Synthetic biology & microdevices: a powerful combination

VENKEN LYN, KU Leuven
MARCHAL KATHLEEN, KU Leuven, UGent
VANDERLEYDEN JOS, KU Leuven

Recent developments demonstrate that the combination of microbiology with micro- and nanoelectronics is a successful approach to develop new miniaturized sensing devices and other technologies. In the last decade, there is a shift from the optimization of the abiotic components, e.g. the chip, to the improvement of the processing capabilities of cells through genetic engineering. The synthetic biology approach will not only give rise to systems with new functionalities, but will also improve the robustness and speed of their response towards applied signals. To this end, the development of new genetic circuits has to be guided by computational design methods that enable to tune and optimize the circuit response. As the successful design of genetic circuits is highly dependent on the quality and reliability of its composing elements, intense characterization of standard biological parts will be crucial for an efficient rational design process in the development of new genetic circuits. Microengineered devices can thereby offer a new analytical approach for the study of complex biological parts and systems. By summarizing the recent techniques in creating new synthetic circuits and in integrating biology with microdevices, this review aims at emphasizing the power of combining synthetic biology with microfluidics and microelectronics.

Categories and Subject Descriptors: J.3 [Computer Applications]: Life and Medical Sciences---biology and genetics; J.6 [Computer Applications]: Computer-aided Engineering---computer-aided design (CAD)

General Terms: Design, Standardization

Additional Key Words and Phrases: synthetic biology, standard biological parts, microfluidics, microelectronics, electrogenic circuits

1. INTRODUCTION

Synthetic biology is an emerging discipline in biological research that aims to create novel behavior in organisms by applying engineering principles to biological systems [Endy 2005; Andrianantoandro et al. 2006]. This research field is expected to have a great impact as already demonstrated by the development of alternative energy resources, new sensor mechanisms and therapeutics based on the engineering of micro-organisms [Martin et al. 2003; Levskaya et al. 2005; Lee et al. 2008]. The synthetic biology approach for the engineering of micro-organisms relies on the assembling of biological parts each performing a specific biological function into a new biological circuit. For this to succeed, libraries of biological parts are needed in which synthetic biologists can search for appropriate biological parts, that when assembled, will perform a desired biological function. To this end, the Registry of Standard Biological Parts (http://partsregistry.org), which is a database of standardized biological parts called BioBricks, was developed. This open-source collection of biological parts provides the synthetic biology community a platform to exchange knowledge and experience as an attempt to increase the speed of the development process of new biological systems. However, biological complexity still hinders the reliable and efficient construction of new genetic circuits, driving synthetic biologists to develop new tools and techniques to simplify the tedious and time-consuming process of creating new genetic circuits [Lucks et al. 2008; Marchisio and Stelling 2009; Clancy and Voigt 2010; Lux et al. 2011; MacDonald et al. 2011]. Mathematical modeling, computer-aided design, standardization and characterization of biological parts are hereby crucial aspects as discussed in this review. Recent techniques in model-based design and parts characterization will also be summarized, accentuating especially the developments that still need to be

Authors' addresses: L. Venken, J. Vanderleyden and K. Marchal, Centre of Microbial and Plant Genetics (CMPG), K.U.Leuven, Kasteelpark Arenberg 20, bus 2460, B-3001 Leuven, Belgium; K. Marchal, Department of Plant Biotechnology and Bioinformatics, UGent, Technologiepark 927, B-9052 Gent, Belgium; email: lyn.venken@biw.kuleuven.be.

achieved in order to improve the design process of genetic circuits. New technologies will thereby be essential to increase our overall understanding of biological parts and systems.

As there is still a lot to be achieved in synthetic biology, this review aims to take one step further by proposing that the integration of microbiology with microfluidics and microelectronics will create new opportunities for the synthetic biology community. Microengineered platforms not only allow maintaining and feeding micro-organisms by providing fluidics and reagents through microchannels, but also allow controlling and monitoring the cellular behavior by signal detection and analysis which can give rise to new analytical approaches for the characterization of biological parts [Weibel et al. 2007; Bennett and Hasty 2009; Gulati et al. 2009; van der Meer and Belkin 2010; Vinuselvi et al. 2011]. Since the scale of microelectronics matches well the physical dimensions of micro-organisms, the processing capabilities of micro-organisms can be further exploited by integrating whole-cell elements into microelectronics [Sayler et al. 2004; Weber et al. 2008]. This review will rather give a biological view on how the combination of microbiology with microfluidics and microelectronics can give rise to new analytical technologies and novel bioelectronic devices, and emphasizes the need to bring biologists, physicists and engineers together in order to enhance and fully exploit the multidisciplinarity of synthetic biology.

2. COMPUTER-AIDED DESIGN OF BIOLOGICAL CIRCUITS

Up to now, most current developed synthetic biological systems are generated by a trial and error based approach, in which a small set of components is assembled and tested *in vivo*, without *a priori* mathematical modeling. This *ad hoc* design process is rather time-consuming and inefficient, limiting the creation of novel synthetic biological systems with a variety of functions. In other engineering disciplines, like mechanical and electronic engineering, design processes of new functional devices are accompanied by computer-aided design (CAD) tools, increasing the speed of the design process and as a consequence decreasing the development cost. Recently, the electronic design automation (EDA) in electronics has been described as an inspiring model for synthetic biology [Gendrault et al. 2011; Lux et al. 2011]. This model contains an iterative design flow in which CAD models are used for the *a priori* design of an electronic circuit to meet the specifications of a desired system. As the engineering of biological systems is proved to be more challenging than anticipated, several groups have been developing CAD tools for synthetic biology in order to establish a similar iterative design process [Goler 2004; Chandran et al. 2009; Czar et al. 2009; Weeding et al. 2010; Beal et al. 2011; Marchisio and Stelling 2011b; Chen et al. 2012].

In this process, the construction of a novel biological system starts by defining certain design specifications. Next, libraries of biological parts are screened for parts that will contribute to attain the design objectives. Out of this selection of usable parts, a circuit is designed guided by specified design rules thereby assembling the selected biological parts into the desired synthetic circuit. The initial output of this CAD process will be a DNA sequence that can be physically fabricated and is used to experimentally validate the designed circuit. Comparison of the experimental data with the former simulations by the CAD tools will clarify if the developed circuit meets the design objectives. This design process will be further iteratively repeated until the aimed biological system is fully developed. The construction of complex

synthetic circuits is thus simplified by including a rational design approach in the whole design process. CAD tools and libraries of reusable biological parts are therefore necessary to increase the predictability of gene network engineering and to decrease the time for *in vivo* construction of the obtained system.

2.1 Drag and drop tools

A number of drag and drop tools are developed in which a user can construct a synthetic circuit by selecting and connecting biological components provided by a library. These components are biological parts that represent a certain biological function, such as promoters and ribosome binding sites, or larger devices consisting of a combination of parts performing a more complex function, such as logic gates, light sensors and protein generators. The tools provide an abstract visualization of the different biological parts which simplifies the construction of a genetic construct *in silico*. The user simply has to add all the components and connections between components which represent the biochemical reactions required to obtain a desired biological function. These tools can subsequently generate an annotated DNA sequence and/or a mathematical description of the designed genetic circuit. The mathematical model will describe a set of biochemical reactions, including transcription and regulation, and can be used to simulate and predict the behavior of the designed genetic circuit. The tools allow to change parameter values and initial conditions in order to analyze the behavior and the influence of changing parameters on static and dynamic systems behavior.

Several recent reviews describe and compare the different drag and drop tools developed for the synthetic biology community in the last few years [Alterovitz et al. 2009; Marchisio et al. 2009; Clancy et al. 2010; MacDonald et al. 2011; Medema et al. 2012]. All of these tools have the same goal, i.e. providing an abstract visualization of the biological parts in order to generate a DNA sequence for quick assembly of the genetic circuit *in vivo* or a mathematical model in order to analyze its behavior *a priori* of any *in vivo* assembling. Although these tools are all developed in order to simplify the design of genetic networks, the practical application of these tools for the design of synthetic circuits in the synthetic biology community is still lacking.

This problem can be mainly attributed to the lack of standardized biological parts with *a priori* characterized static and dynamic behavior which are collected in public available databases [Purnick and Weiss 2009; Kwok 2010]. The first and most widely known database of standard parts is the Registry of Standard Biological Parts, developed at MIT. It maintains and distributes thousands of BioBrick standard biological parts, which are categorized corresponding to their function. Although this database is the most well-known registry of biological parts, standardization of BioBrick parts is currently limited to their physical composition, which is in terms of how individual parts are assembled into multi-component constructs. The standardized assembly is established to increase the modularity and thereby the reusability of the BioBricks. Reusability is however strongly limited by the lack of quality control of the available BioBricks which results in a huge amount of biological parts that do not function as expected [Kwok 2010]. In addition, the majority of the BioBricks lacks proper characterization of their functional and dynamic behavior which hinders selection and screening of parts for a specific design. Intense characterization with experimental data and mathematical models is also required in order to increase the predictability of the BioBricks behavior and consequently the reusability of BioBricks.

Several drag and drop tools provide a connection to this BioBrick library, but all of these tools suffer from the lack of proper characterization of the BioBricks and machine unfriendly mining of the library. This makes the tools strongly dependent on the information manually provided by the user. For instance, BioJADE [Goler 2004] and the Synthetic Biology Software Suite (SynBioSS) [Hill et al. 2008; Weeding et al. 2010] seemed promising by allowing connection to the Registry, but both tools are now outdated. While TinkerCell is regularly updated, it lacks presently a proper connection with public available libraries of biological parts [Chandran et al. 2009]. This makes the tool even more dependent on information provided by the user. Another drag and drop tool is GenoCAD which is an online web application for the graphical design of DNA constructs from genetic parts available in public or user-defined libraries, like the Registry [Czar et al. 2009]. Its main function is focused on the generation of a DNA sequence when parts are connected by the user. Simulation of the behavior of the designed circuit is also possible, though only a few parts which are described by descriptive parameters are available in the provided libraries.

2.2 Circuit optimization tools

In addition to drag and drop tools, several attempts have been made in the last few years to develop tools in which the design of synthetic circuits is guided by computational algorithms [Batt et al. 2007; Rodrigo et al. 2007; Beal et al. 2011; Marchisio et al. 2011b]. These tools will give rise to a faster and more efficient design process because the network design is mathematically optimized to satisfy a desired behavior. Recently, an optimization tool was developed by Beal et al. [2011], which makes use of the biological-oriented programming language Proto [Beal and Bachrach 2008]. The developed platform enables synthetic biologists to express a desired behavior which is subsequently transformed by a compiler into a gene regulatory network. This network will be further optimized by the program and is then translated into an ODE model for the simulation of the network behavior. The optimization program is able to reduce the complexity of the engineered network while preserving and even improving its function.

