On the Feasibility of Informative Biosignature Measurements Using an Enceladus Plume Organic Analyzer

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Abstract

The icy moons of Saturn and Jupiter are high priority locations in which to search for biomarkers of life elsewhere in our solar system. In particular, the ice plumes that jet out through the ice surface at Enceladus provide an enticing opportunity to sample the organic components of its subsurface ocean for possible biosignatures while ameliorating challenges of planetary protection. Extensive high- and hypervelocity light gas gun experiments, the first to model the Enceladus ice plume encounter accurately, have demonstrated that organic-rich ice particles can be efficiently captured using plume transits at and below 3 km s⁻¹ without significant organic destruction. Realistic scenarios for the amount of ice captured and for the ability of organic chemical analysis instruments, especially microfluidic capillary electrophoresis with laser-induced fluorescence, to produce meaningful detection levels for biosignatures are then presented and compared with results from terrestrial models. This analysis indicates that an Enceladus plume fly-by mission carrying microfluidic instrumentation is capable of biosignature measurements with a sensitivity and analytical capability that is highly informative about the extent of extraterrestrial biological processes.

Unified Astronomy Thesaurus concepts: Saturnian satellites (1427); Biosignatures (2018); Impact phenomena (779)

1. Introduction

Examining solar system locations with a history of liquid water is a good guide in the quest for detecting signs of past extraterrestrial life (Voosen 2021), but it is unfortunate that we have not looked aggressively in locations where there is or was recently liquid water in an environment conducive to habitability and to life. Part of this reticence is due to the difficulty of the missions. For example, the polar regions on Mars where one finds frozen water and CO₂ ice have only been examined once (Smith et al. 2008). However, another reason for avoiding these special regions is planetary protection (PP). The polar locations on Mars must be protected from terrestrial contamination of these potentially habitable ecosystems. While important, PP makes missions more difficult, so we are caught in a catch 22: the best places to look for signs of extraterrestrial life are where we impose the highest restrictions on access.

The outer solar system icy moons such as Europa and especially Enceladus offer an opportunity to transcend this problem. Nature and the Cassini mission that explored the Saturn system have conspired to provide us with an enticing opportunity to chemically interrogate the ice layers and/or the underlying ocean at Enceladus while obviating many of the issues of PP by flying through the plume to gather material for analysis. The critical questions are: (1) Can we gather biosignatures embedded in plume ice particles in a high-velocity fly-by without organic destruction? (2) Can we gather enough ice to make meaningful organic biosignature measurements? (3) Will these science measurements be sufficiently sensitive to inform us about the existence of life compared to our only reference state: the level of bioorganic molecules found in the ice sheets and cold ocean regions on Earth? (4) Are there mission scenarios that can provide sufficient sample for a meaningful experiment? This paper discusses our current ability to answer these critical questions.

An extensive line of evidence supports the idea that icy moons such as Enceladus and Europa are promising locations to look for habitability and for life. Our primary focus here is on Enceladus because it has a global water ocean (Postberg et al. 2009; Thomas et al. 2016) that (1) lies under an ice sheet, (2) is in contact with a rocky core (Iess et al. 2014), (3) is understood to have significant salinity (Postberg et al. 2011), and (4) experiences hydrothermal activity at the ocean–core boundary that appears to supply adequate thermal energy to support microbial life forms (Hsu et al. 2015; Sekine et al. 2015; Waite et al. 2017). The hydrothermal activity at Enceladus and the observation of molecular hydrogen in the plume (Waite et al. 2017) are generally believed to be associated with organic compound production and an environment that would support life (Russell et al. 2014; Barge & White 2017). Models have suggested that oxidative processes may also play a role in diversifying the organic molecular complexity (Pasek 2020; Ray et al. 2021). Thus, the Enceladus ocean is thought to have significant organic content, which taken together with the geological context suggests high habitability potential (McKay et al. 2008; Glein & Zolotov 2020). Pristine samples from the subsurface ocean are ejected through warm fractures in the icy crust (Porco et al. 2006; Spencer et al. 2006), forming plumes that extend hundreds of kilometers above the surface (Spahn et al. 2006; Hedman et al. 2009). Simple organic compounds have been observed in the plume material (Waite et al. 2006, 2009; Postberg et al. 2008) and, more recently, complex macromolecular organics have also been detected in salt-rich plume ice particles thought to emanate from the subsurface.
ocean (Postberg et al. 2018; Khawaja et al. 2019), although unambiguous identification of specific species by mass spectrometry remains challenging (Magee & Waite 2017). Thus, Enceladus presents us with a remarkable opportunity to conduct in-depth astrobiological investigations to probe for organic and bioorganic molecules residing in the subsurface ocean that are indicative of at least habitability, but perhaps also extinct or extant life (Neveu et al. 2018). We now explore the critical questions posed above concerning the feasibility of such an investigation.

