HYDROTHERMAL SILICA SINTER AS A REPOSITORY FOR LIPID BIOSIGNATURES DETECTABLE WITH SAM-LIKE THERMOCHEMOLYSIS. L. Kivrak, B. L. Teece, D. Boulesteix, A. J. Williams, J. Havig, J. Curtis, K. Palmer, T. L. Hamilton 1 Department of Geological Sciences, University of Florida, 241 Williamson Hall, PO Box, 112120 Gainesville, FL 32611 (lkivrak@ufl.edu), Jet Propulsion Laboratory, California Institute of Technology 2 Department of Chemical Engineering, CentraleSupélec-Paris-Saclay University, 4 Department of Plant and Microbial Biology, University of Minnesota

Introduction: The search for life on Mars centers around the exploration of organic molecules, their provenance, preservation in the rock record and detectability with current space-flight instrumentation [1]. The Curiosity rover contains the Sample Analysis at Mars (SAM) instrument, a gas chromatograph-mass spectrometer (GC-MS). SAM has detected chlorobenzenes [2] and thiophenic organic compounds [3] in the lacustrine mudstones of Gale Crater. SAM can also perform wet-chemistry experiments using the reagent tetramethylammonium hydroxide (TMAH) [4] to methylate polar organic molecules, such as fatty acids, liberating them from larger macromolecules to increase detectability [5]. Fatty acid methylation produces fatty acid methyl esters (FAMES). Fatty acids are particularly promising as potential biosignatures due to their ubiquity in all known life on Earth and their even-over-odd chain length preference when produced biotically helping to distinguish between biotic and abiotic sources [6].

Analog sample analyses are crucial for understanding data collected on Mars. Hydrothermal siliceous sinter is an especially high priority astrobiological target due to its ability to entomb microbes and preserve organic molecules [7]. Hydrated silica has been detected in many places on the surface of Mars via orbital [8] and in situ [9] observations. In one example, digitate silica structures morphologically similar to biotic stromatolites were observed by the Spirit Rover at Home Plate [10]. Due to the heterogenous nature of hot spring environments, studies of organic molecule preservation in silica sinter should consider multiple pH conditions, and different timescales [11, 12, 13].

Here we perform SAM-like TMAH thermochemolysis and Bligh-Dyer like lipid extractions on a suite of silica sinter samples from Yellowstone National Park (YNP) to better understand the capabilities of current space-flight technology to detect lipid biosignatures. Additionally, this work aims to answer how the earliest stage of diageneses may contribute to loss of organics, and how the spatial and temporal heterogeneity of the hot spring environment may affect biosignature preservation.

Methods: Samples were collected in YNP, an active hydrothermal environment on Earth dominated by silica sinter deposits. Ashed tools and foil were used for the collection of samples to maintain organic cleanliness. The sample suite included three samples from stromatolites along the edge of the Happy Harfer pool (pH ~6). Three distinct layers of stromatolites were sampled – the lowermost consisting of actively precipitating stromatolitic sinter located directly adjacent to the waterline (HH Active), a middle layer consisting of stromatolites that were no longer actively precipitating (HH Mid), and a top layer of the oldest stromatolites (HH Old). Relict sinter samples were also gathered from Steep Cone Geyser (pH ~7.6), at locations like those studied in [12] (SC Mid), and an actively precipitating sample at the hot spring’s edge (SC Active). An actively precipitating digitate silica structure was sampled at Rabbit Creek (RC Dig, pH ~9). Along with a sample from Artesia Geyser (AG Prox) in the Firehole Lake Area (pH ~7.7).

Total organic carbon (TOC) measurements were acquired on the samples using a 50-position automated Zero Blank sample carousel on a Carlo Erba NA1500 CNHS elemental analyzer. Total inorganic carbon (TIC) was measured using a UIC (Coulometrics 5017 CO2 coulometer coupled with an AutoMate automated carbonate preparation device).

Samples were homogenized in an ashed (500°C for ~8 hours) mortar and pestle. Frontier Multi-Shot (EGA/Py-303D) pyrolyzer and Agilent 7890B GC-5975C XL inert MSD GCMS were used for direct pyrolysis-GCMS analyses of analytes evolved from thermochemolysis. 3-5 mg of powdered sample was placed into solvent-washed sample cups along with TMAH in a ratio of 1 µL TMAH to 1 mg sample. 1.5 µL of a C10 internal standard was also added.

Samples were pyrolyzed at 500°C for 0.5 minutes. The GC program ramped from 70°C to 300°C at 15°C min⁻¹ with a 7-minute hold. A modified Bligh Dyer extraction was performed on RC Dig and SC Mid to gain a more complete view of these lipid profiles. A 0.8:2:1 H2O:MeOH:DCM ratio was used for extraction, followed by a medium-acid methanolysis step.

Results and Interpretations: TOC and TIC data confirmed significant carbonate presence in sample AG Prox of 10.7% (Table 1). TOC data showed that the organic content of Happy Harfer samples increased with age, rather than decreased.
SAM-like TMAH thermochemolysis resulted in the detection of a multitude of FAMES. C\textsubscript{16} and C\textsubscript{18} were detected in all samples with high abundances, which is expected due to their ubiquity in bacterial cell membranes.

<table>
<thead>
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<th>Sample Name</th>
<th>Sample Site</th>
<th>TOC</th>
<th>TIC</th>
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<tr>
<td>HH Active</td>
<td>Happy Harfer Pool</td>
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<td>0.01</td>
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<td>HH Mid</td>
<td>Happy Harfer Pool</td>
<td>3.33</td>
<td>0</td>
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<tr>
<td>HH Old</td>
<td>Happy Harfer Pool</td>
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<td>SC Mid</td>
<td>Steep Cone Geyser</td>
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<td>Steep Cone Geyser</td>
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<td>Rabbit Creek</td>
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<td>AG Prox</td>
<td>Artesia Geyser</td>
<td>0.26</td>
<td>10.7</td>
</tr>
</tbody>
</table>

Table 1: Sample names with TOC and TIC data.

A strong even over odd chain length preference was also observed. Even chained FAMES from C\textsubscript{6}-C\textsubscript{18} were detected in AG Prox and SC Active (Fig 1.). Additionally, FAMES from C\textsubscript{20} – C\textsubscript{28} were present in all Happy Harfer samples, but with drastically higher abundance in HH Old (Fig.2). Surprisingly, HH Old included higher abundances of detected FAMES as well higher TOC values. The higher abundance of longer chain FAMES implies input from local vegetation may overprint the initial degradation of FAMES, resulting in an increase in organic carbon content. Water flow on active samples could wash away organics through porous silica [12]. The higher temperatures endured by the active stromatolite could also degrade long chained FAMES. The lipid extractions of SC Mid and RC Dig resulted in the detection of FAMES from C\textsubscript{12}-C\textsubscript{28}, as well as tentative identifications of branched methyl FAMES and iso-anteiso C\textsubscript{15}.

**Conclusions:** TMAH thermochemolysis was highly effective at detecting FAMES in all samples. Bligh Dyer extractions resulted in the detection of more long-chained FAMEs and branched methyl FAMEs. The factors influencing the initial stages of organic matter degradation in modern hydrothermal sinter are complex and more work is needed to understand this. Silica sinter on Mars is a high-priority target for potential biosignatures and the improved detectability of FAMES by extractions emphasizes the need for returned sample science.

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**References:**