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Biocircuits to program cell-cell communications

UPM researchers coordinate PLASWIRES, a European Synthetic Biology project whose objective is to program and build multicellular genetic circuits executed in live bacterial colonies.

How is a genetic circuit being programmed? Is it possible to transfer genetic programs between bacteria? Can bacteria do the work of an engineer? The PLASWIRES project tries to answer these and other issues; it is a European initiative aiming to design and carry out different genetic programs in bacteria and to establish an interbacterial communication protocol.

"This basic research project will have multiple potential applications in biomedicine, biotechnology or, for instance, in the development of antibiotics", Alfonso Rodríguez-Patón, professor at the Escuela Técnica Superior de Ingenieros Informáticos and project coordinator, explains.

The work fits into the European Union's seventh Framework Programme, in the *FET Evolving Living Technologies* Area. With interdisciplinary character, it is supported by funding of over two million and a half Euros and three-year duration. Along with the UPM, it involves researchers from the *University of Cambridge*, the Pasteur Institute and the Universidad de Cantabria.

This project is included in the scope of Synthetic Biology or Biological Systems Engineering, a new area of investigation that considers biology as a technology that can be programmed and with which biological devices can be built and designed. "Biology is a science that has traditionally been limited to trying to explain how living organisms function. In Synthetic Biology, we try to use biology as a hardware to build genetic circuits that have a specific biological function", Rodríguez-Patón explains. "We can not only program a cell but a whole cell population. For instance, programming a bacterial colony to do tasks, communicate and solve problems concurrently and in a distributed manner", he says.

An approach to Synthetic Biology

Synthetic Biology was conceived in the nineties, when biologists and computer scientists began working together in the human genome sequencing. The precursors were Ron Weiss and Tom Knight, researchers at the MIT Artificial Intelligence Laboratory and authors of the early works. "The technological advances made during these years enabled cost reduction in processes such as reading and writing DNA, which made it possible for Synthetic Biology to be approachable to many laboratories", Rodríguez-Patón emphasizes. This, together with other technologies developed around DNA, gave engineers "a good toolbox" to build genetic devices.

A synthetic genetic program is no more than a DNA sequence that contains one or several genes and that is executed when getting inside a cell. All living organisms have in each of their cells their own natural genetic program (genome) with all necessary instructions to live and reproduce. "In the language of computer scientists, a gene is a program that contains the instructions to build a protein", the professor explains. It is a manufacturing process known as gene expression. "A gene is expressed when its DNA sequence is read, it is copied into RNA (which is the working template) and it is translated into protein".

In living organisms, genes have binary behavior: they can be activated, if they are being expressed and they generate its associated protein, or deactivated. Can activation or repression of a gene be controlled? The answer is yes. There are external signs that can control gene expression. "Genetic programs are executed or not depending on the proteins and other biological signs in their environment. Thus, a gene results in its protein, which, in turn, can regulate the activity of other genes. We can custom design this process by building circuits and specific synthetic gene networks", Rodríguez-Patón explains.

In order to do this, researchers execute these genetic programs inside bacteria. One of the most commonly used in laboratories is *Escherichia Coli* (*E. Coli*), which "we know well and it grows very fast, as it is divided every half hour", the UPM professor stresses. In Synthetic Biology, this quality implies that, in only thirty minutes, two processors executing the same genetic program can be obtained from a processor. "We find a parallel computational model that is very fast and cheap", he affirms.

¿How is an experiment in Synthetic Biology finally developed? First of all, researchers design through computer the DNA sequencing belonging to a genetic program, then its production is ordered to a DNA synthesis company and lastly the DNA is introduced into a bacterium in order to execute such program. In order to display the result that the circuit is giving, fluorescent proteins are typically used. The work of engineers in this field consists in the design and the simulations of genetic circuits, that is, simulate how bacterial colonies grow and execute these genetic programs. "We are working in the field of Artificial Intelligence and mathematical modeling of circuits. We run a computer simulation seeing how is the expected execution of these genetic circuits before going to a biology laboratory", Rodríguez-Patón explains.

