Leading Edge
Essay

Cell

Informing Biological Design by Integration of Systems and Synthetic Biology

Christina D. Smolke[1,*] and Pamela A. Silver[2,*]
[1]Department of Bioengineering, Stanford University, 473 Via Ortega, Stanford, CA 94305-4201, USA
[2]Department of Systems Biology and Wyss Institute of Biologically Inspired Engineering, Harvard Medical School, 200 Longwood Avenue, Boston, MA 02115, USA
*Correspondence: csmolke@stanford.edu (C.D.S.), pamela_silver@hms.harvard.edu (P.A.S.)
DOI 10.1016/j.cell.2011.02.020

Synthetic biology aims to make the engineering of biology faster and more predictable. In contrast, systems biology focuses on the interaction of myriad components and how these give rise to the dynamic and complex behavior of biological systems. Here, we examine the synergies between these two fields.

Biology is the technology of this century. The potential uses of biology to improve the human condition and the future of the planet are myriad. Over the last century, humans have used biology to make many useful things, in part based on discoveries from molecular biology. In addition, researchers have redesigned biological systems to test our fundamental understanding of their components and integrated functions. However, the complexity and reliability of engineered biological systems still cannot approach the diversity and richness exhibited by their natural counterparts. It is then the combined promise of systems biology and synthetic biology that may drive transformative advances in our ability to program biological function.

One recent example of the successful engineering of a biological system to address a global challenge in health and medicine is the creation of microbes that produce a precursor to the antimalarial drug artemisinin (Ro et al., 2006). By shifting synthesis from the natural production host (a plant) to one more optimized for rapid production times and inexpensive scale up (a microorganism), researchers were able to develop a process that enabled cheaper supply of this drug, providing a more accessible cure for a disease devastating third world countries. However, the research phase of this project required an investment of over $25 million and 150 person-years of highly trained researcher effort. This investment cannot realistically be replicated for every chemical or material to which we would apply this approach. Instead, imagine a time when a bioengineer designs a system at the computer, orders the necessary DNA encoding the specified system, and then begins the actual experiment of turning it into life. Thus, one overarching goal of synthetic biology is to make the engineering of biology faster, affordable, and more predictable.

Biological systems and their underlying components offer a number of functional parallels with engineered systems. For example, biological sensors are exquisitely sensitive; the olfactory system can detect single odorant molecules and decode them. Biological systems can send and receive signals rapidly and in a highly specific manner. Pathways exist to sense and respond to the environment. Plants and microbes can use sunlight as an energy source. However, biological systems are also uniquely capable of self-replication, mutation, and selection, leading to evolution. Synthetic biologists aim to take advantage of these parallels and develop engineering principles for the design and construction of biological systems. However, an open question is whether we understand biological systems sufficiently to be able to redesign them to fulfill specific requirements.

Engineers enjoy the concept of interchangeable parts and modularity. Biology offers many sources of potential modularity but exhibits nonmodular features as well. For many years the gene was regarded as a fundamental modular unit of biology. As such, a gene is capable of transferring a particular phenotype to the organism. However, we now know that genes display more fine-grained modularity in the form of promoters, open reading frames (ORFs), and regulatory elements. mRNAs contain sequences important for proper intracellular targeting and degradation. Proteins often contain targeting sequences, reactive centers, and degradation sequences. And lastly, entire pathways are modular in that some signaling pathways can be transferred from one organism to another to reconstruct a new state in the engineered organism. This modularity underlies one of the core concepts of synthetic biology—the notion that one can assemble biological systems from well-defined "parts" or modules (Endy, 2005). However, modular assembly approaches have largely remained confounded by the effects of context—that is, the nonmodular aspects of biology. For example, where a gene or an associated regulatory element is located in the genome can impact expression and thus its function. In addition, the location of regulatory elements relative to each other and ORFs can impact their encoded function (Haynes and Silver, 2009). Further analyses provided by systems biology may help to guide the development of standard strategies for assembling genetic modules into functional units.

