

# Synthetic biology meets bioprinting: enabling technologies for humans on Mars (and Earth)

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## Abstract

Human exploration off planet is severely limited by the cost of launching materials into space and by re-supply. Thus materials brought from Earth must be light, stable and reliable at destination. Using traditional approaches, a lunar or Mars base would require either transporting a hefty store of metals or heavy manufacturing equipment and construction materials for *in situ* extraction; both would severely limit any other mission objectives. Long-term human space presence requires periodic replenishment, adding a massive cost overhead. Even robotic missions often sacrifice science goals for heavy radiation and thermal protection. Biology has the potential to solve these problems because life can replicate and repair itself, and perform a wide variety of chemical reactions including making food, fuel and materials. Synthetic biology enhances and expands life's evolved repertoire. Using organisms as feedstock, additive manufacturing through bioprinting will make possible the dream of producing bespoke tools, food, smart fabrics and even replacement organs on demand. This new approach and the resulting novel products will enable human exploration and settlement on Mars, while providing new manufacturing approaches for life on Earth.

## Introducing a new enabling technology for space: life

Imagine a technology that has the following properties. It is programmable like a computer, and modular in design. The technology is self-replicating so multiple units are available for the cost of one. Damage is not an issue as it is self-repairing. It can perform advanced chemical transformations in a tiny form factor and in a non-toxic manner at room temperature and near neutral pH. Its abilities in the field of nanotechnology are unparalleled, with atomic scale precision assembly a nearly constant activity. It can sense as little as a single molecule. Its energy requirements are modest, and never involve petrochemical or electrical sources. In fact, some have built in solar converters whereas others use inorganic energy sources such as hydrogen sulfide (H<sub>2</sub>S), elemental sulfur, ferrous iron (iron II), molecular hydrogen (H<sub>2</sub>), manganese (Mn<sup>2+</sup>) and ammonia (NH<sub>3</sub>). However, some can go for periods of time in excess of a century without any energy input. There are probably over 9 million variants available today [1,2].

This technology is, of course, life.

Once we begin to think of biology as a technology, the potential applications are stunning. For example, the materials that are produced by life have an array of mechanical properties unrivalled by either natural or man-made products. For example, spider silk is reputed to have a tensile strength greater than steel. Although it does fall in the range of steel (0.2–2 GPa), its stiffness is less than steel but its density is almost six times less. As a result, the strength to density ration of spider silk exceeds that of steel

[<http://phys.org/news/2013-06-spider-silk-nature-stronger-steel.html#jCp>]. And then think of other structural marvels of nature, from wood to bone, from fibres to shells.

We are now in an era where we can engineer microbes to make materials that were previously the exclusive domain of larger creatures. This allows us to control the content of the materials through synthetic biology, and position the deposition of the materials through additive manufacturing and cell activation. Bone is wonderful, but production is limited by evolution and ethical sensibilities on the Earth and absent on Mars. But what if bone could be made without animals and laced with spider silk? Spider silk itself has been laced with carbon nanotubes and graphene flakes to produce an enriched spider silk that has a tensile strength greater than that of synthetic fibres such as Kevlar, making it the strongest fibre known [4].

Although such 'not-as-futuristic-as-one-might-think' ideas would no doubt benefit terrestrial industries, they may well be the key to enabling long-term human space exploration and colonization.

## Challenges of humans beyond Earth

How will humans on Mars view planet Earth? Will that tiny dot in the sky (Figure 1) be seen as their *real* home, an ancestral home, a honeymoon destination or something less emotionally laden, like a remote data processing centre? What we do know is that the community will be physically independent.

