Synthetic Biology and Human Health: Potential Applications for Spaceflight

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Synthetic Biology and Human Health: Potential Applications for Spaceflight

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Abstract:

Human space travelers experience a unique environment that affects homeostasis and physiologic adaptation. Spaceflight-related changes have been reported in the musculo-skeletal, cardiovascular, neurovestibular, endocrine, and immune systems. The spacecraft environment further subjects the traveler to noise and gravitational forces, as well as airborne chemical, microbiological contaminants, and radiation exposure. As humans prepare for longer duration missions effective countermeasures must be developed, verified, and implemented to ensure mission success. Over the past ten years, synthetic biology has opened new avenues for research and development in areas such as biological control, biomaterials, sustainable energy production, bioremediation, and biomedical therapies. The latter in particular is of great interest to the implementation of long-duration human spaceflight capabilities. This article discusses the effects of spaceflight on humans, and reviews current capabilities and potential needs associated with the health of the astronauts where synthetic biology could play an important role in the pursuit of space exploration.

Introduction

Space is not nearly an object of commercial exploitation or of scientific investigation, nor an arena limited to engineers, scientists and entrepreneurs. It is indeed, a vast realm into which the human species is expanding physically and intellectually. This expansion not only has the potential to enhance the human condition, but also the power to transform it radically (Finney and Lytkin, 1999). It is in this context that we celebrated, in 2011, the 50th anniversary of the first human in outer space and the first to orbit Earth. This coincides with the 30th anniversary of the first space shuttle mission, and the 10th anniversary of the International Space Station (ISS), by far the most complex and challenging international scientific project in history. Over the past few decades, the National Aeronautics and Space Administration (NASA) has been at the forefront of human spaceflight and space exploration. Accordingly, NASA's mission is to pioneer the future in space exploration, scientific discovery and aeronautics research.

Within that pioneering context, the NASA Ames Research Center has embark upon a synthetic biology initiative in order to harness biology in reliable, robust, engineered systems to support NASA's exploration and science mission, to improve life on Earth, and to help shape NASA's future (Langhoff et al., 2011). This initiative represents a new challenge of designing, constructing, and testing of new methodologies, and ultimately engineered organisms, to perform reliable tasks to sustain human spaceflight and space exploration. Synthetic biology techniques are not only efficient and potentially applicable to space-flight scenarios, but they are also easily minimizable, and do not require use of terribly sophisticated equipment in most cases. The

NASA Ames Research Center and the National Academies Keck Futures Initiative co-sponsored a weekend workshop on the 30th-31st October, 2010 to develop ideas that demonstrate the potential role of synthetic biology in NASA's missions. During the two-day workshop, NASA's experts experienced in the science and engineering of space missions interacted with scientists involved in basic and applied synthetic biology in order to define the future research agenda for synthetic biology within NASA. During the workshop five different working groups where held in parallel, and included biological *in situ* resource utilization, biosensors, biomaterials and self-building habitats, synthetic biology and human health, and life support for long term space travel and habitation.

The current manuscript is a result of that intellectual interaction which took place during the meeting. Our working group focused on the human health perspective of synthetic biology and its potential to sustain long-duration spaceflight. While our group has not exhaustively and comprehensively reviewed all the potential human health applications of synthetic biology, the first section of the manuscript provides a brief introduction to synthetic biology and describes some diagnostic, therapeutic, and nutritional applications. The second section reviews the space environment and its impact on astronaut physiology. Finally, the third section aims to look at potential synthetic biology applications for human spaceflight, and how those could be tested and monitored prior and during future NASA missions.

Synthetic Biology

Synthetic biology is a newly emerging interdisciplinary field which aims to rationally engineer novel biological systems (Andrianantoandro et al., 2006; Ball, 2004, 2007; Benner and Sismour, 2005; Endy, 2005; Keasling, 2008; Rai and Boyle, 2007). Traditional genetic engineering has

had a tremendous impact on biotechnology and relies heavily on naturally-existing templates, such as excising a gene from one DNA molecule with restriction enzymes, and pasting it to another with DNA ligase. In contrast, synthetic biology allows the creation of synthetic sequences from scratch using *de novo* DNA synthesis technology. In essence, it may be possible to design customized metabolic pathways that are not naturally observed in nature (Figure 1).

The field of synthetic biology is developing at an astonishingly rapid pace. The ability to write synthetic DNA is increasing in an exponential fashion and the cost is decreasing (Carlson, 2010). In 1970, it took 20 man-years to synthesize a 75 base pair (bp) DNA sequence, but today researchers can easily order several tens of thousand-bp sequences from DNA synthesis companies at an affordable cost and reasonable turnaround time (Agarwal et al., 1970). A completely chemically synthesized and "booted" *Mycoplasma mycoides* genome, JCVI-Syn1.0 (1.08 Mbp), further validates the enormous power of DNA synthesis for biological engineering, quite literally transforming the landscape of life sciences (Gibson et al., 2010).

With synthetic biology, biological systems and living organisms can effectively be programmed. In general, it can be understood as a catalyst for all biotechnology, including agriculture. Even though synthetic biology is still in a very early stage, it already shows great potential to address many human challenges here on Earth. This section will give some diagnostic and therapeutic examples of the current state of synthetic biology technology applied to human health.

Diagnostic and Therapeutic Applications of Synthetic Biology

Diagnostics and therapeutics based on molecular biology are reshaping our understanding of human health. Advances in synthetic biology as a field may enable a migration from the current paradigm of instruments with biological components to systems with living organisms. Synthetic

biology systems may enable a level of miniaturization and integration not achievable using other approaches, although there remain many unanswered questions about the safety, efficacy, and robustness of synthetic biology systems. Molecular diagnostic approaches are used to identify genetic disorders, and in the diagnosis of infectious diseases and cancer, among other applications. Monoclonal antibodies, perhaps the first wave of biotechnology, have dramatically transformed diagnostic procedures, and are currently on the market as cancer therapeutics, e.g. Herceptin (Alley et al., 2010). Molecular therapeutics such as gene therapy while showing great potential, have yet to result in commercial therapies, in part because of safety concerns but also because of the relative complexity of the *in-vivo* environment (Alexandrova, 2009; Deakin et al., 2010).

Diagnostics

Synthetic biology finds great application in biosensing. Biosensors combine a specific biological recognition element (e.g., cells, enzymes, nucleic acids, etc) with a transducer for signal processing, allowing for visualization or measurement of any biological interaction. One of the most common biosensors is the glucose monitor used in the control of diabetes; another is the disposable pregnancy test. The application space is large, reaching into areas of health, environmental monitoring, epidemiology, and even national defense, but the commercialization of biosensors has lagged in part because of the large up-front investment in R&D (Luong et al., 2008).

Synthetic biology can lower these costs by making the biological components of the detector and reporter easier to engineer, while advances in microfluidics and microelectronics are also contributing to faster and less expensive development. Whole cell biosensors, which use

genetically engineered microorganisms as both the recognition element and transducer, offer many advantages, including self-manufacture and the ability to detect environmental input with high sensitivity, accuracy and reliability (Khalil and Collins, 2010).

Despite the fact that various highly accurate laboratory methods have been developed to detect different kinds of environmental input, whole-cell biosensors are currently a promising solution due to speed of growth, ease of manipulation and high cost effectiveness. To illustrate this point, a team of synthetic biologists from the University of Edinburgh (including one of us, YC) developed a whole-cell biosensor to detect arsenic contamination in drinking water, a serious health problem in developing countries, in particular Bangladesh. The current monitoring system for arsenic contamination in water is the use of a colorimetric chemical test kit based on the Gutzeit method, which involves reductions of arsenite and arsenide to toxic arsine gas, which reacts with mercury (Hg) salts to generate a colored compound. These kits are reportedly insufficiently sensitive and fail to reliably detect levels of contamination below 100ppb (Sarkar et al., 2011). Moreover, Hg is also a toxic heavy metal, leading to potential issues with disposal. The arsenic biosensor developed by the Edinburgh group is composed of three major operons, which respond to three different arsenate concentrations that result in different pH outputs (Figure 2). The system can respond to arsenate concentrations as low as 5 ppb, while the current World Health Organization recommendation is 10 ppb and in many countries a more relaxed limit of 50 ppb is in operation (Aleksic et al., 2007). The future development of this kind of biosensor will lead to a cheap, reliable and foolproof field test device, which will help for example prevent the ongoing chronic arsenic poisoning in Bangladesh (Frisbie et al., 2009).

