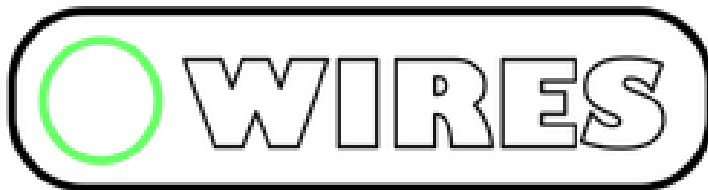
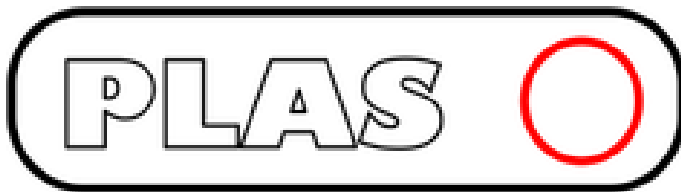




EUROPEAN  
COMMISSION



**Engineering Multicellular Biocircuits:  
Programming Cell-Cell Communication  
Using PLASmids as WIRES**

*A Synthetic Biology FP7 European research project*

**PLASWIRES NEWSLETTER**

**NOVEMBER 2015**

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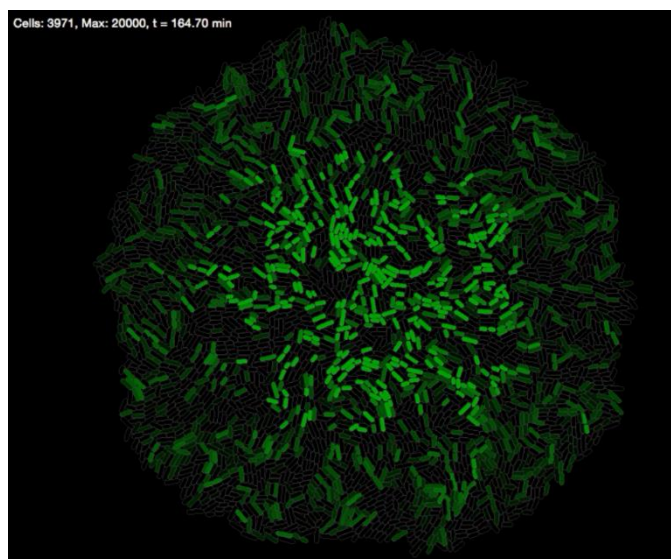
## ***FUTURE ACTIVITIES***



# STATE OF THE PROJECT

## WHAT IS COMING IN PLASWIRES?

UPM is polishing up the simulator GRO and continues to add new functionalities to better explore the spatial effects of multicellular conjugation based circuits. By the end of the project, we hope to have simulated all the proposed designs such as the AND, Multi-And plasmids or the Programmable Logic Array (PLA). We also hope to develop and simulate designs such as the edge detector plasmid or the Smart Task scheduler plasmids. We are still working on a better physical resolution of growth in the simulator GRO, that will be compared to that of our colleagues in Cambridge with Cell Modeller. We expect that the simulator will be shortly ready for a public release and that the rest of the consortium could use it as well.



## NEW RESERARCHERS

### MARCOS RODRÍGUEZ REGUEIRA



Marcos Rodríguez is a Phd student in the Laboratorio de Inteligencia Artificial (LIA), Universidad Politécnica de Madrid.

Marcos completed his undergraduate studies in Aerospace Engineering at Universidad Politécnica de Madrid, at the same time he was finishing his studies in Physics at the Universidad Nacional de Educación a Distancia. After that, he completed a Masters in Industrial Mechanics, where he presented a final thesis on neural networks based modeling.

Simultaneously to his Masters studies, he worked on the Grupo de Investigación de Sistemas Dinámicos, working on the mathematical modeling of dynamic systems.

Nowadays, he is a researcher in the LIA group, working on activities related to the modelling of synthetic biology circuits.

### ANTON KAN



Anton Kan is a PhD student in the department of Plant Sciences, University of Cambridge, working in bacterial synthetic biology. Anton completed his undergraduate studies in Physics at Oxford University (UK), finishing with a Masters in Physics and a thesis on DNA biophysics. After that, he worked on several research projects, the first at the FOM institute AMOLF (Netherlands) modelling information transfer in protein networks, and then at the University of Tokyo (Japan) working with versatile DNA-based logic gates for DNA computing. Since then, he has returned to the UK to work on a PhD in the Haseloff laboratory in Cambridge, where he is currently in his final year. His research in Cambridge focuses on engineering and modelling mechanisms that generate and alter shape and organisation in bacterial colonies.

