CBGP
CENTRO DE BIOTECNOLOGÍA
Y GENÓMICA DE PLANTAS
UPM-INIA

Genetic circuits beyond genes
contextual dependencies as design parameters

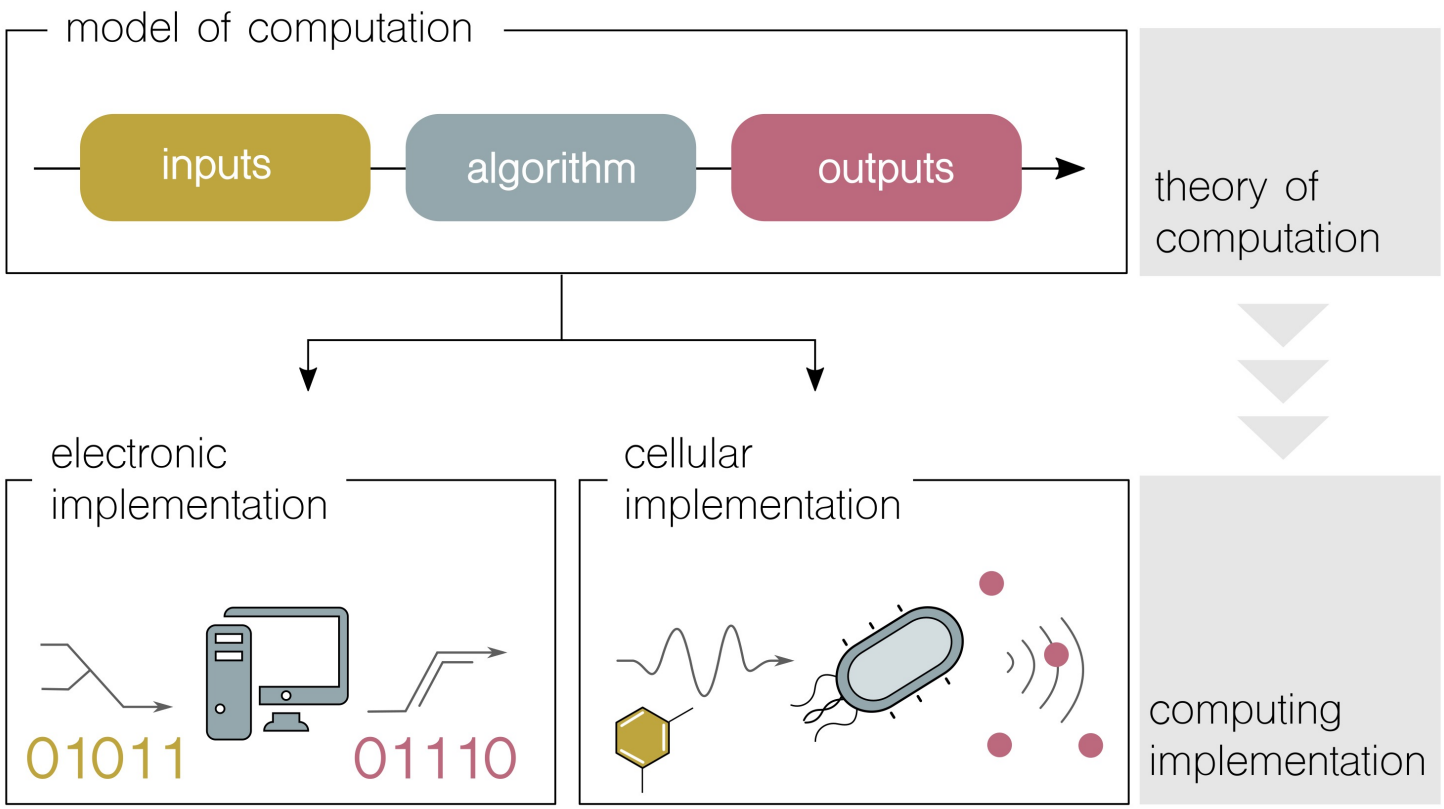
Ángel Goñi-Moreno

 @AngelGMoreno

 angel.goni@upm.es

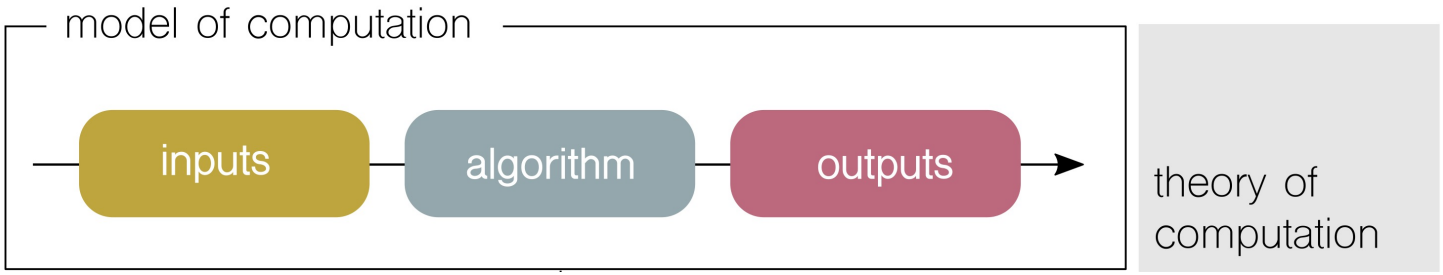
CELLS 2021

The cell as a physical computer



The same theoretical model of computation can be physically implemented in different ways; the nature of the computations remains the same.

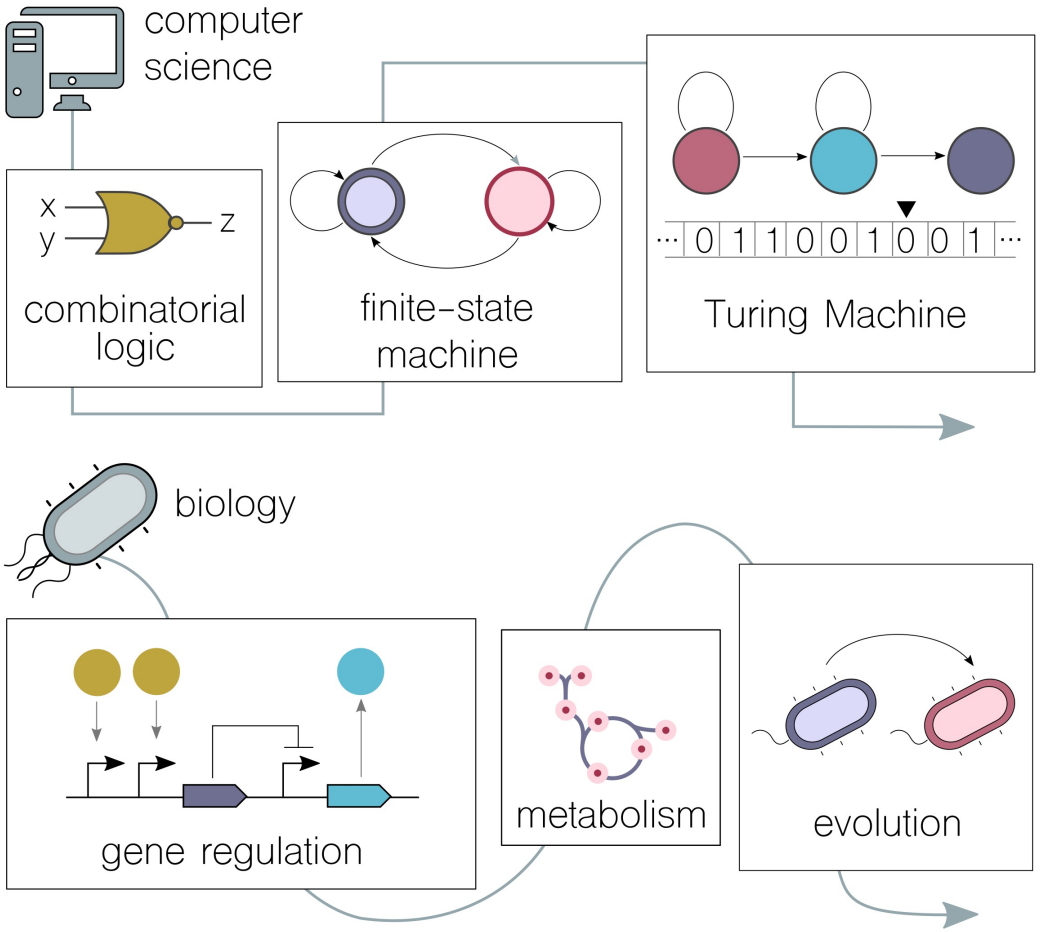
The cell as a physical computer



"… algorithms are concepts that have existence apart from any programming language"

