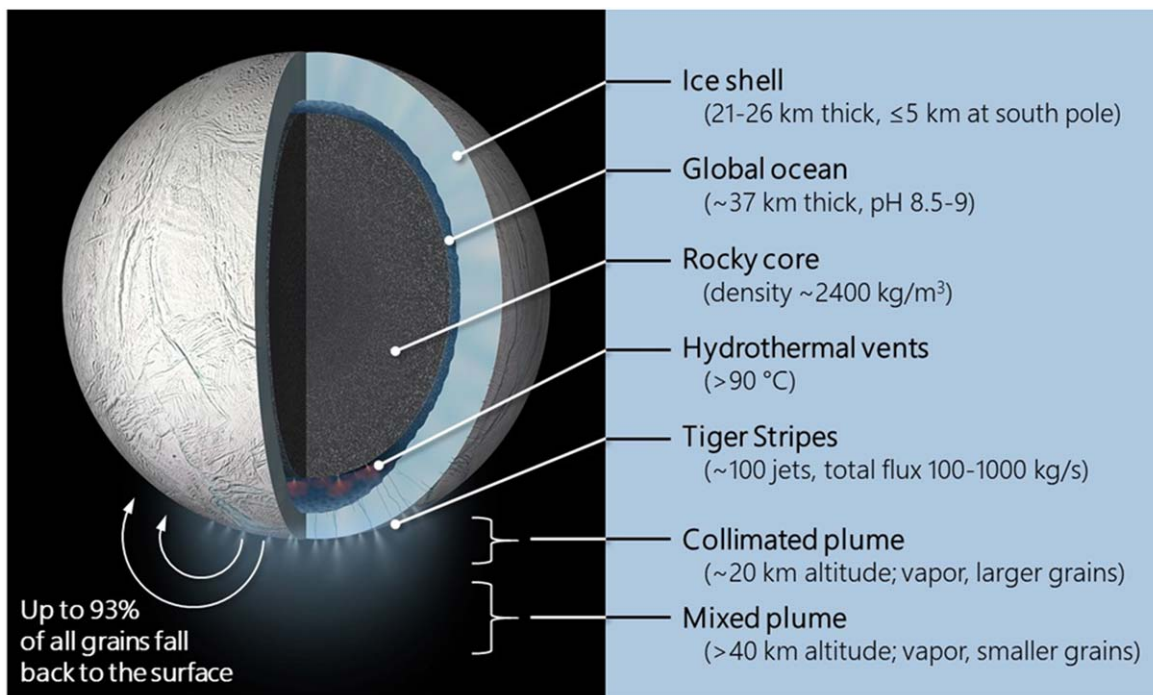
<sup>15</sup> Morgan L. Cable, NASA Jet Propulsion Laboratory, California Institute of Technology, 4800 Oak Grove Drive, Mail Stop 183-601, Pasadena, CA 91109.



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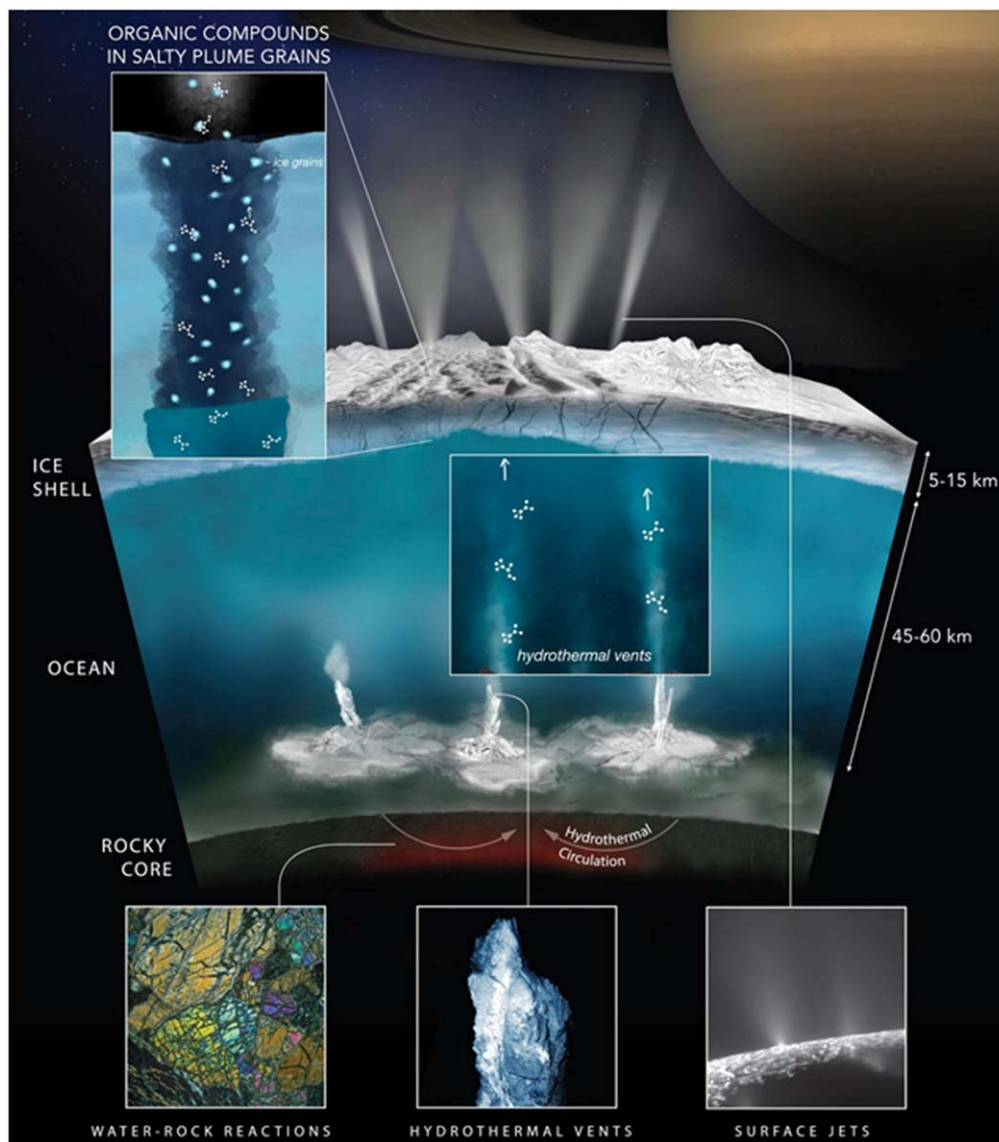
**Figure 1.** The plume of Enceladus is comprised of vapor and water ice particles emitted by about 100 jets (and likely curtain eruptions as well) from four main surface fissures, the “Tiger Stripes,” in the South Polar Terrain. Left image is the first ever sighting of the plume by Cassini on 2005 January 16. It is a highly processed, false-color image taken by Cassini’s narrow-angle camera through a near-infrared filter at a phase angle of  $148^\circ$  with an image scale of  $\sim 1.25 \text{ km pixel}^{-1}$  (Credit: NASA/JPL-Caltech/SSI; Porco et al. 2006). Center image (PIA07759) is a highly processed clear-filter image taken by Cassini’s narrow-angle camera on 2005 November 27 at high phase angle (Credit: NASA/JPL-Caltech/SSI; Porco et al. 2006). Right image (PIA11688) is a two-image mosaic taken by Cassini’s narrow-angle camera during a close Enceladus flyby on 2009 November 21 at a spatial scale of  $81 \text{ m pixel}^{-1}$  (Credit: NASA/JPL-Caltech/SSI; Porco et al. 2014).



