THE EXTRACTOR FOR CHEMICAL ANALYSIS OF LIPID BIOMARKERS IN REGOLITH (ExCALiBR): AN AUTONOMOUS SAMPLE PROCESSING INSTRUMENT TO ENABLE DETECTION OF LIPIDS FOR LIFE DETECTION ON MARS.

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Lipids as Molecular Targets in the Search for Life: In the search for life beyond Earth, lipids are ideal molecular targets [1]. Lipids (i.e., organics soluble in inorganic solvents) comprise the membranes that are universal to all terrestrial life and likely required for putative extraterrestrial life: cellular life requires encapsulation. Simple lipids (e.g., fatty [carboxylic] acids, aliphatic and aromatic hydrocarbons) are also synthesized abiotically and make up >50% of soluble organics in carbonaceous chondrites [2]. Hydrocarbons have been detected in 3 billion-year-old sediments at Gale Crater, Mars, by the Curiosity rover's Sample Analysis at Mars (SAM) instrument [3]. Differences in individual molecular structures (e.g., carbon chain length, unsaturations, branching, cyclization) and overall distributions of preserved lipids can be origin-diagnostic and indicate whether synthesis was biotic or abiotic [1,4]. Lipids have billion-year preservation potential in the geologic record, orders of magnitude longer than other biomarkers (e.g., amino acid enantiomeric excess, DNA, proteins), especially under very arid conditions (**Fig. 1**) [5,6]. The geologic longevity of lipid hydrocarbon cores are on the same order as the age of sediments laid down during the most habitable surface conditions on Mars, making these structures ideal potential indicators of past life on Mars.

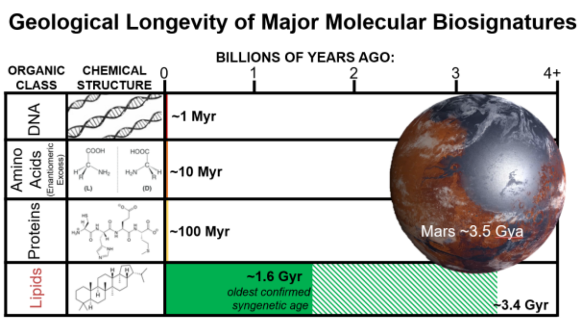

Fig. 1. *Depiction of oldest biomarker recovery age in the geologic record: lipids have the greatest longevity of any detected freely soluble molecular biosignature, outlasting DNA, amino acid enantiomeric excess, and proteins by greater than order of magnitude. This timescale is comparable to the age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

Challenges to *In Situ* Lipid Detection: Challenges to successful detection of origin-diagnostic lipid structures include interference by inorganics during thermal extraction, lipid molecular complexity, and low abundances with heterogeneous distribution.

Inorganic Content and Thermal Extraction. Martian soil contains salts and oxidants that can degrade origin-diagnostic lipid structures during thermal evolution from solid samples (e.g., SAM-like pyrolysis) [7]. Pre-analysis wet chemistry extraction segregates lipids from minerals, avoiding degradation during analysis.

Lipid Structures. In modern terrestrial samples, lipids are often bound in membranes, while ancient samples frequently contain lipids bound in kerogen-like insoluble macromolecules; both require liberation via chemical or thermal modification prior to analysis. However, the soluble fraction contains lipids that are well-preserved via sorption, occlusion, or aggregation [6,8]. Comminution (i.e., grinding) reduces particle size, enabling extraction via organic solvents.

Low Abundance and Heterogeneous Distribution. Lipids preserved in ancient terrestrial and modern extreme Mars analog environments are oft low in abundance and/or heterogeneously distributed. Concentrating extracted samples by an order of magnitude (or more) is often required to achieve a low enough limit of detection (LoD) for successful analysis with laboratory and spaceflight instruments (e.g., gas chromatography-mass spectrometry [GC-MS]). It should be assumed that abundance and distribution of lipids will also present a challenge to analysis of Martian samples (likely <ppb) [3,6]. Extracting and concentrating lipids from larger (multi-cc) samples can be a solution.