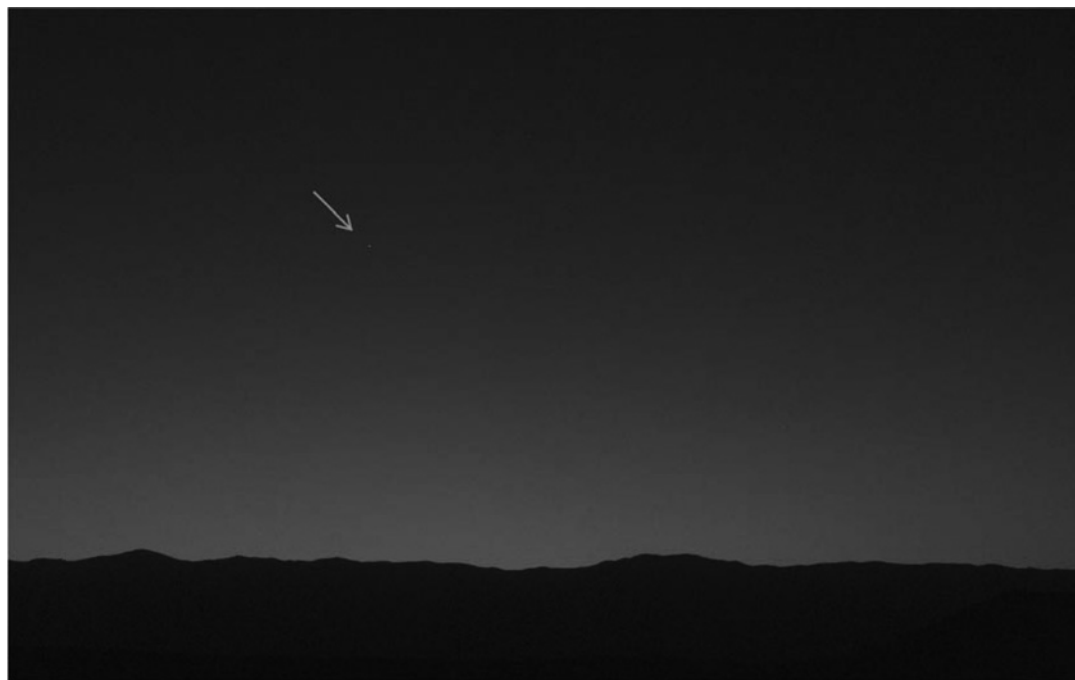
The requirements of a human settlement will need to be met on location. The colonists will need transportation, not only to and from Earth, but on Mars itself for moving themselves,

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### Figure 1 | The view of Earth from Mars

This view of the twilight sky and Martian horizon taken by NASA's Curiosity Mars rover includes Earth as the brightest point of light in the night sky. Earth is a little left of centre in the image indicated by the arrow, and the moon is just below Earth. The left eye camera of Curiosity's Mast Camera (Mastcam) captured this scene approximately 80 min after sunset on the 529th Martian day, or sol, of the rover's work on Mars (31 January 2014). The image has been processed to remove effects of cosmic rays. The distance from Mars to Earth in this photo was  $160 \times 10^6$  km (99 million miles). Image credit: NASA/JPL-Caltech/MSSS/TAMU.



goods and reconnaissance. As with any other settlers, they will need habitats, clothing, food, water, medicines, waste removal and recycling. Although the solar output will be a resource, they will need supplemental sources of power, heat and light. Unlike the Earth, atmospheric oxygen is, for all practical purposes, absent at 0.15% molar fraction. Further, the atmospheric pressure averages 7.5 mbar in contrast with 1.013 bar on the Earth. Even though Mars is one and a half times as far from the sun as the Earth, the absence of an ozone 'shield' means that radiation protection is necessary. And, of course, gravity is far lower, approximately 38% that of Earth. Perchlorate ( $\text{ClO}_4^-$ ) is widespread on the surface, which is a potential source of oxygen as well as a conceivable health hazard [5].

There are challenges to supplying these needs, starting with upmass. Anything launched into space is expensive as it has to overcome Earth's gravity. Today it costs approximately \$10000 to put a pound (454 g) of payload into Earth orbit. To visualize what this means, this is the same weight as a 16 oz can of soda, and it is approximately the official weight of a European (FIFA's *Law of the Game* number 2) or American (NFL Official Playing Rules of the National Football League 2015, rule 2) football. Similarly, 'upvolume' is limited as it too can affect upmass, so volume should be reduced to balance payload needs. Both upmass and upvolume are

