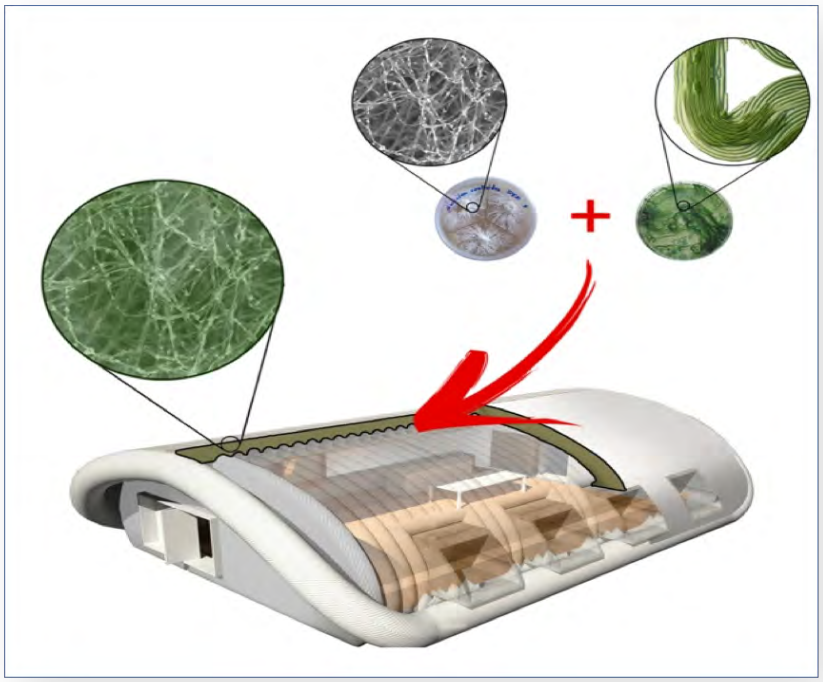
Myco-architecture off planet: growing surface structures at destination NIAC 2018 Phase I Final Report



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Interior of Commons, materials include compacted regolith, and mycotectural building assemblies, textiles, and millwork surfaces fabricated on site from minimal up-massed resources. Credit: Chris Maurer, redhouse

1 Abstract

A turtle carries its own habitat. While it is reliable, it costs energy. NASA makes the same trade-off when it transports habitats and other structures needed to lunar and planetary surfaces increasing upmass, and affecting other mission goals. Imagine a self-pitching habitat made of a light, fibrous material, with excellent mechanical properties. The material could be used dry, wet, frozen with water or as part of a self-produced composite which could allow such enhancements as radiation protection and a vapor seal. It is self-replicating so the habitat could be extended at a future date, and self-repairing. Some form of this material could be used for a habitat at destination, additional buildings, the shell of multiple rovers and furniture. The fibrous material is fungal mycelium, the vegetative structure of fungi consisting of branching, thread-like hyphae. Mycelial materials, already commercially produced, are known insulators, fire retardant, and do not produce toxic gases. Metrics for these materials show compression strengths superior to dimensional lumber, flexural strength superior to reinforced concrete, and competitive insulation values. As mycelia normally excrete enzymes, it should be possible to bioengineer them to secrete other materials on demand such as bioplastics or latex to form a biocomposite. Mycelia are more flexible and ductile than regolith alone. As a standalone material or in conjunction with agglutinated or sintered regolith, a mycotectural building envelope could significantly reduce the energy required for building because in the presence of food stock and water it would grow itself. After the arrival of humans, additional structures could be grown with feedstock of mission-produced organic waste streams. Melanin-rich fungi have the ability to absorb radioactivity suggesting that melanized fungal mycelia could provide radiation protection. Lead found in the regolith, or other radiation blocking materials such as water could accumulate in the mycelia providing additional radiation protection. When protected, the mycomaterials can have a long life, but at the end of its life cycle the material could become fertilizer for mission farming.

Our concept fits within the Mars DRA 5.0 “commuter” scenario, with the major difference being that the habitats and the shells of the rovers would be built at destination. On Earth, a flexible plastic shell produced to the final habitat dimensions would be seeded with mycelia and dried feedstock and the outside sterilized. At destination, the shell could be configured to its final inner dimensions with struts. The mycelial and feedstock material would be moistened with Martian or terrestrial water depending on mass trade-offs, and heated, initiating fungal (and living feedstock) growth. Mycelial growth will cease when feedstock is consumed, heat withdrawn or the mycelia heat-killed. If additions or repairs to the structures are needed, water, heat and feedstock can be added to reactivate growth of the dormant fungi.

The proposed work focuses on filling select key technical knowledge gaps such as the temperature range of mycelial growth, potential for algal feedstock, potential for enmeshed biosensors, mass of inputs and finished product, and material properties of the resulting materials. The potential for enhancing structural and sensing capabilities by the incorporation of the bacterium *Bacillus subtilis*, is novel. Architectural design concepts based on this vision will be examined for use in a mission context including mass trade-offs, and temperature inputs, as well as suggesting new terrestrial routes to infusion where rapidly built, lightweight structures are desired. If successful in developing a biocomposite material that can grow itself, NASA will have a radically new, cheaper, faster lighter material for designing habitats for extended duration lunar missions, Mars missions, and mobile habitats as well as furniture and other structures.

2 Background

2.1 The Problem

A turtle carries its own habitat. While it buys reliability, it costs energy. NASA makes the same trade-off when it transports habitats and other structures needed to lunar and planetary surfaces "on the back" of its missions. Astronauts stayed on the lunar surface for up to 75 h (Apollo 17), so the lunar module (LM) could double as a habitat. An example of the "build it on Earth, launch it into space" approach is the Habitat Demonstration Unit (HDU) Deep Space Habitat, developed by the Habitat Systems Project (NASA AES). The hardware consists of a composite fiberglass resin-infused shell attached to eight steel ribs, providing living and working space for a crew of four. Even with the use of advanced materials, it weighs >14,000 kg. With this approach, upmass and resupply problems will result in minimal surface operations, greater mission risk, loss of productivity and psychological stress.

The solution is an *in situ* resource utilization (ISRU) approach (TA07, Colladay et al., 2012) and sustainable manufacturing (TA12). NASA's Centennial Challenges program sponsors a Mars 3D printed Habitat design challenge (www.nasa.gov/3DPHab) Top designs used *in situ* resources, focusing on agglutinated regolith or even frozen water. A vapor barrier was required and a robotic infrastructure for building. While regolith and ice have advantages as building materials and are compatible add-ons to our concept, regolith has disadvantages including high mass, rigidity, poor thermal insulation, massive energy demand, and a dedicated infrastructure required for production.

2.2 The innovation

Imagine a structure made of a light, fibrous material, with excellent mechanical properties. The material could be used dry, wet, frozen with water or as part of a self-produced composite which would allow such enhancements as radiation protection and a vapor seal. Even better, it is self-replicating so the habitat could be extended at a future date, and thus also be self-repairing. Some form of this material could be used for a habitat at destination, furniture, storage, additional buildings, and the shell of multiple rovers. To source the fibrous material, look no further than a garden for fungal mycelia, the vegetative structure of fungi consisting of branching, thread-like hyphae. "Mycotecture" is the term developed by the artist Phillip Ross[1] to describe building with mycelia. Mycelial materials are known thermal insulators, fire resistant, and unlike plastics, do not outgas. They are more flexible and ductile than regolith alone. The density and material properties are tuned during production. As a standalone material or in conjunction with agglutinated or sintered regolith, a mycotectural building envelope could significantly reduce the energy required for building because in the presence of food stock and water it would grow itself. After the arrival of humans, additional structures could be grown with feedstock of mission-produced organic waste streams including inedible plant or soil components, or human waste. Melanin-rich fungi have the ability to absorb radioactivity (e.g., Dadachova et al., 2007), even at Martian and space levels (Onofri, et al., 2008) meaning that melanized fungal mycelia provide radiation protection. Lead found in the regolith could accumulate in the mycelia providing additional radiation protection. When protected, the mycomaterials can have a long life, but at the end of its life cycle the material could be become fertilizer for mission farming or the production of new mycomaterials.

[1] https://vimeo.com/208513596 for an overview produced by Ross on the capabilities of mycelia, and a popular article extolling the virtues of mycotecture https://gizmodo.com/the-technology-that-will-build-our-future-may-be-found-1693612047.

We would supplement our mycomaterials with bacteria with which they would form a mutualistic relationship. These bacteria would provide three functions. First, they would supplement the structural integrity of the mycotectural envelope through bio-mineralization, polymer production and filament formation. Second, they would act as an intelligent input (biosensor) in the mycomaterial synthesis process detecting pressure and flaws in the mycotectual structural integrity by measuring mechanical strain, and reporting anomalies through color change or fluorescence. Third, they could provide to, and receive from, the fungal mycelia, essential metabolites to speed up the growth of the shell. These bacteria would be a flight hardy spore former such as *Bacillus subtilis*.

If we succeed in developing a biocomposite material that can grow itself, we will provide NASA with a radically new, cheaper, faster lighter material for habitats for extended duration lunar and Mars missions, as well as furniture and other structures. While our habitat shell is designed to be inert, it could be used in a living state participating actively in waste recycling similar to a living roof. The long-term goals of this concept would be to create a living shell that functions beyond structure and warmth; where the organisms can be manipulated to perform tasks like: self-healing, humidity regulation, energy production, nutrient production, and bio-luminescence. Such living architecture was demonstrated by a five-story Bio Intelligent Quotient building in Hamburg, Germany (Wallis, 2013) showing that this approach can scale.

2.3 Credible and Reasonable

Mycoworks®, founded by Ross, and Ecovative Designs® are biomaterial manufacturers. Their products utilize saprotrophic fungi that consume dead matter like saw dust or agriwaste. Fungi digest nutrients by secreting enzymes that break down material for uptake into its cells. Similar to glue in laminated veneer lumber or cement in concrete, the mycelial hyphae act as a binder to the material being digested, and when pressure is applied, a very strong, lightweight building material is created. Such fungi could be bioengineered to secrete other binding agents such as transgenic glues or plastics. Metrics for these materials have shown compression strengths superior to dimensional lumber, flexural strength superior to reinforced concrete, and competitive insulation values. The sample shown to the left is one of many combinations of fungi and organic substrates grown and tested by our team. This 8”x 8”x1” board is composed of *Grifola fondosa* mycelium grown in sawdust (Fig. 1, left). It has strengths comparable to commercially available Oriented Strand Boards or plywoods, but unlike them is fire-resistant and thermally insulating. Ecovative Designs manufactures MycoBoard™, a mycelial wood (density 42.74 lbs/ft^3) whereas fiberglass and polyester composites are 95.04 lbs/ft^3.[2].The panels are strong, machinable, customizable and Class B fire-resistant rated. Current applications are for furniture (work surfaces, molded furniture, seatbacks), architectural panels, door cores, cabinetry. Ecovative produced this material for the chassis of a biodegradable drone for Rothschild’s lab.[3]

[2] http://www.performancecomposites.com/about-composites-technical-info/122-designing-with-fiberglass.html

[3] http://2014.igem.org/Team:StanfordBrownSpelman/Building_The_Drone

Mycofoam is similar in function to styrofoam. Uses include packaging, insulation and acoustic tiles. It can be stained to look like wood, a potential psychological benefit for astronauts on long-duration missions. While mycelial products can be used as is, we envision them also providing fibers for composites. Consumer products include boats, skis, agricultural machinery and cars.[4] Natural fibers have been used in infrastructure.[5] They can replace glass in fiber-reinforced plastics.[6] Thus, mycelial-based composites could add material properties.

Bacillus subtilis makes an ideal natural counterpart to supplement the fungal mycelium. The bacterium is naturally able to produce a range of polymers with adhesive properties, such as levan, which could further bind fungal mycelia together. The organism can also be engineered to produce a fibrous morphology (through deletion of the *yneA* gene) which will provide reinforcing 'cross' fibers to further support the mycelial-based composite. The biocementing ability of the organism is also potentially of use, since the precipitation of calcium carbonate may also enhance the structure properties of the mycelial panels. Whilst the structural properties of *B. subtilis* are attractive, its use as an integral sensor will also be of benefit in reporting the structural integrity of the shell. *B. subtilis* is naturally able to sense oxygen and this ability can be linked to a reporter which will produce a color change when oxygen concentrations are low. Moreover, *B. subtilis* is able to sense pressure through a variety of mechanisms many of which are related to cell wall integrity and stress. The organism can be engineered to report on excess stress and load in the in the shell or lack of pressure and load relating to failures in the shell structural integrity.[7] *Bacillus* is well known for its ability to secrete proteins and small molecules which can act as a nutrient source for its fungal counterpart. A further possibility for fungal nutrition is the triggering of *B. subtilis* cell lysis in response to structure failures, that will promote the growth of fungal mycelium to repair tears and cracks in the structure.

2.4 The Aerospace Architecture Concept

As in Mars Design Reference Architecture 5.0 ([8]), a precursor mission would arrive with the materials for the habitat and rover shells so that it could be in place before the astronauts arrived. We envision a double-layered plastic habitat shell containing dried fungal mycelia and a dried food stock, with a similar process for the shell of the rovers. The shell could later act as a vapor barrier. To comply with planetary protection, the mycelia would be genetically attenuated prior to launch so they could not survive the Martian environment outside of the shell either in the spore or germinated state. The dried food stock could be dried nutrient medium, dried cyanobacteria or algae. The shell would be erected by releasing a lightweight folded frame as in a pop-up tent or inflated with Martian atmospheric gases. The bag would be dampened with Martian water and heated by focusing a Fresnel mirror or by creating exothermic reactions in the medium. The rise in temperature would activate growth. To produce a fruiting body (mushroom), two compatible

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[4] Westman, M.P., Fifield, L.S., Simmons, K.L., Laddha, S.G., and Kafentzis, T.A. 2010. *Natural Fiber Composites: A Review*. US Department of Energy, PNNL-19220

[5] Dittenber, D. B. and GangaRao, H.V.S. 2012. Critical review of recent publications on use of natural composites in infrastructure. *Composites: Part A* **43** 1419–29.

[6] Wambua, P., Ivens, J., and Verpoest, I. 2003. Natural fibres: can they replace glass in fibre reinforced plastics? *Composites Science and Technology* **63**:1259–64.

[7] Waters, S.M., Robles-Martinez, J.A. and Nicholson, W.L. 2014. Exposure of *Bacillus subtilis* to Low Pressure (5 Kilopascals) Induces Several Global Regulons, Including Those Involved in the SigB- Mediated General Stress Response. *Appl. Env. Microbiol.* **80**: 4788–94.

[8] Drake, B.G., ed. 2009. *Human Exploration of Mars Design Reference Architecture 5.0*. NASA/SP–2009–566-ADD

homokaryotic mycelia must join and form a dikaryotic mycelium. Thus, mushroom formation will be prevented by the loading of incompatible spores. The cyanobacteria or algae would grow releasing oxygen and sugar which would be consumed by fungal mycelia. Alternatively, the fungi could live off dry algae or other lightweight nutrient sources. As there would be no organisms in the system to degrade the mycelium, it would grow to fill the space. When the structure is done, the plastic will be tight and the heating removed. If additions or repairs are needed, additional water, heat and feedstock can reactivate growth.

As mycelia normally excrete enzymes, it should be possible to use the tools of synthetic biology to engineer them to secrete other materials on demand such as bioplastics or latex to ultimately form a biocomposite. A habitat could be further strengthened with regolith biocomposites if desired.

Figure 2. Fully encased structure self assembles when water, CO_2 and heat are added by robots.

2.5 Mission Context and Planetary Protection

In the Mars Design Reference Architecture 5.0 (Drake 2009, p. 36) the mobile home, commuter and telecommuter scenarios are imagined, with the "commuter" mission adopted as nominal. This scenario includes a centrally located, monolithic habitat, two small pressurized rovers, and two unpressurized rovers roughly equivalent to the Apollo Lunar Rover Vehicles, LRV. The nominal surface habitat is 29,447 kg (p 259, Table 6-4, Addendum.) The estimated mass of the small and large rovers is 7,500 kg and 15,000 kg respectively, not including the power system mass (Addendum, p 280.) Our concept fits well within this context, with the major difference being that the habitats and the shells of the rovers would be built at destination. We estimate a 200 m^2 (2000 sq ft) habitat, internal volume of 600 m^3, (2000 sq ft) would require a modest 781.5 kg of upmass for the bag and biology.

NASA and COSPAR are undergoing a multi-year process to develop quantitative requirements for human missions.[9] The mycotecture described here, which can be transported as contained

[9] NPI 8020.7: NASA Policy on Planetary Protection Requirements for Human Extraterrestrial Missions, references cited on https://planetaryprotection.nasa.gov/documents. Also see COSPAR Planetary

spores, and activated to grow in double containment on Mars (or other locations) would comply with planetary protection requirements for robotic or human missions, and comply with current COSPAR human mission principles and guidelines. The material could be baked to kill the structures and spores and add structural rigidity, or remain enclosed to protect from contamination. As the materials 'made' on Mars can be inactivated under containment, planetary protection compliance is unlikely to be problematic.

3 Objectives

Objective 1. Fill key technical knowledge gaps

Objective 2. Assess potential enhancements of mycelia.

Objective 3. Design mycelial-based structures including habitats

Objective 4. Identify key knowledge gaps including pathways to implementation

Objective 5. Assess impact of technology for terrestrial applications

4 Approach and Results

The use of mycotecture on Earth, its variable density, lack of outgassing, ability to tune the material, construct it through multiple routes including ones with little to no on-site infrastructure, suggest that the concept is credible for building structures on Mars. However, unknowns remain that are not readily determined, thus Phase I is intended to begin to address these with experiments and paper studies.

4.1 Objective 1. Fill key technical knowledge gaps

4.1.1 Summary

Our work focused on filling major select key technical knowledge gaps, and technical aspects to be addressed in a Phase II proposal were identified such as the secretion of glues or plastics by the fungi or bacteria to form biocomposites. Specifically, we raised the technical TRL by assessing the growth of in-house mycelial-producing strains on potential food substrates and analyzed the advantages and disadvantages to their use off planet. Growth of the mycelia on sawdust and powdered nutrients including dried, powdered algae, was used as a baseline. We measured growth as a function of temperature, tested the relevant material properties of the mycelia products produced, and measured the mass of input and output volume for production per volume of material. Mycotecture was produced in a bag simulating the proposed mission implementation. Post-production such as heat treatment (as is done with terrestrial mycotecture) was assessed.

The material properties of the dried, frozen and baked mycelial outputs included density, strength, thermal insulation and flame retardation. Tensile/compression testing on biocomposite material specimens was performed to obtain three-dimensional elastic constants, as well as to examine deformation and fracture behavior. These mechanical tests were performed on both dormant and activated samples to understand the evolution of the structural material. In addition, experimental data obtained from the mechanical testing was used to build a failure prediction model that accounts for material anisotropy. This aided in future structural design through a quantitative

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Protection Policy as amended 2011 https://cosparhq.cnes.fr/sites/default/files/pppolicy.pdf In February 2018, COSPAR, with support from ESA and NASA, will host the 2nd COSPAR workshop on refining the planetary protection requirements for human missions.
https://planetaryprotection.nasa.gov/COSPARHumans2018

understanding of the mechanical limits of the material. To examine the texture, defects, fracture surfaces of the material, high-resolution microstructural imaging was utilized before and after the mechanical tests. This microstructural analysis informed us about the macro-structural behavior and influenced structural design. Thermal decomposition analysis was also performed to understand the thermal limits.

4.1.2 Mycelium Material & Habitat Development

The primary species of fungus that was used by the Stanford-Brown-RISD iGEM team was *Ganoderma lucidum*, unless otherwise noted. *Ganoderma lucidum* was chosen as advised by PhD Candidate Rolando Perez, of the Drew Endy Lab at Stanford University. Perez noted that of the numerous species of fungus he has worked with, *G. lucidum* has by far produced the strongest mycelial materials, and grew at the quickest rate. Perez provided the Stanford-Brown-RISD Team with the initial *G. lucidum* plate of mycelia as well as a live fungal culture to initiate the team's own mycelium production.

We obtained wild type *Aspergillus oryzae* from the Fungal Genetics Stock Collection (catalog number FGSC #A815) because of its ease of genetic engineering. This was also a result of our collaboration with the 2018 DTU iGEM team, which selected *A. oryzae* as the primary fungal species for their project.

Finally, we purchased *Mucor circinelloides f. circinelloides* (American Type Culture Collection catalog number 20132), a strain known to produce high levels of saturated lipids, compounds rich in hydrogen. As a high concentration of protons should help protect from neutrons and charged particles found in cosmic radiation. Hydrogen or hydrogen-rich materials are ideal materials for radiation shielding because hydrogen does not easily break down to form a secondary radiation source.

4.1.3 Plating and growing cultures of mycelia

Mycelium is able to grow on almost any substrate, so long as the substrate contains sufficient nutrients and is not toxic to the fungus. Though various substrates were tested, a standardized substrate is required to observe the growth of mycelium in different conditions. It has also been found that the growth medium of Potato Dextrose Yeast Agar (PDYA) provides the optimal nutrients for mycelium growth,[10,11] without providing excessive nutrients that will encourage bacterial growth and contamination. The solid PDYA growth medium used in the lab consisted of 2.0% dextrose, 0.10% potato extract, 0.15% yeast extract, 1% agar, and 96.50% water. When growing the mycelium as a liquid culture, the liquid PDY growth medium is composed of 2.0% dextrose, 0.10% potato extract, 0.15% yeast extract, and 97.50% water. The mycelium can then begin to grow on the PDYA medium by extracting a small piece from the live fungus onto the plate, as shown in Figure 3:

[10] Fungal cell wall - an overview | sciencedirect topics. (n.d.). https://www.sciencedirect.com/topics/immunology-and-microbiology/fungal-cell-wall

[11] Hashimoto, M., Ikegami, T., Seino, S., Ohuchi, N., Fukada, H., Sugiyama, J., … Watanabe, T. 2000. Expression and characterization of the chitin-binding domain of chitinase a1 from *Bacillus circulans* wl-12. *Journal of Bacteriology*, 182(11), 3045–3054. https://doi.org/10.1128/JB.182.11.3045-3054.2000.