Similar approaches were developed in which selection and/or assembly of biological parts is guided by the computer [Batt et al. 2007; Rodrigo et al. 2007; Dasika and Maranas 2008; Marchisio et al. 2011b]. All of these tools aim to direct the design of new circuits from a manual trial-and-error assembly to a more automated process. This approach should make the design process less dependent on the *a priori* knowledge of the user on how the design should look like in order to satisfy a specific behavior. These tools, however, lack a proper connection to public-available part or network motif libraries, limiting the functionality of these tools [Beal et al. 2011; Chen et al. 2012].

As the finding of parts that meet specifications of a design is a critical step, new user-friendly databases should be developed that allow computer-guided screening and selection of biological parts. Galdzicki et al. [2011] started the development of a computationally accessible library by standardizing the electronic format of the knowledge of available biological parts. To this end, the Synthetic Biology Open Language (SBOL) (http://sbolstandard.org) is used which is a software standard for the electronic exchange of specifications and descriptions of genetic parts and devices. Although, the SBOL language is a first step to standardize the exchange of knowledge, it does not provide all the information necessary to describe the performance of biological parts [Chen et al. 2012]. Therefore, further efforts have to

be made in order to standardize the experimental characterization and description of biological parts. This is still an important challenge due to the complexity of biology. To this end, techniques and methods are required that allow to construct standard, modular, context-independent and robust biological parts in order to manage this complexity [Endy 2005; Voigt 2006; Lucks et al. 2008; Kwok 2010].

2.3 Will BIOFAB create the new standard for synthetic biology?

In July 2011, a new collection of biological building blocks characterized by BIOFAB International Open Facility Advancing Biotechnology (BIOFAB) (http://biofab.org) was announced. In contrast to the Registry of Standard Biological Parts, the BIOFAB collection aims to provide an extensive characterization and high quality for each biological part in order to overcome the limited reuseability of BioBricks and to stimulate a more predictable design of genetic circuits. The collection contains professional preassembled and validated DNA elements and allows to use these parts for making constructs with Gene Designer. In Gene Designer, genetic elements such as promoters, open reading frames and tags, can be combined to create a synthetic DNA segment through a drag and drop interface [Villalobos et al. 2006]. Besides designing, editing and writing novel sequence information, Gene Designer also enables codon optimization to assure protein expression in any host organism which can be a first attempt to decrease the context dependent performance of biological parts and devices.

For the initial collection, BIOFAB has made and characterized all combinations of most frequently used promoters and 5' UTR in order to quantitatively describe how the genetic part performance varies across changing DNA context. Each part is thereby described by its primary activity, i.e. strength of the promoter, and the quality of the part, i.e. how much promoter strength varies across context. This extensive description of each part allows to better mix and match pre-made DNA parts and results in the reduction of the development time currently spent on combining and validating DNA elements.

BIOFAB also invests in the development of new measurement standards that reduce the variability in parts performance when they are characterized by different researchers or across different environments. For instance, Kelly et al. [2009] describe a measurement tool for the *in vivo* characterization of promoters to reduce the variation of measured promoter activity due to differing experimental conditions and equipment. Promoters characterized by this measurement kit are described by a relative promoter activity which will remain constant across a range of conditions. This results in reliable and comparable data describing the performance of promoters and allows ranking of similar promoters.

In addition, BIOFAB aims to describe its biological parts with electronic datasheets which provide the main characteristics of the static and dynamic performance of the biological parts and devices. When these datasheets are in a machine-readable format, they can be subsequently used to enable computer guided selection of biological parts during the design of synthetic networks. Canton et al. [2008] were the first to fully characterize a biological device by creating a biological datasheet. This datasheet describes the device with a definition of its function and operating context and by the static and dynamic behavior of the device based on experimentally measured characteristics.

Unfortunately, there is at the moment no consequent use of such standard measurement methods or datasheets to describe the performance of new and existing

biological parts and devices. This strongly limits the reusability of biological parts and as a consequence the automated design of genetic circuits. By developing and applying new measurement standards to its parts collection, BIOFAB will not only boost automated design of genetic circuits, but provides also a platform for synthetic biologists to exchange experience and knowledge in a more standardized manner. This should result in a development process of new synthetic circuits that is mainly based on the creation of new functionalities of cells instead of characterizing and optimizing biological parts behavior. In addition, BIOFAB is creating a new CAD tool which will be integrated with its collection. This CAD tool seems promising, because it will be the first tool that is fully integrated with a parts library in which the parts are extensively and reliably characterized in a standardized manner.

Although the ideas and goals of BIOFAB are promising for the field, the BIOFAB collection and techniques are still in the development phase. Success of this new collection and standard methods still need to be demonstrated in order to become the new standard in biology.

2.4 Realizations in synthetic biology through the combined use of computational and experimental design approaches

Although the assembling of a predictable gene network from biological parts is hindered by the inadequate characterization of the available biological parts due to the complexity of biology, several realizations are described in which new synthetic circuits could be successfully assembled *in vivo* with *a priori* guidance of mathematical modeling [Ellis et al. 2009; Salis et al. 2009; Ceroni et al. 2010; Tamsir et al. 2010; Zhan et al. 2010; Wang et al. 2011a].

Salis et al. [2009] constructed a biophysical model that links the DNA sequence of a genetic element to its function inside the genetic system. By combining an optimization algorithm with a model describing translation initiation, the sequence of a synthetic ribosome binding site (RBS) that provides a target translation initiation rate could be predicted. As a result, a RBS sequence can be automatically designed to obtain a desired relative protein expression level with the RBS Calculator [Salis 2011]. This approach was tested by optimizing a complex genetic circuit by combining the RBS design method with a mathematical model of the system. Two synthetic genetic circuits could be successfully connected to obtain a desired functionality based on a rational design approach with only a few mutations and assays [Salis et al. 2009].

As the behavior of biological networks is rather complex, certain engineering principles, i.e. standardization, abstraction, modularity and orthogonality, have to be applied to help synthetic biologists managing this complexity [Endy 2005; Voigt 2006; Lucks et al. 2008]. Abstraction and standardization of the biological parts allow to define a set of reliable characterized and tunable biological parts. A successful approach in creating a set of tunable and predictable promoters is described by Ellis et al. [2009], in which a promoter library consisting of functionally equivalent components with slightly different properties is created. This library is developed by subtly altering the local DNA conformation of each promoter. With this method, promoter function is maintained but the efficiency of the promoters is slightly changed. As small changes to one component of a gene network can have a large impact on the behavior of the entire system, a mathematical model is necessary to accurately design a gene network. To this end, Ellis et al. [2009] built a mathematical model based on the component properties of a single promoter defined

by experimental measurements. This model was tested and approved to be sufficient to predict how the network output will change when the input levels and promoter properties are varied.

Modularity and orthogonality, on the other hand, are both properties necessary to increase the reusability and composability of the individual parts. To this end, Zhan et al. [2010] proposed a strategy to develop reusable designs of transcriptional logic gates, such as NAND, NOR and NOT gates. Combinability of these devices is achieved by the development of different variants of transcription factor-operator pairs that interact with each other in a pairwise-specific manner. This strategy is demonstrated with the development of 5 pairs of the *lac* repressor, LacI, and its operator *O*lac in which each transcription factor is specific for only one of the operator sites. Each logic device can be made with different LacI/*O*lac variant pairs establishing the reusability and combinability of each device. The characteristics of the engineered LacI/*O*lac variants and experimental derived parameters allow constructing a mathematical model that predicts the output response to specific input signals for each logic device.

Another approach to achieve combinability is to isolate genetic constructs in individual cells as described in Tamsir et al. [2010]. In this study, a library of strains each acting as a logic gate was constructed by using different tandem promoters. As a result, sixteen more complex two-input logic gates can be constructed using the library. Each circuit can be constructed by the spatial arrangement of the different library strains. For example, a XOR gate is constructed by the spatial arrangement of three NOR gates and a buffer, each represented by four individual strains. Compartmentalization of the genetic gates in individual cells allows them to be added, removed or replaced simply by changing the spatial arrangement of the strains. This approach avoids the need for any additional genetic manipulation in order to achieve a specific circuit design. In addition, the systems response is robust with respect to distance between colonies and time and density at which they are spotted. Cell-cell variation is also averaged out by the population which prevents propagation of noise to the next layer of the circuit.

An additional study addresses the lack of modularity and reusability of biological parts by developing a modular AND gate that is intensively characterized across different cellular contexts [Wang et al. 2011a]. Non-ideal modularity of biological parts prevent biological parts to behave independently of other parts and their biological context. Although non-modularity of biological parts limits the bottom-up design of circuits, mathematical modeling can still be used to predict the behavior of a designed circuit when the elementary parts are characterized in the proper experimental conditions [Ceroni et al. 2010]. This is demonstrated by Wang et al. [2011a]. A modular AND gate is constructed by *a priori* characterizing a set of components in various contexts. Different promoter/RBS pairs composed out of three promoters, P_{lac}, P_{BAD} en P_{lux}, and six RBSs with various translational efficiencies, were tested across different genetic backgrounds, growth media, temperature and embedded sequences to investigate context dependency of each component. The characterization results highlight that the behavior of a component is dependent on its abiotic and genetic context. To this end, for each component a transfer function model is constructed allowing predicting component behavior in a specific context. In order to design a predictable logic AND gate, the characterization of each component was followed by a model guided design of the AND gate. The modularity and exchangeability of the AND gate was tested by changing inputs of the device while

preserving the logic AND function. In addition, a NAND gate was successfully constructed by connecting the AND gate with a NOT gate guided by an *in silico* design of the circuit. Experimental validation of the NAND gate proves that a forward engineering approach consisting of in-context characterization of biological parts and computational modeling can give rise to predictable synthetic circuits.

All these examples provide specific solutions on how to handle the complexity of biology and provide a useful base for further advances. However, successful rational design will only be boosted when such techniques become standard and redundancy of these tools is reduced. An integration of multiple methods will therefore be necessary to enable the efficient construction of synthetic organisms by computer aided design. Additionally, new methods and technologies have to be developed whereby the development of new computational techniques go hand in hand with the development of new experimental approaches.

