Section I Proposal Cover Page
Proposal Title: *Comparative Capture of E. coli by Antimicrobial-Peptide Biosensors in Microgravity*

Grade Level(s): 14–15

Submitting School: California State Polytechnic University, Pomona

District: AT3

Teacher Facilitator: Michael Pham
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Proposal Summary:
This investigation tests how microgravity affects the initial attachment between antimicrobial-peptide (AMP) coated surfaces and bacteria using an Enzyme-Linked Immunosorbent Assay (ELISA) to quantify bacteria captured. Understanding how microgravity alters this capture process can inform the design of biosensors and surface coatings for spacecrafts, where persistent biofilm formation threatens crew health and equipment reliability.

On Earth, gravity affects how bacteria reach surfaces through sedimentation, buoyant convection, and diffusion. In orbit, sedimentation and buoyancy vanish, leaving only molecular diffusion with active bacteria swimming to move cells toward surfaces. This change in transport physics may reduce bacterial capture rates compared with ground conditions. If microgravity alters attachment rates to AMP-coated surfaces, future spacecraft biosensors will require new designs to maintain reliable performance in orbit.

The investigation will test this hypothesis by comparing bacterial capture by AMP-coated surfaces under microgravity and normal-gravity conditions. Identical sample sets will be exposed for equal durations in flight and on Earth and a post-flight on-coupon sandwich ELISA will quantify the number of *E. coli* cells specifically bound to the immobilized AMPs via electrostatic and hydrophobic membrane interactions, using enzyme-linked antibodies to detect the captured bacteria. Differences between flight and ground results will reveal whether reduced gravity alters the efficiency or extent of AMP-mediated capture.

Findings will clarify how physical transport processes shape the earliest stages of microbial colonization in space and will guide the development of improved antimicrobial coatings for long-duration missions and planetary exploration.

Section II: Student Team Members and Professional Advisors

Principal Investigators

Name: Elizabeth Osborn
Grade level: 15

Co-Principal Investigators

Name: Frank Puga-Raya
Grade level: 14

Name: Steven Picazo
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Name: Jesus Coca
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Co-Investigators

Name: Alejandro Lopez
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Name: Damian Palacios-Rosas
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Name: Maya Ramirez
Grade level: 14

Professional Advisors

Name: Michael Pham
Organization: California State Polytechnic University – Pomona
Contribution to Team: Advised on Proposal and Experimental Process

Name: Dr. Chris Buser
Organization: University of Bridgeport, BioCubic
Contribution to Team: Advised on Proposal and Experimental Process

Section III: Experiment Materials and Handling Requirements

1) Rhodium Fluid Experiment Tube (RhFET-01) Mini-Laboratory Configuration:
Select the proposed mini-lab configuration:

☐ Type-1 Configuration

☐ Type-2 Configuration

☑ **Type-3 Configuration** with interactive microgravity exposure – two valves are opened by the ISS crew to initiate interactions between samples. Allows for activation (e.g., growth or reaction) and later termination (e.g., fixation).

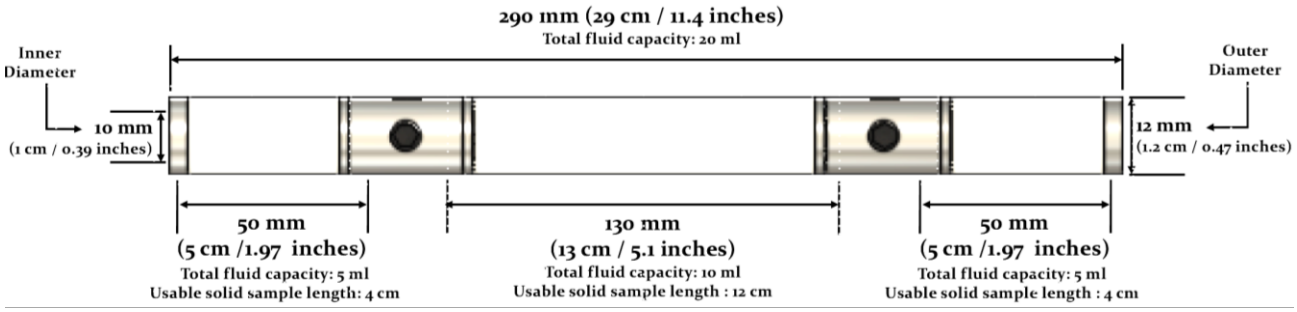

Figure 1: Proposed Mini-Lab to be used with measurements

2) List of Proposed Experiment Samples (Fluids and Solids to be Loaded in the Mini-lab):
We propose the following fluids/solids, and valve configuration (at time of loading):