Within the Plaswires project, Anton has mostly been focussing on creating a model of conjugation within the *CellModeller* bacterial modelling framework, as well as helping with timelapse microscopy to visualise and quantify conjugation events *in vivo*.

# WARWICK SUMMER SCHOOL

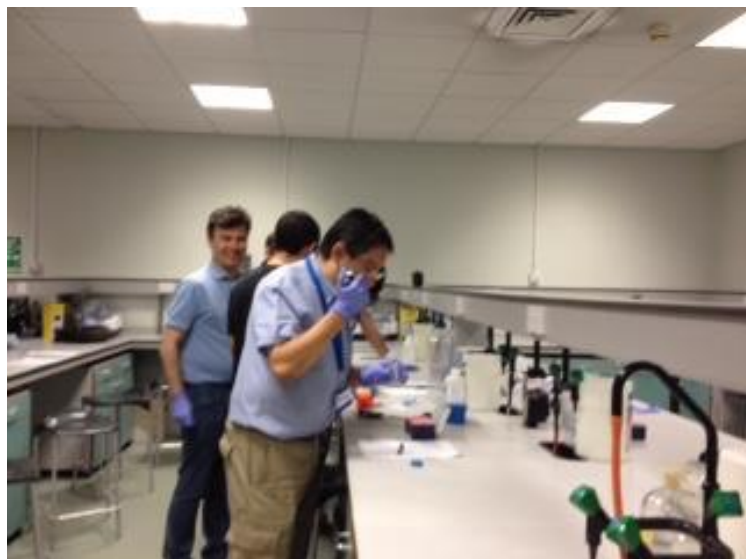
1-3 JULY, 2015

The aim of this Summer School is to deliver a set of lectures and tutorials that will endow the participants with sufficient knowledge to develop experimental designs integrating modelling, genetic circuits and customised labware for Synthetic Biology and Directed Evolution.

The Summer School will also present to the wider community the partners, developments and vision of the EVOPROG and PLASWIRES consortia, with the prospect of generating an open discussion and develop collaborations on the core subjects presented.



*Alfonso Rodríguez-Patón lecturing at the school*



*The school had nice experimental sessions to play with phages*



# WARWICK SUMMER SCHOOL

1-3 JULY, 2015



*Group photo with the majority of the Summer School participants*

## SUMMER SCHOOL PROGRAMME

### DAY 1 - WEDNESDAY

9 AM  
9.30 AM

Welcome and Introduction to the Summer School

Session 1 (Coffee break 11 AM to 11.15 AM)

9.30 AM  
1 PM

- "Simulating multicellular genetic circuits with an individual-based model: new features in GRO simulator" Dr Alfonso Rodríguez-Patón (Universidad Politécnica de Madrid, Spain)
- "Diversity Generating Retroelements" Dr Michal Legiewicz (University of Warwick, UK)
- "Synthesizing Programmable Biomolecular Circuits" Dr Vishwesh Kulkarni (University of Warwick, UK)

1 PM  
2 PM

**Lunch**

2 PM  
5.30 PM

Session 2 (Coffee break 3.30pm to 3.45pm)

"Computational modeling of multicellular bacterial populations" Dr Alfonso Rodríguez-Patón (Universidad Politécnica de Madrid, Spain); Dr John Duncan (University of Warwick, UK); Mr Pakpoom Subsoontorn (University of Cambridge, UK); Mr Martin Gutierrez (Universidad Politécnica de Madrid, Spain)

# WARWICK SUMMER SCHOOL

1-3 JULY, 2015

## DAY 2 - THURSDAY

Session 3 (Coffee break 11 AM to 11.15 AM)

- 9 AM - "New tools for gene evolution in vivo" Dr Yamal González (CSIC, Spain)  
1 PM - "RNA technologies to engineer gene circuits in bacteria" Prof Alfonso Jaramillo (University of Warwick, UK)  
- "Engineering synthetic patterns inspired by development" Dr Mark Isalan (University of Warwick, UK)

1 PM

Lunch

2 PM

Session 4 (Coffee break 3.30pm to 3.45pm)

- 2 PM "Phage production and analysis of the titer" Dr Andreas Broedel (Imperial College London, UK); Dr Yamal González (Consejo Superior de Investigaciones Científicas, Spain); Dr  
5.30 PM Antonia Sagona (University of Warwick, UK)

