# Exploitation of bacterial communication processes for new therapeutic approaches

**Giordano Rampioni**

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**La Sapienza, December 7th, 2023**

# From the asocial existence 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.*

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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."***

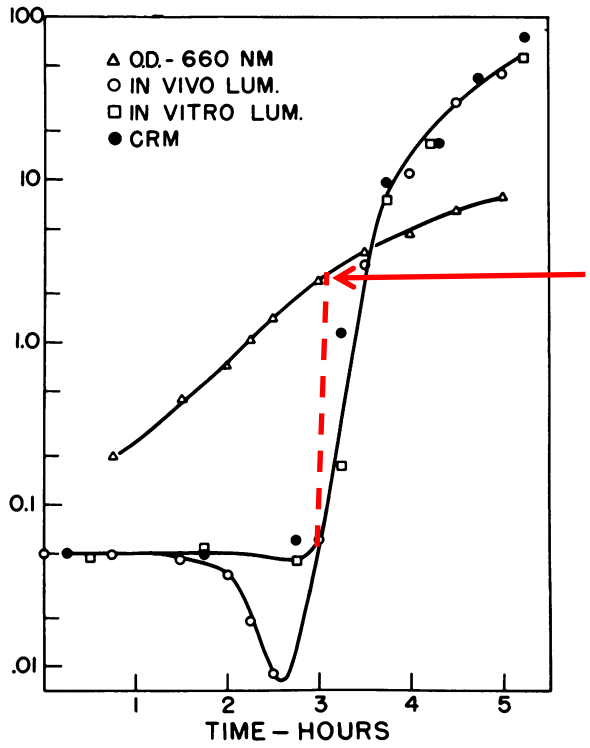
Alexander Tomasz

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

# From the asocial existence 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 suspensions.

**culture of *A. fischeri***


**Bioluminescence is emitted only at high cell density**

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

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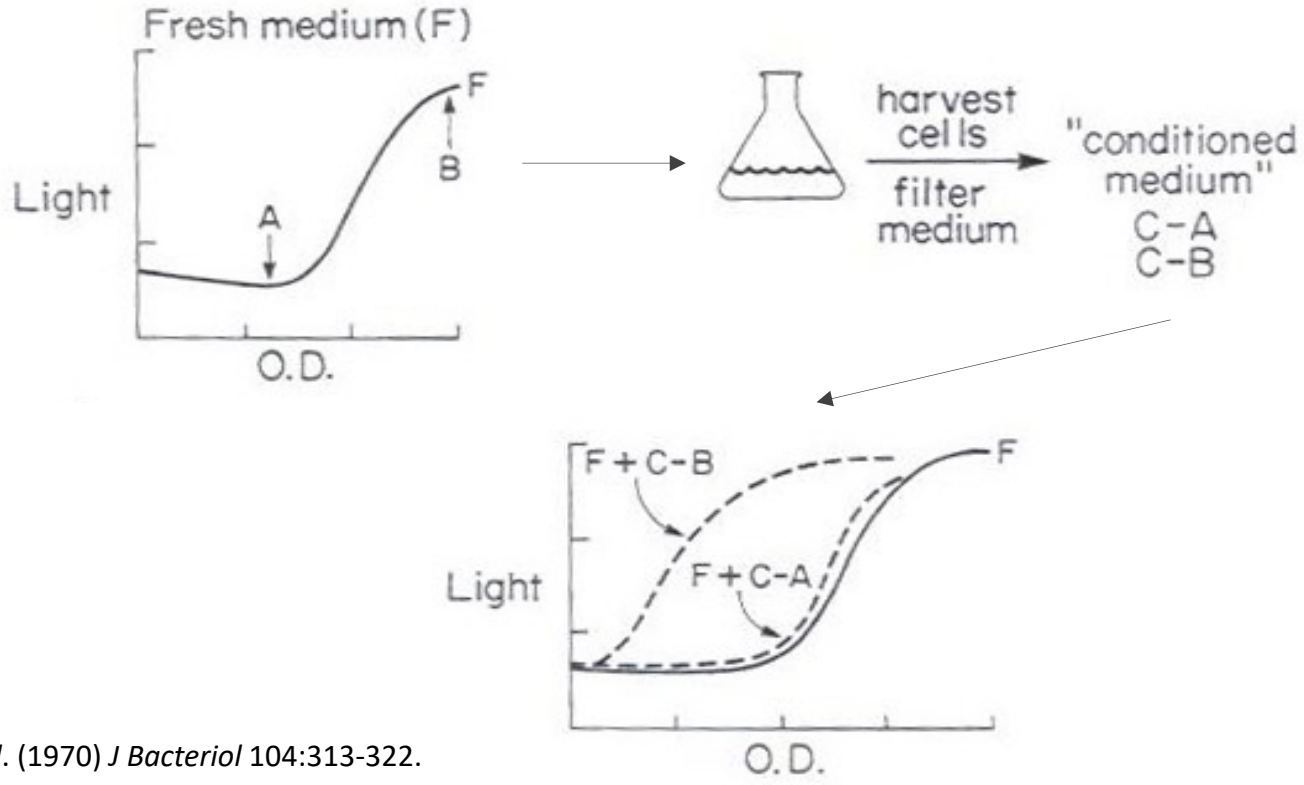
Light production could be induced at low cell density by the exogenous provision of cell-free supernatants from a bacterial culture grown to high cell density.




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

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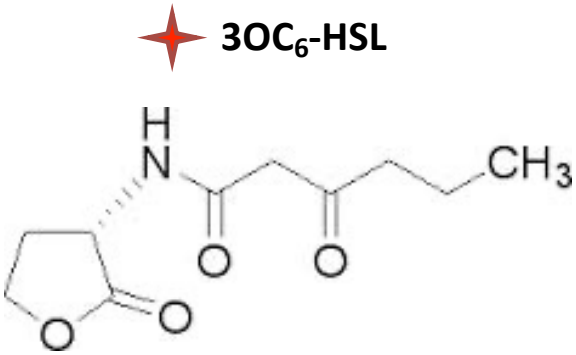
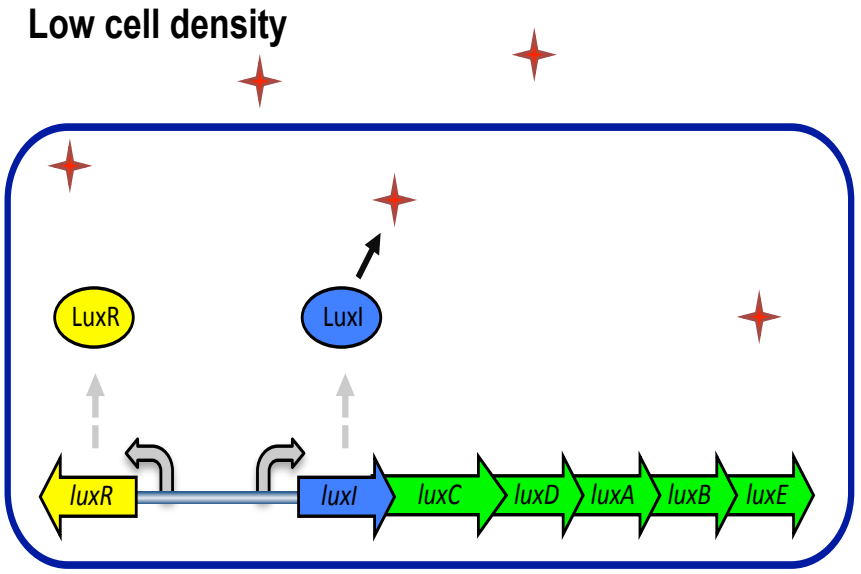
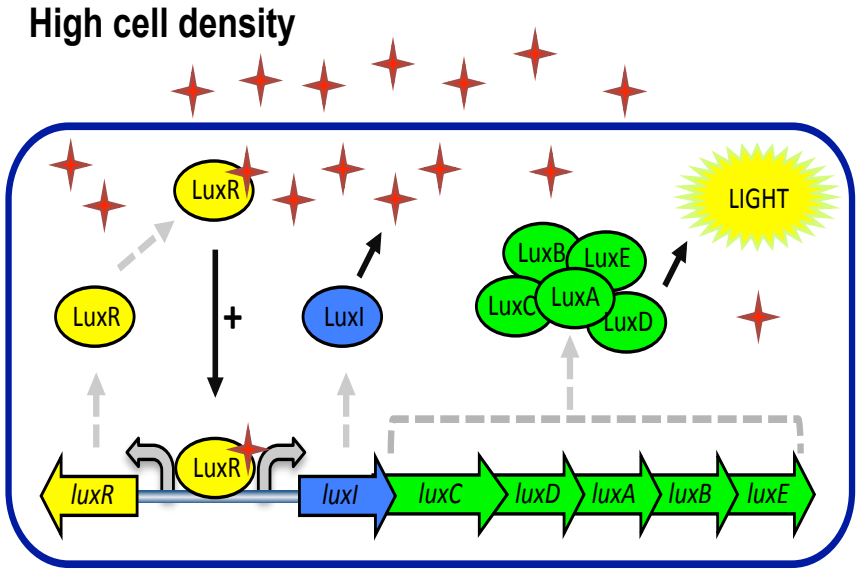
Light emission in controlled by a signal molecule produced by the single cells and released in the environment.

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.



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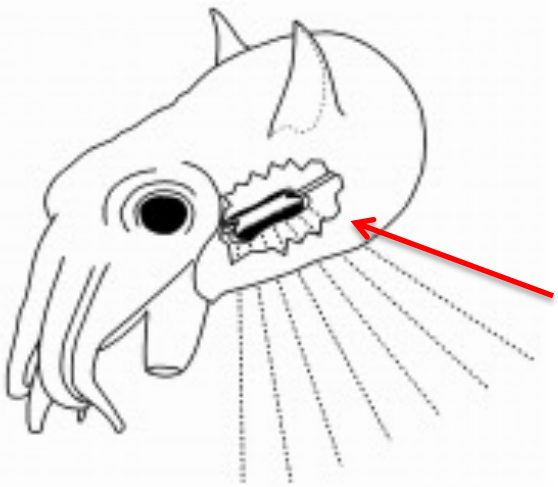
*A. fischeri* is a marine bacterium that colonizes the light organ of the squid *Euprymna scolopes*, an ecological niche rich in nutrients that allows the growth of the bacterial population to high cell density.

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**

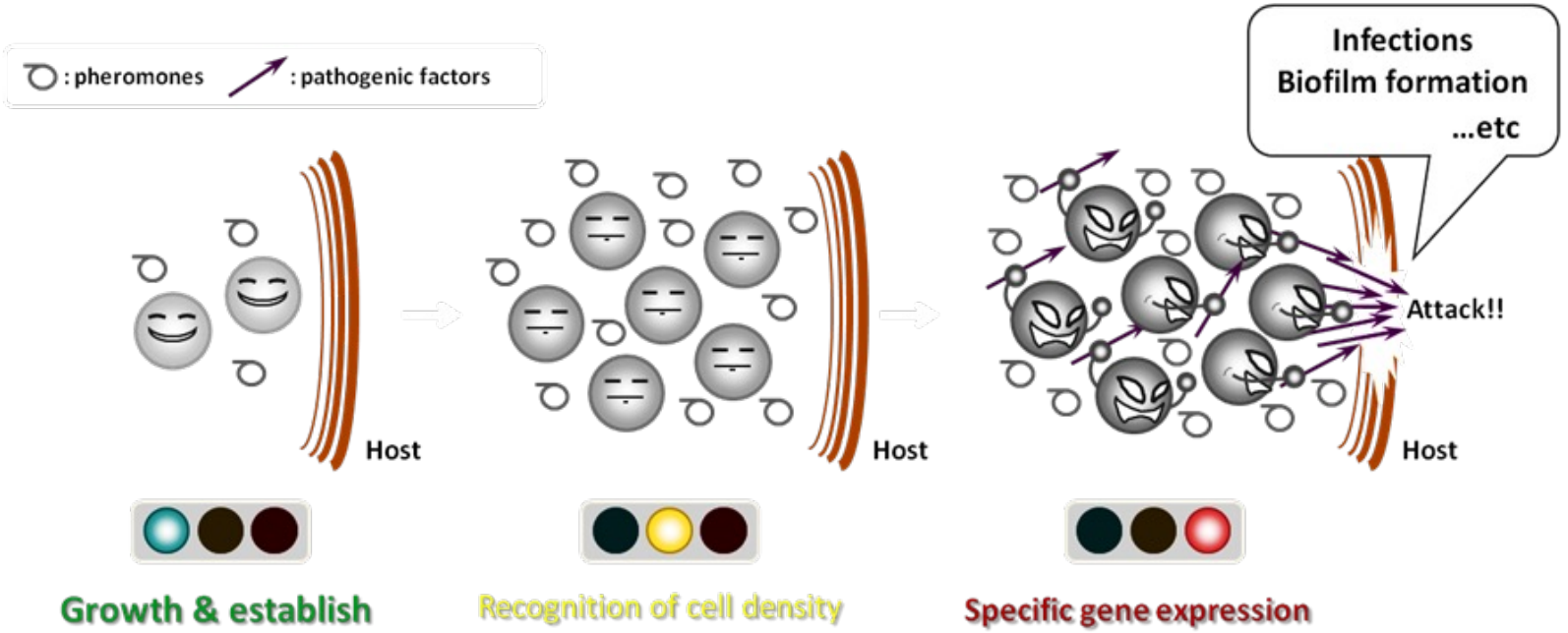


**Light organ colonized by *A. fischeri***

# From the asocial existence of bacteria to socio-microbiology

Many bacterial pathogens control the expression of virulence factors via quorum sensing (QS). It is believed that single bacterial cells producing extracellular virulence factors would be easily defeated by the immune system. So, single bacterial cell inside the host dedicate their energy to increase their population size before producing extracellular virulence factors aimed to damage the host cell and activate the immune system.

Since in many bacterial pathogens key QS-controlled phenotypes are extracellular virulence factors, some researchers proposed that QS did not evolve to sense the population desnity, rather the diffusion of secreated molecules far from the producers (mass transfer).

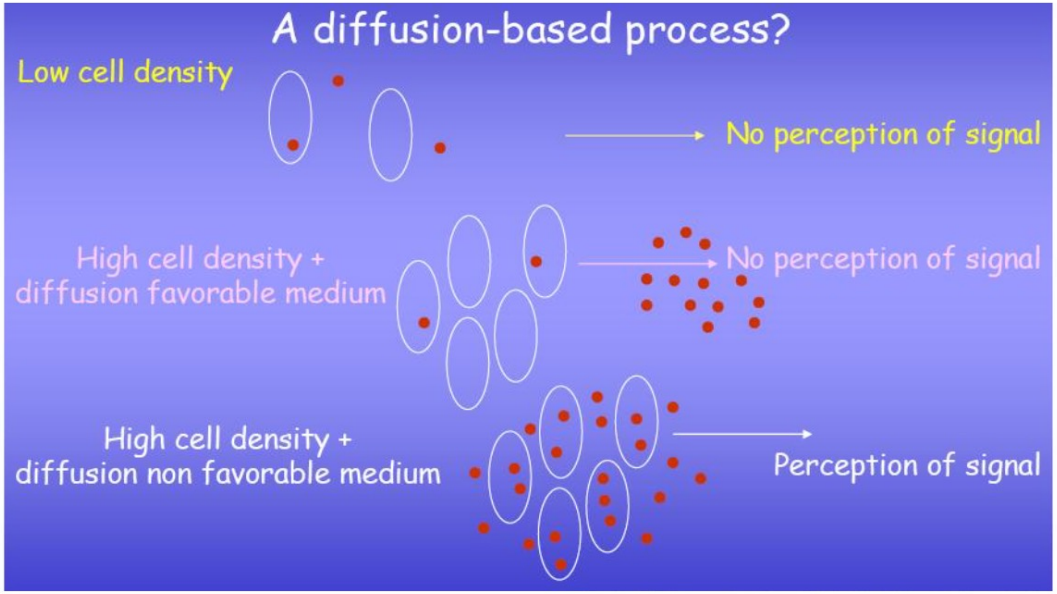


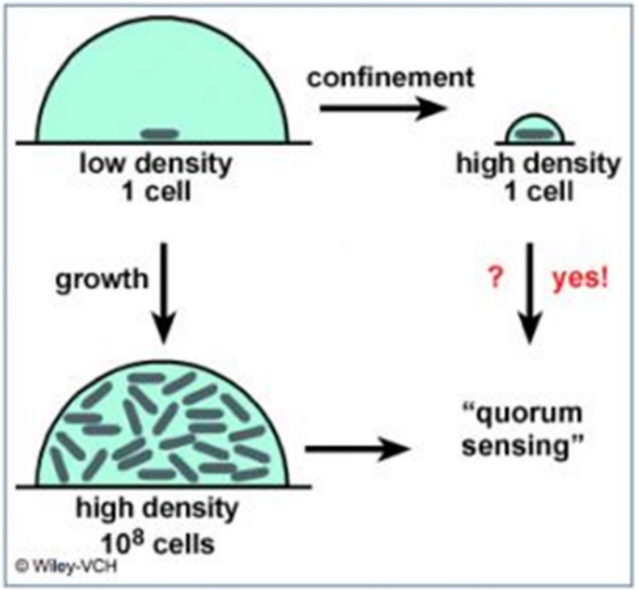
# From the asocial existence of bacteria to socio-microbiology

In this perspective, it has been proposed to rename QS as Diffusion Sensing, *i.e.* the ability to monitor mass transfer via the secretion of small molecules whose production has a limited metabolic cost. Only if the small molecules accumulate in the surrounding of the producer cells, they activate the metabolically expensive expression of multiple secreated virulence factors.

The Diffusion Sensing idea is supported by the evidence that, if confined in a small space, single cells can activate the QS response!

Now it is believed that QS and Diffusion Sensing both contribute to the same phenomenon, that is the ability of bacterial cells to produce extracellular virulence factors once the signalling molecules reach a treshold concentration.



# From the asocial existence of bacteria to socio-microbiology

There are many exceptions to the convention view of QS as a simple cell density dependent regulatory switch. Maybe, the description of QS coming from pioneering studies in *A. fischeri* is not the rule.

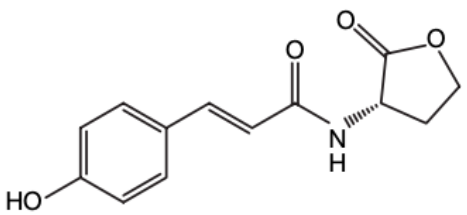
doi:10.1038/nature07088 nature

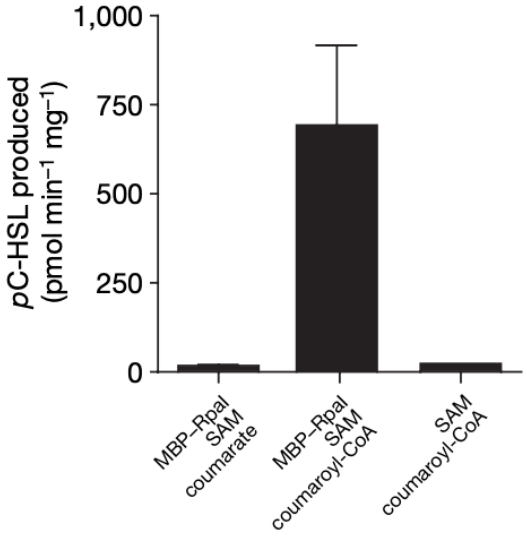
ARTICLES

## A new class of homoserine lactone quorum-sensing signals

Amy L. Schaefer[1], E. P. Greenberg[1], Colin M. Oliver[2], Yasuhiro Oda[1], Jean J. Huang[1], Gili Bittan-Banin[1], Caroline M. Peres[3], Silke Schmidt[4], Katarina Juhaszova[1], Janice R. Sufrin[2] & Caroline S. Harwood[1]

***p*-cumaroyl–homoserine lactone (pC-HSL).**




The QS system of *Rhodopseudomonas palustris* is based on the production of a unique signal molecule, *p*-cumaroyl–homoserine lactone (pC-HSL).

*R. palustris* can produce pC-HSL, and hence activates QS, on at high cell density and if a source of the pC-HSL precursor *p*-cumaroyl is present in the medium.

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nature COMMUNICATIONS

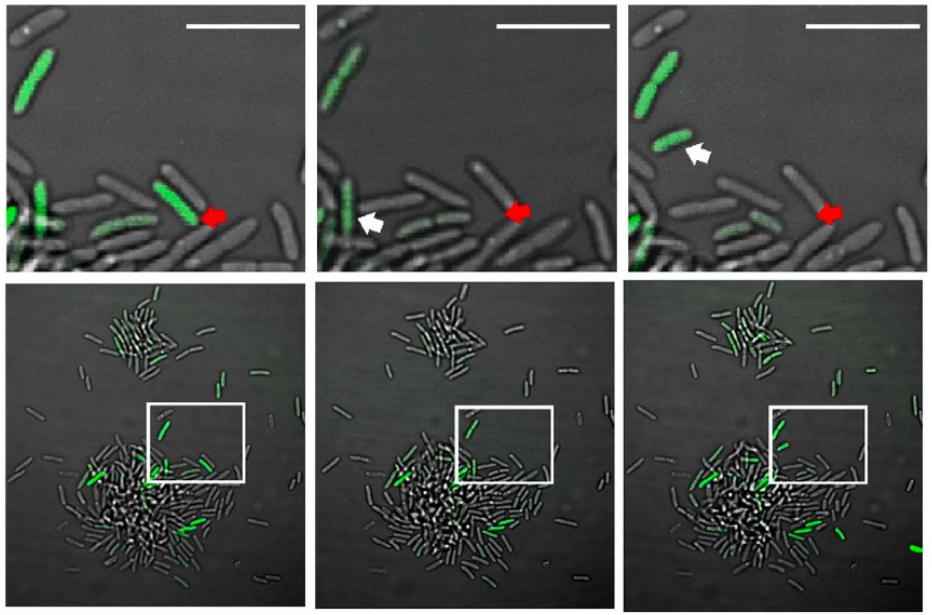
ARTICLE

Received 26 May 2014 | Accepted 24 Nov 2014 | Published 16 Jan 2015

DOI: 10.1038/ncomms6945 OPEN

Quorum sensing triggers the stochastic escape of individual cells from *Pseudomonas putida* biofilms

Gerardo Cárcamo-Oyarce[1,*], Putthapoom Lumjiaktase[1,*,†], Rolf Kümmerli[2,†] & Leo Eberl[1]



In *Pseudomonas putica* PCL1445, single cells activate QS during the early stages of biofilm formation due to stochastic regulatory mechanisms.

QS activation promotes migration of single quorate cells out of the microcolony.

