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Synthetic Biology: opportunities for Chilean bioindustry and education

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ABSTRACT

In an age of pressing challenges for sustainable production of energy and food, the new field of Synthetic Biology has emerged as a promising approach to engineer biological systems. Synthetic Biology is formulating the design principles to engineer affordable, scalable, predictable and robust functions in biological systems. In addition to efficient transfer of evolved traits from one organism to another, Synthetic Biology offers a new and radical approach to bottom-up engineering of sensors, actuators, dynamical controllers and the biological chassis they are embedded in. Because it abstracts much of the mechanistic details underlying biological component behavior, Synthetic Biology methods and resources can be readily used by interdisciplinary teams to tackle complex problems. In addition, the advent of robust new methods for the assembly of large genetic circuits enables teaching Biology and Bioengineering in a learning-by-making fashion for diverse backgrounds at the graduate, undergraduate and high school levels. Synthetic Biology offers unique opportunities to empower interdisciplinary training, research and industrial development in Chile for a technology that promises a significant role in this century's economy.

Key terms: Synthetic biology, bioengineering, education, engineering, biotechnology.

1. WHAT IS SYNTHETIC BIOLOGY?

Over a century ago, a paradigm shift in the physical sciences, triggered mainly by pioneering work from Georg Ohm, Michael Faraday and James Maxwell, resulted in the emergence of the electrical engineering discipline and its applications. In the same way electrical engineers formulated rules and methods to manipulate natural electromagnetic phenomena, synthetic biologists are attempting to establish design principles for the engineering of biological machines and systems.

Synthetic Biology emphasizes the use of well characterized building blocks and mathematical modeling for predictable design, and aims to minimize the need for *ad hoc* approaches, iterative debugging and troubleshooting (Smolke and Silver, 2011). For example, although artificial genetic switches had already been developed in the 1980's (Podhajska et al., 1985), pioneering work by Elowitz et al. (2000) and Gardner et al. (2000) led to the realization of predictable programming of more elaborated dynamical processes in cells (McAdams and Arkin, 2000). The idea that biological systems could be treated as reprogrammable material has led to the exploration of a wide variety of applications. These have ranged from multi-chromatic bacterial photo-films (Levskaya et al., 2005; Tabor et al., 2011) to *in vivo* cell-type classifiers that recognize molecular profiles in cancer cells (Xie et al., 2011). Synthetic Biology is catalyzing new approaches in biotechnology (Khalil and Collins, 2010), medicine (Ruder et al., 2011; Weber and Fussenegger 2011) and scientific research (Elowitz and Lim, 2010; Mukherji and van Oudenaarden, 2012; Nandagopal and Elowitz, 2011). Projects such as the synthesis of artificial genomes (Gibson et al. 2010), genome-wide DNA editing (Esvelt and Wang, 2013); artificial proto-cells (Mansy et al., 2008; Hammer and Kamat, 2012), reprogrammed genetic code (Chin, 2012); *in vivo* numerical computation (Benenson et al., 2011; 2012); nano-robots (Douglas et al., 2012); and digital data storage in DNA (Church et al., 2012); are changing our perception of biology from a mere source of raw materials to a programmable medium for manipulating matter and information.

1.1. Enabling technologies: DNA synthesis and assembly

Whereas traditional genetic engineering uses recombinant DNA technologies for reading, amplifying, cutting and pasting DNA templates from one organism to another, Synthetic Biology exploits large scale chemical synthesis of custom-defined DNA sequences (Carlson, 2009; Baker,2011) and high-throughput methods for DNA assembly (Ellis et al., 2011). These technologies have not only expanded the size of manufactured DNA fragments to the scale of megabases, enough for the synthesis of a whole synthetic genome (Gibson et al., 2010) or a book of 53,426 words (Church et al., 2012), but also provide an unprecedented capability for template-free DNA manufacture that allows arbitrary genetic information to be constructed from the bottom-up. However, these advances have largely surpassed the genetic engineer's ability to rationally design functioning genetic circuits at large scale, creating a "design gap" between the enabling technologies and the knowledge to engineer *de novo* high order biological functions. One of the major challenges of Synthetic Biology is to formulate the methods and design principles that bridge this gap (Smolke and Silver, 2011).

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1.2. Design principles: abstraction, decoupling and modularity.

Abstraction, decoupling and modular design have been key enabling concepts for engineering, allowing the design of systems in which millions of components are combined to produce predictable behaviors. For example at CERN (http://home.web.cern.ch), physicists and engineers have built the LHC (Large Hadron Collider), a 27 km long structure that can accelerate particles close to the speed of light and collide them with a precision equivalent to shooting two needles from 10 km apart. This precision is only possible because the parts are well defined, characterized and modular, allowing their collective behavior to be predicted. Similarly, efforts are beginning to define reliable building blocks for biological engineering (Kosuri et al., 2013; Davis et al., 2011, Mutalik et al., 2013a; 2013b; Liu et al., 2012; Lou et al., 2012; Qi et al., 2012). The Synthetic Biology community has grown around the goal of implementing standardized modular building blocks to construct genetic circuitry (Knight, 2003; Endy, 2005; Shetty et al., 2008; Canton et al. 2008). The aim is to create "off-the-shell" biological parts that can be used to design at a higher level of abstraction. This idea led to the creation of the MIT-based Registry of Standard Biological Parts (www.partsregistry.org) that lists and distributes thousands of widely used genetic building blocks.

