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Section 1: Introduction

1.1 Motivation

The search for extraterrestrial life is an ongoing effort that has captivated scientists for decades. In-situ life detection instruments have been sent on numerous missions to Mars including on rovers such as Viking 1 and 2, Curiosity, and Perseverance, each designed to explore the surface and analyze regolith for signs of extinct or extant life. Future missions, such as Europa Clipper, are planned to investigate the habitability of the ocean worlds on the moons of Jupiter. Recent missions rely on spectroscopy and imaging to observe chemical patterns associated with habitability [1]. These tools help identify promising samples/sites for further investigation by other instruments that are capable of directly detecting evidence of life. Nanopores are nanometer-sized holes that can turn molecular characteristics into electrical signals and have been proposed as a sensor to directly analyse extra-terrestrial biomolecules [2]. This is primarily due to their ability to perform detailed characterization of biological polymers, the formation of which is a key step in the evolution of life. There are two main classes of nanopores. Biological pores are formed by proteins while solid-state nanopores are silicon based. Compared to biopores, solid-state nanopores are suspected to be more robust due to their silicon-based membrane and more flexible in terms of their size and the molecules they can analyse [3]. The strength and versatility of solid-state nanopores make them an ideal candidate for interplanetary life detection missions. However, the stability of solid-state nanopores in harsh environments has never been tested and is an important step to assess and improve their resiliency for space travel. The upper stratosphere provides a valuable analog to the harsh conditions that are found in extra-terrestrial environments. At ~30km in elevation, conditions are similar to the harsh environment on the surface of Mars. Measuring the effects of these conditions on the stability of solid-state nanopores will provide valuable insight for researchers aiming to integrate solid-state nanopores into life detection instruments. Research groups at numerous institutions/organisations including NASA, Georgia Tech, and the University of Ottawa are making advancements in solid state nanopore technologies [4, 5]. Data from environmental stress testing would help these group by providing information on weak points and other factors that could compromise the stability or performance of solid-state nanopores in extraterrestrial environments.

1.2 Novelty of Experiment

Biological nanopores have been the focus of recent extraterrestrial single molecule identification experiments. For this reason, we seek to explore the potential for solid-state nanopores use under extreme environmental conditions. Compared to solid-state nanopores, biological pores have undergone more environmental stress testing, specifically in their ability to sequence nucleic acids in extreme environments such in spaceflight. Most of the studies investigating biological pores in space environments aim to validate the use of nanopore sequencing technology for experiments involving Earth species (ex. investigating human health during spaceflight) [6]. On the other hand, studies using solid-state nanopores in this field are focused on validating their use for extra-terrestrial biomolecule analysis.

In 2016, biological nanopores were tested on the International Space Station [6]. Casto-Wallace et al. investigated ONT's MinION sequencer's ability to read nucleic acids and assemble a genome in a microgravity environment. The authors assessed the stability of the biological nanopore membranes after launch and during the 6 months the membranes spent in orbit. Despite launch vibrations, long storage time, and microgravity, the nanopores exhibited the same effectiveness as the control on the ground and were able to successfully assemble the genome of various earth organisms. This validated the use of the MinION for future DNA sequencing applications in experiments related to the effects of spaceflight on the human body and other organisms. For launch and stowage on the ISS, the membranes were kept in a controlled temperature (4°C) environment and shielded from radiation. The authors noted that biological nanopore membranes are prone to degradation and had stated concerns about their stability over longer missions or on robotic missions (where controlleverd environments are not kept). The others proposed that further development of solid-state nanopore technology may be required for deep-space missions.

Carr et al. developed a low-power heated pressure vessel to shield an ONT MinION device from Mars-like conditions [7]. Due to the protein membrane containing biological nanopores inside of the MinION, stable conditions are necessary to ensure its operation. The vessel was placed in a thermal pressure chamber set at -60°C and at a pressure of 500 Pa. The pressure vessel containing the MinION device was set to 20°C and Earth's atmospheric pressure. The goal of the study was to demonstrate that the MinION could sequence nucleic acids inside of a low-power pressure vessel under Mars conditions. The MinION was able to sequence DNA inside of the heated pressure vessel for around 2 hours until the heat loss of the vessel overpowered the heater, leading to a decline in vessel temperature and a conclusion of the experiment. The authors concluded that with increased insultation and improved computing efficiency, their platform could be improved.

Sutton et al. studied the effects of ionizing gamma radiation on the operation of ONT's MinION and its components [8]. Components were exposed up to 3000 grays of radiation and periodic quality measurements were taken over the course of radiation. The authors found that a exposing the MinION flow cell (containing the nanopore membrane) to over 50 grays resulted in decreased read quality, suggesting damage to the protein membrane. Based on past Mars missions, it was predicted that the flow cell would be able to sustain a flight to Mars and a few months on the surface of the planet before accumulating 50 grays of ionizing radiation exposure. The authors stated that the MinION platform would experience significant loss of performance for a mission to Europa and suggested that solid-state nanopores could be an alternative solution.

To measure the effect of vibrations of the stability of biological nanopores in ONT's MinION device, Carr et al performed sequencing on a parabolic flight trajectory [9]. The study measured vibrational frequencies and intensities during the plane flight and investigated their effects on molecule translocations through the biological nanopores. The authors found no significant difference between the translocations under flight vibrations compared to their control on the ground.

The only study to conduct environmental stress testing of solid-state nanopores is a paper written by Ramírez-Colón et al [10]. To test solid-state nanopore sensing in microgravity environments, the investigators created a setup to run single molecule analysis of various biological molecules under different levels of reduced gravity on a parabolic flight. Their results were inconclusive due to high noise in their measurement systems. Signals caused by molecule translocations through the pores could not be distinguished from random noise. The authors were unable to isolate the cause of the high noise in their system.

Through past studies it is observed that nanopores are prone to the effects of extreme environments, particularly biological nanopores, due to their fragile membranes. In the case of solid-state nanopores, literature on the topic is lacking. It is not yet known how exposure to extreme conditions can affect the stability of the pore and its silicon-based membrane. Environmental stress testing to specifically assess pore stability is necessary to address this knowledge gap. A stratospheric balloon flight provides an accessible analog to the conditions on other planets in our solar system. Measuring the stability of nanopores throughout the extreme temperatures, radiation, vibrations, humidity and acceleration experienced in balloon flight provides vital information to nanopore researchers looking to develop flight-ready nanopore sensing instruments. Novel data collected from this flight can be used to create solutions making nanopore instruments more resilient against extreme conditions.

1.3 Goals

The goals of this project are as follows:

- To assess the effects of extreme environmental conditions and vibrational stresses on the stability of solid-state nanopores and their silicon-based membranes. Specifically, to investigate the effects of temperature, pressure, vibrations, acceleration, and radiation on changes in noise and size of solid-state nanopores.
- To collect environmental data (temperature, pressure and radiation) throughout the stratospheric balloon flight to compare levels to those in extraterrestrial environments.
- To investigate the effects of extreme conditions on the structural integrity of the silicon chips/membranes containing solid-state nanopores.

