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# Determining the “Biosignature Threshold” for Life Detection on Biotic, Abiotic, or Prebiotic Worlds

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## Abstract

The field of prebiotic chemistry has demonstrated that complex organic chemical systems that exhibit various life-like properties can be produced abiotically in the laboratory. Understanding these chemical systems is important for astrobiology and life detection since we do not know the extent to which prebiotic chemistry might exist or have existed on other worlds. Nor do we know what signatures are diagnostic of an extant or “failed” prebiotic system. On Earth, biology has suppressed most abiotic organic chemistry and overprints geologic records of prebiotic chemistry; therefore, it is difficult to validate whether chemical signatures from future planetary missions are remnant or extant prebiotic systems. The “biosignature threshold” between whether a chemical signature is more likely to be produced by abiotic versus biotic chemistry on a given world could vary significantly, depending on the particular environment, and could change over time, especially if life were to emerge and diversify on that world. To interpret organic signatures detected during a planetary mission, we advocate for (1) gaining a more complete understanding of prebiotic/abiotic chemical possibilities in diverse planetary environments and (2) involving experimental prebiotic samples as analogues when generating comparison libraries for “life-detection” mission instruments. Key Words: Prebiotic chemistry—Life detection—Biosignatures—Complexity. *Astrobiology* 22, xxx–xxx.

## 1. Introduction

THE SEARCH FOR signs of life on other worlds is one of the major goals of Astrobiology (NASA Astrobiology Strategy 2015), and many laboratory, field, modeling, and instrument-based efforts have focused on characterizing and differentiating signs of life from abiotic signatures. One general approach for finding life elsewhere is to identify aspects of life as we know it that generally are not observed to happen abiotically. Such phenomena are then defined as potential “biosignatures,” some of which can inform the framework around science goals and instrument development for missions that seek to evaluate potential evidence of biology on, for example, Mars or ocean worlds (Summons *et al.*, 2011; Hand *et al.*, 2017; Mathies *et al.*, 2017; Neveau

*et al.*, 2018; Chan *et al.*, 2019; MacKenzie *et al.*, 2021). However, present-day and geologic/fossil samples from Earth represent examples of biological systems. We argue here that Earth field samples do not preserve the full range of organic chemistry that might be achieved in a purely *abiotic* system because abiotic organic reactivity in terrestrial environments is suppressed and/or overprinted by the widespread biology on our planet. We focus our arguments on the concept of organic complexity; however, similar arguments could be formulated for other proposed biosignatures (*e.g.*, chirality, isotope fractionations, morphology, etc.). Understanding the limits of abiotic organic chemistry in different planetary contexts is critical for assessing any organic signatures that may be detected by a mission on another world.

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On Earth today, biology has permeated nearly all surface and near-surface environments, utilizing a variety of redox niches (Nealson, 2003). Therefore, it is reasonable to assume—if observed in a sample from any Earth field site—that a highly complex organic system is likely the product of life or was at least influenced/overprinted by life sometimes in its geological past. Consequently, abiotic organic chemistry in most terrestrial environments is assumed to be fairly simple based on previous observations: confirmation of abiotically derived organic molecules has included low-molecular-weight compounds such as formate, acetate, amino acids, carboxylic acids, lipids, and short chain hydrocarbons (McCullom *et al.*, 1999; Proskurowski *et al.*, 2008; Sherwood Lollar *et al.*, 2008; Konn *et al.*, 2015; McDermott *et al.*, 2015; Ménez *et al.*, 2018).

This observation (a geological “abiotic organic example”) is also recorded in meteorites, in which a variety of small organic molecules have been detected, including amino acids, carboxylic acids, sugars, and nucleobases (Callahan *et al.*, 2011; Burton *et al.*, 2012; Furukawa *et al.*, 2019). However, given that the geochemical conditions to which asteroids have been exposed are limited (compared with that of a planetary body, which may host a myriad of environments), meteorites also fall short of representing the full complexity that abiotic organic/prebiotic systems may reach. The possible degree of complexity of abiotic organic reactions in a planetary environment would depend on many factors, including: intrinsic characteristics of that body (*e.g.*, age, existence of an atmosphere or liquid water, size of the body, etc.), changing planetary conditions (*e.g.*, those that promote abiogenesis, habitability, or general climate extremes such as global glaciation) (Cantine and Fournier, 2018; Cockell *et al.*, 2019; Sasselov *et al.*, 2020); the prevalence and diversity of life on the planet (Sleep, 2018); and the specific environmental parameters and micro-environments in available reaction settings.

Thus, the question of what the extreme end of “complex abiotic chemistry” might look like in a sample encountered by a mission (either *in situ* or returned) can best be explored in analog experiments in laboratory settings, where the influence of biology is either controlled or eliminated. Herein, we discuss some factors that should be considered when applying results from prebiotic experiments to planetary science and mission-focused life detection efforts, and we argue that, going forward, stronger connections between the origins of life (OOL) and life detection communities are essential for planetary exploration.

## 2. Mission-Relevant Organic Signatures: Possibilities from Prebiotic Chemistry Laboratory Studies

Generally, experimental OOL research aims at replicating versions of biochemical processes, biomolecules, and/or metabolic functions in the laboratory by using only abiotically available organic/inorganic materials (ideally, under conditions simulating the early Earth). The exact degree to which prebiotic processes resembled modern biochemistry is unknown. It has been proposed that certain ancient protein cofactors could reflect organic or inorganic substrates that were originally sourced from geological settings (*e.g.*, iron-sulfur clusters that resemble hydrothermal minerals) (Nitschke *et al.*, 2013; Goldman and Kacar, 2020; Zhao

*et al.*, 2020), and that ancient metabolic pathways could be (even partially) recapitulated abiotically (Wächtershäuser, 1990; Muchowska *et al.*, 2017, 2019). It has also been suggested that some chemical pathways that led to life’s origin do not exactly resemble current metabolism (Lazcano and Miller, 1999; Goldford *et al.*, 2017). Also, there is a plethora of organic chemical reactions that might be possible in a planetary environment, yet that need not be directional toward an Earth-like OOL. Prebiotic/abiotic chemistry laboratory studies have demonstrated a wide variety of organic reactions that—if relevant to life’s emergence on early Earth—may also be informative of processes on other planetary bodies and are thus an important resource when preparing for planetary *in situ* or returned sample analysis.