Abstraction means incorporating detailed information about individual components into simplified representations of their behavior. These abstract parts can then be used to design sub-systems, which are again abstracted (Fig. 1A). For example, genetic elements can be combined to create devices that encapsulate certain biological functions (e.g. invert a signal or communicate with another cell). In this way, hierarchical layers of abstraction enable design of large scale systems (Tabor et al., 2009; Andrianantoandro et al., 2006; Endy, 2005; Canton et al., 2008). The use of these standards and abstractions, in combination with DNA synthesis on demand, would lead to decoupling of high level design tasks from lower level specifications and parts fabrication (Fig. 1A). This approach should allow re-use of components and devices in multiple systems given standards for the definition, description and characterization of genetic building blocks (Endy, 2005) (Fig. 1B).

The concepts of modularity, and hierarchies of modules, have facilitated reverse engineering of naturally evolved biological processes (Lauffenburger, 2000, Nurse, 2008). This is because biological systems naturally exploit some level of modularity (Hartwell et al., 1999). Functional modularity can be identified at the molecular level (Grunberg and Serrano, 2010, Khalil et al., 2012), metabolic level (Alon, 2006) and developmental level (von Dassow et al., 2000; Espinosa-Soto and Wagner, 2010; Gallois et al., 2004, Niehrs and Meinhardt, 2002; Davidson, 2010). This modular organization has been suggested to be an adaptive trait that facilitates evolutionary exploration by allowing rewiring of existing higher level building blocks that perform modular biological functions (Hartwell et al., 1999; Lipson, 2007, Milo et al., 2002; Kashtan and Alon, 2005). This feature is reflected in the scale-free topology of gene regulatory networks (Bray, 2003; Jeong et al., 2000; Albert et al., 2000). There are however, varying degrees of modularity in natural systems, which also exploit crosstalk at different levels of hierarchically nested processes (Vilar, 2006; Weng et al., 1999). Such systems are very difficult to understand and design.

The radical aim of synthetic biology is to minimize such crosstalk and create orthogonal parts and modular functions that would facilitate human design by abstraction

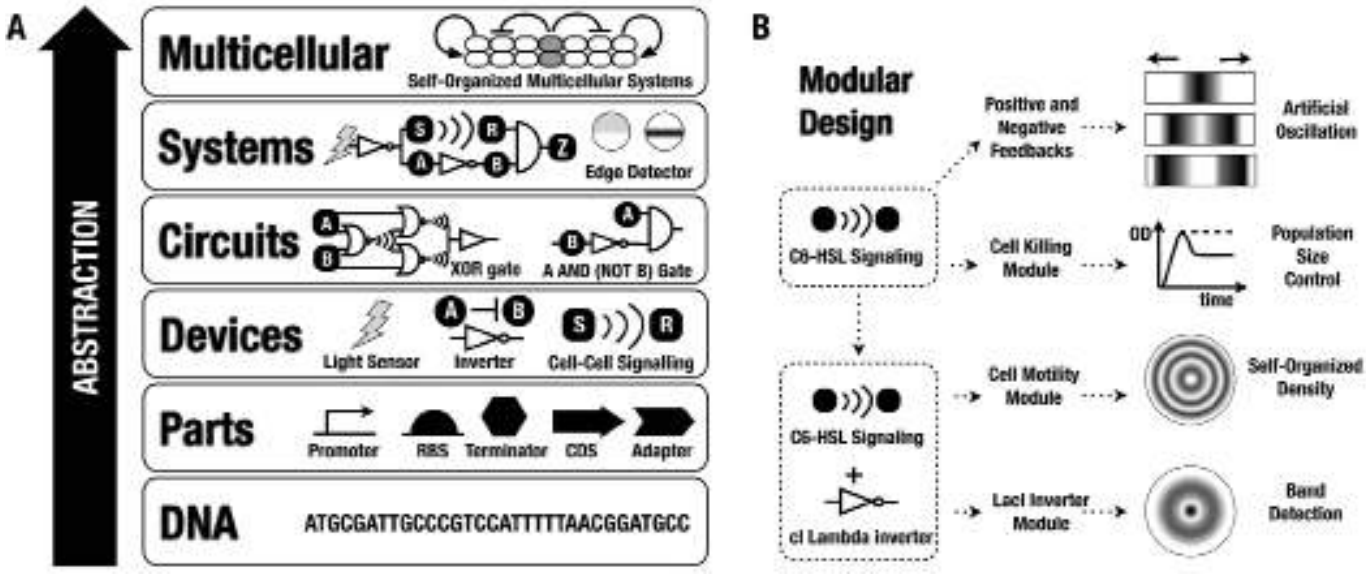

Figure 1. Design principles of Synthetic Biology. A) Abstraction hierarchy from DNA information to engineered multicellular systems. Decoupling and Abstraction (adapted from Endy, 2005). B) Modular design allow re-use of components and devices in multiple systems. For instance, C6 homoserine lactone signaling module has been used in combination with a negative feedback regulation loop for engineering traveling waves and oscillations (Danino et al., 2010), and along with a cell killing module for programming population size regulation by population size-dependent mechanisms (You et al., 2004). The combination of this module and the cI lambda inverter has been used with a cell motility module for artificial cell density regulation in space (Liu et al., 2012), and with a lacI inverter module for the construction of a band detect system (Basu et al., 2005).