**Figure 2.** Discoveries of the Cassini Mission reveal Enceladus to have a global subsurface ocean that contains organic molecules and hosts hydrothermal activity at the underlying sea floor that sustains redox disequilibria. Material from the ocean is expressed into space via the plume, emanating from about 100 jets in the Tiger Stripes of the South Polar Terrain. Background image: PIA20013 (Credit: NASA/JPL-Caltech). Ice shell thickness estimates from Thomas et al. (2016) and Rhoden et al. (2020). Ocean thickness from Hemingway & Mittal (2019). Ocean pH from Glein & Waite (2020). Core density from Iess et al. (2014). Hydrothermal vent temperature from Hsu et al. (2015). Plume flux from Teolis et al. (2017a). Plume grain size estimates can be found in Postberg et al. (2018b) and Kempf et al. (2018). Approximately 68%–93% of all grains ejected in the plume fall ballistically back onto Enceladus’ surface (Ingersoll & Ewald 2011; Porco et al. 2017).

ocean (Figure 2). First, analysis of gravity measurements (Iess et al. 2014; McKinnon 2015; Beuthe et al. 2016; Ćadek et al. 2016) indicates that Enceladus is not in hydrostatic equilibrium, and that it requires isostatic compensation to fit the gravity field and excess topography. The simplest explanation for this is a floating ice shell of variable thickness atop a global ocean. Second, comparison of surface images collected over 7 years of Cassini observations implies that Enceladus’ rotation has a forced physical libration (wobble) too large to be consistent with an icy shell grounded to the rocky core, again only possible if there is a global ocean (Thomas et al. 2016; Hemingway & Mittal 2019).

Enceladus’ global ocean is almost certainly long lived. Recent analysis of the history of tidal dissipation, determined from astrometric observations and the evolution of Enceladus’ orbit (Fuller et al. 2016; Lainey et al. 2020), indicates that the equilibrium heating rate at Enceladus is sufficient to maintain an ocean indefinitely if the ice shell is conductive (Nimmo et al. 2018). A long-lived ocean is relevant for habitability and a de novo origin of life. A global-scale ocean in constant contact with a rocky sea floor maximizes the extent of geochemical interactions leading to the energy and elemental building blocks of habitability, and makes it likely that habitable conditions in the ocean also persisted long enough for life to



**Figure 3.** On Enceladus, the ingredients necessary to sustain life as we know it (liquid water, chemical energy from water-rock reactions, bioessential elements, and organic compounds) can all be sampled via the plume; so might signatures of life. Modified from PIA21442 (Credit: NASA/JPL-Caltech/SwRI) and PIA23173 (Credit: NASA/JPL-Caltech) by M. Neveu.

gain a foothold. This assumes that life can originate within a water ocean without dry land or an atmosphere, and that the time interval after that origin and its rate of development on Enceladus were comparable to that on Earth (for more detail, see Section 5).

## 2. Hydrothermal Activity and Chemical Energy for Metabolic Processes

Life as we know it requires liquid water, organic molecules, and energy originally sourced from either light (for *photosynthesis*), or chemical reagents generating reduction-oxidation (redox) disequilibria (for *chemosynthesis*). Sunlight is not likely to be directly available in the subsurface ocean of Enceladus or any of the other icy moons of the outer solar system, so chemosynthesis, if present at all, is expected to be the dominant mechanism. It is of interest that Enceladus' plume composition appears to be consistent with hydrothermal activity within the underlying sea floor that sustains redox disequilibria (Figure 3 and arguments given below). On Earth,

submarine hydrothermal activity supports a diversity of chemosynthetic microbial communities, starting with the metabolism (reduction or oxidation) of methane, molecular hydrogen, ferrous iron, hydrogen sulfide, and other small molecules, and building on the organic molecular products of these primary producers to support a web of symbionts, carnivores, and scavengers. One particular subset of those vents, the carbonate chimneys of the Lost City hydrothermal field that was only discovered relatively recently, represents an intriguing candidate environment for the origin of life (Martin et al. 2008).

Three key discoveries strongly support the occurrence of contemporary hydrothermal processes at the interface between the ocean and the core of Enceladus. The first is that the Cassini Cosmic Dust Analyzer (CDA) found nanometer-scale dust particles consisting of silica ( $\text{SiO}_2$ ) (Hsu et al. 2015). Particles of this specific composition and limited size range (2–8 nm radius) can most plausibly be inferred to have been generated from hot ( $>90^\circ\text{C}$ ) mineral-laden waters formed by

hydrothermal leaching of silicate minerals issuing from the sea floor and immediately condensing into a colloid upon meeting the cold ( $\sim 0^\circ\text{C}$ ) ocean water (Conrad et al. 2007; Tobler et al. 2009; Tobler & Benning 2013; Hsu et al. 2015). Once formed, those nanoparticles are transported from the sea floor up through the ocean to the fractures in the ice shell by convection and/or diffusive upwelling (Choblet et al. 2017; Steel et al. 2017; Lobo et al. 2021; Kang et al. 2021) and then, by other processes such as pressure-driven upward movement (Manga & Wang 2007; Matson et al. 2012), to the top of the water column, where they are incorporated into the ejected ice grains that join Saturn's E ring and are subsequently released by sputter erosion due to high energy magnetospheric particles.

A second line of evidence for hydrothermal activity comes from the Cassini Ion and Neutral Mass Spectrometer (INMS) plume gas measurements. According to modeling, the relative proportions of measured volatiles are unlikely without a gas input. For example, methane should be preferentially trapped in clathrate hydrates (water-ice cages) and therefore depleted in the plume (Bouquet et al. 2015). One plausible explanation is hydrothermal activity, which could release sufficient methane to match observed levels.

The third piece of evidence is identification by INMS of molecular hydrogen ( $\text{H}_2$ ) in the plume during a “deep dive” flyby (E21, the 21st Cassini flyby of Enceladus) at an altitude of only 48 km above Enceladus' surface (Waite et al. 2017). Molecular hydrogen is a product of the oxidation of reduced iron (Fe(0), Fe(II)) by water. The most familiar process that drives  $\text{H}_2$  production on Earth is serpentinization, a rock alteration process that can occur in a subset of submarine hydrothermal systems where liquid water interacts with ultramafic rocks (igneous rocks with high Mg and Fe contents; German & Seyfried 2014). Thus, this evidence further suggests that the ocean of Enceladus is in contact with, and reacting with, its rocky core through hydrothermal processes.

