



Reprogramming biological functionalities for autonomous therapeutic cells

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MICROBIOME

DENIZENS OF THE HUMAN BODY AND AFFECTS OUR HEALTH



THE HUMAN MICROBIOME

Bacteria, fungi, and viruses outnumber human cells in the body by a factor of 10 to one. The microbes synthesize key nutrients, fend off pathogens and impact everything from weight gain to perhaps even brain development. The Human Microbiome Project is doing a census of the microbes and sequencing the genomes of many. The total body count is not in but it's

600+ SPECIES
In the **mouth, pharynx and respiratory system**

Reprogramming commensal microbes to function as therapeutic units?

1,000 SPECIES
In the **intestines** include:

- Lactobacillus casei
- Lactobacillus reuteri
- Lactobacillus gasseri
- Escherichia coli
- Bacteroides fragilis
- Bacteroides thetaiotaomicron
- Lactobacillus rhamnosus
- Clostridium difficile

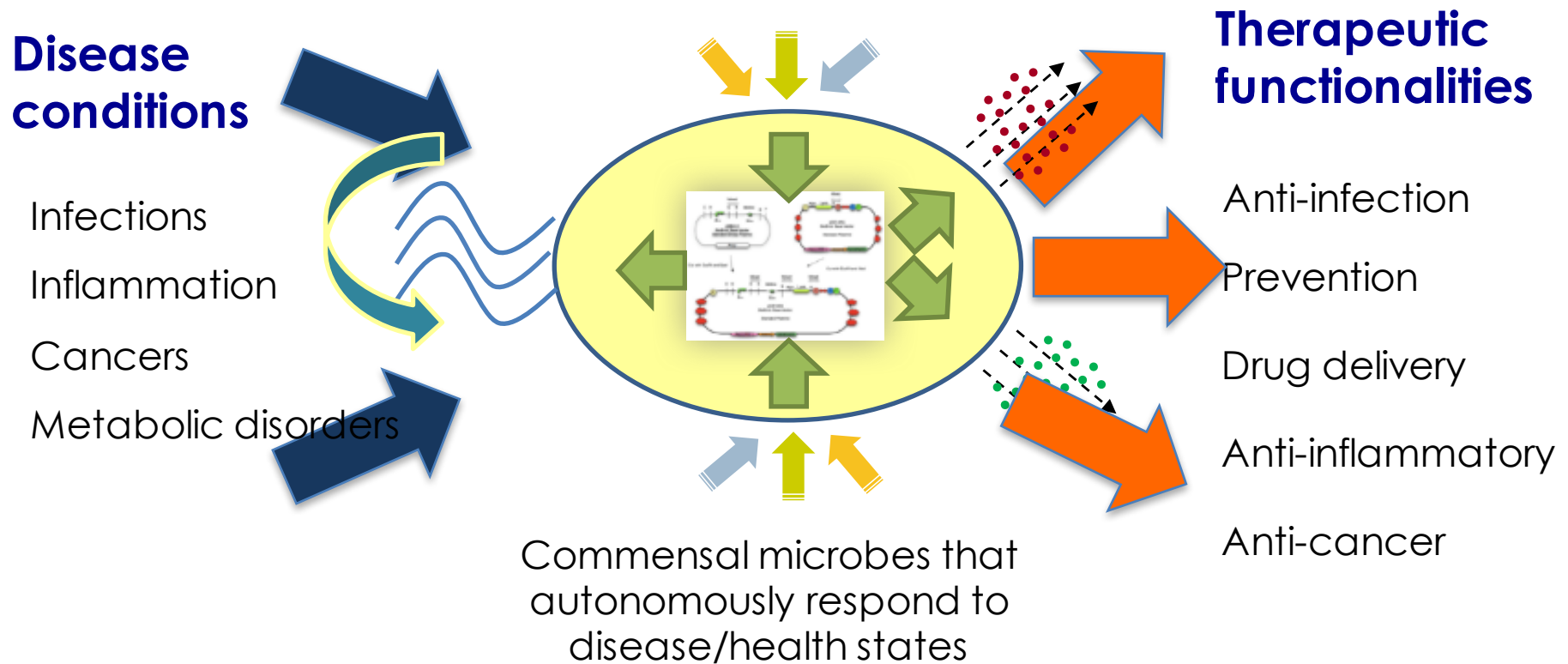
60 SPECIES
In the **urogenital tract** include:

- Trichosporon
- Staphylococcus haemolyticus
- Ureaplasma urealyticum
- Corynebacterium aurimucosum

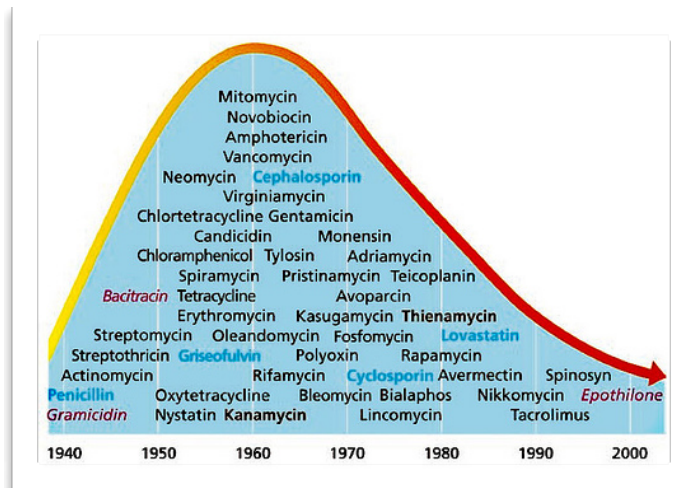
SOURCES: NATIONAL INSTITUTES OF HEALTH, SCIENTIFIC AMERICAN; HUMAN MICROBIOME PROJECT
Dean Tweed • POSTMEDIA NEWS / IMAGE: Fotolia

Functional commensal microbes

Responding to disease conditions in an autonomous manner



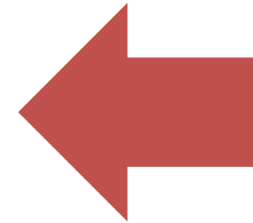
ANTIBIOTIC RESISTANCE: PROBLEM?