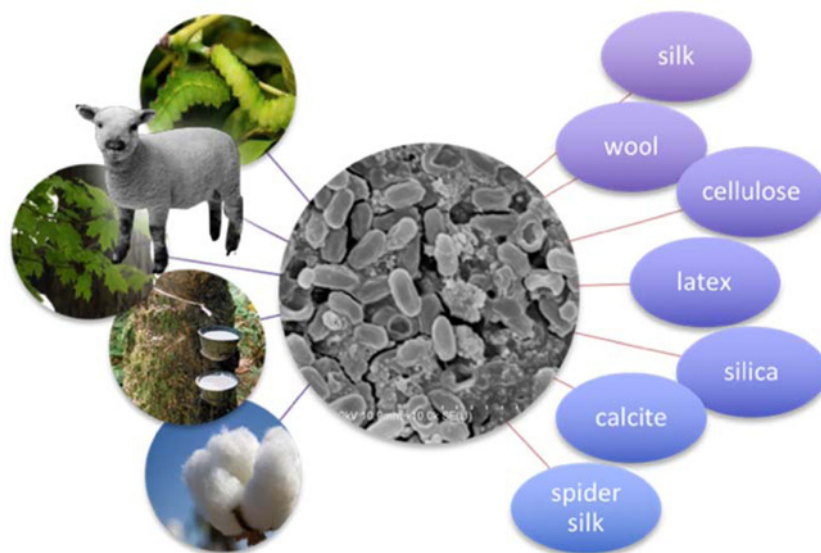
thus tied closely to the cost of the mission. Further, the technologies used must to be stored until needed, flexible in their applications and reliable as resupply will be infrequent.

Biology, and synthetic biology in particular, can overcome many of these challenges. The notion is that upmass and volume will be alleviated, as the organisms 'live off the land' through *in situ* resource utilization (ISRU). Although biology has produced a wealth of potential resources, none has evolved for life on Mars or for the needs of human Mars missions. Rather than wait for evolution, synthetic biology allows us to circumvent evolutionary time scales by producing bespoke organisms.

So what is the 'big idea'? For millennia we have used biology to do chemistry on Earth. In the future, we will use biology to do chemistry beyond Earth, including material synthesis and recycling. We may use synthetically-altered organisms for material production including habitat construction, food, fuel, clothes and drug production, embedding biosensors. We may use materials acquired from Earth or acquired in transit (e.g. from an asteroid or repurposed upmass from missions) or recovered *in situ* through biominer. For exploration we will rely on nanotechnology, and what better way than to exploit the best nanotechnology production platform, living organisms. As we do in the lab today, we will send the information to

**Figure 2 | Biological materials that could in principle be produced by microbes off Earth**

A diverse range of biological material are produced by multicellular organisms. In some cases, such as with cellulose, there are naturally-occurring microbes that can be harnessed to produce this material on or off Earth. In others, such as spider silk, microbes have been – or will be – engineered to produce these compounds. *B. subtilis* spore image courtesy of the 2015 Stanford-Brown iGEM team (<http://2015.igem.org/Team:Stanford-Brown/BioHYDRA>).



synthesize new DNA constructs digitally overcoming the time delay of physical transport so that the DNA can be synthesized on site, allowing the just-in-time production of designer drugs. Life on Earth has chirality with left-handed amino acids and right-handed sugars. Why not produce mirror image cells, a 'Life 2.0', that uses an alternate biochemistry such as right-handed amino acids and left-handed sugars, thus preventing cross-contamination with terrestrial life? Why not use chemical energy to generate and store electricity as does the electric eel, or indeed all organisms with nervous systems?

Our lab at NASA Ames Research Center, located in Silicon Valley, has focused on creating proof-of-concepts as we prepare the way for future human voyages. Starting with 2011, some of these projects have been initiated by the Stanford-Brown iGEM team, a team of undergraduates who design, conduct and present projects for the annual international Genetically Engineered Machine competition (iGEM.org). With the goal of space exploration, we have the incentive to focus on reducing mass, autonomy and systems that operate in extreme environments. Decoupling from the economic and geopolitical realities of current Earth-based economies allows us to pioneer technologies that will revolutionize life for the humans who remain on Earth.

### Biological materials

Biological materials are materials produced or derived from living organisms. This includes an amazing diversity of

resources including biomolecules, biopolymers and hard structures that result from the deposition of minerals by biomineralization. Although some biological materials are useful in solution, such as oils, our focus here is on structural materials which may be created by bulk materials but often by materials arranged in a hierarchical structure.

Biologically-created structural materials have been critical throughout human history, from wood for construction, furniture and heat, to bone for knives and needles, fibres and leather for clothes and so on (Figure 2, left). There is no reason not to continue to use them beyond our home planet. At this point no one is suggesting transporting herds of sheep or setting up sugar cane plantations off planet. However, there is no reason not to take these biological capabilities with us but in a more tractable form factor (Figure 2, centre/right). Thus, rather than harvesting latex for rubber from a forest of 30 m tall *Hevea brasiliensis* trees, why not engineer a yeast cell to produce the rubber hydrocarbons from carbohydrates directly? This also may have the benefit of reducing antigenicity by not producing the additional compounds that are found in natural latex, including proteins, resins, sugars, glycosides, tannins, alkaloids mineral salts and secondary metabolites. Two leading tire manufacturers, Goodyear and Michelin, have entered into partnerships with a handful of biotech companies including Genencor to supply microbially-produced five carbon isoprene to make a synthetic latex similar to rubber [6,7]. Whereas on Earth the microbes will have to prove they are more economical than tree and petrochemical-derived rubbers, on Mars they will be the only option.

