The Fusion of Biology, Computer Science, and Engineering: Towards Efficient and Successful Synthetic Biology

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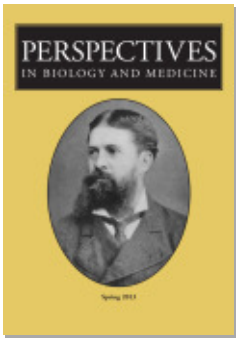

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The Fusion of Biology, Computer Science, and Engineering

towards efficient and successful synthetic biology

Gregory Linshiz,*† Alex Goldberg,‡ Tania Konry,‡ and Nathan J. Hillson*†

ABSTRACT Synthetic biology is a nascent field that emerged in earnest only around the turn of the millennium. It aims to engineer new biological systems and impart new biological functionality, often through genetic modifications. The design and construction of new biological systems is a complex, multistep process, requiring multidisciplinary collaborative efforts from "fusion" scientists who have formal training in computer science or engineering, as well as hands-on biological expertise. The public has high expectations for synthetic biology and eagerly anticipates the development of solutions to the major challenges facing humanity. This article discusses laboratory practices and the conduct of research in synthetic biology. It argues that the fusion science approach, which integrates biology with computer science and engineering best practices, including standardization, process optimization, computer-aided design and laboratory automation, miniaturization, and systematic management, will increase the predictability and reproducibility of experiments and lead to breakthroughs in the construction of new biological systems. The article also discusses several successful fusion

*Fuels Synthesis Division, Joint BioEnergy Institute, Emeryville, CA.
†Physical BioSciences Division, Lawrence Berkeley National Labs, Berkeley.
‡Center for Engineering in Medicine and Department of Surgery, Massachusetts General Hospital, Harvard Medical School, Boston.
Corresponding authors: Gregory Linshiz or Nathan J. Hillson, Joint BioEnergy Institute (JBEI), 5885 Hollis Street, 4th Floor, Emeryville, CA 94608.
E-mail: glinshiz@lbl.gov; njhillson@lbl.gov.

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projects, including the development of software tools for DNA construction design automation, recursive DNA construction, and the development of integrated microfluidics systems.

THE INTEGRATION OF computer science, biology, and engineering has resulted in the emergence of rapidly growing interdisciplinary fields such as bioinformatics, bioengineering, DNA computing, and systems and synthetic biology. Ideas derived from computer science and engineering can provide innovative solutions to biological problems and advance research in new directions. Although interdisciplinary research has become increasingly prevalent in recent years, the scientists contributing to these efforts largely remain specialists in their original disciplines and are not fully capable of covering the many facets of multidisciplinary problems, which impedes the development of truly integrated solutions. It would be beneficial for the scientists working in a multidisciplinary life sciences environment to have hands-on biological experience as well as formal training in computer science or engineering. These "fusion" scientists would be capable of comprehensively analyzing biological systems and would have a deep understanding of interdisciplinary approaches, their integration, and efficient implementation. Fusion scientists would be able to bridge communication gaps between the various research fields and lead interdisciplinary projects. Modern physics, which has fully integrated physics, mathematics, and engineering, presents a successful historical example of fusion science.

A significant obstacle to putting the fusion science approach in to practice is that it is currently difficult to implement a "fusion" scientist training program. Today, few educational institutions offer what could be considered fusion biology training programs. Given that educational institutions have limited amounts of time to train students, the challenge is to develop curricula that provide concomitant depth and breadth. Without breadth, there is no fusion; without depth, it is difficult for trainees to make important new contributions. While there may not be a straightforward means to effectively execute a fusion science training program at present, it will become easier to do so as fusion scientists themselves begin to train students, and as training curricula are no longer forcibly split between different academic departments.

SYNTHETIC BIOLOGY AS FUSION SCIENCE

The development of synthetic biology is an attempt at fusion science. Synthetic biology explores nature not only through observation and systematization, but also through the construction and experimental assessment of new biological systems, such as metabolic pathways, genetic control circuitry, and even entire organisms (Collins 2012; Khalil and Collins 2010). To pursue new complex biological systems in the most effective manner possible, thorough system analysis

should be conducted to present complex problems as hierarchies of abstract layers, and all system components and processes should be standardized, well described, and readily available (Ho-Shing et al. 2012). The standardization of biological processes and components itself enables multiple hierarchical levels of system abstraction (Heinemann and Panke 2006; Müller and Arndt 2012). Such abstraction enables the organization of biological systems in a manner similar to the object-oriented approach taken in computer science. These structured representations of biological systems facilitate the development of laboratory and data management platforms for conducting biological research in a more efficient, methodical, and organized way.

The construction of new biological systems often requires the reprogramming of genetic information and thereby extensive de novo DNA synthesis (Bashor et al. 2010). A deep understanding of a biological system's environmental interactions (i.e., input and output), as well as its intracellular processes is required to determine which genetic modifications are necessary to achieve a given behavior (Gendrault et al. 2011). Computer science can assist in building models to describe these interactions and processes, and the models can be utilized to predict the sets of genetic modifications to be achieved by DNA construction (Shuler, Foley, and Atlas 2012).

DNA Construction Methods

DNA construction technology is at the core of synthetic biology, and there have been several significant breakthroughs over the past few years. Decreases in DNA oligonucleotide synthesis prices along with the development of new DNA assembly technologies, have led to trends in decreasing DNA construction costs (Figure 1A) and increasing DNA molecule lengths (Figure 1B; Carlson 2009, 2011; Ellis, Adie, and Baldwin 2011; Hillson 2011; Mueller, Coleman, and Wimmer 2009). The first synthetic DNA, constructed in 1979, consisted of 207 nucleotides (Khorana 1979). In 2008, the first synthetic microbial genome was reported, consisting of 1.08-Mbp (Gibson et al. 2010).