Approaches to Synthetic Biology

Given that the goals of synthetic biology are to make the engineering of biology faster and more predictable, and to harness the power of biology for the

common good, the development of new approaches that support the design and construction of genetic systems has been a core activity within the field. Although advances have been made in both areas (fabrication and design), our ability to construct large genetic systems currently surpasses our ability to design such systems, resulting in a growing "design gap" that is a critical issue that synthetic biologists must address.

The ability to synthesize large pieces of DNA corresponding to operons, entire pathways, chromosomes, and genomes in a rapid and predictable way is a key approach to system fabrication. Systems biology has provided numerous templates with the abundance of sequenced genomes being deposited daily into publicly accessible databases. Some progress has recently been reported, including the resynthesis of a bacterial genome and its successful insertion into a different bacterial host (Gibson et al., 2010). However, it took researchers nearly 15 years and approximately $30 million to develop various fundamental aspects of this project. Much of this time and cost was methods development that will hopefully reduce the resources needed to carry out such projects in the future. In addition, new high-throughput methods for large-scale DNA synthesis have been recently described (Matzas et al., 2010; Norville et al., 2010; Tian et al., 2009). However, much more work is still needed to develop these technologies to the point where they are accessible to the majority of researchers (that is, in terms of cost and reliability), and systems biology may provide important clues. For example, faster and more reliable ways to synthesize large pieces of DNA may be uncovered by examination of new organisms and thereby reveal new nonchemical methods for DNA synthesis.

A second approach is to develop the methods to generate new component functions that can act as sensors, regulators, controllers, and enzyme activities, for example. These components will in turn extend the set of parts from which synthetic biologists can build genetic devices and systems. Synthetic biologists work not only with design of DNA that encodes genetic circuits but also with molecular design of biomolecules, such as RNAs and proteins, to perform new functions. Substantial efforts in the field of protein engineering have contributed to the diversity of functions exhibited by protein components (Dougherty and Arnold, 2009). However, even with these advances, the diversity of component activities that is currently available as parts has been limited, thus limiting the design of genetic circuits. Systems biology may aid in the development of effective strategies for generating new component functions by providing information on how Nature has evolved different functions for macromolecules.

A third approach is the predictable design of complex genetic circuits that lay the foundation for new biological devices and systems. Many circuits designed and built thus far have relied on our fairly detailed knowledge of how gene transcription is regulated. For example, synthetic circuits have applied concepts of positive and negative feedback to generate systems that sense stimuli, remember past events, and promote cell death in both prokaryotic and eukaryotic cells (Burrill and Silver, 2010; Sprinzak and Elowitz, 2005). However, many of these systems have been built in a fairly ad hoc manner, requiring substantial troubleshooting and iterative design to exhibit desired functions, and lack the robust performance standards one might expect as an engineer. Going forward, synthetic biologists need to better understand the parts underlying system design, how to predict their function in a particular genetic context, and how to predict their integrated function with other system parts (Ellis et al., 2009; Savageau, 2011). This biological understanding will then be integrated with computational models to develop computer-aided design tools.

What Does Systems Biology Mean to Synthetic Biology?

As with synthetic biology, many different types of research have been categorized as systems biology. Broadly speaking, systems biology represents an approach to biological research that focuses on the interactions between components of a biological system and how those interactions give rise to the dynamic behavior of the system in contrast to the more traditional molecular biologists' reductionist approach of studying components in isolation from each other (Alon, 2007). Systems biology has been associated with new technologies and methods that allow for quantitative measures of components and component interactions within biological systems, particularly those that allow for genome-wide measurements. In addition, because many of these technologies result in large datasets, systems biology has also been associated with computational tools that support the integration and analysis of these datasets to identify static relationships and interactions between components. Finally, as one of the ultimate goals of systems biology is to be able to predict a system's dynamic behavior from the component parts, computational tools that can model biological systems-level function from underlying components are associated with this field.