Volume 1

Volume: Load-dry - solids

Sample: *E. coli* K-12 ΔfimA (Keio Collection strain JW1881 or equivalent); Freeze-dried

☐ Open

✓ Close at load

Volume 2

Volume: Solids; Approximately 5mL filled to capacity

Sample: 6 streptavidin-coated glass chips (8mm x 8mm) functionalized with biotinylated antimicrobial peptide (2x LL-37, 2x Magainin I), 2x streptavidin coat only, and 2x no coating in a carrier in Sterile Phosphate-Buffered Saline (pH 7.0) containing 1-5 mM BS3 (bis(sulfosuccinimidyl)suberate) crosslinker

Volume 3

Volume: 5mL; Filled to capacity

Sample: 10% Neutral Buffered Formalin (NBF), with approximately 3.7% formaldehyde in phosphate buffered saline

☐ Open

✓ Close at load

3) Special Handling and Thermal Control During Transportation

Table 1: Request for Thermal Control

		Refrigeration	Ambient
Pre-flight	From Pomona to Rhodium in Houston		**X**
	At Rhodium until Handover to NASA		**X**
Flight	Handover to NASA Until Arrival at ISS		**X**
	Onboard ISS		**X**
Post-flight	From ISS until Arrival at Rhodium		**X**
	At Rhodium to Pomona		**X**

4) Proposed Timeline of Crew Interaction Days (CID) and actions:

Table 2: Requesting Crew Interaction

Allowed Day	Requested Interaction
A=0	None Requested
A+2	None Requested
U-14	None Requested
U–5	Open Valve A. Shake gently for 15 seconds not to exceed 30 seconds
U-2	Open Valve B. Shake gently for 15 seconds not to exceed 30 seconds

Figure 2: Type 3 Configuration: Chamber 1, freeze-dried *E.coli*; Valve A closed; Chamber 2, AMP chips and PBS/BS3 Solution; Valve B closed; Chamber 3, formalin.

Section IV: The Question to be Addressed by the Experiment

Background
Bacterial biofilms are a persistent feature of the International Space Station, with microbial surveys repeatedly detecting surface-associated communities of Staphylococcus, Bacillus, and, Enterobacteriaceae on cabin walls, air filters, and water-handling equipment (Vaishampayan & Grohmann, 2019; Marra, Daniele, et al, 2023). These biofilms exhibit altered growth patterns and increased antimicrobial-resistance compared with Earth controls, contributing to equipment degradation and posing health risks to crew members (Vaishampayan & Grohmann, 2019). Antimicrobial peptides (AMPs)—small, cationic proteins that selectively bind to negatively charged bacterial membranes through electrostatic interactions—are increasingly used as capture molecules in optical biosensors for rapid microbial detection, making them promising candidates for spacecraft contamination monitoring systems (Cuntín-Abal et al, 2024). While AMPs have proven effective in Earth-based biosensors, it is unknown whether bacterial attachment to AMP-functionalized surfaces changes under microgravity conditions where sedimentation and buoyant convection are absent.

Research Question
Does microgravity alter the attachment efficiency of *Escherichia coli* to antimicrobial-peptide biosensor surfaces compared with 1g controls?

Hypothesis
Microgravity will reduce the attachment efficiency of *E. coli* to antimicrobial peptide-coated surfaces compared with 1g controls, because sedimentation and buoyant convection are absent and bacterial transport becomes purely diffusion-limited, supplemented only by flagella-driven swimming.

Rationale
In a liquid environment in 1g, bacteria encounter surfaces through a mixture of gravity and active swimming. AMPs that are immobilized on the surface will bind to bacterial cells upon contact. Bacterial attachment to the AMP surfaces depends on the transport mechanisms that deliver cells to those surfaces. The three mechanisms that affect the transport are sedimentation, buoyancy-driven convection, and molecular diffusion and then supplemented by bacterial swimming. These mechanisms work together to create cell-surface encounter rates in gravity, with sedimentation alone continuously pushing bacteria to horizontal surfaces even in still fluid.

In microgravity, density difference between bacteria and surrounding fluid no longer produces settling forces, and thermal gradients no longer drive bulk fluid circulation. Only two transport mechanisms remain: molecular diffusion and active bacterial swimming. Therefore, bacterial transport in microgravity is effected by passive diffusion with active self-propulsion. We hypothesize the reduction in transport mechanisms alters how quickly bacteria encounter surfaces. Diffusion and swimming alone cannot deliver bacteria to AMP-coated surfaces as efficiently as the combined mechanisms present on Earth, resulting in lower bacterial capture density during identical exposure periods.