and decoupling (Andrianantoandro, et al., 2006). Recently, the NSF-funded International Open Facility Advancing Biotechnology (BioFab, www.biofab.org) was established as the first design-build facility and is specifically addressing this aim. The initiative is inspired by the microelectronics fabrication facilities which propelled the rapid expansion of the electronics industry. BioFab is designing, and performing high-throughput characterization of genetic elements and is making this data freely available to both academic and commercial users, an effort that aims to facilitate open innovation. They have already contributed critical information and solutions for creating modular transcription and translation control elements with minimum interference (Mutalik et al., 2013a; 2013b; Cambray et al., 2013).

1.3. Parts characterization and system design

Abstraction of biological parts relies on precise and accurate part performance and operation measurements covering the range of relevant variation. Reference standards, or special parts referred to as "yardsticks", have been proposed to reduce variation in reported part performance between labs (Kelly et al., 2009; Federici et al, 2012). In this approach, measurements can be made using different methods and equipment, and normalized with respect to the properties of the reference part measured using the same method (Kelly et al., 2009). Normalization with respect to an internal (in vivo, co-localized) reference standard could also significantly decrease variability and artifacts introduced by equipment and operational settings (Federici et al, 2012). These approaches will allow the exchange of part performance data in meaningful and comparable values. Using measurements of reference parts under different conditions and in different strains, it might also be possible to define "dynamic yardsticks" that could allow extrapolation or inference of part performance to untested conditions.

Reference part parameterizations must capture correlations in the behavior of different parts due, for example, to interactions with the cellular environment. This interaction with the cellular context is a major challenge in designing engineered genetic circuits (Cardinale 2012). Even well understood mechanical structural systems can suffer from the unpredictable emergence of context-dependent behaviors (e.g. pedestrian-induced vibration in the Millennium Bridge, London; Dallard et al., 2001). Synthetic circuits can be influenced by extrinsic noise (Elowitz et al, 2002; Swain et al., 2002) arising from context-dependent mechanisms such as metabolic load (Neubauer et al., 2003), fluctuations in rates of component degradation (Cookson et al., 2011) and growth changes (Klumpp et al., 2009). Novel computational methods and genetic techniques (Elowitz 2002, Federici et al., 2012; Hilfinger at al., 2011; Berthoumieux 2013) have been developed to extract the effect of extrinsic noise such as variation arising from growth rates (Berthoumieux 2013). The combination of these measurement techniques with observations in highly controlled micro-fluidic environments (Bennett and Hasty, 2009) could lead to better understanding of extrinsic effects on part performance.

A major motivation for accurate measurement of genetic part behavior is to facilitate predictive mathematical modeling. The use of *in vivo* fluorescent markers, in combination with mathematical descriptions, has been critical to deriving estimates of underlying mechanisms, such as transcription rates (De Jong et al., 2010; Leveau et al., 2001; Kelly et al., 2009; Munsky et al., 2012), parameters of induction/repression, and translation efficiency (Salis et al., 2009). In most cases, these estimates are indirect measurements in arbitrary units because the fluorescence level is used as a proxy for underlying processes that are not accessible to measurement. In some cases, direct measurements can be made, for instance, single molecule fluorescence in situ hybridization (smFISH) has been used to observe mRNA distribution in individual cells and study changes in transcription over time (So et al., 2011). However, in general it is not possible to measure all the parameters of interest, and it is usually necessary to use rough estimates of parameters and consider their variance (e.g. Basu et al, 2005). An alternative is to consider the plausible range of parameters, and to test a large random sampling of these distributions (Ma et al., 2009, Cotterell et al., 2010). This might lead to a design approach which is not strictly predictive, but that guides construction with estimates of the statistical likelihood of a particular system operating as desired.

Mathematical models of genetic parts, parameterized by measurements, can then be used to derive the dynamics of assemblies or systems (Elowitz et al., 2000; Gardner et al., 2000) and incorporate into simulations. Measurement of the composed system in operation then informs further measurement and refined modeling by comparison to simulations. An interesting example is the transcriptional oscillator (Danino et al., 2010). During the development of this system, it was found that delays generated by queueing for degradation (Cookson et al., 2011) were essential to generate oscillations, which led to further investigation and measurement of these effects, as well as incorporation into models.

