

Genes in Space 2023 finalists: Contributing ideas to advance space biology

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INTRODUCTION

All living things that we are familiar with—from humans, to tomato plants, to the bacteria in our yogurt—evolved over millions of years to live and thrive on Earth. To remove an organism from the familiar confines of that environment and send it to space carries considerable biological risk. Damage to DNA, muscle atrophy, vision impairment, and cancer risks are just a few of the ways we know that astronauts are impacted by microgravity and cosmic radiation, but the extent of these complications are still being understood.

We are at a unique point in the history of space exploration. The ongoing Artemis missions are set to return humans to the Moon in 2025, the International Space Station (ISS) is the beacon of human presence in space and will continue to be so throughout this decade, while its successors are being envisioned, and we will likely visit Mars in the next decade. However, if we wish to permanently settle these extraterrestrial bodies—to grow our crops there, raise our families, and thrive there as we have on Earth—new research must be conducted to understand biological uncertainties and develop solutions to overcome these challenges.

Genes in Space is a national science competition for middle school and high school students who are up for such a task. Each year, we invite students to design DNA experiments that can answer the unknowns of space biology. The winning proposal is developed into a flight-ready experiment, launched to the ISS, and conducted by astronauts.

Since the program was co-founded by miniPCR bio and Boeing in 2015, it has launched 10 student experiments. With sponsorship from the ISS National Laboratory and New England Biolabs, the contest has reached thousands of students with dreams of contributing to our understanding of space science.

The students featured on the following pages are the 2023 Genes in Space finalist teams, and their abstracts represent the top five proposals of this year's competition. Their curiosity, hard work, and scientific rigor have made for original and diverse proposals. We trust that you will find them as compelling as we have.



Charlene Chen
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School, Santa Clara,
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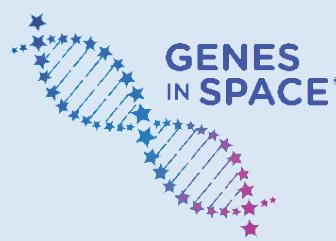
Isabel Jiang
Crystal Springs Uplands
School, Hillsborough,
CA



**Christina Rasa and
Kenneth Koch**
The Tome School,
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Development of a urine RNA aptazyme-based biosensor to detect oxidative DNA damage in space

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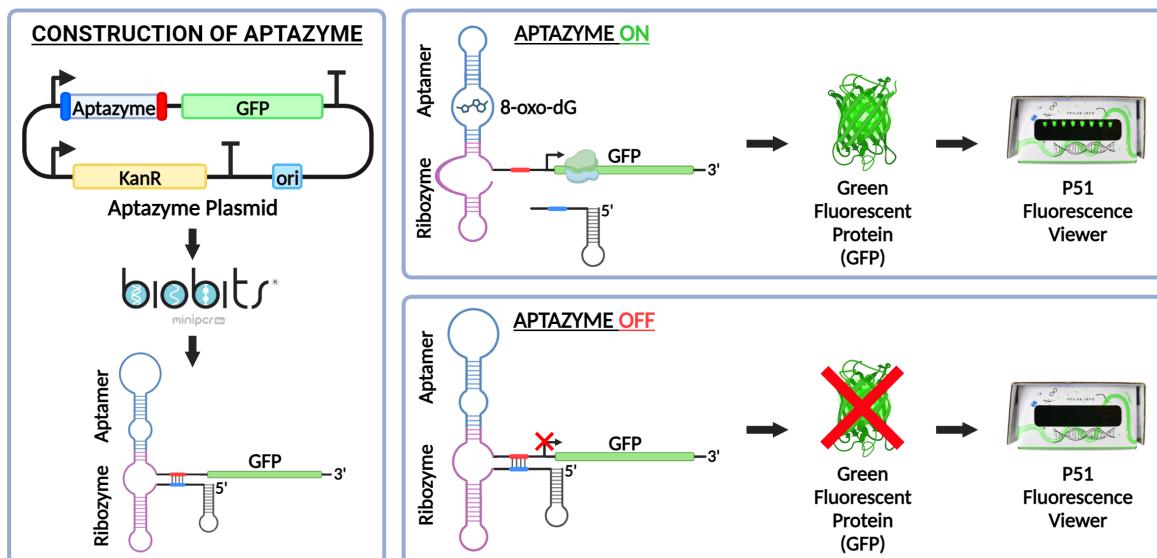
ABSTRACT

