MARTIAN SUB-SURFACE DETECTION OF EARTH-LIKE BACTERIAL SPORES M. L. Cable[1], J. P. Nosanov[2], R. B. Amini, B. P. Trease and A. Ponce, Jet Propulsion Laboratory, California Institute of Technology, 4800 Oak Grove Drive, Pasadena CA 91109, morgan.l.cable@jpl.nasa.gov[1], jeffrey.p.nosanov@jpl.nasa.gov[2], adrian.ponce@jpl.nasa.gov[3]

Introduction: Confirmation of extra-terrestrial life remains a lofty goal for our civilization. Past attempts such as the Viking life detection experiments revealed tantalizing suggestions of extant life on Mars. As a result, the thriving astrobiology community remains polarized regarding whether the Viking landers detected life. We propose to help answer this question by developing a fluorescence instrument package to detect Earth-like bacterial spores beneath the Martian surface.

The wealth of information gained about Mars since the Viking era significantly informs present and future efforts to detect life. For example, the recent discovery regarding ice present near the surface of the mid-latitude regions [1] offers an exciting medium in which to probe in the mid- to long-term (2024-2030).

Though other missions have searched for evidence of water or a "habitable zone" on Mars, the only prior in-situ investigation to specifically target life was that of the two Viking Landers. The Biology Experiments (Pyrolytic Release, Labeled Release and Gas Exchange) involved the addition of ^{14}C-labeled gases and nutrients to Mars soil samples and analysis of organics or gases released via gas chromatography with mass spectrometry and thermal conductivity detection [2].

These approaches were limited by instrument sensitivity and yielded internally contradictory results [3], giving rise to decades of debate and speculation. Further, the recent discovery of perchlorate in the Martian regolith by the Mars Phoenix Lander [4] calls into question any analysis technique where organic material could be destroyed by oxidation prior to reaching the detector. However, very recent work using complexity analysis of the Viking Labeled Release experiments suggests a biological origin for the Labeled Release results [5]. Our knowledge has increased sufficiently since the Viking era to justify another attempt to discover life on Mars.

Current proposed approaches to search for evidence of life on Mars target biomolecules such as amino acids or nucleobases of DNA [6,7]. However, detection of amino acids alone would not conclusively indicate the existence of life. Chiral discrimination is necessary to rule out abiotic sources [8]. Thus far, no in situ technique can, with sufficient sensitivity, detect the L- and D-enantiomers of all 20 amino acids required for life as we know it. An alternate approach is detection of extinct life via DNA detection. Detection of DNA or nucleobases on Mars would be strong evidence for life, though current detection technologies are not sufficiently mature for implementation in an situ instrument.

Bacterial spore detection, however, *can conclusively demonstrate the existence of life as we know it* on Mars. Bacterial spores, also known as endospores, are the toughest form of life known to exist on Earth [9]. These bacteria form a tough spore coat to protect their genetic material in times of stress, and in doing so gain resistance to UV radiation, dessication, pressure and temperature extremes, and the vacuum of space [10-13]. Bacterial spores can remain dormant with no detectable metabolism for potentially millions of years [14-17], and could survive an interplanetary journey [18,19]. Therefore, if we are to discover life as we know it on Mars, it is probable that such life would be in the form of bacterial spores.

Bacterial spores contain ~10^8 molecules of dipicolinic acid (DPA), a unique biomarker [20,21]. Detection of high concentration DPA on Mars would therefore be *direct evidence of life*, specifically of bacterial spores.

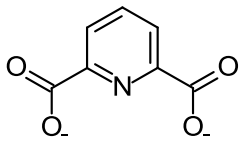

Fig. 1. Chemical structure of dipicolinic acid (DPA)

Method: Detection of DPA is readily performed using a terbium luminescence assay [22]. Terbium is a poor fluorophore alone. However, terbium generates a highly fluorescent complex upon exposure to DPA. This enables not only detection of bacterial spores in a sample, but *quantification*, with limits of detection of ~1000 spores per milliliter of concentrate [23-25].

A terbium luminescence assay for bacterial spores has been proposed previously for Mars, and involved a fluorescence microscopy-based technique to discriminate between 'germinable' and 'ungerminable' spores and determine viability [26]. Unlike that approach, the new approach proposed here could be implemented in an in-situ instrument requiring only a limitied amount of new hardware. Many of the necessary elements and subsystems (heaters, pumps, liquid reservoirs, UV LEDs, etc.) have flown on previous missions. The investigation approach is as follows:

1. An ice sample, filtered or allowed to sublimate, would concentrate bacterial spores while leaving them intact (Fig. 2).

2. Bacterial spore lysis would be effected with heat or acid treatment to release the biomarker (DPA).
3. Addition of a terbium solution and exposure to UV light would allow for rapid quantification of bacterial spores in the sample via luminescence detection using a photomultiplier tube (PMT) or CCD detector.

