Review

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Review

Biomarkers on the Icy Jovian Moons: Can Europa Also Provide Insights into Life's Origin?

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Abstract

Within the payloads of JUICE and Europa Clipper, there are instruments suitable for the search of a specific biosignature. These missions have payloads with mass spectrometers capable of measuring both isotopic abundances, and testing whether ocean worlds could harbor amphiphile mixtures, or populations that would lead to a lipid-first origin of life. We may begin to test whether in the Jovian icy moons there may be large detectable excursions of stable isotopes of chemical elements on the icy surfaces that are substantially shifted from their expected isotopic distributions. The detection of an unambiguous signal would suggest a biogenic origin, provided care is taken to exclude abiotic thermal isotopic fractionation. Our suggested tests should be confirmed independently with other techniques. Stable Isotope Geochemistry on the icy Jovian moons has not yet been thoroughly discussed in the literature. In addition, we enquire whether insights into life's origin could be retrieved from Europa's ocean and its surface, including the question of the first steps in the evolution of life. Special emphasis has been put on an approach to seek on the surface of ocean worlds chemical phenomena that are rather primitive, but nevertheless with published models can predict a path towards life.

Keywords: index of biogenicity; missions to Europa; Europa Clipper; MASPEX; SUDA; biosignatures; early evolution of life; first biomolecules; GARD model; Lipid World hypothesis

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1. Introduction

We discuss firstly, at present the Icy Jovian moons especially Europa, as fertile ground for the search of biosignatures. This is done in Secs. 2-6. In Sec. 7 we consider whether upcoming discoveries would suggest that in the future, life could emerge. This could help to answer the question of the first steps taken by the evolution of life on Earth, such as which were the first biomolecules that led to life as we know it. We emphasize that beyond the interpretation of the data gathered by the Galileo mission, there are preliminary works that point towards new detection of chemical entities, such as ammonia arising from the Europa ocean [1]. Such suggestions open the door for further considerations of new chemical entities, as touched upon in this Review.

2. Probing for Chemical Elements and Their Isotopes on Icy Surfaces of the Jovian Moons

Potential biosignatures as, for instance, the recent report concerning a sample collected by NASA's Perseverance Mars rover from an ancient dry riverbed in Jezero Crater, could preserve evidence of ancient microbial life, but like the biosignature proposal in this work, requires additional studies before such biosignatures could be accepted to be related to living organisms [2]. Extending the isotopic fractionation from the rock record to astrobiology has been attempted by means of the index $\delta^{34}S$, which had been used in the geologic record [3].

We found it profitable to use isotopic fractionation excursions in astrobiology, but in previous research projects attention has been restricted exclusively to the icy surface of the ocean worlds (OWs), which are celestial bodies (planets, or satellites) that contain a hydrosphere with abundant water forming of oceans, often submerging dry land. In the case that concerns us in this review, the Jovian and other outer solar system moons, OWs are also called icy worlds, when the land has been totally submerged, unlike our own planet, where continents are present.

We have not entered in the wider context of isotopic fractionation taking place in planetary science, since that would clearly require a more extensive discussion. For example, the Life Detection Knowledge Base (LDKB) has been developed by a large group of researchers in order to test and evaluate strategies to search for evidence of life beyond Earth. Their emphasis has been on recognizing potential false-positive and false-negative results in the general case of planetology, rather than in the more restricted case of the icy surfaces of ocean worlds, which is exclusively the main topic to which this review has been dedicated [5,6]. The LDKB authors have done a careful analysis for applying life detection criteria to assess isotope abundance patterns as potential biosignatures. They have used previous work [5] for creating a data base to put some order in planning future missions. With LDKB it will be easier to catalog reliable biosignatures, out of the many that are likely to be proposed after JUICE and Europa Clipper begin their measurements. At the same time, they have provided a repository for the acquisition of new data. These authors [5] have introduced a useful language for highlighting reliable biosignatures, "high and low arguments". For instance, isotopic discrimination during biological dissimilatory sulfate reduction can yield delta S values of sulfides that are 15-72%, lower than the reference values $\delta^{34}S$ (‰) of their sulfate source. In section 6 below we shall return to the clear example of such isotopic fractionation due to Wortmann and coworkers.

For the ongoing missions for the Jovian system [7, 8], sulfur would have the most relevant excursions of isotopic fractionation for the detection of traces of biogenic activity, out of other biogenic elements: carbon, hydrogen, oxygen, nitrogen and phosphorous [8]. Sulfur consists of four stable isotopes: ^{32}S (95.02%), ^{33}S (0.75%), ^{34}S (4.21%), ^{34}S (4.21%) and ^{36}S (0.02%). Bacterial sulfate reduction (BSR) is a well-understood process, in which the bacterium involved links atoms of hydrogen with sulfur, taken away from dissolved sulfate (SO_4^{-2}) of seawater to form hydrogen sulfide (H_2S). This gas is enriched in ^{32}S relative to the seawater source [9, 10].

