Bioengineering – Current Applications and Future Perspectives

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***Abstract* — The field of Bioengineering has provoked significant interest both as a theoretical approach towards better understanding of living systems and as compilation of applied methods for improved control over such systems. In the latter aspect significant success has been achieved in decoding biological information formatted as genes and genomes, modeling of biological processes such as metabolic conversion of matter and energy, design and engineering of novel biological parts like synthetic promoters and mutator devices and, finally, development of novel types of bioprocess systems with improved control capability like microfluidics devices. In this work we present the achievements of BioInfoTech Lab in all the above-mentioned applied areas and we elaborate on the future possibilities which the field of Bioengineering provides.**

***Index Terms* — Bioengineering, Genomics, Metabolic modeling, Microfluidics**

I. Introduction

Bioengineering is an immense scientific and applied field encompassing all the efforts towards the technologization of biological and biomedical systems at any organizational level. This field has shaped and thrived in the latter years owing mostly to the recent development of experimental approaches, techniques and instrumentation that allowed for the rigorous and in-depth investigation of biological systems. The latter development, on its hand, led to the accumulation of an immense amount of empirical data, which required novel theoretical apparatus for the sake of the improving our knowledge; and novel practical approaches for the sake of improving our lives. When standard engineering methods were utilized so that useful results could be obtained from our increased knowledge of the biological systems, Bioengineering was born.

As stated above, Bioengineering is an immense filed, since it utilizes all Engineering approaches like control theory, automation, data analysis, computation, material knowledge, miniaturization, industrialization, etc. for the sake of technologization of all biological systems and processes like genes and genomes, molecular biology, metabolism, evolution, bioprocess and biotechnology, biomedicine, etc. All possible combinations of all the above-mentioned entities could give rise to individual and independent disciplines with significant applicability and impact over science, health and economy. In this brief overview we are going to present but a few of the specific tasks from the Bioengineering field that we tackled in the latter years at BioInfoTech lab together with the significance and possible future applications of the broader discipline, which incorporates them.

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II. Genes and Genomes

To decode the biological information stored in the form of genes and genomes, various high-throughput DNA sequencing methodologies have rapidly been developed over the past two decades. Modern sequencing approaches today can provide large volumes of data at a very low cost. [1] This ability opens a lot of new possibilities in the field of Biotechnology and Bioengineering allowing scientists not only to better understand various living systems but also to develop strategies for improved control over such objects.

In general, two major directions of development exist in the modern next-generation sequencing (NGS) field - short-read sequencing and long-read sequencing. The short-read sequencing nowadays unites several so-called "sequencing by synthesis" methods. Most technologies of this type make use of different strategies in which all individual DNA molecules to be sequenced are separately located to millions of individual wells, or attached to specific locations on a solid surface. [2] The separated DNA molecules are next multiplied by different in vitro amplification techniques like PCR or isothermal amplification in order to generate many millions of individual DNA clusters. Each of these clusters is composed of many copies of single initial DNA molecule. Then these groups of DNA molecules are subjected to DNA synthesis reactions in which incorporation of labeled nucleotides, or chemical reactions that are a direct consequence of the incorporation of a particular unlabeled nucleotide, can be detected. This detection allows the generation of up to billions of short DNA sequence reads (in general with a length of 50–300 nucleotides). At the present time, the short-read NGS field is dominated by the various platforms developed by the Illumina Corporation. Their new production scale sequencer named NovaSeq 6000 can generate up to 20 billion short sequencing reads in a single run yielding 6000 Gb of sequencing data. This output is an even more remarkable achievement when is compared to the size of the haploid human genome which is estimated to be approximately 3.1 Gb. [3] Generating such data volumes at low prices allows modern DNA sequencing to re-shape many fields like agricultural biotechnology, genetic and genomic diagnostics, medical and environmental microbiology, forensic science, and many others. [4-6]

One such area of critical importance is the global antibiotic resistance crisis. Following recent improvements

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in sequencing technologies, whole-genome sequencing (WGS) is poised to become an essential tool in the control of antibiotic resistance. The emergence of multidrug-resistant (MDR) pathogenic bacteria has become one of the most serious global public health threats in the last decades. [7, 8] In fact, more than 700 000 people die every year worldwide due to infections caused by pathogens with antimicrobial resistance. If nothing is changed, by 2050, this issue will cause up to 10 million deaths annually and an estimated cost to the world economy of $100 trillion in total. [9] Even more concerning, the global pandemic situation caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is expected to have enormous impacts on the expansion of antimicrobial resistance because of the inappropriate use of large amounts of antibiotics, biocides, and disinfectants. [10]

