# HASP Student Payload Application for 2015

| Payload Title: Organisms Near Space | | |
|---|---|---|
| Payload Class: (check one)<br>X Small ☐ Large | Institution:<br>Arizona State University | Submit Date:<br>12/18/14 |


Project Abstract

The Organisms Near Space (ORGANS) payload proposes a novel approach to expose the common spacecraft contaminant bacterial species, *Bacillus subtilis* and *Escherichia coli*, to the environment at 35km above sea level to determine the effects on microbial viability and mutation. The ORGANS payload will also collect temperature, pressure and UV radiation data. There will be a wild type strain and pressure and temperature sensitive mutant strain of each microbial species sent up on the payload. Once returned to sea level, the ORGANS team plans to analyze effects on microbial viability by attempting to culture the wild type microbial colonies on Luria Bertani (LB) media, an ideal media for bacterial growth, at 30° C. The results of this inoculation will be compared to control colonies also plated on LB media to determine if viability was influenced by the environment. The results of this study will serve to quantify the effects of the stratospheric environment on microorganisms, which can be applied to issues involving forward contamination of extraterrestrial environments and spacecraft sterilization.


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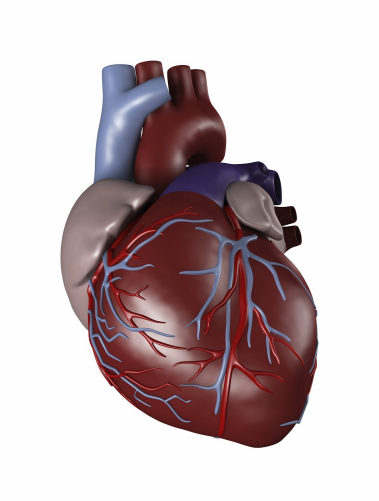
# ORGANS

**(ORGAnisms Near Space)**



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## Abstract

The Organisms Near Space (ORGANS) payload proposes a novel approach to expose the common spacecraft contaminant bacterial species, *Bacillus subtilis* and *Escherichia coli*, to the environment at 35km above sea level to determine the effects on microbial viability and mutation. The ORGANS payload will also collect temperature, pressure and UV radiation data. There will be a wild type strain and pressure and temperature sensitive mutant strain of each microbial species sent up on the payload. Once returned to sea level, the ORGANS team plans to analyze effects on microbial viability by attempting to culture the wild type microbial colonies on Luria Bertani (LB) media, an ideal media for bacterial growth, at 30° C. The results of this inoculation will be compared to control colonies also plated on LB media to determine if viability was influenced by the environment. The results of this study will serve to quantify the effects of the stratospheric environment on microorganisms, which can be applied to issues involving forward contamination of extraterrestrial environments and spacecraft sterilization.

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## Mission Overview

Recent discoveries of terrestrial planets in outer space and evidence of liquid environments even from within the solar system have extended the possibility of the existence of extraterrestrial life. In addition, studies of microorganisms existing and surviving in extreme environments have gradually expanded our definition what is habitable. Based on those studies, the possibility of finding extraterrestrial life or habitable planets has highly increased and missions toward environments deemed in any way hospitable for life (such as Europa) have become very important. However, when launching a spacecraft the possibility of  contamination of life forms from Earth must be addressed. Microorganisms on a spacecraft could potentially contaminate samples collected or act as invasive species in environments outside of Earth. Additionally, microorganisms could potentially mutate in a way that increases their survival rate when they are exposed to extreme conditions. Therefore, sterilization is an essential process for space exploration and better understanding of microbial response to an extreme conditions is needed.

The Arizona State University Organisms Near Space (ORGANS) payload aims to collect data on the resilience of common microorganisms by analyzing their response to atmospheric conditions. The data gathered will provide insight on the effects of these conditions on common gram positive and negative bacteria. Its primary objective is to analyze survivability of microbes and frequency of mutation and secondary objective is to provide better understanding to sterilization methods. The payload consists of sensors to measure temperature, pressure, and UV radiation and four containers in which wild type of strain and loss of function strain bacteria for each gram-positive and gram-negative bacteria are separately placed. After the payload returns, ORGANS team will cultivate these cultures to analyze survivability of microbes. Analyzed data can be applied to increase effects of sterilization and reduce potential of contamination in all kind of space missions.