1.4 Importance to Canada's Space Sector

The objective of this research project aligns with the "Result 1 - Canada remains a leading spacefaring nation" section of the Canadian Space Agency's (CSA) 2024-2025 Departmental Plan [11]. The CSA aims to remain a key player in space science and exploration. With the success of the OSIRIS-REx Laser Altimeter (OLA), the CSA has become an emerging leader in developing scientific instruments for space exploration. Furthermore, the CSA's first lunar rover will house numerous Canadian developed scientific instruments to analyse lunar regolith for its mineralogy and water content. The development of scientific exploration tools a clear priority for the CSA. As the CSA's space missions increasingly push boundaries, research and development into life detection instruments for deep-space exploration is a next step. This research project is a step forward in the development of solid-state nanopores into a future Canadian life detection instrument.

The process of building a flight-ready stratospheric balloon payload will be an invaluable learning experience for the undergraduate students involved in this project. By providing hands-on experience in the development, assembly, and testing of flight-ready instrumentation, this project prepares Canadian students to tackle future challenges in space exploration. In this way, this project actively supports the Canadian aerospace workforce, aligning with broader CSA goals of fostering Canadian talent and expertise in space technologies.

1.5 Relevance to the High-Altitude Environment

The stratosphere provides an extreme environment that mirrors conditions found on the surface of Mars and other extraterrestrial environments. A study conducted by Khodadad et al. recorded environmental conditions at ~32k in altitude and reported pressure values of 0.962 kPa, temperatures of −73.1 °C, < 1% relative humidity, and modeled UV levels totaling 86.6–109 W/m^2 [12]. The temperature, pressure, and radiation levels at this altitude are significantly higher than those found on Earth's surface and are similar to the conditions on the surface of Mars, making it an ideal analog for extraterrestrial conditions [13]. Conducting this experiment via a stratospheric balloon flight is crucial because it allows the simulation of harsh conditions in a controlled manner, enabling real-time observation of solid-state nanopore behavior under relevant environmental stressors. A stratospheric balloon flight also offers a unique opportunity to test nanopore stability and performance in a near-space environment without the need for space missions, significantly reducing costs and logistical constraints.

Ground-based experiments do not fully replicate the combined effects of low temperature, low pressure, high radiation, and varying humidity levels found in extraterrestrial environments. Environmental chambers can simulate some of these conditions but often lack the dynamic changes and gradients present during a balloon flight. A flight provides a continuously changing environment that mirrors the complex and multi-variable nature of space-like conditions. This is essential for identifying failure points or weaknesses in the solid-state nanopores, which may not be apparent in static laboratory settings. As the balloon ascends, the nanopores will be subjected to gradually increasing environmental stressors. During the entire duration of the flight, nanopore stability measurements will be taken, providing valuable insights into their resilience against changes in environmental conditions.

A flight duration of less than 3 hours is sufficient for this experiment. Key phenomena changes in nanopore noise levels and structural integrity of the nanopore membranes due to extreme environmental conditions can be measured within this timeframe. The impacts of temperature, pressure, and humidity could manifest within the three-hour window. Though it is unlikely, the effects of radiation may result depending on the dosage received by the payload.

1.6 Research Hypothesis

1.6.1 Effect of Temperature

The extreme low temperatures may impact the stability and structure of the nanopore. The silicon chip that houses the solid-state nanopore is composed of 2 materials: a 20 nm thick silicon nitride membrane (in which a pore is formed) and a silicon substrate (which frames the silicon nitride membrane) (Figure 1). These materials vary in their coefficient of thermal expansion. Under standard temperature, the tensional stress on an intact silicon nitride membrane is around 100MPa. At -55°C, the tensional stress increases to ~110MPa [14]. Intact silicon nitride thin films can withstand 5.8GPa of tensile stress [15]. However, the presence of a pore within the membrane may disrupt the structural integrity of the membrane. The increased tensional stress may lead to a rupture of the membrane which would result in an observable spike in the current passing through the pore.

Nanopore sensing relies on a constant current of ions though the pore. This is achieved by applying voltage across the nanopore in a salt solution. As the temperature of the payload decreases, the conductivity of the salt solution will decrease as well. This might result in a change in ion current proportional to the temperature of the system.

1.6.2 Effect of vibrations/acceleration

Although silicon nitride membranes can be very thin (~20nm) they are stiff and strong. They can withstand a 5.8 GPa of tensile stress which is around 60 times higher than stress in normal conditions [15]. However, the presence of a pore in the membrane, may weaken the membrane making prone to rupture. During flight, the nanopores may experience events with high acceleration (10g) and large vibrations (ex. payload swinging, ground collision). The silicon nitride membranes may rupture which would result in a measurable shift in pore current after traumatic events.

1.6.3 Effect of Ionizing Radiation

The effect of ionizing radiation on solid-state nanopores may result in an increase in noise. The collision of high-energy photons and charged particles with the silicon nitride membrane may result in the displacement of atoms on its surface. With sufficient irradiation, an accumulation of damage may result in pockets within the silicon nitride membrane that can trap positive charges [16]. A buildup of charges in close vicinity to the nanopore may lead to an increased capacitive load across the nanopore causing an increase in noise. They may also disrupt the flow of ions though pore by vortex formation [17]. However, the dosage of radiation required to observe these charges is far more than the stratospheric payload will likely receive. An estimated $5x10^3$ rads are required for permanent damage to silicon devices while, an estimated $1x10^{-3}$ rads are expected during a 3-hour balloon flight. If any impacts to the silicon nitride membrane occur, they would result in increased high-frequency noise which can be measured by a high bandwidth amplifier (ex. Chimera Instrument VC100 discussed in section 3.2).

1.6.4 Effects of humidity/pressure

Thin film silicon nitride membranes are commonly used in nanomechanical resonators which operate in vacuums. Thus, it is not expected that the low pressure and humidity experiences during balloon flight will affect the nanopore or membrane. The low humidity and pressure experienced during balloon flight would mainly impact the salt solution in the nanopore flow cells. The rate of evaporation from the aqueous solution will likely increase as the balloon rises in altitude. This may cause an increase in molarity of the salt solution leading to shifts in the baseline current though the pore. The magnitude of this affect will be governed by the choice of solution. The salt solution to be used during this flight has not been selected yet and will involve experimentation to find an optimal solution that is compatible with nanopores/flow cells, remains liquid at low temperatures, and is hygroscopic. 10M calcium chloride, 10M magnesium perchlorate, and 1M lithium chloride in ethanol are potential options.