Another example is yeast cells that have been engineered to detect specific chemicals. Dhanasekaran and colleagues developed a *Saccharomyces cerevisiae* strain containing

components from the human olfactory signaling pathway that could detect and report via fluorescence the presence of the odorant 2,4-dinitrotoluene (DNT), a mimic for the explosive trinitrotoluene (TNT) (Radhika et al., 2007). Similar systems could "sniff" out everything from leaking equipment to microbiotic changes in waste processing systems.

One of the promising diagnostic tools is DNA microarrays, a multiplex assay where microscopic spots of oligonucleotide DNA probes of known specific sequence are covalently attached to or synthesized directly on a solid surface, typically a glass slide, silicon chip, or bead (Seidel and Niessner, 2008). Labeled target DNA (or cDNA or RNA) is then quantifiably detected by hybridization to each probe. Hundreds of thousands of probes can thus be rapidly tested in a single experiment. Among the applications for which arrays are employed include 1) the detection of single nucleotide polymorphisms (SNPs) for genetic testing (Eriksson et al., 2010; Pang et al., 2010), 2) changes in gene expression levels (Slonim and Yanai, 2009), e.g., between normal and cancerous cells, and 3) for molecular taxonomy of bacteria or identification of other species or tissues (Huyghe et al., 2008). However, they are still important challenges that need to be considered for successful translation of a microarray based gene profile into a routine diagnostic test implemented to benefit human health (Roepman, 2010).

Finally, DNA or RNA sequencing itself is a very powerful diagnostic tool, and the current generation of sequencers, including those made by 454-Roche (http://www.454.com), Illumina (http://www.illumina.com) and Ion Torrent (http://www.iontorrent.com/) utilize sequencing by synthesis (SBS) mechanisms. The next-generation massively parallel sequencing approaches are increasing the throughput and decreasing the cost of nucleotide-resolution genomics, making whole transcriptomes, exomes, and genomes readily achievable (Shendure and Ji, 2008). Thus, rapidly evolving sequencing technologies have empowered enormous growth in the breadth and

depth of cancer research. However, the technology is still not mature enough for clinical applications and challenges related to computational analysis and interpreting the results of sequencing individual cases remain to be overcome (Cloonan et al., 2010; Taylor and Ladanyi, 2011).

Therapeutics

Synthetic biology is well suited to the manufacture of therapeutics by bringing exceptional control to the manipulation of the genetic code, which in turn directs the manufacture of medically useful output.

A common application is the production of a single protein or enzyme with therapeutic value. Genentech, considered one of the first biotechnology companies, demonstrated the feasibility of this approach when it made bacteria capable of synthesizing human somatostatin, a hormone that inhibits the release of growth hormone, in 1977. Their first marketed compound was synthetic human insulin in 1982, which transformed the way we treat diabetes. Amgen Inc., based in Thousand Oaks, California, used similar techniques to develop human erythropoietin and granulocyte-colony stimulating factor (G-CSF), hormones for, which respectively stimulates, the production of red and white cells.

Antibodies are proteins that are commonly employed both as the sensing component of many assays and biosensors and more recently as therapeutic agents. One advantage for drug development is that most of the antibody molecule remains constant, with only the antigen-binding site changing from drug to drug, reducing pharmacokinetic variation. While used extensively in vaccinations (e.g., hepatitis), monoclonal antibodies have been approved for cancer treatments, treating transplanted organ rejection, and for various autoimmune diseases

(Ball and Broome, 2010; Czaja, 2010; Klipa et al., 2010). Synthetic biology is making engineered antibodies easier to manufacture, a boon to research and the development of new treatments (Flueck et al., 2009).

A powerful case for the successful application of synthetic biology to therapeutics is the engineering of cellular metabolism to produce an antimalarial drug. Malaria is a notorious infectious disease affecting 300-500 million people annually and killing more than 1 million, worldwide, according to estimates by the CDC (Brunette et al., 2009). The majority of malaria patients live in sub-Saharan Africa, where inexpensive drugs would be highly desired. Many malaria species have developed resistance to the more common antimalarials. One of the most effective antimalaria drugs is artemisinin, which is a chemical compound derived from the sweet wormwood plant, used extensively in Chinese traditional medicine. Yet, extracting artemisinin from plants proves to be a significant limiting step in the mass-production of this antimalaria drug. Keasling *et al.* pioneered the engineering of a synthetic *E.coli* to produce amorphadiene, the artemisinin precursor (Ro et al., 2006). The outcome of this synthetic biology application is tremendous – it could create an affordable antimalarial drug and save millions of patients in developing countries. The significance of this achievement using synthetic biology was quickly recognized by the Bill and Melinda Gates Foundation with a $42.6 million award in 2004.

Another exciting area of therapeutic development is aptamers, small pieces of DNA, RNA, or peptide of random sequence which, like antibodies, have features that allow them to be both a sensor and a therapeutic (Cho et al., 2009). Aptamers can be evolved to sense virtually any chemical compound, for example drugs like cocaine (Stojanovic et al., 2001) and theophylline (Ferapontova et al., 2008). Some, like Macugen®, have already been approved by the FDA for clinical use, in this case for macular degeneration. Similar to aptamers are RNA interference

(RNAi), microRNA (miRNA), and small interfering RNA (siRNA) therapeutics able to selectively induce mRNA silence, cleavage, translational repression, and cleavage-independent mRNA decay (Sliva and Schnierle, 2010).

Vaccines are another important therapeutic area for synthetic biology. These typically contain a component of an agent or organism (or attenuated versions of them) that, when administered, will stimulate an immune response. Viruses require a host cell to replicate, and hence the attenuated influenza strains used in the production of the flu vaccine are cultured in chicken eggs. To satisfy the demand for vaccine during a typical flu season, this requires hundreds of millions of eggs and about 6 months lead-time in manufacturing, necessitating the prediction of which flu variant will be prevalent each season. Recently, Synthetic Genomics announced the formation of a vaccine unit in collaboration with Novartis that will utilize synthetic biology and genomics technologies to accelerate the production of the influenza seed strains required for vaccine manufacturing (Kowalski, 2010). The seed strain is the starter culture of a virus, and is the base from which larger quantities of the vaccine virus can be grown. Under this collaboration both groups will work to develop a bank of synthetically constructed seed viruses ready to go into production as soon as the world health organization identifies the flu strains. The technology could reduce the vaccine production time by up to two months.

As with diagnostics, whole cell engineering has application in therapeutics. Yeast cells were engineered to produce alkaloids, a group of natural compounds that includes morphine and other painkiller molecules (Hawkins and Smolke, 2008). Meanwhile, engineered cells could prove important in treating cancers or even spinal damage, acting as miniature biopharmaceutical production plants, or combined with 3D fabrication, even the manufacture of synthetic tissues or organs.

Finally, synthetic viruses could prove to be valuable workhorses for synthetic therapeutics, because of their small genome size (facilitating synthesis), self-assembly processes, and ability to deliver genetic payloads to targeted cells. Many viruses are currently in development as gene therapies, oncolytic cancer treatments, and as antibiotic agents (Khalil and Collins, 2010).