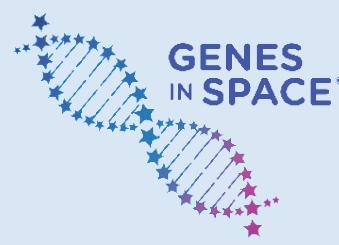
The space environment, mainly microgravity and radiation, poses a threat to astronaut health. Specifically, DNA damage can lead to severe health conditions, such as cancer, premature aging, and reproductive issues. Thus, efficient genome monitoring in space is absolutely imperative, but current methods are impractical due to their cost and complexity.

To address these challenges, we propose the development of an RNA aptazyme-based biosensor to detect space-induced DNA damage. Aptazymes combine aptamers, DNA or RNA molecules able to bind to specific targets, and ribozymes, RNA molecules capable of self-cleavage, to form a molecular detection system. This tool, together with a green fluorescent protein (GFP) reporter system, can be employed to detect 8-oxo-dG, a prominent biomarker of DNA damage, present in human urine samples. In the presence of 8-oxo-dG, the biosensor will activate and allow for the production of GFP. The aptazyme-based biosensor will be synthesized using a template plasmid and the BioBits cell-free protein synthesis system, and fluorescence will be monitored using the P51 Fluorescence Viewer.

To assess the ability of the biosensor to monitor levels of DNA damage, murine urine samples will be collected and tested over time on the International Space Station (ISS) and on Earth. The concentration of 8-oxo-dG present in the samples will be assessed using the proposed aptazyme system and verified by high-performance liquid chromatography. We hypothesize that the mice on Earth will show constant fluorescence levels, while the ISS mice will exhibit an increasing trend of fluorescence due to the accumulation of space-induced DNA damage over time.

The success of this experiment will not only provide astronauts with a simple and cost-effective tool to monitor DNA damage aboard the ISS, but also expand aptamer-based technology for a variety of health monitoring applications.





Microgravity effects on metamorphic protein structure

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ABSTRACT

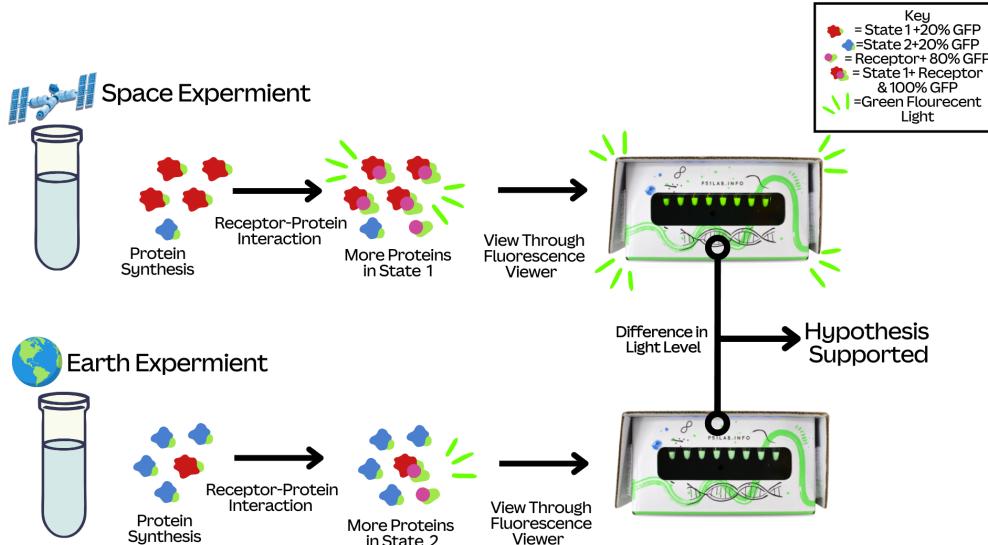
While advancements to successfully establish long-term bases on nearby planets draw closer and closer, self-sufficiency will allow these bases to flourish. Drugs and other medications for medical emergencies in space will also need to be synthesized independently from Earth. Producing properly folded proteins is an important step for the synthesis of many drugs.

