AGNOSTIC APPROACHES TO LIFE DETECTION. S. S. Johnson[1], H. Graham[2], E. Anslyn[3], P. Conrad[4], L. Cronin[5], A. Ellington[3], J. Elsila[2], P. Girguis[6], C. House[7], C. Kempes[8], E. Libby[8], P. Mahaffy[2], J. Nadeau[9], M. R. Rwebangira[10], B. Sherwood Lollar[11], and A. Steele[4] [1]Georgetown University, [2]NASA Goddard Space Flight Center, [3]University of Texas at Austin, [4]Carnegie Institute of Washington, [5]University of Glasgow, [6]Harvard University, [7]Pennsylvania State University, [8]Santa Fe Institute, [9]Portland State University, [10]Howard University, [11]University of Toronto.

Introduction: Current strategies for biosignature detection rely mainly on identification of well-established and widely accepted features associated with terran life and signatures of biologic processes, such as particular classes of molecules and isotopic signatures, enantiomeric excesses, and patterns within the molecular weights of fatty acids or other lipids. Yet as we begin to explore icy moons of Jupiter and Saturn and other destinations beyond Earth, it is important to develop life detection methods that identify unknowable, unfamiliar features and chemistries that may represent processes of life as-yet unrecognized.

Utilizing Existing Approaches in More Inclusive Ways: To cast the widest possible net for life detection, we must broaden not only the range of measurements we make but also the range of interpretations we allow. Part of this can be achieved by utilizing high heritage instrumentation or recently proven techniques with the potential to be developed into space qualified instrumentation in more agnostic ways.

For example, flight capable mass spectrometers have long been flown on spacecraft, designed to search primarily for patterns among the molecular weights of carbon-bearing organic molecules. However, mass spectrometers can also be configured, for example with tandem mass spectrometry [1], to search for chemical complexity of any type of molecule (organic or inorganic) that would be unlikely or impossible to form spontaneously.

Chemical complexity can be thought of in terms of graph theory. What is the simplest way to construct this molecule from its parts, accounting for the simplifying feature of duplication? By encoding a graph and enumerating graph features like subgraphs or walks, generational algorithms can be used to count the operations needed to build complexity graphs out of simpler graphs, thereby computing a Molecular Complexity Index (MCI) [2]. An example of how the algorithm can be expressed chemically is shown in *Figure 1*.

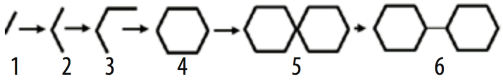

Figure 1. Biphenyl is assigned an MCI of 6.

Among natural products, synthetic drugs, amino acids, metabolites, and other chemical compounds, there appears to be a MCI threshold of 15, above which no molecules tested thus far have an abiotic origin. Without making assumptions about the chemical structures of the molecules, this suggests there may be a threshold beyond which complex molecules are unlikely to form without supporting biological machinery.

Along similar lines, sequencing technologies have been developed as a way to search for nucleic acids based on a shared ancestry hypothesis and monitor terrestrial contamination [3-5].

While this approach is specific to a particular class of molecules (nucleic acids, including those with non-standard bases), recent work is laying the foundation to harness the power of sequencing to explore sample complexity, regardless of whether life is based on nucleic acids. This work (detailed in a forthcoming paper in press at *Astrobiology*) builds on the fact that oligonucleotides naturally form secondary and tertiary structures that can have affinity and specificity for a variety of molecules, from peptides and proteins [6], to a wide variety of small organic molecules [7,8], to inorganics such as mineral surfaces [9] and individual metals [10].

Binding patterns of nucleic acids, independent of their biological function, can thereby be used to probe and report on any chemical environment, opening up a new way to detect agnostic biosignatures. By accumulating large numbers of binding sequences that reflect different compounds in a mixture, statistical data analyses of oligonucleotide sequences and sequence counts enable patterns associated with increasing levels of complexity to be analyzed. This pattern recognition, known as "chemometrics," represents a set of protocols that can be applied to find patterns in chemical data sets [e.g. 11-15], which in turn can be used to fingerprint nonterran biosignatures.