Synthetic Biology and Nutrition

Optimal, balanced nutrition is a major determinant of human health (Gibney et al., 2009). As stated by Hippocrates, the "Father of Medicine" 2500 years ago: "Let thy food be thy medicine and thy medicine be thy food", nowadays nutrition still plays an essential role in prevention of diseases and promotion of human well-being (Shils et al., 2005). With hundreds of millions of food- and nutrition-deprive people globally and the increased awareness and demand of better nutrition strategies with the aging population, we are seeking novel ways to solve the world food crisis and improve human nutrition (Pinstrup-Andersen, 2008). Synthetic Biology again offers a unique and exciting solution to this problem, opening new possibilities into ways of guaranteeing better human nutrition through improving nutrient intake, absorption, and use as well as nutritional monitoring (Benner and Sismour, 2005). Adequate caloric intake is the prerequisite of optimal nutrition (Gibney et al., 2009). Synthetic Biology offers techniques useful in providing a more efficient supply of bio-energy (Marner, 2009; Picataggio, 2009; Wackett, 2008) and improving both quality and quantity of nutrients (Floros et al., 2010). For example, Synthetic Biology can be used to produce novel crops with superior productivity, nutritional quality, and tolerance to stress and environmental extremes than natural plants or to transform inedible waste materials into digestible food (French, 2009). In addition, Synthetic Biology is expected to revolutionize nutritional absorption and biodistribution by producing probiotics as dietary supplements of live organisms that provide health advantages for people (de Vrese and

Schrezenmeir, 2008), and by replacing part of the existing human microbiome with a new microbial community that can facilitate nutrient absorption and use, according to an individual's internal and external environment (Brenner et al., 2008). Another exciting application of Synthetic Biology is to design and construct a microbial community in humans with the capability of producing various proteins or nutraceuticals which can prevent disease (Singer, 2009), e.g., producing micronutrients vitamin C and folate, both of which we depend on from external sources (Leferink et al., 2009; Wegkamp et al., 2005). Synthetic Biology is also a promising avenue for nutrition monitoring by designing and producing family-usable bio-chips which can diagnose an individuals' nutritional status as well as requirements in a quick, efficient and user-friendly way (Langer and Tirrell, 2004). Lastly, synthetic biology can be applied to a wide variety of nutraceuticals to target specific at risks physiologic pathways.

The Space Environment

Space is a unique and challenging environment for human beings. The space environment is composed of 4 key parameters: neutral gas density (near vacuum), extreme temperature variations (hot and cold), weightlessness, and energetic charged particles. The later is one of the main limiting factors for long duration space exploration missions, such as a mission to Mars (Jones and Karouia, 2008).

Weightlessness also has an important impact on organisms. In practice, perfect weightless conditions are very difficult to attain due to many factors including the disturbances from drag, vibrations, constantly changing gravitational vector while in orbit, so the term microgravity is used to describe the actual conditions during space travel. The microgravity environment is responsible for the following main consequences for onboard space system; (i)

no hydrostatic pressure, (ii) no weight, (iii) no sedimentation, (iv) no natural convection, and (v) reduced gravitational effects (Monti and Savino, 1999).

Human Physiology Associated with Spaceflight

The effects of microgravity on human physiology are critically important and are a function of mission duration. They have been the source of extensive investigations to better understand them, and ultimately develop adequate countermeasures as we increase human presence in space. Microgravity effects include; 1) cardiovascular dysfunction, which mainly results in post-flight orthostatic intolerance and reduced exercise capacity (Convertino and Cooke, 2005; Cooke and Convertino, 2005); 2) bone loss, which results in a decrease in bone mineral density at a rate of 1% from the lumbar spine and 1.5% from the hip and femur per month respectively (Lang *et al.*, 2004; McCarthy, 2005); 3) muscle atrophy, which results in a decrease of at least 5-10% in muscle size and over 20% in muscle strength (Adams et al., 2003; Trappe, 2002); 4) hematological factors, which result in decreased plasma volume, blood flow, and blood cell mass (Sonnenfeld, 2005; Zayzafoon *et al.*, 2005); 5a) neurovestibular factors, which result in spatial disorientation, postural and visual illusions and a wide variety of symptoms (best known as space motion sickness or space adaptation syndrome) where post-flight results indicate peripheral and central neural processes are physiologically and functionally altered (Clement *et al.*, 2005); 5b) neurological factors, which result in acute and/or delayed effects on the central nervous system (CNS) neuroplasticity (Vazquez, 1998); and 6) the immune system dysfunction, which result in reduction in T-cell counts and decreases in Natural Killer (NK) cell concentration and functionality (Levine and Greenleaf, 1998; Sonnenfeld and Shearer, 2002), neutrophils have a lower phagocytosis and oxidative burst capacities (Kaur *et al.*, 2004), but there is a decrease in cytotoxicity after returning to Earth (Konstantinova *et al.*, 1995),

monocytes have reduced antimicrobial functions (Kaur *et al.*, 2005), there is an increase in urinary interleukin-6 (IL-6) both in space and after landing (Stein and Schluter, 1994), there is a decreased production of interleukin-2 (IL-2) (Crucian *et al.*, 2000), and shedding frequencies of Epstein-Barr virus (EPV), cytomegalovirus (CMV), and varicella-zoster virus (VZV) increase in astronauts during space shuttle missions (Mehta et al., 2004; Mehta et al., 2000; Payne et al., 1999). Additionally, space radiation exposure may have synergistic effects on the previous factors and could impair the safety of the astronauts by stochastic or deterministic effects (Jones and Karouia, 2008; Manti, 2006; Shearer et al., 2005; Todd et al., 1999).

Microorganisms in the space environment

Previous space flight experience has demonstrated that microorganisms are just as ubiquitous in space habitats as they are on Earth (Pierson, 2001). Accordingly, studies on-board spacecraft, Apollo, Space Shuttle, Salyut, Mir, and the International Space Station (ISS), have also shown the presence of a large variety of bacterial and fungal species (Castro et al., 2004; Ilyin, 2005; Maule et al., 2009; Novikova et al., 2006; Novikova, 2004; Ott et al., 2004; Pierson, 2001; Wong et al., 2010). Several opportunistic organisms that may turn pathogenic have been identified onboard and emphasize that potential conditions for infectious outbreaks on future space missions remain a realistic concern. Furthermore, for the last four decades, multiple spaceflight and simulated microgravity experiments have shown changes in phenotypic microbial characteristics such as microbial growth, morphology, metabolism, genetic transfer, antibiotics and stress susceptibility, and expression of virulence factors (Horneck et al., 2010; Nickerson et al., 2004).

Therefore, space agencies must develop strategies to limit microbial contamination aboard the ISS and future space vehicles, by adequate disinfection and sterilization of space flight materials during assembly and transport to the ISS, in addition to rigorous cleaning procedures. Ultimately, monitoring of biological contamination is imperative and will lead, hopefully, to improvement in countermeasures.

Finally, in addition to their introduction as commensal tourists attached to spacecraft or space habitat surfaces, natural and engineered microbial communities will be intentionally introduced for 1) waste processing in bioregenerative life support systems, 2) biofuels, and 3) *in-situ* resources utilization (Drysdale et al., 2003; Roepman, 2010; Rothschild and Cockell, 1999).