At the BioInfoTech laboratory, we already use short-read sequencing to study the genomes of MDR and Extensively Drug-Resistant (XDR) bacterial pathogens from different hospitals in Bulgaria. So far we have discovered the first vancomycin-resistant *Enterococcus faecalis* isolate in our country [11] as well as the first VIM2-producing *Pseudomonas aeruginosa* strain. [12] Moreover, the short-read sequencing technologies allowed us to characterize an XDR *S. maltophilia* strain carrying the integron-associated gene cassette $bla_{OXA\text{-}74}$*–aac(60)-Ib-cr–cmlA7*, found only in clinical isolates of *P. aeruginosa* before. [4]

The long read sequencing branch unites methods that are capable of sequencing very long individual nucleic acid molecules. At the current moment, two major comercialized technologies exist in this field – Pacific Biosciences (PacBio) with their SMRT (Singe Molecule Real Time) sequencing and the nanopore-based DNA sequencing that was recently commercialized by Oxford Nanopore Technologies. [2] In contrast to the previously mentioned short-read sequencing approaches, the long read sequencing can generate single reads with lengths in the megabase range. This utility is tremendously useful in many applications like *de novo* sequencing of complex genomes and detection of copy number variations (CNVs). Thanks to the PacBio HiFi and Oxford Nanopore ultra-long read sequencing, the Telomere-to-Telomere (T2T) Consortium recently has finished the first truly complete 3.055 Gbp sequence of a human genome that includes gapless assemblies for all 22 autosomes plus chromosome X adding approximately 200 Mbp of novel sequence. [13, 14] Now the sequences of all centromeric satellite arrays and the short arms of all five acrocentric chromosomes are presented for the first time. It is worth mentioning that PacBio and Nanopore technologies also allow simultaneous detection of many (but not all) nucleotides with base modifications like adenine and cytosine methylations. [15, 16]

When even the organisms in all various investigated natural environments cannot provide the sequence encoding a product with specific properties that is needed, it can be designed applying techniques like directed evolution. Directed evolution is a cyclic process that alternates between gene diversification via mutation and screening for/selection of gene variants with improved qualities. [17] A critical step in this method is to create a large number of gene variants. To achieve this, various biological mutator devices can be applied to reduce the danger of the more classical chemical and physical methods for random mutagenesis. Some of them are based on vectors that express a combination of genes known to adversely affect DNA replication fidelity yielding mutation rates 322,000-fold over the basal levels in *Escherichia coli*. [18] Others rely on conditional gene silencing of multiple endogenous genes with known role in DNA reparation processes. [19]

III. Design and Engineering of Novel Biological Parts

Once the biological information stored in the genome of a given organism is read, the next step in the bioengineering paradigm is to use it for the design and engineering of novel biological parts. Such molecular building blocks are quite useful for designing new synthetic gene circuits with desired properties. [20] The standard parts conception relies on three major fundamentals – an existing registry of a large number of well-characterized and easily accessible DNA parts, a simple and reliable method to assemble these elements in any required pre-determined order, and finally, a methodology that can allow fast and efficient generation of new parts. A good example is the BioBrick idea that was developed to introduce the engineering principles of abstraction and standardization into synthetic biology. [21] A large number of BioBrick parts already exist in the so-called Registry of Standard Biological Parts. Furthermore, all of them can be assembled in any required order yielding complex synthetic genetic devices by applying standard gene engineering techniques with just few enzymes. [22] The generation of new parts on the other hand can be easily accomplished via gene synthesis. This allows efficient genome mining of novel enzymes, regulators, and whole biosynthetic gene clusters. [23] Such approach is particularly powerful when a metagenome sequencing data from complex and/or extreme environments is used because the classical cultivation-based recovery of a particular microorganism of interest from these locations is impossible in up to 99% of the cases. [24]

The design and engineering of biological parts is a cornerstone for the construction of Synthetic Biology [25], a discipline which has adopted most directly approaches, techniques and even terminology from Engineering. The definition of Synthetic biology itself is the application of Engineering principles towards biological systems (Genetics for starters) and their *de novo* design and control. Consequently, the requirements for biological parts resemble those for engineering parts; for genetic parts, specifically, those requirements are the same as for parts in Electronics - modularity, characterization, behavior and robustness.

From the latter, modularity requirement has led to the functional and molecular discretization of the genetic function in few separate units – the promoter, the ribosome binding site (RBS), the coding sequence (including the start and the stop codons) and the terminator. Design and characterization of synthetic representatives of all of those classes have been performed successfully and have been already applied in industry (mainly biotech). In short, promoters [26] are the landing pads for the molecular

machinery, which produces ribonucleic acid (RNA) from the coding sequence, they are the places where the whole process of genetic expression commences. The RBS is the part of RNA, which is recognized by the ribosome, the latter on its turn catalyzing the polymerization and production of proteins defined by the coding sequence. Finally, the terminator provides a mechanical break for the process of RNA synthesis and determines the end of the first step of the genetic expression.