2. Plume Fly-by Ice Capture Efficiency

A direct and realistic experimental evaluation of the interaction of a capture surface with micron-sized ice particles containing biological molecules in a hypervelocity impact is difficult. While a number of experiments have been presented as models for this process (Klenner et al. 2020), the recent high- and hypervelocity gas gun experiments (New et al. 2020a, 2020b, 2021) present the most realistic and quantitative evaluation of the efficiency of gathering intact organics in a high- or hypervelocity encounter. Their work employs the hypervelocity light gas gun at the University of Kent, UK, configured to shoot frozen ice projectiles at a capture surface at velocities from 700 m s\(^{-1}\) to 5 km s\(^{-1}\) (Burchell et al. 2014). The capture surfaces (CS) in this study were soft, easily cleaned, inert metals that provide a compliant surface impact to reduce shock and enhance capture and that readily release the captured biomolecules for analysis. Foundational calibration experiments using poly(methyl methacrylate) beads of known diameter defined the relationship between particle size and crater size for the chosen target materials: Al, In, Au, and Ag (New et al. 2020b). A second paper developed the methods for shooting frozen ice projectiles at these targets (New et al. 2020a). The projectile breaks up in flight, peppering the targets with micron-sized ice particles. The resulting crater sizes are then used to determine the size of the impacting ice particle for quantification of capture. The most recent paper focused on the quantification of a fluorescent tracer molecule that is a surrogate for biosignatures (New et al. 2021) using the methods detailed by Kazemi et al. (2021). The amount of fluorescence associated with each crater was used to quantify the capture percentage based on the size of the impacting particle and the known concentration in the initial ice solution.

A representative analysis of one light gas gun ice impact is presented in Figure 1 for aluminum targets impacted with ice particles at 1.7 km s\(^{-1}\) (Kazemi et al. 2021). The crater sizes are used to estimate the volume of the impacting particle and the fluorescence in the crater quantitates the amount of fluorescent dye that resides in the crater after impact, distinct from the background.

Figure 1. Bright-field (left) and humidified fluorescence images (right) of craters in aluminum foils following 1.7 km s\(^{-1}\) velocity ice particle impacts. Fluorescent dye residue, which was doped into the initial ice projectile, is observed in the circled craters, and the corresponding particle diameters and organic capture efficiencies are indicated. The integrated fluorescence intensity in the craters is a direct measure of the capture and survival of the organic tracer molecule in a high-velocity ice impact. Adapted from Kazemi et al. (2021).

Figure 2. Representative organic capture efficiency (%) plotted against particle diameter (μm) from 3 to 25 μm for ice particle impacts into aluminum at 2.2 km s\(^{-1}\). Adapted from New et al. (2021).

Figure 2 examines the dependence of capture efficiency on particle size for aluminum, demonstrating that the efficiency increases as the particle size decreases especially in the 3–10 μm range (New et al. 2021). This is a favorable result, as the Enceladus plume at 50 km height, which has been most thoroughly studied (Porco et al. 2017), is dominated by ice particles below 10 μm in diameter with a distribution median at 6 μm.