Capturing statistical properties, and cell to cell variability of part behavior is important, especially since there are cases where this can lead to unexpected effects (Samoilov et al., 2006,; Neuert et al., 2013). Studies of such effects have been performed using time-lapse microscopy to track reporters in individual cells (Young et al., 2011), using synthetic constructs to analyze the stochasticity in transcription and translation (Swain et al., 2002; Munsky et al., 2012). Stochastic simulations can capture individual cell dynamics, for example using Monte Carlo methods (Gillespie, 1977) or Finite State Projection a more efficient method that estimates the probability distribution of the system state at each time (Munsky et al., 2006; Munsky et al., 2012; Lou et al., 2012). Data from these simulations can be compared to the distributions of reporters within populations, generated by flow cytometry (FACS), to validate underlying models (Lou et al., 2012). Using microfluidic devices, large amounts of data can now also be captured for individual cells over long periods (e.g. Wang et al., 2010; Long et al., 2013), and in combination with inference methods (Neuert et al., 2013) should lead to increasingly predictive models that incorporate stochasticity and other sources of variation.

For predictive modeling, cell growth and division adds another level of dynamics in space and time, which in multicellular systems can contribute significantly to system behavior, e.g. through cell-cell signaling (Danino et al., 2010). Computational modeling of biophysical interactions between cells combined with simple experiments (Volfson et al., 2008; Boyer et al., 2011; Rudge et al., 2012) is beginning to reveal

mechanisms of cellular organization. The recent development of large scale parallel processing architectures (Graphics Processor Units) in low cost, commodity hardware has allowed these models to scale to hundreds of thousands of cells (Rudge et al., 2012). Combined with accurate measurements facilitated by microfluidic devices (Wang et al.,2010) this opens up the possibility of large scale predictive modeling of multicellular systems on desktop computers.

The availability of measured genetic components, cell growth and division, and mathematical models of them, could give rise to effective automated design methodologies (Rodrigo et al., 2011; 2012; Yaman et al., 2012; Beal et al., 2012) that would naturally combine with automated DNA assembly to ease the implementation of genetic designs (Densmore, 2012). For instance, CAD (Computer Aided Design) approaches to compiling high level specification to DNA parts (Chandran and Sauro, 2012; Yaman et al 2012; Beal et al., 2012) are being developed in anticipation of libraries of parts, and could be combined with optimization tools, such as genetic algorithms (Chang et al., 2013).

2. IMPLEMENTATION

Progress on enabling technologies and methods is leading to a transition in the field from the engineering of small proof-of-principle devices such as switches, oscillators and time-delay circuits to the generation of more elaborate systems with a desirable function (Purnick and Weiss, 2009, Lu et al., 2009). This advance can be broadly split in two areas: i) mechanisms for sensing and processing of information, and ii) engineered processes and metabolic optimization as outputs.

2.1. Information processing

The main approach to engineering information processing in biological systems has been boolean logic, concerned with component states that are either "on" or "off" according to certain thresholds (Miyamoto et al., 2012, Weiss et al., 2003). Inspired by the critical role of boolean abstractions in the development of electronics, the resemblance of genetic networks to electrical circuits (McAdams and Shapiro, 1995), and the early application of boolean analysis to genetic regulation (Sugita, 1963; Kauffman, 1969, Thomas, 1973), the synthetic biology field has adopted a similar approach to forward engineering genetic circuits. Although a simplified view, the boolean abstraction has served to understand gene regulatory networks since the early studies on transcriptional regulation pioneered by Jacob and Monod (1961), and continues to be useful today (Peter et al., 2012; Albert and Othmer, 2003; Mendoza et. al., 1999; Espinosa-Soto et al., 2004; Li et al., 2004).

The aim of implementing boolean logic schemes in engineered cells is not to compete with silicon-based technologies, but to program molecular systems to sense, process and respond *in vivo* and at nano scale to certain signal profiles, such as from cancer cells (Douglas et al., 2012; Xie et al., 2011). The approach has led to the design of all possible two-input boolean logic functions (e.g. NOR gates, Tamsir et al., 2010; Win and Smolke, 2008) and higher order information processing functions (Moon et al., 2012). Applications ranging from biosensing (Rinaudo et al., 2007, Wang et al., 2013) to arithmetic operations in mammalian cells (Auslander et al., 2012) have demonstrated the scope and potential of boolean systems for the implementation of human-programmable decision-making networks in cells. Techniques for implementing boolean logic have included transcription and translation regulation (Tamsir et al., 2010, Liu et al., 2012), protein switches (Grunberg and Serrano, 2010; Dueber et al., 2003), as well as recombinase (Siuti et al., 2013) and integrase enzymes (Bonnet et al., 2012; 2013).

