SYNTHETIC BIOLOGY, ASTROBIOLOGY AND THE SEARCH FOR LIFE IN THE UNIVERSE. L. J. Rothschild[1,2], K. Fujishima[3], J. W. Head[2], and I. Paulino-Lima[4]. [1]NASA Ames Research Center, Moffett Field, CA, 94035, USA, Lynn.J.Rothschild@nasa.gov, [2]Brown University, Providence, RI, 02912, USA, James_Head@Brown.edu, [3]Earth-Life Science Institute, Tokyo Institute of Technology 2-12-1- IE-31, Ookayama, Meguro-ku Tokyo 152-8550 Japan, fuji@elsi.jp, 4 Blue Marble Space Institute of Science at NASA Ames Research Center, Moffett Field, CA, 94035, USA, ivan@bmsis.org.

Introduction: Synthetic biology – the design and construction of new biological parts and systems and the redesign of existing ones for useful purposes – has the potential to transform fields from pharmaceuticals to fuels. Our lab has focused on the potential of synthetic biology to revolutionize all three major parts of astrobiology: Where do we come from? Where are we going? and Are we alone?

For the first and third, synthetic biology is allowing us to answer whether the evolutionary narrative that has played out on planet Earth is likely to have been unique or universal. Whereas elucidating the evolutionary history of life on Earth gives us a definitive narrative of what has happened, synthetic biology is what we call, "the art of the possible", the ability to computationally and experimentally test alternatives. This is critical in astrobiology, as it is inconceivable that the origin and evolution of life would occur twice identical in all details due to variations in the chemical and physical environment and biological systems. Thus, what we know about evolution on Earth is the baseline from which synthetic biology allows us to explore what we as yet don't know.

Where do we come from? The evolution of amino acids: As an example of the use of synthetic biology to fill gaps in our knowledge about evolution on planet Earth, we have focused on understanding the evolution of amino acid usage **[1]**. A diversity of amino acids has been found on meteorites and other prebiotic settings including Miller-Urey style simulations, but these are not consistent with the amino acids used by living organisms today. Is this lack of overlap a question of availability? Or chirality, as all ribosome-produced proteins are homochiral, as are the other major biopolymers, DNA and RNA? Some amino acids used today by living organisms are not found in prebiotic settings at all [2]. Worse, the enzymes used to biosynthesize these amino acids may contain the very amino acids that they synthesize. For example, *Escherichia coli* produces cysteine from serine via two enzymes that contain cysteine: serine acetyltransferase (CysE) and O-acetylserine sulfhydrylase (CysK/ CysM). To solve this problem, we substituted alternate naturally-occurring amino acids in CysE, CysK and CysM for cysteine and methionine, which are the only two sulfur-containing proteinogenic amino acids. These substitutions resulted in functioning enzymes, thus providing a possible solution to this "chicken-and-egg" problem.

Exploration of the range of environments in the Solar System and beyond will help to define the range of unanticipated physical and chemical environments and processes associated with habitable environments

Where are we going? Synthetic biology in support of exploration: In the future synthetic biology will play an increasing role in human activities on Earth in fields as diverse as human health and the industrial production of chemicals, materials and novel bio-composites, to metal recovery and nanotechnology. Life as a technology solves so many of the problems of human exploration off planet, starting with the fact that life is programmable, self-replicating and self-repairing and can create a vast array of compounds with inputs found in many planetary bodies. Beyond Earth, we will rely increasingly on biologically-provided life support, as we have throughout our evolutionary history [e.g., 3], with new manufacturing platforms adapted to off-planet production [4].

Our lab has pioneered a range of projects, on the basis of developing synthetic biology-based techniques. For example, metal recovery off planet can involve using naturally-occurring or genetically enhanced organisms to leach or bind metals. We have developed a method where metal-binding peptides are expressed on the surface of organisms which then provide a substrate for highly specific metal extraction [5]. We developing an on-demand astropharmacy [6], where bacteria are pre-programmed to secrete selected biologics on demand and produce doses of medication within 24 hours in space. Wood provides an excellent building compound on Earth, but tree farming is impractical in space. Why not turn to the production of building materials derived from fungal mycelia, so-called "mycotecture"? [7]. We work with planetary geoscientists to define and meet the challenges of future human and robotic exploration.