All modern DNA construction methods assemble small fragments into larger DNA molecules. De novo DNA synthesis begins with a set of short single-stranded DNA oligonucleotides, which are then assembled together into larger double-stranded DNA fragments via ligase chain reaction (LCR) or assembly PCR (Au et al. 1998; Ma, Tang, and Tian 2012; Xiong et al. 2006). Synthetic and natural DNA fragments can then be assembled together with a variety of methods to yield even longer DNA molecules. Several in vitro DNA assembly methods, including BioBricks, BglBricks, and type IIs endonuclease methods (e.g., Golden Gate, MoClo, and GoldenBraid), employ standardized restriction enzyme protocols (Anderson et al. 2010; Engler et al. 2009; Sarrion-Perdigones et al. 2011; Shetty, Endy, and Knight 2008; Werner et al. 2012). A hierarchical method for recursive DNA construction from oligonucleotides and natural fragments that utilizes a divide-and-conquer (D&C) approach is capable of assem-

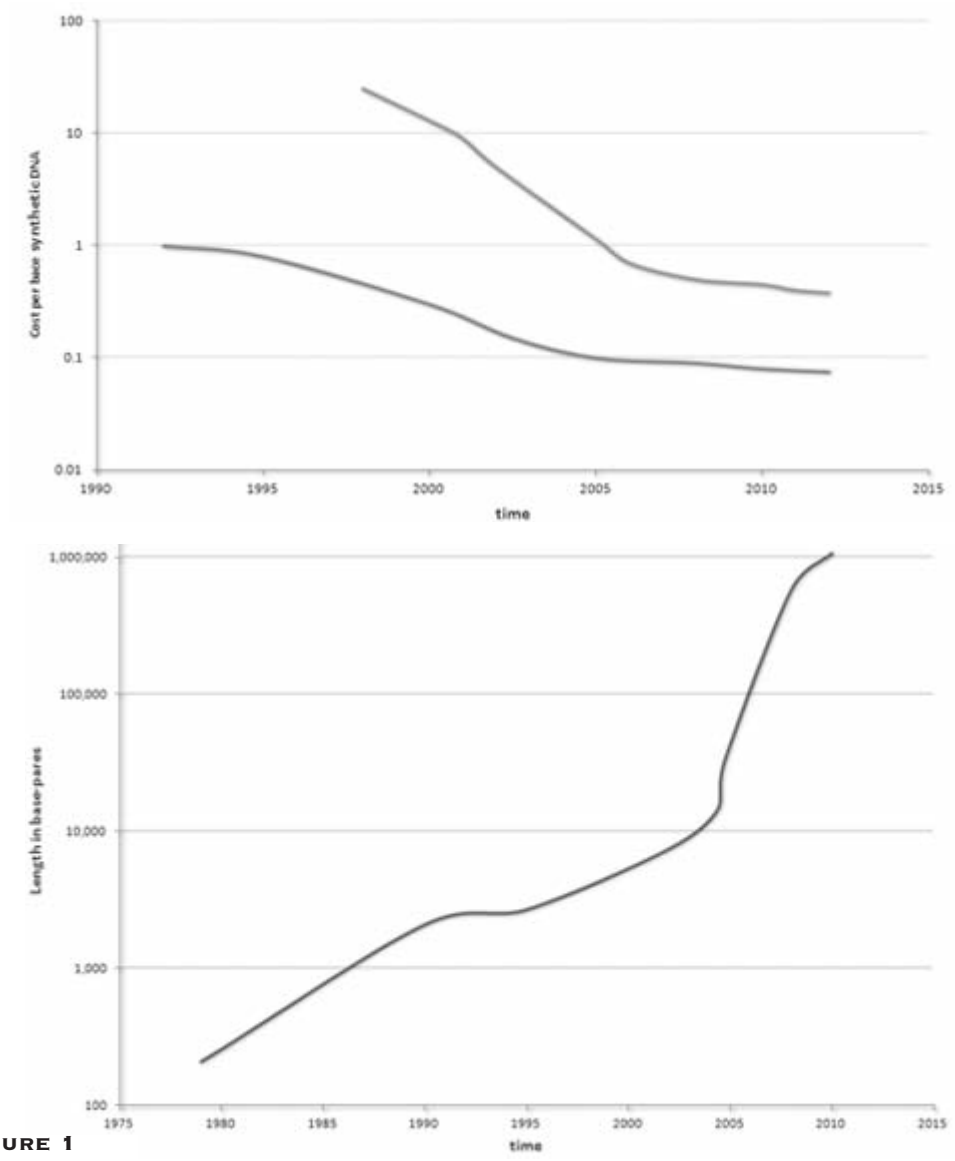

FIGURE 1

*Trends in synthetic DNA price and length. (**Top**) Per base DNA oligonucleotide (lower line) and per base-pair synthetic DNA (upper line) prices. (**Bottom**) Longest published synthetic DNA lengths.*

SOURCE: BAKER 2011; CARLSON 2009, 2011.

bling long error-free DNA molecules from error-prone fragments (Ben Yehezkel et al. 2008; Linshiz et al. 2008). Flanking homology sequence overlap DNA assembly methods, such as the in vitro In-Fusion, SLIC, CPEC, SLiCE, and Gibson methods, as well as the in vivo yeast and *Bacillus* DNA assembly methods, are fairly sequence-independent and capable of constructing DNA molecules as long as an entire microbial genome (Ellis, Adie, and Baldwin, 2011; Gibson 2009; Hillson 2011; Irwin et al. 2012; Li and Elledge 2012; Ohtani et al. 2012; Quan and Tian 2011; Shao, Zhao, and Zhao 2009; Zhang, Werling, and Edelmann 2012).

Continued innovation of DNA construction technology, both methodology and software design automation tools, is required to increase the profitability of the synthetic biology products.

