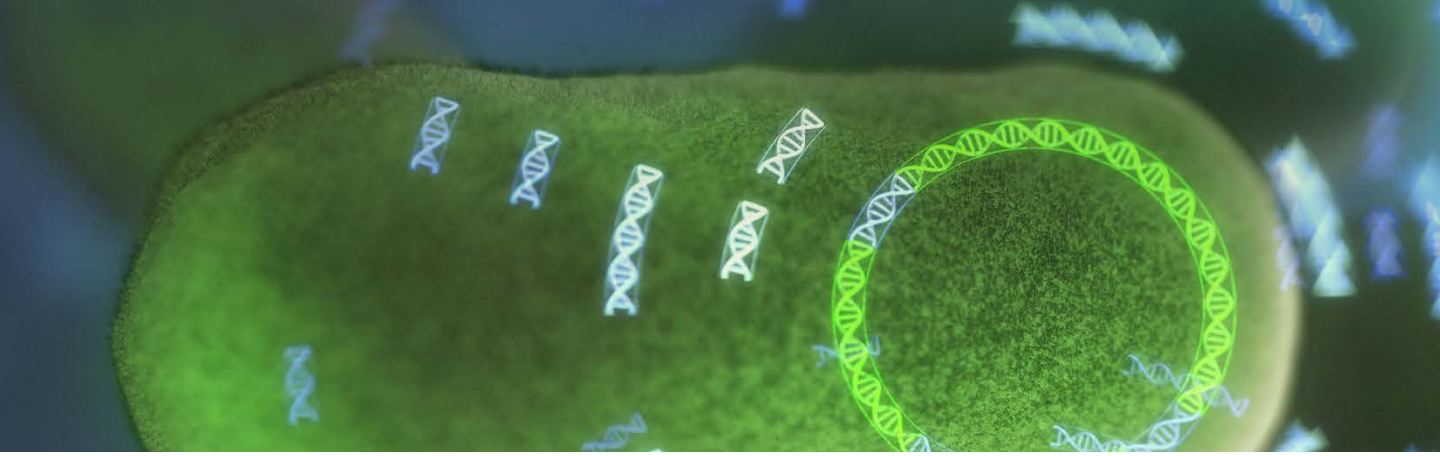

synberc

Building the Future with Biology.

Year 8 Annual Report
February 19, 2014

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2 Project Summary

The vision of the Synthetic Biology Engineering Research Center (Synberc) is to develop the foundational understanding and technologies to build biological components and assemble them into integrated systems to accomplish many particular tasks; to train a new cadre of engineers who will specialize in synthetic biology; and to educate the public about the benefits and potential risks of synthetic biology. In short, we want to make biology easier to engineer.

The Synberc research program is developing the foundational understanding and technologies to build biological components and assemble them into integrated systems to accomplish particular tasks. The Center's specific goals are: 1) to develop a conceptual framework for designing small biological components (parts) that can be assembled into systems (devices) that will perform well-characterized functions under specified conditions; 2) to develop a small number of chassis (stable, robust bacterial hosts with known responses) to host the engineered devices and to assemble several devices into applications to accomplish a larger vision or goal; and 3) to develop a set of standards for the interactions of the parts and devices so that devices can be built more readily and reproducibly. In addition, the Center is 1) catalyzing the development of synthetic biology by designing and facilitating new forms of collaboration among researchers from across disciplines, laboratories, and institutions, and 2) orienting the synthetic biology community toward the goal of benefiting all people and the planet by connecting its techno-scientific goals to broader challenges in safety, security, medicine, energy, and the environment. These goals are being achieved through four thrust areas in 1) Parts and Part Composition, 2) Devices and Device Composition, 3) Chassis Design, Construction, and Characterization, and 4) Practices. Three testbed applications are demonstrating and driving the activity of the thrusts: 1) Glucaric Acid Production, 2) Programmable Organoids, and 3) Nitrogen Fixation. The resulting parts, devices, and chassis are being disseminated through a distributed Web of Registries (including the Synberc Registry) and BioFAB capacities.

Synberc conducts a complementary education program that is 1) creating a new cadre of biological engineers capable of designing and building biological systems, 2) educating the general public about the benefits and risks of synthetic biology, 3) educating and raising awareness among the public, and policy-makers about the benefits and threats of synthetic biology, and 4) educating K-12 students about the opportunities offered by careers in science, engineering, and synthetic biology. Coupled with a strong outreach program to minority institutions and local community colleges and high schools, Synberc is committed to increasing the participation of minority students in this emerging area, and to encouraging high-school students to enter this exciting new field of engineering.

Synthetic biology is transforming the biotechnology, high-technology, pharmaceutical, and chemical industries, as well as suppliers of genetic tools and custom DNA synthesis companies. Synberc is partnering with key companies in these sectors to better understand commercial applications for synthetic biology, to receive advice on our research program,

to speed technology transfer, and to develop Synberc funding. Industrial partners will also provide internships for undergraduate and graduate students.

Intellectual merit. Synthetic biology is transforming the field of biology into an engineering discipline by introducing concepts developed in other fields of engineering into biology: ready access to off-the-shelf parts and devices with standard connections; a substrate onto which one can assemble the parts and devices and a power supply for the devices; standards for the basic components to enable their ready integration into a larger functional system; and open-source availability of parts, devices, and chassis. These developments will make the engineering of biology easier, more efficient, and more predictable. Synberc has been highly successful to date in bringing together many of the pioneers (biologists and engineers from world-class institutions) of synthetic biology to work together to lay the foundation for this nascent field.

Broader impacts. Synthetic biology (as catalyzed by Synberc) is transforming the biotechnology, high-technology, pharmaceutical, and chemical industries, as well as suppliers of genetic tools and custom DNA synthesis companies. Synberc is educating a new cadre of synthetic biologists and biological engineers capable of designing biological parts and useful biological systems. Synberc's integrated Practices thrust is developing responsible innovation as a central and defining part of this growing field, and helping practitioners and the public recognize how this biotechnology will affect our world. Finally, Synberc's education program is providing general information on synthetic biology for the general public, in-depth offerings for public policy professionals, and motivational information on opportunities in higher education for pre-college students.