However, there are currently a number of challenges and limitations facing the field of systems biology. Paramount is determining how to correctly analyze and draw valid conclusions from large amounts of different types of data ranging from genomics and metabolomics to molecular dynamics in many single cells. Effectively addressing this problem may require new mathematical and computer science approaches. A second key challenge is knowing what kind of measurements to make and how accurate these measurements need to be to fully understand a biological system. Effectively addressing this challenge will require a re-evaluation of how measurements have been made over the past 10 years in systems biology (for instance, the movement from two-hybrid interactions to mass spectrometry to measure protein interactions). It will also require the development of even more sensitive strategies to make time-dependent measurements inside many cells simultaneously. Taken together, systems biology is confronted with the problem of both sensitivity and scale.

Does the ultimate goal of synthetic biology of the predictable design, construction, and characterization of biological systems rely on findings and approaches from systems biology? Design, analysis, and understanding are integrally linked in engineering methodology. Therefore, it is reasonable to assume that advances

gained through systems biology in our understanding of how biological components interact to form integrated systems will support efforts in synthetic biology to design engineered biological systems. However, there is a different viewpoint that argues that the design principles that systems biologists elucidate for natural biological systems are products of evolution over many millions of years and thus are limited by the history of what came before. It is possible, then, that the design principles elucidated for natural biological systems may not be necessary or optimal for the engineered systems that synthetic biologists may design from scratch on a computer with less of a restriction of generating new function through evolutionary processes and timescales. Both of these views have merit, and the reality is likely somewhere in between—even if synthetic biologists design biological systems to have certain properties that are not generally found in natural systems (i.e., optimized for troubleshooting, tailoring, reuse, removal, designer identification), a greater understanding of how components interact to form integrated systems will inform and support the design process.

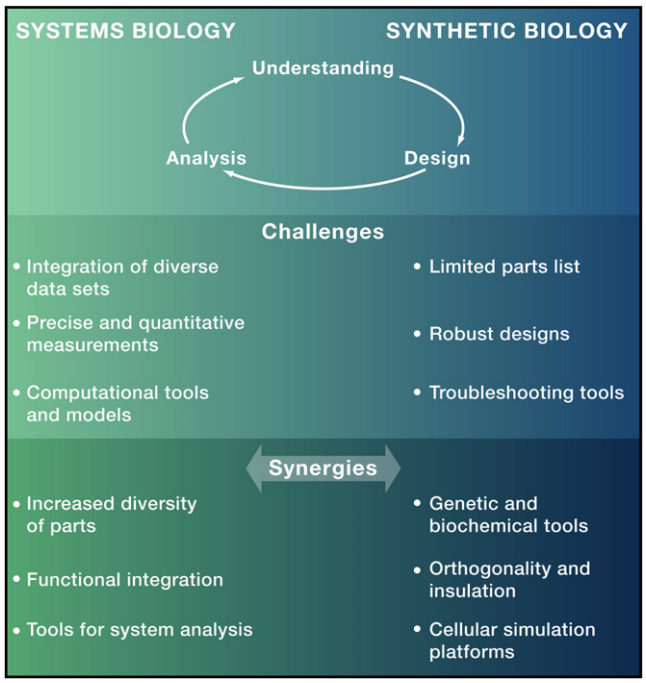

Figure 1. The Challenges and Synergies for Systems and Synthetic Biology

Synergy between Systems and Synthetic Biology

Although synthetic biology did not directly emerge from systems biology, there are important parallels between the two fields. Both systems biology and synthetic biology represent fundamental shifts in approaches from the fields they grew out of. Whereas systems biology represents a shift in the more traditional reductionist approach taken in biological research from studying components in isolation to studying integrated components, synthetic biology represents a shift in emphasizing engineering principles and methodology in building biological systems from more traditional genetic engineering research. In addition, both fields take a bottom-up approach, with systems biology emphasizing the understanding of biological systems from the underlying components and synthetic biology emphasizing building biological systems from modular components.

In examining the parallels between the two fields, it is also useful to examine how the key challenges each field is currently facing relate to one another (Figure 1). The challenges synthetic biologists currently face in engineering genetic systems can be classified as relating to either limitations in understanding biological systems or limitations in technical capabilities to study biological systems. The challenges systems biologists currently face in understanding biological systems are related to the complexities associated with studying natural biological systems and the inadequacies of current computational models to capture the physical properties of biological systems. We see several areas where these two fields can be brought together to effectively address these challenges.