We consider the redistribution of the primordial isotopic mixtures as usual in terms of the sulfur delta parameter [11]:

$$\delta^{34}S = [(^{34}S/^{32}S)_{sa} / (^{34}S/^{32}S)_{st} - 1] \mathrm{x} 10^3 \ [‰, \mathrm{CDM}] \qquad (1)$$

The value of $\delta^{34}S$ is taken to be zero when the sample coincides with the corresponding value of the Canyon Diablo meteorite (CDM), a standard troilite (FeS) that was found in Phoenix, Arizona. The useful parameter $\delta^{34}S$ allows a comparison of a sample (sa) with the standard (st) CDM. The relevant terms are the dominant sulfur isotope (^{32}S) and the next in abundance (^{34}S). In fact, the quotient $(^{34}S/^{32}S)_{st}$ is the average terrestrial fraction of the two most abundant isotopes of sulfur. In a given sample, we obtain a positive value of $\delta^{34}S$ when the quotient $(^{34}S/^{32}S)_{sa}$ exceeds value of the standard CDM; otherwise, we obtain a negative value when the quotient $(^{34}S/^{32}S)_{sa}$ is less than the value of the standard CDM.

Analogously, the biogenic element chlorine has two stable isotopes [12]:

^{35}Cl (75.78%), ^{37}Cl (24.22%),

The Cl isotope fractionation is discussed in terms of $\delta^{37}Cl$, which is defined as we did above with the $\delta^{34}S$ parameter of sulfur. Measurements of the relative abundance of Cl isotopes is reported in the per mil notation (‰) vs. Standard Mean Ocean Chloride (SMOC). SMOC is thought to be isotopically homogeneous to within ± 0.15 ‰ [13].

Interestingly, within our overall objective of searching for reliable indices of biogenicity, the bacterium *Azospira suillum* shows a significant stable isotope fractionation excursion of –15 ‰ [14-15]. Chlorine has been identified on the icy surface of Europa [16,17]. While the model assumes the

Europa's seafloor initial composition to be chondritic Type II has been shown that water-rock cycling at the silicate seafloor is capable of producing a chloride-rich ocean [18-20].

With the sulfur isotopes, it is possible to report three measurable delta values δ33S, δ34S and δ36S. The delta parameter, δ34S, has been highlighted for a very long time in the terrestrial rock record as a valuable index of biogenicity, and only more recently in the context of astrobiological discussions, as in our previous papers reported in this Review**.** The support for δ^{34}S distributions, as indices of biogenicity has been extensively illustrated from sediments throughout the terrestrial rock record. Examples that clearly illustrate such support were to a large extent due to the distinguished Polish condensed matter physicist Manfred Schildowski and co-workers, mentioned above [8]. These examples range throughout the whole evolution of life on Earth, for instance:

(i)Pyrites in banded iron formations (BIF) from the Isua Greenstone Belt dating from the Archaean in the time interval of 3.7-3.8 Gyr BP, which have been assigned δ^{34}S (‰) of – 4 ± 2 [21].

(ii)A second example is from a rock formation in the Pilbara region of Western Australia, in which barite deposits of some 3.47 Gyr BP have been assigned values of δ^{34}S of -5 ± 7) [22].

(iii)A third example is given by pyrites in black shales from 2.7-2.8 Gyr BP from Western Australia that have a δ^{34}S of – 8 ± 7 [23].

Finally, pyrites and other sulfides from the beginning of the Triassic Period from Central Europe, were characterized by δ^{34}S of – 40 ± 20 [24]. We should underline that the above examples range from the earliest manifestation of life in the Archaean to a significantly recent geologic time, namely, the Triassic Period 251.9 Myr BP, when multicellular life was widespread.

In fact, both chemical elements, sulfur and chlorine on the surface of the Jovian moon Europa and whose origin is not of an external source, could have been cycled by oceanic dwelling microorganisms. This hypothesis will be available for testing after the arrival of the above-mentioned NASA and ESA missions, provided that sulfur fractionation, due to metabolic modifications that may have taken place underneath the icy shell render such phenomenon measurable. Fractionated sulfur isotopes may reach the upper side of Europa's icy shell, since for some time we have been aware with telescopic observations of the existence of water vents arising from the Europan ocean. In other words, if microorganisms were responsible for sulfur fractionation in the Jovian OWs, their activity could be detectable from icy shell surficial traces with the sensitivity of the available instrumentation that has been incorporated in the payloads of JUICE and Clipper. To follow this approach for the identification of biosignatures on the icy surface of Europa and other OWs, some instrumental and technical challenges remain. These will be briefly outlined in Section 4 below.