4 Systems Vision and Value Added

4.1 *Systems Vision*

By responsibly leading the field of synthetic biology, Synberc is enabling transformative technologies to meet global challenges and improve lives. This vision is being accomplished by developing the foundational understanding and technologies to build biological components and assembling them into integrated systems to accomplish many particular tasks. In this vision, we include the needs to train a new cadre of engineers who will specialize in synthetic biology and to educate the public about the benefits and potential risks of synthetic biology. In essence, we will build the future with biology.

The richness and versatility of biological systems make them ideally suited to solve some of the world's most significant challenges, such as converting cheap, renewable resources into energy-rich molecules and valuable chemicals; producing high-quality, inexpensive drugs to fight current and emerging diseases; improving the supply and yield of crops to improve food security, detecting and destroying chemical or biological agents; and remediating polluted sites. Over the years, significant strides have been made in engineering microorganisms to solve many of these problems. For example, microorganisms have been engineered to: produce ethanol, bulk chemicals, and valuable drugs from inexpensive starting materials; detect and degrade nerve agents and less toxic organic pollutants; and accumulate metals and reduce radionuclides. However, these biological engineering challenges have long development times, in large part due to a lack of useful tools that would enable engineers to easily and predictably reprogram existing systems, as well as to build new enzymes, signal transduction pathways, genetic circuits, and, eventually, whole cells. The ready availability of these tools would drastically alter the biotechnology industry, leading to less expensive pharmaceuticals, renewable energy, and biological solutions to problems that do not currently have sufficient monetary returns to justify the high cost of today's biological research.

Most of the biological engineering tools currently available to scientists and engineers have not changed significantly since the 1980s: biologists largely use natural, gene expression control systems (promoters with their cognate repressors/activators). The ability to place a single heterologous gene under the control of one of these native promoters and produce large quantities of a protein of interest is the basis for the modern biotechnology industry. While these redesigned biological control systems have been generally effective for their intended purpose (controlling rather roughly the expression of a single gene or a few genes), not surprisingly, they are often inadequate for more complicated engineering tasks (e.g., control of very large, heterologous, metabolic pathways or signal transduction systems). Further, these borrowed "biological parts" retain many of the features that were beneficial in their native form but which make them difficult to use for purposes other than that for which they evolved. Well-characterized standard biological parts and larger devices made from such parts would make biological engineering more predictable and allow construction and integration of larger systems than is currently possible.

In almost every other field of engineering, standards have been developed to allow one to easily assemble components from various manufacturers to build a large integrated system. Biologists and engineers have not yet defined the standards for the various parts that might allow them to build larger biological devices. The design and construction of new devices (genetic control systems, for example) would benefit greatly from a set of standards that would govern how the various parts (regulatory proteins, promoter, ribosome binding site, for example) should interact and be assembled. Setting a standard will, in turn, encourage manufacturing firms to develop parts as well.

The proliferation of genetic parts, devices and chassis raises new opportunities and challenges related to ownership, sharing and innovation in biotechnology. Though many basic parts are not patent protected, some very useful ones, e.g. GFP, are. Use of this part requires acquiring a license which, for small companies, could be prohibitive. On the other hand, the need to get around the patents in general – and on GFP, in particular – has always been the economic incentive for innovation and has led to discovery of new products and applications. Advances in DNA synthesis, automated design, and standards are producing geometric increases in the scale, ease, and complexity of DNA systems and has become the driver of innovation.

There is a need to balance open access with creating economic incentives for innovation. Open access to the basic units of synthetic biology – parts, devices, and ultimately chasses – would lower the barrier of entry to small-scale innovators and encourage the development of novel biological solutions. On the other hand, if we expect innovators to assemble these basic units into new commercially valuable products and services, ample intellectual property rights and economic incentives need to be maintained. To optimize the advance inventions from the lab to the marketplace, we must understand how intellectual property and economic regimes are best applied to synthetic biology.

Synthetic biology is the design and construction of new biological entities such as enzymes, genetic circuits, and cells or the redesign of existing biological systems. Synthetic biology builds on the advances in molecular, cell, and systems biology and seeks to transform biology in the same way that synthesis transformed chemistry and integrated circuit design transformed computing. Synthetic biology is distinguished from traditional molecular and cellular biology by its the focus on the design and construction of core components (parts of enzymes, genetic circuits, metabolic pathways, etc.) that can be modeled, understood, and tuned to meet specific performance criteria, and the assembly of these smaller parts and devices into larger integrated systems that solve specific problems. Just as engineers now design integrated circuits based on the known physical properties of materials and then fabricate functioning circuits and entire processors (with relatively high reliability), synthetic biologists will soon design and build engineered biological systems. Unlike many other areas of engineering, biology is incredibly non-linear and more unpredictable, and there is less knowledge of the parts and how they interact. Hence, the overwhelming physical details of natural biology (gene sequences, protein properties, biological systems) must be organized and recast via a set of design rules that hide information and manage

complexity, thereby enabling the engineering of multi-component integrated biological systems. When this is accomplished, designs of significant scale will be possible.

Synthetic biology arose from four different intellectual agendas. The first is the scientific idea that one practical test of understanding is an ability to reconstitute a functional system from its basic parts. Using synthetic biology, scientists are testing models of how biology works by building systems based on models and measuring differences between expectation and observation. Second, the idea arose that, to some, biology is an extension of chemistry and thus synthetic biology is an extension of synthetic chemistry. Attempts to manipulate living systems at the molecular level will likely lead to a better understanding, and new types, of biological components and systems. Third is the concept that natural living systems have evolved to continue to exist (i.e., "good enough to support necessary function), rather than being optimized for their intended use. By thoughtfully redesigning natural systems, it is possible to simultaneously test our current understanding. It may become possible to implement engineered systems that are easier to study and interact with, and that might exceed the performance of their natural counterparts. Fourth, the idea emerged that biology can be used as a technology, and that biotechnology can be broadly redefined to include the engineering of integrated biological systems for the purposes of processing information, producing energy, manufacturing chemicals, and fabricating materials.