The richness and complexity of engineered genetic networks, which synthetic biologists could build, will be advanced by using the knowledge gained through systems biology research. For example, genome sequencing can provide an increased diversity of biological parts that synthetic biologists can use in their gene circuit designs. More importantly, systems biology will provide not just the physical parts but a fundamental understanding of how these components can be integrated effectively with other components and how biological systems integrate diverse components and regulatory mechanisms to achieve robust information transmission and behaviors. The importance of this contribution is highlighted by the limited diversity of parts and regulatory mechanisms that have been integrated into synthetic gene circuits to date, in which the majority of engineered systems rely on a limited number of transcriptional regulators and do not exhibit robust behaviors over different timescales and environmental conditions (Elowitz and Leibler, 2000; Gardner et al., 2000; Purnick and Weiss, 2009). In order to move toward the design of integrated genetic systems, synthetic biologists will need to design more sophisticated genetic circuits that utilize diverse regulatory strategies (specifically, the integration of posttranscriptional and posttranslational mechanisms), balance energetic load, and dynamically modulate system behavior (Lim, 2010; Win et al., 2009).

Another important contribution of systems biology to synthetic biology is associated with the technologies and tools for analyzing biological systems. Synthetic biologists often spend the bulk of their effort in a design, characterization, and optimization loop, where original designs are modified based on characterization data to achieve the desired system behavior. The tools developed by systems biologists to study components in a system and their interactions can be applied to analyzing synthetic systems and troubleshooting the system performance. This is particularly true in cases in which the synthetic gene network may have

unanticipated effects on native pathways in the cell that may in turn affect system behavior. A common example of this challenge can arise in engineering metabolic pathways, for which synthetic biologists can use genome-wide profiling of transcript, protein, or metabolite levels to identify undesired effects of introducing the synthetic pathway in the host cell on critical functions such as redox balance, cofactor levels, and stress response (Mukhopadhyay et al., 2008). As another example, systems biologists have developed a variety of computational tools for modeling biological systems and sharing information on biological components across different databases. These tools will be useful foundations for synthetic biologists looking to develop methods to standardize and share information across component libraries and develop computer-aided design tools for biological systems.

Advances in synthetic biology will provide key contributions to systems biology research by creating new tools for interfacing with and manipulating biological systems. Research aimed at understanding a biological system often utilizes methods to perturb or manipulate that system and examine the resulting behavior of the modified system. Synthetic biologists are developing novel genetic devices that can be used by systems biologists to interface with native networks and precisely probe and manipulate those systems. For example, synthetic genetic devices have recently been used to rewire signaling pathways and create novel interactions between unrelated cellular components (Culler et al., 2010; Lim, 2010). In addition, synthetic biology can contribute strategies for simplifying and isolating biological components and their interactions through the application of diverse approaches for implementing specific component interactions.

Synthetic biology can also provide new simulation platforms for systems biology. For example, systems biologists currently develop mathematical models to represent the behavior of their systems and use these models to predict the behavior of their systems under different perturbations and environments. However, the development of these models often requires assumptions that are imperfect matches for the physical model of a cell (i.e., hard sphere, dilute gas models), such that the ability of current computational models to capture system behavior is limited at best. The potential advances in constructing genetic systems coming from synthetic biology research may enable systems biologists to shift from computational models to physical models for their systems by implementing simulations inside of cells. Specifically, scalable and affordable DNA synthesis technology can allow systems biologists to build many modified versions of natural systems to test their understanding of those systems.