- ☼ Most major classes of antibiotics developed in 1940's to 1960's
- ☼ Since 1980's, the pharmaceutical industry focusing on chronic diseases that require life-long daily treatment
- ☼ Fewer new classes of antibiotics available

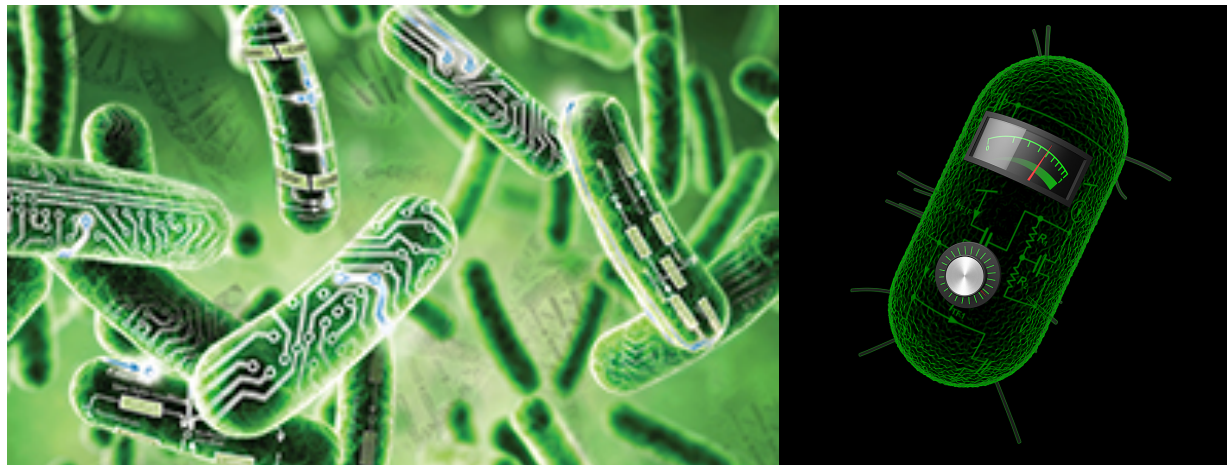
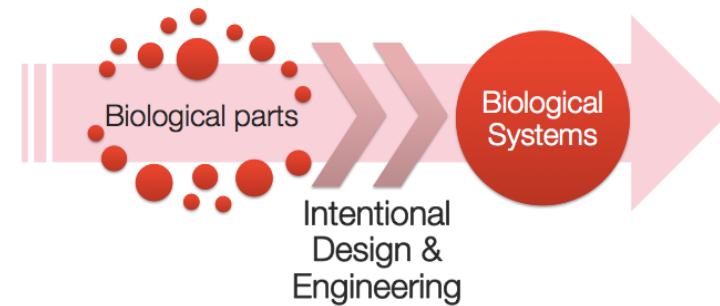
POTENTIAL SOLUTIONS?

- ☀️ Develop new classes of antibiotics
- ☀️ Diversify our antimicrobial strategies
 - Exploiting synthetic biology approaches?



RESEARCH OBJECTIVE

- **Intentional design**
 - Programmable behaviors
 - Sensing, seeking and killing a target pathogen

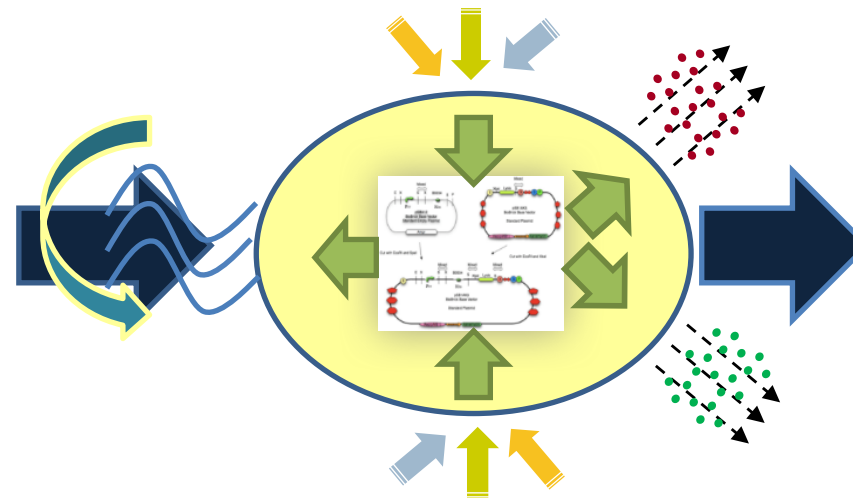


DESIGN: INPUT & OUTPUT

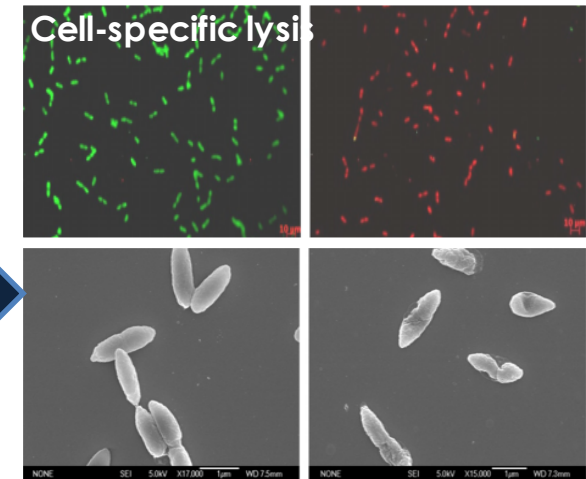
QUORUM SENSING (QS) & BACTERIOCINS

QS molecules

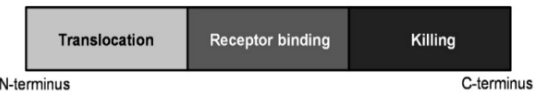
- Intercellular communication
- Transition between cellular lifestyle