Synthetic biology's emergence is motivated by these agendas, though the field's advancement has only been made practical by the more recent advent of two foundational technologies, DNA sequencing and synthesis. Sequencing has increased our understanding of the components and organization of natural biological systems and synthesis has provided the ability to begin to test the designs of new, synthetic biological parts [2-16] and systems [17-28]. While these examples each demonstrate the incredible potential of synthetic biology, they also show routinizing the engineering of biology requires solving many foundational scientific and engineering problems. To meet these foundational challenges, we need to engage many investigators via a coordinated and constructive Center environment. Synberc is devoted to laying the foundation for synthetic biology.

4.2 *Value Added and Broader Impacts*

As part of our goal of laying the foundation for synthetic biology, Synberc is capitalizing on the recently sequenced microbial genomes, the availability of tools to examine and manipulate the molecules of life, extensive biological literature and databases, advances in computing power, and the intellectual resources of the University of California campuses at Berkeley and San Francisco, Massachusetts Institute of Technology, Harvard University, and Stanford University. Through integrated research and education programs, Synberc is training future scientists and engineers in the technologies and methods necessary for engineering biological parts and devices into higher-order systems that may reside in an existing organism or result in the creation of a largely new one. Synberc is also providing a central place for biological engineers to congregate to exchange ideas; establishing a

common infrastructure for those automation and screening technologies in high demand; providing a repository for these open-source basic biological components; and implementing an organizing framework in which to develop specific applications. Synberc's efforts are leading to new synergies, and our collaborative work is achieving what cannot be accomplished through a narrow or individual approach.

The goal of Synberc's research program is to develop the foundational understanding and technologies to build biological components and assemble them into an integrated system to accomplish a particular task. To accomplish this goal, we have developed 1) a conceptual framework and the associated design and database software for designing small biological components (parts) that can be assembled into devices that will perform a well-characterized function under specified conditions; 2) a small number of chassis (assembly substrate and power supply) to host the engineered devices and to assemble several devices to accomplish a larger vision or goal; 3) a set of standards for the interactions of the parts and devices so that the devices can be built more readily and reproducibly; and 4) offered the parts, devices, and chassis as open source to those both formally and informally connected to Synberc. Our research program is supported by four thrusts: 1) Parts and Part Composition, 2) Devices and Device Composition, 3) Chassis, and 4) Practices. Four testbeds are driving the development of the parts, devices, and chassis and demonstrate the usefulness of these cellular components.

We have developed an ambitious complementary education program to educate a new cadre of synthetic biologists (post-doctoral, PhD, and BS-level) capable of designing biological components, just as electrical engineers design and build integrated circuits. Part of this effort includes reaching out to K-12 students to stimulate their interest in careers in science, engineering, and synthetic biology. We promote synthetic biology through design classes and competitions that provide students and teachers with hands-on experience. Target audiences for our educational programs include high schools, colleges and universities, including minority institutions and community colleges. Our programs have been successful in increasing the participation of minority students at all levels in this emerging area. Synberc is also committed to developing outreach programs targeting both the general public and policy makers about the benefits and risks of synthetic biology.

The new discipline of synthetic biology will transform the biotechnology, high technology, pharmaceutical, and chemical industries as well as suppliers of genetic tools and custom DNA synthesis companies. Synberc is partnering with key companies in each of these sectors to invite applications for and advice on our research program. We also work with companies to provide internships for undergraduate and graduate students, and thereby help to position our students will lead these industries in the future. Our industry partnerships also speed technology transfer, and provide financial support for the Center. Through these varied avenues, Synberc's programs stand to make a lasting impact on the evolution of this major new field at the interface between science and engineering.

Synberc's founding investigators include pioneers of the field of synthetic biology from five

leading universities in the U.S. The center has grown to about 500 personnel actively engaged in carrying out the Center's research and education agenda: 33 faculty members (11 of whom are on the leadership team), 105 post-doctoral fellows, 118 graduate students, 119 undergraduate researchers, 17 REU students, and 23 RET teachers. It is important to note that Synberc's impact is felt well beyond its 500 members. This year, Synberc investigators gave 157 seminars and talks to about 20,000 colleagues. In addition, Synberc's education and outreach efforts reached over 8,000 people through programs like iGEM, Synberc workshops, and events especially for college and pre-college students and teachers. To maximize our reach by "teaching the teachers," we focused on developing our RET program. And through iGEM, which has successfully transitioned to an independent non-profit organization, we reached 245 teams of students – as many as 3,000 students. That number is even more impressive when you consider that we engaged them for an entire summer – not simply an afternoon outreach event. None of the numbers above include participants in the associated projects, which is substantial.

Synberc's funding base is impressive. Synberc has approximately $6.0M in funding, 84% of which comes from our NSF base award and supplements, 11% from cost matching by the academic institutions, and 5% from industry. This is roughly in line with the average for other ERCs (Table 1a). Where Synberc stands out among ERCs (and certainly the average for ERCs) is the amount of associated project funding. Synberc PIs have over $11.2M in direct associated project funding. This associated project funding is twice that of the average manufacturing-based ERC. Much of this funding is for biofuels research, an important area where synthetic biology can have far-reaching impact. Synberc's associated funding is an indication of our success in growing the field and helping our partner institutions to successfully develop their own programs. That kind of success is further reflected in the proliferation of synthetic biology centers at Synberc core and affiliated institutions (two new centers came online just this reporting year: the BU Center of Synthetic Biology and the UW Center for Synthetic Biology).

Over the past year, Synberc PIs have published 80 papers in peer-reviewed journals. Of those 80 papers, 36 came directly from Synberc-supported research, the remaining 44 coming from our associated projects. Not only was a Synberc-supported paper published on average about every 10 days, but there were 13 publications with six or more citations at the time of this writing, giving an h-index of 13. For one year, that is quite good. In terms of intellectual property, Synberc PIs filed 11 patent applications and 5 invention disclosures. Synberc spun out another start-up company this year (Lattice Automation, founded by Doug Densmore and focused on bioCAD tools. This brings the total to 6 Synberc start-up companies.