Many of the molecular signatures and chemical processes that we associate with life also transpire in nonliving systems, as seen in laboratory experiments where biological influence is excluded. The prebiotic synthesis of biological building blocks including amino acids, hydroxy acids, nucleotides, fatty acids, and sugars have all been demonstrated via a myriad of processes—as has their oligomerization/polymerization (Table 1) (Ferris and Ertem, 1993; Ferris *et al.*, 1996; Huber and Wächtershäuser, 1998; Simoneit, 2004; McCullom, 2013; Forsythe *et al.*, 2015, 2017; Marín-Yaseli *et al.*, 2015; see Kitadai and Maruyama, 2018 and references therein). The origins of such compounds can generally be traced back to simple starting materials that are ubiquitous in the solar system, such as gases (*e.g.*, CO<sub>2</sub>, CO, CH<sub>4</sub>, N<sub>2</sub>, NH<sub>3</sub>, H<sub>2</sub>S, SO<sub>2</sub>) and transition metal(s) (*e.g.*, Fe, Ni, Mn, Zn, Cu) dissolved or in mineral form (Huber and Wächtershäuser, 1997; Cody *et al.*, 2000; Roldan *et al.*, 2015).

Once organics are generated, they can interact with their environment and react further to form a wide array of compounds through reactions such as reduction, reductive amination, decarboxylation, condensation, aldol reactions, and Michael additions, to name a few (Huber and Wächtershäuser, 2003; Muchowska *et al.*, 2017, 2019; Kitadai and Maruyama, 2018; Barge *et al.*, 2019, 2020; Rodriguez *et al.*, 2019). In regards to cellular processes, the formation of organic vesicles and compartments of various compositions has been accomplished (Maurer and Nguyen, 2015; Aumiller and Keating, 2016; Maurer *et al.*, 2018; Cornell *et al.*, 2019; Jia *et al.*, 2019), experimentally showing that abiotic proto-cells can encapsulate and exchange material, facilitate organic reactions within their interior, grow and divide, and generate a pH gradient (Chen and Szostak, 2004; Adamala and Szostak, 2013; Bonfio *et al.*, 2018; Litschel *et al.*, 2018; O’Flaherty *et al.*, 2018; Saha *et al.*, 2018; Kindt *et al.*, 2020). Moreover, proto-metabolic reactions involving biologically ubiquitous cofactors such as nicotinamide adenine dinucleotide, NAD<sup>+</sup>/NADH (Basak *et al.*, 2021; Weber *et al.*, 2021) can occur in mineral–aqueous mixtures and across proto-cellular membranes (Summers and Rodoni, 2015; Dalai and Sahai, 2020). Another hallmark of biochemical systems is autocatalysis, where the products of a series of reactions include the necessary reactants to start the cycle again; for example, the continuous reactions of the reductive tricarboxylic acid cycle (Smith and Morowitz, 2004), or the formose reaction. Autocatalytic systems are highly sought after in prebiotic chemistry, and various forms of autocatalysis can be

TABLE 1. DEMONSTRATED PREBIOTIC SYNTHESIS OF SELECTED PROPOSED BIOSIGNATURE COMPOUNDS

| <i>Example compound</i>             | <i>Biological uses</i>  | <i>Possible abiotic/prebiotic sources</i>   | <i>References</i>   |
|-------------------------------------|---|---|---|
| Histidine (amino acid)              | Common in metal clusters of enzymes; serves as metal chelator and catalyst  | Erythrose + formamidine + NH <sub>4</sub> Cl + KCN (pH 6.8, 80°C, 15 h)<br>Formaldehyde + hydroxylamine + sea salts (pH 5.5, 105°C, 35 days)  | Shen <i>et al.</i> (1990)<br>Hatanaka and Egami (1977)  |
| Porphyryns/tetrapyrroles (pigments) | UV protection may also protect against desiccation; needed for photosynthesis<br>Oxidized form is a universal precursor for the biosynthesis of tetrapyrroles<br>Note: proposed as prebiotic photocatalysts | Knorr pyrrole synthesis → substituted pyrroles<br>Aminoketones + β-diketones (pH 5–7 at 60–100°C for >6h) → pyrrole → → porphyrin<br>Pyrrole + Paraformaldehyde + Ni/Cu/V (84°C, 3 h) → porphyrin<br>Electric-discharges (CH <sub>4</sub> -NH <sub>3</sub> -H <sub>2</sub> O) for 2–80 h → porphyrin<br><br>Metallation of porphyrins with Mn(II), Fe(II), Co(II), Ni(II), Cu(II), Zn(II), and Pd(II) dissolved/in minerals; incorporation of Mg(II) not observed   | Lindsey <i>et al.</i> (2009), Soares <i>et al.</i> (2012, 2013)<br>Hodgson and Baker (1967)<br>Hodgson and Ponnamparuma (1968), Simionescu <i>et al.</i> (1978)<br>Soares <i>et al.</i> (2013), Pleyer <i>et al.</i> (2018) |
| ATP (nucleotide cofactor)           | Energy currency in metabolism; facilitates nucleotide polymerization  | Adenosine + trimetaphosphate + Ni <sup>2+</sup> + urea + NaBO <sub>4</sub> + NH <sub>4</sub> Cl (pH 7.8) dried at 90°C × 15 h<br>Adenosine + Trimetaphosphate; metals (Mg/Ag/Co/Fe/Li/Ni) are not needed, but they improve yields (pH 7, 37°C, wet-dry cycles, 2 weeks)<br>Adenine + ribose + ethyl metaphosphate (UV, 40°C, 1 h)   | Kim and Benner (2021)<br>Cheng <i>et al.</i> (2002)<br>Ponnamparuma <i>et al.</i> (1963)  |
| Enzymatic polymers                  | Enzymes facilitate metabolic reactions<br>Note: Fe-S clusters bound to peptides are analogous to modern metalloenzymes  | Peptide-bound Fe-S clusters:<br>Fe <sup>3+</sup> + HS <sup>-</sup> + glutathione (RT, pH 7.3–8.1)<br>Fe <sup>3+</sup> + HS <sup>-</sup> + Cys-containing dodecapeptide (RT, pH 8–8.5)<br>Fe <sup>3+</sup> + thioacetate + AA-SH (pH 5.5–7.5, 70°C, 1 h–5 days)<br>Polypeptides, depsiptides, or polyesters:<br><b>4-mers:</b> Glycine thioesters (RT) → polyglycine<br><b>10-mers:</b> AHA + AA (65–85°C, wet-dry cycles) → depsiptides<br><b>12-mers:</b> glycerol + citric acid → polyesters (wet-dry cycles)<br><b>25-mers:</b> glyceric acid condensation (80°C) → polyesters   | Bonfio <i>et al.</i> (2017)<br>Kim <i>et al.</i> (2018)<br>Sanden <i>et al.</i> (2020)<br>Weber and Orgel (1979)<br>Forsythe <i>et al.</i> (2015, 2017)<br>Mamajanov (2019)<br>Weber (1989)                                 |
| Information-storing macromolecules  | Nucleic acids store genetic information; they can catalyze reactions  | RNA:<br><b>50–55-mers:</b> imidazole activated+montmorillonite<br><br><b>17-mers:</b> imidazole activated + Pb + Mg (pH ~6.5, -18°C, 38 days)<br><b>&gt;300-mers:</b> AMP + UMP + NH <sub>4</sub> (pH 2.5, wet-dry cycles, 85°C)<br>PNA: base pairs and stacks like RNA; catalyzes reactions<br>N-heterocycle + acrolein + AAs via Knoevenagel condensation reaction (45°C) → polymers ( <b>MW &gt;10,000 Da; &gt;12-mers</b> ). Also shown to catalyze phosphate ester hydrolysis<br>Proto-RNA Rosettes: base pairs and stacks<br><b>1000s-mers</b> (N-heterocycle = barbituric acid, melamine, 2,4,6-triaminopyrimidine, 2,6-diaminopurine, adenine, cyanuric acid) | Ferris <i>et al.</i> (1996), Joshi <i>et al.</i> (2013)<br>Monnard <i>et al.</i> (2003)<br>Da Silva <i>et al.</i> (2015)<br>Nelsestuen (1980)   |
|                                     |   |   | Cafferty <i>et al.</i> (2013, 2016), Li <i>et al.</i> (2016)  |