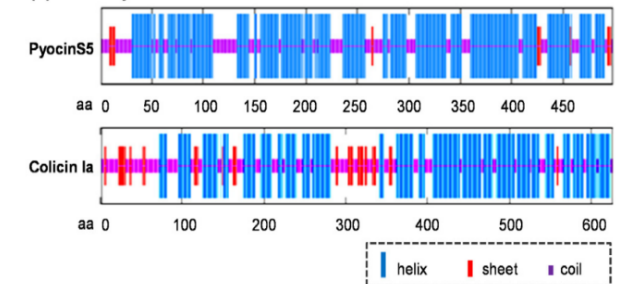
Bacteriocins



(A) Pyocin S5 domains



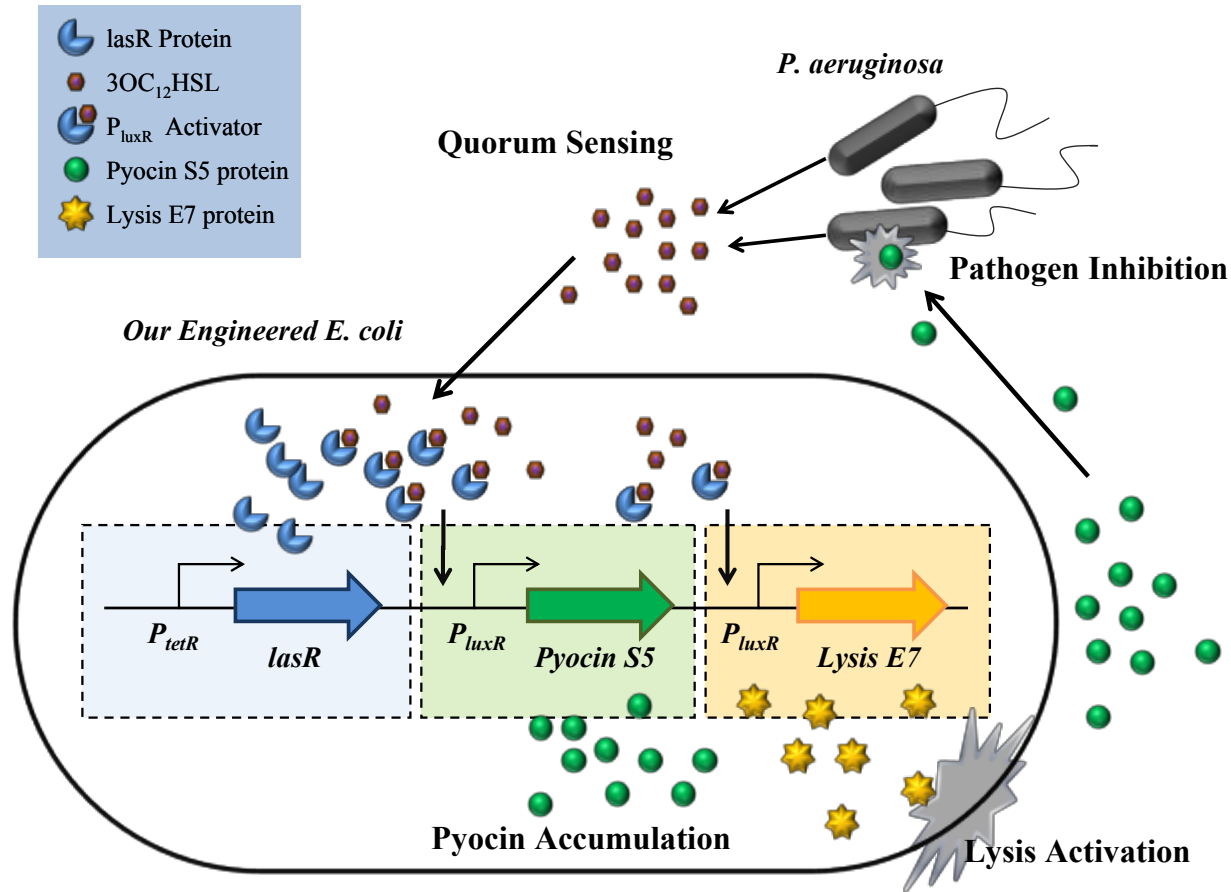
(B) Secondary structure



FEBS Lett (2010); BBRC (2010); Chem Eng Sci (2013); ChemBioChem (2013)

Pathogen-killing microbes

Prototype



Hwang et al. ACS Syn Biol, 2014
Rasouliha et al. ChemBioChem, 2013
Saeidi et al. Mol Sys Biol, 2011
Ling et al. FEBS Lett, 2010

How our synthetic microbes work..

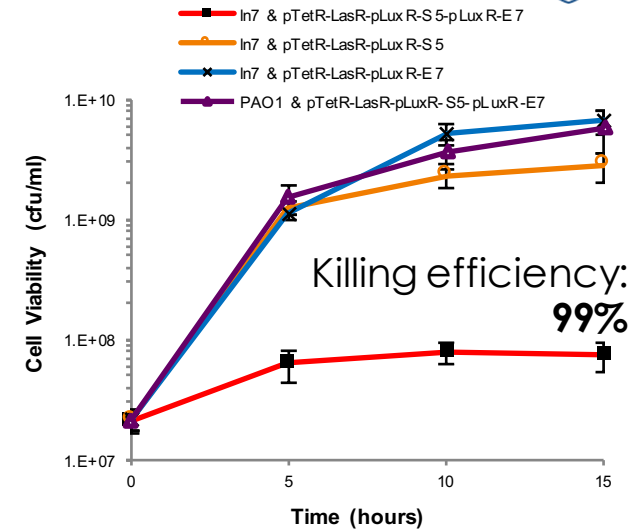
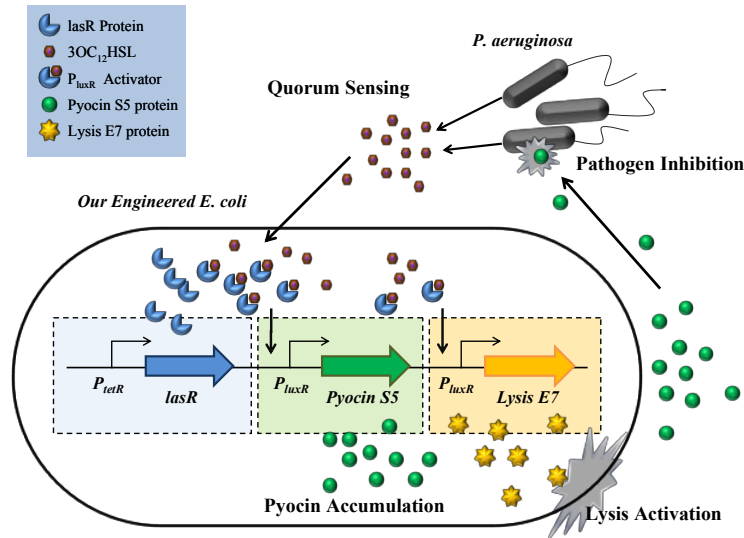
Courtesy of West German Broadcasting (WDR)



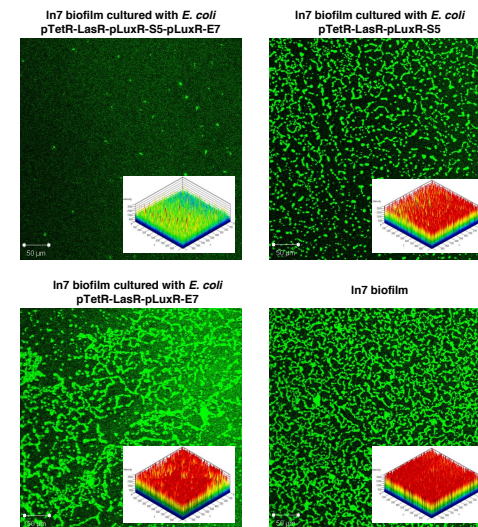
http://www.wdr.de/tv/quarks/sendungsbeitraege/2011/1213/008_bakterien_2.jsp

PATHOGEN-KILLING MICROBES

PROTOTYPES - STILL EVOLVING



- Targeted killing, minimizing negative effects on the microbiome
- Preventive effects