Thus, we have a multi-step process, which is best controlled at the first stage, including from the point of view of resource optimization, namely by the promoters. BioInfoTech lab participated in the development of a number of synthetic promoters, among which the ones utilized to control genetic expression in blue-green algae (Cyanobacteria) [27] raised the highest interest among the Synthetic Biology community. The latter is due to the ongoing interest towards the engineering of eco-friendly sources of hydrogen fuel, of which Cyanobacteria are the most prominent producer.

However, the most important aspect of Synthetic Biology is its final goal – to be able to control living systems in such precise manner that one could create *a la carte* desired biological functions and even complete novel organisms. Amazingly, this is not just a visionary's dream anymore, but actual scientific results. To name just a few of the latter, bioengineers have successfully developed bacteria that oscillate in blue when there is arsenic in the environment [28], other bacteria have been re-designed so that they directly produce bioplastics [29], even specially engineered male mosquitoes [30] whose offspring has a very short lifespan have been created in order to reduce the population of the dengue-carrying subspecies. Next in the line are waste treatment, biofuels production, new materials, and, of course, Mars terraforming.

IV. Metabolic Modeling

Successful design and fabrication of individual synthetic parts represents a great advance towards the goal of complete control in Bioengineering, however, it is far from enough. The reason for the latter is that biological systems are inherently complex in nature. Indeed, even a simple bacterium such as *E. coli* relies for its functioning on more than 300 biochemical reactions, which interconnect in a complicated web more than 500 chemical compounds. In this web it is easy to discover feedback loops (both positive and negative), cycles, shunts, etc. This level of systems complexity is inevitably leading to emergent properties, which are not self-evident *per se* and could only be predicted through careful modeling. Hence, reliable metabolic modeling is of utmost importance for complete understanding of the structure-function interconnection in biological systems.

The general function of the metabolism is to provide the organism with the means (matter and energy) to maintain its physiological state and to grow. While the former is the main goal for higher organisms, for bacteria growth is generally accepted to be the unique focus because of the "winner takes it all" or "survival of the fastest reproducing" principle. In order to achieve optimal growth prokaryotes need to organize the fluxes of matter through the metabolic pathways in a manner, which leads to increased production of certain compounds assumed to be indispensable for biomass accumulation. I.e., cells need to allocate the limited amount of available input resources in order to maximize certain output(s). The latter is a classical optimization problem and is usually solved with classic techniques such as simplex method, which is the essence of the flux balance analysis (FBA) [31] utilized in metabolic engineering.

While FBA was successfully applied for the solution of different theoretical and practical problems mainly in biotechnology, it has the drawbacks of being static (it provides only the steady-state solution) and mechanistically impossible. The first obstacle is being addressed more or less successfully with specific dynamic FBA techniques, but the second one remains a major issue. In a nutshell, simplex method implies that there is a single logical space where the information for all possible resources' allocation methods (i.e. biochemical reactions/metabolic fluxes) is collected, unified and utilized to produce the optimal solution. If it was the case of a farmer deciding how much land to dedicate to which product there is no problem, the farmer is this omniscient being. However, we have no information whatsoever of the existence of such knowledge center in the bacterial (or any other type of) cell.

To tackle this problem, we at BioInfoTech lab assumed a rather different approach. We decided to simulate each of the hundreds of simultaneous biochemical reactions, which take place in the organism, independently by assuming that the direction and rate of each reaction depends only on 2 things: 1) the concentrations of the compounds participating directly in the reaction and 2) the basic thermodynamic principle of Le Chatelier [32]. Each reaction was simulated independently, but all reactions were simulated parallelly for each simulation step (time unit). This way we overcome both the impediments connected to the application of FBA method: the lack of dynamics and the requirement for general knowledge of the system. The only disadvantage of this approach utilizing only basic first principles is that it is far more demanding computationally, however, increasing computational power is rendering that issue less and less important.

We have successfully modeled the complete metabolism of a number of different organisms among which was also the noted *E. coli*. We simulated different growth conditions, including continuous vs. batch cultivation and we observed some significant dynamic patterns, including the optimization of the exhaustion of certain compounds, the reaching of steady state for others and continuous accumulation (optimized production) for metabolites responsible for cellular growth without any limiting factors apart from the first principles (Fig. 1). This way we obtained results similar to simplex method optimization through a type of distributed decision-making.