AA = amino acid; ATP = adenosine triphosphate; MW = molecular weight; RT = room temperature; UV = ultraviolet.

achieved in prebiotic/abiotic systems (Lancet *et al.*, 2018; Blokhuis *et al.*, 2020; Sanden *et al.*, 2020).

In sum, decades of OOL research have demonstrated that it is possible for prebiotic systems to possess some chemical compositions and functionality expected of biological systems—especially from the perspective of mission-relevant instruments, which are limited in their sensitivity and resolution (Table 1). Indeed, abiotic chemistry can give rise to varied and complex arrays of organics, depending on local geochemical conditions, even on extremely short timescales. For example, solutions involving hydroxy acids or nucleotides generate diverse polymer sequence “libraries” (Chandru *et al.*, 2018, 2020) or supramolecular polymers 1000s of units long within hours or days, respectively (Cafferty *et al.*, 2013, 2016; Li *et al.*, 2016); it remains to be seen how such systems would change over longer periods of time.

The last universal common ancestor of life on Earth was likely evolutionarily distinct from the “origins of life” in a prebiotic chemical sense (Cantine and Fournier, 2018). Still, if a mission instrument of the type that we are sending to other worlds could analyze a sample *in situ* on the prebiotic Earth, those organic signatures might appear nearly indistinguishable from an early-life-containing sample taken a (geologically) short time later. These kinds of reactions could also be possible on other planets as long as the driving reaction conditions can be met (*e.g.*, catalytic iron/nickel minerals, cyclic dehydrating mechanisms, geological sources of essential elements, and/or presence or absence of radiation). Many studies have theoretically and experimentally explored the possibility of prebiotic/abiotic organic chemistry on Mars, Enceladus, Europa, and Titan (Levy *et al.*, 2000; Neish *et al.*, 2006; Westall *et al.*, 2013; He and Smith, 2014; Barge and White, 2017; Kahana *et al.*, 2019; Khawaja *et al.*, 2019; Takahagi *et al.*, 2019; Angelis *et al.*, 2020; Sasselov *et al.*, 2020).

Though abiotic syntheses have not generated polymers anywhere near as complex (here defined as large, organized, and functionally efficient) as biological polymers (Hartrampf *et al.*, 2020), the types of molecules and reactions possible in abiotic systems are still sufficiently complex that they could be mistaken for a biosignature on another world (*i.e.*, a false positive). Confounding this problem is the possibility of analyzing samples generated from a niche planetary environment that was host to abiotic conditions that are not very early Earth-like (*i.e.*, high organic concentrations, nonaqueous solvents, abundance of otherwise rare catalytic minerals/metals) that might be extremely favorable for organic synthesis. Although such environments are less likely to be widespread compared with conditions considered typical for rocky worlds, they remain a possibility (*e.g.*, Titan’s hydrocarbon lakes (Hayes, 2016)). The resulting abiotic organic chemistry in such locales could become very complex yet might diverge from what we would normally identify as “prebiotic” (*i.e.*, directional toward an OOL), and perhaps be more akin to what can be achieved in a synthetic chemistry laboratory setting.

### 3. Lessons from Synthetic Chemistry

Prebiotic chemistry focuses on recreating steps that could have led to life on Earth, but that is only a subset of the

possible abiotic organic chemistry that could take place in a planetary setting: early Earth contained many reactive environments in addition to those that actually drove the OOL and other planets contain still more unique sets of conditions (Barge, 2018). In this regard, synthetic organic chemistry and catalysis method development may also be relevant to consider in terms of understanding the limits of abiotic chemistry that might apply to defining the biosignature threshold for a given environment or sample.

