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Biofilm communities, antimicrobials and antimicrobial resistance

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AMHT
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Biodiscovery Institute

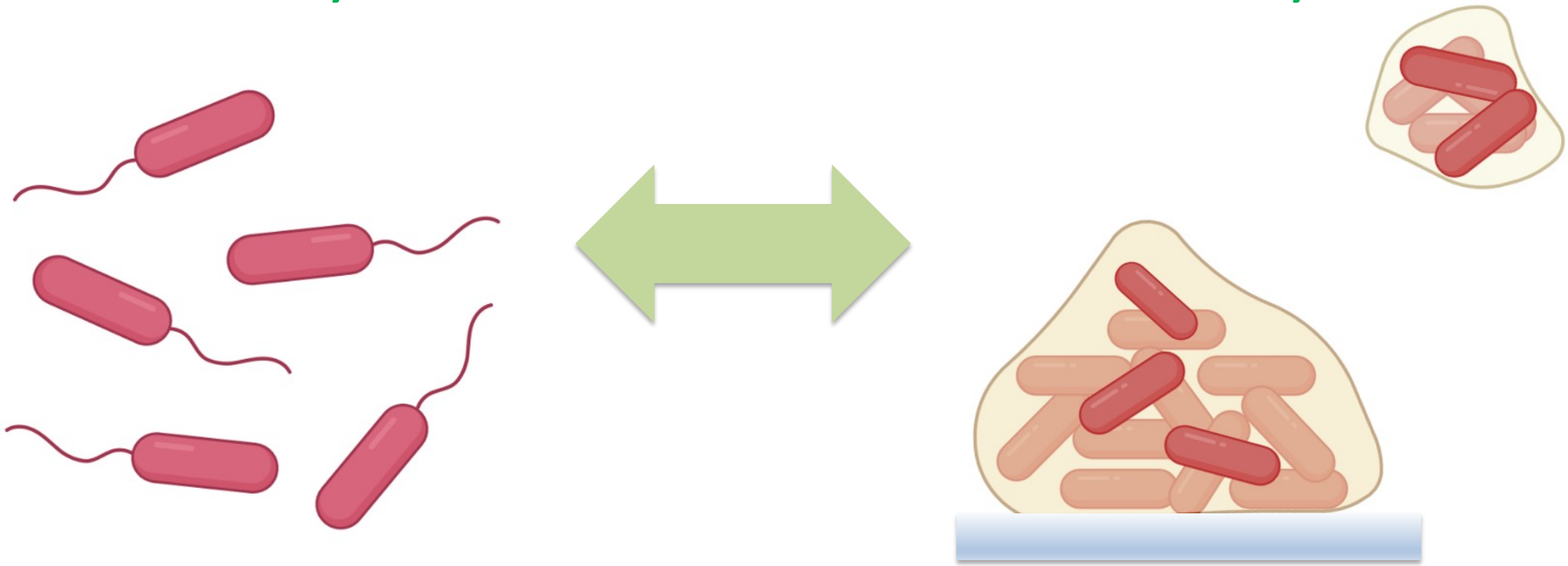
EMPIR EURAMET
The EMPIR initiative is co-funded by the European Union's Horizon 2020 research and innovation programme and the EMPIR Participating States

The University of Nottingham
UNITED KINGDOM · CHINA · MALAYSIA

Bacteria switch between two lifestyles

Solitary

Community



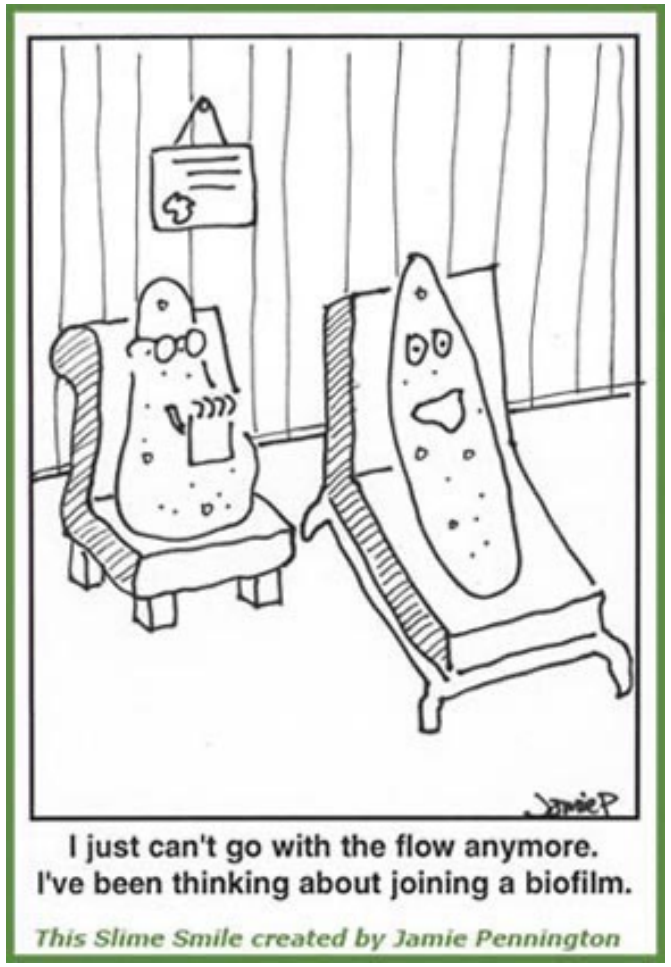
Planktonic

Biofilm

BioRender.com

More bacteria are found in biofilm communities than as free-living cells

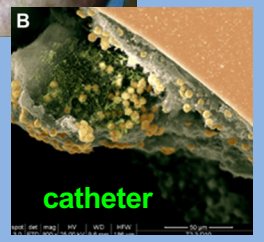
03



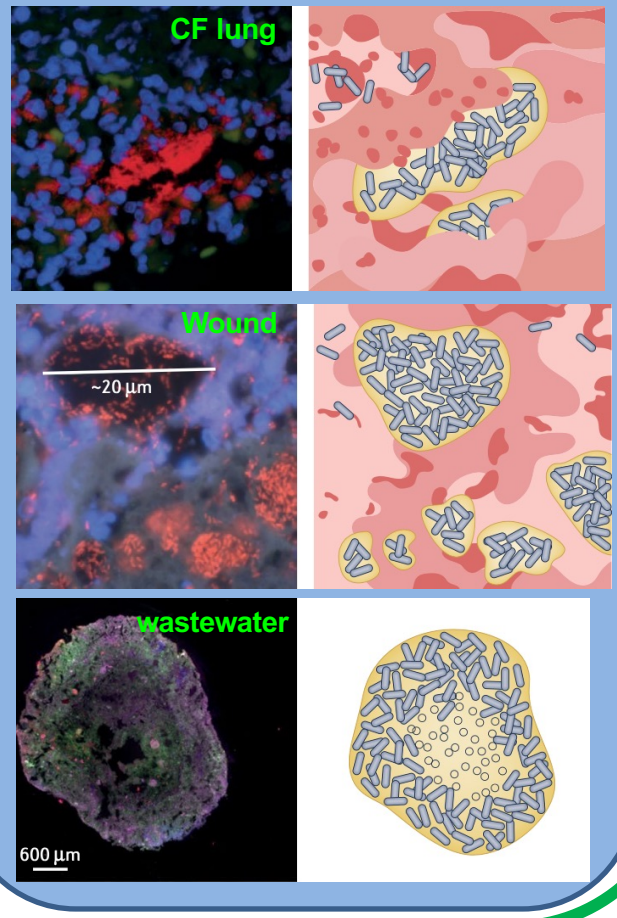
Biofilms are widespread

Biofilms: complex coordinated communities

on surfaces



Suspended aggregates



Sauer et al 2022

Human infections where biofilms are a particular problem

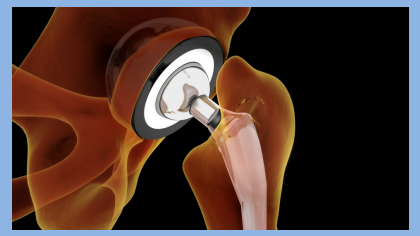
Cystic Fibrosis (>70k people)

$7.5bn, global

78% of Chronic wounds

$281bn, global, 2017

Implants



$7.8bn, global

Indwelling devices

$1bn, global

Add on non-healthcare:

$3967bn, global

Camara et al 2022

Composition of biofilms

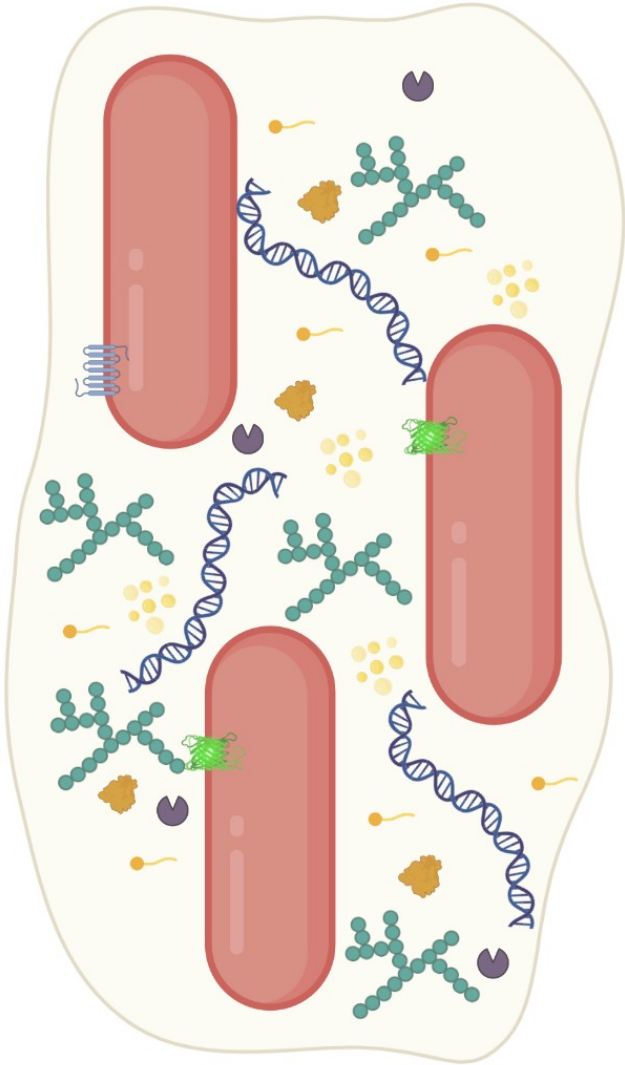
Polysaccharides

Structural Proteins

Enzymes

eDNA

Lipids & biosurfactants



Polysaccharides

- Structural cohesion
- Nutrient source
- Water retention
- Protective barrier
- Absorption of organic compounds and inorganic ions

Structural Proteins

- Structural cohesion
- Nutrient source
- Protective barrier
- Absorption of organic compounds and inorganic ions
- Electron donor/acceptor