Table 1. Environmental variables and their expected impact on solid-state nanopore measurements

Environmental Variable	Potential Impact on Nanopore/Membrane	Resulting Measurement
Temperature	1. Differential shrinkage of the silicon chip resulting in torsional stresses on the silicon nitride membrane. May cause the slow rupture of the silicon nitride membrane holding the nanopore 2. Reduction in conductivity of the salt solution in the nanopore flow cell	1. A large, slow increase in current passing though the pore. An increase greater than 10nA over a few seconds would be expected. If this occurs, it would likely result in the rupture of multiple pores during the extreme low temperature portion of the flight 2. A reduction of current passing though the pore. The trend in pore current would be proportional to recorded temperatures during flight
Vibration/ acceleration	Acute vibrational tresses causing a rupture of the silicon nitride membrane holding the nanopore	A quick breakdown of the silicon nitride membrane would result in a large change in current (greater than 10nA) in less than a second. The breakdown of multiple pores would likely result after a traumatic vibrational event.
Ionizing radiation	Creation of positively charged pockets on the silicon nitride membrane surrounding the nanopore leading to an increase in capacitive load across the membrane.	An increase in capacitance across the membrane would be measurable by an increase in high-frequency noise in a current measurement taken of the nanopore (observable in a power spectral density plot). High dosage of radiation would result in high-frequency noise increases across multiple pores.
Humidity/Pressure	Increased evaporation of salt solution from the nanopore flow cell resulting in an increase in salt concentration	Depending on the solution, an increase in salt concentration would result in an increase or decrease in baseline current though the pore. The rate of change in baseline current would be proportional to the humidity and pressure measurements taken during flight.

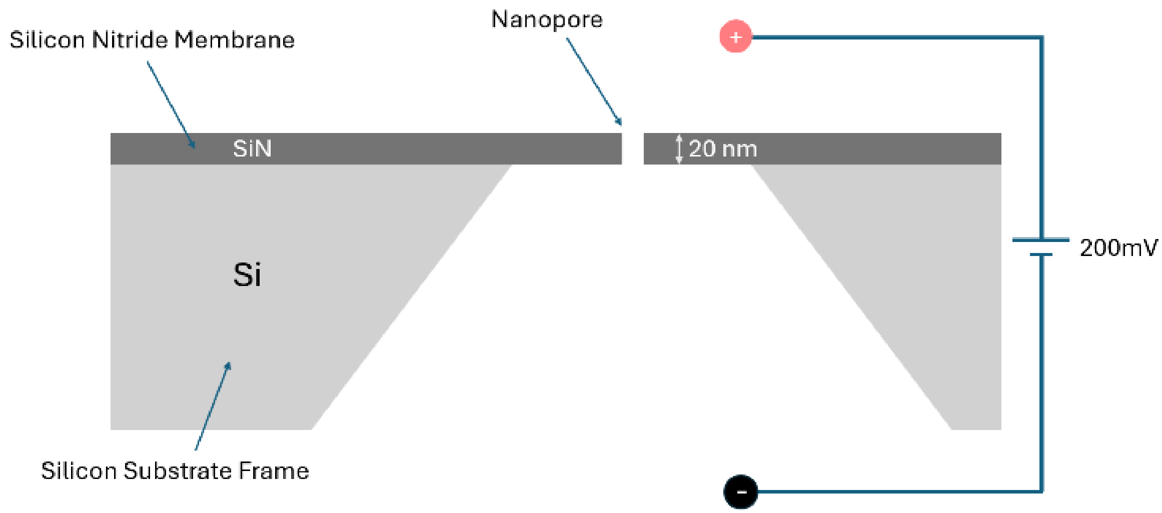

Figure 1. Diagram of a solid-state nanopore.

Section 2: Experiment Concept Design

2.1 Scientific Objectives

- Measure the stability of the solid-state nanopores throughout the balloon flight and correlate changes in stability with variations in environmental conditions
- Take a full suite of nanopore noise/stability measurements (including translocation of DNA through the pore) to characterize the nanopore in detail before and after flight
- Observe changes in the structural integrity of the pores' silicon chip/membrane by microscopy before and after flight
- Measure weather conditions throughout the flight and compare to known conditions in extraterrestrial environments.

2.2 Experimental Design

Multiple replicates and controls will be used to validate experimental conclusions. A total of 8 flow cells will be flown. Two flow cells will contain a nanopore in a salt solution. Another set of two flow cells will contain an intact membrane (no pore) in a salt solution as a control. Current readings from these four flow cells will be collected throughout the flight. Two more flow cells will contain a nanopore without a salt solution. The final set of flow cells will contain an intact membrane without a salt solution. These four flow cells are used to evaluate the structural integrity of silicon chips that house solid-state nanopores. Using these sets of controls and replicates a conclusion of experiment will be easier to reach

2.3 Science Traceability Matrix

Table 2. Science traceability matrix for the proposed nanopore experiment

Scientific Objective	Scientific Measurement		Scientific Instrument	
	Measurement Objective	Measurement Requirement	Instrument	Instrument Requirement
Assess the effects of extreme environmental conditions and vibrational stresses on the stability of solid-state	Damage to the silicon nitride nanopore membrane	Change in the baseline current (nA) passing through the nanopore with a 200mV applied voltage Change in the amount of noise (root mean square and 1/f noise) in the current signal passing though the nanopore	**During flight:** Nanopore Measurement Amplifier PCBs **Pre/post flight:** Chimera Instruments VC100	Range: 0 – 20nA Resolution: 50pA Sampling Rate: 10kHz Range: 0 – 50nA Resolution: 1pA Sampling Rate: 6MHz
Collect flight condition measurements and compare weather data to conditions in extraterrestrial environments.	Observe trends in weather conditions throughout the flight	Track temperature (°C), relative humidity (%), atmospheric pressure (Pa), ionizing radiation (μSv/h), vibrations/acceleration (m/s^2)	**Temperature Sensor:** LM35 **Relative Humidity Sensor:** SHTC3 **Pressure Sensor:** KP212K1409 **Radiation Sensor:** SBM-20 Geiger Tube **Accelerometer:** ISM330DHCX	**Temperature sensor:** Range: -60 – 40°C Resolution: ±0.5°C Sampling Rate: 10Hz **Relative Humidity:** Range: 0 – 100% Resolution: ±1% Sampling Rate: 10Hz **Pressure Sensor:** Range: 10 -2000 mbar Resolution: ±5 mbar Sampling Rate: 10Hz **Radiation Sensor:** Range: $4x10^{-5}$ – $4x10^{-5}$ μSv/h Sampling Rate: 10Hz **Accelerometer:** Range: 0 – 160 m/s^2 Resolution: 0.0005 m/s^2 Sampling rate: 6kHz
Assess the effects of extreme conditions on the structural integrity of silicon chips/membranes containing solid-state nanopores.	Damage to the silicon chips that hold the nanopore membrane	Fractures in the silicon frame and ruptures in the silicon nitride membrane	**Pre/post flight:** Microscope	Range: 40x – 1000X magnification

Mission Requirements
Pre-flight: Nanopore must undergo thorough characterization including, noise, baseline current, and translocation measurements. Silicon nitride chips/membranes must be imaged. During flight: The payload must begin sensor data collection and nanopore measurements at the system power-up before launch. Measurements must be continuously taken throughout the flight and saved for post-flight analysis Post-flight: Nanopores must undergo another round of thorough characterization including, noise, baseline current, and translocation measurements. Silicon nitride chips/membranes must be imaged. Flight data must be recovered for analysis.