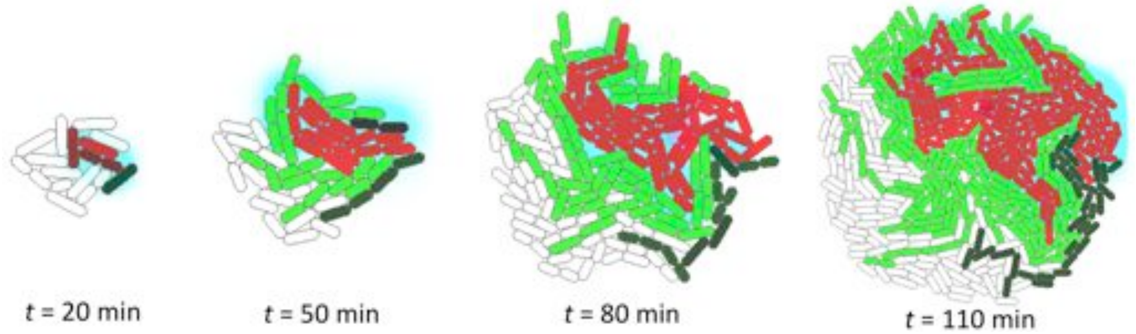
When it comes to working with biological systems, engineers have to take into account that they are living devices that "grow, reproduce and die", entailing some risks, such as mutations, noises or interferences made, bacteria mixing up signs or problems when it comes to connecting the different components, he points out.

Build a "Programmable Bacterial Internet"

The next step in Synthetic Biology lies in executing circuits increasingly complex. In order to achieve this, these circuits can be put into a simple bacterium, or distribute the different parts of the circuit in various bacteria. In such a case, the difficulty lies in getting bacteria communicate with each other.

For an engineer, the UPM professor explains, each bacterium of the colony is a processor (hardware) with an operating system (its genome) that execute an optional and movable genetic program (the genetic software or the "app" of DNA that has received and that biologists call "plasmid"). Bacteria have an intense social life and they communicate exchanging their plasmids by means of an intercellular communication process called conjugation.

Conjugation enables biological processors to exchange genetic information, something very "powerful when it comes to establishing distributed computational models", Rodríguez-Patón states. Using as a basis that conjugation mechanism, the UPM researcher coordinates the European project PLASWIRES (PLASmids-as-WIRES). The objective is to show how to program a distributed and parallel live computer using conjugative plasmids, that is, those that can be transmitted from one bacterium to another.



Computer simulation of a genetic circuit that detects the rim of an infection (bacteria with red plasmid). Bacteria with sensor genetic circuit are dark green. Sensor bacteria emit green fluorescent light by detecting the plasmid.

It involves designing and developing the execution of each of these genetic programs and controlling the communication of plasmids between bacteria. "We are going to program a multicellular live parallel computer where the "wires" that communicate various processors (bacteria) are conjugative plasmids. We will have a type of programmable bacterial Internet", he points out. This parallel, multicellular and live computer consists of a colony of programmed bacteria which, in turn, can "listen" and interact with another colony of natural bacteria and "persuade them", for instance, so that they do not spread or they do not become virulent, preventing them to transmit their worst "apps" to their neighbors.

