THE BIOMOLECULE SEQUENCER PROJECT: NANOPORE SEQUENCING AS A DUAL-USE TOOL FOR CREW HEALTH AND ASTROBIOLOGY INVESTIGATIONS. K. K. John[1], D. J. Botkin[2], A. S. Burton[3] (aaron.burton@nasa.gov), S. L. Castro-Wallace[4], J. D. Chaput[5], J. P. Dworkin[6], N. Lehman[7], M. L. Lupisella[8], C. E. Mason[9], D. J. Smith[10], S. Stahl[11]. C. Switzer[12]. [1]NASA Postdoctoral Program, NASA Johnson Space Center (JSC), Houston, TX, [2]Formerly JES Tech, Houston, TX, [3]Astromaterials Research and Exploration Science Division, NASA JSC, Houston, TX [4]Biomedical Research and Environmental Sciences Division, NASA JSC, Houston, TX, [5]Department of Pharmaceutical Sciences, University of California-Irvine, Irvine, CA, [6]Solar System Exploration Division, NASA Goddard Space Flight Center (GSFC), Greenbelt, MD, [7]Department of Chemistry, Portland State University, Portland, OR, [8]Exploration Systems Projects Office, NASA GSFC, Greenbelt, MD, [9]Department of Physiology and Biophysics, Weill Cornell Medical College, New York, NY, [10]Space Biosciences Division, NASA Ames Research Center, Mountain View, CA, [11]Wyle / NASA JSC, Houston, TX, [12]Department of Chemistry, University of California-Riverside, Riverside, CA.

Introduction: Human missions to Mars will fundamentally transform how the planet is explored, enabling new scientific discoveries through more sophisticated sample acquisition and processing than can currently be implemented in robotic exploration. The presence of humans also poses new challenges, including ensuring astronaut safety and health and monitoring contamination. Because the capability to transfer materials to Earth will be extremely limited, there is a strong need for *in situ* diagnostic capabilities.

Nucleotide sequencing is a particularly powerful tool because it can be used to: (1) mitigate microbial risks to crew by allowing identification of microbes in water, in air, and on surfaces; (2) identify optimal treatment strategies for infections that arise in crew members; and (3) track how crew members, microbes, and mission-relevant organisms (*e.g.*, farmed plants) respond to conditions on Mars though transcriptomic and genomic changes. Sequencing would also offer benefits for science investigations occurring on the surface of Mars by permitting identification of Earth-derived contamination in samples. If Mars contains indigenous life, and that life is based on nucleic acids or other closely related molecules, sequencing would serve as a critical tool for the characterization of those molecules. Therefore, spaceflight-compatible nucleic acid sequencing would be an important capability for both crew health and astrobiology exploration.

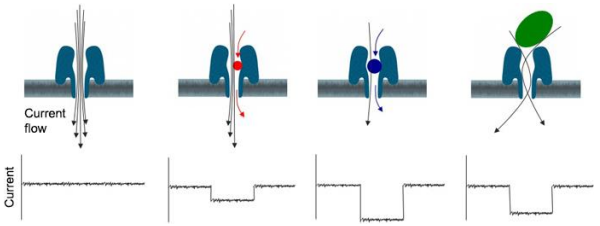

Figure 1. Nanopore-based sequencers measure changes in current caused by DNA strands passing through the pore. The changes in current are diagnostic of the sequence of DNA passing through the pore.

Advances in sequencing technology on Earth have been driven largely by needs for higher throughput and read accuracy. Although some reduction in size has been achieved, nearly all commercially available sequencers are not compatible with spaceflight due to size, power, and operational requirements. Exceptions are nanopore-based sequencers that measure changes in current caused by DNA passing through pores; these devices are inherently much smaller and require significantly less power than sequencers using other detection methods. Consequently, nanopore-based sequencers could be made flight-ready with only minimal modifications.

Figure 2. The MinION™ device (circled) sits atop the Illumina MiSeq sequencer. The MinION™ device is a nanopore sequencer that weighs less than 150 g, and occupies a volume of less than 100 cm^3.

