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Editorial

LINKING BIOLOGY TO ENGINEERING

by Frangiskos Kolisis

Our Infrastructure "OMIC-Engine" is participating in the European Project "**Fostering Synthetic Biology: standarisation through international collaboration (BioroBoost)"** in a consortium along with other 27 Universities, Research Centers and Industries from the European Union, USA, China, Japan and Singapore. The second general assembly of Bioroboost took place on September 3-5 in Ghent and with this letter I would like to present some of the key conclusions derived from this meeting. But, before that I would like to use this opportunity to give some information about the strategic plans and the most important aspects of the project starting with the definition of Synthetic Biology:

Synthetic Biology is an engineering research field aiming at (re)designing biological circuits for applied purposes, or in other words the design and construction of new biological parts, devices, and systems, and the re-design of existing, natural biological systems for useful purposes.

As any other engineering field, it strongly relies on the use of well-defined, universal and robust standard components. But, defining biological standards is a difficult task taking into consideration the crossroad nature of synthetic biology involving mainly biologists/biotechnologists and engineers, whose views on the standardization of living beings tend to differ.

In BIOROBOOST it is planned to overcome cultural issues and to advance in solving technical difficulties by

i) gathering the most relevant stakeholders of all the aspects of standardization in biology in Europe
ii) empirically testing cultural (lab-centric) standardization practices and by promoting a consensus conceptual and technical redefinition of biological standards; and, finally,
iii) fostering a realistic and flexible toolbox of standard biological parts.

The two key aspects of standards which have been chosen are *shareability* and *reusability*. Synthetic biology has promoted a 'culture of sharing' in which researchers are expected to contribute to progress in the field by: 1) Developing well enough characterized standard biological parts that other peers can use; 2) Storing them in digital and

relatively open repositories; 3) Reusing existing parts that were previously developed by other peers.

BIOROBOOST will address the expected impacts by gathering the current knowledge on standardization in biology, by establishing the most complete network of all the relevant stakeholders, by creating a website and resources which will be reference ones in the standardization process. Improving the predictability of biological design demands the development of an ecosystem of standards that will have an impact by:

- Reducing the perceived unreliability of engineering biology, due to the context-dependent and complex emergent character of living systems
- Allowing researchers to build ever more sophisticated devices and systems
- Promoting the translation of SB to socioeconomic and environmental impacts benefitting the whole society.

More, comprehensively, BIOROBOOST will impact on the following areas during and after its lifetime:

- Modelling of bacterial, yeast and mammal cells systems, from the biological parts to the methodological procedures (the project is open also to plants and insects).
- Implementation of niche-adapted bacterial chassis for further fine-tuning as biomachines
- New approaches for the development of novel products
- Development of international research & cooperation plan,
- Participation in EU and Nationally-funded projects

“OMIC-Engine” is participating mainly in WP1 (Standards in Biology, theory development, biological Metrology), WP2 (Chassis in Synthetic Biology) and WP3 (Stardarisation beyond bacteria). According to the project in WP2 (Task 2.1) “OMIC-Engine” is currently developing genetic tools for thermophilic microorganisms to apply SB approaches in the production of metabolites at elevated temperatures. Also, it is involved in the phenotypic characterization in the ColiChassis and other bacterial chassis in extreme environments and in the area of standardizing protein-based parts for SB applications. “OMIC-Engine” activities will focus on the development of a standardized thermophilic bacterial chassis based on *Bacillus* and *Geobacillus* sp. for high-temperature applications, a currently unmet need in SB applications. Also they will work in the phenotypic characterization in the ColiChassis and *Pseudomonas putida* chassis for specific applications in extreme environments, finally in the area of standardizing protein-based parts

for SB applications, as it is expected that in many cases the desired protein-based part will not work in the desired modular fashion, it will aim at applying rational and evolutionary protein engineering approaches to deliver modular protein parts for these challenging cases. Mapping the functional landscape of *Rhizobiales* species (*Rhizobium/Methylobacterium*) with respect to their potential to be applied as chassis in environmental, plant-associated and biotechnological applications will be carried out. Finally, genetic circuitry components, biosensor engineering and building *E. coli*-based microbial cell factories will be mapped.

Conclusions from the Ghent meeting

During the meeting and the extensive discussion that followed, some important conclusions were derived, which can be very useful for all researchers involved in Synthetic Biology. I will emphasize the parts mostly dealing with the definition of what a chassis is and how we can standardized it, as well as the important risk assessment issues we need to keep in mind when constructing and before using it.