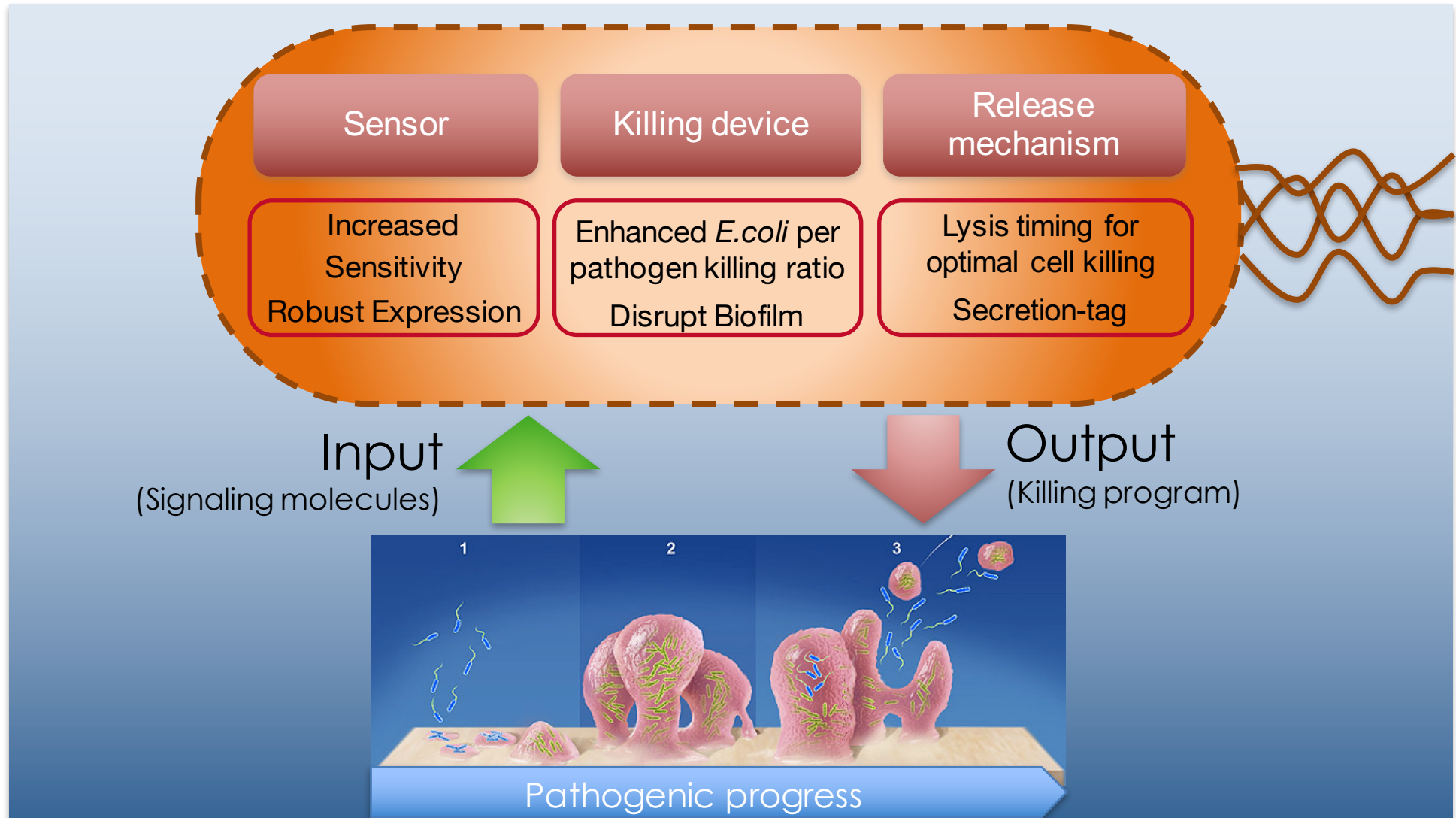


Biofilm inhibition: **90%**

- Molecular Systems Biology (2011), Featured in Reuters, Nature, Science, etc.

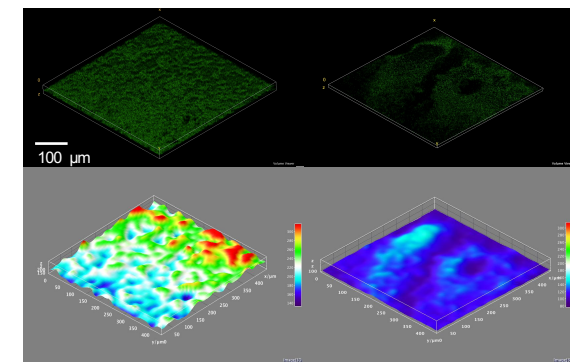
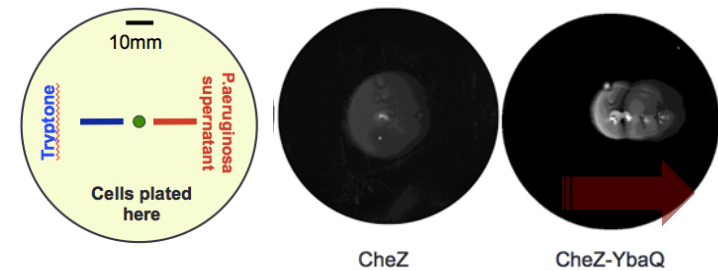
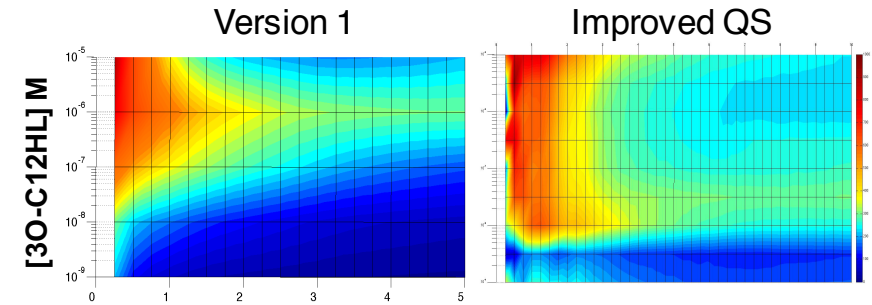
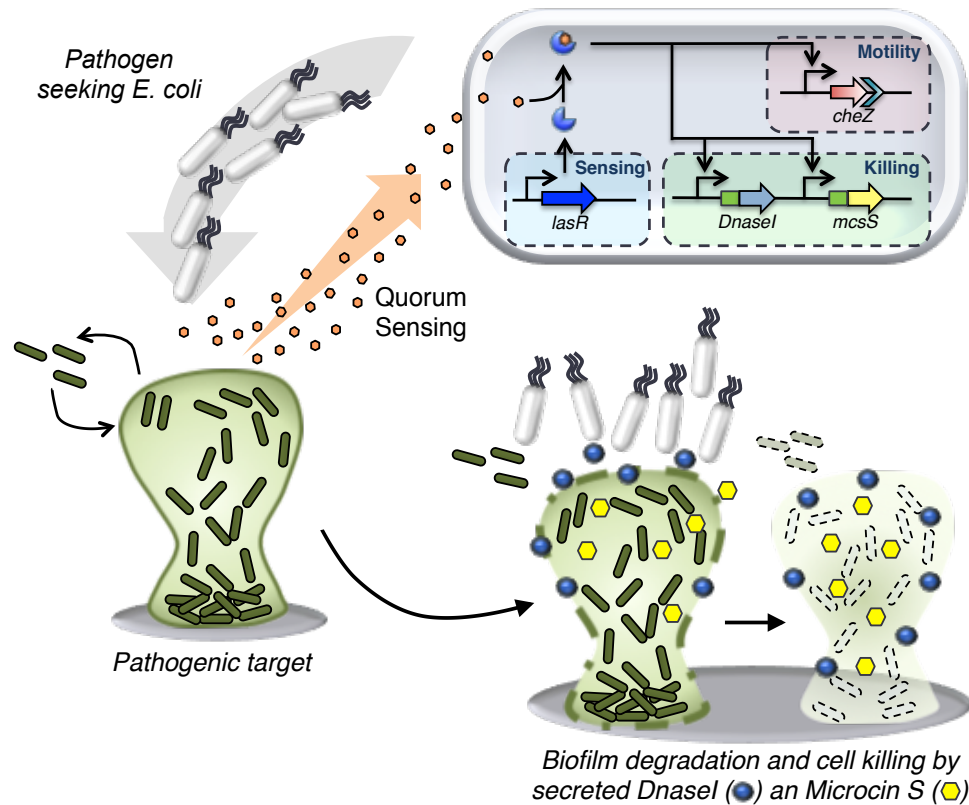
System optimization