2.4 System Architecture

2.4.1 Insulated Control Box

The insulated control box will contain the payload's battery, microcontroller, nanopore measurement amplifier PCBs, a 3-axis accelerometer, pressure sensor, humidity sensor, and a heating element (Figure 2). A 5V power bank will supply power to the main PCB, nanopore amplifier PCBs, and the heating element. Based on power requirement estimates, a lithium-ion battery rated between 4000 and 6000mAh should provide enough energy to power the system for the duration of the 3-hour flight and beyond. The control box will contain a PCB shield designed around the Teensy 4.1 microcontroller. The Teensy will gather data from all sensors, from nanopore stability measurements, and save collected data onto the Teensy's SD card. The Teensy 4.1 has a high clock speed (600MHz) allowing for rapid sampling rate, and the on-board SD card slot simplifies data saving. A 1V linear voltage regulator and a voltage divider will supply a stable 100mV to the Nanopore amplifier PCBs which will collect stability measurements from the nanopores and amplify the signal for the Teensy's analog to digital converter to interpret. An analog 3-axis accelerometer will collect acceleration and vibration flight data. Sensors in the control box will also collect pressure and humidity data. To keep these components in their operatable temperature range, a resistive heating pad will be used to maintain temperatures in the insulated box around 5C°. The external shell of the control box will be 3D printed in polyethylene terephthalate (PETG) (a material resilient to harsh conditions) and will contain four embedded 5mm aluminum rods to reinforce the structure against compression from g-forces. A 1cm layer of insulating foam will surround the inside of the control box. To block communication signals from interfering with sensitive nanopore measurements, a thin aluminum sheet between the shell and insulation of the control box will be used as shielding.

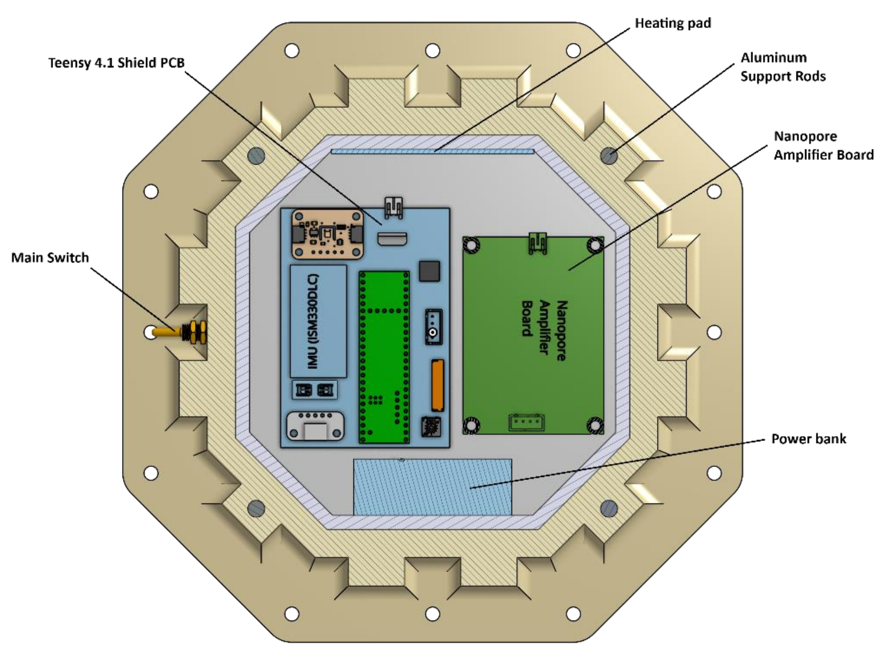

Figure 2. Top view cross section of the control box.

2.4.2 Nanopore Device Holder Box

A compartment above the control box will contain the nanopore device holder and environmental sensors (Figure 3). This compartment will be exposed to the external conditions by vents on the sides of the payload. Each nanopore will be contained in 3D-printed flow cells and a holder will be made to connect 10 nanopore flow cells to a PCB. To collect more environmental data, a sensor suite with a Geiger counter (to measure ionizable radiation levels) and a temperature probe will be used. Sensors that can operate in temperatures as low as -60C° will be used. The structure of this compartment will be 3D printed in PETG and the vents will be machined in aluminum to provide support against compression.

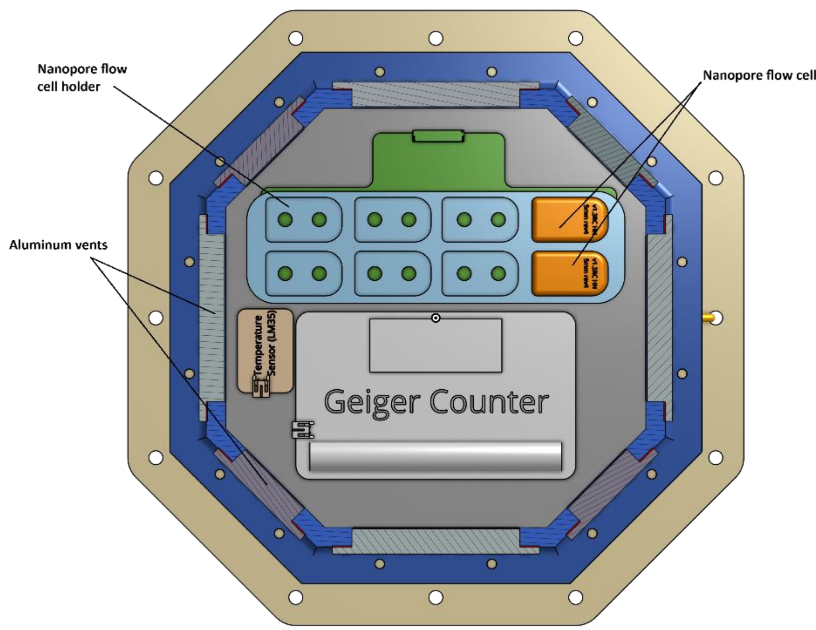

Figure 3. Top view cross section of the nanopore holder box.

2.4.3 Sub-system interfaces

A complete list of electrical components can be found in Appendix B.

Power connections: A 5V (4000 – 6000mAh) power bank will supply power to the Teensy 4.1 shield PCB, environmental sensors, resistive heating pad. The Teensy's on-board 3.3V regulator will power all environmental sensors. The nanopore measurement amplifier will be supplied with a regulated 100mV. The 5V heating pad will be controlled by the Teensy using a MOSFET. A 2A fuse will be used downstream of the battery to protect the payload's electronics. The main switch will be downstream of the fuse and situated on the exterior of the payload.

Signal connections: Nanopore stability will be measured using the nanopore amplifier PCBs which outputs an analog signal for the Teensy's 4.1 12-bit ADC to interpret. Weather condition sensors and the accelerometer will interface with the Teensy 4.1 by digital (I^2C or SPI) or analog signals. The resistive heating pad will be switched by a MOSFET and controlled by PID loop to maintain above freezing temperatures in the insulated control box. Data will be collected from each system at 10kHz and saved to a microSD card on the Teensy 4.1.

