Exploitation of bacterial communication systems in synthetic biology

Giordano Rampioni

Department of Science, University Roma Tre

La Sapienza University – May 24th 2024

From the asocial existance of bacteria to socio-microbiology

The asocial existence of the bacterial cell has been a major paradigm in microbiology. In the 300 years since van Leeuwenhoek's descriptions of the microbial world, bacteria have been regarded as deaf-mute individual cells designed to proliferate but unable to communicate and interact with each other.

"It is perfectly possible to imagine a rather boring universe without sex, without hormones and without nervous system; a universe peopled only by individual cells reproducing ad infinitum. This universe, in fact, exists. It is the one formed by a colture of bacteria."

François Jacob, 1973 – Nobel Laureate for Medicine in 1965.

From the asocial existance of bacteria to socio-microbiology

Actually, in 1965, Alexander Tomasz reported that the ability of a Streptococcus pneumoniae population to acquire exogenous DNA, i.e. the entry into the competent state, is governed by an extracellular factor that is manufactured by Streptococcus itself. This competence factor, which was later shown to be a modified peptide, was described as a "hormone-like activator" that synchronizes the behaviour of the bacterial population.

"Since the activator - a cell-produced chemical - seems to impose a high degree of physiological homogeneity in a pneumococcal population with respect to competence, one is forced to conclude that in this case a bacterial population can behave as a biological unit with considerable coordination among its members. One wonders whether this kind of control may not be operative in some other microbial phenomena also."

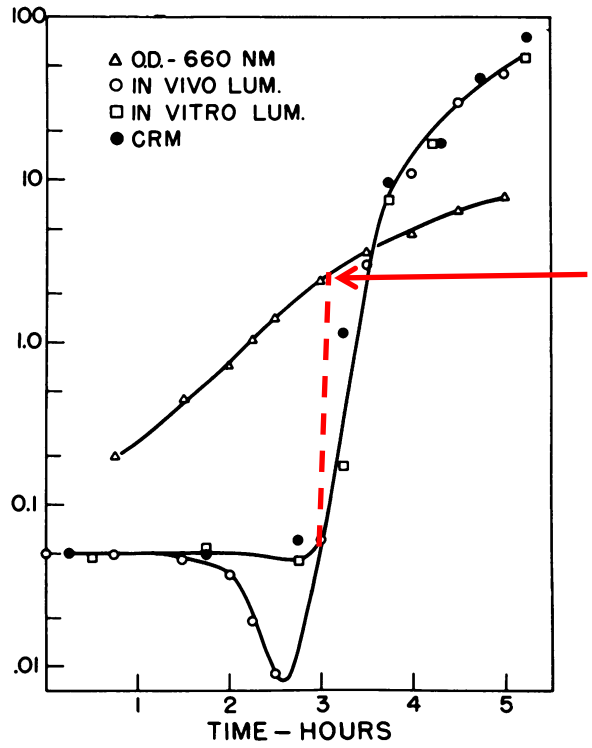
Alexander Tomasz

Tomasz A (1965) *Nature* 208:155-159.

From the asocial existance of bacteria to socio-microbiology

Five years later, Hastings and co-workers noticed that light production in the bioluminescent marine bacterium *Allivibrio fischeri* (previously known as *Vibrio fischeri*) occurred at high cell density but not in diluted bacterial cultures.
Light production could be induced at low cell density by the exogenous provision of cell-free supernatants from bacterial cultures grown to high cell density.

culture of *A. fischeri*



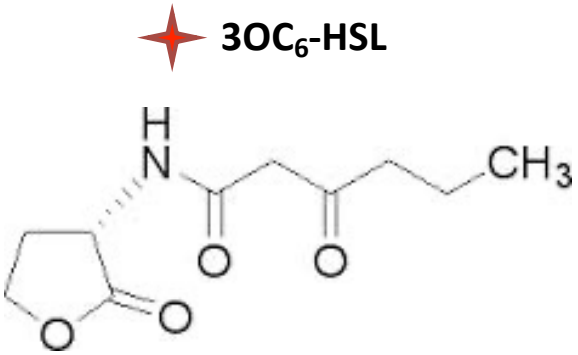
Bioluminescence is emitted only at high cell density

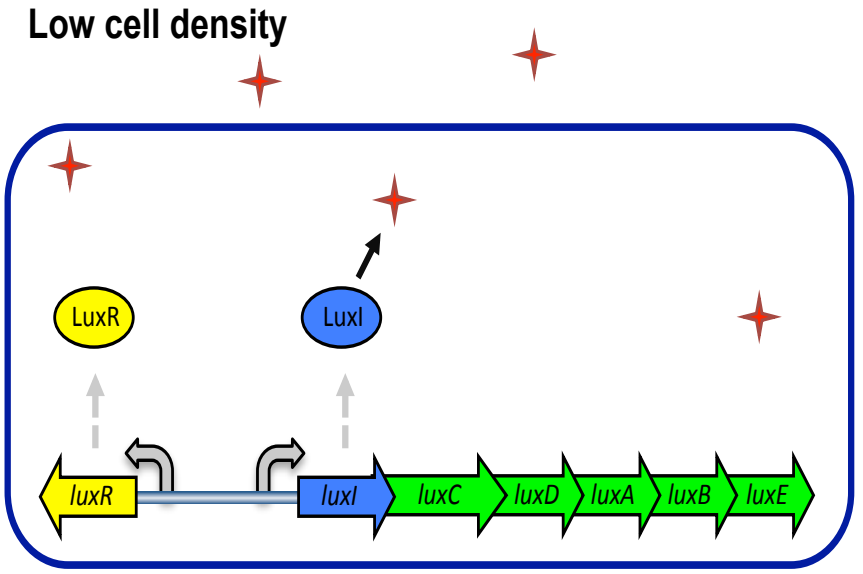
Nealson KH *et al*. (1970) *J Bacteriol* 104:313-322.

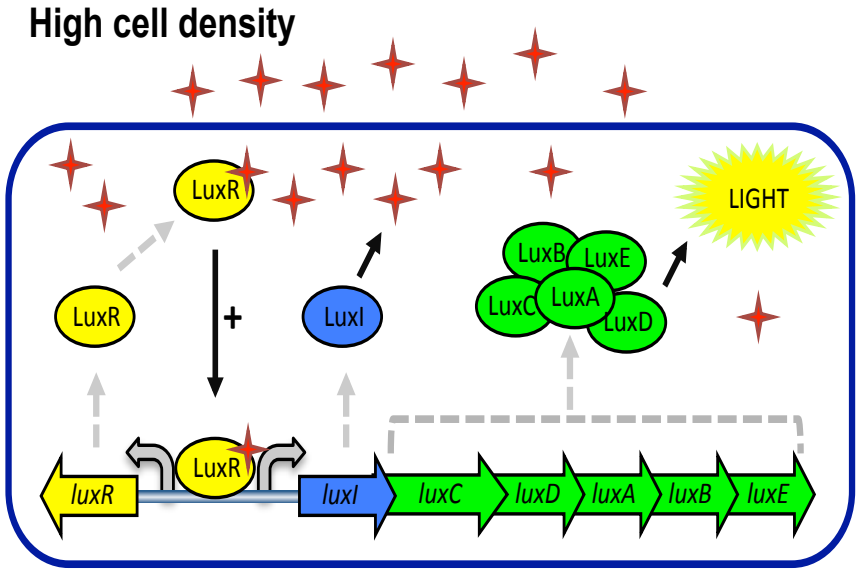
From the asocial existance of bacteria to socio-microbiology

Light emission in controlled by a signal molecule produced by the single cells and released in the environment.

At a certain concentration, corresponding to the *quorum* cell density, the signal molecule binds to and activates a transcriptional regulator, that in turn activates the expression of genes required for light emission.





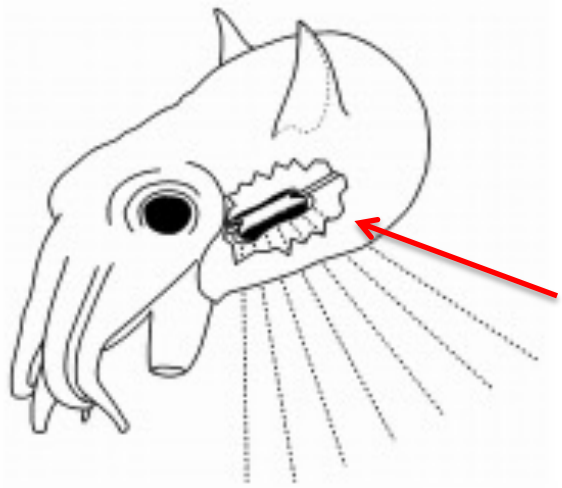


From the asocial existance of bacteria to socio-microbiology

A. fischeri is a marine bacterium that colonizes the light organ of the squid *Euprymna scolopes*, an ecological niche rich in nutrients that allows the growth of the bacterial population to high cell density.

The emission of light by *A. fischeri* is exploited by the squid to mask its shadow when hunting at night, allowing it to escape predation by animals living on the seabed.

***Euprymna* scolopes**

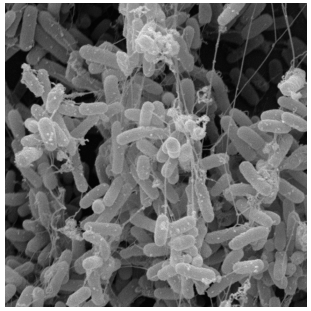


Light organ colonized by *A. fischeri*

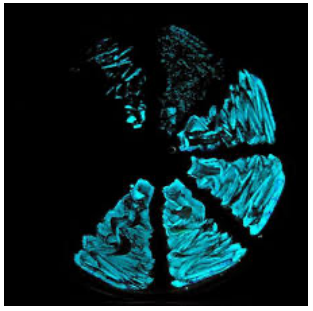
From the asocial existance of bacteria to socio-microbiology

This intercellular communication system, know as **quorum sensing (QS)**, controls group-behaviours in many bacteria.

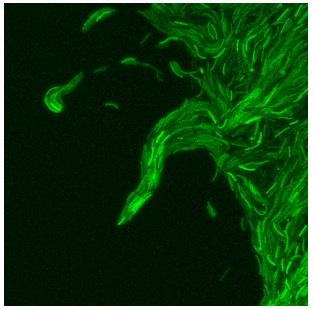
Biofilm

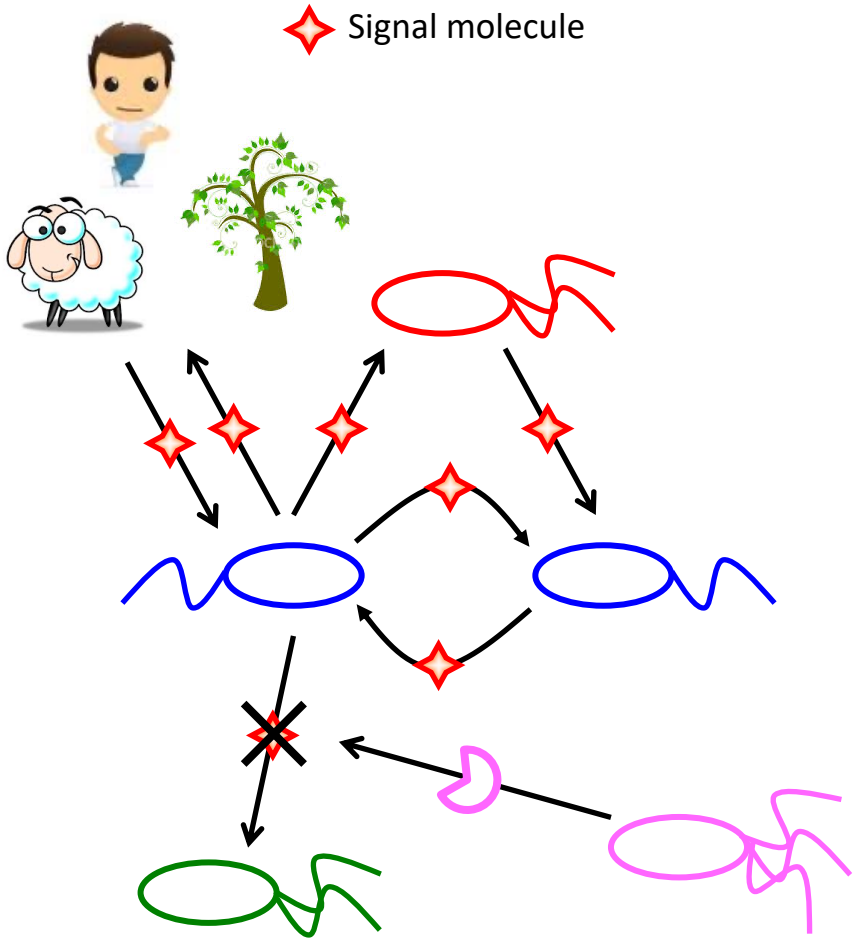


Bioluminescence

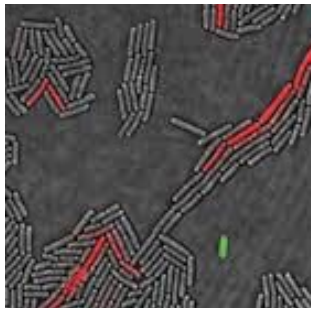


Collective movements



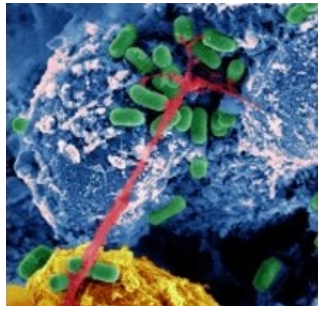


Differentiation



Secondary metabolites

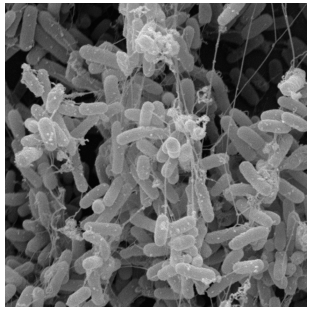
Interaction with the host



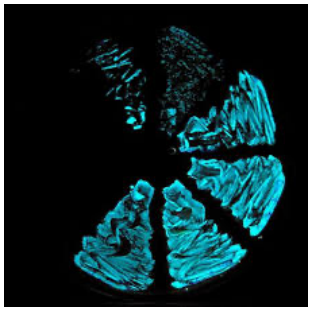
From the asocial existance of bacteria to socio-microbiology

This intercellular communication system, know as **quorum sensing (QS)**, controls group-behaviours in many bacteria.

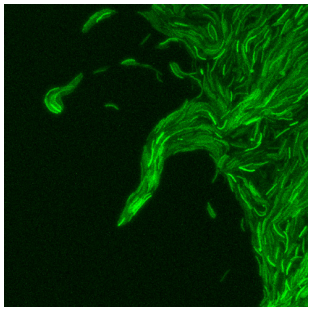
Biofilm

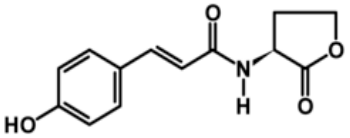


Bioluminescence

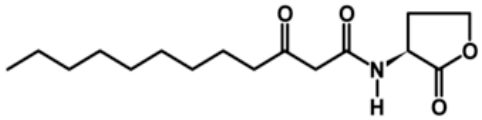


Collective movements

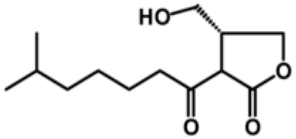




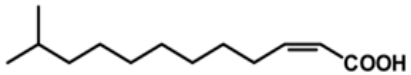
aroyl homoserine lactone
*p*C-HSL (*R. palustris*)



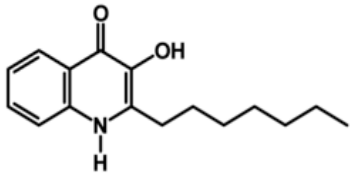
acylated homoserine lactone
3OC12-HSL (*P. aeruginosa*)



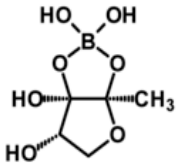
γ-butyrolactone
A-factor (*S. griseus*)



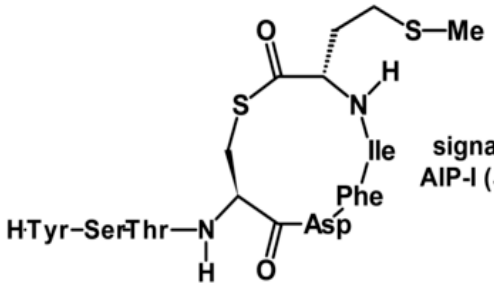
fatty acid derivative
DSF (*X. campestris*)



2-alkyl-4-quinolone
PQS (*P. aeruginosa*)

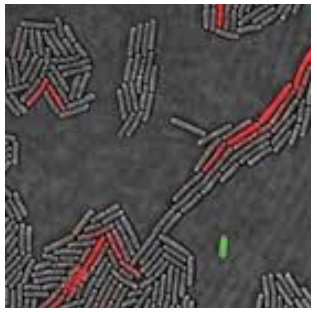


furanone
AI-2 (*V. harveyi*)



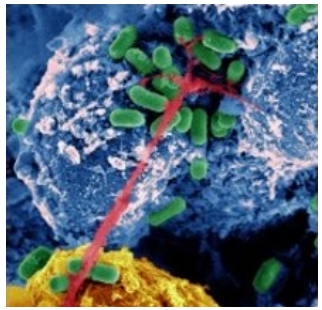
signal peptide
AIP-I (*S. aureus*)

Differentiation



Secondary metabolites

Interaction with the host



Exploitation of QS in synthetic biology

The study of QS elucidates the mechanisms controlling collective behaviours and the evolution of social traits in individual cells.

In the last decade QS has been exploited for many biotechnological applications, including the development of biosensors and new therapeutic approaches.

In some cases, the engineering of bacterial cells with heterologous QS systems follows the principles of synthetic biology.

1) Engineering of non-pathogenic cells to sense and kill bacterial pathogens
2) Generation of new antitumor agents
3) Construction of new whole-cell biosensors
4) Generation of synthetic cells able to interface with natural cells

The principles of synthetic biology

Molecular Systems Biology (2006) doi:10.1038/msb4100073

www.molecularsystemsbiology.com
Article number: 2006.0028

molecular systems biology

Synthetic biology: new engineering rules for an emerging discipline

Ernesto Andrianantoandro[1,3], Subhayu Basu[1,3], David K Karig[1,3] and Ron Weiss[1,2,*]

As early as 2006, synthetic biology was proposed as a new discipline which aims to apply engineering approaches and methods to design and implement new bio-inspired components, systems, and organisms that do not exist in nature.

The difference between genetic engineering and synthetic biology is not the aim (which may be the same), but the approach used to achieve it.

Key principles of synthetic biology are **standardization** of parts, **modularity** in their assembly, and **orthogonality** of processes. As in engineering fields, synthetic biology often makes use of ***in silico* modelling**.

Standardization and modularity

Standardization and modularity are necessary to be able to get to the point of generating complex artificial systems in an engineering-like design mode.

Could you build a skyscraper or aircraft carrier with screws, bolts and beams all of different sizes and whose functional properties you do not know?

Genetic engineering

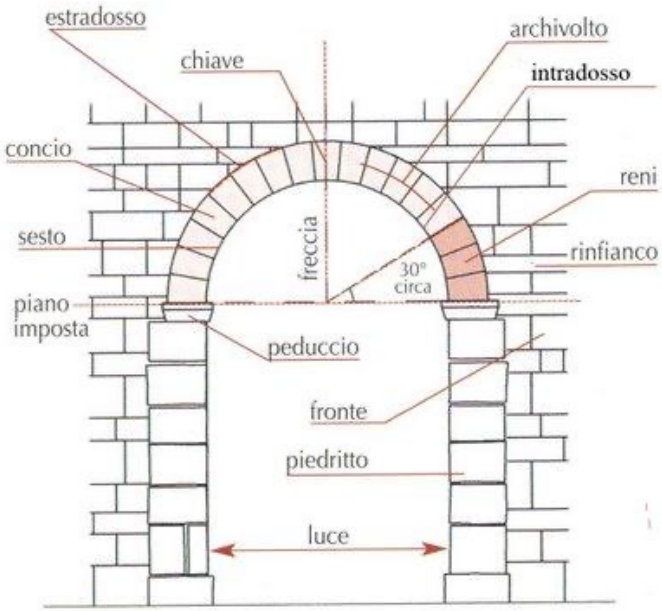
Synthetic biology

Standardization and modularity

Standardization and modularity are necessary to be able to get to the point of generating complex artificial systems in an engineering-like design mode.

Could you build a skyscraper or aircraft carrier with screws, bolts and beams all of different sizes and whose functional properties you do not know?

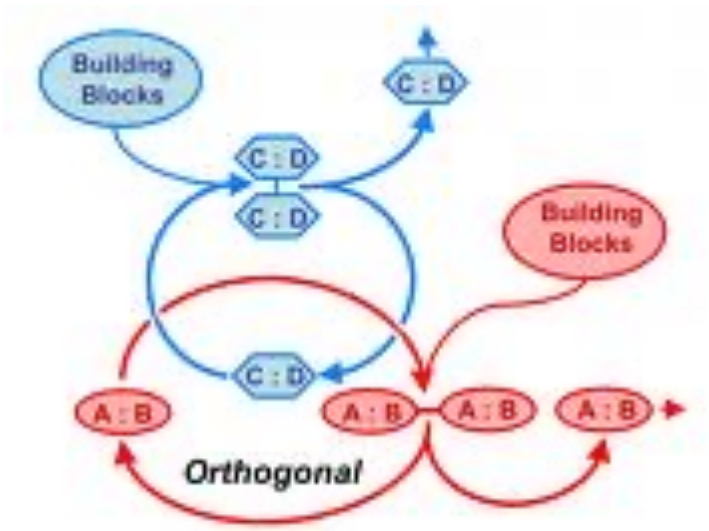
Standard part: Roman arch

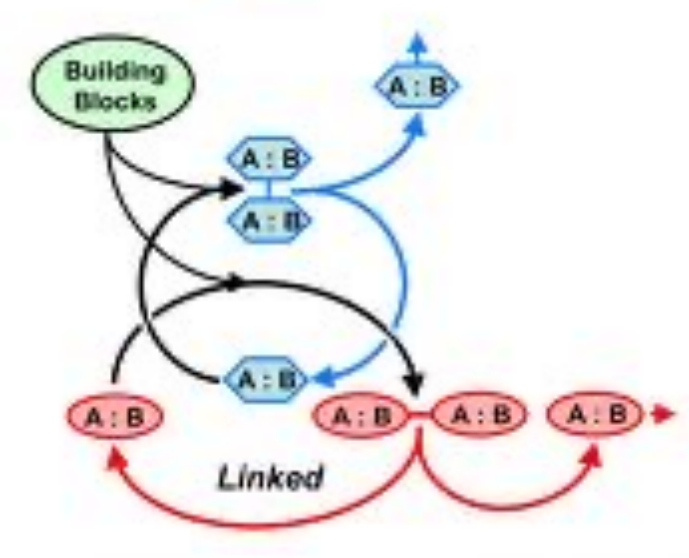


Modular assembly: Roman aqueduct

Orthogonality

Orthogonality, or lack of interaction between various processes, is necessary in order to achieve a controllable and predictable process. What would happen if your process altered other processes that are related to it? Could it in turn be affected by them? How can we predict the performance of a process that interacts with other processes? When interactions become multiple and reciprocal, the system can become chaotic.