Advancing the system towards more effective killing



PATHOGEN-KILLING MICROBES

PROTOTYPES - STILL EVOLVING

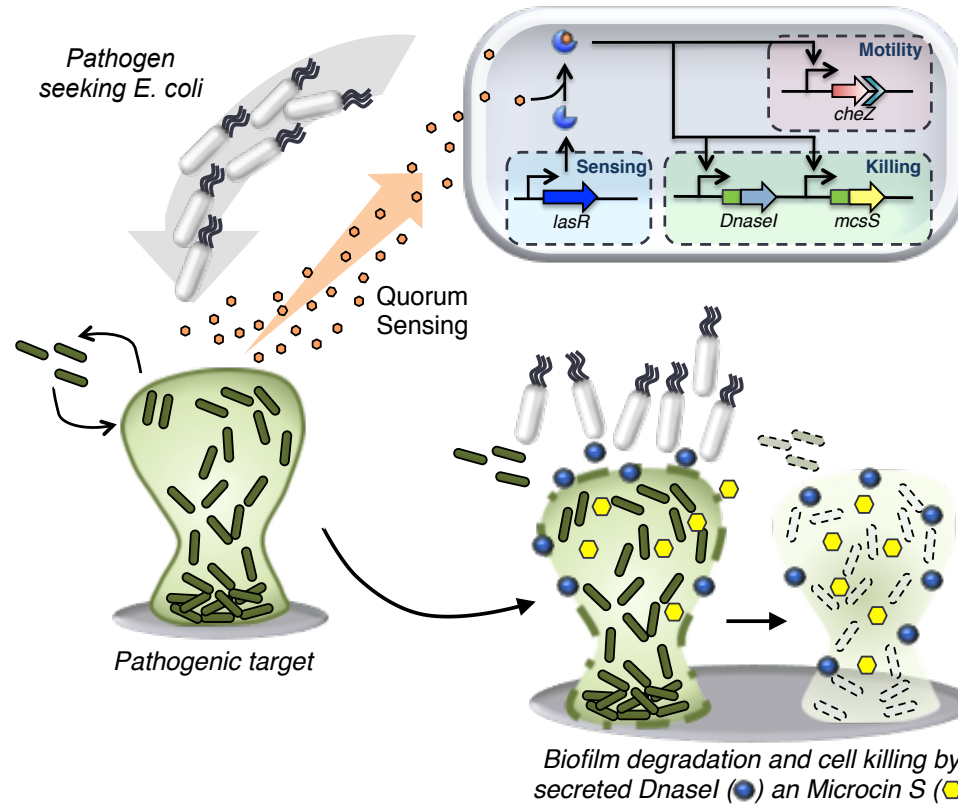


1. Sensitivity and robust expression
2. Target localization (motility)
3. Improved killing efficiency

- ACS Synthetic Biology (2014), Featured in The Economist, Nature News, etc.