Perspective of the Future

Moving forward, the synergy between synthetic and systems biology will drive transformative advances in biotechnology. The impact includes not only further understanding of the complexity of biological systems but the ability to use this information to, for instance, design better drugs, commodity manufacturing processes, and cell-based therapies (Ducat et al., 2011). As one example, the complexity of biosynthesis processes that can be engineered has been recently advanced through the integration of a number of pathway construction and optimization tools, including genomic discovery and engineering (Bayer et al., 2009; Ro et al., 2006; Wang et al., 2009), in vivo screens for enzyme activity (Pfleger et al., 2006), and enzyme localization strategies (Dueber et al., 2009). Future efforts will focus on the development of more advanced tools for bioprocess optimization, such as those enabling noninvasive monitoring of pathway flux (Win and Smolke, 2007), closed loop embedded control of biosynthesis system behavior (Dunlop et al., 2010; Farmer and Liao, 2000), and biosynthesis compartmentalization and specialization. As another example, systems engineering strategies will play key roles in addressing current challenges in cellular therapies by enabling the programming of cell-fate decisions (Culler et al., 2010), differentiated states (Deans et al., 2007), improved engraftment and targeting (Chen et al., 2010), and effective kill switches (Callura et al., 2010). Ultimately, researchers will design systems that incorporate evolution—designing gene circuits that exhibit desired, evolvable behaviors and eventually constructing ecosystems that exhibit dynamic and predictable behavior patterns.

However, it is important to look at history in thinking about the promises and risks of synthetic biology. Molecular biology, and in particular the insertion of foreign genes into microbes, was met with circumspection by both the public and scientific communities. At the time, scientists made promises to the public—for example, the production of human insulin by engineered bacteria—and delivered on at least some of these promises (Villa-Komaroff et al., 1978). So, what can we expect from the interplay between systems biology and synthetic biology in the near and long term? In the near term, we have already seen companies promise to deliver on new fuels and carbon-based products (such as plastics), and in 5 years time this will be a partial reality, thereby starting to take petroleum out of the production loop. We believe that, in 10 years time, many high-value commodities, including drugs, will be produced biologically as the result of synthetic biology efforts. In the much longer time frame of 20 to 50 years, we hope that synthetic biology will lead to new cell-based therapies, the expansion of immunotherapy, synthetic organs and tissues, and rebuilding devastated environments and ecosystems.

These anticipated futures bring us to the controversial areas in synthetic biology. How do we think about a future that could involve the reprogramming of entire organisms? Should we consider engineering ecosystems to support sustainable agriculture, environmental remediation, and pathogen removal and to treat human disease? How far should and can we go in reprogramming life to form new types of cells, tissues, and entire organisms? These are only some of the potential benefits and questions scientists, engineers, policy makers, governments, and, most importantly, the public will need to ponder. Molecular biologists set standards for safe use of engineered organisms over 30 years ago. However, as research in synthetic biology is advancing toward the goals of making biology easier to engineer, the issues of safety and ethical use are being revisited as we write this Essay. In fact,

a recent US government report captures many of the critical issues around public benefits and responsible stewardship (Presidential Commission for the Study of Bioethical Issues, 2010).

Although each field could in principle exist without the other, we instead feel that the natural interplay between design, analysis, and understanding highlights the important relationship between systems biology and synthetic biology. Systems biology brings added layers of information that will further empower future efforts to design synthetic biological systems. Synthetic biology brings new technologies and tools that can be applied to effectively test our understanding of natural biological systems. By integrating the contributions of these rapidly evolving fields, scientists and engineers together will be well positioned to transform health, well-being, and the environment in the years to come.

ACKNOWLEDGMENTS

P.A.S. is supported by funds from the NIH, DOD, DOE, NSF, and the Wyss Institute for Biologically Inspired Engineering. C.D.S. is supported by funds from the NIH, NSF, and the Alfred P. Sloan Foundation. The authors thank Drew Endy and Jeff Way for comments.

REFERENCES

Alon, U. (2007). An Introduction to Systems Biology: Design Principles of Biological Circuits (Boca Raton, FL: Chapman and Hall/CRC Press).

Bayer, T.S., Widmaier, D.M., Temme, K., Mirsky, E.A., Santi, D.V., and Voigt, C.A. (2009). J. Am. Chem. Soc. *131*, 6508–6515.

Burrill, D.R., and Silver, P.A. (2010). Cell *140*, 13–18.

Callura, J.M., Dwyer, D.J., Isaacs, F.J., Cantor, C.R., and Collins, J.J. (2010). Proc. Natl. Acad. Sci. USA *107*, 15898–15903.

Chen, Y.Y., Jensen, M.C., and Smolke, C.D. (2010). Proc. Natl. Acad. Sci. USA *107*, 8531–8536.

