

EUROPEAN
COMMISSION



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

A Synthetic Biology FP7 European research project

PLASWIRES NEWSLETTER

OCTOBER 2016

CONTENT OF THIS ISSUE

THE CONSORTIUM

DISSEMINATION

Conference

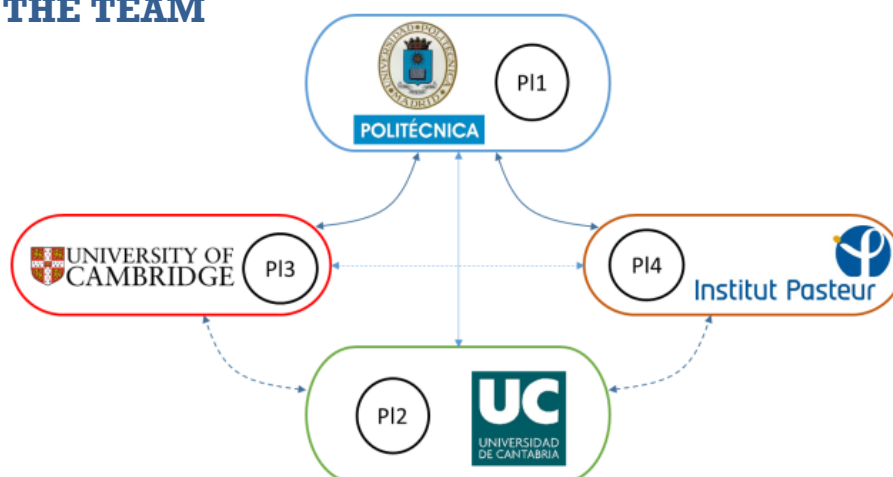
Related Publications

PLASWIRES SUMMARY



THE CONSORTIUM

THE TEAM



PROJECT LEADERS



Alfonso Rodríguez-Patón (Coordinator)
Universidad Politécnica de Madrid (Spain)
arpaton@fi.upm.es
www.lia.upm.es

PI1



Fernando de la Cruz
Universidad de Cantabria (Spain)
fernando.cruz@unican.es
<http://grupos.unican.es/intergenomica/>

PI2



Jim Haseloff
University of Cambridge (UK)
jh295@cam.ac.uk
<http://www.haseloff-lab.org/>

PI3



Didier Mazel
Institut Pasteur (France)
mazel@pasteur.fr
<http://openwetware.org/wiki/Mazel>

PI4

PHAGE BASED TECHNOLOGIES vs ANTIMICROBIAL RESISTANCE

15-16 SEPTEMBER, STRATFORD

This Conference was organised and funded by EVOPROG (Fet Proactive) and INTEGRATE AMR (EPSRC) with the participation of PLASWIRES consortium.

The conference facilitated discussions between specialists who have expertise in phages, virology, directed evolution, microbiology & microbial diversity and encourage collaborations to tackle AMR.

The partners of PLASWIRES contribute to the conference with their vision of conjugation as a cell-cell interaction to fight against antibiotic resistance.

The UPM and Cambridge University showed their models of communication among bacteria while UNICAN and Institute Pasteur presented some new ideas about conjugation and plasmid stabilization.

More info at:

https://www2.warwick.ac.uk/fac/cross_fac/wamic/integrate/phagevsamr/

CONFERENCE PROGRAMME

DAY 1. THURSDAY

13:00-14:00 Lunch and Arrivals

Session 1 – Phage and plasmid tools

14:00-14:10 Prof. Alfonso Jaramillo, University of Warwick, UK. “Opening by conference chair”

14:10-14:50 Prof. Didier Mazel, Institute Pasteur, France. “How to stabilize plasmids in bacteria: lessons from the secondary chromosomes of *Vibrio* species”

14:50-15:30 Prof. Alfonso Rodriguez-Paton, Universidad Politecnica de Madrid, Spain. “Individual-based modeling of horizontal gene transfer with GRO”

15:30-16:10 Dr. Mark Isalan, Imperial College London, UK. “Directed evolution of orthogonal dual transcription factors for multi-input synthetic promoters”