Advanced therapeutic functionalities

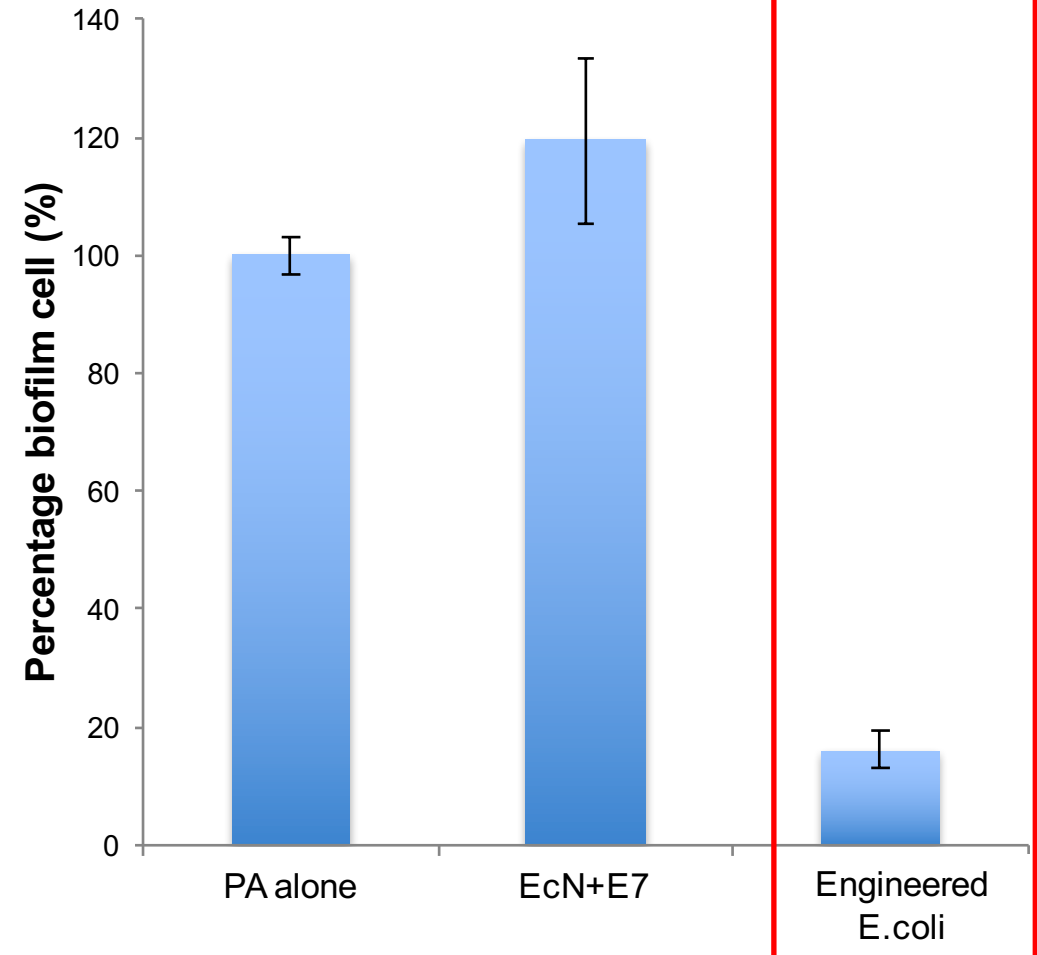
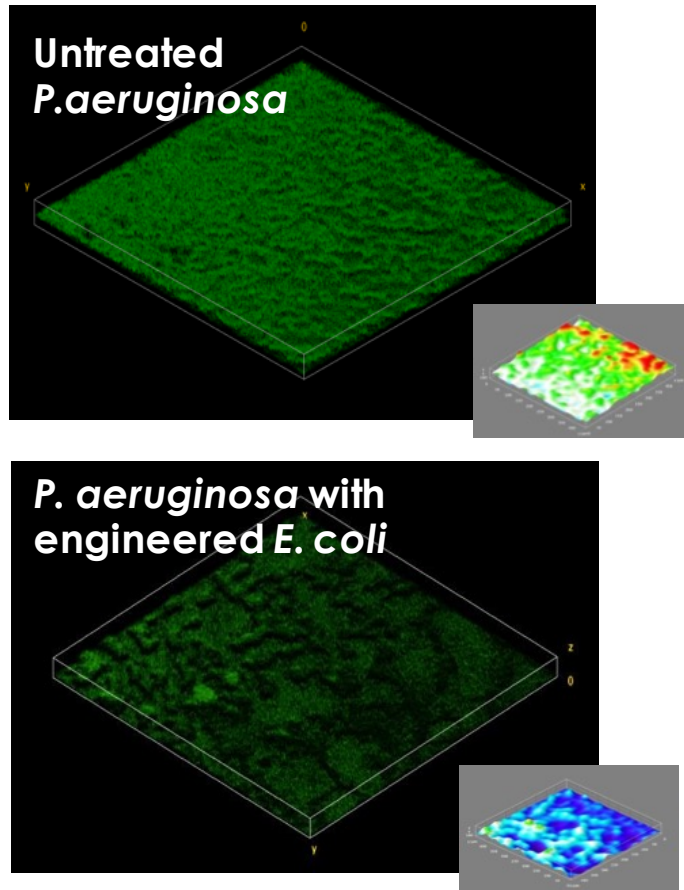
Programmed motility towards a target pathogen



ACS Synthetic Biology (2014), Featured in The Economist, Nature News, etc.

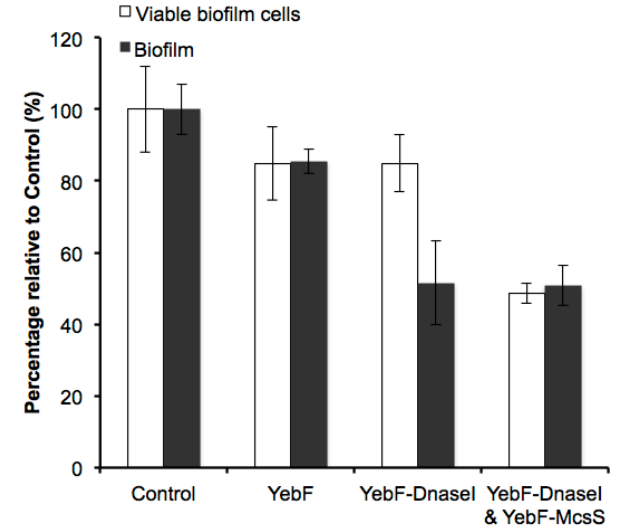
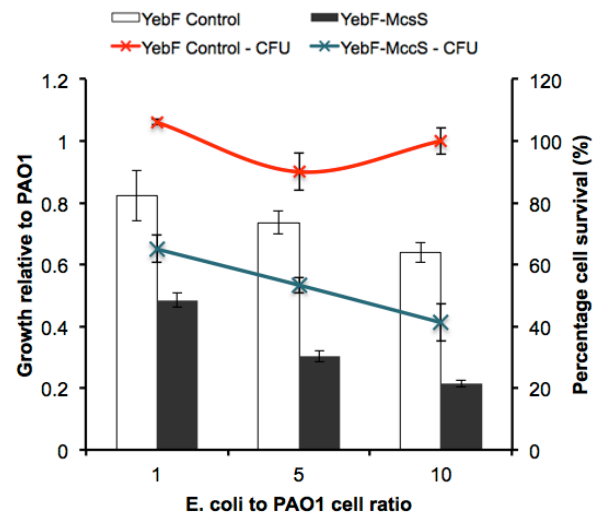
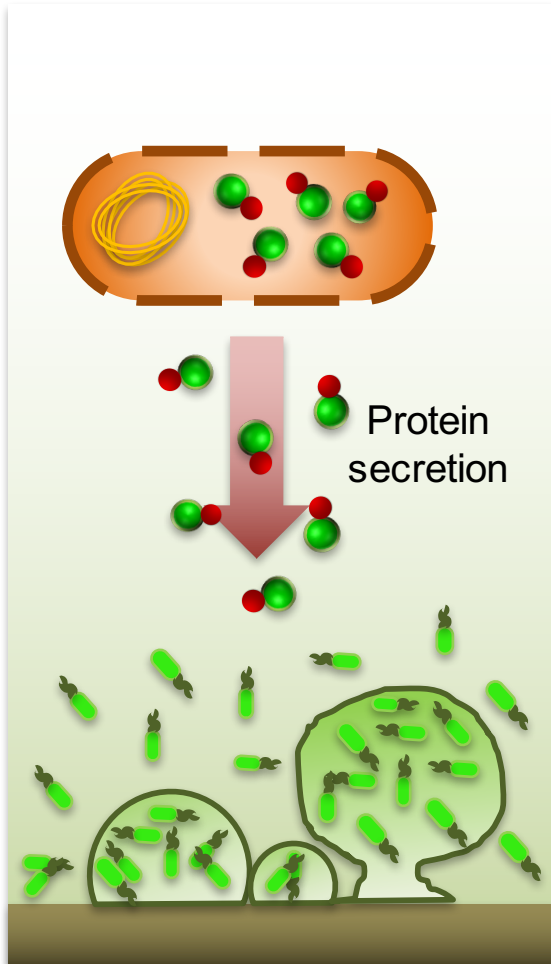
PREVENTION OF INFECTION STRATEGIES