Are we alone? Using synthetic biology to assess the niche space for life: When searching for something, knowing the target is normally a given. Once again we turn to synthetic biology to help understand what other life forms might be like. Can we create organisms that expand the envelope, and possibly even the definition of life? The ability to withstand different environmental variables such as temperature, pH, etc. are shown in

Rothschild & Mancinelli [8] and in the Astrobiology Strategy 2015 [9] Table 3.1. The approximate recorded environmental parameters for metabolic activity are: temperature -20 to 121 °C, pH 0-12, radiation 6000 Gy, UV radiation 5,000 J/m^2, pressure 1100 bars, salinity up to 5.1 M, and desiccation ~60% relative humidity. But something is missing from this approach of looking at the limits for life on Earth as a "Guinness World Records". What good does it do if we know an organism can survive one extreme if it turns out that it cannot survive in combination with other environments that define the extra-terrestrial niche space? For this reason, we searched the literature to define this niche space for life on Earth [9], and we work with planetary geoscientists to define new parameter spaces for possible life off-planet.

The best-studied individual environmental extremes are temperature, pH, salinity, pressure, radiation, desiccation and oxygen stress, but studies that examined multiple stressors in a regulated way were rare. Table 2 [9] is a compilation of temperature and pH growth ranges and optima for over 200 species. From these data, there appears to be a gap when low temperature and either very high or low pH values are combined. Are the gaps highlighting of an artifact of sampling? Or are they indicative of a biological reality? If the latter, does it reflect a lack of environments on present-day Earth, and thus lack of selection pressure, or is there something about the combination of abiotic factors that precludes life? If it is lack of selection pressure, these gaps could potentially be filled by creating synthetic extremophiles (what the 2012 Stanford-Brown iGEM team under our direction called "The Hell Cell Project" [10]), using synthetic biology, directed evolution or both.

But what if there is something about some combinations of environmental factors, that is some abiotic niches, that preclude life as we know it, or even coax it? This would lower the chance that these niches would be the right targets for the search for life without a radically different biochemistry. This would also say something interesting about evolution, and point to studies of the underlying mechanisms of adaptation and evolution to new environments. Exploration of the very wide range of conditions in the Solar System and exoplanetary systems beyond will drive us to becoming less "terracentric" and "acosmic" in our thinking.

As we move forward, efforts are growing to construct cells from biological or abiological components to either mimic natural systems or create new systems. Several consortia exist, including Build-a-Cell, based within the USA, and other international efforts such as BaSyC In the Netherlands

Future prospects: The field of synthetic biology is moving rapidly on an international level, and within the USA has been prioritized by the last several administrations. We anticipate being able to leverage these enormous advances to NASA's needs, most specifically the three main foci of Astrobiology: Where do we come from? Where are we going? Are we alone?

NASA's exploration of inner space (challenges to human physiology in extreme environments; exploration of the full range of Earth environments) and outer space (the Solar System and the explosion of newly discovered exoplanets, soon to exponentially increase with the James Webb Space Telescope) provides the exploration framework for the future!

Acknowledgments: We are indebted to the pioneering projects of a series of iGEM teams in Rothschild's lab in the development of these project, especially Brown-Stanford 2011 (Synbio-enabled Mars Colony), Stanford-Brown 2012 (Astrobiology including life on Venus, Biomining and Hell Cell), Stanford-Brown-Spellman 2013 (BioWires), Stanford-Brown 2018 (Mycotecture) and Brown-Stanford-Princeton 2019 (Astropharmacy), and lab members from Rothschild and Head's groups for mentoring. Please see their wikis for further details. We also gratefully acknowledge support from NASA's NIAC program for biomining, mycotecture, bioprinting and astropharmacy projects, and STMD, Rhode Island, New Jersey and California Space Grant Consortium, and NASA Ames for support for the iGEM teams.

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