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Perspective

An Experimental Approach to Inform Venus Astrobiology Mission Design and Science Objectives

Daniel Duzdevich [1,2,*], Janusz J. Petkowski [3], William Bains [3,4], H. James Cleaves II [5,6], Christopher E. Carr [7], Ewa I. Borowska [8], Armando Azua-Bustos [9,10], Morgan L. Cable [11], Graham E. Dorrington [12], David H. Grinspoon [13], Niels F. W. Ligterink [14], Andreas Riedo [14,15], Peter Wurz [14,15] and Sara Seager [3,16,17]

1 Center for Computational and Integrative Biology, Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114, USA
2 Department of Chemistry, University of Chicago, 5735 S. Ellis Avenue, Chicago, IL 60637, USA
3 Department of Earth, Atmospheric and Planetary Sciences, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA 02139, USA
4 School of Physics and Astronomy, Cardiff University, 4 The Parade, Cardiff CF24 3AA, UK
5 Earth-Life Science Institute, Tokyo Institute of Technology, Ookayama, Tokyo 152-8550, Japan
6 Blue Marble Space Institute of Science, Seattle, WA 98104, USA
7 School of Aerospace Engineering, School of Earth and Atmospheric Sciences, Georgia Institute of Technology, Atlanta, GA 30332, USA
8 The College of Inter-Faculty Individual Studies in Mathematics and Natural Sciences (MISMaP), University of Warsaw, Banacha 2C, 02-097 Warsaw, Poland
9 Centro de Astrobiología (CSIC-INTA), 28850 Madrid, Spain
10 Instituto de Ciencias Biomédicas, Facultad de Ciencias de la Salud, Universidad Autónoma de Chile, Santiago 7500912, Chile
11 NASA Jet Propulsion Laboratory, California Institute of Technology, 4800 Oak Grove Dr., Pasadena, CA 91109, USA
12 School of Engineering, Royal Melbourne Institute of Technology, Melbourne, VIC 3001, Australia
13 Planetary Science Institute, 1700 East Fort Lowell, Suite 106, Tucson, AZ 85719, USA
14 Space Research and Planetary Sciences, Physics Institute, University of Bern, 3012 Bern, Switzerland
15 NCCR PlanetS, University of Bern, 3012 Bern, Switzerland
16 Department of Physics, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA 02139, USA
17 Department of Aeronautics and Astronautics, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA 02139, USA
* Correspondence: duzdevich@molbio.mgh.harvard.edu

Abstract: Exploring how life is distributed in the universe is an extraordinary interdisciplinary challenge, but increasingly subject to testable hypotheses. Biology has emerged and flourished on at least one planet, and that renders the search for life elsewhere a scientific question. We cannot hope to travel to exoplanets in pursuit of other life even if we identify convincing biosignatures, but we do have direct access to planets and moons in our solar system. It is therefore a matter of deep astrobiological interest to study their histories and environments, whether or not they harbor life, and better understand the constraints that delimit the emergence and persistence of biology in any context. In this perspective, we argue that targeted chemistry- and biology-inspired experiments are informative to the development of instruments for space missions, and essential for interpreting the data they generate. This approach is especially useful for studying Venus because if it were an exoplanet we would categorize it as Earth-like based on its mass and orbital distance, but its atmosphere and surface are decidedly not Earth-like. Here, we present a general justification for exploring the solar system from an astrobiological perspective, even destinations that may not harbor life. We introduce the extreme environments of Venus, and argue that rigorous and observation-driven experiments can guide instrument development for imminent missions to the Venusian clouds. We highlight several specific examples, including the study of organic chemistry under extreme conditions, and harnessing the fluorescent properties of molecules to make a variety of otherwise challenging measurements.

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1. Introduction

Earth and its planetary biosphere are interconnected. The many versions of Earth from throughout history would be unrecognizable to humans today, yet biology has co-evolved with the changing planet and thrived. If we think of Earth as an exoplanet, it is not only habitable and inhabited, but the influence of its biosphere functions on a planetary scale [1–3]. Although life on Earth exhibits an expansive superficial variety, modern biological science has shown us that the most fundamental features of all Earth-life are the same. These features include a metabolism based on organic chemistry (being the entirety of life's biochemical networks and reactions), compartmentalization as cells (defining what is outside and what is inside), genetics based on an information-carrying polymer, propagation of the compartments and genetic information, and the coupling of hereditary genotype and expressed phenotype to enable Darwinian evolution.