**Science Objectives**

The objectives of this experiment are as follows

- Analyze the survivability of select species of bacteria under atmospheric conditions at 35 km above sea level.

**Science Requirements - Cultured Species Present on Payload**

| Scientific Classification of Chosen Bacteria | | |
|---|---|---|
| | **Gram Negative** | **Gram Positive** |
| **Domain** | **Bacteria** | **Bacteria** |
| **Phylum** | **Proteobacteria** | **Firmicutes** |
| **Class** | **Gammaproteobacteria** | **Bacilli** |
| **Order** | **Enterobacteriales** | **Bacillales** |
| **Family** | **Enterobacteriaceae** | **Bacillaceae** |
| **Genus** | **Escherichia** | **Bacillus** |
| **Species** | **coli** | **subtilis** |

**Bacillus subtilis:**

The gram-positive bacteria selected for the experiment is *Bacillus subtilis*. *B. subtilis* has a Bio Safety Level of one (BSL 1). Although previously thought to be an obligate aerobe, new research illustrates the species acts as a facultative aerobe (Nakano et. al, 1998). This is pertinent to the experiment since the environment *B. subtilis* is being observed in will have minimal to nonexistent amounts of oxygen, meaning that any results of viability, particularly reproduction, can be attributed to UV radiation. Another interesting aspect of *B. subtilis* that will be observed in the experiment is its' ability to form spores via sporulation (Piggot et. al, 1976). The procedure for sporulation is exceedingly intricate and meticulous, requiring seven distinct steps. However, when formed, these spores are resistant to heat, radiation, chemical processes, and other environmental interactions. Therefore, the spore can survive for longs periods of time in harsh environments. The most common places *B. subtilis* can be found are in soil and the human intestinal tract, where it has a commensal relationship (Hong et. al, 2009). It is generally non-pathogenic to humans, animals, and plants, though it has been identified as a bacteria found in rotting/decomposition. *B. subtilis* grows over a wide range of temperatures; the optimum temperature range being 25-35° C. This will not be a factor in the experiment due to the fact the high altitude environment will hold a temperature of approximately -75° C at cruising altitude.

**Escherichia Coli**

The gram-negative bacteria selected for the experiment is *Escherichia Coli* (E. Coli). E. Coli has a Bio Safety Level of one (BSL 1). *E. Coli*, like *B. Subtilis* is a facultative anaerobe which is important due to the lack of oxygen at extremely high altitudes. Although commonly known to the public for the pathogenic O157 and "shiga-toxin producing" strains (Son et. al 2013), *E. Coli* is a very common bacteria found in the human gastrointestinal tract and other animals. As opposed to *B. Subtillus E. Coli* does not sporulate. It replicates through two distinct forms of reproduction. The first and more common process is Binary fission and the second form is through conjugation in which genetic material is transferred between two Bacterial cells through sex pili. Optimal growth temperature for *E. Coli* is about 37° C which is optimal body temperature. However it can grow, at slower rates, in environments ranging from 10°-45°C and some experiments have seen growth in temperatures as high as 49°C (Fotador et.al 2005). Since it is a very common bacterium in humans, it is important to study its behavior in the near-space environment for future manned spaceflights. It is for these reasons that we have chosen it to represent gram-negative bacteria in our experiment.