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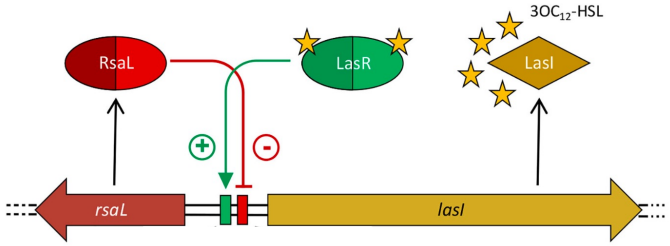
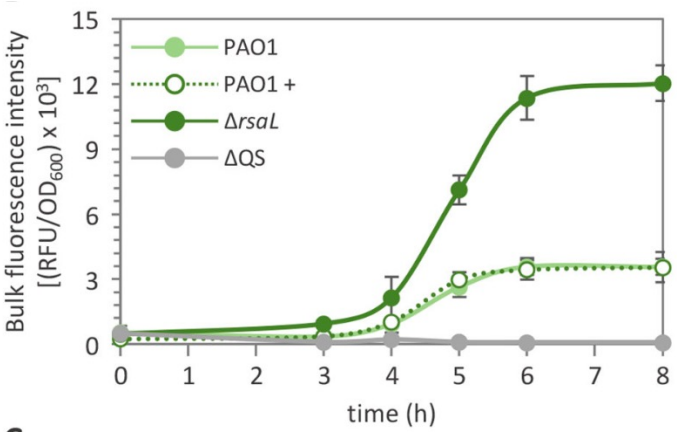
AMERICAN SOCIETY FOR MICROBIOLOGY | mBio

Genetics and Molecular Biology | Research Article

**RsaL-driven negative regulation promotes heterogeneity in *Pseudomonas aeruginosa* quorum sensing**

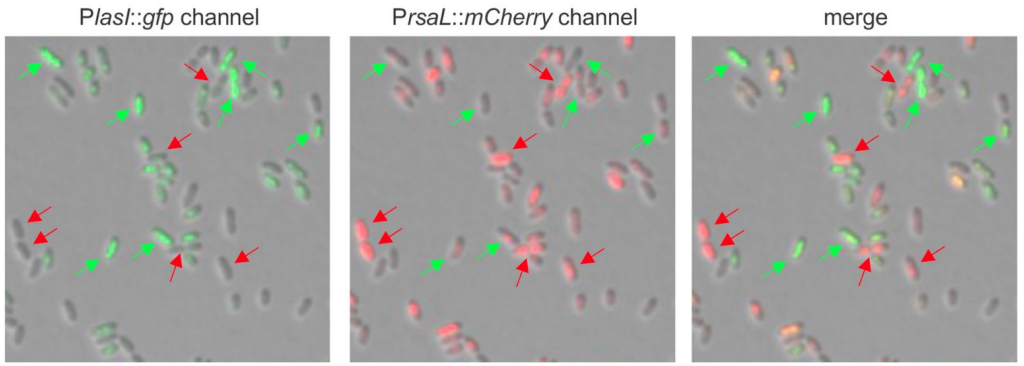
Marta Mellini,[1] Morgana Letizia,[1] Lorenzo Caruso,[1] Alessandra Guiducci,[1] Carlo Meneghini,[1] Stephan Heeb,[2] Paul Williams,[2] Miguel Cámara,[2] Paolo Visca,[1,3,4] Francesco Imperi,[1,3,4] Livia Leoni,[1] Giordano Rampioni[1,4]




In *Pseudomonas aerugionosa* PAO1 QS activation is graded and bimodal.

Single cells of the population do not activate QS in a synchronous way at a given cell density. Distinct sub-population of cells become quorate during growth. About 20% of cells remain in a non-quorate state also at high cell density due to a stochastic regulatory switch controlled by the negative regulator RsaL.



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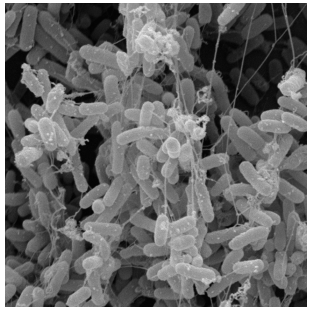
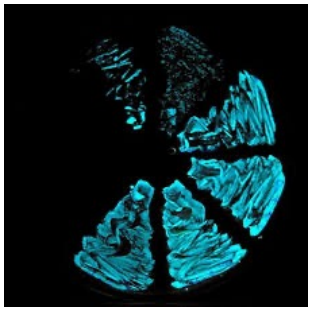
This intercellular communication system, know as ***quorum sensing (QS)***, controls group-behaviours in many bacteria.

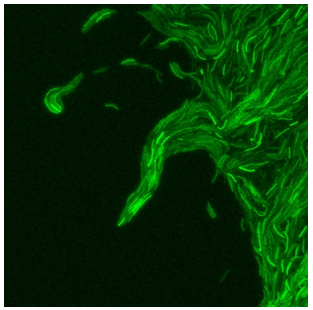
Biofilm

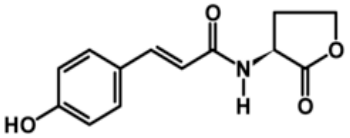


Bioluminescence

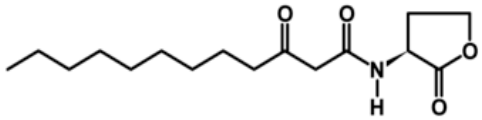


Collective movements

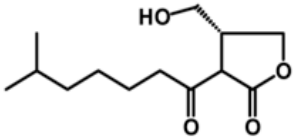




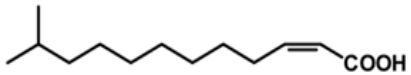
aroyl homoserine lactone
*p*C-HSL (*R. palustris*)



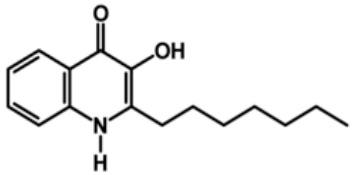
acylated homoserine lactone
3OC12-HSL (*P. aeruginosa*)



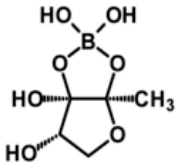
γ-butyrolactone
A-factor (*S. griseus*)



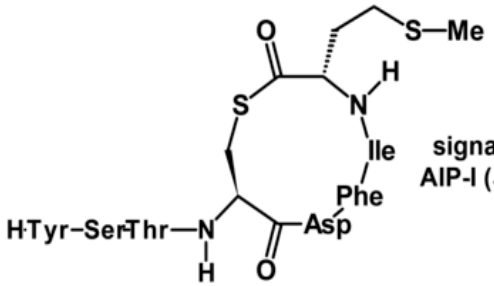
fatty acid derivative
DSF (*X. campestris*)



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

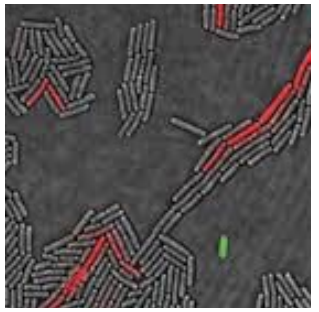


furanone
AI-2 (*V. harveyi*)



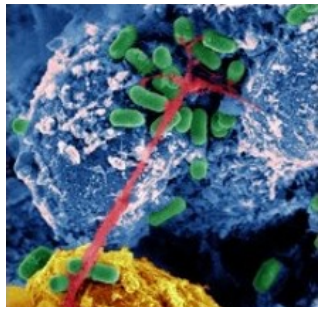
signal peptide
AIP-I (*S. aureus*)

Differentiation



Secondary metabolites

Interaction with the host



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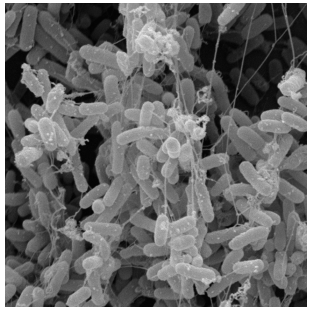

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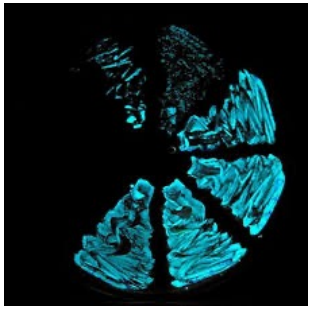
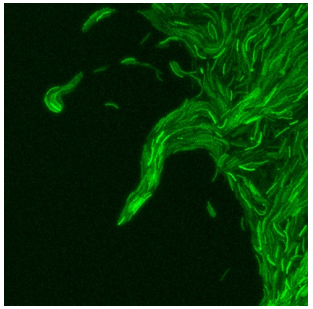
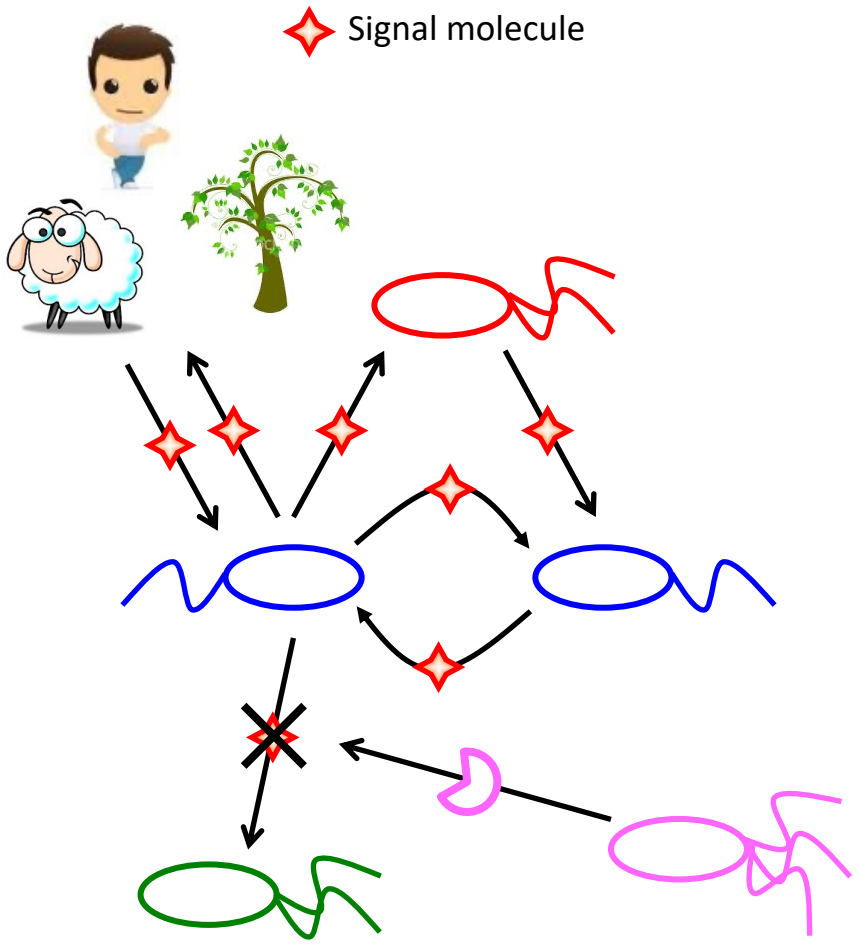


Collective movements



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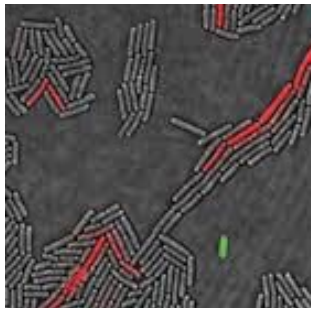

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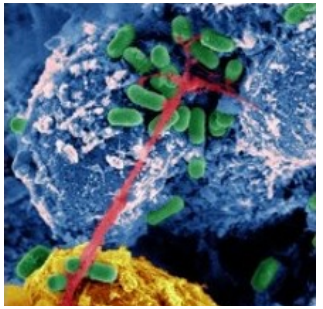


# Exploitation of QS for new therapeutic approaches

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 new therapeutic approaches.

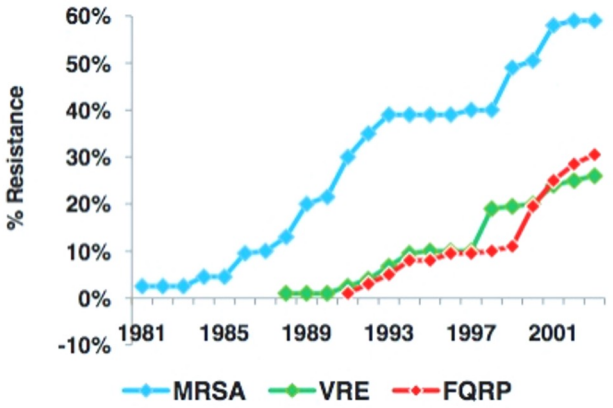
1) Inhibition of QS in bacterial pathogens (***anti-virulence approach***).
2) Use of QS signal molecules as molecular markers to detect pathogens (***biotic antibacterials***).
3) Generation of engineered bacteria able to synchronize their activities at the population level (***biotic antitumor agents***).
4) Generation of synthetic cells able to interface with natural cells (***soft-nanorobots***).

**ECDC/EMEA** JOINT TECHNICAL REPORT

# The bacterial challenge: time to react

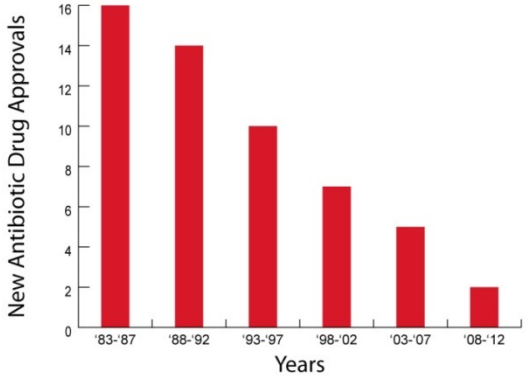
*A call to narrow the gap between multidrug-resistant bacteria in the EU and the development of new antibacterial agents*

Antibiotic resistance increases




BUT

The antibiotic pipeline is running dry




We are clearly facing the possibility of a

**FUTURE WITHOUT EFFECTIVE ANTIBIOTICS**

(WHO/EDM/PAR/2004.7. Priority Medicines Europe and the World)

# Inhibition of virulence as an alternative antibacterial strategy

NATURE REVIEWS | MICROBIOLOGY 300 | APRIL 2014 | VOLUME 12

ANTIBIOTIC ALTERNATIVES — OPINION

## Targeting virulence: can we make evolution-proof drugs?

*Richard C. Allen, Roman Popat, Stephen P. Diggle and Sam P. Brown*

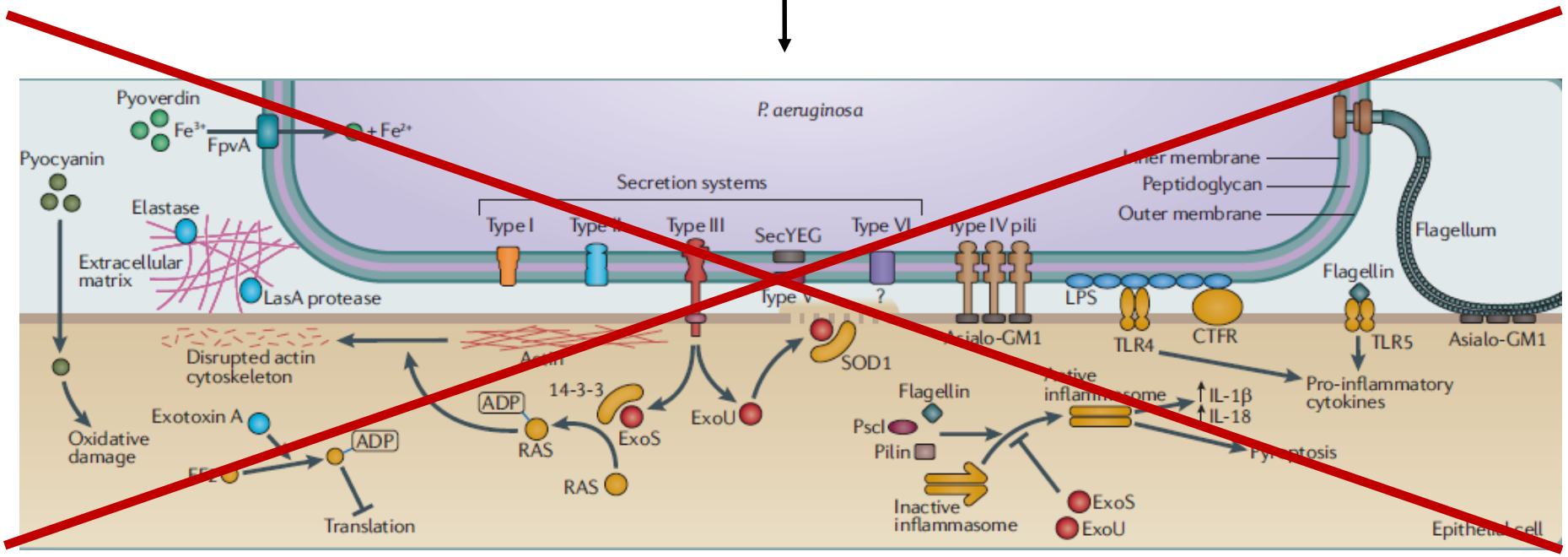
**Antivirulence drugs disarm bacteria without affecting growth**

- prevent/inhibit the establishment of the infection
- reduce the capability of pathogens to cause damage to the host
- should have less adverse effects on host microbiota (?)
- should impose weaker selective pressure for drug resistance

Rasko and Sperandio (2010) *Nat Rev Drug Discov* 9:117-128; Rampioni *et al.* (2017) *Emerg Top Life Sci* doi:10.1042/EBC20170018

# In many bacterial pathogens QS controls secreted virulence factors, hence QS is an ideal target for anti-virulence drugs

QS inhibitor ⊣ **QS**




Rasko and Sperandio (2010) *Nat Rev Drug Discov* 9:117-128; Hauser and Ozer (2011) *Nat Rev Micobiol* Poster

# Furanone C-30, the first QS inhibitor

The Australian red algae *Delisea pulchra* shows reduced bacterial colonization on its surface. This inhibition was found to be mediated by some secondary metabolites, called furanones.


***Figura 6 a*** – *Delisea pulchra.*

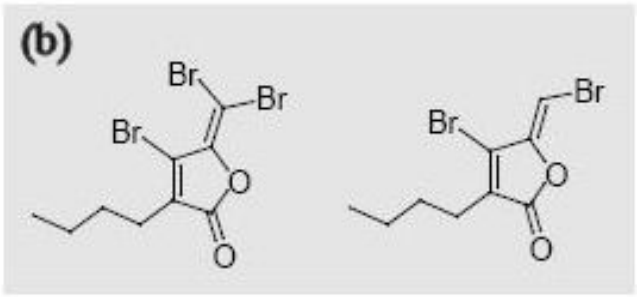
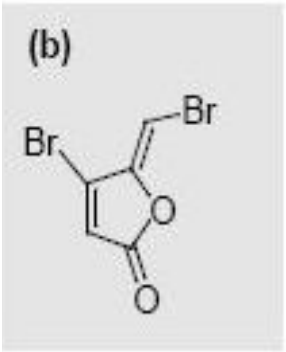



***Figura 6b*** – Furanoni naturali (modificata da Suga e Smith, 2003).

*D. pulchra* produces at least 30 different furanones which are contained in special vesicles and released at the surface level of the thallus. The concentration of furanones is inversely proportional to the degree of bacterial colonization. Furanones inhibit QS because, by binding to LuxR-like receptors, they induce their rapid degradation.

# Furanone C-30, the first QS inhibitor

Structural analogs of furanones produced by *D. pulchra*, such as furanone C-30, have been synthesized in Prof. Givskov's laboratory in Denmark.




Furanone C-30 reduces the production of virulence factors in *P. aeruginosa* without affecting its growth.

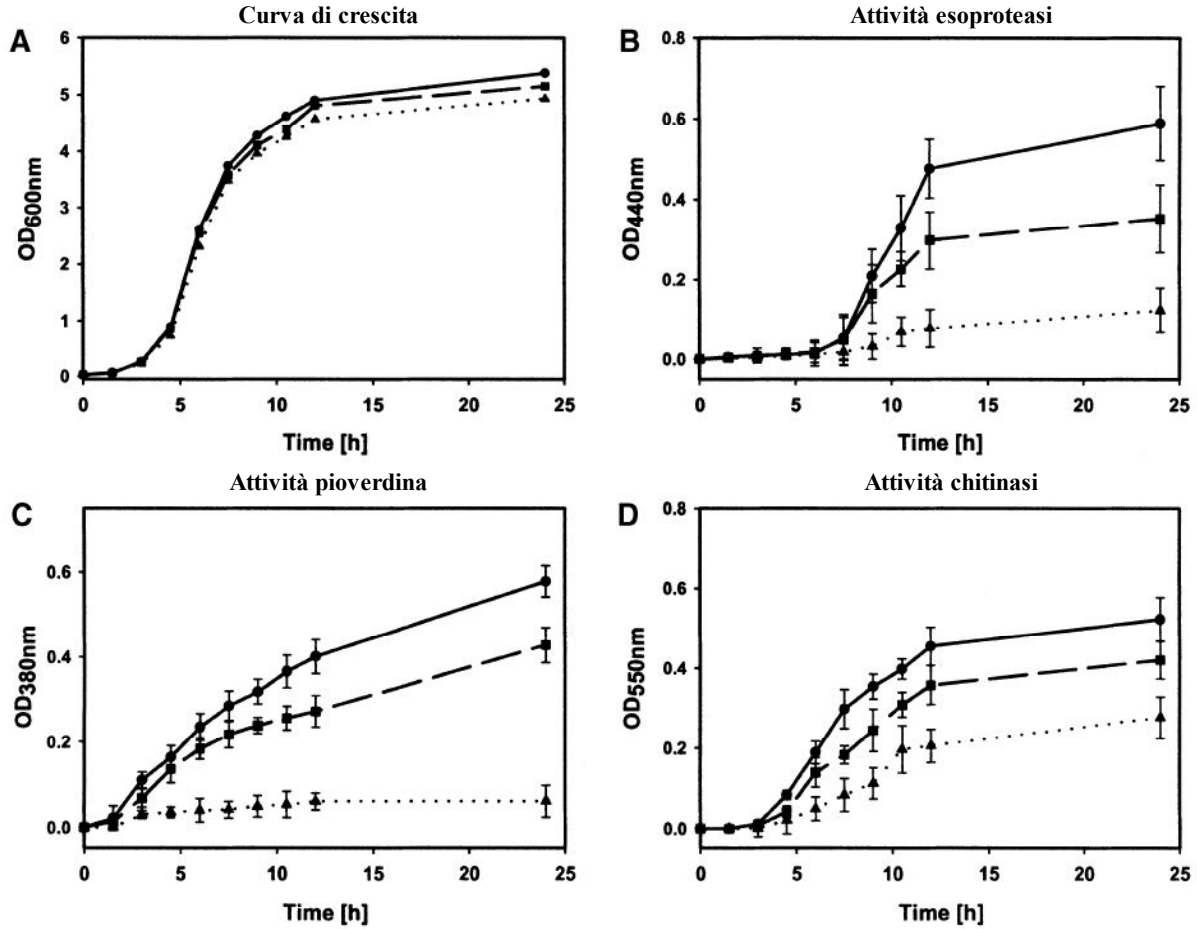



***Figura 8*** – Influenza del furanone C-30 sulla crescita e la produzione di fattori di virulenza di *P. aeruginosa* PAO1. La linea continua indica colture cresciute in assenza di inibitore, la linea tratteggiata quelle cresciute con 1 μM e quella punteggiata colture cresciute con 10 μM di furanone (modificata da Hentzer *et al.*, 2003).

# Furanone C-30, the first QS inhibitor

Microarray analyses showed that furanone C-30 represses the transcription of about 90 genes *in P. aeruginosa*.

About 80% of these genes are known to be activated by QS.