The search for life beyond Earth must acknowledge that we only possess one example of life, and an imperfect understanding of how life modifies planetary changes over billions of years, in the context of stellar evolution over this same time frame. We presently cannot rigorously extrapolate how biology may influence detectable atmospheric biosignatures on other astronomical bodies, especially those with substantially distinct histories and environments [4].

We argue that our one example of life is still informative in the hunt for exobiology. First, a methodology informed by the fundamental features of Earth-life can teach us as much about the limits of our biology as about the possibility of life elsewhere (e.g., [5]), and is therefore an inherently worthwhile approach to astrobiology even if we never discover exobiology or if it proves to be different from Earth-life. Second, we cannot rule out that some or even all of Earth-life's fundamental features are in fact universal [6–9].

Third, these concrete features inspired by Earth-life enable us to design rigorous experiments and generate valuable data for space mission instrument design. (We emphasize that this conception of what is most fundamental to Earth-life is not necessarily about any specific chemistry, and we do not imply that the search for life should be limited to the molecular biology we know). We are motivated by the understanding that instrument payloads make specific and inherently limited measurements, and we require a set of practical conceptual and experimental tools to try and determine the optimal measurements to make for any given mission and to generate useful data on the ground to accurately interpret data from space.

It is useful to contextualize the search for past or present life in the solar system with respect to the field of astrobiology as a whole. Statistical analyses of current exoplanet datasets indicate that most stars host at least one planet, which come in a huge variety of types and arrangements [10]. The gross physical properties of an exoplanet can be inferred from mass and radius measurements and knowledge of host star energy flux, while information about atmospheric composition—the main target of biosignature studies—can be gleaned from the absorption spectra of stellar light that has passed through the planet's atmosphere on its path to an observing telescope. Although such atmosphere measurements are exceedingly difficult for Earth-like planets, we can expect new insights from the next generation of observations, especially by the James Webb Space Telescope. Observations by the Atacama Large Millimeter Array (ALMA) have provided glimpses of how planetary systems form, and this has put the history of our solar system in a cosmic perspective for the first time [11]. Earth-life takes on a new significance in light of these discoveries: we can now begin to consider how the trajectory of our solar system as a whole and our planet, in particular, may have affected the emergence of life, how biology has managed to successfully interact with its dynamic host planet over deep time, and how it can affect detectable planetary features. This growing mass of data is fueling the development of astrobiology because it continuously generates constraints against which we can challenge our knowledge of life science.

The emergence and trajectory of life on Earth are intimately connected to planetary history. A robust biosphere with enough biological abundance and diversity to allow it to survive for billions of years, occasionally going through cataclysmic environmental changes, likely requires strong coupling between biological and geological processes (e.g., [12]). Such planetary biology is also implicitly required for life detection. The biology of a planet must have significant, long-term, planet-scale effects to generate a remotely observable physical signal [13]. The mechanisms by which planet and biosphere interact on long timescales are the subject of Earth Systems Science, but remain poorly understood. Regardless of how biology and planet modulate one another, the implications for biosignature detection [14] raise a crucial question for the astrobiology community: What are the requirements for the formation and maintenance of a planetary biosphere? To begin understanding this question we can turn to the example of Earth, which evidently hosts a resilient biosphere. We can also examine other bodies in our solar system because they provide alternative environments that are physically accessible to us. Especially relevant are the icy moons of the gas giants, Mars, and even more exotic environments, like the methane/ethane seas of Titan or clouds of Venus [15]. It is unclear whether any solar system body could harbor trace life at a persistent level, or had life—even a planetary biosphere—in the past, but we are on the verge of exploring these worlds closely enough to find out. We advocate for a strong, sustained, and international program of robotic space probes to systematically explore the solar system and provide the observations needed to push astrobiology into a new experimental era. It is unlikely we will be able to physically visit exoplanets, but our findings over the coming decades of whether life in the solar system in any form or at any scale is unique to Earth will be invaluable to evaluating the prevalence of planetary-biosphere life in the galaxy. The discovery of a second biology in the solar system—even at a trace level—would be monumental (though we do not ascribe any probability to this, only that it cannot be ruled out). However, even if we find no evidence for any life elsewhere in the solar system, we will still gain extremely important insights by then considering why Earth specifically hosts life, but these other habitats do not [16]. The search for life in the solar system is therefore tremendously informative in our search for life in the galaxy, regardless of what we find.