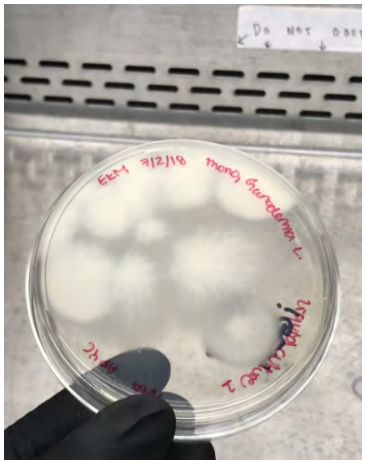
A  B  C 

Figure 3. A, Live culture of Ganoderma lucidum *with fruiting bodies. B, Mycelium plate of* G. lucidum*. C, Microscopic image of* G. lucidum *mycelium, taken by Leo Penny & Lynn Rothschild.*

Once a culture of mycelium is well established, pieces of mycelium may be extracted from a single source plate and transferred to other plates of PDYA. This reduces the potential for contamination of mycelium plates as it reduces the chances of bacteria from the live fungus to be transferred onto the plates of PDYA.

Two forms of *G. lucidum* were grown; one was the monokaryon, and the other was the dikaryon. A monokaryon is a fungal cell which has only one nucleus. In contrast, a dikaryon is a fungal cell that has two genetically distinct nuclei, although they are allelically-compatible. These two species were provided courtesy of Perez. Based on the analysis done by Perez's lab the dikaryon variant grows at greater speeds and produces composite materials with greater strength. The monokaryon was grown in addition to the dikaryon as it was easier sample to isolate a sample as it was grown in a petri dish, while in contrast the dikaryon first had to be grafted from a live mushroom that was gifted to us.

4.1.4 .Substrate Tests

To further investigate the potential for using mycelium to grow materials and structures both on and off planet, the growth of mycelium on varied substrates was tested. Noteworthy substrates with exceptional material and environmental promises are yard waste, sawdust, used ground coffee beans, and other forms of food waste. Even more importantly, the mycelium successfully grew on lunar and martian regolith simulant with minimal nutrients added in the form of PDY. A series of images with all the various substrates that the *Ganoderma lucidum* mycelium was grown on is shown in Figure 4.

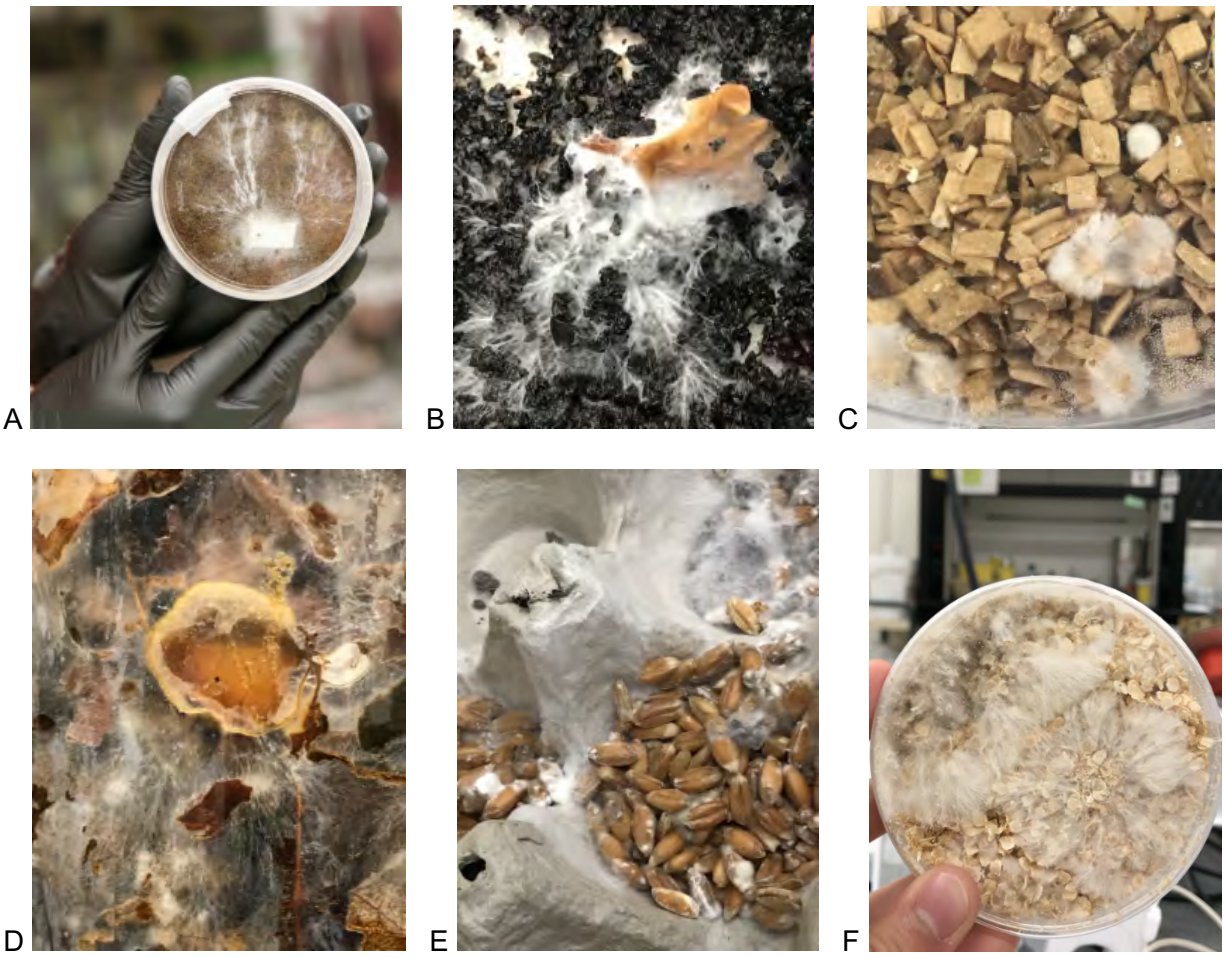
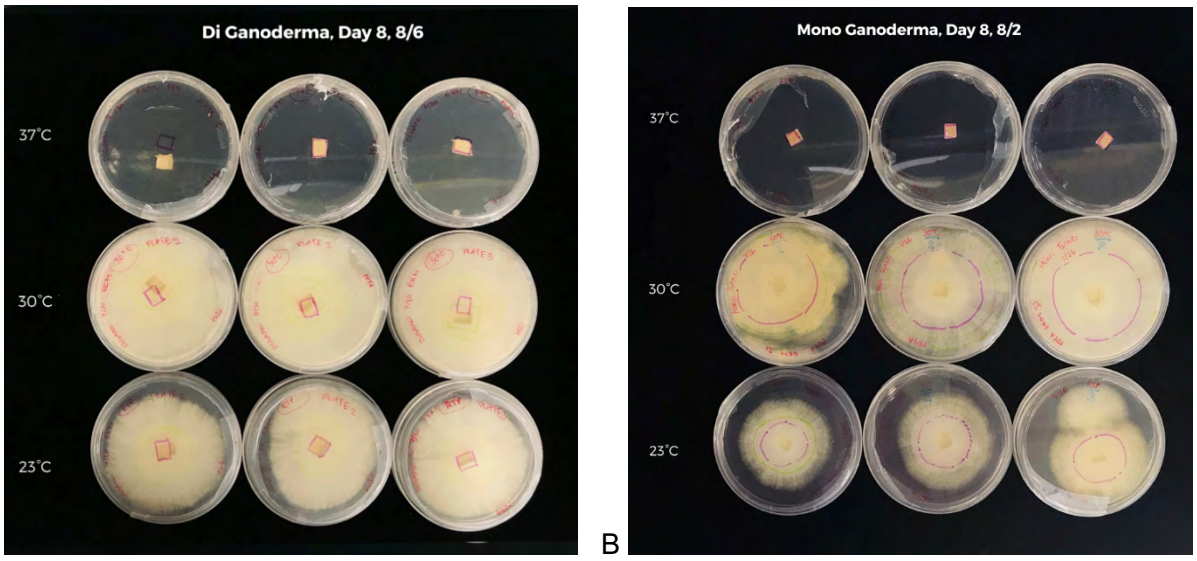


Figure 4. Growth of Ganoderma lucidum *on various substrates: A, Martian regolith simulant. B, Used coffee grounds. C, Discarded wood chips. D, Yard Waste. E, Mixed seeds in an egg carton. F, Quinoa flakes.*

The results of our substrate tests indicate that the mycelium can grow on any organic material—even Martian regolith mixed with supplemental nutrients. We did find, however that there were factors that impacted the success of mycelium colonization. First the moisture level greatly impacted how quickly the mycelium could grow and whether it entered a dormant state or not (entering it if the material is too dry). Second the particle size also impacted colonization rate; with particles that were too fine or too large being harder to colonize than particles of a medium size.This information was important to formulating our mission architecture; helping us to determine what could be used as a substrate on the Moon and on Mars and how coarse or fine the particles of the substrate would need to be.

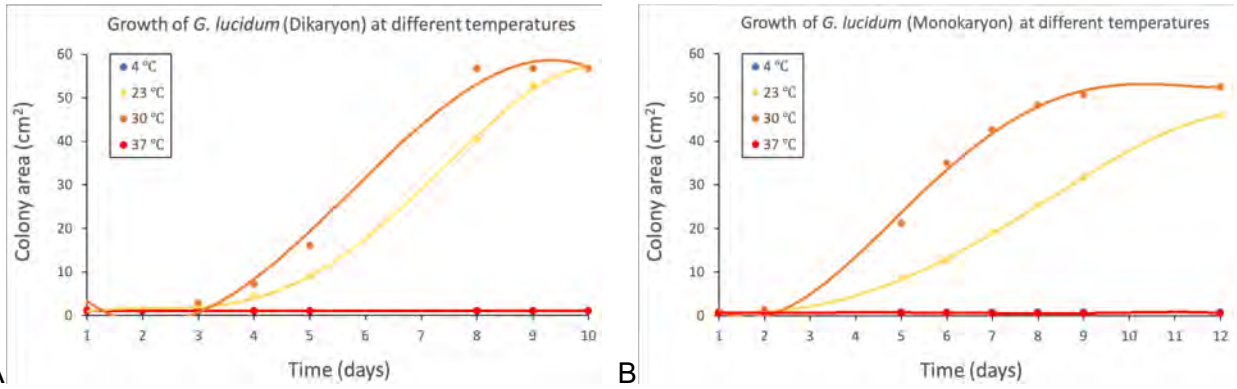
4.1.5 Temperature Tests

To further understand the growth properties of the mycelium and predict its growth patterns in different environments (e.g., the Moon or the Martian environment), temperature tests were conducted. Based on already existing literature on mycelium, it has been suggested that optimal temperature conditions range from 23°C to 30°C. With this in mind, the growth rate of mycelium at the following temperatures were measured: 4°C, 23°C, 30°C, and 37°C. The surface area of each plate of mycelium in each temperature condition measured each day over the course of 20 days.



A B

Figure 5. Mycelial growth of Ganoderma lucidum *dikaryon (A) and monokaryon (B) on PDYA were all plated on the same day and put at each temperature condition.*



A B

Figure 6. Growth dynamic of Ganoderma lucidum *Dikaryon (A) and Monokaryon (B) under different temperatures, as measured by colony area (cm^2).*

Based on the results of our temperature test (Figs. 5 and 6), we concluded that the optimal growth temperature (based on the temperature settings available to us) was 30 °C and that the dikaryon variant grew faster than the monokaryon variant. The dikaryon grew at approximately 7.7 cm^2 per day at 30 °C while it only grew 7 cm^2 per day at 23 °C. The monokaryon grew at approximately 5.6 cm^2 per day at 30°C while it only grew 4.2 cm^2 per day at 23 °C. Our results also allowed us to conclude that at temperatures close to 4°C and 37°C growth is limited, as there was little to no change in area. This information was important as it also informed our mission architecture. Because of the low temperatures on the Moon and on Mars and the slower growth rate of mycelium at low temperatures, we realized we needed to configure a heating system into the design of the habitat during the growth period.

4.1.6 Growing Mycelia in Molds

We grew our mycelium products using a procedure we developed and standardized through running of a variety of different experiments and conducting preliminary research with DIY (Do-It-

Yourself) mycelium growth kits that are sold by Ecovative on their website[12]. The kits we purchased were aspen, hemp, and kenaf.

Our procedure began with the preparation of the substrates for inoculation with mycelium. To begin, we ground the substrate into a loose particles (if it did not come in that form). We then sterilized the substrate and any containers we planned to use with the autoclave.

After the autoclave process was complete we moved the substrate, the containers, sterilized scalpels, Whirlpack® bags which are supplied sterile and sealed, and Petri dishes of mycelium under the laminar flow hood. We then divided the substrate among the Whirlpack® bags. We then sealed the bags and removed them out from under the hood. After removal we weighed the mass of the bags and recorded it on the front of the bag. After re-sterilizing with ethanol, the bags were placed back under the laminar flow hood. We then calculated and added a specific amount of PDY (Potato-dextrose-yeast) to each of the bags containing the substrates (this was done to provide the mycelium with an easy substrate to break down - in the case of Ecovative they used regular flour). We experimented with different ratios between weight and PDY to find the best amount to add - while we were unable to generate a specific ratio by the end of our experimental time frame, the general consensus was the material should be damp but not soaked. Once the PDY is added to the substrate plates of mycelium were added. These plates were first divided into grids before being mixed in with the substrate in the bag. The larger the amount of substrate the larger the number of plates were added.

Once the bags were filled, they were sealed - making sure to leave plenty of air within the bag - and removed from the autoclaves. The bags were then placed in the incubator at 30 °C for 1-2 weeks, depending on the rate of growth of mycelium into the substrate. Once there was a decent amount of mycelium grown the material was remixed under the laminar flow hood, in order to distribute the mycelium more evenly, and then packed into molds (note that some bricks were also grown just leaving the mycelium to grow until the form was filled). These bricks were then left to fit, some in the 30 °C incubator and others in a room temperature growth chamber, for another 1-2 weeks.

Once the mycelium had digested the substrate enough to form a solid block, it was removed from the mold, weighed, and baked at 120 °C for several hours. Once the mycelium block was 30% of its original weight it was considered finished material.

Using the procedure described above combined with the results of the previous tests we were able to produce several bricks and small pieces with different material substrates (Figure 7).

[12] Ecovative GIY. Grow It Yourself. https://giy.ecovativedesign.com/

A 

B 

C 

D 

Figure 7. A, stool after 2 weeks of growth, prior to being baked. B, iGEM team member Emilia Mann sitting on the stool after it has been baked. C, iGEM team member Javier Syquia holding the first prick produced by the team. D, pile of bricks produced with mycelium and yard waste, wood chips.

4.1.7 Mycelium Glue

In designing our mycelium specific bioadhesive candidates, we looked for protein-based adhesive molecules that could be easily produced in *E. coli* and *B. subtilis*. We excluded highly glycosylated adhesive proteins as well as proteins with other significant post translational modifications (PTMs) because we wanted to start with simpler ones. The last component of our criteria for identifying mycelium specific bioadhesive candidates revolved around the composition

of mycelium itself. All fungus, mycelium included, contains an outer cell wall composed of chitin, which makes up the surface that would interface with the adhesive.[13]

In nature, various organisms have evolved chitin-binding-domains (CBDs) that allow them to bind tightly to the fungal cell wall.[14] One of these organisms is *Bacillus circulans* from which we borrowed a CBD sequence.[15] Our first glue candidate uses this CBD in a fusion protein of four CBD's with GSGGSG linkers in between each; we expected that this could act as a mycelium specific "cross linker" in a sense. Our construct also included a six residue polyhistidine tag and a Lumio fluorescent sequence (CCPGCCGAGG) for detection with Thermo Fisher Scientific's Lumio Detection Kit.[16]

Another protein-based adhesive candidate we investigated was csgA, the major subunit of the bacterial curli system which is secreted in monomers and self-assembles into longer polymer fibers that contribute to the biofilm formation.[17] The csgA protein has been known to be highly adhesive and have amyloid-forming properties, so it was reasoned that it would be a good starting point for bioadhesive proteins. Furthermore, the manner in which csgA fusion proteins express the functionality of the introduced polypeptides while retaining their self-assembly behavior has made them a molecule of extreme interest in engineering functional biomaterials.[18]

Our third glue candidate was a combination of the first two: a csgA-CBD fusion protein. We wanted to take advantage of the self-assembling nature of the csgA as well as its known tendency to aggregate under isoelectric conditions[19] The rationale for this fusion protein was that csgA has a strong affinity for binding its own monomers and CBD of course has a strong affinity for binding chitin. We theorized that a fusion protein with both of these domains would bond tightly to itself and to the mycelium surface.

Lastly, we decided to test the mussel foot fusion protein fp151 developed and described by researchers at Pohang University of Science and Technology.[20] The fp151 fusion protein is comprised of repeats of the most adhesive segments of marine mussel adhesive proteins (MAPs)

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[13] Fungal cell wall - an overview | sciencedirect topics. (n.d.). https://www.sciencedirect.com/topics/immunology-and-microbiology/fungal-cell-wall

[14] Hashimoto, M., Ikegami, T., Seino, S., Ohuchi, N., Fukada, H., Sugiyama, J., … Watanabe, T. (2000). Expression and characterization of the chitin-binding domain of chitinase a1 from bacillus circulans wl-12. Journal of Bacteriology, 182(11), 3045–3054. https://doi.org/10.1128/JB.182.11.3045-3054.2000.

[15] Hashimoto, M., Ikegami, T., Seino, S., Ohuchi, N., Fukada, H., Sugiyama, J., … Watanabe, T. (2000). Expression and characterization of the chitin-binding domain of chitinase a1 from bacillus circulans wl-12. Journal of Bacteriology, 182(11), 3045–3054. https://doi.org/10.1128/JB.182.11.3045-3054.2000.

[16] Lumio green in-cell detection kit - thermo fisher scientific. (n.d.). https://www.thermofisher.com/order/catalog/product/12589057.

[17] Barnhart, M. M., & Chapman, M. R. (2006). Curli biogenesis and function. ?Annual Review of Microbiology, 60, 131–147. https://doi.org/10.1146/annurev.micro.60.080805.142106.

[18] Nguyen, P. Q., Botyanszki, Z., Tay, P. K. R., & Joshi, N. S. (2014). Programmable biofilm-based materials from engineered curli nanofibres. Nature Communications, 5, 4945. https://doi.org/10.1038/ncomms5945.

[19] Zhong, C., Gurry, T., Cheng, A. A., Downey, J., Deng, Z., Stultz, C. M., & Lu, T. K. (2014). Self-assembling multi-component nanofibers for strong bioinspired underwater adhesives. Nature Nanotechnology, 9(10), 858–866. https://doi.org/10.1038/nnano.2014.199.

[20] Hwang, D. S., Gim, Y., Yoo, H. J., & Cha, H. J. (2007). Practical recombinant hybrid mussel bioadhesive fp-151. Biomaterials, 28(24), 3560–3568. https://doi.org/10.1016/j.biomaterials.2007.04.039.

1 and 5.[21] MAPs are the proteins responsible for adhering marine mussels to the rocks and other hard surfaces they live on in marine ecosystems. It is thought that much of MAPs adhesion properties' can be attributed to the high number of 3,4-dihydroxyphenyl-alanine (L-DOPA) residues, which require the hydroxylation of tyrosine. To facilitate this post translational modification, we ordered an additional DNA construct encoding the tyrosinase enzyme, which would be able to hydroxylate tyrosine residues *in vivo*. We also ordered mushroom tyrosinase which has been shown to be able to hydroxylate tyrosine in vitro post-purification.[22]

Once all five constructs were ordered, we ligated the linear DNA constructs to the PSB1C3 iGEM backbone using Gibson Assembly Mastermix 2x, transformed NEB T7 competent *E. coli* cells with our new plasmids, and plated the colonies on chloramphenicol selective LB plates. The colonies were incubated at 37 ℃ overnight or until there were distinct, visible colonies and never longer than 72 hours. The existence of our DNA constructs in the colonies were confirmed using verification primers in a colony PCR. We then performed his-tag purification on crude cell extracts from our colonies using Thermo Scientific HisPur Ni-NTA spin columns. Following protein purification with the Thermo Scientific HisPur Ni-NTA spin columns, we confirmed the presence of our protein in the final elution using Thermo Fisher Scientific's Lumio Tag Protein Detection Kit. We then performed a standard BCA Assay to determine our total protein concentration in each elution.

4.1.8 Mechanical testing

To test quantitatively the strength of each purified glue candidate we performed a lap-shear test using the Instron 5565 in the Stanford Soft & Hybrid Materials Facility. To do this, we grew *G. lucidum* mycelium on sawdust into the specified dimensions of the ASTM D3163 rectangle, which is used as a standard for lap-shear adhesive tests.[23] We also cut cardboard into these dimensions to use as a rough control that would help us assess whether or not our glue candidates were truly specific to mycelium material. Before applying our various glue candidates to the mycelium and cardboard specimens, we incubated the purified protein solutions at their isoelectric points at 4 ℃ for 2 days, spun them down, and discarded the supernatant. This was done to allow aggregate forming proteins (csgA, csga-CBD) to form amyloid fibers and for all the proteins (CBD4x, fp151) to reach their optimum functional pH.[24]

As shown in Figure 8, all of our purified bioadhesive proteins successfully bound to baked mycelium substrate and produced non-negligible adhesive strengths. The CsgA-CBD and CBD4x fusion proteins were the most successful, with both reaching strengths of 28.2 kPa which is comparable to the tested strength of Elmer's glue on mycelium at 32.1 kPa. Non-fusion CsgA was slightly weaker at 27.1 kPa on, and fp151 gave the poorest adhesive strength on mycelium at 16.8 kPa.

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[21] Hwang, D. S., Gim, Y., Yoo, H. J., & Cha, H. J. (2007). Practical recombinant hybrid mussel bioadhesive fp-151. ***Biomaterials,*** 28(24), 3560–3568. https://doi.org/10.1016/j.biomaterials.2007.04.039.

[22] Marumo K, Waite JH. Optimization of hydroxylation of tyrosine and tyrosine-containing peptides by mushroom tyrosinase. Biochemistry Biophysics Academy. 1986;872:98–103. https://www.ncbi.nlm.nih.gov/pubmed/3089286.