Donal Knuth

More than logic circuits

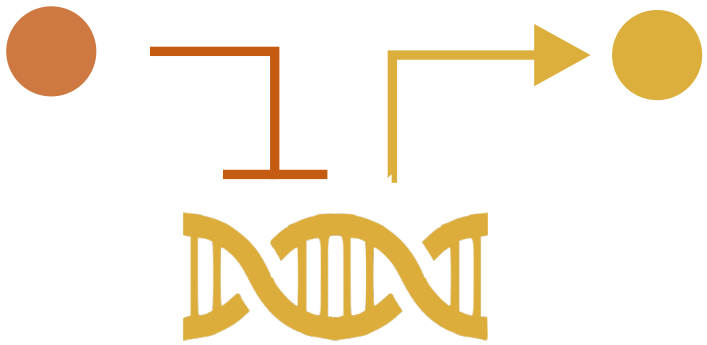


Grozinger et al. Pathways to the cellular supremacy in biocomputing. Nature Communications (2019)

- The logic circuit paradigm is an extremely conservative approach for engineering cellular computations.
- Computer science has developed models of computation that are far more powerful than combinatorial logic, such as finite-state machines or the Turing Machine.
- Similarly, living systems have evolved a variety of computational processes to allow cells to process information. New solutions!

DNA-based logic

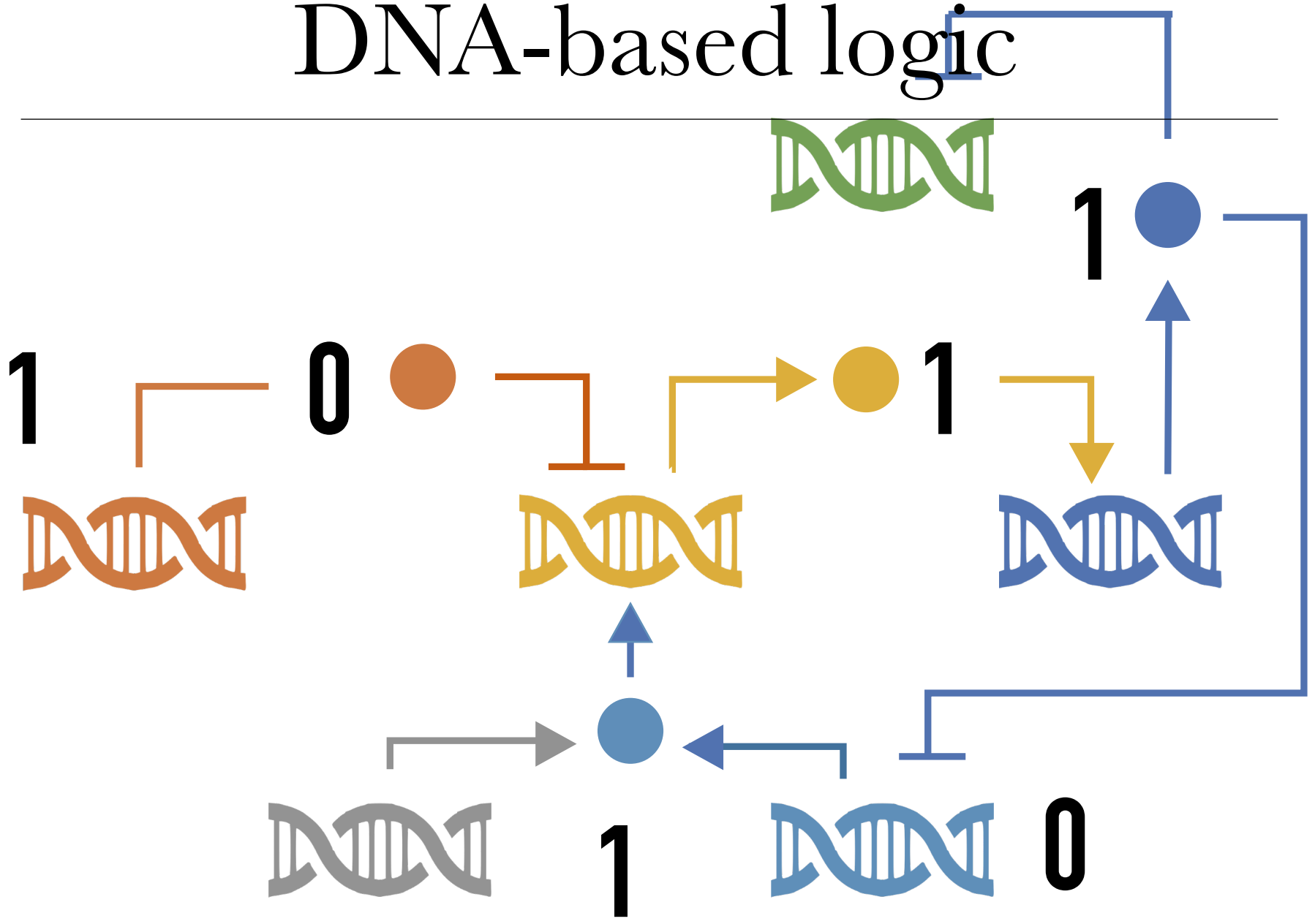
DNA-based logic



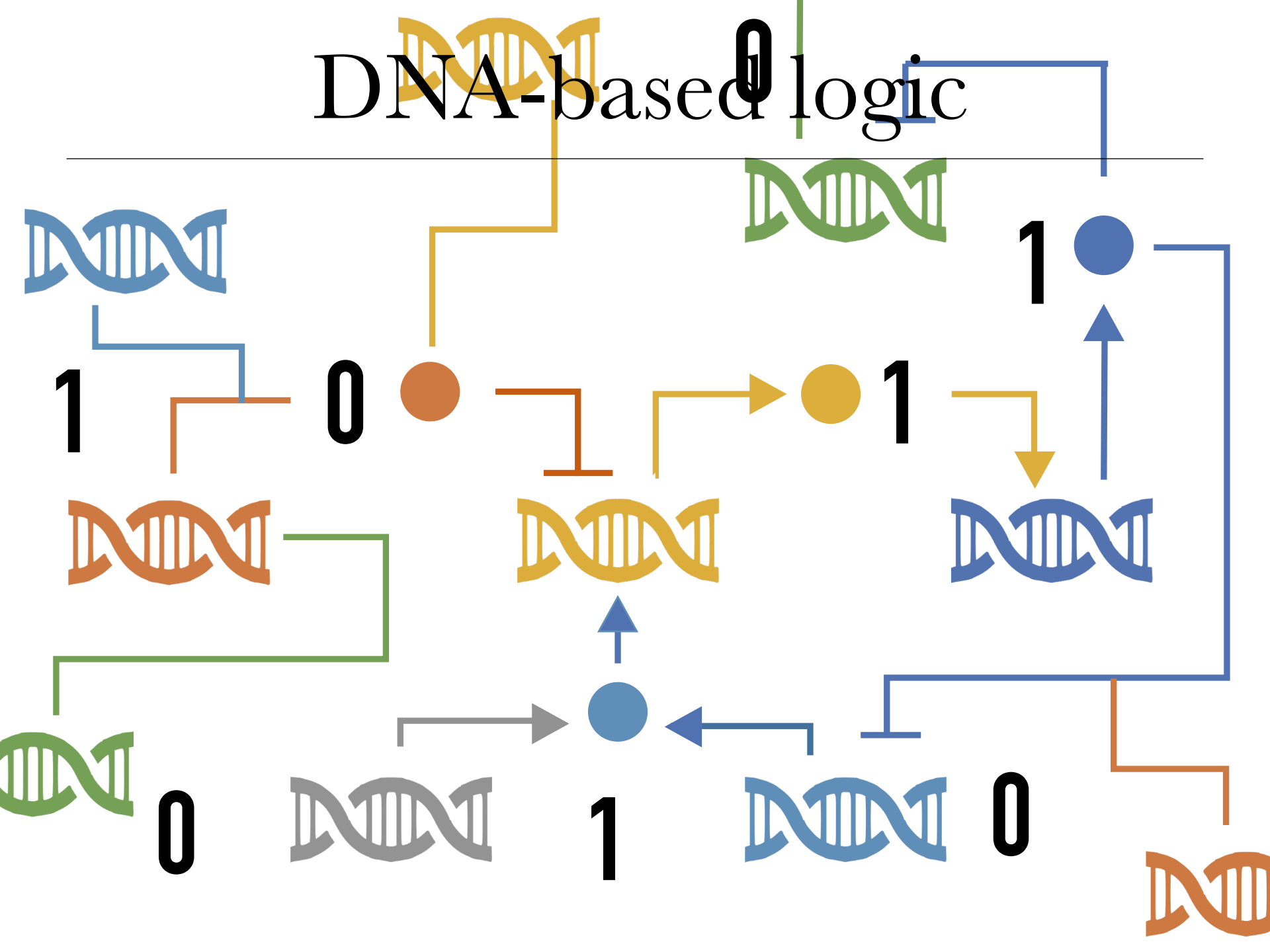
DNA-based logic

DNA-based logic

DNA-based logic



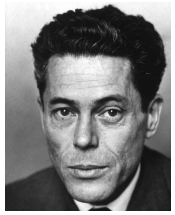
DNA-based logic



Origins of DNA-based logic