2. Venus: A Challenging Environment for Life as We Know It

Venus is an "exoplanet next door" [17], a venue for testing hypotheses in astrobiology that would otherwise be entirely inaccessible [18]. Venus and Earth are often called sister planets; however, such labeling can be misleading. Although the two are of similar mass and size, their surface and atmospheric conditions differ significantly (Table 1). The surface of contemporary Venus is completely uninhabitable, with temperatures reaching 465 °C and atmospheric pressure reaching 92 bars. However, ~50 km above the surface, in the clouds, the temperatures and pressures are much lower: ~60 °C and ~1 bar, according to [19] (see also [20] for a discussion of the thermal structure of the Venusian atmosphere and its variability) (Table 1). The permanent cloud decks are the most striking feature of Venus. While on Earth clouds are transient and fragmented, often forming and disappearing within minutes, on Venus they are permanent and continuous. This characteristic, in contrast to Earth, makes the Venusian cloud environment stable and predictable. Due to its stability and clement temperatures the Venusian cloud environment has been considered as a potentially suitable abode for life (e.g.,: [13,21–26]) and is a target for astrobiologically motivated space missions [27–29], such as the Rocket Lab mission to Venus [30] or the Venus Life Finder [31–35]. The clouds of Venus nonetheless pose severe and unique environmental challenges for Earth-like life [24]. The Venusian clouds are not made from small droplets of liquid water as are clouds on Earth. Instead, the dominant liquid is believed to be concentrated sulfuric acid. The highly acidic composition of the Venusian clouds remains to be confirmed and should be a major target measurement of any upcoming missions [36–39].

If current understanding is correct, then these chemical conditions are incredibly harsh, orders of magnitude more acidic and extreme than any inhabited environment on Earth (e.g., [24,39–41]). The existence of acidophiles on Earth indicates that life can adapt to quite acidic conditions, though not nearly as acidic as concentrated sulfuric acid. This suggests that a potential selective pressure may be the local neutralization of sulfuric acid [39]. The cloud particles have about 50 to 100 times less available water than the Atacama Desert environment, one of the driest places on Earth, far less than the limits needed for life as we know it [24,41]. The water activity in the droplets is extremely low because the water is tightly bound to the sulfuric acid. This condition also suggests a potential selective pressure to retain water rather than allow it to equilibrate with the environment. To survive in the clouds, organisms would have to be adapted to an extremely chemically aggressive environment: one that is highly acidic, and with low water activity.

Table 1. Earth and Venus at a glance. The basic characteristics of both planets compiled based on [42–51].

	Earth	**Venus** *
	Basic Planetary Parameters	
Mass (⊕)	1.0	0.82
Radius (⊕)	1.0	0.95
Surface gravity (g)	1.0	0.9
Year length (Earth days)	365	225
Day length; one rotation on its axis (Earth days)	1	243
Atmospheric superrotation (Earth days)	n/a	4
	Surface conditions	
Surface temperature (°C)	15	465
Surface pressure (bar)	1	92
Volcanism	active	active
Form of crust	Plate tectonics	"Jostling" crustal tectonics
	Atmospheric conditions	
Main atmospheric gases	78% N_2, 21% O_2, 1% Ar	96.5% CO_2, 3.5% N_2
Dominant liquid	H_2O	concentrated H_2SO_4
Clouds—main composition	H_2O	85% H_2SO_4, 15% H_2O (putative)
Clouds—avg. altitude range (km)	0–20 (variable)	48–70 (stable) *
Clouds—temp. range (°C)	40 (surface)–(−73) (20 km)	100 (at 48 km)–0 (at 60 km)
Clouds—pressure range (bar)	1 (surface)–0.1 (20 km)	2 (at 48 km)–0.4 (at 60 km)

* The characteristics given for Earth and Venus are average values. In reality specific characteristics (e.g.,: the cloud deck altitudes [52], atmospheric thermal structure [20], or the overall abundance of atmospheric N_2 [53]) are variable. For example, the cloud decks of Venus are stable in comparison with the clouds on Earth, but the altitude of Venus's clouds does vary. The clouds extend up to ~74 km above the mean surface in low latitudes and to ~67 km in polar regions [52]. See also [51].

If there is life in Venus's clouds with some of the fundamental features of Earth-life, then it would require adaptations that have no parallel on Earth simply because a parallel environment has never existed on Earth [15,39]. Similarly, the Venusian atmosphere has changed throughout its history, allowing for the possibility of life having emerged under distinct conditions and subsequently having adapted to the current environments [54]. Many organic compounds, especially those we associate with life on Earth, are not stable under such extremely acidic conditions [55,56].