The dependence of capture efficiency on velocity is also an important parameter. For example, Figure 3 presents a summary of the median capture efficiency for indium and aluminum for 3–10 μm diameter ice particles that are appropriate for Enceladus at a pass height of 50 km. The indium capture is very low at 3 km s\(^{-1}\) but rises dramatically to 17% below 1 km s\(^{-1}\) and is still rising at 800 m s\(^{-1}\) the lowest velocities observed. Aluminum is more effective at 3 km s\(^{-1}\) and rises to a maximum capture of 17% at 2 km s\(^{-1}\) then falling to 5% at lower velocities. It is thus demonstrated that relatively efficient 17% or higher capture of intact organics can be achieved below 3 km s\(^{-1}\) with indium and/or aluminum capture surfaces.

3. Total Ice that Can be Gathered

Based on these data we have the ability to calculate the amount of organic molecules in ice grains that will be captured in a variety of transit scenarios. For this comparison we focus on a capture surface area up to 1 m\(^2\) (MacKenzie et al. 2021) that is the largest currently being considered and velocities <3 km s\(^{-1}\). This limitation excludes single-pass high Enceladus transit velocity nonorbital trajectories through the Saturn system.
However, it has recently been simulated that transit velocities in the 4–6 km s$^{-1}$ range would be appropriate for detecting fragmented organics in ice plumes by impact ionization (Jaramillo-Botero et al. 2021). For intact organic capture, we will focus our discussion on mission scenarios including Saturn orbits and Enceladus orbits that enable plume pass velocities below 3 km s$^{-1}$.

An Enceladus orbiter is the most effective plume-sampling format for gathering significant amounts of organic-rich ice particles. The escape velocity for Enceladus is $\sim$240 m s$^{-1}$ so these orbits must have a plume transit velocity of only a few hundred m s$^{-1}$. The experimental ice particle data in Figure 3 extend down to only 800 m s$^{-1}$ where the peak capture efficiency for indium is 17%. However, it is clear that the capture efficiency is rising dramatically with reduced velocity in Figure 3. For purposes of this calculation, we will use the measured capture percentage of 17% with the realization that the capture efficiency is likely significantly higher at lower velocities. Particle size distribution also plays a role in these estimates. Experimental data extend down to 3 $\mu$m diameter ice particles, which span the 6 $\mu$m diameter median of the Enceladus ice particle distribution at 50 km closest approach (Porco et al. 2017). The capture efficiency rises strongly with smaller particle size as demonstrated in Figure 2 for the reasons previously discussed (New et al. 2020a). However, these capture percentages are lower limits because they were determined with a vertical foil target from which weakly adhering particles will fall off due to gravity. Using a plume density of 2 $\mu$l m$^{-2}$ pass$^{-1}$ (Porco et al. 2017) and a conservative 17% capture efficiency, we predict the capture of 0.34 $\mu$l (0.34 mg) of ice per m$^2$. The amount of harvested ice can be increased linearly with multiple passes that are very feasible in an Enceladus orbital format. Because plume density varies temporally and thins at increasing altitude (Ingersoll & Ewald 2017), it is advantageous to use a multipass collection approach rather than attempting to perfectly time a single transect.

For the Saturn orbital trajectories, we perform a similar calculation for a 2.2 km s$^{-1}$ encounter on an aluminum target that also provides a capture efficiency of $\sim$17% and an ice capture of 0.34 $\mu$l m$^{-2}$ for a single plume transit. Again, multiple passes are also possible for a Saturn orbital trajectory, but the total feasible number of passes is restricted by the longer Saturn orbital period (Reh et al. 2016).