Expected Result and Impact
The experiment will produce actionable findings regardless of outcome.

I. If microgravity significantly reduces bacterial capture efficiency of immobilized AMPs, it demonstrates that diffusion-limited transport constrains surface colonization rates in orbit. This finding would require spacecraft biosensors to be redesigned with longer exposure times, higher AMP surface densities, or modified detection thresholds to compensate for reduced cell-surface encounter rates. It would also inform contamination-control models for predicting biofilm formation rates and guide prevention strategies for long-duration missions.
II. If bacterial capture remains statistically equivalent across gravity conditions, it validates that AMP-based biosensors maintain their detection performance in microgravity without recalibration. This outcome would eliminate the need for gravity-specific calibration and support immediate deployment of current Earth-tested biosensor designs for spacecraft contamination monitoring. It would also suggest that bacterial swimming compensates adequately for the loss of sedimentation and convection.

Either result provides essential data for developing reliable microbial detection and biofilm prevention strategies in reduced-gravity environments, directly addressing a documented threat to crew health and mission success.

Section V: Experiment Design & Procedures

Hardware
Rhodium RhFET-01 Type-3 configuration with three isolated chambers and two crew-operated valves, enabling precise temporal control over bacterial activation and chemical fixation entirely in microgravity.
Chamber 1 contains freeze-dried *E. coli* K-12 ΔfimA bacteria loaded as a dry solid. The bacteria remain metabolically inert during pre-launch and ascent, ensuring activation begins only after Valve A opens on orbit.
Chamber 2 houses 6 streptavidin-coated glass chips functionalized with biotinylated antimicrobial peptide (2x LL-37, 2x Magainin I), 2x streptavidin coat only, and 2x no coating in a carrier four immersed in sterile PBS containing 1-5 mM BS3 (bis(sulfosuccinimidyl)suberate) crosslinker. BS3 is a water-soluble, amine-reactive crosslinker that forms stable covalent bonds between the cationic primary amines on the AMPs and the primary amines on the bacterial surface proteins. The chips remain stationary throughout the mission, providing stable surfaces for bacterial attachment.
Chamber 3 contains formalin as 10% Neutral Buffered Formalin (NBF), which contains approximately 3.7 % formaldehyde in phosphate buffered saline, filled to capacity. When Valve B opens on Day U-2, formalin flows into Chamber 2, killing bacteria and cross-linking surface-bound cells in place. Formalin fixation preserves the microgravity attachment state during descent and post-flight handling, preventing gravitational sedimentation or additional attachment events from altering the experimental outcome.
Valve A (between Chambers 1 and 2) opens on Day U-5 to initiate bacterial rehydration and mix with biosensor chip with shaking for 15 seconds
Valve B (between Chambers 2 and 3) opens on Day U-2 to introduce fixative with shaking for 15 seconds, preserving samples for the return transport.

Precise optimal duration (e.g., shake gently for 20 seconds) as determined during pre-flight Activation and Fixative Efficacy tests outlined below.

Antimicrobial Peptide Capture Chip Design
Glass chips (8mm x 8mm) are prepared with three layers:

Streptavidin Attachment: Glass chips are coated with Streptavidin which covalently attaches to the glass. This creates a biotin binding layer that can immobilize a dense layer of AMPs.
AMP Capture Layer: AMPs tagged with biotin molecules are attached to the streptavidin surface via biotin-streptavidin interaction, a very strong non-covalent biological bond. High AMP surface density is achieved because each streptavidin molecule presents four biotin-binding sites.
Anti-Fouling Backfill After AMP attachment, remaining streptavidin binding sites are blocked by adding biotinylated polyethylene glycol (PEG), preventing non-specific bacterial adhesion to the chip surface.

Chip Configuration
Chip 1-2: AMP-coated chips with different peptide sequences (test conditions)
Chip 3: Scrambled peptide control (non-functional sequence, measures non-specific binding)
Chip 4: PEG-only control (no peptides, defines baseline background)

Bacteria Strain Selection Rationale
The *E. coli* K-12 ΔfimA strain lacks the fimA gene and cannot produce type-1 fimbriae—adhesive protein appendages that enable bacterial attachment independent of AMP binding. Without fimbriae, observed bacterial attachment occurs exclusively through AMP-mediated interactions. This strain allows the experiment to isolate microgravity as the independent variable by eliminating fimbriae attachment, a variable unrelated to AMP-coated surfaces.