Further, if we posit some form of compartmentalization and water-based biochemistry, such cell-like structures would require an energy-intensive mechanism to accumulate water otherwise complexed with sulfuric acid. In principle, life could exist in an aqueous droplet inside liquid concentrated sulfuric acid cloud particles. However, the energy required to maintain the internal cellular environment and to counteract leakage of water out of the cell (or sulfuric acid into it) could be substantially larger than that used by Earth's halophiles to survive the disruptive effects of extreme salinity. A need for selective retention of water, and for modulating the permeability of sulfuric acid-stable membranes pose a formidable challenge, but are in principle testable.

There is no close analog for a Venus cloud environment on Earth, but that does not require us to dismiss the possibility that life could evolve to exist under such conditions, nor that we cannot draw on the fundamental features of Earth-life to consider how non-Earth-life may contrive to occupy such an alien ecological niche. Using this general approach, we next outline laboratory experiments that could generate valuable insights and datasets to guide instrument development for an astrobiological mission to Venus's clouds (Table 2).

3. The Astrobiological Exploration of Venus Can Start in Earth's Laboratories

Astrobiological conceptions of the Venusian clouds require grounding in the behavior of organic chemistry in the presence of concentrated sulfuric acid. This is such an unusual and harsh solvent that existing data may be limited and spread across the literature, and targeted data that accounts for sulfuric acid in microscopic droplet form is entirely absent. Conjectures about chemistry in the Venusian clouds, and, importantly, interpretation of in situ measurements that detect organics, require a database of how organic molecules react in sulfuric acid [57]. Many complex organic molecules, and almost all those we associate with life (see below), are relatively quickly destroyed by concentrated acid. Nonetheless, there may be numerous organic molecule types that are resistant to acid, and it would be useful to know what reactions may be kinetically and thermodynamically possible among them, especially when exposed to UV irradiation at levels and wavelengths found in the Venusian clouds and across different levels of acidity. Instruments meant to probe Venus's cloud chemistry should account for the following questions: What degree of organic chemical complexity can be found in concentrated sulfuric acid? This will directly inform the evaluation of instrument detection limits in organic poor or rich environments; both extremes may be problematic and confounding depending on instrument sensitivity. How similar are sulfuric acid resistant organic molecules, regardless of the starting materials, and how does the profile of the starting material, whatever its source, affect the final distribution of detectable organics? Are certain functional groups especially prevalent, and if so, how do they relate to the capabilities of specific instruments? The initial development of such a database has already begun with a deep search of existing literature [57], but ultimately, a comprehensive program of experimentation is needed. Mission data on organics detected in Venus's clouds will not be interpretable without a well-developed body of organic chemistry experiments in sulfuric acid and sulfuric acid droplets.

A related set of experiments should consider putative de novo synthesis of organics in sulfuric acid, such as may be occurring spontaneously in Venus's clouds. There could be sources of chemistry on Venus relevant to a space mission that are exogenous to the sulfuric acid solvent, such as Earth microbes from the spacecraft or historical meteoritic transfer [58,59], space dust and meteoritic infall, or surface chemistry that exchanges with the cloud deck. These sources would generate acid decay products on exposure to the cloud droplets and yield potentially characteristic distributions of molecules. The sulfuric acid environment itself may also be a source of unique synthetic chemistry. Understanding any such phenomena is indispensable to interpreting in situ measurements of cloud organics. We are especially interested in whether the two sets of products—decayed from complex starting mixtures or synthesized de novo—are fundamentally distinct in some systematic and identifiable way. This would significantly further mission goals because it would enable instrument development to target one set or the other. To assess this type of synthetic sulfuric acid solvent chemistry, we suggest a series of Miller–Urey type experiments [60,61] under model Venusian cloud conditions. Generally, this would involve exposing sulfuric acid under a carbon-dioxide and nitrogen atmosphere to an energy source such as electric discharges mimicking lightning, which likely occurs on Venus (e.g.,: [62]). Additional variables could be the use of UV light, and a physical mechanism to generate sulfuric acid droplets inside the reaction chamber. From an astrobiological perspective, this type of experiment would reveal which categories of organic molecules could in principle be produced abiotically in liquid concentrated sulfuric acid and therefore serve as markers of abiotic organic chemistry in the Venusian cloud droplets. This would provide a baseline of background chemical diversity, that is, an "abiosignature" [63], against which other signals could be compared. The material generated this way would also be an ideal test substance for instrument evaluation [64]. Finally, such work will be essential to evaluating a significant confounding issue: the possibility of forward contamination.