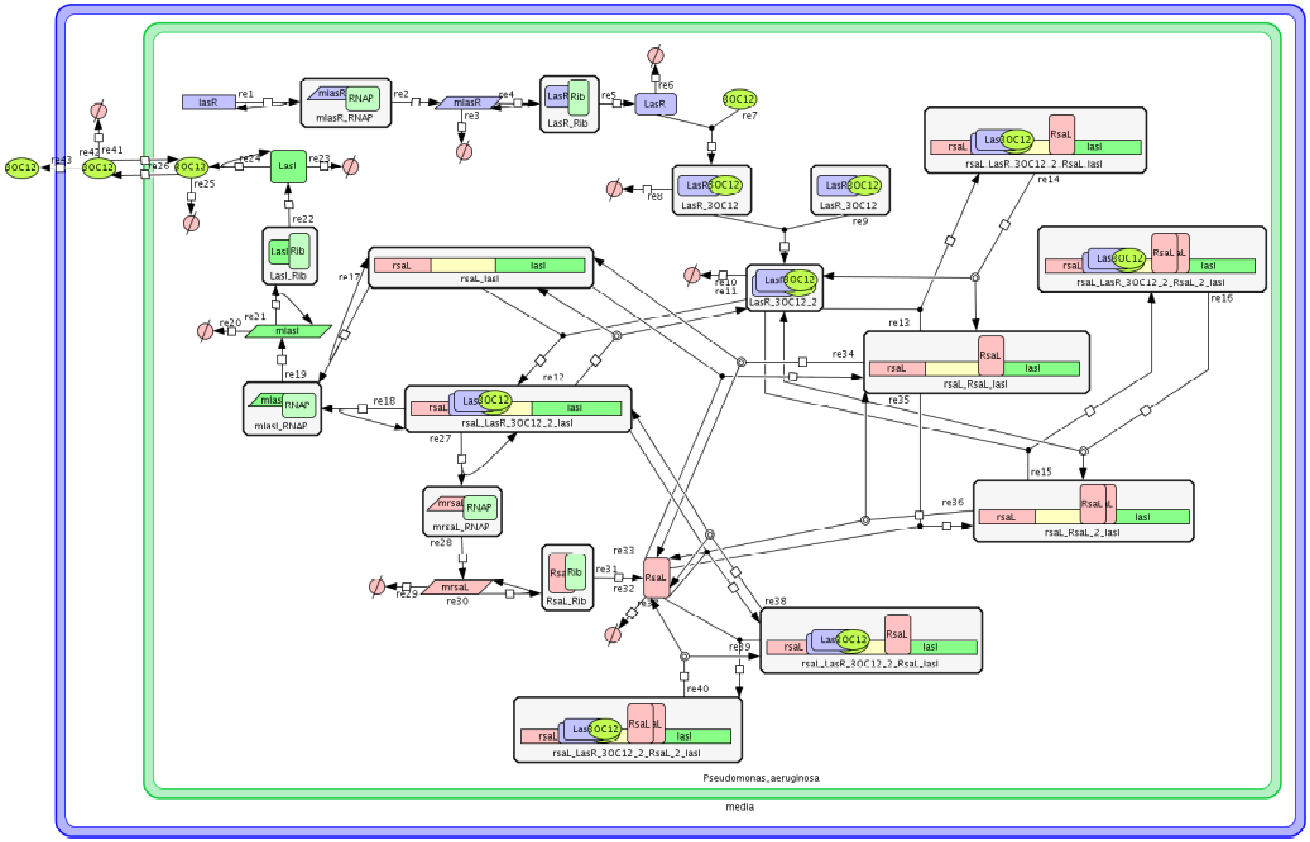


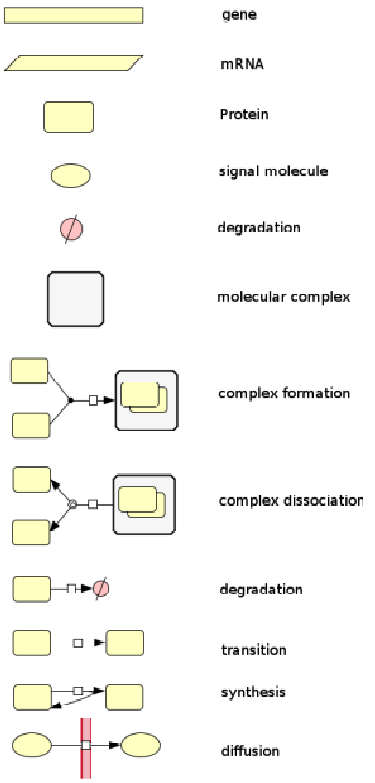


It is important to consider in what cellular background you want to put a synthetic genetic circuit so that it does not interfere with endogenous processes. A “chassis” must be defined!

In silico modelling

To predict the behavior of new genetic circuits generated to functionalize cells once they are placed inside a chassis, synthetic biology often makes use of computer simulations (*in silico* modelling).





Exploitation of QS in synthetic biology

The study of QS elucidates the mechanisms controlling collective behaviours and the evolution of social traits in individual cells.

In the last decade QS has been exploited for many biotechnological applications, including the development of biosensors and new therapeutic approaches.

In some cases, the engineering of bacterial cells with heterologous QS systems follows the principles of synthetic biology.

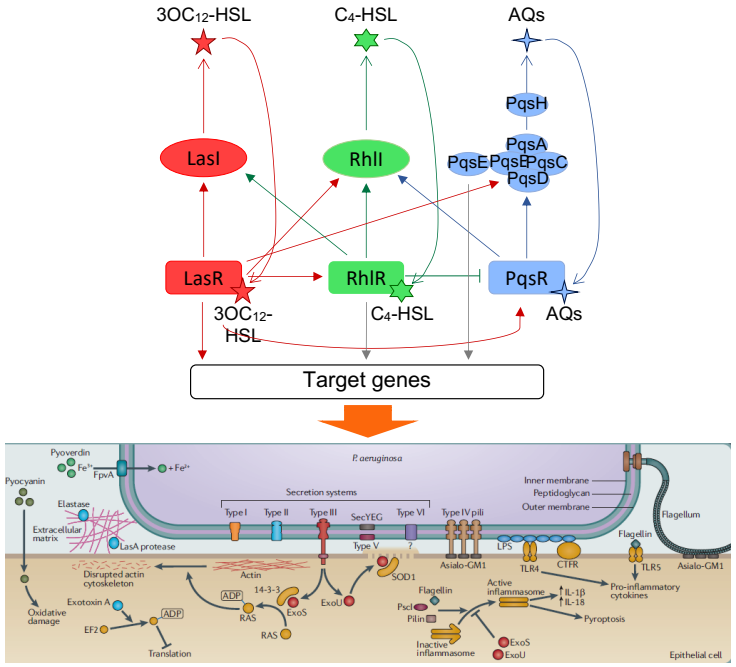
1) Engineering of non-pathogenic cells to sense and kill bacterial pathogens
2) Generation of new antitumor agents
3) Construction of new whole-cell biosensors
4) Generation of synthetic cells able to interface with natural cells

Many bacterial pathogens control the expression of virulence traits *via* QS systems

During certain bacterial infections, the level of QS signal molecules correlates with clinical status.

As an example, the QS signal molecules alkyl-quinolones (AQs) produced by *Pseudomonas aeruginosa* are detectable in sputum, blood and urine of ca. 80% of cystic fibrosis (CF) patients suffering with *P. aeruginosa* chronic lung infections.

Levels of the AQ molecule NHQ increased at the start of a pulmonary exacerbation and positively correlated with quantitative measures of *P. aeruginosa* cells in the lung.



Machan *et al*. (1992) *J Antimicrob Chemother* 30:615-623; Collier *et al.* (2002) *FEMS Microbiol Lett* 215:41-46; Barr HL *et al*. (2015) *Eur Respir J* 46:1046-1054

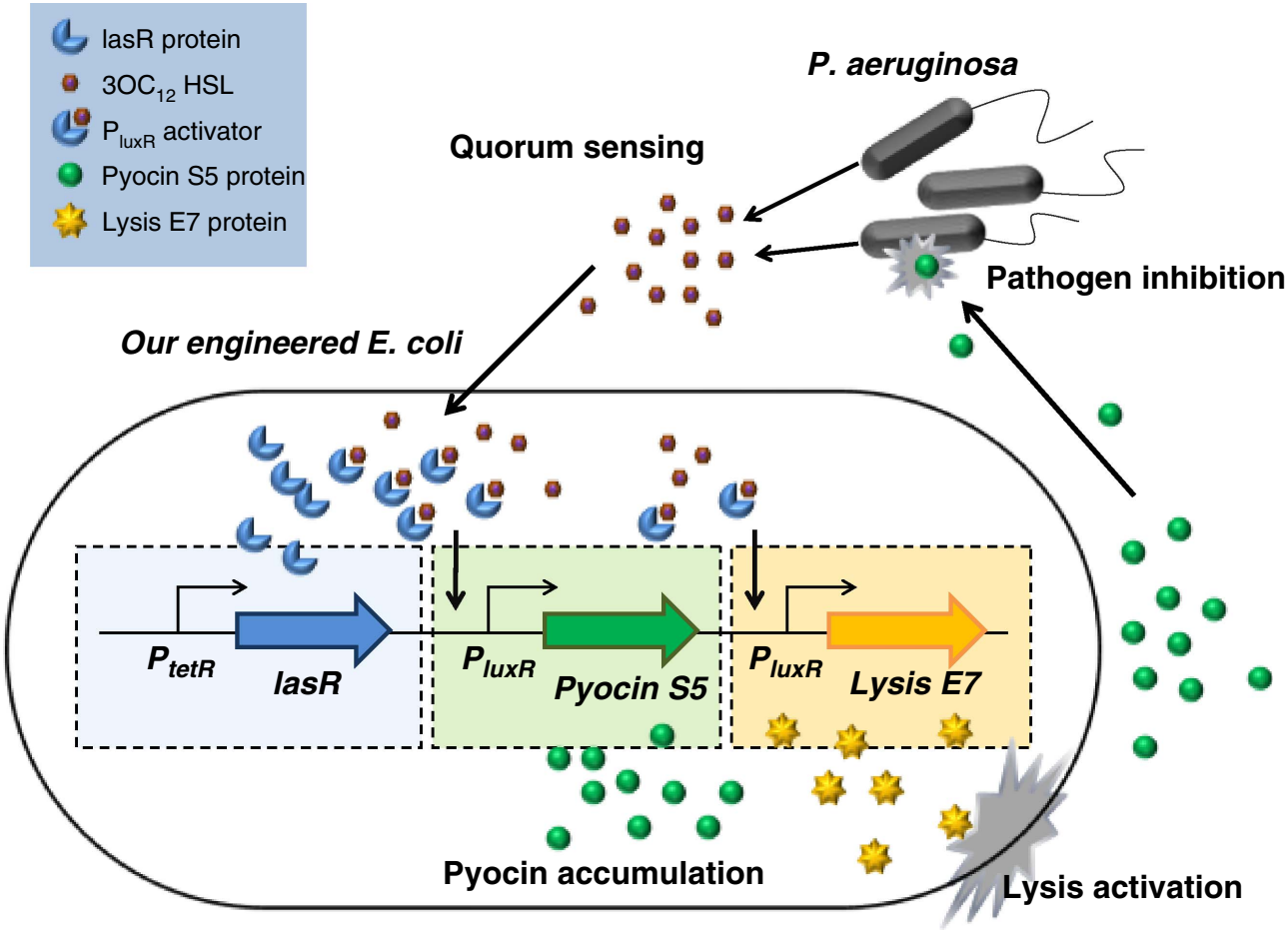
Molecular Systems Biology 7; Article number 521; doi:10.1038/msb.2011.55
Citation: *Molecular Systems Biology* 7:521

www.molecularsystemsbiology.com

molecular systems biology

Engineering microbes to sense and eradicate *Pseudomonas aeruginosa*, a human pathogen

Nazanin Saeidi[1], Choon Kit Wong[1], Tat-Ming Lo, Hung Xuan Nguyen[2], Hua Ling, Susanna Su Jan Leong, Chueh Loo Poh* and Matthew Wook Chang*



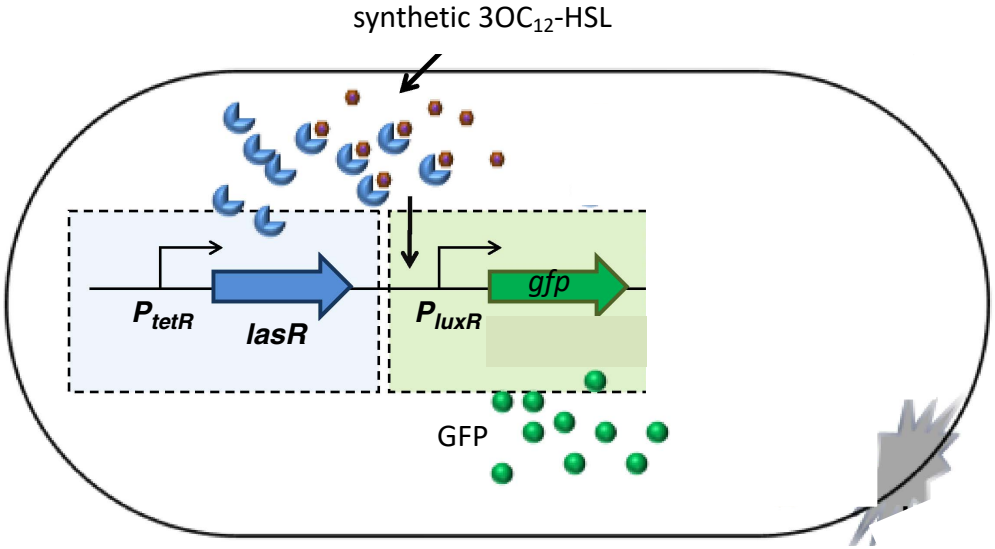
Molecular Systems Biology 7; Article number 521; doi:10.1038/msb.2011.55
Citation: *Molecular Systems Biology* 7:521

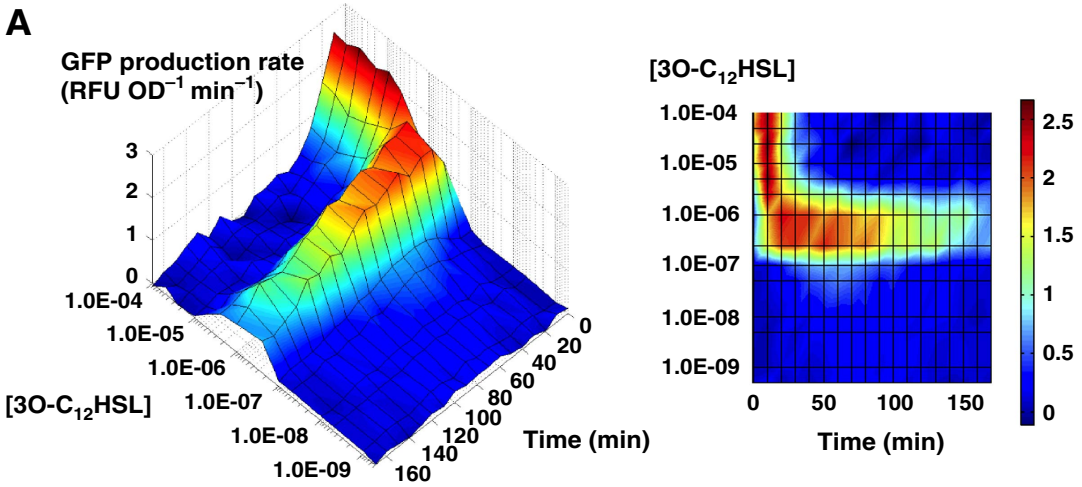
www.molecularsystemsbiology.com

molecular systems biology

Engineering microbes to sense and eradicate *Pseudomonas aeruginosa*, a human pathogen

Nazanin Saeidi[1], Choon Kit Wong[1], Tat-Ming Lo, Hung Xuan Nguyen[2], Hua Ling, Susanna Su Jan Leong, Chueh Loo Poh* and Matthew Wook Chang*





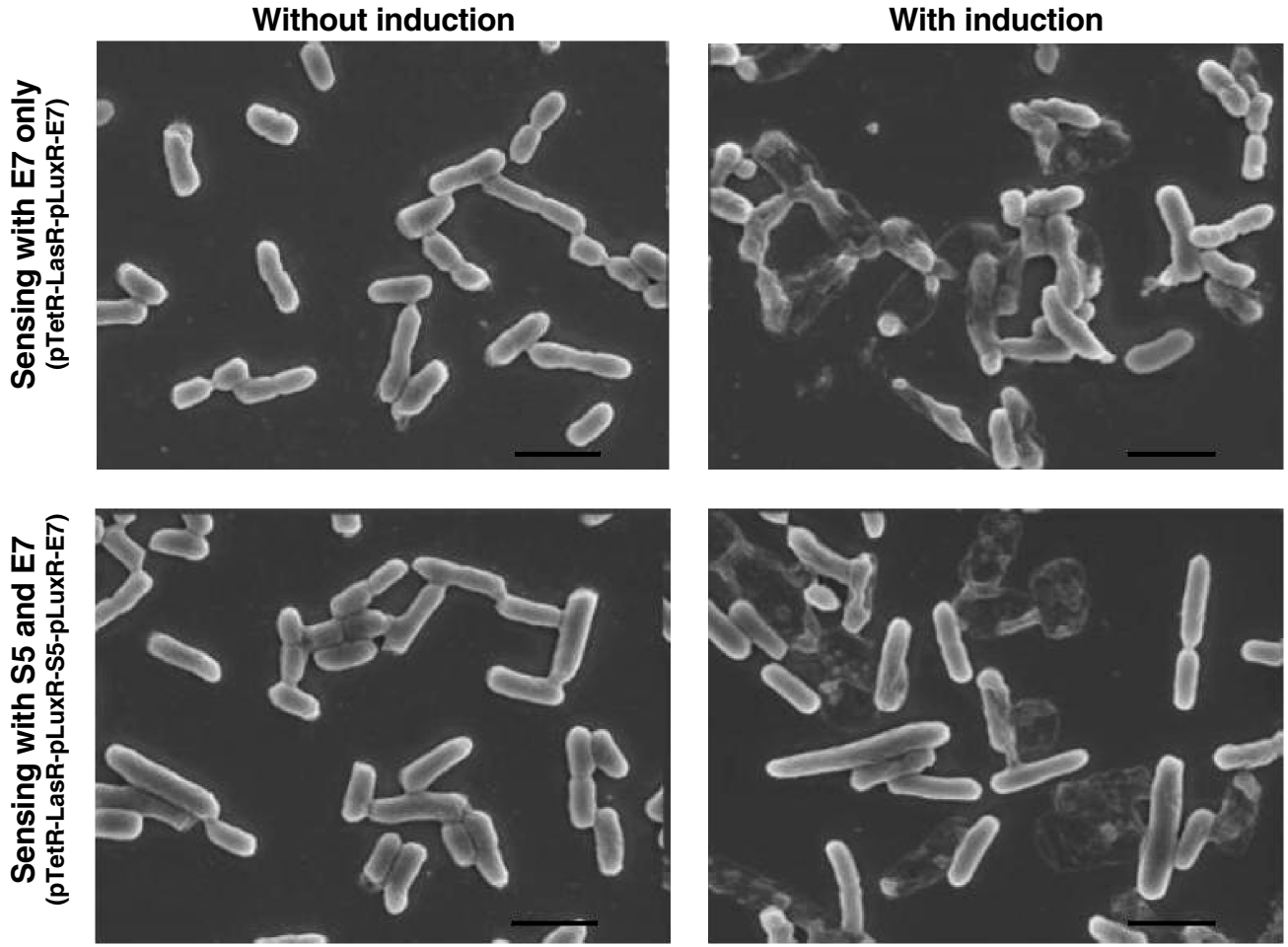
Molecular Systems Biology 7; Article number 521; doi:10.1038/msb.2011.55
Citation: ***Molecular Systems Biology* 7:521**

www.molecularsystemsbiology.com

molecular systems biology

Engineering microbes to sense and eradicate *Pseudomonas aeruginosa*, a human pathogen

Nazanin Saeidi[1], Choon Kit Wong[1], Tat-Ming Lo, Hung Xuan Nguyen[2], Hua Ling, Susanna Su Jan Leong, Chueh Loo Poh* and Matthew Wook Chang*



Molecular Systems Biology 7; Article number 521; doi:10.1038/msb.2011.55
Citation: *Molecular Systems Biology* 7:521

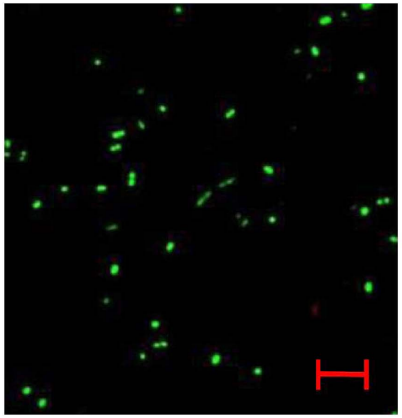
www.molecularsystemsbiology.com

molecular systems biology

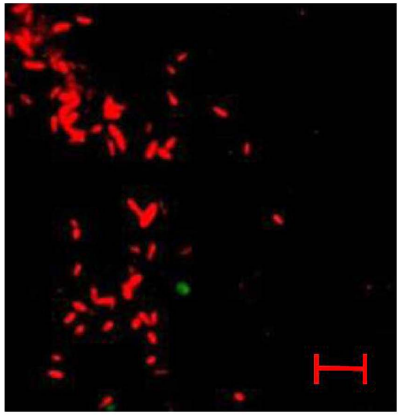
Engineering microbes to sense and eradicate *Pseudomonas aeruginosa*, a human pathogen

Nazanin Saeidi[1], Choon Kit Wong[1], Tat-Ming Lo, Hung Xuan Nguyen[2], Hua Ling, Susanna Su Jan Leong, Chueh Loo Poh* and Matthew Wook Chang*

Exposed to supernatant of wild-type *E. coli*



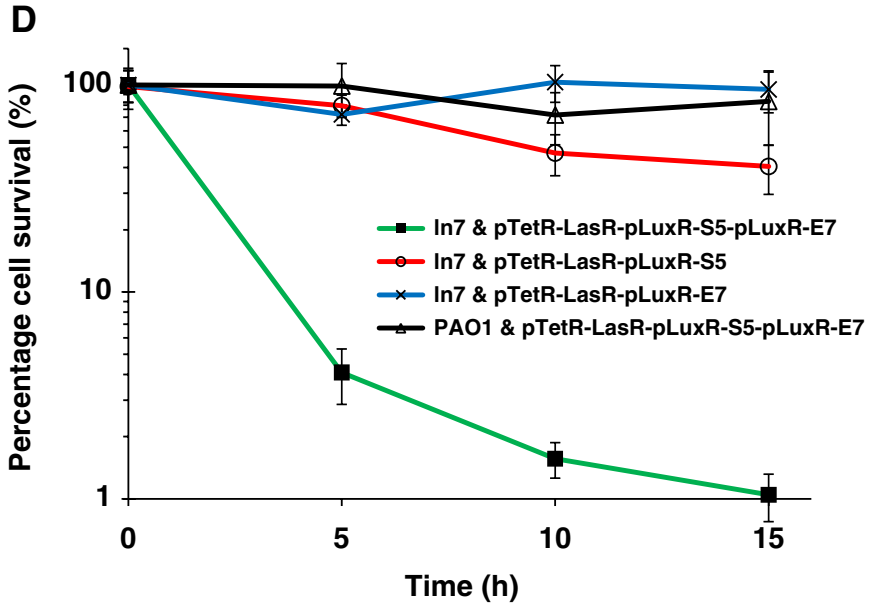
Exposed to supernatant of engineered *E. coli* induced with native $3OC_{12}HSL$



This engineered bacterium can be also considered as an intelligent drug delivery vehicle

P. aeruginosa cells imaged with LIVE/DEAD staining.

The engineered *E. coli* strain can detect and kill *P. aeruginosa*.



The same approach can be used to engineer probiotics

ACS Synthetic Biology

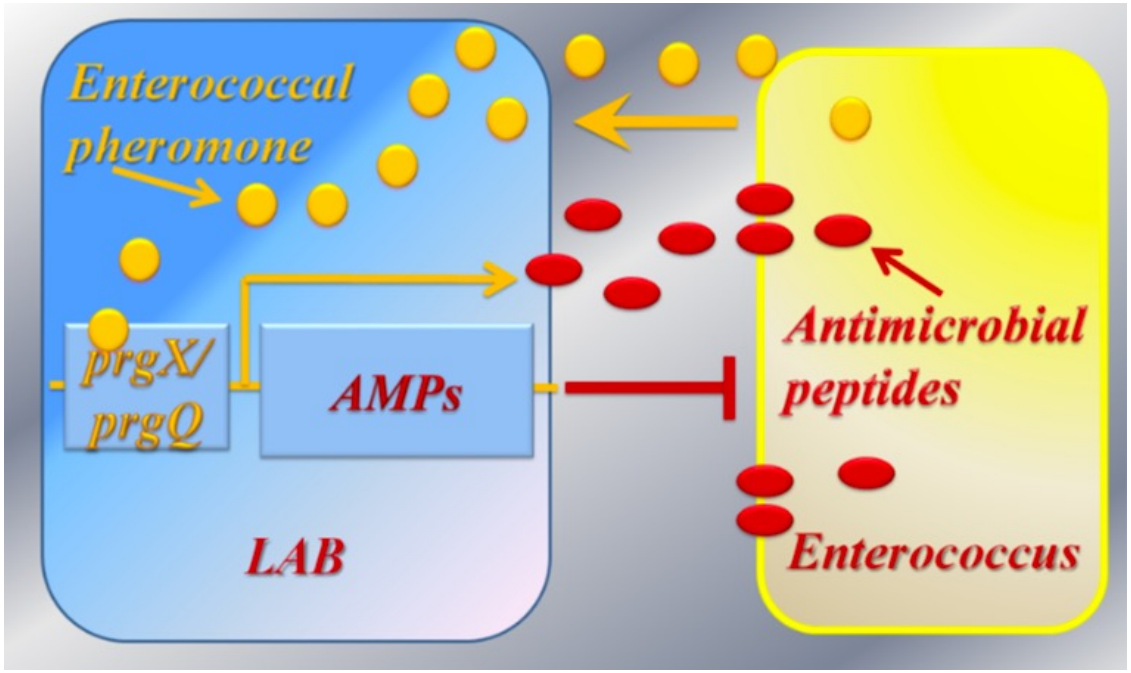
Research Article

pubs.acs.org/synthbio

Modified Lactic Acid Bacteria Detect and Inhibit Multiresistant Enterococci

Juan Borrero,[†] Yuqing Chen,[‡] Gary M. Dunny,[‡] and Yiannis N. Kaznessis*[,†]

[†]Department of Chemical Engineering and Materials Science, [‡]Department of Microbiology, University of Minnesota, Minneapolis, Minnesota 55455, United States



Exploitation of QS in synthetic biology

The study of QS elucidates the mechanisms controlling collective behaviours and the evolution of social traits in individual cells.

In the last decade QS has been exploited for many biotechnological applications, including the development of biosensors and new therapeutic approaches.

In some cases, the engineering of bacterial cells with heterologous QS systems follows the principles of synthetic biology.