3. The Identification of Fractionation Due to Abiotic Processes

Thermochemical Processes is a first topic to keep in mind, regarding the Jovian icy moons from the point of view of stable isotope geochemistry. Our recent attempts have been restricted to anticipate how instrumentation could be used for the search for habitable ecosystems in the exploration of Europa and Ganymede [25]: We have kept in mind the basic discussions how living processes can be distinguished from abiotic processes [26]. Our previous discussion followed the standard approach [27], as briefly described in this section.

In the fractionation of stable isotopes, there are two mutually exclusive regimes: for sulfate reduction in the range 60–80°C that should be compared with high temperature in the range of 150–200 °C. These higher temperatures are generally larger than the cases of biogenicity. However, the above two thermal regimes do overlap when aqueous sulfate can be reduced by organic compounds at temperature, close to the water boiling point [28]. Abiotic fractionations are irrelevant for Europan ocean considerations, where abiotic sulfate reductions are larger than biogenic ones.

More specifically, experiments have only yielded fractionations of 10–20 ‰ for temperatures in the range of 100–200 °C. Even though there is some ocean-seafloor contact, hence raising the possibility of abiotic fractionation, due to the exposure to the temperatures of hydrothermal vents. However, we should keep in mind that, at the seafloor, the emergence of microorganisms would be subject to thermochemical sulfate reduction, but globally this would be largely negligible from the experimental evidence of fractionations being smaller than about 20 ‰ [29].

When a chemical element is reduced, due to heat, rather than by microorganisms, we should insist in the use of the abbreviated phrase "thermochemical sulfate reduction (TSR)". This adoption allows us to distinguish this abiotic phenomenon from the following independent sources of abiotic fractionation. Next in importance are the hydrothermal processes. First consider seafloor hydrothermal vent sulfides that are characteristic by low Δ^{33} values, where the capital delta parameters are defined as follows:

$$\Delta^{33} = \delta^{33}\ S - 0515\ x\ \delta^{33}\ S \tag{2}$$

$$\Delta^{36} = \delta^{36}\ S - 1.90\ x\ \delta^{34}\ S \tag{3}$$

The capital delta parameters measure deviations from mass-dependent relationships [30], compared with biogenic sulfides. It should also be remembered that there are small contributions, or even null contributions, from microbial sulfate reduction into hydrothermal sulfides at sediment-free mid-oceanic ridge systems. Besides measurements of δ^x parameter (1), where x = 33, 34, 36 were carried out in four high temperature seafloor hydrothermal vents along sediment-free ridge systems. The sulfur systematics suggest isotopic exchange between sulfate and sulfide at T = 400 ° C. The systematics can decouple microbial sulfur cycling at ocean crust-seawater interfaces, even when δ^{34} values lead to non-unique interpretations [31].

Photochemical processes should also be taken into account. Returning to the above two thermal regimes, there is some overlap between them, but these cases are mostly irrelevant for our main interest that concerns the two OWs that are nearest to planet Jupiter. We should also keep in mind that at the seafloor, the possible origin of life would be subject to TSR, but this would tend to be true, since such fractionations have been verified to be smaller than about -20 ‰ [32].

A careful experimental survey of SO_2 photolysis caused by a series of different UV wavelengths have uncovered a relationship between such wavelengths and sulfur isotope fractionation in the geological record as in the above work of Farquhar and co-workers. These experiments have demonstrated that there is a given wavelength for which SO_2 photolysis coincides with certain Archaean samples; other wavelengths in these experiments differ from the observed geological record. This can be used to retrieve information from the terrestrial atmosphere. To sum up, there is a certain wavelength-sensitive mass-independent abiotic sulfur isotope fractionation effect during SO_2 photolysis.

It is interesting to highlight the fact that biotic processes that are characteristic of hydrothermal environments can simulate isotopic discrimination exhibited by sulfate-reducing enzymes. For instance, in hydrothermal systems, differences in $\delta^{34}S$ values between sulfate and sulfide of -14‰, to -21‰ have been shown to occur via geochemical (abiotic) processes [33]. These values are too small to be relevant, for if observed, the magnitude of the $\delta^{34}S$ to be significant ($\delta^{34}S$ > -60 to -80 ‰) would in this case be suggestive of biogenic origin.

4. Fractionation from the Rock Record to the Jovian Moons

Our initial suggestion was to point out the relevance of transferring to the astrobiological context the successful application of geochemistry of the stable sulfur isotope fractionation from the early rock record on earth. Our motivation included in this work we have addressed several questions, the main ones being [34]:

Why should sulfur isotopes be the main objective of the search for biosignatures?