Enzymatic dispersal of mature biofilm

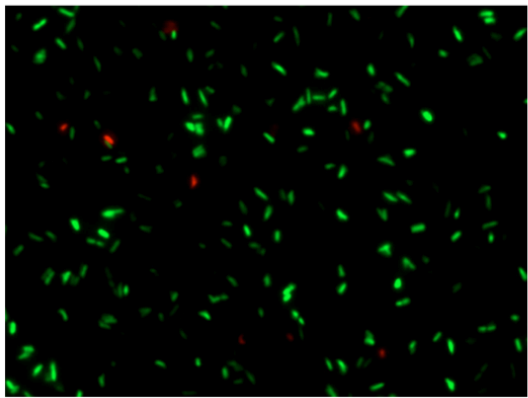


DELIVERY STRATEGIES

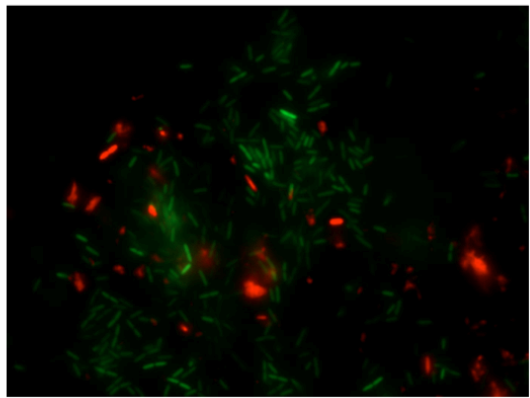
Targeted killing by protein secretion



PAO1 treated with E.coli supernatant



PAO1 treated with YebF-McsS supernatant

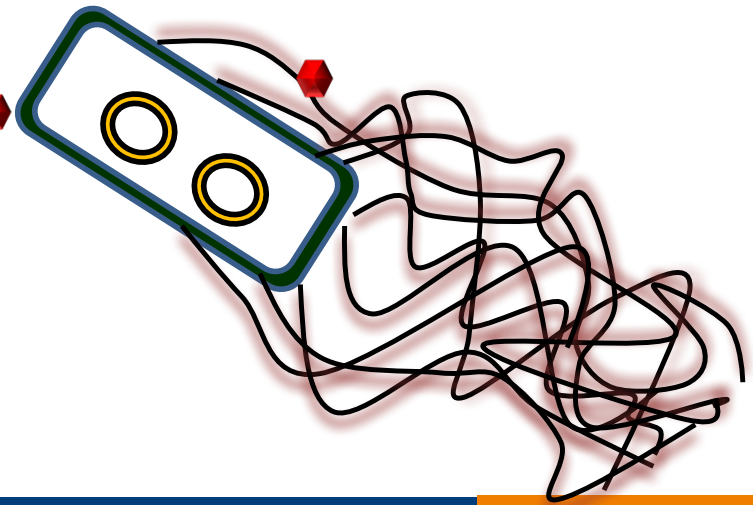
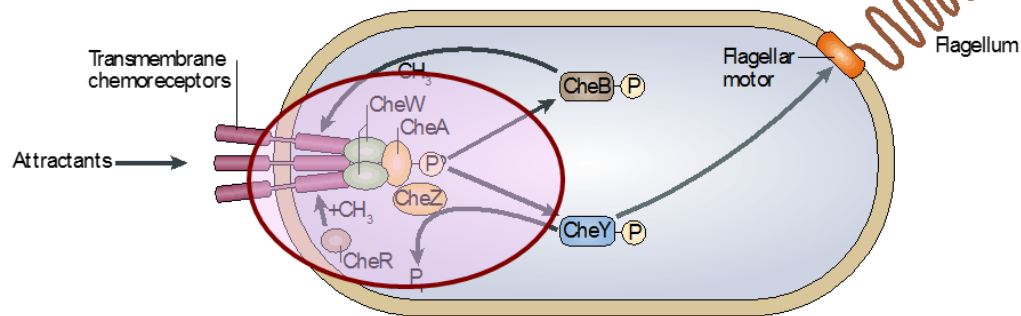
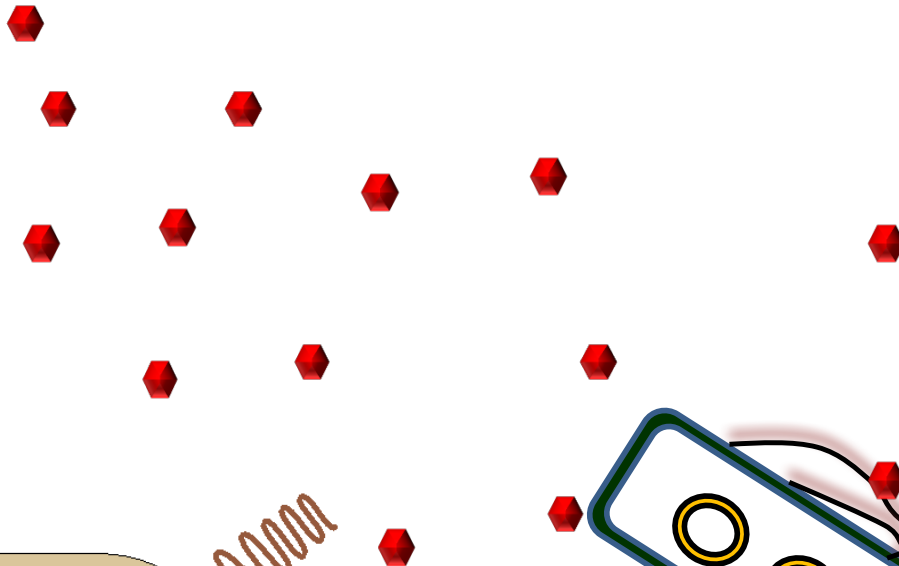