16:10-16:40 Coffee Break

16:40-17:20 Prof. Alfonso Jaramillo, University of Warwick, UK. “Synthetic gene circuits by evolutionary technologies”

17:20-18:00 Prof. Victor de Lorenzo, CNB-CSIC, Madrid, Spain. “Learning from the enemy: spreading beneficial traits through the global environmental microbiome”

18:00-18:40 Prof. Fernando de la Cruz, Universidad de Cantabria, Spain “Bacterial conjugation and the fight against antibiotic resistance”

18:40-19:20 Prof. Jim Haseloff and Dr. Pakpoom Subsoontorn, Cambridge University, UK. “Dynamic models of cell-cell interactions in synthetic biofilms”

19:45- Dinner at the Rooftop Restaurant of the Royal Shakespeare theatre

PHAGE BASED TECHNOLOGIES vs ANTIMICROBIAL RESISTANCE

15-16 SEPTEMBER, STRATFORD

DAY 2. FRIDAY

Session 2 – Phages against AMR

09:00-09:40 Prof. Andrzej Gorski, Polish Academy of Sciences, Poland. *“Phage therapy: today treating complications, tomorrow targeting diseases”*

09:40-10:20 Prof. George Salmond, University of Cambridge, UK. *“The triumph of death: how bacteria control phage infection by altruistic suicide”*

10:20-11:00 Prof. Stephen Abedon, Ohio State University, USA. *“Phage therapy from a pharmacological perspective: various perspectives on how to improve the art”*

11:00-11:30 Coffee Break

11:30-11:50 Prof. Ramesh Wigneshweraraj, MRC Centre for Molecular Bacteriology & Infection, Imperial College London, UK. *“Phage-inspired inhibition of the bacterial transcription machinery.”*

11:50-12:10 Dr. Antonia Sagona, University of Warwick, UK. *“Developing an in vitro model system for phage therapy against pathogenic E.coli”*

12:10-12:30 Dr. Akos Nyerges, Hungarian Academy of Sciences, Hungary. *“Deciphering the Achilles’ heel of multidrug resistant bacteria by novel genome engineering tools”*

12:30-13:30 Lunch

13:30-14:30 Social activity: Royal Shakespeare Theatre Tour

Session 3 – Towards Synthetic Phages

14:30-15:10 Prof. Ian Molineux, University of Texas at Austin, USA. *“A molecular basis for phage infection specificity of bacteria”*

15:10-15:50 Dr. David Harper, Evolution Biotechnologies, UK. *“Bacteriophages as biological control agents”*

15:50-16:30 Dr. Mark van Raaij, CNB-CSIC, Madrid, Spain. *“Structural analysis of bacteriophage receptor-binding proteins: towards phages with designed host specificity”*

16:30-17:00 Coffee Break

17:00-17:40 Prof. Martha Clokie, University of Leicester, UK. *“Developing Clostridium difficile phages as novel therapeutics”*

17:40-18:20 Dr. Udi Qimron, Tel Aviv University, Israel. *“Bacteriophages programmed to reverse antibiotic resistance of bacteria”*

18:20-18:40 Dr. Freya Harrison, University of Warwick, UK. *“Building & dismantling chronic infection biofilm”*