- Jacob and Monod established, in 1961, that the *lac* operon facilitates switching in a manner that may be interpreted as a simple Boolean circuit.

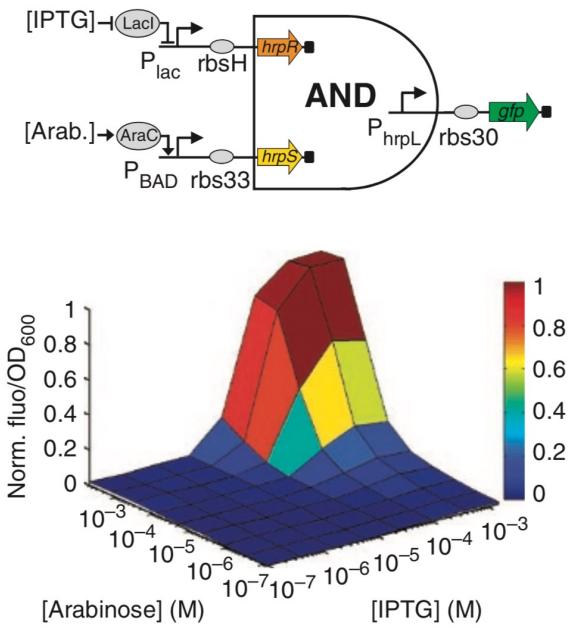
- Monod makes this connection explicit in 1971:



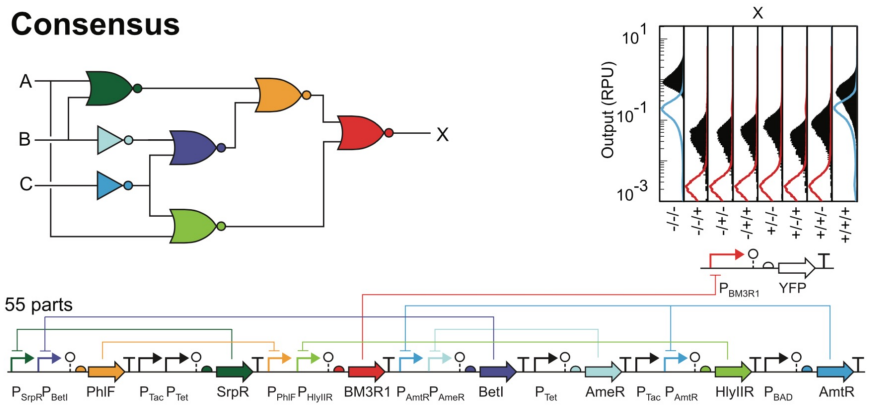
Jacques Monod

"The logic of biological regulatory systems abides not by Hegelian laws but, like the workings of computers, by the propositional algebra of George Boole"

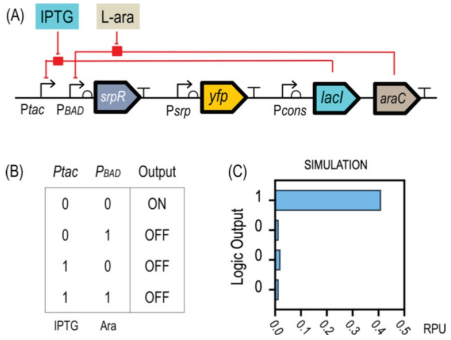
Genetic Logic Gates



Wang, B. Engineering modular and orthogonal genetic logic gates for robust digital-like synthetic biology. Nature Communications (2011) DOI: 10.1038/ncomms1516



Nielsen, A. et al. Genetic circuit design automation. Science (2016).

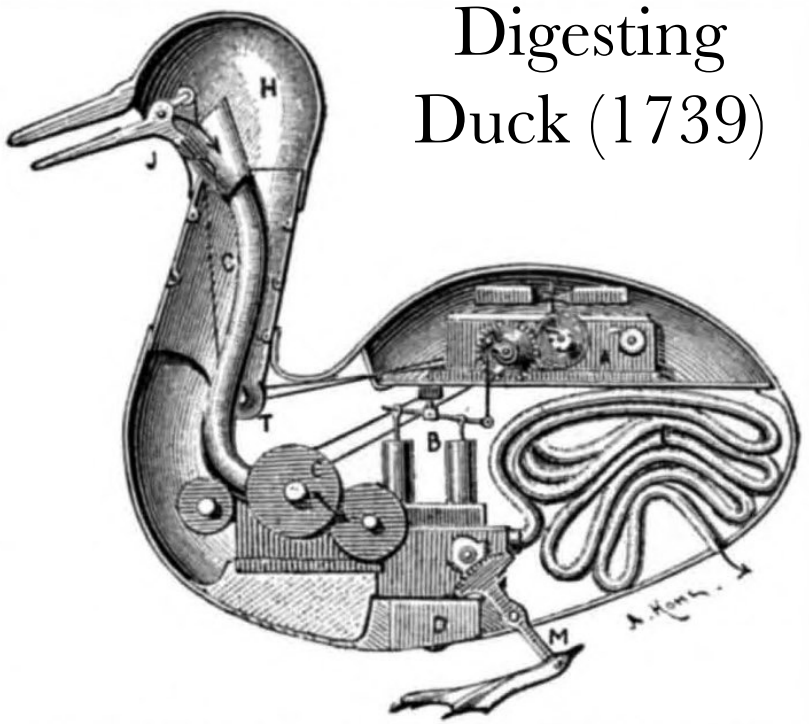


Huseyin Tas, Lewis Grozinger, Angel Goñi-Moreno, Victor de Lorenzo. Automated design and implementation of a NOR gate in *Pseudomonas putida*. Synthetic Biology (2021)

Reductionism—warning!

