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Toward biotechnology in space: High-throughput instruments for *in situ* biological research beyond Earth

Fathi Karouia[a,b,c], Kianoosh Peyvan[d], Andrew Pohorille[a,b,*]

[a] University of California San Francisco, Department of Pharmaceutical Chemistry, San Francisco, CA 94158, USA
[b] NASA Ames Research Center, Exobiology Branch, MS239-4, Moffett Field, CA 94035, USA
[c] NASA Ames Research Center, Flight Systems Implementation Branch, Moffett Field, CA 94035, USA
[d] Peyvan Systems Inc., Seattle, WA 98119, USA

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ABSTRACT

Space biotechnology is a nascent field aimed at applying tools of modern biology to advance our goals in space exploration. These advances rely on our ability to exploit *in situ* high throughput techniques for amplification and sequencing DNA, and measuring levels of RNA transcripts, proteins and metabolites in a cell. These techniques, collectively known as "omics" techniques have already revolutionized terrestrial biology. A number of on-going efforts are aimed at developing instruments to carry out "omics" research in space, in particular on board the International Space Station and small satellites. For space applications these instruments require substantial and creative reengineering that includes automation, miniaturization and ensuring that the device is resistant to conditions in space and works independently of the direction of the gravity vector. Different paths taken to meet these requirements for different "omics" instruments are the subjects of this review. The advantages and disadvantages of these instruments and technological solutions and their level of readiness for deployment in space are discussed. Considering that effects of space environments on terrestrial organisms appear to be global, it is argued that high throughput instruments are essential to advance (1) biomedical and physiological studies to control and reduce space-related stressors on living systems, (2) application of biology to life support and *in situ* resource utilization, (3) planetary protection, and (4) basic research about the limits on life in space. It is also argued that carrying out measurements *in situ* provides considerable advantages over the traditional space biology paradigm that relies on post-flight data analysis.

1. Introduction

Biotechnology is a major scientific, technological and economic driver that holds promise not only to change permanently our lives on the Earth, but also to advance, and perhaps even facilitate, long-term human exploration of space. To apply biotechnology in space we have to meet a number of challenges, both scientific and technical, not encountered in terrestrial setting. Terrestrial organisms away from Earth confront hostile environments characterized by multiple stresses. Some of these stresses, such as markedly reduced gravity or energetic, charged particles are unfamiliar, nearly impossible to protect against and unreliably reproduced on the ground. Other, such as very low pressures, temperature variations, high levels of UV radiation, desiccation or nutritional deprivation, although encountered in some terrestrial ecosystems, are often more extreme in space. To survive and thrive in space, living systems must cope with all these stress factors simultaneously. To what extent and how they are able to do so are central questions in space biology research. These questions are both profound and difficult to answer because they involve a combination of desired biological traits that mostly have not been a subject of natural

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Abbreviations: cDNA, Complementary DNA; CE-MS, Capillary Electrophoresis Mass Spectrometry; DMR, Diagnostic Magnetic Resonance; DNA, Deoxyribonucleic acid; ECD, Electrochemical readout; ESI-MS, Electrospray Ionization Mass Spectrometry; GEMM, Gene Expression Measurement Module; ISS, International Space Station; LC-MS, Liquid Chromatography Mass Spectrometry; LEO, Low Earth Orbit; LLMDA, Lawrence Livermore Microbial Detection Array; LLM, Light Microscope Module; MALDI-MS, Matrix Assisted Laser Desorption Ionization Mass Spectrometry; mRNA, messenger RNA; MS, Mass Spectrometry; NASA, National Aeronautics and Space Administration; NMR, Nuclear Magnetic Resonance; μNMR, Miniaturized Nuclear Magnetic Resonance; O/OREOS, Organism/Organic Exposure to Orbital Stresses; PCR, Polymerase Chain Reaction; PCR/MA, PCR and DNA melting analysis; RNA, Ribonucleic acid; RT-PCR, Reverse Transcription Polymerase Chain Reaction; RT-qPCR, Real Time quantitative Polymerase Chain Reaction; SAU, Sample Analysis Unit; SETG, Search for Extra-Terrestrial Genomes; SOLID, Sign Of Life Detector; SPU, Sample Preparation Unit; STS, Space Shuttle mission; TRL, Technology Readiness Level; UPLC, Ultra Performance Liquid Chromatography

[*] Corresponding author at: NASA Ames Research Center, Exobiology Branch, MS239-4, Moffett Field, CA 94035, USA

E-mail addresses: fathi.karouia@nasa.gov (F. Karouia), kia@peyvansystems.com (K. Peyvan), andrew.pohorille@nasa.gov (A. Pohorille).

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selection on the Earth. Therefore, clues to engineering or reinforcing them that can be obtained from modern organisms or evolutionary studies are only very limited. Progress in this area is inextricably connected with our ability to explore space permanently and safely.

To control effects of space-related stressors on living systems one needs to understand how these stressors impact organisms at the cellular and molecular level. In the half-century of space exploration, multiple lines of evidence have accumulated to state with near-certainty that effects of space environments are not limited to a small number of genes or a single subcellular component, but instead influence many gene products and cell functions (Cervantes and Hong, 2016; Fernandez-Gonzalo et al., 2017; Foster et al., 2014; Najrana and Sanchez-Esteban, 2016; Taylor, 2015). These diverse effects can be understood only by taking a global, integrative approach that parallels an approach used to deal with consequences of terrestrial stresses, such as environmental pressures or states of disease (Buescher and Driggers, 2016; Jozefczuk et al., 2010; Nielsen, 2017). The approach relies heavily on developing, cost-effective techniques for monitoring the identity and activity of genes, proteins and metabolites in organisms or their consortia, and interpreting them in terms of global, complex interactions within biological systems (Mousavian et al., 2015; Pulido et al., 2015). These techniques belong respectively to research areas of genomics, transcriptomics, proteomics and metabolomics, collectively known as “omics”. Even though “omics” approaches are relatively new, they have already produced many important insights to biology and medicine (Egea et al., 2014; Li et al., 2016; Schmidt and Goodwin, 2013).