The flux of hydrothermally sourced  $\text{H}_2$  into a  $\text{CO}_2$ -containing ocean appears to provide sufficient redox chemical disequilibrium to support life that would make methane to obtain usable energy (Waite et al. 2017). This metabolic process (methanogenesis) is among the most primitive known microbial metabolisms on Earth (Reveillaud et al. 2016). Other metabolic processes (e.g., sulfate reduction) that rely on stronger oxidants derived from the radiation-induced breakdown (radiolysis) of water molecules may also contribute to the supply of chemical energy (Ray et al. 2021). The pH of the Enceladus ocean is mildly alkaline, likely in the range of 8.5–9.0, based on recent geochemical interpretations of Cassini mass spectrometry data (Glein & Waite 2020). While previous studies reported wider ranges reaching higher pH values (pH 8.5–10.5, Hsu et al. 2015; pH 11–12, Glein et al. 2015, 2018), the more constrained pH range of 8.5–9.0 is based on a self-consistent analysis of two different data sets from INMS (Waite et al. 2017) and CDA (Postberg et al. 2009) and is considered the current best estimate. This geochemical interpretation is consistent with long-term buffering by a sea floor containing quartz, talc, and carbonate minerals. Together with the silica-rich nanograins and the detection of molecular hydrogen, these results suggest a heterogeneous rocky core featuring chemical gradients between a carbonate-rich upper layer and a serpentinizing interior (Glein et al. 2018). Ultramafic-hosted hydrothermal systems on Earth such as Lost City in the Mid-Atlantic Ocean (Kelley et al. 2005) and Von

Damm on the Mid-Cayman Rise (McDermott et al. 2015) are rich in  $\text{H}_2$ , and may represent the closest Earth analogues to fluids associated with alteration processes below Enceladus' sea floor. Intriguingly, evidence suggests that de novo abiotic organic synthesis is occurring at these terrestrial submarine hydrothermal vents (Proskurowski et al. 2008; Lang et al. 2010; McDermott et al. 2015), an important step in generating the conditions believed to be suitable for life to emerge in a planetary subsurface. Indeed, recent laboratory work supports the generation of hydrothermal chimneys—and associated abiotic organic synthesis within them—in conditions similar to the Enceladus sea floor (Angelis et al. 2021). Cassini evidence, therefore, suggests that conditions in the ocean of Enceladus are consistent with a specific class of hydrothermal systems on Earth, where abiotic organic synthesis can be sustained and where redox chemistry supports some of the most primitive known forms of microbial life on Earth. Could similar hydrothermal systems be actively hosting chemosynthetic life on Enceladus?

### 3. Chemical Building Blocks and Availability of CHNOPS

Cassini's CDA and INMS instruments characterized the composition of ice grains and vapor, respectively, emanating from the plume. These measurements indicate that the Enceladus ocean hosts a range of organic compounds, including diverse low- and high-mass compounds comprised of the biologically essential light elements carbon, hydrogen, oxygen, and nitrogen (CHON; Waite et al. 2009; Postberg et al. 2018a; Khawaja et al. 2019), which could serve as building blocks for, or be the byproducts of, life. Intriguingly, a subset of plume particles ( $\sim 4\%$ ) contain organic molecules with masses larger than 200 u (u = atomic mass units). These high-mass organic molecules are found in relatively high abundance ( $>1\%$  by mass) in these ice particles and are consistent with unsaturated (i.e., containing double and triple carbon-carbon and carbon-nitrogen bonds) and partially aromatic (i.e., containing ring-shaped substructures) molecules (Postberg et al. 2018b). Importantly, analysis of CDA spectra collected at different velocities indicates that detected organics are probably fragments of even larger organic molecules (Postberg et al. 2018b); this interpretation is supported by detection of diverse organics in the vapor phase at the fastest spacecraft flyby velocities ( $\sim 18\text{ km s}^{-1}$ ) (Waite et al. 2009). Cassini's CDA also obtained evidence for a different class of low-mass volatile organic compounds (VOCs), such as acetic acid and acetaldehyde (Khawaja et al. 2019). These compounds further demonstrate the richness of organic carbon sources on Enceladus. However, the INMS and CDA instruments lacked the mass range and mass resolution to characterize these fascinating organics in any greater detail, so their sources (abiotic or biotic) are currently unknown.

Evidence suggests that the size and productivity of a putative biosphere on Enceladus would not be limited by the availability of carbon, nitrogen, or probably sulfur. In addition to the high-mass organic molecules, sources of biologically available nitrogen ( $\text{NH}_3/\text{NH}_4^+$  and amines) have also been found in the plume (Waite et al. 2009; Khawaja et al. 2019) and as plume-derived nitrogen ions in Saturn's inner magnetosphere (Smith et al. 2008). These nitrogen-rich species could act as amino acid precursors. Observed abundances of  $\text{CO}_2$  and  $\text{NH}_3$  in the plume from Cassini's INMS (Waite et al. 2017) exceed biological requirements to support the average cell abundance

of Earth's oceans ( $10^6$  cells  $\text{cm}^{-3}$ ; Fagerbakke et al. 1996) by 5–6 orders of magnitude (Cable et al. 2020). While the reported detection of hydrogen sulfide ( $\text{H}_2\text{S}$ ) in the plume by INMS (Waite et al. 2009) is ambiguous due to the limited mass resolution of the instrument (Magee & Waite 2017), this sulfur-containing species is predicted by geochemical models to be present in the ocean, and hydrothermal leaching of iron sulfides (e.g.,  $\text{FeS}$ ) would increase the flux of dissolved sulfide ( $\text{H}_2\text{S}$ , or  $\text{HS}^-$ ) to the ocean (Zolotov 2007). Both tentative in situ evidence and predicted concentrations from the models indicate that the abundance of sulfur in the ocean would exceed the biological requirement by 1–5 orders of magnitude (Cable et al. 2020). Sulfate ( $\text{SO}_4^{2-}$ ) could also contribute to the S budget depending on the fate of radiolytically produced oxidants ( $\text{O}_2$ ,  $\text{H}_2\text{O}_2$ ) in the ocean (Ray et al. 2021).

Phosphorus has been suggested as the limiting bioessential element of a possible Enceladan biosphere (Lingam & Loeb 2018). Phosphine ( $\text{PH}_3$ ) was not definitively identified in the vapor phase in any plume flythroughs by Cassini (though INMS data show nonresolvable peaks in this mass range; Waite et al. 2009), nor was phosphate ( $\text{PO}_4^{3-}$ ) detected in any ocean-derived plume grains. However, ambiguities in the data and/or limitations in instrument sensitivities and implementations (Cassini's CDA, for example, was sensitive only to cations) mean that an appreciable abundance of these or other phosphorus-containing species cannot be ruled out. In terms of bulk elemental inventory based on chondritic building blocks of Enceladus, sufficient phosphorus should be present at Enceladus (Cable et al. 2020); the questions are, what are the forms of P, and how are they spatially distributed inside Enceladus? This will impact the availability of P to putative life in the ocean. Physical-chemical modeling of water-rock interactions and fluid compositions at Enceladus (Zolotov 2007) suggests equilibrium phosphate concentrations in the Enceladus ocean would meet the biological requirement to support an average cell density of  $10^4$  cells  $\text{cm}^{-3}$ , a value comparable to some cell density estimates (Section 5). Phosphorus availability may also have implications for a potential origin of life on Enceladus. However, too little is known about the specific requirements for the origin of life, including P chemistry, that present focus is better placed on the ocean's "carrying capacity" for extant life as we know it, which can be more concretely constrained by observations and modeling. Given the similarities between terrestrial hydrothermal systems and the conditions that may be present at the sea floor of Enceladus, such estimates using life as we know it may be more apropos for this particular ocean world than others, where geochemical conditions may be quite different and less Earth-like (e.g., the ammonia-rich subsurface water ocean of Titan). Future in situ measurements of sulfur and phosphorus species in the plume would greatly improve our understanding of the availability of elements needed for life as we know it (CHNOPS) in the Enceladus ocean, and what possible cell densities these element abundances might support.