[23] Lap shear strength astm d3163 and lap shear adhesion astm d5868. (n.d.). http://www.ptli.com/testlopedia/tests/lap_shear-d3163.asp.

[24] Zhong, C., Gurry, T., Cheng, A. A., Downey, J., Deng, Z., Stultz, C. M., & Lu, T. K. (2014). Self-assembling multi-component nanofibers for strong bioinspired underwater adhesives. ***Nature Nanotechnology,*** 9(10), 858–866. https://doi.org/10.1038/nnano.2014.199.

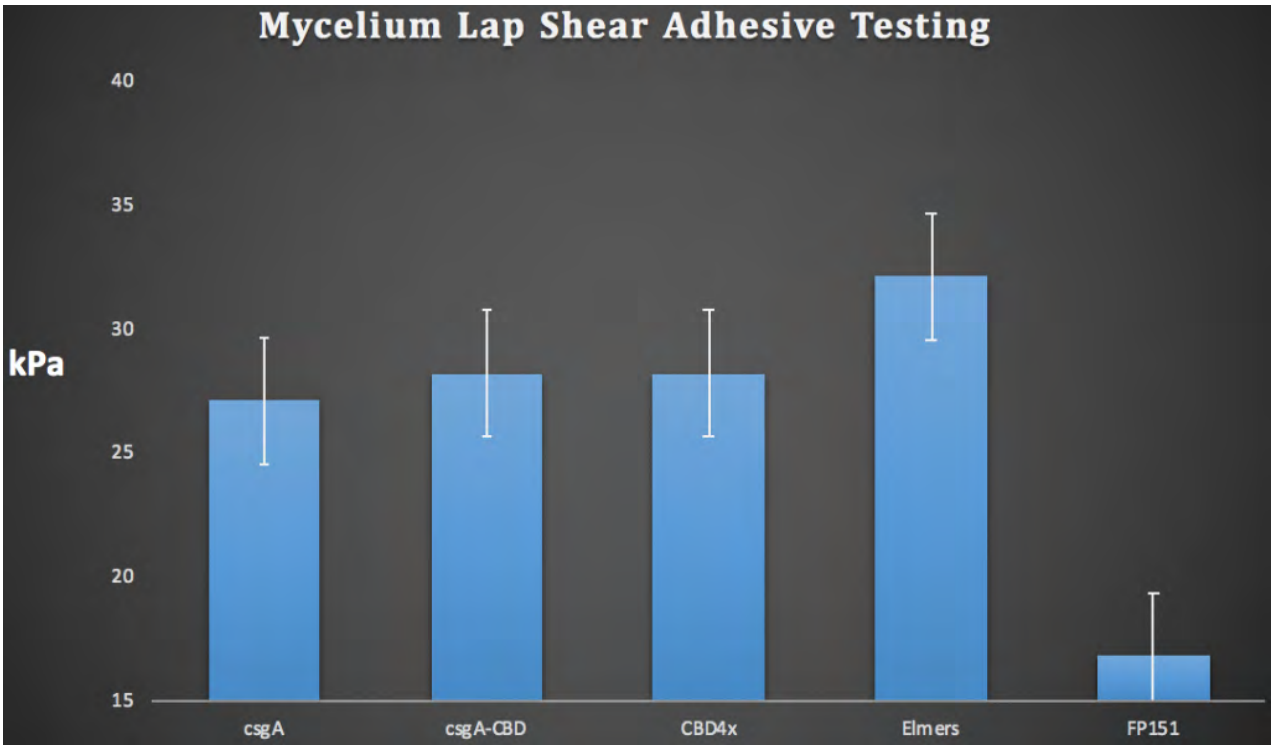

Figure 8. Lap Shear Adhesive Strength. Different bioadhesive candidates where applied to mycelium samples that were pressed together and allowed to set. Using the Instron 5565 at Stanford, the force necessary to separate the mycelium samples was then measured and converted to kPa accounting for the area of overlap between the mycelium samples.

On a cardboard substrate (Figure 9) there are several notable differences from the mycelium substrate testing. Elmer's Glue was again the strongest and more than doubled its bonding strength from the mycelium substrate test, to 78.1 kPa. CBD4x was surprisingly stronger than on mycelium, improving to 46.5 kPa. The fp151 protein also more than doubled in strength, reaching 40.6 kPa in the cardboard testing. Csga-CBD was weaker in the cardboard testing, at 22.7 kPa. The sample of csgA bound to cardboard was unfortunately dropped and was not salvageable for lap-shear testing.

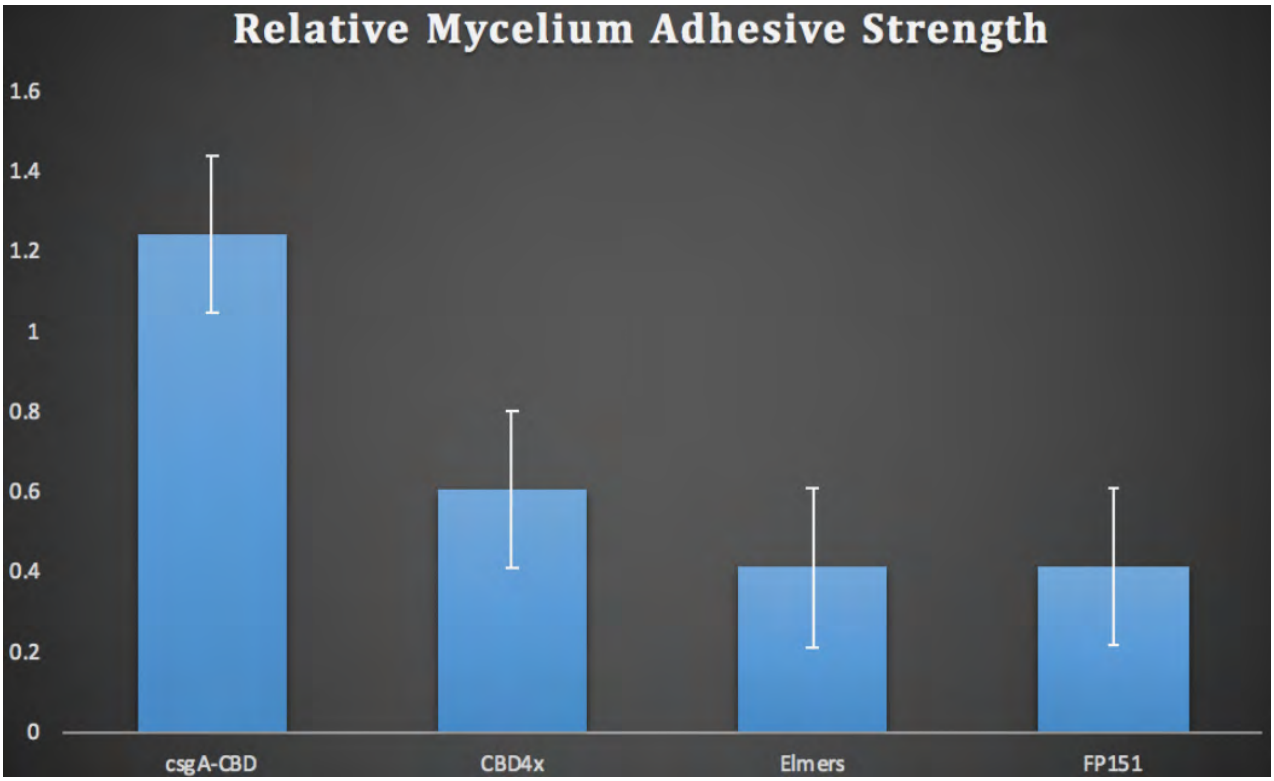

Figure 9. Relative Mycelium Adhesive Strength. After testing the adhesive strength of each bioadhesive candidate on mycelium, we tested each candidate again on cardboard. We then took the ratio of adhesive strength on mycelium to adhesive strength on cardboard to determine the relative efficiency of the two substrates.

By taking the ratio of adhesive strength on mycelium to the adhesive strength on cardboard, we can find the relative efficiency of the adhesives on the two substrates. Every adhesive except for csgA-CBD bonded stronger on cardboard substrate than the mycelium substrate. This could be due to cardboard having a more uniform surface and thus allowing better molecular bonding and glue function. This makes it very notable that csgA-CBD was a stronger adhesive on mycelium, and provides evidence that by adding the chitin binding domain to csgA we were able to successfully make the protein a mycelium-specific glue. CBD4x, while still performing better on cardboard, did not decrease in efficiency as much as Elmer's glue or fp151 which shows, albeit less dramatically, that chitin binding domains can be functionalized as mycelium specific bioadhesives.

Once we identified csgA-CBD as our best mycelium specific bioadhesive, we collaborated with the DTU-Denmark iGEM Team[25] to test our glue on their mycelium grown from a different fungus. The DTU-Denmark Team's mycelium was grown from the fungus *Aspergillus oryzae* while ours was grown from *G. lucidum*. When we performed a lap shear test using our csgA-CBD glue on DTU's mycelium samples that were shipped to us in the mail, the maximum adhesive strength reached was 6.7 kPa. While this was still significant adhesion, it was lower than the 28.2 kPa reached with *G. lucidum*. The difference in adhesive strength, however, provided useful information about the variability in surface composition of mycelium grown from different species of fungus.

[25] http://2018.igem.org/Team:DTU-Denmark

In total, from the lap shear testing data it can be concluded that the fusion proteins that were designed for this project, CBD4x and csgA-CBD, successfully act as biological adhesives for mycelium materials. By outperforming fp151, which has been reported to be an extremely strong bioadhesive,[20] and almost reaching the adhesive strength of Elmer's glue both csgA-CBD and CBD4x show promise in future use as structural binders of mycotecture in Mars or other extraplanetary colonization efforts. As Elmer's glue requires raw materials like natural gas and petroleum that are plentiful on Earth but are often rare to non-existent on other planets, the slight adhesive advantage it shows in comparison is greatly outweighed by the mass producibility of our bacterially synthesized bioadhesives.[24] In further research, the characterization of alternate csgA-CBD fusion proteins implementing different spatial conformations and/or multiples of csgA of CBD polypeptides could yield even better mycelium-specific bioadhesives. The inclusion of a third type of polypeptide in the fusion protein could theoretically create multipurpose mycelium-specific glue proteins, able to bind mycelium together while also performing some other useful task such as molecular sensing.

4.2 Objective 2. Assess potential enhancements of mycelia.

4.2.1 Summary

Natural mycelium can be enhanced by adding other microbes or inert materials to make a biocomposite. However, during the course of the project, we realized that mycelial materials were the answer to putting Rothschild's prior 2016 Phase 1 NIAC "Urban biomining meets printable electronics: end-to-end at destination biological recycling and reprinting", into practice. Thus a project was conceived and conducted to link the metal binding peptides from the biomining NIAC to the mycelium growing in this NIAC, leading the way to both a metal recovery and water purification system. Both a patent disclosure and manuscript resulted from this work, both currently under review.

4.2.2 Biofiltration: From Concept to Prototype

Clean water is vital. A self-assembling habitat for extraterrestrial exploration is simply not stable without a sustainable, regularly filtered water supply, and access to clean water remains one of the largest challenges humanity will face in the upcoming future, whether it's in space or on Earth. Another challenge is metal acquisition, whether from waste electronics or martian regolith. We developed a cost-effective, scalable, completely biodegradable filtration system for waste metal recovery from aqueous solution, using copper as a proof of concept. We first modified metal binding domains through computationally-aided rational design, and tested multiple in tandem to increase the binding capacity of our peptides. We then created metal binding domain - chitin binding domain (CBD) fusion proteins, using the CBD as a platform for functionalizing the mycelium surface and enhancing its properties. We quantitatively characterized and modeled the binding affinity of the individual domains to rigorously understand the molecular underpinnings of the filter. We finally created a tangible, functioning prototype composed of filter strips of pure mycelial material that removes >92% of the copper from a >300 μM aqueous solution within 30 minutes through surface adsorption and tangential flow, demonstrating the viability and utility of our novel process for filtration and biomining applications, both on Earth and in space.

4.2.3 Design of a Copper-binding Biofilter

When designing our biofilter, we had two guiding questions in mind: how can we best bind metals on a molecular level, and how can we use synthetic biology to create a platform for functionalizing mycelial material? We also considered the advantages of our platform in comparison to previous efforts, such as flagella-based or cellulose filtration tools.[26,27] One of the largest benefits of using

[26] Eckhard et al. 2017. Discovery of a proteolytic flagellin family in diverse bacterial phyla that assembles enzymatically active flagella. *Nature Communications*, 8(1). doi:10.1038/s41467-017-00599-0

mycelium material is that it leverages the concept of economies of scale, and presents an entirely feasible option of scale-up of our technology to a level that could be successfully implemented on a space mission and on Earth in developing countries with poor access to clean water. Fungi are capable of displaying growth on an enormous variety of biomass types, and grow at a rate that is unparalleled by other biological agents used in synthetic biology today.[28]

4.2.4 Chitin Binding

We sought to find ways to utilize mycotecture and enhance its properties, and in the process realized that chitin is ubiquitous in nature in organisms ranging from fungi to insects - in fact, it's the second most abundant biopolymer on earth.[29] Methods to take advantage of this fact using synthetic biology are limited - we established a part collection using chitin binding domains as a novel platform to functionalize chitin-containing surfaces, with almost unlimited potential for modifying regions of organisms. We demonstrated the utility through designing novel biological cross-linking glues, fluorescent dyes, but most directly, filtration tools. Our use of the CBD in fusion protein production forms an important pipeline for future teams and the global scientific community for numerous applications, and here we focused on its use for metal recovery from mixed aqueous solution.

4.2.5 Copper Binding

We then sought to optimize the actual binding interaction between our peptides and metals. We chose copper as our proof of concept as a metal that is both biologically and economically important. When looking at past iGEM teams working with metal binding domains, we found that very few, if any, focused on optimizing the manner in which the metals were bound, instead choosing to simply obtain a binding domain from nature and attempting to implement it. We thought we could do better. We recently developed a 20 amino acid long peptide (referred to as HHTC-Re (HNLGMNHVLQGNRPLVTQGC), that is a modified peptide with substituted amino acids adapted from the peptide HHTC designed by Kozisek et al..[30] We wondered whether we could apply this in tandem with repeats to bind multiple copper atoms to the same biomolecule, and if we could use chitin binding domains (CBD's) to create fusion proteins that could bind copper atoms and then also bind to chitin on our mycelium material.[31]

4.2.6 Construct Design and Modeling

To test whether we could bind multiple metal ions on the same protein and if this relationship would be monotonically increasing, we designed peptides with one, two, and three copper binding domains in tandem (1xHHTC-Re HNLGMNHVLQGNRPLVTQGC, 2xHHTC-Re HNLGMNHVLQGNRPLVTQGCHNLGMNHVLQGNRPLVTQGC, 3xHHTC-Re

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[27] Shipovskaya et al. 2003. Physicochemical Modification of Cellulose Acetate for Manufacturing Films, Membranes, and Biofilters. *Russian Journal of Applied Chemistry*, 76(9), 1514–1518. doi:10.1023/b:rjac.0000012678.32530.c3

[28] Cavka et al. 2014. Comparison of the growth of filamentous fungi and yeasts in lignocellulose-derived media. *Biocatalysis and Agricultural Biotechnology*, 3(4), 197–204. doi:10.1016/j.bcab.2014.04.003

[29] Domard, A. 2011. A perspective on 30 years research on chitin and chitosan. *Carbohydrate Polymers*, 84(2), 696–703. doi:10.1016/j.carbpol.2010.04.083

[30] Kožíšek et al. 2008. Molecular Design of Specific Metal-Binding Peptide Sequences from Protein Fragments: Theory and Experiment. *Chemistry - A European Journal*, 14(26), 7836–7846. doi:10.1002/chem.200800178

[31] Watanabe et al. 2000. Expression and Characterization of the Chitin-Binding Domain of Chitinase A1 from *Bacillus circulans* WL-12. *Journal of Bacteriology* 182(11), 3045–3054. doi:10.1128/jb.182.11.3045-3054.2000

HNLGMNHVLQGNRPLVTQGCHNLGMNHVLQGNRPLVTQGCHNLGMNHVLQGNRPLVTQGC; see biobrick page for sequence information). These three peptides were synthesized by Elim Biopharmaceuticals (Hayward, CA), and provided as a lyophilized powder at >98% purity. They were also modified to possess N-terminal acetylation/C-terminal amidation to avoid having a charged peptide.[32] 10 mM MES pH 5.5 and the Pierce BCA assay were used for reconstitution and concentration determination, respectively.[33]

To check whether the proteins would retain their respective conformations when added in tandem, we used a tool produced by the Zhang lab (University of Michigan) for *de novo* protein structure prediction (QUARK).[34] Based on the structures, we were able to hypothesize that the HHTC-Re domains would each be able to bind copper atoms, even when restricted spatially by ordering them sequentially without spacing.

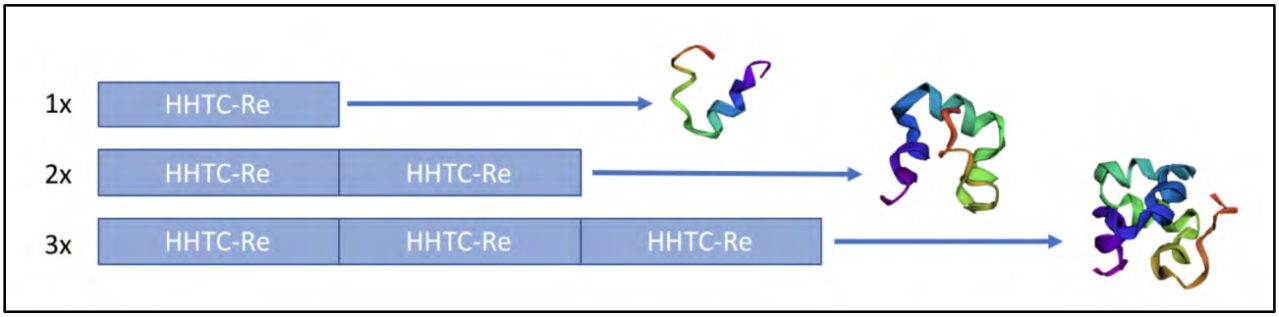

Figure 10. Depiction of the design of the HHTC-Re repeating peptides, along with predicted ab initio structures as per the QUARK database and algorithms. Individual HHTC-Re domains retain their conformation, even in the 3x peptide.

We also developed 3 fusion proteins: CBD-2xHHTC, CBD-3xHHTC, and CBD-6xHHTC for use in the actual filter, comprised of a CBD, and HHTC metal binding repeats interspaced with a GSGGSG flexible linker. These constructs also included a Lumio tag for downstream verification of protein production, and a 6x His tag for protein purification. A challenge incurred when designing these parts was that the His tag maintains some affinity for metals, including copper. This would interfere with downstream modeling and experimental analysis of the interaction between copper and the HHTC domain. We therefore added the Mxe GyrA Intein between the Lumio/His tag and the rest of the protein, as this intein could be cleaved through addition of 50 mM DTT through thiol-mediated cleavage after protein purification.[35] After DTT was added, we were able to obtain the fusion proteins ready for downstream application (as per Figure 11).

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[32] Yi et al. 2010. A Highly Efficient Strategy for Modification of Proteins at the C Terminus. Angewandte Chemie International Edition, 49(49), 9417–9421. doi:10.1002/anie.201003834

[33] Huang et al. 2010. Competitive Binding to Cuprous Ions of Protein and BCA in the Bicinchoninic Acid Protein Assay. *The Open Biomedical Engineering Journal* 4:271-278. doi:10.2174/1874120701004010271.

[34] Zhang et al. 2015. Integration of QUARK and I-TASSER for *Ab Initio* Protein Structure Prediction in CASP11. *Proteins: Structure, Function, and Bioinformatics* 84, 76–86. doi:10.1002/prot.24930

[35] Marshall et al. 2014. An Evolved Mxe GyrA Intein for Enhanced Production of Fusion Proteins. *ACS Chemical Biology* 10(2), 527–538. doi:10.1021/cb500689g

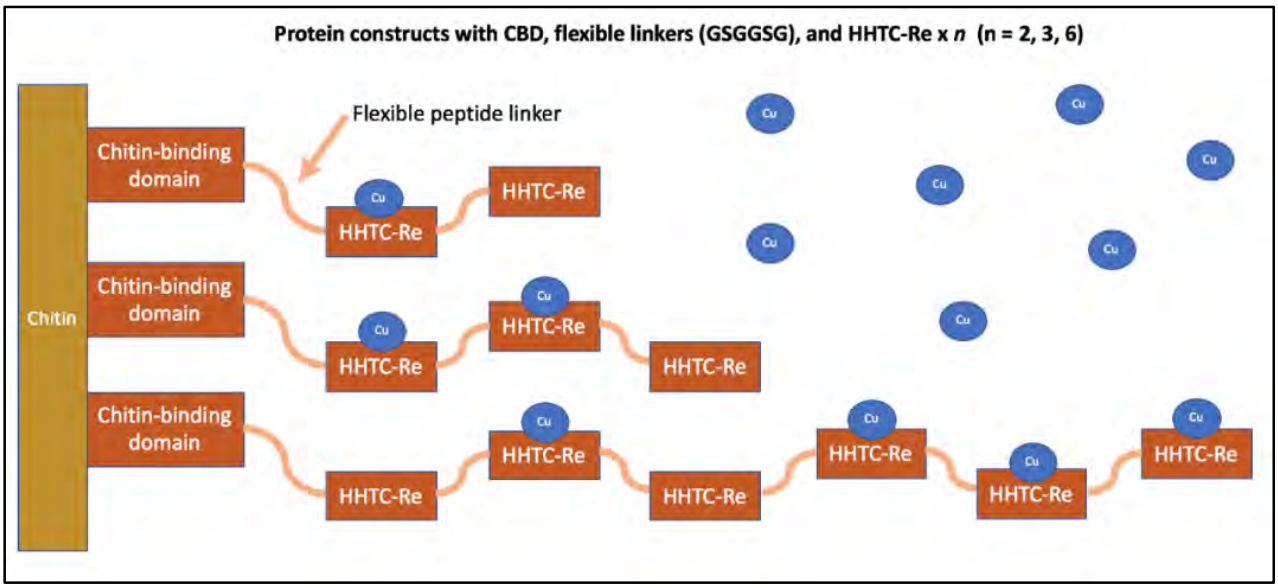


Figure 11. Schematic of the design of the fusion proteins (HHTC-Re x n) and the method in which they bind copper.