**Theory and Concepts -**

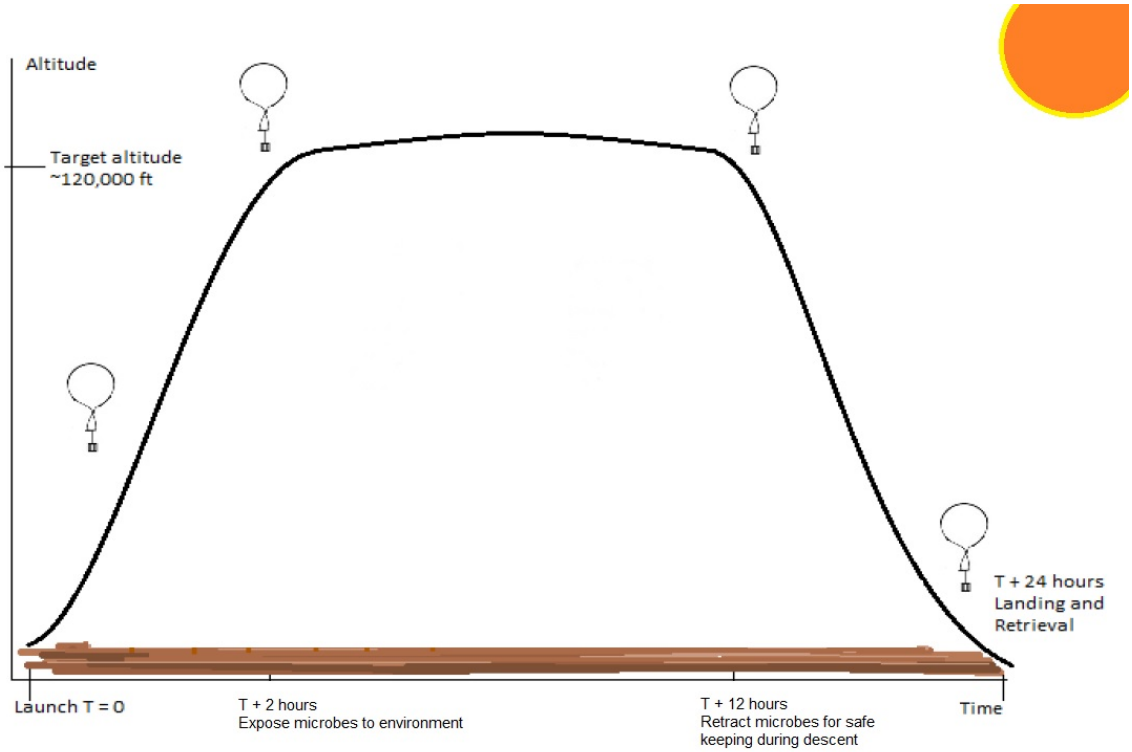
The search for life on other worlds is certainly an interesting and important endeavor. However, this search is confounded by two main issues scientifically. The first issue of concern is the initial difficulty in discovering life elsewhere without Earth life contaminating the sample and measurements (Rummel, 2001). To get around this issue, it will require careful consideration from scientists on how to best sterilize spacecrafts in order to minimize risks of exporting Earth life to an environment where it could grow and thrive (Rummel, 2001). This phenomenon of bringing Earth life to new worlds where it could invalidate the science being done as well as threaten a possible alien ecosystem is known as forward contamination (Rummel, 2001). The second confounding issue deals with the handling of samples of possible alien life that could be harmful to Earth life. This phenomenon is known as reverse contamination. Neither forward nor reverse contamination are a trivial matter for interplanetary exploration and have made way for planetary protection policy and protocol. Studies done during the Apollo era focused on the identification of microbial contaminants on both manned and unmanned spacecraft found that around 70% of microbial contaminants found were indigenous to the human microbiome and around 20% were microorganisms indigenous to soils (Nicholson et al., 2005). The Committee on Space Research (COSPAR) has set limits on microbial bioloads for unmanned surface missions to Mars at 300 spores/$m^2$ and $3 \times 10^5$ spores/vehicle, although these numbers drop significantly when life detection missions are being conducted (Nicholson et al., 2005). In the bioloads detected on previous missions, the gram-positive, spore-forming microorganism *Bacillus subtilis* has been found to represent up to 10% of the bioload, and is found along with the gram-negative microorganism *Escherichia coli*. These two microorganisms will serve as model species for better understanding the effects of the environment at 35km on viability as they are ubiquitous microorganisms often found on human skin and in clean rooms. *B. subtilis* also serves an important role in that it is a spore forming bacteria, which is a mechanism for increased survival in harsh

environments. It is possible that *B. subtilis* might form spores during the HASP flight, but changes in pressure and temperature could possibly inhibit this evolutionary adaptation to harsh environments.