It should also be noted that both of these calculations are based on a 50 km pass height and the estimated density at this elevation. The particle size increases at lower elevation, providing more material, but the capture efficiency in our experiments drops off with increased particle size. The amount of ice captured per particle increases almost 100-fold in going from 1 to 5 $\mu$m particles to 21–25 $\mu$m particles even considering the large drop in capture efficiency (New et al. 2021). The increased volume of the larger particles more than compensates for the reduced capture efficiency. Thus, in an orbital format, if the closest approach distance ranges from 20 to 65 km, the closer passes may contribute significantly more material to the harvest than calculated here. However, Guzman et al. (2019) performed detailed additional modeling of plume ice capture considering pass heights from 20 to 60 km and predicted the capture of 1.6 $\mu$l m$^{-2}$ pass$^{-1}$ (uncorrected for capture efficiency), which is indistinguishable from the values presented earlier by Porco et al. (2017).

### 4. Biosignature Detection Capabilities

There is a direct relationship between the amount of ice sample gathered, the volume of the processed sample solution, and the detection sensitivity of the analyzer that determines the lowest level of biosignatures that can be detected in a given ice sample. The instrumental detection sensitivity in solution experiments is concentration limited, indicating that gathering

![Figure 3](image-url)
more plume sample and processing it in the smallest possible volume are critical. The advantage of using microfluidic sample processing systems is their low volume capabilities. While microfluidic processing can be performed at the nanoliter level, it is more typical and practical to perform processing, transport, and analysis of volumes on the order of 10 μL or larger (Landers 2007). Small 10 μL volumes would enable high-sensitivity analysis by a single instrument while larger volumes of ~100 μL volumes would enable multiple types of analyses and transport to other instruments in the spacecraft.

The second factor is the detection sensitivity of the analytical instrument. We consider the circumstance where the analyte is labeled with a functional-group-specific reactive dye such as fluorescein, fluorescamine, or Pacific Blue succinimidyl ester and detected by laser-induced fluorescence (LIF). These reagents have been extensively used to label and detect amines and amino acids using lab-based prototype microfluidic capillary electrophoresis devices (Skelley & Mathies 2003; Chiesl et al. 2009; Creamer et al. 2017). The sensitivity limits for these reagents have been characterized in detail, producing detection limits in the nanomolar (nanomole/liter, nM) range for fluorescein (Skelley et al. 2005), from micromolar to 5 nM for fluorescein (Creamer et al. 2017), and extending this range to 100 picomolar (pM), or 0.01 ppb assuming nominal 100 Dalton molecular mass using Pacific Blue (Chiesl et al. 2009). These detection limits are fundamentally limited by the water Raman background scattering and its associated shot noise. Optimized LIF systems have demonstrated fluorescence dye sensitivity of <10 pM extending down to a few hundred femtomolar with more exotic detection systems (Dovichi et al. 1983; Peck et al. 1989). Thus, by using LIF detection, solution-phase organic detection limits in the low pM range are a reasonable expectation.

Microfluidic instruments combining fluorescence labeling of analytes, high-performance capillary electrophoresis analysis, and sensitive LIF detection (CE-LIF) are in advanced development for in situ biomarker analysis (Bada et al. 2008; Creamer et al. 2017; Mathies et al. 2017; http://eoa.ssb.berkeley.edu). CE-LIF was first developed as a laboratory breadboard (Skelley et al. 2005) followed by maturation of a portable integrated prototype that was successfully field-tested in the Atacama Desert (a Mars analog site) in 2005 demonstrating 10 ppb sensitivity for amino acids (Skelley et al. 2005, 2007). More recently, the CE-LIF mission focus has shifted to the icy moons of Saturn and Jupiter with the development of technology such as the Enceladus Organic analyzer (EOA, Mathies et al. 2017), a flight-capable design currently being matured to TRL 6–7 (http://eoa.ssb.berkeley.edu). In fact, the experiments presented below on terrestrial models of icy moons were performed with a flight design confocal detection system with a detection sensitivity of better than 50 pM, limited only by water Raman scattering. We therefore adopt a routine fluorescence sensitivity detection limit of 50 pM for CE-LIF systems with the prospect of improvements to 10 pM (0.001 ppb). It should be noted that time-of-flight impact ionization MS operates at the ~100 ppb limit of detection (LOD)—a limit that does not improve with sample accumulation (Brockwell et al. 2016). GCMS does benefit from sample volume accumulation improving from 1000 to 10 ppb with multimilligram samples (Goessmann et al. 2017). Thus, liquid-based CE-LIF is more than 100-fold more sensitive for the detection of biologically relevant organic molecules than current mass spectrometric approaches. CE-LIF also provides a complementary analysis that will help to disambiguate the many possible organic isomers that are degenerate in mass spectrometric data, as highlighted by Ingersoll & Ewald (2017).