Jaques de Vaucanson

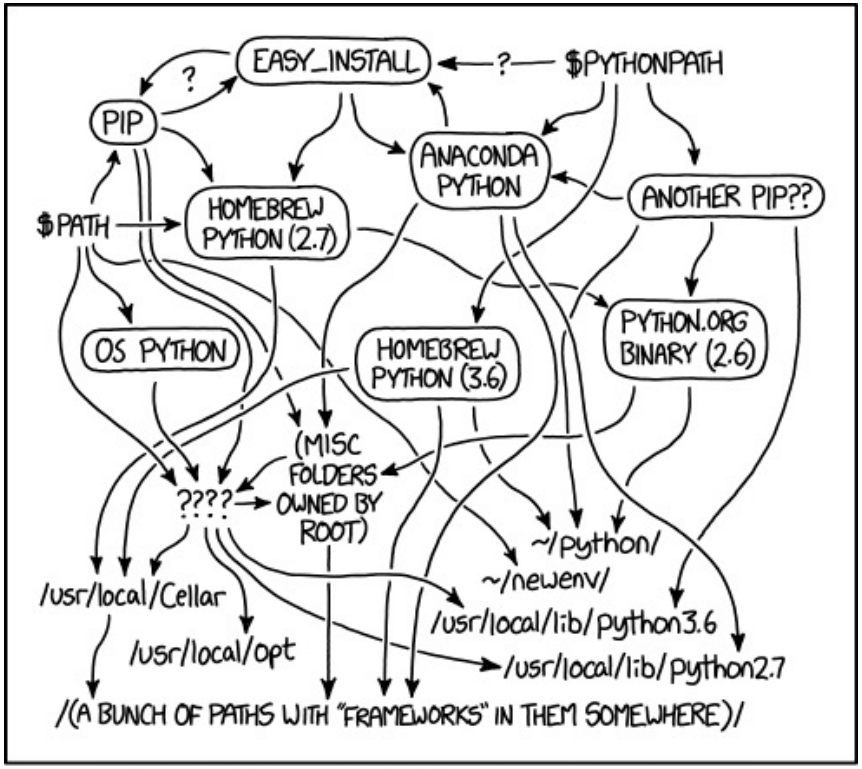
Digesting Duck (1739)



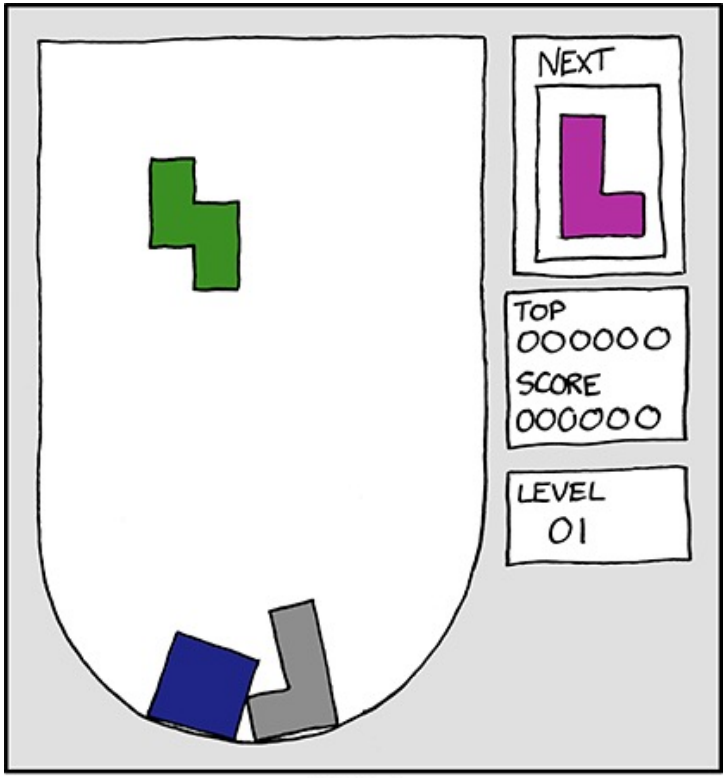
INTERIOR OF VAUCANSON'S AUTOMATIC DUCK.

A, clockwork; *B*, pump; *C*, mill for grinding grain; *F*, intestinal tube; *J*, bill; *H*, head; *M*, feet.

Dependency Hell



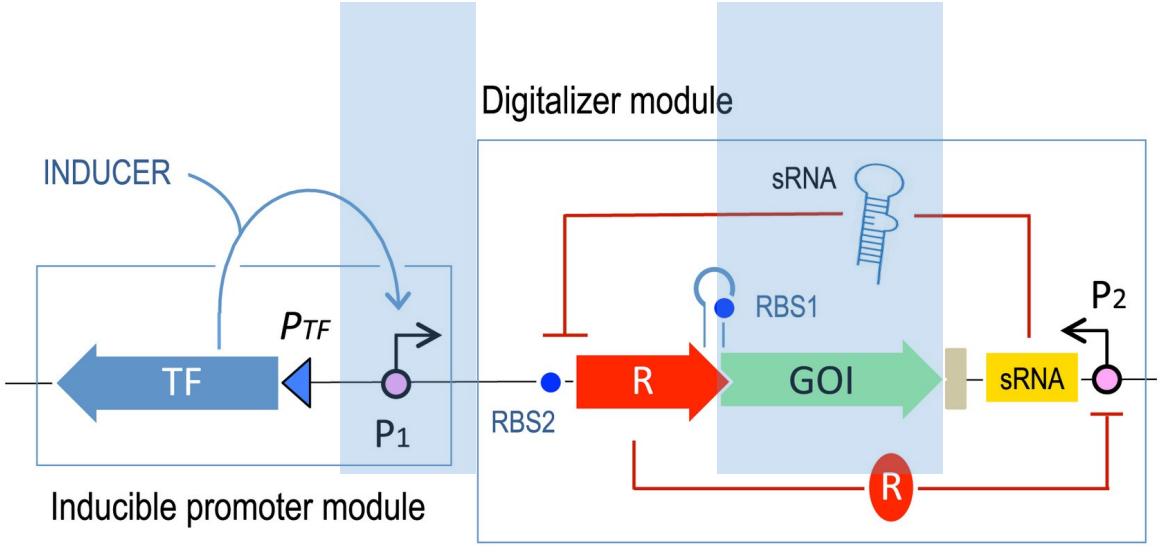
MY PYTHON ENVIRONMENT HAS BECOME SO DEGRADED THAT MY LAPTOP HAS BEEN DECLARED A SUPERFUND SITE.



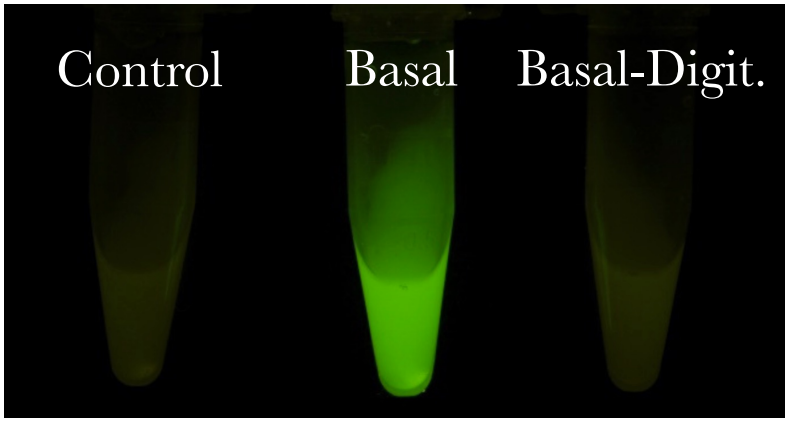
HELL

- **Genomic dependencies**
- **Spatial dependencies**
- **Metabolic dependencies**
- **Host dependencies**