Biomolecule Sequencer Project: As a first step towards evaluating the suitability of nanopore-based sequencing as a tool for human exploration, the Biomolecule Sequencer project [1] will test the commercially available MinION™ device (Oxford Nanopore Technologies) aboard the International Space Station (Figure 2). The initial flight test of the MinION™ will evaluate how the device performs after

flight to the ISS and under continuous microgravity conditions. The samples to be sequenced, containing lambda bacteriophage, *Escherichia coli* and mouse genomic DNA, will be prepared for sequencing on the ground and stored frozen until they are analyzed in-flight in parallel with ground control samples. Immediately prior to sequencing, the crew member will thaw a frozen sample, inject the thawed sample into a new flow cell, and initiate sequencing. After ~5 GB of sequence data have been acquired, sequencing will be terminated and the data downlinked to Earth for analysis. The concept of operations, from sample preparation to termination of sequencing, was successfully tested aboard a parabolic flight [2]. Certification of the flight hardware is expected to be completed by the end of March 2016. The Biomolecule Sequencer payload is expected to be launched in June 2016, and in-flight testing is expected to be completed by the end of 2016. Should in-flight sequencing prove feasible, the next milestone will be demonstrating end-to-end in-flight sequencing, from sample collection to data analysis.

Sequencing as a tool for astrobiology: Nucleic acid sequencing is well-known as a versatile tool for Astrobiology. Sequencing can place extremophiles in evolutionary context [e.g., 3-5], provide insights into the origin and evolution of the ribosome itself [e.g., 6, 7], and give key information regarding organismal metabolism [e.g., 8]. Sequence data have been successfully obtained from samples including: DNA from bones that were hundreds of thousands of years old [9,10]; insects trapped in amber over 100 million years old [11]; and archaea entrained in halite crystals over 400 million years old [12]. This remarkable preservation potential for DNA coupled with the amount of organismal information that can be obtained from even partial genomic sequences, provides strong support for the inclusion of nucleic acid sequencing as an important component of a life detection mission.

However, a major limitation of traditional DNA or RNA sequencing methods for life detection elsewhere in the solar system is that they can sequence only DNA or RNA. If life existed elsewhere but used informational molecules other than DNA or RNA, conventional sequencers would not be able to obtain sequence data, even from closely related nucleic acid analogs such as arabinose or threose nucleic acids (Figure 3). Because nanopore based sequencers such as the the MinION™ measure changes in current based on the polymer passing through them, they can also read RNA, proteins, and other polymers as well [13]. Thus, nanopore-based sequencing provides much greater versatility as a life detection tool than would sequencers that require nucleic acid synthesis or other detection methods.

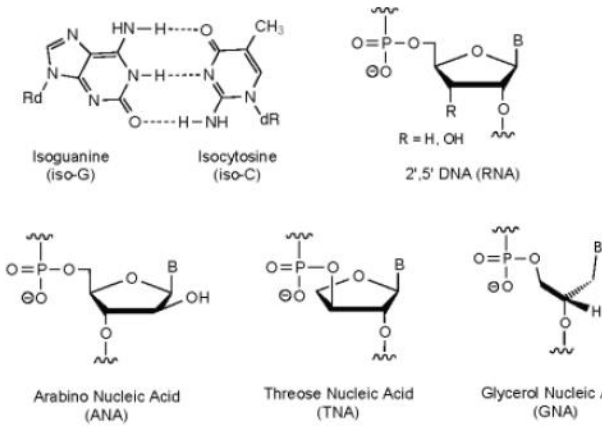

Figure 3. Examples of nucleic acid-like molecules that could support life. Nanopore-based sequencers have the potential to detect these kinds of molecules, whereas sequencers that require synthesis or incorporation of chromophores would not.

References:

[1] "Sequencing DNA in the palm of your hand"; http://www.nasa.gov/mission_pages/station/research/news/biomolecule_sequencer; September 29, 2015 [2] McIntyre et al., Nanopore Sequencing in Microgravity, bioRxiv: http://dx.doi.org/10.1101/032342 [3] Ciccarelli F. D. et al. (2006) *Science* 311, 1283-1287. [4] Puigbò P. et al. (2009) *J. Biol.* 8, 59 – 59. [5] Wolf Y. I. et al. (2002) *Trends Gen.* 18, 472-479. [6] Fox G. E. (2010) *Cold Spr. Harb. Persp. Biol.* 2, a003483 [7] Petrov A. S. (2014) *Proc. Natl. Acad. Sci. USA* 111, 10251-10256. [8] Oren A. (2008) *Sal. Syst.* 4, 2-2. [9] Dabney J. et al. (2013) *Proc. Natl. Acad. Sci USA* 110, 15758-15763. [10] Orlando L. et al. (2013) *Nature* 499, 74-78. [11] Cano R. J. et al. (1993) *Nature* 363, 536-538. [12] Park J. S. et al. (2009) *Geobiology* 7, 515-523. [13] Oukhaled A. et al. (2012) *ACS Chem. Biol.* 7, 1935-1949.