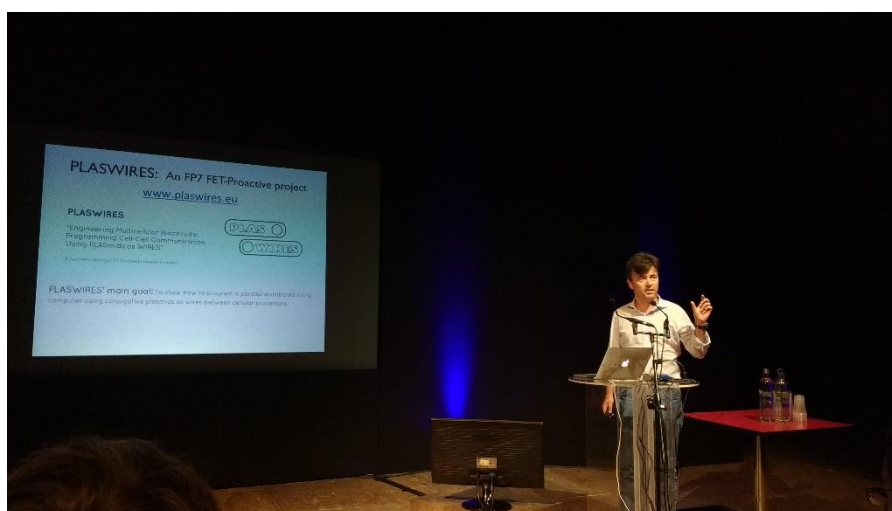
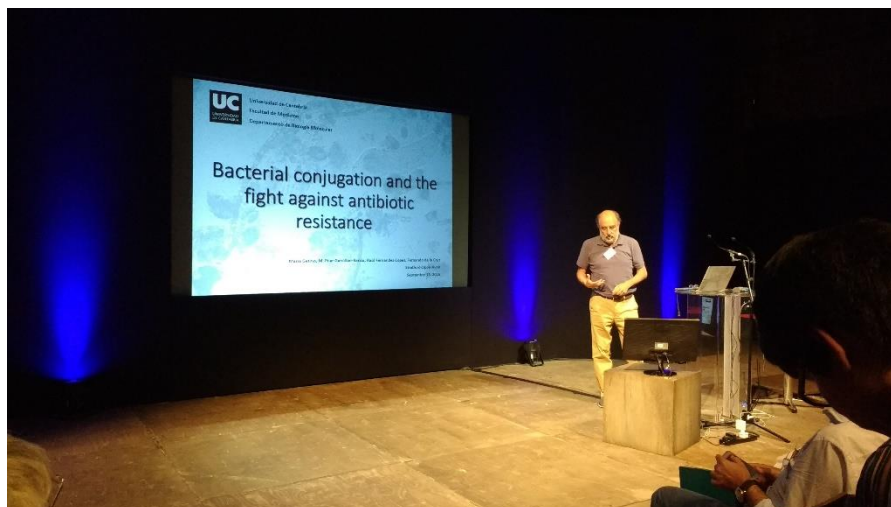
18:40-19:00 Dr. Meera Unnikrishnan, University of Warwick, UK. *“Innovative tools for probing complex host-microbe interaction”*

19:00-19:10 Close by conference chair

19:45- Dinner at the Rooftop Restaurant of the Royal Shakespeare theatre

PHAGE BASED TECHNOLOGIES vs ANTIMICROBIAL RESISTANCE

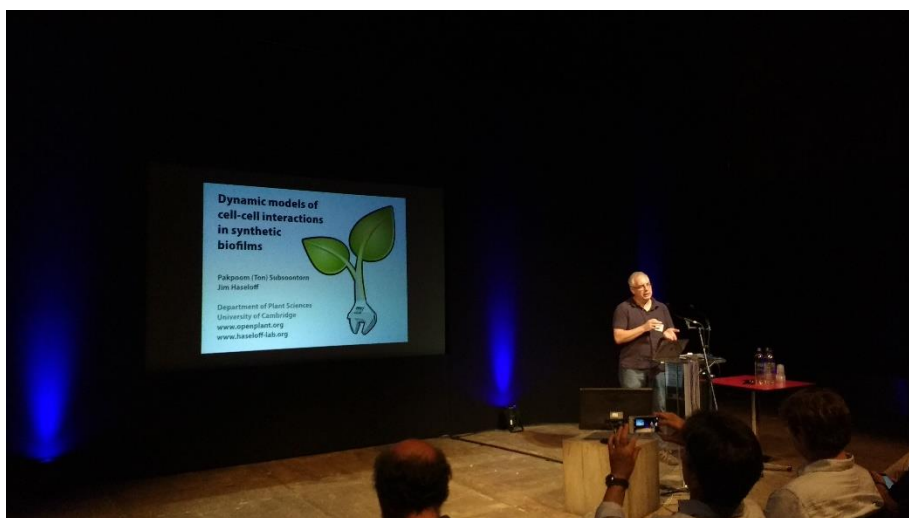
15-16 SEPTEMBER, STRATFORD



Fernando de la cruz and Alfonso Rodríguez Patón giving his talks about “Bacterial conjugation and the fight against antibiotic resistance” and “Individual-based modeling of horizontal gene transfer with GRO”.