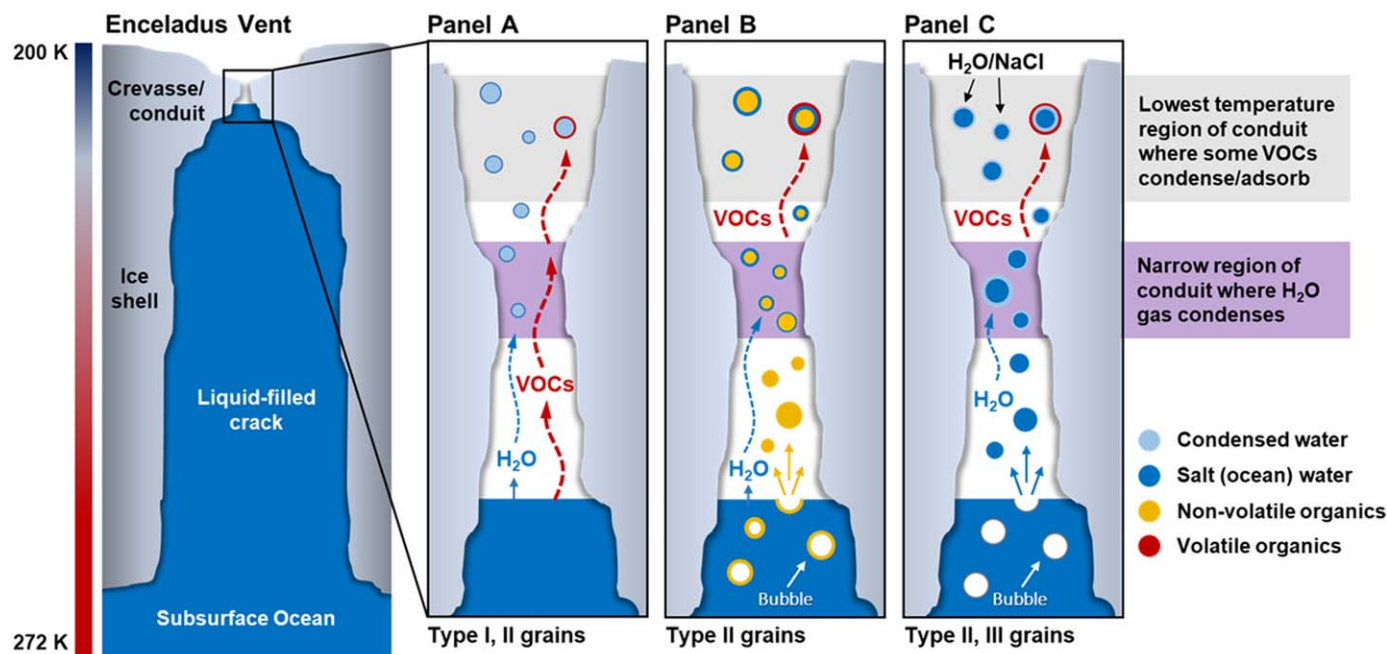
Beyond CHNOPS, life as we know it also utilizes metal ions (e.g., Mg, Fe, Ni, Zn) to catalyze key biochemical reactions (Maret 2016). Whether or not life at Enceladus would need the same element suite as Earth life is not known, but hydrothermal vents on Earth provide a continuous supply of metals (as free dissolved ions, minerals, and organic-metal complexes; Sander & Koschinsky 2011) which may indicate that availability of such metals would not be limiting for putative life in this

environment. We note that on Earth, decreased solubility of metals in alkaline conditions at the sea floor leads to precipitation of chimneys (i.e., Lost City) that concentrate minerals and focus redox and other chemical gradients, potentially acting as incubators for biochemistry leading to life (Barge & White 2017). Many metals could also be enriched (on average) in rocks on Enceladus compared with rocks on Earth if Enceladus has a bulk chondritic composition, but lacks a metal core that sequesters these elements.

#### 4. Enrichment of Organics

Processes may be occurring within the water column, between any site of sea floor venting and the underside of the South Polar Terrain, in the ocean that could increase the concentration of organic materials in emitted plume particles, and therefore the likelihood of detecting trace species (such as biomolecules) and even cells. Volatile exsolution, boiling, and any turbulence in the liquid-filled conduits leading from the ocean to the surface would create bubbles that could scavenge organic material and even microorganisms via attachment to the bubbles' surfaces as they rise. The organics and cells would then be released in a spray when the bubbles burst at the water's upper surface (Porco et al. 2017). This bubble-scrubbing process is most efficient with hydrophobic molecules and surfactants, and has been shown to increase organic and microbial cell concentrations in the resulting sea spray by up to three orders of magnitude on Earth (e.g., Carlucci & Williams 1965; Blanchard & Syzdek 1970, 1972). On Earth, the most energetic spray can be lofted into the atmosphere; on Enceladus, it would presumably freeze upon encountering the vacuum of space (Figure 4) and generate many if not most of the larger organic- and salt-rich icy particles forming the plume (Postberg et al. 2018b). Some fraction of the smaller plume particles likely form via homogeneous nucleation from the vapor phase, and can also accumulate volatile organic compounds via condensation/adsorption as they move through the plume conduit (Postberg et al. 2009; Bouquet et al. 2019; Khawaja et al. 2019), though their smaller size means they carry a smaller organic load. Indeed, the sizes of film droplets formed when bubbles shatter (0.02–4  $\mu\text{m}$  diameter), and jet droplets formed when the bubble cavities collapse (2–100  $\mu\text{m}$  diameter), are consistent with the particle size distribution of the plume (De Leeuw et al. 2011; Veron 2015; Ingersoll & Nakajima 2016).

A result of this scavenging process is that the less energetic spray that falls back to the water's surface, as well as the bubbles that fail to burst, can under certain conditions form an organic-rich film at the top of the water column, similar to the sea surface microlayer seen on Earth's oceans, especially after a storm. In the cold polar regions on Earth, the wind-induced bursting of air bubbles at the sea surface microlayer generates a sub-micron sea spray aerosol containing ice particles that are organic-rich and salt-poor (Burrows et al. 2014). These ice particles are similar in organic and salt content to the organic-rich plume grains (Figure 4) detected by Cassini (Postberg et al. 2018b); these terrestrial particles are also enriched 10–1000-fold in organic material compared with Earth's average ocean concentrations (Burrows et al. 2014). It is not clear if conditions at the top of the water column at Enceladus would allow the formation of an organic-rich film; further experimental work, theoretical modeling, and in situ measurements are needed. It should be noted that the bubble-scrubbing