We also produced an RFP-CBD fusion protein to visualize the distribution of chitin on the surface of a piece of mycelium, as well as a proof of concept for aesthetic design purposes.

4.2.7 Wet-lab Experiments: Fusion Protein Production

Once all three constructs were ordered (IDT gBlocks), we ligated the linear DNA constructs to the PSB1C3 iGEM backbone using Gibson Assembly, transformed NEB T7 competent *E. coli* with our new plasmid, and plated the colonies on chloramphenicol selective LB plates. The colonies were incubated at 37℃ overnight or until there were distinct, visible colonies, and never longer than 72 hours. The existence of our DNA constructs in the colonies was confirmed using verification primers (VF2 and VR) in a colony PCR. We then performed His-tag purification on crude cell extract from our colonies using Thermo Scientific HisPur Ni-NTA spin columns. Following protein purification with the spin columns, we confirmed the presence of our protein in the final elution using Thermo Fisher Scientific's Lumio Tag Detection Kit.[36] The standard protein purification protocol was modified by introducing a buffer containing 50 mM DTT for on-site cleavage, so the desired fusion protein could be eluted with the His-tag removed. We then performed a BCA Assay to determine our total protein concentration in each elution.

4.2.8 ITC Testing

Isothermal titration calorimetry (ITC; MicroCal iTC200) was employed to determine the association equilibrium constant (Ka), enthalpy (ΔH), and the number of ions bound per ligand (n). Ka describes the affinity of a ligand for its substrate, and we used it to quantitatively characterize the interaction between our peptide and copper.[37] All binding parameters for the test were within the specifications determined by the manufacturer. We used 10 mM, 2-(N-morpholino)-ethanesulfonic acid (MES) buffer for testing because it does not cause metal ion

[36] Lumio green in-cell detection kit - Thermo Fisher Scientific. (n.d.). https://www.thermofisher.com/order/catalog/product/12589057.

[37] Freyer, M. W., & Lewis, E. A. 2008. Isothermal Titration Calorimetry: Experimental Design, Data Analysis, and Probing Macromolecule/Ligand Binding and Kinetic Interactions. *Methods in Cell Biology* 79–113. doi:10.1016/s0091-679x(07)84004-0

interference, and has a stable pKa over a wide temperature and pH range. Experiments were conducted at pH 5.5 to prevent copper precipitation, and pre-made copper stock solutions of known concentration were used.

Peptides were prepared for ITC by dissolving lyophilized protein (powder) in MES buffer, and ITC experiments were run at 25°C and set to deliver 20, 0.5 – 1 µL injections of Cu at 150 second intervals. The metal solution in the syringe was titrated into the peptide solution in the cell, and interactions were measured. Raw data were corrected by subtracting the heats of dilution, and collected data were fit with a one-site binding model using the Origin-7™ software.

4.2.9 Prototype and Phen Green Assay for Bulk Adsorption

To test the peptides, strips of pure mycelium from the species Ganoderma lucidum were incubated in purified CBD-2xHHTC protein (suspended in 6 mL of 10 mM pH 5.5 MES buffer, with a fusion protein concentration of 0.45 mM) that had been cleaved with DTT on a flatbed shaker for 72 hours. These pieces were then cut into uniform 1 cm2 squares (assuming chitin assumes an approximately regular distribution, as this is difficult to control for and testing with RFP-CBD led us to believe this was the case). As a control, we also incubated untreated mycelium in 10 mM pH 5.5 MES buffer for the same time period. We then prepared a Cu stock solution in MES, and the Cu concentration in our starting solution was 325 (+/-25) µM. Three environments/types of trials were run: one with the treated mycelium filter, one with untreated mycelium, and one with just the Cu solution and no mycelial material. These took place in 6 mL of the Cu stock solution in sterile 15 mL falcon tubes on a flatbed shaker. All experiments were run in triplicate, for a total of 9 trials. Two hundred µL samples were collected after 0 minutes, 30 minutes, and 72 hours for surface adsorption testing.

To quantify the amount of copper in the samples that were collected, we used Phen Green SK dye, which is proportionally quenched in the presence of copper.[38] Phen Green SK dye was prepared in a stock solution in PBS (28 µM final concentration). Two hundred µL of this stock solution was added to each well in a 96-well plate to which 25 µL of sample was added. All experiments were done in triplicate, and a standard curve was generated for Cu and PGSK to determine the amount of Cu in the tested solutions.

We developed a cost-effective, scalable, completely biodegradable filtration system for waste metal recovery from aqueous solution, using copper as a proof of concept. We first modified metal binding domains through computationally-aided rational design, and tested multiple in tandem to increase the binding capacity of our peptides. We then created metal binding domain - chitin binding domain (CBD) fusion proteins, using the CBD as a platform for functionalizing the mycelium surface and enhancing its properties. We quantitatively characterized and modeled the binding affinity of the individual domains to rigorously understand the molecular underpinnings of the filter. We finally created a tangible, functioning prototype composed of filter strips of pure mycelial material that removes >92% of the copper from a >300 µM aqueous solution within 30 minutes through surface adsorption and tangential flow, demonstrating the viability and utility of our novel process for filtration and biomining applications, both on earth and in space.

4.2.10 ITC Analysis: Data, Modeling, and Binding Parameters

The results from the ITC experiment to determine binding parameters for peptides 1x-, 2x-, and 3x-HHTC-Re alongside the predicted structure (QUARK Ab Initio program) follows [2]. This

[38] Wehbe, M., Malhotra, A. K., Anantha, M., Lo, C., Dragowska, W. H., Dos Santos, N., & Bally, M. B. 2018. Development of a copper-clioquinol formulation suitable for intravenous use. *Drug Delivery and Translational Research* 8(1), 239–251. http://doi.org/10.1007/s13346-017-0455-7

program uses Monte Carlo simulations and knowledge of atomic force fields to construct the most probable structural conformation of a protein from just the linear amino acid sequence.

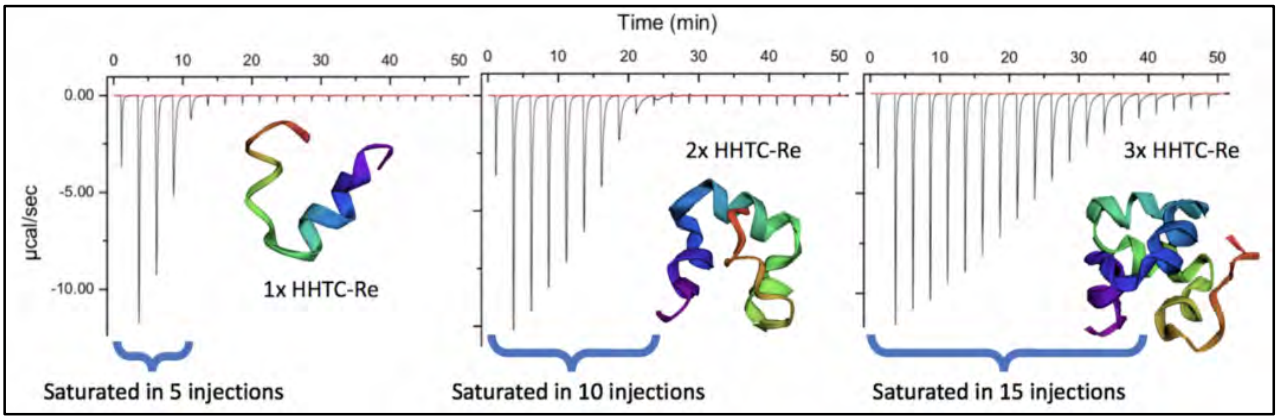


Figure 12. ITC data for the three HHTC-Re peptides. The binding affinity (Ka) was assessed to determine the strength of the interaction, and the results follow: 1x-HHTC-Re Ka = (1.55 ± 0.21) x 106 M-1; 2x-HHTC-Re Ka = (3.73 ± 0.53) x 105 M-1; 3x-HHTC-Re Ka = (1.50 ± 0.05) x 105 M-1. Figure design credits to Jesica Urbina.

The binding affinity values in Figure 12 are largely comparable to one another, and we do see a linear trend between the number of HHTC-Re repeats and injections taken to reach saturation, which means that each peptide can bind a linearly increasing amount of copper proportional to the HHTC-Re repeats. This validates our hypothesis underlying our construct design for our fusion proteins, allowing us to bind multiple metals on the same biomolecule. In Figure 13, we can see how the specific subunits maintain proper folding (predicted by QUARK), supporting the notion that they would retain their original functions.

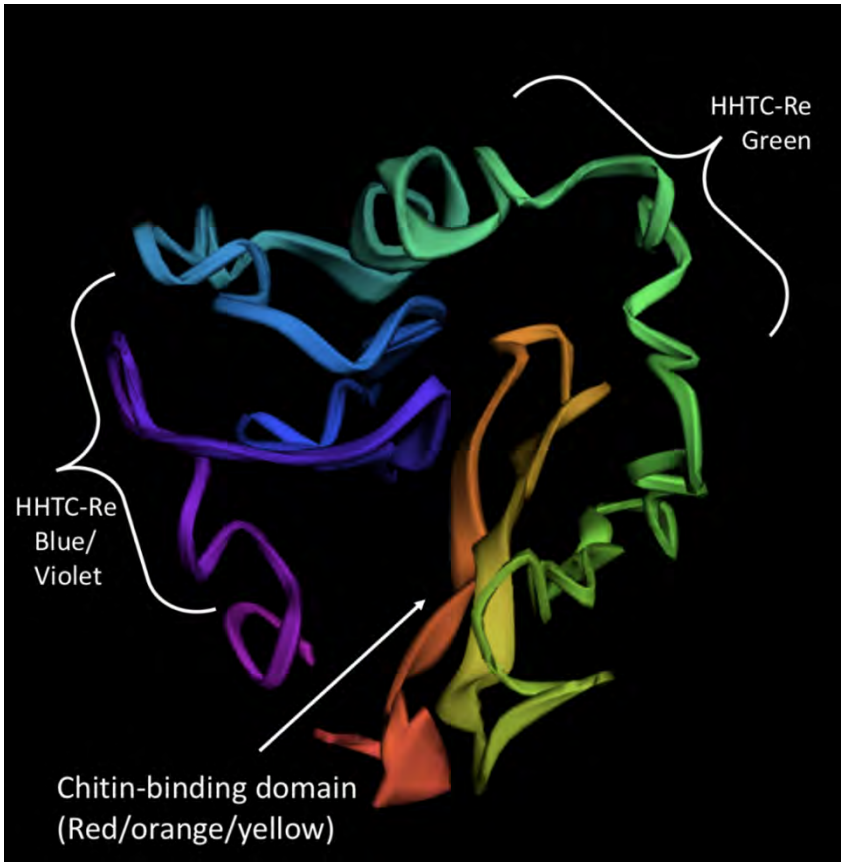


Figure 13. Quark ab initio model of 2x-HHTC-Re with chitin binding domain. Domains within the fusion protein have been annotated to display the conformation and spatial orientation.

After confirming the copper binding of the individual HHTC-Re x n peptides, we then needed to assess whether our fusion protein could bind copper and chitin, and whether it could do so when already saturated with the other substrate. Figure 14 depicts two experiments a) Raw data and b) isotherm for 2x-HHTC-Re-CBD + NaDg and Cu. In this experiment, N-acetyl D-glucosamine (“NaDg”; analogous to a chitin monomer and widely used in the literature

for assessing chitin binding) was first titrated into 2x-HHTC-Re-CBD and no isotherm was calculated because binding sites were not saturated by the ligand. Cu was then titrated into the 2x-HHTC-Re-CBD + NaDg complex and this resulted in a Cu affinity at Ka= 7.61 +/- 1.49x 106 M-1 that is largely comparable to 2x-HHTC-Re (no CBD) and lowerby an order of magnitude than 2x-HHTC-Re-CBD without bound NaDg. Data show 20 1 µL injections. 2x-HHTC-Re-CBD was selected as the candidate for testing because it displayed the most consistent and strong results during protein purification procedures, and seemed most promising for downstream applications (such as our filter). Work is in progress on optimizing the 3x and 6x fusion proteins and creating prototypes (thus far, protein production as been successful).

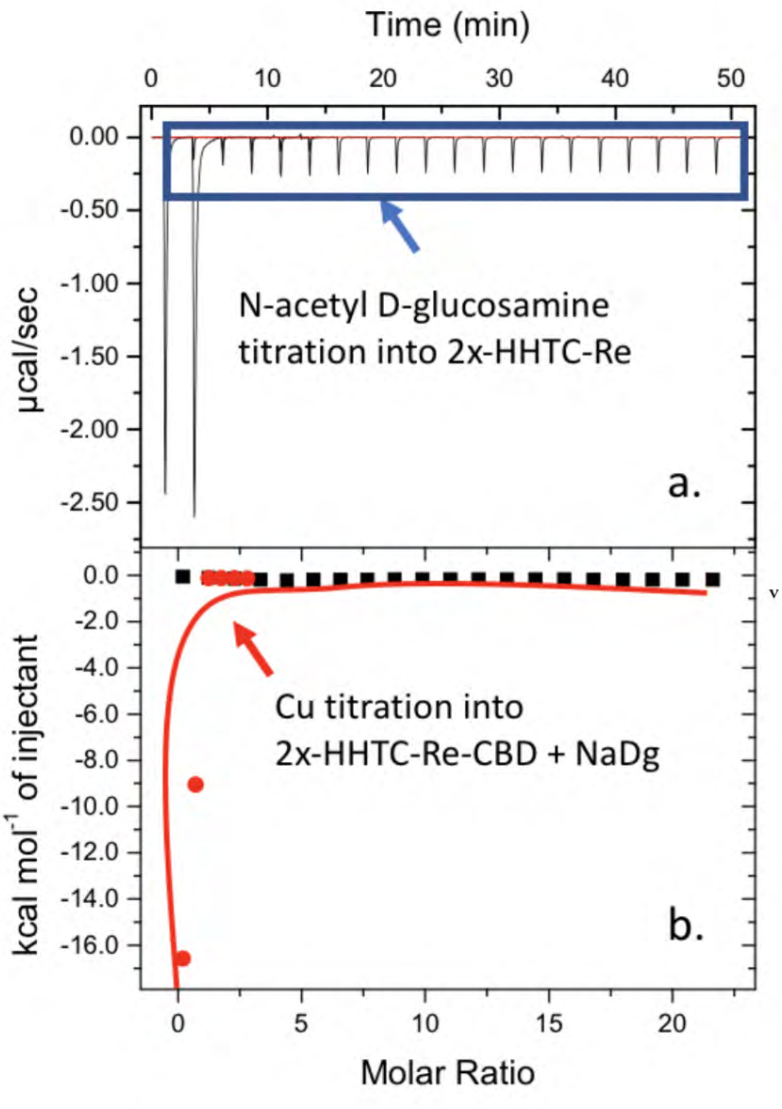


Figure 14. Isotherm and data produced by ITC for assessing the binding affinity of our fusion protein (2x-HHTC-Re-CBD) for chitin (represented by N-acetyl D-glucosamine) and Cu.

4.4.10 Tangential Flow Prototype Creation: Material Properties and Design Considerations

The first consideration when thinking about creating a mycelium biofilter had to do with the qualities and characteristics of the mycelium material. Would it be waxy? Would the chitin be exposed? Such questions and more could significantly impact the approach we picked for functionalizing any material produced. The first test we did was comprised of a simple dye being poured on a piece of mycelium to observe the hydrophobicity of the material and whether it could be easily penetrated (as seen in the video below).

It is evident that the mycelium surface is highly hydrophobic. While this may appear to be an undesirable trait for a water filter, we found that subsequent incubation of mycelium in moving water for a period of 48 hours allowed for the material to become permeable. In an interesting twist of synergy, the fusion protein we were attempting to produce was highly hydrophobic as well, and therefore probably showed good affinity for the mycelium material (Figure 15).

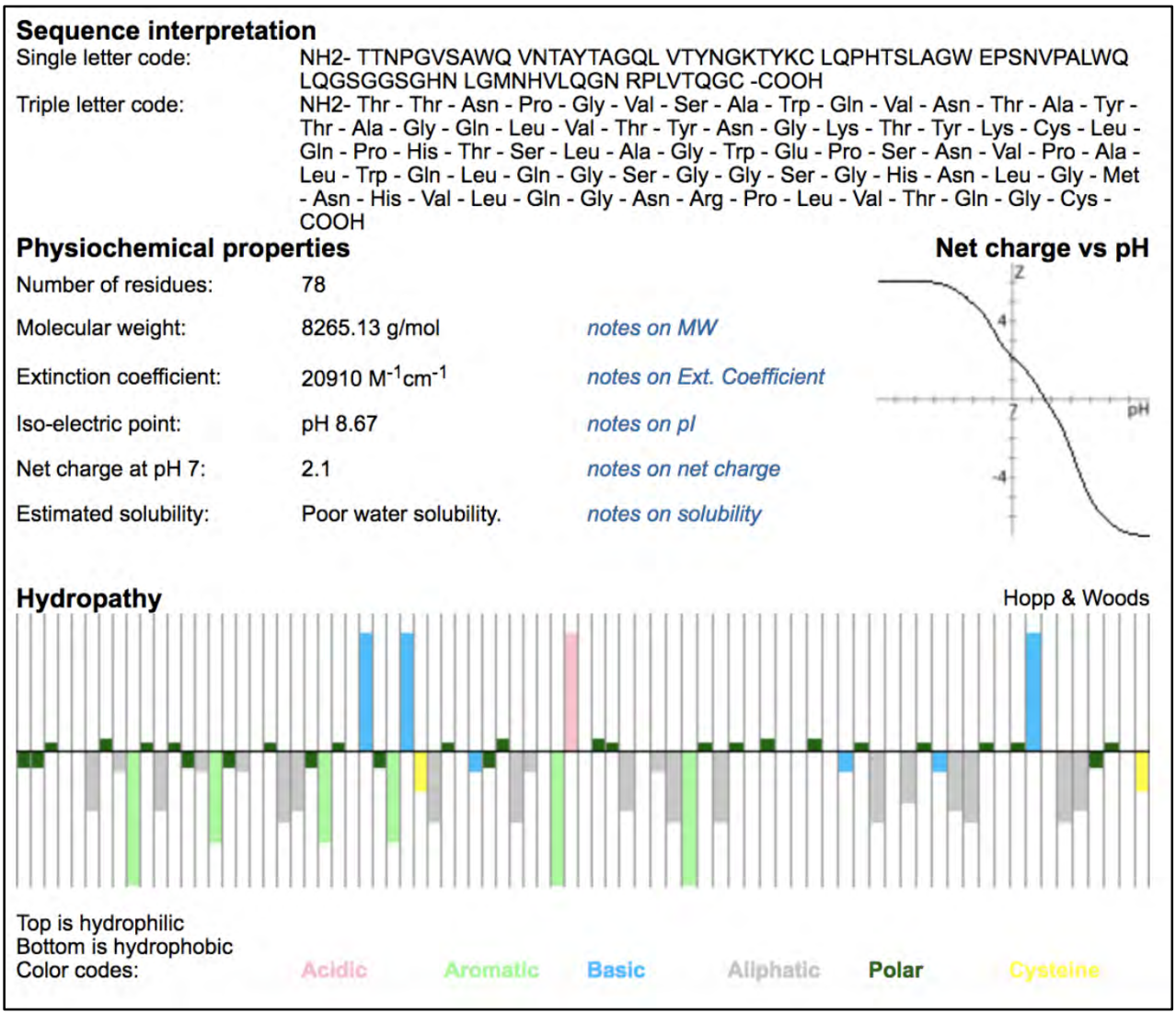


Figure 15. Biochemical properties and hydropathy of a CBD-HHTC-Re fusion protein. The fusion protein displays significant hydrophobic tendencies. While this was initially thought to be a problem, it synergized well with the nature of the mycelium surface and most likely allowed the protein to obtain a proximity close enough for chitin binding to occur. The simulation to produce the graphs above was done by LifeTein.

We then also produced RFP-CBD fusion protein following a regular His-Tag purification protocol and analogous transformation/cloning steps as our other fusion proteins to stain mycelial material with, and as a cool visual tool/probe (Figure 16).

A  B 

Figure 16. Purified protein (A) and liquid cultures (B) of T7 E. coli *cells producing the RFP-CBD fusion protein.*

The next factor to contemplate was the manner in which the filter could be implemented. Our first thought was to grow fungus inside a syringe on wood chips and treat the mycelium with our fusion protein, and then run water through this. We discovered that the prototypes created, however, were not very permeable, and applying pressure to push water through washed out the mycelium itself, leaving only substrate behind (Figure 17).

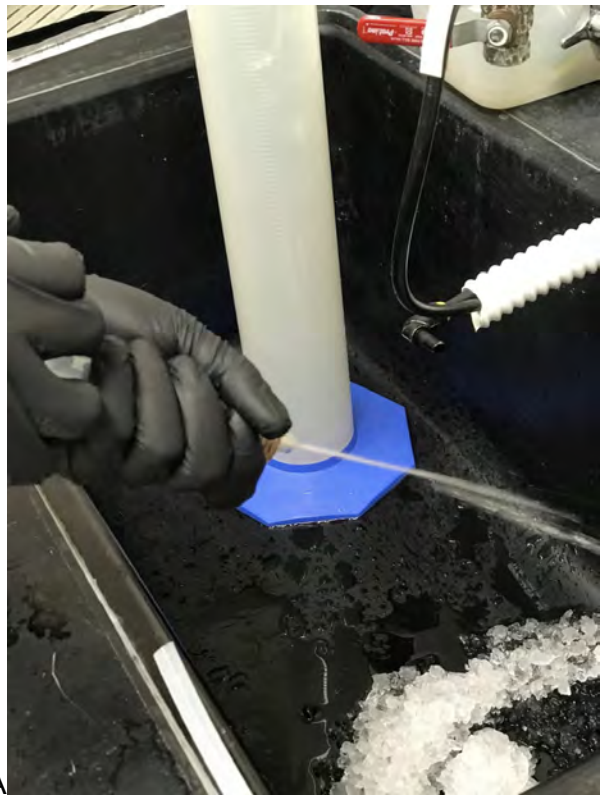
A  B 

Figure 17. Pressure applied to the syringe (A) and mycelium lost in the process (B).