Towards a definition of SynBio chassis:

In general sense, a chassis is a defined, reusable biological frame where non-native components can be plugged in and out for creating new functionalities.

In a realistic sense, a chassis is a biological host amenable to and optimized for holding designed genetic circuits and deploying their encoded properties under specified environmental conditions.

What extra info makes a host to become a chassis:

- Genome edited for efficacy in target scenario
- Stability (no HR), durability
- Ab-sensitive, phage sensitivity
- Genetic tools

Standardized chassis:

- Defined energy metabolism
- Stress resistance
- Traceability (unique identifiers)
- HGT (both as recipient & donor)
- Safety (GRAS/FDA vs QPS/EFSA)
- Containments achievable if required

Risk assessment issues for SynBio Agents:

1. Can: SBAs colonized and eventually takeover natural microbial communities?
2. Is there a chance that SBAs enter new niches that natural bacteria cannot?
3. Could SBAs go into a stage of uncontrolled growth?
4. What are the chances of horizontal transfer of the synthetic genes to novel recipients?
5. Is there a trade-off between safety and biotechnological efficacy of SBAs?
6. Could traits engineered in SBAs evolve towards viruses or other deleterious behavior?
7. Are there scenarios where SBAs are capable of changing life or property?
8. What is the environmental fate of synthetic genes?
9. Should SBAs be endowed with traits to increase their safety and predictability?

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Seed project: A (photo)synthetic biology platform for triterpenoid expression

By Konstantinos Vavitsas
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Cyanobacteria are a promising platform for the renewable production of high value-added chemicals, as they combine photosynthetic growth with simple cellular organization. And they come with an added advantage: they are excellent hosts to express complicated plant pathways. The synthetic biology toolbox of cyanobacteria has not been fully explored. But some recent works make the engineering of cyanobacteria easier. The synthetic biology kit CyanoGate is one toolbox that can be used in generating a lot of genetic constructs to transform a variety of cyanobacterial species

Cyanobacteria growing in a multi-cultivator. Image by Dave Thomas, CC BY-NC 2.0

In this work, supported by OMIC-Engine seed funding, we plan to express a complex triterpenoid pathway in the model cyanobacterium *Synechococcus elongatus*. Triterpenoids are specialized plant molecules. They consist of a skeleton of 30 carbon atoms, which can be "decorated" with hydroxyls by a specialized enzyme group call cytochrome P450s. Combining the strengths of our photosynthetic hosts, we plan to express a triterpenoid pathway and improve its expression and yield using genetic engineering. This project is a

collaboration between a group from the National & Kapodistrian University of Athens and the University of Thessaly. The research team consists of Dr C. Garagounis and Assoc. Prof. Kalliope Papadopoulou from UTH, and Assoc. Prof. Dimitris Hatzinikolaou and I from AUA.

We are grateful for the financial report from Omic-Engine that allows us to initiate the project and test its feasibility. Stay tuned for the first results!

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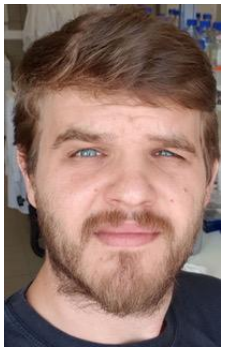
Interviewing Researchers of OMIC-Engine

In this section we will present you the researchers of the OMIC-Engine Research Infrastructure



Dr Costas Garagounis holds a degree in Biochemistry and Biotechnology from the University of Thessaly, he was awarded a DPhil in Plant Sciences, focusing on the microcompartmentation of enzymes in plant metabolism, from the University of Oxford and has since worked as a post-doctoral researcher for the FP7, EU-funded project TriForC and as a contracted lecturer at the University of Thessaly. Costas was recruited by OMIC-Enginge as a post-doctoral researcher in synthetic biology to establish *in vitro* culture techniques and transformation methods of the non-model legume fenugreek, with a view to developing it as a metabolic engineering platform for plant specialized metabolite production.

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Question: Kosta tell us a few words about the research project you are working on in OMIC-Engine?

I am currently working on a couple of different projects, both of which focus on deciphering the biosynthetic pathways for specific specialized chemical compounds made in certain members of the bean family and in developing synthetic biology pipelines for their production. Such compounds often have strong potential to be used for medicinal applications, natural food preservatives and food additives, and many other applications. As such, there is a big interest both in finding out how plants produce these compounds and in developing the Synthetic Biology-based methods to produce these natural products in quantity in a reliable and eco-friendly manner.