## DAY 3 - FRIDAY

Session 5

- 9 AM - "Synthetic biology in Cyanobacteria" Dr Rocío López (Institut Pasteur, France)  
10.45 AM - "Designing Portable Gene Expression for Cross-species Application" Dr Michal Legiewicz (University of Warwick, UK)  
- "Synthesizing Programmable Biomolecular Circuits" Dr Manish Kushwaha (University of Warwick, UK)

10.45 AM  
11 AM

Coffee break

Session 6 (Lunch 1pm to 6 pm)

- 11 AM "Creation of reproducible custom components for the lab" Dr Rui Rodrigues (University of  
3.30 PM Warwick, UK) ; Dr Soichiro Tsuda (University of Glasgow, UK) ; Mr Ismael Garcia (Universidad Politécnica de Madrid, Spain)

3.30 PM  
4 PM

Final notes on the Summer School

# MEETING IN MADRID

17 – 18 MARCH, 2015



*Alfonso Rodríguez-Patón talking with Fernando de la Cruz and Rocío López in the Plaswires meeting in Madrid*



# MEETING IN INSTITUTE PASTEUR

23 JUNE, 2015

The group from Pasteur Institute in Paris held a meeting for the PLASWIRES consortia on the 23th of June, 2015. The meeting consisted on different updates of the project progress from the different union members.

After lunch in the Institute Restaurant (Canard et Macarons), the group visited the Pasteur Museum where besides a collection of scientific objects illustrating the scientist's work, there is a crypt where Pasteur is buried. The meeting continued in the early afternoon with contributions from Martin Gutierrez and Jim Haseloff.



## RELATED PUBLICATIONS

### **Rebooting the genome: The role of negative feedback in horizontal gene transfer.**

*Mobile genetic elements*, 2015 Feb 23, vol. 4, no 6, p. 1. DOI: 10.4161/2159256X.2014.988069

Fernandez-Lopez R., de la Cruz F.

#### **Abstract**

Horizontal Gene Transfer (HGT) is one of the key mechanisms driving bacterial evolution. Conjugative plasmids are fundamental vehicles for HGT in bacteria, playing an essential role in the spread of antibiotic resistances. Although the classical view has stressed the instrumental role of these mobile genetic elements in the dissemination of antibiotic resistance genes, plasmids contain a rich physiology devoted to horizontal and vertical reproduction. This particular lifestyle imposes specific constraints and trade-offs on plasmid physiology, and plasmids have evolved dedicated circuits to balance the opposing demands of vertical and horizontal reproduction. Recent studies on the transcriptional networks of IncW plasmids and other incompatibility groups have unveiled common architectures in the regulatory networks of different plasmid groups. Comparative studies show that negative feedback loops (NFLs) with strong gains are preferred, opening the question of a possible convergent evolution dictated by certain adaptive properties of this particular network motif. System analysis of NFLs with strong feedback gains indicate that this architecture exhibits transient overshooting after horizontal gene transfer. Since plasmid burden is dependent on the expression of plasmid functions, transcriptional overshooting results in a transient increase of the burden immediately after conjugation. We discuss the possible implications of this phenomenon on plasmid propagation, and the regulatory networks that plasmids have evolved to counteract the detrimental side effects of transient overshooting.

### **Towards an integrated model of bacterial conjugation.**

*FEMS Microbiology Rev.* 2015 Jan;39(1):81-95. DOI: 10.1111/1574-6976.12085. Epub 2014 Dec 4.

Cabezón E, Ripoll-Rozada J, Peña A, de la Cruz F, Arechaga I.

#### **Abstract**

Bacterial conjugation is one of the main mechanisms for horizontal gene transfer. It constitutes a key element in the dissemination of antibiotic resistance and virulence genes to human pathogenic bacteria. DNA transfer is mediated by a membrane-associated macromolecular machinery called Type IV secretion system (T4SS). T4SSs are involved not only in bacterial conjugation but also in the transport of virulence factors by pathogenic bacteria. Thus, the search for specific inhibitors of different T4SS components opens a novel approach to restrict plasmid dissemination. This review highlights recent biochemical and structural findings that shed new light on the molecular mechanisms of DNA and protein transport by T4SS. Based on these data, a model for pilus biogenesis and substrate transfer in conjugative systems is proposed. This model provides a renewed view of the mechanism that might help to envisage new strategies to curb the threatening expansion of antibiotic resistance.

## RELATED PUBLICATIONS

### Characterization of intrinsic properties of promoters.

*ACS Synthetic Biology*. 2016 Jan 15;5(1):89-98. DOI: 10.1021/acssynbio.5b00116. Epub 2016 Jan 7.