Enzymes

- Catalytic conversions
- Nutient source

eDNA

- Structural cohesion
- Nutrient source
- Exchange of genetic information

Lipids & biosurfactants

- Nutrient source

BioRender.com

Pseudomonas aeruginosa is a well studied biofilm former

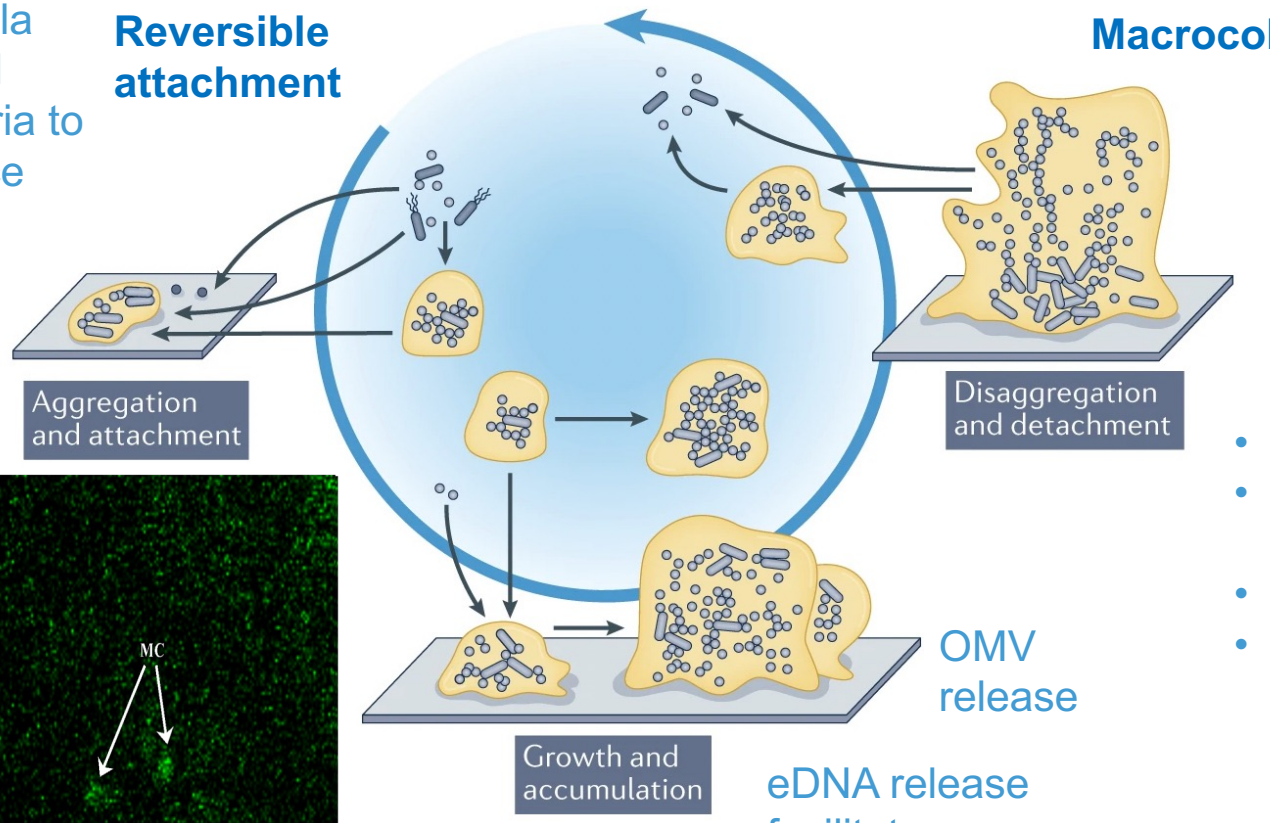
- Gram negative bacterium
- Opportunistic pathogen: Cystic Fibrosis, wounds, eyes
- Acute and chronic infections
- Ubiquitous: many reservoirs of infection
- Intrinsically resistant to many antibiotics
- Numerous virulence factors with complex regulatory controls

The complex biofilm structure is formed in co-ordinated way

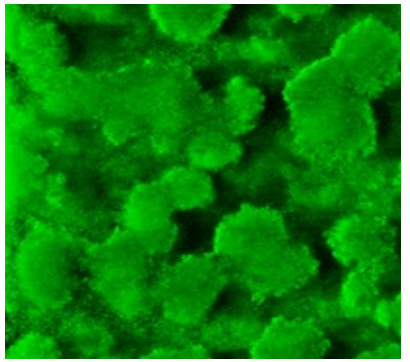
Flagella propel bacteria to surface

Reversible attachment

Irreversible attachment



Macrocolony



Flagella/surfactants facilitate swarming over surface

Type IV pili extend/retract to tug across surface (twitching)

Extracellularpolysaccharides (EPS) & proteins create matrix

OMV release

eDNA release facilitates clumping

- Matrix composition alters,
- water channels/anoxic pockets exist
- persister cells emerge
- Secretion machinery & flagellum expression changes

P. aeruginosa

Sauer et al. Nat Rev Micro 2022

Example mechanisms underlying biofilm formation

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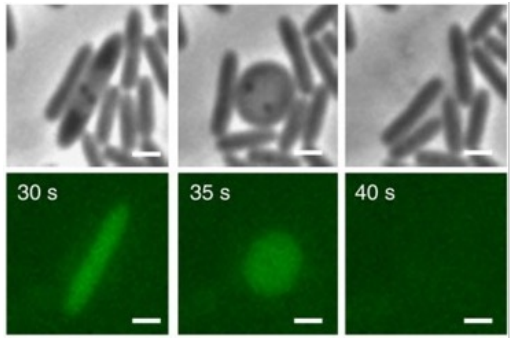
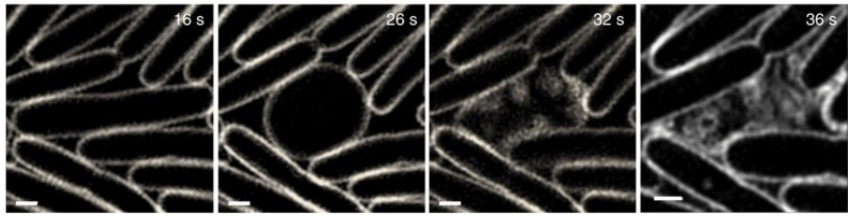
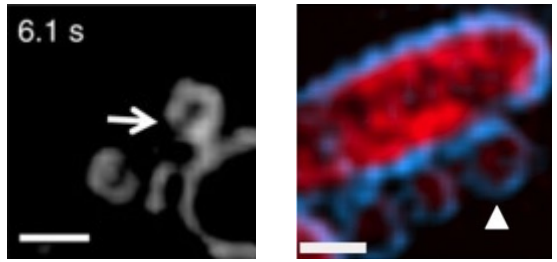
Release of eDNA and OMV by explosion



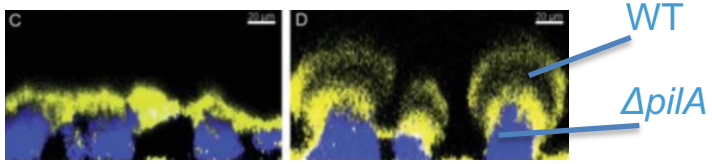




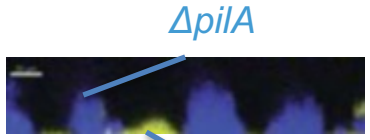
Turnbull *et al* Nat Commun 2016 14;7:11220
(Cynthia Whitchurch)

Structured assembly

Type IV pili, flagellum-mediated motility and quorum sensing-controlled DNA release are involved in the formation of mature *P. aeruginosa* multicellular structures



+ DNAase - DNAase



Barken et al Environ Micro 2008 10:2331
(Tim Tolker-Nielsen)

- ***Polysaccharide release***
- ***Stress responses***
- ***Metabolic changes***
- ***Co-ordinated communication***