Mechanical interfaces: The payload will consist of two vertically stacked compartments. One compartment will contain the insulated control box, while the other will be vented and will house the nanopore device holder and weather sensors. The structure of the payload can be 3D printed in PETG allowing for quick prototyping with durable material. The control box will be fastened to the base of the payload gondola using M4 stainless steel bolts and washers. To interface the control box and the nanopore device holder compartment, stainless steel M3 bolts will be fastened into embedded nuts within the 3D printed structure. Data and power cables will be fed though the compartment divider to connect the nanopores and sensors to the control box. Any PCB or wire interfaces will be connected by JST connectors to create secure contacts against vibrations. The total payload mass is estimated to be less than 1kg.

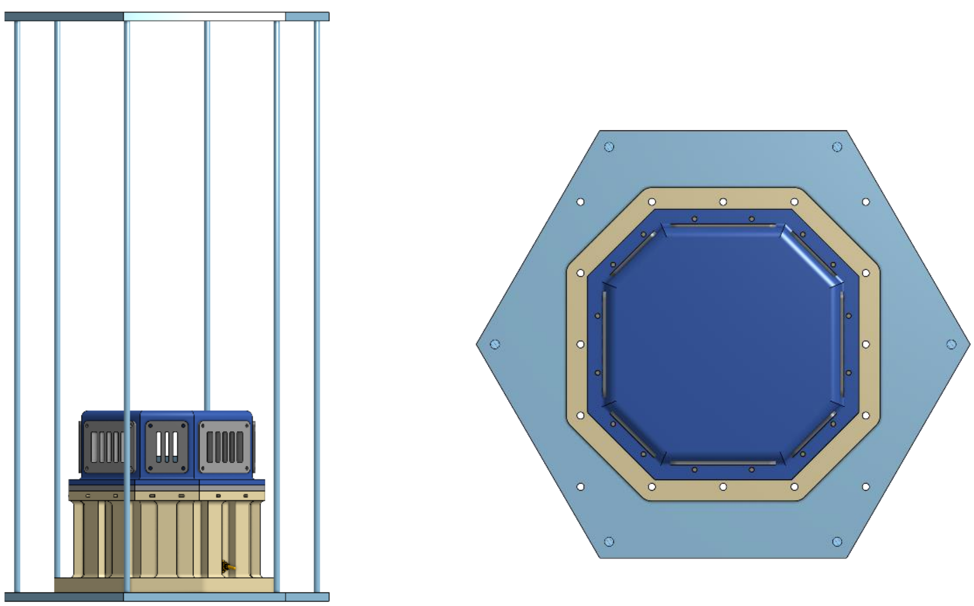

Figure 4. Side view and top view of payload on the gondola

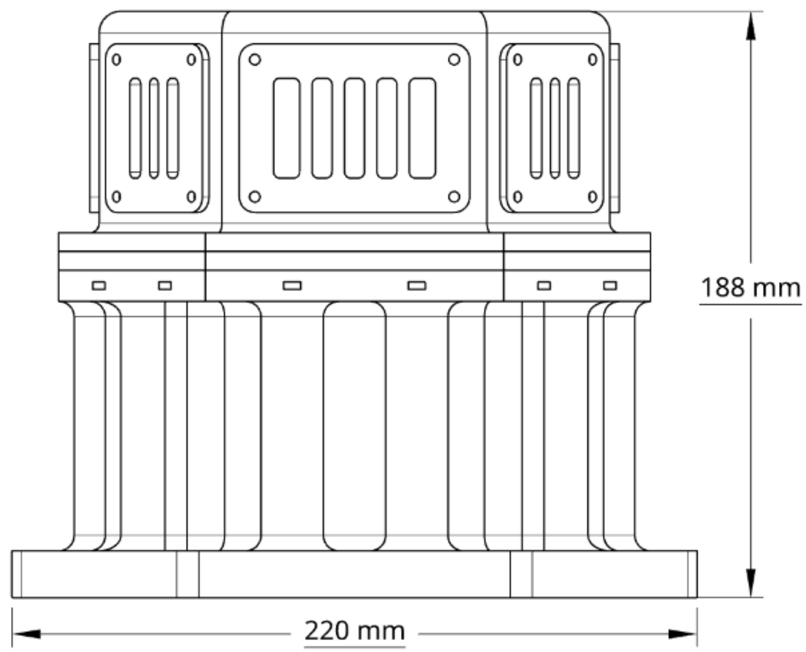

Figure 5. Dimensions of the payload

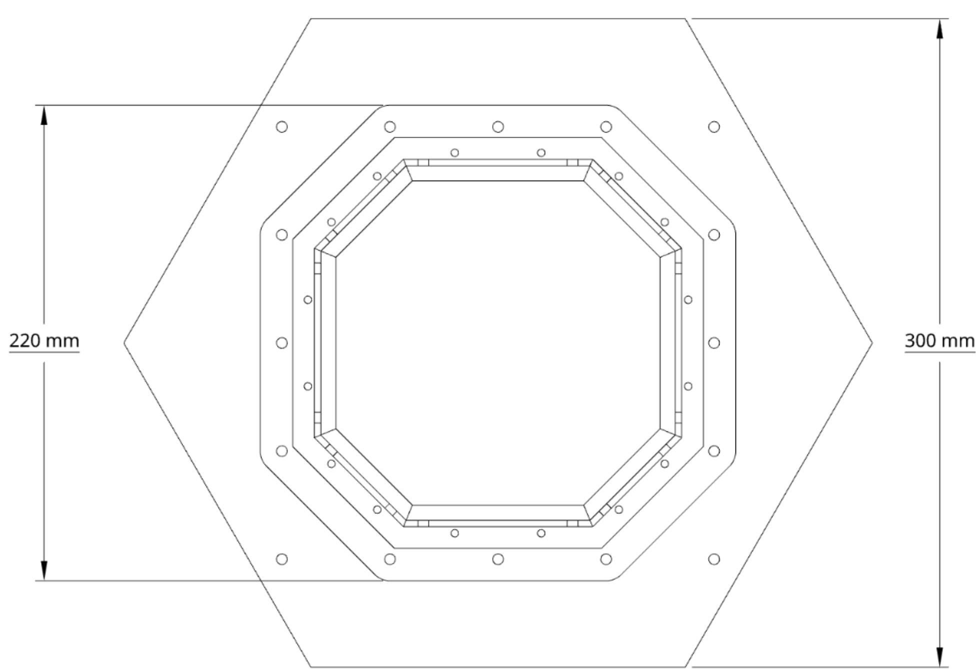

Figure 6. Top view of the payload and gondola base with dimensions.