Rudge TJ, Brown JR, Federici F, Dalchau N, Phillips A, Ajioka JW, Haseloff J.

#### Abstract

Accurate characterization of promoter behavior is essential for the rational design of functional synthetic transcription networks such as logic gates and oscillators. However, transcription rates observed from promoters can vary significantly depending on the growth rate of host cells and the experimental and genetic context of measurement. Further, in vivo measurement methods must accommodate variation in translation, protein folding and maturation rates of reporter proteins, as well as metabolic load. The external factors affecting transcription activity may be considered extrinsic, and the goal of characterization should be to obtain quantitative measures of the intrinsic characteristics of promoters. We have developed a promoter characterization method that is based on a mathematical model for cell growth and reporter gene expression and exploits multiple in vivo measurements to compensate for variation due to extrinsic factors. First, we used optical density and fluorescent reporter gene measurements to account for the effect of differing cell growth rates. Second, we compared the output of reporter genes to that of a control promoter using concurrent dual-channel fluorescence measurements. This allowed us to derive a quantitative promoter characteristic ( $\rho$ ) that provides a robust measure of the intrinsic properties of a promoter, relative to the control. We imposed different extrinsic factors on growing cells, altering carbon source and adding bacteriostatic agents and demonstrated that the use of  $\rho$  values reduced the fraction of variance due to extrinsic factors from 78% to less than 4%. This is a simple and reliable method for quantitative description of promoter properties.

### Orthogonal intercellular signaling for programmed spatial behavior.

*Molecular Systems Biology*. 2016 Jan 25;12(1):849. DOI: 10.15252/msb.20156590

Grant PK, Dalchau N, Brown JR, Federici F, Rudge TJ, Yordanov B, Patange O, Phillips A, Haseloff J.

#### Abstract

Bidirectional intercellular signaling is an essential feature of multicellular organisms, and the engineering of complex biological systems will require multiple pathways for intercellular signaling with minimal crosstalk. Natural quorum-sensing systems provide components for cell communication, but their use is often constrained by signal crosstalk. We have established new orthogonal systems for cell-cell communication using acyl homoserine lactone signaling systems. Quantitative measurements in contexts of differing receiver protein expression allowed us to separate different types of crosstalk between 3-oxo-C6- and 3-oxo-C12-homoserine lactones, cognate receiver proteins, and DNA promoters. Mutating promoter sequences minimized interactions with heterologous receiver proteins. We used experimental data to parameterize a computational model for signal crosstalk and to estimate the effect of receiver protein levels on signal crosstalk. We used this model to predict optimal expression levels for receiver proteins, to create an effective two-channel cell communication device. Establishment of a novel spatial assay allowed measurement of interactions between geometrically constrained cell populations via these diffusible signals. We built relay devices capable of long-range signal propagation mediated by cycles of signal induction, communication and response by discrete cell populations. This work demonstrates the ability to systematically reduce crosstalk within intercellular signaling systems and to use these systems to engineer complex spatiotemporal patterning in cell populations.

# PLASWIRES IN THE MEDIA

## HORIZON. The EU Research & Innovation



**HORIZON**  
*The EU Research &  
Innovation Magazine*

ICT

### Growing computer chips from slime mould and bacteria

16 February 2015

by Rex Merrifield



*In its vegetative state, slime mould is one large cell. Shutterstock - Torsten Lorenz*

**Scientists are developing computing devices built from living organisms such as slime mould and bacteria, in order to harness their problem-solving and programmable properties.**

**FULL ARTICLE :** [http://horizon-magazine.eu/article/growing-computer-chips-slime-mould-and-bacteria\\_en.html](http://horizon-magazine.eu/article/growing-computer-chips-slime-mould-and-bacteria_en.html)

## FUTURE ACTIVITIES



This workshop is an interdisciplinary meeting that will bring together researchers from various domains with interests in synthetic biology, programmable biology, DNA computing, natural computing and any other *in vivo* cellular computing paradigm. We want to join engineers and computer scientists with biologists to foster collaboration, and to imagine new ways of programming cells.

We invite the submission of long papers (12 pages maximum), or extended abstracts (3 pages maximum) to the workshop EasyChair site.

### Important Dates:

- Submission deadline: **4 April 2016**
- Notification of acceptance: **9 May 2016**
- Final versions due: **30 May 2016**
- Workshop: one day within **July 11 - 15, 2016**

### Contact.

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*Ángel Goñi Moreno* (email: [agoni@cnb.csic.es](mailto:agoni@cnb.csic.es))



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Project number 612146.

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