**Figure 4.** The different paths an ice grain can take: various mechanisms lead to the formation of different types of ice grains in Enceladus' vents. Enceladus Vent (left): ocean waters ( $\sim 272$  K) likely fill  $\sim 90\%$  of the crack due to equilibrium elevation of the water table height (Matson et al. 2012; Ingersoll & Nakajima 2016); the temperature decreases along the open conduit, reaching approximately 200 K at the surface. Panel (A): Type I grains (Postberg et al. 2009) are formed via nucleation of water vapor from the gas phase, which occurs most readily at the narrow region of the conduit (purple shaded region) where water vapor becomes sufficiently supersaturated (Schmidt et al. 2008). Volatile organic compounds (VOCs) can condense/adsorb onto these grains in the upper region of the conduit (Bouquet et al. 2019), where temperatures are lower (gray shaded region); this forms a subset of Type II grains (Khawaja et al. 2019). Panel (B): bubbles disrupt the surface via exsolution, boiling or upwelling, aerosolizing the organics that are transported from ocean depths via bubble scrubbing and concentrated at the ocean surface, for example forming ice grains which contain compounds that produce High Mass Organic Cations (HMOCs) in mass spectra (Postberg et al. 2018b), a subset of organic-rich, salt-poor Type II grains. Panel (C): flash-freezing of ocean waters due to surface disruption leads to the formation of the Type III salt-rich grains (Postberg et al. 2009). Water vapor or VOCs can condense/adsorb onto these HMOC and salt-rich grains as well. Note that all of these processes depicted in Panels (A)–(C) are likely occurring simultaneously, to varying degrees across the vents. Not shown: water vapor and un-adsorbed VOCs also escape to form part of the gas phase of the plume. Ice shell thickness and conduit height not to scale. Figure by M. Cable after Khawaja et al. (2019).

process (as described in the previous paragraph) does not require the presence of an organic-rich surface film to yield ice particles enriched in organics/cells up to 1000-fold.

If such enrichment processes are occurring on Enceladus, the potential for detecting organic biosignatures in the plume is much higher than may be expected based on estimates of bulk concentrations in the ocean alone (Porco et al. 2017; Cable et al. 2020).

## 5. Constraints, Outstanding Questions, and Needed Developments

Significant experimental and theoretical efforts have been (and are currently being) undertaken to improve our understanding of Enceladus by contributing to the interpretation of existing data and informing future missions and observations. These interdisciplinary studies include, but are not limited to, work to constrain plume physical and chemical properties, investigations to predict and detect abiotic and biologically formed organic molecules in ice grains in situ, simulations of sample collection and analysis from plumes and surfaces of icy airless bodies, laboratory and theoretical exploration of the geochemical reactivity space for ocean-rock interactions in hydrothermal systems, laboratory work replicating various environmental gradients for putative life, and theoretical modeling of Enceladus' ocean circulation, biogeochemical processes in the ocean, and interior structure and composition. For a detailed review of these and other studies, please see Taubner et al. (2020), and references therein. We highlight

below a few areas of recent progress and/or where significant questions still persist.

Estimates of the ages of Enceladus and its liquid water ocean were previously debated in the literature. An analysis of its orbital evolution (Ćuk et al. 2016) originally suggested that Enceladus may be only about 100 Myr old, but this work relied on the assumption of equilibrium tides. More recent analyses—both theoretical and observational—support an age as old as the solar system (Fuller et al. 2016; Nimmo et al. 2018; Neveu & Rhoden 2019; Lainey et al. 2020). Modeling indicates Enceladus' ocean is likely to be long lived and perhaps as old as Enceladus itself (Section 1), though at present we lack empirical information on the duration and long-period variability of its hydrothermal activity and plume outgassing. Whether these chronological uncertainties influence the estimated probability that life has taken hold there depends on how long it takes for life to emerge given suitable conditions and processes, and this is also weakly constrained and highly variable, depending on what assumptions are made.

Estimates of timescales for the emergence of life are also poorly constrained. On Earth, biogenic carbon-bearing compounds tend to be depleted in the heavier stable isotope of carbon,  $^{13}\text{C}$ , relative to the light stable isotope,  $^{12}\text{C}$ , due primarily to kinetic isotope effects associated with biochemical reactions such as carbon fixation during photosynthesis (e.g., Park & Epstein 1960). As such, the highly depleted carbon isotopic signatures of organic molecules preserved in the geologic record on Earth are often interpreted as evidence of

**Table 1**  
Reported Estimates of Bioavailable Energy Flux and Biological Potential in the Enceladus Ocean

Bioavailable Energy Flux ( $W$ )	Microbial Concentration (cells $cm^{-3}$ )			Reference
	Vent Fluids	Bulk Ocean	Plume Grains	
1.1e6–2.9e6 <sup>a</sup>	...	0.6–890 <sup>b</sup>	...	Vance et al. (2016)
6.1e3–1.9e6 <sup>a</sup>	8.5e8 <sup>c</sup>	0.004–340 <sup>b</sup> ; 80–4250 <sup>d</sup>	8.5e7 <sup>e</sup>	Steel et al. (2017)
10.1–390 <sup>a</sup>	...	6e–6–0.12 <sup>b</sup>	...	Taubner et al. (2018)
...	1e5 <sup>f</sup>	...	1e4 <sup>e</sup> –1e7 <sup>e,g</sup>	Porco et al. (2017)
2.6e6–1.8e7 <sup>h</sup>	...	0.026–0.18 <sup>i</sup>	...	Ray et al. (2021)

**Notes.** Modified from Cable et al. (2020) and Porco et al. (2017).

<sup>a</sup> Calculated based on reported flux of  $H_2$  from the reference cited, and an assumed value of  $\Delta G$  for methanogenesis ( $-120$  to  $-45$   $kJ\ mol^{-1}$ ; Waite et al. 2017).

<sup>b</sup> Calculated as a bulk ocean average based on assumed values for methanogen biomass yield ( $1.3$ – $7.2$   $g\ mol^{-1}\ CH_4$ ; Thauer et al. 2008), maintenance energy ( $1.2e-19$  to  $6e-17$   $W\ cell^{-1}$ ; Tjihuis et al. 1993; Hoehler & Jørgensen 2013), average cell mass ( $2e-14$   $g$ ; Fagerbakke et al. 1996) and ocean volume ( $2.7e16$   $m^3$ ; Steel et al. 2017).

<sup>c</sup> Calculated assuming  $7.8$   $mM$   $H_2$  concentration in vent flows (Lost City analogy), 10% efficiency of biomolecule synthesis as a fraction of energy expended on growth (McCollom & Amend 2005), and a value of  $\Delta G = -125$   $kJ\ mol^{-1}$  for methanogenesis.

<sup>d</sup> Calculated assuming an annual biomass production of  $4e4$ – $2e6$   $kg\ yr^{-1}$ , 100% efficient biotic production from hydrothermally produced  $H_2$ , and a 1 kyr period of cell destruction (Steel et al. 2017).

<sup>e</sup> Assumed dilution from vent fluids by a factor of 10 due to mixing between vent outflow and ambient ocean (Steel et al. 2017). But note that, in Earth’s hydrothermal plumes, vent fluids are diluted by a factor of  $1e4$  within timescales of an hour (German & Seyfried 2014).

<sup>f</sup> Estimated based on tidal energy dissipation and heat flux into ocean from the south polar sea floor (Choblet et al. 2017; Lainey et al. 2017; Liao et al. 2020), leading to similar microbial concentrations as in Earth hydrothermal systems (Brazelton et al. 2006).

<sup>g</sup> Assumed increase in concentration of cells by a factor of  $1e2$  to  $1e3$  due to bubble scrubbing (Porco et al. 2017).

