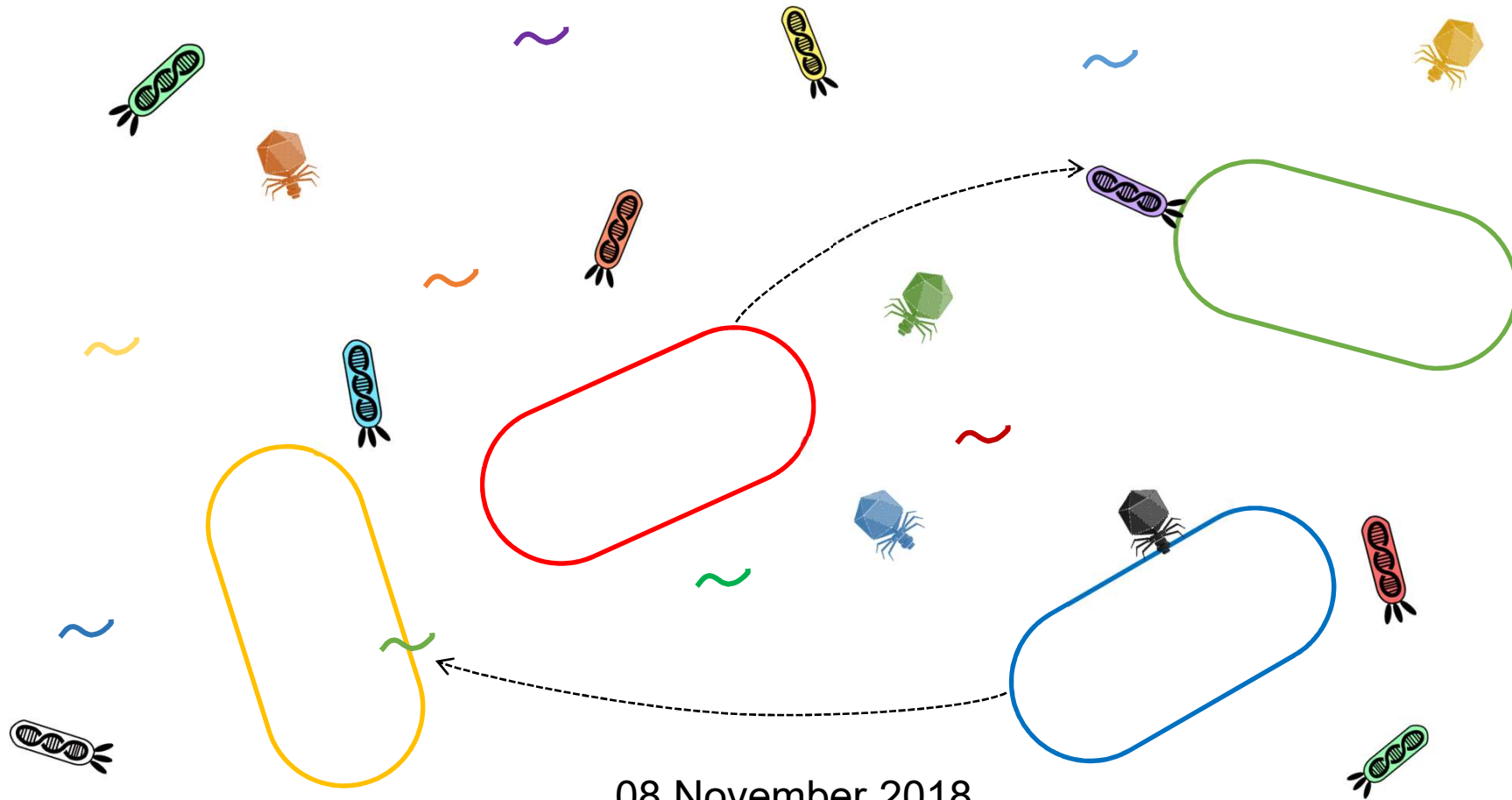


# Exploiting bacteriophage-derived signals as external wires for biological computing

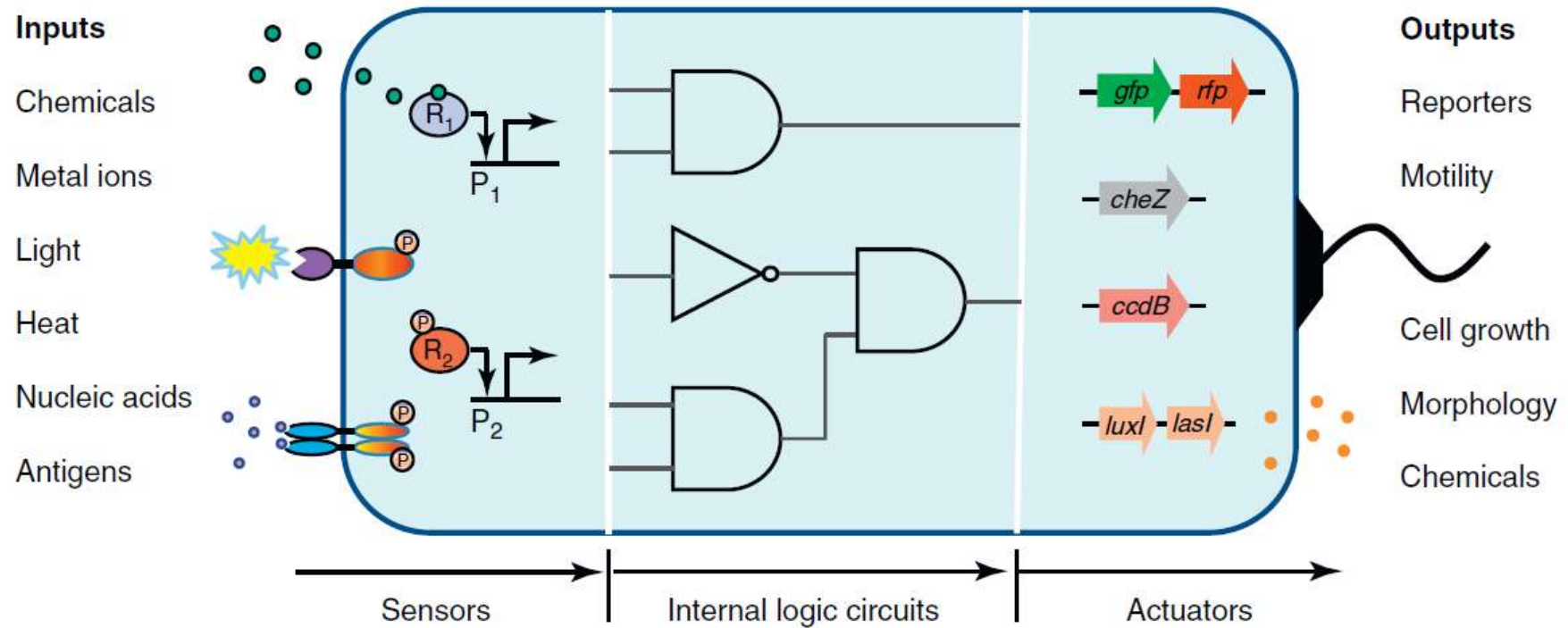


08 November 2018

Manish Kushwaha

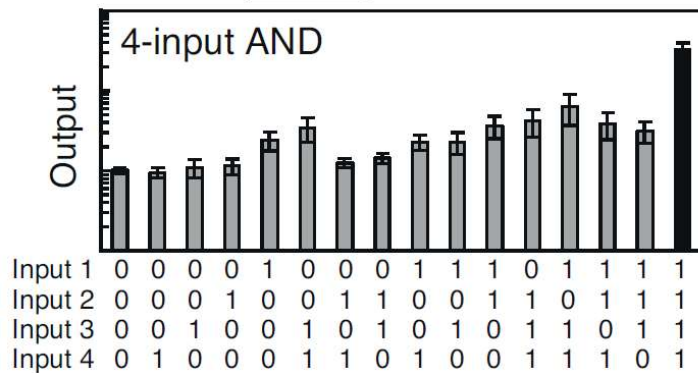
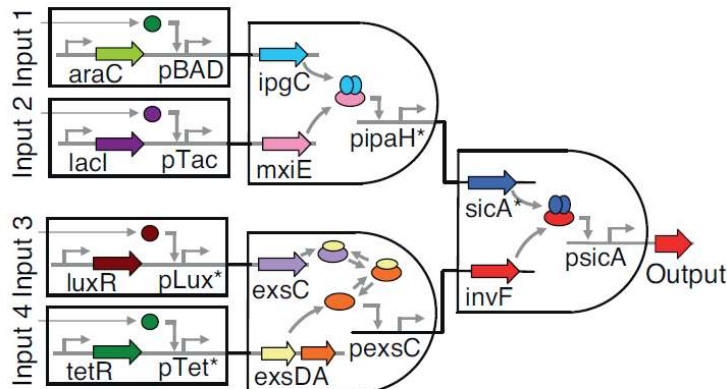
MICALIS Institute, INRA, Jouy-en-Josas