Although the field of organic chemistry often focuses on the synthesis of natural products or important organic molecules (*i.e.*, for pharmaceutical, agricultural, or materials needs) (Baran, 2018), many of the reactions and structures overlap with reactions that are of interest to prebiotic chemists (Humphrey and Chamberlin, 1997; Hartrampf *et al.*, 2020). For example, amino acids syntheses are sought after in development of natural products and pharmaceuticals as well as prebiotic chemistry (Ivanov *et al.*, 2013; Vale *et al.*, 2018). While synthetic chemistry often uses conditions that are not relevant to geological settings, many organic syntheses are “biomimetic,” that is, inspired by the routes that nature uses to access these materials. Usually, the purpose of synthetic chemistry is to increase yield/selectivity of a desired product, and so studies aim at optimizing and constraining reactions by using organic solvents and/or very specific and complex ligands. For example, focusing on maximizing yield and purity alone have allowed for synthetic chemists to abiotically synthesize chains of more than a 100 amino acids to make artificial proteins (Hartrampf *et al.*, 2020), and 100-unit-long sugar polymers (Joseph *et al.*, 2020). Generally, we should consider synthetic organic chemistry the true “extreme” end of what is abiotically possible, since these reactions have undergone optimization beyond what may be possible (or likely) in a geological setting.

One example of where synthetic chemistry can help inform the biosignature threshold for a planetary environment is by understanding the role of metals in organic synthesis. Organometallic chemistry aims at understanding specific mechanisms by which metals catalyze or mediate organic reactions; so even if a synthetic chemistry study may not involve organics that are “prebiotically relevant,” these mechanistic studies can still provide valuable information about what metal/mineral reactions might be observable in planetary samples.

Metals such as Fe, Pd, Ni, Cu, and others are attractive catalysts for enhancing organic reactivity as they can easily exchange electrons with other molecules, exist in a variety of oxidation states, and form complexes with organics (Brandsma *et al.*, 1999; Tasker *et al.*, 2014; Rana *et al.*, 2021). Individual catalytic reactions driven by transition metals are an active field of research; for example, cross-coupling is an important way to connect two materials in a C-C or C-X bond (where X is a noncarbon atom, *i.e.*, O, N) (Nicolaou *et al.*, 2005; Tasker *et al.*, 2014; Campeau and Hazari, 2019). Multiple abiotic organometallic cycles can be joined together and/or with autocatalytic photochemical cycles. For example, a topic of interest in organometallic chemistry is nickel-photoredox dual catalysis: reactions of this nature link a nickel catalytic cycle along with a photocatalyst (*i.e.*, material whose electrons are excited on absorption of light such as ZnO, TiO<sub>2</sub>, or Ru/Ir complexes)

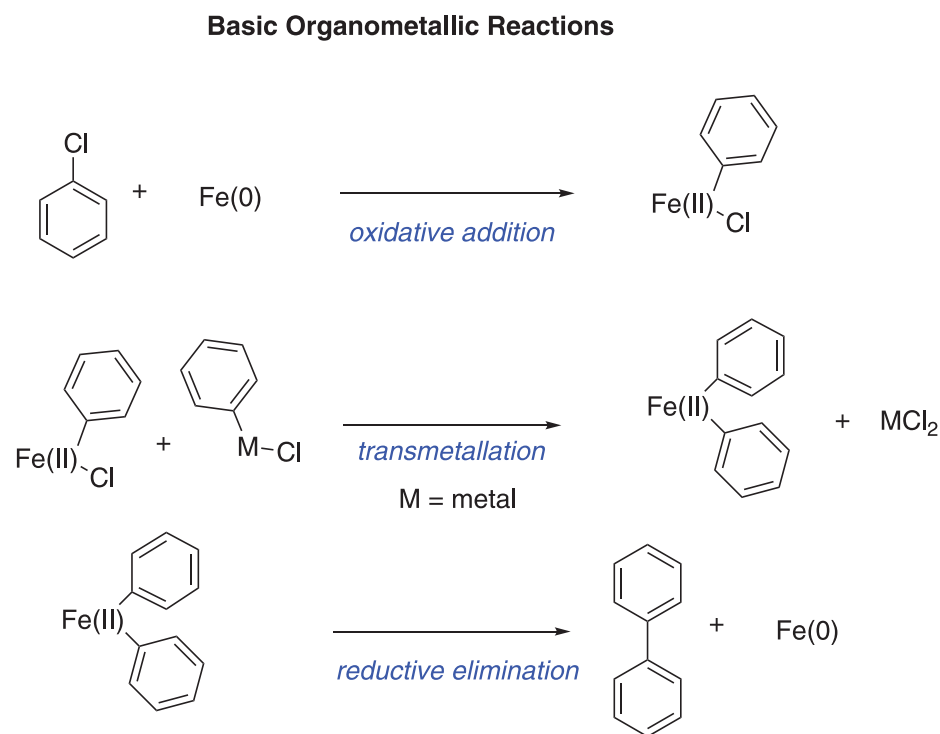
that allows the Ni to reach higher oxidation states such as  $\text{Ni}^{3+}$  or  $\text{Ni}^{4+}$  (Zuo *et al.*, 2014; Zhu *et al.*, 2020).

Various forms of metallic catalysts could be present on other planets, for example, in Fe/Ni meteorite material, in chondrite-rich interiors of ocean worlds, and/or from serpentinizing systems that form native metals and metal alloys (Ashley *et al.*, 2011; Schwarzenbach *et al.*, 2014; Sekine *et al.*, 2015; Pasek, 2017; Preiner *et al.*, 2018; Lawley *et al.*, 2020). Some transition metal catalyzed reactions can have low catalyst loadings (5–0.1%) (Nicolaou *et al.*, 2005; Tasker *et al.*, 2014), meaning that metals/minerals do not have to be extremely abundant to impact organic reactivity. In many cases, ligands help to stabilize the metal in solution and control its reactivity (Fig. 1) (Tasker *et al.*, 2014); a plethora of prebiotic molecules (including *N*-heterocycles) are also capable of acting as ligands in this fashion, and similar processes might be observed in a geological metal- and organic-containing environment.