Experiment Design

Preflight
Pre-flight testing under normal gravity will confirm that every critical step—activation, fixation, and measurement—works reliably before the experiment is integrated for launch. Five short validation tests establish baseline performance and analytical specificity for the full system.

Biological Viability and Activation: The freeze-dried E. coli K-12 ΔfimA strain will be rehydrated in PBS/BS3 solution to confirm that the cells return to a viable state and reach population levels consistent with laboratory controls.
Fixative Efficacy: This test determines how long the neutral buffered formalin (NBF) must remain in contact with the samples to completely inactivate the bacteria while preserving their attachment to the AMP surface.
BS3 Crosslinking Optimization: The optimal concentration of BS3 (1-5 mM range) and exposure duration will be determined to maximize AMP-bacteria covalent bonding.
Antifouling and Specificity Validation: AMP-coated chips will be compared directly with PEG-only control chips to confirm that any signal measured after ELISA processing comes from AMP-specific capture rather than nonspecific adhesion to the surface.
Detection Probe Optimization: The concentrations of the primary anti-E. coli antibody and the enzyme-linked detection reagents will be adjusted to produce the highest signal-to-noise ratio while keeping background low.
Standard Curve Establishment: Finally, known concentrations of E. coli will be applied to AMP-coated chips and processed through the complete ELISA workflow. Measuring the resulting optical density at 450 nm creates a reference curve linking color intensity to the actual number of bacteria attached. Using this relationship, post-flight absorbance readings can be converted into bacterial surface density (cells per cm^2).

Completion of these five tests will verify that activation, fixation, and analytical quantification meet acceptable tolerances and that the experiment is fully qualified for microgravity execution.

In-flight
In-Flight Procedure: Chips remain fully immersed in the rehydration and crosslinking solution throughout the capture interval. Bacteria are released and introduced to solution, then fixed with a fixative at least two days before undocking. The experiment uses identical AMP-coated chips and PEG-only control chips that will be analyzed using a sandwich ELISA.

Ground Control isolates the effect of gravity by replicating identical tests on earth. Ground control FME unit is loaded with the same bacterial strain, AMP-coated chips, buffer, and

formalin concentration as flight unit. Ground controls are kept in the same liquid configuration and all handling procedures, exposure duration, fixative timing, and mixing protocols are kept the same, Exact duration subject to pre-flight validation.

Post-flight Data Collection
The chips are removed from each mini-lab and fixed in separate wells on a microplate. Bacterial density on each chip is measured using a two-antibody sandwich ELISA with biotin-streptavidin amplification.