# Typical Organization of a Synthetic Genetic Network

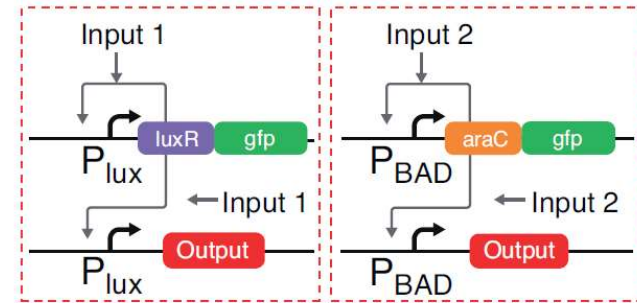


(Wang & Buck, 2012. Trends in Microbiology)

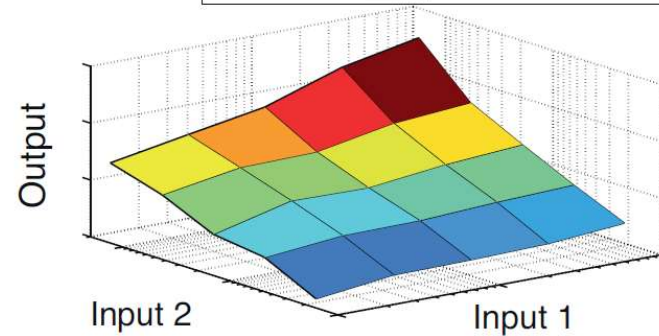
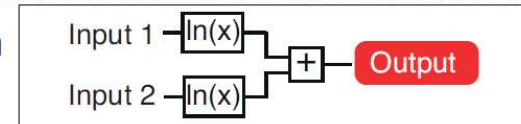
# Biological computation in Synthetic Biology



**Digital computation**  
(Moon *et al.*, 2012. Nature.)



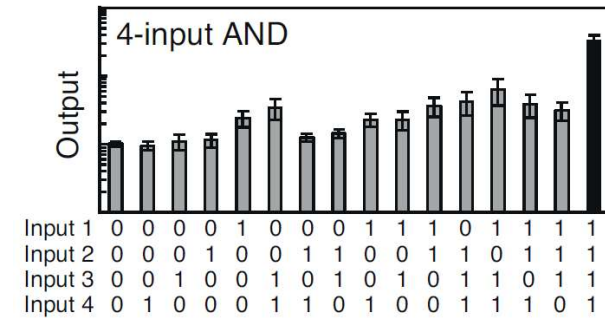
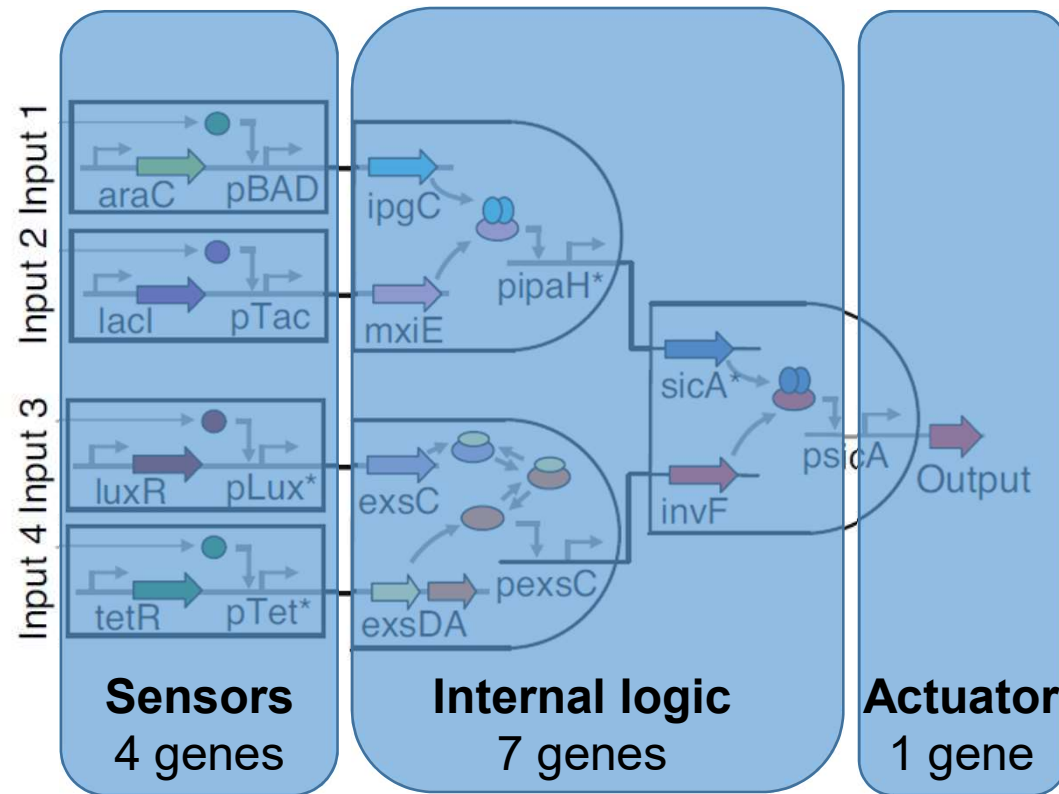
Log-domain  
addition



**Analog computation**  
(Daniel *et al.*, 2013. Nature.)

- Synthetic genetic circuits can process digital and analog information
- Their construction requires the careful wiring of different genetic components

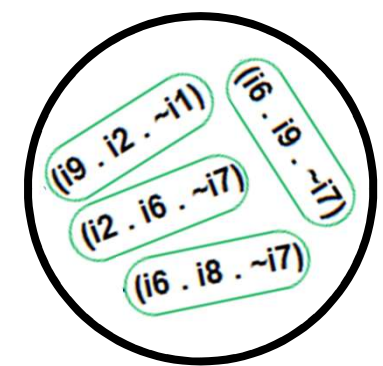
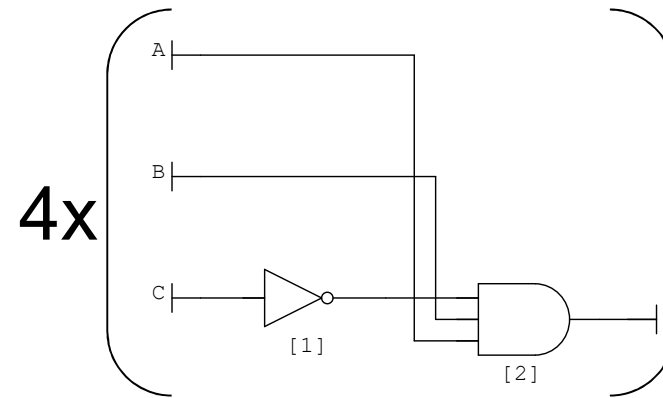
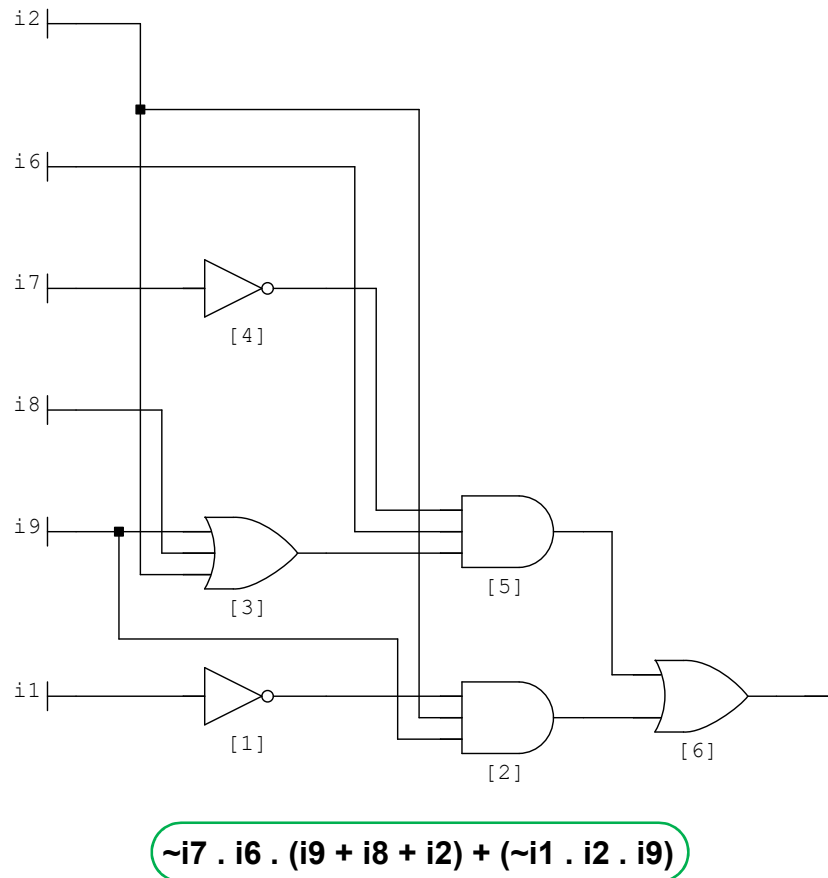
## Connecting wires: Expenses add up