3. WHAT CAN MICROFLUIDICS OFFER TO SYNTHETIC BIOLOGY ?

As discussed in the previous section, the main challenge of synthetic biology is to define a systematic and robust way to characterize biological parts. To this end, the synthetic biology community has recently incorporated microfluidic devices as an approach to develop novel technologies allowing automated and multiplexed analytical measurements [Bennett et al. 2009; Gulati et al. 2009; Vinuselvi et al. 2011]. Microfluidics provides platforms that can manipulate, process and control small volumes of fluids due to miniaturization of the analytical system. Several reviews describe in detail the recent developments in microfluidics and give an extensive overview of what microfluidics can specifically offer to synthetic biology [Weibel et al. 2007; Ingham and van Hylckama Vlieg 2008; Bennett et al. 2009; Dufva 2009; Gulati et al. 2009; Vinuselvi et al. 2011]. This section rather aims to give a short description of the advantages of using microfluidics for synthetic biology and to give some examples of successful synthetic biological designs due to the integration with microfluidic devices.

By analyzing a large number of small volume samples simultaneously, microfluidics offers a new analytical approach that will reduce cost and work of experiments and improve resolution and precision of the experimental results. Since microfluidics technology can provide dynamic controlled micro-environmental conditions, new sensitive and robust experimental approaches can be developed to quickly characterize complex biological systems [Gulati et al. 2009; Vinuselvi et al. 2011]. In addition, microfluidics can stimulate the synthetic design process by offering time-lapse experiments where cells can be monitored in real-time and at the single-cell level.

These features of microfluidic devices are demonstrated in the study described by Balagaddé et al. [2008], where a synthetic predator-prey system is constructed between two engineered *E. coli* populations. The two populations communicate and regulate each others density through quorum sensing. Two quorum sensing modules, LuxI/LuxR from *Vibrio fischeri* and LasI/LasR from *Pseudomonas aeruginosa*, are implemented into the two populations (see Figure 1a). This enables a two-way communication where the prey population will suffer from the growth of the predator population and the latter benefits from the growth of the former. As the proper functioning of this system is highly dependent on the complex behavior of each bacterial population, the dynamics of the cells have to be accurately monitored. To this end, a microchemostat platform is used to perform high-throughput screening of

the dynamics of the cells in order to experimentally validate the synthetic system (see Figure 1b). The microchemostat can inexpensively perform rapid characterization of synthetic circuits under a variety of conditions with long-term, non-invasive measurements of the microbial population properties [Balagaddé et al. 2005]. By parallelizing the reactors on the chip and automating single-cell fluorescence measurements, a higher throughput is achieved. This study describes also that minimizing the bacterial population size results in the stabilization of a population during long-term culturing by slowing down microbial evolution, giving an additional advantage of using microfluidic systems in microbiology [Balagaddé et al. 2005; Balagaddé et al. 2008]. Modeling and experimental data are also combined to study the effect of changing system parameters on the dynamics of the system.

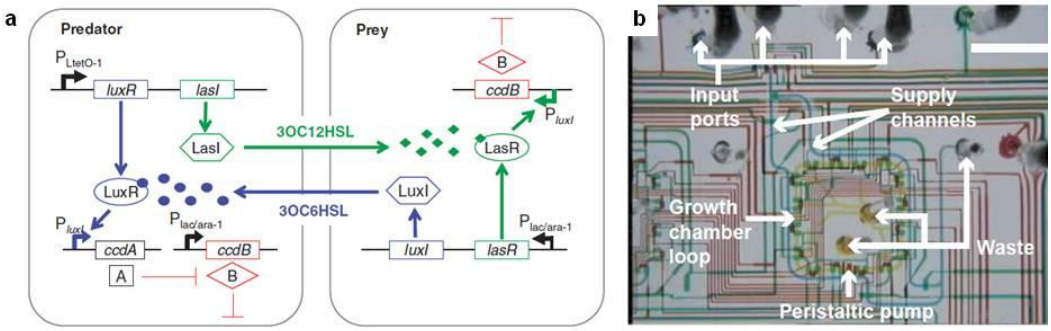

Fig. 1: *a)* The synthetic predator-prey system is constructed between two *E. coli* populations that can communicate and regulate each others density through two quorum sensing modules: LuxI/LuxR from Vibrio fischeri and LasI/LasR from Pseudomonas aeruginosa [Balagaddé et al. 2008]. *b)* A microchemostat platform is used to perform high-throughput screening of the dynamics of the cells in order to experimentally validate the synthetic system. (Figures adapted from [Balagaddé et al. 2005; Balagaddé et al. 2008])

Microfluidic systems can additionally offer **tight control of transport** of cells and nutrients or **spatial gradients** of specific chemicals. The controlled movement of components is due to the fact that the flow in microchannels is in laminar rather than turbulent conditions favoring predictable and controllable flow. **Fine control of growth conditions** with microfluidic devices allows to reduce the variability due to random fluctuations that arise from variation in growth between individual cells, which will consequently increase the reliable functioning of a synthetic system [Kwok 2010].

As demonstrated in Danino et al. [2010] and Prindle et al. [2011], synchronized behavior over a population of cells can be established by the use of microfluidic devices and the intercellular coupling of cells. The cells in the populations are coupled by the introduction of an autoinducer. This network motif whereby an activator activates its own repressor enables oscillatory behavior of cells [Stricker et al. 2008; Tigges and Fussenegger 2009]. Further, synchronized oscillations in the population are established by implementing quorum sensing elements creating intercellular communication (see Figure 2a) [Danino et al. 2010]. As synchronized oscillations of cells requires a specific cell density, microfluidic devices are used in these studies to modify the cell density and monitor the oscillatory behavior of the cells. The devices consist of a main channel providing rectangular trapping chambers with a constant supply of nutrients or inducers (see Figure 2b). Cell density can therefore be maintained for more than 4 days. The scale of synchronization could be

further increased by introducing communication between colonies in different chambers based on the exchange of gas molecules (see Figure 2d) [Prindle et al. 2011]. As a consequence of the properties of polydimethylsiloxane (PDMS), i.e. the material used to construct the microfluidic device, the gas molecules can pass the walls of the chambers and diffuse to nearby chambers, thereby establishing intercolony synchronization (see Figure 2c). This study demonstrates that microfluidic devices can not only be used to control cell culturing and monitor cellular behavior, but can establish more complex cellular behavior through active interaction with the used materials. This microfluidic device also allows easily changing device parameters rather than redesigning the underlying genetic circuit in order to analyze the influence of changing systems parameters on the oscillations of the cell. Mathematical modeling was subsequently used in order to understand and explain how the synchronized oscillations can be tuned by changing systems parameters.

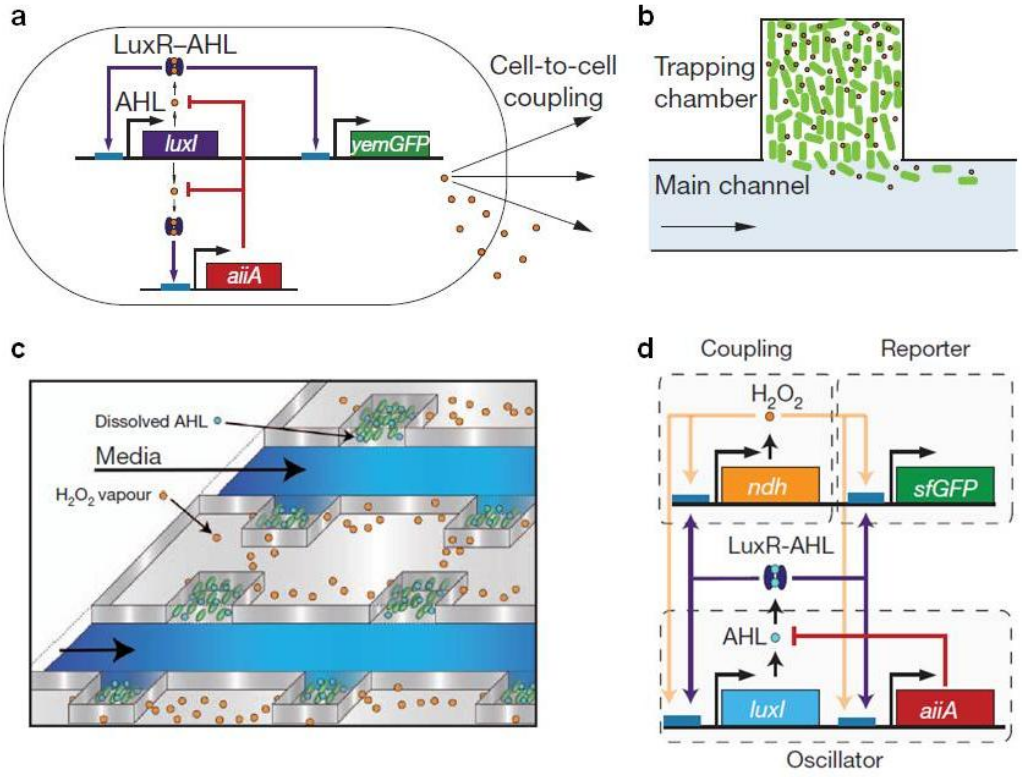

Fig. 2: *a)* The engineered genetic network which gives rise to synchronized oscillation in a cell population. LuxI synthase is responsible for the production of an acyl-homoserine lactone (AHL), which is a small molecule that will diffuse across the cell membrane. AHL will form a complex with LuxR which is constitutively produced. The LuxR-AHL complex is a transcriptional activator of the *luxI* promoter controlling the expression of *luxI*, *aiiA* en *yemGFP* genes. Finally, AiiA is a negative regulator of the circuit by degrading AHL [Danino et al. 2010]. *b)* The microfluidic device consists of a main channel providing rectangular trapping chambers a constant supply of nutrients or inducers [Danino et al. 2010]. *c)* The microfluidic device is constructed with polydimethylsiloxane (PDMS) which allows the diffusion of gas molecules across different chambers [Prindle et al. 2011]. *d)* In order to establish synchronized oscillations between colonies in different chambers, intercellular coupling is introduced based on the exchange of gas molecules. Therefore, the cells were engineered by placing a gene coding for NADH hydrogenase II (*ndh*) under control of an additional *lux* promoter [Prindle et al. 2011]. (Figure adapted from [Danino et al. 2010; Prindle et al. 2011])

Both of the described studies provide a good example of how the combination of synthetic biology with microfluidics and mathematical modeling can provide a better understanding of natural biological systems which leads ultimately to a better design of synthetic biological networks. Microfluidic chips can also be integrated with microelectronics in such a way that not only cell handling and reagents transport can be controlled, but that also an array of different detectors for studying cell responses is provided. This allows to develop fully integrated systems to control and monitor cellular behavior.