We will use metamorphic proteins to investigate how gravity affects protein conformation. A metamorphic protein can fold into multiple stable conformations, depending on environmental factors. One such protein is XCL1 which has two different states: State 1 binds to chemokine receptors, while State 2 does not. We hypothesize the lack of gravitational forces in space will influence protein synthesis to favor one metamorphic conformation.

We will tag the metamorphic protein with exon 11 from green fluorescent protein (GFP) and the chemokine receptors with exons 1–10 from GFP. When State 1 interacts with the chemokine receptors, the two parts of the GFP will join and fluoresce green, which can be seen through a fluorescence viewer. A high intensity of green fluorescence would correlate to a high proportion of State 1. We plan to normalize the fluorescence from the metamorphic proteins to a standard curve generated by varying ratios of proteins known to interact.

To ensure GFP is not driving protein interactions, we would tag two proteins that are known to not interact with two fragments of GFP. If no glowing occurs, the proteins do not interact, and thus, GFP does not drive protein interactions in space. When conducting this experiment in space and Earth, any difference in light intensity would signal a difference in protein structure resulting from a difference in gravitational forces.

From the results, we will gain a better understanding of how different gravitational environments affect protein conformation, lay the foundations for future developments in drug synthesis, and eventually lead to the creation of a safer, more reliable medical application base for space travel throughout the cosmos.





Detection and treatment of LINE1 retrotransposon activation in space

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ABSTRACT

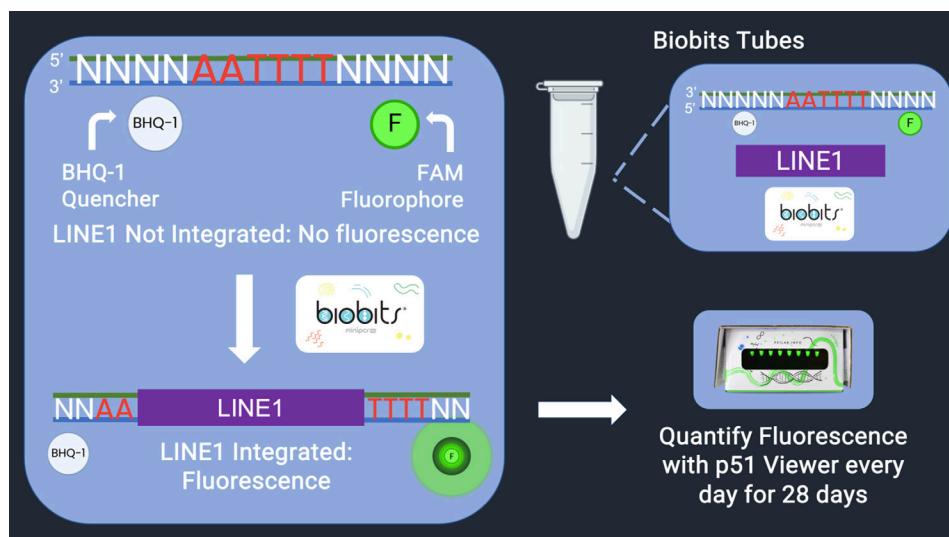
In space, astronauts experience heightened reactivation of latent herpes viruses such as chickenpox. However, the activity of endogenous, retrovirus-like retrotransposons in space is unknown.