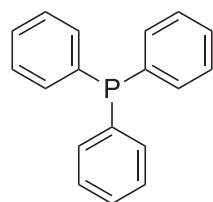
Organometallic/catalytic chemistry is often optimized in nonaqueous solvents. However, green chemistry—which aims at reducing the use of organic solvents in abiotic organic syntheses (Horváth and Anastas, 2007; Li and Trost, 2008)—is another field that can inform astrobiologists of the potential complexity that abiotic organic chemistry can achieve under more geologically realistic conditions. As green chemistry is done by using relatively mild aqueous solutions, the field effectively brings organic/organometallic chemistry closer in condition space to that which may have existed on early Earth or other planetary environments.

#### 4. Identifying the “Biosignature Threshold”

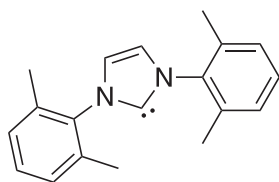
Where then should we place the biosignature threshold between “abiotic” and “biotic” in a mission context? The difficulty for life detection is that this is not a constant



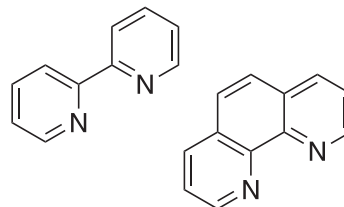
Ligands excluded in reactions for clarity, but could include:



phosphine compounds,



N-heterocyclic carbene compounds,



bipyridine/phenanthroline compounds

**FIG. 1.** Examples of simple organometallic reactions that could synthesize complex organic products and possible ligands for chelation to metals (see Tasker *et al.*, 2014 for more information on elementary organometallic steps).

answer and cannot be defined for other worlds by comparison to Earth field samples or Earth life properties. Rather, the boundary between abiotic organic chemistry and a “biosignature” should be specific to the environment being examined, as well as a planet’s overall organic chemical state; and thus, the biosignature threshold should vary depending on the planet being studied.

As an example, consider a planetary mission conducting *in situ* organic analysis of a sample from a terrestrial planet with an Earth-like geobiological history (*i.e.*, a planet where life emerged, diversified, and globally spread). Depending on the point in the planet’s history that this hypothetical mission took place, the “life detection” interpretation would have to be quite different, even though similar instrument measurements might be obtained.

For example, from a sample taken early in the planet’s history (before life emerged) from an environment where prebiotic chemistry was ongoing, one might observe protocellular membranes—containing peptides or nucleic acid oligomers—that sustain and couple intracellular pH gradients with proto-metabolic reactions involving molecular precursors of protein cofactors (all of which has been demonstrated separately under prebiotic conditions; see Section 2 for details and references). Such a sample would yield organic signatures that—if compared only with the biosignature threshold for an Earth field sample—would likely be classified as a potential biosignature; however, this would be erroneous since there is no life on this hypothetical planet. In this “prebiotic world,” the biosignature threshold for identifying an organic detection as life would be very high, since extremely complex abiotic organic chemistry could still exist (Fig. 2).

At some point, for a biotic world, there would then be a transition from prebiotic chemistry to life/biochemistry (Nowak and Ohtsuki, 2008); however, the timescales of these transitions are unknown. Once this transition occurs, life and prebiotic/abiotic chemistry would likely not coexist long, with biochemistry dominating the environment. A mission analyzing a sample several billion years after the emergence and spread of life on this same world might also observe components of organic membranes, peptides, oligomers, metabolites, and redox reactions; however, in this case, those detections would actually be from life, since abiotic organic chemistry would be much more suppressed once a biosphere is dominant. In this “biotic world,” the biosignature threshold for identifying signs of life would be lower since abiotic chemistry would not be expected to produce such phenomena (Fig. 2). Similar to the “dynamic habitability” concept (NASEM, 2019), as planetary conditions evolve, the biosignature threshold on a planet should also evolve.

To facilitate correct interpretation of organic signatures from a planetary mission, it might be more useful—instead of structuring instrument requirements around life-detection aspects from Earth samples—to design mission science frameworks (or Science Traceability Matrices) around a question such as, “What are the *planetary organic chemical states* that might exist for this world, and what would they look like to this mission payload?” This is important to avoid false biosignature identifications: if any organic material of chemical complexity approaching that expected for Earth life were detected on another world with no life (or

