Krassimir Markov, Vitalii Velychko,
Lius Fernando de Mingo Lopez, Juan Casellanos
(editors)

New Trends in Information Technologies

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BACTERIAL TECHNOLOGY TO BUILD COMPUTERS: A SURVEY

Paula Cordero, Sandra Gómez, Rafael Gonzalo

***Abstract**: Synthetic biology is an emerging discipline that combines knowledge from various disciplines including molecular biology, engineering, and mathematics to design biological devices whose goal is to extend or modify the behavior of organisms and engineer them to achieve desired functions for broad applications. This paper provides a brief survey about the methods for altering the behavior of individual elements and the construction of complex networks in single-cell. In this review, we shown and analyze the recent advances in synthetic biology towards engineering complex living single-cell by designing genetic circuits to perform new tasks in bacteria behavior.*

***Keywords**: Synthetic biology, bacterial engineering, bacterial computation, natural computing*

***ACM Classification Keywords**:*

Introduction

It has been observed for several years the emergence of synthetic biology, a new area of biological research that aims to engineer biological devices with desired functions for broad applications. A large set of advances in this field highlight the potential of this discipline to impact diverse areas, including environment, bioremediation and computation. Synthetic biology aims to create novel behaviors through the engineering of genetic elements and the integration of basic elements into circuits that implement more complex functions that do not occur in nature [Weiss, 2005]. The design of synthetic biology applications includes living organisms, hardware and software components representing the instruments and the application to acquire and process signals generated by the biological component of the system. Early efforts aimed at altering the behavior of individual elements have now evolved to focus on the construction of complex networks in single-cell and multicellular systems. Synthetic biology needs to develop more sophisticated design strategies because offers valuable quantitative insight into naturally occurring information processing activities.

From the information codification point of view, synthetic biology has tackled a great change of concept in the field of computing. Molecular computing consists of representing the problem's information with organic molecules and making them react within a test tube in order to solve a problem. This concept is based on the work made by Leonard M. Adleman, where the first DNA computation based on Operations to hard combinatorial problem solved using desoxirribonucleic acid molecules [Adleman, 1994]. In this point, all the computations carried out in vitro based the codification of the problem solutions on representing them into organic molecules and as a result it was required the presence of a scientist to execute the experiments and give meaning to the solution obtained in the context of the problem domain. Here, the great difference provided by synthetic biology appears. In particular, in the area of bacteria computing the codification of the information is implemented at genetic level, this way the organisms are equipped with autonomous computing abilities due to the information have biological functionality in this case. At higher level, these computations have a biological meaning for organisms that carry out it and as a result applications in complex systems with specific purposes can be applied; this is the main aim of synthetic biology.

This paper is structured in the following way: firstly, a background about engineering bacteria is shown in order to present a framework of the concepts and the importance of this field. Secondly, a set of prominent developments are introduced to show the fast growth of the synthetic biology and the revolutionary change of concept that is

producing in the field of computing. Finally a set of perspectives and conclusions about the potential applications and synthetic biology areas for improving are shown.

Background: Engineering Bacteria

Cells are an important element of nature that serves as a model to abstract efficient and complex functions. Cells can be programmed by identifying three functional layers: an input layer, an information processing layer, and an output layer [Kobayashi, 2004]. In the input layer, they are inherently able to detect small concentrations of chemicals or combinations of chemicals in their environment to process in the processing layer and respond usually with an amplified signal across the output layer. This abstraction allows viewing this functionality as a device that can be manipulated and programmed and therefore performed as a computational device. Therefore, one of the most important aims of the synthetic biology is to design bacteria in order to achieve miniature computers, which could be programmed by designing genetic circuits into bacteria with specific functionalities.