P. aeruginosa

P. aeruginosa

The polysaccharides and eDNA in the biofilm matrix are structured

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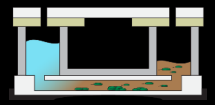
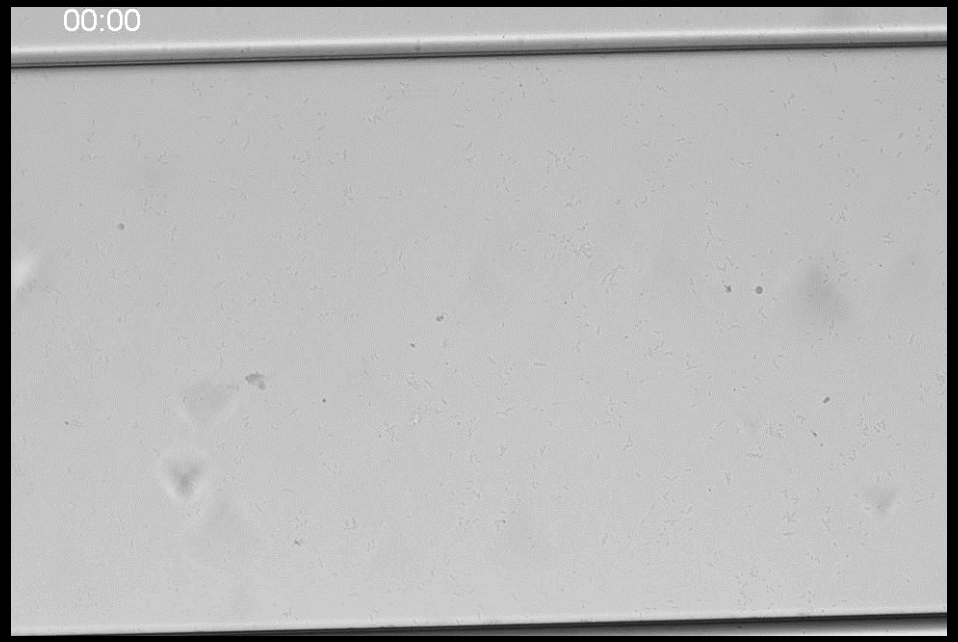
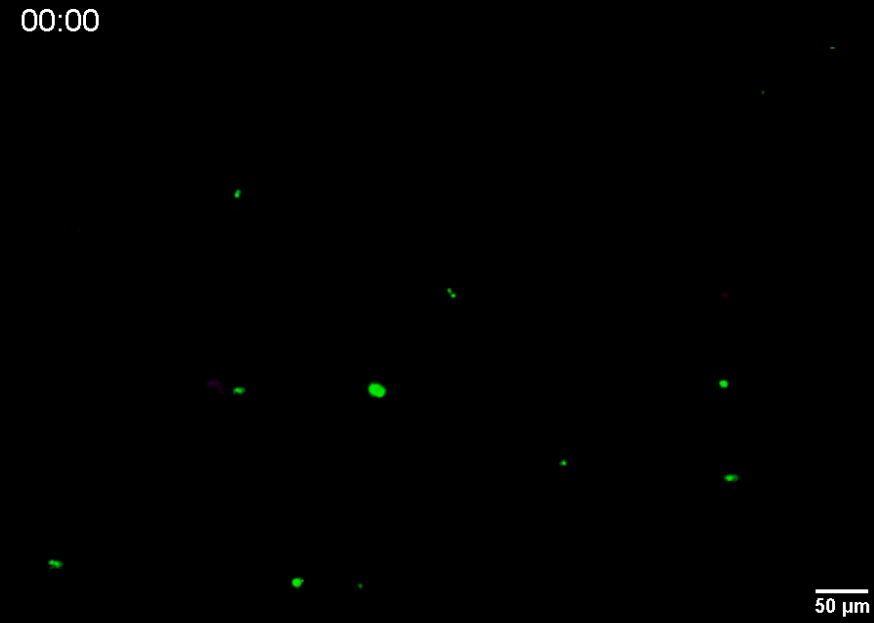
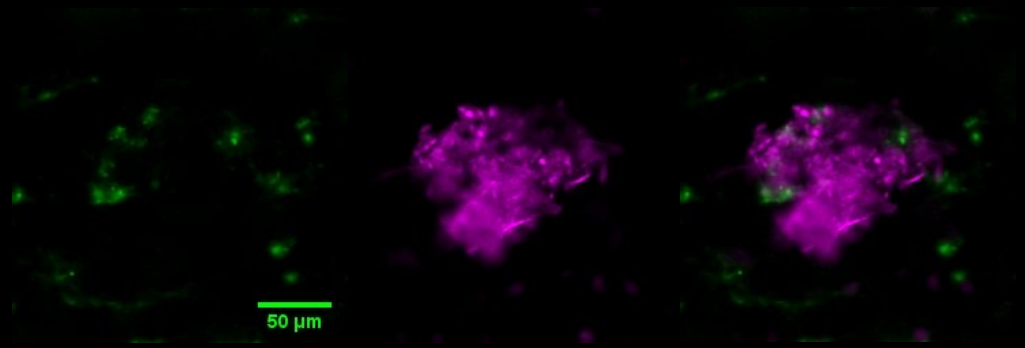
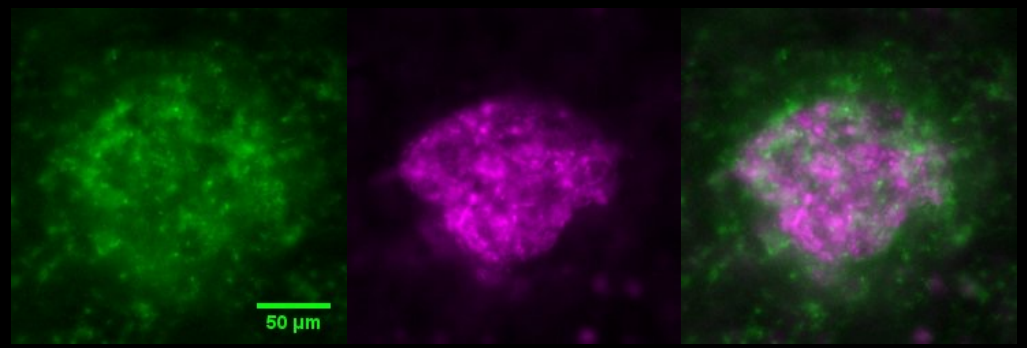








YOYO-1 (eDNA) ConA (EPS)

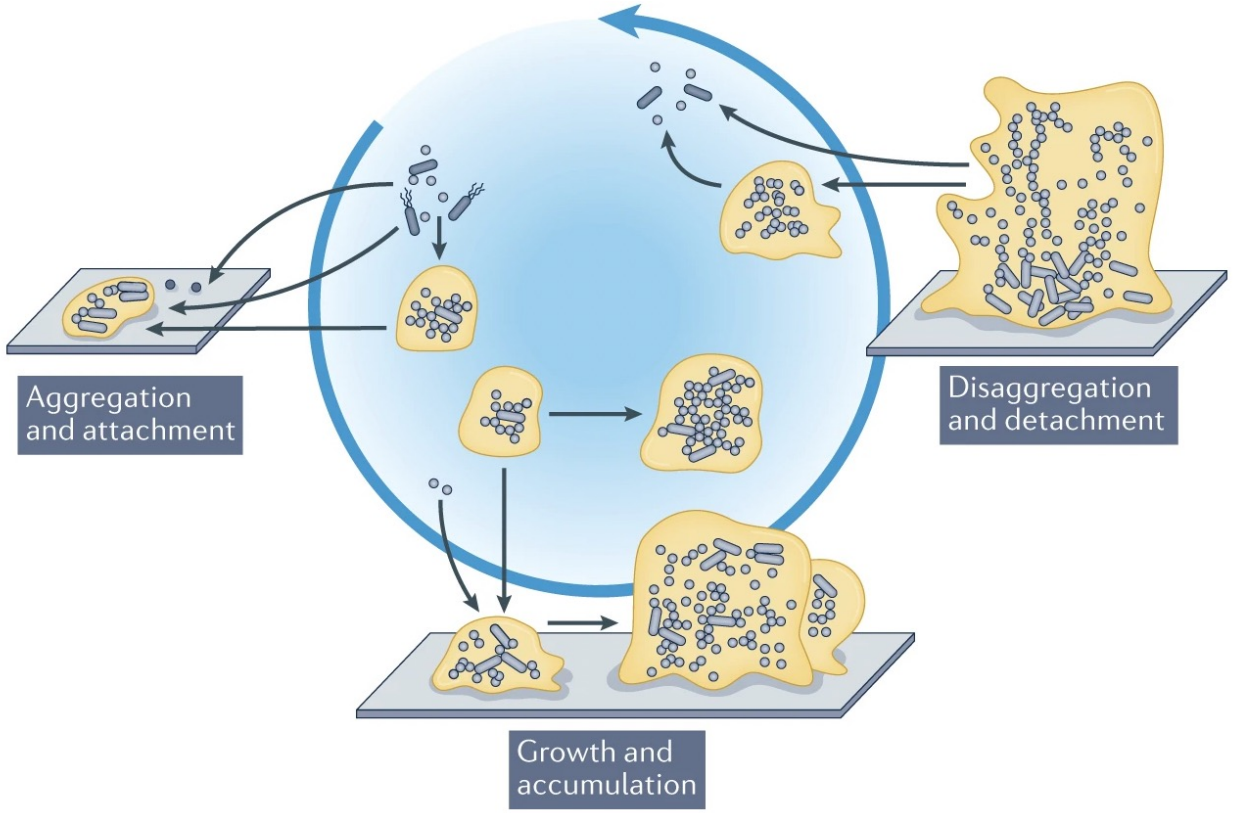


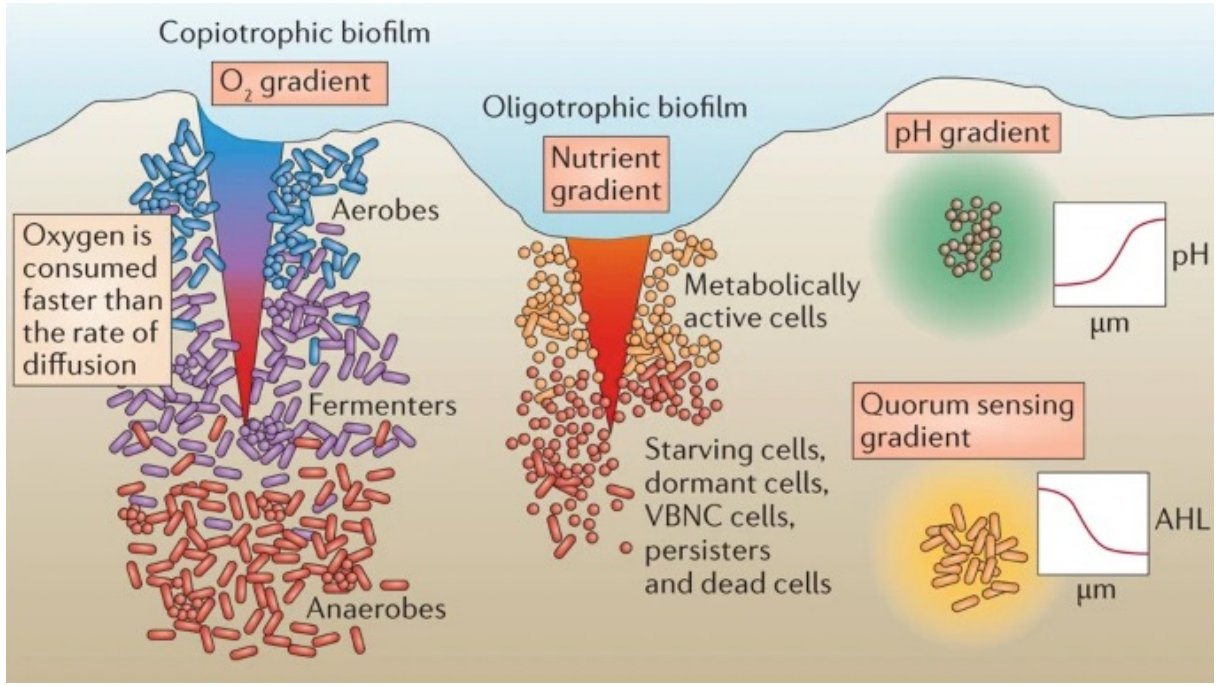
YOYO-1 (eDNA) ConA (EPS)