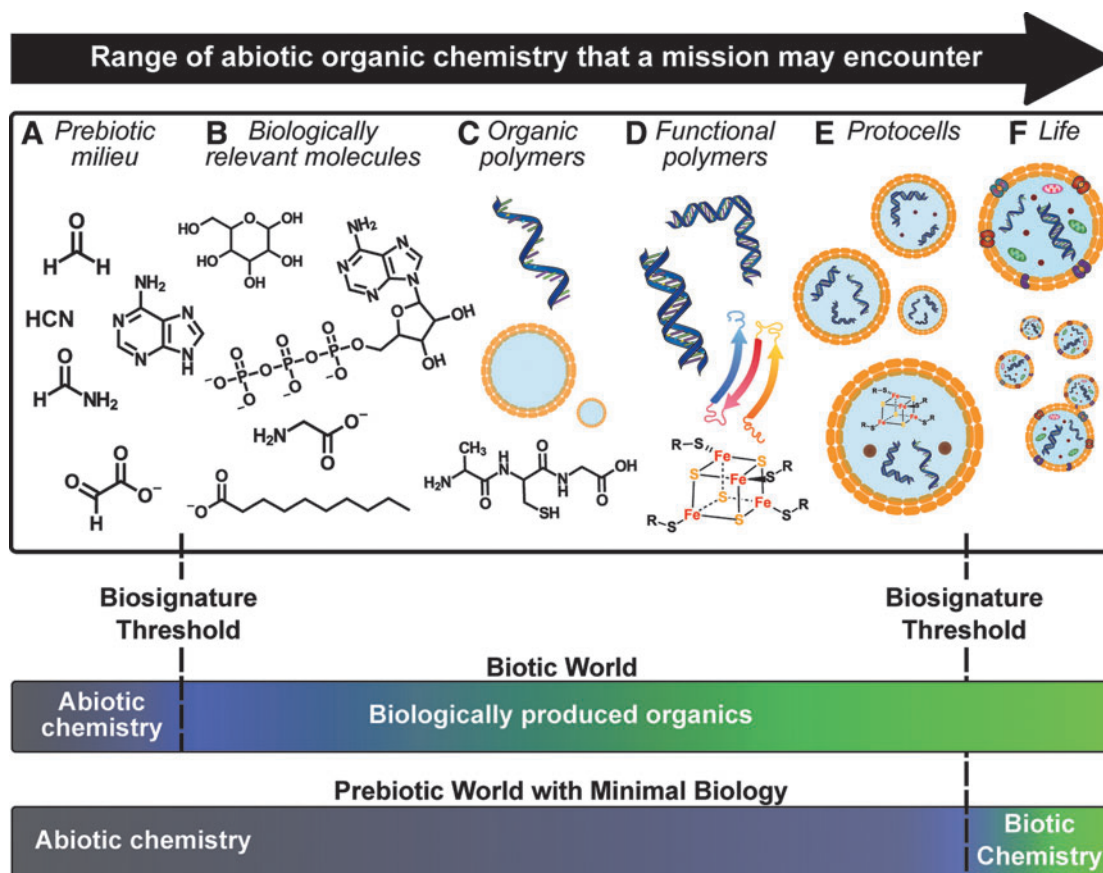
less prevalent life), it could give a false positive for life detection if we compare it with an Earth-centric biosignature threshold instead of the appropriate biosignature thresholds for other environments.

For example, organics have been detected on Enceladus (Postberg *et al.*, 2018), and some have suggested that prebiotic chemistry may still be ongoing on Enceladus today (Kahana *et al.*, 2019). If Enceladus does currently harbor complex prebiotic/abiotic organic chemistry, then, comparing its organic detections with Earth biological systems to define a biosignature threshold would not be appropriate. For Mars, whether life ever emerged there is unknown, but many studies have proposed habitable environments that may exist or have existed (Cockell *et al.*, 2012; Westall *et al.*, 2013; Tarnas *et al.*, 2018; Ojha *et al.*, 2020) and various mission-relevant techniques have been proposed and applied to analyze organic signatures in soil or rock samples (Goesmann *et al.*, 2017; Eigenbrode *et al.*, 2018; Stevens *et al.*, 2019; Farley *et al.*, 2020).

In a general laboratory context, it may be possible to distinguish between prebiotic chemistry and biotic chemistry in a sample by evaluating the degree of chemical complexity present, for example, via the molecular assembly theory (Marshall *et al.*, 2017, 2021). It should be noted, however, that the assembly theory has only been tested on a limited set of prebiotic mixtures; it remains possible that on geologic timescales (and under planetary settings that are more diverse) such mixtures could favor the formation of chemically complex molecules that, via the assembly theory or any other measurement of complexity, could lead to false positives.

Moreover, in a mission context, there are additional difficulties that make this analysis more challenging, including organic alteration/degradation on a planetary surface, lack of ability to chemically process the sample, and limited analytical techniques on a spacecraft payload that could accurately distinguish between molecular isomers of higher weight compounds. It is, therefore, imperative for mission-related experimental efforts to (1) create abiotic organic analog samples according to the high complexity observed in OOL and abiotic synthesis experiments and (2) characterize these abiotic/prebiotic samples with mission instruments to develop analytical strategies that would minimize the possibility of mistakenly identifying these abiotic samples as false positives for life.

One previously proposed aspect to distinguish biological from abiotic systems in a mission context is the distribution of organic compounds by their molecular weight or number of carbons (rather than just detection of a specific product) (Lovelock, 1965; Dorn *et al.*, 2011; McKay, 2011; Creamer *et al.*, 2017; Georgiou, 2018; Klenner *et al.*, 2020), the idea being that biological systems have efficient mechanisms to generate the compounds they need in high yields, and their organic distributions are thus distinct. In comparison, prebiotic systems are sometimes thought to be directionless and instead form increasingly larger compounds in a systematic step-wise fashion—thus, their pattern follows a bell-shaped distribution (*i.e.*, the distribution curve appears “smooth”; Fig. 3). This may be true in Earth-like biological versus abiotic systems. However, the threshold for what is abiotically possible on other worlds could be quite different and



**FIG. 2.** The biosignature threshold (boundary between “abiotic” and “biotic” organic chemistry) can shift depending on the geobiological state of the planet. In a biotic world, such as modern-day Earth, the threshold where an organic detection is likely to be from life is low since abiotic chemistry is suppressed. In a prebiotic world with advanced abiotic organic processes and minimal (if any) biology, the threshold to identify “life” would be higher. To determine whether an organic detection from a mission is a biosignature, we need a more complete understanding of where the abiotic–biotic boundary lies for the world or environment of interest. Organic molecules shown and references demonstrating their prebiotic synthesis are as follows: **(A)** various organics (formaldehyde, cyanide, formamide, pyruvate) formed under prebiotic conditions or detected in prebiotic environments, including meteorites, the interstellar medium, and comets (Schlesinger and Miller, 1983; Ziurys and Turner, 1986; Ehrenfreund and Charnley, 2000; Cleaves, 2008; Guzman and Martin, 2009; Kitadai and Maruyama, 2018; Smith *et al.*, 2019); **(B)** adenine, glycine (Callahan *et al.*, 2011; Burton *et al.*, 2012; McCollom, 2013; Kitadai and Maruyama, 2018), glucose (Likholobov *et al.*, 1978; Omran *et al.*, 2020), ATP (Table 1), and fatty acids (Nooner and Oro, 1979; McCollom *et al.*, 1999; Rushdi and Simoneit, 2001); **(C)** ribonucleotide polymers (Table 1), prebiotic vesicles (Maurer and Nguyen, 2015; Maurer *et al.*, 2018), and polypeptides (Table 1); **(D)** catalytic ribonucleotides and amyloid polypeptides (Rufo *et al.*, 2014; Mutschler *et al.*, 2015; Maury, 2018), base pairing and macro supramolecular nucleotide structures (Table 1) (Todisco *et al.*, 2018), and proto-metalloenzymes (Table 1); **(E)** encapsulation of ribonucleotides, polypeptides (Apel *et al.*, 2002; Hanczyc *et al.*, 2003; Tang *et al.*, 2014), and proto-metalloenzymes (Bonfio *et al.*, 2017, 2018). **(F)** Shows biological cells with intermembrane proteins and higher order structures, including organelles.