Question: Which opportunities did your work in Omic-Engine offers to you in terms of training, networking and personal development?

In the course of this work I have had the opportunity to both conduct my own lab work and further enhance my knowledge and understanding of plant metabolism, something which is of great interest to myself and is also a field for future scientific research in general. I've also had many opportunities to meet and interact with colleagues and senior scientists in Greek academia and this has been a great experience for me as well

as the starting point for at least two research project collaborations with people I had never met prior to OMIC-Engine. I am also mentoring an undergraduate team in the 2019 iGEM competition with the help and support of OMIC-Engine and this is also a wonderful experience and a chance to hone my mentoring and teaching skills. In all this has been a very positive experience for me.

Question: What do you think will be the impact in your future career?

Well, we can never be certain of the future, but as I mentioned above OMIC-Engine has given me the chance to continue some interesting paths in research as well as start some new research avenues, these have already led to a couple poster presentations and at least two manuscripts for publication. It has also helped me develop my research network within Greece. All of these are positive for developing an academic career in future.

Question: Has this experience matched your expectations so far?

I would say, as far as research is concerned, it has been similar to my past experiences as a doctoral-candidate and a post-doctoral researcher. The challenges have been similar, if anything it's been slightly tougher. I have been pleasantly surprised by the new contacts I've made under OMIC-Engine, these certainly exceeded my initial expectations. Hopefully, they will last long after OMIC-Engine. The other really important thing, which I'm really happy about, is the acquisition of new equipment under OMIC-Engine. It has been a huge boost to my host-department and I'm sure it's the same for the other participating institutions. Off-course you can never have enough new equipment, but this was certainly a necessary and timely, if anything slightly belated, update package in the equipment available for synthetic biology research in Greece. Overall, I guess you could say it's been better than I expected in the beginning.

Question: Three words that sum up your experience within the OMIC-Engine infrastructure.

Challenging, Interesting, Exciting, in that order of priority!

iGEM Thessaly 2019

The iGEM Competition

The iGEM Competition, stands for international Genetically Engineered Machine and is the biggest Synthetic Biology competition in the world that was initiated by the MIT and takes place every year in Boston. The very concept of iGEM has spread across the globe and as a result this year more than 350 student groups, from universities and schools all over the world, will flock to Boston in order to present their research projects. The research projects are based on the principles of Synthetic Biology and mainly aim to propose solutions in local and global problems.

iGEM Thessaly 2019

The spark that lighted up the creation of our team was the Synthetic Biology course that is taught in an undergraduate level for the first time in Greece at the Department of Biochemistry & Biotechnology of the University of Thessaly. iGEM Thessaly 2019, is the first interdisciplinary team of the University of Thessaly to take part in the iGEM 2019 competition. Our team is comprised of 10 undergraduate students from 4 different Science Departments of the University of Thessaly. Vasiliki, Leandros, Afroditi, Nikoleta, Nikos, Thodoris and Athina studying at the Department of Biochemistry and Biotechnology, Xenia from the Department of Mechanical Engineering, Maria from the Department of Architecture and Eleftheria from the Department of Computer Science and Biomedical Informatics. During iGEM we develop many skills including research in real life conditions, science communication, website development, modelling and fundraising.

Our project – ODYSSEE

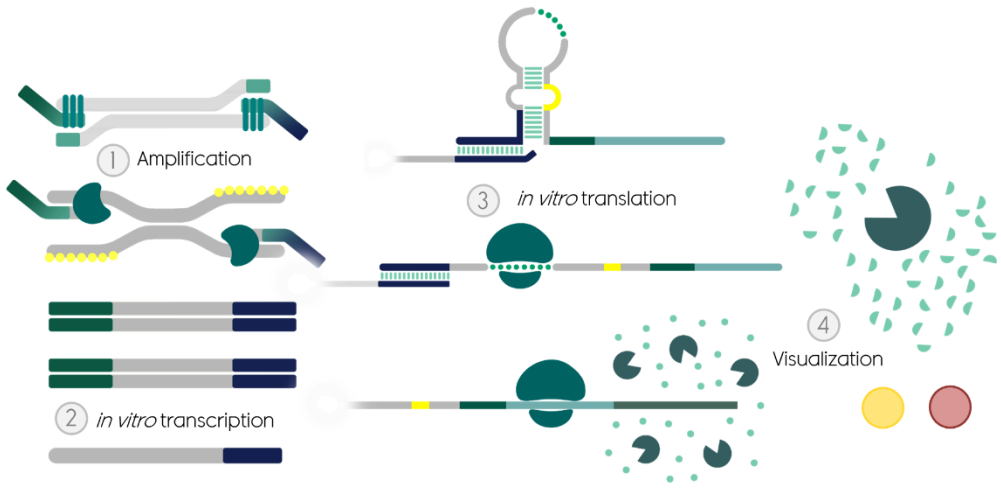
ODYSSEE aims for the fight against the communicable disease Tuberculosis (TB), a major threat for populations affected by crises such as refugees. Refugees and migrants are entitled to the same universal human rights and fundamental freedoms as all people, which must always be respected, protected, and fulfilled. More than 85% of refugees flee from and stay in countries with a high burden of TB.