<sup>h</sup> Calculated for methanogenesis based on  $1e9$  to  $5e9$   $mol\ yr^{-1}$   $H_2$  production rate in the ocean (Waite et al. 2017).

<sup>i</sup> Calculated based on total cells from reference cited and ocean volume of  $2.7e16$   $m^3$  (Steel et al. 2017).

biogenicity. Most recently, the depleted carbon isotopic composition of graphite inclusions in a single 4.1 Gyr terrestrial zircon has been interpreted as putative evidence for the presence of a biosphere  $\sim 400$  Myr after Earth’s accretion (Bell et al. 2015). Prior investigations have also invoked the depleted carbon isotopic composition of mineral-hosted graphite as a possible “chemical fossil” of life’s emergence on Earth  $\sim 3.8$  Gyr ago (e.g., Mojzsis et al. 1996; Rosing 1999; McKeegan et al. 2007). However, these claims remain controversial given that abiotic processes such as Fischer–Tropsch-type synthesis of organic compounds or diagenetic or metamorphic alteration of existing carbonaceous matter can result in carbon isotopic signatures depleted to an extent comparable to those produced via biologic processes (e.g., Eiler et al. 1997; McCollom & Seewald 2006). Evidence for the oldest cellular life—in the form of preserved microfossils and, in some cases, their associated carbon isotopic compositions—suggest a perhaps less controversial upper limit for the emergence of life sometime before  $\sim 3.5$  Gyr ago (Tice & Lowe 2004; Schopf et al. 2018). Given the large uncertainties in timescales both for the origin of life on Earth ( $\leq 1$  Gyr) and the length of time Enceladus’ ocean has supported habitable conditions, current evidence cannot unambiguously support or refute the possibility of a genesis of life on Enceladus. It is worth noting that the transition of a system such as Enceladus from habitable (but uninhabited) to inhabited is likely to occur in a stepwise process, starting with prebiotic synthesis of building blocks and increasing in system complexity to reach evolutionary innovation and finally a complete, living organism (Sutherland 2017). Future missions to Enceladus should consider payloads that could assess where the ocean might lie on this spectrum (Section 6).

Conversely, the search for life on Enceladus could enable significant progress toward resolving currently intractable

debates between surface versus subsurface scenarios for the origin of life. Enceladus provides a natural laboratory for investigating a hydrothermal origin of life (Barge & White 2017), similar to the case often made that Titan provides a natural laboratory for the study of prebiotic chemistry on a global scale (Sagan et al. 1992).

The terrestrial analog for sea floor hydrothermal activity outlined above (Section 2) is based on a premise that reduced chemical species released from seafloor water-rock interactions are the rate-determining reactants in most limited supply for redox disequilibria. Hence, the metabolic energy for life that they provide would be concentrated at the sea floor, where the formation of the silica nanoparticles is predicted to occur (Hsu et al. 2015). It could also be plausible that chemically reduced species are more abundant in Enceladus’ ocean, fueling redox gradients where oxidants may be introduced from above during recycling of the young outer crust (Teolis et al. 2017b; Ray et al. 2021). In this case, chemosynthetic life might instead be concentrated at the ice-ocean interface. Further kinetics studies of abiotic redox equilibration (i.e., the  $H_2$ – $O_2$ – $H_2O_2$  system with an excess of  $H_2$ ) under conditions more analogous to the Enceladus ocean would better elucidate the net concentrations and distributions of species that chemosynthetic life could utilize.

Should life exist in Enceladus’ ocean, the quality and abundance of evidence thereof will depend on the balance of factors that control the production, transport, and destruction of organic matter as well as cells and their remnants. Published estimates of cell densities span many orders of magnitude (Table 1), owing in part to differences in the basis on which those estimates are made, in part to uncertainties in the resource fluxes that Enceladus may provide, and in part to different assumptions in how those resource fluxes might translate into cell abundance. Research to better constrain both energy fluxes

into the ocean and biomass-energy relations (Ray et al. 2021) would better inform the expected detection limits of biosignatures. The longevity and preservation of biosignatures (such as amino acids; Truong et al. 2019) is also a critical aspect that should be taken into account and further work is required to fully understand how this varies with the conditions unique to Enceladus and other ocean world environments (Europa, Titan, etc.).

Laboratory and field work studying the limits of life as we know it on Earth can also provide context for astrobiological investigations and challenging real-world samples to test instrument thresholds (e.g., Klenner et al. 2020a, 2020b). Such investigations, coupled with modeling efforts, can provide guidance for future payloads and sampling architectures at Enceladus and other ocean worlds. Additionally, investment in strategies and technologies to minimize the risk of contamination would help in two areas: first, reducing contamination risk for ultrasensitive life-detection payloads could lead to increased confidence that analytical results would reflect true biosignature detections (McKay et al. 2020); second, minimizing contamination to the ocean world environment would open to exploration regions previously declared off-limits in the interest of planetary protection, ultimately enabling more capable and wider-reaching astrobiology mission architectures.

## 6. Strategies to Search for Life

While remote sensing techniques can provide valuable information about surface composition, geology, topography, internal structure, the plume and the geysers that compose it, the relationship of geysers to thermal emission, etc., ultimately we anticipate that in situ sampling will be required to more effectively address the life question. Current remote sensing instruments (such as ultraviolet or near-infrared spectrometers) cannot unambiguously identify biomolecules (e.g., fatty acids or a biopolymer) from orbit at the sub-ppm limits of detection, especially in complex mixtures expected in the potentially energy-constrained environment of Enceladus' ocean (Cable et al. 2020). However, it should be noted that some spectroscopy-based flight instruments in development, such as the Europa Raman Spectrometer for Ocean worlds (ERSO; Phillips-Lander et al. 2019) may be able to achieve ppb limits of detection for amino acids and other biomolecules collected from orbit via use of cavity-enhanced Raman spectroscopy by the end of the 2020s.

At Enceladus, in situ measurements could be performed via a variety of mission architectures: flythroughs from Saturn or Enceladus orbit (i.e., the Enceladus Life Finder mission concept; Lunine et al. 2015; Reh et al. 2016) or the Enceladus Life Signatures and Habitability mission concept (Eigenbrode et al. 2018), which would sample the plume vapor and grains as Cassini already did to a limited extent, landed architectures (i.e., the Enceladus Orbilander mission concept; MacKenzie et al. 2020, 2021), and sample return missions (Tsou et al. 2012; Neveu et al. 2020). Any mission with life detection as its primary science objective should include multiple, independent, and orthogonal tests for life, as well as thorough environmental characterization to provide context (Neveu et al. 2018). Multiple payload combinations could achieve this goal to varying degrees and have been discussed elsewhere (e.g., Europa Lander Study 2016; Dachwald et al. 2020; MacKenzie et al. 2020). Impact speeds of plume particles encountered in flythrough missions (orbiting Saturn) are