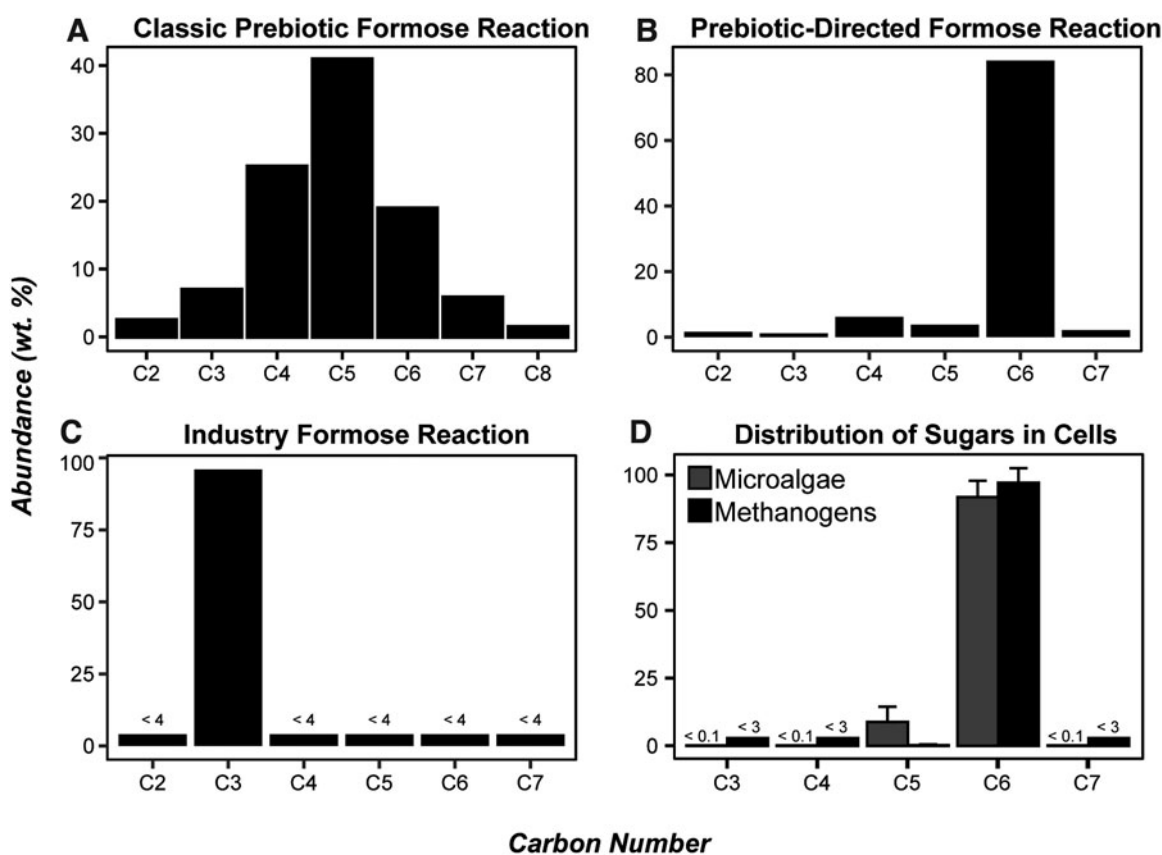
perhaps more difficult to distinguish from a biological system, and it would be useful to consider abiotic samples simulated under conditions that encompass a larger geochemical parameter space.

For example, principal component analysis (PCA) of mass distribution patterns from organics extracted from various samples showed that prebiotic (laboratory generated, limited parameter space explored) and petroleum samples (derived from metamorphosed biological materials) clustered together, as did meteorites (abiotic chemistry in a natural setting) and cellular cultures—demonstrating that organic distributions of abiotic samples can appear similar to those derived from biological materials depending on the geochemical conditions

to which they were subjected (Guttenberg *et al.*, 2021). Although Guttenberg *et al.* (2021) were ultimately successful in developing a machine-learning model to classify abiotic from biotic samples (92% accuracy using 15 PCA components), the authors noted that the chemical diversity of the abiotic samples used was inherently limited and thus does not represent the full diversity that abiotic chemistry could achieve, especially under varying geochemical conditions over geologic time scales; importantly, they noted that geochemical processing could result in abiotic samples chemically evolving that confound their existing classification model.

Indeed, abiotic/prebiotic chemistry need not yield only bell-shaped distributions, as these systems can be directed/