## Biocomposites

Biocomposites are defined as composite materials formed by the reinforcement of a matrix by natural fibres. But in a general sense, they could be considered materials that combine biological and non-biological components. Examples include the agglutinated tests (shells) of some amoebae (e.g. members of the Foraminifera and the rhizopod *Heleopera petricola*) which are composed of foreign particles glued together with a calcareous or organic cement. The organo-sedimentary laminated structures that make up stromatolites also could be considered biocomposites.

Harnessing the ability of organisms to create concrete has suggested a way to make building materials based primarily on minerals found on the surface of Mars, the moon or Earth. The bacterium *Sporosarcina pasteurii* (previously *Bacillus pasteurii*), can raise the pH of a solution of urine, sand and calcium chloride, thus inducing calcite precipitation. The microbially-induced calcite precipitation (MICP) has been used to create bricks. At least one company, bioMASON, produces bricks commercially by this procedure. The 2011 Brown-Stanford iGEM team [<http://2011.igem.org/Team:Brown-Stanford/REGObricks/Biocementation>], under our direction, pioneered the concept of using this approach to agglutinate the Martian regolith (surface material) to produce a brick. With that buildings could be built to provide wind and radiation protection. Even if the buildings then require additional infrastructure such as a vapour barrier, much of the mass of the structures would have been created using *in situ* materials.

## Biological structures and how to make them

Biological materials may be present in bulk or in aqueous suspensions such as blood and urine, but many biological materials derive their structural features by the organization of the components in a hierarchical structure based on a few polymers. For example, keratin is a protein in a family of fibrous structural proteins that are a major component of such diverse structures as skin, hair, nails, hooves, horns and teeth. They are intermediate filaments (average diameter of 10 nm) that derive properties from their hierarchical structure [8].

Seven features distinguish biological materials from their synthetic counterparts [9]. These are:

1. Self-assembly.
2. Multifunctionality where many components serve more than one function in the organism such as feathers serving for warmth, camouflage, sexual display and flight.
3. Hierarchical structure where the nano- to ultra-scale creates unique properties because of their relationship.
4. Hydration, a property nearly absent from materials such as ceramics, but the general rule for biology where water is the solvent for life. As a practical consequence, mechanical

properties such as strength are decreased by hydration, whereas toughness is increased.

5. Mild synthesis conditions compatible with life, which for most organisms mean at temperatures between 10 and 40°C, and pH near neutral.
6. Evolutionary and environmental constraints which means that although some structures may have been optimized as the result of selection, evolution does not test all options nor is the optimal functional design of a feature necessarily selectively advantageous to the organism as a whole. To re-iterate the Jacob's point: evolution is a tinkerer, not an engineer [10].
7. Self-healing, a property critical to the Darwinian success of organisms.

Nearly of these properties can be retained, or some cases enhanced, by moving production into a microbial form factor if it does not already exist. The one exception is that microbial production cells in bulk do not produce large scale hierarchical structures. How can a microbe produce a feather, which demands macroscale assembly? Or the hierarchical structure of a toucan's beak [11] or of ivory [12]? One solution our laboratory has developed is to print the production cells in a pre-determined array, and then induce the cells to produce the structural material, as described in [13]. In theory, cells could be printed that then produced a sheet of bird's beak, a fabric with embedded biosensors or even a complete tool. Once this is realized, novel 'synthetic biomaterials' could be produced that will have no analogue in the living world, at any scale, anywhere. Imagine a yeast cell depositing a metallic filament, surrounded by a 5–10 μm layer of microbial cellulose, surrounded by cross-linked keratin, surrounded by silicate – a structure never found in nature but in theory could be produced with such a system.