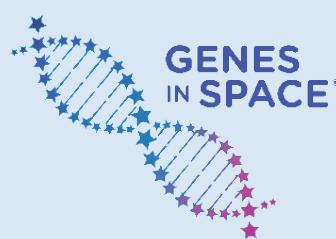
Retrotransposons are mobile DNA elements that occupy about 40% of the human genome. Among these, only one retrotransposon, called LINE1, can become autonomously active. LINE1 comprises approximately 17% of the genome. On Earth, LINE1 is suppressed by strict epigenetic control, but if control mechanisms are dysregulated, LINE1 will be activated and use a reverse transcriptase enzyme to "copy and paste" itself randomly throughout the genome. Such random insertions lead to gene mutations and can cause cancer and neurodegeneration.

In space, radiation and microgravity dysregulate epigenetic control. Therefore, we hypothesize that LINE1 is more activated in space than on Earth and drugs that inhibit reverse transcriptase, specifically nucleoside reverse transcriptase inhibitors (NRTIs), can serve as a countermeasure.

To test this, we will use a fluorescence-based assay. First, we will synthesize an oligonucleotide with consensus AATTTC sequences flanked by the fluorescence quencher BHQ-1 ("B") and the fluorophore FAM ("F"), depicted as 3'-(B)-AATTTC-(F)-5'. The fluorescence emitted by FAM is absorbed by BHQ-1 due to their proximity. If there is no LINE1 insertion, there will be no fluorescence. We will then add this oligonucleotide and a LINE1 expression cassette to the BioBits system. Prior to the addition of the LINE1 cassette, one group of oligonucleotides will be treated with NRTIs to assess the efficacy of the prophylactic treatment. The expression of the proteins encoded in LINE1 and the subsequent formation of the LINE1-ribonucleoprotein complex takes place in the BioBits system. After the integration of LINE1 into the consensus sequence AATTTC, depicted as 3'-(B)-AA[LINE1]TTTC-(F)-5', BHQ-1 will no longer be in functional proximity to FAM, enabling FAM to emit fluorescence, measurable by the P51 fluorescence viewer.

It is critical to understand the genetic changes in astronauts, especially in deep-space missions. Through this experiment, we hope to define LINE1 activity in space and evaluate NRTIs as an intervention.





A photosynthetic therapy for animal cells to reverse the cause of osteoarthritis in space

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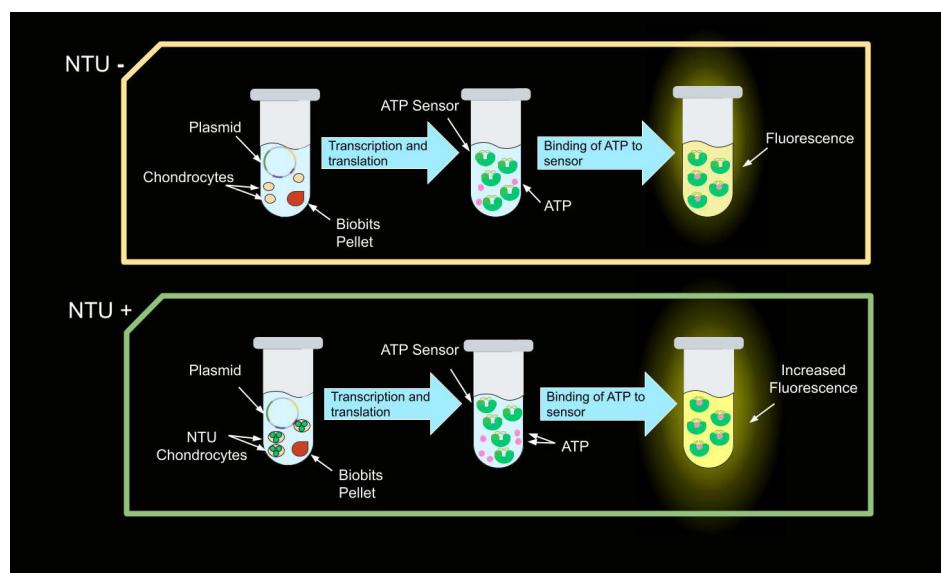
ABSTRACT