**Digital computation**  
(Moon *et al.*, 2012. Nature.)

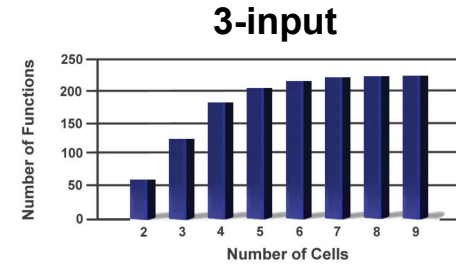
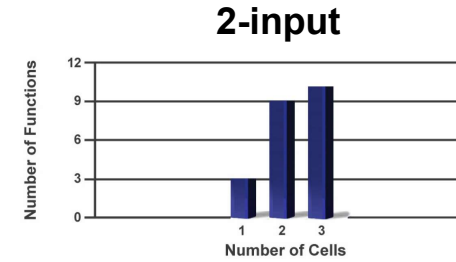
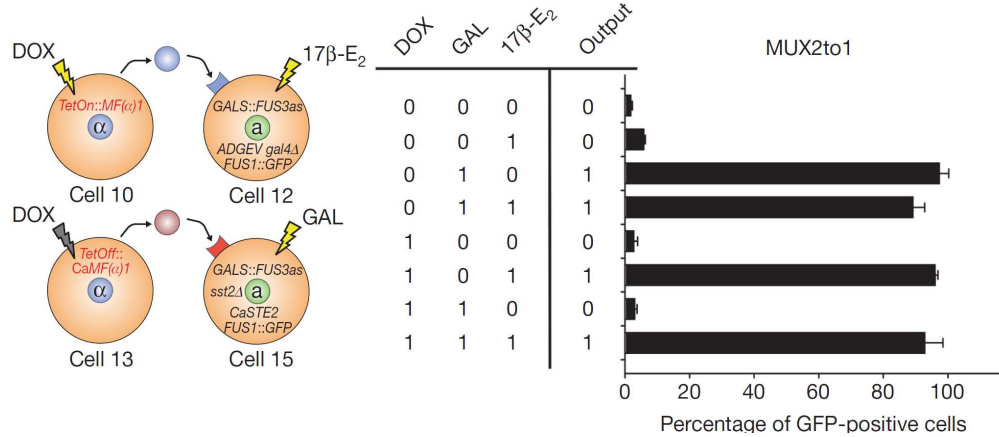
- Adding multiple “wires” is expensive for the cell
- Multiple internal circuit layers often dissipate the signal

# Single versus multiple cells for Biological computing

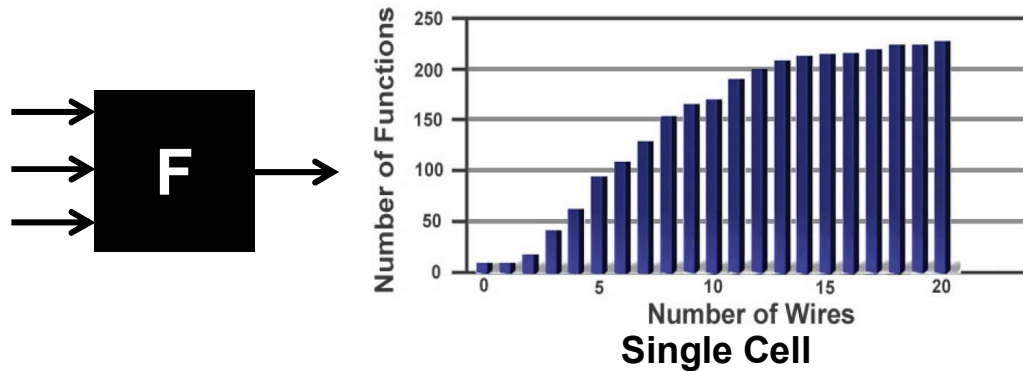


- “Distributed computing” could allow decomposition of a complex problem into multiple smaller parts that can be solved by different computers

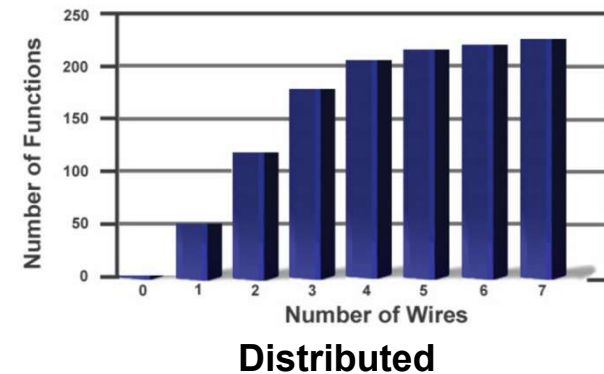
# Distributed Computing



(Regot *et al.*, Nature 2011)

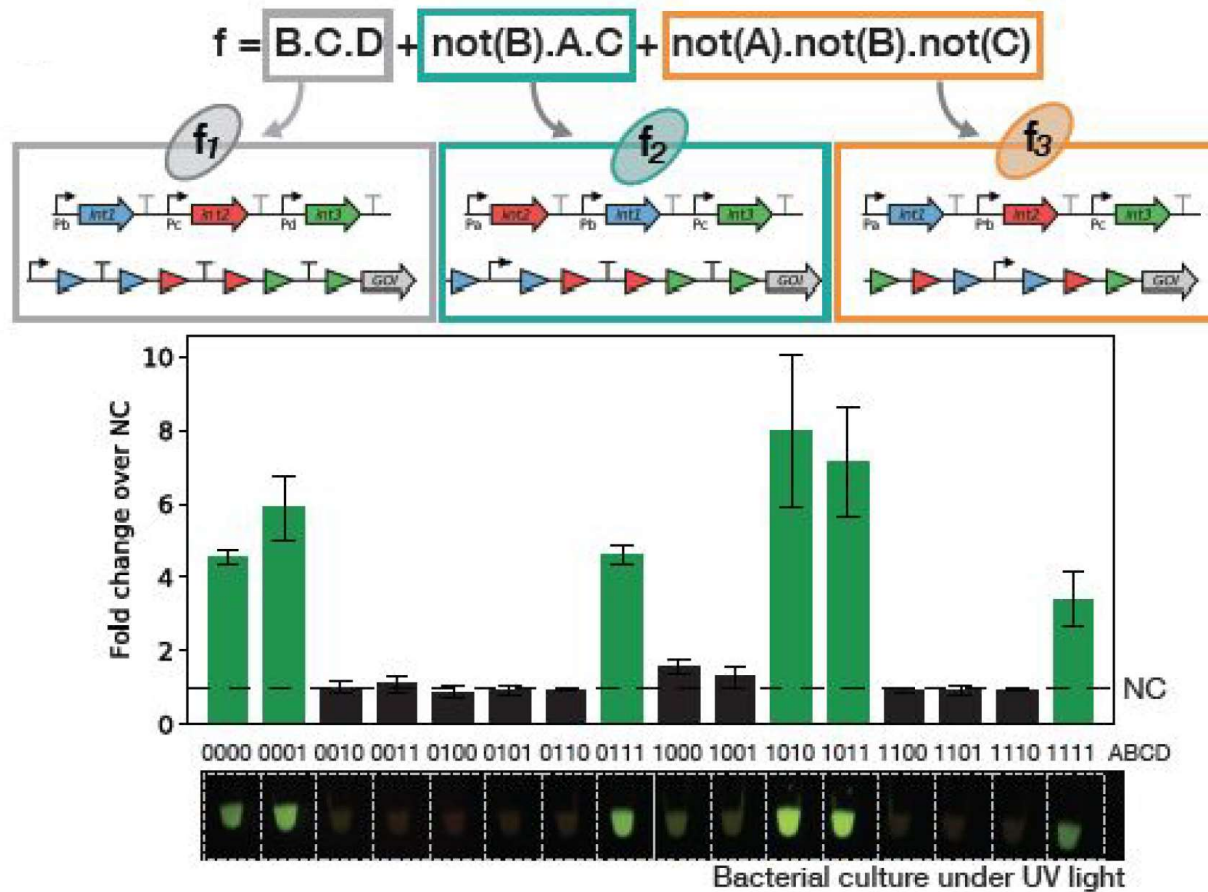


### 3-input NOR only logic



- As circuit complexity increases, the number of genetic parts and their connecting “wires” increases rapidly
- Distributed computing significantly **reduces wiring requirements** and enables **re-use of sub-circuits**