Although, the use of microchips can offer a lot to microbiology researchers, the availability of these devices in the field is too limited. This is due to the lack of validated and easy-to-use commercially available chips. Therefore, it is necessary to bridge the knowledge and culture gap between microbiologists, physicists and engineers in order that new technologies will arise from interdisciplinary cooperations.

4. MICROELECTRONICS

The potential of chip devices is however not limited to controlling cell culturing and analyzing biological behavior. To fully exploit the possibilities with miniaturized devices, synthetic biology can give rise to more possibilities in interfacing microbiology with microdevices resulting in the development of novel high-technology applications. Standardization and characterization of biological systems will hereby boost our knowledge about biology and how to engineer it, giving rise to new application by combining living and non-living systems. In the following sections, recent studies are described in which the optical and/or electrical interaction between micro-organisms and microelectronic devices are investigated. Although some of these studies are still in their infancy, the potential of interfacing microbiological systems with microelectronic devices is promising as these hybrid systems will combine the strength and diversity of the biological world with the speed of computational processing. Some of these studies also illustrate that in addition to the genetic engineering of the micro-organisms, a model prediction of their behavior is an crucial asset in order to optimize the systems performance.

4.1 Microchip-based biosensors

A biosensor is an analytical device integrating a biological recognition element with a physical transducer to generate a measureable signal that is proportional to the concentration of specific analytes [D'Souza 2001; Belkin 2003; Lei et al. 2006; Yagi 2006]. The transducer element in the biosensor will convert the biological response to a detectable signal which can be optical, mechanical or electrical (see Figure 3). In synthetic biology, many research projects study the possibilities in engineering and optimizing biosensor devices by genetically engineering sensing and reporter circuits [Khalil and Collins 2010; van der Meer et al. 2010; Marchisio and Rudolf 2011a; Zhang and Keasling 2011]. The applicability of biosensors will further increase by the incorporation of biosensor cells onto microengineered platforms. These provide not only a solid support for cell containment and long-term maintenance, but also contain microchannels for sample and reagent transport. In addition, signal analysis, temperature control, communication capacities or other control devices can be included, hereby increasing the functionality of the biosensor [van der Meer et al. 2010]. The smaller dimensions elicited by the miniaturization of the biosensor give

also rise to an improved signal to noise ratio, faster response time and increased sensitivity of the sensing devices [Popovtzer et al. 2006]. The features of microchip-based biosensors is further highlighted by the description of recently developed microchip-based biosensor integrating both optical as electrochemical biosensors to a microelectronic device.

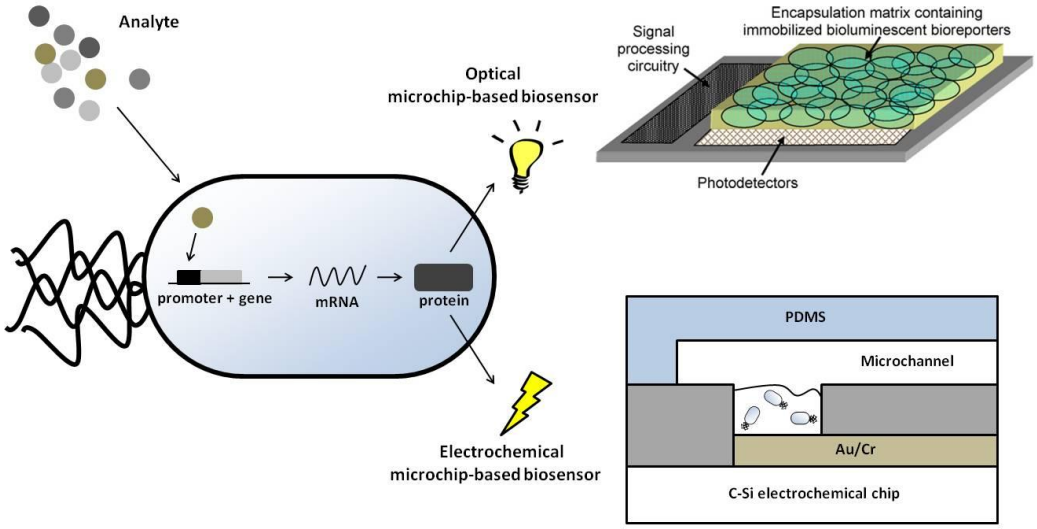

Fig. 3: A biosensor is an analytical device integrating a biological recognition element with a physical transducer to generate a measurable signal that is proportional to the concentration of specific analytes. A common used strategy to create whole-cell based biosensors, is based on transcriptional control of a gene that codes for a reporter protein, which can give rise to an optical or electrochemical signal. By integrating the biosensor cells with microelectronic devices, microchip-based biosensors can be created which can process detection signals produced by the biosensor cells. (Figures adapted from [Vijayaraghavan et al. 2007; Ben-Yoav et al. 2009a])

4.1.1 Optical microchip-based biosensors. The bioluminescent-bioreporter integrated circuit (BBIC) was one of the first developments in which genetically engineered bacteria are interfaced with an electronic circuit and has been reported many times in the last decade since its first development [Simpson et al. 2001b; Bolton et al. 2002; Ripp et al. 2003; Nivens et al. 2004; Vijayaraghavan et al. 2007]. In these studies, a biosensor, i.e. *Pseudomonas fluorescens*, was created by fusing the *luxCDABE* gene cassette from *Vibrio fischeri* to a regulatory gene system responsive for salicylate and naphthalene. This gene fusion will give rise to an increased *lux* gene expression in the presence of naphthalene or the metabolite salicylate, resulting in the generation of bioluminescence. As a result, the bacterial biosensors are producing blue-green light, with a maximum intensity at 490 nm, of which the magnitude is correlated with the concentration of the detected analytes. Therefore, a CMOS microluminometer, i.e. BBIC, was developed to detect and process the bioluminescence signal in order to quantify the concentration of the analytes. The BBIC contains two main components: photodiodes for transducing the optical signal into an electrical signal, and a signal processing circuit for managing and storing the information derived from the optical signal (see Figure 5).

In order to accomplish an accurate calibration between the analyte concentration and the optical signal detected by the microchip, mathematical modeling is also

necessary. To this end, a model is developed to describe the kinetic process of the bioluminescence response of bacteria to the presence of an analyte [Daniel et al. 2008]. In addition, mathematical modeling can be used to investigate the relationship between system geometry, bacterial concentration and optical measurements [Ben-Yoav et al. 2009b; Shacham-Diamand et al. 2010]. These models were experimentally validated and can be subsequently used to optimize light collection and detection of the microchip in order to establish more efficient and sensitive microchip-based biosensors.

4.1.2 Electrochemical microchip-based biosensors. The electrical signal produced by electrochemical biosensors consists of the production of electro-active compounds undergoing redox reactions which can be subsequently detected by several electrochemical techniques. The most widely used technique in biosensors is amperometry which allows quantifying the concentration of electroactive species in both aqueous and complex biological samples. In amperometry, the biosensor operates at a fixed potential between a working and a reference electrode. The current arising from the oxidation or reduction of species at the surface of the working electrode is subsequently measured and gives information about the concentration of these species in a solution. This electrochemical technique provides a highly sensitive detection and a wide dynamic range [Lagarde and Jaffrezic-Renault 2011; Su et al. 2011].

Biran et al. [2000] were able to develop an amperometric biosensor that enables electrochemical detection of nanomolar cadmium concentrations. In *E. coli*, a cadmium responsive promoter was fused to a promoterless *lacZ* gene, resulting in increasing ß-galactosidase activity with increasing cadmium concentrations [Biran et al. 1999]. This reporter enzyme reacts with the substrate *p*-aminophenyl-ß-D-galactopyranoside (PAPG) resulting into two different products, electrochemical active p-aminophenol (PAP) and inactive ß-D-galactopyranoside. The electrochemical product, PAP, will be oxidized at an electrode when subjected to a constant potential. The current generated by the oxidation can be subsequently measured and represents the concentration of cadmium in the sample.

The electrochemical method used in this biosensor does not require any pretreatment of the samples or complex instrumentation and enables the creation of *in situ* or disposable biosensor devices. In contrast to biosensors generating an optical signal, electrochemical biosensors are not hindered by turbid samples [Paitan 2003]. This biosensor also allows on-line monitoring since the bacterial response was obtained within minutes. In addition, very small volumes can be used for the measurements as the electrical output does not depend on the reaction volume [Ronkainen et al. 2010]. The electrical readout can be subsequently linked to an electronic device that can process, store or display the measurement results. Such electronic devices are relatively inexpensive to make and are suitable for miniaturization thereby increasing the portability and applicability of microbial biosensor. Miniaturization of analytical devices is therefore a growing trend in order to make small, portable, autonomous and inexpensive sensors for *in situ* and on-line monitoring. Finally, the electrical readout can be complemented to other available output signals, such as an optical readout, enabling the parallel detection of several analytes.

In the last decade, similar biosensors were developed and integrated with microfluidic chips in order to analyze multiple samples simultaneously in a fast and

sensitive manner (see Figure 3) [Matsui et al. 2006; Popovtzer et al. 2006; Ben-Yoav et al. 2009a]. These studies illustrate that by scaling down electrodes to a micro or nano scale, sensitive miniaturized biosensor devices can be developed. As described in Popovtzer et al. [2007], further optimization of this system can be done when the experimental characterization of the microchip-based biosensor is accompanied by a mathematical model. In this study, an experimental validated model was developed in order to investigate the influence of electrode geometry and culturing chamber structure on the response time of the biosensor. As a result, the response time of the biosensor was highly dependent on the system parameters demonstrating the need for a multidisciplinary approach to optimize such hybrid systems on both the biological and the electronic level.

These studies highlight the potential of electrochemical biosensors whereby synthetic biology can provide new approaches for the development of new electrochemical reporter strains thereby giving rise to novel sensitive microchip-based biosensors.