Figure 4 depicts the fundamental relationships between plume sample volume, the key instrument analytical parameters of detection sensitivity and processed sample volume, and the level of biomarkers in the input sample. The amount of plume ice gathered ranges from 3.4 nl to 0.34 μL per plume pass for a capture area that ranges from 100 cm² to 1 m². This calculation is based on a plume transect density of ~2 μL ice per m² at 50 km pass height (Porco et al. 2017), a particle diameter less than 10 μm, and a mean capture efficiency of 17% as presented above. If we first consider a single pass through the plume and an instrument with 10 pM detection sensitivity, then the desired 1 nanomole/liter or nM biomarker detection goal indicated by the horizontal dashed line is easily achieved with a 1 m² detector gathering 0.34 μL. In fact, a smaller capture area of 0.6 m² would still meet the 1 nM goal by gathering ~100 nl. Examination of the diagonal traces shows that instruments with detection sensitivities poorer than ~34 pM will not be able to meet the biomarker detection goal of 1 nM (Hand et al. 2017) in a single pass. It is evident that with careful design and optimization of spaceflight CE-IF technology, even a single pass through the Enceladus plume can provide a sufficient sample for high-sensitivity analyses of biosignatures.

While this is a promising result, if we exploit an orbiter to integrate 10 passes through the plume, the blue shaded
detection space in Figure 4 shifts one log to the right. In this case instruments with ∼34 pM detection sensitivity, which is within current capabilities, could extend the sample concentration detection limits to ∼100 pM, which may be necessary for a meaningful measurement (see below). These calculations indicate a significant advantage of the accumulative capture surface approach. By performing multiple passes through the plume accumulating additional ice for analysis, the CE-LIF detection limits can be extended and/or more sample can be provided to additional complementary instruments.

5. Comparison to Terrestrial Reference States

The Europa Lander SDT report (Hand et al. 2017) did an excellent job of assembling the measurement requirements for an icy moon mission. They examined data on terrestrial oceans and ices to establish an organic measurement sensitivity goal. Based on dissolved free amino acid (DFAA) measurements of icy moon analog sites, they proposed a science measurement goal of 1 nM as the baseline for the characterization of habitability and the search for biomarkers of potential extraterrestrial life. The five most common amino acids in model aquatic deep ocean ecosystems (glycine, serine, alanine, aspartic acid, and glutamic acid) range in concentration from 2 to 3 ppb with concentrations from 17 to 38 nM (Moura et al. 2013). More studies are needed to examine a wider range of terrestrial models for Enceladus to determine the range of amino acid concentrations, the amino acid compositions in different types of environments, the chirality of these biomarkers, and the relationship of these results with the biological ecosystem and geological environment. It will also be important to examine organic biomarkers containing other functional groups such as amines, carboxylic acids and aldehydes, and ketones. How realistic is this choice of 1 nM for samples at Enceladus? To explore this question, we are now performing an examination of amine biomarkers in a variety of terrestrial ocean and ice environments to develop more knowledge of the terrestrial reference state.

