FINGERPRINTING NONTERRAN LIFE. S. S. Johnson[1], A. D. Ellington[2], E. V. Anslyn[2], H. V. Graham[3], and P. R. Mahaffy[3], [1]Georgetown University, 37th and O Streets NW, Washington, DC, 20057 (sarah.johnson@georgetown.edu), [2]The University of Texas at Austin, [3]NASA Goddard Space Flight Center

Introduction: An entirely new set of missions with astrobiological relevance is now coming into the realm of possibility: the idea of visiting the icy moons of Jupiter and Saturn. Reaching these Ocean Worlds, an order of magnitude further away than our near neighbor Mars, has long been cost prohibitive, but advanced solar electric propulsion technologies are paving the way for their exploration. Seeking life there will be more challenging, and the possibilities for that life are more diverse. These distant moons are far farther from the Earth and far more foreign than Mars. For instance, Europa, orbiting Jupiter, and Enceladus, orbiting Saturn, hold their seas beneath icy crusts [1]. There are geysers on both worlds, which mysteriously fluctuate, and clear evidence of tidal warming and geologic activity [1-4]

As we design instrumentation for future missions to these worlds, we can, should, and will apply traditional biosignature approaches: looking for isotopic signatures, particular classes of molecules, evidence of enantiomer excess, and patterning within the molecular weights of fatty acids or other lipids. Taking along a nucleic acid sequencing device has also been proposed, in part as a highly specific way of monitoring and identifying potential contamination brought from Earth that may interfere with readings from other instruments. Rapid advances in miniaturization have led to stand-alone versions of sequencing technology, like the Oxford Nanopore MinION, which is half the size of a pack of cards. Sequencing technology could also be used to look for nucleic acids based on a shared ancestry hypothesis, though shared ancestry is arguably less tenable as we proceed deeper into the Solar System. Yet we believe that sequencing has a potentially broader and more useful application than just detecting RNA/DNA-based life, or monitoring for contamination: sequencing could also be used as a tool for detecting chemometric patterns associated with non-Terran biosignatures.

Oligonucleotides naturally form secondary and tertiary structures and can have extremely high affinity and specificity for binding other molecules, be they other nucleic acids, proteins, or small organic compounds [5] (See Fig. 1). DNA sequences as short as 15 base pairs in length can form complex structures that, like antibodies, will bind to analytes in a sample. However, unlike antibodies, oligonucleotides can be directly sequenced, enabling recovered sequences to serve as "images" of binding chemistry, regardless of sample source.

This same concept is at the heart of SELEX, or Systematic Evolution of Ligands by Exponential Enrichment, a combinatorial chemistry technique with deep heritage that is popularly used to select high-affinity single-stranded DNA or RNA, also known as aptamers, to target a wide variety of small and large molecules including surface proteins on tumor cells and other biomarkers [6]. But instead of selecting for oligonucleotide sequences that specifically bind to preselected target ligands, millions of randomly generated single-stranded DNA sequences can be introduced to a tiny liquid sample. A small portion of these random oligonucleotides, dependent upon the concentration of the analytes present, will then bind to analytes, such as the surfaces of cellular compartments, and the full binding patterns that result in the presence of the entire library can be analyzed. This pattern recognition approach is known as "chemmetrics." It represents a set of statistical analysis protocols that can be applied to find patterns in chemical data sets, which in turn can be used to fingerprint nonterran biosignatures.

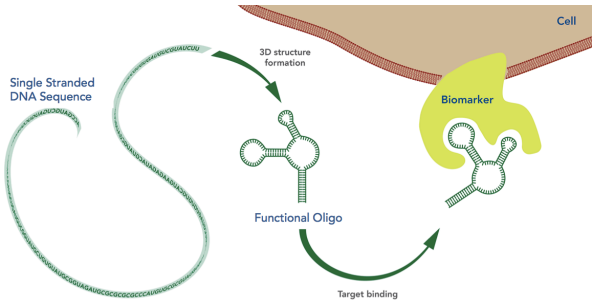

Figure 1: Secondary and tertiary structure transform single stranded DNA sequences into functional olionucleotides, which will bind to a variety of analytes. Adapted from [7].