SYNTHETIC BIOLOGY: HYPE AND EXPECTATIONS

The global synthetic biology market totaled over $1.6 billion in 2011 and is expected to reach $10.8 billion by 2016, increasing at a compound annual growth rate (CAGR) of 45.8% (BCC Research 2011). The synthetic biology market can be broken down into three segments: enabling technologies, core products, and enabled products. Enabling technologies drive the development of the synthetic biology industry, while core products, including standardized DNA parts, synthetic genes, and chassis organisms, form the basis of the cellular factories and systems that enable production. Enabled products, including pharmaceuticals, diagnostic tools, chemicals, biofuels, and agricultural products, target large downstream market opportunities. Since each of these downstream markets is annually worth tens of billions of dollars, there are optimistic expectations for the CAGR of synthetic biology.

The Gartner hype cycle characterizes the progressive developmental stages that each nascent technology passes through, from emergence to adoption (Figure 2). Synthetic biology began to emerge in earnest around the turn of the millennium. Expectations for the success of the synthetic biology approach are currently very high, especially after the publication of the synthesis of first synthetic cell genome and the construction of a synthetic pathway for the production of a precursor to the anti-malarial drug artemisinin (Baker 2011; Gibson et al. 2010; Ro et al. 2006). Synthetic biology has recently achieved high visibility in the popular media, and the development of successful synthetic biology applications capable of solving health-care problems and providing solutions for biofuel production and other major challenges are eagerly anticipated (Weber and Fussenegger 2012). According to the Gartner hype cycle, successful synthetic biology applications should appear in the coming years, but not before a period of public disillusionment. To ensure that synthetic biology successfully matures to its plateau of productivity, we suggest in the following sections a framework for the development of synthetic biology based on the fusion science approach.

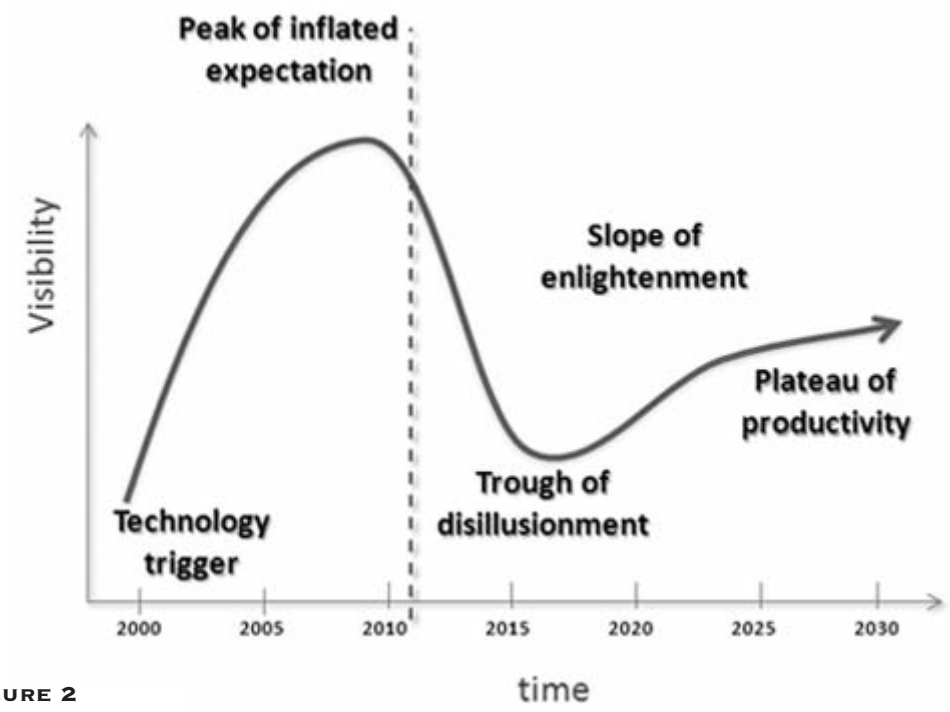

Figure 2

Gartner hype cycle for synthetic biology.

Laboratory and Project Management

Scientific Information Cloud

Modern research requires multi-level project management and the accurate recording of protocols and results (Prilusky et al. 2005; Vu et al. 2012). Inaccurate project management and imprecise protocol recording lead to experimental data loss, irreproducible experiments, production of redundant data, and a lack of scientific transparency. Pharmaceutical companies and scientists from academia report that more than half of academic research is not reproducible (Begley and Ellis 2012; Ioannidis et al. 2009; Mullard 2011).

As an overall solution, we envision the development of a "scientific information cloud" for the management and documentation of research projects. This system would allow scientists to define projects at a high level of abstraction and would facilitate the division of projects into subtasks assigned to team members. Group leaders and lab members would define which protocols would be used at each step of the project according to project analysis, available technical resources, and prior experience. All protocols would be stored in the system, and each change in a protocol tracked by version control. Results from each experiment would be stored in the database and linked to the exact protocol that has produced the results. Security policies would govern which people have access

to which scientific data, allowing for quick online data access, efficient collaboration, and research transparency. The system would produce reports linked to primary data, enhancing manuscript preparation efficiency and scientific reliability, while reducing occurrences of irreproducible experiments. Manuscripts would provide links to the scientific information cloud for public access to primary data and protocols, contributing to the standardization of experimental protocols, laboratory device input/output, and data representation. The scientific information cloud would gradually emerge as a scientific social network and a comprehensive solution for concentrating scientific knowledge.

Communication Tools

The success of fusion science depends on efficient interdisciplinary communication, which may require the development of new expressive languages. Examples of lingua franca that provide communication bridges between biology and computer science include script-based and graphical design tools for combinatorial DNA libraries, such as the Eugene (any resemblance to *eugenics* is purely coincidental) biological design specification language and the DeviceEditor visual biological CAD canvas (Bilitchenko et al. 2011; Chen et al. 2012). Both Eugene and DeviceEditor enable abstract, high-level definitions of DNA constructs and provide easy, compact, and flexible means of representing combinatorial DNA libraries, making the logic and rationale behind the DNA library easy to understand. Not only does a defining DNA library with Eugene or DeviceEditor serve as a communication tool that helps computer scientists better understand the biologists' needs, it also provides a platform for more inspired and creative biological thinking.