Within our education programs, Synberc PIs developed one new synthetic biology courses in 2012, while an impressive 34 courses were offered this year that include ERC content. A number of these are freely available on OpenWetWare (www.openwetware.org) for use by Synberc and non-Synberc universities to develop their own synthetic biology curricula. This year, we graduated one Masters student (four in all years) and thirteen PhDs (57 for

all years). Of these graduates, 28 have gone to industry (including six this year) and 37 to academic positions (thirteen this year alone). Finally, nine Synberc students have had industry internships.

Synberc has a strong and rapidly growing industry program. We now have 32 member companies (doubled since last year's report) and commitments from 4 others. These represent 13 large corporations (>500 employees), 5 midsized companies (61-500 employees), 5 small companies (11-60 employees), 11 startups (1-10 employees), and 2 not-for-profit organizations. Members come from all sections of the bioeconomy, including industrial biotechnology, agro-biotechnology, healthcare, and tools, software, and services. Synberc's Industry Liaison Officer continues to aggressively grow this alliance, focussing on large companies and the healthcare sector. Synberc PIs and alumni have spun-out seven startups. The center's mentorship program now pairs 34 students and postdocs with industry mentors. Working relationships between Synberc PIs and member companies, sponsored projects, and licensing of Synberc-funded IP have become commonplace.

As mentioned, many large-scale programs and institutions are growing up around Synberc (the Synthetic Biology Center at MIT, the Center for Systems and Synthetic Biology at UCSF, the Synthetic Biology Institute at UC Berkeley, and the iGEM Foundation, to name a few). This suggests that Synberc has had tremendous impact in catalyzing this new field, with Synberc researchers and institutions at the center. We have built an infrastructure that supports collaboration, constructive competition, and the production of tools and knowledge needed to responsibly and productively exploit synthetic biology. Synberc's value is in no small measure as a venue that convenes and orients this growing community and all its resources to tackle the key technical and social challenges of synthetic biology on a scale that no other venue can match. In doing so, we are leading the field in creating the transformative technologies needed to meet global challenges and improve lives.

5 Nuggets of Significant Achievement and Impact

5.1 *Research and technology highlights*

5.1.1 Creating new components for genetic circuits

Synberc researcher Christopher Voigt and his MIT colleagues describe in the February 2014 issue of Nature Chemical Biology their new components that can be used in genetic circuits. This greatly expands researchers' capacity to engineer microorganisms to execute complex tasks such as sensing and responding to their environment, tracking time to control gene expression and even memory and simple algorithms.

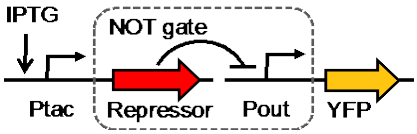

Figure 1: NOT gates are created by inducing a repressor which then binds to its target sequence in a promoter, preventing transcription.

The ability to program a responsive biological system has been hindered by the lack of highly reproducible control elements. Therefore, identifying these elements remains a high priority for synthetic biologists.

Voigt and colleagues mined microbial genomes and found 16 genes that encoded proteins that could very specifically bind a DNA sequence and turn off gene expression downstream. This DNA:protein pair can function in a NOT/NOR gate, which is a fundamental component of circuits. When used in combination, these 16 components could create $>10^{54}$ circuits, greatly increasing the ability to engineer microorganisms for more complex tasks.

Genomic mining of prokaryotic repressors for orthogonal logic gates. Stanton BC, Nielsen AA, Tamsir A, Clancy K, Peterson T, Voigt CA. Nat Chem Biol. 2014 Feb;10(2):99-105. doi: 10.1038/nchembio.1411. [Stanton, 2014 #918]

5.1.2 Creating a CRISPR way to turn off gene expression

The ability to control endogenous gene expression in an organism is an essential goal of synthetic biology. Synberc researchers Lim (UCSF), Church (Harvard), and Arkin have collaborated with a number of investigators to tailor a naturally occurring genome editing system named CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) to interfere with the expression of multiple endogenous genes. Reported in the February 2013 issue of Cell, this system, CRISPRi, is compact and can be used in both bacterial and mammalian cells.

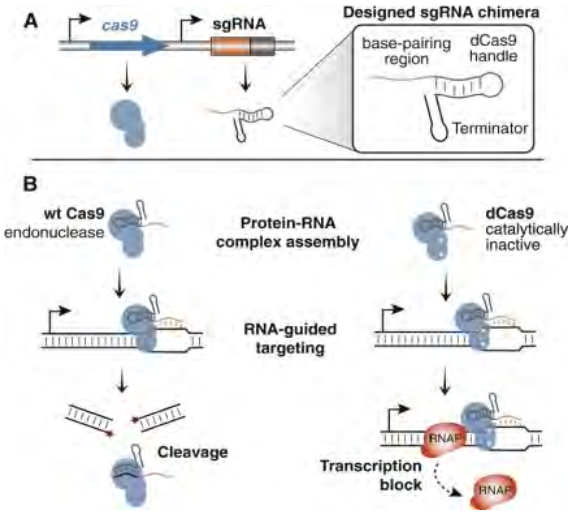

Figure 2: (A) The Cas9 protein (blue) binds to a sgRNA to guide it to a specific DNA sequence. (B) The wild-type Cas9 normally cleave the DNA at the target sequence. Researchers modified the Cas9 to be unable to cut DNA, now called dCas9. Instead, dCas9 blocks RNA polymerase (RNAP, red) from moving along the DNA and transcription is stopped.

The Synberc investigators altered a CRISPR component, Cas9, to home to a specific genomic sequence via associated RNA and then "park" on the target gene, preventing it from being transcribed by RNA polymerase and, hence, effectively silencing gene expression.

The investigators also showed that CRISPRi can be made inducible and that it is remarkably specific in *E. coli*. The CRISPRi system can be used in a general genetic programming platform that is suitable for a variety of applications, including genome-scale functional profiling, microbial metabolic engineering, and cell reprogramming.

Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. Qi LS, Larson MH, Gilbert LA, Doudna JA, Weissman JS, Arkin AP, Lim WA. Cell. 2013 Feb 28;152(5):1173-83. doi: 10.1016/j.cell.2013.02.022 [Qi, 2013 #917]

5.1.3 An novel alternative to standardization and prediction: screen for regulatory elements

"You can observe a lot by watching." - Yogi Berra's quote is a great description of a new method Synberc researchers (lead by Church, Arkin, Endy, and Kosuri) devised to discover new regulatory elements in arbitrary settings.

Using standardized elements or prediction-based design often fails to yield regulatory components that function in the same way under different conditions or in combinations. This is a potential problem for synthetic biologists, where multicomponent circuits and pathways are necessary to generate products that are often superior to non-biological alternatives.

The researchers took a comprehensive look at the behavior of a library of 12,563 combinations of common regulatory elements and simultaneously measured DNA, RNA, and protein levels from the entire library. They quantified how often simple measures of promoter and RBS strengths can accurately predict gene expression when used in combination.

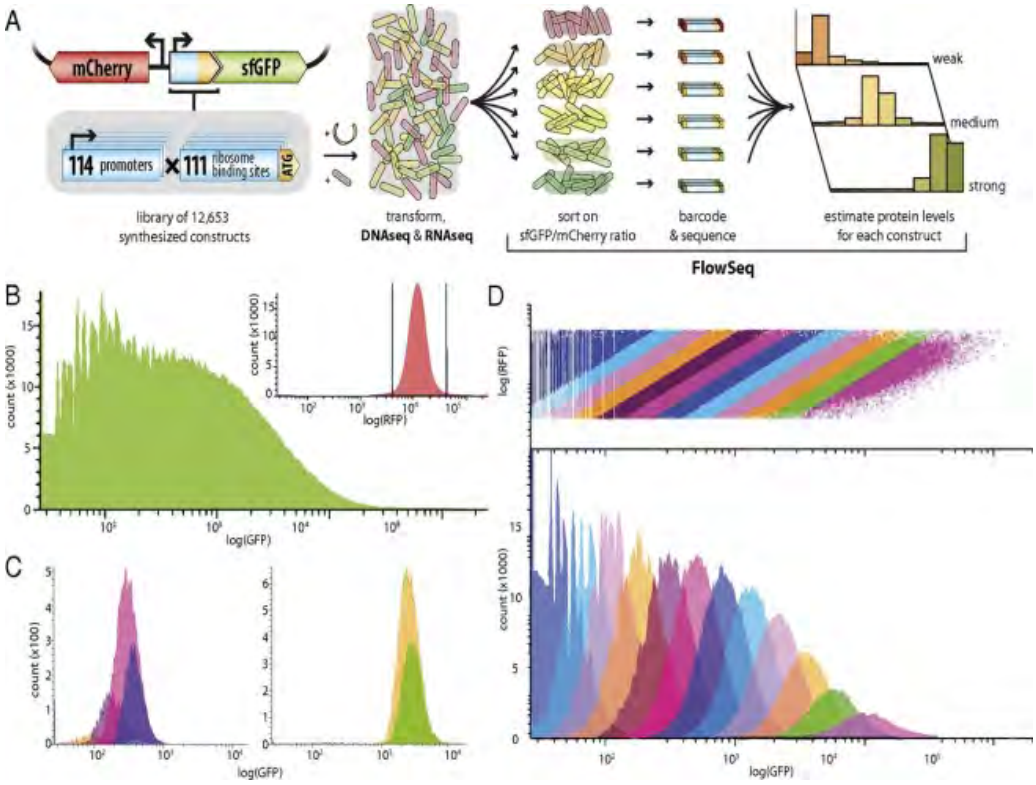

Figure 3: By simultaneously observing the activity of a massive library of regulatory elements, researchers can identify elements of the desired strength under any

The resulting treasure trove of data provides a resource for other researchers seeking to

achieve particular expression levels. The ease and scale this of approach indicates that rather than relying on prediction or standardization, synthetic libraries can be screened for desired behavior.

Composability of regulatory sequences controlling transcription and translation in *Escherichia coli*. Kosuri S, Goodman DB, Cambray G, Mutalik VK, Gao Y, Arkin AP, Endy D, Church GM. Proc Natl Acad Sci U S A. 2013 Aug 20;110(34):14024-9. doi: 10.1073/pnas.1301301110. Epub 2013 Aug 7. [29]

5.1.4 Genomically recoded organisms for increased safety and utility

How can we create a safer and more useful microorganism for synthetic biology? The canonical genetic code is nearly universal, allowing natural organisms to share beneficial traits via horizontal gene transfer. Genetically modified organisms also share this code, rendering them susceptible to viruses and capable of releasing recombinant genetic material [e.g., resistance genes] into the environment.

By redefining the genetic code as they described in Science, Synberc researchers Church and Kosuri hope to produce genomically recoded organisms that are safe and useful. In addition to being isolated from nature, radically altered genetic codes will also allow for genomically recoded organisms that have expanded chemical capabilities such as non-standard amino acids.