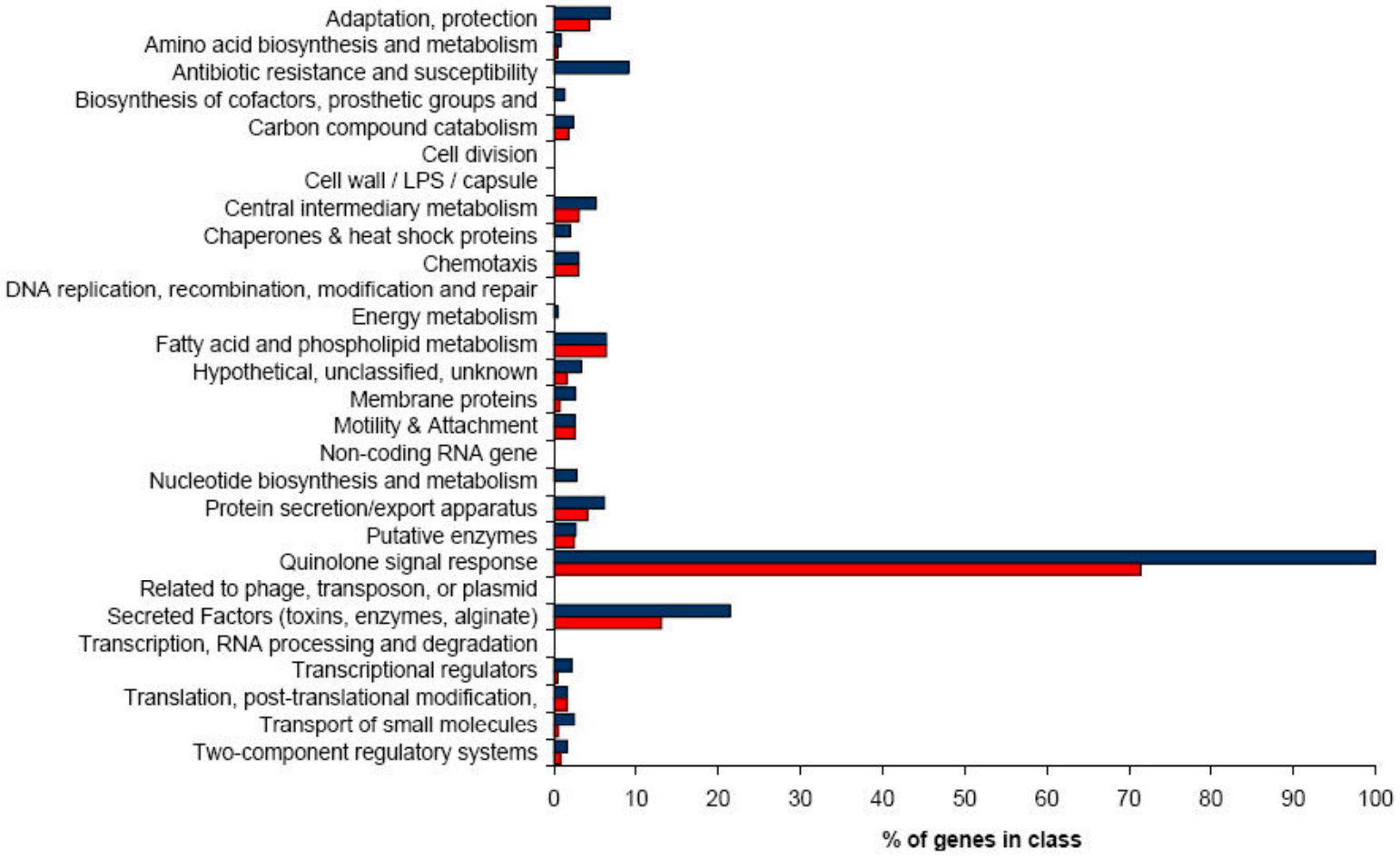



***Figura 10*** – Percentuale dei geni attivati da QS (blu) e repressi da C-30 (rosso) suddivisi in gruppi funzionali (Hentzer *et al.*, 2003 - suppl. data).

# Furanone C-30, the first QS inhibitor

Furanone C-30 increases the sensitive to antibiotics of *P. aeruginosa* biofilma (live cells, green – dead cells, red).

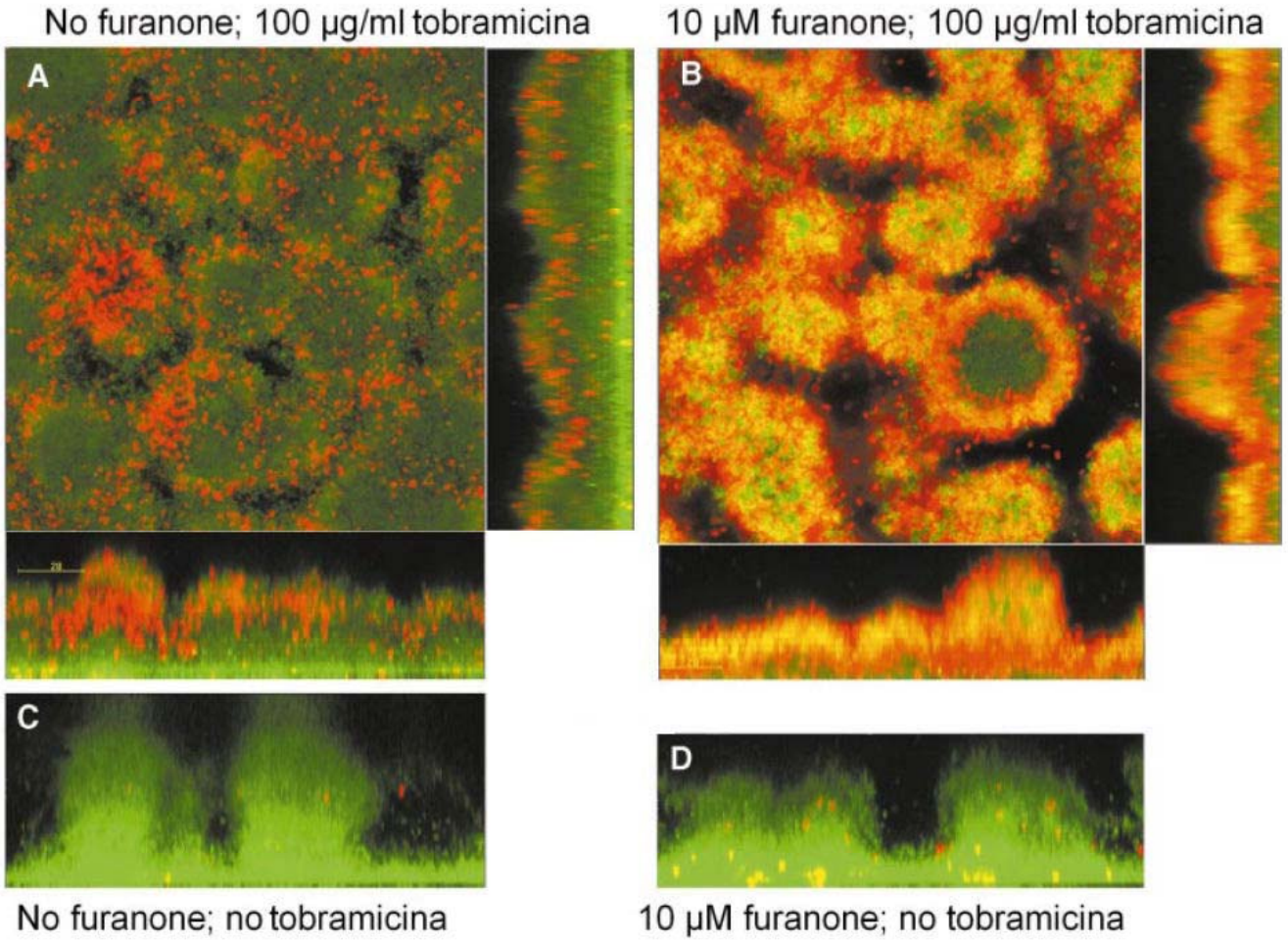
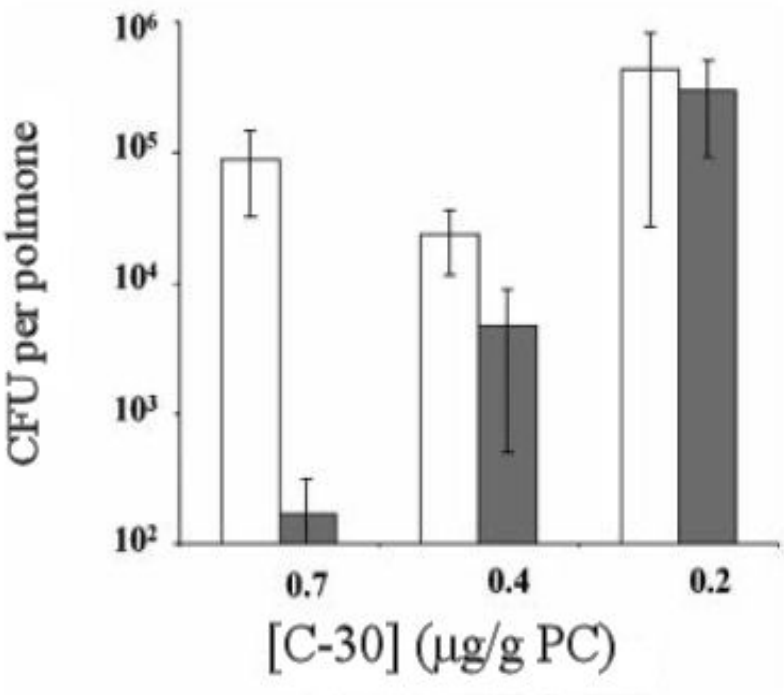


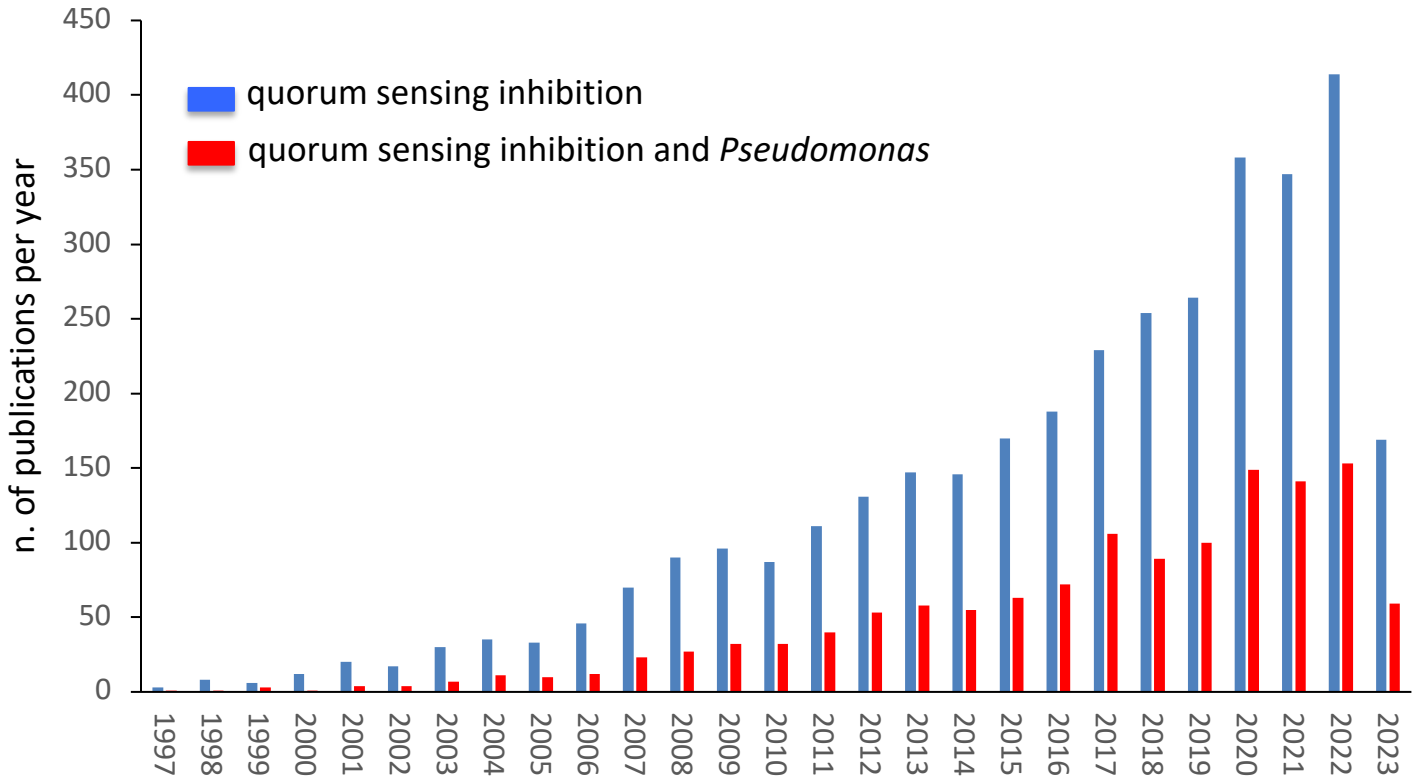

***Figura 12*** – Sensibilità alla tobramicina del biofilm di PAO1. Dopo 3 giorni di crescita i biofims vengono esposti a 100 µg/ml di tobramicina per 24 ore. La vitalità delle cellule è stata rilevata usando un LIVE/DEAD *Bac*Light Bacterial Viability Kit: le aree rosse sono cellule morte e le aree verdi sono cellule vive. (A) 100 µg/ml di tobramicina, (B) furanone 10µM e tobramicina 100 µg/ml, (C) assenza di furanone e tobramicina e (D) furanone 10µM. Immagini ottenute al SCLM, vedi testo (modificata da Hentzer *et al.*, 2003)

# Furanone C-30, the first QS inhibitor

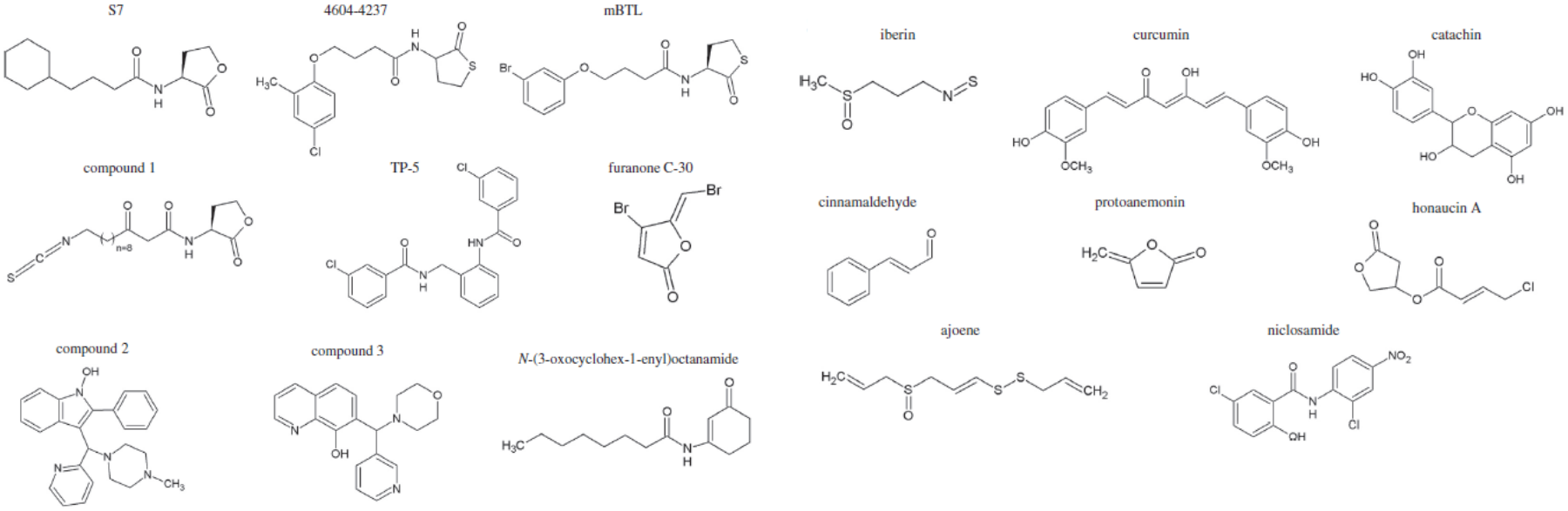
Twenty mice were infected with *P. aeruginosa* PAO1 at day zero and divided into two groups of ten individuals. The two groups of mice were treated with injections of furanone C-30 (~ 0.7 µg/g PC) or saline (placebo), respectively, at 8-hour intervals for the next three days. Seven days after infection, the lungs were removed, homogenized and plated for CFU determination. Animals treated with furanone C-30 showed a three orders of magnitude reduction in the bacterial load relative to the controls. The efficacy of the treatment was directly related to the concentration of the inhibitor, as shown by two other similar experiments perfoermd with ~0.4 µg/g PC and ~0.2 µg/g PC furanone C-30.



# More than 100 papers/year published on QS inhibition since 2012, most of which focused on inhibition of QS in *P. aeruginosa*



# Many QS inhibitors have been described so far

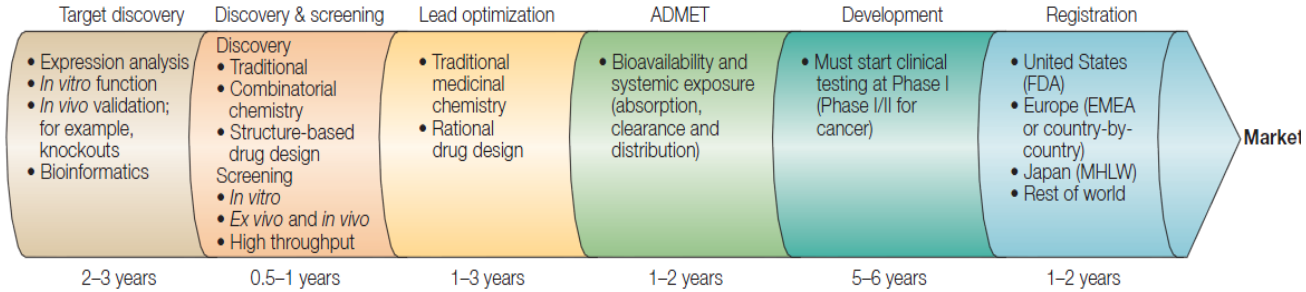



# Unfortunately, most of them have unfavourable pharmacological properties

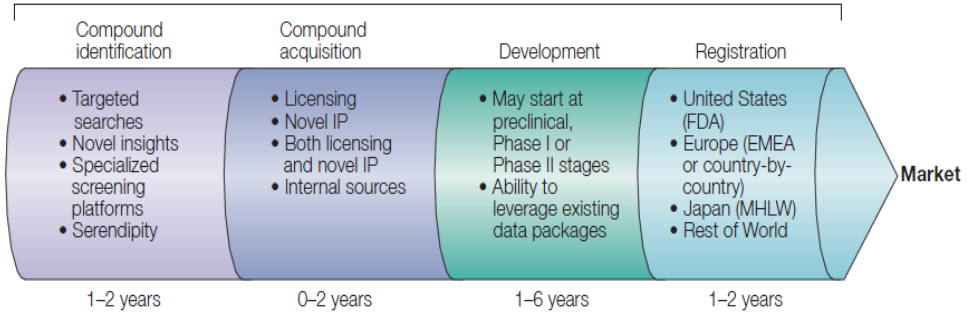
Rampioni *et al.* (2014) *Biorg Chem* 55:60-68

# Drug repurposing

This strategy is based on the use of “old” drugs already approved for use in humans to treat different (*out of target*) diseases.



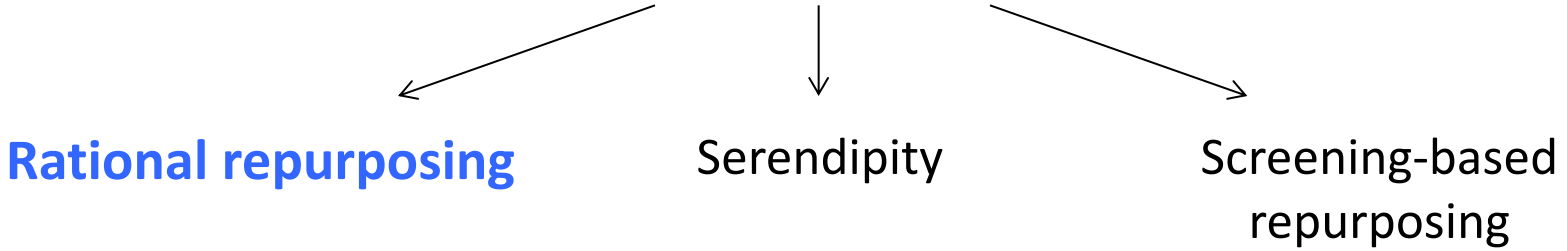

***De novo* drug development (10-17 years)**




**Drug repurposing (3-12 years)**

Ashburn and Thor (2004) *Nat Rev Drug Discov* 3:673-683.

## Drug repurposing




Alteration of uracil metabolism reduces biofilm formation in *P. aeruginosa*.

This led the researchers to screen uracil analogues for biofilm-inhibitory activity against *P. aeruginosa*.

Results highlighted a potent anti-biofilm effect of 5-fluorouracil, a drug currently used in the therapy of solid tumours.

Ueda *et al*. (2009) *Microb Biotechnol* 2:62-74.

# Drug repurposing

Rational repurposing

**Serendipity**

Screening-based repurposing

**Sildenafil** [developed in 1980s as a drug for angina pectoris (chest pain)]

The desired cardiovascular effects were not observed in clinical trials,

but participants described **AN INTERESTING SIDE EFFECT** !!

# Drug repurposing

Rational repurposing

Serendipity

**Screening-based repurposing**

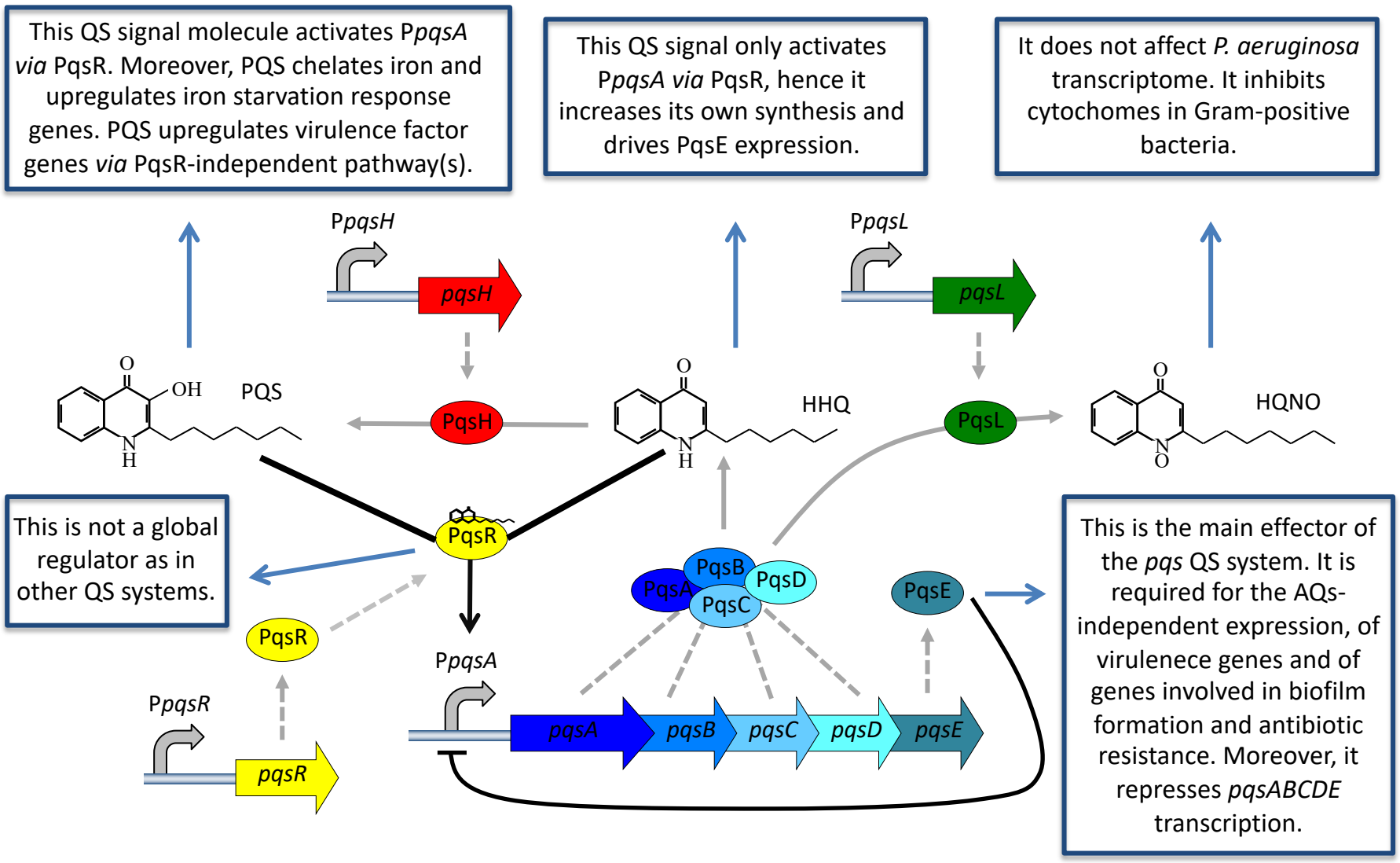
A collection of highly diverse drugs is screened for side activities of interest

↓

The hit compound(s) could be either directly tested in clinical studies or used as a starting point for specific drug optimization programs.