V. Microfluidics

Up till now we have discussed matters concerning engineering and optimization of different types of biological units at different level – genes, genomes, metabolism, whole organisms. However, Bioengineering encompasses also the

larger field of Bioprocess engineering, to which biological systems and their activity are merely one of the many steps of complicated industrial or scientific processes. At present the problems with macro-scale instrumentation and its proper control are solved and we seem to have reached the technological maximum of this approach. However, just like with electronics, miniaturization has offered a number of remarkable novel features of the technology like improved control, lower cost, parallelization, multiplication and seamless integration with microelectronics.

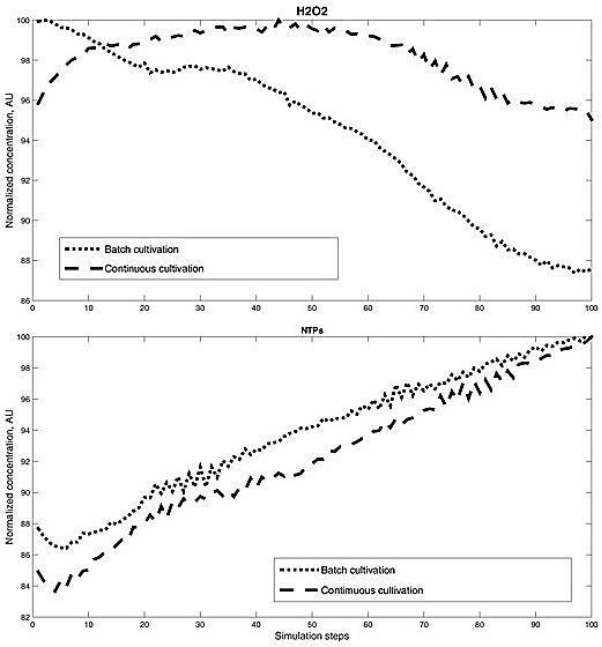

Figure 1. Metabolic simulation of batch and continuous cultivation of E. coli. The difference between the two cultivation regimes is clearly visible for the side-product hydrogen peroxide (above). However, the key-for-growth building blocks for ribonucleic acid (below) are continuously synthesized regardless of cultivation regime.

The technology that allowed for the successful miniaturization of biological processes is called Microfluidics [33]. Microfluidics devices consist of systems of fluidic channels and reaction chambers. The strict definition a microfluidic device is a fluidic device with at least one dimension within the micron scale. The smaller device dimensions result in a very low ratio between the inertial and friction forces (Reynolds number). Consequently, the fluid flow within a microfluidics device is strictly laminar with no observable spontaneous mixing. The latter is extremely important, since it provides the opportunity for precise direction of reagents with strict chemical composition towards specific parts of the microfluidic chip this way imitating spatial separation that usually takes place when macro-scale reactions are performed. In short, Microfluidics allows for the reducing the size of a whole biological lab or biotech plant to a chip sized 2x5 cm.

The practical applications of Microfluidics in Bioengineering are countless: molecular biology, biotechnology, medical diagnostics, organ-on-chip, etc. We, at BioInfoTech lab have assembled a dedicated lab for low-cost microfluidics chips prototyping, which consists of resin-based 3D-printing, elastomer molding, plasma-cleaning and attachment and temperature curing. Currently, we work on a number of projects involving microfluidics including a rapid antibiotics susceptibility test, on-chip cell-lysis system [34], vacuum-loading system for continuous cultivation (Fig. 2).

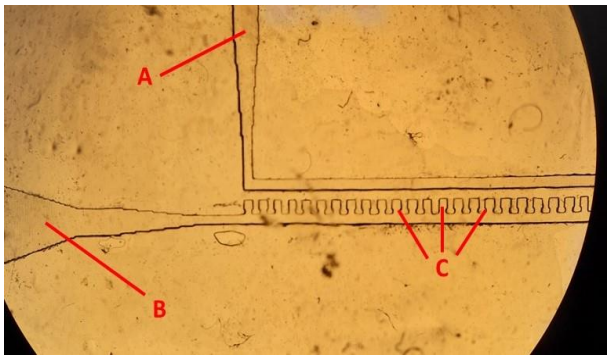

Figure 2. A microscope image of a vacuum-loaded microfluidics device for continuous cultivation of bacteria. The vacuum line (A) does not contact directly the supply line (B), but rather utilizes the material's porosity in order to suck the air from the growth chambers (C). The latter function as micro-chemostats (width is 50 μm).

VI. Conclusion

In this paper we have briefly reviewed a minuscule part of the immense variety of theoretical topics and practical problems addressed by modern Bioengineering. Indeed, the complete flourishment of this vast field is yet to come, since we are just entering the era of synthetic food, strive for longevity and better quality of life and, why not, space conquest and terraforming. All of those rely heavily on the accomplishments of Bioengineering and the complete control of the existing and the development of novel life forms it promises. Hence, this huge scientific and technological area is an indispensable part of any Engineering institution aiming to prosper in the nearer and further future.

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