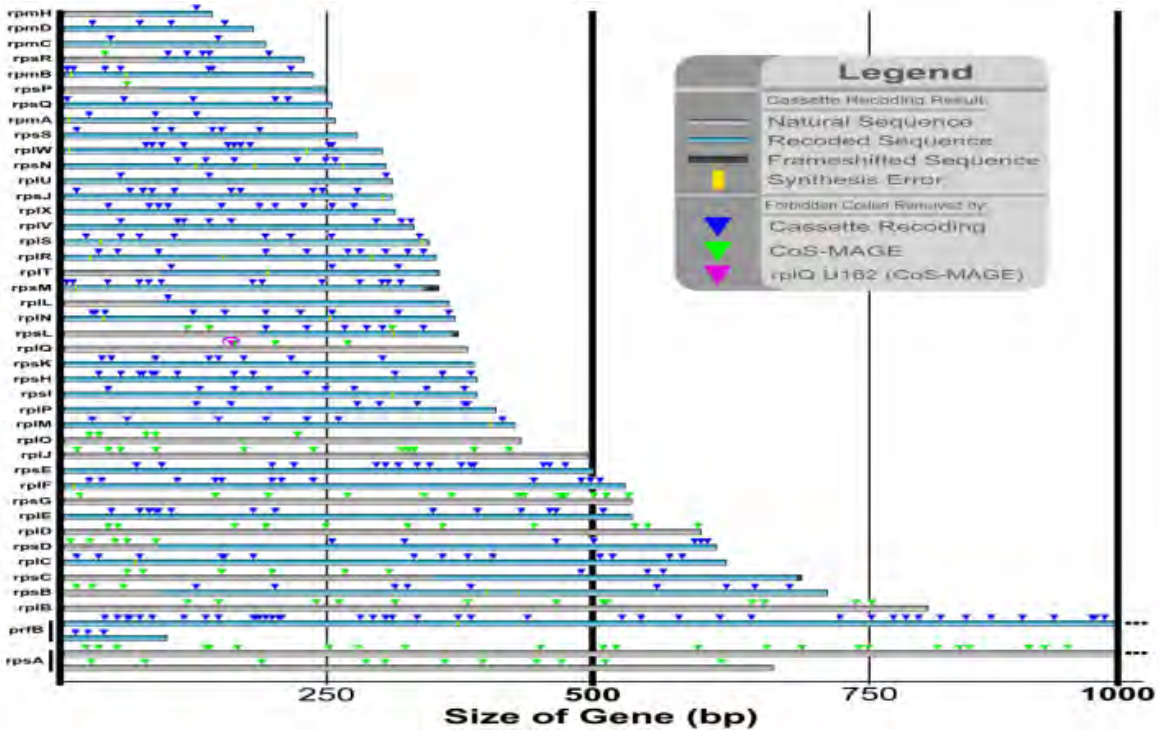

Figure 4: To assess the feasibility of radically altering the genetic code, the researchers selected a panel of 42 highly expressed essential genes for modification. Across 80 *Escherichia coli* strains, they removed all instances of 13 rare codons from these genes and attempted to shuffle all remaining codons. Their results suggest that the genome-wide removal of 13 codons is feasible; however, several genome design constraints were apparent, underscoring the importance of a strategy that rapidly prototypes and tests many designs in small pieces.

Probing the limits of genetic recoding in essential genes. Lajoie MJ, Kosuri S, Mosberg JA, Gregg CJ, Zhang D, Church GM. Science. 2013 Oct 18;342(6156):361-3. doi: 10.1126/science.1241460. [Lajoie, 2013 #919]

5.1.5 Channeling stress for greater industrial yield

Just as a factory can generate pollution and a health risk, microbes engineered to synthesize a useful product, such as a drug or biofuel, can create toxic intermediates on the way to the final product that can limit production.

Synberc researcher Jay Keasling and colleagues reported a novel solution to this problem in Nature Biotechnology: harnessing the natural stress mechanisms of *E. coli*. They screened the genome of *E. coli* and identified promoters that responded to toxic intermediates. Using such promoters to regulate pathway expression in response to the intermediates creates a link between the cell's metabolic state and the expression of the metabolic pathway.

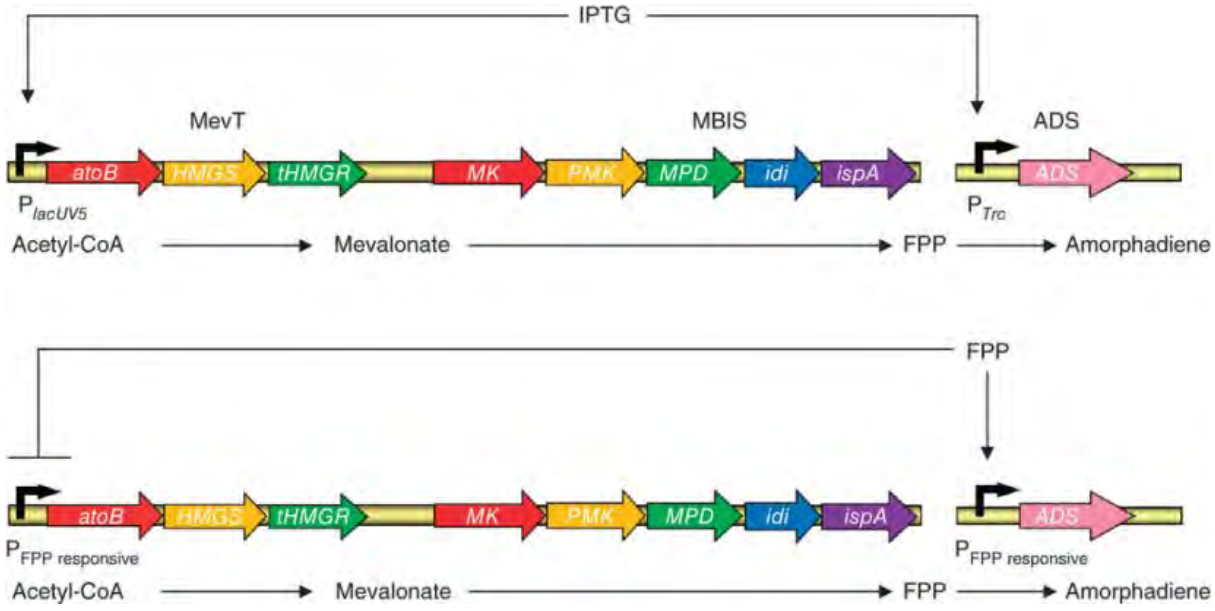

Figure 5. Top: regulation using inducible promoters. Bottom: regulation using the cell's response to heterologous pathway expression and metabolite (FPP here) toxicity.

This approach increased the titer of an isoprenoid by twofold over that achieved from inducible or constitutive promoters, eliminated the need for expensive inducers and improved growth.

Harnessing an organism's natural response machinery to regulate heterologous pathways in an inducer-free manner is a simple yet powerful solution to what has been a significant roadblock to an industrial application of synthetic biology.

