MICROFLUIDIC LIFE ANALYZER (MILA)

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Overview:

MILA is a newly initiated planetary instrument development effort funded under the NASA-PICASSO Program for the period Sept 2014 - Sept 2017. The goal of this new effort is to enable future NASA missions to perform powerful chemical analyses of fundamental interest to astrobiology and planetary science, which aid in the search for life on other worlds and in the broader need to characterize fundamental carbon chemistry and processes throughout the solar system.

MILA focuses on the analysis of organic acids using microfluidic technology. Measurements of chiral amino acid distributions in samples encountered during in situ missions would inform us of not only the prebiological potential of those environments, but also would provide powerful evidence for the existence of life, should a chiral signature be found.[1] By measuring the carboxylic acid content and carbon chain length distributions over a wide length range (1-30 atoms), MILA could search for signatures highly suggestive of lipids or other biological structures.[2-5] Furthermore, these two chemical analyses are most likely not possible using existing gas phase techniques used on past missions due to fundamental aspects of chemistry and mineralogy.[6]

Introduction:

Some of the highest level goals of NASA exploration are addressed by the chemical search for signs of life on other worlds. By analyzing extraterrestrial samples for the presence and distribution of organic functional groups, it is possible not only to infer the presence of extant life, but also extinct life, as well as habitability and the potential for the future emergence of life.[7] This chemical search, typically performed using gas chromatography coupled to mass spectrometry detection, would be drastically enhanced by the implementation of liquid-based analyses, which can also be used to efficiently extract organics from minerals or sediments prior to analysis. The use of microfluidic systems is ideally suited for this for type of investigation, due to their low mass/volume/reagent requirements and extreme sensitivity.

Chemical analysis of organic acids, namely of carboxylic acids and amino acids, is particularly interesting for future astrobiology missions. These compounds form essential components of all life as we know it and serve as a useful starting point in the search for life, prebiotic chemistry, and habitable environments. MILA not only detects and quantifies the presence of these compounds but also determines the distribution of key molecular properties that can be used to inform upon the chemical origin of samples studied. For amino acids, this property is chirality, or the geometric arrangement of functional groups around the central carbon atom in the molecule. Amino acids, regardless of their origin, exist in one of two possible mirror-image forms. Measurement of an excess of either chiral form would serve as strong evidence for living processes [1]. And for carboxylic acids, carbon chain length is a highly diagnostic of their origin. Biological carbon chains are generally built up two atoms at a time, in order to create a host of biological structures. These products include the phospholipid fatty acids (PLFAs), which have specific chain lengths and give cellular membranes their structure, serving as useful indicators of life. [2-5]

Approach:

As a new initiative funded under the NASA PICASSO program (September 2014 project start date) we will advance the TRL of the MILA instrument concept from TRL 3 to TRL 5. Although these analyses require liquid samples, for the PICASSO-funded duration of this project we do not include development efforts in the area of liquid extraction, but rather use the Automated Sample Processing System (ASPS) developed by Beegle for extraction of organics from solid samples.[8] During a follow-on MatISSE effort in the 2017-2020 timeframe we will merge MILA technology with solid sample handling and liquid extraction and also explore the possibility of interfacing with other instrument concepts as well.

This newly initiated effort builds upon over a decade of technological and scientific background initiated at UC Berkeley by Prof. Richard Mathies[9], which was matured during development of the Urey Instrument for the exploration of Mars[10], and, most recently, extended further with ASTID funded development of microfluidic chemical analyzers for the exploration of Titan.[11,12]

These previous efforts demonstrated the viability of microfluidic analysis for in situ astrobiology investigations and solved a host of technological problems[13 and references therein], including for example com-

plete automation of amino acid analysis, [14] novel protocols for analysis of a range of other organic functional groups[15 and references therein] and demonstration of analysis at low temperatures. [11,12] Nonetheless, methods for a simultaneous chiral analysis of more than five amino acid types, the analysis of fatty acids, and a reliable method for the long-term storage of the chemical reagents which underly this measurement technique remain. These primary issues form the basis of the MILA development approach. As this project is only just beginning, at the time of writing we have preliminary data pertaining only to the analysis of fatty acids, which was directly enabled by our Titan based work in the low-temperature microchip non-aqueous capillary electrophoresis (μNACE) of long-chain aliphatic amines.[11,12] We will present our intial results in that area next.