Wet Chemistry Extraction for Laboratory Analysis of Terrestrial Geolipids: Solvent extraction techniques for lipid analyses are well-established and have been used successfully on tens of thousands of samples for over 70 years [4,9]. To understand commonly-used techniques for translation to spaceflight application, we conducted a review of the literature (221 papers and 1574 individual samples) and catalogued techniques used to detect and analyze origin-diagnostic distributions for two classes of acyclic lipids (fatty acids and acyclic hydrocarbons) preserved in biotic (i.e., terrestrial) and abiotic (i.e., meteorite) natural samples [4]. Solvent extraction was used to liberate lipids for 83% of the samples, and analysis with GC-MS was used to identify lipids in 90% of the samples. The five most common extraction techniques leverage organic solvents in tandem with other sample processing steps that can vary with the apparatus, pressure, temperature, sonic energy, and/or solvent type and ratio. In no particular order, they include: (1) Modified Bligh and Dyer (2:2:1.8 [v/v/v] ratio of methanol, water, and chloroform or dichloromethane), (2) solvent extraction (an individual, sequence, or cocktail of organic solvent without reporting use of commercial instrumentation, refluxing, or ultrasonication), (3) ultrasonic extraction (organic solvents with the ultrasonic energy), (4) Soxhlet (organic solvent and sample refluxed), and (5) accelerated solvent extraction (ASE) (organic solvent introduced under high temp and pressure via a commercial instrument) **(Fig. 2)**.

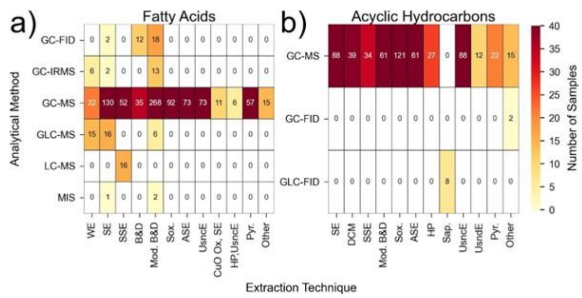

Fig 2. *Extraction techniques and analytical methods used to characterize 1574 acyclic lipid samples, as reported in 221 published studies on **(a)** fatty acids and **(b)** acyclic hydrocarbons, show that solvent-based extraction coupled to GC-MS are most common. The 5 most frequent techniques include Modified Bligh & Dyer (Mod. B&D), Accelerated Solvent Extraction (ASE), Soxhlet, sequential solvent extraction (SSE), and solvent extraction (SE). Other methods include water extraction (WE), ultrasonic extraction (UnscE), CuO oxidation (CuO Ox, SE), hydros pyrolysis (HP,UnscE; HP), pyrolysis (Pyr), dichloromethane (DCM), saponification (Sap.), and other (Other) methods (< 3 individual counts/sample)s. GC-MS is the most common analytical method used to characterize lipids. Color corresponds to counts, with red indicating higher frequency and yellow indicating lower.*

ExCALiBR: Translating Benchtop Lipid Extraction for Spaceflight: To build upon SAM's successful detection of organics, our team of organic geochemists and chemical, mechanical, and electrical engineers with expertise in spaceflight microfluidics has developed the Extractor for Chemical Analysis of Lipid Biomarkers in Regolith (ExCALiBR), an autonomous sample processing unit optimized for lipids and hydrocarbons on Mars (**Fig. 3**). ExCALiBR translates benchtop solvent extraction techniques to overcome aforementioned analytical challenges. Previously, these laborious techniques were ill-suited for spaceflight given challenges in fluid handling. ExCALiBR enables *in situ* analysis of Martian samples by (1) conserving origin-diagnostic lipid structures and patterns by maintaining them in the liquid phase, using the organic solvents required for optimal extraction, for delivery to an analytical system; (2) reducing signal interference by extracting lipids from mineral matrices and filtering out minerals; (3) increasing the signal by concentrating lipids by > 1000x to ensure detection in lean samples; and (4) maintaining a sample flow path with contamination below analytical instrument LoD. Our instrument has been tested with standards and natural samples, and performance validated via comparison to benchtop extractions performed manually.

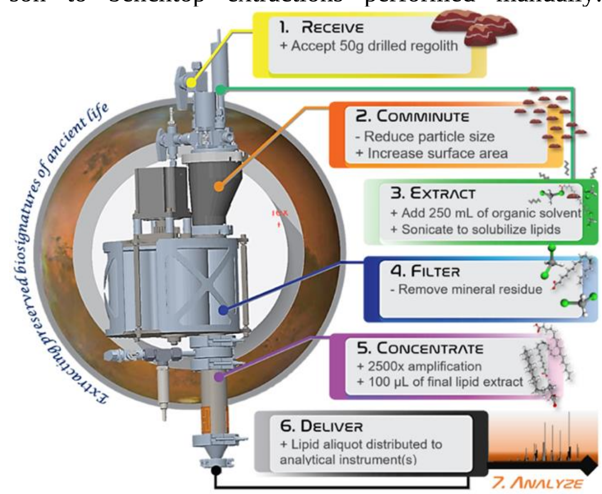

Fig 3. *The ExCALiBR fluidic system will enable six key steps required to extract potential biomarkers from rock samples on Mars and deliver extracts to instruments for analysis. Functions and corresponding enabling subunits shown.*

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