University of Nottingham
UK | CHINA | MALAYSIA

James Brown, Paul Williams

Biofilm formation and microniches are inconsistent and dynamic

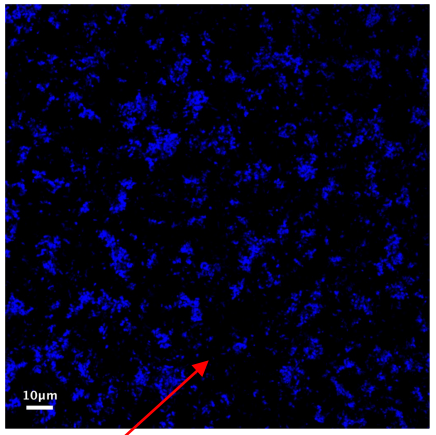




Flemming et al. Nat Rev Micro 2016

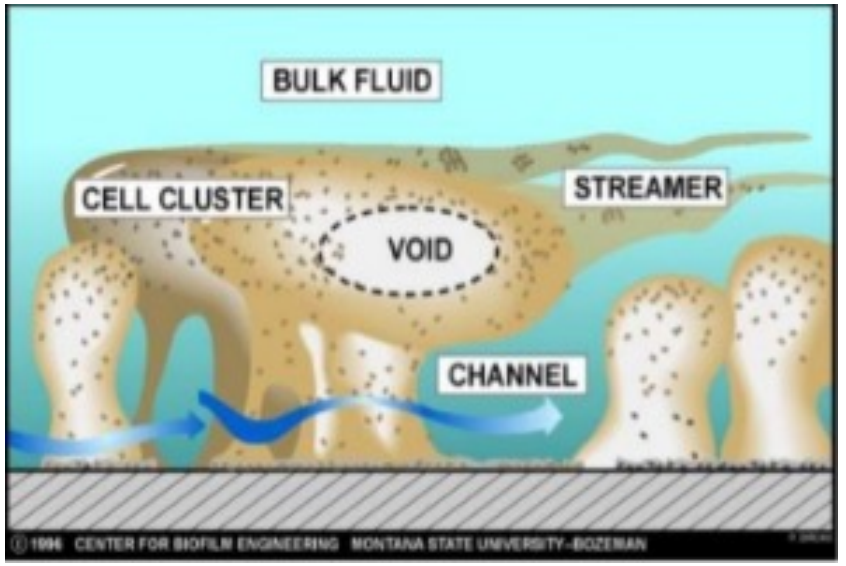
Sauer et al. Nat Rev Micro 2022

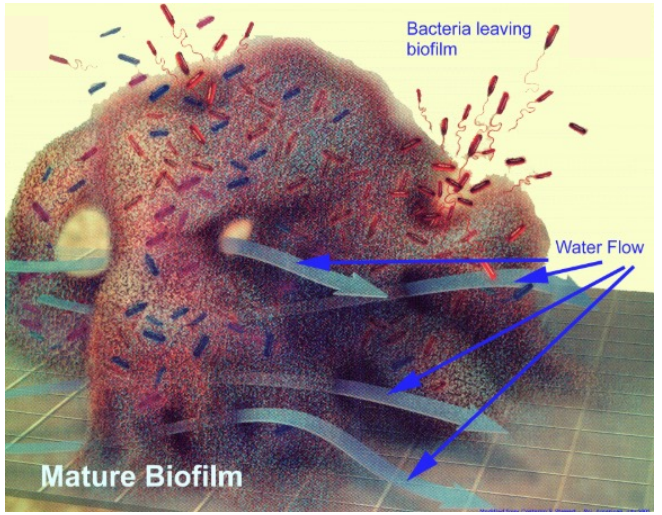
There are channels within biofilms



Gaps between microcolonies in static biofilms create channels

Flow creates channels





Optical nanosensors can probe biofilm architecture and geography in real time to inform antimicrobial delivery.

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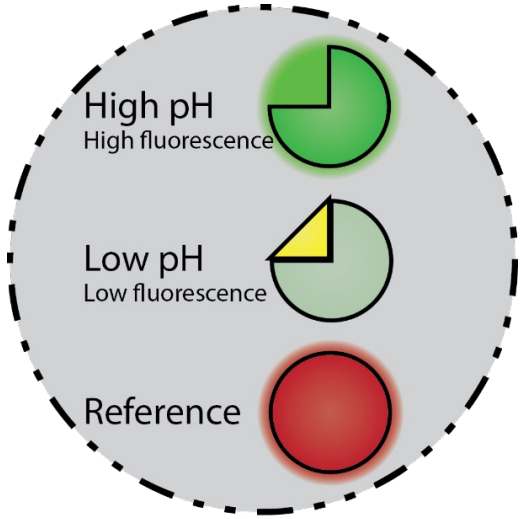
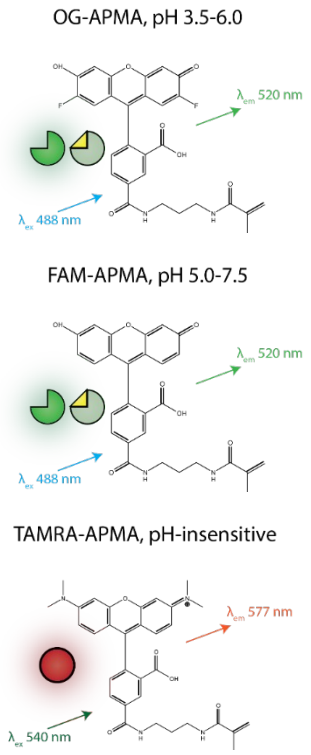
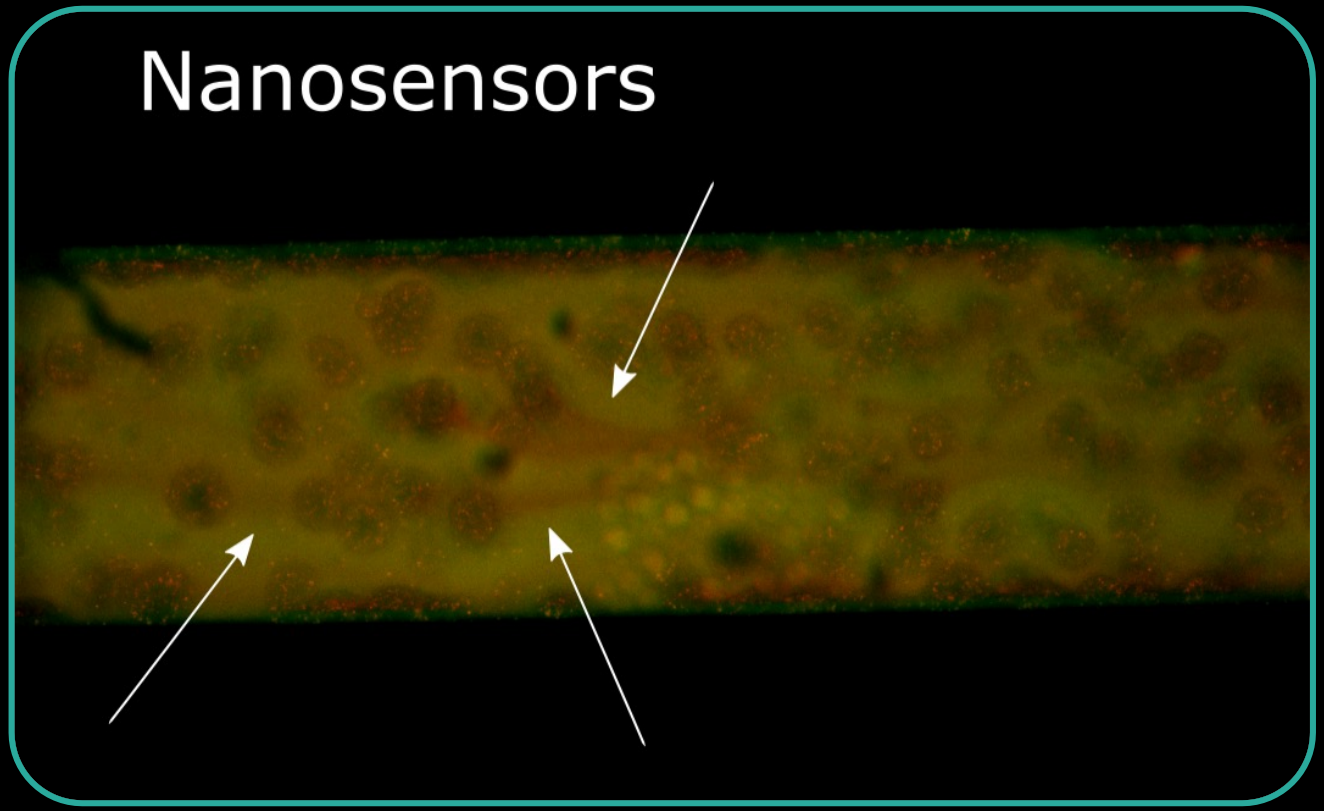
optical nanosensors



(Adapted from Chauhan *et al.* 2013, ASC NANO)





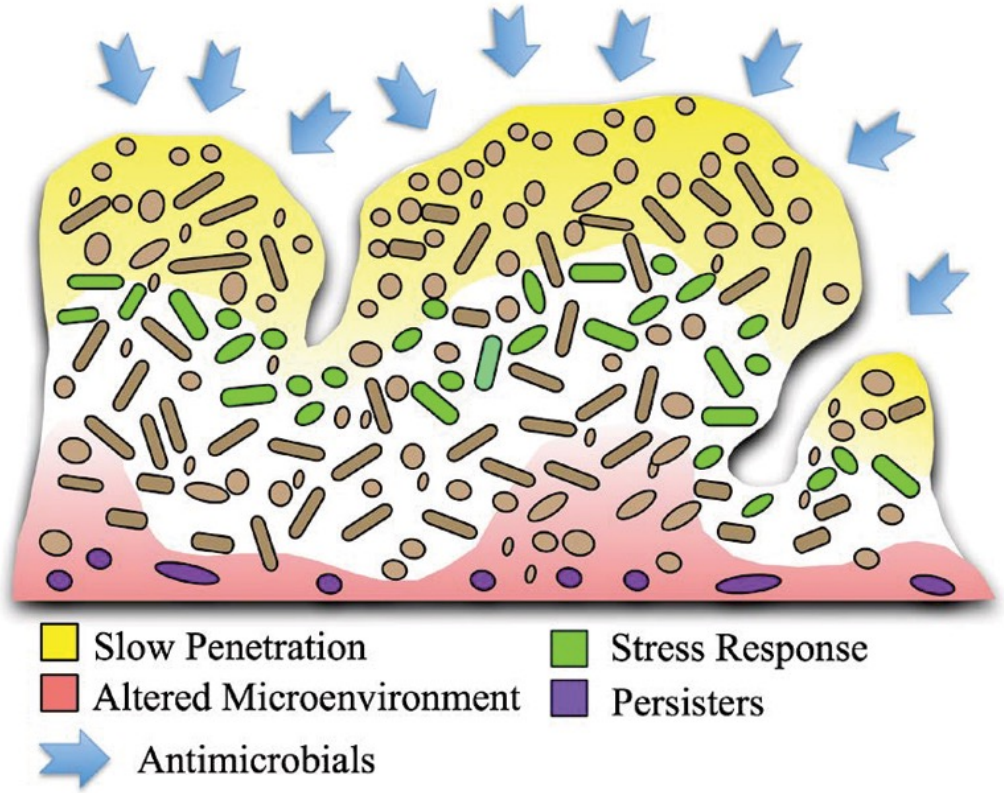
high
pH
low

P. aeruginosa

Hollmann et al. npj Biofilms and Microbiomes2021

Birte Hollmann, Mark Perkins, Jon Aylott

Biofilm features impede antimicrobial effectiveness

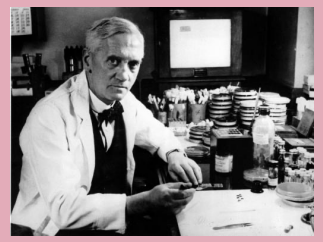


- barriers to antimicrobial penetration
- Not all bacteria actively growing/susceptible to antimicrobial
- conditions may inactivate the antimicrobial