Laboratory Evolution: Automation and Scaling Down

Robot Programming Language

The development of biology-friendly robotic platforms and software tools is a crucial step towards laboratory process automation. The current lack of such tools is a major obstacle for the modernization of the life sciences. Many well-funded research laboratories are equipped with liquid-handling robots that aim to accelerate research, save time, and provide high-throughput solutions. While proudly shown to visitors during laboratory tours, these robots frequently remain underutilized with very low duty cycles. A simple explanation for this is that it often requires a researcher more effort and time to instruct a robot to perform a new task than the robot saves the researcher in performing the task. In other words, it is a net loss for the researcher to use the robot. Since liquid-handling robotic companies have largely targeted the lucrative, highly repetitive industrial operations market, there has been little effort devoted to developing easy-to-use programming tools targeted at dynamic (non-repetitive) research environments.

To help close this gap, we are developing a new biology-friendly high-level language in our laboratory. The syntax and compiler for the language are based on computer science principles and a deep understanding of biological needs. Our language allows researchers to use liquid-handling robots effectively, enabling a plethora of new experiments that would not have been considered previously. After minimal training, a biologist can independently write relatively complicated protocols for a robot within a half an hour. This is a good example of how biology-friendly programming languages can give a real boost to research and open new horizons for innovative scientific directions.

From Macro to Micro

Liquid-handling robots, though a good step towards laboratory automation, are expensive and have large footprints, and they are well outside of the budgetary reach of many laboratories. Furthermore, the minimum accurate pipetting volumes for these robots (frequently 2–5 μL) result in the inefficient utilization of costly reagents. Performing automated laboratory operations on small scales and increasing experiments throughput, using miniaturized microfluidic lab-on-a-chip (LOC) devices, is the next step forward in biotechnology (Mark et al. 2010).

Microfluidic devices integrate and scale down laboratory processes to a microchip format by allowing fluid manipulation in tiny channels and micro reactors instead of traditional test tubes. Microfluidic devices are used in a wide array of biological and analytical applications, including rapid pathogen detection, clinical diagnostics, forensic science, electrophoresis, flow cytometry, blood chemistry analysis, and protein and DNA analysis (Gulati et al. 2009; Haeberle and Zengerle 2007). Furthermore, LOC has the potential to offer point-of-care diagnostic abilities that could revolutionize medicine (Chin, Linder, and Sia 2007). The fusion of microfluidic technology, computer science, and synthetic biology should lead to the development of new applications, such as DNA construction and high-throughput screening within a single microfluidic device. The design and fabrication of microfluidic systems is a complex task that requires a multidisciplinary approach. Computer-aided design is critical in the development of successful microfluidic devices, facilitating the design of optimal fluidic channel topology, modeling fluid flow, controlling reagent and information flow, and supporting data collection and processing (Amarasinghe, Amin, and Thies 2009; Chakrabarty and Zeng 2006; Mark et al. 2010; Vangelooven and Desmet 2010).

From Micro to Nano

Although microfluidics itself is a recent and advanced technology, the next step would be to take biological research to the nano level (Jain 2003; Kumar 2010). Nanotechnology refers to understanding and control of matter at the atomic, molecular or macromolecular levels, at the length scale of approximately 1–100 nanometers. Until recently, nanotechnology concentrated almost entirely

on electronics, computers, telecommunications, and materials manufacture. Now, biomedical nanotechnology has emerged, in which bioengineers construct tiny nano-scale bio-structures that combine inorganic and biological materials (Hurst 2011). Recently very interesting and complex nano-scale structures have been built from biopolymers, such as DNA molecules and proteins (Yeates 2011; Zhang et al. 2011). The manipulation of biological materials on the nano-scale level opens new perspectives for the creation of new biosensor applications, measurements of single molecule–level biochemical reactions, high-throughput DNA sequencing, and the construction of new nano biomaterials with unique properties (Kumar et al. 2011).

Standardization

The standardization of components and processes has led to major advances in mature engineering disciplines such as mechanical and electrical engineering. The capacity to quickly and reliably engineer multi-component systems from libraries of standardized interchangeable parts is a hallmark of modern technologies. However, to a great extent, the design and construction of engineered biological systems remains an ad hoc process for which costs, completion times, and probabilities of success are difficult to estimate accurately. The standardization of protocols, tools, biological parts, and processes management is a basic principle of synthetic biology (de Lorenzo 2010; Müller and Arndt 2012).

The standardization of biological protocols would help produce more reliable and reproducible experiments. Ideally, experiments would be performed using standardized labware, so that each experiment could be conducted with the same (or at least comparable) devices across different laboratories. The protocols would use standardized input (definition of experiments) and output (the results) formats that reference standard lab devices. These protocols would evolve until ultimately only the most successful protocols, validated across laboratories, are accepted as community standards.

Another crucial step is the standardization of computational tools, as well as data processing, storage, and representation. The Synthetic Biology Open Language (SBOL) is a major community-based initiative in this direction that seeks to standardize not only the data representation but also the visual depiction of biological components (Galdzicki et al. 2011). For monitoring and managing research projects, the development of a multi-level transparent system, such as the scientific information cloud described above, is required.

Reuse of standardized biological parts, such as promoters, ribosome binding sides, coding regions, and terminators, is another very important component of standardization. These biological parts are informatically as well as physically deposited in, and accessible from, community repositories such as the Registry of Standardized Parts, the JBEI-ICE repository platform, the DNASU plasmid repository, and AddGene (Cormier et al. 2010; Ham et al. 2012; Herscovitch et al.

2012; Müller and Arndt 2012). The standardization of biological parts enables the rapid and inexpensive construction of sophisticated pathways and new biological systems.