This biological devices or miniature computers represents genetic circuits composed of biochemical reactions and genes of the same manner those electronic circuits work with boolean logic signals and gates. These devices can be engineered with sets of one or more biochemical reactions and are based on inducible promoters which allow us to switch on/off the expression of a certain gene. Every gene is a long double DNA strand which codifies particular information. Gene expression is the main principle of synthetic biology. This is the process by which information from a gene is used in the synthesis of a functional gene product. These products are often proteins, but in non-protein coding genes the product is a functional RNA. Several steps in the gene expression process may be modulated, including the transcription, and post-translational modification of a protein. The initiation of gene transcription is controlled by two sequences located upstream of the gene, the promoters. RNA polymerase recognizes these promoters as a signal to start transcription. This initiating step is the main site at which the rate of gene transcription is controlled. The ability of RNA polymerase to recognize and bind promoters can be altered by accessory proteins which can be activators or repressors. By controlling this process it is possible to achieve biological devices with a broad spectrum of applications.

Synthetic biology can be inspired by chemistry or by engineering to synthesize biomolecules, to develop synthetic analogs and to apply them in the framework of biological systems; and to design of new, complex, bioinspired systems respectively [Pleiss, 2006]. In this field bacteria computing is developed and in order to define these biological devices is necessary a design strategy. As in the design of computer systems, the strategy consists of dividing the complex biological system in a set of parts or reusable modules with defined properties and functions. Each of these parts represents a biological device. Several devices can be assembled in even more complex devices until the final system is constructed. This architecture allows a high level of abstraction because it allows separation of design and construction.

Therefore, complex systems with predictable properties such as computers can be constructed from only a small number of different, standardized basic parts. In accordance, the programmed bacteria could be able to communicate with others, and as a result to operate in a coordinate manner. For instance, Escherichia coli bacteria are designed with quorum-sensing proteins that allow emitting or receiving signals when the concentration level exceeds a certain limit. These useful techniques are applied in the design of communication systems in order to achieve more complex architectures.

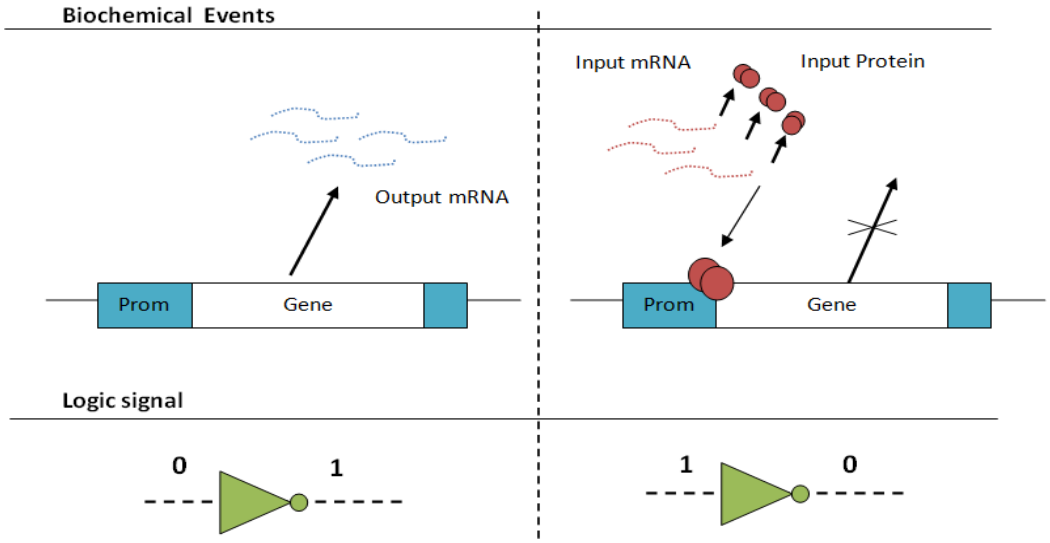

Figure 1: Genetic circuit's modules. A logic signal is represented by a certain mRNA concentration. The gate has a single input signal, the mRNA. Firstly the mRNA is absent and as a result the gene is transcribed into mRNA as signal output. Secondly, when the mRNA input signal is presented a protein is traduced, this protein binds to an operator of the gene's promoter, and RNA polymerase is prevented from transcribing the gene. Applying Boolean logic to the system, where high protein concentrations represent '1' and low concentrations represent '0', it can be seen that the above system is behaving like an inverter. The gate is used to determine the intracellular state of the cell. Source: [Weiss, 2003]