Any Earth-life contaminants on a probe passing through the Venusian clouds and exposed to concentrated sulfuric acid are expected to quickly degrade or become significantly altered into a distribution of organic compounds that would be distinct from the native mixture of organics [56]. Establishing the fingerprints of such distributions is essential to interpreting in situ organics measurements, especially using mass spectrometry, because these signals would function as unwanted noise in attempts to ascertain the chemical components of the clouds. As a positive control, we suggest a systematic mass spectrometry analysis of the material generated by exposing various microbes [65–68] to concentrated sulfuric acid. Major variables would include the type and density of cell cultures, acid concentration and temperature, and acid exposure time. We expect that certain classes of molecules will always appear regardless of these variables and reflect protein, RNA, and biologically universal small molecule degradation products, whereas others may be specific to the source or exposure condition. We must also establish the threshold of contamination needed to foul in situ measurements, and in some cases that threshold may be exceedingly low [64]; such experiments would provide invaluable sample material for instrument evaluation. A similar approach can be applied to testing the stability of small organic molecules in concentrated sulfuric acid, again across relevant temperature and concentration gradients. These reactivity experiments will help in the interpretation of any potential future detections of organic molecules by the in situ probes sent to the clouds of Venus.

It is difficult to predict the exact combination of forward contaminants or expected organics, and all experimental data about putative chemical diversity in the Venusian clouds based on current data will be incomplete. An in silico method to evaluate molecular stability in sulfuric acid is therefore extremely desirable [56,57]. There is extensive literature about the reactivity of various organic molecules and functional groups in concentrated sulfuric acid, and a deep meta-analysis of available literature would enable us to predict which molecules, or classes of molecules, will be resistant to sulfuric acid, or otherwise how they will degrade [57]. Expanding such a database with new chemicals and their reactivity would be useful for interpreting experimental data and evaluating hypotheses of which organic mixtures may exist in the Venusian clouds and why.

The experiments discussed so far assume that any putative Venusian life would be measurably distinct in its organic composition from potential terrestrial contaminants and background abiotic environmental chemistry, and possess some form of biochemistry to generate organics in the first place. Additional experiments can be inspired by the fundamental life feature of compartmentalization, which may be especially important if we assume that any Venusian biochemistry would have to be protected from an outside environment of concentrated sulfuric acid. In that case, a strong barrier between the inside and outside of a cell would be needed, though as with Earth-life, one that would still somehow allow for the regulated exchange of nutrients and especially the selective accumulation of water.

Earth-life employs lipid membranes as a cellular barrier, but there is extensive variety among the constituents of membranes—much more than with other fundamental classes of biomolecules such as nucleic acid and protein building blocks—and significant scope for variation [69]. We suggest that the data generated by the experiments and analyses above could enable evaluation of the stability of classes of lipids that we expect or measure to be sulfuric acid resistant. This may be somewhat easier for lipids than with other organic molecules because their salient feature is a carbon tail that will be relatively intransigent to acid hydrolysis if saturated. Lipids can spontaneously form vesicles in polar solutions [70,71], and their presence and stability can be measured by non-invasive optical techniques such as phase contrast microscopy or dynamic light scattering (DLS). Further, the presence of lipid molecules from any source could affect in situ measurements. For example, the hydrophobic environment in lipid bilayers or films could change the fluorescence of organic molecules [72], or change the profile of organics accessible to a mass spectrometer. Therefore, such experiments could not only establish whether a lipid-based compartmentalization system is possible in principle, but also guide instrument development by highlighting how acid-resistant lipids may affect in situ measurements.