Standardization facilitates the description and annotation of these new biological systems. Furthermore, since the behavior of each standardized system component is known from previous experimental data, standardization also enables the simulation of new biological systems and pathways. Reliable in silico simulation, a key portion of the engineering design-build-test cycle, promises to reduce the amount of requisite biological wet lab experiments, thereby lowering the cost of new biological systems development, as experiments are much more expensive than simulation.

System Optimization

One of the major goals of synthetic biology is to optimize biological application development, reducing the time and cost to product. Even with standardized tools for engineering new biological systems, the time to reaching functionality (the time to product) is very long, since reprogramming and debugging biological systems is a complicated and resource-intensive process (Figure 3A). Engineering disciplines widely utilize iterative approaches for system optimization, and there is a broad range of iterative algorithms that help solve challenging optimization problems. The adoption of an iterative approach in synthetic biology would help reduce the time to product and make the development of biological systems fast, inexpensive, and robust (FIRst; Figure 3B).

The *iterative protein design cycle* applies the iterative approach to the optimization of biological systems. This method utilizes efficient automated DNA construction and high-throughput screening and identifies promising bio-catalysts through an iterative semi-rational design and directed evolution process (Figure 4). Each iteration includes four stages: the design of the DNA library, the construction of the DNA molecules, the expression and screening of the proteins, and the evaluation of the results. At the stage of DNA synthesis, the hierarchical structure of synthesis methods enables the creation of granular combinatorial libraries that contribute to efficient protein optimization. The main power of the approach lies in the reuse of DNA sequence parts, created in previous rounds, for the construction of the sequences required in the next iteration of the cycle. This significantly reduces the cost and time of the DNA construction phase. Screening rationally designed libraries can dramatically reduce the number of selection rounds and the number of activity assays in each round, which are a major cost in the development of new enzymatic properties.

In the iterative semi-rational approach, the identification of candidate proteins is initially derived from multiple sequence alignments (producing a set of putative permissive mutations) and from protein structure insights. The results from

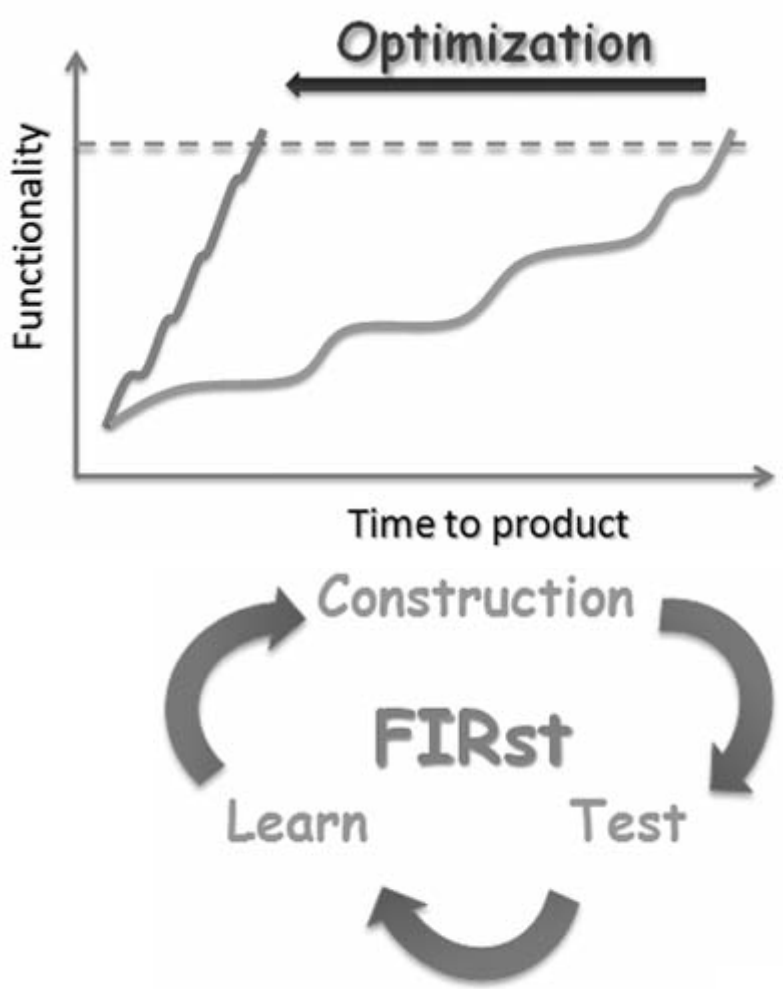

Figure 3

Time to product and the adoption of the fast, inexpensive, and robust (FIRst) iterative approach in synthetic biology.

each round of experiments progressively lead to a better understanding of the connection between sequence, protein structure, and function. These results feed in to the design of the next round of candidates, according to a scoring function that maps sequence to a protein function and optimization algorithms. After a few rounds of rational design, random mutagenesis is performed on the most promising candidates. This provides a sampling of the sequence landscape surrounding the promising candidates, adding genetic diversity for next iteration.

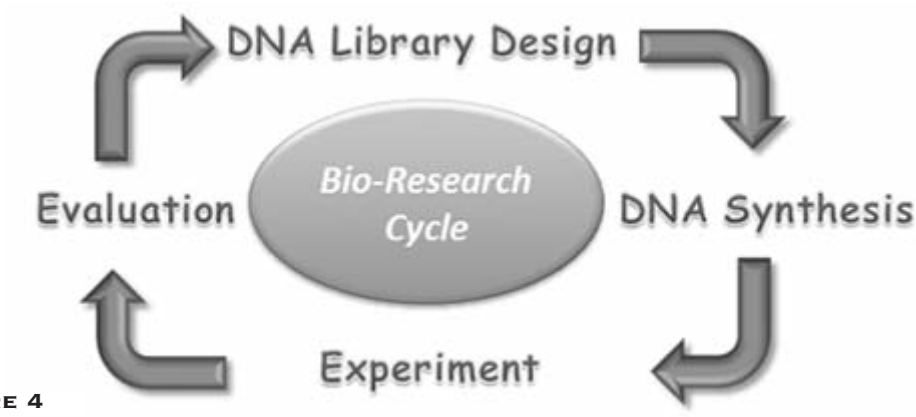

FIGURE 4

Iterative bio-research cycle.