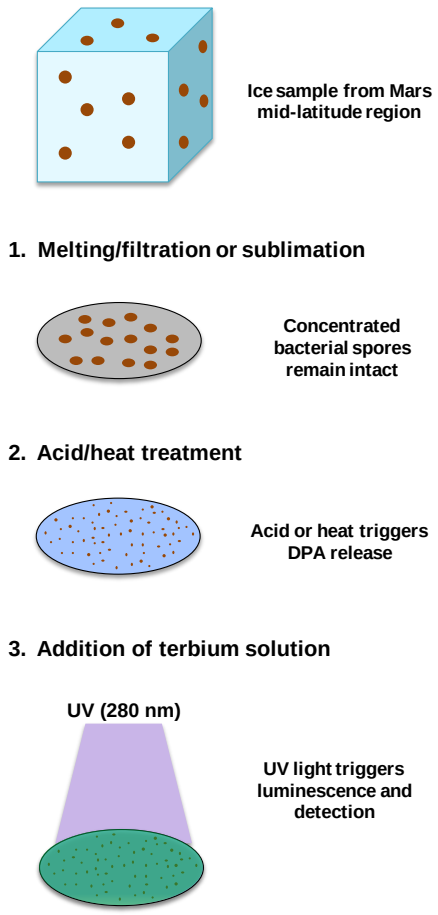

Fig. 2. Schematic of experimental design

Due to the fact that terbium luminescence is long-lived (0.6-2.0 ms), interference from other fluorescent species and minerals with much shorter lifetimes (ps to ns) could be avoided using time-gated detection [24].

This investigation could be included as part of a fluorescence instrument package of a dedicated mission using a high-heritage lander system such as the Mars Phoenix lander or its recently-proposed descendent, the InSight lander. InSight would include a probe capable of penetrating the Martian regolith to a depth of five meters. Performing bacterial spore analysis to that depth would be a wholly different environment than the Viking lander life detection experiments, and a depth profile would validate the assay against the possibility of contamination.

Detection of bacterial spores using this assay is straightforward and capable of leveraging hardware with flight heritage. *A positive result for high concentration DPA in micron sized spots on Mars would be the first direct evidence of life on another body*, and could inform future missions using non-Earth-centric techniques to investigate other possible forms of life. Further, this investigation could be included as part of a more comprehensive instrument for general fluorescence studies, expanding the science return to include mineralogical analyses as well as life detection.

Investigation of Martian life can (1) answer fundamental questions about life itself, (2) inform future human exploration of Mars by investigating the biological hazards of a potential landing site, (3) inform future sample return missions by investigating a potential sample site, (4) continue the work of current Mars missions such as MER and MSL, and (5) reinvigorate the public in and increase congressional support of Mars exploration. Such knowledge would revolutionize our view of the Red Planet and greatly inform future manned and unmanned missions to this new frontier.

References: [1] Byrne S. et al. (2009) *Science, 325*, 1674-76. [2] Klein H. P. et al. (1976) *Nature, 5563*, 24-7. [3] Klein H. P. (1977) *JGR, 82*, 4677-80. [4] Hecht M. H. et al. (2009) *Science, 325*, 64-7. [5] Bianciardi G. et al. (2012) *IJASS, 13*, 14-26. [6] Skelley A. M. et al. (2005) *PNAS, 102*, 1041-6. [7] Skelley A. M. et al. (2006) *Astrobiology, 6*, 824-37. [8] McKay C. P. (1997) *Orig Life Evol B, 27*, 263-89. [9] Roberts T. A. and Hitchens A. D. (1969) in *The Bacterial Spore*; Gould G. W. and Hurst A. Eds, Academic Press, 611-70. [10] Byrne A. F. et al. (1960) *J Bacteriol, 80*, 139-40. [11] Yung P. T. and Ponce A. (2008) *Appl Env Microbiol, 74*, 7669-74. [12] Bisset K. A. (1950) *Nature, 166*, 431-2. [13] Nicholson W. L. et al. (2000) *Microbiol Mol Biol Rev, 64*, 548-72. [14] Church B. D. and Halverson H. (1959) *Nature, 183*, 124-5. [15] Berg P. E. and Greez N. (1970) *J Bacteriol, 103*, 517-9. [16] Cano R. J. and Borucki M. K. (1995) *Science, 268*, 1060-4. [17] Vreeland R. H. et al. (2000) *Nature, 407*, 897-900. [18] Nicholson W. L. (2003) *Orig Life Evol B, 33*, 621-31. [19] Shafaat H. S. and Ponce A. (2006) *Appl Env Microbio, 72*, 6808-14. [20] Gould G. W. and Hurst A. (1969) *The Bacterial Spore*. [21] Gerhardt P. and Marquis R. E. (1989) in *Regulation of prokaryotic development*; Smith I. et al. Eds., American Society for Microbiology, 43-63. [22] Rosen D. L. et al. (1999) *Rev Anal Chem, 18*, 1-21. [23] Pellegrino P. M. et al. (1998) *Anal Chem, 70*, 1755-60. [24] Cable M. L. et al. (2007) *JACS, 129*, 1474-75. [25] Yung P. T. et al. (2007) *Biotech Bioeng, 98*, 864-71. [26] A. Ponce et al. (2008), *ASTID*, Biodosimetry Instrument for Monitoring Microbial Survival Beyond the Magnetosphere.