SEEKING SIGNS OF LIFE ON MARS:

IN SITU INVESTIGATIONS AS PREREQUISITES TO A SAMPLE RETURN MISSION

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SCOPE

A campaign of in situ analytical surface exploration missions are needed to identify sites on Mars with unequivocal biosignature presence. A Mars sample return mission would then be the required and justified follow-on to provide for scientific discovery beyond that provided by in situ capability. Detection of organic compounds as biosignature remnants or as evidence of prebiotic chemistry would best be realized though a Follow the Nitrogen strategy for in situ exploration. Instruments with exquisite sensitivity and nondestructive extraction techniques would be required, and landing site selection must emphasize sequestration potential. This input is intended to contribute to the development and selection criteria for potential Mars Astrobiology missions designed for the detection of organic compounds or seeking signs of extraterrestrial life.

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INTRODUCTION

“Seeking Signs of Life” was introduced at the most recent MEPAG meeting as the new theme for Mars exploration, replacing the “Follow the Water” strategy that has carried us through the last decade. This shift reflects the latest Astrobiology Goals document [Des Marais 2008], and is a welcome evolution both within the planetary science community and among the public at large. To realize this ambitious goal, we must focus our search on markers that either indicate an environment conducive to prebiotic synthesis or directly reveal extinct or extant life. It would take little more than detection of a particular set of organic compounds to demonstrate that Mars was once a cradle for biochemistry as we know it on Earth.

Two strategies have been suggested for seeking signs of life on Mars: The aggressive robotic pursuit of biosignatures with increasingly sophisticated instrumentation vs. the return of samples to Earth (MSR). While the former strategy, typified by the Mars Science Laboratory (MSL), has proven to be painfully expensive, the latter is likely to cripple all other activities within the Mars program, adversely impact the entire Planetary Science program, and discourage young researchers from entering the field.

In this White Paper we argue that it is not yet time to start down the MSR path. We have by no means exhausted our quiver of tools, and we do not yet know enough to intelligently select samples for possible return. In the best possible scenario, advanced instrumentation would identify biomarkers and define for us the nature of potential sample to be returned. In the worst scenario, we would mortgage the exploration program to return an arbitrary sample that proves to be as ambiguous with respect to the search for life as ALH84001.

A robust robotic program to seek signs of life should reach beyond MSL in several respects. Supported by orbital remote sensing, advanced mobility and precision landing technology should be applied to access potentially habitable environments such as the subsurface, gullies, layered sediments, and ice sheets. Microanalytical techniques should be applied in situ to identify physical textures and surface chemical signatures of rocks, soils and ices. In this paper, we concentrate on the next generation of a Follow the Nitrogen approach [Capone 2006] using exquisitely sensitive microfluidic techniques such as micro-capillary electrophoresis to identify and determine the concentrations of amino acids, nucleobases, amino sugars, and other essential organic molecules.

We argue here that when in situ methods have definitively identified biomarkers, or when all reasonable in situ technologies have been exhausted, it would be time for MSR. We are not yet at that crossroad.

1. MARS SAMPLE RETURN (MSR) SHOULD ONLY BE SUPPORTED AFTER THE PRESENCE OF BIOMARKERS HAS BEEN CONFIRMED.

Mars sample return missions would eventually allow for high precision measurements to be conducted with higher sensitivity, accuracy, and greater scope than is possible with in situ instrumentation. The major scientific drawbacks of such mission architectures would be the low achievable sample return masses (~350 grams), the fact that any returned sample(s) would only probe a minute geographical area on Mars, and the fact that no unequivocal evidence of biosignatures has yet been obtained to ensure that the returned sample would address the potential detection of extraterrestrial life. Current MSR strategies do not address major goals of the Mars scientific community such as surface geochemical microscale and macroscale heterogeneity, nor would the sparse returned sample allow for any relative characterization of spatial variability. Our current state of knowledge regarding the surface geochemical diversity of Mars is not so much hindered by instrument resolution as the lack of appropriately diverse samples for investigation. MSR would not help to address this issue. Sample return missions would have the drawback of being prohibitively expensive, exceeding the cost of 3 NASA flagship missions. Therefore, the central criterion for the implementation of a Mars sample return mission becomes: what would be an acceptable scientific return to justify such a large expenditure of engineering and capital resources? We argue that a credible and sustainable sample return mission must necessarily be preceded by the in situ detection of organic compounds to justify such an expenditure.
Recent successful robotic missions and in situ experiments include the Mars Exploration Rovers (MERs) and the chemical results of Phoenix [Hecht 2009]. Opportunities to incorporate advanced in situ flight instruments on future missions would allow for thorough investigations of regional variability of habitability potential via the search for organic compounds (e.g. Sample Analysis at Mars, SAM, aboard MSL) and the concentrations of oxidants within the Mars regolith.