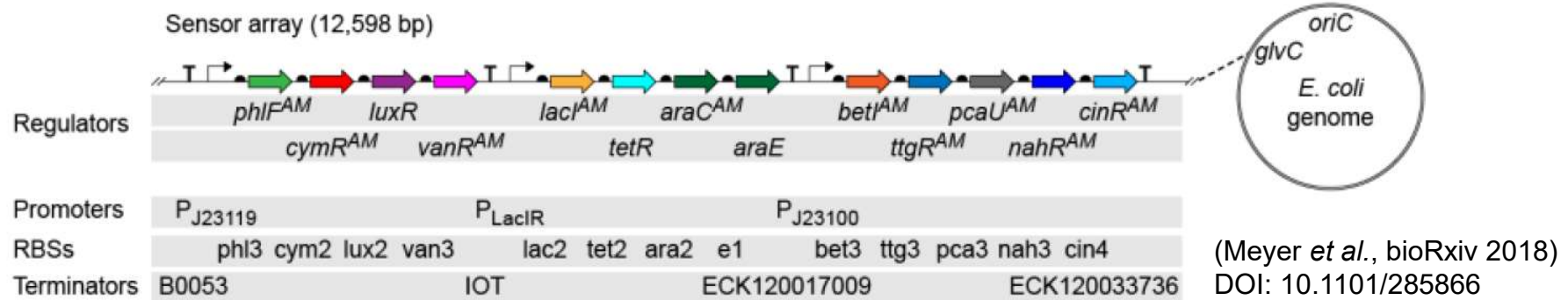
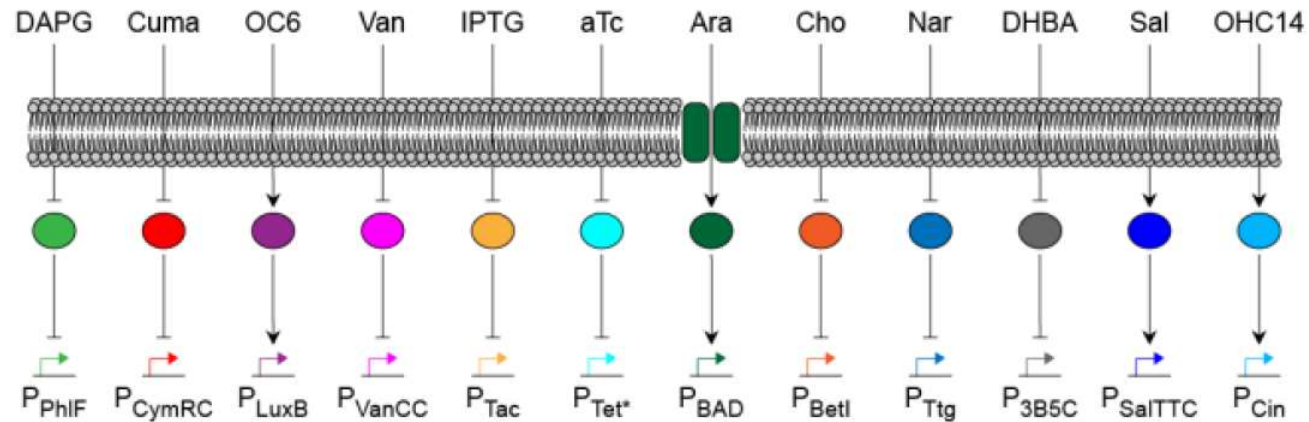
# More Multicellular Computing



(Guiziou *et al.*, bioRxiv 2018)  
DOI: 10.1101/390823

- Combining recombinase “state machine” logic with multicellular computing

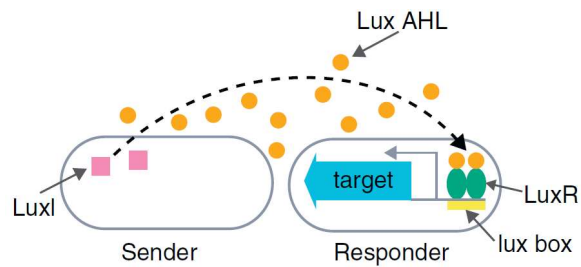
# More Internal Wires



- Recent work has built strains with 12 different “sensor” modules

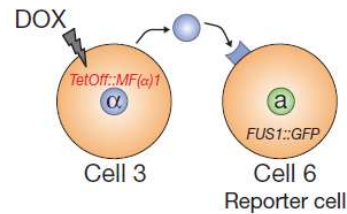


# Limited external wires



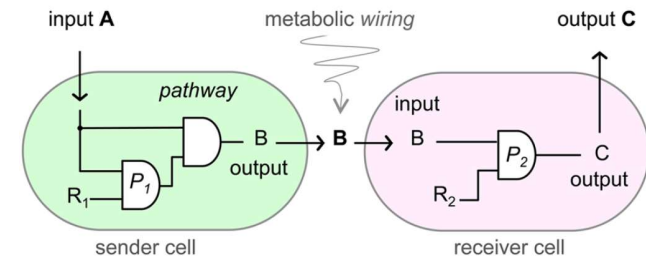
**Quorum sensing molecules**

(Macia *et al.*, Trends in Biotech. 2012)



**Hormone molecules**

(Regot *et al.*, Nature 2011)



**Metabolites**

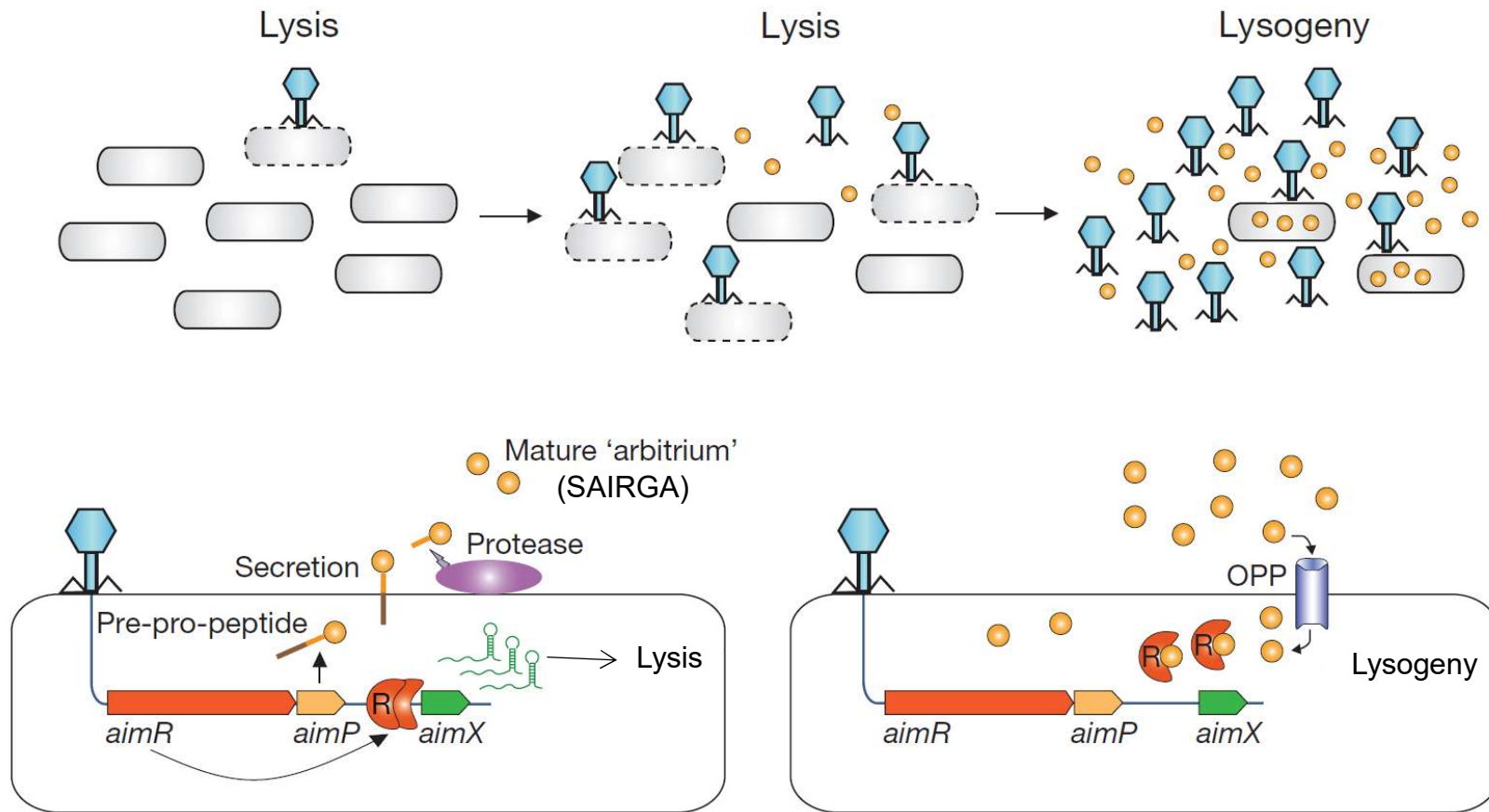
(Silva-Rocha *et al.*, ACS SynBio. 2013)

- A limited set of orthogonal external wires exist for cell-to-cell communication