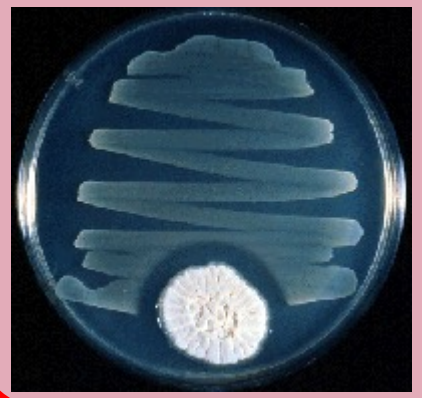
Biofilms are difficult to eradicate

Takenaka et al 2016

Antibiotics have transformed our lives



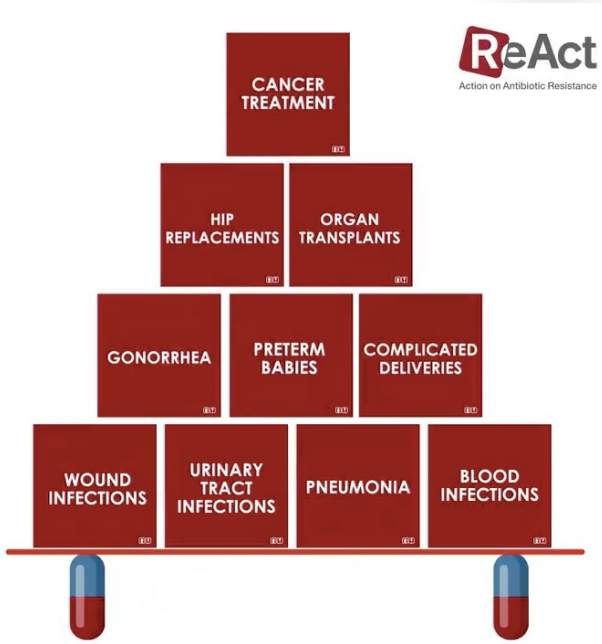
Alexander Fleming discovers antibiotic penicillin being made by fungus killing bacteria



1928 → Structure/Production/purification/testing → 1945

Dorothy Hodgkin, Ernst Chain, Sir Howard Florey

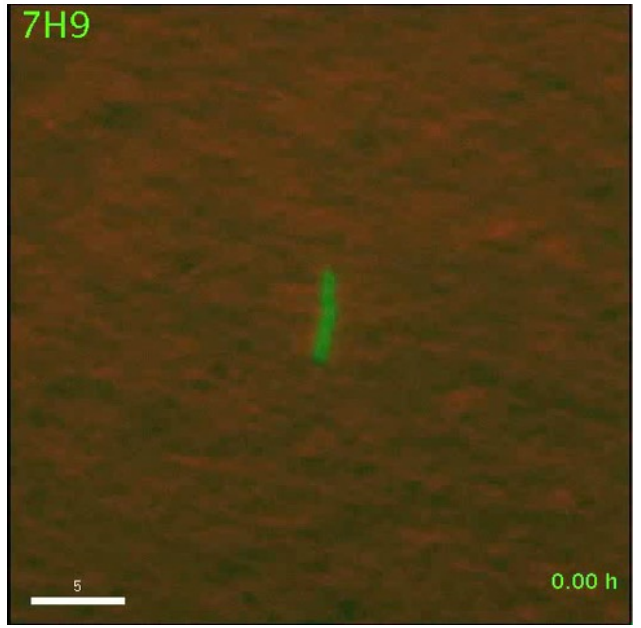
Vital medicines



https://twitter.com/i/status/1198681249105223680

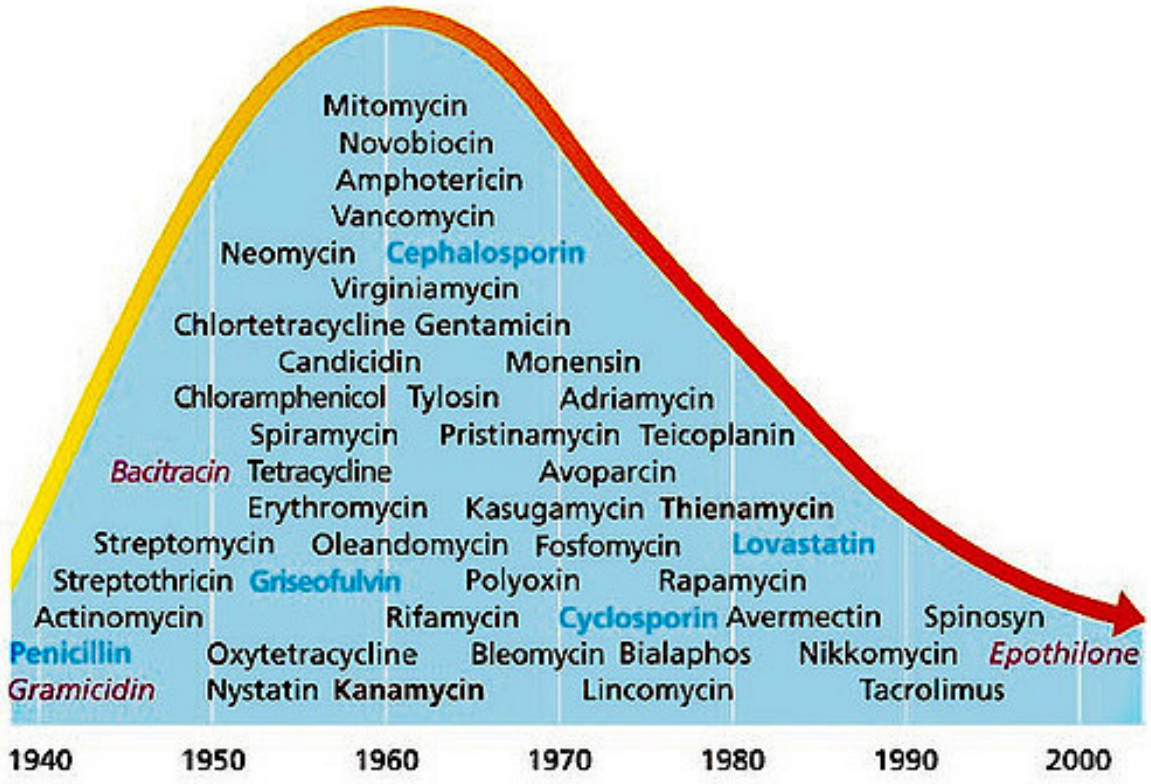
Bacteria dying in presence of antibiotic:

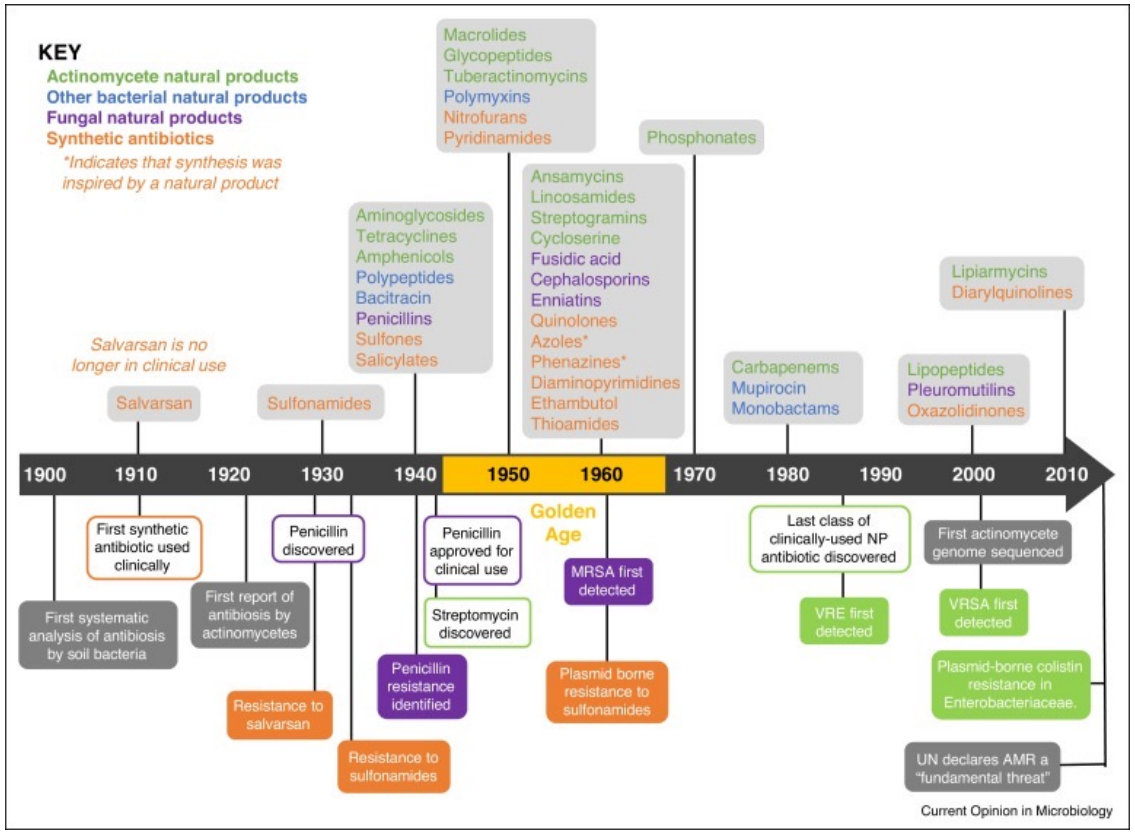
Lights turning out



http://www.youtube.com/watch?v=2Vq3B5ExMrE

Many antibiotics with different chemistries discovered





Gonzalez-Zorn International Microbiology 2012

Hutchings et al. Current Opinion Micro 2019

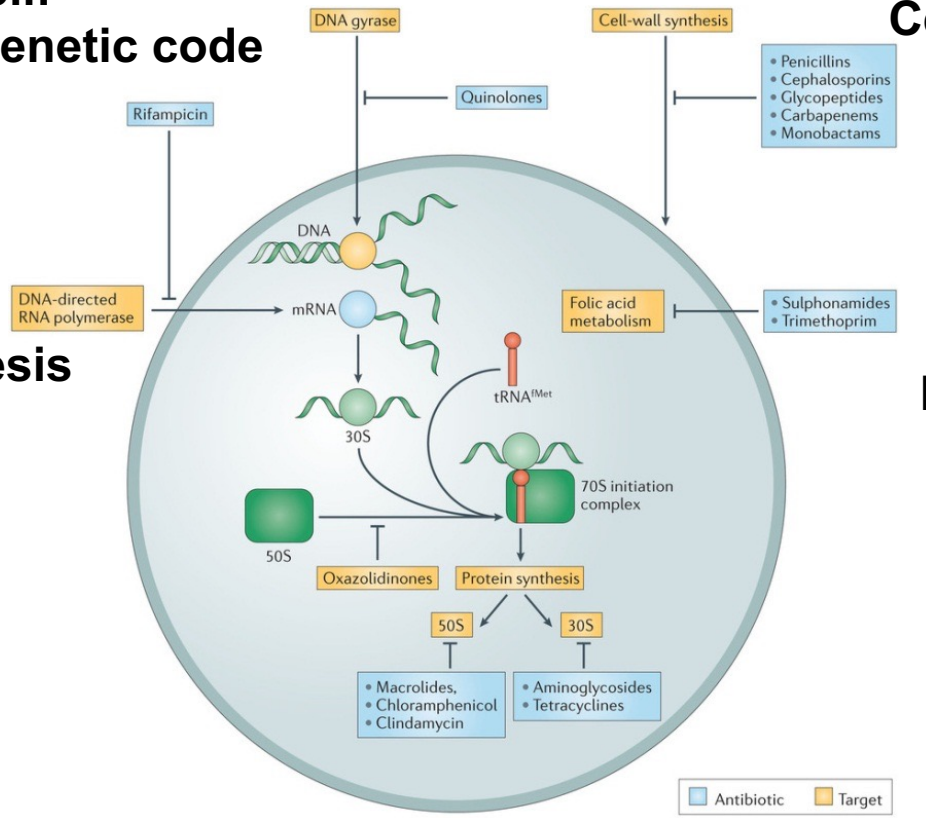
Antibiotics target different cell locations