The approach that we selected in the end was to make use of filter-based design in which we could take advantage of tangential flow as opposed to direct head-on flow. Figure 18 panel A shows the mycelium strips being incubated with purified CBD-HHTC-Re 2x protein for 72 hours with DTT for cleavage (the protocol we designed for creation of the filters). These were realistically implemented by inserting a thin sheet of glass fiber at the bottom of a syringe to prevent any mycelial material from flowing through. The mycelium material could also be shredded to increase surface area for absorption of metal and display of chitin. The strips were then added on top and water could be flown through this setup. The filter strips were cut into 1 cm x 1 cm squares for prototype testing. Various iterations of this process can be seen in the images that follow.

Figure 18. Use of filter-based design taking advantage of tangential flow (A, C and D) as opposed to direct head-on flow (C).

4.2.11 Tangential Flow Prototype Testing: Bulk Surface Adsorption Using Phen Green

The Cu concentration in the initial copper solution was 325 (+/-25) μM Cu. After 30 minutes of tangential flow, interestingly, the untreated mycelium absorbed about 23% of the copper in solution, revealing a fascinating synergy in that the mycelium possesses some inherent metal sequestration properties (Figure 19). The treated mycelium (filter prototype) was able to sequester ~92% of the available Cu in solution - validating its efficacy and potential utility.

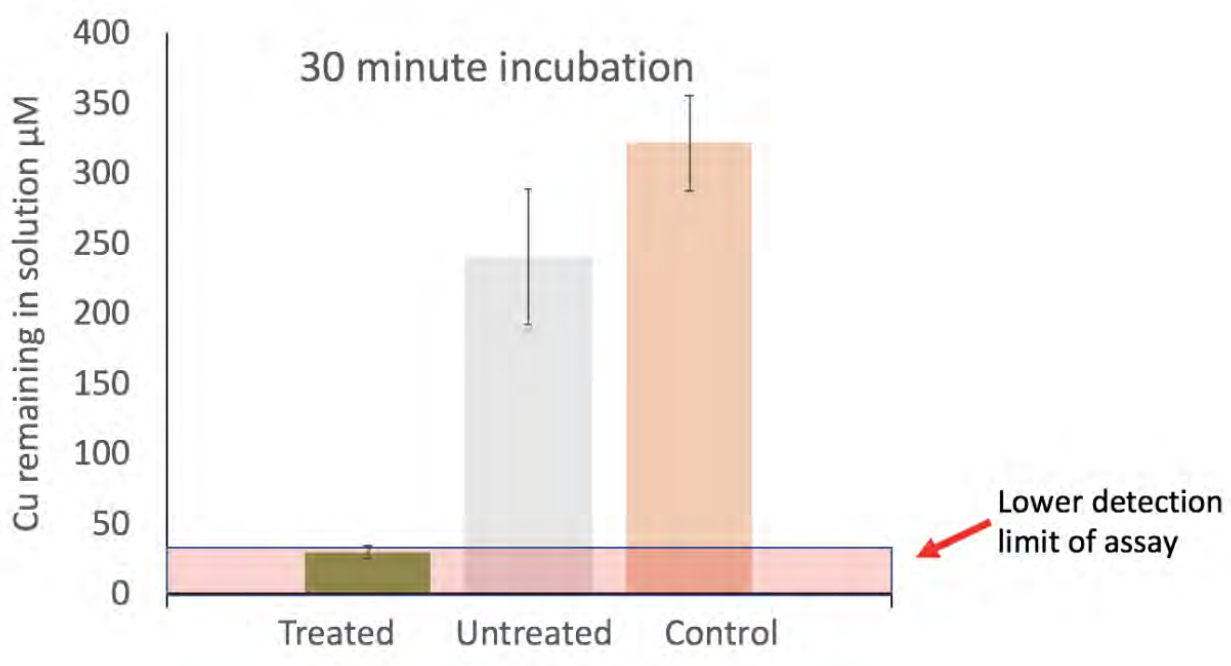


Figure 19. Cu (μM) remaining in solution for n = 3 samples after incubation for 30 minutes while undergoing tangential flow on a flatbed shaker (Figure design credits: Jesica Urbina). Experimental conditions were maintained as described previously.

We also tested the filters over a period of 72 hours and measured the copper concentrations at the end in each of the three experimental trials. The filter was able to bind nearly all of the copper in solution to almost undetectable levels, and the plain mycelial material displayed remarkable properties as well (Figure 20).

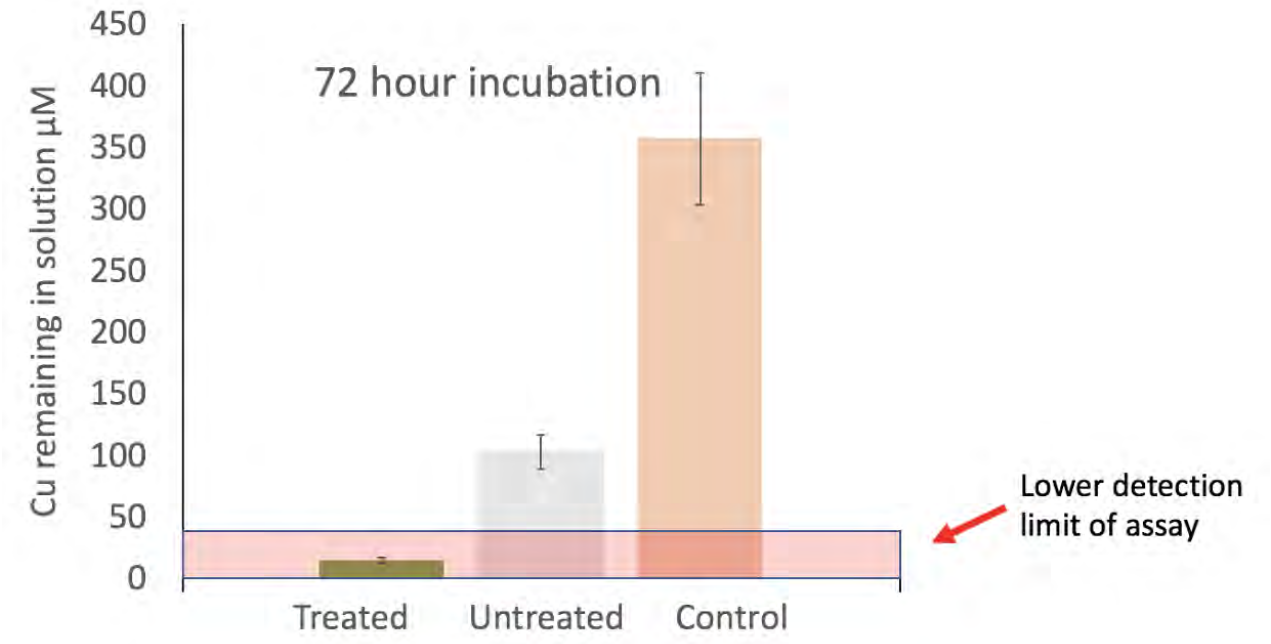


Figure 20. Cu (µM) remaining in solution for n = 3 samples after incubation for 72 hours while undergoing tangential flow on a flatbed shaker. Experimental conditions were maintained as described previously.

4.2.12 Summary and Conclusion

We were able to rationally design, produce, prototype, and extensively characterize our vision for a mycelial water filtration and biomining system that is fueled by synthetic biology and protein engineering. We created tangible filter strips to demonstrate how our methodology could feasibly be applied in actuality, going beyond just a proof-of-concept phase. These prototypes were able to bind >92% of copper in solution (at >300 µM Cu concentration) within 30 minutes. They are relatively inexpensive, completely biodegradable with ease of metal extraction, and require low, less complex upmass in comparison to bacterial or cellulose-based filters (which require complex sugars for sustenance). A patent disclosure was submitted to NASA on 10/16/18. Our results demonstrate the utility of our end-to-end filtration system in solving water and biomining-related challenges both on earth and in space.

4.3 Objective 3. Design mycelial-based structures including habitats

4.3.1 Summary

On another planet, mycelia could grow and expand to provide structural integrity for the house, inside which the astronauts will live. However, the mycelia requires a substrate (food) and oxygen to grow. Where will these supplies come from? Cyanobacteria.

Two groups worked on the design of habitats semi-independently: Co-I Maurer, an architect, and several of the iGEM students, in particular Emilia Mann, a dual degree Brown/ Rhode Island School of Design (RISD) undergraduate on design, and Santosh Murugan, a Stanford undergrad, on the experimental work.

Cyanobacteria are oxygenic photosynthetic organisms that could convert water and the carbon dioxide from the Martian atmosphere into oxygen and biomass. This oxygen can be used to grow the mycelia, as well as to keep astronauts healthy and alive inside the habitat. Moreover, it has been shown that mycelia can use cyanobacteria as a substrate (food) to grow (at normal Earth

gas concentrations). Our goal in this experiment is to demonstrate and quantify the oxygen production capabilities of a specific strain of cyanobacteria, known as *Anabaena variabilis*. Achieving Earth-like percentages of oxygen (~20%) solely from the *Anabaena*, in combination with existing knowledge, would demonstrate the feasibility of cyano-based mycelial growth and Astronaut sustenance. These experiments and results are given below under Mission Architecture, Deployment at Destination.

4.3.2 redhouse studio

4.3.2.1 Introduction

The architectural designs and deployable *in situ* construction methodologies were developed at redhouse studio (Fig. 21). redhouse studio has developed plans, 3d models, section details, and animations of various designs and building processes. The team looked at many ways to deploy bio-composites off planet including lightweight formwork, masonry, additive manufacturing, but arrived at a sealed bag deployment as best to control the environment for growth, develop the shape of the shelter, and protect the Martian and Lunar environments from potential contamination.

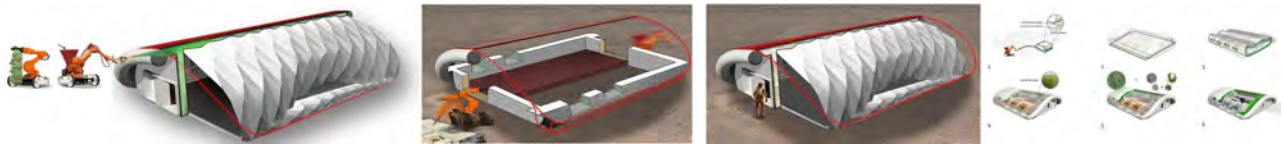


Figure 21. From left to right, additive manufactured bio-composite, masonry bio-composite construction, cast in place with folded formwork, sealed bag that grows in place.

The sealed bag concept will allow the bio-composites to self-assemble in multiple layers of membranes that can provide redundant protection, channel nutrients, and create warm habitable spaces within. This can be achieved at many scales and could be utilized as a platform technology for building any mission structure or object. Figure 22 shows three different structures utilizing the same process.

Figure 22. From left to right: Initial concept for self-assembled bio-composite in unfolding bag, Larger mission building with integrated algal bio-reactor to create biomass and energy, bioHAB 3.0 integrated bio-reactor allows fenestration.

4.3.2.2 Architectural Concept Evolution

The design concept started as deployable habitat shell that would grow like a living organism at destination with the aid of *in situ* resources. It was meant to compete with lava-printed or regolith-sintered shells under review by NASA.[39] This would be less energy intensive and leave a smaller planetary footprint then mining or melting surface material. As with all organisms the concept has grown and evolved new multi-functional facets. The biological functions that enable growth of the

[39] Khoshnevis, B.. Carlson, A.. Leach, N.. Thangavelu, M. 2012. *Contour Crafting Simulation Plan for Lunar Settlement Infrastructure Build-Up*. NASA NIAC Phase 1 Final Report.

materials also bestow such benefits as oxygen production and may be used to generate heat and electricity as well. We now see this biomimetic and bio-utilitarian option as competing with the comforts of up-massed prefabricated structures that come fully outfitted.

The biological analogy of the turtle that carries its home, or the bird that builds at destination assumes an a/b trade off, of reliability or speed. We submit that you can have it both ways. The necessary attributes like plumbing lines, stovetops, and floormats can be folded into the form plugged-in ready to go and the floors walls, windows, and furniture can be grown in place such that Mars grown building has all the comforts of an Earth fabricated structure.

The trick will be packing.

Many of the domestic utilities, scientific equipment, furnishings, and fixtures can be built directly into the expandable shell. Figure 23 shows the building transforming from shell to living space with interior walls and furniture. These self-contained modules can be wrapped in the larger structure and secondarily deployed once robotic enabled construction of the shell is finished.

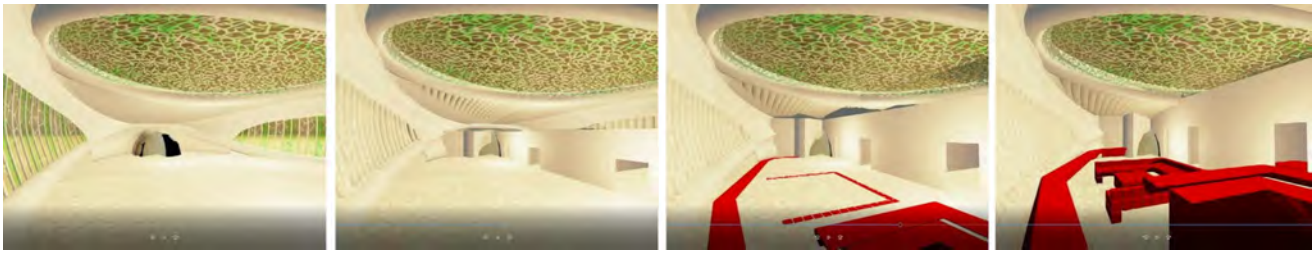


Figure 23. Screenshots from an animation of interiors growing in situ from already grown building envelope.

4.3.2.3 Biomimetic Design

Similar to living organisms, the bioHAB utilizes a circulatory system to deliver nutrients to pre-seeded microbes and spores embedded within “cells” or modules to grow in place (Fig. 24). This circulatory system can be reused after the materials are fully developed to deliver nutrients and gasses for secondary processes discussed in later in this section. The cells create a compartmentalization that ensure the inflated cavities become filled with the bio-composite. This method also provides redundancy, should a problem exist in any of the cells. Further, it removes the issue of dispersal of embedded spores or other additions to the mycelia by compartmentalization. Finally, it allows the use of this material to build other structures at destination.

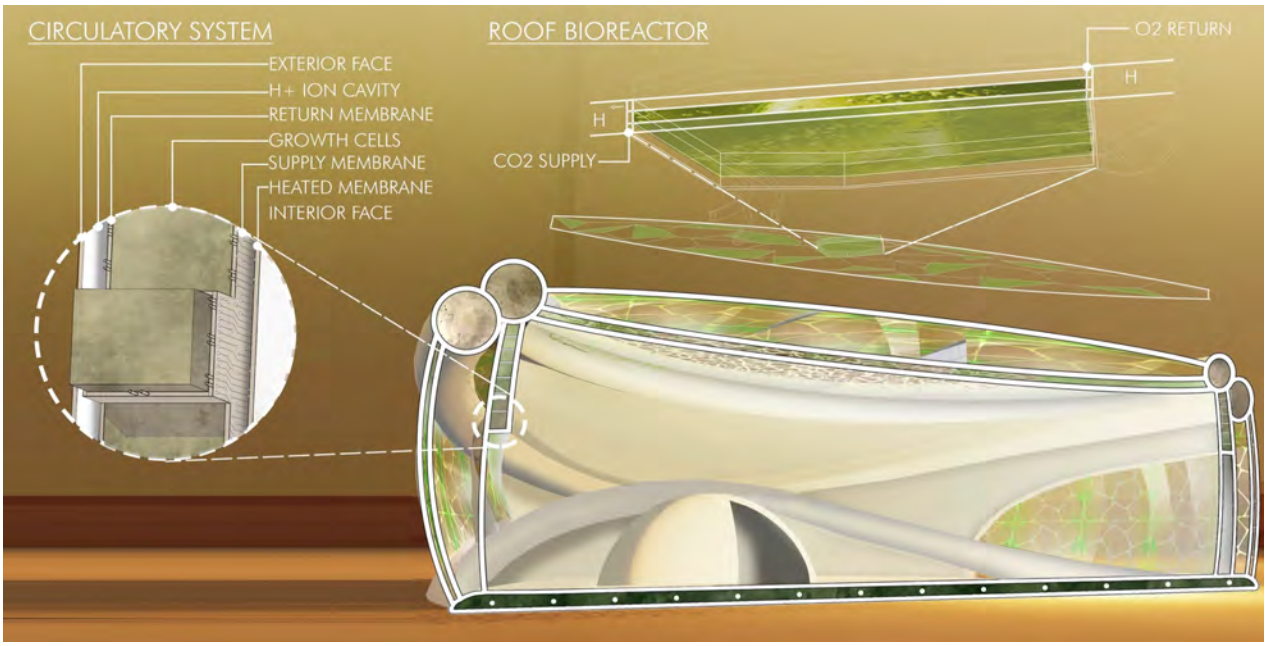


Figure 24. The image shows a building section and details of integral bioreactor that can supply oxygen for breathing and hydrogen for fuel and radiation protection, and biomass for food or more building materials.

4.3.2.4 Materialization

The process of making fungal composites includes growing filamentous saprophytic fungi on biomass substrates that can become fused at a cellular level. Our team has demonstrated composites that have structural characteristics superior to wood framing, thermal resistance characteristics superior to fiber-glass batt insulation, and fire resistance equivalent to type-X gypsum board, that is, construction industry standards.[40] Commercial building material applications exist for "mycoterials" including insulation and sound attenuating materials made by Ecovative Design.[41] Table 1 shows the comparison of our bio-composites with other ISRU suggested materials.

Table 1.

Material	Regolith (Comp)	Sintered Basalt	Lunar Regolith	Sulfer Concrete	Ice	Mycrete
Project	Chow et al.	PISCES - HI	NASA Khoshnevis	NASA Khoshnevis	Icehouse-SEArch	redhouse
Photo						
Modulus of Rupture	40 MPa	40 MPa			3 MPa	19 MPa
Ultimate Compression	40 MPa	206 MPa	53.5 MPa	17.24 MPa	4.9 Mpa	6.7 MPa
Modulus of Elasticity					5100 Mpa	5334 MPa
R-value (per inch)		0.05r/in.			0.45 r/in.	3.8/in.
Tensile					1MPa	TBD
Temperature to Produce		1400C	1025C	130C	> 0C	15-30C
Thickness for radiation sheilding		3 meters	3.5 meters		30 cm	TBD

[40] Bar-Cohen, Yoseph Editor. 2018. *Advances in Manufacturing and Processing of Materials and Structures.* CRC Press/Taylor & Francis Group, LLC.

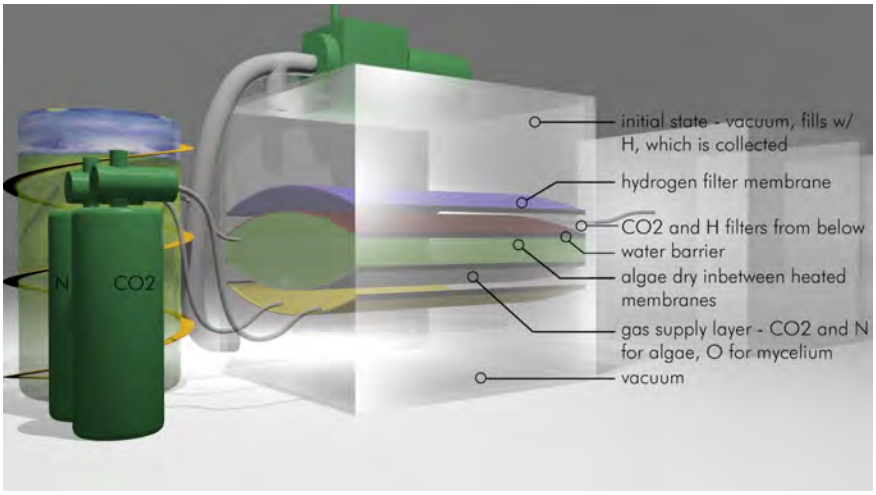
[41] https://ecovativedesign.com/

Table 1. The above table compares material made by our team with materials under consideration for ISRU construction. Sources: [42,43,44,45,46,47]

The challenge of this investigation is to develop bio-composites that can be completely grown off-planet, such as where no bio-waste exists. We've successfully grown mycelium on *Spirulina*, a cyanobacterium that we believe can be axenically grown with the circulatory and cell system described above. The below microscopy shows the bio-composites referenced in Table 1 and Figure 25 and the new composites made using *Spirulina* (a cyanobaterium) as the substrate.

Figure 25. Mycelial composites made by redhouse NIAC team.

The illustration in Figure 26 shows a prototypical "cell" that might be developed in subsequent phases to test mutual growth. It is a chamber that allows the axenic growth and dehydration of micro-algae that can then be myceliated. A vacuum is formed on both sides of the cell to mimic pressure differentials. Heated membranes can be added to the prototype later to test growth in sub-zero environments.



[42] Bar-Cohen, Yoseph Editor. 2018. *Advances in Manufacturing and Processing of Materials and Structures.* CRC Press/Taylor & Francis Group, LLC.

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[45] Khoshnevis, B.. Carlson, A.. Leach, N.. Thangavelu, M. 2012. *Contour Crafting Simulation Plan for Lunar Settlement Infrastructure Build-Up.* NASA NIAC Phase 1 Final Report.

[46] Voitkovscii, K F. 1960. *The Mechanical Properties of Ice.* Air Force Cambridge Research Laboratory, Geophysics Research.

[47] S, Keith and Stack Exchange Community. 2013. *What Thickness/depth of water would be required to Provide Radiation Shielding in Earth Orbit?* Stack Exchange. Space Exploration beta. https://space.stackexchange.com/questions/1336/ what-thickness-depth-of-water-would-be-required-to-provide-radiation-shielding

Figure 26. Unit cell designed for testing simulated *in situ* growth.

4.3.2.5 Construction Methods: Building Envelope

The below diagrams (Fig. 27, 28) illustrate the concepts developed in Phase I, which we would like to build upon in Phase II.