To achieve engineered logic-based decision making as complex as natural systems would require a very large set of orthogonal components-parts which do not interfere with each other. As opposed to electronic circuits where crosstalk is avoided by an spatial distribution of components; cells rely on chemical specificity and differential affinity for chemical isolation (Hartwell et al., 1999). The distribution of elements in the cell forces the components to be chemically isolated from each other. This problem has been tackled by separation of gates in different bacterial colonies and the use of diffusible signals to link them (Tamsir et al., 2010), and inspiration from distributed computing (Regot et al., 2011). Recent work (Liu et al., 2012) has developed orthogonal RNA-based transcription and translation regulators, and used them to compose NOR gate-based regulatory functions in single cells. The number of orthogonal parts could also be increased by computational protein design (Van der Sloot et al., 2009; Jiang et al., 2008; Röthlisberger et al., 2008); protein shuffling (Grunberg and Serrano, 2010); directed evolution (Moon et al., 2012), rational design of DNA-protein interactions (e.g.TAL effectors, Garg et al., 2012; Boch et al., 2009; Blount et al., 2012), RNA-based protein-DNA interaction (e.g CRISPR, Farzadfard et al, 2013) and complete orthogonal transcription and translation mechanisms (An and Chin, 2009).

DNA computing is another approach with promising prospects for implementing information processing in cells (Benenson, 2011). Based on DNA displacement reaction mechanisms, this method has been used to perform logical and numerical operations (Qian and Winfree, 2011), and can achieve modularity and orthogonality through DNA sequence specificity. DNA molecules also have the advantage of coupling information processing and mechanical behaviour, allowing sensing, processing and actuation to be performed by the same type of molecules at nano-scale. This feature has been exploited to create nano-structures whose cargo release depends on DNA sequence-specific unlocking (Andersen et al., 2009) and aptamer-gated control (Douglas et al., 2012).

A complementary approach to tackle issues of orthogonality and number of parts is the use of feedback mechanisms in simpler designs inspired by naturally evolved systems. Simpler circuitry, in combination with cell-cell signaling, nonlinear reactions and the stochastic nature of gene expression, give rise to the emergence of complex biological phenomena such as morphogenesis (Turing, 1952, Gierer and Meinhardt, 1972) and cell differentiation (Suel et al., 2006: Maamar et al., 2007; Cagatay et al., 2009). Small genetic regulatory circuits exhibiting nonlinear behavior and multiple states, such as those found in metabolic networks (Ozbudak et al., 2004) and morphogen interpretation (Kicheva et al., 2012), could aid in the engineering of complex information processing schemes with simpler implementations. The use of feedbacks, a critical mechanism for many biological processes (Ferrel et al., 2002); has already enabled the development of complex systems such as oscillators and traveling waves of

gene expression (Danino et al., 2010), switches (Gardner et al., 2000), and excitable media (Suel et al., 2006). Information processing challenges in other fields have already found inspiration in the non-linear, highly parallel and self-organizing mechanisms of biological systems such as social insect behavior (Bonabeau et al., 2000), neural organization (Hopfield,1982), tissue patterning (Afek et al., 2011), and slime mold foraging strategies (Tero et al., 2010, Fratzl et al., 2007, Adamatzky and Jones, 2010).

Re-applying these concepts to the engineering of biological systems, engineering biology with biology, could shed light on the engineering of decentralized decision-making mechanisms, self-assembly and robustness in engineered systems. For instance, the dissection of input-output relationships in bacterial chemotaxis (Barkai and Leibler, 1997; Alon 1999; Yi et al., 2000) and animal development (Goentoro et al., 2009; Goentoro and Kirschner 2009; Cohen-Saidon et al., 2009) could provide a blueprint for implementing fault-tolerance in man-made biological systems. Systems Biology is starting to catalog the motifs (Shen-Orr et al., 2002) and identify the topologies that underlie robust behavior (Hartwell, 1997; Yi et al., 2000; Kitano, 2007; Shinar and Feinberg, 2010; Barkai and Leibler, 1997; Stelling 2004; Jeong et al., 2000; Albert et al., 2000; Balaji et al., 2006). These could be abstracted and used as modular core functions in engineered higher order implementations (Lim et al., 2013) and rewired under certain objective function constraints to direct the evolution of desired performance. A recent innovation is the application of principles from analog computing to engineer regulatory networks to perform continuous valued (rather than digital or binary) transformations of inputs such as arithmetic operations (Ramiz et al., 2013).

2.2. Output

Complete sense-and-respond functional systems and applications have emerged from the interfacing of the signal processing mechanisms described above with output processes of interest. For instance, cells have been engineered to maintain uric acid homeostasis (Kemmer et al., 2010). target human pathogens (Saeidi et al., 2011) and invade cancer cells (Anderson et al., 2006).

An area of increasing interest is the programming of emergent behavior and self-organizing processes in multicellular systems and consortia (Shong et al., 2012). The implementation of artificial cell-cell communication mechanisms in bacteria (Bulter et al., 2004; Brenner et al., 2007; Basu et al., 2005), yeast (Chen and Weiss, 2005) and mammalian cells (Wang et al., 2008) has been exploited to engineer collective behavior (You et al., 2004; Liu et al.; 2011; Danino et al., 2010). For instance, Wu et al. (2013) engineered mammalian cancer cells to produce a quorum sensing signal (AI-2), and concurrently created a strain of AI-2 responsive *E coli*. The bacteria were further engineered by coupling the AI-2 response to their native chemotaxis system, causing them to migrate towards nearby mammalian cells, and also produce a fluorescent protein signal. Hence two natural bacterial behaviors (quorum sensing and chemotaxis) were combined with engineered regulatory networks to produce a useful functional output - aggregation of fluorescent bacteria on cancer cells.