necessarily higher ( $>4 \text{ km s}^{-1}$ ) than either missions that would orbit ( $\sim 200 \text{ m s}^{-1}$ ) or those that would land on Enceladus and gather falling particles (plume particles falling back to the surface of Enceladus hit at  $\sim 150 \text{ m s}^{-1}$ ; Porco et al. 2017). Potential biosignature molecules (e.g., amino acids, fatty acids, and peptides; Klenner et al. 2020a, 2020b; Jaramillo-Botero et al. 2021) and even larger biomolecules (polypeptides; Ulibarri et al. 2018) survive hypervelocity impacts and could be sampled at speeds up to  $5\text{--}6 \text{ km s}^{-1}$  (Table 2); these sampling speeds have been confirmed by both experimental (Klenner et al. 2020a, 2020b) and theoretical (Jaramillo-Botero et al. 2021) work, and importantly are validated by in situ data from Cassini. High mass organic cations (HMOC) were only observed with Cassini CDA between  $5 \text{ km s}^{-1}$  and  $15 \text{ km s}^{-1}$  in Enceladus plume and E ring flythroughs (Postberg et al. 2018b). This  $5 \text{ km s}^{-1}$  threshold is likely due to the fact that the large HMOC parent molecules did not ionize and fragment to create the characteristic defining HMOC pattern of mass lines below 200 u. Above  $15 \text{ km s}^{-1}$ , more severe fragmentation probably created smaller organic molecules that were no longer consistent with the characteristic HMOC pattern. In addition, CDA data indicate that efficient fragmentation of the HMOC parent molecules only occurs above  $8 \text{ km s}^{-1}$  (Postberg et al. 2018b), suggesting that  $5\text{--}8 \text{ km s}^{-1}$  is the best window to ionize, but not fragment, these species. Similarly, mass lines allowing for the identification of O-bearing organics with CDA (acylium ( $\text{C}_2\text{H}_3\text{O}^+$ , 43 u) and  $\text{C}_2\text{H}_5\text{O}^+$  (45 u) only occurred at speeds below  $\sim 8.5 \text{ km s}^{-1}$  (Khawaja et al. 2019). Aromatic organics (phenyl cation ( $\text{C}_6\text{H}_5^+$ , 77 u) and benzenium ( $\text{C}_6\text{H}_7^+$ , 79 u)), appeared more robust to fragmentation, as they were identified with mass lines observed up to  $15 \text{ km s}^{-1}$  (Khawaja et al. 2019), in agreement with the upper HMOC speed threshold. Various mass spectrometers (the Enceladus Icy Jet Analyzer; Srama et al. 2015a, 2015b and the MAss Spectrometer for Planetary EXploration; Brockwell et al. 2016) and other microfluidics-based instruments (the Enceladus Organic Analyzer; Mathies et al. 2017) have been proposed to perform hypervelocity measurements of the Enceladus plume. These molecular surveys could address significant science questions regarding the search for biosignatures, conditions within the ocean, and other investigations at Enceladus. Examples of biosignature tests include the abundance pattern of amino acids, the number of carbon atoms in lipids, and the isotopic distribution in alkanes (Reh et al. 2016). Examples of geochemistry science objectives include measuring abundances of neutral species indicative of thermal alteration (and hence chemical evolution) in the ocean (Reh et al. 2016), detailed quantitation of mixing ratios of neutrals and ions indicative of redox disequilibria ( $\text{H}_2$ ,  $\text{CH}_4$ ,  $\text{O}_2$ ,  $\text{H}_2\text{O}_2$ ,  $\text{CO}_2$ ,  $\text{HCO}_3^-$ ,  $\text{H}_2\text{S}$ ,  $\text{SO}_4^{2-}$ ; Ray et al. 2021), and measure of D/H in  $\text{H}_2\text{O}$  and other isotopic ratios in plume volatiles to determine the physico-chemical environment of the ocean (MacKenzie et al. 2020).

While in situ detection of biosignature molecules and other species can be accomplished with hypervelocity sampling from a Saturn orbiter, a richer set of life, geochemical, and geophysical investigations can be undertaken with Enceladus orbiters and/or landers. Orbiters can get quite low in the plume, well below the lowest altitude at which Cassini sampled plume materials (50 km) and where the larger particles, not lofted as high as the smaller grains, will be found. These more massive grains could well be where most of the intact and more complex biosignatures would be found: microbes the size of



**Table 2**  
Recommended Plume Sampling Velocities for Various Biosignature and Organic Molecules of Interest, Based on In Situ Data, and Experimental and Theoretical Work

Experiment Type	Recommended Plume Flythrough Velocity ( $\text{km s}^{-1}$ ) <sup>a</sup>				Notes	Reference(s)
	Aromatic	Amino Acid	Fatty Acid	Peptide		
In situ data from Cassini Cosmic Dust Analyzer (CDA)	5–15 <sup>b</sup>	...	...	...	Enceladus plume and E ring measurements by Cassini CDA at velocities up to $17 \text{ km s}^{-1}$ . Ice grains impact a rhodium target and cations are accelerated to the detector.	Postberg et al. (2018b), Khawaja et al. (2019)
Laser-induced liquid beam ion desorption (LILBID)	...	4–10	3–6	4–8	IR laser impacts a water beam, generating ions and charged and neutral water clusters; reproduces data from Enceladus plume measurements by Cassini CDA.	Klenner et al. (2020a, 2020b)
Reactive molecular dynamics (RMD) simulations	Bare: 3–5	Bare: 3–5 In ice grains: 4–6	Bare: 3–5 In ice grains: 4–6	...	High-fidelity in silico simulations (>100,000 per molecule) varying velocity, impact angle, molecular structure, impact surface material and presence/absence of ice (Ih) shells (up to 25.6 nm diameter).	Jaramillo-Botero et al. (2021)
Ballistic impact	1–2 <sup>c</sup> , 4.9 <sup>c</sup>	1.9 <sup>c</sup>	4.2 <sup>c</sup> , 4.9 <sup>c</sup>	...	Ice projectile containing organic compound(s) fired into targets of indium or water ice using light gas gun, or steel spheres fired into organic-laden water ice target (aromatics and fatty acids); aqueous solution of organics (amino acids) shocked by impact with a steel projectile plate.	Burchell et al. (2014), Bowden et al. (2009), New et al. (2020), Blank et al. (2001)

**Notes.**

<sup>a</sup> Recommended sampling velocity to maximize ionization and/or detection of the target organic(s) while minimizing molecular fragmentation.

<sup>b</sup> Aromatic organics were identified with mass lines observed up to  $15 \text{ km s}^{-1}$  (Khawaja et al. 2019); other organics (O-bearing) were identified only at speeds below  $\sim 8.5 \text{ km s}^{-1}$  (Khawaja et al. 2019).

<sup>c</sup> Speed at which the organic molecule(s) survived after collection from craters and/or debris post-impact; the actual velocity threshold for molecular fragmentation may be higher.

viruses or nanobacteria may be present in particles 1  $\mu\text{m}$  in diameter, but more complex microorganisms, the size of bacteria, would necessarily be found in particles larger than 1  $\mu\text{m}$ . In addition, a mission orbiting Enceladus rather than Saturn would have a more rapid plume flythrough cadence, providing for much greater accumulation of plume material over multiple passes, and would also have slower plume flythrough velocities ( $\leq 200 \text{ m s}^{-1}$ ), enabling collection of intact plume grains rather than in situ volatilization and ionization of molecules in grains as with hypervelocity encounters. Collection and analysis of plume grains requires more complex sample processing, but is achievable with current technology and enables far more sensitive analyses of trace species, with concomitantly greater confidence in the results obtained.