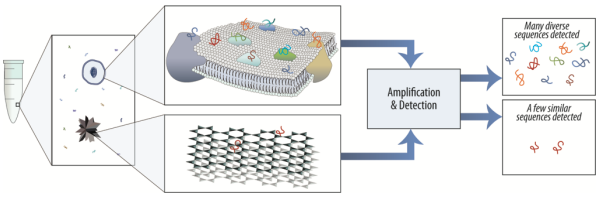

Figure 2: A new concept for life detection harnesses the power of DNA sequencing for detecting life that is not based on nucleic acids. 1) DNA strands are mixed with samples. 2) Diverse folded oligonucleotides bind to complex surfaces and far fewer bind to a simple, repeating, structures. 3) Bound sequences can be amplified and sequenced, revealing the diversity of binding sites within a sample.

The approach could distinguish samples with chemistries suggestive of biology—to "read" patterns of molecules that arise from the vast amount of information stored on the surface of a primitive microbial cell, and to do it with great sensitivity. By utilizing the power of PCR, this technique could be capable of amplifying the signal associated with an exceedingly small input.

Pursuing New Leads: Other concepts remain at a nascent stage. While biological phenomena, from biomolecular production to growth and biosynthesis, have indelible "biosignatures," it is also true that these compounds and processes are, in essence, well-coordinated chemical reactions. Metabolically active organisms, by necessity, maintain themselves at chemical disequilibrium from the environment. This disequilibrium can be detected and the biogenicity of this signal assessed. Redox reactions are typical mechanisms for terran organisms to create energy and terran life can use organic carbon as a reductant and a diversity of soluble oxidants including oxygen, nitrate, sulfate and carbon dioxide. An agnostic approach to life detection would not limit bioelectrochemical observations to just these compound pairs though. Rather, disequilibrium redox chemistries that are inconsistent with abiotic redox reactions could be used as an indicator of active metabolism.

As an example, many microbes can utilize solid-phase minerals as an electron acceptor, e.g. insoluble Fe(III) oxides. An agnostic means of detecting this microbial activity is to use an inert, conductive electrode (e.g. graphite) in the environment. The current density and other electrical attributes produced by microbes are notably distinct from abiotic oxidation; thus this signal could be used as an agnostic biogignature. An example of these observations are the results reported in [15]. This experiment "fed" microbial communities iron sulfide mine tailings and found a marked and sustained increase in voltage (and thus net coulombs recovered) in the reactor with a microbial community compared to a sterile control. These reactions can also be divorced from observations of cellular activity. Microbial extracellular electron transfer (EET) has been observed in terran life, where organic redox-active molecules shuttle electrons to insoluble mineral oxides [16,17].

Probabilistic Approaches to Data Analysis: While it is necessary to broaden our scope and design inclusive life detection strategies, these approaches may be less definitive than, say, uncovering a hopane or DNA sequence. A data interpretation scheme that considers expectations and likelihoods and establishes critical thresholds for life detection based upon probabilistic models is thereby key. Life detection may best be viewed along a spectrum of certainty, more refined than a binary "life" versus "no life" model.

Modern space missions typically include instrument packages with results considered in tandem. No single signature or measurement will serve as unequivocal evidence for extraterrestrial life, rather a combination of data from a variety of approaches will be required. The efficacy and the optimal measurement combinations can be determined statistically. A Bayesian network for which the output is the probability there is a biosignature in a measurement set (*i.e.,* P(biosignature | Data) can be utilized to assess the probability of life, and thus convert measurements into likelihoods and thresholds.

Expectations for abiotic signals can be set by developing challenging null models. For instance, models of nonterran physical and physiological environments can generate a large space of synthetic data representing a wide variety of possibilities for life. These models, which do not pre-suppose terran chemistry, heritage, or physiology, can help the community build "life-relevant" expectations for our collected data. Theoretical models can also inform the limits of biology in foreign environments, anticipate necessary trade-offs indicative of alternate life strategies, and help us to understand minimum sample sizes necessary to provide robust statistical analysis for the results. A theoretical approach that focuses on combining inclusive principles with physical and chemical laws to define feasibility regimes. Studies that carefully consider the abiotic mimics of biosignatures and what tools and metrics can distinguish them from life are also of critical importance.

Conclusions: A key challenge in astrobiology is the development of life detection methods that inform our search for life without presupposing any particular molecular framework. Agnostic biosignature detection concepts need to be advanced individually but also joined in a unified data interpretation program informed by theoretical models.

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