Would it not be better to rather have a combination of sulfur and carbon isotope anomalies?

Sulfur is unique amongst the biogenic elements since in extraterrestrial material (lunar fines and meteorites) sulfur, rather than carbon, shows in isotopic fractionation a narrow range of values about zero per mil. An additional question in favor of sulfur isotopic fractionation is*:*

Can you accept some contribution of sulfur from Io, and still find the biogenic fraction in those sulfur deposits?

In this case, since we are assuming from the data that only biogenic processes alter the null values of the isotopic sulfur fractionation, the contribution from the exogenous (Io) sulfur, if it were the product of bacterial metabolism, would not give the significant biogenic signal, that would otherwise be produced by endogenous sulfur.

We have attempted to go from fractionation in the rock record to Galilean moons as follows: Besides long-term planning for the exploration of the moons of the Jovian system, additional search for biosignatures should focus firstly on two of the Galilean moons: Europa and Ganymede. Subsequently, Callisto would also deserve a separate mission too. Fortunately, both missions by ESA and NASA that are already on the way to the Jovian System are equipped with appropriate instrumentation [35,36]. In the short term, such instrumentation makes it feasible to explore the exoatmospheres of the Jovian OWs. Since generally Europa's surface composition determines the composition of its atmosphere and can yield information on surface components, most surficial trace species will be present in the extended atmosphere [37].

In view of the many instruments that are in the payloads of the forthcoming missions to the Jovian system, with the $\delta^{34}S$ index of biogenicity, it is convenient to further comment on only a few of them that would be relevant from the point of view of the biogeochemical biosignatures. Firstly, there are mass spectrometers: In JUICE (the PEP Package with its NIM instrument); secondly, in the Europa Clipper we have MASPEX and SUDA. The NIM instrument is intended to measure the exospheres of the Jupiter satellites during their flybys [38].

In the present context we could raise the question whether the bioastronomy of OW biosignatures is relevant, for we still do not have a reliable SETI signal, let us now consider some of its consequences. As a working hypothesis, we could assume that the evolution of life in the universe can be explained in terms of evolutionary forces that we experience today. In that case, once the living process has started, it will continue due to evolutionary pressures, as it was explained in detail in the Hawaii Bioastronomy conference [39].

We can devise experiments addressing specifically the search for the evolution of microorganisms, especially from prokaryotes to eukaryotes, the latter being a first step towards the evolution of intelligence, as we have witnessed on earth. Indeed, several arguments militate in favor of the ubiquity of eukaryogenesis in habitable environments in other worlds, as argued in the San Juan Capistrano Conference No. 5 [40]. We had argued that in the Drake Equation a factor f_e should be included, which denotes the fraction of planets or satellites, where eukaryogenesis occurs [41].

For the distribution of life in the universe: one approach is the search for microorganisms to test for the onset of eukaryogenesis in the solar system. Alternatively, the direct approach is a major aim of bioastronomy, namely the time-honored SETI search of (intelligent) life in other solar systems, as proposed by Frank Drake, when in 1960 he set out to search for extraterrestrial intelligence as a legitimate and doable scientific endeavor [42], which was subsequently widely accepted by the bioastronomy community, in terms of the Drake Equation. Sadly, as underlined at the beginning of this Section, this approach has still not produced a reliable signal, suggesting that alternatives should be followed up, such as searching for OW biosignatures as indicated in this Review.

5. The Future of Jovian Ocean World Exploration

In an astrobiological context of biosignatures from the Europan ocean, the minor S isotopic biosignatures are not corrections of an order of magnitude comparable with the biosignatures in the terrestrial cases, where there are definite large fractionation excursions of up to 72 ‰ that have been caused by *in-situ* microbial sulfate reduction [43]. Provided the result of the orbital Jovian measurement gave sufficient large fractionation excursions (> 50-60 ‰, or larger), the measurements of large $\delta^{34}S$ could be interpreted as a biosignature. We have insisted that measurements of the Jovian orbital missions ought to search for the presence of significant large values of $\delta^{34}S$.

With the two ongoing probes for Europa or Ganymede, or with a subsequent lander on any of the OWs of the Jovian system, the isotopic abundances discussed in the present review should be complementary with other searches for life. Eventually, the measured anomalous isotopic abundances could be a first step towards an early identification of the existence of other life away from planet earth, possibly when JUICE and Clipper begin gathering data in the 2030s, or subsequently.

A Europa lander even as a mission concept, an eventual Europa lander remains an appealing project of NASA [44]. This is not a new concept, since early proposals were made at the end of the Twentieth Century, in which a probe called a cryobot capable of penetration through the icy shall of the Jovian moon, provided with a miniature submarine probe (hydrobot) could attempt exploring the Europan ocean.