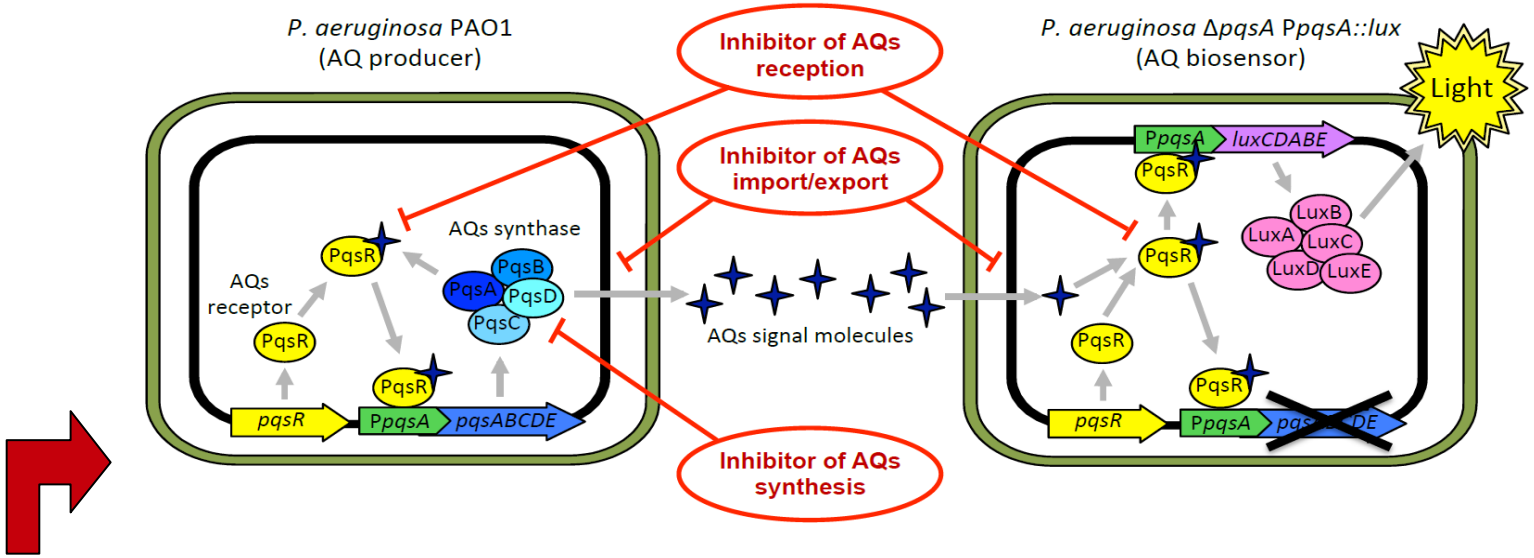
To use this strategy a feasible biosensor biosensor system ins required.

# Schematic model of the *pqs* QS system

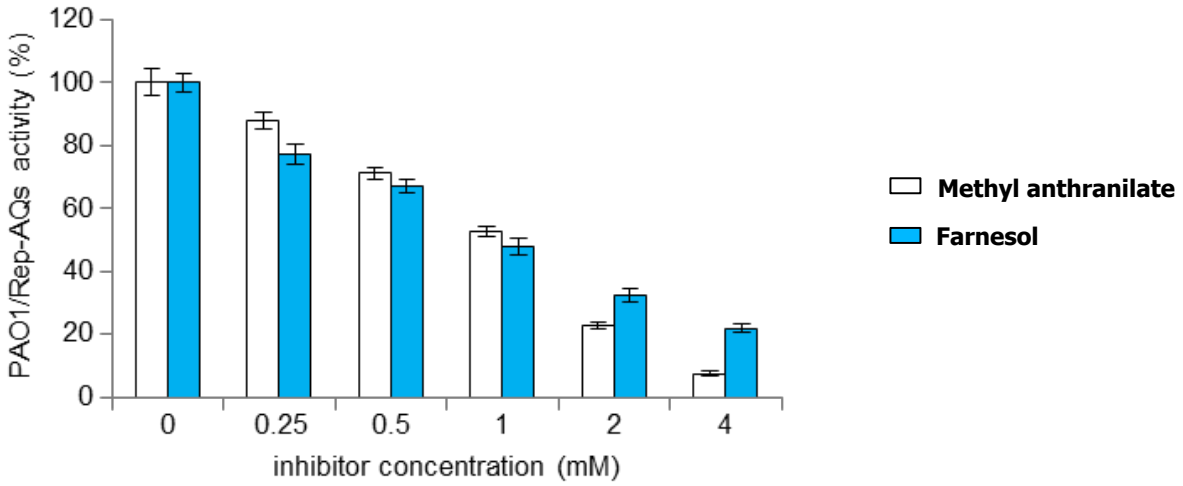



Heeb *et al.,* (2010) *FEMS Microbiol Rev* 35:247-274; Rampioni *et al.,* (2010) *Environ Microbiol* 12:1659-1273; Rampioni *et al*. (2016) *PLoS Pathog* 12:e1006029.

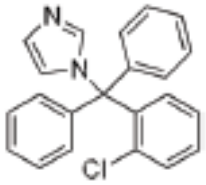
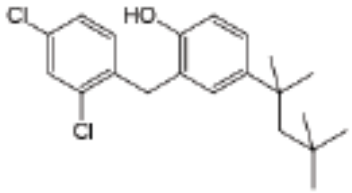
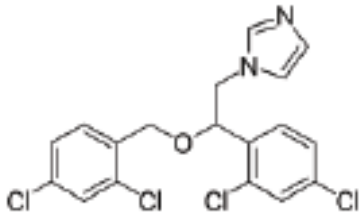
# Development of a coculture-based system for monitoring *pqs* signalling activity



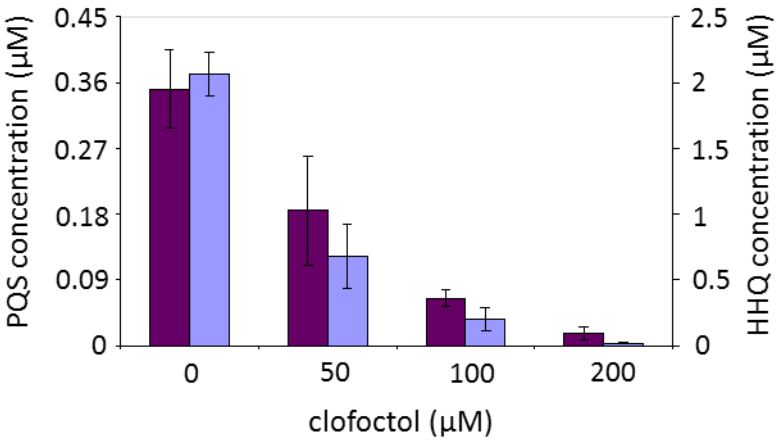

PHARMAKON
PHARMACEUTICALS

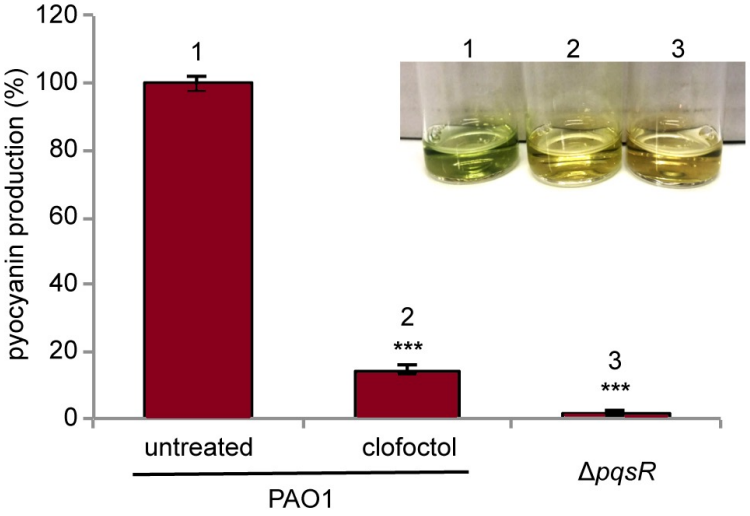


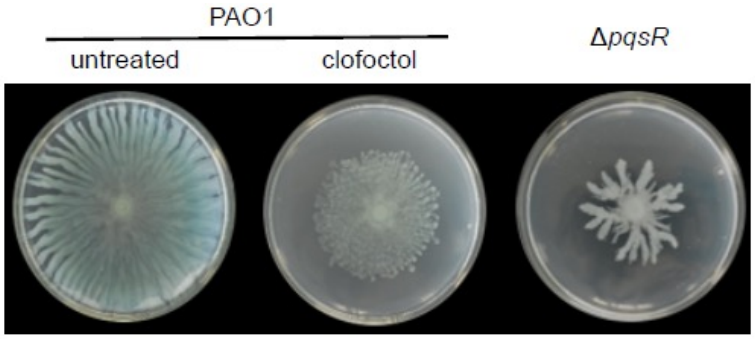
# Clofoctol is the best hit

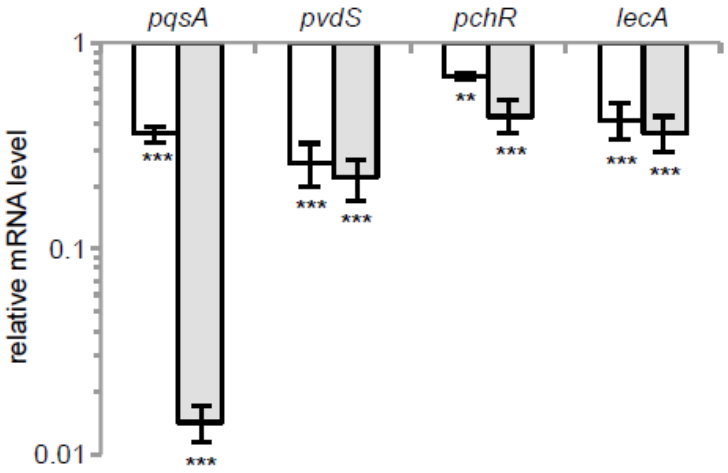
| Drug name | Property | Structure | $IC_{50}$ | ΔG |
|---|---|---|---|---|
| Clotrimazole | Antifungal |  | 39 | -8.4 |
| Clofoctol | Antibacterial |  | 20 | -9.8 |
| Miconazole | Antifungal |  | 27 | -8.5 |

# Clofoctol inhibits the expression of *pqs*-controlled virulence phenotypes

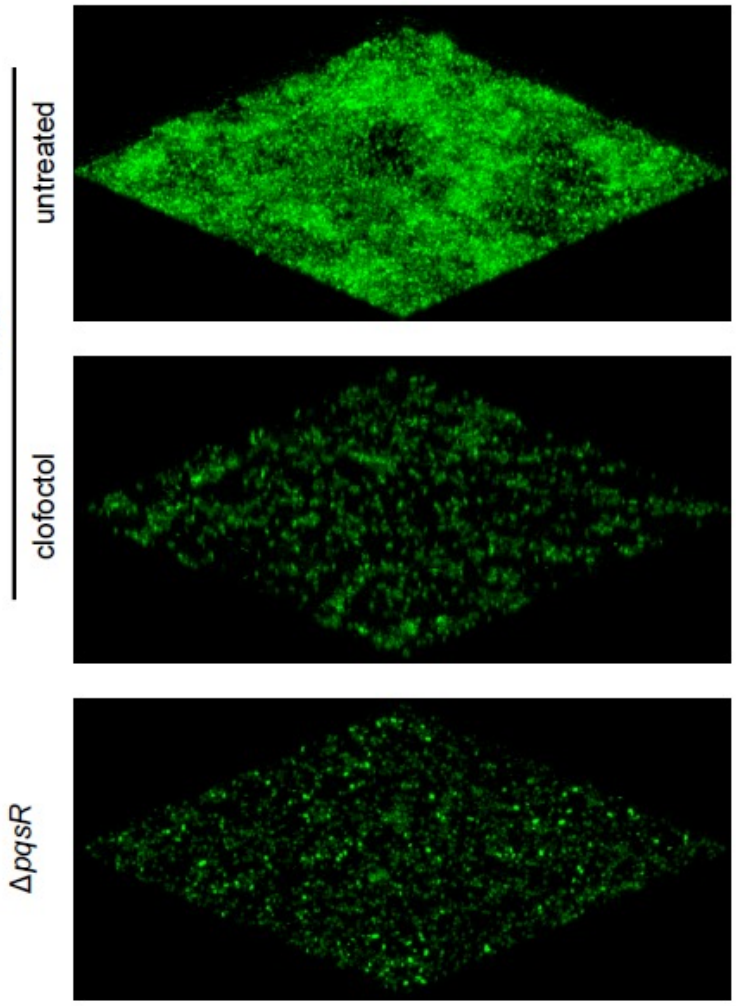


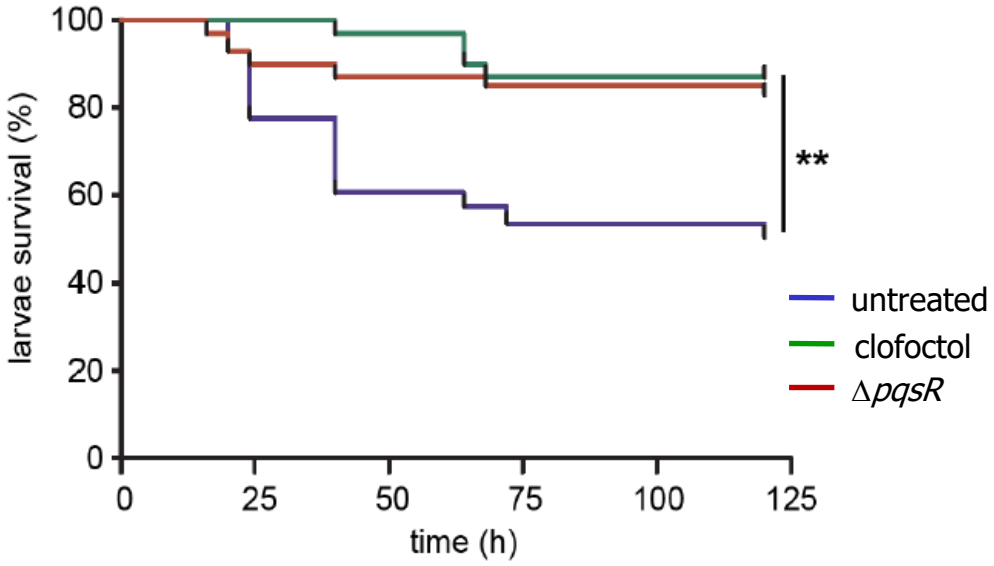



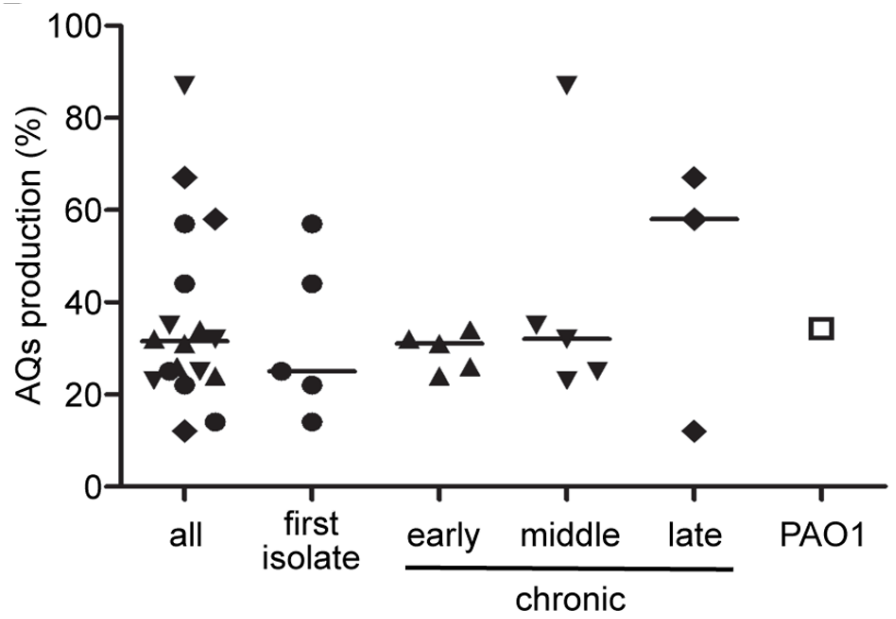



# Clofoctol inhibits biofilm formation and alleviates *P. aeruginosa* infection in *Galleria mellonella*



# Clofoctol inhibits the *pqs* QS system in *P. aeruginosa* cystic fibrosis clinical isolates





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MECHANISMS OF ACTION: PHYSIOLOGICAL EFFECTS



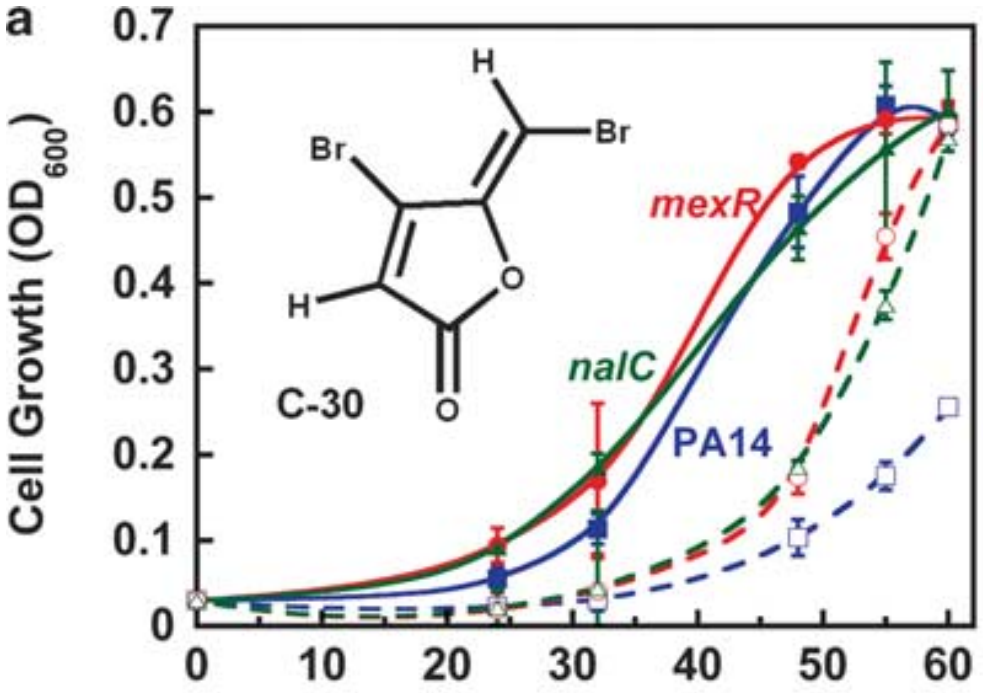
## Identification of FDA-Approved Drugs as Antivirulence Agents Targeting the *pqs* Quorum-Sensing System of *Pseudomonas aeruginosa*


Francesca D'Angelo,[a] Valerio Baldelli,[a] Nigel Halliday,[b] Paolo Pantalone,[b] Fabio Polticelli,[a,c] Ersilia Fiscarelli,[d] Paul Williams,[b] Paolo Visca,[a] Livia Leoni,[a] Giordano Rampioni[a]

# Is QS a good target for evolution-proof drugs?

## Quorum quenching quandary: resistance to antivirulence compounds

Toshinari Maeda[1,2,8], Rodolfo García-Contreras[3,4,8], Mingming Pu[1,8], Lili Sheng[1,5], Luis Rene Garcia[6], Maria Tomás[7] and Thomas K Wood[1,6]




In *P. aeruginosa* the enzyme involved in the degradation of adenosine (nucleoside hydrolase) is regulated by QS. In the presence of a QS inhibitor, such as furanone C-30, this enzyme is not expressed and *P. aeruginosa* cannot grow in minimal medium with adenosine as the sole carbon source.

Some mutations (*e.g. nalC* or *mexR*) restore the ability of *P. aeruginosa* to grow on adenosine as the sole carbon source.

# Is QS a good target for evolution-proof drugs?

## Quorum quenching quandary: resistance to antivirulence compounds

Toshinari Maeda[1,2,8], Rodolfo García-Contreras[3,4,8], Mingming Pu[1,8], Lili Sheng[1,5], Luis Rene Garcia[6], Maria Tomás[7] and Thomas K Wood[1,6]

Conclusion: Bacteria can develop resistance to QS inhibitors.

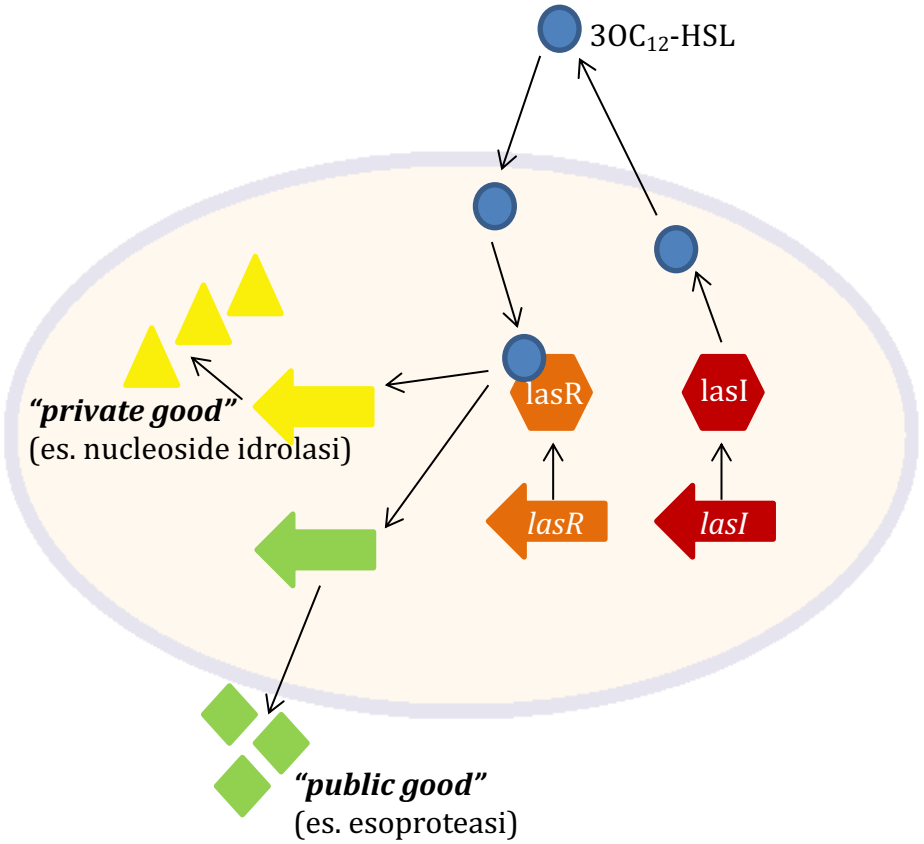
Perhaps, however, we should evaluate an important aspect of this experimental approach: adenosine is degraded intracellularly by the cytoplasmic enzyme nucleoside hydrolase, therefore if a bacterium manages to degrade adenosine even in the presence of furanone C-30, this is the only one capable of growing in the entire population. Being the resistance to the QS inhibitor genetically determined, after a while the resistant clone will generate a population of bacteria resistant to the anti-QS compound.

Under such experimental conditions, what is the difference between a QS inhibitor and a traditional antibiotic? Obviously none. The experimental system adopted imposes a strong selective pressure for the emergence of resistant strains, which can take advantage of their ability to grow in a population of cells sensitive to furanone C-30.

