EXTRACTION INSTRUMENTS TO ENABLE DETECTION OF ORIGIN-DIAGNOSTIC LIPIDS FOR LIFE DETECTION. M.B. Wilhelm[1], A.J. Ricco[1,2], D.K. Buckner[3,4,1], T. Boone[5,1], M. Chin[1], J.L. Eigenbrode[6], L. Jahnke[1], R. Williams[7,6], C. Lee[8,9], M. J. Anderson[8,1], T. McClure[1], T. Hoac[5,1], K. Sridhar[1], A. Rademacher[5,1], T. Chinn[1], L. Friend[1], A. Southard[8,6], X. Li[7,6], C. Vidal[10], L. Kivrak[3], Anamika[11], S. Wisnosky[12]; [1]NASA Ames Research Center, Moffett Field, CA 94035 (marybeth.wilhelm@nasa.gov), [2]Stanford University, [3]University of Florida, [4]Blue Marble Space Institute for Science, [5]Millennium Engineering and Integration, Co., [6]NASA Goddard Space Flight Center, [7]University of Maryland, [8]Universities Space Research Association, [9]Lunar and Planetary Institute, [10]Iowa State University, [11]Universisty of North Dakota, [12]University of Miami

Lipids as Targets in the Search for Life: Lipids are key molecular targets to search for evidence of life, as they can reveal information about life processes and their chemical origins [1]. Essential for terrestrial life and likely required for putative extraterrestrial organisms, lipids make up the membranes that separate cell contents from an external environment. They are well preserved in the geologic record, some recording the activity of organisms that lived over a billion years ago, an order of magnitude longer than other types of molecular biosignatures (**Fig. 1**). Lipids are also synthesized abiotically, making up ~60% of soluble organics detected in carbonaceous chondrite meteorites [2].

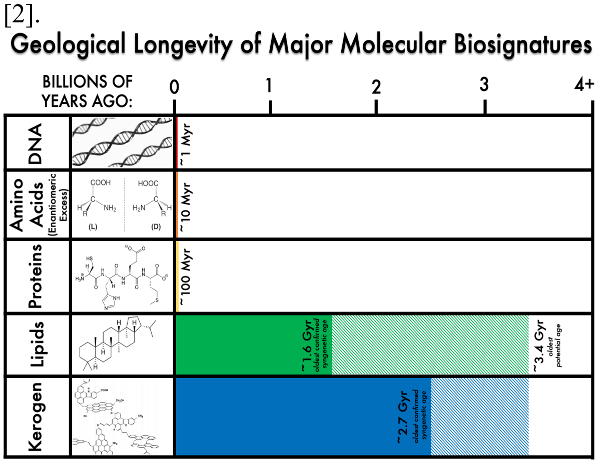

Fig 1. *Depiction of oldest biomarker recovery age in the geologic record: lipids and kerogen have the greatest longevity of any detected molecular biosignature, outlasting DNA, amino acid enantiomeric excess, and proteins by greater than an order of magnitude. The timescale of their preservation is comparable to the age of the ancient, putatively habitable Martian surface.*

Origin-diagnostic molecular features and patterns reveal if lipids were synthesized biotically or abiotically (e.g., [3]). Therefore, to determine whether life ever arose on another planetary body using lipid biomarkers, one must discern not only the presence of lipids, but also their structure. Patterns in chain length or carbon number of certain lipid classes may only be observable through identification of trace constituents in extracted lipids. For example, in biological settings, often the most abundant fatty acids present are $C_{16:0}$ and $C_{18:0}$, but significant information about the microorganisms present is lost if poor limits of detection (LoD) precludes detection of other lower abundance fatty acids. Diagnostic information that distinguishes biotically synthesized lipids from abiotic includes patterns in overall distribution in carbon number, as well as structural features in individual lipid molecules, like the position of branches, functional groups, cyclization, and/or molecular conformation.

Key Challenges to *In Situ* Lipid Detection: There are numerous challenges to the successful detection of relevant lipids, including interference by inorganics present in samples, molecular complexity of the lipids, limitations on their solubility, and low lipid abundance.

Sample Inorganic Content. Samples from Mars, Europa, and Enceladus contain inorganics that will impact the expected chemical structure of lipids, their preservation over geologic time, and their detectability with conventional instrumental approaches. Lipid extraction in each of these astrobiologically significant environments must be tailored to the sample's mineral content, solute type, salt presence and concentration, and oxidant chemistry. Together, these factors dictate the reactions that may occur during sample processing, some of them deleterious to lipid structures present.