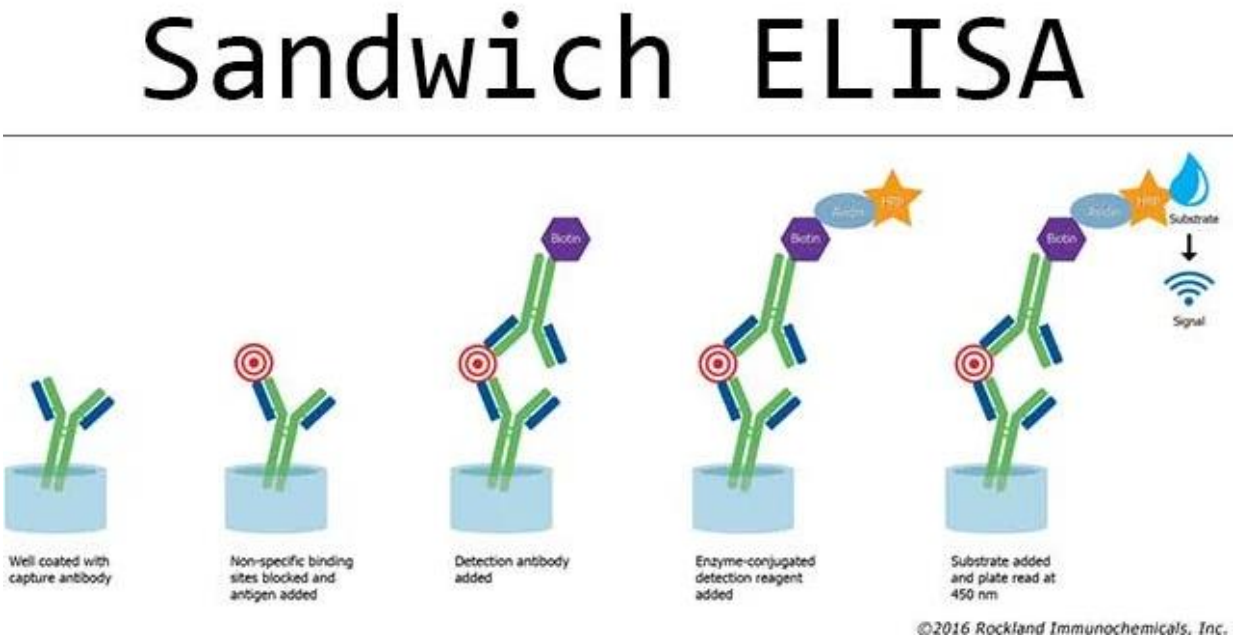

Figure 1: Aryal, Sagar, PhD. "Sandwich ELISA- Steps and Advantages." *Microbe Notes*, 10 Aug. 2021, microbenotes.com/sandwich-elisa-steps-and-advantages.

Chips are processed using a sandwich ELISA with streptavidin-HRP amplification. An anti-E. coli primary antibody binds to LPS on captured bacteria, followed by a biotinylated secondary antibody. Streptavidin-HRP conjugate then binds to the biotin tags (the streptavidin coating on the chip surface does not interfere as all biotin-binding sites were saturated during pre-flight preparation with biotinylated AMPs and PEG). TMB substrate is added, producing a blue color that converts to yellow upon addition of stop solution. Absorbance is measured at 450 nm, with optical density directly proportional to bacterial capture density. Unbound reagents are removed by washing between each step.

This sandwich ELISA approach ensures highly sensitive, specific, and quantitative detection of bacteria captured by AMP surfaces, enabling detection of differences in bacterial attachment between microgravity and 1g conditions.

Post-Flight Data Analysis
Quantification is measured by light absorbance at 450 nm using a plate reader. Optical density is proportional to the number of bacteria attached to the chip. Background signal from PEG-only control chips is subtracted. Corrected optical density values are converted to bacterial capture density (cells/cm^2) using the pre-established standard curve.

Success Criteria
Success is defined by either (1) statistically significant difference in bacterial capture density between microgravity and 1g, indicating transport physics affects AMP-mediated attachment, or (2) statistical equivalence, indicating AMP biosensors perform reliably across gravity conditions. PEG-only controls must show low background signals relative to AMP-coated chips, confirming measured signal reflects AMP-specific bacterial capture.

Design Rationale
Using the ELISA provides a sensitive method to quantify bacterial capture, with absorbance at 450 nm directly reflecting the number of bacteria attached to AMPs during microgravity exposure. The design isolates microgravity as the independent variable by ensuring all other factors remain constant: identical AMP-coated chips, bacterial strain and concentration, PBS composition, exposure duration, and temperature are maintained across flight and ground samples. The only difference is the gravitational environment. Both the BS3 solution and the fixative act as crosslinkers between the bacteria and the AMPs. These preservation methods lock captured bacteria in place throughout transport between microgravity and Earth environments, ensuring microgravity remains the only variable affecting the samples.

Section VI: References

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Section VII: Letter of Certification by the Teacher Facilitator

11/4/2025

I certify that the student team designed the experiment described herein and authored this proposal, and not a teacher, parent, or other adult. I recognize that the purpose of this letter is to ensure that there was no adult serving to lead experiment definition and design, or write the proposal, and thereby provide content and/or professional expertise beyond that expected of a student-designed and student-proposed experiment.

I also understand that NCESSE recognizes that facilitation of thinking across the student team through advice and counsel by the team's Teacher Facilitator, other teachers, and local area and national researchers, is not only to be encouraged but is absolutely vital if students are to receive the necessary guidance on the process of scientific inquiry, experimental design, how to do background research in relevant science disciplines, and on writing the proposal. I also understand that it is appropriate for the Teacher Facilitator and other teachers to provide editorial comment to the student team on their proposal drafts before proposal submission.

I also certify that the samples list and the special handling requests listed in this proposal are accurate and conform to the requirements for SSEP Mission 21 to ISS. I confirm that the team, after reviewing their procedure and budget for obtaining the samples for the experiment, is certain that they will be able to obtain the necessary samples for their experiment in time to meet the deadline for shipping the flight-ready RhFET-01 to Rhodium.

Finally, I certify that the student team will have access to the proper facilities and equipment to prepare the RhFET-01 for flight and to analyze the samples after the flight.

Michael Pham
Teacher Facilitator