# Is QS a good target for evolution-proof drugs?

It is important to underline that *in P. aeruginosa*, as in many other bacteria, QS regulates the expression of both intracellular enzymes and proteins (such as the nucleoside hydrolase necessary to degrade adenosine), and of extracellualr factors that are secreted and act outside the cell (such as the exoprotease needed to degrade BSA).

Since intracellular enzymes confer an advantage only on the bacterium that produces them, they are referred to as **"private goods"**. On the contrary, exo-products are usable by all members of the population, and are therefore defined as **"public goods"**.



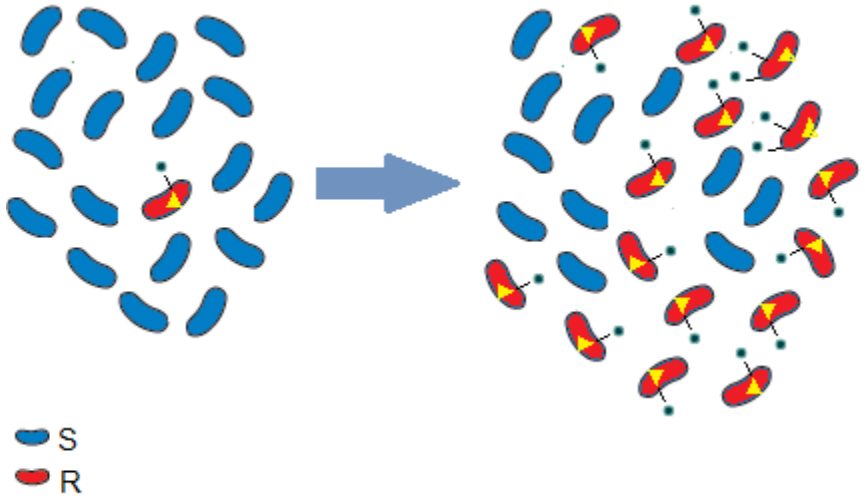
# Is QS a good target for evolution-proof drugs?

## The Sociomicrobiology of Antivirulence Drug Resistance: a Proof of Concept

**Brett Mellbye and Martin Schuster**

Department of Microbiology and Molecular and Cellular Biology Program, Oregon State University, Corvallis, Oregon, USA

In this medium, QS inhibitor-resistant strains have a reproductive advantage over susceptible members of the population. Therefore, resistant strains, even if present in small numbers within the population, tend to emerge.




Growth in a medium containing adenosine as the sole carbon source requires the production of QS-regulated **"private goods"**.

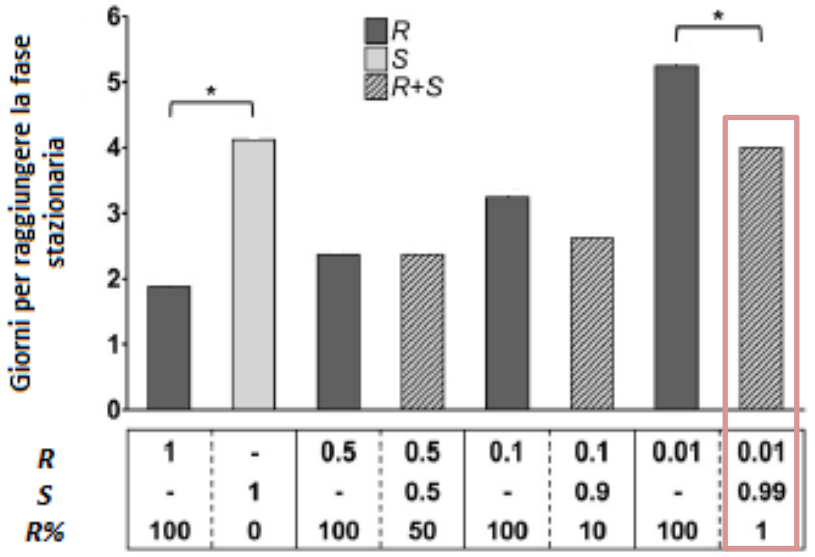



Immagine modificata da Mellbye e Schuster, (2011)

# Is QS a good target for evolution-proof drugs?

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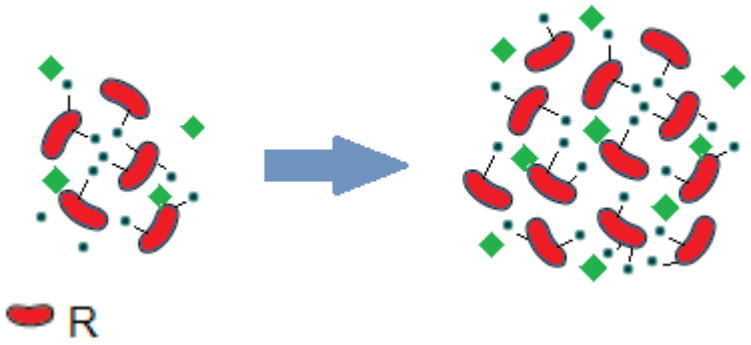
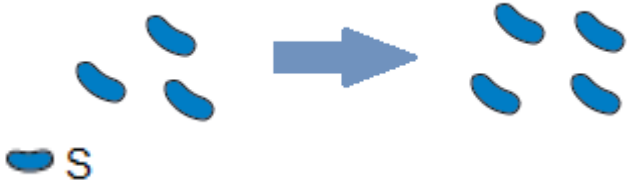
**Monoculture of the QS inhibitor resistant strain.**
It grows because it can produce the exoproteases needed to degrade BSA extracellularly.




**Monoculture of the QS inhibitor sensitive strain.**
It does not grow because it cannot produce the exoproteases needed to degrade BSA extracellularly.




Growth in a medium containing BSA as sole carbon source requires the production of **QS-regulated "public goods"**.

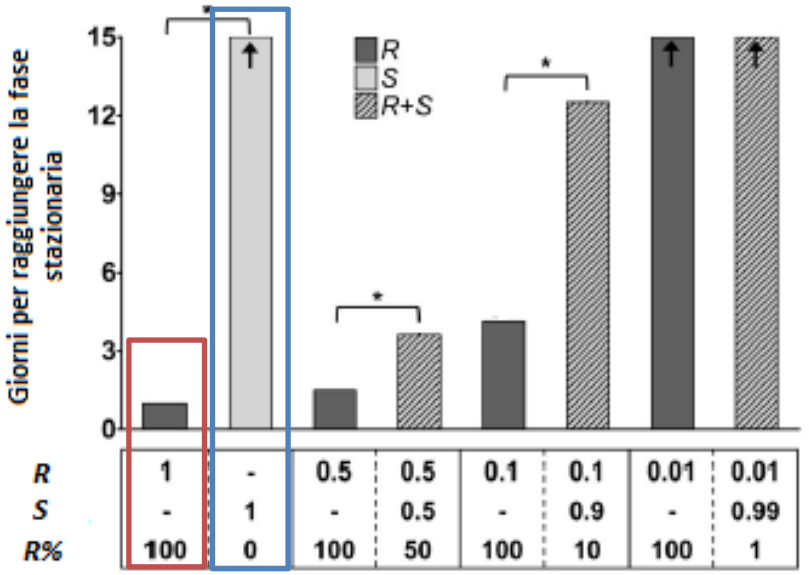



Immagine modificata da Mellbye e Schuster, (2011)

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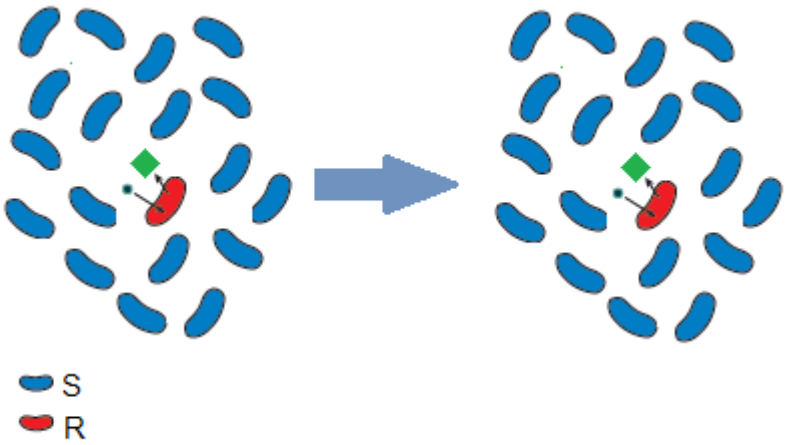
When the QS inhibitor-resistant strain is present in a small percentage within the co-culture (99% inhibitor-sensitive individuals and 1% inhibitor-resistant individuals), the ”public goods" produced by the resistant strain are not sufficient to support population growth.
Thus, the resistant strain has no reproductive advantage and does not emerge within the population.




Growth in a medium containing BSA as sole carbon source requires the production of **QS-regulated “public goods”**.

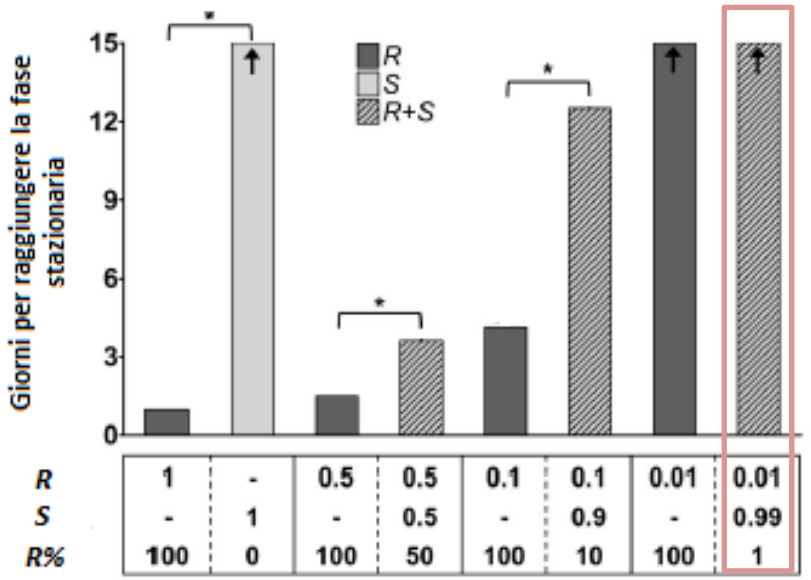



Immagine modificata da Mellbye e Schuster, (2011)

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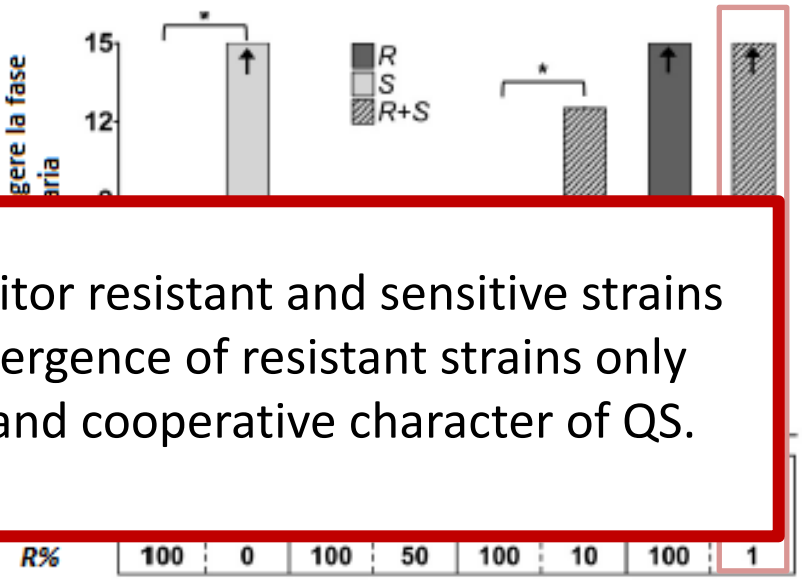


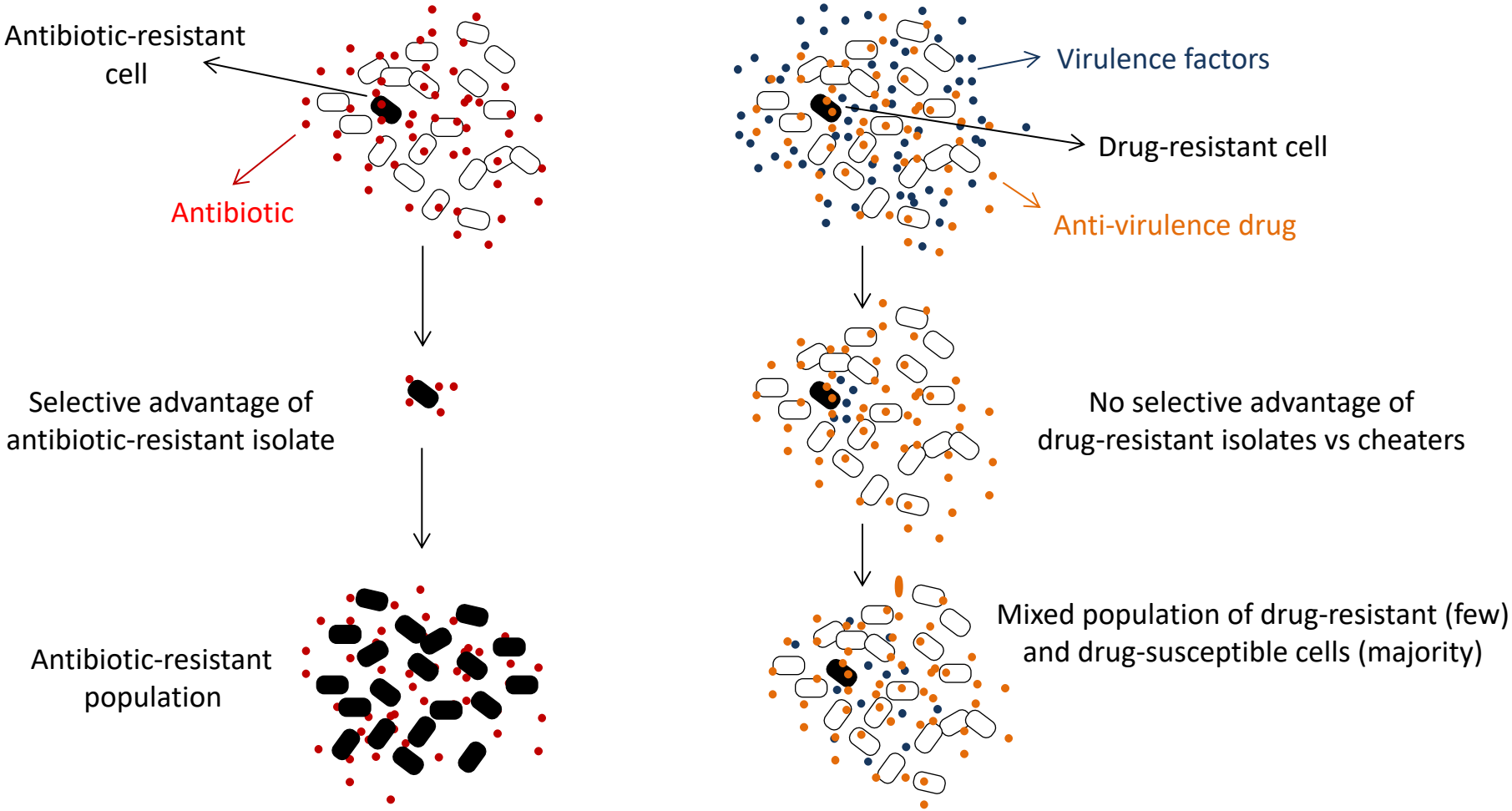

The “social conflict” between QS inhibitor resistant and sensitive strains has a relevant role in limiting the emergence of resistant strains only when the inhibitors affect the social and cooperative character of QS.


Immagine modificata da Mellbye e Schuster, (2011)

# Conventional antibiotics

*vs*

# Anti-virulence drugs




Mellbye and Schuster (2011) *mBio* 2(5):e00131-11

# Exploitation of QS for new therapeutic approaches

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 new therapeutic approaches.

1) Inhibition of QS in bacterial pathogens (***anti-virulence approach***).

2) Use of QS signal molecules as molecular markers to detect pathogens (***biotic antibacterials***).

3) Generation of engineered bacteria able to synchronize their activities at the population level (***biotic antitumor agents***).

4) Generation of synthetic cells able to interface with natural cells (***soft-nanorobots***).

# QS signal molecules can serve as diagnostic markers

QS signals are produced by bacterial pathogens.
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.

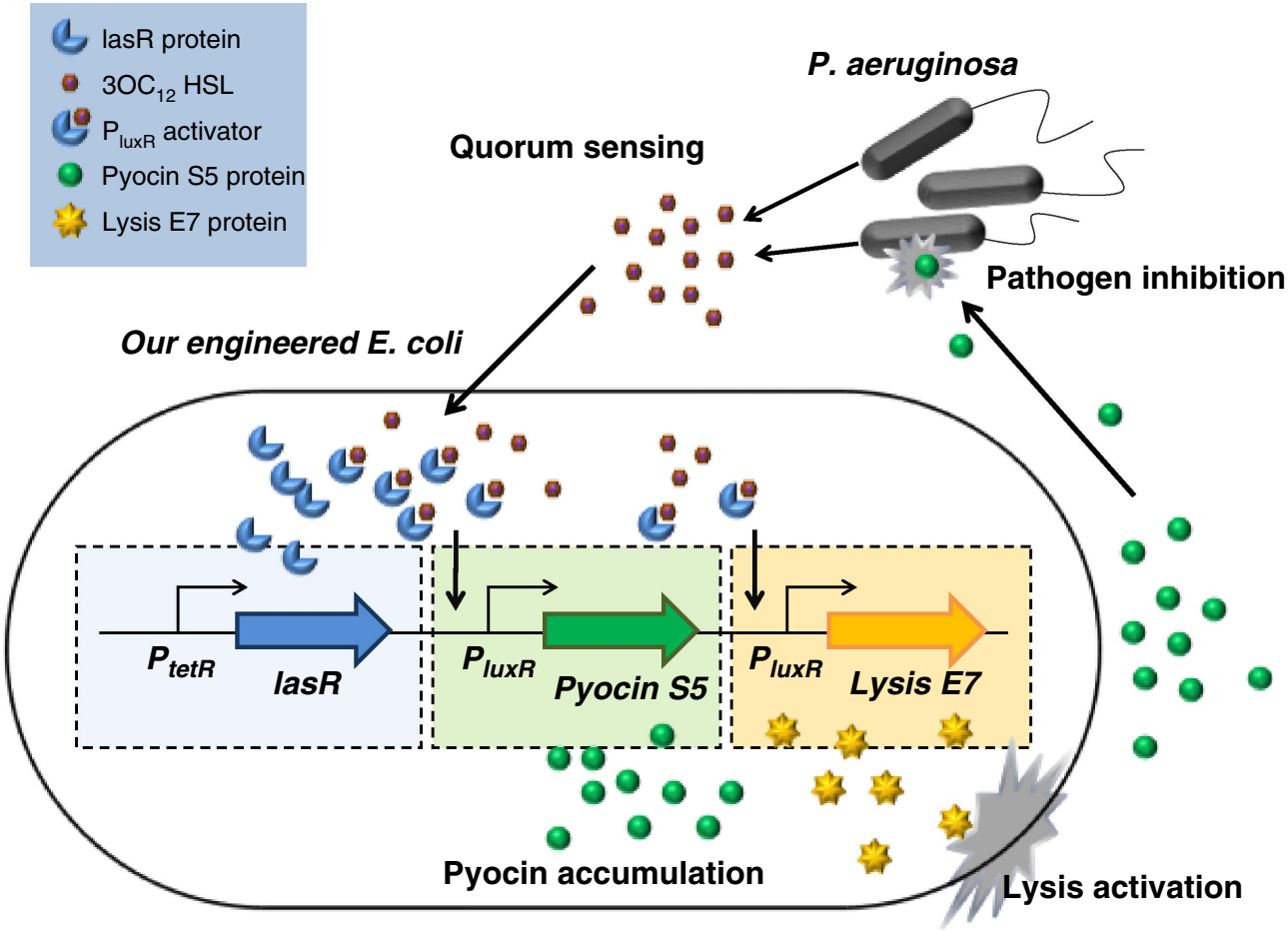
Molecular Systems Biology 7; Article number 521; doi:10.1038/msb.2011.55
Citation: *Molecular Systems Biology* 7:521

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*



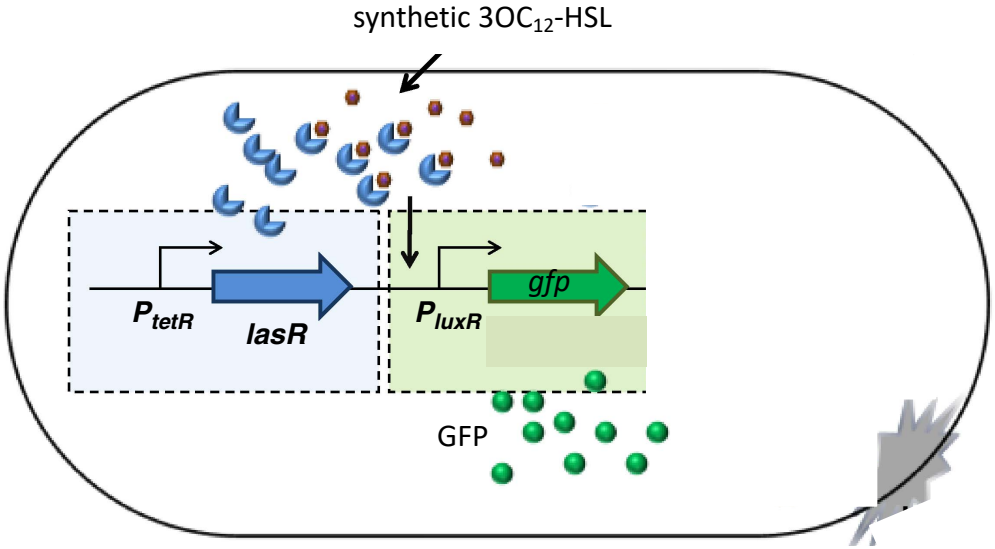
Molecular Systems Biology 7; Article number 521; doi:10.1038/msb.2011.55
Citation: *Molecular Systems Biology* 7:521