**Part-1:**  
**Bacteriophage-derived signals as  
External Wires**

**Small Signaling Peptides**

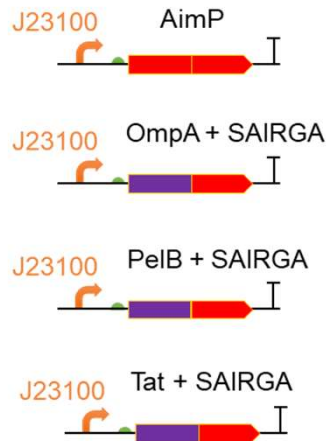
# Phage Quorum sensing system



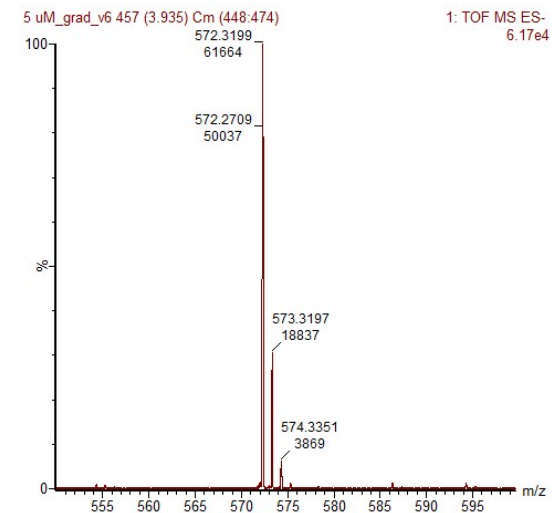
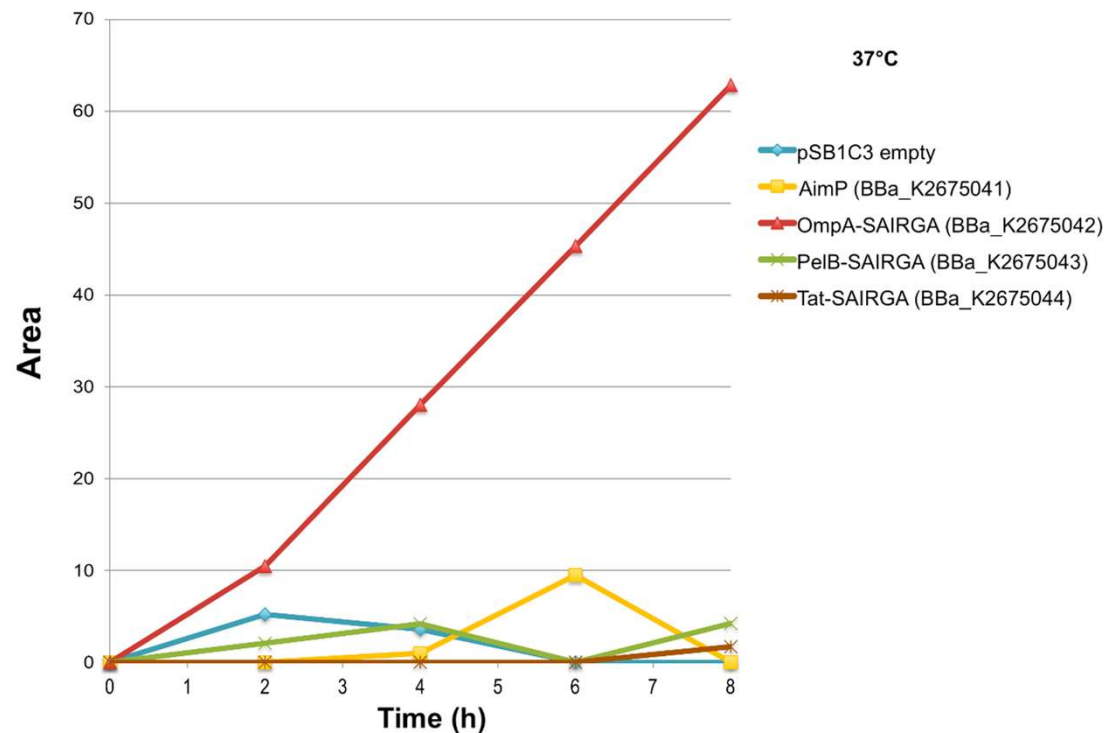
(Erez *et al.*, Nature 2017)

- Quorum sensing system of *Bacillus* phage phiT3 uses a hexapeptide as signaling molecule

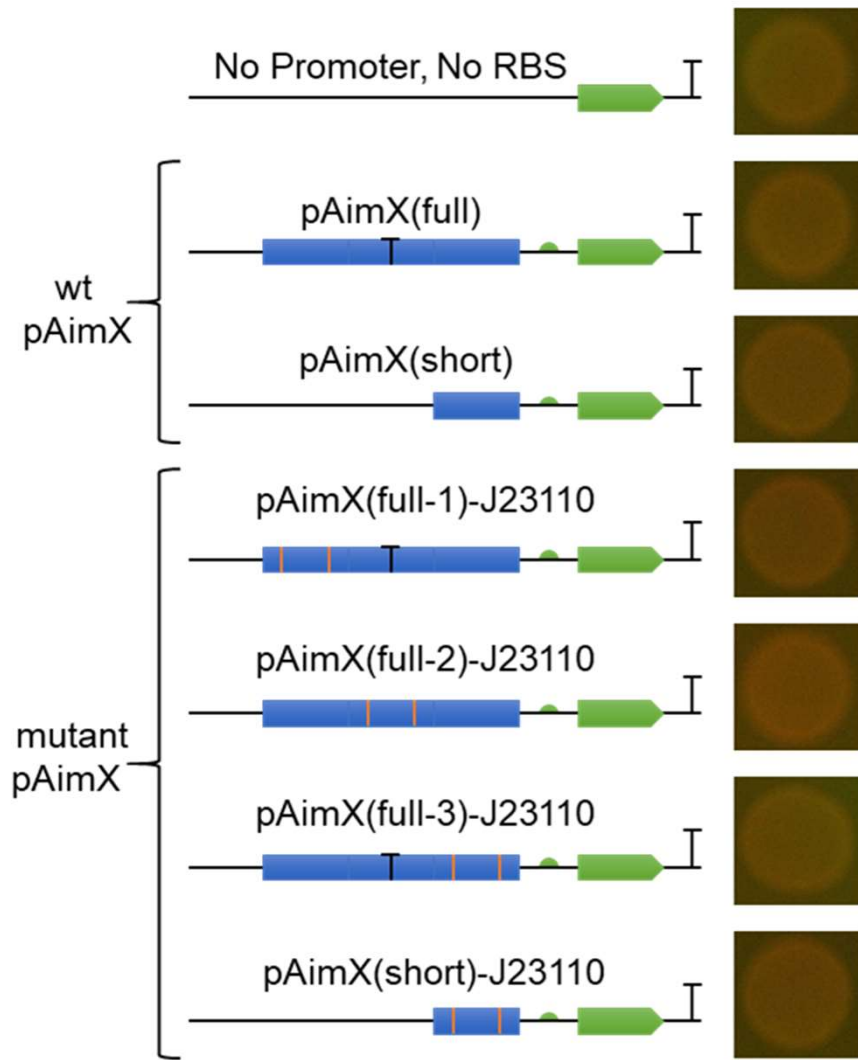
# Re-engineering peptide secretion



- While one system was characterized, genomic searches revealed at least 17 different peptide signals and their receptors
- This repertoire can be expanded by directed evolution of the peptide-receptor pair
- We decided to adapt this system for peptide secretion in *E. coli*

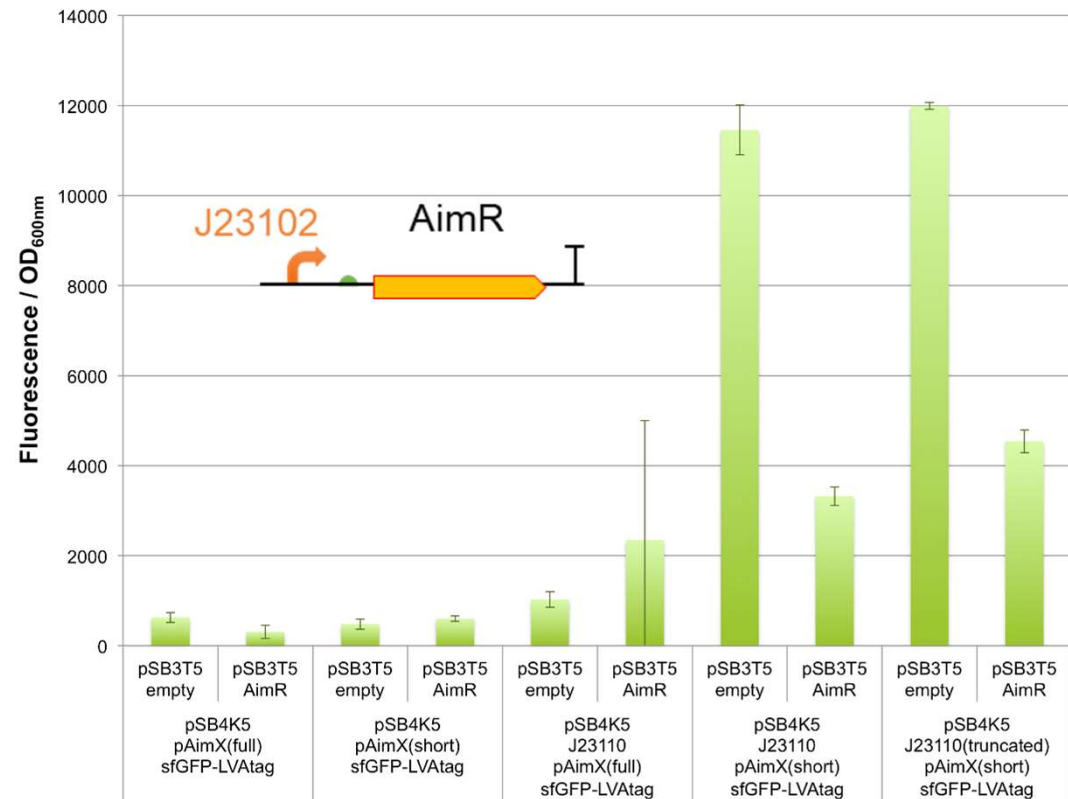
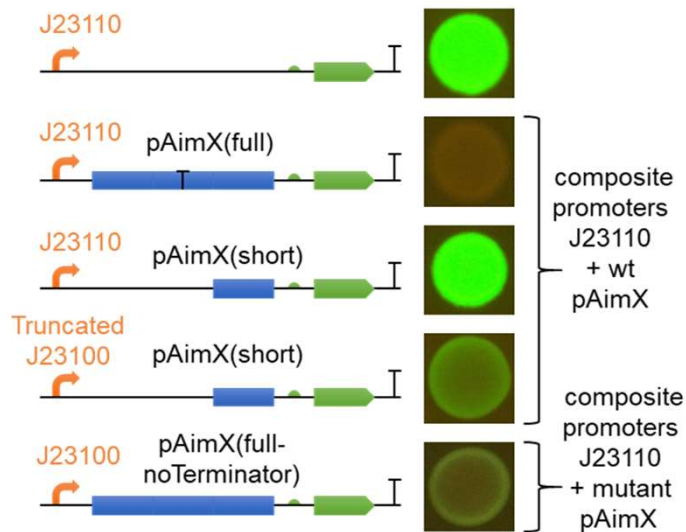