Such a proposal initiated at JPL, was appealing to some of us at that time [45]; but at present, it is not clear whether a Europa lander would be capable of carrying a hydrobot in its payload and deliver it into the ocean. With our present technology and, especially with the current limited financial support, this possibility is definitely to be excluded in the short term, but it should not necessarily be discarded in the long term. Yet more recently some more advanced proposals have been tentatively explored with significant financial support, such as an extension of the cryobot miniature surficial probes such as PRIME, the comprehensive cryobot architecture for accessing Europa's ocean [46].

To conclude with Europa landers, some recent more realistic proposal proposes to focus on a Europa lander instrument. It is suggested to couple a mass analyzer with sample introduction/laser ablation/desorption ion source (ORIGIN instrument).

This instrument is more like LMS with the laser ablation/desorption ion source [47]. But the work of Pitesky and Hand summarize a further series of ideas that ought to be followed up after the "post-Europa Clipper" era.

6. Instrumentation Challenges, Uncertainties and Implicit Hypotheses

INSTRUMENTATION CHALLENGES. It is intuitive to consider especially the instruments on JUICE and Europa clipper but there remains the challenge to demonstrate how these instruments could determine the sulfur isotopic ratios and if the results would be accurate enough. For example: NIM on JUICE is expected to have only a mass resolution of around 800.

In the long term, it is uncertain whether penetrating instruments (cryobots) may not be an option to get further insights of the chemical nature of the ocean to constrain the distribution of chemical elements on the OWs icy surfaces.

UNCERTAINTIES. A mass resolution of around 800 is not sufficient to differentiate, e.g., between H_2O_2, $^{18}O^{16}O$,^{34}S, and H_2S, which all have the nominal mass 34. Radiolysis/photolysis species could be expected in realistic observation scenarios and thus should be considered. The mass resolution of SUDA is even less than that of NIM. Only MASPEX has a sufficient resolution to allow delineation of the species on nominal mass 34. Our suggestion is only to be interpreted as a first step towards potential reliable biosignatures

IMPLICIT HYPOTHESES. Related is the question of how the (heavy isotope enriched) sulfate anions reach these mass spectrometers. For SUDA, which has the lowest mass resolution, it seems intuitive. It is less obvious for NIM or MASPEX.

Another implicit hypothesis is whether all the sulfur on the icy surfaces of the ocean worlds, including specific case of Europa, is exogenic. There remains the question whether the sulfur that could emanate from hydrothermal vents could reach the lower side of the icy shell and eventually contaminate the exogenic sulfur, whose source could be due exogenic, possibly emanating from the Jovian moon Io's significant volcanic eruptions.

Tentatively there has been a report of the detection of a characteristic NH_3 absorption feature at 2.20 ± 0.02 μm on Europa's icy surface by careful studies of the NIMS Spectrometer. that suggests NH_3-hydrate and NH_4-chloride are the most likely candidates. The implication is that there has been emplacement from the underground (or shallow subsurface). The paper suggests transportation to the surface via cryovolcanism during recent geological past [1]. The presence of ammoniated compounds implies a thinner ice shell [49] and a thicker, chemically reduced, high-pH subsurface ocean on Europa [50]. Besides reporting the detection of NH_3-bearing components, this study also presents the first evidence of a N-bearing species on Europa, an element that is intimately related to life as we know it on earth, especially for the synthesis of more complex biomolecules such as amino acids, nucleotides and phospholipids.

7. Discovering Evidence for Future Life on Ocean Moons

There are in fact two quite different approaches for finding chemical entities on planets, or their satellites. The first is the attempt, as in the above Secs 2-6, to discover chemical phenomena suggesting that life is currently existing on the planet or their moons, or whether life existed there in the past. The second approach, to be reviewed in the present Section 7, consists in seeking chemical phenomena that are rather primitive, but with published models, can predict a path towards life. In the previous sections, we discussed evidence for past or present life on Icy Jovian moons, especially Europa, as fertile ground for the search of biosignatures. Here, instead, we consider whether upcoming discoveries from the current missions to the Jovian system or later, would suggest that life could emerge. This could help to answer questions about the early stages of life’s emergence on Earth.

In line with the Lipid World hypothesis [51], which is realized and quantitated by the GARD model, a group of amphiphile aggregates, e.g., micelles or vesicles, may be protocell precursors [52]. The most important finding portrayed by computer simulations show that some of these aggregates can undergo self-reproduction [53, 54] (Figure 1).