There are serious community reservations about a rush to commit valuable scientific resources and funding to MSR until a valid scientific discovery has been made to justify investment – the in situ detection of localized biosignatures and an attempt at characterization of spatial variability as a function of depth or mineralogy would make a strong case as a valid scientific rationale on which to pursue expensive sample return ambitions. We feel that organic detection efforts over the next two decades via investment into advanced in situ robotic instrumentation are fundamental in support of a future intelligent MSR mission. Currently, MSR is regarded by much of the scientific community as largely weighted towards a technology demonstration as the rationale for good astrobiology will not be apparent until we discover more about our neighboring planet.

2. ASTROBIOLOGY INSTRUMENTATION MUST INCLUDE SPECIFIC CAPABILITIES FOR IDENTIFICATION OF UNEQUIVOCAL BIOSIGNATURES FOR INVESTIGATION INTO THE PRESENCE OF ORGANICS WITH RESPECT TO LIFE DETECTION.

We feel that the high costs and program implications associated with a potential MSR mission must be preceded by the in situ detection of organic compounds on Mars. The term biosignature can apply to the presence of morphological features, molecular organic compounds, or the presence of inorganic phases (e.g. mineralogical) that are present as a result of biological activity. Here we focus on organic molecular biosignatures because they are necessary to supplement any morphological biosignatures in order to provide unequivocal evidence of life [Cady 2003; Westall 2008].

The search for organics on Mars remains a central goal in near future Mars exploration. To date, most efforts for chemical biosignature detection have centered on a Follow the Carbon approach [Farmer & Des Marais 1999]. It has been argued that while there are numerous abiotic pathways to carbon chemistry, the presence of nitrogenous compounds is specifically diagnostic for biogenicity on Mars, a planet that lacks widespread inorganic nitrate deposits [Capone 2006]. We endorse here a variation of the Follow the Nitrogen approach in which chemical characterization of highly specific nitrogenous compound classes would provide a method for unequivocal biosignature detection.

In our view, the relative fitness of a biomarker for life detection depends primarily on the following criteria:

1) UBQUITOUS MOLECULAR COMPONENT OF MICROBIAL LIFE;

2) ALLOWS FOR DISCRIMINATION BETWEEN BIOGENIC AND ABIOTIC SYNTHETIC INVENTORIES;

3) BIOMARKERS MUST BE STABLE OVER GEOLOGICAL TIMESCALES.

The largest fractions by dry mass of microbial cells are proteins and nucleic acids (Fig. 1A), both of which are labile organic compounds. Of these reservoirs, amino acids (protein components) and the nucleobases cytosine, adenine, and guanine (nucleic acid components) are organic primary amines and have been recommended amongst the highest priority molecular compounds for astrobiological investigation in a recent ESA study [Parnell 2007]. Furthermore, >80% of the total dry weight of E. coli is composed of amine containing organics (ACOs). Not only is the detection of these compounds important as molecular building blocks of life as we know it, these compounds offer ability to discriminate between their biogenic or abiotic origin – a critical requirement for a definitive and unequivocal life detection approach.

Amino acids, which are present in proteins in exclusively levorotary configurations, can be discriminated from any abiotic reservoir (formed via prebiotic chemistry on the Martian surface or from exogenous
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delivery) through chirality measurements [Kvenvolden 1973]. Likewise the determination of the chirality of sugars, present as microbial cell wall components in exclusively the dextrotary configuration, has also been suggested as an effective method for biosignature detection [Westall 2000a]. The detection of these primary amine components (i.e. amino acids, amino sugars) and determination of their chirality offers unequivocal determination of the presence of biosignatures on Mars. Extracellular polymeric substances (EPS), primary secretions constituting microbial biofilms [Meyer 1999], contain extremely high levels of protein ranging from 14-35% (Fig. 1B) and would also be detectable as robust biomarker compounds by probing the chirality of their component amino acids. In this robust nitrogen-centric life detection strategy, it is necessary to probe small molecular components (e.g. amino acids from proteins and amino sugars from microbial cell walls) in order to determine a biogenic origin.