Lipid Structure. In modern terrestrial samples, the majority of lipids are bound in membranes (i.e., phospholipids) and only a fraction occur freely in the environment that are readily detectable with gas chromatography-mass spectrometry (GC-MS). To analyze bound lipids, chemical or thermal modifications are required to make them GC-amenable. Similarly, ~99.5% of amino acids are bound in peptides/proteins/humic complexes in terrestrial soils [4], requiring hydrolysis before detection.

Further, lipids are often bound in large, complex, insoluble macromolecular structures called kerogen that requires significant chemical or thermal treatment to liberate lipids prior to analysis. Kerogen typically forms early during sedimentary diagenesis and makes up ~75-90% of the organic inventory of terrestrial and extraterrestrial materials. While most of the organics contained in geologic samples are bound in kerogen, the soluble fraction contains some free lipids, and some bound to minerals (e.g., via sorption, occlusion, or aggregation) [5]; these are also well preserved. Each kerogen macromolecule is unique and complex, consisting of smaller fragments that can contain diagnostic information about their origin (biotic or abiotic) and diagenetic history.

Low Abundance of Lipids. In ancient terrestrial samples and in modern extreme environments, it is often necessary to concentrate extracted lipids an order of magnitude or more to achieve sufficiently low LoDs. Since low abundance of biomass is a common challenge in analysis of Mars analog samples [6,7]; it must be assumed that low concentrations will also be a challenge on Mars (likely < ppb) and other astrobiological targets where additional degradation processes would drive the concentration of organics even lower, such as irradiation and the presence of oxidants (e.g., the high abundance of hydrogen peroxide on Europa).

Translating Traditional Laboratory Lipid Extraction to Spaceflight Instruments: Laboratory lipid characterization techniques are well established and used successfully in terrestrial laboratories for over 70 years (e.g., [8-11]). The efficacy of these techniques at overcoming the abovementioned challenges and the breadth of published data from a diverse range of sample ages and mineralogies make them attractive for spaceflight applications. Previously, these laborious techniques were ill-suited for *in-situ* analysis on Mars or Europa given challenges in fluid handling.

Our team of organic geochemists and chemical, mechanical, and electrical engineers with expertise in spaceflight microfluidics [12-16] has adapted traditional wet chemistry techniques for soils to a TRL-4 system called ExCALiBR (Extractor for Chemical Analysis of Lipid Biomarkers in Regolith). ExCALiBR (**Fig. 2**) will enable lipid detection by coupled mass spectrometry by (1) conserving origin-diagnostic lipid structures and patterns by maintaining them in the liquid phase, using the organic solvents required for optimal lipid extraction, and delivering them to an analytical system unadulterated; (2) reducing signal interference by extracting lipids from mineral matrices and filtering out minerals, which are known to interfere with sample analysis [17,18]; (3) increasing the signal by concentrating lipids by > 1000x to ensure detection in organic-lean samples; and (4) maintaining a sample flow path with contamination below analytical instrument LoD.

Additionally, our team has developed two TRL-2 concepts (1) KAMELOT (Kerogen And Macro-molecule Extractor Liberating Organics Thermolytically) to liberate lipids bound in kerogen in Martian samples and (2) SOWILD (Search in Ocean Worlds Ice for Lipid Derivatives) to target soluble lipids in low-volume icy samples with high concentrations of sulfur-bearing compounds and reactive oxidants such as hydrogen peroxide. These concepts lay the framework for future instrumentation development expanding lipid extraction technology for planetary science to a broader range of sample types and lipid chemistries.

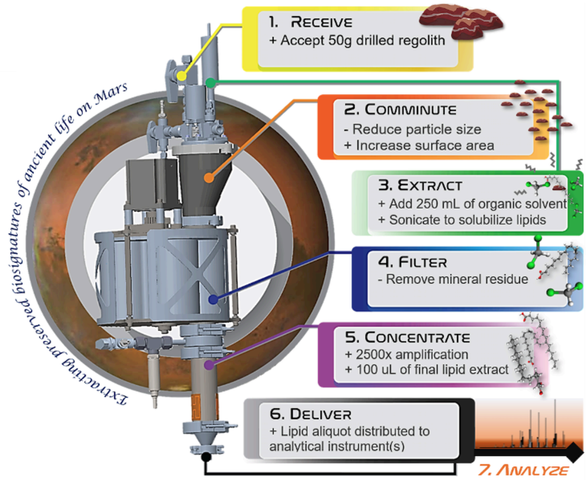

Fig 2. *The ExCALiBR fluidic system will enable six key steps required to extract potential biomarkers from rock powders on Mars and deliver extracts to instruments for analysis. Functions and corresponding enabling subunits shown.*

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