The engineering of multicellular populations could lead to the programming of pattern formation, artificial cell consortia and synthetic ecosystems (Brenner et al., 2008, Brenner and Arnold, 2011, Shou et al., 2007; Wintermute and Silver, 2010; Chuang, 2012). The establishment and maintenance of distinct cohorts of cells might be used for example to facilitate the optimization of metabolic pathways by separating inhibitory intermediate metabolites. Multicellular and colonial systems exploit cell differentiation for the compartmentalization of tasks, for instance the C4 photosynthesis system improves enzymatic efficiency by delegating different steps to distinct leaf cell-types. The use of artificial cell-cell communication, symmetry-breaking mechanisms (Turing, 1952; Gierer and Meinhardt 1972), cell-type domain maintenance (Perales and Reddy, 2012), mechanical cell interaction (Rudge et al, 2012; Boudaoud et al., 2010), and growth (Liu et al., 2011) could lead to the development of self-organized, distributed systems such as morphogenesis. The rewiring of modular cellular behaviors in multicellular systems would add an extra layer of abstraction to synthetic biology (Fig. 1A).

3. MODELING BIOLOGY WITH BIOLOGY: AN APPROACH TO ACCELERATING BIOLOGICAL RESEARCH

In addition to the benefits for technology, Synthetic Biology is helping biological researchers to gain a better understanding of living organisms (Bashor et al., 2010; Isaacs et al., 2003). Synthetic circuits have already provided insights into the functioning of natural processes such as mechanisms of evolution and selection (Chuang et al., 2009; Chuang et al., 2010; Sekine et al., 2011); population dynamics (Weber et al., 2007; Balagadde et al., 2008; Tanouchi et al., 2012); stochastic processes in molecular networks (Pedraza and van Oudenaarden, 2005; Elowitz et al., 2002; Blake et al., 2003); robustness and performance of gene regulation (Damle and Davidson 2012; Becskei and Serrano, 2000; Cantone et al., 2009; Isaacs et al., 2003); animal physiology (Chow and Boyden, 2011); signaling mechanisms in immune cells (Schamel and Reth, 2012); and genome-wide network evolvability (Isalan et al., 2008). Reconstituting natural systems; modeling biology with biology, is refining reverse engineering tools (Cantone et al., 2009) and helping in the study of fundamental question in biology (Liu and Fletcher, 2009). The assembly of dynamical biological systems will aid the transition from research focused on individual molecules, which dominated twentieth-century biology, to a more holistic perspective such as the approach proposed by Systems Biology (Hartwell et al., 1999).

A profitable relationship is already growing between Systems and Synthetic Biology (Lanza et al., 2012) (Fig. 2). Both fields share a view of biology based on dynamical systems, rather than isolated genes, and see similar challenges in obtaining accurate measurements, recognizing the important parameters to measure, and integrating these values into higher-order abstractions (Smolke and Silver, 2011). The use of forward and reverse engineering approaches in combination with machine learning tools (Bongard et al., 2006; Bongard and Lipson, 2007; Schmidt et al., 2009; Schmidt et al., 2011) could lead to new ways of modeling and understanding biological phenomena as well as refining the design principles of Synthetic Biology. Applying the design-build-test cycle of synthetic biology to Systems Biology would benefit both hypothesis-driven experiments and forward engineering of