Previous work by Schuerger et al. (2003) provides insight about the survivability of *B. subtilis* endospores when exposed to a high UV radiation, low temperature, low pressure environment that simulated the surface of Mars. Schuerger et al. (2003) found that it took no less than 15 minutes of exposure to the UV radiation dose present on Mars to inactivate 100% of the *B. subtilis* endospores. This has implications for the survival of microorganisms in space which suggest that the space environment might be enough to prevent forward contamination. Diaz et al. (2006) studied the effect of a high UV radiation, low temperature, low pressure environment on *E.coli* cells, however, these cells were in saltwater soil media, freshwater soil media, and seawater. Diaz et al. (2006) found that the environmental conditions reduced the viability of *E.coli* but found less of an effect in the microorganisms suspended in seawater, suggesting that water and external media dampen the effects of environmental stress. The Organisms Near Space (ORGANS) payload aims to determine the survivability of these representative microorganisms after they have been exposed to the environment at 35km above sea level by taking a novel approach not explored in the literature. To alleviate the potential for water and/or media to dampen the signals from environmental stress, sterile paper will be used for inoculation of microorganisms.

**Concept of Operations**

To ensure that microorganisms get proper exposure to environmental conditions, several precautions need to be taken. The first precaution deals with the novel approach to inoculation. Since water and/or media can dampen environmental stress, sterile paper strips will be inoculated with bacterial cultures. This will be done in triplicate for each culture. These papers will be placed in a clear, walled, plastic tray and will be tightly wrapped in thin plastic wrap, in order to ensure maximal UV exposure with minimal risk of contamination. On the flight, nothing will be done to disturb the natural environmental conditions experienced by the microorganisms. Along with wild type strains of the bacterial microorganisms being used, strains which are pressure and temperature sensitive mutants. These bacterial microorganisms will be inoculated on papers in their own plastic trays and kept separate from the wild type strains.




**Expected Results**

Based on previous research done by Schuerger et al. (2003) and Diaz et al. (2006), it is expected that some inactivation of microbial cells will be seen. It is possible that some cells will recover when placed in their optimal environments, but previous studies have shown a delay in this reactivation. The ability of the microbial cells to survive the conditions will be compared against measurements of temperature, pressure, and radiation exposure to determine if any factors, or combination of factors, appear to be significantly detrimental to the microorganisms. We expect most of our cells to not return to viability due to the extended period of exposure. It is also speculated that *B. subtilis* should form spores, as this environment is not one favorable to cell growth. However, it is possible that the environmental conditions could interfere with the sporulation process. Depending on the rate of temperature and pressure decrease experienced by *B. subtilis*, key enzyme functions could be inhibited, or possibly enhanced.

# Payload Design Details

## Requirements

### HASP Requirements

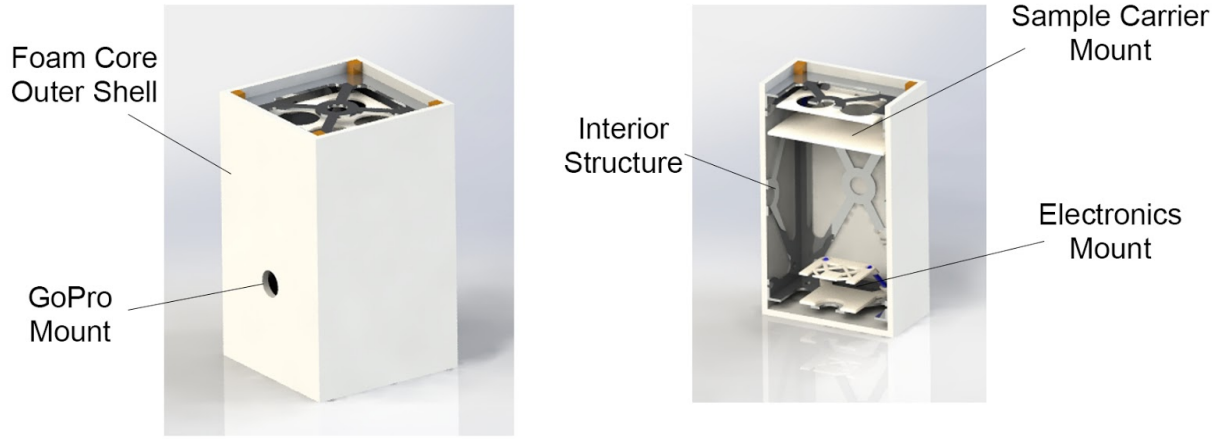
- The payload shall not exceed 3 kilograms in mass
- The height of the payload shall not exceed 30 cm
- The maximum footprint shall not exceed 15 cm x 15 cm
- The payload shall be capable of withstanding shock forces of 10g vertical and 5g horizontal
- The payload shall operate on independent battery power
- The payload shall be capable of proper operation under pressures as low as 3 millibar.
- The mounting plate shall adhere to HASP specifications