Developing new and more sophisticated design techniques becomes necessary, as synthetic biology evolves. From this point of view, as electronic development carries out systematic comparisons of different possible designs based on user specifications in order to determine an optimal design, the need to conduct the same design process review in the field of synthetic biology comes up. An important design decision is the division of the features implemented at hardware/software level to make the study of the problems arising from the convergence of technologies in heterogeneous systems easier. In the context of synthetic biology applications, the design of wetware components, living organisms, and software/hardware components is required due to the need of obtaining at software level the signaling processing generated by the biological hardware components involved. It is recognized at higher level that the component wetware is heterogeneous due to transcription, translation and proteomic components handled in this kind of devices represent different areas of design. In this sense, it is necessary to use electronic engineers' knowledge about design methods of complex heterogeneous systems in order to optimize its efficiency, achieve more flexible biological applications and reduce the production costs of devices.

In the work developed by the group of David A. Ball in 2010 [Ball, 2010] three distinct solutions to a specification, namely the detection of distinct combinations of chemical signals. Figure 1. In the first option, hybrid promoters that contain binding sites for transcription factors responsive to the inputs are used to control the expression of fluorescent proteins. As the input signals of devices increase, the number of promoters needed to model de system increase also, this situation could be a disadvantage. The number of components needed to detect the different combinations of inputs may require more promoters which can be arranged. The second proposal implements the logic design at the protein level. This is accomplished by coupling each input to the expression of a non-fluorescent fragment of a fluorescent protein. This will get more flexibility to get larger and more complex

devices. However, the number of fluorescent proteins used in this modeling is also limited. By using protein self assembly, the logic design at this level provides to the device with a lower activation response at the transcription level which may be interesting, it depends on the device required. Finally the third level focuses on the study of the fluorescence spectrum for the identification of the input molecules. This option is to embed the logic in the electronic layer. In this case each input directly activates the expression of one of the different fluorescent proteins and the inputs present are determined by processing the pattern of fluorescence that is obtained. By implementing the logic outside of the biological system, the number of molecules possible to distinguish between is greatly increased, limited only by the number of transduction mechanisms and reporters.

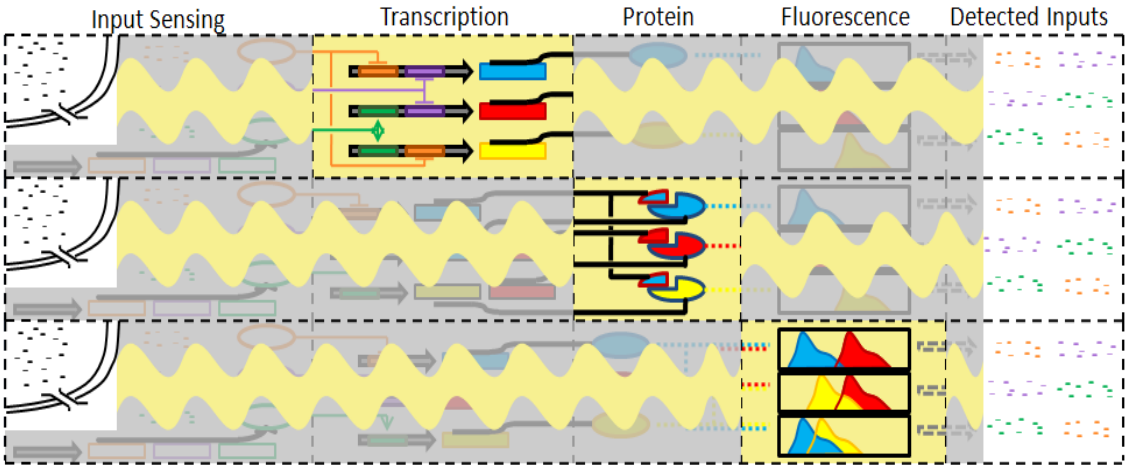

Figure 2. Implementation of logic in different design domains. The figure gives an overview of how each approach processes the environmental inputs. Wavy yellow lines indicate signal transduction, and yellow boxes highlight where the logic occurs. Source: [Ball,2010]