**Fig. 1.** Dry weight composition of (A) a typical E. coli bacterial cell [Neidhardt 1990], and (B) extracellular polymeric substances, EPS [Tsundeda 2003], primary components of bacterial biofilms.

[LPS=lipopolysaccharides; PG=peptidoglycan; asterisk denotes compounds containing primary amines]

Among the most refractory cellular material present within microbial cell walls are structural components that compose ~15% of the total mass of microbial cells by dry weight [Fig. 1A]. These compounds include fatty acids (e.g. lipids), sugars, and peptidoglycans (murins). Of these compounds, components of peptidoglycan may be the most reliable biomarker because it allows for greater preservation of amino acids over geological timescales by effectively sequestering these compounds within a more refractory matrix [Grutters 2002]. Nitrogenous primary amine compounds, particularly amino acids and amino sugars, are present in high concentrations within peptidoglycans. Microbial biofilms have also been suggested as refractory targets for the detection of remnants of extraterrestrial microbial life [Westall 2000b; Boston 2001]. The potential for mineralization of the biofilm residue offers strong preservation potential for the molecular constituents. Amino acids are also present at significant concentrations within desert varnishes at hundreds of parts-per-billion (ppb) levels and these refractory materials have been suggested to form through microbial processes [Perry 2003].

ACOs, including proteins and their component amino acids, are generally classified as labile materials compared to more refractory molecular classes such as fatty acids [e.g. Eglinton 1991]. An empirical study of amino acid exposure to galactic cosmic rays (GCR), fluxes of which are much greater on Mars than on Earth, estimates that catalytic degradation diminishes with depth and is completely attenuated at depths of ~3 meters [Kminek & Bada 2006]. However, kinetic models of the survival of primary amines conclude that amino acids could have potentially survived over 3.5 billion years of Mars’ history [Kanavarioti & Mancinelli 1990]. The degradation of amino acids within evaporitic sulfate minerals inferred by the detection of amine decarboxylation products also suggests preservation over these timescales [Aubrey 2006]. Similar models of the diagenetic process of racemization, which is usually
much slower than degradation, concluded that amino acid chiral signatures should persist over billions of years and that these lifetimes are enhanced with low water activity and low temperatures, both conditions of which are satisfied on Mars [Bada & McDonald 1995]. The oldest indigenous amino acids claimed to have been measured were detected in late Precambrian aged samples [Akiyama 1982]. Nitrogenous biomarkers from proteins and peptides sequestered in soils and sediments are suggested to be preserved over geological timescales [Knicker 2004].

3. **MINERALOGICAL ENVIRONMENTS TARGETED FOR ASTROBIOLOGICAL INVESTIGATION MUST SHOW HIGH ORGANIC SEQUESTRATION POTENTIAL.**

The strategy for the detection of extraterrestrial life is similar to the search for the earliest evidence of terrestrial life. The major challenge in this search is detecting biosignatures that have not been destroyed over time by metamorphic or diagenetic processes. This is an easier task on Mars than earth because of the lack of tectonic activity and observations of Noachian aged phyllosilicates exposed on the Martian surface [Bibring 2006]. While theoretical studies suggest that organic compounds degrade to heterocyclic carbon compounds over billion year timescales via oxidative pathways [Benner 2000], the total loss of all nitrogen even over geological timescales is unreasonable. Nitrogen is preferentially lost from the rock record compared to carbon as C:N ratios increase with time via diagenetic pathways [Ertel 1983]. However, analyses of terrestrial Archean aged rocks still show low amounts of nitrogen [Ueno 2004] and elevated amounts of ammonium [Honma 1996].

While not only a function of exposure temperature and time, the degradation of ACOs is strongly a function of the sequestration within mineral matrices [Farmer & Des Marais 1999]. Amino acids can be effectively encapsulated within minerals during their formation process. These processes could also act to isolate labile organics from surface oxidative processes such as oxidation by GCR [Aubrey 2006]. Under optimal depositional environmental conditions, for instance with rapid burial, it is possible for these compounds to be stable over geological timescales. Therefore there is a paramount need to identify astrobiologically relevant minerals that show potential for preservation over long timescales [Martinez-Frias 2006].