4.2 Microbial fuel cells : microbial production of electricity

Microbial fuel cells (MFC) are devices that establish an electrical link between specific bacteria and an insoluble electron donor or acceptor. The most extensively described MFCs enable the microbial production of electricity. In these systems, organic compounds, serving as electron donor, are oxidized by the bacteria. Electrons gained by the metabolization of these organic compounds are subsequently transferred by the bacteria to the anode of the MFC, which serves as an electron acceptor (see Figure 4). The gained electrons flow from the anode to a cathode through an external electrical connection, consisting of a resistor, a battery or other electronic devices. At the cathode, electrons are subsequently consumed by strong electron acceptors, such as oxygen. Finally, the electrical circuit is closed by the diffusion of cations through an ion-selective membrane.

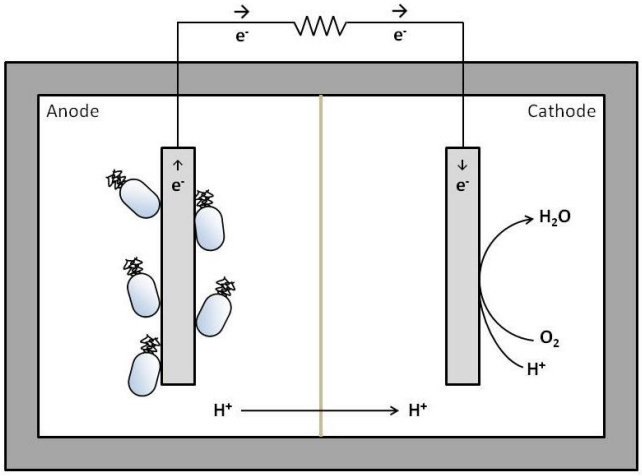

Fig. 4: In microbial fuel cells, organic compounds, serving as electron donor, are oxidized by electrode-reducing bacteria, e.g. *Geobacter sulfurreducens* and *Shewanella oneidensis*. Electrons gained by the metabolization of these organic compounds are subsequently transferred by the bacteria to the anode of the MFC, which serves as an electron acceptor. The gained electrons flow from the anode to a cathode through an external electrical connection. At the cathode, electrons are subsequently consumed by strong electron acceptors, such as oxygen. The electrical circuit is closed by the diffusion of cations through an ion-selective membrane.

The production of electricity through this mechanism is possible when electrode-reducing micro-organisms are used which can donate electrons to solid materials, such as electrodes [Rabaey and Verstraete 2005; Lovley 2006; Lovley 2008]. In contrary, electrode-oxidizing micro-organisms are capable of accepting electrons from a cathode, resulting in the consumption of electrons [Gregory et al. 2004; Gregory and Lovley 2005; Clauwaert et al. 2007; Thrash et al. 2007; Thrash and Coates 2008]. However, little is known about the mechanisms of this microbial consumption of electrons from electrodes. As the microbial production of electricity by electrode-reducing microorganisms has gained much more interest in order to develop alternative energy resources, most research is consequently done on unraveling the electron transfer mechanism of these bacteria. Therefore, *Geobacter sulfurreducens* and *Shewanella oneidensis* are intensively studied because of their ability to directly transfer electrons from the cell interior to electrodes in the extracellular environment [Lovley 2006; Rabaey et al. 2007; Lovley 2008].

Genetic studies of these bacteria revealed that these bacteria are able to transfer electrons to electrodes via *c*-type cytochromes displayed on the outer membrane of the bacteria [Holmes et al. 2006; Bretschger et al. 2007; Kim et al. 2008]. Electron transfer is also established by electrical conductive pili, so called microbial nanowires [Reguera et al. 2005; Gorby 2006; Reguera et al. 2006; El-Naggar et al. 2010]. In addition, *S. oneidensis* can establish indirect electron transfer by the excretion of a soluble electron shuttle, riboflavin [von Canstein et al. 2007; Marsili et al. 2008; Brutinel and Gralnick 2011]. Although the electron transfer mechanisms of these bacteria are slowly unraveled, much optimization of the microbial fuel cells is still required to develop applications based on the microbial production of electricity. To this end, genetic engineering in combination with *in silico* metabolic modeling can yield strains with more efficient and enhanced power production [Izallalen et al. 2008].

In addition, the miniaturization of MFCs is intensively studied by creating MFC arrays or on-chip bioenergy devices [Wang et al. 2011b]. Although, these microsized MFCs are limited in their power production, they offer unique features such as large surface to volume ratio, short electrode distance, fast response time and low Reynolds numbers. These advantages due to the microscale of the MFC allow developing high-throughput screening devices to study the current limits and future potential of MFCs. As a result, optimization of MFCs can be done by screening for microorganisms with higher electricity generation capabilities or by studying the influence of electrode materials and structures on electricity generation [Hou et al. 2009; Qian et al. 2009; Wang et al. 2011b].

By isolating the gene clusters responsible for electron transfer in *G. sulfurreducens*, *S. oneidensis* or other electrogenic bacteria, non electro-active bacteria can be engineered and provided with an electrical capacity [Agapakis and Silver 2010; Fischbach and Voigt 2010]. Since the electron transfer mechanism of *S. oneidensis* is intensively studied and one of the best understood electrogenic pathways, a genetic cassette containing a part of the electron transfer chain of *S. oneidensis* can be used to create an electron conduit in *E. coli*. Expression of the *mtrCAB* genes of *S. oneidensis* in *E. coli* gives *E. coli* the ability to reduce both metal ions and solid metal oxides by transfer of electrons along a well-defined path from the cell interior to extracellular inorganic materials [Jensen et al. 2010]. This study demonstrates that through a synthetic biology approach, cells can be engineered to

obtain electrical communication between living cells and non-living systems. When the genetic engineering of such bacteria is further combined with materials and electronic engineering, the electron transfer between the engineered cells and electrodes can be optimized to achieve efficient, predictable and controllable electron transfer.

4.3 Electrical stimulation of micro-organisms, triggering an optical response

In contrast to the knowledge about electrical stimulation of neurons and muscle cells, the response of micro-organisms to electrical impulses is still open for research. In order to exploit the possibilities of stimulating cells, better understanding is necessary to take full advantage of the interaction between electronic devices and biological components. A preliminary search for current-inducible promoters in *E. coli* was performed by Simpson et al. [2001a]. This was done by subjecting the cells to a current during a time period of 30 minutes. By comparing the gene expression of these cells with microarray analysis, induced or repressed genes due to the current exposure could be identified. Although this initial screening resulted in the experimental finding of 8 induced genes and 42 repressed genes of 1512 genes of *E. coli* investigated, no further report on the identification of these genes has appeared. This may suggest that electricity-based gene expression is not involved to be used as a control mechanism in micro-organisms or that it is still an undisclosed research field in microbiology. Control of gene expression with an electrical current or voltage would be however an ideal biological device for the creation of hybrid bioelectronic devices.

Recently, Vilanova et al. [2011] were the first to report electrically induced increase of intracellular calcium in the yeast, *S. cerevisiae*. A genetically engineered *S. cerevisiae* strain, expressing the calcium indicator, aequorin, will emit light when electrically triggered. Although, the exact mechanism of increased intracellular calcium levels after electrical stimulation is not defined, this study proved that electrical stimulation of yeast was reproducible in a short time frame and not harmful for the cells. Although more studies are needed to fully understand the electrical triggering of yeast and other micro-organisms, this study is a first step towards new synthetic biology applications in which the electrical capabilities of cells are exploited. For instance, organisms can be engineered to create novel bio-electronic lighting devices or electrically controlled behavior of organisms.

4.4 Optical stimulation of micro-organisms, triggering an electrical response

In contrast to electrically induced light emission, the reverse mechanism is also a topic of current research. Cyanobacteria are investigated for their capability to transfer electrons to the extracellular environment in response to illumination [Pisciotta et al. 2010]. This light-dependent electrogenic activity was, in contrast to the electrogenic activity of intensively studied chemotrophic bacteria such as *G. sulferreducens*, observed in the absence of any exogenous organic fuel and was driven entirely by the energy of light [Lovley 2008; Pisciotta et al. 2010]. Although the biological function of this electrogenic activity is not yet clear and the yield of the electron harvesting has to be improved, the light-dependent electrogenic pathway appears to be an important electrical conduit between microorganisms and electronic devices. Evidently, an efficient biological conversion of solar energy to electrical energy will have a significant impact on a global scale with many kinds of applications.

4.5 Electrical stimulation of micro-organisms, triggering an electrical response

A synthetic electrogenic device using electricity-induced expression of specific transgenes is recently developed in mammalian cells [Weber et al. 2008]. This device allows creating an interface managing mutual exchange of information between mammalian cells and electronic processing units (see Figure 5). Electronic controlled gene expression was developed in engineered mammalian cells by linking electrochemical oxidation of ethanol to acetaldehyde with acetaldehyde-inducible transgene expression. This electrical input signal triggers the production of human placental SEAP, which can be subsequently detected by an enzymatic-optical process consisting of a photodiode that produces a dose-dependent electrical signal. Both input and output parts of this electro-genetic device create an electronic-cell interface allowing linking engineered mammalian cells to integrated electronic circuits. By miniaturizing the electro-genetic input device, power consumption of the device is reduced. As a first example of modulation of transgene expression in response to electricity, this study highlights that our molecular understanding of how electricity impacts biological functions remains too limited and should be improved to define novel interfaces between microelectronic and biological transcription circuits.