1) Engineering of non-pathogenic cells to sense and kill bacterial pathogens
2) Generation of new antitumor agents
3) Construction of new whole-cell biosensors
4) Generation of synthetic cells able to interface with natural cells

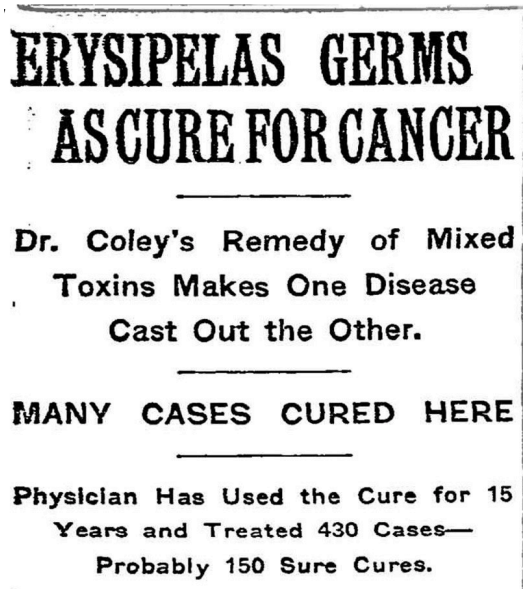
Bacteria are promising anti-tumour agents

In 1868 Karl David Wilhelm Busch intentionally provoked erysipelas infection in a young girl with a big solid tumour on the neck. The tumour mass significantly decreased in few days.

In 1882 Friedrich Fehleisen isolated the etiological agent of erysipelas, *Streptococcus pyogenes.* He injected *S. pyogenes* in 7 patients with solid tumours and described complete tumour regression in 3 patients.

In 1893 William Bradley Coley described the anti-tumour effect of the "Coley toxic", an injectable medication based on filtered *S. pyogenes* e *Serratia marcescens* cultures.

In 1936 Coley published a manuscript reporting complete regression of solid tumours in hundreds of patients treated with the "Coley toxic".



ERYSIPELAS GERMS AS CURE FOR CANCER

Dr. Coley's Remedy of Mixed Toxins Makes One Disease Cast Out the Other.

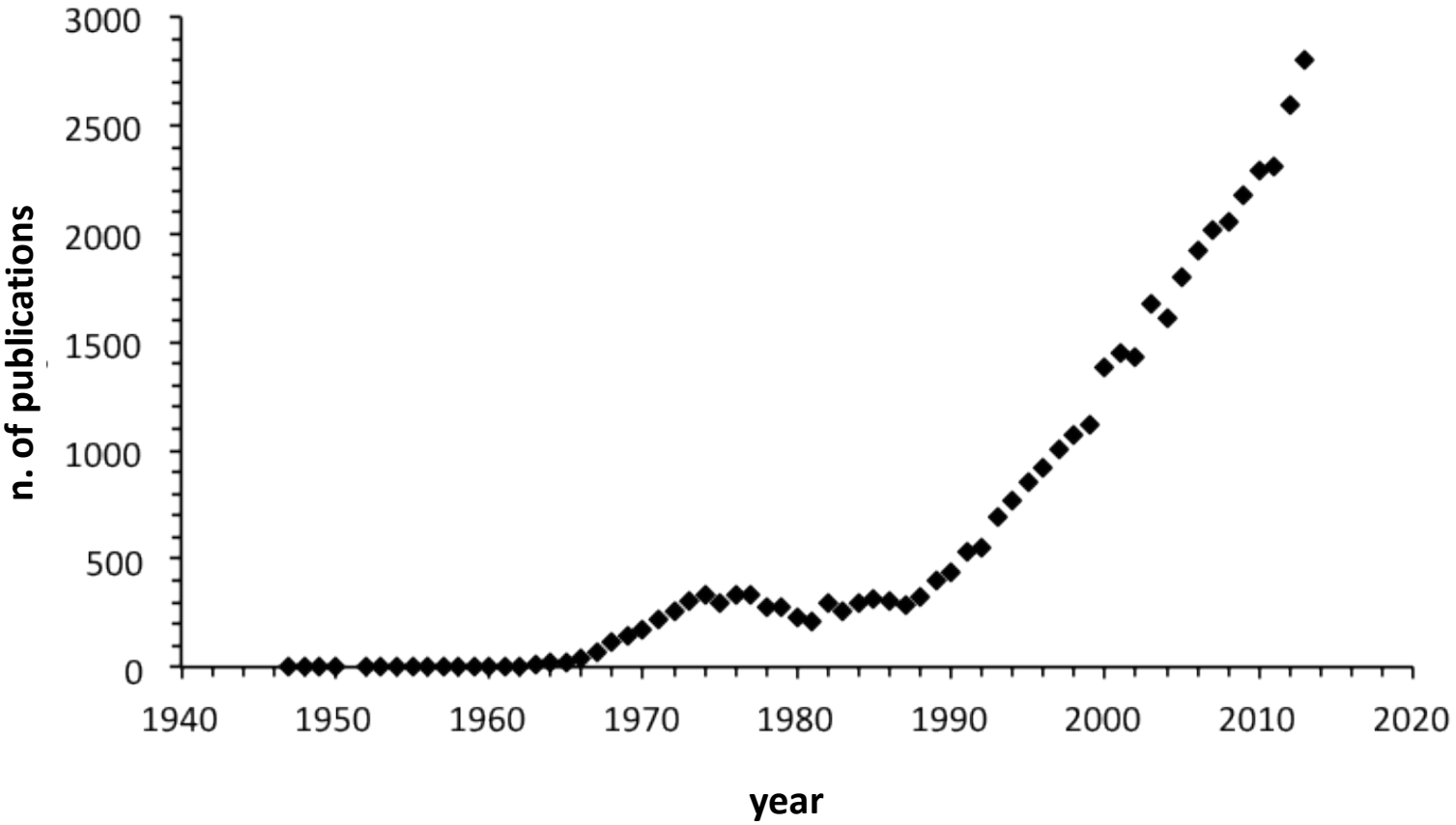
MANY CASES CURED HERE

Physician Has Used the Cure for 15 Years and Treated 430 Cases— Probably 150 Sure Cures.

New York Times, July 29th, 1908

Bacteria are promising anti-tumour agents

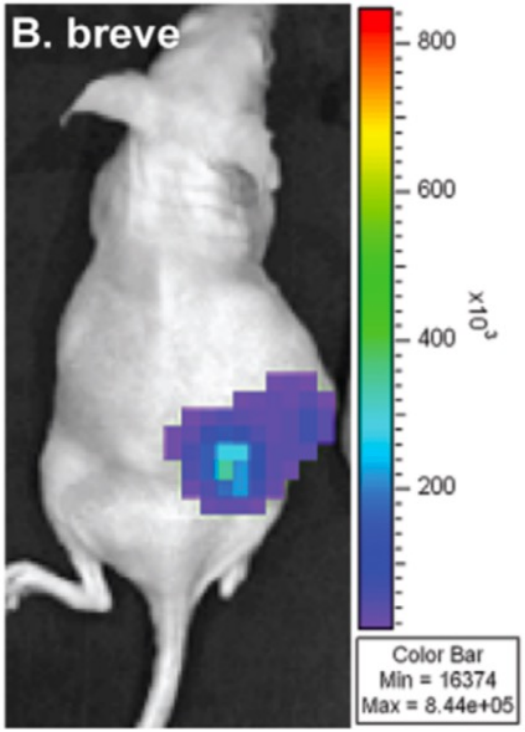
Scientific manuscripts retrieved in Pubmed (www.ncbi.nlm.nih.gov/pubmed) with the query "*bacteria AND tumour AND therapy*".

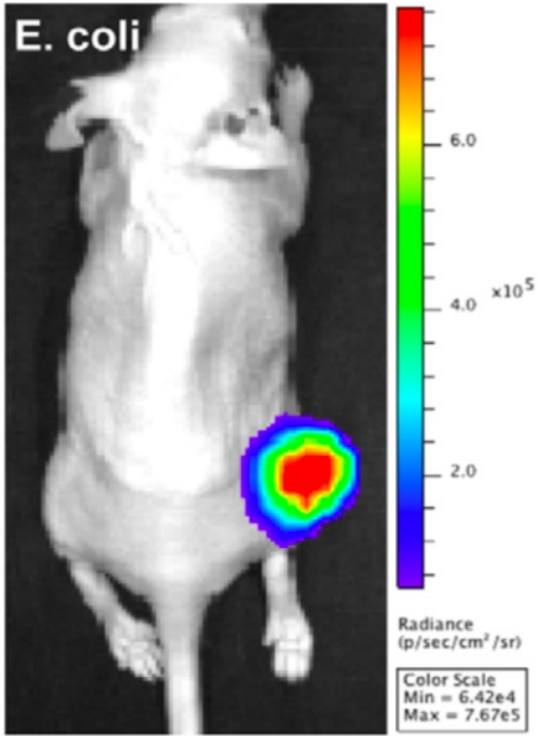


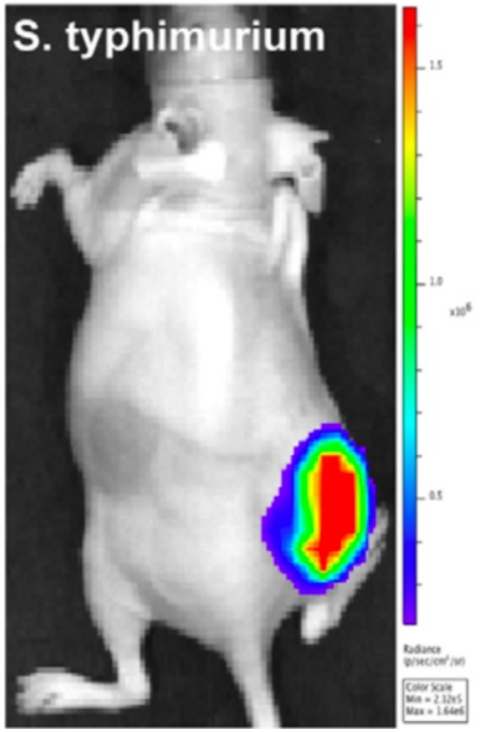
High Resolution *In Vivo* Bioluminescent Imaging for the Study of Bacterial Tumour Targeting

Michelle Cronin[1], Ali R. Akin[2], Sara A. Collins[1,3], Jeff Meganck[2], Jae-Beom Kim[2], Chwanrow K. Baban[1], Susan A. Joyce[4], Gooitzen M. van Dam[5], Ning Zhang[2], Douwe van Sinderen[4], Gerald C. O'Sullivan[1], Noriyuki Kasahara[3], Cormac G. Gahan[4,6], Kevin P. Francis[2], Mark Tangney[1,3]*

Many genera of bacteria have been shown to preferentially accumulate in tumours, including *Salmonella*, *Escherichia*, *Clostridium* and *Bifidobacterium*. Bacteria administered by tail vein injection co-localize with solid tumours.



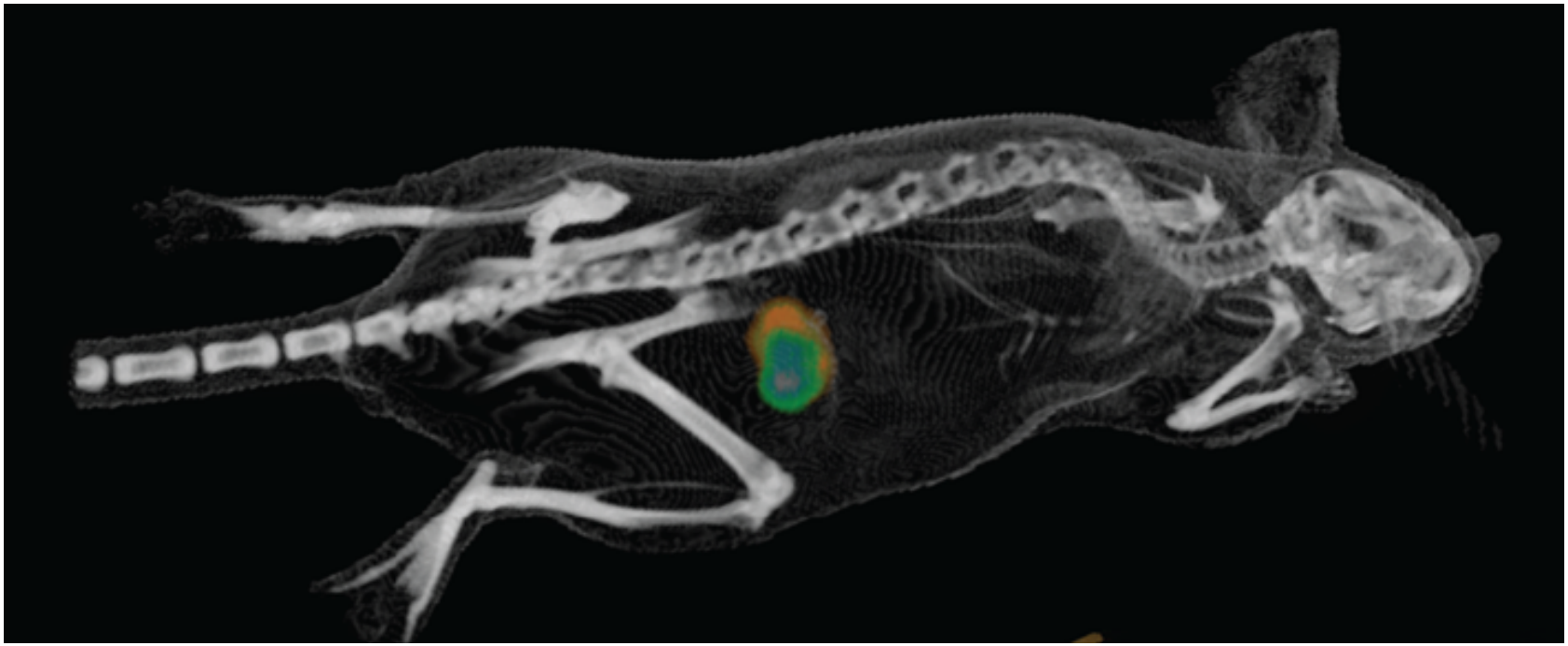




High Resolution *In Vivo* Bioluminescent Imaging for the Study of Bacterial Tumour Targeting

Michelle Cronin[1], Ali R. Akin[2], Sara A. Collins[1,3], Jeff Meganck[2], Jae-Beom Kim[2], Chwanrow K. Baban[1], Susan A. Joyce[4], Gooitzen M. van Dam[5], Ning Zhang[2], Douwe van Sinderen[4], Gerald C. O'Sullivan[1], Noriyuki Kasahara[3], Cormac G. Gahan[4,6], Kevin P. Francis[2], Mark Tangney[1,3]*

Many genera of bacteria have been shown to preferentially accumulate in tumours, including *Salmonella*, *Escherichia*, *Clostridium* and *Bifidobacterium*. Bacteria administered by tail vein injection co-localize with solid tumours.

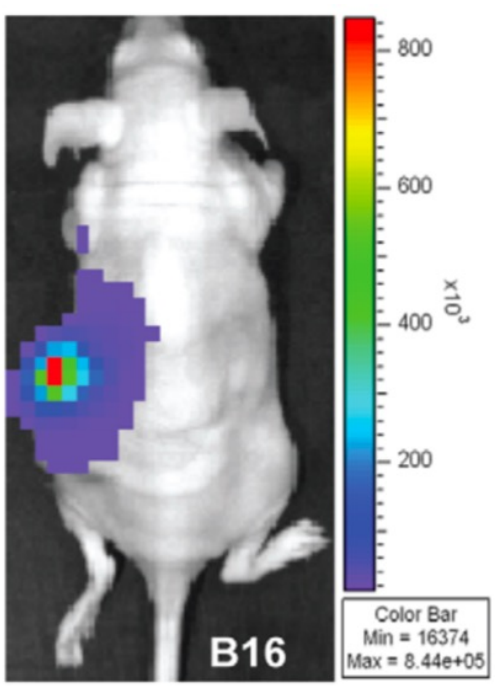


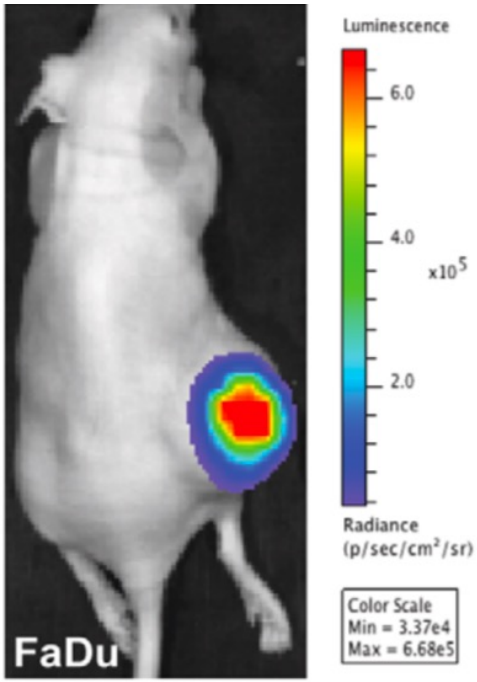
High Resolution *In Vivo* Bioluminescent Imaging for the Study of Bacterial Tumour Targeting

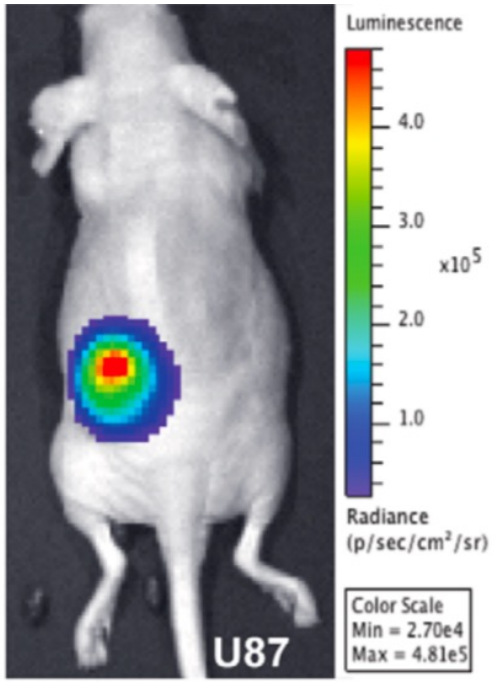
Michelle Cronin[1], Ali R. Akin[2], Sara A. Collins[1,3], Jeff Meganck[2], Jae-Beom Kim[2], Chwanrow K. Baban[1], Susan A. Joyce[4], Gooitzen M. van Dam[5], Ning Zhang[2], Douwe van Sinderen[4], Gerald C. O'Sullivan[1], Noriyuki Kasahara[3], Cormac G. Gahan[4,6], Kevin P. Francis[2], Mark Tangney[1,3]*

Bacteria co-localize with different tumour types.

e.g. E. coli MG1655 co-localization with melanoma B16, carcinoma FaDu, e glioblastoma U87.



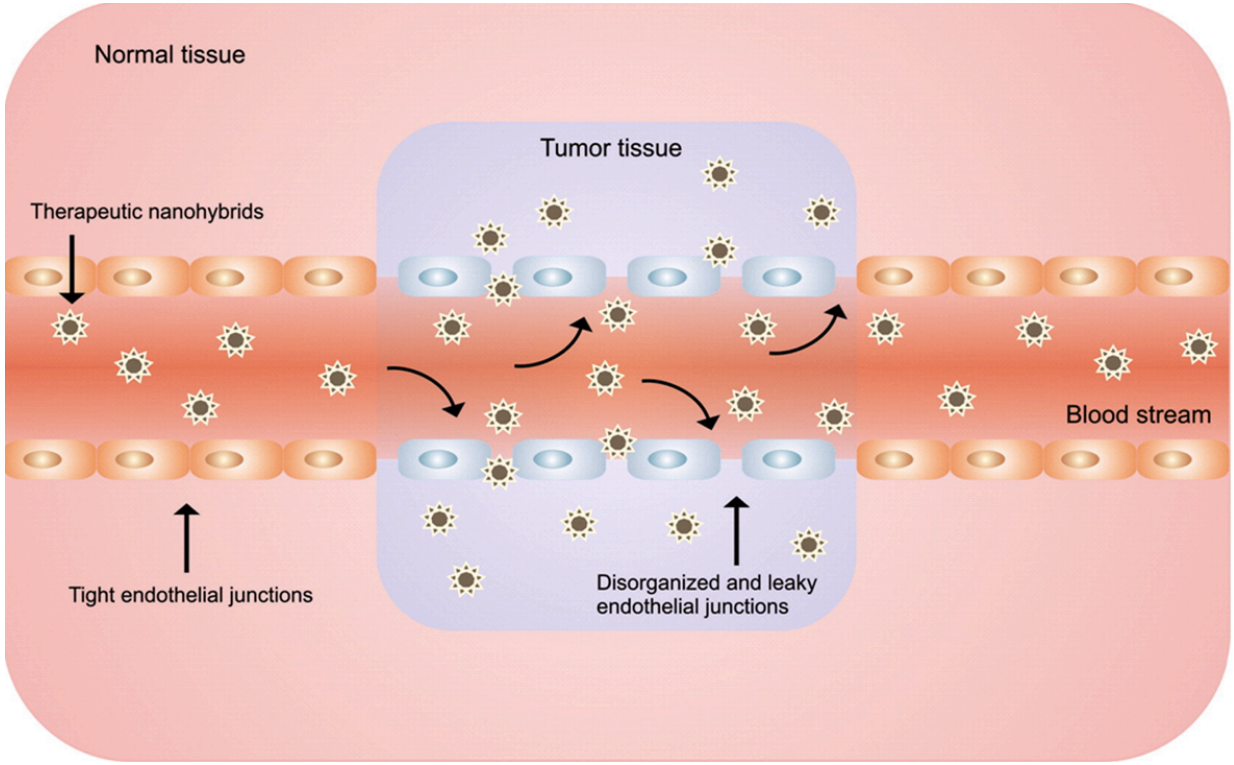




High Resolution *In Vivo* Bioluminescent Imaging for the Study of Bacterial Tumour Targeting

Michelle Cronin[1], Ali R. Akin[2], Sara A. Collins[1,3], Jeff Meganck[2], Jae-Beom Kim[2], Chwanrow K. Baban[1], Susan A. Joyce[4], Gooitzen M. van Dam[5], Ning Zhang[2], Douwe van Sinderen[4], Gerald C. O'Sullivan[1], Noriyuki Kasahara[3], Cormac G. Gahan[4,6], Kevin P. Francis[2], Mark Tangney[1,3]*

This ability is partly due to the "*enanched permeability and retention effect*".

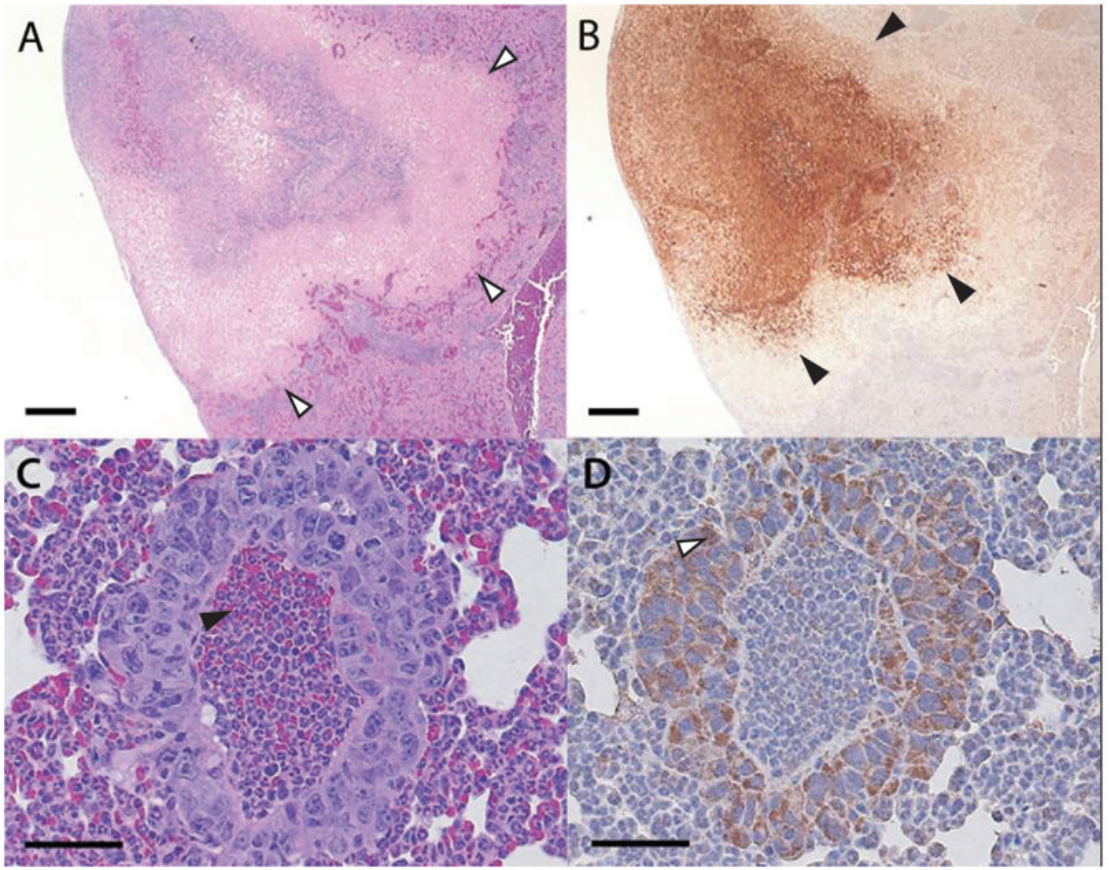


In tumors *Salmonella* migrate away from vasculature toward the transition zone and induce apoptosis

Sabha Ganai[1,2], **Richard B. Arenas**[1,2,3], **Jeremy P. Sauer**[4], **Brooke Bentley**[3], and **Neil S. Forbes**[2,3,4,*]

Cancer Gene Ther. 2011 July ; 18(7): 457–466.

Bacteria also co-localize with lung and liver metastasis.