PHAGE BASED TECHNOLOGIES vs ANTIMICROBIAL RESISTANCE

15-16 SEPTEMBER, STRATFORD



Jim Haseloff and Didier Mazel giving his talks about “Dynamic models of cell-cell interactions in synthetic biofilms” and “How to stabilize plasmids in bacteria: lessons from the secondary chromosomes of *Vibrio* species”

RELATED PUBLICATIONS

Tanzawaic Acids, a Chemically Novel Set of Bacterial Conjugation Inhibitors

PLOS ONE, 2016 Jan 26, vol. 11, no 1. DOI: 10.1371/ journal.pone.0148098

María Getino , Raúl Fernández-López , Carolina Palencia-Gándara , Javier CamposGómez , Jose M. Sánchez-López , Marta Martínez , Antonio Fernández , Fernando de la Cruz

Abstract

Bacterial conjugation is the main mechanism for the dissemination of multiple antibiotic resistance in human pathogens. This dissemination could be controlled by molecules that interfere with the conjugation process. A search for conjugation inhibitors among a collection of 1,632 natural compounds, identified tanzawaic acids A and B as best hits. They specially inhibited IncW and IncFII conjugative systems, including plasmids mobilized by them. Plasmids belonging to IncFI, IncI, IncL/M, IncX and IncH incompatibility groups were targeted to a lesser extent, whereas IncN and IncP plasmids were unaffected. Tanzawaic acids showed reduced toxicity in bacterial, fungal or human cells, when compared to synthetic conjugation inhibitors, opening the possibility of their deployment in complex environments, including natural settings relevant for antibiotic resistance dissemination.

Design of Novel Relaxase Substrates Based on Rolling Circle Replicases for Bioconjugation to DNA Nanostructures

PLOS ONE. 2016 March; vol 11, no 3. DOI: 10.1371/journal.pone.0152666

Sandra Sagredo, Fernando de la Cruz , Gabriel Moncalián

Abstract

During bacterial conjugation and rolling circle replication, HUH endonucleases, respectively known as relaxases and replicases, form a covalent bond with ssDNA when they cleave their target sequence (*nic* site). Both protein families show structural similarity but limited amino acid identity. Moreover, the organization of the inverted repeat (IR) and the loop that shape the *nic* site differs in both proteins. Arguably, replicases cleave their target site more efficiently, while relaxases exert more biochemical control over the process. Here we show that engineering a relaxase target by mimicking the replicase target, results in enhanced formation of protein-DNA covalent complexes. Three widely different relaxases, which belong to MOB_F, MOB_Q and MOB_P families, can properly cleave DNA sequences with permuted target sequences. Collaterally, the secondary structure that the permuted targets acquired within a supercoiled plasmid DNA resulted in poor conjugation frequencies underlying the importance of relaxase accessory proteins in conjugative DNA processing. Our results reveal that relaxase and replicase targets can be interchangeable *in vitro*. The new Rep substrates provide new bioconjugation tools for the design of sophisticated DNA-protein nanostructures.

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 (ρ) 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 ρ 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