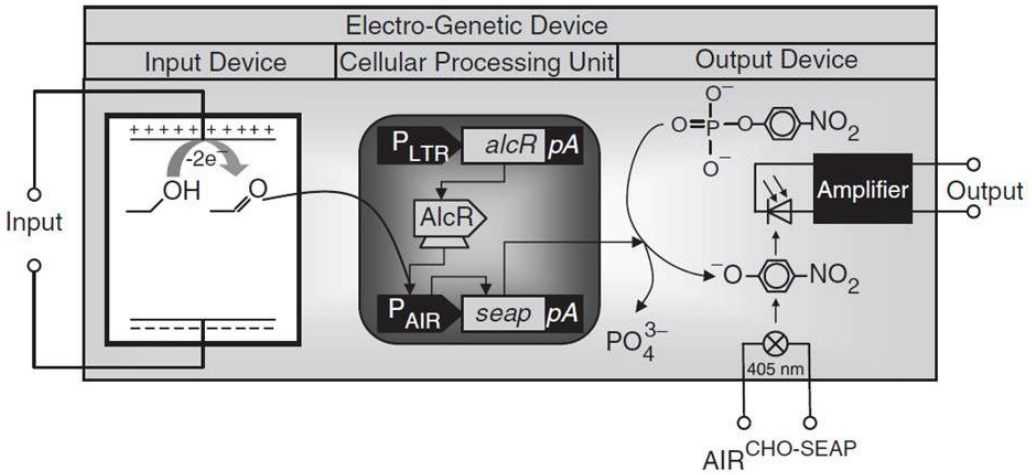

Fig. 5: Electronic controlled gene expression was developed in engineered mammalian cells by linking electrochemical oxidation of ethanol to acetaldehyde with acetaldehyde-inducible transgene expression. This electrical input signal triggers the production of human placental SEAP, which can be subsequently detected by an enzymatic-optical process consisting of a photodiode that produces a dose-dependent electrical signal. (Figure adapted from [Weber et al. 2008])

4.6 Optical stimulation of micro-organisms regulated by an *in silico* closed-loop control system

By integrating optical stimulation with computational modeling, Milias-Argeitis et al. [2011] combined the features of both techniques to implement an *in silico* feedback control mechanism to tightly regulate gene expression at a desired level in a robust manner. To illustrate the robust optical control of cellular behavior, two constructs were made wherein the photoreceptor chromoprotein (PhyB) is fused to a GAL4 DNA-binding domain and the phytochrome interacting factor (PIF3) is fused to a GAL4 activation domain. Expression of the YFP reporter is under control of the

Gal1 promoter which contains Gal4 binding sites. As a result, the interaction of PhyB with PIF3 is under control of red (650 nm) and far-red (730 nm) light pulses, thereby controlling the production of the YFP reporter protein. A computational model was subsequently developed that describes the dynamics of the Phy/PIF/Gal system. This model could be used to develop a strategy to regulate YFP fluorescence to a desired level or set-point.

In this strategy, the model is used to predict unmeasured states of gene expression based on recent fluorescence measurements and knowledge of light pulse history. Based on this information, a train of light pulses is calculated and applied in order to minimize the deviation between the model-predicted YFP expression and the desired fluorescence output. This process is repeated at several time point, whereby new control signals are calculated based on new fluorescence measurement. The light control is consequently continuously updated and tuned as the process evolves. Due to this online computation of the control signal based on real-time measurements, the desired set point will be achieved despite modeling errors, biological fluctuations, cellular variability and unknown initial conditions. As a result, this approach allows robustly controlling cellular behavior.

This model predictive control mechanism is commonly used in chemical, electrical and other industrial processes to achieve a predefined working point in a robust and efficient manner by controlling several system inputs. Applying this method to control cellular behavior will increase the controllability and predictability of synthetic circuits and will as a consequence boost the development of biotechnological applications.

5. CONCLUSIONS

The reuse of biological components in synthetic biology research is a key through which synthetic biologists can more easily engineer and construct new biological systems with increased complexity. For this to succeed, better libraries with modular biological parts characterized in both a mathematical as an experimental manner are required. This should transform the design process in synthetic biology from an expensive, time-consuming and unreliable process to a fast, automated and efficient design method. Although a lot of techniques and methods are available in synthetic biology, they are not yet systematically used in the design and characterization of new biological circuits. This demonstrates the need for a better general framework that defines generic techniques for the standardization and characterization of biological parts in both an experimentally as a mathematically manner. A compilation of recently developed and novel methods will therefore be necessary enabling to manage the complex behavior of biological systems.

Although, there is still a lot to be achieved in synthetic biology, the International Genetically Engineered Machine (iGEM) (http://igem.org) competition clearly demonstrates the current power of standardization in biology to stimulate innovation and creativity [Goodman 2008]. This power arises from the Registry of Standard Biological Parts which can be further maintained and improved when a quality control of the BioBricks is included in order to maximize the benefits for its users [Peccoud et al. 2008]. In addition, the reusability of biological parts will also be slightly improved by systematically disclosing annotated sequence information when reporting synthetic gene networks in research articles, as recently addressed by Peccoud et al. [2011]. As a consequence, a combination of such small changes and

new technologies will ultimately encourage the successful design of novel synthetic biological systems.

As described in this review, new analytical and bioelectronic technologies can be created by interfacing microbiology with microfluidics and microelectronics. Microfluidics and microelectronics are promising but rather unexplored fields in synthetic biology. The development of new microfluidic and microelectronic devices will not only boost our knowledge about biology, but will also improve current standardization and characterization techniques to describe biological systems. As a consequence, new opportunities in biological, environmental and medical research will arise.

REFERENCES

AGAPAKIS, C. M. AND SILVER, P. A. 2010. Modular electron transfer circuits for synthetic biology: insulation of an engineered biohydrogen pathway. *Bioeng Bugs 1,* 6, 413-418.

ALTEROVITZ, G., MUSO, T. AND RAMONI, M. F. 2009. The challenges of informatics in synthetic biology: from biomolecular networks to artificial organisms. *Briefings in Bioinformatics 11,* 1, 80-95.

ANDRIANANTOANDRO, E., BASU, S., KARIG, D. K. AND WEISS, R. 2006. Synthetic biology: new engineering rules for an emerging discipline. *Molecular Systems Biology 2,*

BALAGADDÉ, F. K., SONG, H., OZAKI, J., COLLINS, C. H., BARNET, M., ARNOLD, F. H., QUAKE, S. R. AND YOU, L. 2008. A synthetic Escherichia coli predator–prey ecosystem. *Molecular Systems Biology 4,*

BALAGADDÉ, F. K., YOU, L., HANSEN, C. L., ARNOLD, F. H. AND QUAKE, S. R. 2005. Long-term monitoring of bacteria undergoing programmed population control in a microchemostat. *Science 309,* 5731, 137-140.

BATT, G., YORDANOV, B., WEISS, R. AND BELTA, C. 2007. Robustness analysis and tuning of synthetic gene networks. *Bioinformatics 23,* 18, 2415-2422.

BEAL, J. AND BACHRACH, J. 2008. Cells Are Plausible Targets for High-Level Spatial Languages. 284-291.

BEAL, J., LU, T. AND WEISS, R. 2011. Automatic compilation from high-level biologically-oriented programming language to genetic regulatory networks. *PLoS One 6,* 8, e22490.

BELKIN, S. 2003. Microbial whole-cell sensing systems of environmental pollutants. *Current Opinion in Microbiology 6,* 3, 206-212.

BEN-YOAV, H., BIRAN, A., PEDAHZUR, R., BELKIN, S., BUCHINGER, S., REIFFERSCHEID, G. AND SHACHAM-DIAMAND, Y. 2009a. A whole cell electrochemical biosensor for water genotoxicity bio-detection. *Electrochimica Acta 54,* 25, 6113-6118.

BEN-YOAV, H., ELAD, T., SHLOMOVITS, O., BELKIN, S. AND SHACHAM-DIAMAND, Y. 2009b. Optical modeling of bioluminescence in whole cell biosensors. *Biosensors and Bioelectronics 24,* 7, 1969-1973.

BENNETT, M. R. AND HASTY, J. 2009. Microfluidic devices for measuring gene network dynamics in single cells. *Nature Reviews Genetics 10,* 9, 628-638.

BIOFAB INTERNATIONAL OPEN FACILITY ADVANCING BIOTECHNOLOGY (BIOFAB), 2012. http://biofab.org/

BIRAN, I., BABAI, R., LEVCOV, K., RISHPON, J. AND RON, E. Z. 2000. Online and in situ monitoring of environmental pollutants: electrochemical biosensing of cadmium. *Environ Microbiol 2,* 3, 285-290.

BIRAN, I., KLIMENTIY, L., HENGGE-ARONIS, R., RON, E. Z. AND RISHPON, J. 1999. On-line monitoring of gene expression. *Microbiology 145 (Pt 8),* 2129-2133.

BOLTON, E. K., SAYLER, G. S., NIVENS, D. E., ROCHELLE, J. M., RIPP, S. AND SIMPSON, M. L. 2002. Integrated CMOS photodetectors and signal processing for very low-level chemical sensing with the bioluminescent bioreporter integrated circuit. *Sens Actuators B Chem 85,* 1-2, 179-185.

BRETSCHGER, O., OBRAZTSOVA, A., STURM, C. A., CHANG, I. S., GORBY, Y. A., REED, S. B., CULLEY, D. E., REARDON, C. L., BARUA, S., ROMINE, M. F., ZHOU, J., BELIAEV, A. S., BOUHENNI, R., SAFFARINI, D., MANSFELD, F., KIM, B. H., FREDRICKSON, J. K. AND NEALSON, K. H. 2007. Current Production and Metal Oxide Reduction by Shewanella oneidensis MR-1 Wild Type and Mutants. *Applied and Environmental Microbiology 73,* 21, 7003-7012.

BRUTINEL, E. D. AND GRALNICK, J. A. 2011. Shuttling happens: soluble flavin mediators of extracellular electron transfer in Shewanella. *Applied Microbiology and Biotechnology*

CANTON, B., LABNO, A. AND ENDY, D. 2008. Refinement and standardization of synthetic biological parts and devices. *Nature Biotechnology 26,* 7, 787-793.

CERONI, F., FURINI, S., GIORDANO, E. AND CAVALCANTI, S. 2010. Rational design of modular circuits for gene transcription: A test of the bottom-up approach. *Journal of Biological Engineering 4,* 1, 14.

CHANDRAN, D., BERGMANN, F. T. AND SAURO, H. M. 2009. TinkerCell: modular CAD tool for synthetic biology. *J Biol Eng 3,* 19.

CHEN, J., DENSMORE, D., HAM, T. S., KEASLING, J. D. AND HILLSON, N. J. 2012. DeviceEditor visual biological CAD canvas. *Journal of Biological Engineering 6,* 1, 1.

CLANCY, K. AND VOIGT, C. A. 2010. Programming cells: towards an automated 'Genetic Compiler'. *Current Opinion in Biotechnology 21,* 4, 572-581.

CLAUWAERT, P., RABAEY, K., AELTERMAN, P., DE SCHAMPHELAIRE, L., PHAM, T. H., BOECKX, P., BOON, N. AND VERSTRAETE, W. 2007. Biological denitrification in microbial fuel cells. *Environ Sci Technol 41,* 9, 3354-3360.

Czar, M. J., Cai, Y. and Peccoud, J. 2009. Writing DNA with GenoCADTM. *Nucleic Acids Research 37,* Web Server, W40-W47.

D'Souza, S. F. 2001. Microbial biosensors. *Biosens Bioelectron 16,* 6, 337-353.

Daniel, R., Almog, R., Ron, A., Belkin, S. and Diamand, Y. S. 2008. Modeling and measurement of a whole-cell bioluminescent biosensor based on a single photon avalanche diode. *Biosensors and Bioelectronics 24,* 4, 882-887.