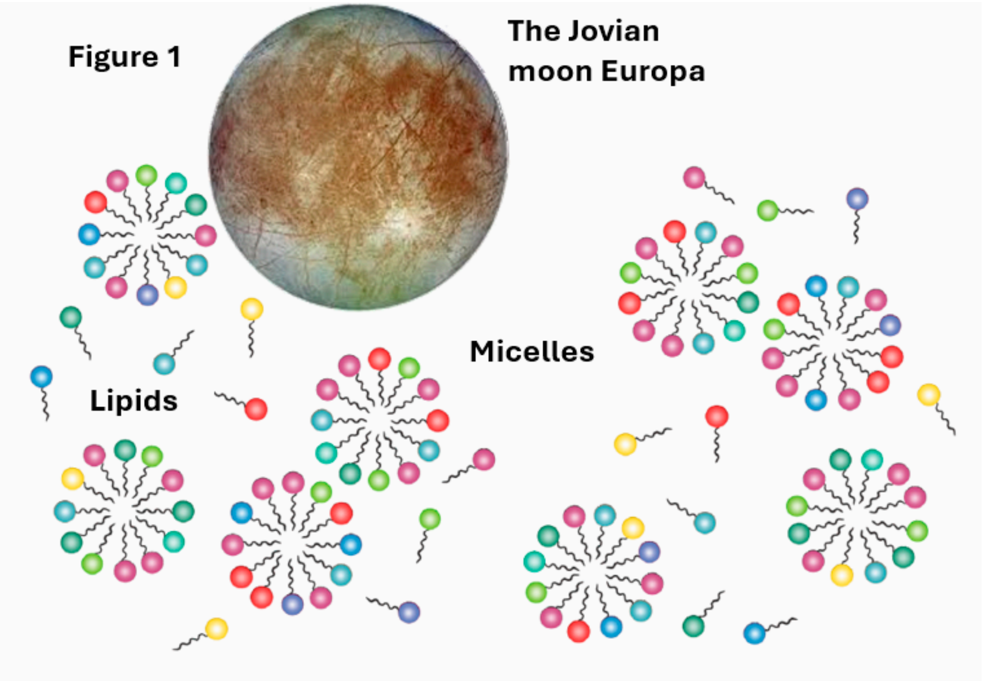

Figure 1. On planet Earth, with a repertoire of 100 lipid types, an assessment computes a huge library of 10^{33} micelles (nanoscopic protocells), each with a different composition [54]. With a 4-fold smaller diameter of the surface, the micellar library size will be about one order of magnitude (~10^{32}). As the GARD model of micellar self reproduction [54] is computed as $P=10^{-12}$ [55], a yet astronomic number of 10^{20} micelles on Europa might undergo compositional self-reproduction. The instrumentation on future expected Europa missions (Figure 3) would be able to detect micelles and measure their composition. This will allow to find out if in limited environments in the ocean of Europa there are proto-species with numerous micelles having similar composition

(with only a few mutations) [56]. Such an observation would possibly indicate a seed of Darwinian evolution at life's origin on Europa.

We now intend to discuss how the instrumentation currently included in Europa Clipper's payload can support the Lipid World hypothesis. The Clipper's Mass Spectrometer for Planetary EXploration (MASPEX) can generally measure volatile and organic molecules in Europa's sputtered and radiolytically processed exosphere as well as ocean plumes, at very high sensitivity and resolution [57]. This is the best shot at detecting amphiphilic organics, beginning with fatty acids, and ending with rather complex lipid-like molecules that can aggregate into micelles and vesicles. The SUrface Dust Analyzer (SUDA), which is a dust/ice grain impact time-of-flight mass spectrometer, analyzes ice grains/ejecta and is particularly strong at salts and organics in grains, which has importance for reporting counterions, and salinity that set aggregation behavior i.e. critical micelle concentration (CMC) [58] (Figure 2).