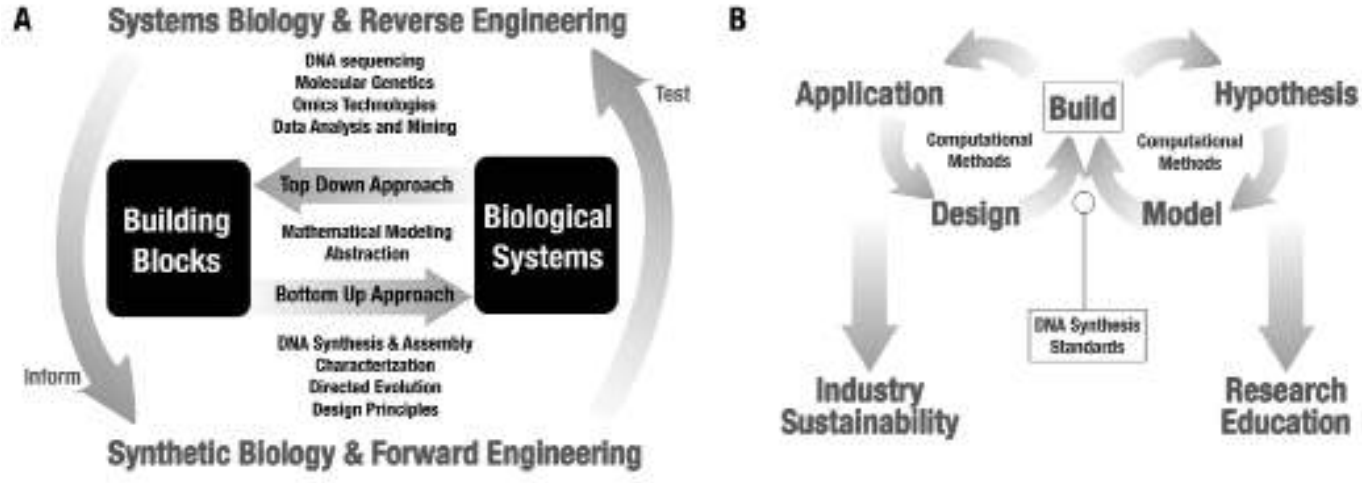

Figure 2. Synergy between Synthetic and Systems Biology. A) Systems Biology and its methods can inform the design process of Synthetic Biology and provide new parts and network motifs; whereas Synthetic Biology and its tools can benchmark Systems Biology tools and test its models by rewiring and refactoring natural systems. Abstraction and mathematical modeling are key tools for the systematic analysis of naturally evolved systems as well as the engineering of artificial biological functions. B) The advent of large scale DNA synthesis technologies and the development of standard for bioengineering will accelerate forward and reverse engineering cycles through faster and more predictable ability to build large genetic systems. These improvements will boost the industry, educational programs and scientific research.

biology (Fig. 2B). Thus, Systems and Synthetic Biology can both gain from each other's resources and methods (Smolke and Silver, 2011; Lim et al., 2013).

4. LEARNING BY MAKING: A NEW PERSPECTIVE FOR TEACHING BIOLOGICAL SCIENCES AND BIOENGINEERING

With decreasing costs in DNA manufacturing technologies, innovation and entrepreneurship in Synthetic Biology becomes accessible not through infrastructure, but mainly through knowledge and human capital. Therefore, developing Chile's future human capital through new educational programs results crucial to play a major role in the future development of bio-economies. Synthetic Biology and its more reliable methods are already transforming educational programs (Elowitz and Lim, 2010). With a radical shift towards "learning by making", the refactoring of biological sub-systems represents a more engaging approach for teaching Biology and Bioengineering. This idea has already given rise to an innovative educational program, namely the international Genetically Engineered Machine competition (iGEM) (Smolke, 2009). This is an international contest in Synthetic Biology for undergraduate students, with participants from more than 180 different universities. iGEM is held in the spirit of robotics competitions in engineering fields, except that the students face the challenge of conceiving, designing and implementing a synthetic biological system and operating it in living cells. Open access to a collection of genetic parts, robust assembly methods for the construction of devices, user experience information regarding device implementations and parts performance, and a community repository of experimental protocols (www.openwetware.org) has significantly eased the construction of genetic systems. More efficient methods of DNA assembly and the abstraction of lower level DNA details allow students to focus on system design and modeling, and not on manufacturing the basic building blocks. A clear indication of the potential of these enabling tools is the fact that in just 12 weeks, students with no previous experience in molecular biology have assembled novel biological systems such as bacterial photo-films (Levskaya et al., 2005) and spatiotemporal patterning mechanisms (Liu et al., 2011). P. Universidad Católica de Chile sponsored the first Chilean iGEM team in 2012 (www.igemuc.cl), which was selected in the Latin American regional final to compete in the grand final at MIT. More Chilean teams are expected this year and further support to these initiatives will undoubtedly contribute to the creation of the human capital and infrastructure required for engaging Chile with a promising technology and its nascent economy.

5. LEGAL, ETHICAL AND SOCIAL CONSIDERATIONS

The application of Synthetic Biology will produce recombinant organisms that could be used in large-scale contained use, biomedical therapies or for field release. The new technology has great potential to provide substantial benefits for human health, nutrition and sustainable industrial practices. However, as our ability to manipulate DNA and engineer new biological systems advances, so does concern about the impact (due to unforeseen consequences or human error) that new organisms may have on our environment. Most surveys of the potential risks associated with Synthetic Biology (e.g. New Directions: The Ethics of Synthetic Biology and Emerging Technologies. 2010; http://bioethics.gov/synthetic-biology-report) have recognized the similarity of the technology with existing approaches that use organisms with genetic modifications (e.g. transgenic crops). As for genetically modified organisms, the main areas of concern are (i) the dangers of environmental release of genetically modified organisms due to invasive growth or predation, (ii) unforeseen problems for human or

animal health through consumption or exposure to genetically modified products, and (iii) inequities that might arise through concentration of ownership and control of the new technologies by a few corporations. At this time, anticipated Synthetic Biology products fall within the scope of existing regulations and practices for handling genetically modified organisms.

However there is a recognized need to maintain a watching brief on development of the technology, and to ensure that existing regulatory systems are revised to take account of future developments in the field. Those who find the use of genetically modified organisms unacceptable, find similar concerns with the application of Synthetic Biology. A coalition of non-governmental organizations have called for a moratorium on the release and commercial use of Synthetic Biology products (The Principles for the Oversight of Synthetic Biology, 2012). In contrast, corporations and their lobbyists are keen to explore applications of the technology, and vigorous debate is in progress. The international scientific community has played a substantive role in this debate, promoting discussion of the practical potential and ethical implications for the technology, and contributing to policy development internationally. This healthy debate is likely to continue, as the field continues to develop, and we can better evaluate its potential contribution to improvement of sustainable practices for agriculture, production and conservation, and balance these factors with risks due to the new technology, and existing unsustainable practices. Chile should promote similar discussions and debate to generate an adequate legal framework and regulatory system for Synthetic Biology as well as other technologies that use genetically modified organisms.