Geological targets for the detection of bioorganic compounds on Mars during future *in situ* missions should be focused on the following high priority mineralogical targets:

1. **Evaporitic Materials (Sulfates, Halites)** – Evaporitic minerals show a high capacity for organic encapsulation during terrestrial formation, which effectively traps organics and sequesters them from the elements, and there is no reason that these processes shouldn’t extend to Mars [Mancinelli 2004]. Minerals such as halite or gypsum provide ideal media for molecular trapping and remain stable over geological timescales. The preservation of organics, specifically primary amines [Aubrey 2006; Bada 2008] and enzymes [Tehai 2002], should be enhanced by sequestration. Fluid inclusions have been suggested to offer protection from oxidative and UV-catalyzed degradation and prevent dehydration, potentially preserving extant bacteria for hundreds of millions of years [Satterfield 2005].

2. **Phyllosilicates** – The suggestion of clays as a prime target for the detection of biosignatures and organic preservation [Ehlmann 2008a] arises from the fact that these materials are traditionally associated with high organic loads and sorptive properties that effectively sequester and preserve organic materials [Keil 1994; Mayer 2004]. Archean materials that are enriched in ammonium ion (NH$_4^+$) associate the high nitrogen levels with authigenic clays [Honma 1996], thereby acting to preserve organics over long timescales. Primary amines have been observed to show strong sorptive properties with clay minerals due to their high available surface area and charged surfaces [Keil 1998] and this capacity has been demonstrated in numerous empirical studies using kaolinite, montmorillonite, goethite, and illite [Wang & Lee, 1993; Ding & Heinrichs, 2002; Hedges & Hare, 1987; Greenland 1964].
3. **Carbonates** – Data from the Compact Reconnaissance Imaging Spectrometer for Mars (CRISM) has recently been interpreted to show strong evidence of carbonate deposits (~5%) in close association with phyllosilicate-rich areas [Ehlmann 2008b]. Carbonates provide an interesting target to probe for biomarkers and are often associated with marine or lacustrine evaporitic environments, therefore it may have provided a past habitable environment in the early Noachian. Terrestrially, these materials show good preservation of precambrian morphological biosignatures [Farmer & Des Marais 1999] and might be expected similarly to preserve chemical biosignatures.

4. **Polar Layered Deposits (PLDs)** – An important target for astrobiological investigation includes the Mars Polar Layered Deposits (PLDs). It has been demonstrated that harsh surface conditions on Mars lead to enhanced organic degradation due to GCR [Kminek & Bada 2006] and oxidative processes [Benner 2000]. The reason PLDs are attractive targets for *in situ* instrument investigation is that the samples would be delivered for analysis in the aqueous state and deep access to ice reservoirs through sample drilling and/or melting would allow investigation into the variation with depth of nitrogenous biomarkers.

From a life detection standpoint, the most relevant facies on Mars are lacustrine and fluvial deposits which are particularly attractive because their presence indicates water activity. These sediments include evaporitic sulfates as well as phyllosilicate-rich areas, which are suspected to be products of aqueous activity over basaltic material. Halites and sulfates may have extended the presence of water in the liquid state on the surface of Mars because of freezing point depression and slower evaporation rates experienced in these brines [Altheide 2009]. These layered fluvial and lacustrine materials (sulfates, phyllosilicates, carbonates) are primary targets for future astrobiological missions as these types of terrestrial facies are strongly associated with high biodensities on Earth.

### 4. **Requirements for in situ Life Detection Instrumentation Capabilities**

If organics are present on the surface of Mars, they are expected to be present at extremely low concentrations (ppb), orders of magnitude below the sensitivities of most flight instrument technologies. Therefore, it is necessary to assure that the techniques of extraction and analysis degrade as little as possible of the labile organic inventory that may be present in the Mars regolith. Extraction methods must demonstrate that they are non-destructive with respect to target biomarker compound classes while analytical methods must be focused on the detection of extremely low levels of biomolecular compounds. Over the last decade the development of the *Urey* instrument for organic and oxidant detection on Mars has succeeded in addressing many of the aspects of primary concern for effective detection of biosignatures on Mars [Aubrey 2008].