Figure 2

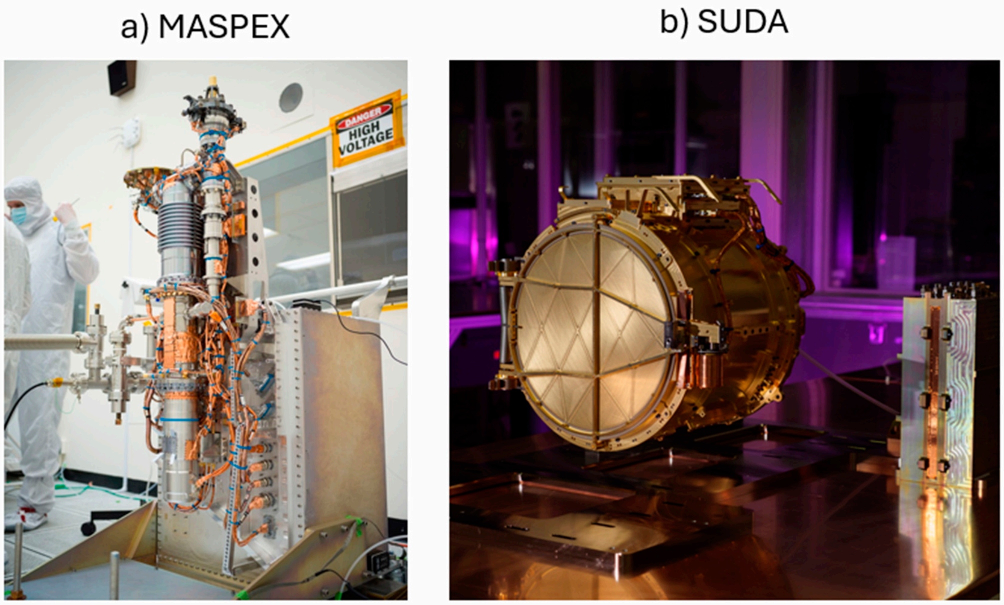
a) MASPEX
b) SUDA

Figure 2. This instrumentation is carried on the Europa Clipper mission. a) MASPEX (Mass Spectrometer for Planetary EXploration) measures volatile and organic molecules, including amphiphilic lipids in Europa's sputtered exosphere, as well as ocean plumes. b) SUDA (SUrface Dust Analyzer) is a dust/ice mass spectrometer, analyzing salts and organics in grains. The results would possibly support the Lipid World origin [REF Lipid world] (see Figure 1).

Taken together, both technologies can also help find chain-length distributions and headgroup diversity that can define amphiphile populations rather than random trace organics. The above information allows determining a whole spectrum relevant to protocellular chemistry. Careful analyses of the data and discovering amphiphiles in non-equilibrium ratios should provide further robustness. All in all, the data collected could provide support for the idea that ocean worlds could harbor amphiphile mixtures, or populations that would lead to a lipid-first origin of life. Results from the current mission could also guide future landers, including sample-return missions.

To demonstrate reproduction or heredity, or infer specific structures (e.g., micelles or vesicles) directly there is a need to show high similarity of composition and that they are progeny of a composome (attractor-like reproducing state predicted by the GARD model) reproducing with only a few mutations. Such groups may be considered protospecies, hence may be viewed as a seed of life, capable of undergoing natural selection, the prerequisite for Darwinian evolution.

A central requirement for evaluating compositional heredity in Europa samples, in future endeavors, is the ability to resolve the molecular composition of lipid micelles after controlled growth–dilution cycles, allowing detection of composomes. This necessitates instrumentation capable of handling microliter-scale melted-ice aliquots, separating complex amphiphile mixtures, and quantifying their relative abundances with sufficient resolution to distinguish non-random chemical organization from stochastic assembly. Space-qualified microfluidic capillary electrophoresis (CE) platforms, most notably the "Chemical Laptop", a fully automated CE–LIF (capillary electrophoresis - laser-induced fluorescence) system for high-resolution organic separations [59, 60], and the more recent Microfluidic Organic Analyzer (MOA) [61], which integrates a programmable microfluidic handler with a precision CE wafer and is explicitly targeted to ocean-world missions, provide robust separation of amphiphiles by charge and size, converting each micelle into a measurable electrophoretic composition vector. Complementary detection can be achieved using miniature laser-desorption mass spectrometry (LDMS) [62], in which micelles are disrupted and their components resolved by mass-to-charge ratio, yielding quantitative stoichiometric spectra. Front-end sample preparation—including melting, filtration, derivatization, reagent addition, and aliquoting—is supported by monolithic lab-on-chip processors such as SPLIce [63], while auxiliary techniques such as contactless conductivity microfluidics for ionic and amphiphile profiling [64] and digital microfluidics for controlled mixing, splitting, and serial growth–dilution cycles [65] provide additional operational flexibility. Together, these microfluidic and spectrometric technologies form a realistic, flight-deployable analytical architecture in which micellar compositions—whether random or convergent—can be reconstructed across multiple aliquots and compared statistically to the compositional clustering expected for GARD-type composomes.

In parallel, new technologies are being utilized in terrestrial settings that have, with modification, potential to be employed in extraterrestrial settings as well. One such technology, termed CytoFLEX nano flow cytometer, is currently in use to profile extracellular vesicles [66, 67] and lipid nanoparticles (LNPs) [68, 69]. While at this point in time, the CytoFLEX nano flow cytometer is better suited for > 80 nm micelle profiling, in the near future this might be honed down to also suit smaller sizes. Providing it is further fine-tuned to a space-qualified nanoscale cytometer, the advantage of such instrumentation would be direct detection of single-particle biosignatures as opposed to bulk composition detection with CE-LIF. Although the sensitivity for micelles is borderline, the next step in evolution, i.e. fusion of micelles to much larger vesicular protocell precursors (0.1-10μm) [53], and that both micelles and vesicles can show the emergence of protospecies, could be significantly supported by the above-mentioned technology (Figure 3).