Digitalizing gene expression



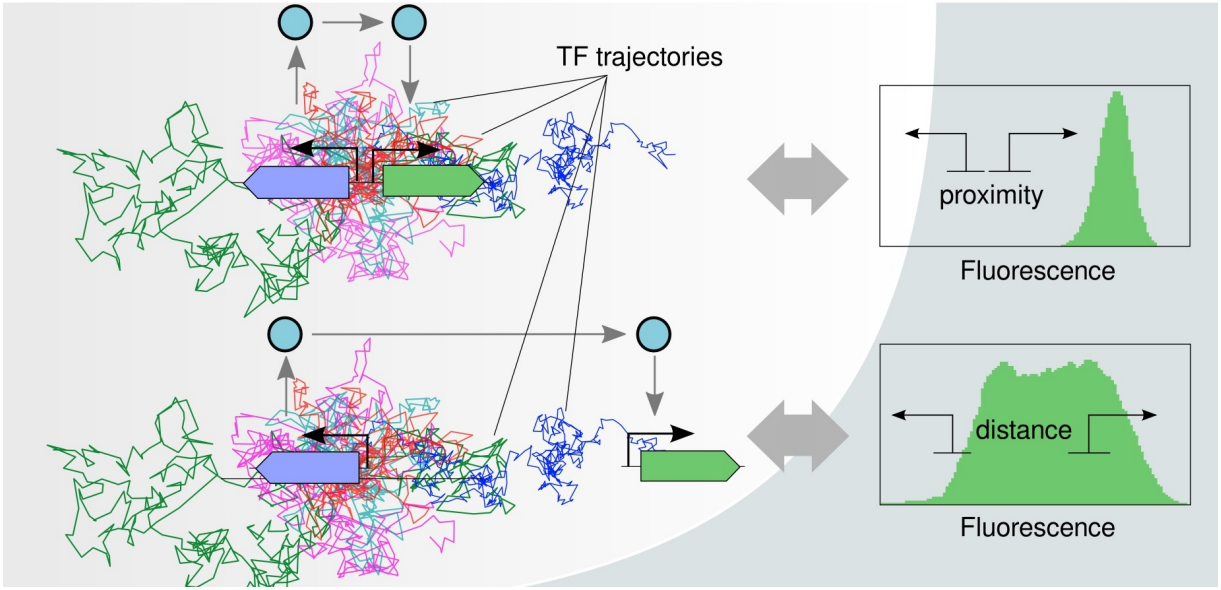
Calles et al. Digitalizing heterologous gene expression in Gram-negative bacteria with a portable ON/OFF module. Molecular Systems Biology (2020)



We provide an environment that interacts with the promoter-gene pair of choice in a pre-designed way.

- Genomic dependencies
- Spatial dependencies
- Metabolic dependencies
- Host dependencies

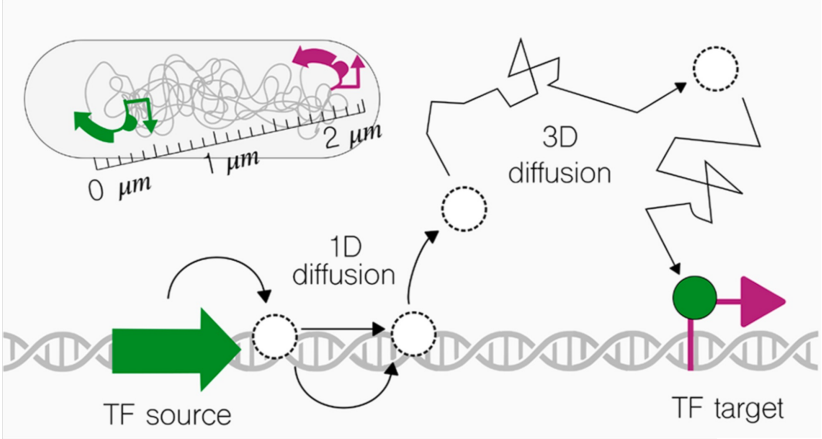
Separation between genes



Angel Goni-Moreno et al. Deconvolution of Gene Expression Noise into Spatial Dynamics of Transcription Factor-Promoter Interplay. ACS Synthetic Biology (2016).

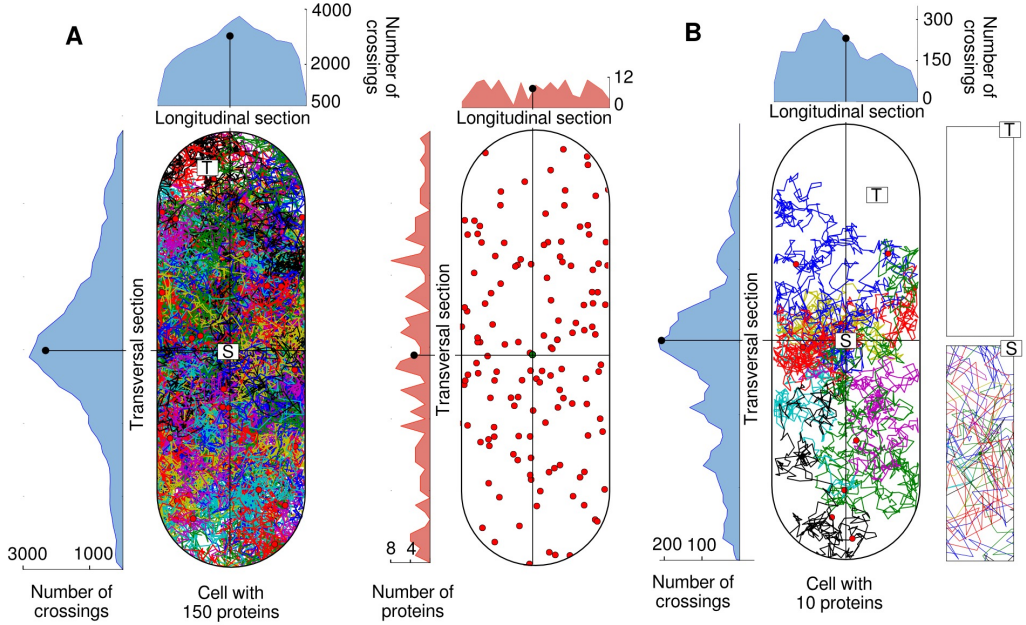
- Gene expression noise correlated to intracellular distance.
- Expression sharp when components close.
- Expression noisy when promoters far.
- Reusability of designs!