**FIG. 3.** Organic distribution patterns from prebiotic, directed-prebiotic, synthetic, and biological systems, using the example of sugars. **(A)** A classic prebiotic formose reaction has a sugar distribution that is bell-shaped. The data shown here are approximate values derived from Pallmann *et al.* (2018); the sugars were generated from a schreibersite ( $\text{Fe}_3\text{P}$ ) facilitated formose reaction (0.125 M  $\text{CH}_2\text{O}$ , 0.125 M glycolaldehyde, 1 g/mL  $\text{Fe}_3\text{P}$ , 24 h, 80°C). **(B)** Higher temperatures temper the diversity of formose products at 18% formaldehyde conversion (generated at 1.82 M  $\text{CH}_2\text{O}$ , 0.112 M  $\text{CaCl}_2$ , 98°C, <1 min), favoring the formation of C6 sugars (84.3 wt %), with glucose being the dominant product (75.4 wt %). For longer durations and higher conversions, selectivity for C6 sugars remained, but was reduced slightly (68 wt %); similarly, decreasing temperature diminished selectivity for hexoses. Data for the plot were derived from Likhobolov *et al.* (1978). **(C)** A formose reaction optimized for the selective production of the C3 sugar, dihydroxyacetone (96 wt %) via formose reaction in dioxane solvent (6 M  $\text{CH}_2\text{O}$ , 0.3 M 3-ethylbenzothiazolium bromide, 0.3 M trioctylamine, 100°C, 1 h); abundance of other sugars (C2, C4, C5, C6, C7) was not reported and is, thus, shown to have an abundance of <4% after accounting for that from dihydroxyacetone. The histogram was made with data derived from Matsumoto *et al.* (1984). **(D)** Percentage of various sugars (by their carbon number) in polysaccharides extracted from microalgae and methanogens shows that biological matter selectively incorporates C6 and, to a minor extent, C5 sugars. C6 sugars on average made up 91.8 and 97.1 wt % of sugars in microalgae and methanogens, respectively; C5 sugars on average made up 8.8 and 0.2 wt % in microalgae and methanogens, respectively. Data were derived from Brown (1991) (extracted from 16 microalgae species) and Veiga *et al.* (1997) (sugars in extracellular polysaccharides extracted from two species of methanogens grown in different mediums). To generate the histogram, sugars reported from each analyzed sample were grouped by carbon number and then averaged over all samples; error bars represent the standard deviation of each. Importantly, in both studies, only C5 and C6 sugars were detected (thus, C3, C4, and C7 sugars are reported as their maximum theoretical abundance [*i.e.*, the amount of sugars unaccounted for after considering the amounts of C5 and C6 detected]).

selective when organic catalysts, metals, minerals, and fluctuating environmental conditions are involved. For example, in reductive amination reactions, the specific properties of the mineral reactants (as well as pH and ammonia concentration) determine the distributions of organics that result (Novikov and Copley, 2013; Barge *et al.*, 2019, 2020). Varying organic product distributions have also been observed in formose reactions where minerals, dissolved salts, pressure, and temperature can all impact the resulting sugar products (Likhobolov *et al.*, 1978; Lambert *et al.*, 2010; Kopetzki and Antonietti, 2011; Imai *et al.*, 2016; Colón-Santos *et al.*, 2019).

Figure 3 shows several examples of the distribution of sugars that form in abiotic formose reaction conditions, including: the bell-shaped distribution that occurs with classic prebiotic formose reactions (here facilitated by schreibersite and glycolaldehyde, 24 h, 80°C; Fig. 3A) (Pallmann *et al.*, 2018), a temperature-directed prebiotic formose reaction (98°C,  $\text{Ca}(\text{OH})_2$ ; Fig. 3B) (Likhobolov *et al.*, 1978), and an industry formose reaction with trioctylamine and 3-ethylbenzothiazolium bromide catalyst (100°C, 1 h, in dioxane solvent; Fig. 3C) (Matsumoto *et al.*, 1984)—all compared with sugar distributions in microalgae and methanogens (Fig. 3D) (Brown, 1991; Veiga *et al.*, 1997). To



pursue biosignature detection based on these sugar distributions, an organic detection spacecraft instrument would need to distinguish not just between Fig. 3A and D, but also between Fig. 3B and D, which could be more challenging. Similarly, other types of organic distributions proposed as life detection strategies require more thorough testing of the range of abiotic possibilities beyond just what is observed in meteorites or in Earth field conditions, but also incorporating a full range of analog samples from prebiotic and synthetic chemistry (Fig. 3C).

## 5. Conclusions

The fields of prebiotic chemistry and synthetic chemistry provide many examples of what could be possible for abiotic organic systems, and these are the analog samples that should provide the “abiotic extreme” when testing mission-relevant instruments that aim at detecting signs of life on other worlds. It is possible that the same chemical signature observed on two different worlds could be biological in one and have an abiotic origin in another, depending on the geobiological state of those worlds—in other words, the “biosignature threshold” could vary depending on the planetary body of interest.

The burden of proof for classifying a chemical signature as biological is higher for other worlds, since many of the chemical signatures that in an Earth sample would be identified as biological cannot be used unambiguously as indicators of life in a world where we do not know the extent of complex abiotic chemistry. The need for facilitating studies of prebiotic/abiotic organic standards with mission instruments means that connections between the OOL community and life detection community will be essential for planetary exploration in upcoming decades.

It is difficult to define measurable differences between prebiotic chemistry and signs of extant or extinct biochemistry, when we still do not have a complete understanding of the requirements of life or its origin, or what other abiotic chemistry possibilities might exist on other worlds. Life (or “Lyfe”) (Bartlett and Wong, 2020) on other geological bodies might look chemically different from life on Earth, yet it might still fulfill fundamental qualities that we define as “alive.” The chemical signatures of a prebiotic system would also likely vary depending on the stage of prebiotic evolution at which it was observed. Though abiotic/prebiotic reactions do not necessarily require minerals (Stubbs *et al.*, 2020), minerals or metals can drive selection and concentration of particular organic products (Novikov and Copley, 2013; Muchowska *et al.*, 2017, 2019; Barge *et al.*, 2019, 2020; Haas *et al.*, 2020). This adds another layer to the difficulty of biosignature identification, since to interpret an organic signature from a mission we also must understand how “environmental tuning” could impact abiotic/prebiotic reactions, and how predictable the chemical end states are given the initial geochemical conditions.

As a community, astrobiologists should invest in developing a sample collection of complex abiotic organic standards, drawing from OOL experiments and from the world of synthetic chemistry, as well as experiments targeted at simulating abiotic organic chemistry on other worlds under conditions that are not Earth- or early Earth-like. Defining mission instrument requirements relative to detection limits

of molecules, minerals, or other measurements of interest can still be advantageous. However, it is important to keep in mind that the “life detection” threshold for an organic chemical measurement may be specific to a planetary body’s environment and geologic history and can therefore not be resolved by observation of any specific molecule or organic distribution unless the abiotic possibilities for that world are well understood.

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#### Abbreviations Used

MW = molecular weight  
 OOL = origins of life  
 PCA = principal component analysis  
 UV = ultraviolet