Applications

The main motivation of synthetic biology arises from the notion of programmable cells capable of resolving highly complex problems. Basic elements, for example promoters, ribosome binding sites and transcriptional repressors were combined to form small behaviors with specified modules. Devices as logic gates, switches or oscillators have been Implemented by using this kind of techniques. These and other modules can be used to regulate gene expression, protein function, metabolism and cell-cell communication.

In the last few years many challenges have been tackled in order to achieve the main objectives of synthetic biology. Many developments and efforts have been combined to design and build approaches to use in fields such as bioremediation, biomedical therapies, molecular fabrication of biomaterials, sustainable energy production etc.

A first interesting work was proposed in [Weiss, 1999]. Weiss et al., presents a design paradigm for gene-expression based digital logic implemented in vivo. The proposed modular abstraction enables the construction of complex digital logic circuits into genetic regulatory networks using a library of interchangeable components. The chemical activity of such genetic network in vivo implements the computation specified by the digital circuit. Logic signals are implemented by rates of DNA binding proteins since they can function as transcriptional repressors. This manner the flow of logical information is represented as the effect of one protein on the transcription rate of

another. Gates are represented by structural genes which codifies output proteins. These genes are fused to promoter/operator regions that are regulated by input proteins.

An implementation of a specific device, an oscillator denominated the “repressilator”, was presented in [Elowitz, 2000]. This approach is composed of two plasmids consists to oscillating network in Escherichia coli bacteria that use three transcriptional repressor systems. The network periodically induces the synthesis of green fluorescent protein (GFP). The larger plasmid contains the oscillatory circuit of the repressors (LacI, tetR and CI). The first repressor inhibits the transcription of the second repressor gene which in turn inhibits the expression of a third gene completing the cycle, as is illustrated in the Figure 3. The observation of GFP permits monitoring the repressilator, which oscillates at a regular interval. The periodicity of the GFP cycle was much longer than the periodicity of cell division by the bacteria, which indicates the signaling mechanism outlived the lifetime of any given cell [Campbell, 2005].

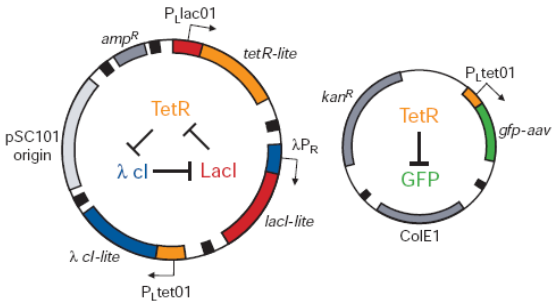

Figure 3: The repressilator network: cyclic negative-feedback loop composed of three repressor genes and their corresponding promoters, as shown schematically in the centre of the left-hand plasmid. Source: [Elowitz, 2000]

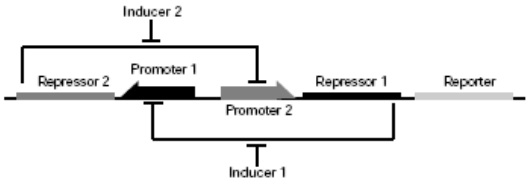

Figure 4: Toggle switch design: repressor 1 inhibits transcription from Promoter 1 and is induced by Inducer 1. Repressor 2 inhibits transcription from Promoter 2 and is inducedby Inducer 2. Source: [Gardner, 2000]

Another interesting work based on logic circuit, published in the same year and that uses an Escherichia coli was published in [Gardner, 2000]. This work presents the design and construction of a genetic bistable toggle switch in Escherichia coli. Its design is simple: two promoters and two constitutive promoters. When the black gene is active, the gray gene and the reporter gene are silenced as illustrated int the Figure 4. In this sense the toggle exhibits bistability.