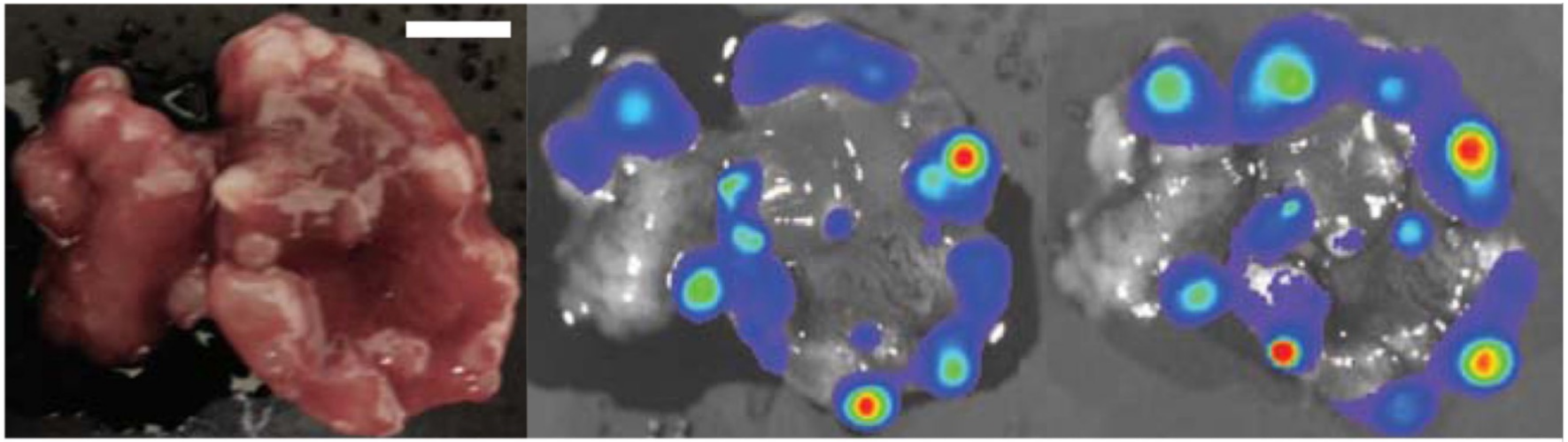
Published in final edited form as:

Sci Transl Med. 2015 May 27; 7(289): 289ra84. doi:10.1126/scitranslmed.aaa3519.

Programmable probiotics for detection of cancer in urine

Tal Danino[1,*], **Arthur Prindle**[2,*], **Gabriel A. Kwong**[1,†], **Matthew Skalak**[1], **Howard Li**[2], **Kaitlin Allen**[1], **Jeff Hasty**[2,3,4,‡], and **Sangeeta N. Bhatia**[1,5,6,7,8,§,‡]

Co-localization of the orally administered probiotic strain *E. coli* Nissle 1917 with liver metastasis in mouse.



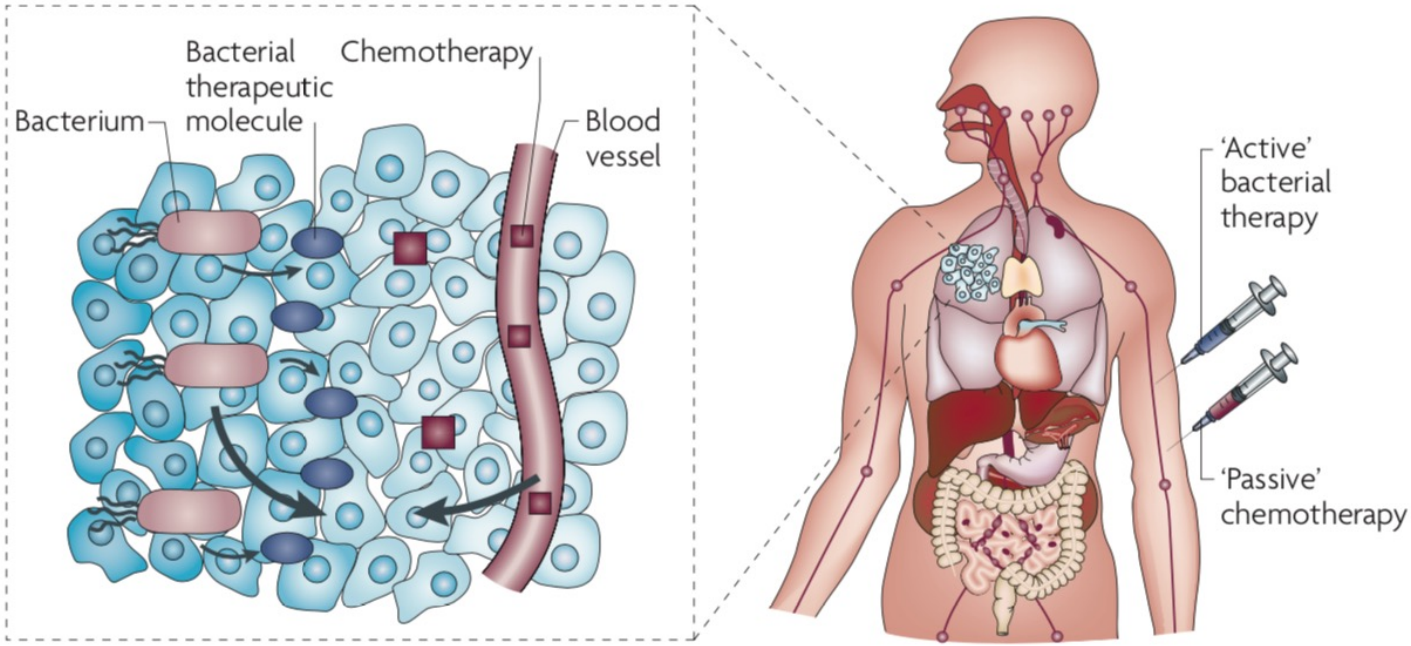
Excised liver | Tumor luminescence | Bacterial luminescence

INNOVATION

Engineering the perfect (bacterial) cancer therapy

Neil S. Forbes

Some bacteria, especially *Closdtridium* sp., are endowed with oncolythic activity. Bacteria can be used in combination with “passive” chemotherapy.



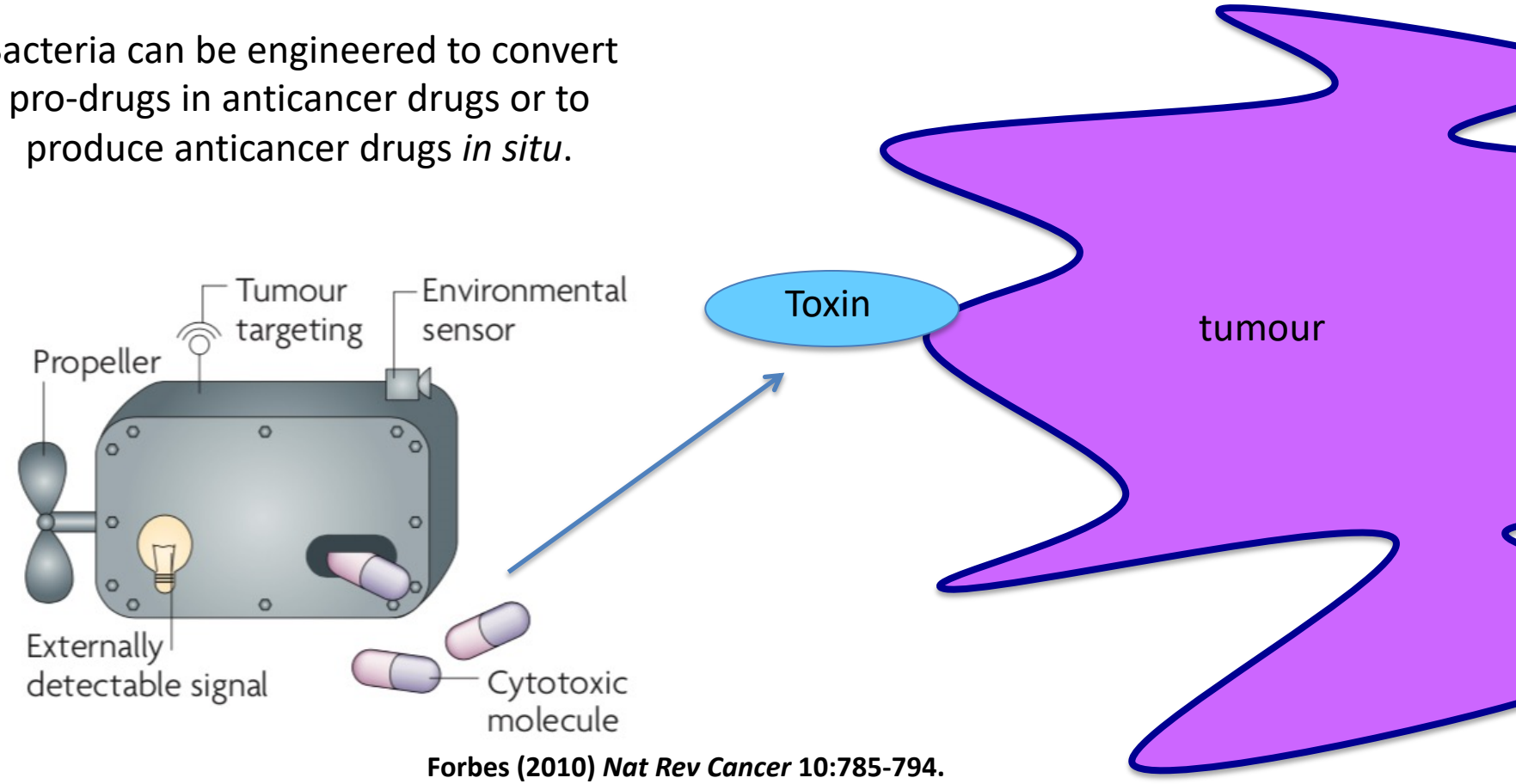
Forbes (2010) *Nat Rev Cancer* 10:785-794.

INNOVATION

Engineering the perfect (bacterial) cancer therapy

Neil S. Forbes

Bacteria can be engineered to convert pro-drugs in anticancer drugs or to produce anticancer drugs *in situ*.



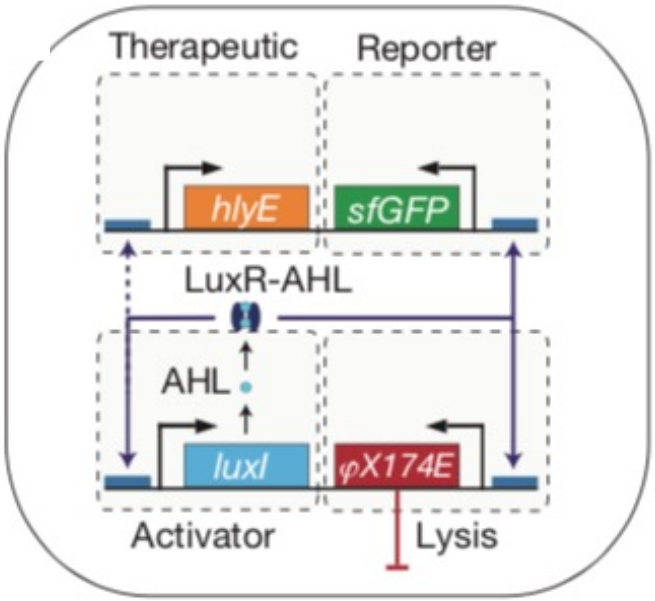
Forbes (2010) *Nat Rev Cancer* 10:785-794.

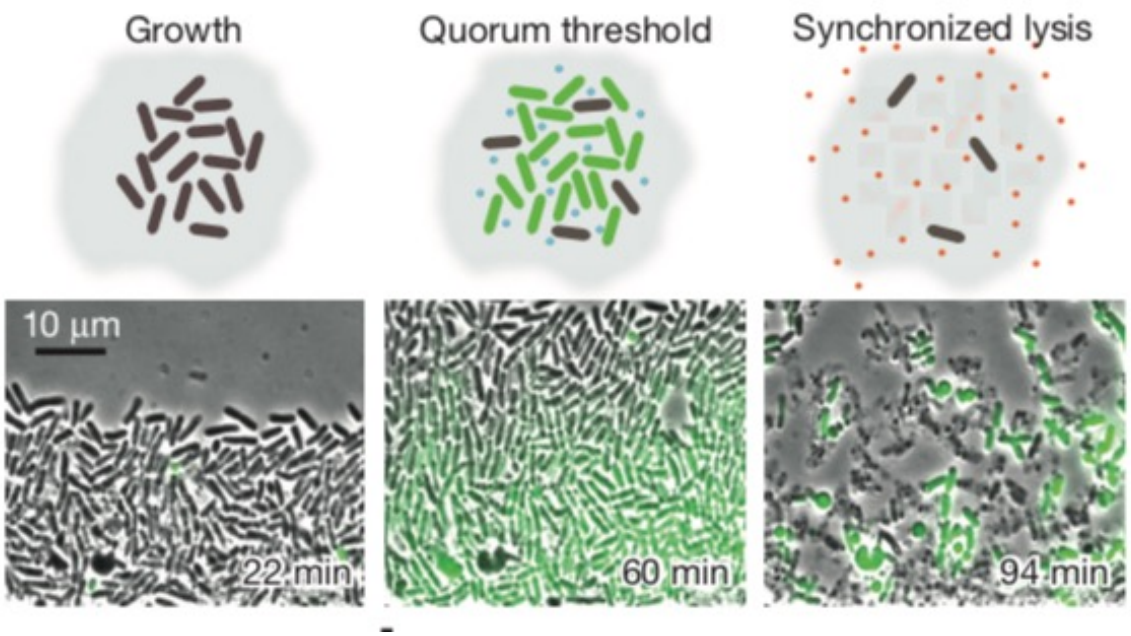
LETTER

doi:10.1038/nature18930

Synchronized cycles of bacterial lysis for *in vivo* delivery

M. Omar Din[1]*, Tal Danino[2]†*, Arthur Prindle[1], Matt Skalak[2], Jangir Selimkhanov[1], Kaitlin Allen[2], Ellixis Julio[1], Eta Atolia[2], Lev S. Tsimring[3], Sangeeta N. Bhatia[2,4,5,6,7,8]§ & Jeff Hasty[1,3,9]§





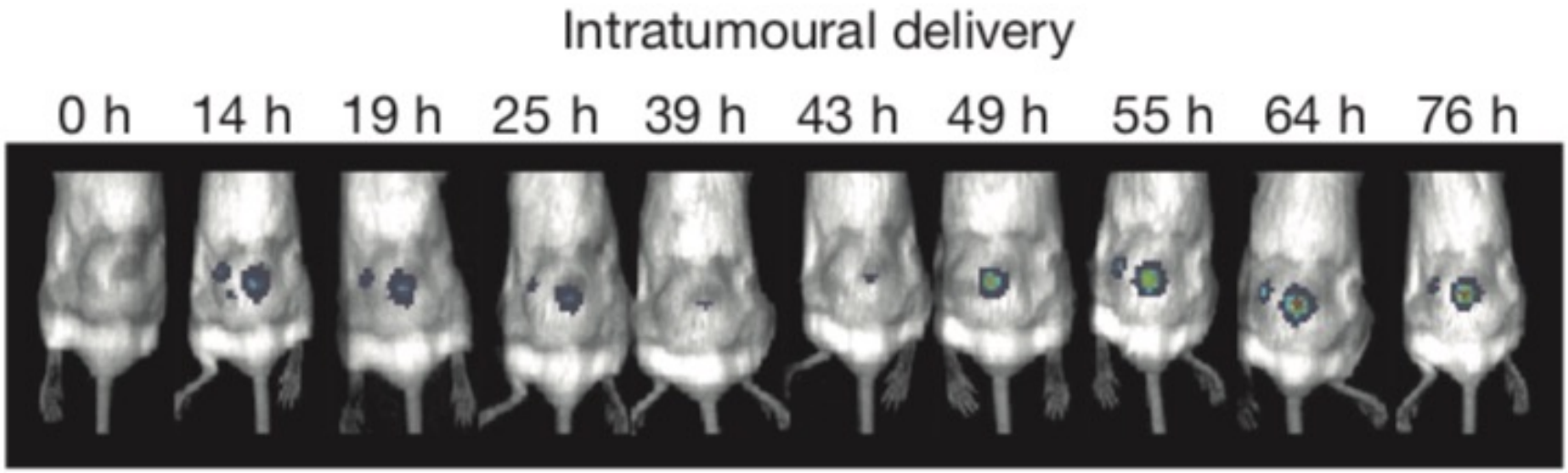
Din *et al*. (2016) *Nature* 536:81–85

LETTER

doi:10.1038/nature18930

Synchronized cycles of bacterial lysis for *in vivo* delivery

M. Omar Din[1]*, Tal Danino[2]†*, Arthur Prindle[1], Matt Skalak[2], Jangir Selimkhanov[1], Kaitlin Allen[2], Ellixis Julio[1], Eta Atolia[2], Lev S. Tsimring[3], Sangeeta N. Bhatia[2,4,5,6,7,8]§ & Jeff Hasty[1,3,9]§



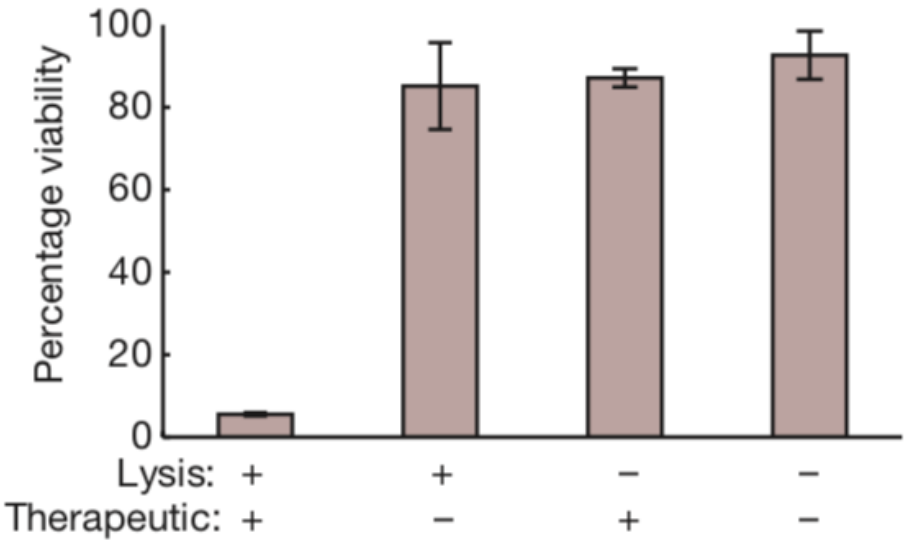
Din *et al*. (2016) *Nature* 536:81–85

LETTER

doi:10.1038/nature18930

Synchronized cycles of bacterial lysis for *in vivo* delivery

M. Omar Din[1]*, Tal Danino[2]†*, Arthur Prindle[1], Matt Skalak[2], Jangir Selimkhanov[1], Kaitlin Allen[2], Ellixis Julio[1], Eta Atolia[2], Lev S. Tsimring[3], Sangeeta N. Bhatia[2,4,5,6,7,8]§ & Jeff Hasty[1,3,9]§



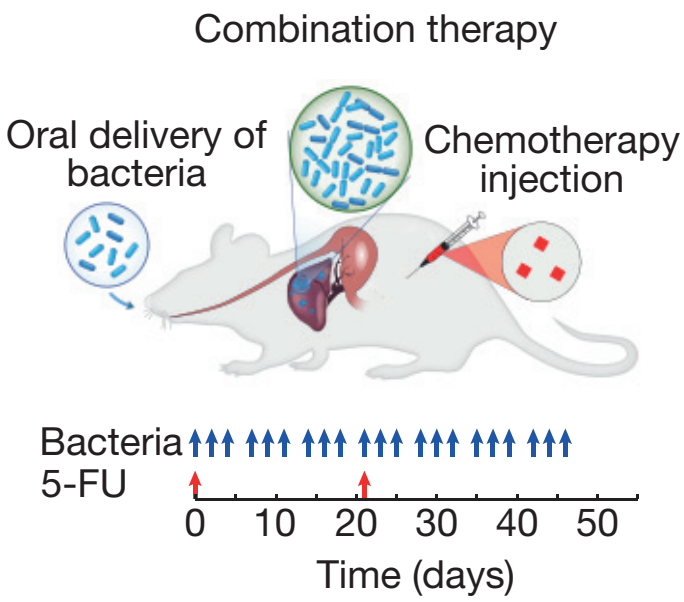
Din *et al*. (2016) *Nature* 536:81–85

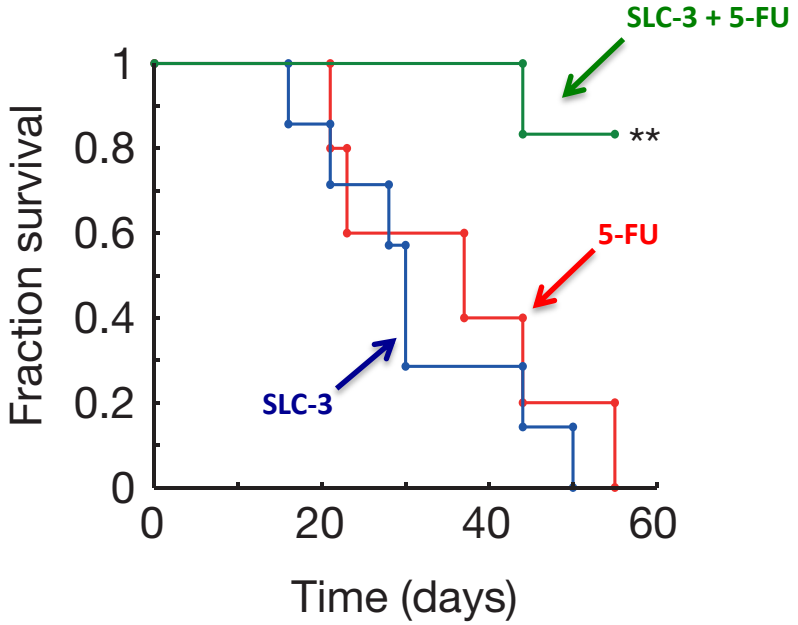
LETTER

doi:10.1038/nature18930

Synchronized cycles of bacterial lysis for *in vivo* delivery

M. Omar Din[1]*, Tal Danino[2]†*, Arthur Prindle[1], Matt Skalak[2], Jangir Selimkhanov[1], Kaitlin Allen[2], Ellixis Julio[1], Eta Atolia[2], Lev S. Tsimring[3], Sangeeta N. Bhatia[2,4,5,6,7,8]§ & Jeff Hasty[1,3,9]§





Din *et al*. (2016) *Nature* 536:81–85

Exploitation of QS in synthetic biology

The study of QS elucidates the mechanisms controlling collective behaviours and the evolution of social traits in individual cells.

In the last decade QS has been exploited for many biotechnological applications, including the development of biosensors and new therapeutic approaches.

In some cases, the engineering of bacterial cells with heterologous QS systems follows the principles of synthetic biology.

1) Engineering of non-pathogenic cells to sense and kill bacterial pathogens

2) Generation of new antitumor agents

3) Construction of new whole-cell biosensors

4) Generation of synthetic cells able to interface with natural cells

A genetic oscillator

Some negative feedback loops generate oscillations. Circadian rhythms are based on this kind of network motif.

Nature. 2010 January 21; 463(7279): 326–330. doi:10.1038/nature08753.