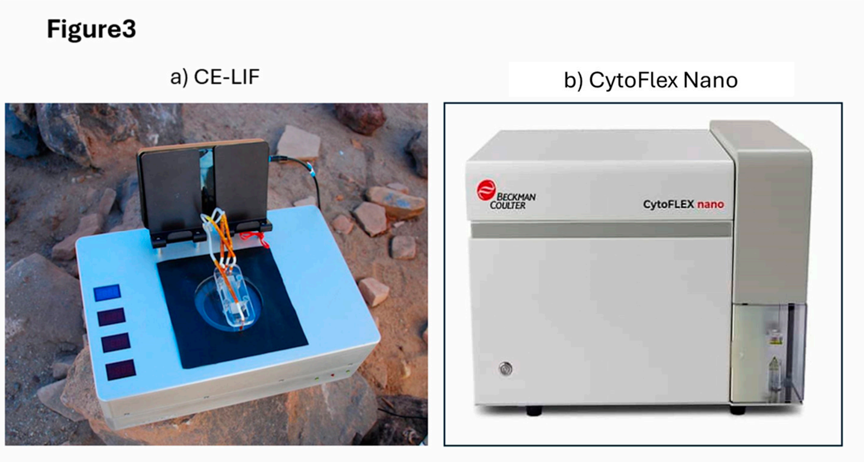

Figure 3. a) A portable instrument using Capillary Electrophoresis Laser-Induced Fluorescence (CE-LIF) technology, is being developed and tested by NASA's Jet Propulsion Laboratory (JPL) for future missions to

search for evidence of extraterrestrial life. b) CytoFLEX is a nano-type flow cytometer that would analyze potential living cells, and importantly also nanoscopic reproducing micellar protocells [52, 54] (see Figure 1).

8. Conclusion

We have suggested that the search for biosignatures on Europa's icy surface should aim at the search for a High Argument signal, in the LDKB terminology, by identifying a definite S-isotopic excursion. Such potential biosignatures, if detected, should be supplemented soon by independent evidence to produce complementary evidence of truly a biogenic origin. In other words, the ongoing Jovian Orbital Missions in due course could search for large values of $\delta^{34}S$ for the possible detection of meaningful values.

This could occur either orbitally, or eventually by means of a lander. Thus, such results would be a first step for the detection of a possible biosignature, to be confirmed by independent measurements. Some possibilities have been explored by looking into the early stages of life on earth. The astrobiological experimentation described in Sec 7 would be useful for discovering chemical entities on oceanic moons, protospecies that could be evidence for on the surfaces of Europa and Ganymede that would be a route to future life's emergence.

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Abbreviations

The following abbreviations are used in this manuscript:

BIF	Banded Iron Formation Before the present.
BP	Before the present.
BSR	Bacterial sulfate reduction.
CE	Capillary electrophoresis.
CDM	Canyon Diablo meteorite.
CMC	Critical Micelle Concentration
CytoFLEX	Nano flow cytometer.
ESA	European Space Agency.
GARD Model:	Graded Autocatalysis Replication Domain.
LIF	Laser-induced fluorescence detection.
LDKB	Life Detection Knowledge Base.
LDMS	Laser-desorption mass spectrometry.
LIMS	Laser ablation ionization mass spectrometry.
LMS	Laser-Abation Time-of-Flight Mass Spectrometer.
LNPs	Lipid nanoparticles.
MASPEX	MAss SPectrometer for Planetary EXploration/Europa (Europa Clipper).
MOA	Microfluidic Organic Analyzer.
MSR	Microbial sulfate reducers.
NASA	National Aeronautics Space Administration.
NIM	Neutral Ion Mass Spectrometer (JUICE).
NIMS	Near Infrared Mapping Spectrometer (Galileo).
ORIGIN	ORganics Information Gathering Instrument.
OW	Ocean world.
PEP	Particle Environment Package (JUICE).

PRIME	Probe using Radioisotopes for Icy Moons Exploration.
SETI	Search for Extraterrestrial Intelligence.
SIG	Stable Isotope Geochemistry.
SMOC	Standard mean ocean chloride.
SPLIce	Sample Processor for Life on Icy Worlds.
SRM	Sulfate reducing microorganisms.
SUDA	SUrface Dust Analyzer (Europa Clipper).
TSR	Thermochemical sulfate reduction.

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