#### (A) *Astrobiology in situ Instrumentation Must Include Specific Capabilities for Identification of Nitrogenous Organic Compounds for Life Detection Investigations.*

The advantage of a *Follow the Nitrogen* approach to biosignature detection is strengthened by the intrinsic chirality of amino acids and amino sugars that are incorporated into terrestrial life. Likewise, extraterrestrial life would be expected to exhibit similar characteristics [Pace 1997]. Primary amines are detected with extraordinarily high sensitivity after derivatization with highly specific fluorophores and analysis by laser-induced fluorescence (LIF), a central theme for detection of ACOs utilized by the *Urey* instrument.

The *Urey* Instrument is an advanced *in situ* instrument suite designed to assess the presence of organics and oxidants on the Martian surface [Aubrey 2008]. The biomolecules targeted by *Urey* include amino acids, nucleobases with exocyclic amine groups (e.g. cytosine and adenine), amino sugars, diamines and
other primary amine degradation products that may be present due to extinct or extant life [Skelley 2006]. Resolution of enantiomers using a micro-capillary electrophoresis (µCE) analytical chip, called the Mars Organic Analyzer (MOA), allows for positive life detection results [Skelley 2007]. This lab-on-a-chip microanalysis system provides for a wide variety of analytical chemical techniques in a compact low mass structure [Skelley 2007].

(B) Extractions Methods for the Isolation of Organic Compounds from Mars Regolith Need to Preserve the Target Molecular Compounds with Low Amounts of Degradation.

The null detection of organics by the Viking lander resulted in the hypothesis that powerful surface oxidants overwhelmed the accumulation of any organics on the surface of Mars [Klein 1979]. Subsequent studies suggest that the Viking gas-chromatograph-mass spectrometer (GCMS) would not have detected organics at low parts-per-million (ppm or µg/g) levels [Navarro-Gonzalez 2006] and the GCMS would not have been sensitive to microbes present in soils at levels ~10^7 cells/gram [Glavin 2001]. In the presence of oxidants, measurements of Atacama Desert soils indicate that GCMS detection limits are no greater than 150 ppb in analyses of Atacama Desert soils [Navarro-Gonzalez 2009]. Studies further suggest that oxidants interfere with thermal volatilization (TV) extraction approaches, including the methods utilized by the Phoenix Thermal and Evolved Gas Analyzer (TEGA), the MSL Sample Analysis at Mars (SAM) instrument, and the ExoMars Mars Organic Molecule Analyzer (MOMA). The recent detection of high levels of perchlorate at the Phoenix landing site by the Mars Electrochemistry and Conductivity Analyzer (MECA) at levels of 0.6% [Hecht 2009] make this danger extremely relevant. While enhanced degradation of organics in the aqueous phase is insignificant at perchlorate concentrations up to 0.1 M [Abdullah 1990], rapid catalytic oxidative degradation of reduced carbon is observed in solid samples [Patai & Hoffman 1950] at temperatures of 367°C. This is in accord with recent results from the Phoenix Thermal Evolved Gas Analyzer (TEGA) team that show copious CO_2 evolution at low temperatures from mellite acid decomposition due to the presence of perchlorate [Ming 2009].

To address these problems, recent developments have demonstrated non-destructive aqueous extraction and analytical methods for future astrobiological instrumentation. Wet chemical extraction techniques under investigation include subcritical water, SCWE [Amashukeli 2007], aqueous microwave assisted extraction, MAE, and organic solvent extraction [Buch 2003]. Complementary to solvent extraction methods is the development of miniaturized analytical space flight instruments that require aqueous extracts including microfluidic capillary electrophoresis chips, µCE [Skelley 2005], liquid-chromatography mass-spectrometrometers, LC-MS [Liu 2008], and Life Marker Chips, LMC [Sims 2008]. Based on the scientific rationale above, it appears that TV approaches place a severe risk of degradation during extraction while aqueous extraction methods are non-destructive and would be best suited for the extraction of trace levels of organic materials from Mars regolith samples.