Deep subglacial lakes, circumpolar deep water, and glacial ice sheets provide valuable environments to probe for biosignatures at the icy cold limits of life on Earth (Barbaro et al. 2017). Examples include Lake Vostok, Lake Vida, the West Antarctic Ice Sheet, and the Greenland Ice Core. As an initial test, we examined ice samples from the well-studied West Antarctic Ice Sheet (WAIS) core samples. WAIS core samples consist of deposited and frozen compacted snowfall that extends to 3404 m deep (68,000 yr BP); the core has been very well characterized and should exhibit some of the lowest DFAA concentrations (Buizert et al. 2015; Barbaro et al. 2017; Sigl et al. 2016). We present here results from a 12,500 yr BP WAIS samples from −2024 m depth. The analysis in Figure 5 used the Enceladus Organic Analyzer (Mathies et al. 2017) microfabricated capillary electrophoresis chip and LIF for the detection of the Pacific Blue labeled amines and amino acids in this sample. The key result is that the WAIS sample exhibits very low levels of dissolved free amino acids. Careful examination of the expanded traces in the 55–75 s range reveals that the WAIS sample exhibits various amino acid peaks (glycine, alanine, and serine and peaks labeled 1–7 shaded in red) at the few nanomolar levels as well as a small number of amino acids (peaks 1 and 4) just above the water and
reagent blank at the 200 pM level. This result demonstrates that detection sensitivities of 100 pM or better will be needed to fully characterize such terrestrial model ice samples.

WAIS ice samples that have been deposited in an open environment on Earth and sequestered for thousands of years exhibit very low levels of amino acid concentrations. If this is the case for Enceladus, then a measurement capability of 100 pM or better will be necessary to obtain a result that tells us whether these or other important biomarkers are present at levels comparable to those found on analogous environments on Earth. It has been proposed that a process called bubble scrubbing in the plume generation process can produce enhancement of amino acids in the plume by factors of 5–50 thereby improving detectability (Guzman et al. 2019). Our results demonstrate that even without bubble scrubbing, efficient capture surfaces, high-sensitivity LIF detection, and microfluidic processing can be combined to perform scientifically meaningful experiments both at Enceladus and at Europa. While this discussion has focused on amino acid biomarkers, it will also be important to examine amine, carboxylic acid, and aldehyde and ketone containing biomarkers in these reference samples by CE-LIF using the methods that we have previously presented (Chies et al. 2009; Stockton et al. 2010, 2011).

6. Conclusions

A focused mission to sample the Enceladus plumes with organic analysis and plume characterization instruments is feasible and, if performed, would likely produce valuable scientific measurements that address one of NASA’s most important goals—the search for life in our solar system. The extensive work of New and coworkers (New et al. 2020a, 2020b, 2021) has experimentally demonstrated that there are well-defined capture surfaces that can efficiently and nondestructively gather organic-containing ice particles at velocities from a few hundred m s$^{-1}$ up to 3 km s$^{-1}$. By using capture surfaces with an area from 100 cm$^2$ to 1 m$^2$, it is possible to gather significant amounts of plume ice for analysis. By exploiting the unique capability of the capture surface format to integrate plume particle capture through multiple passes, these capture surfaces can dramatically enhance the total amount of plume ice for analysis by 10-fold or more thereby improving the detection limits to below the 1 nM level proposed as a science measurement requirement for the Europa Lander (Hand et al. 2017). Further enhancement of analysis sensitivity through more efficient particle capture, increased number of plume passes, efficient low volume dissolved sample transport, and enhanced instrument detection capability of CE-LIF instruments will all ensure that the overall measurement capabilities are at least good enough to detect organic biosignatures if they are present at the concentrations found in the most challenging cold ocean analog environments on Earth. Furthermore, the Planetary Protection challenges of an Enceladus orbiter are easier to solve because there is no need to contact the ice surface while making scientifically valuable measurements on the plume. Detailed analysis of the chemical content of the Enceladus plume for potential intact bioorganic molecules will dramatically advance our understanding of the limits of habitability and of the potential for life elsewhere in our solar system by directly probing one of the most interesting possible gestation sites currently accessible.

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