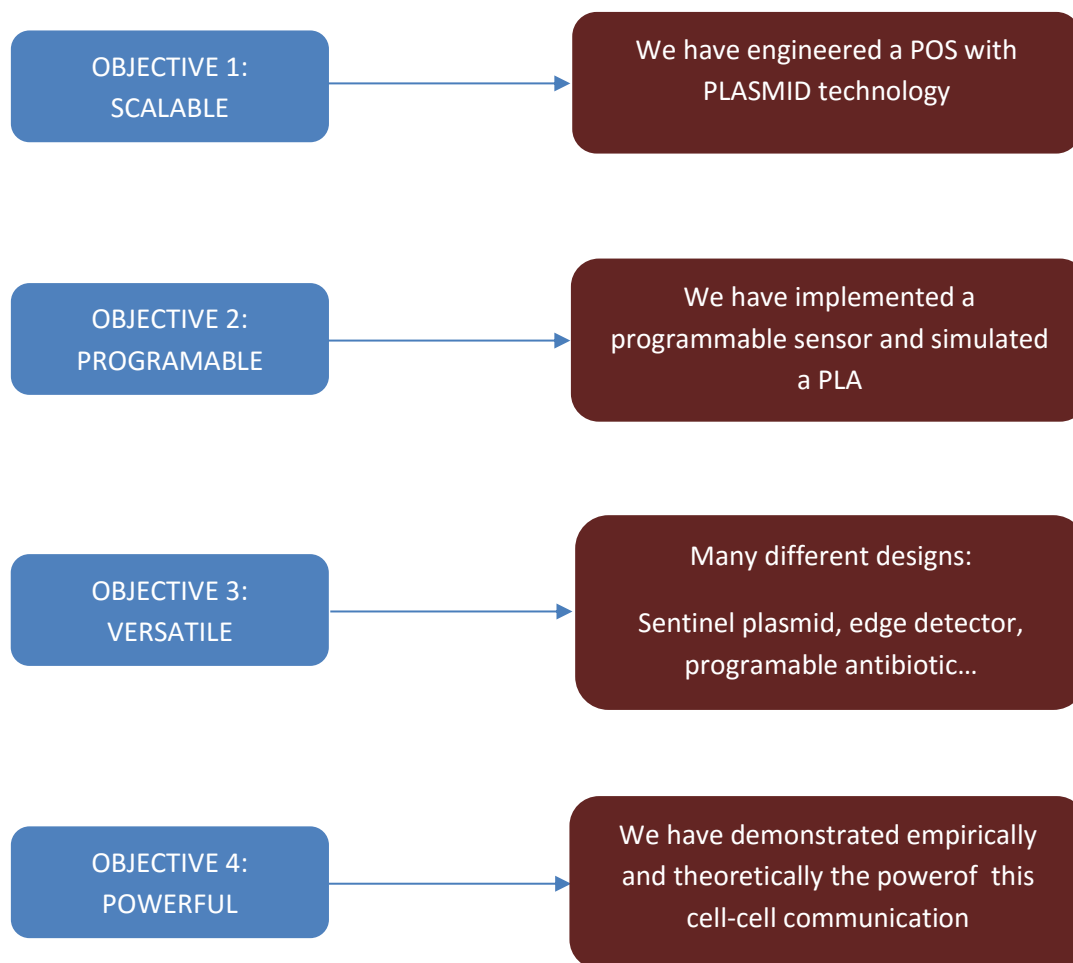
Grant PK, Dalchau N, Brown JR, Federici F, Rudge TJ, Yordanov B, Patange O, Phillips A, Haseloff J.

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.

SUMMARY OF THE PROJECT

PLASWIRES HAS ALMOST ACCOMPLISHED ALL THE OBJECTIVES



CONTACT INFORMATION

PLASWIRES WEBSITE: <http://www.plaswires.eu>

COORDINATOR: Alfonso Rodríguez-Patón Aradas

ADDRESS: Facultad de Informática. Universidad Politécnica de Madrid (UPM)
Campus de Montegancedo s/n, Boadilla del Monte, 28660, Madrid

LIA SITE: www.lia.upm.es

E-MAIL: arpaton@fi.upm.es

TWITTER: @LIA_UPM @synbio_papers

PLASWIRES is supported by the European Commission, funded under the Seventh Framework Programme:

Future and Emerging Technologies (FET) Proactive: Evolving Living Technologies (EVLIT) (ICT-2013.9.6)
Project number 612146.

This document reflects only the views of the author(s), and the European Commission is not liable for any use that may be made of the information contained therein.



**EUROPEAN
COMMISSION**



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

A Synthetic Biology FP7 European research project