2.4 Block Diagram

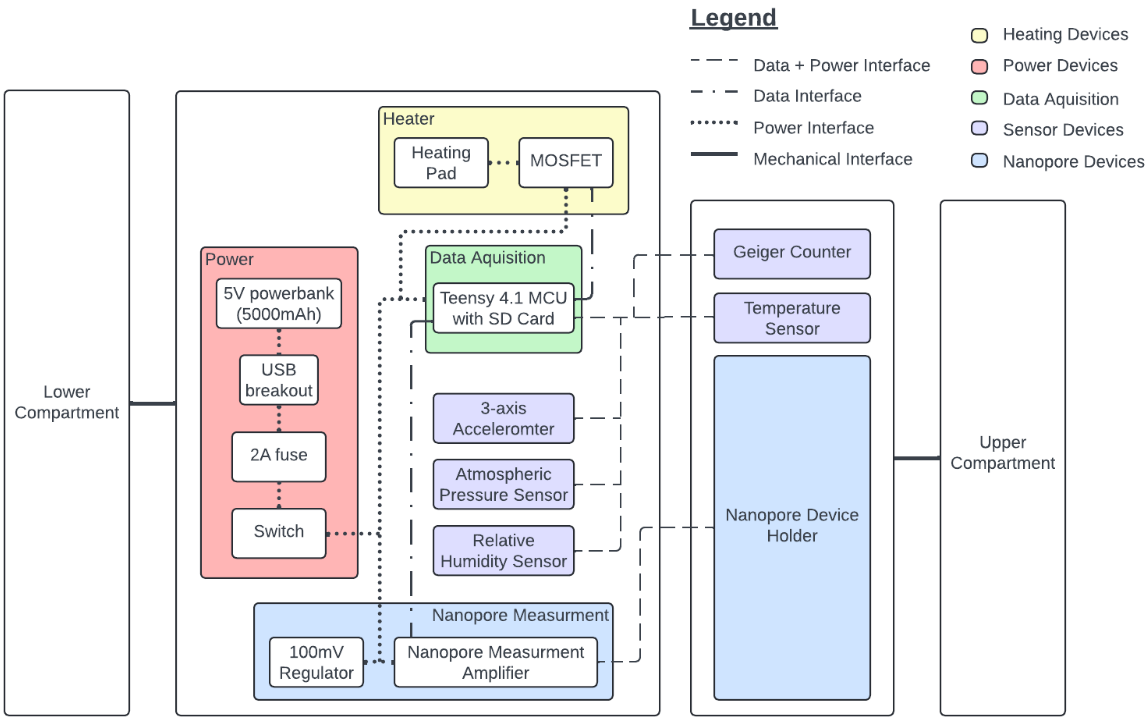

Figure 7. Block diagram of the interfaces of the payload.

Section 3: Concept of Operation

3.1 Equipment Requirements

3.1.1 Nanopore Measurement Amplifier PCBs

Amplifier PCBs will be used to take nanopore stability measurements over the course of the flight. These are specialized low-noise circuits than can measure nanoamperes of current passing through nanopores. The stability of the current passing through the pore is indicative of the quality of the nanopore. The effects of environmental/flight conditions on the stability of the current passing though the pore will be observed. These amplifier PCBs will be provided by our faculty advisor.

3.1.2 Microscope

To observe any effects on the structural integrity of the silicon chips/membranes that contain the solid state nanopore, a compound light microscope can be used. The chips will be imaged before and after the flight using a microscope provided by the faculty advisor.

3.1.3 Chimera Instruments VC100

Before and after flight, a full suite of noise/stability measurements will be taken. Additionally, DNA samples with known characteristics will be passed through each nanopore. These measurements will provide very detailed information about the flight's effect on the pore's stability and quality. A high bandwidth amplifier (Chimera Instruments VC100) will be used to take these measurements. The Chimera Instruments VC100 will be provided by the faculty advisor.

3.1.4 Nanopore Fabrication Units

To fabricate a nanopore, an increasing voltage is applied across a 20nm thick silicon nitride membrane. Once the voltage across the membrane reaches a critical point, the silicon nitride membrane undergoes a dielectric breakdown producing a nanometer sized pore. The pore can be enlarged to a desired diameter with additional applied voltage. The faculty advisor's lab has developed specialized instruments to fabricate nanopores and we are permitted to use them to fabricate nanopores for the payload.

3.1.5 Silicon Chips and Nanopore Flow Cells

Solid-state nanopores will be fabricated in silicon chips mounted inside 3D-printed flow cells (Figure 8). Both are designed and provided by the faculty advisor's lab.

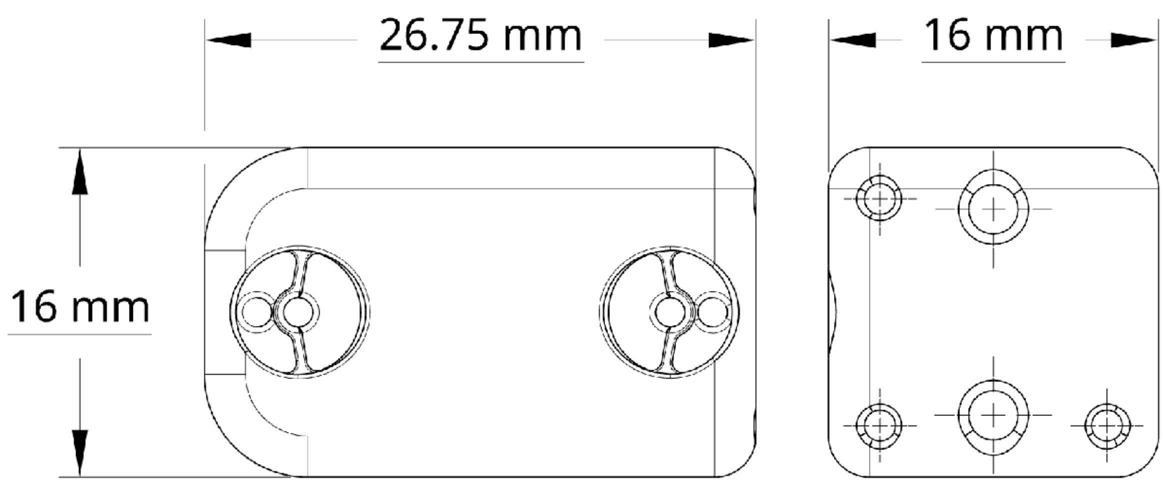

Figure 8. Dimensions of the nanopore flow cell

3.2 Environmental Requirements

3.2.1 Risks at the Integration Site

- Mechanical handling during integration
    - Risk: During assembly, integration, and transportation of the payload, rough handling or improper mounting can damage sensitive components, particularly the nanopores and sensor systems.
    - Mitigation Strategy: The payload will be transported and handled in shock-absorbing containers. The assembly team will follow a detailed integration procedure to ensure proper mounting and connection of all subsystems. Pre-launch tests will confirm mechanical integrity.
- Electrostatic discharge (ESD) during integration
    - Risk: Sensitive electronic components, including the nanopore amplifiers, are vulnerable to ESD, which could result in permanent damage or signal interference.
    - Mitigation: Store components in anti-static bags when not in use. Handle sensitive electronics with ESD-safe mats and wrist straps