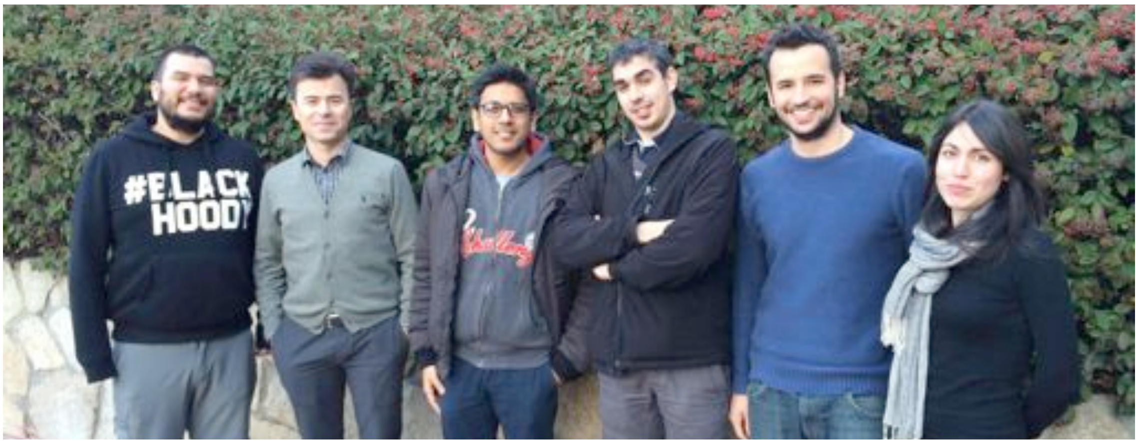
Drug development and biotechnology, potential applications

Synthetic Biology represents an emerging area of basic research, even though some applications are already starting to materialize. Jay Keasling, researcher of the *University of California*, Berkeley, is working on the production of a synthetic anti-malaria drug as an alternative to its natural variant with high cost that is obtained from the tropical shrub *Artemisia*. The American researcher funded by the Gates Foundation and the pharmaceutical Sanofi works to develop a synthetic version of the vaccine enabling cost price reduction.

On the other hand, some laboratories work in the design of microbes that produce ethanol. "There is still no mechanism that achieves efficiency in the production of ethanol to compete on prices with the current fuels, but there is great interest by oil companies to work on this", the Spanish Rodríguez-Patón points out.

According to researchers, Synthetic Biology will have dozens of potential applications, from drug production to the development of the best chemical compounds or the field of biotechnology. In the words of Jay Keasling, "everything that a plant can produce will also be able to be produced with a microbe as well".

So far, most work done has involved the design and execution of simple genetic circuits, such as logic gates (AND, OR, etc.), bistables (two genes that mutually inhibit), oscillators (a circuit with three genes that mutually suppress) or *feedbacks* (a gene that is suppressed or that is self-activating). "We are at a similar level as electronics in the 60's. Despite the tremendous strides in genetic engineering, biology is still not easy to be programmed. It is a new field of research, multidisciplinary and fascinating in which biologists, engineers, physicists and mathematicians have started working together. This new engineering of biological systems progresses quickly and nobody doubts its huge impact on the future. However, let us be realistic, from design to execution of a circuit there is a yawning gap: executing basic biological circuits in a cell still today represents a major challenge", the UPM researcher concludes.



Researchers of the LIA group. From left to right: Martín Gutiérrez, Alfonso Rodríguez-Patón, Vishal Gupta, Ismael Gómez, Guillermo Pérez del Pulgar and Paula Gregorio.

ALFONSO RODRÍGUEZ-PATÓN

B.A in Physics specialized in Electronics (1992), PhD in Computer Science since 1999. His doctoral thesis was focused on Computation with DNA, and later carried out his postdoctoral research activities in Turku (Finland) and at Harvard

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His lines of research cover Synthetic Biology, Systems Biology and Biomolecular Computation. At present, his works focus on the analysis and synthesis of multicellular genetic devices, with the goal of finding out how cells process information and make decisions. He takes part in two European research projects, one as a coordinator (*FP7-FET Proactive* named *PLASWIRES: Engineering Multicellular Biocircuits: Programming Cell-Cell Communication Using PLASmids as WIRES*)and another one as chief researcher (*EVOPROG: General- Purpose Programmable Evolution Machine on a Chip)*. He is also chief researcher of the national research project *TIN2012-36992: Engineering and Programming Biocircuits: Design and In Silico Modeling.* He is the author of 83 publications in the *Web of Science*.

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