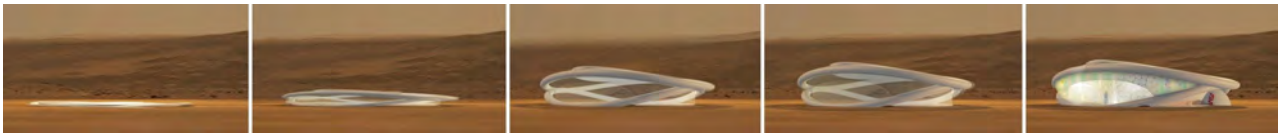

Figure 27. Building envelop grows as three pneumatic rings provide initial structure. The circulatory system then delivers nutrients to the building membrane cells.

An intersection of three rings inflates to set the structural form. This can be delivered by compressed gasses from the Martian atmosphere, from Lunar water, or from compressed canisters embedded within the rings. Rovers can be used to deliver the *in situ* resources, or the mechanisms could be embedded within the folded skin. The rings will later be filled bio-composites, but as air filled tubes they initially and immediately as scaffolding to let the micro-organism begin permanent construction. The building's circulatory system feeds the membrane cells to grow the bio-composite structure. Cyanobacteria embedded within the cells are fed water, nitrogen, carbon dioxide, and other nutrients sourced *in situ.*

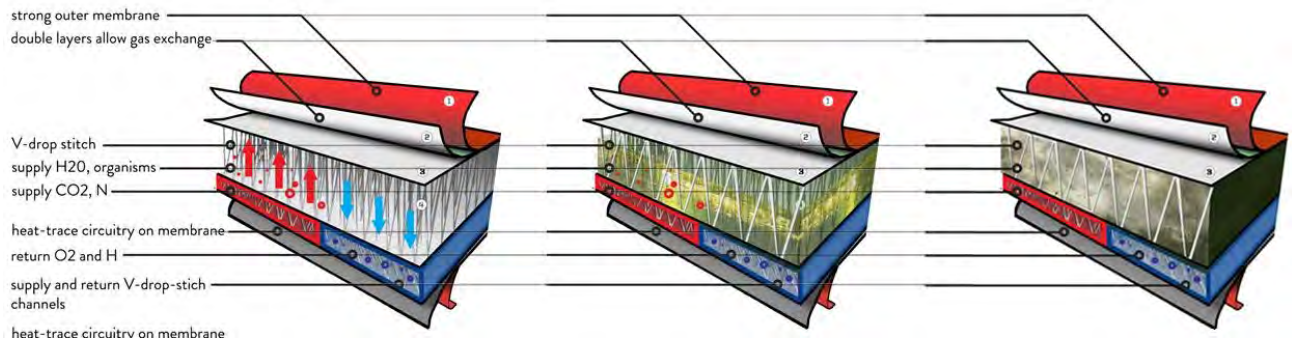

Figure 28. Multilayered system shows cavity in "dropstitch: inflatable. The bioreaction occurs in cavity and biomass fuses with stitches.

Heat is supplied to the cells creating the right conditions for growth. The organisms grow releasing oxygen that is stored within a special bladder. Once the cyanobacteria have reached a critical biomass, the nutrient rich substrate is dehydrated to a level that would support myceliation by saprophytic fungi. Oxygen is then released back into the cells and the fungi can feed on the oxygenated algal biomass. The fungi branch between the algal cells and begin to devour them by secreting enzymes and converting the external dissolved cellulosic material into chitin within the fungal cell walls.

The algal biomass becomes fused with the mycelium at a cellular level and is heated and compressed by the heat-traced pneumatic membranes. The multilayer system allows for redundant protection and separation of materials in various states of matter. The hydrogen produced allows fenestration while providing radiation protection. (see Figures 31 and 32)

4.3.2.6 Interiors: Embedded FFE (Furnishings, Fixtures, And Equipment)

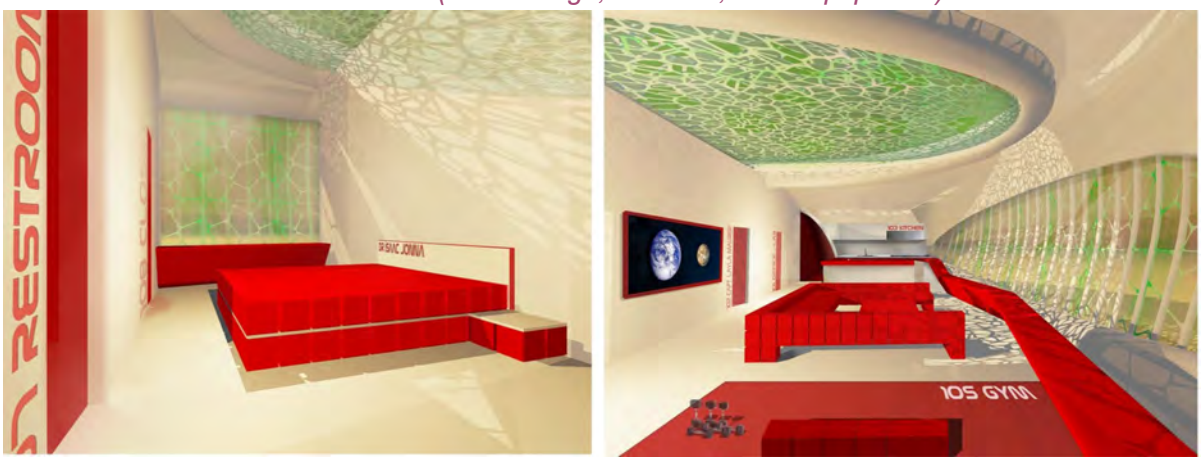

Figure 29. Interiors of habitat with furniture and fixtures ready for use. The furniture is grown, unfolding to reveal pre-packed equipment like stoves and sinks. The bio-reactor creates a stained-glass effect and creates hydrogen to filter ionizing radiation

Inner modules can be folded with the outer form to grow fractally once the exterior envelope is fully developed and the interior conditions are favorable for growth. Bathroom units can be grown with plumbing lines in place leading to processing chambers (to be developed in later phases) Kitchen units can be designed with cooking equipment and utensils pre-folded and pre-seeded in place. Scientific labs can be seeded that include equipment specially designed to be grown. In this regard the biomimetic habitat concept becomes more of platform than a product. Can we grow a toothbrush? Of course. Complex machinery will need to be pre-embedded, but tools and even simple machines like telescopes or hammers can be grown in ever-smaller scales down to the finer details.

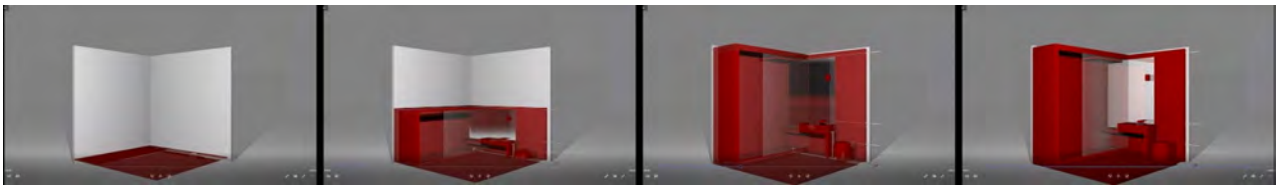

Figure 30. A bathroom grows within the allotted space. Pipes and fixtures are made flexible and the room grows the same way the building envelop does.

Figures 23 and 30 illustrate this fractal self-assembly at various scales. An animation is provided at redhouse's website:
www.redhousearchitecture.org/redplanet/v2q5ajy60j1xm6bzlo4fb6u46krc2f
There is an interactive model provided which shows what bioHAB might look like before crewed missions arrive, including cabinets, desks, beds, oxygen(!) and even display monitors.[48]

4.3.2.7 Building systems

The MEP systems (mechanical, electrical, and plumbing) can be integrated into the folded membranes as well. Flexible plastic cabling can be embedded within the folded structure for plumbing and electrical conduits and smart home systems and display monitors can be prewired with space-ready electronics. Plumbing fixtures such as toilets with p-traps and shower heads can be designed to be grown using the same membrane and bio-composite technology as the

[48] https://app.walkthruit.com/mc181207v2/

overall structure. Human waste should be utilized for compost and/or power generation. The "Waterwall" system proposed by Flynn et al. during a 2013 NIAC (NASA-ARC) discusses the possibility of recycling human waste as a nutrient for bioreaction.[49] It is suggested that nuclear power will power Lunar and Martian settlements.[50] We demonstrate that the bioreactors may enable electric production for heating membranes in Figure 32. It may be possible to power the entire structure with this effort, and only rely on nuclear power as a backup. Redundant systems for waste disposal, electrical production, and carbon ventilation should be developed as fail-safes.

4.3.2.8 Radiation protection

Ionizing radiation is a show-stopper for long-term crewed space missions.[51] Most architectural concepts for Mars construction require building below ground in lava tubes or piling three meters + of regolith on the outside of structure. This is because of the dangerous levels of ionizing radiation, including particle radiation (alpha and beta) and electromagnetic (UV, x-ray, and gamma) can damage crew DNA. Heavy materials like lead (Pb) and aluminum are effective but costly to transport. water is an option but are in demand for other uses on crewed missions. NASA's 3D printing challenge led to an innovative proposal by SEA-rch that utilized ice as a building material, claiming that only 30 cm of the material could block the ionizing radiation.[52] It was subsequently found it would require much more material, however, and the delicate nature of ice may also prove too challenging.

There are a few ways we can leverage the biological processes for making materials to create radiation shielding materials and possibly even radiation transduction to harvestable energy.[53] The first is the production of hydrogen. Hydrogen is a known shield for ionizing radiation because of the low atomic mass. The second is little studied phenomenon known as radiosysnthesis. Researchers have studied the processes of radiosynthesis- the natural transduction of ionizing radiation into benign forms of energy - for over a decade.[54] They've observed that melanin-rich fungi can shield from ionizing radiation, including electromagnetic and particle radiation, at efficacies comparable to lead (Pb) and twice as effective as charcoal.[55] The organisms can be extremely radioresistive,demonstrating survival at 1.6 million times a dose considered lethal to humans.[56]

Hydrogen and H+ ion production and utilization. Hydrogen gas, liquid hydrogen, stabilized hydrogen proton ions, or hydrogen infused polymers may be good options for radiation protection.[57]

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[49] https://www.nasa.gov/sites/default/files/files/Flynn_2012_PhI_WaterWalls.pdf

[50] Drake, B.G., ed. 2009. *Human Exploration of Mars Design Reference Architecture 5.0.* NASA/SP–2009–566-ADD

[51] NASA | Radiation Shielding Materials Containing Hydrogen, Boron, and Nitrogen. Quotation: Sheila Thibeault, Senior Research Physicist NASA LaRC: https://www.youtube.com/watch?v=ADA-FtQ_Vno

[52] https://www.nasa.gov/directorates/spacetech/centennial_challenges/3DPHab/index.html

[53] https://onlinelibrary.wiley.com/doi/abs/10.1111/1462-2920.13753

[54] https://www.sciencedirect.com/science/article/pii/S1369527408001306?via%3Dihub

[55] https://www.ncbi.nlm.nih.gov/pubmed/18426412

[56] https://onlinelibrary.wiley.com/doi/abs/10.1111/1462-2920.13753

[57] https://onlinelibrary.wiley.com/doi/abs/10.1111/1462-2920.13753

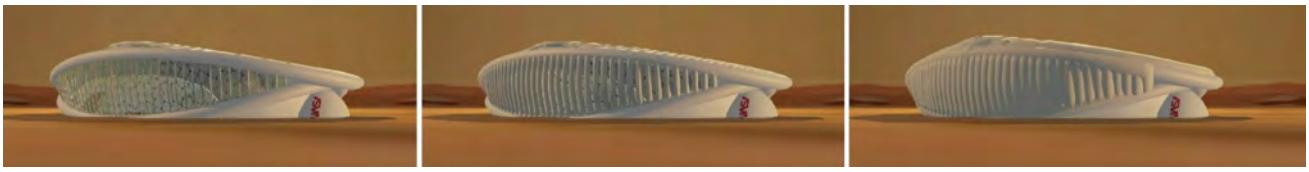


Figure 31. Extra radiation shield can be deployed through hydrogen bladders when solar wind is forecasted.

The processes that we submit here for developing building materials within a pre-seeded bag- such as the life-giving circulatory system - may have secondary benefits of allowing a portion of the building to remain alive post-bio-construction. Should the circulatory system be allowed to feed an embedded algal bioreactor, the algae could scrub carbon dioxide from the cabin and convert that into oxygen

and/or hydrogen.[58] The oxygen could be used for cabin air and the hydrogen could be used to provide a clear radiation shield that could serve as fenestration. By utilizing proton exchange membranes the hydrogen could be split into electrons to power heat membranes (see Figure 32), and the separated H^+ ions can used as the radiation shield. Excess hydrogen could be stored to power hydrogen fuel cells and deployed in special protective bladders that inflate during forecasted solar wind events deliver higher levels of ionizing radiation (see Figure 31).

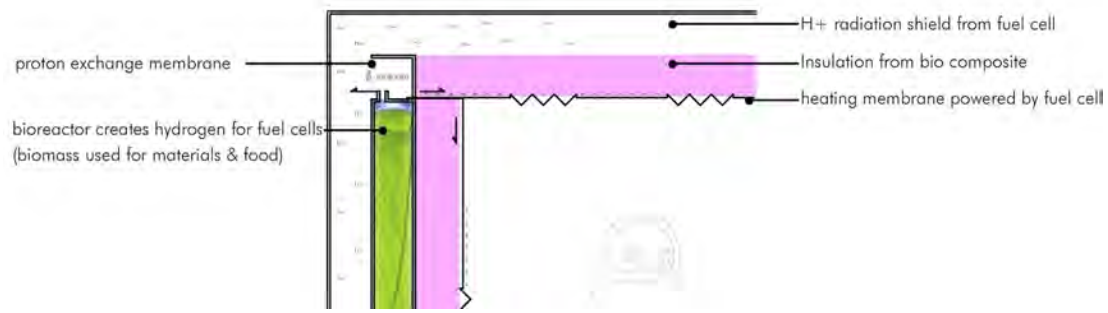
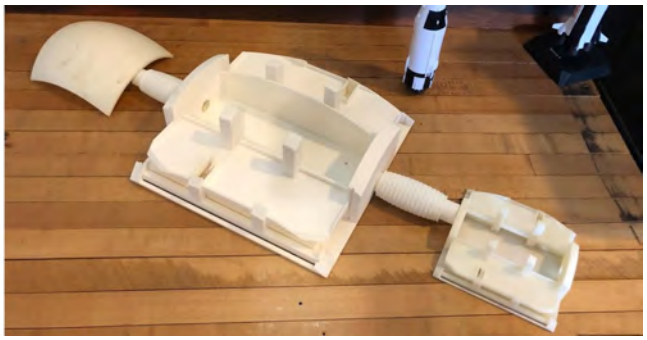


Figure 32. The schematic shows how hydrogen protons can be separated, utilized, and later recombined with electrons used for electrical current and heating with a proton exchange membrane. This principal can be applied to all surfaces.



[58] https://s3.amazonaws.com/academia.edu.documents/40725052/Bio-hydrogen_production_from_waste_materials.pdf?AWSAccessKeyId=AKIAIWOWYYGZ2Y53UL3A&Expires=1550688950&Signature=gd5wk2T9tFLOOG%2FYm5UobHr5Lic%3D&response-content-disposition=inline%3B%20filename%3DBio_hydrogen_production_from_waste_mater.pdf

Figure 33. Physical models, 3D printed.

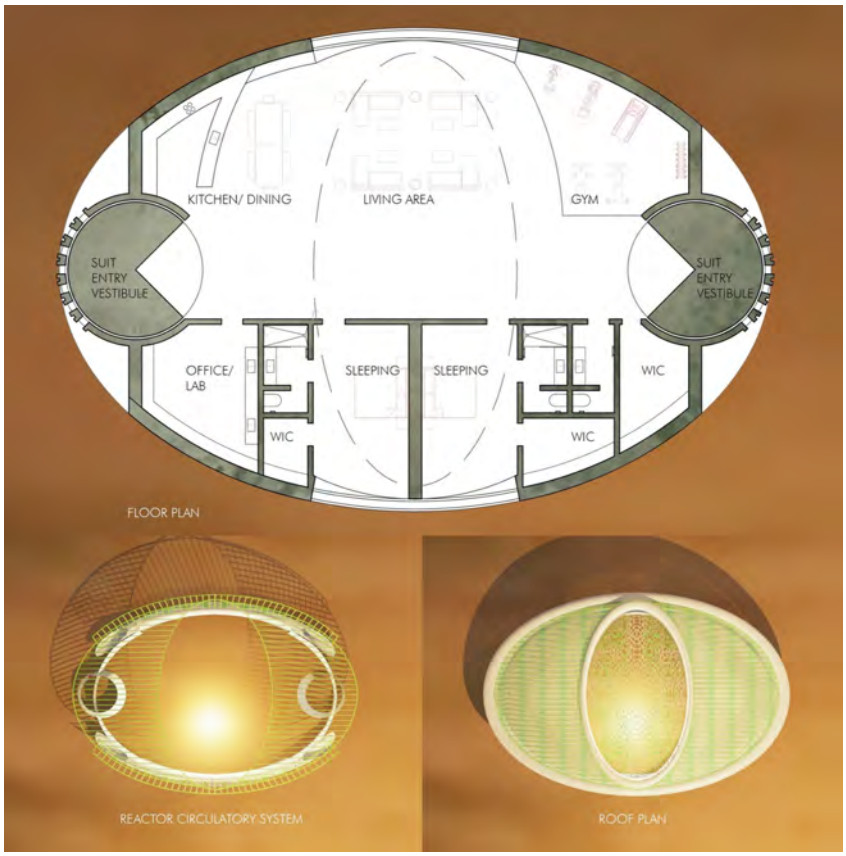


Figure 34. Floorplan, roof plan, and reactor circulatory system plan. (to left)

4.3.3 iGEM design

The habitat is based around a three-layered plastic dome, of which the outer layer is a layer of water which will freeze into ice, another shell encloses the cyanobacteria, and lastly the layer of mycelium. Each layer provides its own benefits. The ice water layer will be drawn from a pump which will melt subsurface ice water from our landing site, this layer provides insulation as well as providing radiation protection. Lastly this layer can be partially melted to provide water to our living cyanobacteria and fungus. The layer of cyanobacteria, suspended in a water solution will receive the sunlight which passes through the ice and use this energy it to break down carbon dioxide as well as molecular nitrogen, and create biologically available carbohydrates and nitrogen. We considered a design for a system that would ensure the cyanobacteria was well supplied and that could feed the products into the mycelium matrix. The source of the nutrients required for cyanobacteria growth would in part be provided by the martian regolith as well as the atmosphere and would be supplemented with any lacking nutrients by fertilizer brought from earth. The mycelium layer provides the main functionality in the system, providing structural stability, radiation protection and insulation and can be functionalized to provide additional benefits such as water filtration. The mycelium from the species we used in our experiments as well as many others produces edible mushrooms which astronauts could eat. Our design requires some robotic assembly.

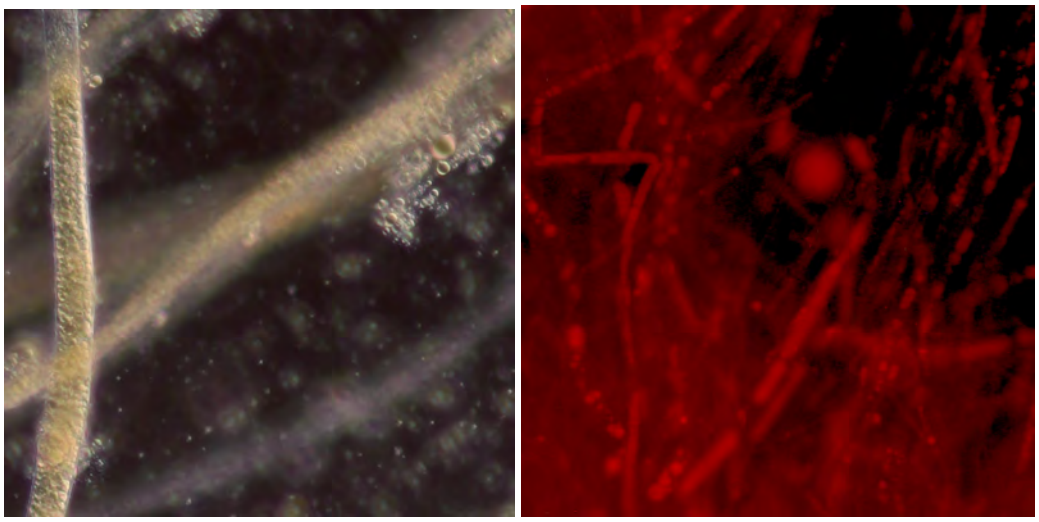
4.3.4 Radiation Protection

Our group also considered the radiation environment on Mars as well as some possible solutions. Since Mars has little atmosphere nor a magnetic field to protect it from radiation, solar flares, solar ultraviolet radiation and galactic cosmic rays can bombard the surface and could cause serious damage to life forms[59]. For comparison, the amount of total radiation incident on an astronaut over the course of 180 day transit and 500 days on the martian surface is almost 200

[59] Zeitlin, C. et al. 200). Overview of the Martian radiation environment experiment. https://mepag.jpl.nasa.gov/topten.cfm?topten=10

times as much as a person who remained on earth would receive over an equal amount of time.[60] This amount of radiation incident is enough to rapidly kill any life form as we know it, and is thought to have sterilized the surface and some of the subsurface of Mars.[61] If humans are ever to live on the surface of Mars, the problem of radiation must be addressed.