EXAMPLES OF FUSION SCIENCE PROJECTS

Recursive DNA Construction

Recursive DNA construction, integrating ideas from biology, computer science, engineering, and robotics, is a striking example of a Fusion project. This method utilizes a divide-and-conquer (D&C) approach, which is a widely used algorithm design paradigm in computer science, often implemented using recursions (Ben Yehezkel et al. 2008; Linshiz et al. 2008). We have proposed using the D&C approach for construction of complex error-free objects from the error-prone components in a biochemical system. This DNA synthesis technology employs a biochemical protocol to enable in vitro synthesis and error correction of long DNA molecules in an automated manner, and can accurately produce large rationally designed combinatorial DNA libraries with pre-specified combinations of interest (Linshiz, Ben Yehezkel, and Shapiro 2012). The D&C DNA construction technology is based on the computer-aided design (CAD) and manufacturing (CAM) of DNA molecules, making new use of information technologies to accelerate progress and bolster efficiency in key areas of research and development.

In some respects, genetic programming is akin to computer programming. However, unlike the straightforward composition of computer programs with text editors, the design, construction, and editing of DNA in a programmatic fashion remains a slow, expensive, and labor-intensive process (Shabi et al. 2010). The vision of CAD/CAM, applied to DNA construction in particular and to synthetic biology in general, is to replace labor-intensive manual laboratory operations, which are today carried out by thousands of skilled lab workers around the world, with efficient laboratory management, computational design, high-throughput process automation, and optimization (Ben Yehezkel et al. 2011). In the semiconductor industry, the analogous approach has enabled a rev-

olution in computers, internet, and telecommunication, and we anticipate a similar effect on biology and biotechnology.

Software Tools for DNA Construction Design

In addition to experimental DNA construction methodology development, there are active research efforts into the development of software tools that automate the design and robotic execution of optimal DNA construction protocols for these newly emerging techniques. Algorithms that optimize the reuse of BglBrick assembly intermediates have been coupled with liquid-handling robotics to execute the resulting protocols (Densmore et al. 2010; Leguia et al. 2011). Complementary algorithms have been developed to design cost-optimal (leveraging DNA synthesis when cost-effective to do so) scar-less combinatorial type IIs endonuclease and flanking homology sequence overlap DNA assembly methods, which can similarly be executed with robotics platforms (Hillson, Rosengarten, and Keasling 2012). Beyond saving researcher effort and DNA construction costs, the further development of these software design automation tools will be crucial for scaling up DNA construction efforts beyond that possible with manual protocol design alone. Genomic-scale DNA design and construction software tools have recently enabled the report of the first partially synthetic eukaryotic (yeast) chromosome (Dymond et al. 2011; Richardson et al. 2012).

Microfluidics Implementation: An Integrated Microfluidics System

Microfluidic devices offer powerful techniques for cell interrogation with rapid processing speeds and low cost. These techniques can be combined with monoclonal antibodies (mAb) developed against cellular markers to identify and capture cells with high specificity, and with a highly specific and sensitive screening method for the detection of protein markers based on rolling circle amplification (RCA) to understand cellular heterogeneity (Konry et al. 2011a, 2011b).

The use of nano-liter reaction volumes and parallel sample processing offered by droplet-based microfluidic devices make them ideally suited for total chemical and bioassay analyses, ultra-high throughput screening applications, and other cases where samples and reagents are available in limited quantities. Single-cell droplet technology provides a cost-effective method to gain sequence information and protein expression from individual cells that have been sorted for phenotype. Thus, both phenotypic and genomic information can be obtained from droplet-encapsulated individual cells. For cell encapsulation, droplet microfluidics uses a two-phase system in which live cells and assay reagents can be compartmentalized in an aqueous microdroplet (of 1 pL to 10 nL in size) surrounded by immiscible oil.

The advantages of this droplets-based technique include the physical and chemical isolation of droplets eliminating the risk of cross-contamination, the fast and efficient mixing of the reagents within droplets, the ability to digitally

manipulate droplets at a very high throughput, and the ability to incubate stable droplets off-chip and reintroduce them into the microfluidic environment for further processing and analysis. Using a custom-designed optical system for interrogation of fluorescent signal within the droplets, one can then determine the secretion pattern in the nanoliter droplets in a time-dependent fashion and sort the cells that secrete specific molecules to establish the heterogeneity in the population. Thus, live-cell secretion and surface monitoring can be carried out in distinct microenvironments, utilizing a microfluidic approach merged with microsphere sensors. Previously, this was only possible using complicated and multi-step in vitro and in vivo live-cell microscopy, combined with immunological studies of the secretion outcomes of cellular interactions. This system can also operate as a droplet-based fluorescence-activated cell sorting (FACS), interrogating the entire reaction volume and sorting cells based on the results. However, unlike a traditional FACS, the cells remain encapsulated in droplets and therefore can be identified individually post-sorting.

CONCLUSION

Over the course of human history, scientific progress has taken a path of increasing specialization. To explore nature, scientists have frequently utilized a reductionist approach, breaking the world into smaller and smaller fragments. As a result, distinct scientific disciplines, concentrated on specific aspects of nature, have emerged. Recently, there has been a powerful resurgence of integrative multidisciplinary research, in which experts from different fields work together on common projects. However, if scientists remain within the boundaries of their own disciplines, this greatly constrains progress.

The fusion science approach moves beyond simple collaboration and attempts to integrate concepts from multiple disciplines. Fusion science research requires that researchers gain a depth of understanding in multiple disciplines and become fluent in their disparate languages and technologies. Fusion scientists break down traditional disciplinary barriers and would be able to bridge communication gaps between the various research fields and lead interdisciplinary projects.