Engineering dynamic pathway regulation using stress-response promoters. Dahl RH, Zhang F, Alonso-Gutierrez J, Baidoo E, Batth TS, Redding-Johanson AM, Petzold CJ, Mukhopadhyay A, Lee TS, Adams PD, Keasling JD. Nat Biotechnol. 2013 Nov;31(11):1039-46. doi: 10.1038/nbt.2689. Epub 2013 Oct 20. [Dahl, 2013 #920]

5.1.6 A data-driven approach to defining opportunities and risk in synthetic biology

Synthetic biology can be used to engineer metabolic pathways of an organism to produce a biochemical product. But how do we begin to chart the pathway we want to engineer? Synberc researcher J. Christopher Anderson and his Berkeley colleagues are developing a computational tool called the Act Synthesizer (http://act.berkeley.edu/synthesizer) that generates a plausible enzymatic pathway to the target biochemical product.

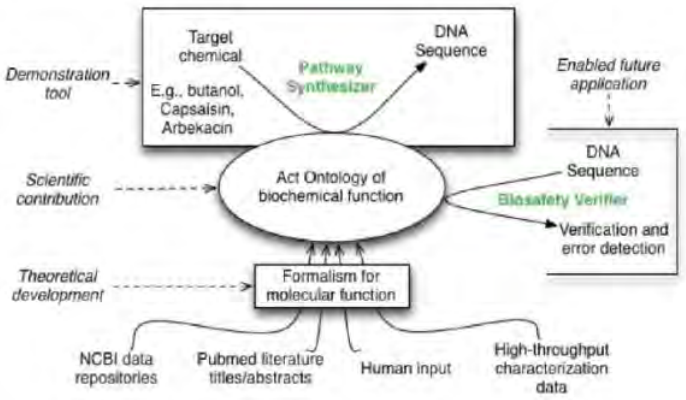

Figure 6: The Act Ontology is populated by data repository and literature mining. It also includes a function to detect biosafety issues.

The Act Synthesizer functions by using the Act Ontology, a structural framework for organizing biochemical knowledge. Anderson's team populates the Act ontology by aggregating enzymatic data across various sources and deriving biochemical rules from them. When provided a natural or unnatural chemical target, the Act Synthesizer can provide an enzymatic pathway to that product in a cell as well as alternate pathways.

Not only does the Act Synthesizer greatly accelerate the rational design of novel pathways, it has a very strict encoding of biosafety. It is prohibited from exploring known harmful patterns in chemicals and known harmful families.

5.1.7 Beyond Containment: Design, Testing and Demonstration for Biosafety

A team lead by Synberc Practices Investigator Ken Oye seeks to address biosafety risks before rather than after problems arise. It focuses on biosafety beyond containment, in cases where accidental release may be anticipated and/or where physical containment is not an option. This several workshops focused on assessment and governance of risks associated with a biosensor and early versions of the *rE.coli* chassis.

These Synberc and Sloan funded workshops resulted in a NSF grant to MIT and the Wilson Center to Develop a Synthetic Biology Research agenda to help realize potential ecological effects of synthetic biology.

The grant is supported jointly by three units within the NSF, the Division of Cellular and Molecular Biology, the Division of Environmental Biology and the Engineering Directorate. The grant will fund development of an interdisciplinary research agenda to improve understanding of potential ecological effects of commercial uses of synthetic biology. The research agenda will be developed through consultations amoung synthetic biologists, evolutionary biologists, ecologists and environmental scientists. It will be based on near- and medium term applications of synthetic biology, with scenarios based on the intentional and unintentional release of engineered organisms.

Todd Kuiken of the Wilson Center leads a discussion at JBEI on environmental release of engineered organisms.

5.2 *Education and training highlights*

5.2.1 Increasing diversity in synthetic biology: Synberc scholars

Initiated in 2014, the Synberc Scholars Program is designed to encourage greater diversity and participation of undergraduates in faculty labs across the ERC. Scholars are nominated by a Synberc PI or affiliate and must be from a group underrepresented in the sciences. Students perform research during the school year, and attend one Synberc retreat per year where they present a poster of their research results. In addition, each student will be paired with a graduate student at another campus who meets with them periodically to provide advice and mentorship. We are currently piloting the program with five Synberc Scholars at UC Berkeley and Arizona State University.

5.2.2 Creating education resources and infrastructure for synthetic biology with BioBuilder

Figure 7: Biobuilder creator Natalie Kuldell (center) and BioBuilder master teachers.

Directed by Synberc's Associate Education Director Natalie Kuldell, BioBuilder.org is a Synberc-sponsored program that develops and provides interactive, web-based classroom and laboratory lessons. Kuldell strives to ensure that these lessons are connected to real-world research questions and engage students in active learning through experiments. To complement this novel content, the organization provides teacher certification that enable educators to grow professionally and actively collaborate with a growing teaching community.

In 2013, 125 teachers received training in one-day and five-day workshops, in addition to 75 from the previous year- bringing the Biobuilder curriculum to over 200 classrooms around the country. To ease implementation of Biobuilder, Synberc provided seed funding for an online Biobuilder Guidebook, which will include workshop content, diagrams and charts, and teacher-written unit plans.

http://www.Biobuilder.org

5.2.3 Learning about the industrial path: Synberc IAB Mentoring program

The highly successful Industry Advisory Board (IAB)- Synberc Mentoring Program was launched in fall 2013 under the co-leadership of the Synberc Student Postdoc Association (SPA) and the Director of Industry Relations. In the initial round of applications, 31 graduate students and postdocs signed up to be mentored by 17 industry partners, including Dow Agrosciences, Amyris, Agilent, Ginkgo Bioworks, Dupont, BP, and Synthetic

Genomics. Through the program, students learn about the methodologies used by industry to assess research progress and gain insight into how research fits within a company's larger interests. Mentors use their knowledge of industry-based research to discuss students' research progress and help students to sharpen their professional skills. The understanding gained through the program enables students and postdocs to build strong relationships with industry leaders, and helps them make informed career choices as they prepare to leave the academic environment.

