Team name and photography - De Novo Synthetic Biology Lab

Brief description - The therapeutics of tomorrow will be individualized, specific, and self-regulated. This could be achieved by synthetic organisms, once we know how to engineer them. The simplest and safest is the bacteriophage (phage). We aim to experimentally construct a new phage (de novo) that could be useful against antibiotic-resistant bacteria.

Another aim is to experimentally create artificial intelligence in living cells. For this, we propose a new way to engineer general-purpose gene circuits without a predefined final behaviour. The circuit dynamics will adapt through reinforcement learning to acquire the desired phenotype only once the cell activates it. We have demonstrated this with E. coli, where we have empowered the cells with the ability to learn board games such as tic-tac-toe. This adaptation is inherited to the offspring but it is not genetic.

We will pursue those aims by developing general methodologies to design new biological molecules, viruses and cells with completely new functions. These methodologies rely on automated computational and/or experimental procedures. We have developed computational methodologies to design de novo: RNA, proteins and whole-cell transcriptional circuits. We have also developed automated methodologies to design biomolecules based on experimental procedures. They rely on the combination of phage transduction, evolution and continuous culture bioreactors to implement an accelerated evolution in living cells, which mimics our computational optimisation algorithms. We work at the interface of experimental molecular biology, combinatorial optimisation, microfluidics and directed evolution.

Group Members:

Alfonso Jaramillo - PI

Mariel Montesinos - PM

Cristina Ramos - PostDoc

Álvaro Barrera - Research Associate

Paula Pastor - Research Associate

Esther Marín - Research Assitant

Sofia Jiménez - Research Associate

Anabel Vargas - Research Assistant

Claudia Pascual - Research Associate

Research topics: Our lab aims to develop new general methodologies enabling future scientists to engineer living cells with completely new capabilities *de novo*. We want to restrict ourselves to two main applications: 1) de novo engineering of organism-specific antibiotics, and 2) de novo engineering of living artificial intelligence.

Recent articles:

Tridgett, M., Ababi, M., Osgerby, A., Ramirez Garcia, R. & Jaramillo, A. Engineering Bacteria to Produce Pure Phage-like Particles for Gene Delivery. *ACS Synthetic Biology* 10, 107-114 (2021).

2 Cunliffe, T. G., Parker, A. L. & Jaramillo, A. Pseudotyping Bacteriophage P2 Tail Fibers to Extend the Host Range for Biomedical Applications. ***ACS Synthetic Biology*** **11, 3207-3215 (2022).**

3 Ababi, M., Tridgett, M., Osgerby, A. & Jaramillo, A. Scarless ssDNA Recombineering of Phage in the Lysogenic State. ***Methods in molecular biology (Clifton, N.J.)*** **2479, (in press) (2022).**

4 Tridgett, M., Ababi, M. & Jaramillo, A. Lambda red recombineering of bacteriophage in the lysogenic state. ***Methods in molecular biology (Clifton, N.J.)*** **2479, (in press) (2022).**

5 Tridgett, M., Ababi, M., Osgerby, A., Ramirez Garcia, R. & Jaramillo, A. Engineering Bacteria to Produce Pure Phage-like Particles for Gene Delivery. ***ACS Synthetic Biology*** **(2020)**

6 Ababi, M., Tridgett, M., Osgerby, A. & Jaramillo, A. Scarless ssDNA Recombineering of Phage in the Lysogenic State. ***Methods in molecular biology (Clifton, N.J.)*****, (in press) (2021**

7 Tridgett, M., Ababi, M. & Jaramillo, A. Lambda red recombineering of bacteriophage in the lysogenic state. ***Methods in molecular biology (Clifton, N.J.)*****, (in press) (2021**

8 Grigonyte, A. M. ***et al.*** **Comparison of CRISPR and Marker-Based Methods for the Engineering of Phage T7.** ***Viruses*** **12, 193 (2020).**

9 Broedel, A. K., Rodrigues, R., Jaramillo, A. & Isalan, M. Accelerated evolution of a minimal 63-amino acid dual transcription factor. ***Sci Adv*** **6, eaba2728 (2020**

10 Broedel, A. K., Isalan, M. & Jaramillo, A. Engineering of biomolecules by bacteriophage directed evolution. ***Current Opinion in Biotechnology*** **51, 32-38 (2018**

11 Broedel, A. K., Jaramillo, A. & Isalan, M. Intracellular directed evolution of proteins from combinatorial libraries based on conditional phage replication. ***Nature Protocols*** **12, 1830-1843 (2017).**

12 Broedel, A. K., Jaramillo, A. & Isalan, M. Engineering orthogonal dual transcription factors for multi-input synthetic promoters. ***Nature Communications*** **7, 13858 (2016**

13 Sagona, A. P., Grigonyte, A. M., MacDonald, P. R. & Jaramillo, A. Genetically modified bacteriophages. ***Integr Biol (Camb)*** **8, 465-474 (2016**

14 Brdel, A. K., Jaramillo, A. & Isalan, M. Engineering orthogonal dual transcription factors for multi-input synthetic promoters. ***Nature Communications*** **7, 13858 (2016)**

Research projects:

Project Title and reference	Funding source
Adaptive gene circuits (GeneCircuits++). PID2020-118436GB-I00	Ministerio de Ciencia e Innovación
Photosynthetic electron focusing technology for direct	Horizon Europe EIC

efficient biohydrogen production from solar energy (PhotoSynH2). 101070948	
Accelerated evolution of high-weight protein complexes for precision antimicrobials (EvoPunch). HR22-00405	**CaixaResearch Health**
Engineering bacteria to communicate through Morse code (BACMORSE). KCAQGZUD98X1	**ONRG (USA)**

Name	University	Dates	Number of publications
Maria Ababi	Warwick (UK)	2018-2021	3
Alexis Mercadal	Warwick (UK) & Monash (Australia)	2019-2022	0
Robert Ramirez	Warwick (UK) & Monash (Australia)	2017-2021	1
Tabitha Cunliffe	Warwick (UK) & Cardiff (UK)	2018-2021	0
William Rostain	Warwick (UK)	2013-2017	6
Satya Prakash	Warwick (UK)	2014-2018 later as postdoc until 2021	5
Eduardo Goicoechea	Warwick (UK)	2016-2020	0
Paul MacDonald	Warwick (UK)	2015-2019	3
Aurelija Grigonyte	Warwick (UK)	2016-2019	2
Boris Kirov	Evry (France)	2008-2012	6
Filipe Pinto	Porto (Portugal)	2007-2011	3
Daniel Camsund	Upsala (Sweden)	2007-2011	2
Javier Carrera	UPV (Spain)	2007-2011	25
Thomas Landrain	Evry (France)	2008-2012	7
Guillermo Rodrigo	UPV (Spain)	2007-2011 later as postdoc until 2013	32

Web site

https://de-novo-sb.csic.es/