# Re-engineering promoters



- *Bacillus* promoters were not functional in *E. coli*, with or without the AimR activator

# Re-engineering promoters



- So, activatable promoters were re-engineered as repressible promoters in *E. coli*


# Presented at the Giant Jamboree



Evry Paris-Saclay  
iGEM



Boston  
October 24-28th 2018



# PEPTalk

Students: Esteban Letrun, Axel Radin, Suzanne Phengsay, Paul Akhai, Adèle Mazelin, Yasmine Aji, Yann Bourgois, Paul Del Riccon, Solène Castaner, Angelo Cardoso Balista, Marine Podévin, Sara Gadaï-Aghamiri, Léa Raucher, Sambavi Marandu, Azim-Berly Besya, Camille Morlet, Charlotte Denaux, Nade Farjaoui, Khawla Achoch, Advisors: Stef Horemans, Nazim Garcia, Supervisors: Ioana Popescu, Manish Kushwaha

BIOLOGICAL ENGINEERING HAS TURNED BACTERIA INTO BIOSENSORS, NANO-ROBOTS AND PRODUCTION FACTORIES. AS THESE APPLICATIONS ARE ORGANISED FOR HIGHER-LEVEL TASKS, MULTIPLE DIFFERENT BACTERIA WILL BE NEEDED TO WORK IN A CONSORTIUM EACH WITH THEIR OWN ASSIGNED SUB-TASK. HOWEVER, SUCH DIVISION OF LABOUR CAN ONLY BE EFFICIENT IF THE BACTERIA CAN COMMUNICATE WITH EACH OTHER USING UNAMBIGUOUS LANGUAGE. THE AIM OF OUR PROJECT IS TO BUILD A SYNTHETIC COMMUNICATION SYSTEM WITH AN EXPANDABLE PEPTIDE VOCABULARY SO THAT BACTERIA CAN SEND DIFFERENT AND SPECIFIC SIGNALS TO COMMUNICATE DIFFERENT THINGS.

### COMMUNICATION IS KEY

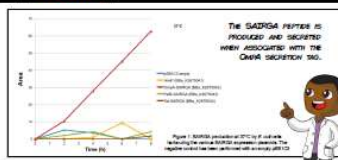
TO HAVE EFFICIENCY IN COMMUNICATION, IT IS BETTER TO HAVE "ORTHOGONAL" OR "SIMPLE" WORDS THAT DESCRIBE SINGLE THINGS. IF THE LANGUAGE USED TO COMMUNICATE HAS LIMITED VOCABULARY, OR IF THE INDIVIDUALS ARE NOT ABLE TO UNDERSTAND THE WORDS, IT'S HARD TO HAVE ANY MEANINGFUL CONVERSATION.

...CAN LEAD TO CROSS-TALK WITH OTHER WORDS ALREADY EXISTING.

WE DEVELOP AN EFFICIENT COMMUNICATION SYSTEM...

WITH A LARGER VOCABULARY WHERE WORDS HAVE SINGLE MEANINGS FOR UNAMBIGUOUS COMMUNICATION.

### RESULTS



THE GAZDRA PEPTIDE IS PRODUCED AND SECRETED WHEN ASSOCIATED WITH THE OTHER SIGNALING TAG.

AMAR CAN ONLY ACTIVATE THE TRANSCRIPTION OF E. COLI BECAUSE IT DOES NOT INTERFERE WITH RIBU AS IT DOES IN E. COLI.

WE COMBINED DIFFERENT PRODUCTION TOOLS AND DEVELOPED A BETTER WAY TO PROTECT PROTEIN STRUCTURE.

AMAR REPRICES THE GFP EXPRESSION IN CONSTRUCTIONS CONTAINING THE COMPOSITE PROTEIN.

THE PEPTIDE SLIGHTLY REMOVES THE REPRESSION EFFECT OF AMAR.

### DESIGN

IT STARTS REPLICATING AND PRODUCES A PEPTIDE WHICH IS RELEASED TO THE OUTSIDE. THEN, IT LISTENS THE CELL AND LOOKS FOR ANOTHER ONE TO INTERACT.

WE RE-PURPOSE THE PEPTIDE BASED SIGNALING SYSTEM OF BACILLUS BACTERIOFORMIS FOR APPLICATION IN E. COLI.

IF THE PEPTIDE CONCENTRATION IS HIGH IN THE NEWLY INFECTED CELL, THE PHAGE ENTERS THE LYSOGENIC PHASE.

TO IMPLEMENT THE SYSTEM IN E. COLI WE HAVE DESIGNED SEVERAL CONSTRUCTIONS WITH THE PEPTIDE, THE RECEPTOR AND THE PROMOTER WHICH ARE INVOLVED IN THE COMMUNICATION PROCESS.

### EDUCATION AND PUBLIC ENGAGEMENT

THE GENERAL PUBLIC IN FRANCE SEEMS TO ASSOCIATE BIOLOGY WITH THE OBSERVATION OF FLORA AND FAUNA. OUR AIM WAS TO CHANGE THAT PERCEPTION BY POPULARIZING SYNTHETIC BIOLOGY. WE SPOKE TO A LARGER TARGET AUDIENCE OF HIGH SCHOOL PROFESSORS AND SOCIAL SITUATIONS.

WE MADE SCIENTIFIC EXPERIMENTS WITH TEENAGERS AND SYNTHETIC BIOLOGY.

WE TALKED ABOUT SYNTHETIC BIOLOGY WITH SENIORS.

WE ARE CREATING AN OPEN-LAB CALLED BIOCLUB WHERE PEOPLE WILL BE ABLE TO PRACTICE AND LEARN SYNTHETIC BIOLOGY AND GIVE A NEW LIFE TO OUR PREVIOUS iGEM PROJECTS.

### FUTURE APPLICATIONS


IN THE FRAMEWORK OF ALIENI PRACTICES, WE INITIALLY BEGAN WITH MEETING PROFESSIONALS IN THE RESEARCH COMMUNITY TO FIND THE APPLICATIONS OF OUR PROJECT. PEPTALK, AS A FOUNDATIONAL ADVANCE, AIMS TO TRANSCEND DISCIPLINES. IT HAS THE ADVANTAGE OF BEING ABLE TO AFFECT MANY APPLICATIONS, ESPECIALLY CHEMICAL SENSOR APPLICATIONS.

TO ENHANCE THE POTENTIAL OF OUR PROJECT, WE PLAN TO CHARACTERISE A LIBRARY OF GDS MOLECULES TO CREATE A MOLECULAR TOOLBOX.

THE TOOLBOX SHOULD BE USED FOR MANY APPLICATIONS SUCH AS DISTRIBUTED LOGIC GATES...

...COMPLEX BIOSENSORS...

...AND CO-CULTURES.