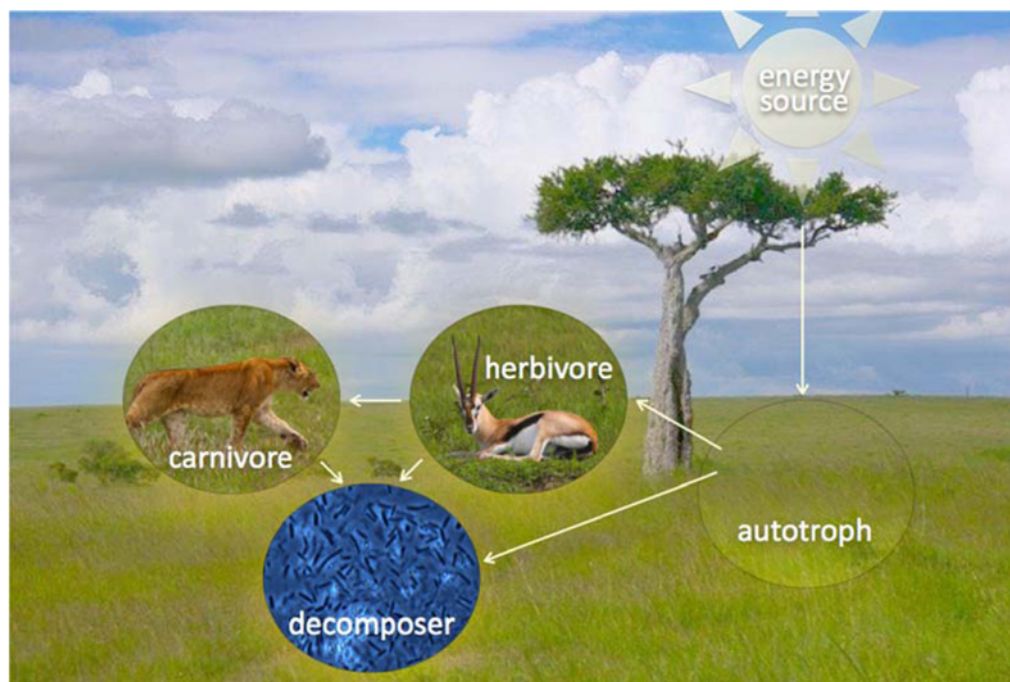
## Feeding the production organisms

The microbes that will serve as the production organisms for biological materials off planet will need inputs in order to power their metabolism. The basic nutritional types are primary producers (autotrophs), herbivores who eat the autotrophs, and carnivores who in turn eat the herbivores. The decomposers degrade biological materials produced by the first three. All four nutritional types are found among the microbes, and all four produce materials of use (Figure 3).

On Mars the primary inputs available are atmospheric gasses, primarily CO<sub>2</sub> (95%) with some N<sub>2</sub> (<2%), water mostly in the form of ice, surface minerals and sunlight. But, for the most part, the microbial 'chassis' organisms used in synthetic biology are decomposers and thus require an external source of organic carbon. We suggest that, as on Earth, a photosynthetic organism will be the interface between the raw materials and a food source for the production organisms. Diazotrophic (nitrogen fixing) cyanobacteria are ideal as they can convert N<sub>2</sub> to NH<sub>3</sub> as

**Figure 3 | Basic food web**

The arrows indicate the flow of energy. The source of energy for the majority of life on Earth is the sun [14] Autotrophs such as plants, algae, cyanobacteria and other photosynthetic bacteria use this energy to produce reduced (fixed) carbon compounds from atmospheric or dissolved CO<sub>2</sub> or, in the case of some bacteria, CO. Herbivores, such as the impala shown here, graze on the autotrophs, whereas the carnivores – whether lions, squid or ciliates – are predators who feed on the herbivores. Ultimately organic carbon from these nutritional groups is preserved, degraded by physical and mechanical forces, or metabolized by decomposers such as fungi and many bacteria. Photos taken on the Massai Mara, 7 January 2007.



well as convert CO<sub>2</sub> to organic material. In 2011 the Brown-Stanford iGEM team suggested that this cyanobacterium would function as the metabolic power centre for the biological infrastructure, thus providing the inspiration for its name, PowerCell (<http://2011.igem.org/Team:Brown-Stanford/PowerCell/Introduction>).