Separation between genes

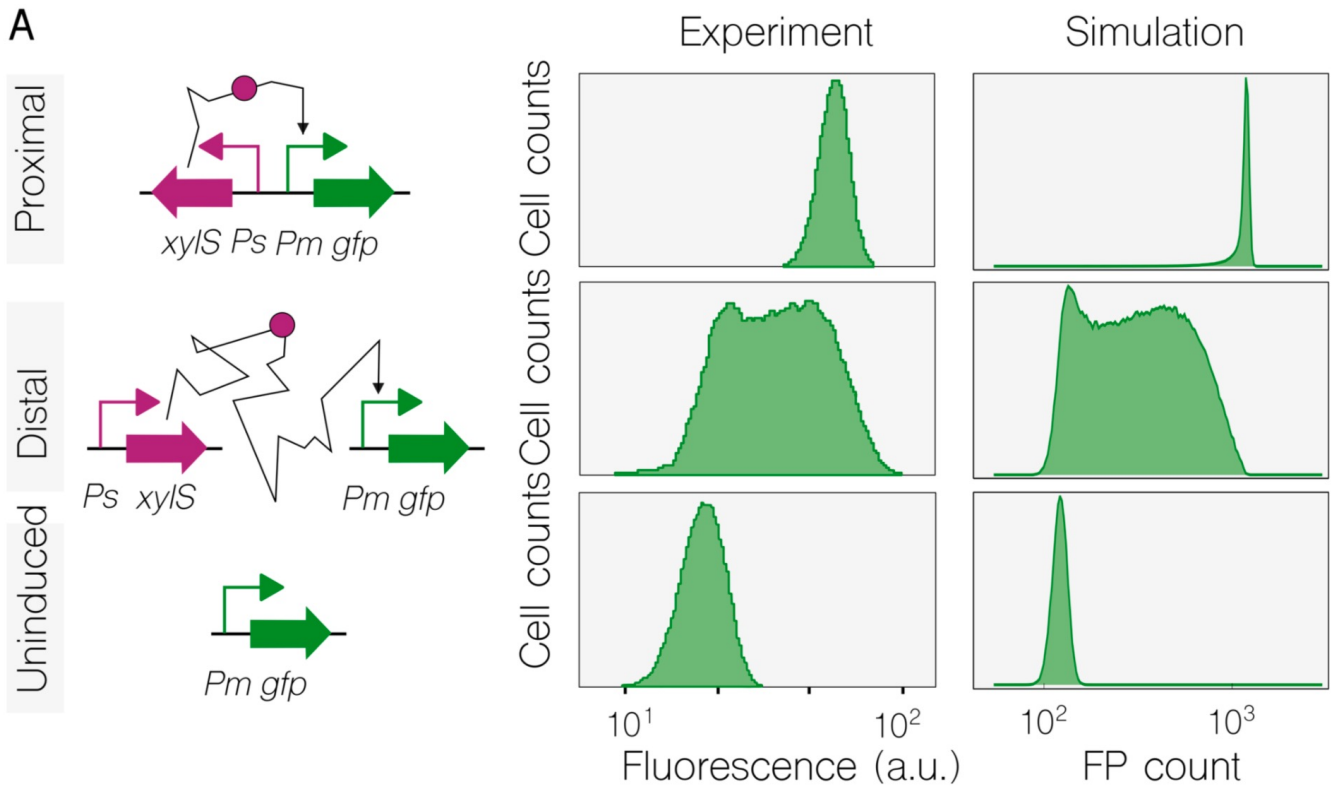


Ruud Stoof et al. A Model for the Spatiotemporal Design of Gene Regulatory Circuits. ACS Synthetic Biology (2019).

Regulators diffusing through the cell would generate heterogeneous occupancy regions.

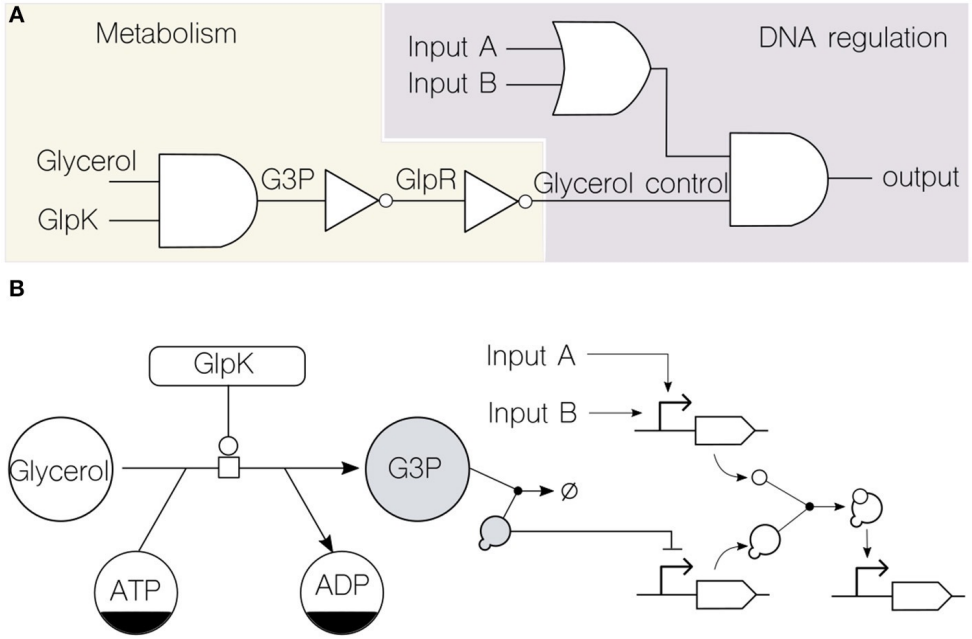


Distance as a design parameter



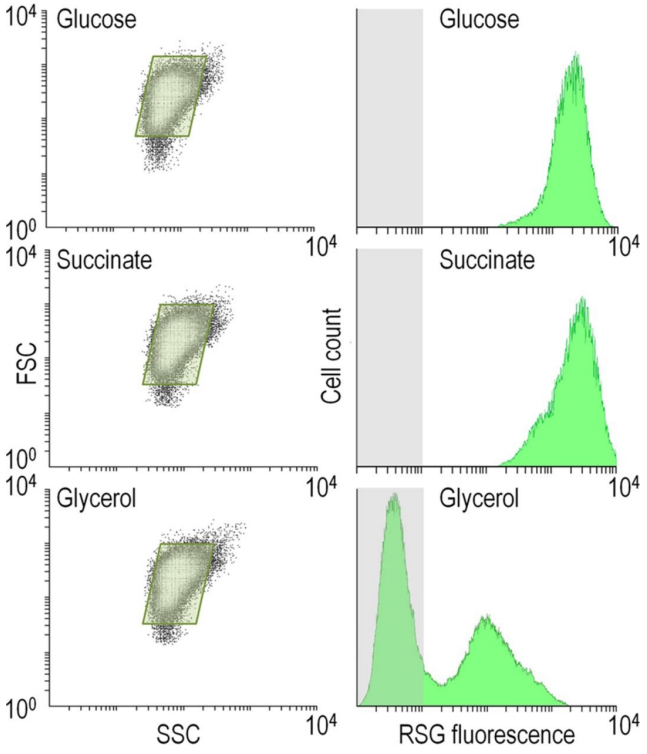
- Genomic dependencies
- Spatial dependencies
- Metabolic dependencies
- Host dependencies

Hybrid transcriptional-metabolic circuits



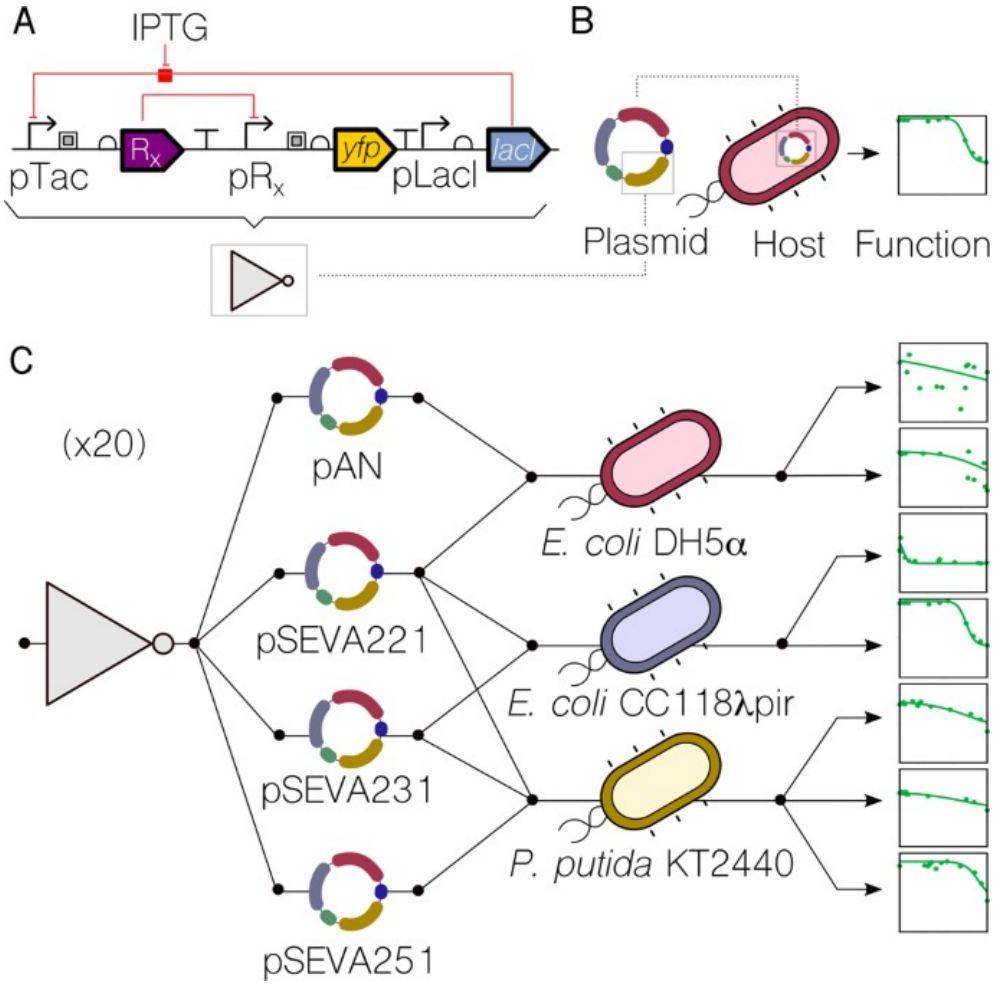
Coñi-Moreno, A., Nikel P. High performance biocomputing in synthetic biology—integrated transcriptional and metabolic circuits. Frontiers in Bioengineering and Biotechnology (2019)