Incredibly high sensitivity to primary amines would be achieved by the proposed Urey instrument which involves extraction of bound organics from mineral matrices using subcritical water extraction (SCWE). SCWE treatment has been demonstrated to liberate organics from Mars regolith analogs using dielectric tuning of a single ultra-high purity solvent - water. Another important aspect of SCWE extraction is that it simultaneously hydrolyzes proteins into component amino acids which can then be resolved using the MOA analytical chip. The SCWE operating conditions have been optimized for extraction of amino acids from soils collected in Atacama Desert [Amashukeli 2007] - one of the best Mars regolith analog sites [Banin 2005]. The capabilities of SCWE are not limited to the extraction and analysis of primary amines and it has been demonstrated as a front-end extraction method for analytical determination of polycyclic aromatic hydrocarbons (PAHs), fatty acids, and other more complex biomarker compounds [Becker 2006].
Diagenetic pathways may have acted to degrade organic compounds over time due to the oxidizing surface conditions on Mars [Benner 2000]. For this reason, it is paramount that technologies exist for highly specific quantification of the target biomolecular compounds at trace levels, equivalent to low parts-per-billion (ppb) or parts-per-trillion (ppt) sensitivity. Field studies carried out in 2005 as part of Urey instrument development efforts have shown that in extremely arid locations like the Atacama Desert, variations in biodensity are incredibly pronounced on both the macroscale and microscale [Skelley 2007]. If similar levels of biological heterogeneity were expected at one time on Mars, then it is probable that biosignatures could remain elusive during in situ investigation if instruments with inadequate sensitivity were utilized. Similarly, selection of a limited sample size could result in a null result for life detection during MSR missions and poses a high risk of ultimate failure.

The nitrogen-centric life detection approach allows for highly sensitive detection limits via laser induced fluorescence (LIF) detection of primary amines after derivatization using fluorophores as highly specific probes for extremely low-level quantification [Skelley & Mathies 2003]. After wet chemical extraction (e.g. SCWE), detection limits in the picomole (pM) to femtomolar (fM) range are achieved with μCE-LIF quantification [Chiesl 2009; Skelley 2005]. The techniques utilized for these high sensitivities are appropriate for all primary amines including protein fragments with a terminal amine group. The high performance of the Urey instrument was demonstrated under a NASA Goddard comparative instrument performance investigation of Mars analog mineralogical analogs in the Astrobiology Sample Analysis Program (ASAP) [Glavin 2009].

Still higher sensitivities can be achieved through concentration methods such as sublimation [Glavin 2001] or solid-phase extraction (SPE) concentration of an aqueous extract. The combination of non-destructive aqueous extraction methods (SCWE) combined with inline concentration and LIF detection allows for the most sensitive methods to probe trace amounts of organics during in situ investigations. These methods allow for sub-ppb sensitivities that correspond to ~10^3 cell equivalents per one gram of soil. Urey also includes the Mars Oxidant Instrument (MOI) for the detection of soil and atmospheric oxidants in the same sample in which organic biomarker concentration was analyzed. In this way the oxidative chemical context can be correlated with biomarker levels found.

**CONCLUSION**

The contents of this paper closely align with the objectives set forth in the 2008 Astrobiology Roadmap [Des Marais 2008], specifically: **Objective 2.1 – The Detection of Life on Mars**, and **Objective 7.2 – The Search for Biosignatures in Nearby Planetary Systems**. Westall [2000a] has argued that the search for life must “be based on a coordinated strategy to search for visible, biochemical and geochemical signs of life in a suitable environment”. In this white paper, we address the biogeochemical perspective and scientific rational behind a Follow the Nitrogen strategy towards organic and biomarker detection. The ability to chemically resolve biosignatures using primary amine distributions and chirality at high sensitivity could enhance our knowledge of the range of conditions suitable for prebiotic synthesis and perhaps allow for the first detection of organics on Mars – possibly evidence of a second genesis. Extremely high sensitivity is necessary due to the harsh environmental conditions on Mars and the fact that only the top few meters of regolith will be accessible. Drilling to the greatest depth possible will allow for the greatest chance of success of detecting organics and potential biosignatures on Mars [Kminek & Bada 2006]. The 2011 MSL includes capabilities to drill centimeters deep within surface rocks while ExoMars, set to launch in 2018, includes a sample drill that will provide capability to probe up to ~2 meters depth within the regolith. Instruments most well suited for investigation of organics on Mars that follow this strategy include the Urey instrument for organic and oxidant detection [Aubrey 2008].
References