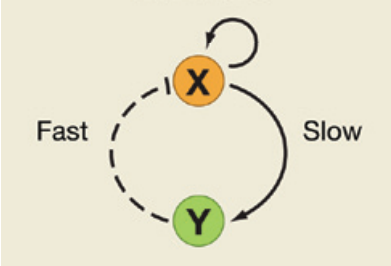
A synchronized quorum of genetic clocks

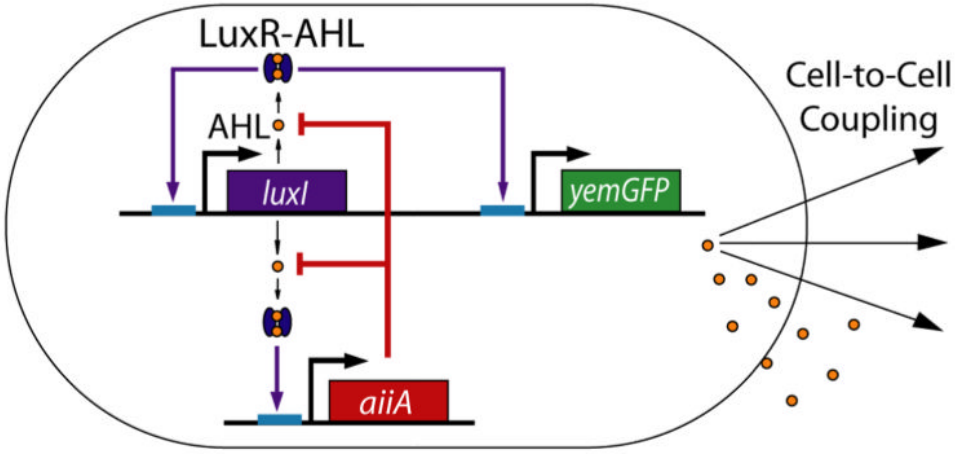
Tal Danino[1,*], **Octavio Mondragón-Palomino**[1,*], **Lev Tsimring**[2,†], and **Jeff Hasty**[1,2,3,4,†]

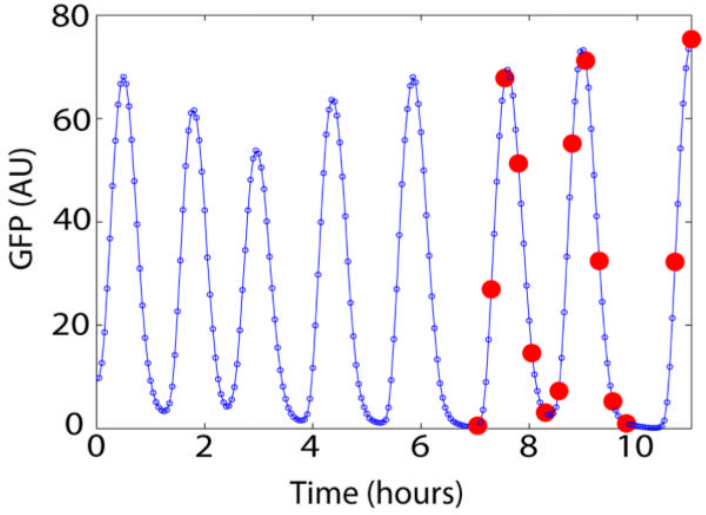
[1]Department of Bioengineering, University of California, San Diego, La Jolla, California, USA

[2]BioCircuits Institute, University of California, San Diego, La Jolla, California, USA

[3]Molecular Biology Section, Division of Biological Science, University of California, San Diego, La Jolla, CA 92093, USA



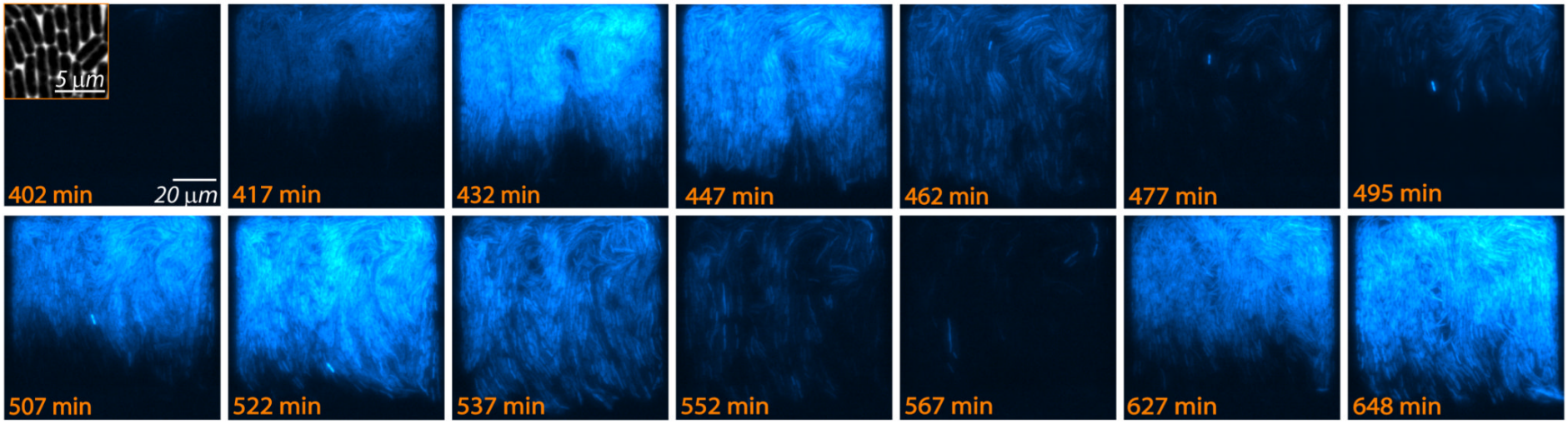




A genetic oscillator

Some negative feedback loops generate oscillations. Circadian rhythms are based on this kind of network motif.

Fluorescence emission from bacteria containing the oscillating control system during time. These pictures are from a single microcell (or biopixel) of a microfluidic device in which the bacteria are contained.



Use of a synchronized genetic oscillator to generate a new biosensor system for arsenite

A sensing array of radically coupled genetic 'biopixels'

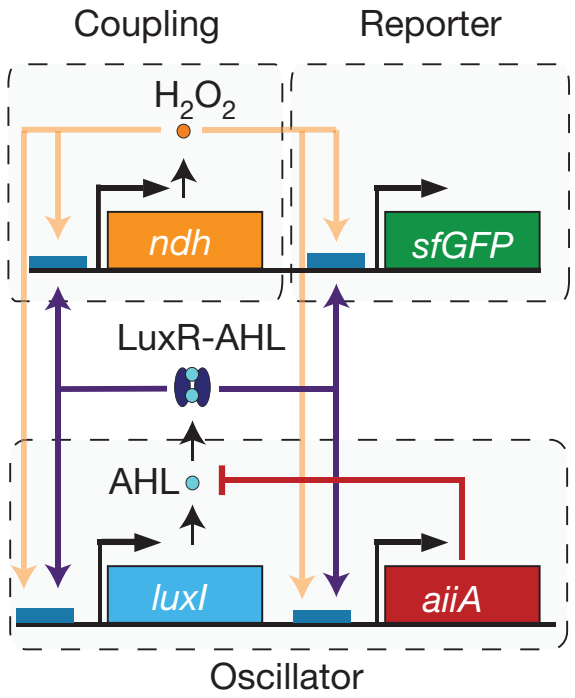
Arthur Prindle[1]*, Phillip Samayoa[2]*, Ivan Razinkov[1], Tal Danino[1], Lev S. Tsimring[3] & Jeff Hasty[1,2,3,4]

In this study, published in *Nature*, researchers want to generate a biosensor system that relies on frequency variations of the emitted signal, rather than on the amplitude of that signal. Frequency variations can be easily monitored, transferred and digitized. In addition, frequency variations are less sensitive to differences in the readout instrument and do not require frequent calibration.

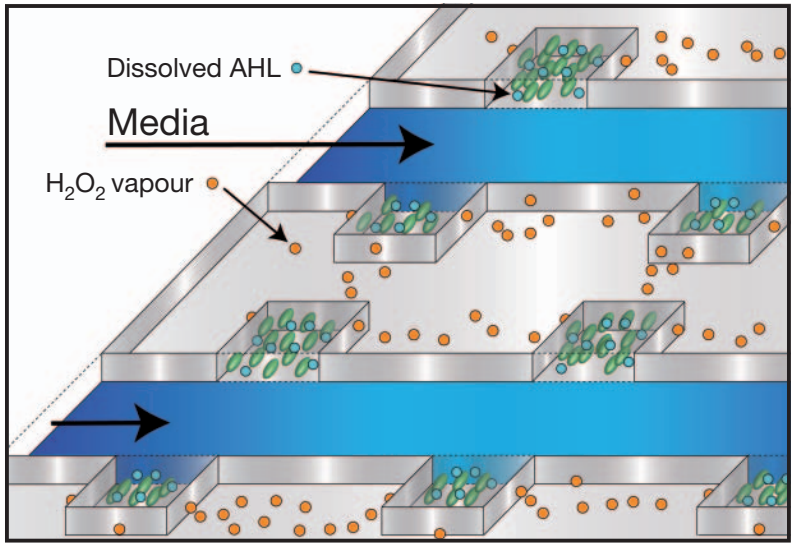
To this aim, they want to construct a microfuidic divice in which fluorescence emission by hundreds of biopixels is synchronized, and in which the frequency of signal oscillation is modulated by a pollutant.

Use of a synchronized genetic oscillator to generate a new biosensor system for arsenite

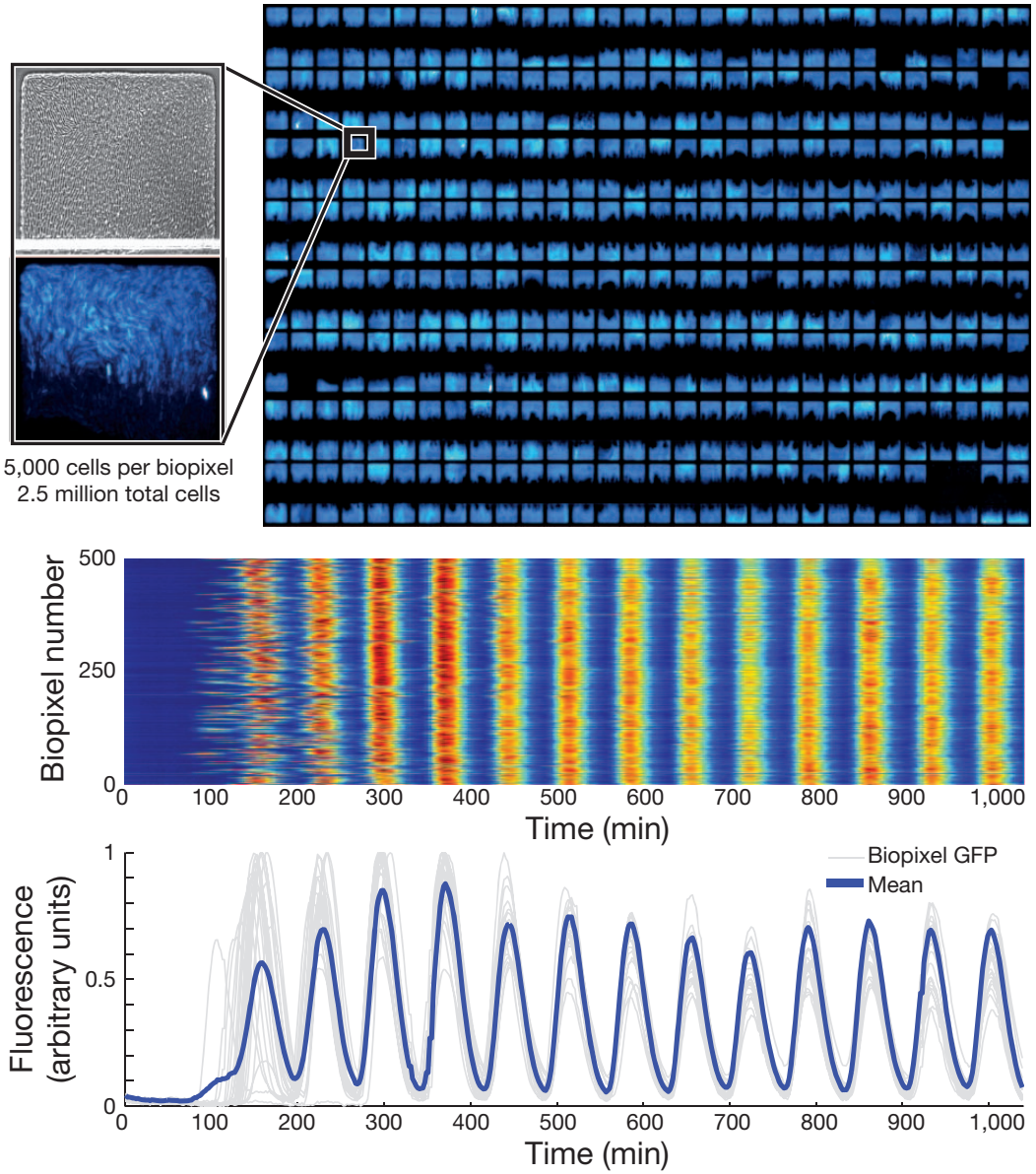
Fluorescence emission by different biopixels is not synchronized due to slow diffusibility of the QS signal molecule at the macroscopic scale. To solve this problem, the researchers coupled the QS-based oscillating system with an intercellular signaling system based on the production and sensing of H_2O_2. This molecule can rapidly diffuse from one biopixel to another in the microfluidic device, and therefore can synchronize oscillation in individual biopixels.



The material that divides the biopixels, polydimethylsiloxane (PDMS), is H_2O_2 permeable.

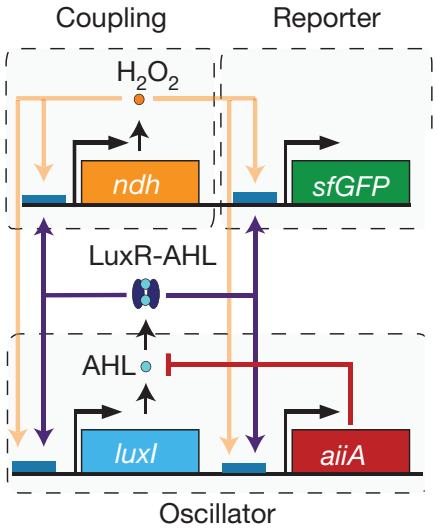


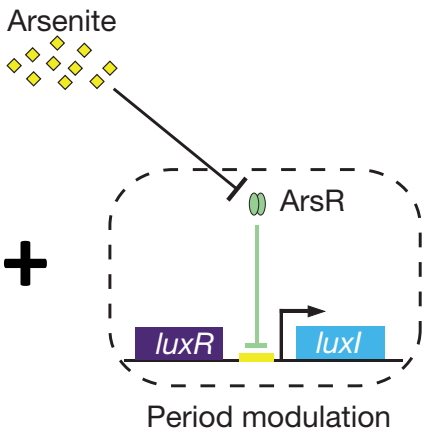
Use of a synchronized genetic oscillator to generate a new biosensor system for arsenite

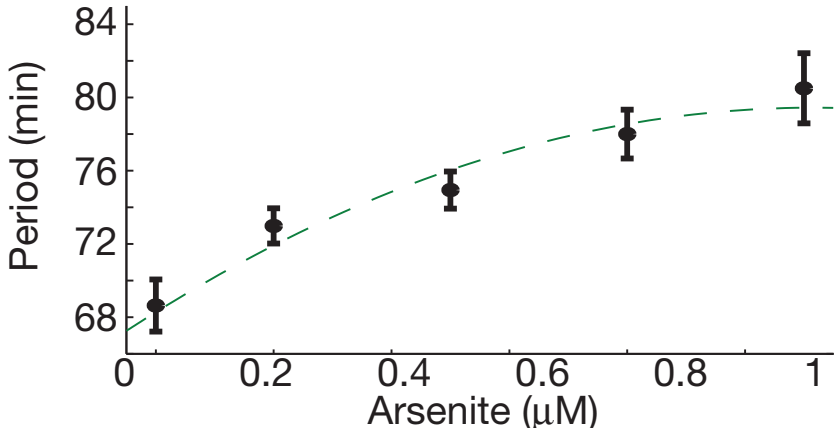


Use of a synchronized genetic oscillator to generate a new biosensor system for arsenite

The researchers inserted an additional element into the system, an second *luxI* gene under the control of a promoter repressed by ArsR. The constitutively expressed ArsR regulator represses the expression of the second *luxI* gene, and thus the synthesis of additional QS signal molecule, unless arsenite is present in the growth medium. In the presence of arsenite, ArsR will no longer be able to repress the expression of the second *luxI* gene, and this will lead to an increase in the levels of QS signal molecule produced. This effect is detectable as an increase in the oscillation period.







Use of a synchronized genetic oscillator to generate a new biosensor system for arsenite

In the final chip (24 mm x 12 mm; 12,000 biopixels) the synchronization of oscillations and the arsenite-induced frequency change are maintained.

This chip can be “read” with a simple optical instrument containing an LED to excite GFP, a photodetector and a processor that transduces the light signal into an image (commercially available for 50 USD).

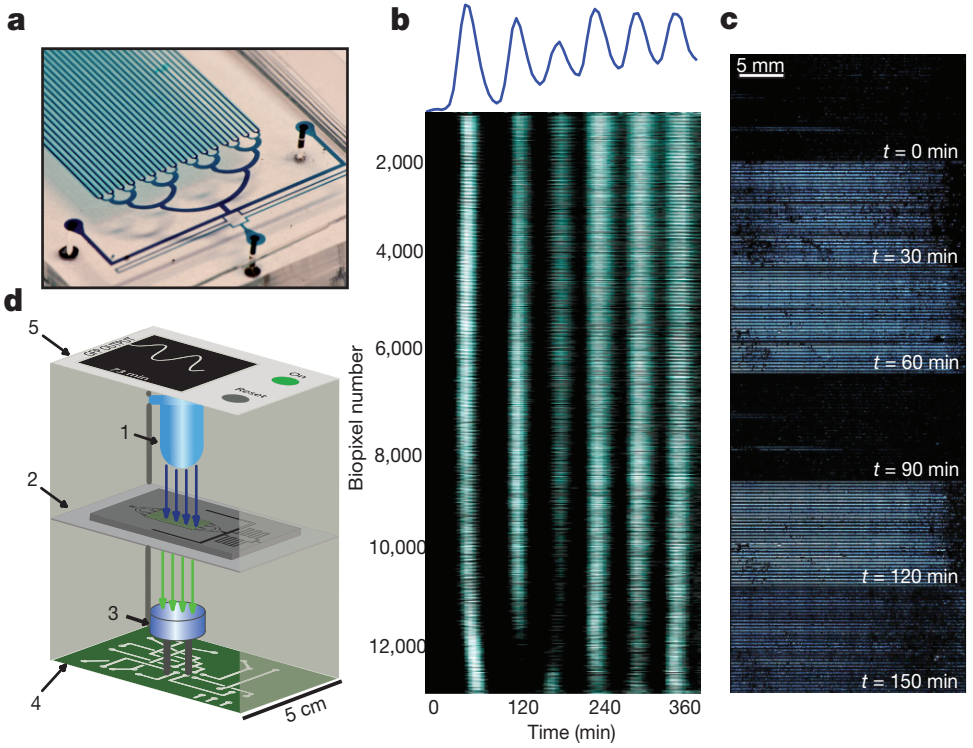


Figure 4 | Radical synchronization on a macroscopic scale. **a**, The scaled-up array is 24 mm × 12 mm and houses over 12,000 biopixels that contain approximately 50 million total cells when filled. **b**, Global synchronization is maintained across the array. Heat map of individual trajectories of all 12,224 oscillating biopixels. **c**, Image series depicting global synchronization and oscillation for the macroscopic array. Each image is produced by stitching 72 fields of view imaged at ×4 magnification. **d**, Schematic diagram illustrating our design for a handheld device using the sensing array. An LED (1) excites the array (2) and emitted light is collected by a photodetector (3), analysed by an onboard processor (4), and displayed graphically (5).

Exploitation of QS in synthetic biology

The study of QS elucidates the mechanisms controlling collective behaviours and the evolution of social traits in individual cells.

In the last decade QS has been exploited for many biotechnological applications, including the development of biosensors and new therapeutic approaches.

In some cases, the engineering of bacterial cells with heterologous QS systems follows the principles of synthetic biology.

1) Engineering of non-pathogenic cells to sense and kill bacterial pathogens

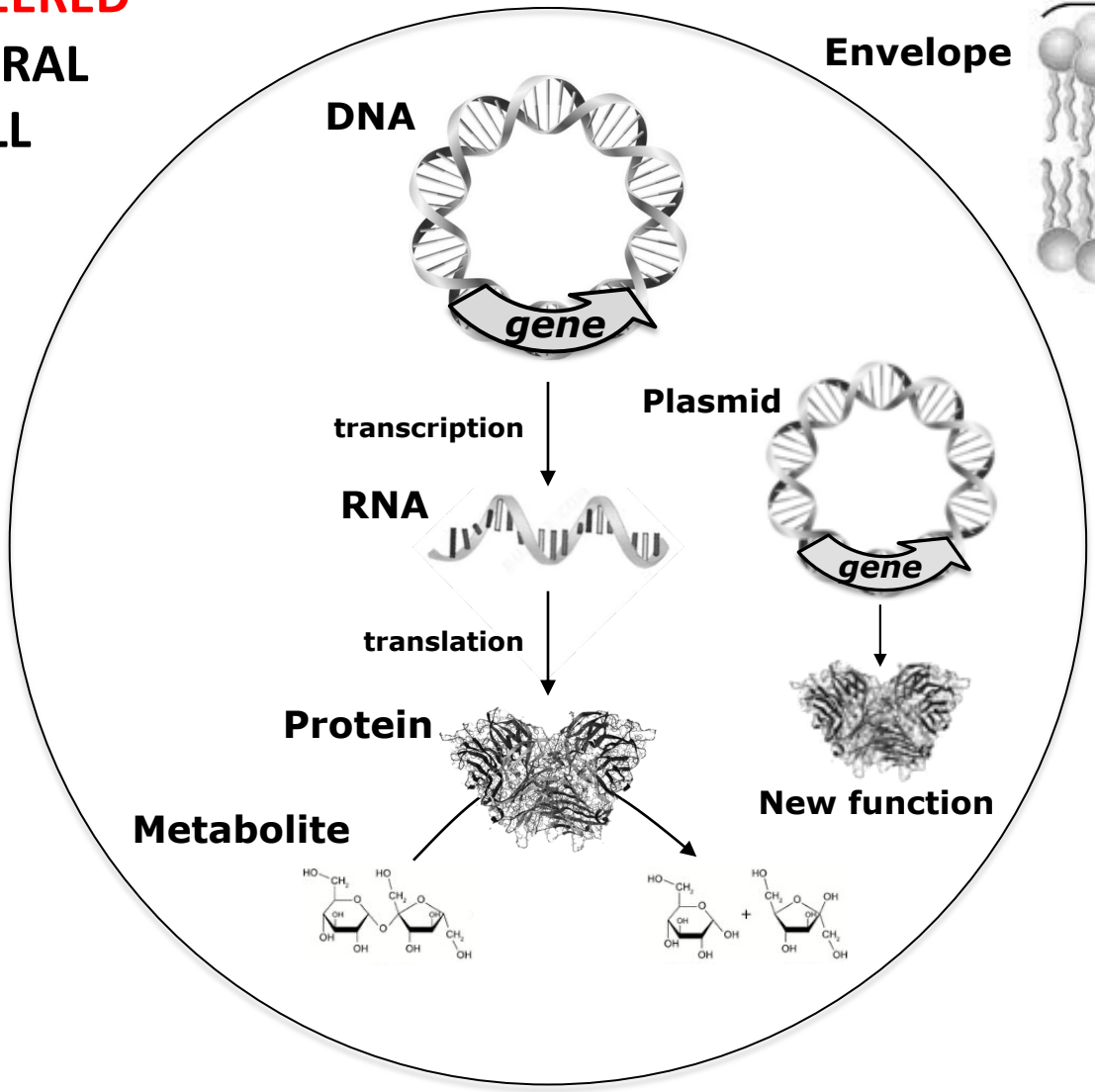
2) Generation of new antitumor agents

3) Construction of new whole-cell biosensors

4) Generation of synthetic cells able to interface with natural cells

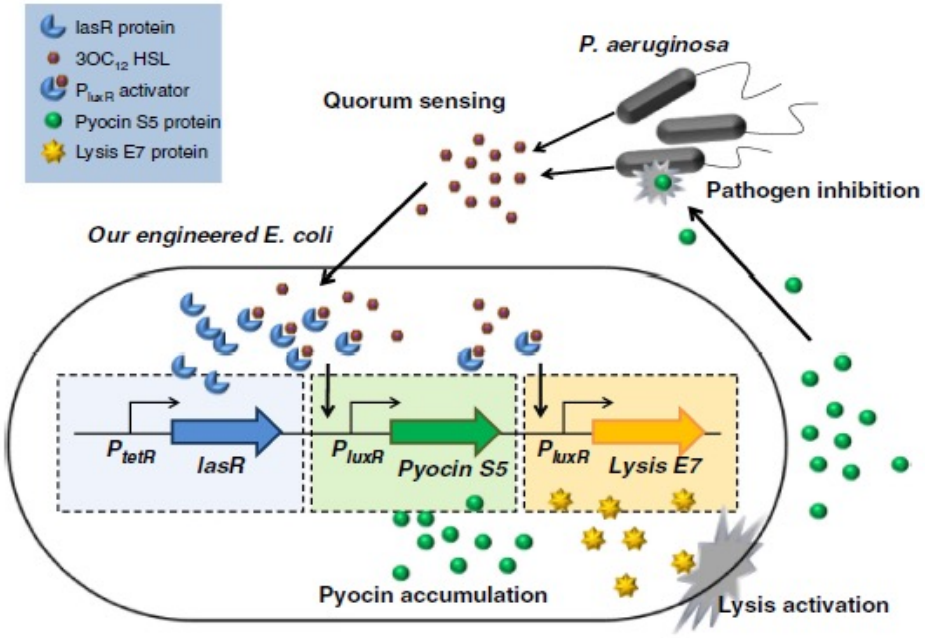
ENGINEERED

NATURAL CELL

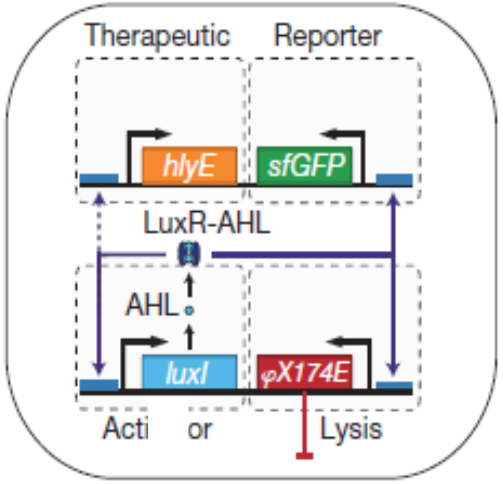


Engineered cells are useful in many fields

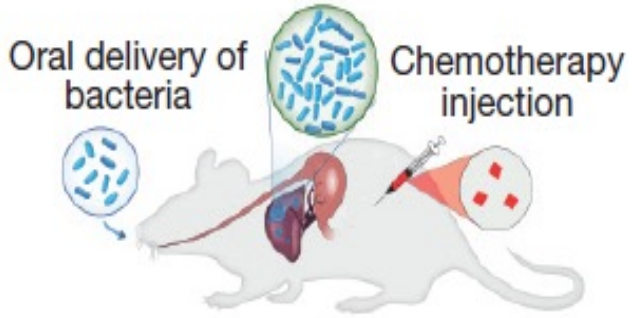
- Production of fine chemicals, drugs, biofuels
- Bioremediation
- Generation of biosensors
- Biomedical applications
- etc…