5.2.4 Incorporating biosafety and bioethics into online synthetic biology learning

In fall 2013, Synberc PIs Chris Anderson and Terry Johnson completed two online biosafety modules: Biological Risk Assessment, and Responsible Conduct in Synthetic Biology. The modules use video, content from the UC Berkeley Synthetic Biology Lab Course (BioEng 140L), materials created for iGEM, and methodology developed by Synberc's Practices Group to explain the technical, social, and ethical dimensions of biosafety, both real and perceived, and are now freely available through the Synberc website, U-Tube, and iGEM. In 2014, both modules will be incorporated into the Synthetic Biology Learning Trail. The modules fulfill the biosafety requirement for the Synthetic Biology Undergraduate Certificate program.
http://qb3.berkeley.edu/Synberc/courses-college landing page.html

5.2.5 LEAP: Synthetic Biology Leadership Excellence Accelerator Program

The details of the 2012 pilot Synthetic Biology Leadership Excellence Accelerator Program (LEAP) and the resulting white papers in a downloadable eBook were made public on the new LEAP website (http://synbioleap.org/). The site will be used to provide updates on past participants and also to document and share future meetings and whitepapers.

Figure 8: Pilot LEAP workshop participants Karmella Haynes (left) and Sarah Munroe (right).

Megan Palmer, the program's director, has secured a grant from the Alfred P. Sloan Foundation to develop a year-long accelerator program. The program will include the workshop as in the pilot but will also include seed-grants for promising projects that arise from the program. In addition developing a sponsorship strategy for the long-term sustainability of the program, the program will also extend participation internationally.

5.3 *Technology transfer highlights*

5.3.1 BioBuilder.org enters licensing agreement with VWR/Ward

Started in 2007 at MIT, BioBuilder was created under the direction of Natalie Kuldell in response to countless requests for synthetic biology learning materials from policy makers, environmental groups, and members of the media needing to know more about the basic biology involved, as well as scientists wanting to know more about engineering aspects of the field. This 2012, BioBuilder became an independent non-profit foundation.

This year Natalie Kuldell entered a licensing agreement with VWR/Ward to distribute kits to provide consumable and perishable components that teachers need to run biobuilder's investigative synthetic biology lab activities with students in secondary and post-secondary classrooms around the country.

5.3.2 Survey of enabling technologies in synthetic biology

In order to realize the full potential of synthetic biology, enabling technologies must be continually developed as well as policies and practices to ensure these technologies remain accessible for research. Monitoring the enabling technologies of synthetic biology will facilitate the systematic investigation of property rights coupled to these technologies and help shape policies and practices that impact the use, regulation, patenting, and licensing of these technologies.

Linda J. Kahl and Drew Endy conducted a survey among a self-identifying community of practitioners engaged in synthetic biology research to obtain their opinions and experiences with technologies that support the engineering of biological systems.

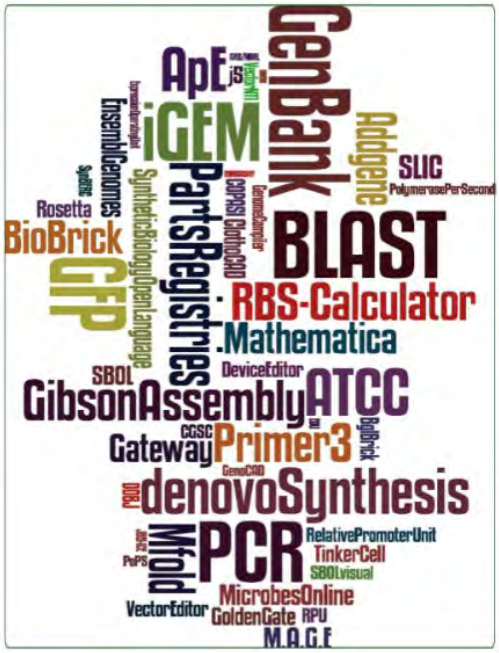

Figure 9: Word cloud of enabling technologies in synthetic biology.

The set of enabling technologies compiled from this survey provide insight into the many and varied technologies that support innovation in synthetic biology. Many of these technologies are widely accessible for use, either by virtue of being in the public domain or through legal tools such as non-exclusive licensing. Access to some patent protected technologies is less clear and use of these technologies may be subject to restrictions imposed by material transfer agreements or other contract terms.

A Survey of Enabling Technologies in Synthetic Biology. Linda J. Kahl and Drew Endy. J Biol Eng 2013; 7:13

5.4 *Infrastructure highlights*

5.4.1 Synberc sustainability

In order to sustain the work that Synberc and others have done to lead the field after NSF support ends in 2016, Synberc had received funding from the Sloan Foundation to conduct a one-year feasibility study and strategic planning process designed to support Synberc's evolution into a sustainable organization that meets the needs of the research community and its public stakeholders.

To carry out this effort, Synberc created a Sustainability/Business Planning Team composed of Synberc research and domain experts, industrial and strategic advisers, and external business and financial experts. The team was co-lead by Jay Keasling and Nancy J. Kelly & Associates. This team acted in concert to develop a feasibility study and strategic plan for the development of a sustainable organizational model for synthetic biology. As part of its existing NSF mandate, Synberc researchers and advisers provided a vision for the future of synthetic biology, to include not only technological aims but also an actionable strategy for earning the public support needed for synthetic biology to thrive.

Figure 10: Synberc members meet at MIT in September 2013 to kick-off the sustainability project with Nancy J. Kelley & Associates.

5.4.2 The Synberc Web of registries: Extending JBEI-ICE to the community

Synberc investigators at the Joint BioEnergy Institute reported the creation of new registry software, Joint BioEnergy Institute Inventory of Composable Elements (JBEI-ICE), to manage biological constructs. It is an information repository of plasmids, strains, part libraries and Arabidopsis seeds.

This work completes a specific aim of a NSF Cyberinfrastructure supplement to develop a distributed software platform to accelerate the sharing of composable biological elements (genes, plasmids, etc.) across the synthetic biology research community.

Figure 11: The primary activities of the Web of Registries project

JBEI-ICE's utility to the broad synthetic biology community lies in several features: open source software; it is independent of installation; it handles legacy parts that do not conform to a particular standard as well as having a high degree of interoperability with other software; development tools and third-party libraries used by ICE are carefully chosen for their open source licenses and permissive redistribution terms; and the development process is open to participation by the community.