**The vision for the future**

The overall vision for a biology-enabled Mars colony consists of several components (Figure 4), alone or in combination. Solar radiation supplies the energy for PowerCell to convert *in situ* resources (CO<sub>2</sub>, N<sub>2</sub>, water and minerals) into organic compounds such as sugars and proteins. The products of PowerCell in the form of excreted organics or a cell lysate are then used as the feedstock for production organisms. These are microbes that have evolved to produce products of use such as the production of microbial cellulose by the bacterium *Gluconacetobacter xylinus*, previously known as *Acetobacter xylinum* and since reclassified as *Komagataeibacter xylinus*. Alternatively, the production organisms may have been genetically engineered for transgenic production, as in the microbial production of spider silk. If a hierarchical structure is desired, the production cells could be printed

in specified arrays and then production and secretion of the product initiated. The resulting products could range from clothing to aircraft parts, construction tools to medical devices.

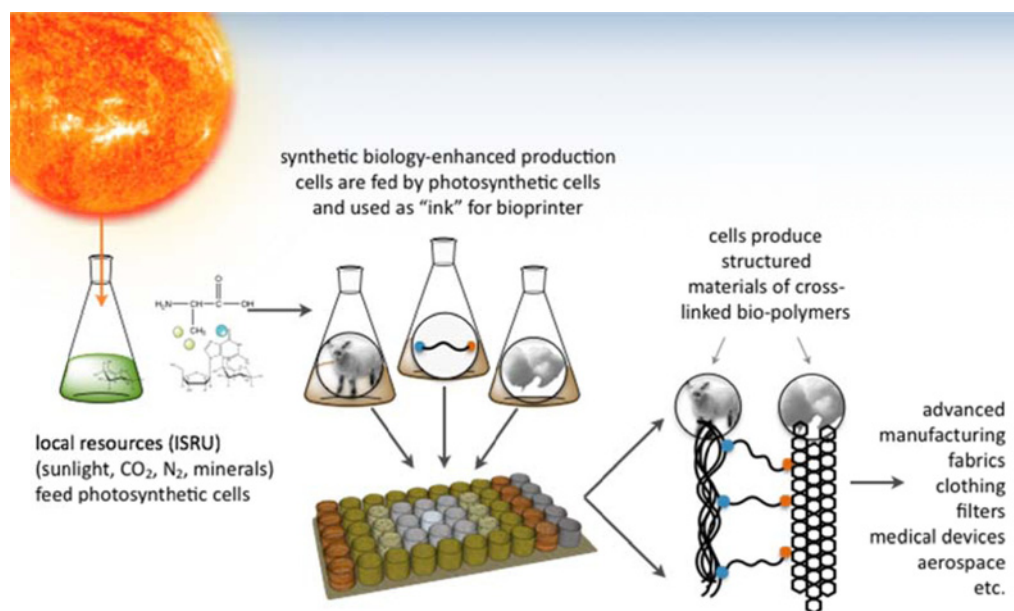
It should be noted that although the focus of this article has been on production by microbes, surely small plants could be pressed into service. There has even been a suggestion of bringing small animals such as silkworm on crewed missions to Mars, both for material production and for food [15].

**The first steps in space: the EuCROPIS satellite mission**

Currently the transit time from Earth to Mars is approximately 7 months. In addition to all the other constraints, organisms making the journey will be subjected to Earth gravity (1 × *g*), microgravity en route and approximately 0.38 × *g* at destination. How will this affect the vision of a synthetic biology-enabled settlement? Will we have to compensate for gravity when we conduct genetic engineering beyond Earth? How will gravity affect the efficacy of PowerCell? Production organisms? Bioprinting?

To address all but the last question, we will fly the PowerCell payload on the DLR (German Space Center)

Figure 4 | The grand vision



EuCROPIS satellite, with a scheduled launch of March 2017. During the mission the satellite has periods of time when the rotation rate will mimic microgravity, lunar gravity and finally, Martian gravity. Our NASA secondary payload will consist of two types of experiments, both conducted in microfluidic cards remotely. The PowerCell experiment will test the ability of the diazotrophic cyanobacterium *Anabaena* to feed a production bacterium, *Bacillus subtilis*, which will act as a reporter system as originally conceived by the 2013 Stanford-Brown iGEM team (<http://2013.igem.org/Team:Stanford-Brown/Projects/EuCROPIS>). The second set of experiments will consist of a basic transformation of the *B. subtilis* in space. As the samples will need to be loaded, stored dry with a trip from California to Germany and back prior to launch, systems have been developed for long-term stasis. With the engineering constraints on the payload, transformation protocols have been developed that obviate the need for electroporation or temperature changes.

Thus, by the end of the decade, we will have taken the first steps towards realizing the vision of a synthetic biology-enabled future off planet.

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