Despite increases in notifications of TB, progress in closing detection and treatment gaps is slow and large gaps remain. The goals of the World Health Organization's End TB strategy will not be achieved without new tools to fight TB. For this reason, we are developing ODYSSEE, a rapid, reliable and safe test for early

diagnosis of Tuberculosis that would be applied in refugee camps in Greece, as well as worldwide, wherever is needed.

The test will work on urine samples. Once the **Mycobacterium tuberculosis,** that causes the disease, dies in a patient's lung, it releases DNA fragments (cell-free DNA - cfDNA) into the blood as it breaks down. cfDNA's small size allows for it to cross the kidney barrier and appear in the urine. The biomarker we selected is the IS6110 gene (1355 bp), which is located in the genome of the **Mycobacterium Tuberculosis** (MTB) and encodes for a putative transposase. IS6110 belongs to the family of insertion sequences (IS) of the IS3 category and is most commonly used for the detection of MTB because it is highly conserved.

The detection workflow contains 4 steps of amplification of the target gene. It begins with isothermal DNA amplification of the MTB DNA fragment, with the incorporation of two universal sequences, at 5' and 3' end respectively. An *in vitro* transcription of the amplicon follows with the combination of these two steps enabling addition and amplification of a universal trigger sequence, which is transcribed to RNA. This trigger RNA enables the *in vitro* translation of a toehold switch, a biosensor that encodes for a b-lactamase. b-lactamase is an enzyme that hydrolyzes a chromogenic substrate nitrocefin, which then turns from yellow to red. The colorimetric readout will enable naked eye detection of the result.



Tuberculosis detection is just the beginning. We aim to create a universal tool able to identify other communicable diseases as well. The key component to achieve this is the trigger RNA that is designed by the team's wet lab and added to the reverse primer for the first step amplification. This can be achieved by just changing the primer set and keeping the overhangs that contain the universal trigger, as well as the following path, the same, targeting this way different pathogenic agents.

The ultimate goal is to supplement conventional diagnostics by providing a modular, universal diagnostic platform for various diseases so that all patients have access to innovative tools and services for rapid diagnosis and care.

iGEM Thessaly's project brought to life

Omic-Engine's vision to enhance Synthetic Biology efforts throughout the Greek Academic Community is fully aligned with our goals for the iGEM Competition. The Greek Infrastructure for Synthetic Biology has offered great support to our team basically by sponsoring our laboratory provisions without which we could have not conducted our experiments.

Although, the support we are receiving from Omic- Engine doesn't stop there. For starters, our PIs Kalliope Papadopoulou and Kostas Mathiopoulos have been supporting us since the very beginning. Their laboratories at the Department of Biochemistry & Biotechnology of the University of Thessaly, are providing us with lab space, supplies, and instrumentation while helping us with troubleshooting during our experiments.

Other Omic- Engine's members have offered us their valuable help as well. Professors Karpouzas, Moutou, Skretas, Leonidas and their labs have supported our project by providing us with reagents crucial for our experiments. Not only that but, back in January while we were considering our project design, we came in contact with researchers from Stamatis Lab at the Department of Biological Applications & Technologies of the University of Ioannina, that shed light on the use of microfluidics in biological applications.

Omic- Engine Infrastructure's support makes the opportunities we can have limitless and for that we are really thankful to its members. We hope to contribute to the effort of spreading the word of Synthetic Biology to Greece through our journey to Boston.

iGEM Thessaly's research project is supported by the research infrastructure Omic-Engine, the State Scholarships Foundation (IKY), the Research Committee of the University of Thessaly, Hellenic Petroleum, Novartis and ELPEN.