Danino, T., Mondragón-Palomino, O., Tsimring, L. and Hasty, J. 2010. A synchronized quorum of genetic clocks. *Nature 463,* 7279, 326-330.

Dasika, M. S. and Maranas, C. D. 2008. OptCircuit: an optimization based method for computational design of genetic circuits. *BMC Systems Biology 2,* 1, 24.

Dufva, M. 2009. Microchips for Cell-Based Assays. *509,* 135-144.

El-Naggar, M. Y., Wanger, G., Leung, K. M., Yuzvinsky, T. D., Southam, G., Yang, J., Lau, W. M., Nealson, K. H. and Gorby, Y. A. 2010. Electrical transport along bacterial nanowires from Shewanella oneidensis MR-1. *Proceedings of the National Academy of Sciences 107,* 42, 18127-18131.

Ellis, T., Wang, X. and Collins, J. J. 2009. Diversity-based, model-guided construction of synthetic gene networks with predicted functions. *Nature Biotechnology 27,* 5, 465-471.

Endy, D. 2005. Foundations for engineering biology. *Nature 438,* 7067, 449-453.

Fischbach, M. and Voigt, C. A. 2010. Prokaryotic gene clusters: A rich toolbox for synthetic biology. *Biotechnology Journal 5,* 12, 1277-1296.

Galdzicki, M., Rodriguez, C., Chandran, D., Sauro, H. M. and Gennari, J. H. 2011. Standard biological parts knowledgebase. *PLoS One 6,* 2, e17005.

Gendrault, Y., Madec, M., Lallement, C., Pecheux, F. and Haiech, J. 2011. Synthetic biology methodology and model refinement based on microelectronic modeling tools and languages. *Biotechnology Journal 6,* 7, 796-806.

Goler, J. A. 2004. BioJADE: A Design and Simulation Tool for Synthetic Biological Systems. *Cambridge, MA: MIT*

Goodman, C. 2008. Engineering ingenuity at iGEM. *Nat Chem Biol 4,* 1, 13.

Gorby, Y. A. 2006. Electrically conductive bacterial nanowires produced by Shewanella oneidensis strain MR-1 and other microorganisms. *Proceedings of the National Academy of Sciences 103,* 30, 11358-11363.

Gregory, K. B., Bond, D. R. and Lovley, D. R. 2004. Graphite electrodes as electron donors for anaerobic respiration. *Environ Microbiol 6,* 6, 596-604.

Gregory, K. B. and Lovley, D. R. 2005. Remediation and recovery of uranium from contaminated subsurface environments with electrodes. *Environ Sci Technol 39,* 22, 8943-8947.

Gulati, S., Rouilly, V., Niu, X., Chappell, J., Kitney, R. I., Edel, J. B., Freemont, P. S. and deMello, A. J. 2009. Opportunities for microfluidic technologies in synthetic biology. *Journal of The Royal Society Interface 6,* Suppl_4, S493-S506.

Hill, A. D., Tomshine, J. R., Weeding, E. M., Sotiropoulos, V. and Kaznessis, Y. N. 2008. SynBioSS: the synthetic biology modeling suite. *Bioinformatics 24,* 21, 2551-2553.

Holmes, D. E., Chaudhuri, S. K., Nevin, K. P., Mehta, T., Methe, B. A., Liu, A., Ward, J. E., Woodard, T. L., Webster, J. and Lovley, D. R. 2006. Microarray and genetic analysis of electron transfer to electrodes in Geobacter sulfurreducens. *Environmental Microbiology 8,* 10, 1805-1815.

Hou, H., Li, L., Cho, Y., de Figueiredo, P. and Han, A. 2009. Microfabricated microbial fuel cell arrays reveal electrochemically active microbes. *PLoS One 4,* 8, e6570.

Ingham, C. J. and van Hylckama Vlieg, J. E. T. 2008. MEMS and the microbe. *Lab on a Chip 8,* 10, 1604.

International Genetically Engineered Machine (iGEM) competition, 2012. http://igem.org/Main_Page

Izallalen, M., Mahadevan, R., Burgard, A., Postier, B., Didonato, R., Sun, J., Schilling, C. H. and Lovley, D. R. 2008. Geobacter sulfurreducens strain engineered for increased rates of respiration. *Metabolic Engineering 10,* 5, 267-275.

Jensen, H. M., Albers, A. E., Malley, K. R., Londer, Y. Y., Cohen, B. E., Helms, B. A., Weigele, P., Groves, J. T. and Ajo-Franklin, C. M. 2010. Engineering of a synthetic electron conduit in living cells. *Proc Natl Acad Sci U S A 107,* 45, 19213-19218.

KELLY, J. R., RUBIN, A. J., DAVIS, J. H., AJO-FRANKLIN, C. M., CUMBERS, J., CZAR, M. J., DE MORA, K., GLIEBERMAN, A. L., MONIE, D. D. AND ENDY, D. 2009. Measuring the activity of BioBrick promoters using an in vivo reference standard. *Journal of Biological Engineering 3,* 1, 4.

KHALIL, A. S. AND COLLINS, J. J. 2010. Synthetic biology: applications come of age. *Nature Reviews Genetics 11,* 5, 367-379.

KIM, B. C., POSTIER, B. L., DIDONATO, R. J., CHAUDHURI, S. K., NEVIN, K. P. AND LOVLEY, D. R. 2008. Insights into genes involved in electricity generation in Geobacter sulfurreducens via whole genome microarray analysis of the OmcF-deficient mutant. *Bioelectrochemistry 73,* 1, 70-75.

KWOK, R. 2010. Five hard truths for synthetic biology. *Nature 463,* 7279, 288-290.

LAGARDE, F. AND JAFFREZIC-RENAULT, N. 2011. Cell-based electrochemical biosensors for water quality assessment. *Analytical and Bioanalytical Chemistry 400,* 4, 947-964.

LEE, S. K., CHOU, H., HAM, T. S., LEE, T. S. AND KEASLING, J. D. 2008. Metabolic engineering of microorganisms for biofuels production: from bugs to synthetic biology to fuels. *Current Opinion in Biotechnology 19,* 6, 556-563.

LEI, Y., CHEN, W. AND MULCHANDANI, A. 2006. Microbial biosensors. *Analytica Chimica Acta 568,* 1-2, 200-210.

LEVSKAYA, A., CHEVALIER, A. A., TABOR, J. J., SIMPSON, Z. B., LAVERY, L. A., LEVY, M., DAVIDSON, E. A., SCOURAS, A., ELLINGTON, A. D., MARCOTTE, E. M. AND VOIGT, C. A. 2005. Synthetic biology: engineering Escherichia coli to see light. *Nature 438,* 7067, 441-442.

LOVLEY, D. R. 2006. Bug juice: harvesting electricity with microorganisms. *Nature Reviews Microbiology 4,* 7, 497-508.

LOVLEY, D. R. 2008. The microbe electric: conversion of organic matter to electricity. *Current Opinion in Biotechnology 19,* 6, 564-571.

LUCKS, J. B., QI, L., WHITAKER, W. R. AND ARKIN, A. P. 2008. Toward scalable parts families for predictable design of biological circuits. *Current Opinion in Microbiology 11,* 6, 567-573.

LUX, M. W., BRAMLETT, B. W., BALL, D. A. AND PECCOUD, J. 2011. Genetic design automation: engineering fantasy or scientific renewal? *Trends in Biotechnology*

MACDONALD, J. T., BARNES, C., KITNEY, R. I., FREEMONT, P. S. AND STAN, G.-B. V. 2011. Computational design approaches and tools for synthetic biology. *Integrative Biology 3,* 2, 97.

MARCHISIO, M. A. AND RUDOLF, F. 2011a. Synthetic biosensing systems. *The International Journal of Biochemistry & Cell Biology 43,* 3, 310-319.

MARCHISIO, M. A. AND STELLING, J. 2009. Computational design tools for synthetic biology. *Curr Opin Biotechnol 20,* 4, 479-485.

MARCHISIO, M. A. AND STELLING, J. 2011b. Automatic Design of Digital Synthetic Gene Circuits. *PLoS Computational Biology 7,* 2, e1001083.

MARSILI, E., BARON, D. B., SHIKHARE, I. D., COURSOLLE, D., GRALNICK, J. A. AND BOND, D. R. 2008. Shewanella secretes flavins that mediate extracellular electron transfer. *Proceedings of the National Academy of Sciences 105,* 10, 3968-3973.

MARTIN, V. J., PITERA, D. J., WITHERS, S. T., NEWMAN, J. D. AND KEASLING, J. D. 2003. Engineering a mevalonate pathway in Escherichia coli for production of terpenoids. *Nat Biotechnol 21,* 7, 796-802.

MATSUI, N., KAYA, T., NAGAMINE, K., YASUKAWA, T., SHIKU, H. AND MATSUE, T. 2006. Electrochemical mutagen screening using microbial chip. *Biosensors and Bioelectronics 21,* 7, 1202-1209.

MEDEMA, M. H., VAN RAAPHORST, R., TAKANO, E. AND BREITLING, R. 2012. Computational tools for the synthetic design of biochemical pathways. *Nature Reviews Microbiology 10,* 3, 191-202.

MILIAS-ARGEITIS, A., SUMMERS, S., STEWART-ORNSTEIN, J., ZULETA, I., PINCUS, D., EL-SAMAD, H., KHAMMASH, M. AND LYGEROS, J. 2011. In silico feedback for in vivo regulation of a gene expression circuit. *Nature Biotechnology 29,* 12, 1114-1116.

NIVENS, D. E., MCKNIGHT, T. E., MOSER, S. A., OSBOURN, S. J., SIMPSON, M. L. AND SAYLER, G. S. 2004. Bioluminescent bioreporter integrated circuits: potentially small, rugged and inexpensive whole-cell biosensors for remote environmental monitoring. *Journal of Applied Microbiology 96,* 1, 33-46.

PAITAN, Y. 2003. On-line and in situ biosensors for monitoring environmental pollution. *Biotechnology Advances 22,* 1-2, 27-33.

PECCOUD, J., ANDERSON, J. C., CHANDRAN, D., DENSMORE, D., GALDZICKI, M., LUX, M. W., RODRIGUEZ, C. A., STAN, G. B. AND SAURO, H. M. 2011. Essential information for synthetic DNA sequences. *Nat Biotechnol 29,* 1, 22; discussion 22-23.