CELLULAR DRUG DELIVERY STRATEGIES

Motility via chemotaxis pathway

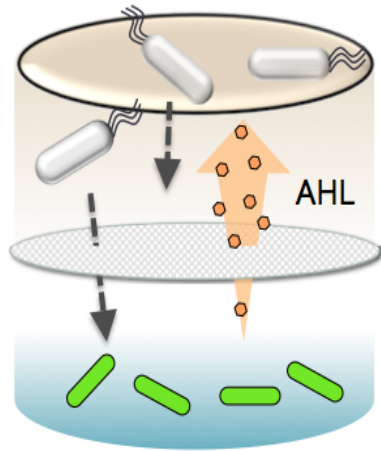


Pseudomonas aeruginosa

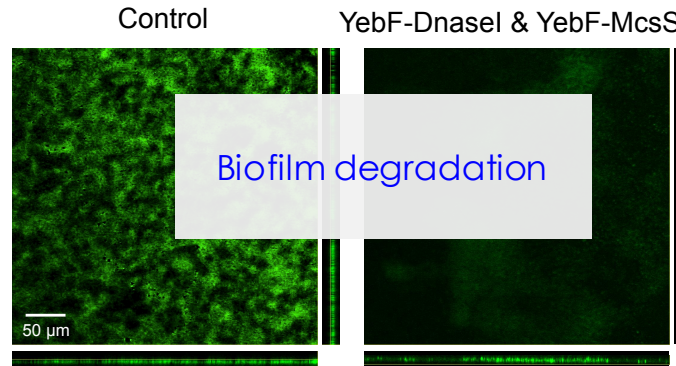
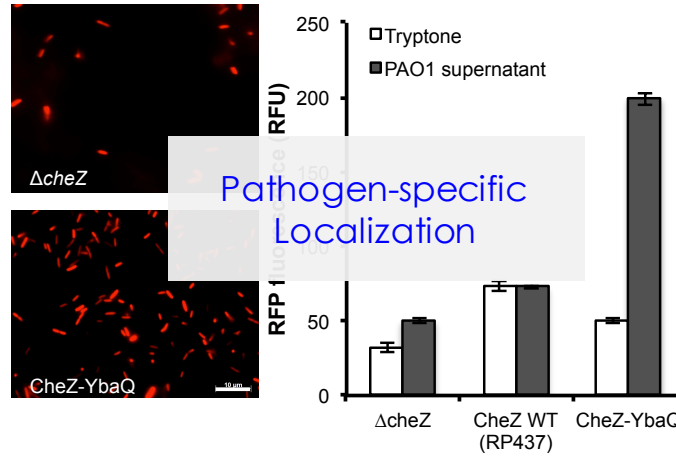


FULLY FUNCTIONAL MODEL

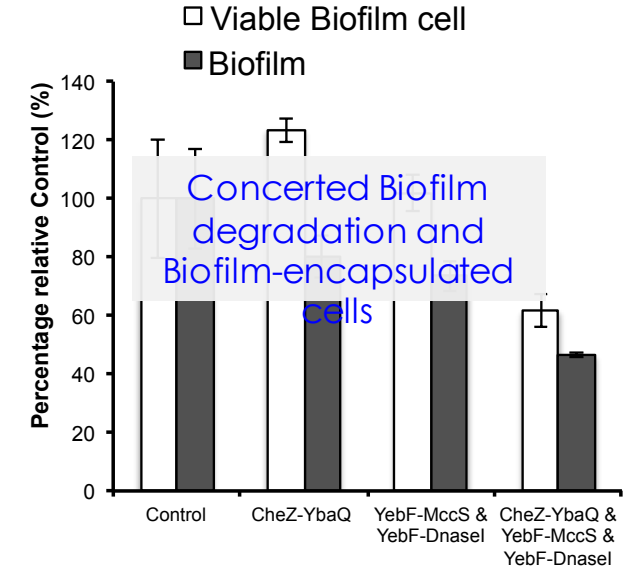
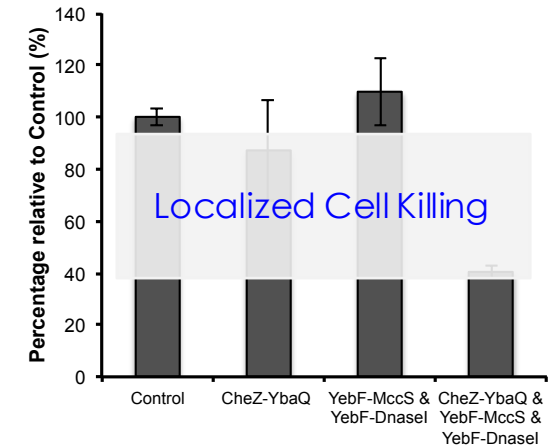
PROGRAMMED MOTILITY-DRIVEN KILLING



Reprogrammed *E. coli*
(+ killer enzymes)
swims towards target cells



Viable PAO1 cells (CFU)



ACS Syn Biol (2014)

TEST MODELS

Killing effects in eukaryotic models (*Mus musculus*)

P.aeruginosa infection

Treatment

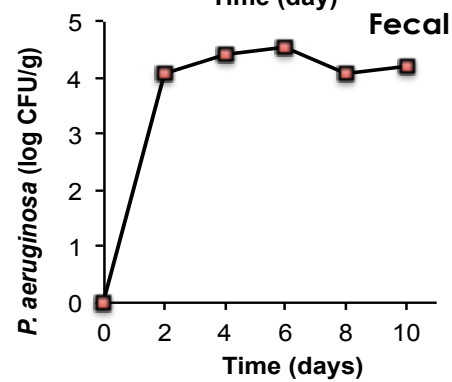


Antibiotic
treatment

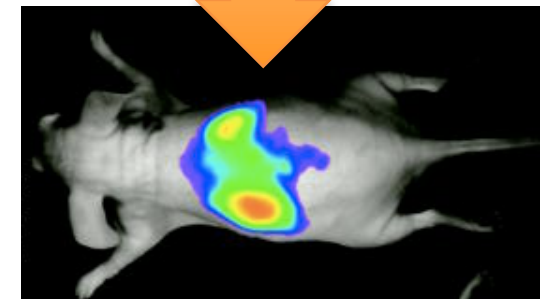
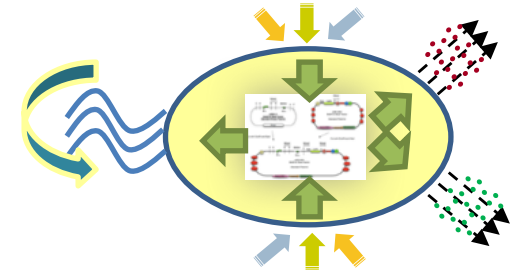


infection

gut colonization



Programmed
therapeutic cells



ALL-IN-A-PAGE

What we have learned so far and what will come