Saeidi *et al.* (2011) *Mol Syst Biol* 7:521

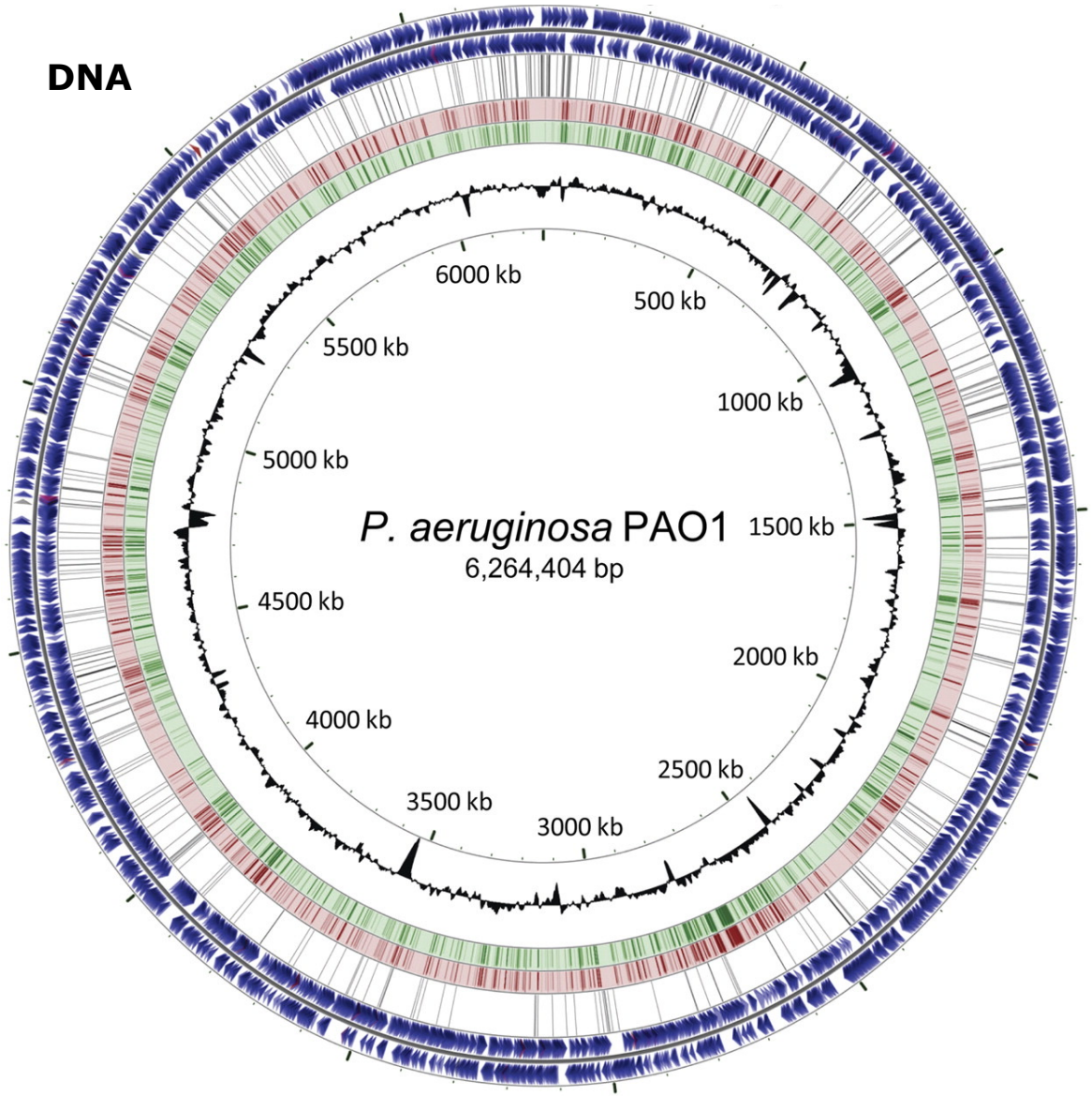


Combination therapy

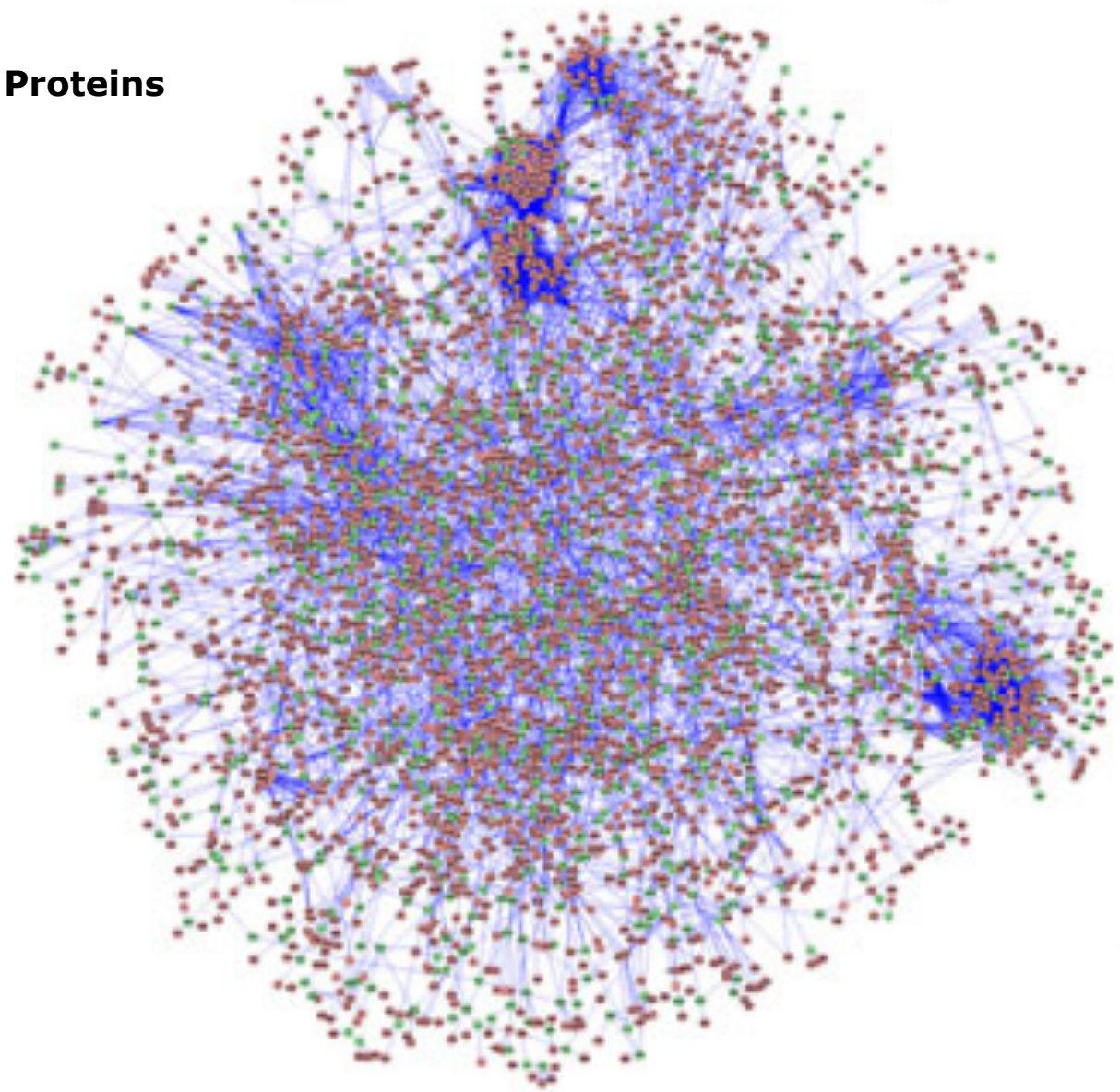


Din *et al.* (2016) *Nature* 536:81-85

The complexity of modern cells limits our understanding of their functionality

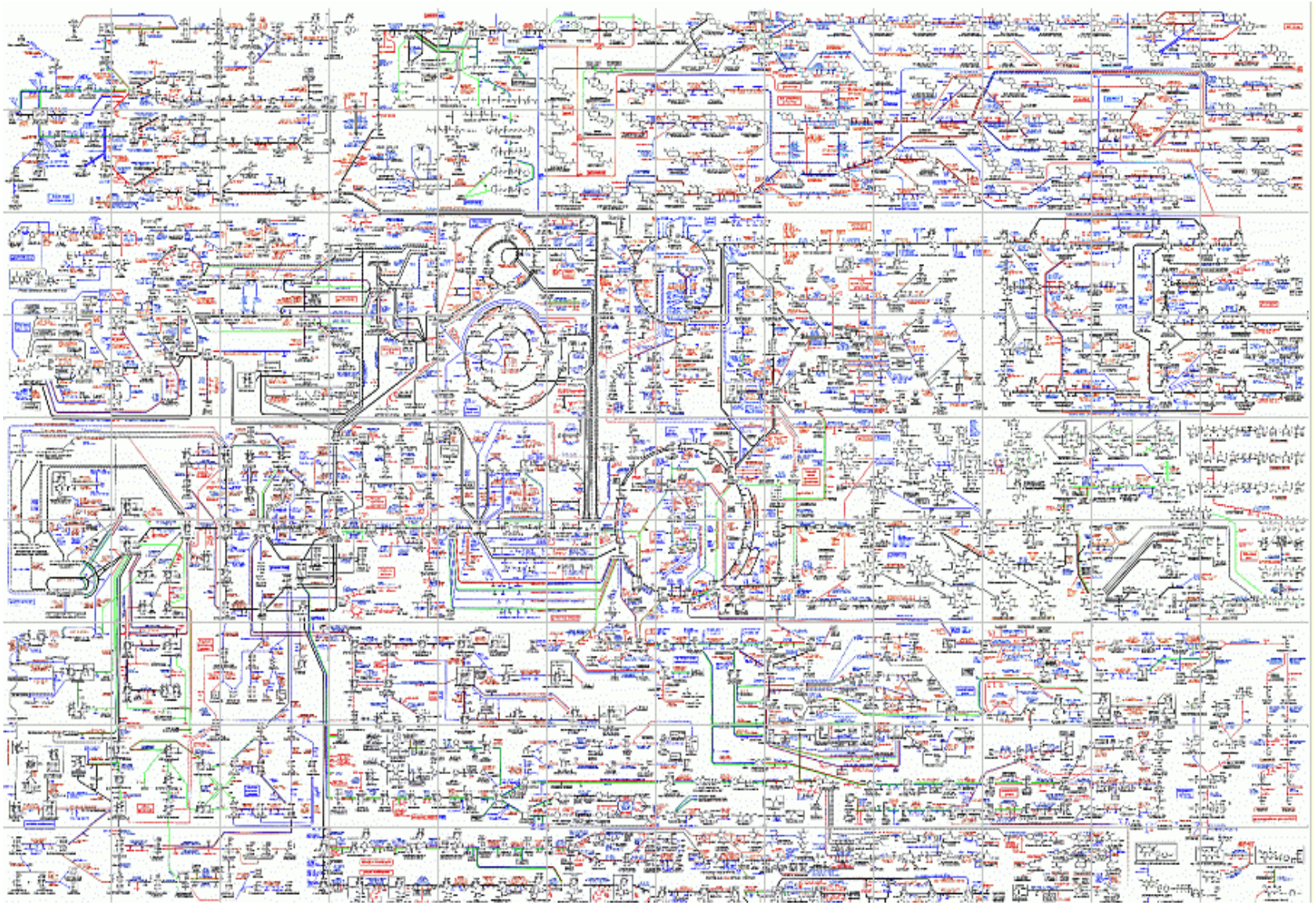


The complexity of modern cells limits our understanding of their functionality



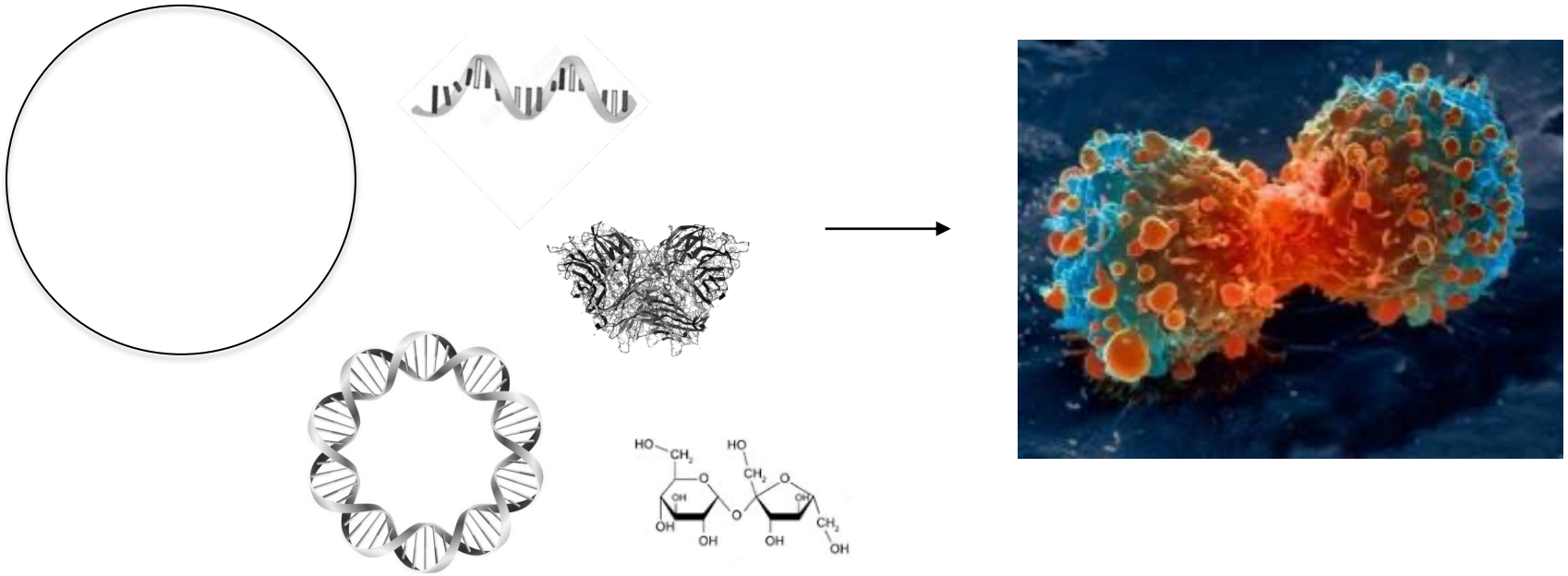
The complexity of modern cells limits our understanding of their functionality

Metabolites



The complexity of modern cells limits our understanding of their functionality

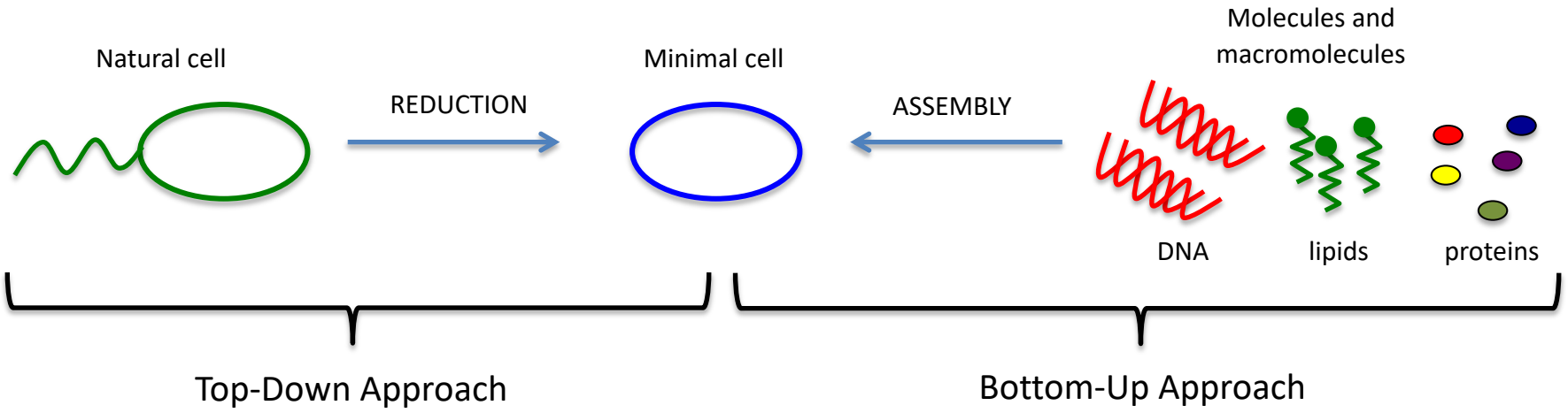
We cannot predict possible interactions between synthetic gene circuits and endogenous cellular functions, hence orthogonality is not guaranteed.



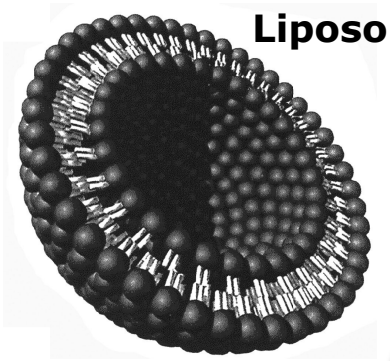
The complexity of modern cells limits our understanding of their functionality

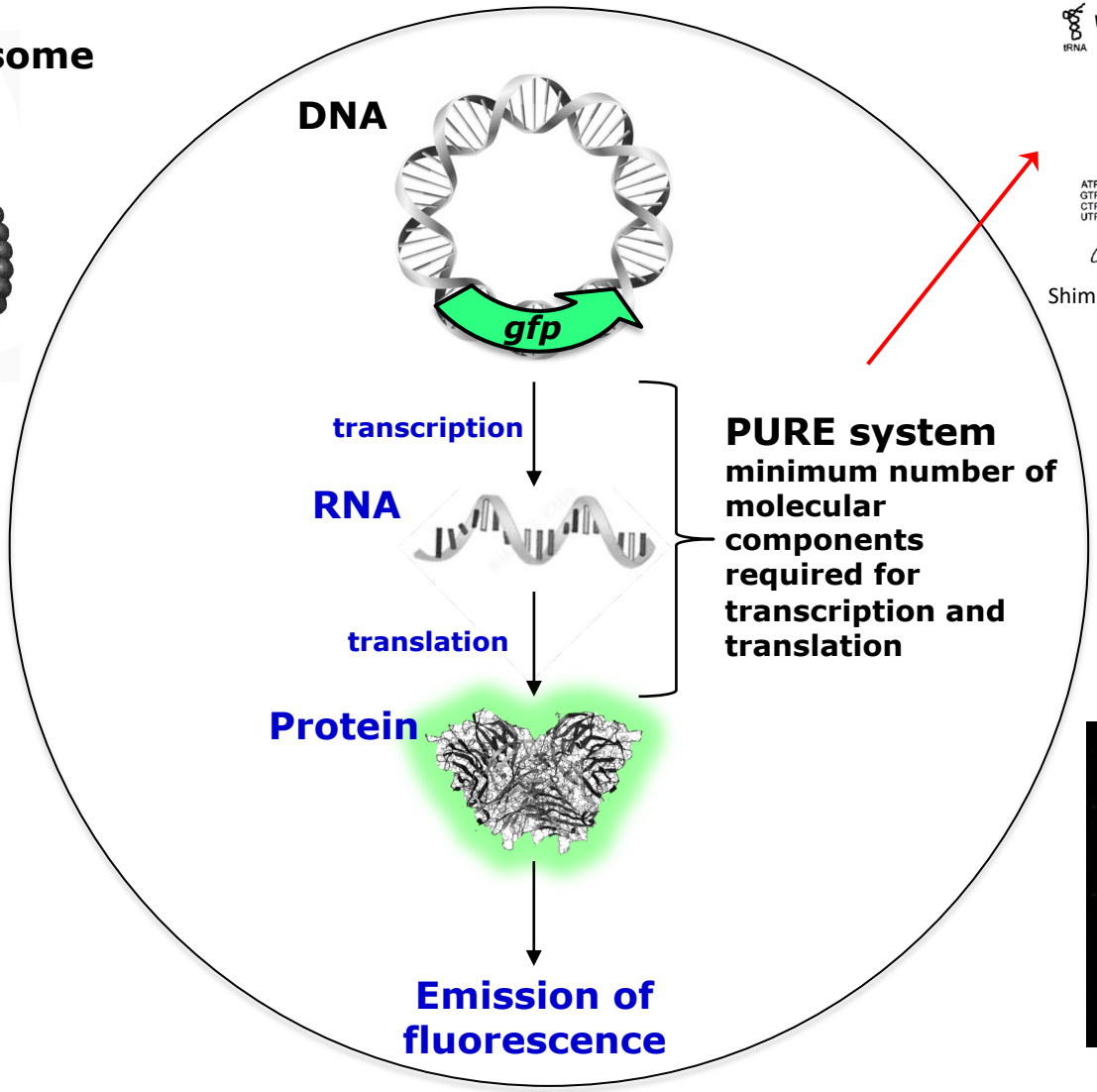
We cannot predict possible interactions between synthetic gene circuits and endogenous cellular functions, hence orthogonality is not guaranteed.

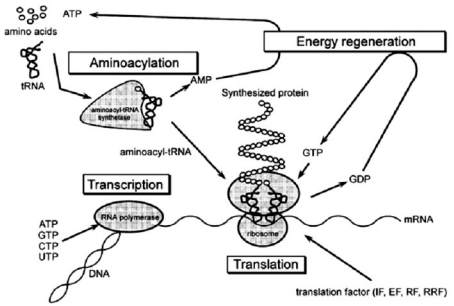
To solve this problem, synthetic gene circuits could be used in minimal cells.



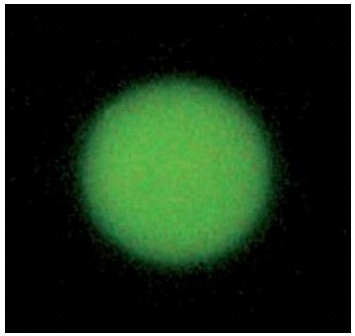
Synthetic cells are models of primitive/simplified cells







Shimizu *et al*. (2001) *Nat Biotechnol* 19:751-755.

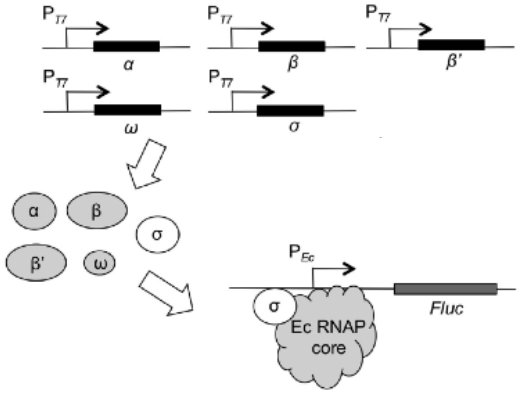


Synthetic cells can be programmed to accomplish specific tasks

Drive gene transcription

***In vitro* genetic reconstruction of bacterial transcription initiation by coupled synthesis and detection of RNA polymerase holoenzyme**

Haruichi Asahara and Shaorong Chong*

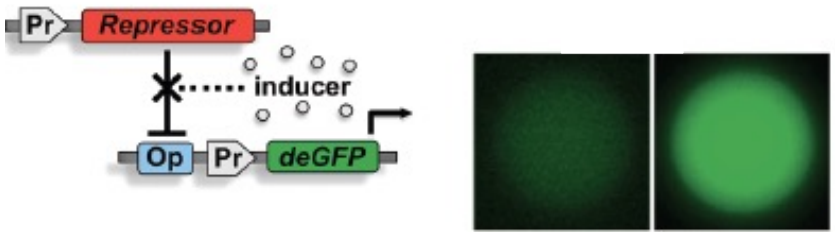


Asahara and Chong (2010) *NAR* 38:e141.

Regulate gene transcription

An *E. coli* Cell-Free Expression Toolbox: Application to Synthetic Gene Circuits and Artificial Cells

Jonghyeon Shin and Vincent Noireaux*

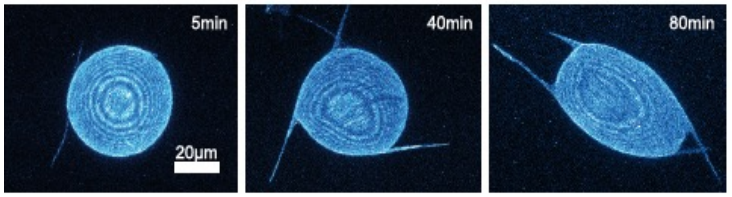


Shin and Noireaux (2012) *ACS Synth Biol* 1:29-41.