Inside cell:
maintenance of genetic code

Cell wall/membrane structure

Inside cell:
Protein building block synthesis

Inside cell:
Metabolism

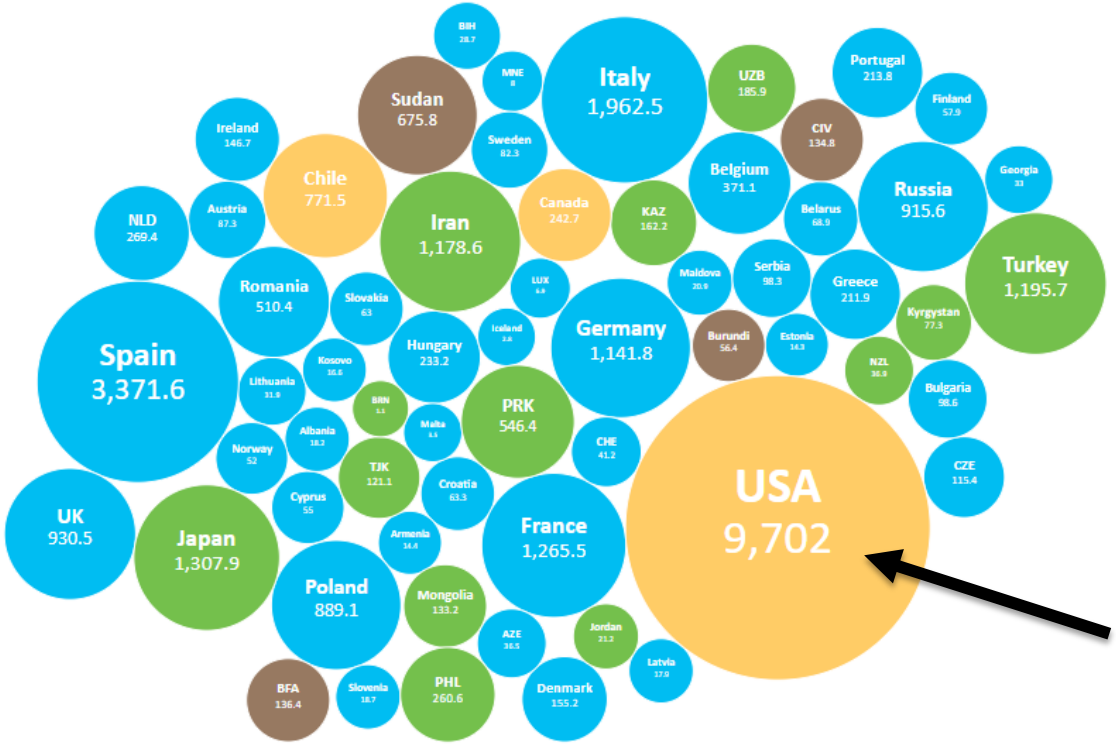


Kim Lewis *Nature Reviews Drug Discovery* **12**, 371–387 (2013)

Globally our antibiotic footprint is large

All sectors:

- Human consumption
- Community
- Hospital
- Animal agriculture

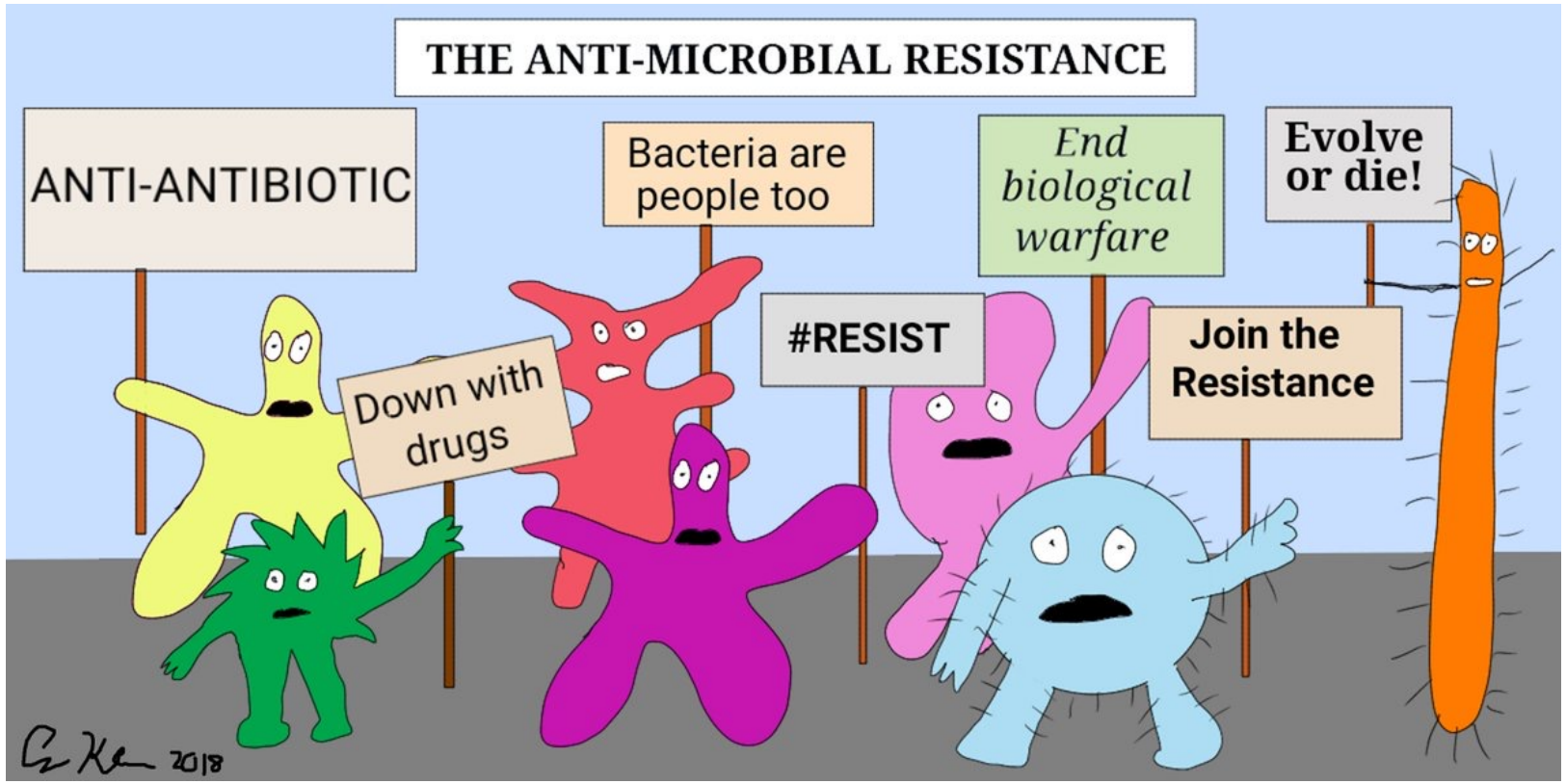


Animal agriculture

Metric Tonnes

https://www.antibioticfootprint.net/infobox.aspx?pageID=101&lang=en-GB

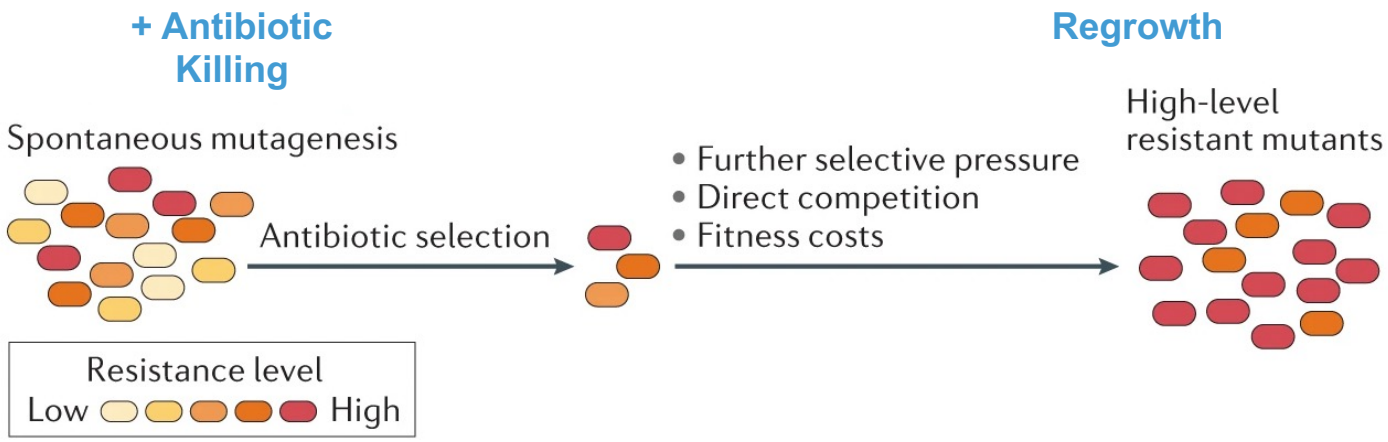
Antibiotic usage selects AMR

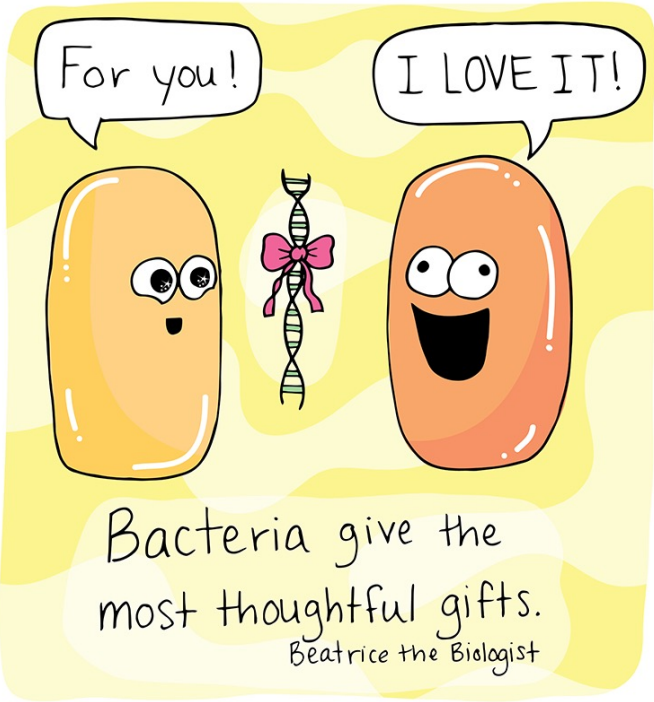


Antibiotics kill sensitive bacterium: large clear zone

Less killing of AMR bacteria

Natural selection of AMR

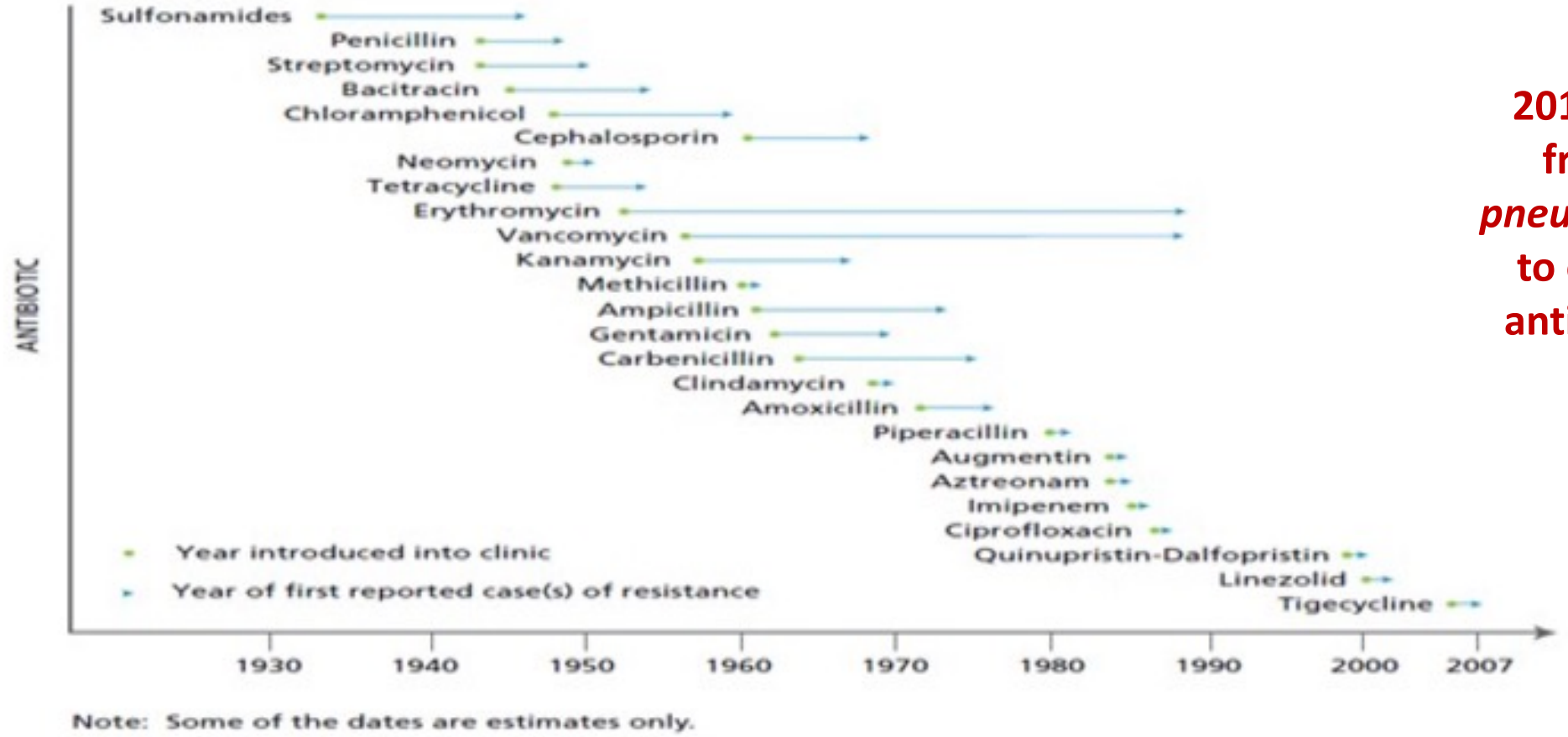




AMR occurs naturally e.g. in soil

Ciofu et al. Nat Rev Micro 2022

In the clinic: Antibiotic resistance emergence threatens infection control



Note: Some of the dates are estimates only.

2017: Patient died from *Klebsiella pneumoniae* resistant to every available antibiotic in the US

http://slideplayer.com/slide/8850471/

AMR spreads fast

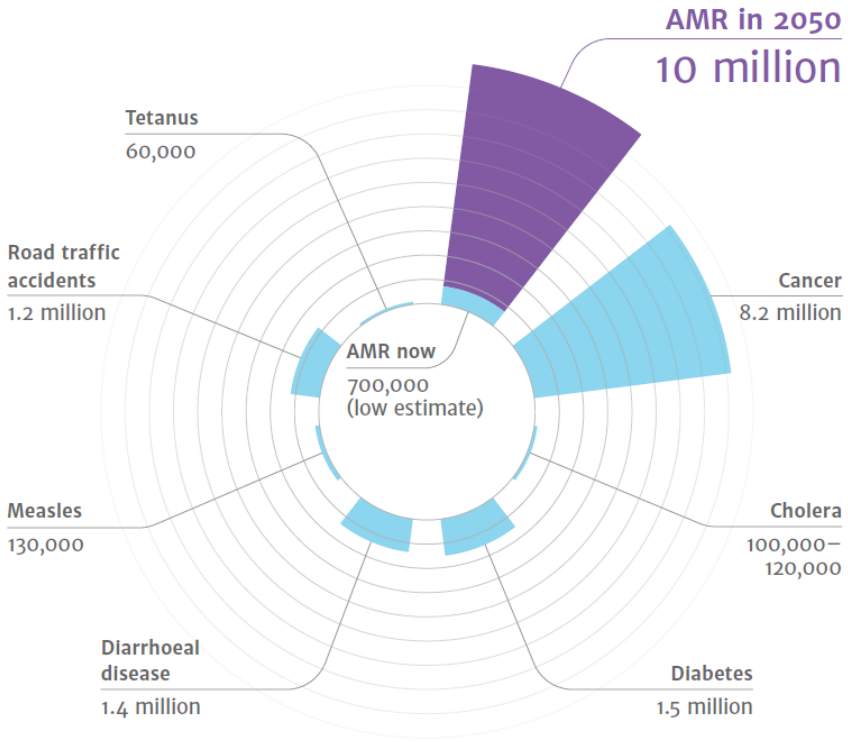
Enzyme that makes bacteria resistant to a broad range of beta-lactam antibiotics including the carbapenem family

New Delhi metallo-beta-lactamase 1 (NDM-1) spread to more than 80 countries in just a few years after its initial identification in the mid-2000s

It was on a short-cut through the hospital kitchens that Albert was first approached by a member of the Antibiotic Resistance.

https://www.pewtrusts.org/en/research-and-analysis/articles/2017/10/10/superbugs-dont-respect-borders

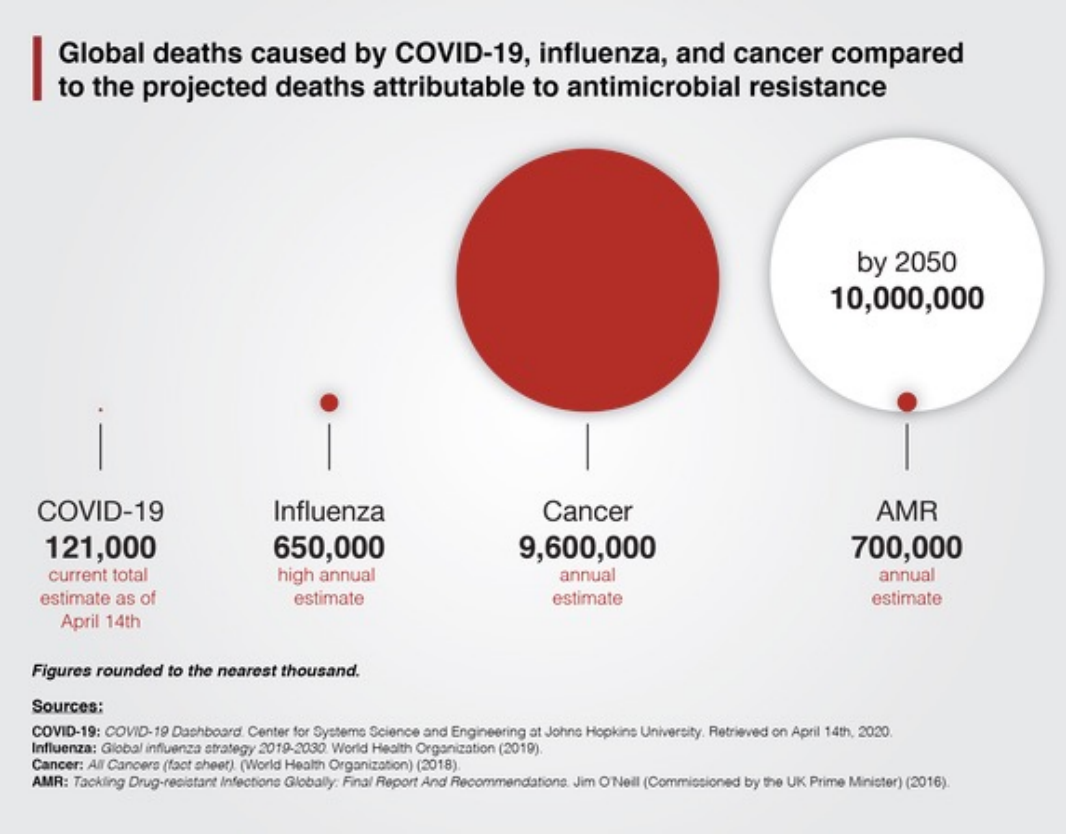
AMR affects treatment

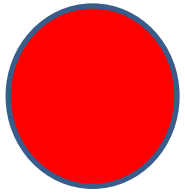


AMR:

- **reduces how effective antibiotics are in treating human and animal health**
- **is responsible for >700,000 deaths per year across the globe and by 2050 it is predicted this figure will reach 10 million per year (O'Neill report, May 2016, Tackling drug-resistant infections globally)**

How these numbers compare to deaths due to the pandemic





COVID-19

6,540,000

September 27th 2022

Lori L. Burrows
Professor of Biocchemistry and Biomedical Sciences, McMaster University

https://theconversation.com/drug-resistant-superbugs-a-global-threat-intensified-by-the-fight-against-coronavirus-135790

Antibiotic use/AMR during the pandemic

Increased use of antimicrobials during the pandemic

- Number of people in intensive care & unknown cure
- Secondary bacterial infections
- Increase in telephone GP consultations