The engineering of living systems has raised issues around ownership of the technology. Current business practices in biotechnology promote patent protection of all key innovations. The securing of intellectual property is crucial for company investment, licensing income and freedom to operate in the market. Restrictive licensing practices and patent protection are often used as tools to secure market share, as well as to protect a company's investment in innovation. Synthetic Biology offers the prospect of large-scale reprogramming of living systems, but this will require access to relatively large numbers of components. This is in contrast to existing GM products that contain one or a few components. There is growing requirement for libraries of well characterized routine components that can be shared for the construction of a variety of systems, where small companies would be ensured freedom to operate. This is essential to foster the kind of innovation seen at the emergence of other new technologies such as microelectronics and software development.

Accordingly, a substantial part of the Synthetic Biology field has promoted open standards and sharing of data and resources, inspired by the open source software movement. The educational community has embraced the open source principle in the establishment of the MIT Registry of Standard Parts, which is widely and freely distributed internationally, and grows year-on-year. The BioBricks Foundation is a non-profit organization which has roots in the academic community, and which has promoted a legal framework which would allow protection of Synthetic Biology applications and key activities, but which would facilitate sharing of parts. The UC Berkeley-Stanford BioFab recently made the sequences and measurement data for its collection of parts available online.

These kinds of resources are precious, enabling freedom to operate for individual inventors, entrepreneurs and small companies. However these resources are also fragile, and as commercial interest increases, the pressure to restrict access to innovations and new resources will rise. It is therefore important to consider the legal framework for protection of intellectual property and the types of institutions gathering and storing data on biological resources, in order to foster open innovation. Maintaining a decentralized, globally accessible, and open database of parts would foster access to these new technologies outside elite research institutions and large biotechnology corporations. Advances in DNA synthesis technology, that decouple DNA-encoded biological information from its physical DNA substrate, allow engineers to reconstitute functional genetic devices anywhere in the world by 'compiling' digital information. Maintaining open access to this digital information would make the technology inherently low cost; leading to a knowledge-based economy with obvious applications in developing countries.

6. FUTURE PROSPECTS FOR CHILE: PROGRAMMING BIOLOGY AND POTENTIAL APPLICATIONS TO CHILEAN INDUSTRY

Synthetic biology could benefit Chile and other Latin American countries by boosting existing bio-industrial platforms with more reliable tools and methods, and opening a new horizon for biotechnological applications. The World Economic Forum placed synthetic biology at the forefront of the coming century's economic agenda (World Economic Forum Global Agenda Council on Emerging Technologies, 2012). It has also been made a priority by the US government as a key area for the transition to the bio-economy (National Bioeconomy Blueprint). Recent developments such as the production of antimalarial drug precursor artemisinin in microbes (Ro et al., 2006; Peplow, 2013), the refactoring of a nitrogen fixation pathway (Temme et al., 2012) and the production of biofuel and chemicals (McEwen and Atsumi, 2012) with engineered microbes have shown the potential of synthetic biology for the generation of drugs, new compounds as well as renewable resources. Advances in the design of robust genetic circuits and systems will lead to faster, affordable and more efficient development of such applications.

Most biotechnological developments rely on the modification of existing natural components such as the recent generation of genome editing tools based on mechanisms of *Xanthomona's* infection of plants (Garg et al., 2012; Boch et al., 2009; Moscou et al., 2009; Blount et al., 2012). Chile has a unique opportunity to contribute to expanding this repertoire of biological components, processes and chassis by tapping into its genetic biodiversity. Chile's geography constitutes an advantage by spanning a wide range of climates and environments from antarctic to desert ecosystems. For instance, the Atacama desert, the driest desert on earth, contains unique microorganisms and plants adapted to harsh environmental conditions (Navarro-Gonzalez et al., 2003; Drees et al., 2006; Villagran et al., 1981). Mining this biodiversity for the development of novel sensors, actuators, effectors and other parts is possible if funding programs for exploratory or catalog type research projects are established in Chile. The re-engineering of these natural biological functions could generate new industries and have a great economic

impact in the region. A single engineered enzyme used in laundry detergents that can reduce hot water energy use by the equivalent of 100.000 barrels of oil a day in the US (http://democrats.energycommerce.house.gov/documents/20100527/Endy.Testimony.05.27.2010.pdf). Chile is currently supporting innovation and entrepreneurship (Orellana, 2004), however a great deal of work is necessary to generate the tools and building blocks necessary for new Synthetic Biology-based biotechnologies. We currently have significant support for basic and applied research programs. Complementing these efforts with technology funding programs will guarantee the generation of sufficient parts, devices and circuits for developing Synthetic Biology applications.

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