Nikel et al. The Glycerol-Dependent Metabolic Persistence of Pseudomonas putida KT2440 Reflects the Regulatory Logic of the GlpR Repressor. mBio (2015)



- **Genomic dependencies**
- **Spatial dependencies**
- **Metabolic dependencies**
- **Host dependencies**

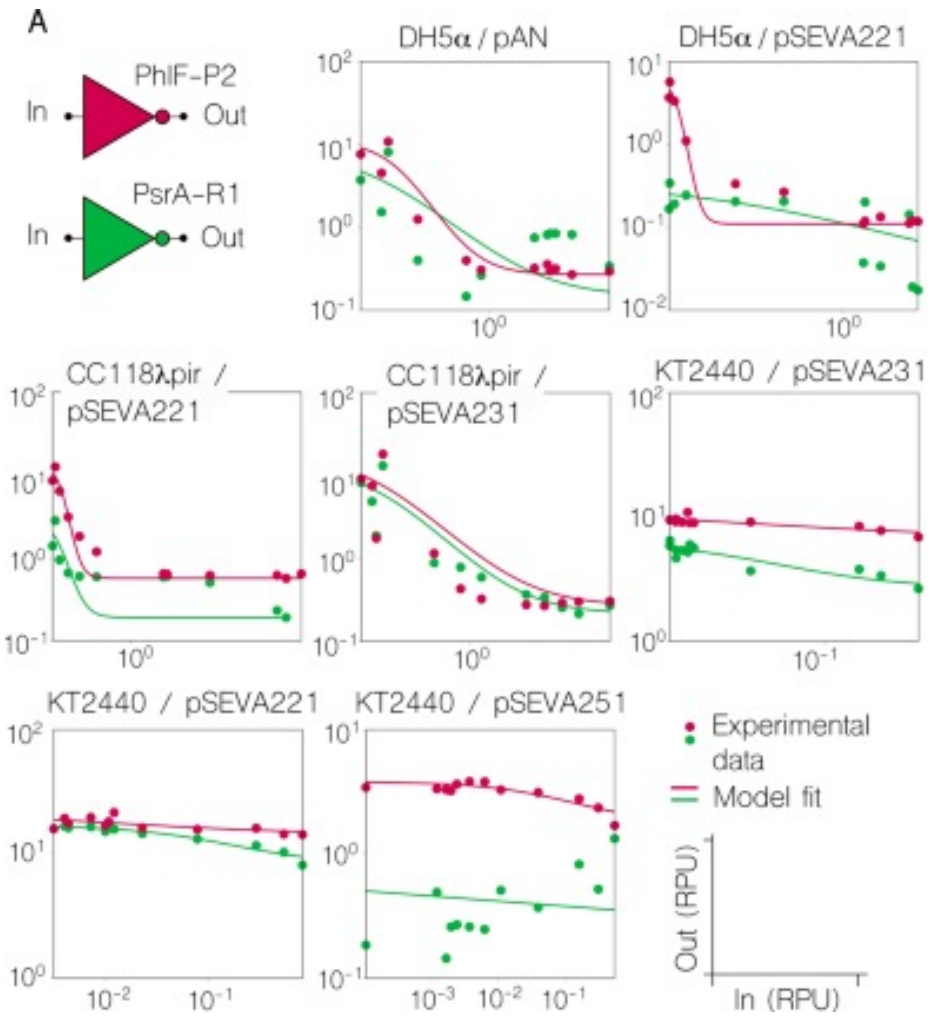
Plasmid + host dependencies



From a library of 20 genetic inverters, we built a library of 135 contextual genetic inverters.

Huseyin Tas et al. Contextual dependencies expand the reusability of genetic inverters. Nature Communications (2021)

Nonlinearities



- Two gates change performance in different ways.
- Very difficult to build predictions

Huseyin Tas et al. Contextual dependencies expand the reusability of genetic inverters. Nature Communications (2021)

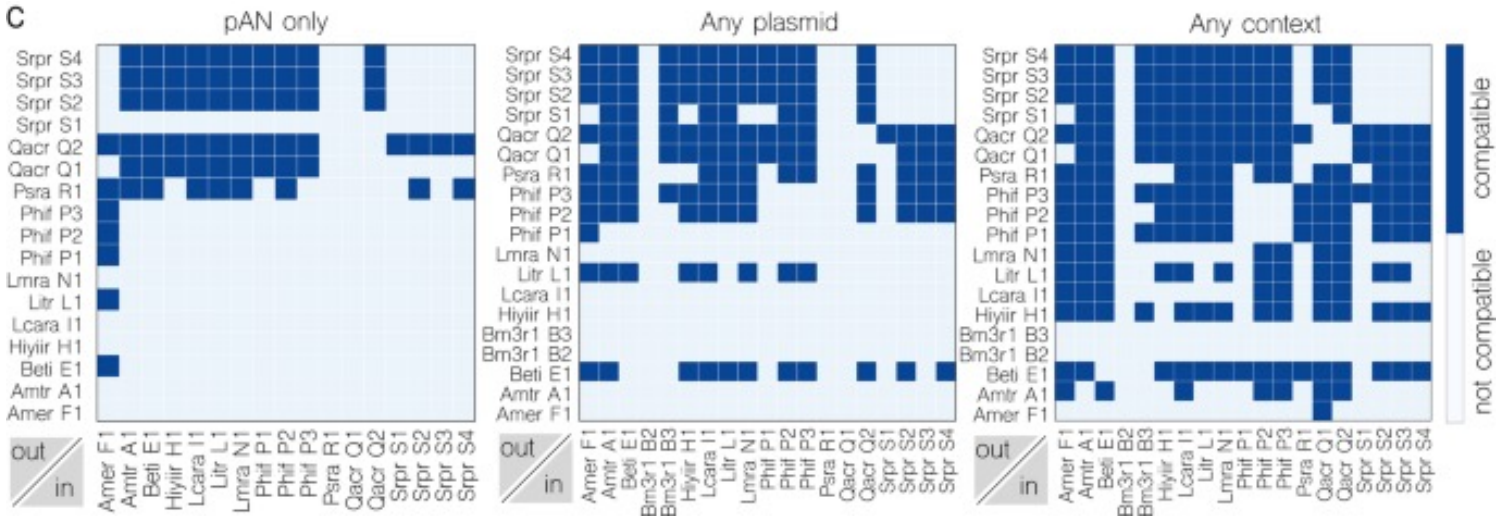
Compatibility

Compatible gate pairs (i.e., output of the first gate is compatible with the input of the second) increase when dependencies are customizable.