A landed mission would allow a significantly larger abundance of plume material (by several orders of magnitude or larger, depending on mission duration), as well as surface material, to be collected (Spilker et al. 2009; Porco et al. 2017; Hendrix et al. 2019; Choukroun et al. 2021), enabling a greatly increased number and diversity of measurements, possibly even pre-concentration of plume material prior to analysis, improved detection limits and confidence levels, and the identification of intact cells and/or cell debris, should there be any.

Regarding potential cells, whether intact microorganisms within ocean water droplets would survive rupture from freezing as they are ejected in the plume depends on several factors, such as cell size and freezing rate (Mazur & Schmidt 1968; Dumont et al. 2004), neither of which are clearly defined for Enceladus. We note that hardy organisms such as bacterial spores are known to survive extreme treatment such as the temperature, pressure, and radiation conditions of space (Horneck et al. 1994), and thermophilic bacterial spore communities isolated from marine sediments have been demonstrated to survive freezing down to  $-80^\circ\text{C}$  (Cramm et al. 2019). As for pressure, typical bacteria-elimination protocols (e.g., in the food industry) require pressure cycling at much greater differentials than are likely to exist at Enceladus to kill microorganisms. Given the unknowns, biosignature searches targeting cells should also take into account how to identify inactivated cells or cell debris, in addition to intact/living organisms.

Finally, studying the geophysical and geochemical context of habitability at Enceladus is important, both to inform interpretation of biosignature measurements and to advance understanding of Enceladus' current activity and its surface and interior evolution (German et al. 2021). Geophysical characterization of Enceladus' gravity field can be achieved from flyby, orbital, and landed architectures, the last of which can include an extensive orbital phase prior to landing (Spilker et al. 2009; MacKenzie et al. 2020). We note that Enceladus orbiters/landers would provide the best measurements for determining the higher gravity moments and internal mass distribution of this moon, and some geophysical measurement techniques such as seismometry—important to assess hydrodynamical conditions within the near-surface of the South Polar Terrain ice shell—would be uniquely achieved by a landed mission.

The search for life at Enceladus is an important part of a broader quest to understand the relationship between habitability and life in ocean worlds throughout the solar system (comparative oceanography; Hand & German 2018), and will

likely require an exploration strategy akin to the Mars program with strongly coupled technology development and missions spanning decades (Sherwood 2016; Hendrix et al. 2019; Hand et al. 2020).

## 7. Leveraging NASA Investments

A quantitative, robust search for life beyond Earth can be carried out given our current knowledge of Enceladus and flight-ready technology. Through technology development programs like Planetary Instrument Concepts for the Advancement of Solar System Observations (PICASSO), Maturation of Instruments for Solar System Exploration (MatISSE), Concepts for Ocean Worlds Life Detection Technology (COLDTech), and Instrument Concepts for Europa Exploration 2 (ICEE-2; Simons et al. 2019), instruments key to life detection have advanced in technology readiness level (TRL), including those capable of making complementary and orthogonal biosignature measurements as well as sample ingestion, preparation, and contamination mitigation. Furthermore, NASA has invested in programs specifically targeting ocean worlds and life detection. These community-building efforts include establishing research coordination networks focused on themes foundational to astrobiology: developing life-detection technologies (Network for Life Detection), understanding the pathways of prebiotic chemistry (Prebiotic Chemistry and Early Earth Environments), exploring ocean worlds (Network for Ocean Worlds; Howell et al. 2020), and investigating the evolution of cellular life (From Early Cells to Multicellularity). Coordination between projects under these themes is poised to continue improving how we strategize, execute, and interpret the results of the search for life. Thanks to NASA investments in the last decade, the available technology and community expertise are ready to tackle one of the biggest scientific questions of our time, and one that reaches beyond planetary science to impact us all at the civilization scale: Is there life beyond Earth?














## 8. Conclusions

The Enceladus plume provides direct access to pristine ocean-derived materials in the ejecta or as surface deposits. In response to the seminal discoveries of the Cassini mission, the next strategic step is to search for biosignatures in Enceladus' ocean materials. This is a key objective of the 2018 NASA Strategic Plan, tackling an overarching question of the Vision & Voyages Decadal Survey: "Beyond Earth, are there contemporary habitats [...] and do organisms live there now?" Thanks to 13 years of observation by Cassini, the subsurface ocean of Enceladus is the only confirmed and most well-studied habitable environment beyond Earth with strong evidence supporting the presence of the ingredients considered necessary for life as we know it: liquid water, chemical building blocks including organics, and energy sources (e.g., Des Marais et al. 2008; McKay et al. 2008, 2014; Hendrix et al. 2019; Cable et al. 2020). While there are several candidate habitable environments in the solar system (including environments within Europa, Titan, and Mars, among others), none of them has been demonstrated to meet as many foundational requirements for habitability as Enceladus, and Cassini's findings constitute at present the highest standard of evidence for a habitable world. In situ detection of biosignature molecules can be accomplished with hypervelocity sampling of the plume from a Saturn orbiter, but a richer set of life,

geochemical, and geophysical investigations can be undertaken with an Enceladus orbiter or lander. Current mature instrumentation and measurement strategies can enable the detection of trace quantities of key features expected to be universal to life (membrane-forming molecules, information-storing polymers, etc.; Reh et al. 2016; MacKenzie et al. 2020), while also measuring the possible breadth of abiotic or prebiotic compositions if life is not present. The search for extant life, presently living or recently dead, in Enceladus' ocean materials is possible with today's technology, and ongoing investments are continuing to improve the maturity and sensitivity of these instruments. The ability to sample the subsurface ocean via the plume, without the need to drill or dig, makes Enceladus the most accessible habitable zone in the solar system and particularly alluring as the next target for a dedicated search-for-life mission.

C.R.G. (WHOI) acknowledges support from the Exploring Ocean Worlds (ExOW) project (NASA Award: 80NSSC19K1427). C.R.G. (SwRI) acknowledges internal support from Southwest Research Institute. M.N. acknowledges support from NASA award number 80GSFC17M0002. We also thank two anonymous reviewers whose comments greatly improved this manuscript. A portion of this research was carried out at the Jet Propulsion Laboratory, California Institute of Technology, under a contract with the National Aeronautics and Space Administration (80NM0018D0004).

### ORCID iDs

Morgan L. Cable  <https://orcid.org/0000-0002-3680-302X>  
 Christopher R. German  <https://orcid.org/0000-0002-3417-6413>  
 Shannon M. MacKenzie  <https://orcid.org/0000-0002-1658-9687>  
 Marc Neveu  <https://orcid.org/0000-0002-6220-2869>  
 Amy E. Hofmann  <https://orcid.org/0000-0001-6869-5118>  
 Amanda R. Hendrix  <https://orcid.org/0000-0002-0435-8224>  
 Frank Postberg  <https://orcid.org/0000-0002-5862-4276>  
 Linda J. Spilker  <https://orcid.org/0000-0002-5696-6125>  
 Nozair Khawaja  <https://orcid.org/0000-0001-8237-1523>  
 J. Hunter Waite  <https://orcid.org/0000-0002-1978-1025>  
 Peter Wurz  <https://orcid.org/0000-0002-2603-1169>  
 Ariel Anbar  <https://orcid.org/0000-0002-6015-7750>  
 Jean-Pierre de Vera  <https://orcid.org/0000-0002-9530-5821>

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