### Payload Requirements

- The payload shall contain cultures of selected microbial species
- The payload shall measure the temperature experienced by the microbes
- The payload shall measure the pressure experienced by the microbes
- The cultures shall be analyzed when returned to the ground for resilience to atmospheric conditions

## Mechanical System

### Design Drawing




The above figure shows a rendering of the initial design of the team's payload. The exterior dimensions of the payload are 15cm x 15cm x 25cm. The exterior structure is composed of lightweight high-density foam core sheets. The payload has an interior CNC milled aluminum structure which provides reinforcement and mounting points for the interior components. The Arduino microcontroller is seated on a 3D printed vibration dampening mount, and attached to the bottom of the payload. The samples being flown are seated in a sample carrier plate, which which is attached to the interior

structure via a vibration dampening mount. The horizon imager is directly mounted to the interior wall of the payload.

**Mass Budget**

| Part | Quantity | Mass(g) | Total Mass(g) |
|---|---|---|---|
| Outer Structure | 1 | 250 | 250 |
| Inner Structure | 1 | 1500 | 1500 |
| Arduino Vibration Mount | 1 | 150 | 150 |
| Sample Vibration Mount | 1 | 200 | 200 |
| Electronics | 1 | 400 | 400 |
| **Total** | | | **2500** |

**Risks and Hazards**

The primary risk to the payload is the impact upon landing after the flight. By incorporating a robust inner structure to act as a "roll cage" for the payload, we can mitigate the risk of impact-related events potentially damaging the payload. These events are not limited to the shock of landing, but also include any post-landing movement of the gondola and the uncertain terrain of the landing environment. This inner structure will be tested using structural analysis tools in SolidWorks, as well as with real-world crush and drop testing. The payload does not include any hazardous materials or introduce any extra risk to the HASP platform.

## Electrical System

**Power Distribution**

The main requirements for power in this experiment are the BMP180 pressure sensor, the TMP36 temperature sensor, the ML8511 UV sensor, and the Arduino Uno microcontroller. The BMP180 requires a maximum of 3.60 V and 5.00 µA at standard resolution with 1 sample per second. For the TMP36, requires between 2.70-5.50 V and a current of 50.0 µA. The ML8511 UV sensor requires 300 µA and 3.30V. The Arduino Uno requires 5.00V to operate, and a maximum current of 130 mA for our purposes. The maximum total current is 130 mA. Over the total flight time, the temperature, radiation, and pressure sensors need to run the entire time so as to obtain measurements of the environment for the experiment throughout the flight (18 hours). This means that for the BMP180 the total current of 90 µAh (5.00 µA*18h = 90.0 µAh); the TMP36 requires 900 µAh (50.0 µA*18h = 900 µAh); the ML8511 UV sensor requires 5.40 mAh (300 µA*18h = 5.40 mAh) and the Arduino Uno requires 1.62 Ah (130

mA*18h = 1.62 Ah). These add together for a combined subtotal of 1.63Ah, an additional 20% allowance provides a total of 1.96Ah needed at absolute maximum. All of this power shall be supplied by the HASP power bus throughout the duration of the flight, and this is all of the power requirement for the payload. This source is reliable as it has been in use on numerous payloads historically as a main power source, and is still provided to this experiment.