The development of synthetic biology is an attempt in fusion science, accomplished by blurring traditional lines between biology, computer science, and engineering. The adoption of mature engineering discipline best practices, such as laboratory management, automation, standardization, system analysis, and optimization, has contributed to synthetic biology becoming an efficient and successful fusion science, enabling scientists from different fields to join efforts and collaborate fruitfully on the development of novel creative solutions for the construction of new biological systems.

References

Amarasinghe, S., N. Amin, and W. Thies. 2009. Computer-aided design for microfluidic chips based on multilayer soft lithography. In *Proceedings of the 2009 IEEE International Conference on Computer Design*. Lake Tahoe: Institute of Electrical and Electronics Engineers.

Anderson, J. C., et al. 2010. BglBricks: A flexible standard for biological part assembly. *J Biol Eng* 4(1):1.

Au, L. C., et al. 1998. Gene synthesis by a LCR-based approach: High-level production of leptin-L54 using synthetic gene in Escherichia coli. *Biochem Biophys Res Commun* 248(1):200–203.

Baker, M. 2011. Synthetic genomes: The next step for the synthetic genome. *Nature* 473(7347):403, 405–8.

Bashor, C. J., et al. 2010. Rewiring cells: Synthetic biology as a tool to interrogate the organizational principles of living systems. *Annu Rev Biophys* 39:515–37.

BCC Research. 2011. Synthetic biology: Emerging global markets. *BCC Research* (report code BIO066B).

Begley, C. G., and L. M. Ellis. 2012. Drug development: Raise standards for preclinical cancer research. *Nature* 483(7391):531–33.

Ben Yehezkel, T., et al. 2008. De novo DNA synthesis using single molecule PCR. *Nucleic Acids Res* 36(17):e107.

Ben Yehezkel, T., et al. 2011. Computer-aided high-throughput cloning of bacteria in liquid medium. *BioTechniques* 50(2):124–7.

Bilitchenko, L., et al. 2011. Eugene: A domain specific language for specifying and constraining synthetic biological parts, devices, and systems. Ed. Diego Di Bernardo. *PloS One* 6(4):e18882.

Carlson, R. 2009. The hanging economics of DNA synthesis. *Nat Biotechnol* 27(12): 1091–94.

Carlson, R. 2011. Cost per base of synthetic DNA2. http://www.synthesis.cc/assets_c/2011/06/carlson_synthesis_cost_per_base_june_2011.html.

Chakrabarty, K., and J. Zeng. 2006. Design automation methods and tools for microfluidics-based biochips. *ACM J Emerg Technol Comput Syst* 1(3):403.

Chen, J., et al. 2012. DeviceEditor visual biological CAD canvas. *J Biol Eng* 6(1):1.

Chin, C. D., V. Linder, and S. K. Sia. 2007. Lab-on-a-chip devices for global health: Past studies and future opportunities. *Lab Chip* 7(1):41–57.

Collins, J. 2012. Synthetic biology: Bits and pieces come to life. *Nature* 483(7387):S8–S10.

Cormier, C. Y., et al. 2010. Protein structure initiative material repository: An open shared public resource of structural genomics plasmids for the biological community. *Nucleic Acids Res* 38(database issue):D743–D749.

de Lorenzo, V. 2010. Synthetic biology: Something old, something new. *BioEssays* 32(4): 267–70.

Densmore, D., et al. 2010. Algorithms for automated DNA assembly. *Nucleic Acids Res* 38(8):2607–16.

Dymond, J. S., et al. 2011. Synthetic chromosome arms function in yeast and generate phenotypic diversity by design. *Nature* 477(7365):471–76.

Ellis, T., T. Adie, and G. S. Baldwin. 2011. DNA assembly for synthetic biology: From parts to pathways and beyond. *Integr Biol* 3(2):109–18.

Engler, C., et al. 2009. Golden Gate shuffling: A one-pot DNA shuffling method based on type IIs restriction enzymes. *PloS One* 4(5):e5553.

Galdzicki, M., et al. 2011. Standard biological parts knowledgebase. *PloS One* 6(2): e17005.

Gendrault, Y., et al. 2011. Computer-aided design in synthetic biology. In *Proceedings of the 4th International Symposium on Applied Sciences in Biomedical and Communication Technologies—ISABEL '11*, 1–7. New York: ACM Press.

Gibson, D. G. 2009. Synthesis of DNA fragments in yeast by one-step assembly of overlapping oligonucleotides. *Nucleic Acids Res* 37(20):6984–90.

Gibson, D. G., et al. 2010. Creation of a bacterial cell controlled by a chemically synthesized genome. *Science* 329(5987):52–56.

Gulati, S., et al. 2009. Opportunities for microfluidic technologies in synthetic biology. *J R Soc Interface* 6(suppl. 4):S493–S506.

Haeberle, S., and R. Zengerle. 2007. Microfluidic platforms for lab-on-a-chip applications. *Lab Chip* 7(9):1094–110.

Ham, T. S., et al. 2012. Design, implementation and practice of JBEI-ICE: An open source biological part registry platform and tools. *Nucleic Acids Res* 40(18):e141.

Heinemann, M., and S. Panke. 2006. Synthetic biology: Putting engineering into biology. *Bioinformatics* 22(22):2790–99.

Herscovitch, M., et al. 2012. Addgene provides an open forum for plasmid sharing. *Nat Biotechnol* 30(4):316–17.

Hillson, N. 2011. DNA assembly method standardization for synthetic biomolecular circuits and systems. In *Design and analysis of biomolecular circuits: Engineering approaches to systems and synthetic biology*, 295–20. Dordrecht: Springer-Verlag.

Hillson, N. J., R. D. Rosengarten, and J. D. Keasling. 2012. J5 DNA assembly design automation software. *ACS Synth Biol* 1(1):14–21.

Ho-Shing, O., et al. 2012. Assembly of standardized DNA parts using BioBrick ends in E. coli. *Methods Mol Biol* 852:61–76.