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New Projects coming in the OMIC-Engine Network

In this section we will update you on research activities and new project coming in the research network of the OMIC-Engine Research Infrastructure

- A new project entitled "*Environmental fate and interactions of the veterinary antibiotics ceftiofur and tiamulin with the soil microbial community: resistance, biodegradation or ecotoxicity?*" was accepted for funding in the frame of the second call for the Support of Researchers with emphasis on Young Researchers. The project will take place in the Laboratory of Plant and Environmental Biotechnology, Department of Biochemistry and Biotechnology, University of Thessaly under the supervision of Prof. Dimitrios G. Karpouzas. Dr C. Perruchon and PhD student E. Katsivelou will be working on the project in collaboration with Dr. S. Sotiraki researcher in the Veterinary Research Institute of HAO-Demeter. The project aims to unravel the complex interactions between veterinary antibiotics, ending up in agricultural soils through manuring, and soil microbes leading to dispersal of novel antibiotic resistance traits, enhanced biodegradation by the soil microbiota or toxicity to the soil microbiota. The factors that select which will of the above will be outcome of this interaction, and the mechanisms driving the evolution of resistance or biodegradation capacities by the soil microbiota will be determined. The project will commence in November 2019 and will last for 15 months.

- Two new projects entitled "*Structure guided design of glycogenolysis modulators to develop new therapeutic agents*" and "*Investigation of the role of poly(A)-specific ribonuclease in circadian gene expression*" were accepted for funding in the frame of the second call for the Support of Researchers with emphasis on Young Researchers. The two new projects will take place in the Laboratory of Structural and Functional Biochemistry, Department of Biochemistry and Biotechnology, University of Thessaly and supervised by Prof. Demetres Leonidas (Assis. Prof Anna-Maria G. Psarra will act as deputy supervisor) and Assist. Prof. N. Balatsos, respectively. Both projects will commence in November 2019 and last for 15 months.

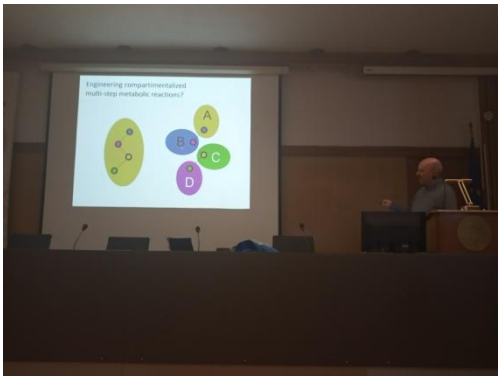
  The structural project on glycogenolysis modulators will be implemented by Dr C. Drakou and PhD student A. Tsagarakou. Three enzymes with central role in the human liver process of glycogenolysis (glycogen phosphorylase, glycogen debranching enzyme and glycogen phosphorylase kinase) will be studied using enzyme kinetics and X-ray crystallography and cellular methods to discover new small molecules modulators of their activity. The new compounds will be assessed for their pharmacodynamic properties and their effect on the metabolic profile of whole cells by NMR methods, in collaboration with Prof. Spyroulias and his team in the Department of Pharmacy at the University of Patras.

  Dr Zoi Arsenopoulou and PhD student Rafailia Beta will work on the project on the circadian gene expression. The project aims to unravel the complex mechanisms of the regulation of circadian gene expression focusing on the stability of circadian mRNAs. More specifically, it will investigate the role of poly(A)-specific ribonuclease, PARN, an enzyme with well-established roles in mRNA degradation, but unexplored roles in circadian gene expression. In this direction, we will search for factors interacting with PARN, as well as for microRNAs that may act in concert with the enzyme to regulate the stability of circadian mRNAs and overall circadian gene expression.

OMIC-Engine Seminars

Here we will keep you updated about seminars that are organized in the different hubs of OMIC-Engine

Victor de Lorenzo, member of the advisory board of OMIC-Engine, was invited by Dr G. Skretas, member of the research infrastructure, to give a talk on his pioneering work on the implementation of synthetic biological approaches to environmental clean-up. Dr de Lorenzo gave a fascinating overview on the development of a range of synthetic biology tools utilized on chassi microorganisms like *Pseudomonas putida* KT2440. He described the implementation of these tools for the development of novel engineered bacterial strains and consortia of engineered bacteria, attached to carriers, able to attain full conversion of the organic pollutants in an optimized mode.



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