Workshop Session: Synthetic Biology and Human Health

During the meeting, five different working groups where held in parallel which included, 1) biological *in situ* resource utilization, 2) biosensors, 3) biomaterials and self-building habitats, 4) synthetic biology and human health, and 5) life support for long term space travel and habitation. Group number 4, composed of researchers and scientists from academia, the biotechnology industry, research institutes and governmental agencies; focused on the human health aspects of synthetic biology and its potential applications for human spaceflight. Some of the discussion initially centered on the need within the next 5 years to test synthetic biology systems in microgravity on the ISS, in order to assess its potential use for space application. Additionally, some mentioned the utility of developing a repertoire of sensing elements incorporated into synthetic organisms and the value of undertaking a comprehensive microbial ecology assessment of the biome onboard the ISS, i.e. the astrobiome, and the crew's microbiome. Projecting the potential of synthetic biology within the next 15 years led to some ideas in drug delivery

capabilities and radioprotectants. Accordingly, the development of therapies such as reprogrammable drug delivery patches, the engineering of probiotics as radiation protectants, and therapeutics for acute radiation exposure were discussed. Finally, within a 30-year timeframe, the use of gene knock-out, RNAi, and stem cell therapies might be implemented as countermeasures to the physiological changes induced in humans by the space environment. Similarly preventive reprogramming of the crew genome was also discussed. Finally, the need to perform ground studies identifying the basis for the crew's individual susceptibility to disease and variability in effects and reactions to disease and the testing of large-scale genetic circuits, *i.e*. DNA sequence of defined structure and function, for their susceptibility to the space environment were also discussed. The astronaut selection based on individual susceptibility to disease still remains controversial and difficult to implement for obvious ethical reasons. However population-based studies of genetic susceptibility or sensitivity to disease likely will prove of value in assessing long-term health risks (Brackley et al., 1999).

Potential Synthetic Biology Applications for Human spaceflight

As we have already seen, synthetic biologists have successfully engineered a wide range of functionality into artificial gene circuits. Some of these engineered gene networks have been applied to perform useful tasks such as population control, decision making for whole cell biosensors, genetic timing for fermentation processes, image processing, and have begun to be used to address important medical and industrial problems (Lu et al., 2009). The latter is of great interest to sustain and implement long-duration human spaceflight capabilities. We are reporting here, not exhaustively, some potential synthetic biology therapeutics delivery and treatment applications that could be beneficial for human spaceflight. The manuscript summarizes some research findings known to be related in some way to some of the issues faced by astronauts

during long-duration spaceflight. These different synthetic biology initiatives may one day contribute to the development of suitable countermeasures for problems associated with human spaceflight.

Therapeutics delivery and treatment:

Synthetic biologists have made good progress in mimicking some of the most basic features of regulatory networks and cellular pathways for the controlled delivery of drugs as well as gene therapy (Mukherji and van Oudenaarden, 2009). Synthetic oscillator circuits, genetic circuitry, and programmed time-delay circuits offer the autonomous capability of the synthesis and release of chemical compounds for suitable health applications (Atkinson et al., 2003; Stricker et al., 2008; Tigges et al., 2009; Weber et al., 2007). For example, a recent study in cultured cells and in animal models has demonstrated the sophisticated kinetic control of synthetic hormones which may yield therapeutic advantages and reduce undesired side effects (Stavreva et al., 2009). Accordingly, attention to the kinetics of therapy in patients with glucocortinoid-responsive diseases such as rheumatoid arthritis might provide a new approach to decreasing the glucocorticoid side effects, while maximizing the benefit afforded to patients by these therapies (Schacke et al., 2002).

RNA interference/Gene Silencing

RNA interference (RNAi) is a process by which the presence of double-stranded RNA (dsRNA) induces the selective or catalytic degradation of the homologous mRNA by an endogenous cell machinery (Lund et al., 2010). Small interfering RNA (siRNA) is capable of knocking down targets in various diseases *in vivo* (de Fougerolles et al., 2007). Cancer associated cellular pathways such as oncogene and anti-apoptotic gene expression and regulation are effective

targets for this technology, as are genes that play a role in host-tumor interactions, and those that confer resistance to radio- and chemotherapy (Ellis and Hicklin, 2009). Accordingly, *in vivo* investigations and successful treatment of bone and ovarian cancer has been reported with siRNA (Halder et al., 2006; Takeshita et al., 2005). Over the past few years, an important role of micro RNA (miRNA) in cancer pathogenesis has emerged and many studies have reported a link between cancer and unregulated expression of miRNAs (Calin and Croce, 2006). Therefore, synthetic biology might be a suitable system to tackle many of the obstacles related to carcinogenesis and tumor progression. Furthermore, synthetic circuits, by their ability to silence, activate, and tune the expression of desired genes, provide a more suitable approach to gene therapy. As such, a genetic switch that couples transcriptional repressor proteins and an RNAi module for tight, tunable, and reversible control systems over the expression of desired genes in mammalian cells models has been developed (Deans et al., 2007). This type of an approach is particularly attractive given its efficacy. Application of these techniques towards gene silencing had shown to be extremely promising with a yield > 99% for target gene repression.

Recombinant Adeno-Associated Virus (rAAV)

In terms of applying some of these technologies to the field, an ideal gene transfer vector should be efficient, safe, economic, and have ease of use (Monahan and Samulski, 2000). Viral vectors, *e.g.* retrovirus, lentivirus, adenovirus, and adeno-associated virus are considered to be an effective delivery system as they are efficient, associated with higher infection efficiency, and provide more pre-clinical and clinical utility than non-viral vectors (Bonadio and Cunningham, 2002). Accordingly, recombinant adeno-associated virus (rAAV) holds promise as a gene therapy vector for a multitude of genetic disorders.

For example, promising findings include the use of rAAV as a gene therapy vector to treat hemophilia, cystic fibrosis and the muscular dystrophies (Schultz and Chamberlain, 2008). Additionally, rAAV vector is one of the most promising systems for the correction of bone defects, cartilage lesions, and rheumatoid arthritis (Dai and Rabie, 2007). More recently, a combination of lyophilized rAAV and biomaterials, *e.g.* hydroxyapatite, presents a promising strategy for bone regenerative medicine (Nasu et al., 2009).

Some of the major drawbacks of cancer therapies are problems with inefficient drug delivery, drug resistance, and the undesired effects some drugs have on normal cells (a lack of tumor specificity). Replicating viruses could overcome these drawbacks and be designed to selectively replicate in and lyse tumor cells, while leaving normal tissue unaffected. Interestingly, p53 and E2F transcription factor pathways are altered frequently in human cancers and are among regulatory targets for cancer therapy (Polager and Ginsberg, 2009). Accordingly, an rAAV has been engineered to contain a novel regulatory circuit to synergize replication of the virus to the state of the p53 pathway in human cells (Ramachandra et al., 2001). Thus, usual expression of p53 in normal cells resulted in the inhibition of viral replication, whereas wild type viral replication levels and subsequent cell killing were observed in tumors cells, where the p53 expression is altered. Similarly, cancer-targeting *E. coli* were engineered so their ability to target cancer cells was specifically linked to sensing heterologous environmental signals from the malignant cell microenvironment (Anderson et al., 2006). Thus, the method could be used to engineer bacteria to sense the microenvironment of a tumor and respond by selectively killing cancerous cells, thru the release of cytotoxic agents.

Synthetic Matrix

Regenerative medicine is a new therapeutic strategy that promotes the healing of diseased or damaged organ, *e.g.* skin. Advances over recent years have been a result of collaborative research among biomaterial, pharmaceutical, and biological fields (Huang and Fu, 2010). Accordingly, research has shown that optimal healing environments are induced by moist wound healing. This resulted in the development of various occlusive synthetic dressings that improved acute and chronic wound environments. These new synthetic polymer dressings maintain an ideal moist wound environment, help autolytic debridement, prevent infection, and speed up granulation and epithelialization (Limova, 2010). Similarly, a large subset of biologically active materials and synthetic skin substitutes has been developed to address the various challenges of wound healing in general and minimize problems created by fluid loss and infection (Shores et al., 2007).

Antimicrobial Agents

Synthetic biology provides new grounds to circumvent drug development problems by offering promising antimicrobial strategies. For example, a bacteriophage engineered to over-express cellular proteins and attack gene networks that are not directly targeted by antibiotics has been created (Lu and Collins, 2009). This work revealed that suppressing the SOS network, *i.e.* regulatory network induced by DNA damage, in *E. coli* by the non-lytic M13 phage enhanced the killing by three major classes of antibiotics both *in vitro* and in murine models. Thus, engineered bacteriophage targeting non essential gene networks can be implemented to supplement other antimicrobial strategies to reduce the incidence of antibiotic resistance and enhance bacterial killing.