This review is focused on high-throughput instruments of “omics” that hold potential to advance qualitatively research in space biology. Even though such instruments are indispensable for basic science and biotechnology they have not been yet permanently used in spaceflight. In large part, this is because deploying “omics” tools onboard spacecraft, even those that are based on mature technologies, such as Polymerase Chain Reaction (PCR) and measurements of gene expression, poses significant technical difficulties and might require substantial reengineering of their ground-based counterparts. The purpose of such effort is to meet the needs for miniaturization, automation, compatibility of protocols and materials with spacecraft or space habitats, reliability in the absence of gravity, and, at least in some cases, ruggedness and low power. This might appear to be a complex, time consuming and costly task. However, as we will argue in this review, with sufficient programmatic commitment and leveraging commercial partnerships, this task can be accomplished at reasonable costs in the next 2–3 years.

In the next section, we briefly discuss selected space biology research that has benefitted or will benefit from the application of “omics” tools. Subsequently, we review high-throughput ‘omics” technologies. We focus on information that these technologies provide, their advantages and disadvantages, and potential adaptations that might be required for space applications. This section may be particularly useful to experts in space exploration who are interested in the potential of biotechnology to advance this endeavor. Next, on-going efforts to develop flight instruments capable of carrying out “omics” measurements are described in detail. Since these instruments are not stand-alone devices, we also discuss supplementary capabilities needed to create “biological laboratories in space”. We close with conclusions and outlook for future investigations. In particular, we discuss why and under what circumstances “omics” analysis should be carried out *in situ* rather than post-flight in ground based laboratories.

2. Biological research in space

Biological research in space has a long history, the full account of which is beyond the scope of this review (Barratt and Baker, 2017; Nickerson et al., 2016; Nicogossian et al., 2016). However, in order to appreciate the type of investigations that are currently being conducted in space, it is important to review briefly the uniqueness of the space environment and how it impacts biological systems. Subsequently, we include here a few representative examples of studies that are closely related to biotechnology. Informally, this research can be divided into four areas: (1) biomedical and physiological studies, which mainly deal with effects of space on humans and animal models, (2) research on biological systems that support exploration, primarily in the areas of life support and *in situ* resource utilization, (3) planetary protection, and (4) astrobiological studies aimed at explaining how organisms exposed to space environments respond to stresses.

2.1. *The space environment*

The space environment is characterized by four key parameters: neutral gas density (near vacuum), extreme temperature variations, weightlessness, and energetic charged particles. The last one of a crucial limiting factor for long duration human space exploration missions, and especially for missions to Mars (Jones et al., 2017a; Jones et al., 2017b).

Space radiation sources consist of a variety of particles that have a wide range of energies and both temporal and spatial variations (Jones et al., 2017b). For practical purposes, the space radiation environment can be considered in two distinct categories: Low Earth Orbit (LEO) and deep space. For missions in LEO, such as those on the Space Shuttle, Mir, and now the International Space Station (ISS), the two main sources of radiation exposure are galactic cosmic rays and bands of geomagnetically trapped particles (the Van Allen belts) consisting of mostly protons and electrons. On average, the skin dose received by an astronaut per day is equivalent, depending of the solar cycle, to 5 to 10 times the exposure from a typical chest X-ray (Jones et al., 2017b). Earth's geomagnetic field lines protect the planetary surface from incident cosmic and solar radiation by deflecting a fraction of charged particles, but high-energy transient particles, in addition to the trapped particles from the Van Allen belts, remain to create local hazard for LEO missions. In deep space missions that extend beyond the relative protection of the geomagnetic fields, such as lunar or interplanetary space flights, the primary sources of exposure are galactic cosmic radiation and solar particle events. The projected dose received during a Mars mission (6 months of travel each way and 2 years of surface stay) will result in the total cumulative dose equivalent close to 1 Sv, which corresponds to the astronaut career limits (Hassler et al., 2014; Zeitlin et al., 2013). It has been shown that radiation increases risks of carcinogenesis, acute in-flight and late risks to the central nervous system (impairments in mental function, motor coordination, and strength), degenerative risk such as cardiovascular, and acute radiation syndromes (Jones et al., 2017a; Jones et al., 2017b). Secondary radiation can also be produced when the primary particles interact with the materials of the spacecraft or the constituents of the rarefied upper atmosphere in LEO.

Weightlessness also has an important impact on living species. In practice, perfect weightless conditions are impossible to attain due to disturbances from drag, vibrations, *etc*., and so the term microgravity is used to describe the actual conditions. The suppression of gravitational force, or its strong reduction, is responsible for the following main consequences 1) no hydrostatic pressure, 2) no weight, 3) no sedimentation, and 4) no natural convection (Monti and Savino, 1999; Nickerson et al., 2016).

Other space related parameters that significantly impact life are pressure and temperature (McKay, 2014). These parameters are intrinsically dependent of locations within the solar system. In LEO, pressures vary from 10^{-7} to 10^{-4} Pa and the temperature directly outside the ISS varies from -120 to $+120$ °C as a function to direct exposure to the sun (Horneck et al., 2010). On Mars, data received from rovers and orbiters indicate that temperatures vary from 20 °C (noon, equator, summer) to -153 °C (poles) and pressure is over 100 times lower than on Earth (Moissl-Eichinger et al., 2016b). In general