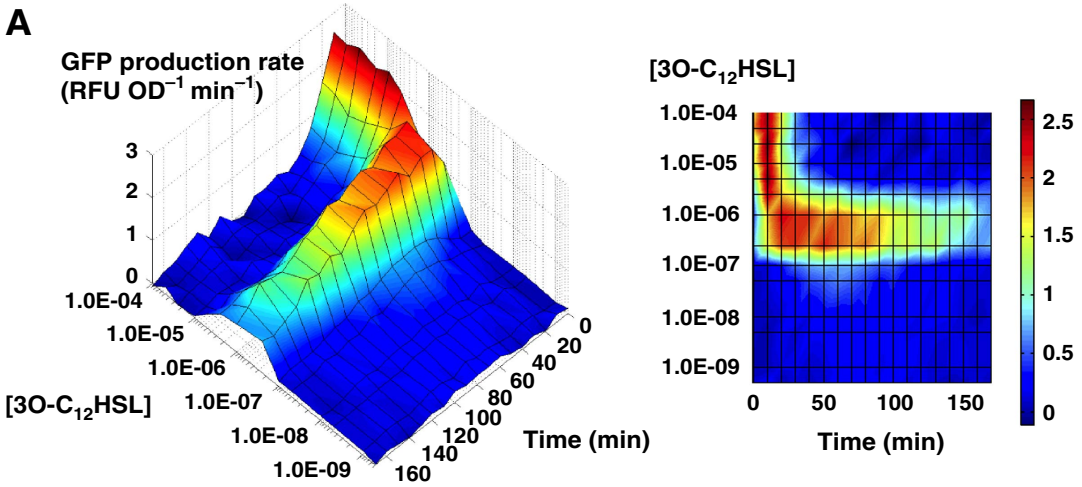
www.molecularsystemsbiology.com

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*



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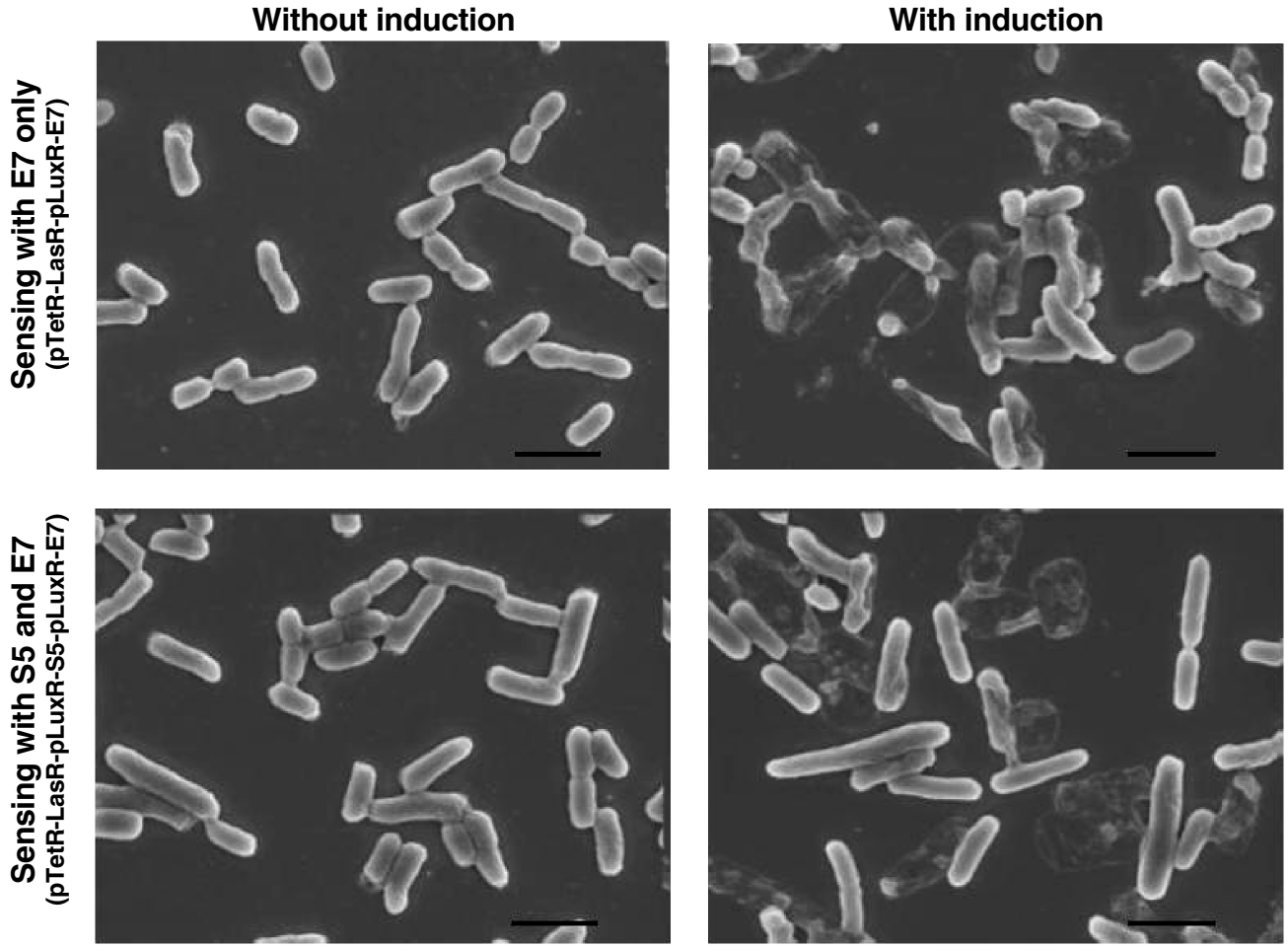

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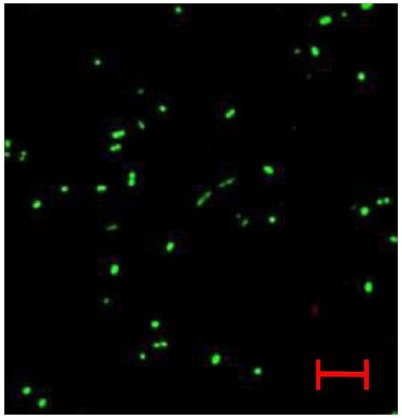
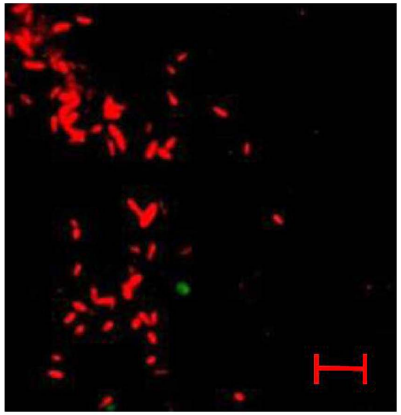
**Exposed to supernatant of wild-type *E. coli***



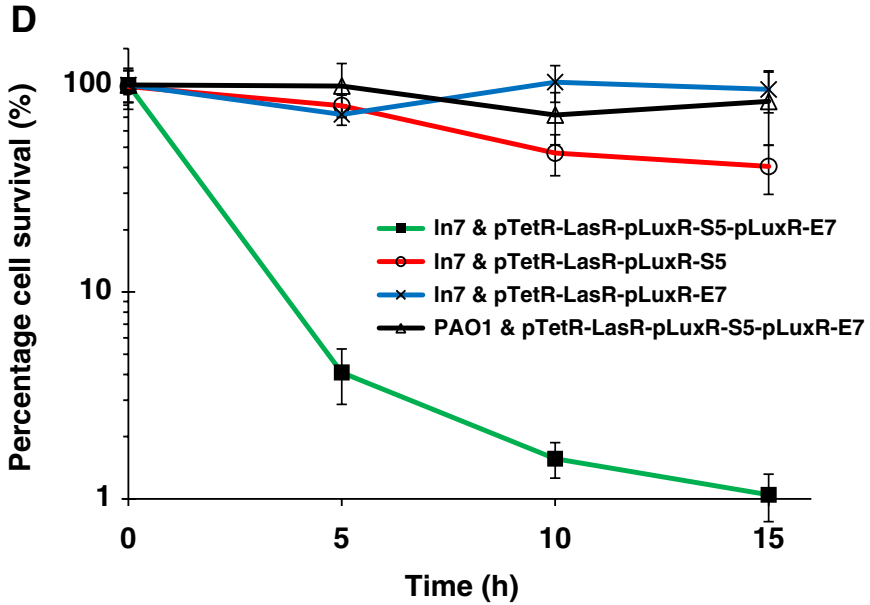
**Exposed to supernatant of engineered *E. coli* induced with native $3OC_{12}HSL$**



**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

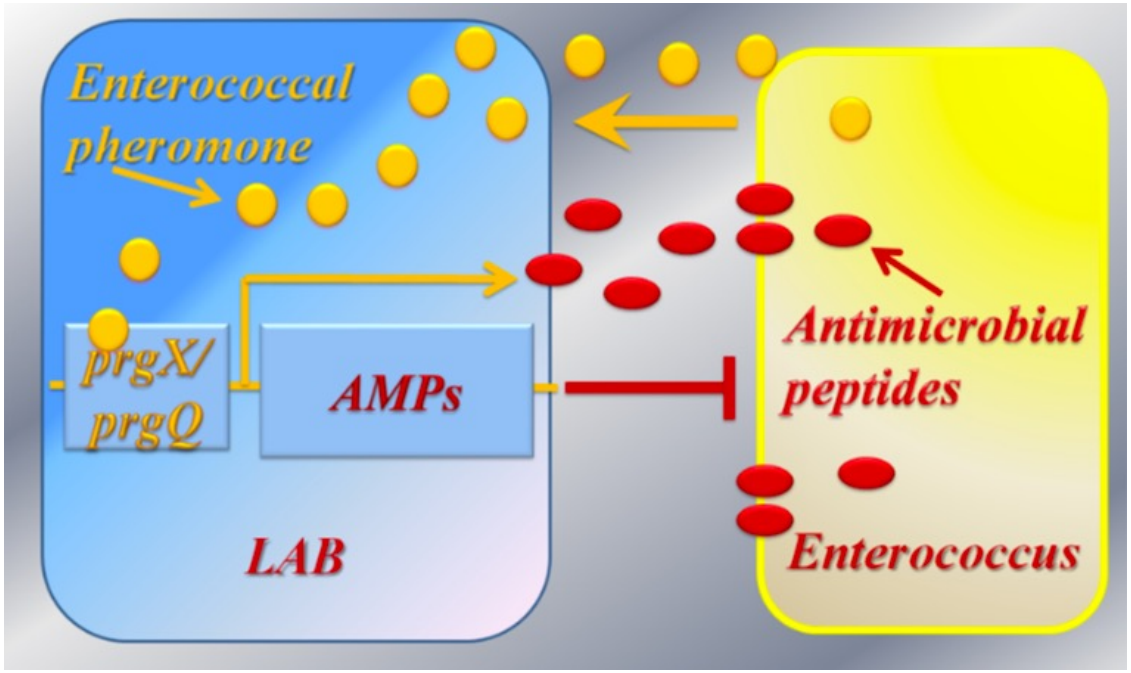
Research Article

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



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1) Inhibition of QS in bacterial pathogens (***anti-virulence approach***).

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3) Generation of engineered bacteria able to synchronize their activities at the population level (***biotic antitumor agents***).

4) Generation of synthetic cells able to interface with natural cells (***soft-nanorobots***).

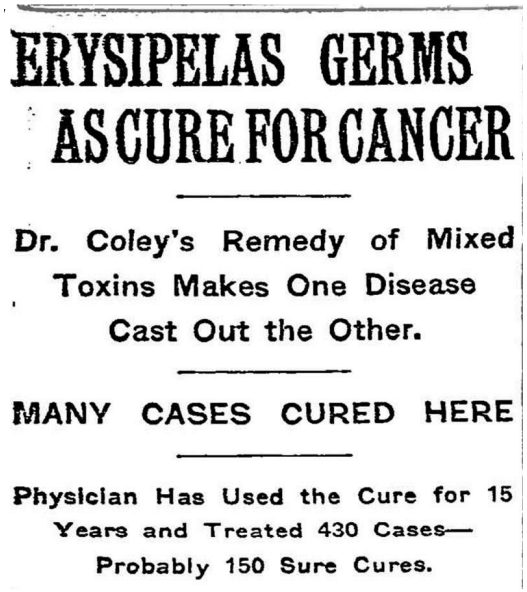
# Bacteria are promising anti-tumour agents

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* and *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.

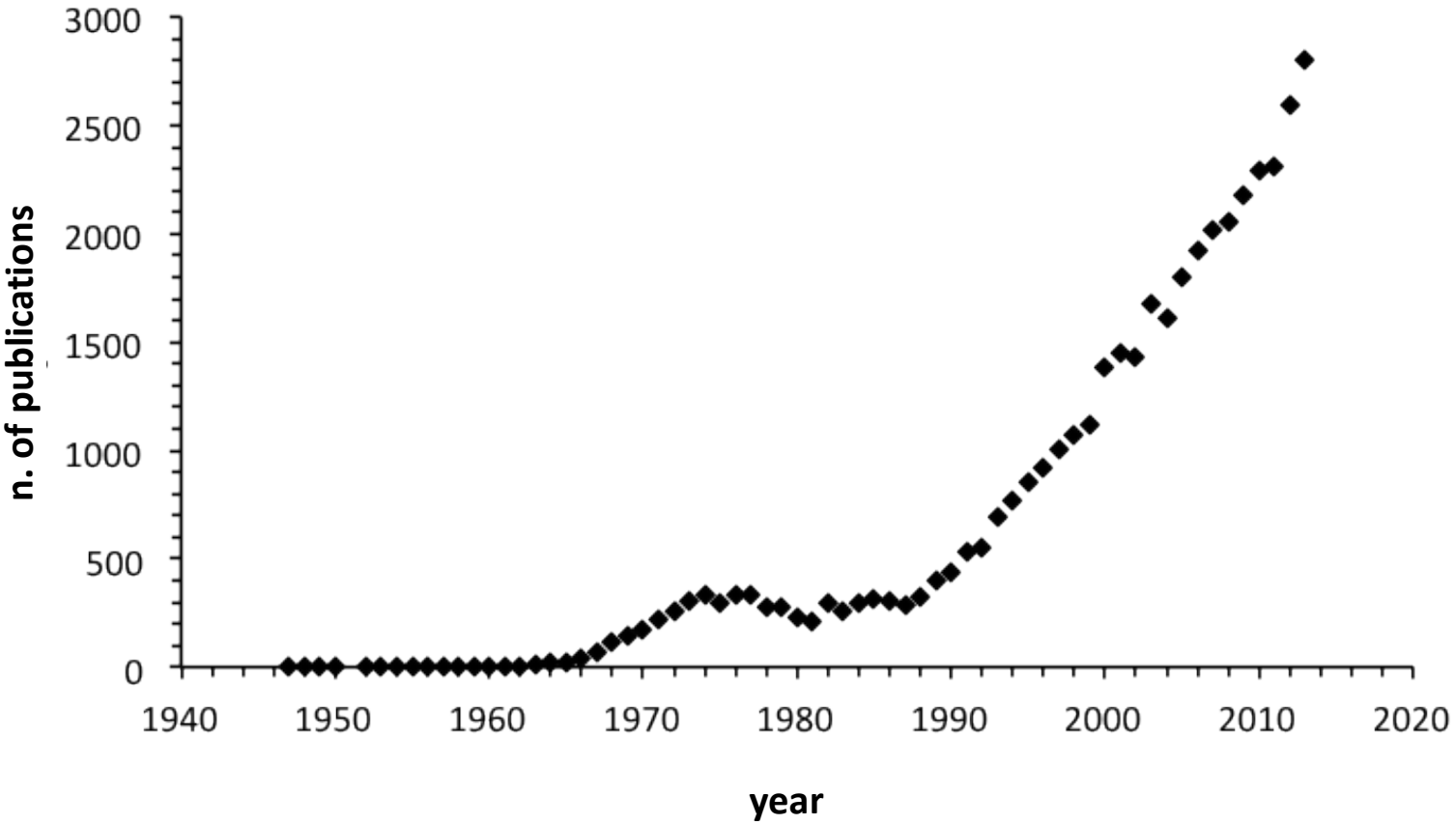
MANY CASES CURED HERE

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

***New York Times, July 29th, 1908***

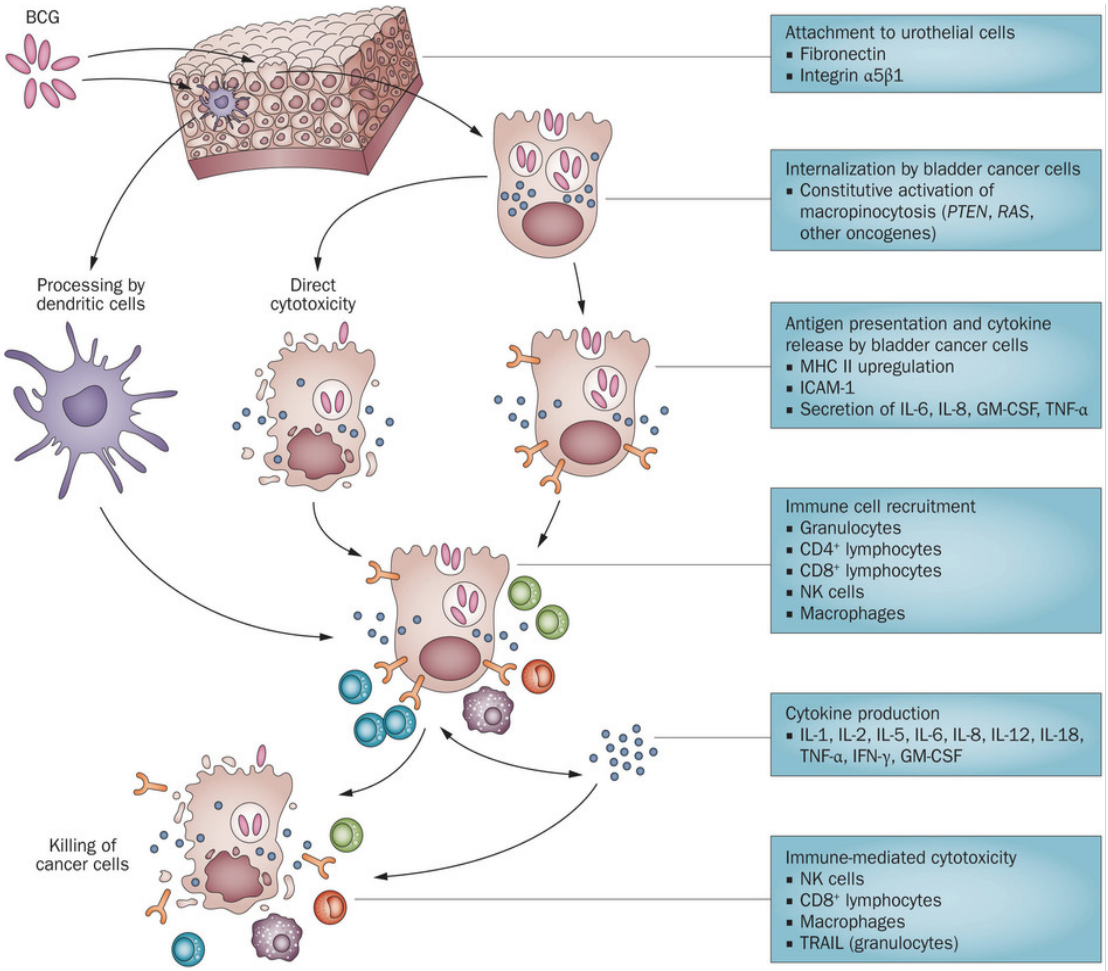
# Bacteria are promising anti-tumour agents

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



# Few bacteria are currently used to treat cancer

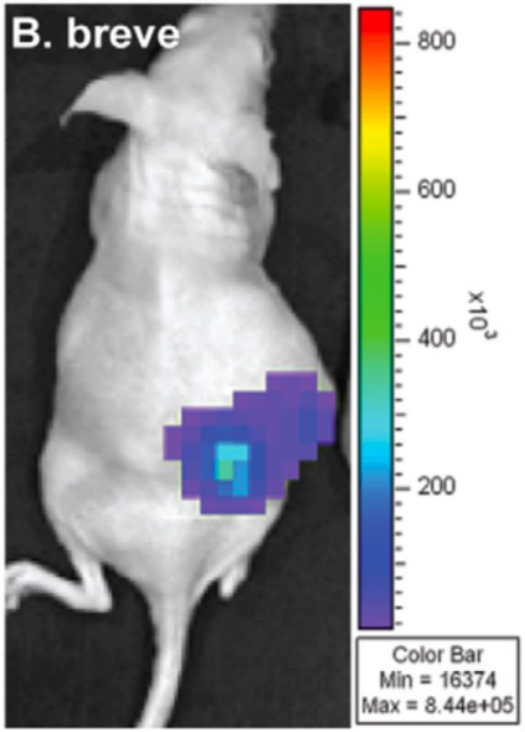
Some bacteria activate a antitumor immune response, as in the case of *Bacillus Calmette-Guerin*, used to treat bladder cancer.

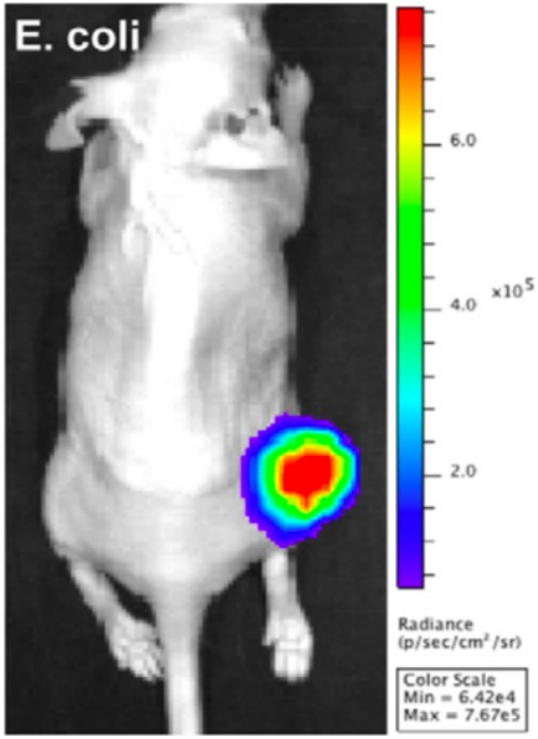


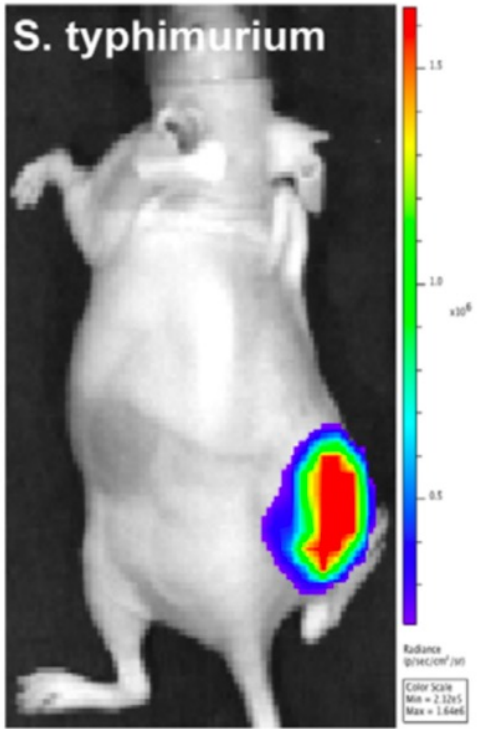
# High Resolution *In Vivo* Bioluminescent Imaging for the Study of Bacterial Tumour Targeting

**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.



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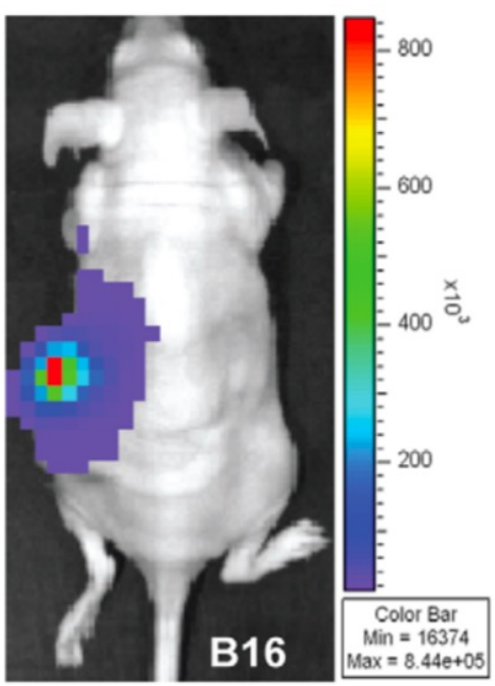
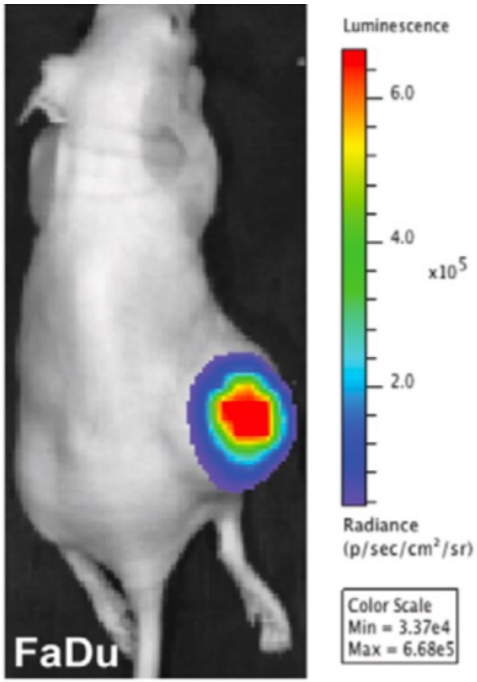
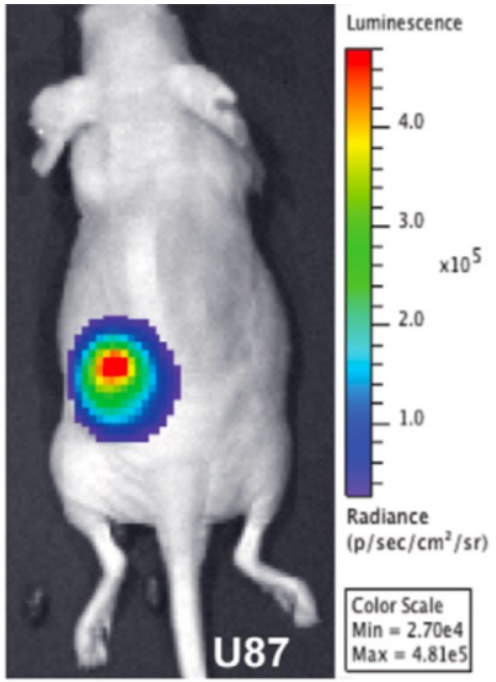
Bacteria co-localize with different tumour types.