Biofilms

Bacterial biofilms are differentiated communities of sessile cells. Cellular encapsulation and attachment is provided by the expression of extracellular matrix components curli fimbriae and cellulose (Wang et al., 2006). The large collection of cells and the materials that hold them together make them naturally resistant to antibiotics, as the core cells do not typically come in contact with the antibiotic. Biofilms are also difficult to remove from the surface they attach to. Infections involving biofilms play a major role in diverse medical conditions presenting a significant threat to human health. Biofilms were common problem on the Russian space station MIR, as were astronaut skin reactions (Jones et al., 2004; Pierson, 2001). Therefore, the development of novel, efficient, and cost-effective treatments are needed to impede the current trend of antibiotic resistance and biofilm formation (Hoffman et al., 2005). Specially engineered bacteriophage have been used to express a biofilm degrading enzyme to simultaneously attack the bacterial cells in the biofilm and the biofilm matrix upon infection (Lu and Collins, 2007), resulting in over 99% removal of biofilm bacterial cells. The applicability of synthetic biology, by engineering bacteriophage, paves the way in helping to circumvent important medical and industrial problems.

Testing of Synthetic Biology

Synthetic biology offers tremendous new opportunities to benefit all aspects of life, and human health in particular. In order to assess the applicability of synthetic biology for the space program scientists will have to test those systems under the stringency of the space environment. This will help to reduce the uncertainties associated with cellular and organismal responses to microgravity and the space environment. Scientific experiments involving spaceflight are particularly difficult due to relatively infrequent flight opportunities, restrictions on up and down mass, significant planning expenses, and limitations in crew time. Some measures have been

taken to address these limitations by modeling space environment conditions right here on Earth. To investigate cellular alteration under simulated microgravity, NASA's Johnson Space Center developed a new type of cell culture instrument, the Rotating Wall Vessel (RWV) that is based on the principle of clinorotation (Klaus et al., 1998). The underlying principle is the nullification of gravitational forces by a slow rotation around one axis which creates a low shear environment (Figure 3). This new technology paralleled the efforts of the European Space Agency in the development of a Random Positioning Machine (RPM), which uses a two-axis system. Although some reports point towards similar effects on cells, it remains controversial whether one is superior to the other (Patel et al., 2007; Villa et al., 2005).

The RWV was initially developed for the purposes of cell culture in simulated microgravity. Previous studies on bacteria, revealed that the conditions of either a Low Shear Modeled Microgravity (LSMMG) or spaceflight provoque an environmental signal that induces significant molecular changes on the organism (Nickerson *et al.*, 2003; Nickerson *et al.*, 2004; Wilson *et al.*, 2007). As such, 1) bacterial growth kinetics are usually increased under an LSMMG environment as compared to Earth gravity controls, 2) simulated microgravity does not have a universally negative effect on secondary metabolites, 3) simulated microgravity confers bacterial resistance to environmental stresses and antibiotics, and 4) simulated microgravity environment increases the virulence of pathogenic bacteria. Furthermore, it seems that the bacterial response to simulated microgravity is species-specific, and thus a comprehensive assessment of the applicability of synthetic biology altered microorganisms to the space environment will have to be undertaken.

Such efforts will pay off, however, both helping to ensure mission success and improving life on this planet in the process. As is often the case, materials and technologies developed for the

space mission context have practical applications on Earth. The possible developments of synthetic biology systems for space applications carry important considerations for Earth-based pathologies and clinical applications. The use of microgravity or simulated microgravity may alter cell behavior including growth pattern, apoptosis and gene expression, in order to take advantage of some of these changes for a particular application. Hence, one could think of this concept as using physical properties of the environment to bring or alter a cell's behavior for a certain function. A few examples are presented below:

- use of 3-D culture of human small intestinal epithelial cell line to infect human cells by nanovirus, which was previously not possible in other cell cultures (Straub et al., 2007),
- *in vivo* use of cartilage created using the RWV to heal injuries (Yoshioka et al., 2007),
- use of RWV in combination with extracellular matrix to influence embryonic stem cell differentiation (Chen et al., 2007b; Philp et al., 2005),
- use of engineered myocardial patch to replace myocardium following infarction (Bursac et al., 2007),
- use of the RWV environment to produce stroma for corneal tissue engineering (Chen et al., 2007a),
- culturing of pancreatic islets in RWV to improve the success of islet transplantation in diabetic patients (Rutzky et al., 2002; Stepkowski et al., 2006),
- use of the simulated microgravity environment to make use of a unique growth pattern of endothelial cells, which could potentially have an impact for angiogenesis and cardiovascular disease (Grimm et al., 2010).

Overall, in addition to spaceflight applications, the use of simulated microgravity for cell biology presents new venues to treat diseases on Earth, particularly with tissue engineering. This has been recognized in the past decades by the scientific community (Abbott, 2003). It will be interesting to see where this exploration takes us in the future.

Monitoring of synthetic biology

Monitoring of synthetic biology systems is a prerequisite for the use of these systems in the space environment. Monitoring would provide a level of assurance that SB systems remain functional and safe, even in unpredictable situations. Critical monitoring functions could include: 1) an ability to detect the presence/absence of SB organisms including the ability to sequence their genetic material, and 2) the ability to monitor their performance, whether at the level of gene expression, protein expression, or other measures.

NASA is currently funding early development of the Search for Extra-Terrestrial Genomes (SETG) instrument, which would have the capability to isolate, detect, and sequence DNA or RNA (Isenbarger et al., 2008). SETG is based on the hypothesis that life on Mars, if it exists, may be related to life on Earth, and hence have common genetic systems. The approximately one billion tons of rock thought to have been transported between these planets, due to meteorite impacts mainly between 3.5-4 Gya, may have carried viable microbes (Javaux, 2006). If so, such microbes may continue to survive on Mars today, or we may all be Martians. Thus, SETG will target life-as-we-know-it by isolating, detecting and sequencing RNA or DNA *in situ* from soil, ice, or brine samples on Mars (Figure 4). The most viable approaches for automating these instrument functions require biological components, including nucleotides, DNA primers, and enzymes such as DNA polymerase and reverse transcriptase. These components, while far from some definitions of synthetic biology, are already synthetic: primers are synthesized from nucleotides and are often modified in both novel ways, and those that exist in nature. Polymerases have been widely engineered to improve, remove, or add functionality (Wang et al., 2004). Other instruments including some already slated for flight, depend upon similar biological components. On a related note, antibodies are a critical component of the Life Marker Chip, developed for the ExoMars mission; this instrument will use an immunoassay

approach in an attempt detect specific organic molecules (Parnell et al., 2007; Thompson et al., 2006). These components must withstand a number of challenging aspects of the space environment including potential large temperature fluctuations, and a more intense and diverse radiation environment. Understanding how to safely store these components over long periods of time and how to protect them from the extreme conditions in space will support the development of space systems with increased biological heritage. If these systems ultimately utilize living synthetic organisms, the ability of these organisms to survive in the space environment will also inform our search for the origin of life on Earth and in particular of the probability that life has been spread via meteoritic transfer, *i.e.* the panspermia hypothesis (Mileikowsky et al., 2000). SETG's intended application is to search for life on Mars that could be ancestrally related to life on Earth (or *vice versa*), but it can also be used in planetary protection, space medicine, and environmental health applications. It may also have a role in monitoring space applications of synthetic as well as natural biology.