Preliminary Fatty Acid Results:

We demonstrated μNACE of the full range of short to long saturated fatty acids using a new custom-fabricated dye for labeling the acids and rendering them fluorescent, enabling detection via laser-induced-fluorescence detection following illumination at 405nm. [16] Pacific Blue hydrazide (PB-NH_2) labels the carboxylic acid in a two-step, one-pot reaction (Fig.1). Labeling is performed in dimethylformamide (DMF) using 1 mM PB-NH_2, 2 mM O-(1H-6-chlorobenzotriazole-1-yl)-1,1,3,3-tetramethyl-uronium hexafluorophosphate (HCTU) coupling agent, and 50 mM DIEA.

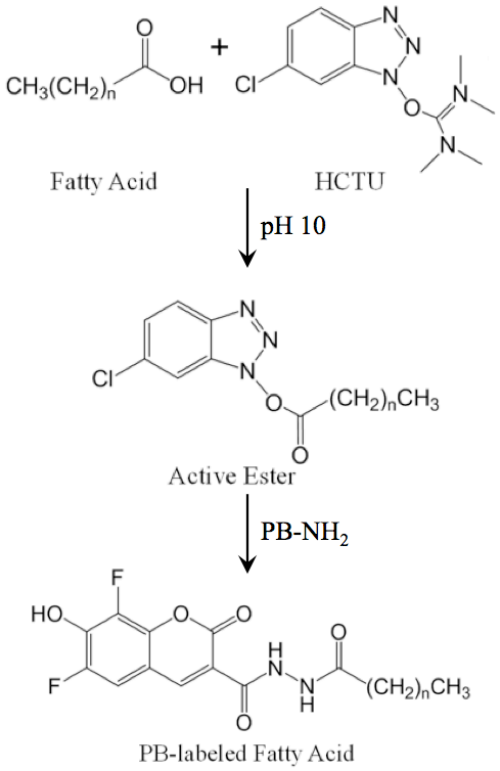

Figure 1. Labeling reaction employed using a new dye (PB-NH_2). Reproduced from Reference [16] with permission from The Royal Society of Chemistry.

The optimized protocol for analysis of fatty acids enables microchip electrophoretic separation of acids containing between 2 and 30 carbon atoms (Fig.2). Limits of detection for C10 to C30 fatty acids ranged from 0.9 to 5.7 μM. Separations in ethanol allowed for resolution of both short and long chain fatty acids, with those differing by two methyl units baseline resolved. To our knowledge, this is the first separation of fatty acids up to C30 on a microfluidic device. In order to demonstrate analysis on a real terrestrial sample simulating what could possibly be encountered in sediments on planetary missions, fatty acids were detected and quantified in a sediment sample from the 'Snake Pit' hydrothermal system of the Mid-Atlantic Ridge (Fig. 3 and Fig.4).

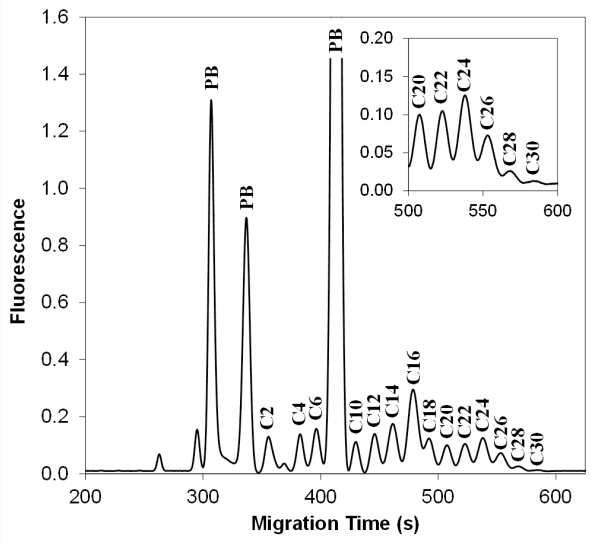

Figure 2: Optimized μNACE separation of C2 to C30 fatty acids (2 μM C2-C26, 5 μM C28-C30) in ethanol. *Inset:* Long-chain fatty acids. Reproduced from Reference [16] with permission from The Royal Society of Chemistry.