Hurst, S. J. 2011. Biomedical nanotechnology. *Methods Mol Biol* 726:1–13.

Ioannidis, J. P. A., et al. 2009. Repeatability of published microarray gene expression analyses. *Nat Genet* 41(2):149–55.

Irwin, C. R., et al. 2012. In-fusion® cloning with Vaccinia virus DNA polymerase. *Methods Mol Biol* 890:23–35.

Jain, K. 2003. Nanodiagnostics: Application of nanotechnology in molecular diagnostics. *Expert Rev Mol Diagn* 3(2):9.

Khalil, A. S., and J. J. Collins. 2010. Synthetic biology: Applications come of age. *Nat Rev Genet* 11(5):367–79.

Khorana, H. 1979. Total synthesis of a gene. *Science* 203(4381):614–25.

Konry, T., et al. 2011a. Droplet-based microfluidic platforms for single T cell secretion analysis of IL-10 cytokine. *Biosens Bioelectron* 26(5):2707–10.

Konry, T., et al. 2011b. Ultrasensitive detection of low-abundance surface-marker protein using isothermal rolling circle amplification in a microfluidic nanoliter platform. *Small* 7(3):395–400.

Kumar, C. S. S. R. 2010. *Microfluidic devices in nanotechnology: Fundamental concepts*, vol. 1. Hoboken: John Wiley.

Kumar, H., et al. 2011. Biopolymers in nanopores: Challenges and opportunities. *Soft Matter* 7(13):5898.

Leguia, M., et al. 2011. Automated assembly of standard biological parts. *Methods Enzymol* 498:363–97.

Li, M. Z., and S. J. Elledge. 2012. SLIC: A method for sequence- and ligation-independent cloning. *Methods Mol Biol* 852:51–59.

Linshiz, G., et al. 2008. Recursive construction of perfect DNA molecules from imperfect oligonucleotides. *Mol Syst Biol* 4:191.

Linshiz, G., T. Ben Yehezkel, and E. Shapiro. 2012. Recursive construction of perfect DNA molecules and libraries from imperfect oligonucleotides. *Methods Mol Biol* 852: 151–63.

Ma, S., N. Tang, and J. Tian. 2012. DNA synthesis, assembly and applications in synthetic biology. *Curr Opin Chem Biol* 16(3-4):260–67.

Mark, D., et al. 2010. Microfluidic lab-on-a-chip platforms: Requirements, characteristics and applications. *Chem Soc Rev* 39(3):1153–82.

Mueller, S., J. R. Coleman, and E. Wimmer. 2009. Putting synthesis into biology: A viral view of genetic engineering through de novo gene and genome synthesis. *Chem Biol* 16(3):337–47.

Mullard, A. 2011. Reliability of "new drug target" claims called into question. *Nat Rev Drug Discov* 10(9):643–44.

Müller, K. M., and K.M. Arndt. 2012. Standardization in synthetic biology. *Methods Mol Biol* 813:23–43.

Ohtani, N., et al. 2012. Serial Assembly of thermus megaplasmid DNA in the genome of Bacillus subtilis 168: A BAC-based domino method applied to DNA with a high GC content. *Biotechnol J* 7(7):867–76.

Prilusky, J., et al. 2005. HalX: An open-source LIMS (laboratory information management system) for small- to large-scale laboratories. *Acta Crystallogr D Biol Crystallogr* 61(pt. 6):671–78.

Quan, J., and J. Tian. 2011. Circular polymerase extension cloning for high-throughput cloning of complex and combinatorial DNA libraries. *Nat Protoc* 6(2):242–51.

Richardson, S. M., et al. 2012. Design-a-gene with GeneDesign. *Methods Mol Biol* 852: 235–47.

Ro, D.-K., et al. 2006. Production of the antimalarial drug precursor artemisinic acid in engineered yeast. *Nature* 440(7086):940–43.

Sarrion-Perdigones, A., et al. 2011. GoldenBraid: An iterative cloning system for standardized assembly of reusable genetic modules. *PloS One* 6(7):e21622.

Shabi, U., et al. 2010. Processing DNA molecules as text. *Syst Synth Biol* 4(3):227–36.

Shao, Z., H. Zhao, and H. Zhao. 2009. DNA assembler, an in vivo genetic method for rapid construction of biochemical pathways. *Nucleic Acids Res* 37(2):e16.

Shetty, R. P., D. Endy, and T. F. Knight. 2008. Engineering BioBrick vectors from BioBrick parts. *J Biol Eng* 2:5.

Shuler, M. L., P. Foley, and J. Atlas. 2012. Modeling a minimal cell. *Methods Mol Biol* 881: 573–610.

Vangelooven, J., and G. Desmet. 2010. Computer Aided design optimisation of microfluidic flow distributors. *J Chromatogr A* 1217(43):6724–32.

Vu, T. D., et al. 2012. A laboratory information management system for DNA barcoding workflows. *Integr Biol (Camb)* 4(7):744–55.

Weber, W., and M. Fussenegger. 2012. Emerging biomedical applications of synthetic biology. *Nat Rev Genet* 13(1):21–35.

Werner, S., et al 2012. Fast track assembly of multigene constructs using Golden Gate cloning and the MoClo system. *Bioeng Bugs* 3(1):38–43.

Xiong, A.-S., et al. 2006. PCR-based accurate synthesis of long DNA sequences. *Nat Protoc* 1(2):791–97.

Yeates, T. O. 2011. Nanobiotechnology: Protein arrays made to order. *Nat Nanotechnol* 6(9):541–42.

Zhang, Y., U. Werling, and W. Edelmann. 2012. SLiCE: A novel bacterial cell extract-based DNA cloning method. *Nucleic Acids Res* 40(8):e55.

Zhang, Z., et al. 2011. Self-assembly-based structural DNA nanotechnology. *Curr Org Chem* 15(4):14.