Some of the stress factors, microgravity and space radiation, have a tremendous impact on expanding our needs for space exploration and could benefit our understanding of the limits of life. Real-time microbial gene expressions analyses during exposure to the harsh environment of space can provide new means and insights into cellular processes. However, current protocols to assess how the space environment alters cellular activities, performed either on the ISS, other spacecrafts (*e.g.* small satellites), and high altitude balloons, require fixing the cells in space after short- or long-term exposure, then conducting wet laboratory studies in ground facilities. This is due to the lack of flight qualified analytical instruments for biological studies. Thus, the development of an instrument that could perform *in situ* microbial gene expression analysis for small satellites and the ISS is needed.

Accordingly, NASA is also funding the development of a space-based instrument for measuring *in situ* gene expression of microorganisms. This miniaturized fluidic system will automate all the steps involved in growing microorganisms, extracting RNA from these organisms, and hybridizing nucleic acids to DNA microarrays (Pohorille et al., 2010). The prototype under development in collaboration with CustomArray Inc. (Mukilteo, WA) will be a small integrated and automated system part of the nanosatellite platforms developed by NASA's Ames Research Center's Small Spacecraft Office. The system will perform the automated cultivation and measurements of gene expression of the photosynthetic bacterium *Synechococcus elongates* (a cyanobacterium) exposed to polar orbit for a proposed period of 6 months. *S. elongatus* and other cyanobacteria in general, exhibit remarkable metabolic diversity and resilience to adverse conditions (Gao et al., 2009; Lopez-Gomollon et al., 2009; Olsson-Francis et al., 2010; Rothschild and Mancinelli, 2001). For this reason, NASA considers cyanobacteria to be ideal model organisms for research on the adaptation of terrestrial organisms to the space environment. Subsequently, understanding the underlying mechanisms of adaptation of these microorganisms to microgravity and ionizing radiation, as provided by long-term cultivation in a small satellite, can provide details about the suitability of cyanobacteria and other microorganisms (including those manipulated by synthetic biology) for space applications. Once developed, the system can be used with minor modifications for multiple experiments on different platforms in space, including extensions to higher organisms and microbial monitoring. Thus, the instrument will support multiple objectives in astrobiology, fundamental biology and biomedical space sciences.

Finally, monitoring of organics from natural or synthetic biology systems could be accomplished using tools such as the Mars Organics Analyzer (MOA) (Benhabib et al., 2010).

The multichannel MOA, a portable instrument for the sensitive microchip capillary electrophoresis analysis of organic compounds such as amino acid biomarkers and polycyclic aromatic hydrocarbons has been developed as part of the quest to search for signs of past or present extraterrestrial life on the Martian surface . The instrument includes all the necessary microfluidics, optics, and electronics for *in situ* capillary electrophoresis analysis of a variety of organic molecules (Stockton et al., 2010). Interestingly, the MOA is one of the analytical elements of the Urey Instrument (Figure 5), which was developed for the Pasteur payload of the ExoMars rover (Aubrey et al., 2008).

No doubt there are numerous other instruments and programs that would also contribute to our ability to monitor and control synthetic biology systems. While we have not exhaustively reviewed them here, we feel such monitoring is useful from a verification perspective, as it is also enabling by combining synthesis tools with monitoring tools which could facilitate *in situ* synthetic biology, such as engineering a synthetic biology system to produce a medicinal compound identified after the start of a spacecraft mission.

These tools also highlight a potential path to development of synthetic biology systems for spaceflight: initially, such systems will be conventional instruments with synthetic or biological components, later, such components may be replaced with more-integrated synthetic components and ultimately synthetic organisms. Whether such systems can achieve the robustness required for space operations remains to be seen. Additionally, the systems presented are complementary and could be part of an integrated microbial monitoring system. Such systems could support planetary protection efforts, hardware reliability, and help to sustain crew health during exploration class missions (Venkateswaran et al., 2010).

Recommendations and conclusions:

The current manuscript is a brief snapshot of a workshop held at the NASA Ames Research Center where experts involved in the science and engineering of space missions were interacting with scientists involved in basic and applied synthetic biology research in order to frame the potential research agenda for synthetic biology within NASA missions. During the meeting five different working groups where held in parallel. The synthetic biology in human group was primarily looking at the human health aspect of synthetic biology and its potential applications for human spaceflight. A broad spectrum of human health applications have promising potential for human spaceflight and will help circumvent some of the physiological challenges encountered by astronauts during long-duration spaceflight, thus helping to ensure mission success. Some of the applications discussed herein are promising technologies that have direct applications to many facets of space travel. Their practicality in the harsh and stressful space environment, however, remains to be demonstrated. It is the opinion of this panel, however, that the development and implementation of trial experimentation and assessment should be considered in the very near future.

NASA Ames initiative

The NASA Ames Research Center (ARC) synthetic biology initiative is intended to assess, develop, and implement the potential use of synthetic biology for space applications(Langhoff et al., 2011). Such applications could gravitate around potential development of biosensors to understand the astrobiome, conduct experiments to help us tailor life off the planet using appropriate platforms, *e.g.* ISS and nanosatellites, and research the production of bioproducts to sustain long-duration spaceflight. These initiatives will be implemented by the development of a

synthetic biology program at ARC that will include opportunities for research fellows to perform cutting edge research that has a direct impact on NASA missions with NASA scientists as mentors, and the possibility to accommodate groups of talented undergraduate students over the summer to work on a particular synthetic biology research project. It will be important, within NASA to acknowledge the potential technical and economical benefits associated with the synthetic biology initiative to implement the current missions and programs, and subsequently expand towards the more challenging goals of space exploration. A goal for NASA also would be to develop low-cost, low-power, automated, and miniaturized, space-qualified microarray and sequencer instruments for environmental monitoring and health diagnostic applications to sustain and implement space exploration. These could be implemented by providing new sources of funding to the scientific community to tackle those particular challenges.

One interesting opportunity worth considering is the International Genetically Engineered Machine (iGEM) Competition. Since 2004, iGEM provides undergraduates with an opportunity to design and create unique synthetic biology research projects. The competition is held each year at the Massachusetts Institute of Technology (Cambridge, MA) and gives an opportunity to the students to present their work and be recognized through different prizes for their efforts and achievements (Purnick and Weiss, 2009). As a result of this competition, a plethora of research projects have significantly contributed to the field of synthetic biology, many of which have led to publications in the field. NASA has a unique opportunity given its community role, the agency and its mission inspire the public in general, and the youth in particular, to undertake the initiative to host groups of students that will participate in the iGEM competition. Furthermore, it might also be a good initiative for NASA to sponsor a space related prize within iGEM, where research projects contributing to NASA missions could be recognized.

Synthetic biology has emerged as a new and vibrant discipline that has the potential to impact how we interact with the environment and how we approach human health. Subsequently, we will need to consider both the potential benefit and harms to Earth and our society. Thus, synthetic biology raises several ethical issues that will have to be addressed. For example, the issues associated with the risk that knowledge from synthetic biology will be misused in biological terrorism or warfare is of great concern (Douglas and Savulescu, 2010). Furthermore, as noted during the workshop, the negative public perception of synthetic biology and the creation of artificial life in particular are still very strong. These concerns will have to be addressed and the scientific community could certainly play an important role in reaching out through the traditional media, teaching courses at all levels, presenting public seminars, and the use of social media to present a case for these techniques and the potential synthetic biology benefit to the environment and human health. Finally, the requirements for space applications are different from those on Earth and therefore will have to also be addressed with the international NASA partners through COSPAR (committee on Space Research).

The aim of this manuscript was to give an introduction of synthetic biology in the context of human health, present some of the challenges that the astronauts have to face during long-duration spaceflight, and give some examples of current synthetic biology initiatives that might impact human spaceflight. The latter provides a summary of the literature on particular synthetic biology topics related to human spaceflight. It is clear that synthetic biology offers tremendous potential to both Earth based medicine and space crew health to help address some of the risks associated with humans venturing into extreme environments.

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Legends for figures:

Fig. 1. Schematic illustration of synthetic biology.