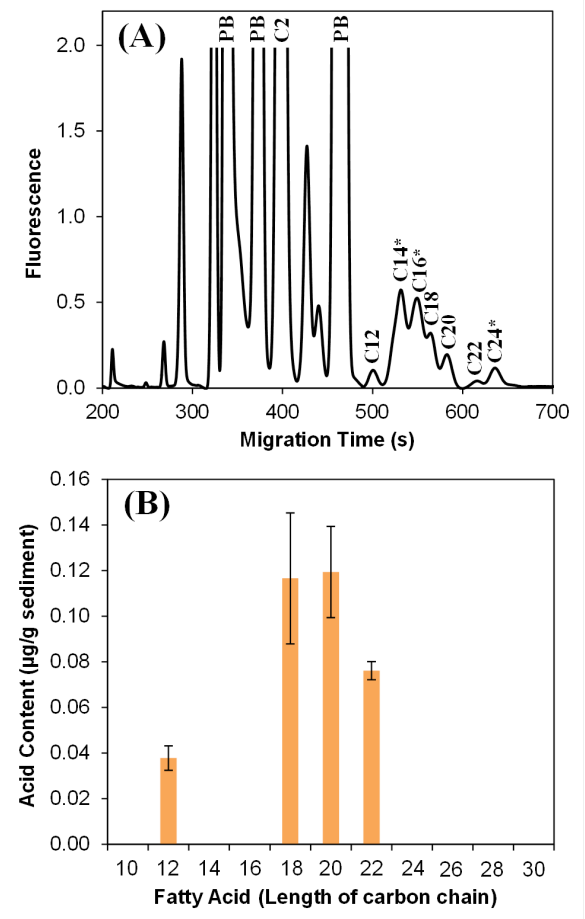

Figure 3. Fatty acids present in a sediment sample collected from the Snake Pit hydrothermal vent system. (A) Separation of sediment extract yields several long chain fatty acids (starred acids were also present in procedural blank). (B) Fatty acids reported in μg per g of sediment prior to extraction. Reproduced from Reference [16] with permission from The Royal Society of Chemistry.

Several long chain fatty acids were detected and quantified, including C12, C18, C20 and C22 fatty acids, which serve as biomarkers of microbial ecosystems. The size distribution and relative abundance of fatty acids provides a highly diagnostic means for differentiating between abiotically produced organics (e.g., those found in meteorites) and those produced by biological processes (e.g., those derived from microbes or living organisms). Hence this method would provide invaluable chemical information in the search for life on other worlds.

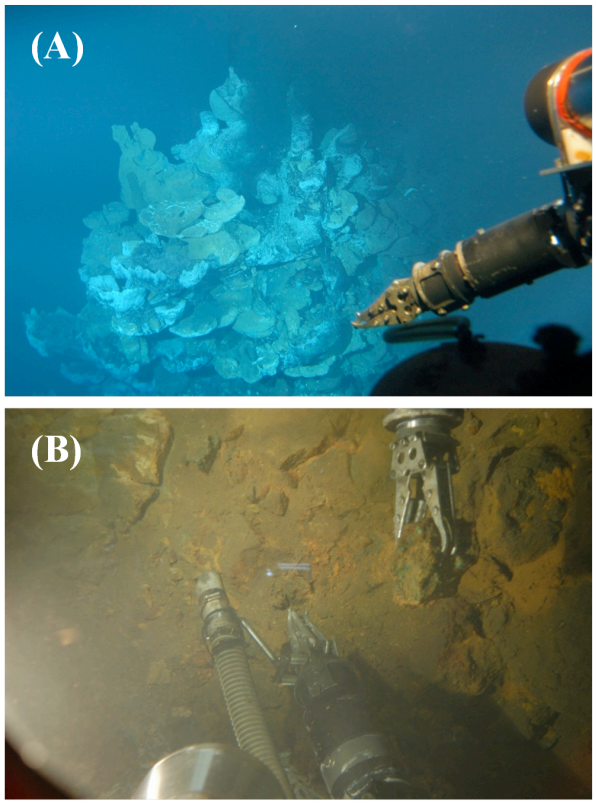

Figure 4. (A) A view of the Snake Pit hydrothermal vent field from the MIR 2 submersible. The diameter of the mound shown is 15-20 m. The sample site was at the base of the structure. (B) Sediments analyzed were collected at the base of the mound using a water vacuum connected to a sample chamber. Photos by K. H. Hand. Reproduced from Reference [16] with permission from The Royal Society of Chemistry.

Summary:

The MILA instrument employs microfluidic technology to perform two fundamentally important organic analyses for the detection of extant or extinct life on planetary missions, enabling the search for the presence, distribution, and properties of amino acids and fatty acids. Preliminary data from a newly developed non-aqueous microchip CE method for fatty acids have been acquired using a custom fabricated dye. This fatty acid analysis method supports the analysis of species not accessible using water solutions, and also open up the range of temperatures that can be used for analysis. The non-statistical distribution and abundances of the fatty acids measured in seafloor sediment samples suggests a biogenic source for this material; such information would be essential in determining a biotic/abiotic origin for fatty acids or other organics detected beyond Earth.[7] Future work (2014-2017) concerns the development of methods for the simultaneous chiral analysis of all 20 of the terrestrial amino acids at enhanced sensitivity (50pM) as well as validation of long-term storage of reagents appropriate for spaceflight application.

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