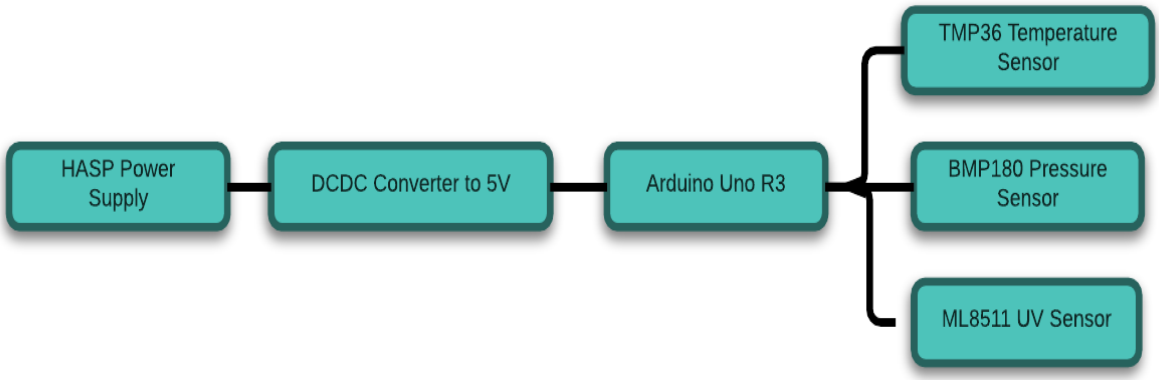
**Power Budget**

| System Component | Voltage | Current | Power Requirement |
|---|---|---|---|
| BMP180 pressure sensor | 3.60 V | 5.00 μA | 1.80E-5 W |
| TMP36 temperature sensor | 5.50 V | 50.0 μA | 2.75E-4 W |
| ML8511 UV Sensor | 3.30 V | 300 μA | 9.90E-4W |
| Arduino Uno microcontroller | 5.00V | 130 mA | 6.50.E-1 W |
| **Total** | | | 6.50E-1 W |

**Risks to Electrical System**

There is little to no additional risk to the Arduino, temperature, or radiation sensors at the altitudes, pressures, and temperatures to be achieved during experiment flight, as they have been used repeatedly and are rated to endure such environments. The largest risk within the electrical design comes from the BMP180 pressure sensor which is not rated for the low pressures that will be achieved during the payload flight. This risk is mitigated because this sensor has been successfully used on payloads that have achieved similar altitudes and pressures. This is the only major risk associated with our equipment given the structure and needs of our experiment. Risks will also be mitigated through comprehensive pre-flight testing of both temperature and pressure.