Fig. 2. Schematic diagram of the complete arsenic biosensor system. The system is controlled by two repressor proteins, ArsR (responding to low concentrations of arsenate or arsenite) and AsrD (responding to higher concentrations). In the presence of lactose, but absence of arsenate, urease is induced and the pH rises. When low amounts of arsenate are present, an ArsR-repressed promoter is induced, leading to expression of lambda cI repressor, switching off urease production. Thus the pH remains neutral. If higher amounts of arsenate are present, *LacZ* expression is induced through an ArsD-responsive promoter, leading to a fall in pH.

Fig. 3. Overview of the Operating Orientations of the RWV and the Effects of RWV Rotation on Microbe Suspension. A. Picture of the High Aspect Rotating Vessel (HARV) attached to the rotating/aerating platform (Synthecon Inc., Houston, TX). **B.** The two operating orientations of the Rotating Wall Vessel are depicted. In the Low Shear Modeled Microgravity (LSMMG) orientation (i), the axis of rotation of the RWV is perpendicular to the direction of the gravity vector. In the normal gravity orientation (ii), the axis of rotation is parallel to the gravity vector. **C.** Effects of RWV rotation on cell suspension. When the RWV is not rotating or in the normal gravity orientation (i), the cells in the apparatus will sediment and settle on the bottom of the RWV due to gravitational force. When the RWV is rotating in the LSMMG position (ii), cells are continually suspended in the medium. The medium within the RWV rotates as a single body, and the sedimentation of the cells due to gravity is offset by terminal velocity.

Figure 4. The Search for Extra-Terrestrial Genomes (SETG) instrument. This 2.5 centimeter by 2.5 centimeter (or 1 inch by 1 inch) microfluidic chip is part of the SETG instrument prototype, which performs amplification and detection of DNA. Tiny channels feed in the samples to be analyzed and control the fluidic circuitry on the chip. Blue light excites fluorescent dyes that help identify DNA within 3072 cubic chambers, each about the width of a human hair, or one billionth of a liter in size. Image credit: C. Carr.

Figure 5. Computer Assisted Design of the Urey Instrument. The Urey instrument uses a Sub-Critical Water Extractor (SCWE), the Mars Organic Detector (MOD), and a Micro-Capillary Electrophoresis (μCE) analyzer to investigate the target organic compounds in samples collected during the ExoMars rover activities. Samples will be analyzed to detect the presence of organic compounds and to determine their composition. If detected, amino acids are analyzed further to determine their chirality. *Image courtesy of NASA*

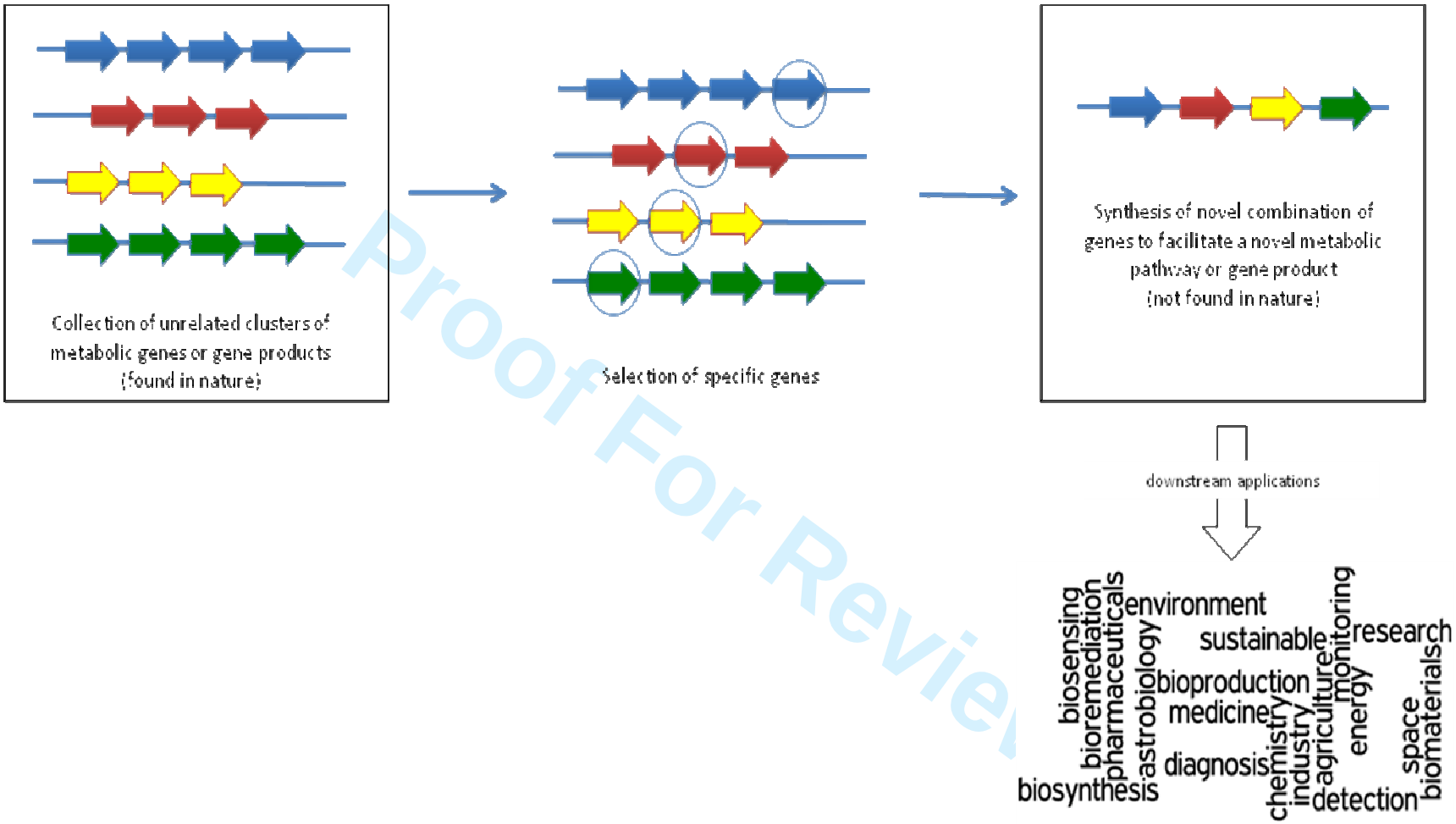

Fig. 1.

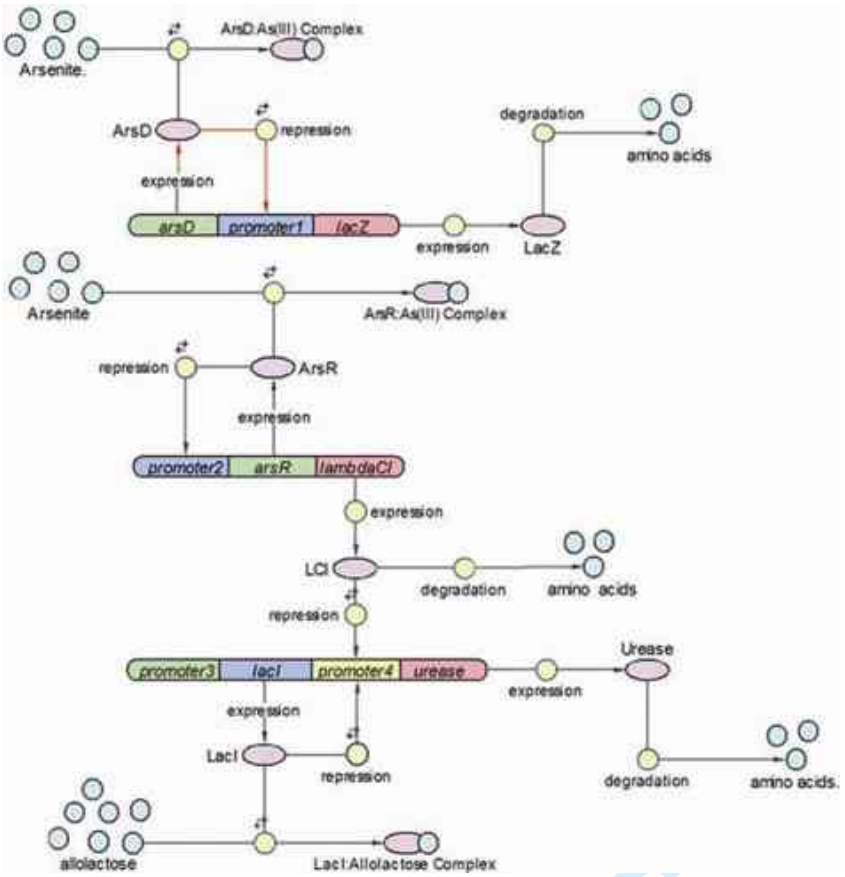

Fig. 2.