1 Erez et al., Nature (2017), 541, 488-493.

## Next Steps



- 1) Test the effect of peptide on the repressible promoters
- 2) Test the other 16 peptide-receptor pairs similarly
- 3) Perform directed evolution of the peptide-receptor pair to expand repertoire

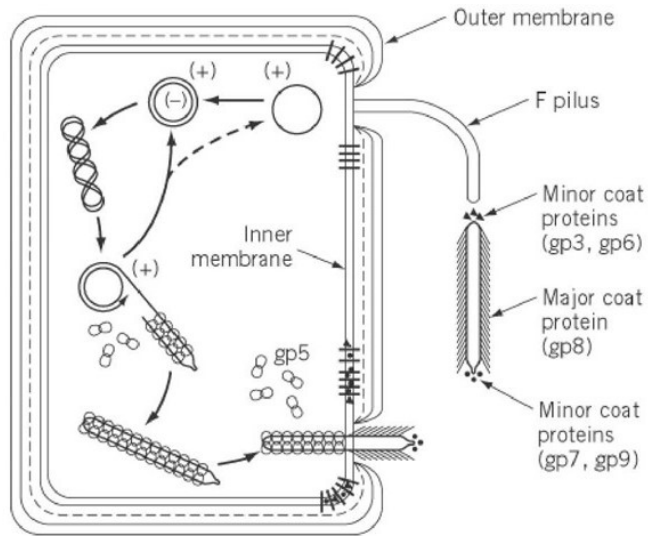
(iGEM Evry Paris-Saclay 2018.  
[https://2018.igem.org/Team:Evry\\_Paris-Saclay](https://2018.igem.org/Team:Evry_Paris-Saclay))



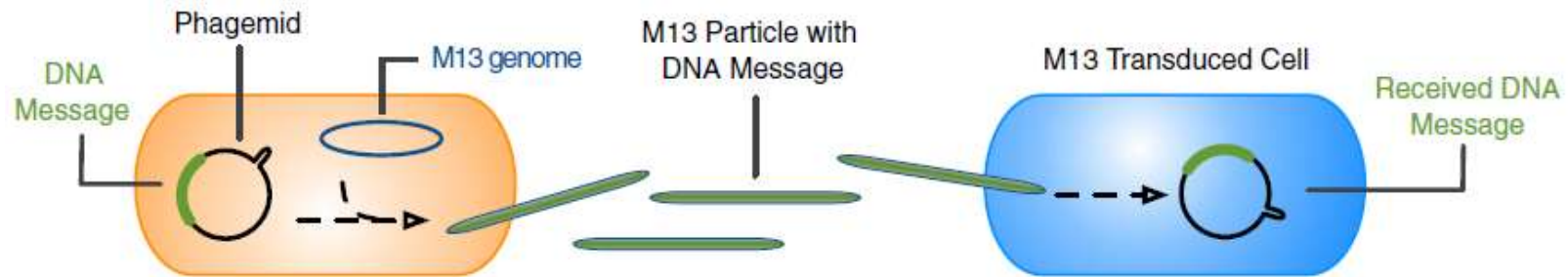
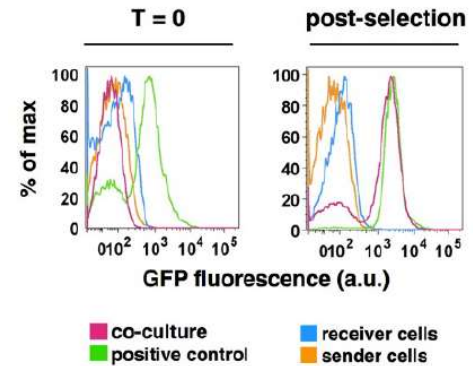
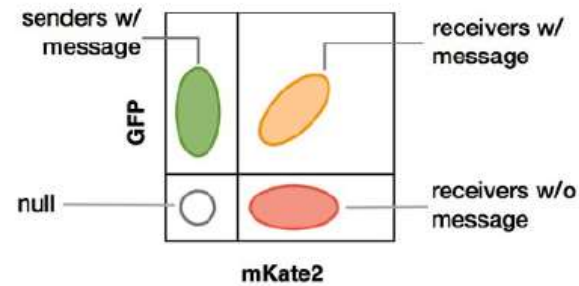
**Part-2:**  
**Bacteriophage-derived signals as  
External Wires**

**Packaged DNA**

# Engineering Filamentous bacteriophages for messaging



- Filamentous phages are non-lytic phages that reproduce in bacterial cells

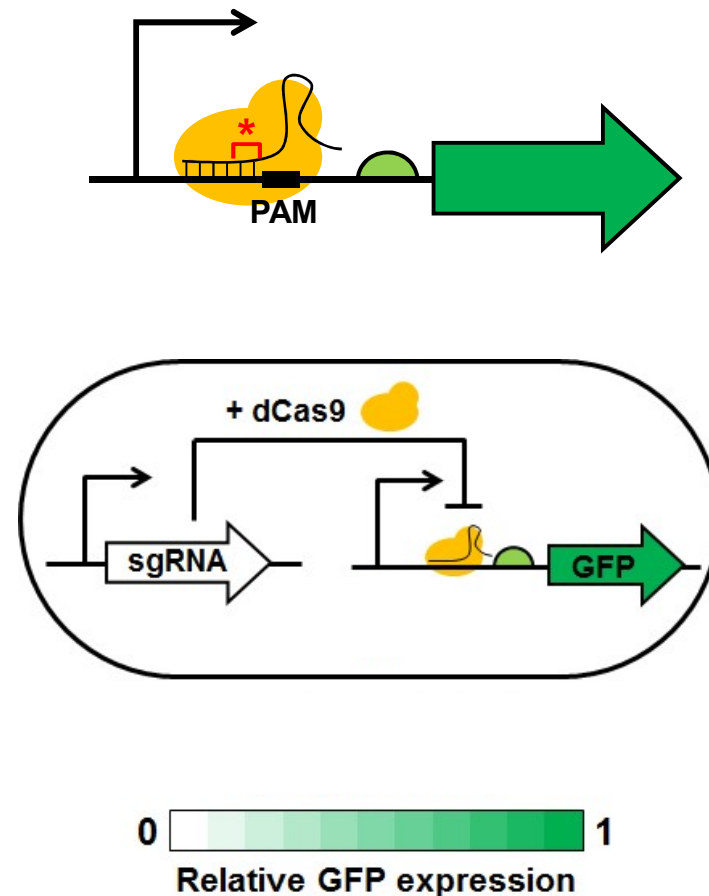
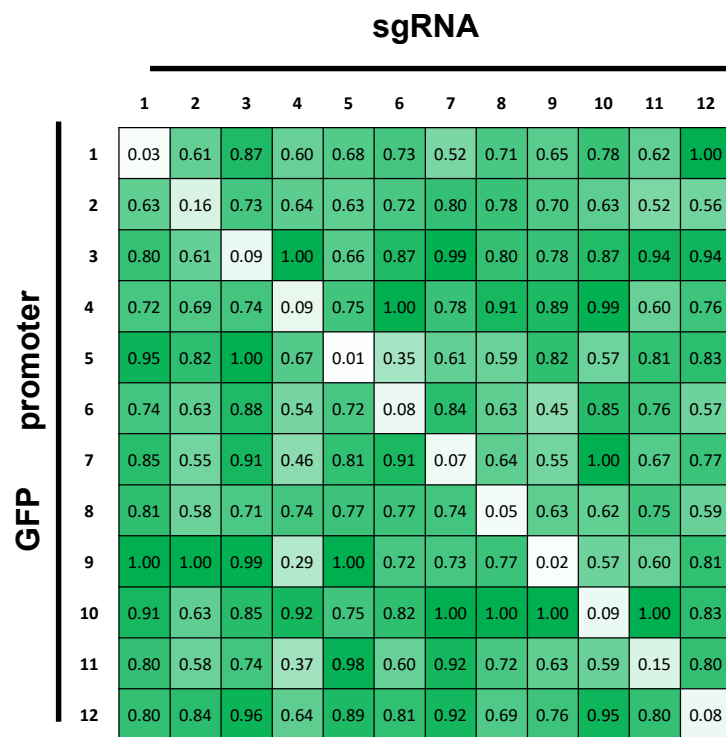


(Ortiz & Endy. J. Biol. Engg. 2012)

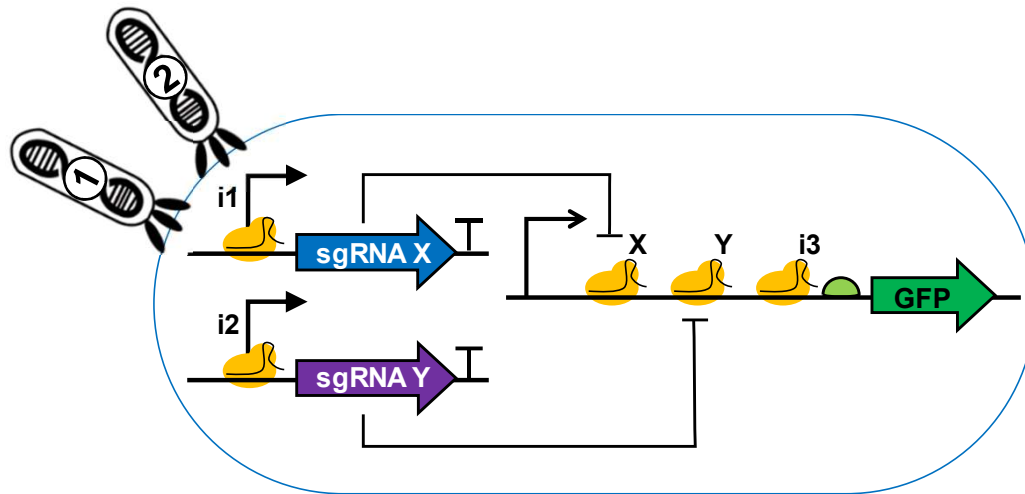
- They can be engineered to send “phagemid” messages between cells
- The Sender cells have the secretion machinery, while the Receiver cells have the surface receptors