Noninvasive measurements that rely on optics rather than physical contact with target samples are highly preferred because of the extremely corrosive nature of sulfuric acid. A straightforward but powerful measure of organics is autofluorescence, which requires laser excitation and, in principle, no direct contact with the material being interrogated [73]. An experimental approach to assessing the usefulness of autofluorescence for detecting organics in Venus's atmosphere would first determine whether organic compounds can fluoresce in concentrated sulfuric acid, and then catalog the fluorescent features associated with different types of target molecules. It is also desirable to determine optimal wavelengths for laser excitation of fluorescent species. Work on this has already been completed [73] for the Autofluorescence Nephelometer (AFN) instrument selected for the Rocket Lab Mission [30]. It is already known that the majority of organic compounds react in concentrated sulfuric acid to yield yellow colored fluorescent species, often referred to in the literature as "red oil," conjunct polymers, humic acids, humines, or humic-like acids (e.g., [74–76]). The coloration and fluorescence behavior of organic compounds results from the formation of conjugated organic molecules. If there is organic carbon in the Venusian atmosphere, it may react with concentrated sulfuric acid in the cloud droplets [77], resulting in colored, strongly UV-absorbing, and fluorescent products that can be detected by the AFN instrument selected for the Rocket Lab Mission [30,73]. Here, as above, it will be essential to determine the possibility of false positives from forward contamination and to formulate hypotheses about how any in situ biology could, in principle, affect fluorescence measurements. Interestingly, if any Venusian cloud biology has evolved structures and mechanisms to shield its biochemistry from the surrounding sulfuric acid, then most detection methods would require an initial extraction of the organics from the cellular structures to enable the measurement of otherwise encapsulated molecules. It is interesting to consider whether this applies to a fluorescence approach: if the compartmentalization is not opaque to the excitation or emission wavelengths under consideration, then the internal organics could generate unique fluorescence signatures distinct from what would be expected on exposure to concentrated sulfuric acid, and in conjunction with the compartmentalization experiments suggested above, this is an experimentally testable possibility.

Table 2. Chemistry and biology experiments to inform Venus Life Finder (VLF) Mission science and instruments [32,33].

VLF Biology Experiment	Objective	Connection to VLF Mission Science	References
Reactivity of Organic Molecules in Concentrated Sulfuric Acid			
Assessment of Chemical Stability and Reactivity of Organics in Concentrated Sulfuric Acid	1. Assess which classes of organic molecules are reactive and which are stable in concentrated sulfuric acid, and to what degree. 2. Develop a comprehensive predictive database of sulfuric acid reactivity, with a focus on chemical functional groups.	Inform instrument range and target capabilities, and enable data interpretation.	[30,56,57,73,77]

Table 2. *Cont.*

VLF Biology Experiment	Objective	Connection to VLF Mission Science	References
Fluorescent Properties of Organics in the Venusian Atmosphere	1. Determine the categories and properties of autofluorescent organic compounds dissolved in concentrated sulfuric acid.	Inform the design of the AFN instrument for the Rocket Lab mission by identifying optimal wavelength(s) for laser excitation of fluorescent organic compounds potentially present in the Venusian atmosphere.	[30,73]
Possibility for Life			
Vesicle Formation in Concentrated Sulfuric Acid	1. Assess whether Earth-life-like bilayer membranes are stable, and can form vesicles in concentrated sulfuric acid. 2. Assess whether sulfuric acid-stable membranes can sequester canonical (Earth-like) biochemistry or water.	Enable the testing of instrumentation tolerance if lipid molecules are encountered in concentrated sulfuric acid, and inform future hypothesis-driven experiments about the limits of compartmentalization inspired by Earth life.	
False Positives and Forward Contamination			
Miller–Urey Type Experiments in Concentrated Sulfuric Acid	1. Assess whether complex organic chemistry can be generated in sulfuric acid with relevant input energy. 2. Determine which organic molecules could in principle be produced abiotically in liquid sulfuric acid and therefore serve as baseline markers.	False positive assessment: organic molecules produced during such high-energy reactions need not be made by life.	
Degradation Products of Cellular Material in Concentrated Sulfuric Acid	1. Assess which cellular components of model Earth microbial life, if any, survive in concentrated sulfuric acid, for how long, and otherwise characterize the molecular profile of the resultant hydrolyzed/reacted material.	Forward contamination assessment.	

4. Conclusions

We hope that the approaches suggested here for the exploration of Venus will encourage and inspire bold thinking about astrobiology, but tempered by rigorous experimentation. When target environments are as alien as the Venusian clouds, and so unlike any habitable terrestrial environments, can we expect to find a role for life as informed by Earth-biology? We argue that this is possible by using the fundamental features of life as we know it to guide the design of laboratory experiments. Such experiments will inform mission design directly, and successful missions will in turn iteratively refine future experiments. We further argue that exploring solar system bodies from an astrobiological perspective is not only about the possibility of discovering life, but rather evaluating our understanding of life as a planetary, and planetary-system, phenomenon. We can therefore use what we know about biology and what we currently know about solar system bodies to prompt experimentally testable questions. Such a research program can encompass (i) broad long-term questions of fundamental importance, such as the emergence of life and Darwinian evolution, even in environments different than Earth, (ii) the essential properties of biomolecules, (iii) and much more specific questions that relate to particular planetary scenarios, such as the current environment of the Venusian clouds.

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