Life in the clouds of Venus? An experimental synthetic biology approach. L. J. Rothschild[1], I. G. Paulino-Lima and the 2012 Stanford-Brown iGEM team "Venus Life" team (D. Amatya[3], B. Bajar[3], B. Geilich[4], J. Hu[4], C. J. Jackson[3], [1]NASA Ames Research Center, Moffett Field, CA, USA, 94035-1000, Lynn.J.Rothschild@nasa.gov, [2]NPP Fellow at NASA Ames Research Center, Moffett Field, CA, USA, 94035-1000, [3]Stanford University, Stanford, CA, USA, 94305-2160, [4]Brown University, Providence, RI 02912, USA.

Introduction: The surface of Venus constitutes the most hellish and biologically inhospitable planetary surface in our solar system, boasting a pH of 0, blistering winds that can melt lead, and pressures of 60 atm. However, during the earlier years of the solar system, without the runaway greenhouse effect that has plagued the planet, Venus potentially housed oceans and perhaps even life. There is a possibility that microbes could have retreated into hospitable niches in the atmosphere, as suggested by Carl Sagan as early as 1967 [1]. For example, 50 km above the raging hell of the Venusian surface, exists a relatively temperate environment that might serve as reservoir for life.

This astrobiology project seeks to explore life at the extremes and to theorize whether microbial communities could not only survive but also reproduce in the Venusian atmosphere. Specifically, we ask: are aerosols viable microbial environments? But before we can test for life in the clouds, we have to develop a proper reporter to visualize cell growth *in situ*. For this purpose, we aimed to develop cell-growth dependent reporters to serve as remote biosensors for cell growth. We developed two using the polA promoter, a DNA-replication dependent promoter, and nrd operon promoter, a cell-cycle dependent promoter.

Using these cell-growth reporters, the next step is to aerosolize microbes expressing these reporters in a suspension chamber adapted from a Millikan Drop Apparatus to assay reproduction in an aerosolized environment. Better yet is to test the reproduction of microbes in a microgravity regime such as on ISS.

Approach: We engineered two cell-cycle dependent genetic reporters. One was the polA promoter which codes for DNA Polymerase I, a gene active in DNA replication [2]. The other was the nrdP. The activation of ribonucleotide reductase reduces ribonucleotides into deoxyribonucleotides and is involved in the bacterial cell cycle [3]. This promoter began activation during the initiation of DNA replication and is cell-cycle dependent [4]. These promoters were fused to a GFP reporter, transformed into *E. coli*. The constructs were deposited in the iGEM registry as

- K847210: *Escherichia coli* DNA-replication dependent polA promoter
- K847211: *Escherichia coli* cell-division dependent nrd promoter

Results: Our constructs displayed fluorescence when transformed into NEB-5alpha competent cells. While nrdP-E0840 displayed sufficient fluorescence as verified by fluorescent microscopy, the original polAP-E0840 construct (which uses mut3b GFP) exhibited low expression; while fluorescence was visible under the microscope, the signal was too weak for the camera to recognize. The polA promoter was therefore digested with EcoRI and SpeI then ligated into plasmid pNCS containing a RBS, Clover, and a terminator. Clover is a highly engineered green fluorescent protein that exhibits extreme brightness [5] Fluoresence time course data demonsrated that the genes were induced in a cell cycle dependant manner [6]. Our assays via microscopy and the bulk assay shows that our promoters are functional as cell cycle reporters.

Conclusions: The application of such tools are widespread and not limited to astrobiology; nrdP could be used to determine doubling times empirically and could possibly extrapolate DNA content from intensity of signals expressed by polAP. However, we are primarily interested in its use in astrobiology. We plan to use these promoters in reporters expressed in bacteria that we will aerosolize cells into a flight unit on ISS to test for cell division while suspended. The presence of similar oscillations in fluorescence that we observed in the bulk assay would suggest that bacteria can reproduce in aerosol and therefore inhabit a niche in the clouds. Thus, we suggest promoter tools like nrdP and polAP present a broad range of possibilities to researchers, especially in the new application of synthetic astrobiology.

References: [1] Morowitz, H., and Sagan, C. (1967) *Nature* 215, 1259-260. [2] Quiñones A., Wandt G., Kleinstauber S., Messer W. (1997) *Molecular Microbiology, 23*, 1193-1202. [3] Sun L., Jacobson B., Dien B., Srienc F., Fuchs J.(1994) J. Bacteriology 176, 2415-2426. [4] Sun L., Fuchs J (1992) *Molecular Biology of the Cell, 3*, 1095-1105. [5] Lam A., St-Pierre F., Gong Y., Marshall J.D., Cranfill P.J., Baird M.A., McKeown M.R., Wiedenmann J., Davidson M.W., Schnitzer M., Tsien R.Y., Lin M.Z. (2012) *Nature Methods*, *9*, 1005-1012. [6] (http://2012.igem.org/Team:Stanford-Brown/VenusLife /Biosensing)