Culler, S.J., Hoff, K.G., and Smolke, C.D. (2010). Science *330*, 1251–1255.

Deans, T.L., Cantor, C.R., and Collins, J.J. (2007). Cell *130*, 363–372.

Dougherty, M.J., and Arnold, F.H. (2009). Curr. Opin. Biotechnol. *20*, 486–491.

Ducat, D.C., Way, J.C., and Silver, P.A. (2011). Trends Biotechnol. *29*, 95–103.

Dueber, J.E., Wu, G.C., Malmirchegini, G.R., Moon, T.S., Petzold, C.J., Ullal, A.V., Prather, K.L., and Keasling, J.D. (2009). Nat. Biotechnol. *27*, 753–759.

Dunlop, M.J., Keasling, J.D., and Mukhopadhyay, A. (2010). Syst. Synth. Biol. *4*, 95–104.

Ellis, T., Wang, X., and Collins, J.J. (2009). Nat. Biotechnol. *27*, 465–471.

Elowitz, M.B., and Leibler, S. (2000). Nature *403*, 335–338.

Endy, D. (2005). Nature *438*, 449–453.

Farmer, W.R., and Liao, J.C. (2000). Nat. Biotechnol. *18*, 533–537.

Gardner, T.S., Cantor, C.C., and Collins, J.J. (2000). Nature *403*, 339–342.

Gibson, D.G., Glass, J.I., Lartigue, C., Noskov, V.N., Chuang, R.Y., Algire, M.A., Benders, G.A., Montague, M.G., Ma, L., Moodie, M.M., et al. (2010). Science *329*, 52–56.

Haynes, K., and Silver, P.A. (2009). J. Cell Biol. *187*, 589–596.

Lim, W.A. (2010). Nat. Rev. Mol. Cell Biol. *11*, 393–403.

Matzas, M., Stahler, P.F., Kefer, N., Siebelt, N., Boisguerin, V., Leonard, J.T., Keller, A., Stahler, C.F., Haberle, P., Gharizaden, B., et al. (2010). Nat. Biotechnol. *28*, 1291–1294.

Mukhopadhyay, A., Redding, A.M., Rutherford, B.J., and Keasling, J.D. (2008). Curr. Opin. Biotechnol. *19*, 228–234.

Norville, J.E., Derda, R., Drinkwater, K.A., Leschziner, A.E., and Knight, T.R. (2010). J. Biol. Eng. *4*, 17.

Pfleger, B.F., Pitera, D.J., Smolke, C.D., and Keasling, J.D. (2006). Nat. Biotechnol. *24*, 1027–1032.

Presidential Commission for the Study of Bioethical Issues. (2010). http://www.bioethics.gov/.

Purnick, P.E., and Weiss, R. (2009). Nat. Rev. Mol. Cell Biol. *10*, 410–422.

Ro, D.K., Paradise, E.M., Ouellet, M., Fisher, K.J., Newman, K.L., Ndungu, J.M., Ho, K.A., Eachus, R.A., Ham, T.S., Kirby, J., et al. (2006). Nature *440*, 940–943.

Savageau, M. (2011). Ann. Biomed. Eng. Published online January 4, 2011. 10.1007/s10439-010-0220-2.

Sprinzak, D., and Elowitz, M.B. (2005). Nature *438*, 443–448.

Tian, J., Ma, K., and Saaem, I. (2009). Mol. Biosyst. *5*, 14–22.

Villa-Komaroff, L., Efstradiadis, A., Broome, S., Lomedico, P., Tizard, R., Naber, S.P., Chick, W.L., and Gilbert, W. (1978). Proc. Natl. Acad. Sci. USA *75*, 3727–3731.

Wang, H.H., Isaacs, F.J., Carr, P.A., Sun, Z.Z., Xu, G., Forest, C.R., and Church, G.M. (2009). Nature *460*, 894–898.

Win, M.N., and Smolke, C.D. (2007). Proc. Natl. Acad. Sci. USA *104*, 14283–14288.

Win, M.N., Liang, J.C., and Smolke, C.D. (2009). Chem. Biol. *16*, 298–310.