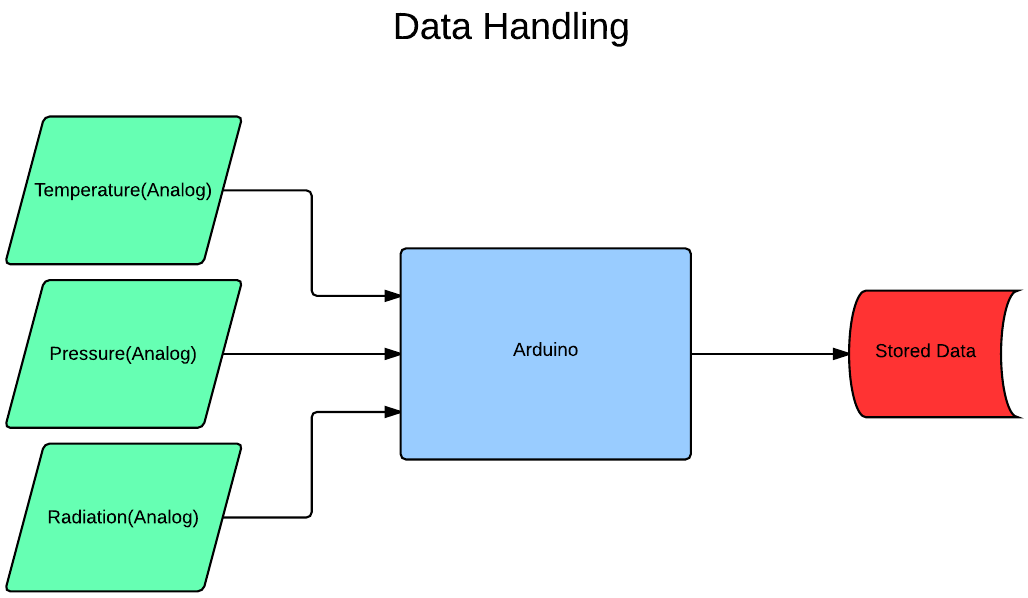
**Electric Flow Diagram:**




## Software System

### Flight Procedures

The payload software architecture will be based on the Arduino programming language. An Arduino Uno will be used to collect the input data from the temperature, pressure, and UV radiation sensors. No onboard manipulation of this data will occur, and the data will therefore be output directly to the SD card in the data logger for post-flight analysis.



**Risks and Hazards**

The software system is a low risk component of the payload. The nature of the experiment allows for raw data to be directly stored and manipulated post flight. Software will be tested with real input data to ensure it is being stored correctly.

## Thermal Management

The thermal environment of the flight is well characterized from previous years HASP flights. The thermal conditions have a large flux during the flight, from 40C to -70C. These cold temperatures could impact the functionality of the electronics. Environmental testing will be performed prior to launch and the results of these tests will determine the need for increased insulation or heating elements to ensure electronic functionality. The samples contained within the payload will be fully exposed to the exterior environment and as such, the temperature is not a concern.

Temperature will be a measured variable throughout data collection. An attached thermometer will record the temperatures experienced by the culture. This temperature will be considered when discussing the atmospheric conditions and its affects on our cultures.

## Systems Testing

The system will be tested locally in addition to compliance with H.A.S.P testing requirements. All components of the payload system will be individually tested cost efficiently with air circulated cooler filled with dry ice to bring down temperatures to approximately -50˚C to simulate extreme cold temperatures of high earth altitude. Local full system payload tests will be performed in facilities available in the labs of faculty in School of Earth and Space Exploration.

**Environment Simulation**

The high altitude environment the payload will experience is extreme. The system will encounter temperatures as low as -70C and pressures as low as .001 millibar for a duration of the flight. To ensure our system will survive in these conditions, testing will take place in a simulated mission environment for a period of time no shorter than the estimated duration of the flight. This testing will include both thermal and vacuuming testing to ensure the integrity of the structure as well as the electronics.

Additionally, the payload will experience a high G impact at the end of the flight. A combination of Solidworks simulation tools and drop testing will ensure that our payload will remain intact after a landing event.

**Anticipated Integration Procedures**

The payload will require no modification to HASP and will be constructed for easy integration with the platform.

**Risk Analysis**

The thermal environment adds risk to the electronics, because low temperatures can cause failure of components. These components will all be tested for extended duration in the anticipated thermal environment and if any anomalies occur, increased insulation or resistive heaters can be added

to mitigate the in-flight risk. The microcontroller is the main failure point of the electronics which would jeopardize the mission. This risk is partially mitigated by the extensive flight heritage of the microcontroller, but additionally, the system will be tested extensively to ensure functionality.

Data collected from the initial testing of the payload in thermal and vacuum conditions of the flight will further inform our design and any necessary changes can be made.