While there are many proposed solutions to mitigating this radiation for astronauts or other life forms on the surface, one strategy that has not yet been studied extensively is the use of lipids to block radiation. Lipids could be especially useful at blocking galactic cosmic rays because of their high hydrogen density. According to Jonathan Pellish, a space radiation engineer at NASA's Goddard Space Flight Center, "The best way to stop particle radiation is by running that energetic particle into something that's a similar size."[62]. Because galactic cosmic rays consist of high energy protons and neutrons, the best way to block it would be with hydrogen which has a nucleus similar in size. The high hydrogen content of plastics is already being investigated at NASA where plastics and other trash is being compacted into tiles with the hope that they will provide increased radiation protection. Lipids are similar in structure and hydrogen density to plastics and so we investigated the possibility of creating lipids to shield our habitat from galactic cosmic rays. To this end our team identified a species of filamentous fungus which produces more lipids than other species, *Mucor circinelloides*.[63] We considered incorporating this species of fungus into our structure to increase the radiation protective abilities. While we were able to obtain the strain from the American Type Culture Collection (strain 20132), grow it at NASA Ames, and stain it with Nile Red[64] to confirm high lipid content (Fig. 35).



[60] Hassler, D.M. and the MSL team 2014. Mars' Surface Radiation Environment Measured with the Mars Science Laboratory's Curiosity Rover. *Science* **343**:1244797. DOI: 10.1126/science.1244797

[61] Dartnell, L. 2011. Ionizing radiation and life. *Astrobiology* **11**: 551–582 https://doi.org/10.1089/ast.2010.0528

[62] Frazier, S. 2015. Real Martians: How to Protect Astronauts from Space Radiation on Mars. https://www.nasa.gov/feature/goddard/real-martians-how-to-protect-astronauts-from-space-radiation-on-mars

[63] Xia, C., Zhang, J., Zhang, W. & Hu, B. 2011. A new cultivation method for microbial oil production: cell pelletization and lipid accumulation by *Mucor circinelloides*. Biotechnology for Biofuels 2011, 4:15 http://www.biotechnologyforbiofuels.com/content/4/1/15

[64] Kimura, K., Yamaoka, M. & Kamisaka, Y. 2004. Rapid estimation of lipids in oleaginous fungi and yeasts using Nile red fluorescence. *J. Microbiol. Methods* **56**: 331-8.

Figure 35. Mucor circinelloides*. The light micrograph on the left was taken under DIC. Note the yellowish lipid globules packing the mycelia on the left, and the lipid vesicles that have been released from broken mycelial to the upper right. The image on the right was stained with Nile Red and the image taken with fluorescence microscopy using a Cy3 filter. Again note high quantity of lipid droplets in the mycelia.*

4.4 Objective 4. Identify key knowledge gaps including pathways to implementation

4.4.1 Key knowledge gaps

Technical for mycotecture

While we grew several prototypes, we still are not confirmed on optimal fungi, enhancements, growth conditions, packing, shipping, growth at destination or post-processing.

Habitat Architecture

Several designs were produced but we have yet to prototype with mycelia and deploy. Embedded systems also need consideration.

Mission Architecture

A mission architecture for Mars was developed, but one for the Moon is needed.

4.4.2 Pathway to implementation for Mars[65]

4.4.2.1 Habitat Design

Beginning on Earth, the first task involves manufacturing the plastic shell for the habitats. Our habitat is designed as a three-layered plastic dome as described above. The outer layer is designed to hold H_2O, which can provide some measure of temperature insulation (high specific heat) and radiation protection (due to the high hydrogen-composition, which interacts with harmful primary radiation, while producing less secondary radiation than compounds composed of heavier elements).[66,67,68] The supply for the H_2O layer will be drawn via melted subsurface ice water at the landing site.

The middle layer is designed to hold cyanobacteria, which can fix compressed carbon dioxide and molecular nitrogen to produce oxygen for our main biomass (the mycelia), as well as for our astronauts to breathe. In addition, these diazotrophic cyanobacteria can produce organic carbon and fixed (biologically usable) nitrogen. In some of our experiments, we show that additional nutrients for cyanobacteria growth can be provided by Martian regolith and small quantities of fertilizer brought from Earth. The light for photosynthesis passes through the topmost layer to reach the cyanobacterial layer.

The final layer is designed to hold the mycelia, which provide the structural integrity and additional radiation protection for the habitat. This mycelia can also be functionalized for several

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[65] http://2018.igem.org/Team:Stanford-Brown-RISD/MissionArchitecture

[66] USGS. Heat Capacity of Water, www.water.usgs.gov/edu/heat-capacity.html

[67] Mars Architecture Steering Group. Human Exploration of Mars Design Reference Architecture 5.0 Addendum. NASA, July 2009, www.nasa.gov/pdf/373665main_NASA-SP-2009-566.pdf.

[68] Space Faring: The Radiation Challenge. NASA, www.nasa.gov/pdf/284275main_Radiation_HS_Mod3.pdf.

other purposes, which you will see in other sections: primarily MycoGlue (to assemble smaller objects), and Filtration (to provide clean water for astronauts).

Note that, between each layer are one-way valves allowing water to flow into the cyanobacteria layer, and cyanobacteria to flow into the mycelial layer (keeping each organism alive).

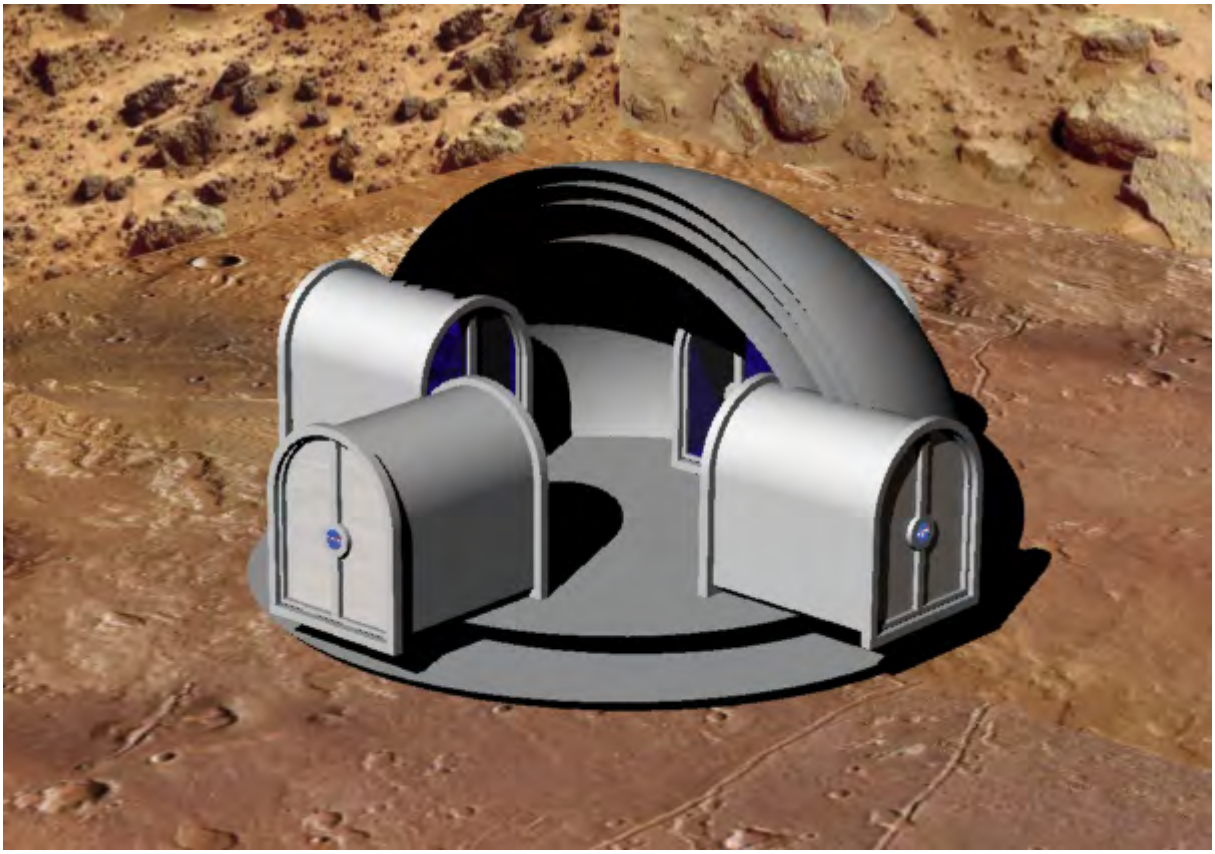

Figure 36. Habitat Design. Design and figure created by team member Emilia Mann using Rhino 3D software.

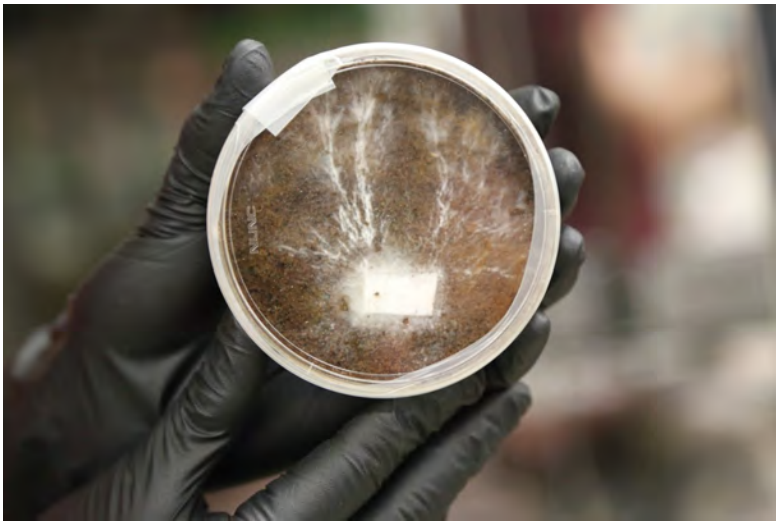

Figure 37. Mycelia growing on Martian regolith simulant. They also grew on lunar regolith simulant. (special thanks to Joe Minafra of the Solar System Exploration Virtual Institute-SSERVI for supplying us with the simulants.)

4.4.2.2 Launch, Travel, and Landing

Once the habitat is designed, built and loaded with mycelial starter filaments and dried cyanobacteria or spores (akinetes), we need a way to get the structures from Earth to another destination (e.g., the

Moon or Mars). There are a few rockets we can choose from: the Falcon Heavy from SpaceX,[69] and the newly developed Space Launch System from NASA are potential options. The launch site will likely be NASA Kennedy Space Center in Florida.
In talking to Engineering Prof. Rick Fleeter from Brown University (an industry-veteran and consultant to the Italian Space Agency), he suggested allocating at maximum 80% of the actual rocket payload to the weight of our structures - providing us with a ballpark estimate of how much material we can carry. As other infrastructure is needed for a human settlement, we aimed to be substantially lower in mass.

Once in space, estimated travel time to Mars is approximately 6-7 months, in the closest approach between Earth and Mars (which occurs no less than ~1.5 years apart).[70]
Numerous factors need to be considered when choosing a Martian landing site. These include equatorial proximity (to reduce temperature fluctuations on the order of hundreds of degrees between day/night), low altitude (thicker atmosphere allows for easier spacecraft deceleration, and meaningful quantities of useful gases), and availability of shallow sub-surface water (for use by astronauts).

4.4.2.3 Landing site selection

We first spoke to Dr. Lisa Pratt, NASA's Planetary Protection Officer, to better understand the regulations regarding which types of landing sites are off-limits, or may require additional precautions. Then, based on consideration of these factors, and in discussions with Co-I Jim Head, we chose as our landing site Deuteronilus Mensae;[71,72,73] this site (Figs 25 and 26) was among the very top sites in NASA's First Landing Site (LS)/Exploration Zone (EZ) Workshop for Human Missions to the Surface of Mars[74] and is known to have abundant near-surface ice/water deposits on the basis of Mars Reconnaissance Orbiter penetrating radar results.[75] Deuteronilus Mensae is nearer the equator so there is less seasonal temperature flux. It is still far enough from the equator that it can still recieve ice and dust accumulation and currently there is a strong hope that there is a large body of ice water within 10 m of the surface in this area according to Head. The region also has a lower elevation and thus thicker atmosphere which will provide more

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[69] "Falcon Heavy." SpaceX, SpaceX, 16 Nov. 2012, www.spacex.com/falcon-heavy

[70] Mars Architecture Steering Group. Human Exploration of Mars Design Reference Architecture 5.0 Addendum. NASA, July 2009, www.nasa.gov/pdf/373665main_NASA-SP-2009-566.pdf

[71] Head, J. W., D. R. Marchant, M. C. Agnew, C. I. Fassett, and M. A. Kreslavsky 2006. Extensive valley glacier deposits in the northern mid-latitudes of Mars: Evidence for Late Amazonian obliquity-driven climate change, *Earth Planet. Sci. Lett.*, 241, 663-671, doi: 10.1016/j.epsl.2005.11.016.

[72] Baker, D. M. H., and J. W. Head III 2015. Extensive middle Amazonian mantling of debris aprons and plains in Deuteronilus Mensae, Mars: Implications for the record of mid-latitude glaciation, *Icarus*, 260, 269-288, doi: 10.1016/j.icarus.2015.06.036.

[73] Morgan, G. A., J. W. Head, and D. R. Marchant 2009. Lineated valley fill (LVF) and lobate debris aprons (LDA) in the Deuteronilus Mensae northern dichotomy boundary region, Mars: Constraints on the extent, age and episodicity of Amazonian glacial events, *Icarus*, 202, 22-38, doi:10.1016/j.icarus.2009.02.017.<

[74] First Landing Site/Exploration Zone Workshop for Human Missions to the Surface of Mars. USRA. 30 October 2015. https://www.youtube.com/watch?v=is9B7EM4UN0&t=0s&list=PLQ7WzZtg-qMBAKEHnjfoTR3vPtMSnoM-D&index=10

[75] Plaut, J. J., A. Safaeinili, J. W. Holt, R. J. Phillips, J. W. Head, R. Seu, N. E. Putzig, and A. Frigeri 2009. Radar evidence for ice in lobate debris aprons in the mid-northern latitudes of *Mars Geophys. Res. Lett.*, 36, L02203, doi: 10.1029/2008GL036379.

nutrients and protection to our system as well as helping slow down any lander we send to the area.

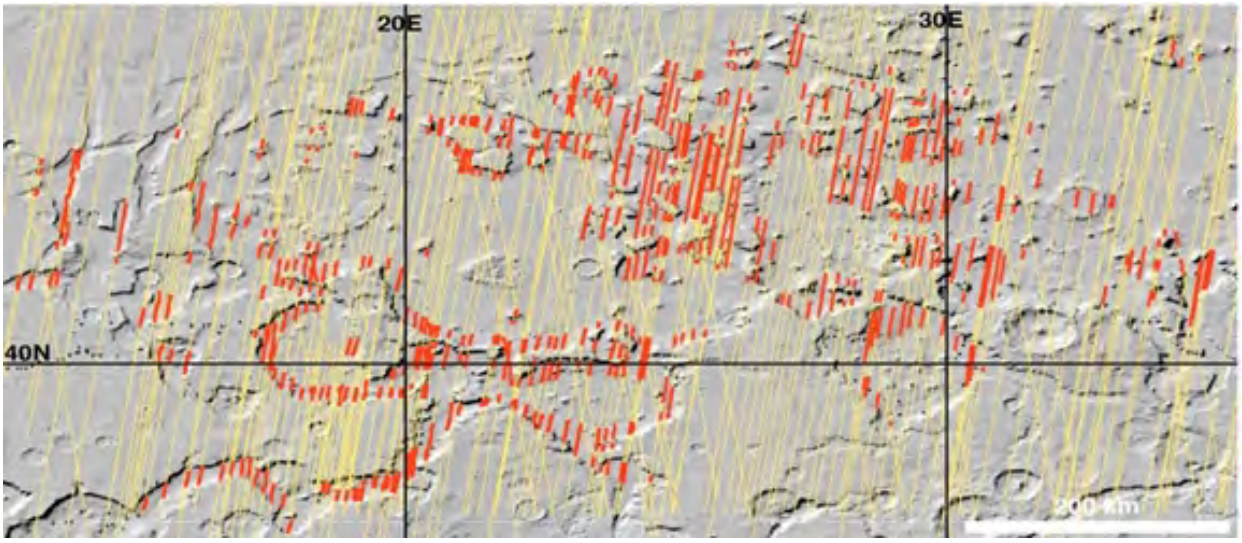
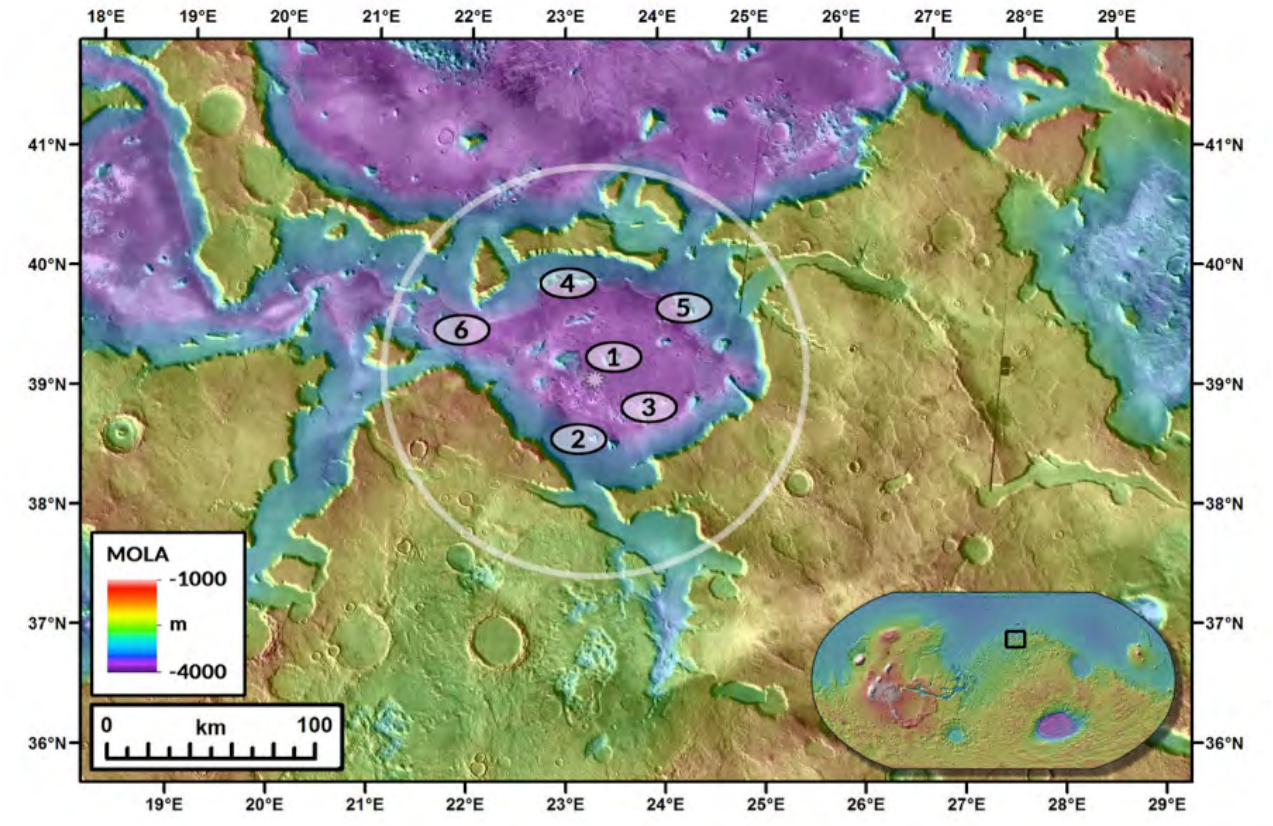


Figure 38. "MOLA shaded relief map of the Deuteronilus Mensae area, with the groundtracks of SHARAD observations shown as yellow lines." Red indicates potential ice detections.[76]

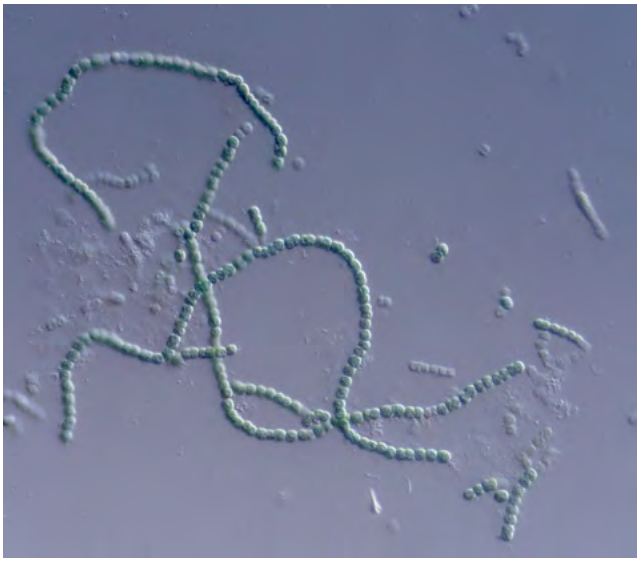


[76] Plaut, J J, et al. "Thick Ice Deposits In Deuteronilus Mensae, Mars: Regional Distribution From Radar Sounding." 41st Lunar and Planetary Science Conference (2010), Mar. 2010.

Figure 39. The Deuteronilus Mensae Region of Mars: Circle represents landing region and numbered ovals represent candidate landing sites that have access to shallow glacial ice. Inset map shows location on Mars. Color represents Mars Orbiter Laser Altimeter topography (purple low, red high).

4.4.2.4 Deployment at destination

At destination, our habitats will assemble with the help of robots (the mycelial growth is effectively rate-limited by access to feedstock) on the order of weeks, and is designed to provide a safe living environment for our astronauts or to enable human colonies on other planets.



Cyanobacterial Oxygen-Production Validation. Cyanobacteria play an important role in our structure: serving as feedstock for the mycelia, and producing oxygen for use by astronauts. We wanted to quantify the ability of cyanobacteria to provide sufficient oxygen to sustain a habitat. We chose the strain ***Anabaena*** 7120 mainly because it is diazotrophic and photosynthetic, in addition to other favorable qualities for use on other planets.[5]

Figure 40. Differential Interference Contrast Microscopy Image of **Anabaena** *7120. Credit: Lynn Rothschild.*

Using 425 ml of ***Anabaena*** in liquid culture, we used wireless oxygen and carbon dioxide sensors (Vernier Go Direct Series) in an airtight environment to quantify the production of these gases on a short time-scale. We used BD Anaerobic Sachets to achieve a <0.5% oxygen environment, and a 1627 mL Nasco Whirl-Pak® airtight bag to maintain a suitable environment.