An original application “bacteria ‘photograph” was reached by Levskaya et al., in [Levskaya, 2005]. In this work is showed a smart a light pattern as a high-definition chemical image. This system is switched between different states by red light and consists of a synthetic sensor kinase that allows a lawn of bacteria to function as a biological film, such that the projection of a pattern of light on to the bacteria produces a high-definition two-dimensional chemical image. This spatial control of bacterial gene expression could be used to ‘print’ complex biological materials, for example, and to investigate signaling pathways through precise spatial and temporal control of their phosphorylation steps.

To encourage the development of applications in this field and attract engineers to form interdisciplinary groups was created the iGEM - Synthetic Biology Summer Competition. This competition consist of teams of multidisciplinary groups of students whose goal is to design a genetic circuit with an interesting feature with its mathematical model and verify its operation with an implementation experimentally in the laboratory. This initiative was born of SynBioComm, an organization that aims to create a community of researchers in synthetic

biology through conferences such as the European Conference on Synthetic Biology and the participation in the iGEM, which is also promoted annually by the Massachusetts Institute of Technology.

In this context, the Pico-Pumbler project shows the results obtained by the iGEM. Pico-Pumbler presents a set of plumbers bacteria [PicoPlumber, 2009]. In this development E. coli is engineered to detect the breach responding to an inducer molecule (IPTG) released from this site. Bacterium is sensed by the inducer IPTG and swim towards breach so that can repair it. Pipes released he inducer only in the case of a breach in the wall. To do this, is used quorum sensing. This mechanism of some microorganisms permits that a bacterium can detect the presence of other bacteria in the neighborhood. The density of bacteria increases substantially close to the leak. Hence, by the quorum sensing signal, bacteria know that they have reached the leaking site, and therefore, they can start producing the glue proteins. Bacterium lyses after a certain time interval, during which they have produced a sufficient amount of the glue proteins. This project is designed in a modular architecture that permits that individual gene modules can be mathematically modeled and their design improved, before being built and tested and of this manner ensures a challenging research project.

Perspectives and Conclusions

The synthetic biology strategy consists of applying the knowledge of biological systems in order to design new biological devices with new and improved properties. This strategy is similar than the challenges that allowed organic chemistry to develop new organic compounds with interesting non-natural properties. This challenge concerns the adaptation of engineering practices to the design of biologically-inspired systems and the improvement of its development and standardization in order to design more complex system with a large set of functionalities. The development of practical synthetic biology devices will require a system-level analysis and a design approach that have yet to be explored.

A new meaning is given to the concept of information codification; the information is encoded into organic molecules with meaning as long the scientist as the organisms which are the support of the computation. As a result it is possible to design bacteria with the ability of autonomous computing in order to apply these functionalities in different fields like environment, new materials, industrial processes, energy and biomedicine. These applications are the main challenges of synthetic biology and in order to achieve them it is necessary to improve same of the synthetic biological devices design techniques. Most of synthetic biology efforts have to be focused on the following aspects: the identification, characterization and design of synthetic systems with programmable and controlled behavior. The development of efficient practices in order to integrate modules in a more complex system, the capacity to replace natural genomes by synthetic genomes is one of the main challenges to achieve. The characterization and standardization of biological modules in order to obtain engineers specialized in both areas: the design of basics modules and complex systems and finally the improvement of the no controlled noise of the biologically-inspired systems.

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Authors' Information

Paula Cordero *– Natural Computing Group. Universidad Politécnica de Madrid, Boadilla del Monte, 28660 Madrid, Spain: e-mail: p.cordero@alumnos.upm.es*

Sandra Gómez - *Natural Computing Group, Universidad Politécnica de Madrid, Carretera de Valencia Km 7., Madrid, Spain: email: sgomez@eui.upm.es*

Rafael Gonzalo *– Artificial Intelligence Department. Facultad de Informática. Universidad Politécnica de Madrid, Boadilla del Monte, 28660 Madrid, Spain. e-mail: rgonzalo@fi.upm.es*