PECCOUD, J., BLAUVELT, M. F., CAI, Y., COOPER, K. L., CRASTA, O., DELALLA, E. C., EVANS, C., FOLKERTS, O., LYONS, B. M., MANE, S. P., SHELTON, R., SWEEDE, M. A. AND WALDON, S. A. 2008. Targeted Development of Registries of Biological Parts. *PLoS One 3,* 7, e2671.

PISCIOTTA, J. M., ZOU, Y. AND BASKAKOV, I. V. 2010. Light-dependent electrogenic activity of cyanobacteria. *PLoS One 5,* 5, e10821.

POPOVTZER, R., NATAN, A. AND SHACHAM-DIAMAND, Y. 2007. Mathematical model of whole cell based bio-chip: An electrochemical biosensor for water toxicity detection. *Journal of Electroanalytical Chemistry 602,* 1, 17-23.

POPOVTZER, R., NEUFELD, T., RON, E., RISHPON, J. AND SHACHAMDIAMAND, Y. 2006. Electrochemical detection of biological reactions using a novel nano-bio-chip array. *Sensors and Actuators B: Chemical 119,* 2, 664-672.

PRINDLE, A., SAMAYOA, P., RAZINKOV, I., DANINO, T., TSIMRING, L. S. AND HASTY, J. 2011. A sensing array of radically coupled genetic 'biopixels'. *Nature 481,* 7379, 39-44.

PURNICK, P. E. M. AND WEISS, R. 2009. The second wave of synthetic biology: from modules to systems. *Nature Reviews Molecular Cell Biology 10,* 6, 410-422.

QIAN, F., BAUM, M., GU, Q. AND MORSE, D. E. 2009. A 1.5 µL microbial fuel cell for on-chip bioelectricity generation. *Lab on a Chip 9,* 21, 3076.

RABAEY, K., RODRÍGUEZ, J., BLACKALL, L. L., KELLER, J., GROSS, P., BATSTONE, D., VERSTRAETE, W. AND NEALSON, K. H. 2007. Microbial ecology meets electrochemistry: electricity-driven and driving communities. *The ISME Journal 1,* 1, 9-18.

RABAEY, K. AND VERSTRAETE, W. 2005. Microbial fuel cells: novel biotechnology for energy generation. *Trends in Biotechnology 23,* 6, 291-298.

REGISTRY OF STANDARD BIOLOGICAL PARTS, 2012. http://partsregistry.org

REGUERA, G., MCCARTHY, K. D., MEHTA, T., NICOLL, J. S., TUOMINEN, M. T. AND LOVLEY, D. R. 2005. Extracellular electron transfer via microbial nanowires. *Nature 435,* 7045, 1098-1101.

REGUERA, G., NEVIN, K. P., NICOLL, J. S., COVALLA, S. F., WOODARD, T. L. AND LOVLEY, D. R. 2006. Biofilm and Nanowire Production Leads to Increased Current in Geobacter sulfurreducens Fuel Cells. *Applied and Environmental Microbiology 72,* 11, 7345-7348.

RIPP, S., DAUMER, K. A., MCKNIGHT, T., LEVINE, L. H., GARLAND, J. L., SIMPSON, M. L. AND SAYLER, G. S. 2003. Bioluminescent bioreporter integrated-circuit sensing of microbial volatile organic compounds. *J Ind Microbiol Biotechnol 30,* 11, 636-642.

RODRIGO, G., CARRERA, J. AND JARAMILLO, A. 2007. Genetdes: automatic design of transcriptional networks. *Bioinformatics 23,* 14, 1857-1858.

RONKAINEN, N. J., HALSALL, H. B. AND HEINEMAN, W. R. 2010. Electrochemical biosensors. *Chemical Society Reviews 39,* 5, 1747.

SALIS, H. M. 2011. The ribosome binding site calculator. *Methods Enzymol 498,* 19-42.

SALIS, H. M., MIRSKY, E. A. AND VOIGT, C. A. 2009. Automated design of synthetic ribosome binding sites to control protein expression. *Nature Biotechnology 27,* 10, 946-950.

SAYLER, G., SIMPSON, M. AND COX, C. 2004. Emerging foundations: nano-engineering and bio-microelectronics for environmental biotechnology. *Current Opinion in Microbiology 7,* 3, 267-273.

SHACHAM-DIAMAND, Y., BELKIN, S., RISHPON, J., ELAD, T., MELAMED, S., BIRAN, A., YAGUR-KROLL, S., ALMOG, R., DANIEL, R., BEN-YOAV, H., RABNER, A., VERNICK, S., ELMAN, N. AND POPOVTZER, R. 2010. Optical and electrical interfacing technologies for living cell bio-chips. *Curr Pharm Biotechnol 11,* 4, 376-383.

SIMPSON, M. L., SAYLER, G. S., FLEMING, J. T. AND APPLEGATE, B. 2001a. Whole-cell biocomputing. *Trends Biotechnol 19,* 8, 317-323.

SIMPSON, M. L., SAYLER, G. S., PATTERSON, G., NIVENS, D. E., BOLTON, E. K., ROCHELLE, J. M., ARNOTT, J. C., APPLEGATE, B. M., RIPP, S. AND GUILLORN, M. A. 2001b. An integrated CMOS microluminometer for low-level luminescence sensing in the bioluminescent bioreporter integrated circuit. *Sens Actuators B Chem 72,* 2, 134-140.

STRICKER, J., COOKSON, S., BENNETT, M. R., MATHER, W. H., TSIMRING, L. S. AND HASTY, J. 2008. A fast, robust and tunable synthetic gene oscillator. *Nature 456,* 7221, 516-519.

SU, L., JIA, W., HOU, C. AND LEI, Y. 2011. Microbial biosensors: A review. *Biosensors and Bioelectronics 26,* 5, 1788-1799.

SYNTHETIC BIOLOGY OPEN LANGUAGE (SBOL), 2012. http://sbolstandard.org

TAMSIR, A., TABOR, J. J. AND VOIGT, C. A. 2010. Robust multicellular computing using genetically encoded NOR gates and chemical 'wires'. *Nature 469,* 7329, 212-215.

THRASH, J. C. AND COATES, J. D. 2008. Review: Direct and indirect electrical stimulation of microbial metabolism. *Environ Sci Technol 42,* 11, 3921-3931.

THRASH, J. C., VAN TRUMP, J. I., WEBER, K. A., MILLER, E., ACHENBACH, L. A. AND COATES, J. D. 2007. Electrochemical stimulation of microbial perchlorate reduction. *Environ Sci Technol 41,* 5, 1740-1746.

TIGGES, M. AND FUSSENEGGER, M. 2009. Recent advances in mammalian synthetic biology—design of synthetic transgene control networks. *Current Opinion in Biotechnology 20,* 4, 449-460.

VAN DER MEER, J. R. AND BELKIN, S. 2010. Where microbiology meets microengineering: design and applications of reporter bacteria. *Nature Reviews Microbiology 8,* 7, 511-522.

VIJAYARAGHAVAN, R., ISLAM, S. K., ZHANG, M., RIPP, S., CAYLOR, S., BULL, N. D., MOSER, S., TERRY, S. C., BLALOCK, B. J. AND SAYLER, G. S. 2007. A bioreporter bioluminescent integrated circuit for very low-level chemical sensing in both gas and liquid environments. *Sensors and Actuators B: Chemical 123,* 2, 922-928.

VILANOVA, C., HUESO, Á., PALANCA, C., MARCO, G., PITARCH, M., OTERO, E., CRESPO, J., SZABLOWSKI, J., RIVERA, S., DOMÍNGUEZ-ESCRIBÀ, L., NAVARRO, E., MONTAGUD, A., DE CÓRDOBA, P. F., GONZÁLEZ, A., ARIÑO, J., MOYA, A., URCHUEGUÍA, J. AND PORCAR, M. 2011. Aequorin-expressing yeast emits light under electric control. *Journal of Biotechnology 152,* 3, 93-95.

VILLALOBOS, A., NESS, J. E., GUSTAFSSON, C., MINSHULL, J. AND GOVINDARAJAN, S. 2006. Gene Designer: a synthetic biology tool for constructing artificial DNA segments. *BMC Bioinformatics 7,* 285.

VINUSELVI, P., PARK, S., KIM, M., PARK, J. M., KIM, T. AND LEE, S. K. 2011. Microfluidic Technologies for Synthetic Biology. *International Journal of Molecular Sciences 12,* 6, 3576-3593.

VOIGT, C. A. 2006. Genetic parts to program bacteria. *Current Opinion in Biotechnology 17,* 5, 548-557.

VON CANSTEIN, H., OGAWA, J., SHIMIZU, S. AND LLOYD, J. R. 2007. Secretion of Flavins by Shewanella Species and Their Role in Extracellular Electron Transfer. *Applied and Environmental Microbiology 74,* 3, 615-623.

WANG, B., KITNEY, R. I., JOLY, N. AND BUCK, M. 2011a. Engineering modular and orthogonal genetic logic gates for robust digital-like synthetic biology. *Nature Communications 2,* 508.

WANG, H.-Y., BERNARDA, A., HUANG, C.-Y., LEE, D.-J. AND CHANG, J.-S. 2011b. Micro-sized microbial fuel cell: A mini-review. *Bioresource Technology 102,* 1, 235-243.

WEBER, W., LUZI, S., KARLSSON, M., SANCHEZ-BUSTAMANTE, C. D., FREY, U., HIERLEMANN, A. AND FUSSENEGGER, M. 2008. A synthetic mammalian electro-genetic transcription circuit. *Nucleic Acids Research 37,* 4, e33-e33.

WEEDING, E., HOULE, J. AND KAZNESSIS, Y. N. 2010. SynBioSS designer: a web-based tool for the automated generation of kinetic models for synthetic biological constructs. *Briefings in Bioinformatics 11,* 4, 394-402.

WEIBEL, D. B., DILUZIO, W. R. AND WHITESIDES, G. M. 2007. Microfabrication meets microbiology. *Nature Reviews Microbiology 5,* 3, 209-218.

YAGI, K. 2006. Applications of whole-cell bacterial sensors in biotechnology and environmental science. *Applied Microbiology and Biotechnology 73,* 6, 1251-1258.

ZHAN, J., DING, B., MA, R., MA, X., SU, X., ZHAO, Y., LIU, Z., WU, J. AND LIU, H. 2010. Develop reusable and combinable designs for transcriptional logic gates. *Molecular Systems Biology 6,*

ZHANG, F. AND KEASLING, J. 2011. Biosensors and their applications in microbial metabolic engineering. *Trends in Microbiology*