Move

Topology and dynamics of active nematic vesicles

Felix C. Keber,[1,2*] Etienne Loiseau,[1*] Tim Sanchez,[3*] Stephen J. DeCamp,[3] Luca Giomi,[4,5] Mark J. Bowick,[6] M. Cristina Marchetti,[6] Zvonimir Dogic,[2,3] Andreas R. Bausch[1†]

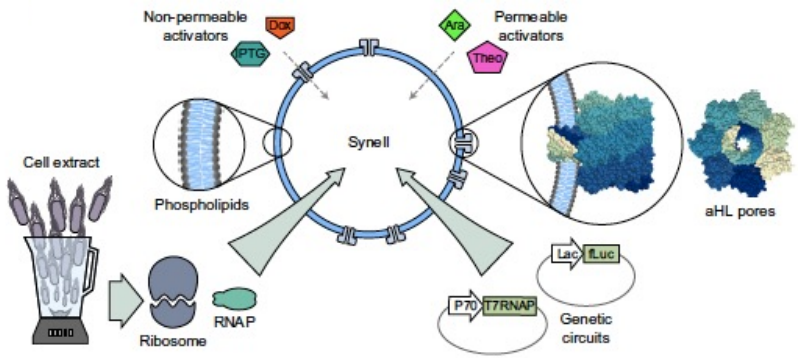


Keber *et al*. (2014) *Science* 345:1135-1139.

Exchange information with the environment

Engineering genetic circuit interactions within and between synthetic minimal cells

Katarzyna P. Adamala[1†‡], Daniel A. Martin-Alarcon[2‡], Katriona R. Guthrie-Honea[1] and Edward S. Boyden[1,2,3*]



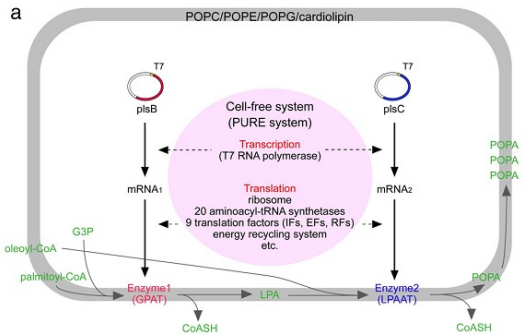
Adamala *et al*. (2017) *Nat Chem* 9:431-439.

Synthetic cells can be programmed to accomplish specific tasks

Synthesize lipids

A synthetic biology approach to the construction of membrane proteins in semi-synthetic minimal cells

Yutetsu Kuruma [a], Pasquale Stano [a, b], Takuya Ueda [c], Pier Luigi Luisi [b]

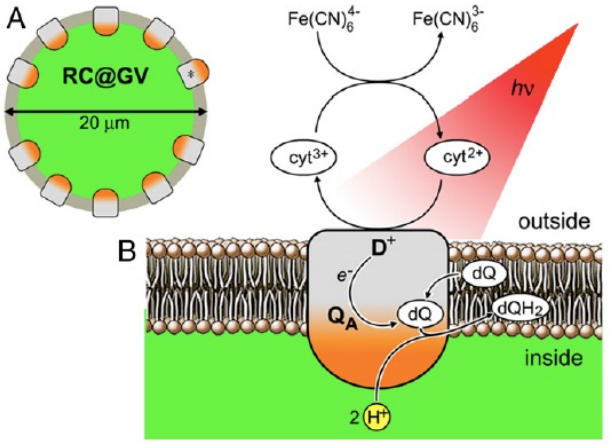


Kuruma *et al*. (2009) *Biochim Biophys Acta* 1788:567-574.

Produce energy

Highly oriented photosynthetic reaction centers generate a proton gradient in synthetic protocells

Emiliano Altamura[a], Francesco Milano[b], Roberto R. Tangorra[a], Massimo Trotta[b], Omar Hassan Omar[c], Pasquale Stano[d,1], and Fabio Mavelli[a,2]

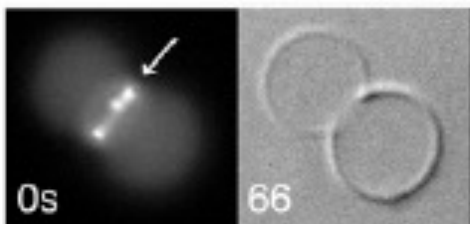


Altamura *et al*. (2017) *PNAS* 114:3837-3842.

Divide into two synthetic cells

Liposome division by a simple bacterial division machinery

Masaki Osawa (大澤正輝)[1] and Harold P. Erickson



Osawa and Erickson (2013) *PNAS* 110:11000-11004.

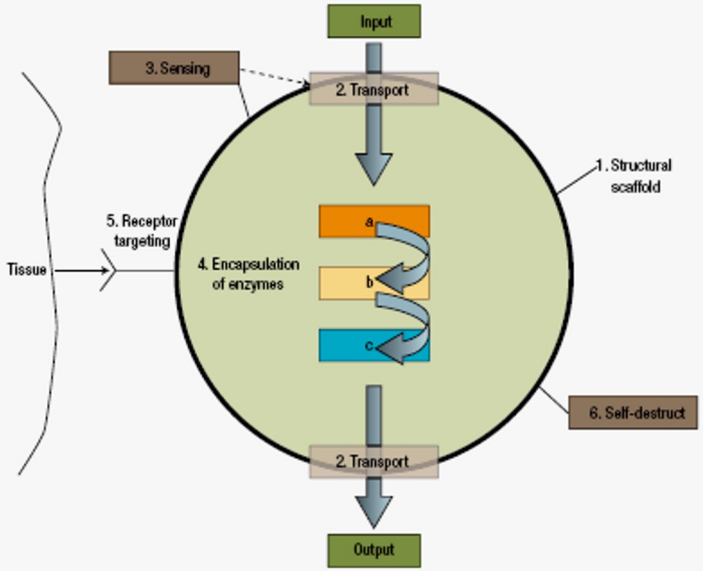
Can we generate synthetic cells interfacing with natural cells?

Synthetic cells able to process external stimuli and to consequently react (*i.e.*, to interface with natural cells) could be employed as **"soft nano-robots"** for future intelligent drug delivery approaches, as biosensors, as cell-free nanofactories, etc...

Towards an *in vivo* biologically inspired nanofactory

PHILIP R. LEDUC[1*], MICHAEL S. WONG[2*], PLACID M. FERREIRA[3], RICHARD E. GROFF[4], KIRYN HASLINGER[5], MICHAEL P. KOONCE[6], WOO Y. LEE[7], J. CHRISTOPHER LOVE[8], J. ANDREW McCAMMON[9], NANCY A. MONTEIRO-RIVIERE[10], VINCENT M. ROTELLO[11], GARY W. RUBLOFF[12], ROBERT WESTERVELT[13] AND MINAMI YODA[14]

LeDuc *et al*. (2006) *Nature Nanotech* 2:3-7.



Notably, liposomes are already used for drug delivery.

Liposomes as drug carriers

Liposomes are used as delivery systems in diverse medical fields, including **anti-cancer**, **anti-fungal** and **anti-inflammatory** drugs.

In 1995, liposomal **doxorubicin** (Doxil™) was first introduced in U.S., to treat ovarian cancer and AIDS-related Kaposi's sarcoma.

DaunoXome® was developed by NeXstar Pharmaceuticals (Boulder, CO, USA) for the delivery of **daunorubicin**, and was FDA approved in 1996 for the management of advanced HIV-associated Kaposi's sarcoma.

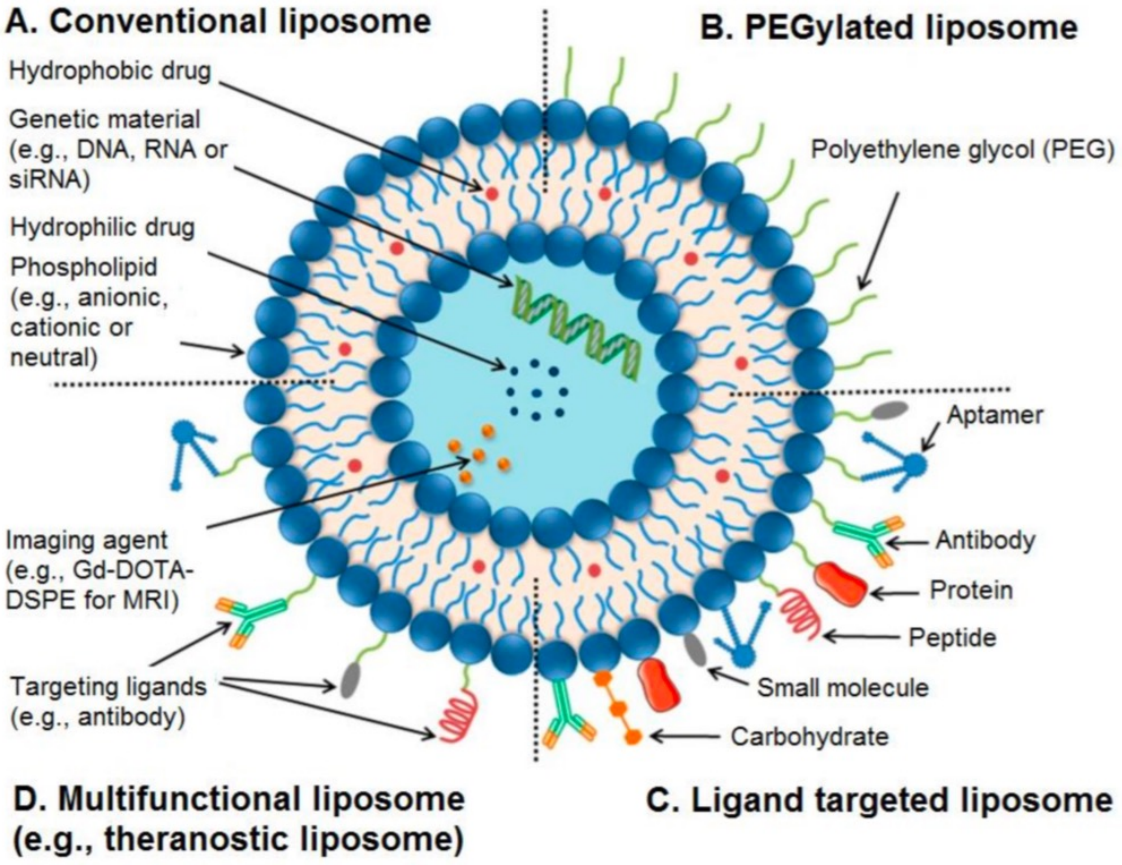
Other anticancer-liposomal products: Mepact® by Takeda Pharmaceutical (Deerfield, IL, USA), DepoCyt® by SkyPharma Inc. (Belgravia, London, UK), Marqibo® by Talon Therapeutics (San Francisco, CA, USA) and a fluorouracil, leucovorin combination with liposomes (Merrimack Pharmaceuticals Inc., Cambridge, MA, USA), Myocet® by Elan Pharmaceuticals (San Francisco, CA, USA).

Liposomal products were also developed for other diseases such as **fungal infections (Amphotec® and AmBisome®)**. Liposomes have become an important carrier systems for vaccine development leading to the development of **vaccines such as Epaxal® and Inflexal V®** for hepatitis and influenza, respectively.

Lamichhane *et al*. (2018) *Molecules* 23:288.

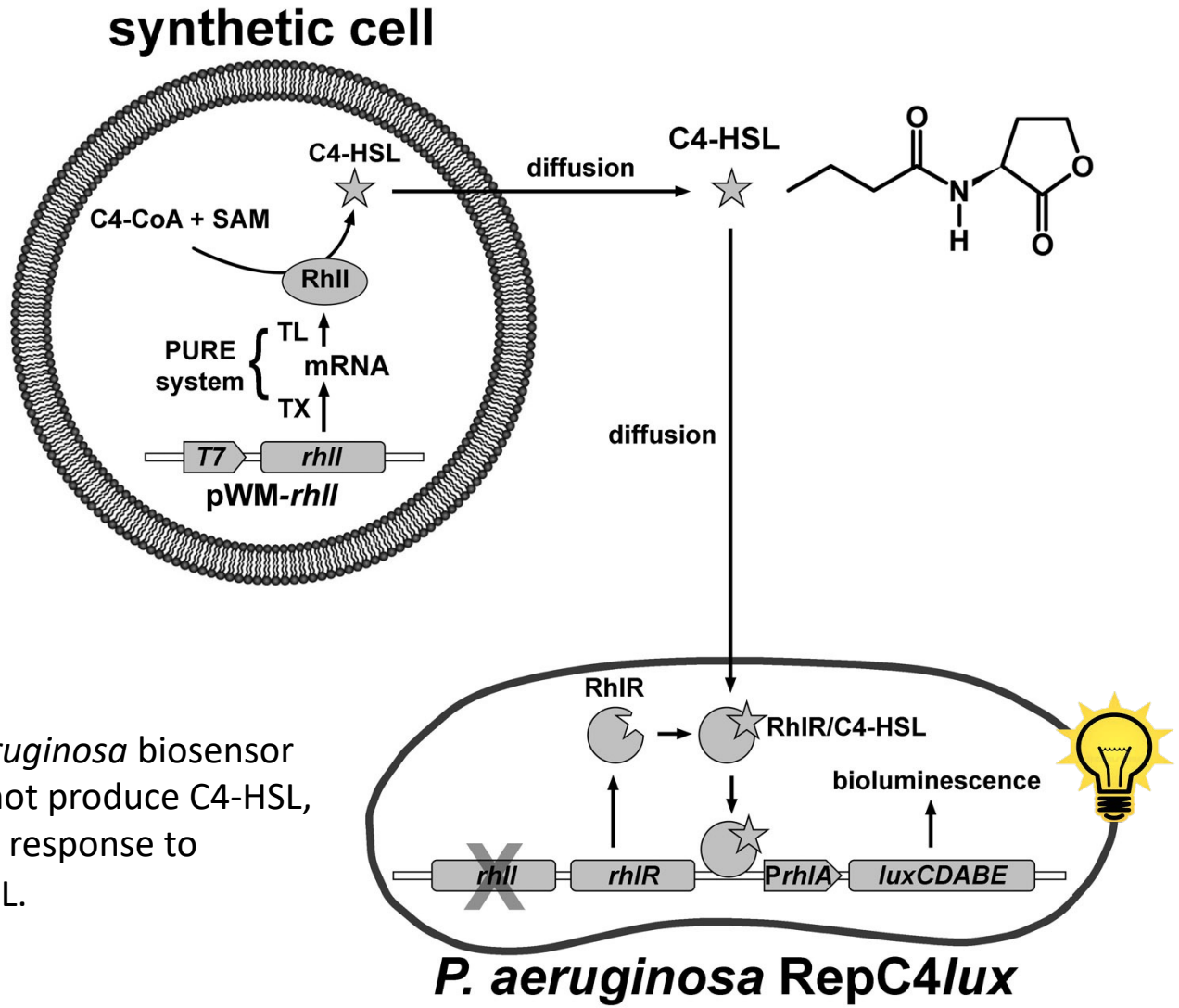
Liposomes as drug carriers

Liposomes are biocompatible, they are naturally nontoxic, non-immunogenic, and biodegradable. They have a role in enhancing drug solubility, providing targeted drug delivery, reducing the toxic effect of drugs, providing protection against drug degradation, enhancing circulation half-life.



Olusanya *et al*. (2018) *Molecules* 23:907.

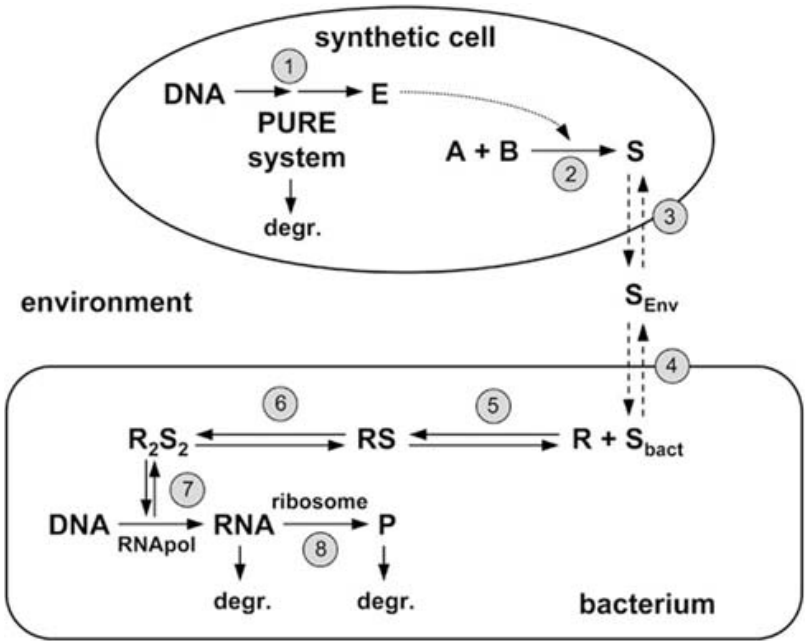
Quorum sensing-based communication between synthetic cells and *Pseudomonas aeruginosa*



Engineered *P. aeruginosa* biosensor strain that does not produce C4-HSL, and emits light in response to exogenous C4-HSL.

Generation of synthetic cells interfacing with bacteria
1) *in silico* modelling

Schematic representation of the communication process



Kinetic differential equations used in the model

I. Synthetic cell

1 $$\frac{d[E]}{dt} = (k_{TXPS}t)\cdot k_{TLPS}\exp(-k_{inactPS}t)$$

2 $$\frac{d[A]}{dt} = \frac{d[B]}{dt} = -k_{cat}[E]\frac{[A]}{K_{MA}+[A]}\frac{[B]}{K_{MB}+[B]}$$

3 $$\frac{d[S]_{sc}}{dt} = k_{cat}[E]\frac{[A]}{K_{MA}+[A]}\frac{[B]}{K_{MB}+[B]} - \frac{\sigma_{sc}\wp}{V_{sc}}([S]_{sc}-[S]_{env})$$

II. Environment

3,4 $$\frac{d[S]_{env}}{dt} = N_{sc}\frac{\sigma_{sc}\wp}{V_{sc}}([S]_{sc}-[S]_{env}) + N_{bact}\frac{\sigma_{bact}\wp}{V_{bact}}([S]_{bact}-[S]_{env})$$

III. Bacterium

4,5 $$\frac{d[S]_{bact}}{dt} = -\frac{\sigma_{bact}\wp}{V_{bact}}([S]_{bact}-[S]_{env}) - k_{on}[R][S]_{bact} + k_{off}[RS]$$

5,6 $$\frac{d[R]}{dt} = -k_{on}[R][S]_{bact} + k_{off}[RS]$$

5,6 $$\frac{d[RS]}{dt} = k_{on}[R][S] - k_{off}[RS] - 2k_{\dim}[RS]^2 + 2k_{diss}[R_2S_2]$$

6 $$\frac{d[R_2S_2]}{dt} = k_{\dim}[RS]^2 - k_{diss}[R_2S_2]$$

7 $$\frac{d[mRNA]}{dt} = \frac{1}{3L}k_{TX}C_{RNApol}\frac{C_{DNA}}{K_{MTX}+C_{DNA}}\cdot\frac{[R_2S_2]^n}{K^n_{MR_2S_2}+[R_2S_2]^n} - k_{\deg,mRNA}[mRNA]$$

8 $$\frac{d[P]}{dt} = \frac{1}{L}k_{TL}C_{rib}\frac{[mRNA]}{K_{MTL}+[mRNA]} - k_{\deg,P}[P]$$

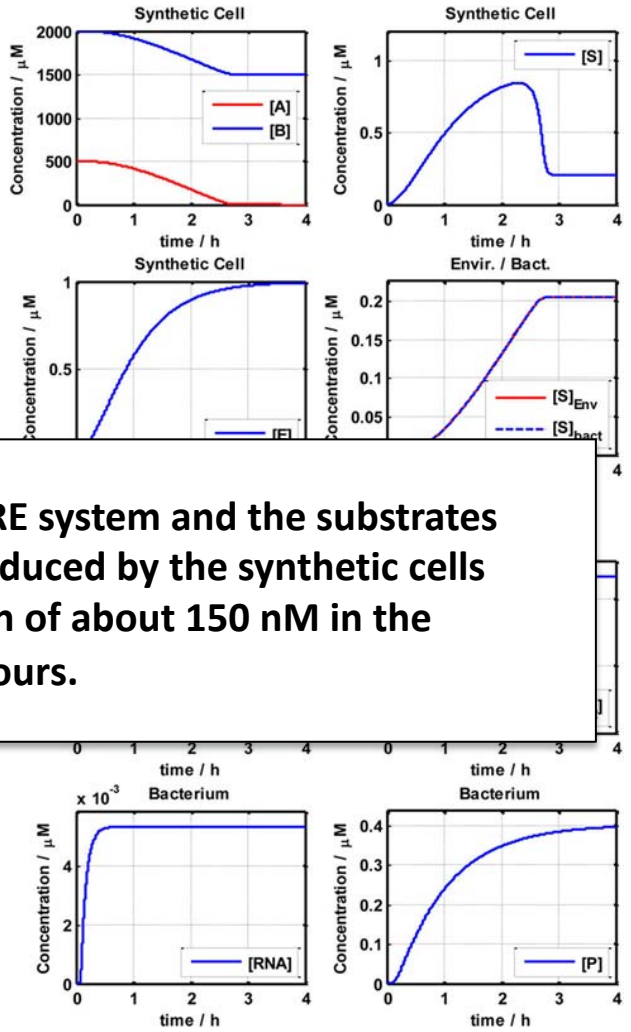
Rampioni *et al*. (2006) *Nat Comput* doi:10.1007/s11047-014-9425-x

Generation of synthetic cells interfacing with bacteria
1) *in silico* modelling

Physical parameters, thermodynamic and kinetic constants used in the model

Symbol	Meaning	Value	Units
V	Reaction volume	$2\ 10^{5}$	μm^3
N_{sc}	Number of synthetic cell in V	1	
N_{bact}	Number of bacteria in V	320	
	Synthetic cell radius	2.7	μm
σ_{sc}	Synthetic cell surface	91.6	μm^2
V_{sc}	Synthetic cell volume	84.2	μm^3
V_{bact}	Bacterium volume	1	μm^3
σ_{bact}	Bacterium surface	4.8	μm^2
k_{TXPS}	Transcription rate (PURE system)		
k_{TLPS}	Translation rate (PURE system)		
k_{TXPS} k_{TLPS}	Product of TX-TL rates (PURE system)	$2.8\ 10^{-7}$	$\mu M\ s^{-2}$
$k_{inactPS}$	Translation inactivation constant (PURE system)	$5.3\ 10^{-4}$	s^{-1}
k_{cat}	Catalytic constant of the enzyme E	0.1	s^{-1}
K_{MA}	Michaelis–Menten constant for A	10	μM
K_{MB}	Michaelis–Menten constant for B	200	μM
K_{MTX}	RNA polymerase/DNA binding constant	0.5	μM
K_{MR2S2}	Hill affinity constant of R_2S_2/DNA promoter	$2.5\ 10^{-5}$	μM
n	Hill cooperative coefficient	1.5	
$k_{deg\text{-}mRNA}$	mRNA degradation rate constant	$3\ 10^{-3}$	s^{-1}
C_{RNApol}	RNA polymerase concentration	$6\ 10^{-2}$	μM
C_{DNA}	Promoter/reporter gene concentration	$2\ 10^{-3}$	μM
L	Length of the reporter protein P	250	aa
k_{TL}	Translation rate	15	$aa\ s^{-1}$
K_{MTL}	Ribosome/mRNA binding constant	0.1	μM
$k_{deg\text{-}P}$	Protein degradation rate constant	$3\ 10^{-4}$	s^{-1}
C_{rib}	Ribosome concentration	0.04	μM

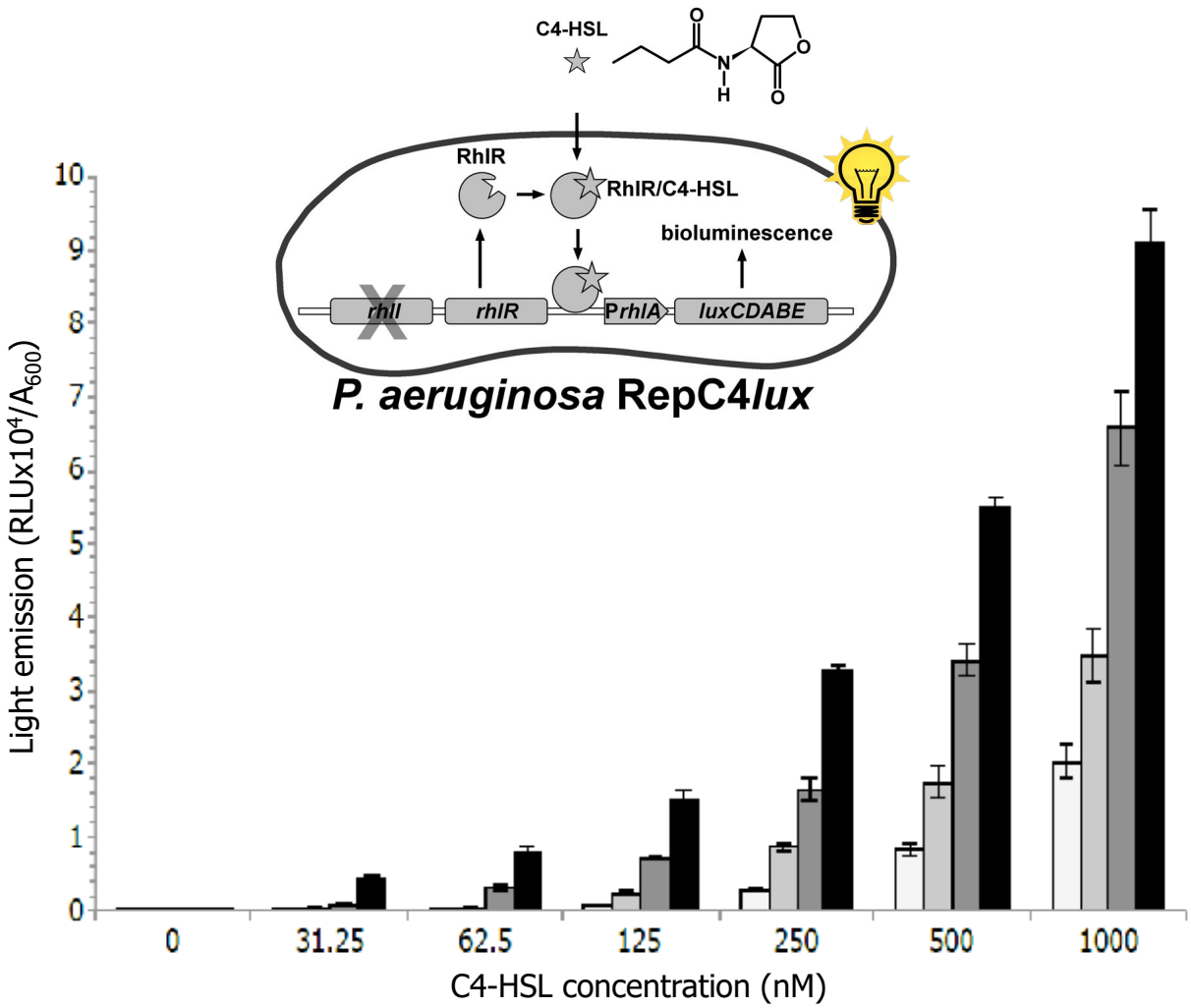
Results of numerical integration



If containing a plasmid with the *rhlI* gene, the PURE system and the substrates SAM and C4-CoA, the C4-HSL signal molecule produced by the synthetic cells expressing RhlI should reach the concentration of about 150 nM in the environment within 2.5 hours.