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



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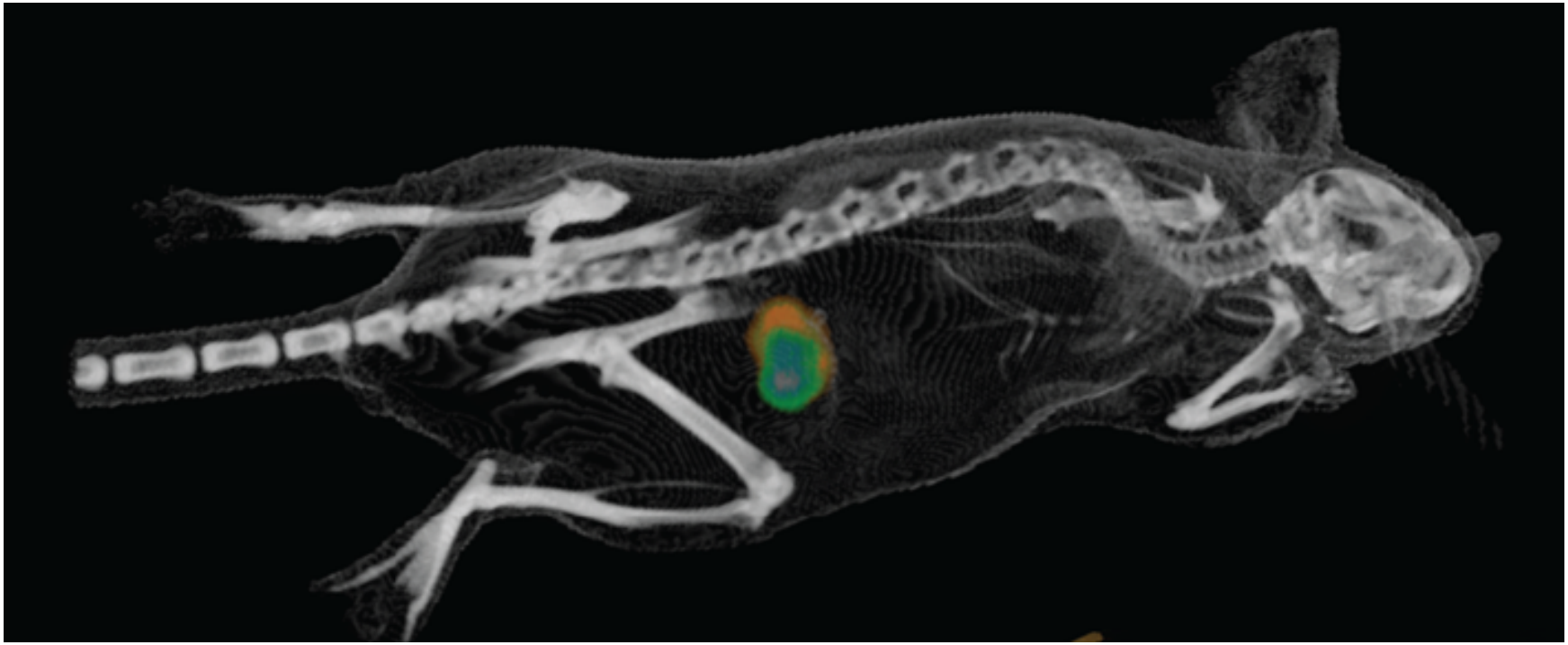
Tumor cells (green-blue) – *E. coli* cells (orange)



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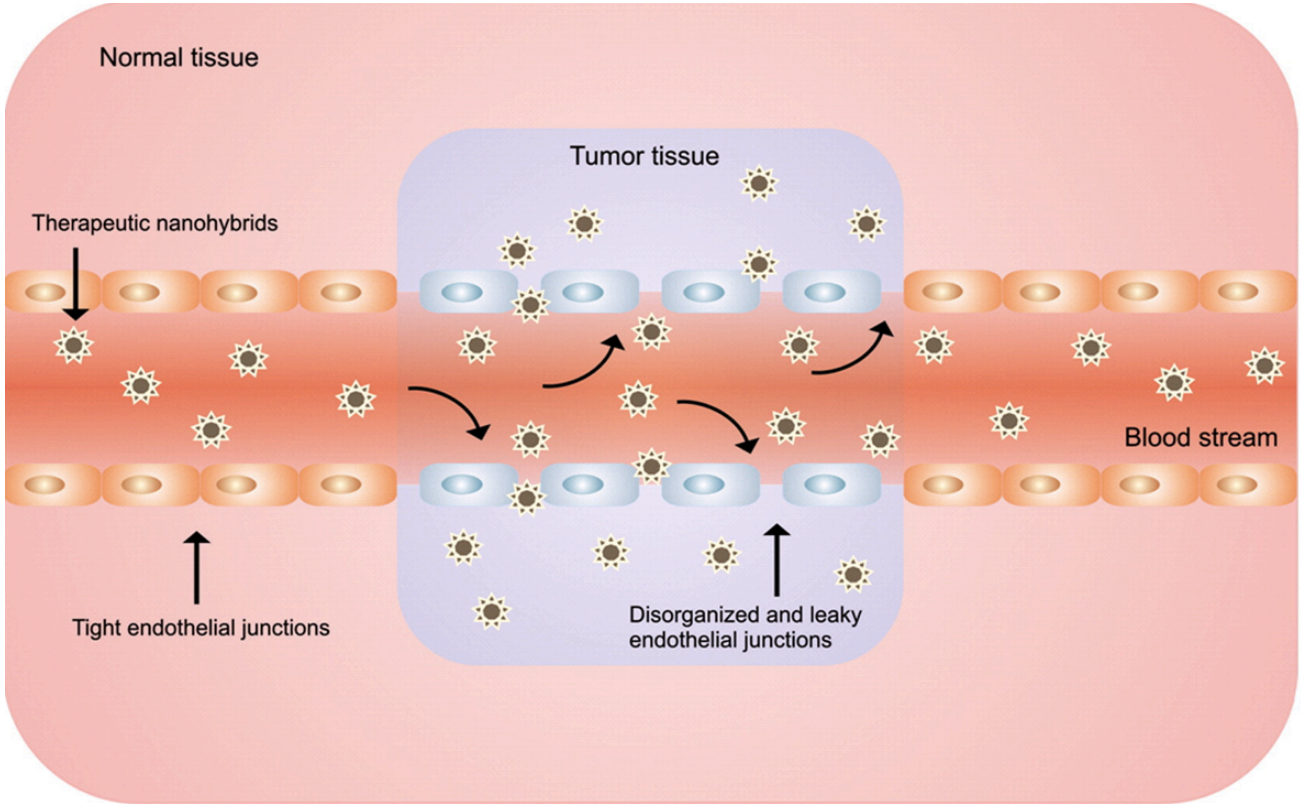
## Enhanced permeability and retention effect

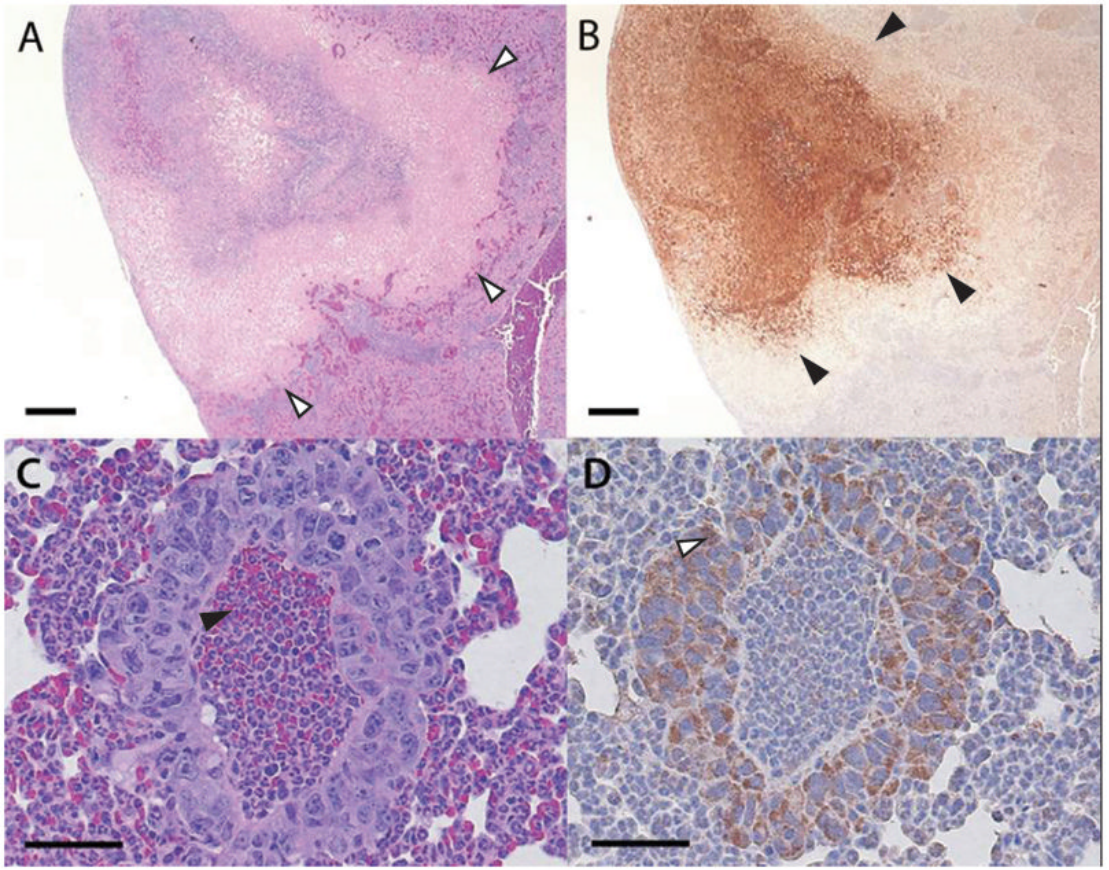


# In tumors *Salmonella* migrate away from vasculature toward the transition zone and induce apoptosis

**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.



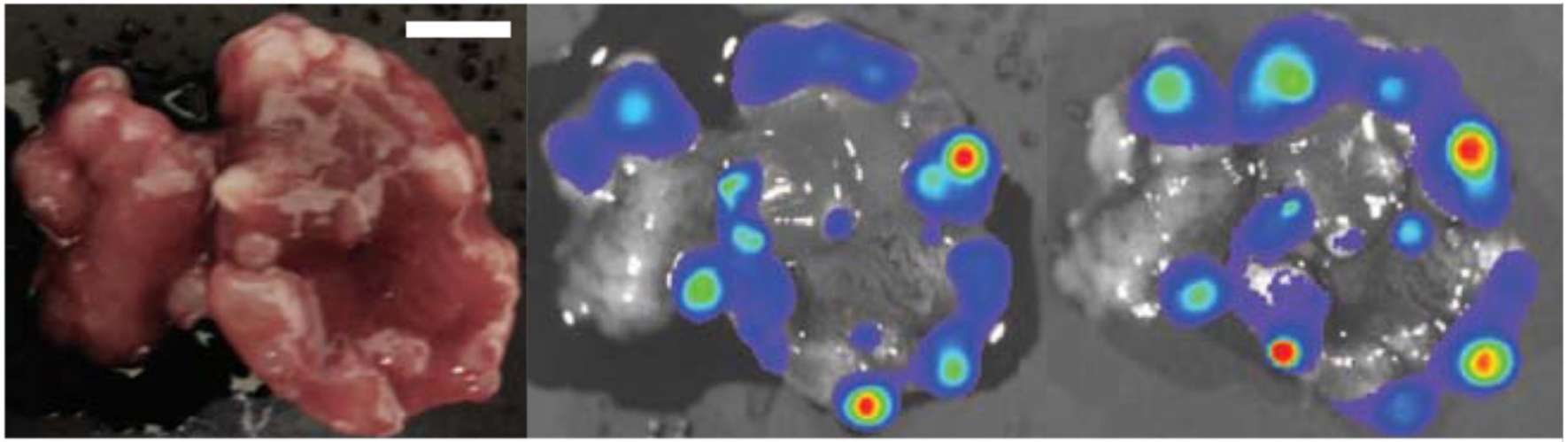
Published in final edited form as:

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


## Programmable probiotics for detection of cancer in urine

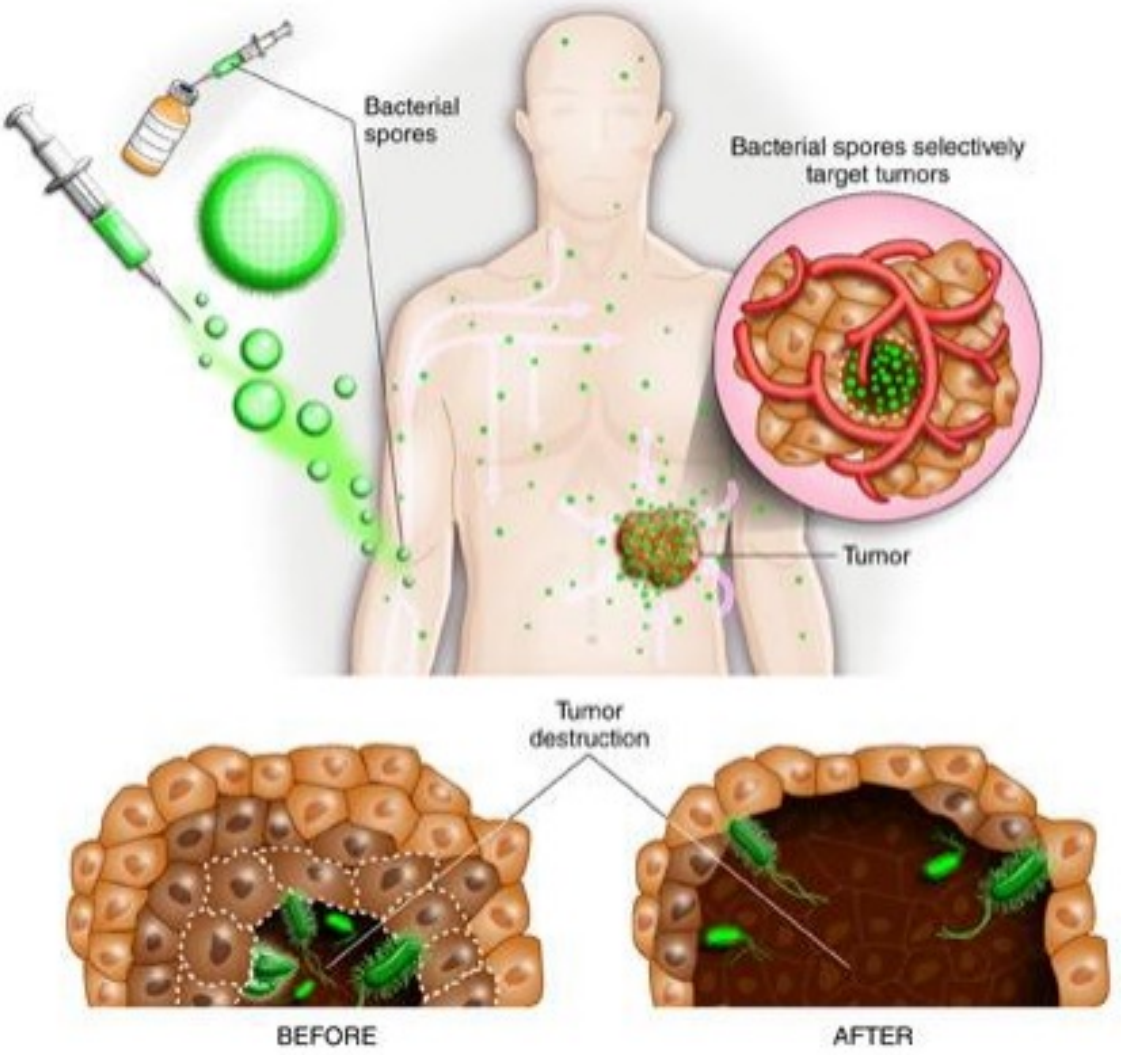

**Tal Danino**[1,*], **Arthur Prindle**[2,*], **Gabriel A. Kwong**[1,†], **Matthew Skalak**[1], **Howard Li**[2], **Kaitlin Allen**[1], **Jeff Hasty**[2,3,4,‡], and **Sangeeta N. Bhatia**[1,5,6,7,8,§,‡]


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



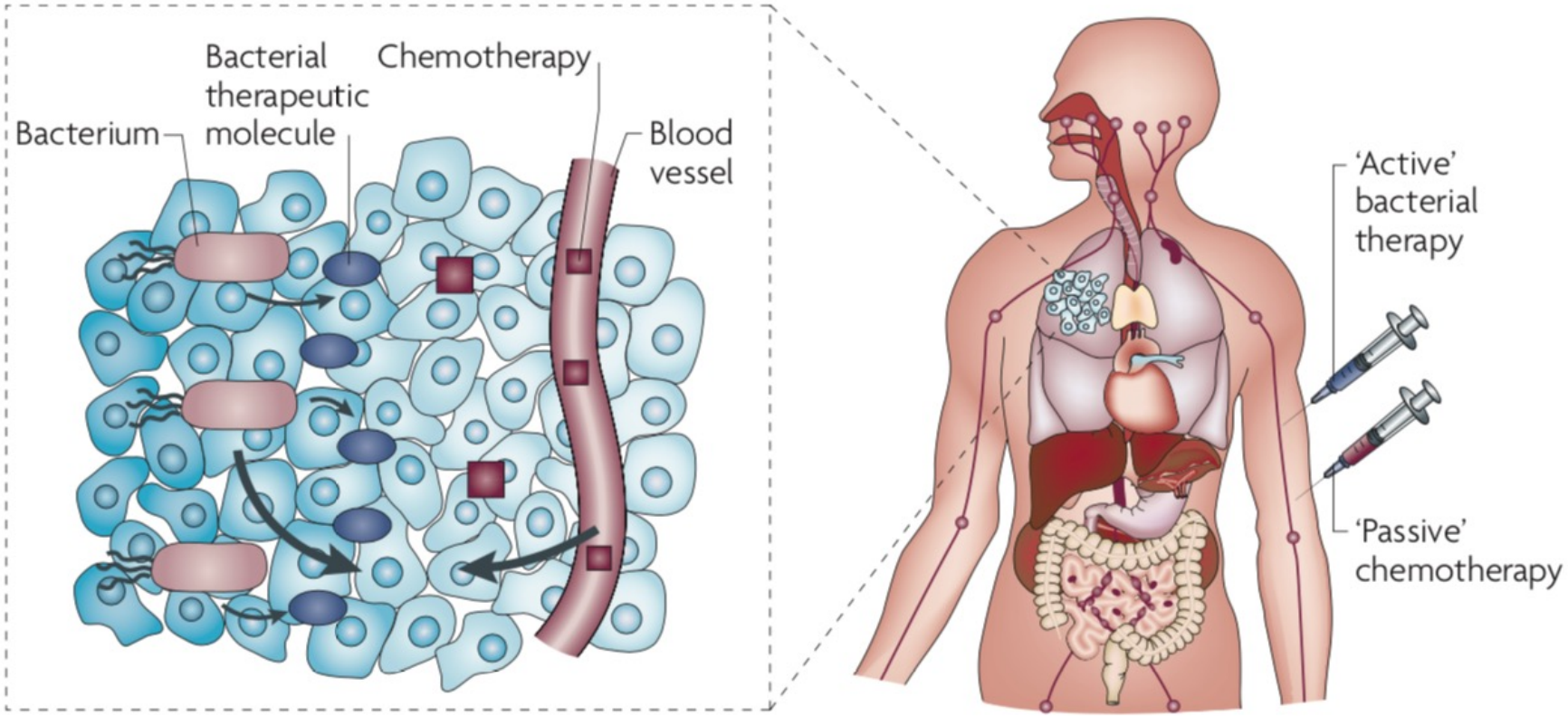
Excised liver | Tumor luminescence | Bacterial luminescence

**Clostridia spores can germinate only in the internal anoxyc part of solid tumors. Germinated Clostridia cells display an oncolytic activity.**



# COBALT therapy

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




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

# Engineered cells of *Clostrium sporogenes* can convert a non-toxic prodrug in a chemotherapeutic agent inside the tumore

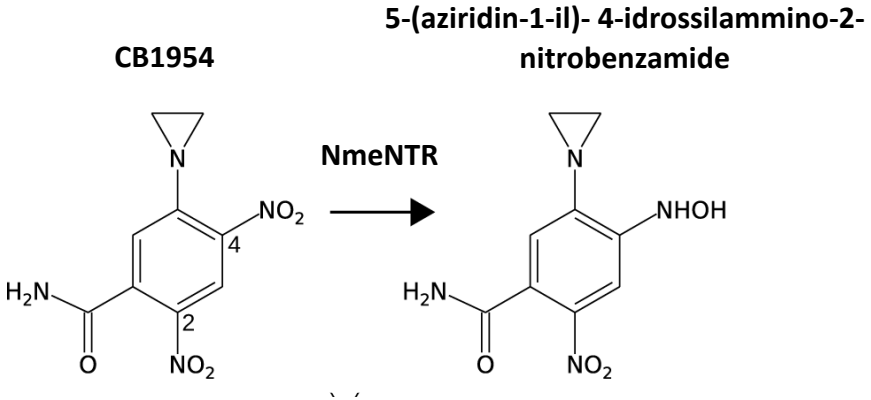
www.impactjournals.com/oncotarget/ Oncotarget, Vol. 5, No. 7

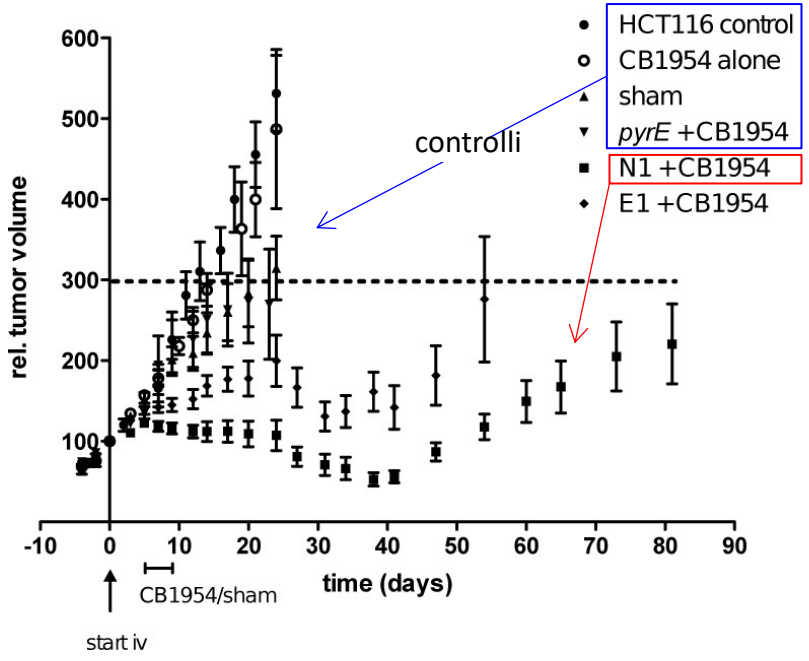
**Spores of *Clostridium* engineered for clinical efficacy and safety cause regression and cure of tumors *in vivo***

John T. Heap[1,5,*], Jan Theys[2,*], Muhammad Ehsaan[1], Aleksandra M Kubiak[1], Ludwig Dubois[2], Kim Paesmans[2], Lieve Van Mellaert[3], Richard Knox[4], Sarah A. Kuehne[1], Phillipe Lambin[2] and Nigel P. Minton[1]

*Clostridium sporogenes* has been engineered to express a nitroreductase from *Neisseria meningitidis* (NmeNTR). This enzyme converts the non-toxic prodrug CB1954 into the chemotherapeutic agent 5-(aziridin-1-il)- 4-idrossilammino-2-nitrobenzamide.