Given the empirically observed rate of oxygen production from our liquid culture, we reason that it will take ~92 hours (4 days) to achieve 20% oxygen, almost mimicking Earth's atmosphere. This time-frame is certainly reasonable in the context of our mission architecture. (For more details, please refer to the Results page.)

Figure 41. Laboratory setup to quantify oxygen production. In a separate experiment, we also attempted to grow ***Ganoderma lucidum*** (a strain of fungus) directly on ***Arthrospira platensis*** (a species of cyanobacteria). A separate flask of ***Anabaena*** provided the oxygen supply. Unfortunately, we have thus far not been successful in showing measurable ***Ganoderma*** growth. We believe the Arthrospira culture which was being used as feedstock for the

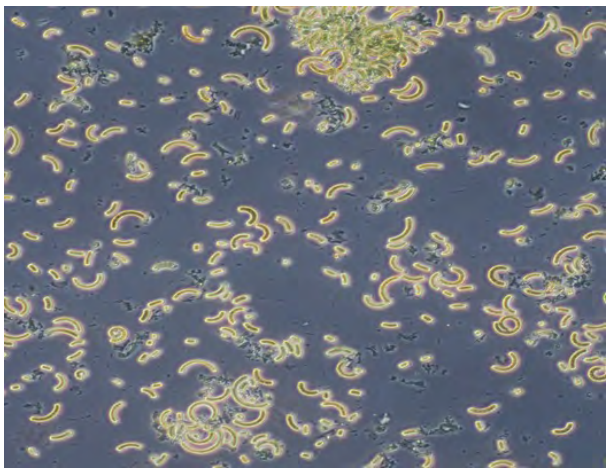
Ganoderma may have had too much water, and the ***Anabaena*** culture may also have been too dilute. Future experimentation will hopefully yield more optimal results (one of our mentors, Co-I Maurer, was successful in growing mycelia on ***Arthrospira***.) Credit: Santosh Murugan.

Figure 42. Phase contrast microscopy image of the cyanobacterium **Arthrospira***. Credit: Lynn Rothschild.*

4.5 Objective 5. Assess impact of technology for terrestrial applications

4.5.1 Summary

Terrestrial applications include potential ones for NASA (field supplies, packing and shipment of delicate mechanical and electronic parts and even “green buildings”.) Other terrestrial applications are in architecture, packing materials, and the quick production of structures in remote locations. Others are exploring the use of mycelial-based substitutes for leather. See 4.9.2.



4.5.2 Results

The impact of our project and research conducted to bring it to fruition, will reach all the way from Earth to Mars. For that reason, the Stanford-Brown-RISD 2018 iGEM team decided to define human practices (applications of their project) in two different ways: how it will effect Mars and how it will affect the Earth.

For Mars, unfortunately, because there are no users on Mars (yet!) it was impossible for us to engage with end users of our habitat. For that reason we decided the most effective approach would be to discuss with the experts who would be utilizing this design. During our interviews we discussed the parameters of our design and what cautionary steps we would need to talk in our design to not only protect travelers to Mars but the planet as well from contamination.
For earth we explored that technological implications of mycelium composites and how they could be used in sustainable industrial products and housing.
Below is how we engaged with experts and members of the community who had a stake by our research, for “Martian” and human practices.

4.5.3 Martian Habitats

The idea of people living on Mars is an inherently exciting prospect, and it can be a great deal of fun to dream of what the homes, or even cities, of a future martian colony would be like--will they be giant geodesic domes filled with lush greenery that dot the martian landscape and are connected by underground tunnels? Or will they be totally underground, an endless maze that will shield its inhabitants from the harsh cosmic rays? Or perhaps even crafted from ice--creating glistening ice sculptures upon the martian surface?

However, for the Stanford-Brown-RISD iGEM team just as alluring and fun as the imaginative aspect of designing for Mars is--which we certainly explored--was the challenge of designing our habitat to be a highly cost-effective, protective, and functional design.

There are a lot of moving parts that go into generating a design like this. We needed to understand what are the basic functions that a general habitat needs to fulfill (i.e. places to rest, to work, and to socialize. Certain degrees of privacy and lighting are also requirements) and what new functions a habitat on Mars would need to fulfill (for example protection from the harsh environment and radiation). We also had to investigate the different options for Martian habitats that exist already, craft the mission architecture, and consider the laws and regulation of planetary protection that would certainly impact our project.

In order to get the best possible answers to these questions we engaged directly with experts planning the exploration of Mars, and overseeing the protection of the planet.
Our first contact was Oliver Morton, a British science writer and former editor of Nature. We presented our rough ideas about our project to him, and he gave us guidance on how to present the information more effectively and what areas we needed to explore more. And, perhaps most importantly, he also questioned our motives. He asked why we wanted to design a Martian habitat in the first place, when we could also easily focus on the earth and probably have a greater immediate impact. It was a difficult question that required us to be introspective about a notion that seemed so obvious at first. After a lot of dialogue we realized, while designing a Martian Habitat may not benefit the maximum number of people, designing in Space gives us the freedom to explore new ideas and materials without having to worry about the constraints of the market and competition on Earth. After our first interview with Morton, we had a better understanding of the direction we needed to go in and why. We were now ready to discuss with specialists in Martian and space travel.

Figure 43. Team Members Emilia Mann, Leo Penny, Arvind Veluvali, and Advait Patil with Oliver Morton.

We interviewed Dr. Michael Meyer, the lead scientist of NASA's Mars Exploration Program (https://www.nasa.gov/mission_pages/mgs/michael-meyer.html), to answer some of our primary questions about what the design of a human mission to Mars would like. We asked about which options for martian habitats are currently being explored by NASA--he shared two, both 3D printed structures, one created from a composite made from Martian regolith the other ice--as well as questions about the longevity that is required of the mission, the estimated budget, the kinds of experiments that would be run, the benefit of having people on Mars versus and Rover, and how does NASA define acceptable risk.

Figure 44. iGEM Team Members Emilia Mann, Leo Penny, and Javier Syquia meeting with Dr. Michael Meyer.

The iGEM team also spoke with Dr. Lisa Pratt, NASA's planetary protection officer, about concerns that may arise from the fact that we are using a biological material as our main building component. Pratt informed us that we needed to ensure that no spores would be able to form from our mycelium blocks, and to ensure that there will not be the creation of a superorganism due to the interaction between the mycelium and the substrate it eats. We were also reminded that there are specific parameters in regards to harvesting water from the Martian surface. But most importantly, Pratt emphasized that many of these regulations will be subject to change as real human missions to Mars are planned and that we therefore must remain adaptive and anticipate any potential issues.

Jim Head, a professor at Brown University in the Department of Geoscience, gave us excellent recommendations on potential landing sites for Mars as well as other important logistical information key to the mission architecture of our design.

Lastly, Amy Kronenberg, Biophysicist Staff Scientist at the U.S. Department of Energy Lawrence Berkeley Labs, as well as a member of the NASA Innovative Advanced Concepts advisory board, gave us great insight and critique on our radiation protection. We had initially planned to use melanin, but she encouraged us to focus on finding ways to increase the number of protons (hydrogen) present to protect from cosmic radiation as melanin is only useful for UV radiation which the mycelial structures themselves should do. As a result we shifted our focus to creating a design that contains a thin outer layer of water. She also provided us with important literature.[77]

The information and feedback we received from these experts greatly informed our mission architecture—helping us to design a mission that not only fits NASA's requirements but also protects the planet in order to ensure the integrity of scientific missions to Mars.

4.5.4 Sustainable industrial products

We initially gravitated towards using mycelium as the core material in the design our habitat for Mars because of its self-perpetuating property--it can grow, given a substrate to bind, into the shape of any mold it is placed in. The results of our work demonstrated that we could produce a cost-effective, replicable habitat for Martian exploration.

However, as our research progressed and we spoke to more experts working with the material, we learned there was so much more to mycelium than its self-growing properties.

Phil Ross, one of the founders of MycoWorks, a company working to produce a more humane and sustainable leather from mycelium, was one of our first points of contact. He showed us a material produced from mycelium that behaved and looked just like leather. It was so unlike any of the solid mycelium bricks we had produced. He emphasized to us the ways in which the mechanical properties of mycelium can be manipulated by how it is grown and what is grown on.

This sentiment was echoed by Rolando Perez, a Ph.D. student focused on mycelium at Stanford University in the Drew Endy Lab.He also added that the strain of the mushroom can affect its material properties, and for that reason he is currently working on creating a fungal database in order to make information about mycelium more accessible to the public and other researchers.

Eben Bayer, one of the co-founders of Ecovative, a company that got its start producing mycelium based styrofoam)emphasized in our interview with him how he first got interested in mycelium because of how it can act as a natural glue, binding together diverse substrates to create new material that is completely biodegradable. As a company they have conducted many material tests and found the material to be strong and flame retardant.

Through these conversations it became clear to us that mycelium has the potential to revolutionize product manufacturing on earth, by introducing a completely biodegradable material that is also self-growing. These key characteristics will allow manufactures to cut back on the cost, time, energy, and environmental impact involved with traditional production methods. And the ability of manipulate the mechanical properties of the materials ensures that mycelium could be used as a substitute for a variety of products—from furniture to clothing to medical devices.

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In response to this exciting realization we began exploring the ways mycelium could be used to create other products that might be necessary for our habitat--ranging from furniture, to scientific instruments, to a filtration system, to even a Rover chassis. We even generated some designs and prototypes for a few of these ideas, fully flushing out the idea of an entire living space created from one material.

One of first designs was a modular hexagonal stool that could be assembled into a larger surface through many units or separated into individual seating. We grew this stool by crating a hexagon mold from recycled cardboard and then stuffing the stool with aspen chips coated with mycelium. Within two weeks we had grown a fully functional stool! Images of us crafting, baking, and testing the tool can be seen below.

However, in order to have a holistic approach, we also wanted to understand what were the challenges of introducing this material to the public, something we questioned these pioneers on. They all echoed similar statements regarding some social resistance to the use of fungus in products. And perhaps the even greater challenge is shifting policy to favor the production of more sustainable products over cheaper, more environmentally damaging products. However, as Eben Bayer noted, opinions about mycelium are shifting. What was at first perceived as strange and alien, is now the center of growing excitement and research. People are thrilled by mycelium and all the potential it has to help create a better, more sustainable future.

4.5.5. Sustainable Housing

While exploring the design of our habitat for Mars, we realized that the short-time frame for construction of the house combined it's self-growing and eco-friendly nature might make our habitat useful on the Earth.

Figure 45. Chris Maurer with team member Emilia Mann at the NIAC Symposium in Boston, September 2018.

To further expand our ideas we consulted with Architects Chris Maurer, from redhouse studio in Ohio and a key collaborator on the NIAC with Dr. Lynn Rothschild, and Maria Aiolova, the co-founder of Terreform One, a non-profit that is focused on envisioning what a truly environmental city would like. Both these designers have done work exploring the potential of mycelium in an architectural setting.

Maurer emphasized the sustainable nature of the material, and their relative affordability--he speculated that some of the structures he has designed using mycelium would only cost around $8000 to produce. He also spoke about the many properties mycelium has that makes it an excellent building material--fire resistant, insulating, etc. He has also incorporated the remediating process of fungi in his designs to help clean up waste. Aiolova also noted the material's excellent building properties--highlighting that it has great acoustics and could also create an invigorating natural microbiome in interior spaces. She also cautioned, however, that when we design with mycelium we should be conscious of the material constraints—highlighting that multi-story building made solely of mycelium are unlikely and that rather it the creation of composite materials with mycelium that will create successful buildings.

With this information in mind, we began by brainstorming a variety of housing needs. At first we considered adapting the design of the house for refugees--a pressing issues, with the United Nations Human Rights Council estimating that in 2016, 65.6 million people were forcibly displaced from their homes, with 22.5 million of those people falling under the category of "refugees".[78] However after reading reports about the crisis and discussions with designers involved with refugee design we concluded that the development of another "pop up" house would not address the route of the problems--which have much more to do with policy.[79]

Next we looked inwards, focusing on the local issues of homelessness in San Francisco. California, as a whole, hosts 12% of the population of the United States, but 22% of the homeless population.[80] Again, however, deeper exploration revealed that while a shortage of affordable housing was a component, it was not addressing the core of the problem.

Turning once again to the drawing board, we decided to revisit what Maurer and Aiolova had said, this time focusing of the idea of sustainability and affordability. Construction is a major contributor to global warming and pollution; the construction of buildings alone is responsible for up to 40% of carbon emissions.[81] And in the US alone there are 50 million tons of demolition produced annually.[82] According to the US Environmental Protection Agency, there are a myriad of benefits to reducing the protection of demolition--ranging from cutting down on the number of demolition facilities needed to preventing the construction of more landfills.

We propose that our martian habitat, which is grown in an inflatable mold (which on earth, unlike on Mars, could be removed and reused), would be a great new form of sustainable housing. In addition it could be cost effective and reduce population by taking advantage of the ability of a fungus to absorb many toxins.

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[78] (2016) UNHRC Global Trends--forced displacement in 2016 http://www.unhcr.org/globaltrends2016/

[79] Fairs, Marcus (2017) "Don't Design Another Shelter" for Refugees, Says Experts. https://www.dezeen.com/2017/12/18/dont-design-shelter-refugees-kilian-kleinschmidt-rene-boer-good-design-bad-world/

[80] Hart, Angela (2017). How California's Housing Crisis Happened. https://www.sacbee.com/news/politics-government/capitol-alert/article168107042.html

[81] Chris Maurer. Biocycler. http://www.redhousearchitecture.org/biocycler/zgyxrogjln0fzs6ux2ognrnzuohzgb\

[82] Sustainable Management of Construction and Demolition materials. https://www.epa.gov/smm/sustainable-management-construction-and-demolition-materials

Figure 46. Production process including crafting, baking, and testing the stool. http://2018.igem.org/Team:Stanford-Brown-RISD/Human_Practices

5 Technical Risks and Suggested Mitigation Strategies

1. Self-assembly of structure requires too much mass/infrastructure. *Unknown without study.*
2. Algae are poor feedstock for fungi. *Co-I Maurer has grown mycelia on the cyanobacterium* Spirulina*. It's slow growing but is showing promise for a building material. New strains will be tested, and a low mass alternative such as sawdust or powdered nutrients will also be tested.*
3. Fungi do not have sufficient oxygen in the bags. *Live algae should produce sufficient O_2 even if not used as a food source for the fungi. Gas permeable plastics may be an alternative.*
4. Material properties do not meet standards. *Testing of multiple fungal strains, feedstocks, ratios with* B. subtilis*, processing parameters (water, temperature) and post-processing (e.g., baking.)*

6 Deliverables from proposal

1. A survey of a selected mycelial strains for suitability, as described in section 4.1.2 "Mycelium Material & Habitat Development. Biomass production of several feedstock

types for the mycelia, demonstrated in section 4.1.4 "Substrate Tests". Growth rates determined as mass and volume as a function of temperature over time, as shown in section 4.1.5 "Temperature Tests". Material properties of the dried, frozen and baked mycelial, described in section 4.1.6 "Growing Mycelia in Molds".

2. Identification and analysis of potential enhancements to the mycelia. This includes the synthesizing fusion proteins that linked a chitin binding domain (CBD) to various different functional proteins. The CBD binds chitin, which makes up the outer cell wall of fungus and our mycelium material, thus allowing CBD fusion proteins to bind to our mycelium. Among our most successful fusion proteins were the the CBD fused to the bacterial biofilm adhesion protein csgA, described in section 4.1.7 "Mycelium glue". CBD fused to three copper binding domains, described in section 4.2.5 "Copper binding", which enabled mycelium filter's ability to bind 92% of the available copper in 30 minutes. In addition, one patent disclosure was begun and a manuscript submitted (currently in review in *Scientific Reports*) on the use of fungal mycelia for a filter, work which also leveraged results from Rothschild's prior Phase I NIAC "Urban biomining meets printable electronics: end-to-end at destination biological recycling and reprinting" NNH17ZOA001N (2017).
3. Designs of a mycelium-based habitat, described in sections 4.3.2.2 "Architectural Concept Evolution" and 4.3.2.3 "Biomimetic Design. Advantages/disadvantages compared to alternatives, described in section 4.3.2.4 "Materialization".
4. Identify key knowledge gaps including paths to implementation, described in section 4.4 "Key knowledge gaps".
5. Assess impact of technology for terrestrial applications, described in section 4.5.
6. Programmatic deliverables include this final report.

7 Benefits of the study

7.1 To NASA

7.1 Human exploration

The primary benefit to NASA is the ability to deliver low mass building materials that can grow on site as a renewable resource. The possibilities for off planetary building with such materials are numerous. With the little upmass requirements of a few organisms, their ability to reproduce with *in situ* resources, and the multi-functionality of the materials; these processes are very promising for space.

Various forms and applications of the materials were tested. Specifically, we focused on building materials that could be used for wall assemblies, roof assemblies, arches, domes, floors, and furniture will be tested and several strains may be identified as ideal for specific purposes. A prototype was made of a stool. The second application tested was using the fungal mycelia as a substrate for a water filter, something that had not been envisaged in the original proposal.

7.2 Workforce development.

Although not originally proposed, the 2018 Stanford-Brown-RISD iGEM team, under Rothschild's direction, jumped at the chance to participate in the NIAC work. iGEM is the international Genetically Engineered Machine competition, the premier student competition in synthetic biology. Students identify and conduct a project, with the culmination being a competition (Jamboree) in Boston in the autumn where the students are judged on their project through the wiki, poster and 20 minute oral presentation.

iGEM team

2018 Stanford-Brown-RISD iGEM team, "Myco for Mars" (http://2018.igem.org/Team:Stanford-Brown-RISD) runner up, best new composite part, best in manufacturing, bronze medal

April 2018 Rothschild met with the nascent DTU (Technical University of Denmark) iGEM team when they were brainstorming projects. As their advisor, Chris Workman, has expertise in filamentous fungi, she suggested a complementary project. Thus, team DTU conducted a project became "Fungal building materials for extreme environments".[83] This allowed collaboration between Stanford-Brown-RISD in Rothschild's lab and DTU. This has resulted in Workman being invited to participate in the Phase II project.

7.3 Public Outreach, Rothschild

Rothschild's Public Talks that discussed the NIAC Phase I

- TEDx Beacon Street @ WGBH Boston (Oct 29)
- lecturer for STEAM week, Los Altos High School (Oct 11)
- Boston Museum of Science, "From Science Fiction to Science Fact" (Sept 22)
- taped, podcast, Copenhagen with TechFestival
- podcast, How Could Genetic Engineering Affect Space Exploration? - Offworld Episode 12 (YouTube series on Tested,) https://www.youtube.com/watch?v=Cc0HvlmAuAY published Oct 15
- ComiCon Panelist, "Pack Your Bags: We're Moving to Mars! (Eventually)", Silicon Valley Comic Con, Jason Lederman (Pop Sci), moderator. http://www.svcomiccon.com, https://www.nasa.gov/svcc2018

And professional talks:

- MIT Earth and Planetary Science Department (Sept 2018)
- Blueyard Capital, Berlin (Nov 2018)
- CUBES seminar, Berkeley (Feb 2019)

7.2 Other Benefits

In addition to the benefits to space exploration, the building processes optimized here may have a profound effect on the building industry on earth. The concept of a rapidly deployable, self-building self-healing structure potentially with embedded biosensors and eco-friendly in that it would be biodegradable and emit no toxic volatiles, is appealing. In the developing world, the techniques can be used to build quickly deployable warm safe shelter with little carbon footprint to house the hundreds of millions of refugees experts anticipate by midcentury due to climate change and political forces. Similarly, we envisage a use in the rapid building of mobile military and exploration units. Currently the building industry is responsible for 40% of the carbon emissions on the planet. The processes proposed here use the waste stream from algal production, which can be used to absorb carbon emissions. In developed markets the materials and processes could lead to cleaner greener buildings that can be composted instead of landfilled at the end of their lifecycle. Uses of the technology without the building application includes areas being explored by the commercial sector such as insulation and packing materials, but with the addition of new feedstocks and embedded sensing capabilities could be that much more useful. With the right material properties, new applications include lightweight protective gear, buoys and even custom grown shoes in areas where access to clothing that fits may be limited. Imagine an expedition where the potential to build habitats, furniture, boxes and shoes can be made quickly on location by the addition of water.

The idea of off planet mycotecture is something that should capture the attention of the public. We envision producing miniature versions of the inflatable-grow-your-own mycotecture habitat for students and the public to grow their own at home, museums or in schools.

[83] http://2018.igem.org/Team:DTU-Denmark

8 The Team

The team consisted of a space scientist/microbiologist/synthetic biologists at NASA, an architect with expertise in mycotecture, a synthetic biologist in the UK, a materials expert and iGEM students and their advisors:

- **Lynn Rothschild (PI),** is a pioneer in using synthetic biology for NASA, and a leader in astrobiology. She will oversee the project, supervise the microbiology and contribute to the white papers.
- **Christopher Maurer (Co-I),** is an architect and professor of architecture. He has designed and managed many innovative projects in limited resource environments. He will manage the architecture, engineering, graphic representation, and form prototyping.
- **Debbie Senesky (Collaborator)** is faculty in Aeronautics and Astronautics at Stanford. Senesky will provide guidance on materials characterization, as well as the potential for utilizing existing equipment located in the Soft & Hybrid Materials Facility and other facilities at Stanford.
- **James Head III** (Co-I), is a planetary scientist at Brown University with extensive expertise in human space exploration. He advised the team in general, in particular on mission architecture.
- **Anil Wipat (Collaborator)** specializes in engineering *B. subtilis* to make useful products. He will provide engineered *B. subtilis*, and advise on their use and project objectives.
- **Ivan Paulino-Lima (Co-I),** is a microbiologist and radiation expert, and will conduct the biological work.
- Jesica Urbina (not listed on the proposal but key to the prior biomining NIAC award), finished her Ph.D. from UCSC on biomining in Rothschild's lab during the project, and was instrumental in advising the biofiltration project.
- **iGEM students** from Brown University, Stanford University, and two dual degree students from Brown and the Rhode Island School of design were team members of the 2018 Stanford-Brown-RISD iGEM team. They conducted all the lab work at Ames and material testing at Stanford under the direction of Senesky and her staff. Team advisors included Drs. Jesica Urbina, Nils Averesch, and Tomasz Zajkowski.