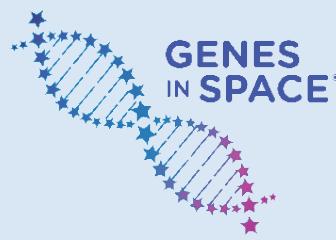
As long-term spaceflight missions are in developmental stages, it is critical to protect the well-being of those on board. The microgravity and radiation experienced in space are known to cause osteoarthritis in astronauts, so if they are to remain in space for long durations, a preventative treatment is essential.

Osteoarthritis occurs when cartilage-producing cells called chondrocytes do not have enough energy in the form of ATP. Recently, a revolutionary photosynthesis-based method of increasing chondrocytes' ATP production successfully reversed osteoarthritis in an animal model. The researchers who made this discovery found that inserting photosynthetically competent Nano-Thylakoid Units (NTUs) into the chondrocytes of mice provided them with enough ATP to sustain cartilage production. We propose to test the efficacy of NTU treatment on chondrocytes in space and hypothesize that photosynthesis of the NTUs will increase their ATP production.

To test this hypothesis, we will use BioBits cell-free technology to produce a biosensor for ATP based on the ATP-binding protein, malonyl coenzyme A synthetase. When two reporter fluorophores are attached to this protein, they will fluoresce in the presence of ATP. Once cells are lysed by a mild detergent, the levels of ATP in the sample can be measured by the amount of fluorescence, allowing us to compare ATP levels of chondrocytes with and without NTUs.

Higher fluorescence in the NTU-chondrocytes compared to normal chondrocytes would indicate that NTUs are capable of increasing cellular ATP production in space. If our hypothesis is supported, NTU treatment could be tested in an animal model on the ISS to see if the heightened energy of chondrocytes would be sufficient to regenerate cartilage and reverse osteoarthritis. This technology of combining plant and animal cells is groundbreaking, and its application would allow for monumental discoveries that will benefit humanity as a whole.





Uncovering the role of spaceflight in the autoreactivity of thymocytes

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ABSTRACT

In an era where space exploration is on the rise, it is imperative we understand how exposure to microgravity can influence autoimmunity. The immune system is essential for protecting us against invading germs, but it is also crucial that it does not react against our own cells. The ability to identify self-antigens from non-self-antigens, also known as central tolerance, is the foundation of thymocyte, or T-cell, training.

During this, medullary epithelial thymic cells (mTECs) express self-antigens to the thymocytes in order to "train" them not to react against cells from the body. AIRE encodes for a transcription factor that controls the expression of these peripheral antigens. If the thymocyte binds to a presented antigen with a high affinity, it is deemed autoreactive and is deleted.

Previous studies have showcased reduced T-cell production for astronauts in space as well as lower AIRE expression in simulated microgravity, but none have delved into the ratio of autoreactive cells escaping apoptosis. Thus, we propose using an autoimmune mouse model aboard the ISS to first assess the number of autoreactive cells developed and second, measure the level of AIRE expression in T-cells in space. Thymocytes will be collected from the mice and mixed with ovalbumin-specific fluorescent tetramers. Autoreactive thymocytes that have not been deleted would bind to tetramers, resulting in more fluorescence. After using the P51 fluorescence viewer, brighter fluorescence in the samples from space as compared to Earth would indicate more autoreactive cells are escaping deletion. In addition, AIRE expression will be measured in the mTECs by qPCR, and we expect lower expression in space, suggesting that autoreactive cells are evading immune checkpoints.

The two objectives of this experiment would thus function side by side to infer the correlation between lower AIRE expression and higher T-cell autoreactivity in space. This allows discussion to prevent the body's natural defense from sabotaging the space explorer and provides insight into how aging on Earth, which mimics thymic involution in microgravity, could impact autoreactivity.