3.2.2 Risks During the Stratospheric Balloon Flight

- Temperature extremes
    - The extreme cold at high altitudes (~-55°C) could impair the function of electronics in the payload and reduce battery performance.
    - Mitigation: An insulated control box with a resistive heating pad will be used to maintain the payload's electronics and nanopore measurement system at operational temperatures around 5°C. The heating element will be regulated by a PID control system, ensuring that the internal temperature remains within safe limits.
- Measurement interference from radio communication signals
    - Risk: The nanopore amplifier PCB can be sensitive to radio communication signals
    - Mitigation: The control with the amplifier board will be shielded with a layer of aluminum which should offer sufficient protection and will lower measurement noise.
- Vibration and high acceleration during flight.
    - Impact: Vibrations and shocks during balloon ascent, payload swinging, or upon landing could damage sensitive electronics or cause a loss of structural integrity.
    - Mitigation Strategy: A shock-absorbing insulating layer will be used between sensitive components and the external shell of the payload to dampen vibrations. The payload structure will include reinforced materials (e.g., aluminum rods and PETG) to withstand mechanical stresses. Pre-flight tests simulating high acceleration and vibrations will be conducted to ensure the design's robustness.

3.3 In-flight Operations

3.1.1 Pre-flight Procedures

1. Using a nanopore fabrication unit, 10 nanopores will be fabricated 1-2 days before flight
2. A detailed characterization of noise, baseline current, and translocation measurements of each pore will be taken 1-2 hours before flight. These measurements will be taken using the Chimera Instruments VC100.
3. Each silicon chip will be imaged and recorded by a microscope
4. Out of the 10, 8 nanopores will be selected for flight based on their quality as characterized by the Chimera Instruments VC100
5. Payload components will be assembled and nanopores will be loaded pre-flight.
6. The system will be powered on by a switch on the exterior of the payload activating weather/nanopore measurement acquisition
7. Lift-off of the payload

3.1.2 Post-flight Procedures

1. Payload recovery
2. Nanopore flow cells will be removed and stored for analysis
3. MicroSD will be recovered from the control box and stores for analysis
4. A detailed characterization of noise, baseline current, and translocation measurements of each pore will be taken as soon as possible. These measurements will be taken using the Chimera Instruments VC100.
5. Each silicon chip will be imaged and recorded by a microscope
6. Analysis of flight data

This experiment relies on pre/post-flight nanopore measurements as well as flight data saved locally on the payload. Thus, recovery of the payload is vital to conclude the experiment. Without recovered flight data, analysis of the experimental results is not possible.

Section 4: Project Plan

4.1 Funding Strategy

Funding Sources and Application Plans:

Tabard-Cossa Nanoscale Biophysics Lab, University of Ottawa

- **Support:** Provision of nanopore reading equipment and development of new nanopore related tools to comply with flight requirements

- **Status:** Confirmed
- **Eligibility:** Project must be conducted within the realms of nanophysics and contribute to ongoing research themes in the T-Cossa Lab.
- **Conditions:** Regular project updates to the lab director and participation in lab meetings to discuss progress.
- **Assigned Team Member:** Raghav Bhargava

uOttawa Engineering Endowment Fund (EEF)

- **Support:** Travel expenses, funding to purchase materials and required components
- **Amount:** $2,500
- **Application Procedure:**
  - **Preparation:** Gather all necessary documentation and endorsements. Prepare a detailed presentation highlighting the project's alignment with EEF goals.
  - **Status:** Awaiting opening of application window (Winter session).
- **Eligibility:** Must be an engineering related project with an educational or innovative component (fulfilled).
- **Deadline:** Application window expected to open in early Winter 2025.
- **Conditions:** Funding is contingent upon demonstrating how the project aligns with the advancement of engineering education or contributes to technological innovation.
- **Assigned Team Member:** Alejandra Carolina González González

University of Ottawa Centre for Entrepreneurship and Engineering Design

- **Support:** Free access to 3D printers and specialized filaments
- **Status:** Confirmed
- **Eligibility:** Must be an engineering-related project involving a University of Ottawa engineering student
- **Assigned Team Member:** Alvin (Phone) Thant Htet

SolidWorks

- **Support:** In-kind donation of CAD software licenses and technical support
- **Status:** Initial discussions underway.
- **Justification:** Essential for extensive CAD work required for the project
- **Eligibility:** N/A
- **Conditions:** Continued use of SolidWorks for project designs and promotional mentions in project presentations and reports.
- **Assigned Team Member:** Emma Saunders

PiShop.ca

- **Support:** Sponsorship; Discounted electronics and sensor components required for the payload.
- **Status:** Planning to initiate discussions.
- **Justification:** PiShop is a local Ottawa supplier that provides essential electronics, crucial for the technological components of our experiment.
- **Conditions:** Project must demonstrate the use of provided components in the final design and experiments. Promotional mentions in project presentations and reports.
- **Assigned Team Member:** Nada Kerrouri

Ottawa Makerspace (Hack613) Grants

- **Support:** Funding for the project's materials and components
- **Amount:** $400
- **Application Procedure:**
    - Provide a detailed project description and estimated budget
- **Eligibility:** Grant must be used to fund local Ottawa engineering projects
- **Deadline:** N/A
- **Assigned Team Member:** Raghav Bhargava

Fundraising Events on the uOttawa Campus

- **Support:** Funding for the project's materials and components
- **Amount:** ~$100
- **Description:** Organize a GoFundMe, bake sale and merchandise sales to garner support and funding for the project from the general public and the uOttawa community
- **Assigned Team Member:** Ashayana McNeilly

4.2 Outreach Strategy

The primary focus of our outreach activities will be to engage the next generation of scientists, engineers, and STEM enthusiasts. Locally, we will reach out to our city's chapter of Let's Talk Science to help organize and produce a series of educational activities geared towards school-aged children. We aim to produce one activity monthly over the course of the academic year, starting in October and finishing in June. Each activity will include a short presentation on the theory behind the instructed concept, such as planetary exploration, astrobiology, atmospheric science, as well as a hands-on component. Such activities could include craft sundials, balloon cars, small Arduino projects, DIY spectroscopes, etc. In the final session, we will host a mini design day where students will put their cumulative knowledge of the scientific process and problem solving to the test as we challenge them to create their own miniature balloon launch. This will encourage collaboration between the students, fostering scientific inquiry as they work together to approach this challenge. Also in this final session, we will ask all the students to write their names or a note that we will include in the payload on its stratospheric journey. This will personalize our project for the students, allowing them to feel as if they are a part of our team.

To reach the general public, we plan to use social media platforms including Instagram, Facebook, and TikTok. On these pages, we will regularly post educational content related to our project. In these posts, we will present basic scientific concepts in a succinct and digestible manner, that any member of the public, regardless of scientific knowledge, would be able to understand. As well, we aim to post regular updates of the design and testing process throughout the duration of our project, providing viewers with an insider's view of the tasks required to create a successful balloon launch. We aim to make these posts highly interactive and engage the public by hosting frequent question and answer sessions as well as incorporating a design competition for artwork to be included within our vessel. We will also use these platforms to promote our planned events including movie nights, demonstrations, as well as a presentation of our project. These larger public events we plan to produce every two months. Posts on these platforms will be shared every two weeks starting upon acceptance to the program up to launch day. Beyond social media, we will reach out to local libraries and community centres to advertise our events, allowing our events to be accessible to all members of the public.