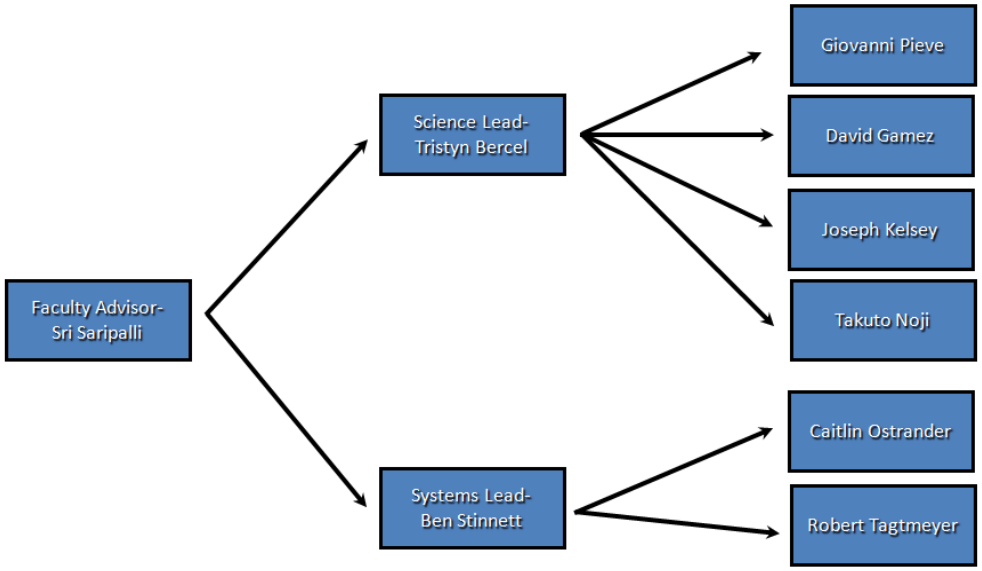
## Data Analysis

To determine the viability of the returning microorganisms, each paper will be swabbed and plated on Luria Bertani (LB) nutrient agar, which is a nutrient agar that can support growth of bacterial microorganisms. Each of the triplicate experiments will be plated on its own agar in order to control for minor differences between starting cultures and exposure during flight. These plates will be incubated at optimal temperature for growth of each individual species, 30 C, for 2 weeks, to account for any delay in growth. The growth, if any, will additionally by streaked on plates following standard dilution for cell count techniques to determine a measure of Colony Forming Units per mL (CFU/mL), and this number will be compared with control samples of each microorganism to determine a difference in microbial density and therefore a change in viability of the cells (Leboffe et al., 2010).

Along with the agar plate inoculations for *B. subtilis*, a swab will also be taken of each triplicate paper and transferred to unique slides, where they will be stained using a malachite green stain and a safranin counterstain following standard procedures in Leboffe et al. (2010) for visualization of endospores.

## Project Management

### Work Breakdown Structure



A large portion of the team will be available in Fall 2015 to perform the post-flight data analysis and document the flight results in the final report. This work will be headed by Ben Stinnett, who will be graduating Fall 2015. The laboratory where this work will be performed will be Professor Jason Raymond's lab located in ISTB4 on ASU Campus. Scientific advising on *E.coli* and *B.subtilis* strains and experimental design is provided by Professor Yixin Shi, faculty member in the ASU School of Life Sciences.

**Cost Budget**

| Component | Quantity | Cost (each) | Cost (Total) | Supplier |
|---|---|---|---|---|
| **TMP36 Temperature Sensor – SEN 10988** | **3** | **$1.50** | **$4.50** | **Sparkfun.com** |
| **BMP180 Pressure Sensor – SEN 11842** | **3** | **$9.95** | **$29.85** | **Sparkfun.com** |
| **Arduino Uno R3 – DEV – 11021** | **3** | **$24.95** | **$74.85** | **Sparkfun.com** |
| **ML8511 UV Sensor** | **3** | **$12.95** | **$38.85** | **Teledynemicro.com** |
| **Foam Core Board** | **1** | **$10.00** | **$10.00** | **TBD** |
| **3D Printing** | **1** | **$300.00** | **$300.00** | **TBD** |
| | | **Total Cost Estimate** | **$458.05** | |

**Schedule**

| | |
|---|---|
| January 15 - February 15 | Part acquisition<br>Order TMP36 Temperature Sensor<br>Arduino Uno R3 DEV 11021<br>ML8511 UV Sensor<br>BMP180 Pressure Sensor |
| February 15-March 15 | Component Testing<br>Order TMP36 Temperature Sensor<br>Arduino Uno R3 DEV 11021<br>ML8511 UV Sensor<br>BMP180 Pressure Sensor |
| March 15-April 15 | System Construction and Impact Testing |

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