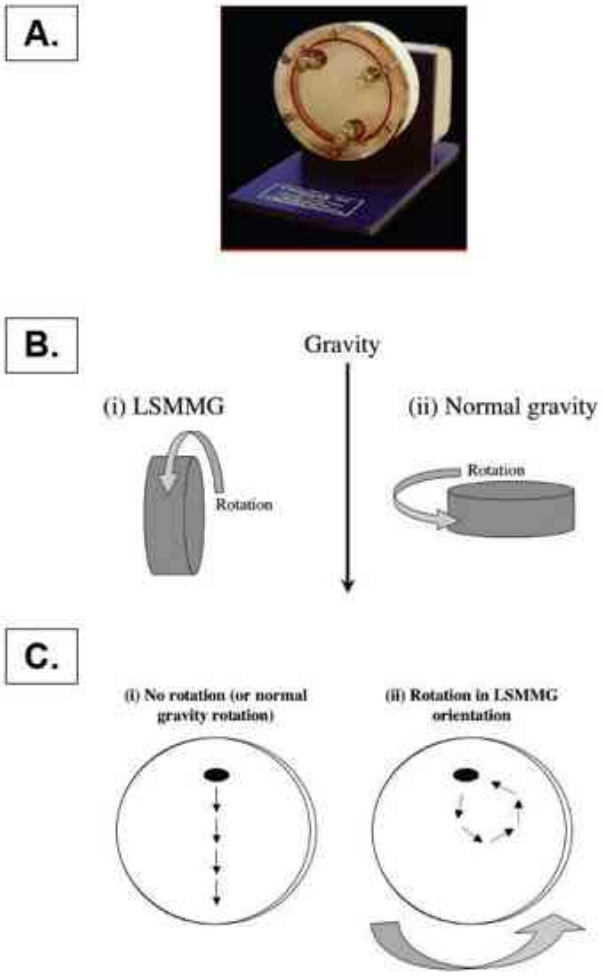
A.
B.
Gravity
(i) LSMMG
Rotation
(ii) Normal gravity
Rotation
C.
(i) No rotation (or normal gravity rotation)
(ii) Rotation in LSMMG orientation

Fig. 3

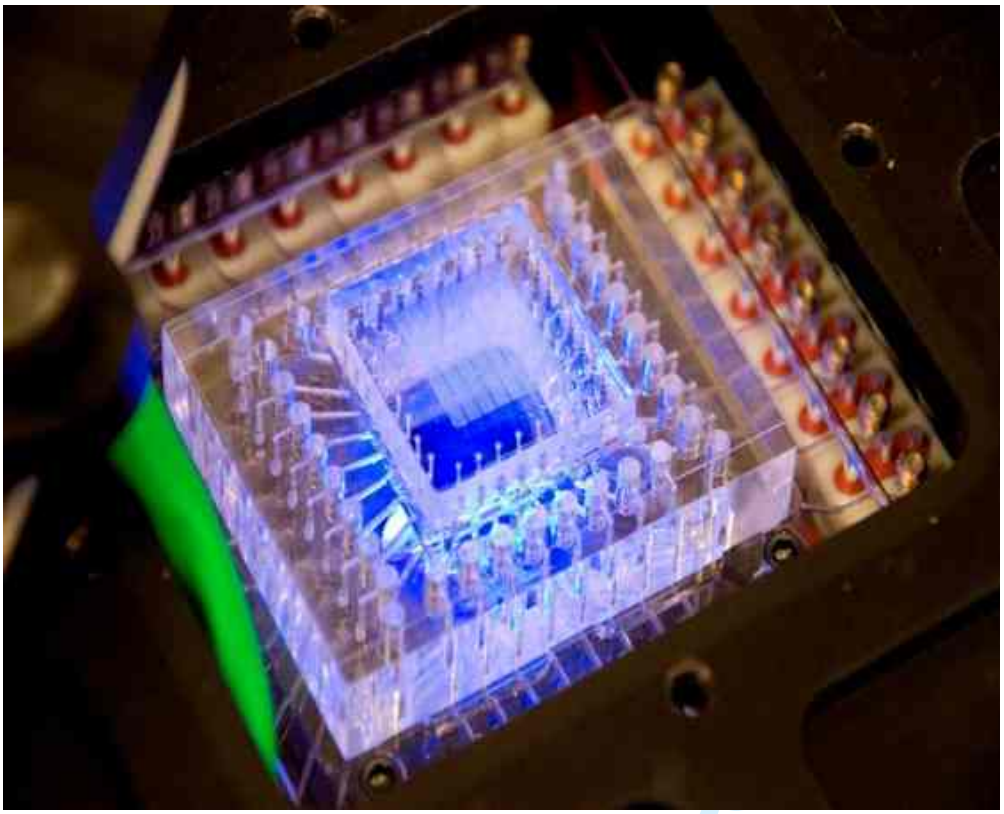

Fig. 4

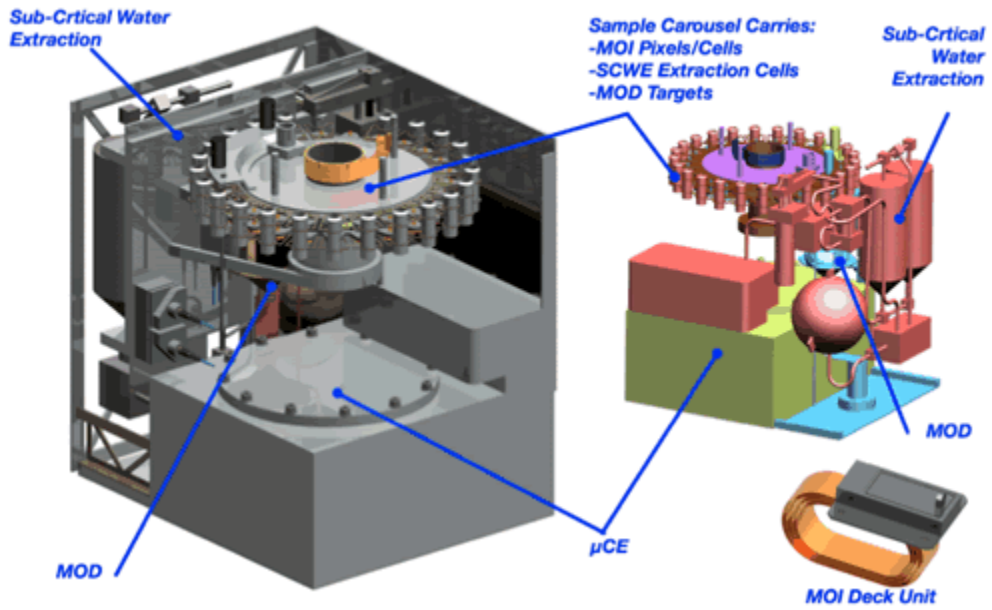

Fig 5.