FUTURE APPROACHES TO LIFE DETECTION ON MARS. S. S. Johnson[1], H. Graham[2], E. Anslyn[3], W. Brinckerhoff[2], P. Conrad[4], L. Cronin[5], A. Ellington[3], J. Elsila[2], P. Girguis[6], A. Grubisic[2], C. House[7], C. Kempes[8], X. Li[2], E. Libby[8], P. Mahaffy[2], J. Nadeau[9], A. Roussel[1], B. Sherwood Lollar[10], 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]University of Toronto.

Introduction: Current strategies for biosignature detection on Mars rely mainly on identification of 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, it is plausible that life on Mars may have originated independently from than life on Earth. It is equally plausible that detection of these aforementioned targets are suboptimal for life detection on Mars. We posit that developing "agnostic" life detection methods—approaches that do not presuppose a specific underlying biochemistry—provides a robust means of life detection.

Chemical Complexity: The recently formed Laboratory for Agnostic Biosignatures (LAB) project is pioneering the use of existing chemical analyzers, such as the Sample Analysis at Mars (SAM) gas chromatograph mass spectrometer (GCMS) instrument [1] and the Mars Organic Molecule Analyzer (MOMA) instrument in development for the 2020 Rosalind Franklin (ExoMars), to search for chemical complexity of any type of molecule (organic or inorganic) that would be unlikely or impossible to form spontaneously. Algorithms are being developed to describe pathway complexity [3] and link it, using a mapping of the mass spectroscopic fragmentation pattern, to the complexity of a given molecule. This promising methodology has begun to enable the search for different complexity distributions. LAB is currently testing the correlation between structure and complexity on a variety of abiotic and biotic samples to build a robust complexity index database (see **Figure 1**).

Chemometrics: Sequencing technologies have also been developed as a way to search for life [4-6]. While this approach primarily targets nucleic acids, including those with nonstandard bases, recent work has laid the foundation to harness the power of sequencing to explore sample complexity, regardless of whether life is based on nucleic acids [7]. This research 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 [8], to a wide variety of small organic molecules [9,10], to inorganics like mineral surfaces [11] and metals [12].

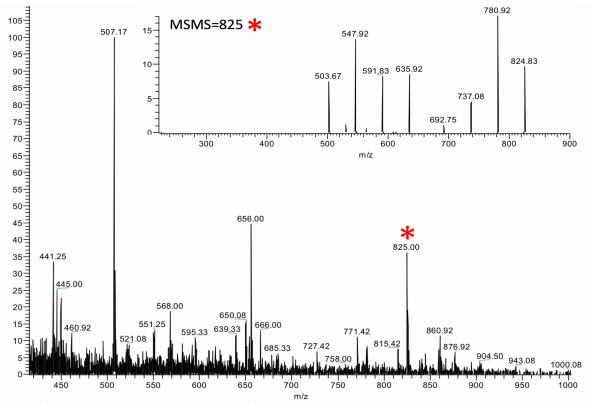

Figure 1. MS/MS data from the 825 Da paraformaldehyde ion shows repeating common mass losses, indicative of a polymer with high molecular weight, but low complexity.

Binding patterns of nucleic acids, independent of their biological function, can thereby be used to probe and report on any chemical environment. 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. 13-17], which in turn can be used to fingerprint nonterran biosignatures. 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.

Disequilibrium Redox Chemistries: While biological phenomena, from biomolecular production to growth and biosynthesis, have indelible "biosignat ures," it is also true that these compounds and processes are, in essence, well-coordinated chemical reactions. Metabolically active organisms, by necessity, maintain

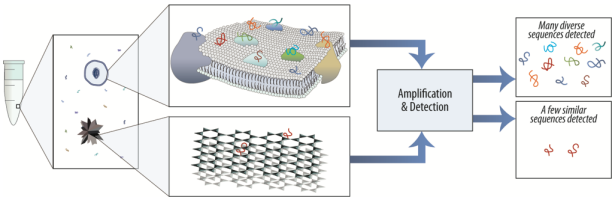

Figure 2: A new concept for life detection harnesses the power of DNA sequencing for detecting life that is not based on nucleic acids. 1) DNA strands are mixed with samples. 2) Diverse folded oligonucleotides bind to complex surfaces and far fewer bind to a simple, repeating, structures. 3) Bound sequences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

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, such as a subglacial brine lake [18]. 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 [19].

Chemical Fractionation: Living cells are universally distinct from their local environment in their elemental and isotopic composition. Further, driven by metabolism, the gross compositions of biological cells are far from geochemical equilibrium. Using this concept as the basis for the detection of life also provides an agnostic view that distills the complexity of biology into an observable phenomena—discrete, metastable entities that are geochemically substantially distinct from their local environment. The LAB team is currently laying the foundation for this new analytical approach by deploying and analyzing cells collected on deep mine biosamplers.

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 therefore key to progress. Life detection may best be viewed along a spectrum of certainty, more refined than a binary "life" versus "no life" model.

Expectations for abiotic signals can also 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 different environments on Mars, 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.

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