Rampioni *et al*. (2006) *Nat Comput* doi:10.1007/s11047-014-9425-x

Generation of synthetic cells interfacing with bacteria
2) wet-lab experiments

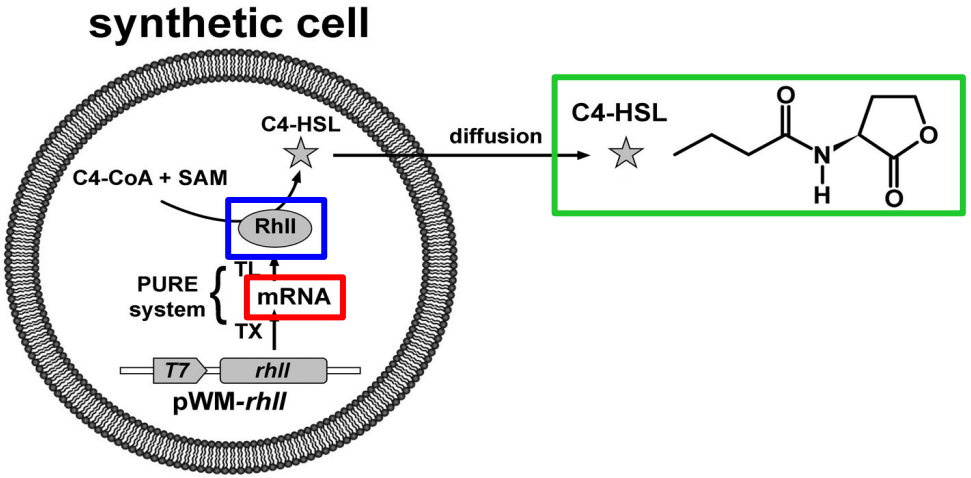


Growth conditions

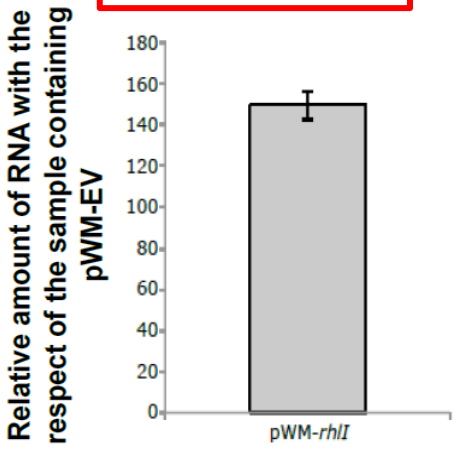
- Kanamycin 200 µg/mL, 37 °C, shaking, low cell density.
- Kanamycin 200 µg/mL, 37 °C, shaking, low cell density, depletion of $3OC_{12}$-HSL.
- Kanamycin 500 µg/mL, 37 °C, shaking, blow cell density, depletion of $3OC_{12}$-HSL.
- Kanamycin 500 µg/mL, 30 °C, static, high cell density, depletion of $3OC_{12}$-HSL.

Rampioni *et al*. (2018) *Chem Commun (Camb)* 54:2090-2093.

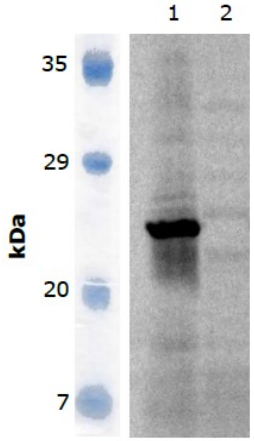
Generation of synthetic cells interfacing with bacteria
2) wet-lab experiments



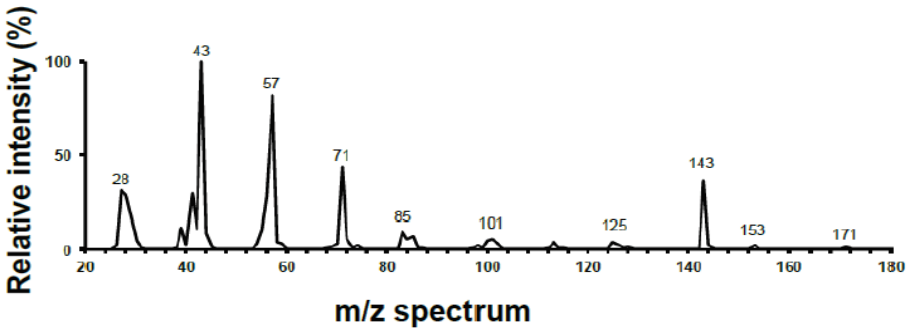
Transcription of the *rhlI* gene



Expression of the RhlI enzyme

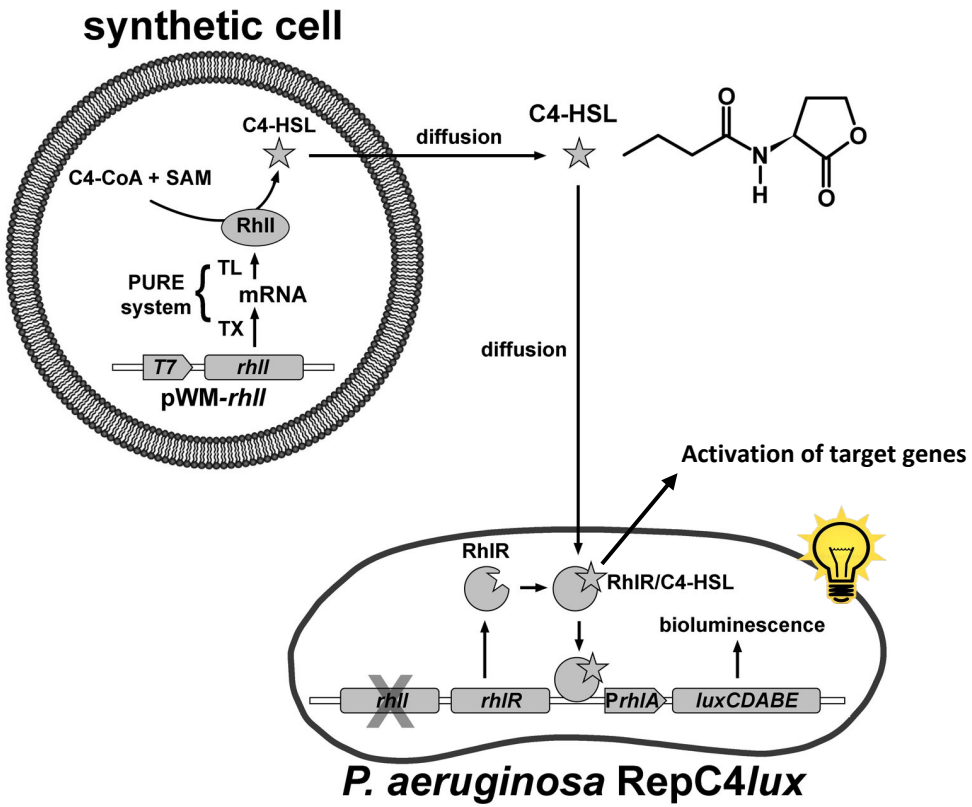


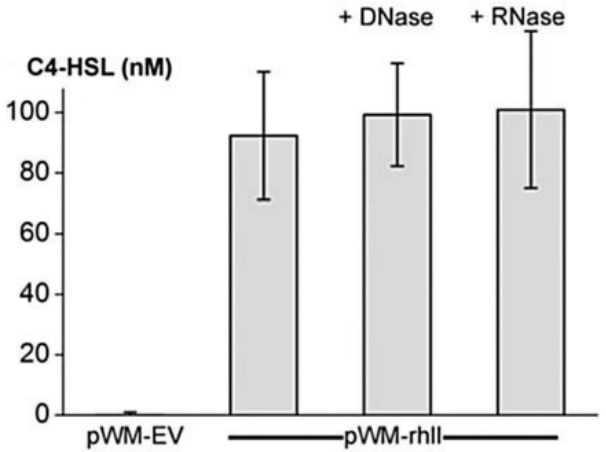
Synthesis of the C4-HSL signal molecule

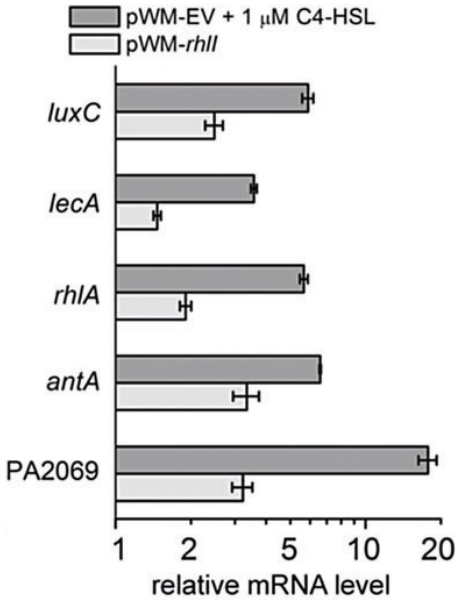


Rampioni *et al*. (2018) *Chem Commun (Camb)* 54:2090-2093.

Generation of synthetic cells interfacing with bacteria
2) wet-lab experiments

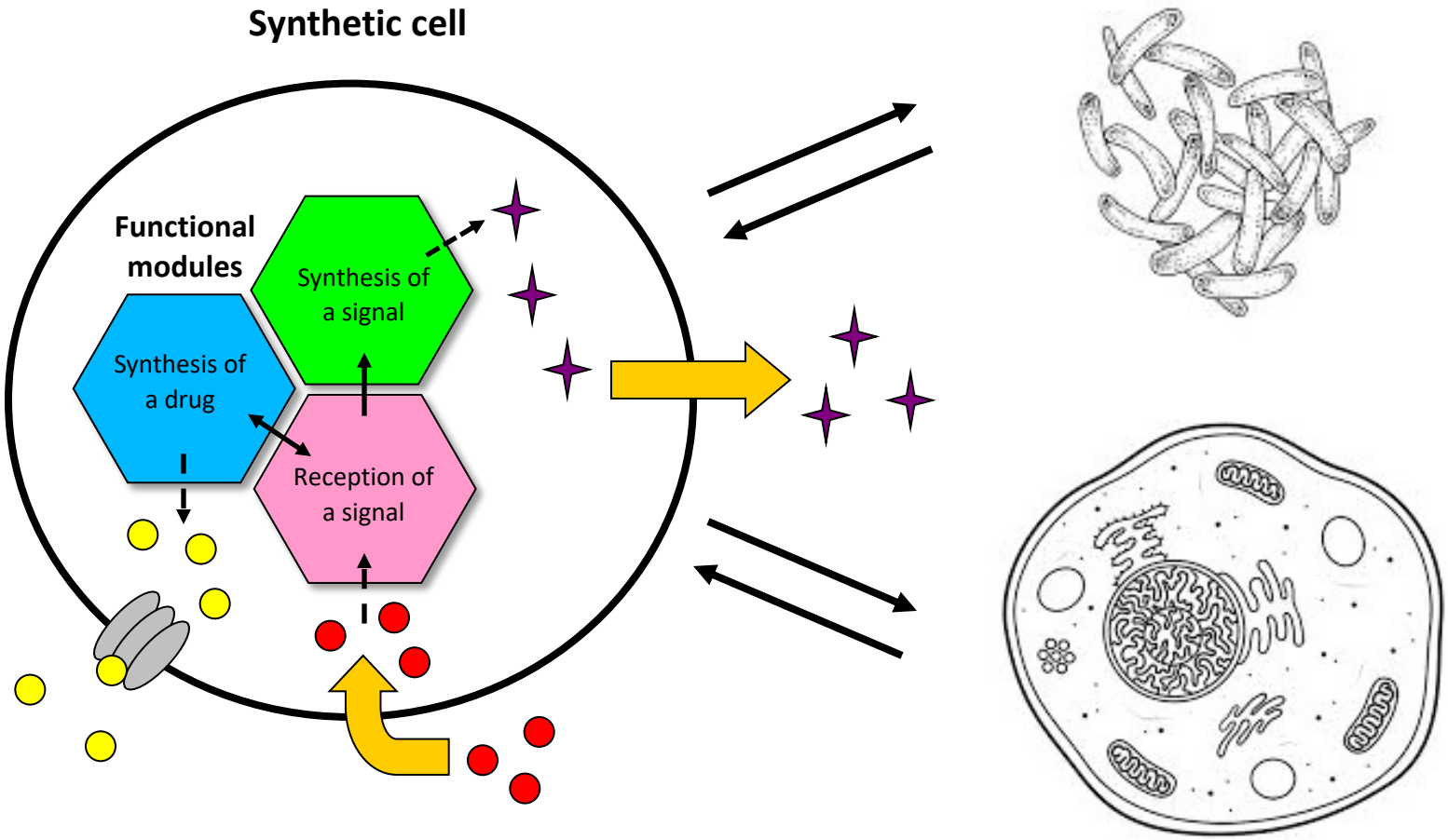






Rampioni *et al*. (2018) *Chem Commun (Camb)* 54:2090-2093.

Generation of synthetic cells interfacing with bacteria to develop innovative drug delivery approaches



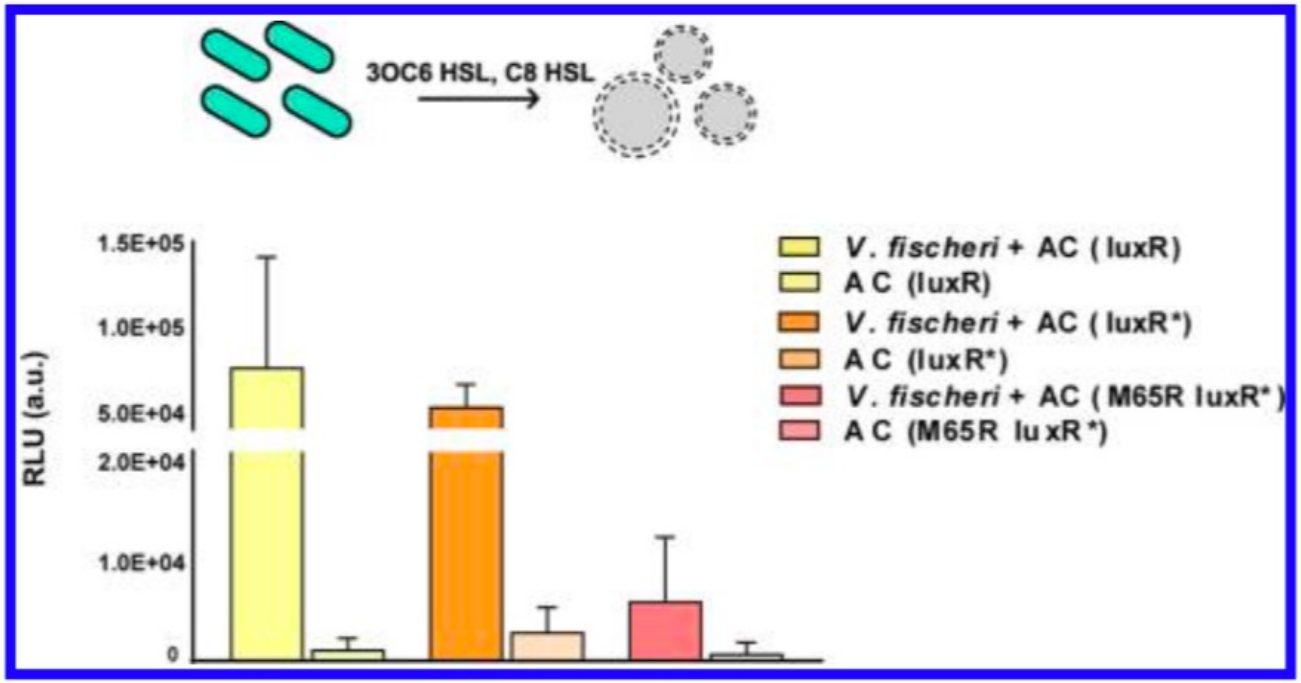
ACS central science

Research Article

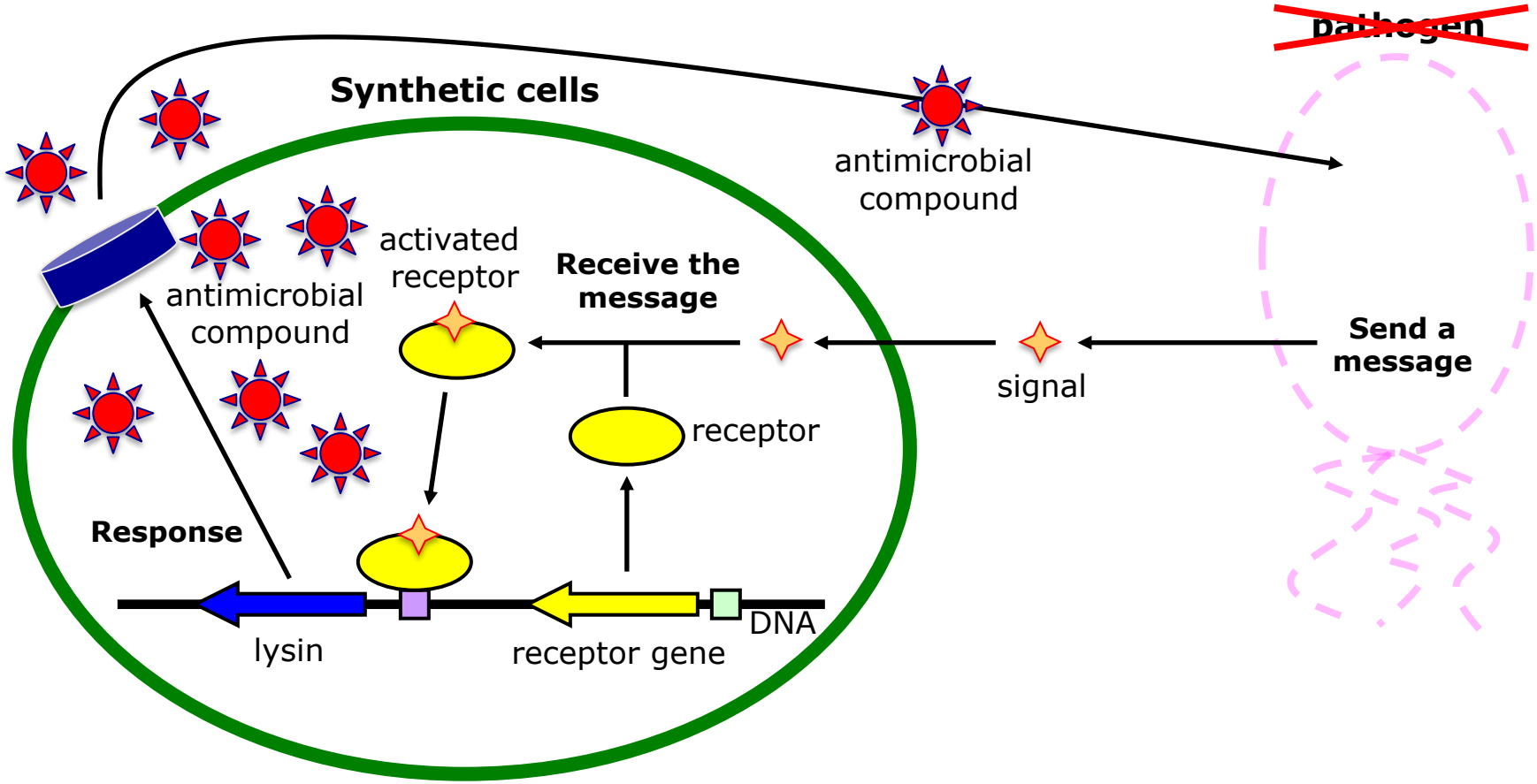
http://pubs.acs.org/journal/acscii

Two-Way Chemical Communication between Artificial and Natural Cells

Roberta Lentini,[†,‡] Noël Yeh Martín,[†,‡] Michele Forlin,[†] Luca Belmonte,[†] Jason Fontana,[†] Michele Cornella,[†] Laura Martini,[†] Sabrina Tamburini,[†] William E. Bentley,[§] Olivier Jousson,[†] and Sheref S. Mansy*[,†]



Generation of synthetic cells interfacing with bacteria to develop innovative drug delivery approaches



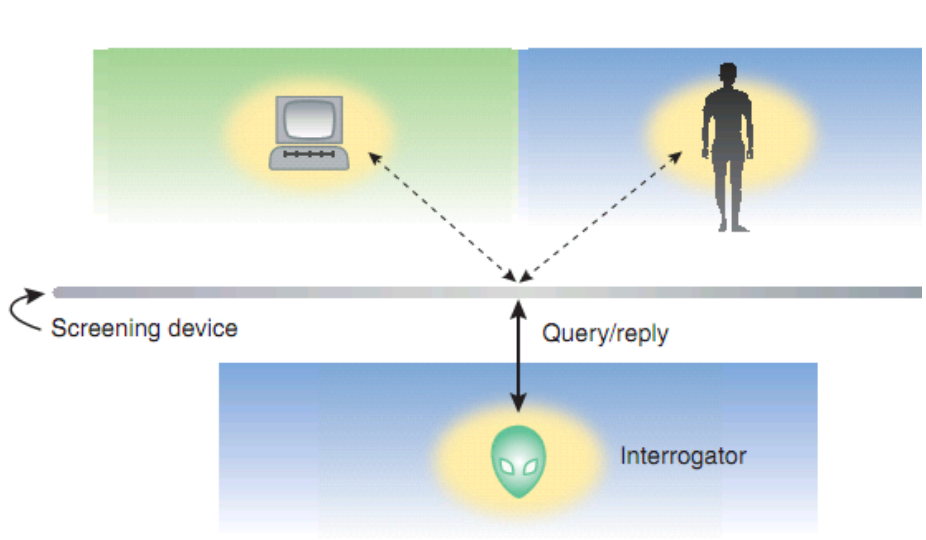
The Imitation Game

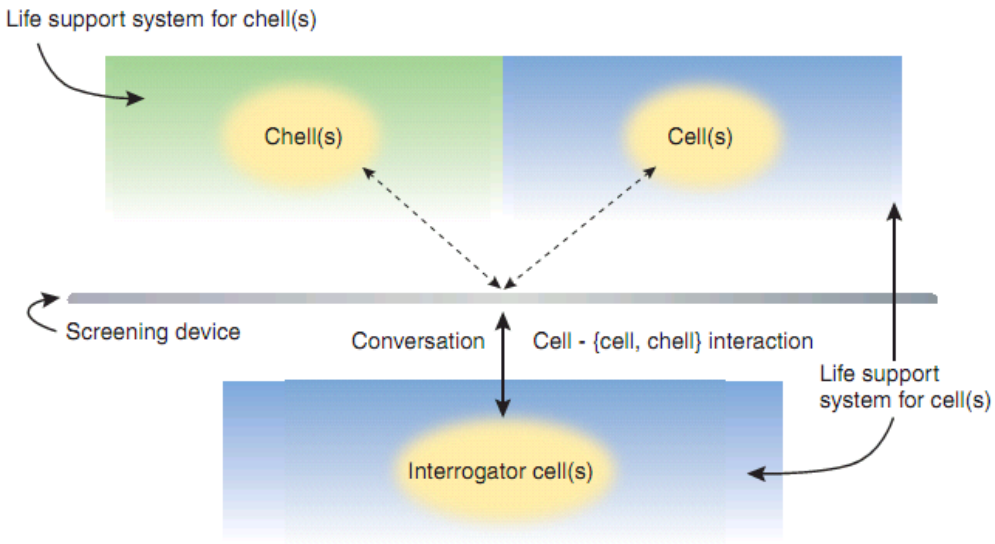
The generation of minimal cells interfacing with natural cells has interesting implications also in theoretical-philosophical fields.

The imitation game—a computational chemical approach to recognizing life

Leroy Cronin, Natalio Krasnogor, Benjamin G Davis, Cameron Alexander, Neil Robertson, Joachim H G Steinke, Sven L M Schroeder, Andrei N Khlobystov, Geoff Cooper, Paul M Gardner, Peter Siepmann, Benjamin J Whitaker & Dan Marsh

When is an artificial cell alive? A Turing test–like method may provide the answer.






Thank you for the attention