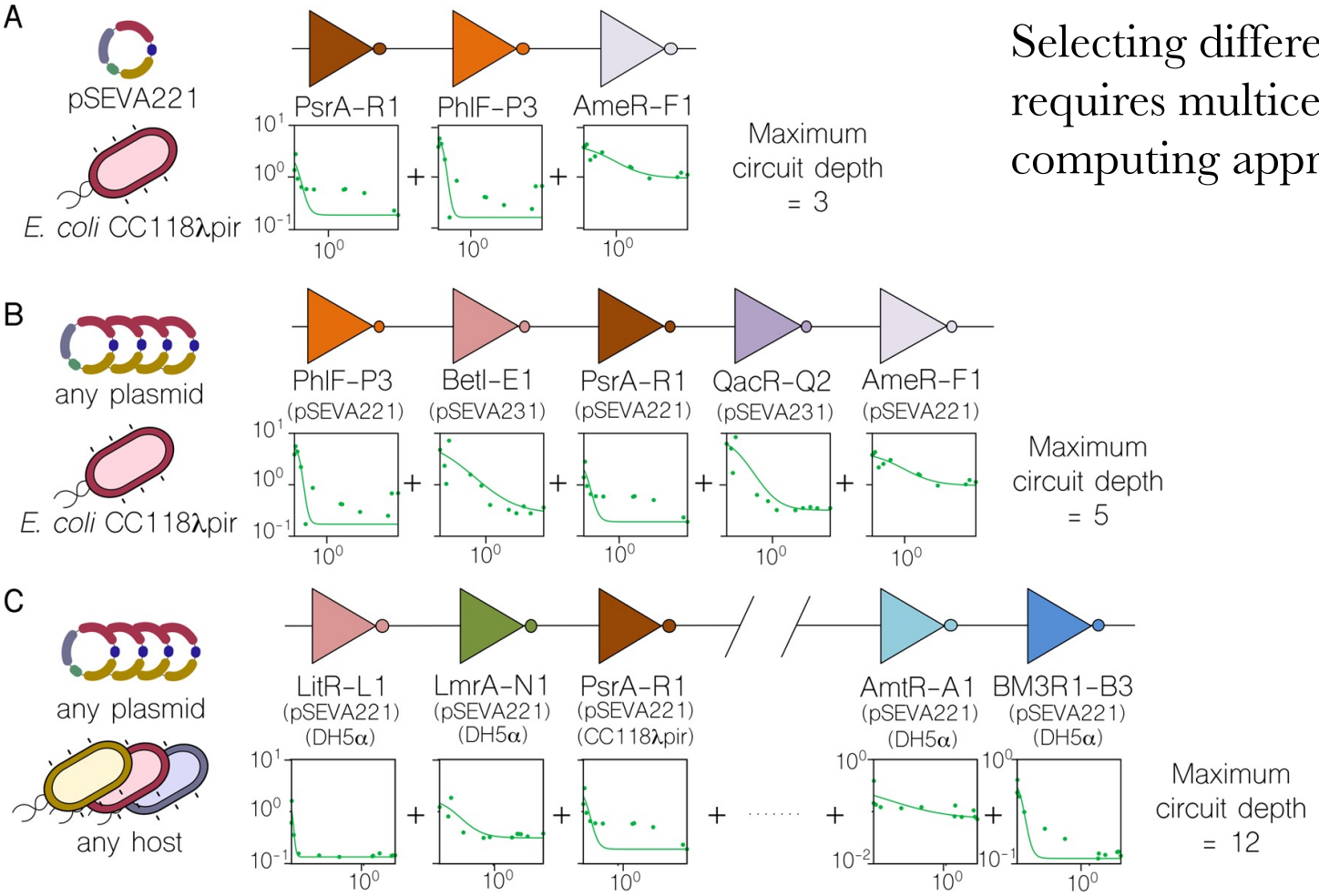
Compatible pairs same plasmid/host

Compatible pairs same host

Compatible pairs [no constraints]

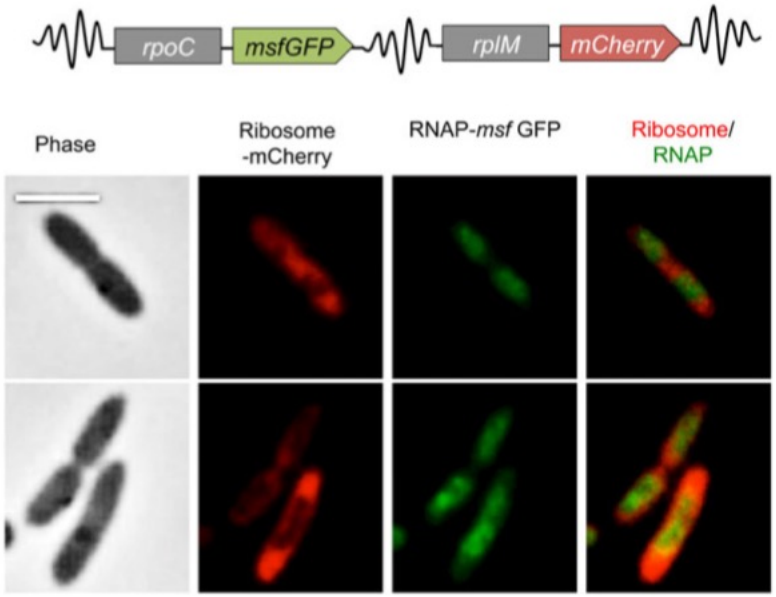


Compatibility

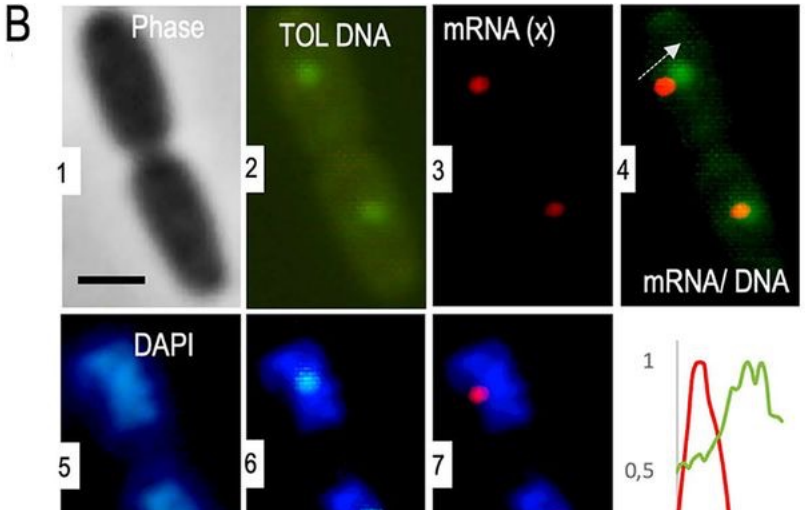


Selecting different hosts requires multicellular computing approaches

Hardware (vs. software)



Kim J., et al. Spatial organization of the gene expression hardware in Pseudomonas putida. Environmental Microbiology (2019)



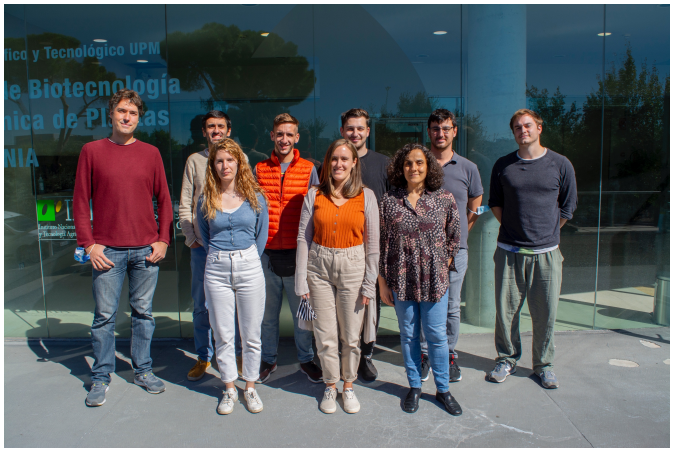
Kim J., et al. Subcellular architecture of the xyl gene expression flow of the TOL catabolic plasmid of Pseudomonas putida mt2. mBio (2021)

Conclusion

- We can modify / fine-tune genetic performance by programming contextual dependencies
- We could potentially build more complex bio-computations if we designed devices where DNA is just part of them.

Acknowledgements

Thanks!



Also to: Ruud Stoof, Juhyun Kim, Huseyin Tas, Pablo Nikel, Víctor de Lorenzo…

CBGP
CENTRO DE BIOTECNOLOGÍA Y GENÓMICA DE PLANTAS
UPM-INIA

EXCELENCIA SEVERO OCHOA

EPSRC
Engineering and Physical Sciences Research Council

Comunidad de Madrid

European Commission
Horizon 2020
European Union funding for Research & Innovation

GOBIERNO DE ESPAÑA
MINISTERIO DE CIENCIA E INNOVACIÓN