In short, chemometrics can harness the power of DNA sequencing—but not to detect DNA or RNA. One principle advantage of this approach is its sensitivity. It is likely that the amount of biomass produced lithoautotrophically on the ocean worlds of Europa and Enceladus would be extremely small compared to the biomass produced photosynthetically on Earth [8]. If a plume flyby were to only collect the equivalent of a few tens to a few hundreds of cells, it would require exquisite sensitivity within the suite of life detection instruments. By utilizing the polymerase chain reaction, or PCR, the signal associated with an exceedingly small input can be amplified a billion fold within a few hours.

Signal Detection: There are multiple possibilities for signal detection, including several commercial-off-the-shelf (COTS) detection technologies. Examples include handheld nanopore sequencers as well as DNA microarrays, where, upon hybridization, data in the form of a pattern of fluorescence can be simply and quickly optically imaged. The former is of particular relevance to Mars, where data transfer limitations are less stringent and detailed sequence information might also be utilized for testing a shared ancestry hypothesis (coupled to phylogenic analysis, a nanopore sequencing capability could readily distinguish between modern microbial contaminants and novel, deeply branching DNA-based life forms, similar to but long isolated from terrestrial life in terms of evolution). The latter better approximates the technical limitations of an Ocean Worlds mission.

An electrochemical chip could also be utilized, such as short hairpins that terminate in an electrochemical reporter like methylene blue or ferrocene (in this case, when an amplicon binds to the loop portion of the molecular beacon, the electrochemical reporter will move away from the surface of the chip, resulting in an electrochemical signal that can be easily read).

Chemometric Pattern Identification: It is not any one binding event but the pattern of interactions that specifies the binding "fingerprint" for non-Terran samples. The variations in binding chemical complexity can be analyzed using a variety of statistical tools, including Principle Coordinate Analysis (PCA), Linear Discriminate Analysis (LDA), Support Vector Machines (SVMs), and Artificial Neural Networks (ANNs) [for examples of chemometric analyses describing the analysis of very complex mixtures for specific chemical identities, as well as mixture consistency and complexity, see 9-15].

Contamination Monitoring: This approach also provides an in-built way to monitor potential sources of contamination, a key challenge for any life detection approach. In the run up to a mission launch, analyses could be carried out with washes of clean room surfaces, providing the mission team with an idea of what characteristic patterns are associated with common clean room contaminants, such as *Bacillus atrophaeus* [16]. During or shortly after a mission, the randomly generated oligonucleotide library could be exposed to synthesized laboratory mixtures of new or unusual minerals/organics detected by other instruments abroad the spacecraft, testing to see if patterns match these abiotic materials. Amplifiable oligonucleotides could even be painted onto the surfaces of sample compartments or other spacecraft components to monitor for cross-contamination.

Implications: The discovery of extraterrestrial ocean worlds in our own solar system motivates development of techniques to search for evidence of biosignatures that are agnostic of terrestrial biochemistry. On other planetary bodies, particularly those isolated from exchange with terrestrial material, life may not have originated along the same pathway as it did on Earth. While highly definitive, techniques such as DNA and RNA sequencing that precisely detect terrestrial biology may not be the best approach for missions that collect material from plumes of Enceladus or Europa, or that land on the cold icy surfaces of these moons to search for evidence of life. Yet with chemometrics, a sequencing approach to life detection would enable the science community to not only look for biosignatures associated with life similar to our own (i.e. nucleic acid based life, based on DNA, RNA, and/or nonstandard bases), but also life that is vastly different from life on Earth, probing for forms of biochemistry that may be unimaginable within the confines of our current thinking.

Without presupposing any particular molecular framework, this chemometric life detection approach could be used from Mars to the far reaches of the solar system, all within the framework of a miniaturized chip drawing little heat and power. It would enable truly agnostic biosignature detection, enabling us to fingerprint intricate patterns of binding chemistry.

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