The prodrug is converted into the by chemotherapeutic agent only inside the tumore, as *C. sporogenes* NmeNTR spores germinates only in the internal anoxyc part of the tumor.



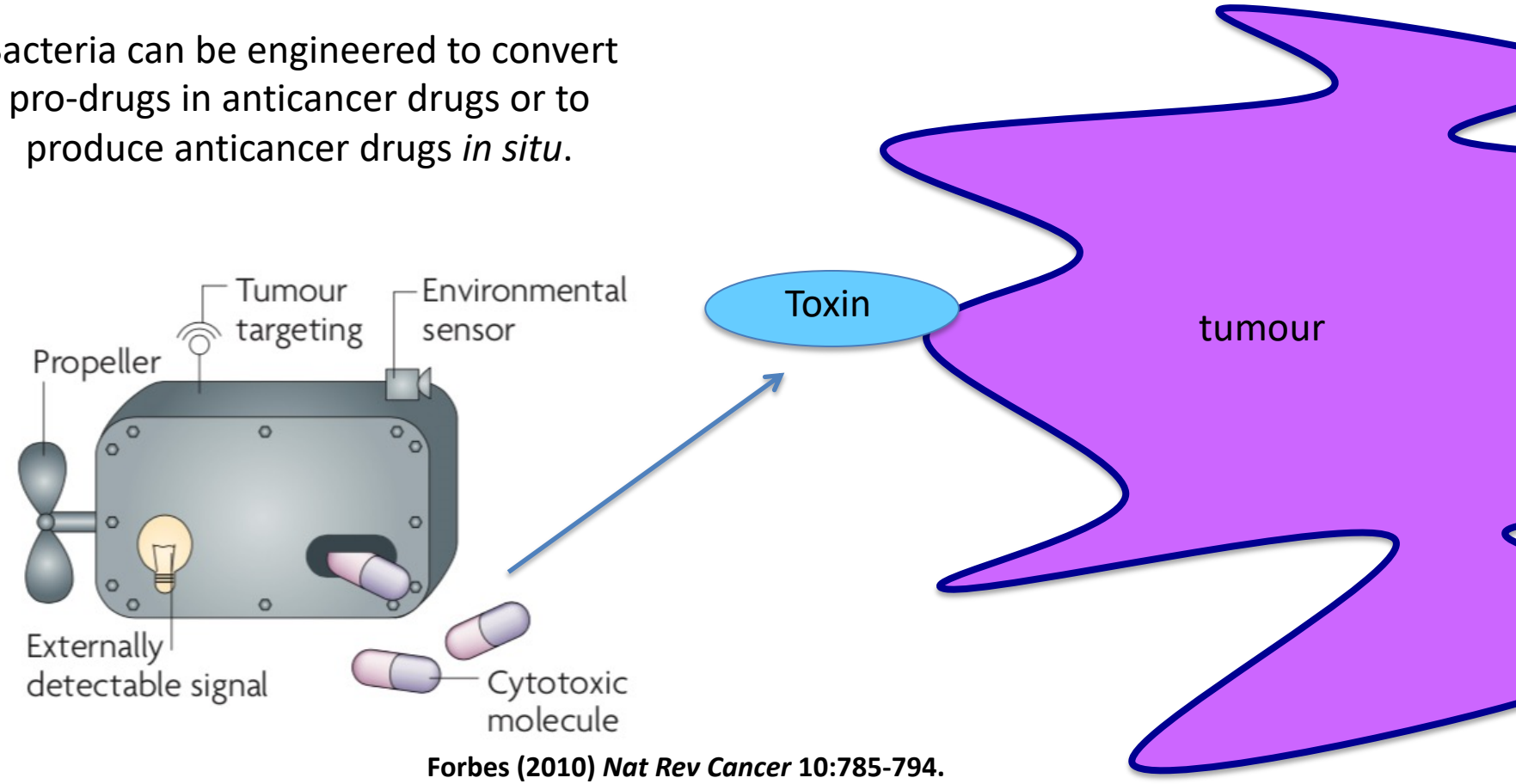



INNOVATION

# 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*.

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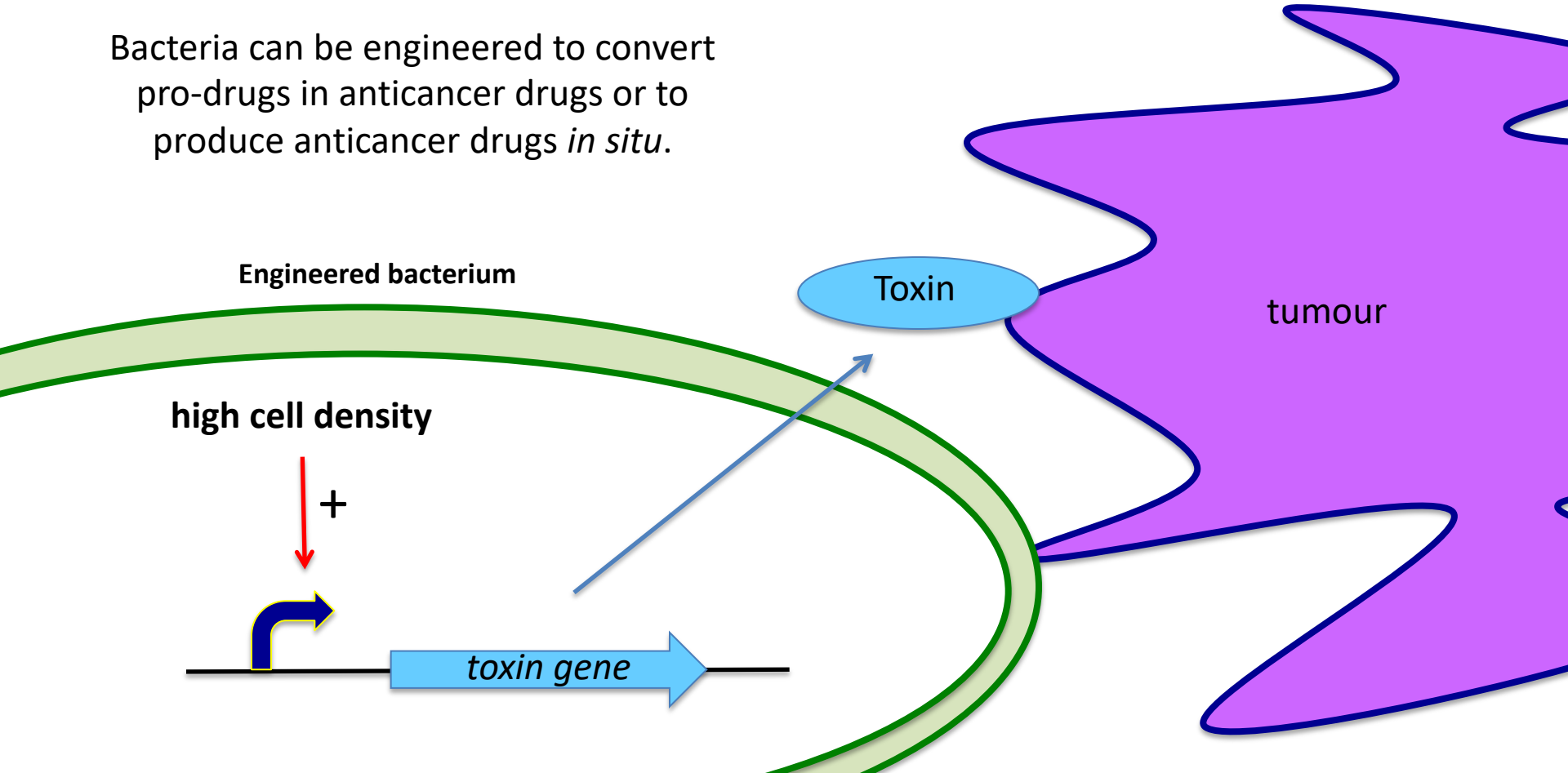

LETTER

doi:10.1038/nature18930

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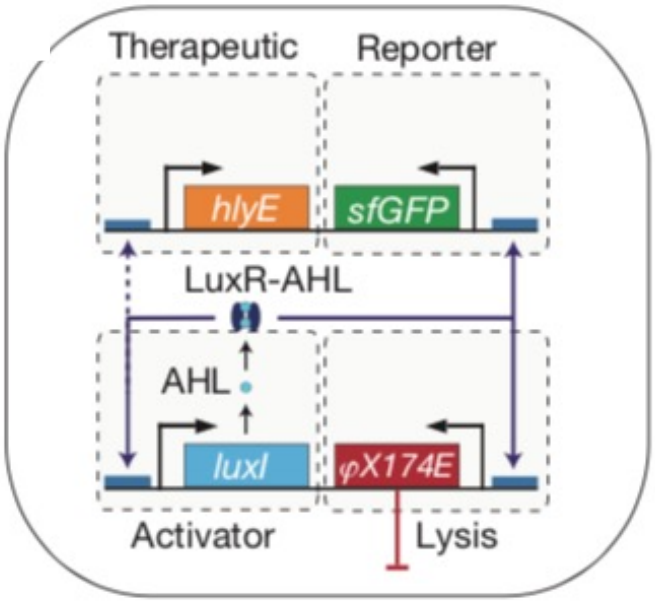


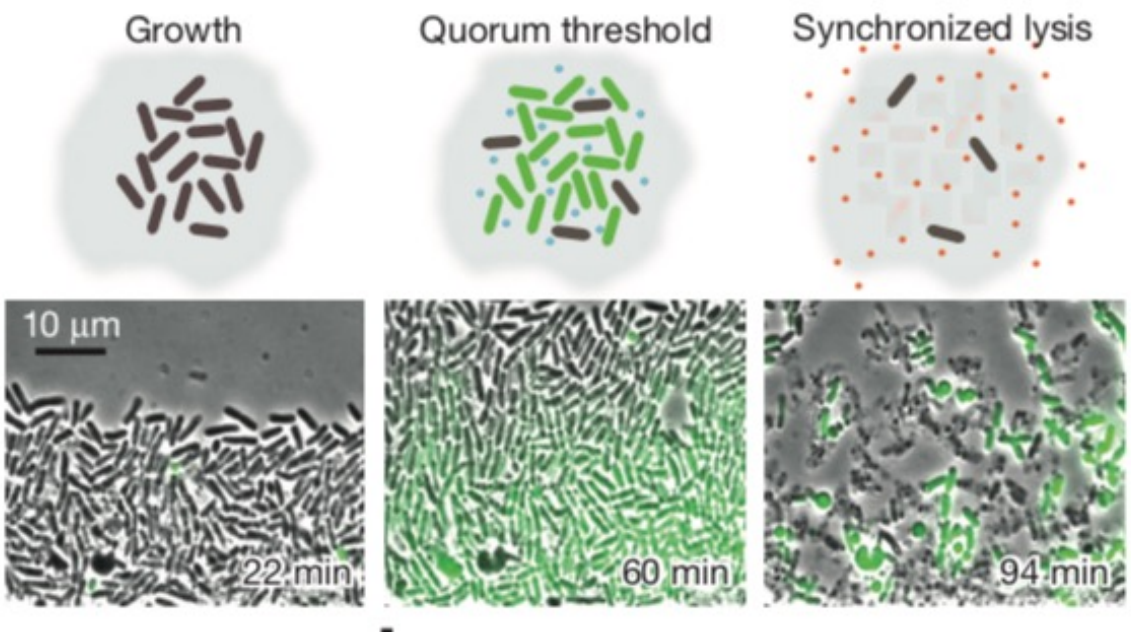


Din *et al*. (2016) *Nature* 536:81–85

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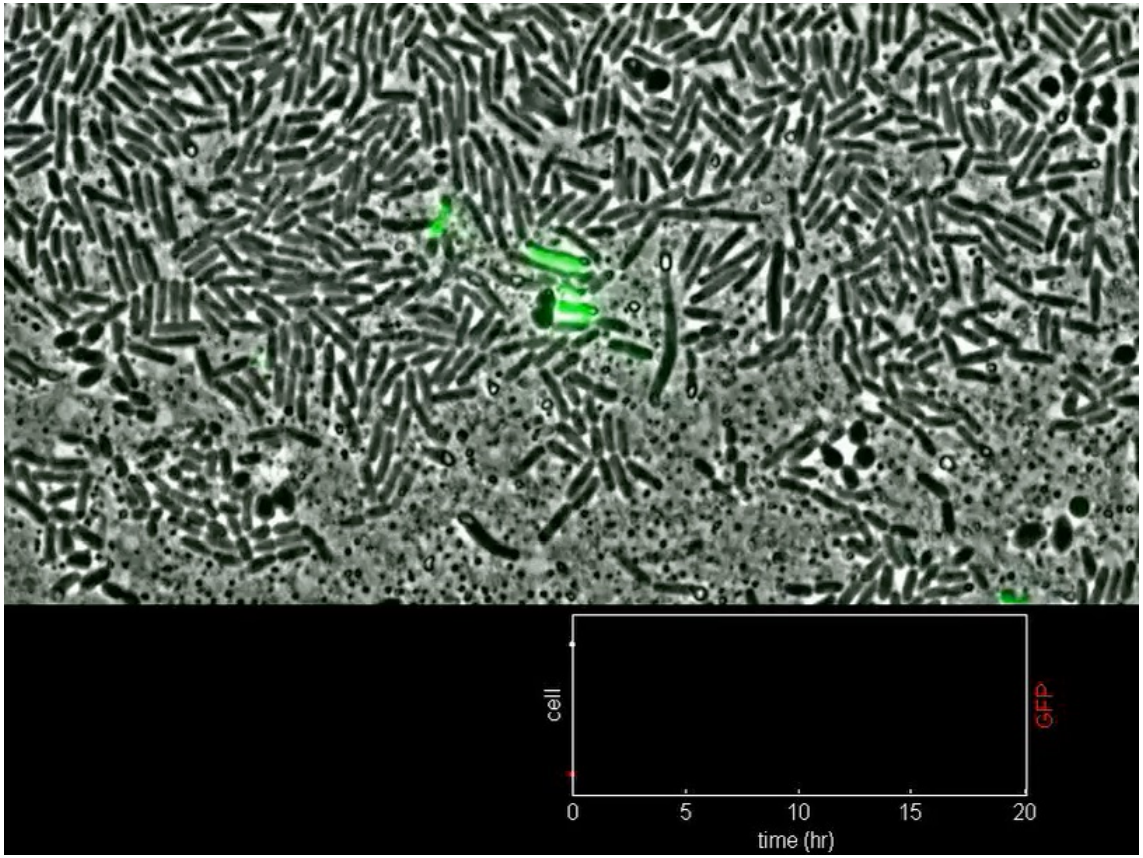

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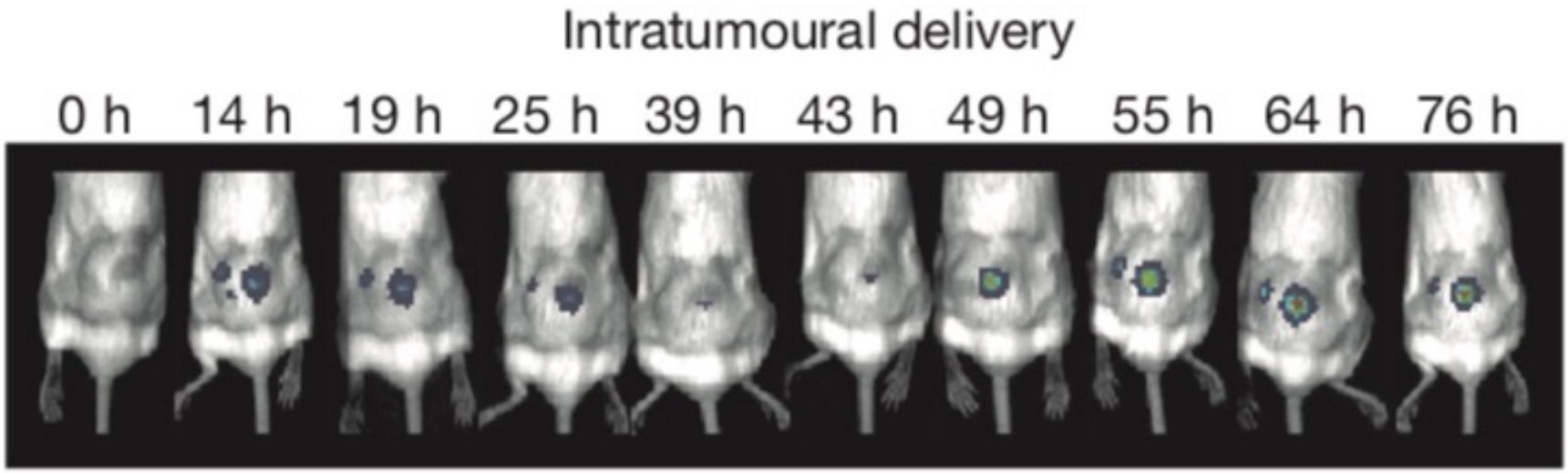


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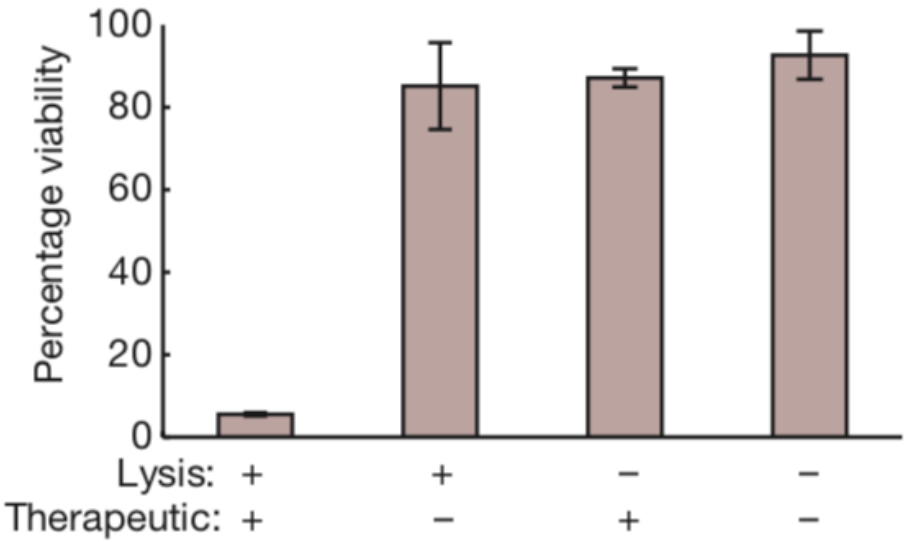


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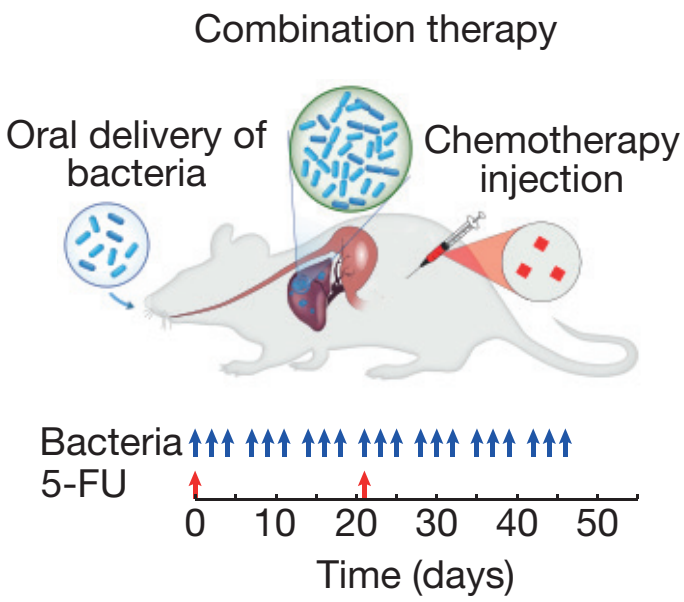


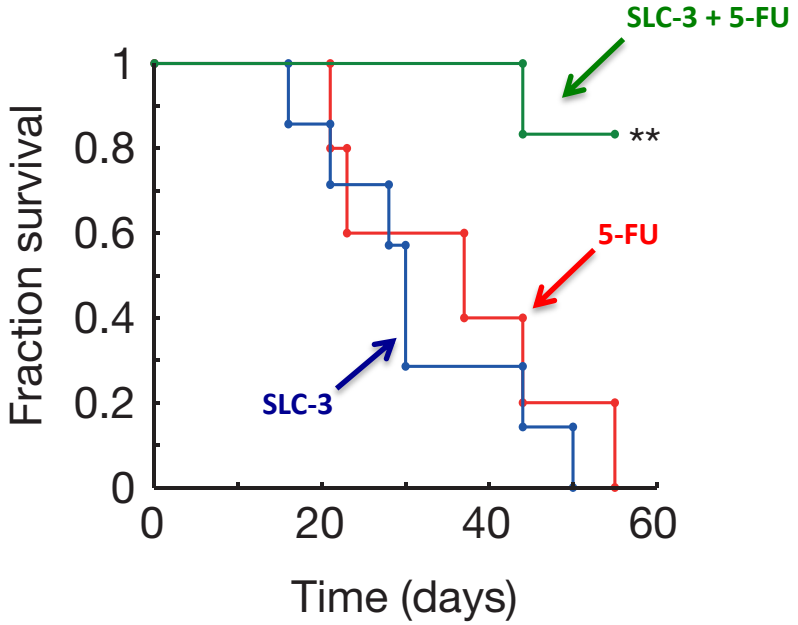


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***For the next lesson.***

# Letture consigliate


- Allen RC, Popat R, Diggle SP, Brown SP (2014) Targeting virulence: can we make evolution-proof drugs? *Nat Rev Microbiol* 12:300-308.
- D'Angelo F, Baldelli V, Halliday N, Pantalone P, Polticelli F, Fiscarelli E, Williams P, Visca P, Leoni L, Rampioni G (2018) Identification of FDA-approved drugs as antivirulence agents targeting the *pqs* quorum sensing system of *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 62(11).
- Hense BA, Schuster M (2015) Core principles of bacterial autoinducer systems. *Microbiol Mol Biol Rev* 79:153-169.
- Rampioni G, Leoni L, Williams P (2014) The art of antibacterial warfare: Deception through interference with quorum sensing-mediated communication. *Bioorg Chem* 55:60-68.
- Rampioni G, Visca P, Leoni L, Imperi F (2017) Drug repurposing for antivirulence therapy against opportunistic bacterial pathogens. *Emerging Topics in Life Sciences* 1:13-22.
- Forbes NS (2010) Engineering the perfect (bacterial) cancer therapy. *Nat Rev Cancer* 10:785-794.
- Gurbatri CR, Arpaia N, Danino T (2022) Engineering bacteria as interactive cancer therapies. *Science* 378:858-864.


Thank you for
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