Outreach to the academic community will be centered around students and faculty at our home institution, the University of Ottawa. We will collaborate with our university's engineering and physics student societies to host events and post information regarding our project. These events will mirror those offered to the general public, such as movie nights and demonstrations. A scientifically enriched presentation about our project and its design is also planned. As well, we intend on producing a poster to be presented at the undergraduate science student research symposium, taking place in March 2025. These presentations and posters will allow us to expose students to stratospheric balloon experiments that novel within our institution. It will also give us a platform to discuss the scientific background of the project with more rigour as we engage with likeminded individuals.

Appendix

Appendix A: References

Bibliography

[1] Keigo Enya, A. Yamagishi, K. Kobayashi, Y. Yoshimura, and E. J. Tasker, “A Comparative Study of Methods for Detecting Extraterrestrial Life in Exploration Missions to Mars and the Solar System II: Targeted Characteristics, Detection Techniques, and Their Combination for Survey, Detection, and Analysis,” *Astrobiology*, vol. 23, no. 10, pp. 1099–1117, Oct. 2023, doi: https://doi.org/10.1089/ast.2022.0148.

[2] K. Bywaters *et al.*, “Development of Solid-State Nanopore Life Detection Technology,” *ECS Meeting Abstracts*, vol. MA2019-02, no. 2476, 2019, doi: https://doi.org/10.1149/ma2019-02/57/2476.

[3] F. Rezzonico, “Nanopore-Based Instruments as Biosensors for Future Planetary Missions,” *Astrobiology*, vol. 14, no. 4, pp. 344–351, Apr. 2014, doi: https://doi.org/10.1089/ast.2013.1120.

[4] Z. Xia, M. Patchin, C. P. McKay, and M. Drndić, “Deoxyribonucleic Acid Extraction from Mars Analog Soils and Their Characterization with Solid-State Nanopores,” *Astrobiology*, vol. 22, no. 8, pp. 992–1008, Aug. 2022, doi: https://doi.org/10.1089/ast.2021.0051.

[5] C. E. Carr *et al.*, “Solid-State Single-Molecule Sensing with the Electronic Life-Detection Instrument for Enceladus/Europa (ELIE),” *Astrobiology*, vol. 23, no. 10, pp. 1056–1070, Oct. 2023, doi: https://doi.org/10.1089/ast.2022.0119.

[6] S. L. Castro-Wallace *et al.*, “Nanopore DNA Sequencing and Genome Assembly on the International Space Station,” *Scientific Reports*, vol. 7, no. 1, Dec. 2017, doi: https://doi.org/10.1038/s41598-017-18364-0.

[7] C. E. Carr *et al.*, “Nucleic acid sequencing under mars-like conditions,” in *IEEE Aerospace Conference*, 2019, pp. 1–7. doi: https://doi.org/10.1109/AERO.2019.8741543.

[8] M. A. Sutton *et al.*, “Radiation Tolerance of Nanopore Sequencing Technology for Life Detection on Mars and Europa,” *Scientific Reports*, vol. 9, no. 1, Mar. 2019, doi: https://doi.org/10.1038/s41598-019-41488-4.

[9] C. E. Carr, N. C. Bryan, K. N. Saboda, S. A. Bhattaru, G. Ruvkun, and M. T. Zuber, “Nanopore sequencing at Mars, Europa, and microgravity conditions,” *npj Microgravity*, vol. 6, no. 1, Sep. 2020, doi: https://doi.org/10.1038/s41526-020-00113-9.

[10] RamírezColón, José L *et al.*, “Nanogap SolidState SingleMolecule Detection at Mars, Europa, and Microgravity Conditions,” *bioRxiv. To be published.*, p. 2024.02.29.582359, Jan. 2024, doi: https://doi.org/10.1101/2024.02.29.582359.

[11] "2024–25 Departmental plan," *Canadian Space Agency*, Feb. 29, 2024. https://www.asc-csa.gc.ca/eng/publications/dp-2024-2025.asp

[12] Christina *et al.*, "Stratosphere Conditions Inactivate Bacterial Endospores from a Mars Spacecraft Assembly Facility," *Astrobiology*, vol. 17, no. 4, pp. 337–350, Apr. 2017, doi: https://doi.org/10.1089/ast.2016.1549.

[13] D. J. Smith and M. B. Sowa, "Ballooning for Biologists: Mission Essentials for Flying Life Science Experiments to Near Space on NASA Large Scientific Balloons," *Gravitational and space research : publication of the American Society for Gravitational and Space Research*, vol. 5, no. 1, pp. 52–73, 2017, Available: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6662212/

[14] T. Larsen *et al.*, "Ultrasensitive string-based temperature sensors," *Applied Physics Letters*, vol. 98, no. 12, Mar. 2011, doi: https://doi.org/10.1063/1.3567012.

[15] W.-H. Chuang, R. K. Fettig, and Reza Ghodssi, "An electrostatic actuator for fatigue testing of low-stress LPCVD silicon nitride thin films," *Sensors and Actuators A: Physical*, vol. 121, no. 2, pp. 557–565, Jun. 2005, doi: https://doi.org/10.1016/j.sna.2005.03.026.

[16] E. H. Snow, A. S. Grove, and D. J. Fitzgerald, "Effects of ionizing radiation on oxidized silicon surfaces and planar devices," *Proceedings of the IEEE*, vol. 55, no. 7, pp. 1168–1185, doi: https://doi.org/10.1109/PROC.1967.5776.

[17] H. W. Kraner, "Radiation damage in silicon detectors," *Nuclear Instruments and Methods in Physics Research*, vol. 225, no. 3, pp. 615–618, 1984, doi: https://doi.org/10.1016/01675087(84)901145.

Appendix B: Electrical Components List

Electrical Components	Part Name	Source
Control Box		
Teensy 4.1	Teensy 4.1	link
MicroUSB connector	475900001	link
Fuse Holder	3568	link
Accelerometer/Gyroscope	ISM330DHCX	link
Relative Humidity Sensor	SHTC3	link
Pressure Sensor	MS560702BA03-50	link
Toggle Switch	ADA-3218	link
1V 150mA regulator	MIC5376	link
MOSFET	IRLB8721PbF	link
2A Fuse	MIN2BP	link
Power Bank	BBPB-J115-BK	link
Heating Pad	ADA-1481	link
Nanopore Amplification PCBs	NA	
Nanopore Holder Compartment		
Temperature sensor	LM35	link
Geiger Counter	SBM-20	link
12-pin JST cable	A12SR12SR30K102A	link
12-pin JST connector	SM12B-SRSS-TB	link
2-pin JST connector	B2B-XH-A	link
4-pin JST connector	B4B-XH-A	link

Appendix C: Exploded View of the Payload