# Orthogonal RNA signals encoded in phagemid particles

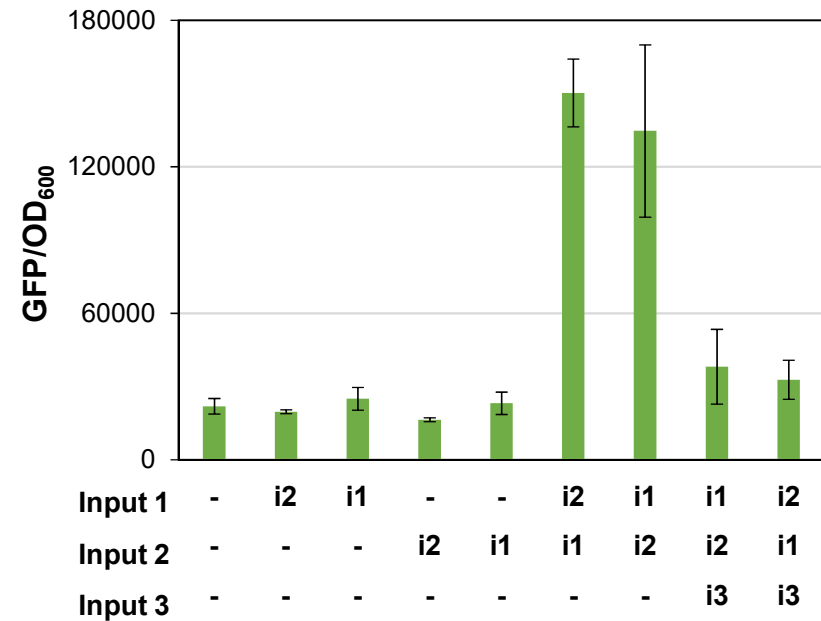
- dCas9 protein is an RNA-programmable repressor
- Computational design was used to generate a panel of orthogonal gRNAs
- Orthogonality was experimentally tested



# Logic gates based on phage-delivered signals

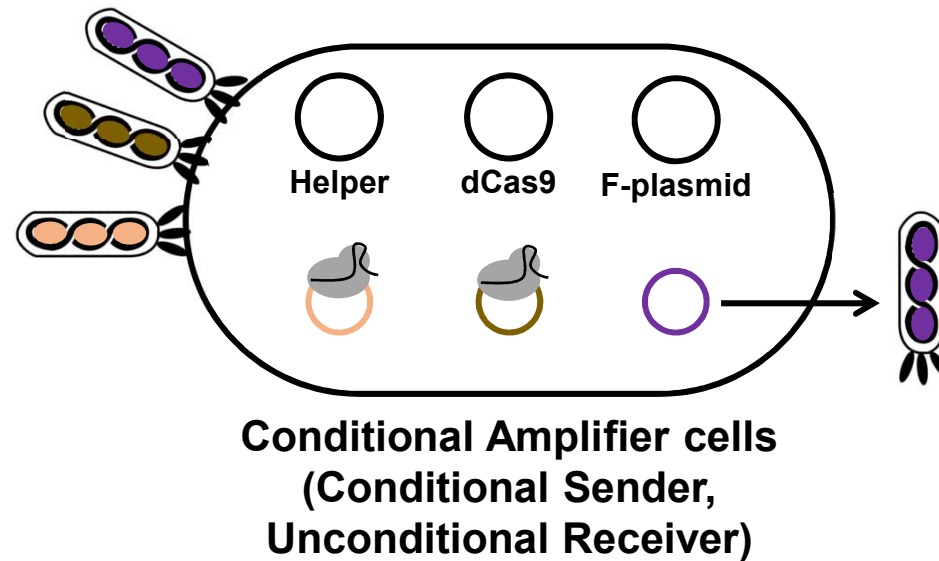
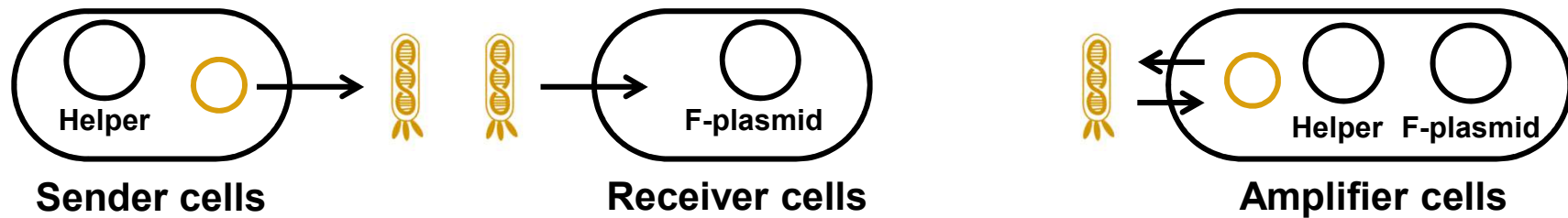


$$\begin{aligned}
 &(i1 \cdot i2 \cdot \sim i3) \\
 &= \sim(\sim i1 + \sim i2 + i3) \\
 &= \sim(X + Y + i3)
 \end{aligned}$$



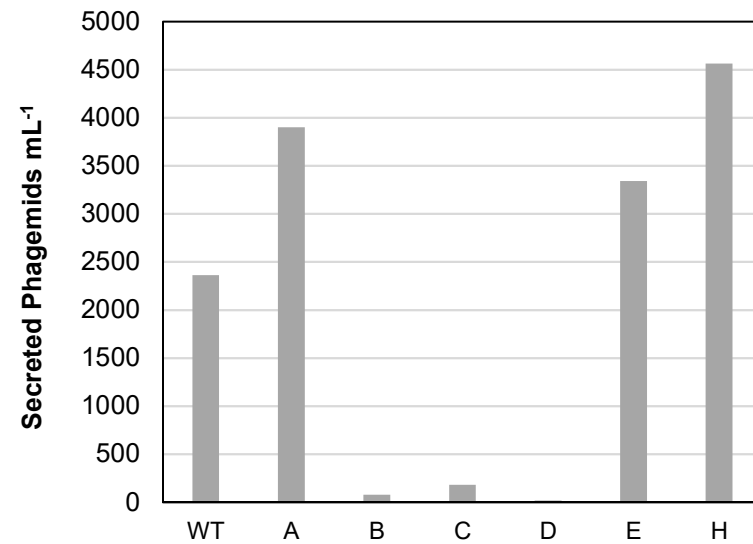
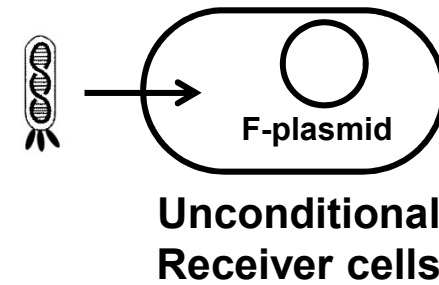
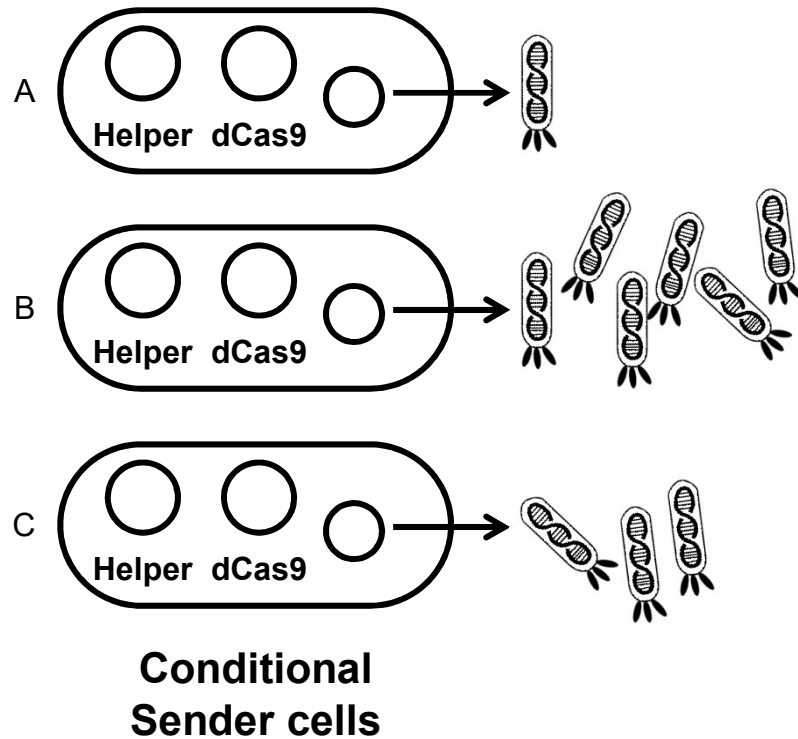
- de Morgan's rule allows re-coding of AND-AND-NOT logic
- Two internal gRNAs (X and Y) were used as inverters (NOT gates)
- The AND-AND-NOT gate was implemented using a gRNA-only strategy

## Conditional amplification of phagemid signals



- A conditional amplification system is designed to prevent packaging of some phagemids, while allowing packaging for others

# Conditional amplification of phagemid signals



- Conditional Senders can select which phagemids to package for secretion

## Next Steps

- 1) Phagemids B, C and D will be tested for Conditional amplification
- 2) Cell-to-cell signaling cascades will be tested for multiple layers
- 3) The cascades will be coupled with metabolic pathways to optimize production



# Acknowledgments



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