Interrupted antibiotic supply chain during pandemic

- China and India major suppliers

Resources directed away from AMR surveillance

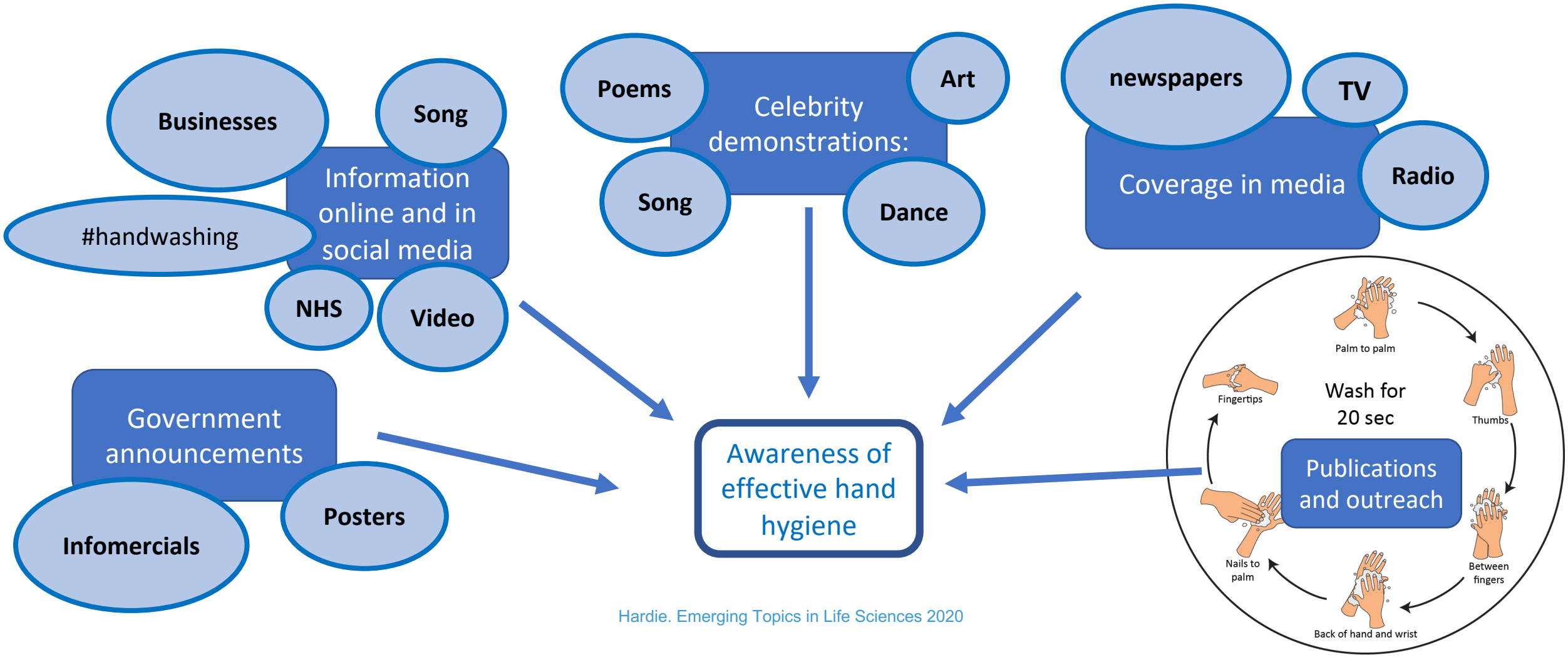
Delays in AMR legislation

USA withdrawal of support for WHO

Low public awareness of AMR

Crisis fatigue

Could pandemic-led public awareness of effective hygiene curb spread AMR?



Hardie. Emerging Topics in Life Sciences 2020

Unclear if hand hygiene compliance altered pre- versus post- pandemic

Pandemic initially increased healthcare worker HHC

New Medical students more aware of HHC

Initial analysis indicates pandemic did not generate sustained increase in healthcare worker HHC, and unstudied in community

- **Denmark (Sandbol et al., American J Inf Control July 2022)**
- **USA (Makhni et al., JAMA internal Medicine April 2022)**

AMR threat increased by pandemic

80% of COVID patients received at least 1 antibiotic (2020, CDC)

>15% increase (2019-20) nosocomial AMR infections (USA)

- **Carbapenem-resistant A*ctinetobacter* up by 78%**
- **Multi-drug resistant *Pseudomonas aeruginosa* up by 32%**
- **Multi-drug resistant *Candida auris* up by 60%**

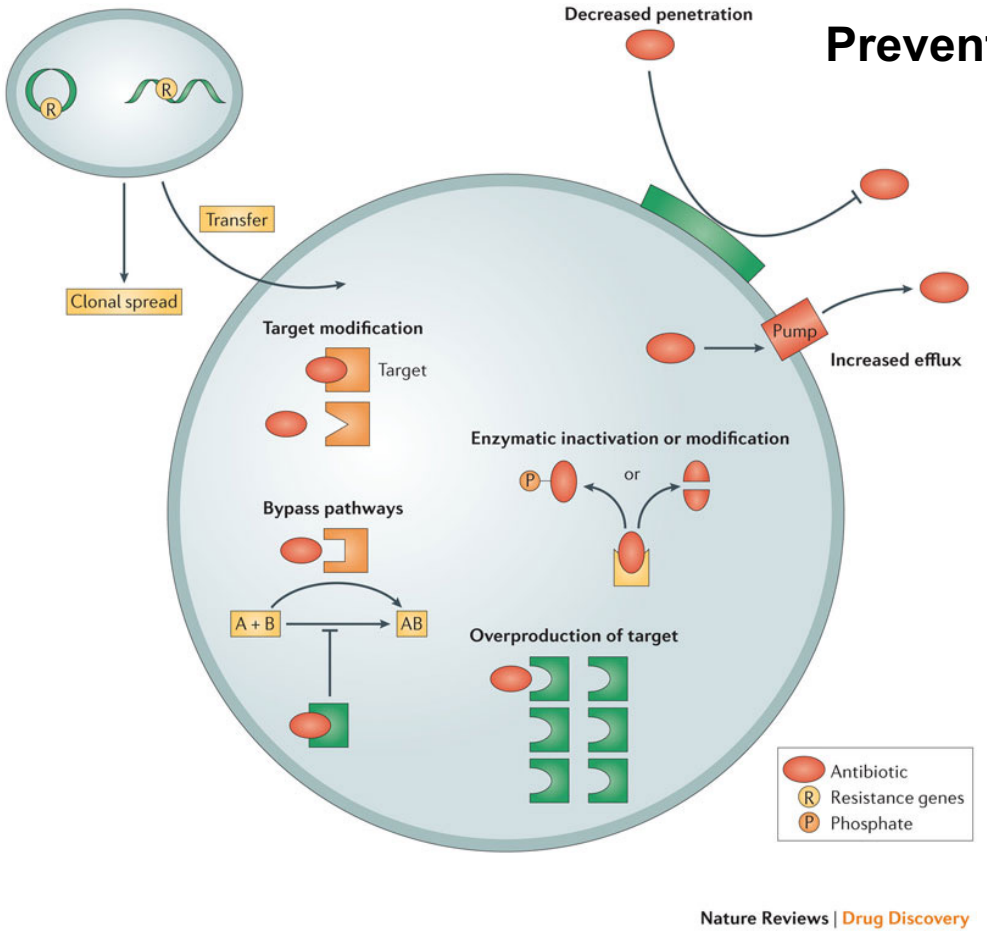
>15% increase (2019-20) AMR resultant deaths (USA)

CDC report July 2022

https://www.cdc.gov/drugresistance/covid19.html

Many AMR mechanisms known

Change target



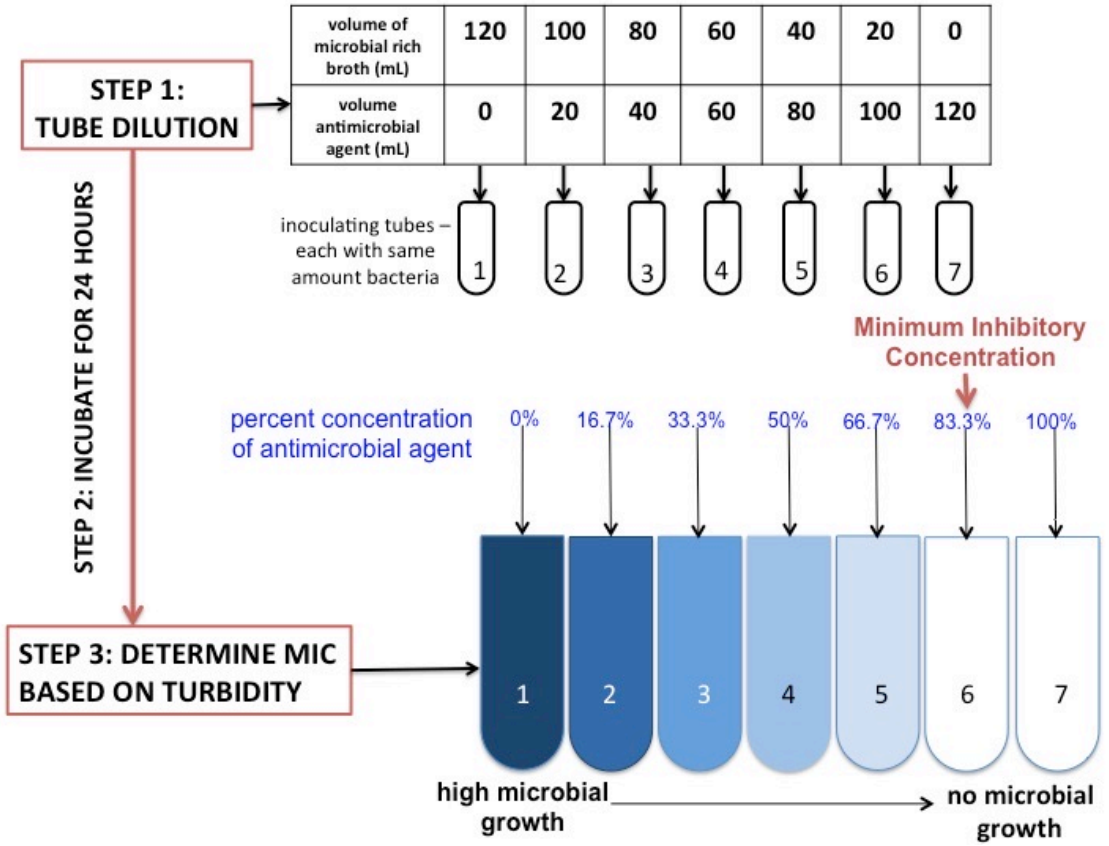
Prevent entry

Pump out

Inactivate antibiotic

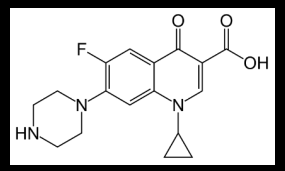
Kim Lewis *Nature Reviews Drug Discovery* **12**, 371–387 (2013)

AMR assessed clinically using free-living microbes

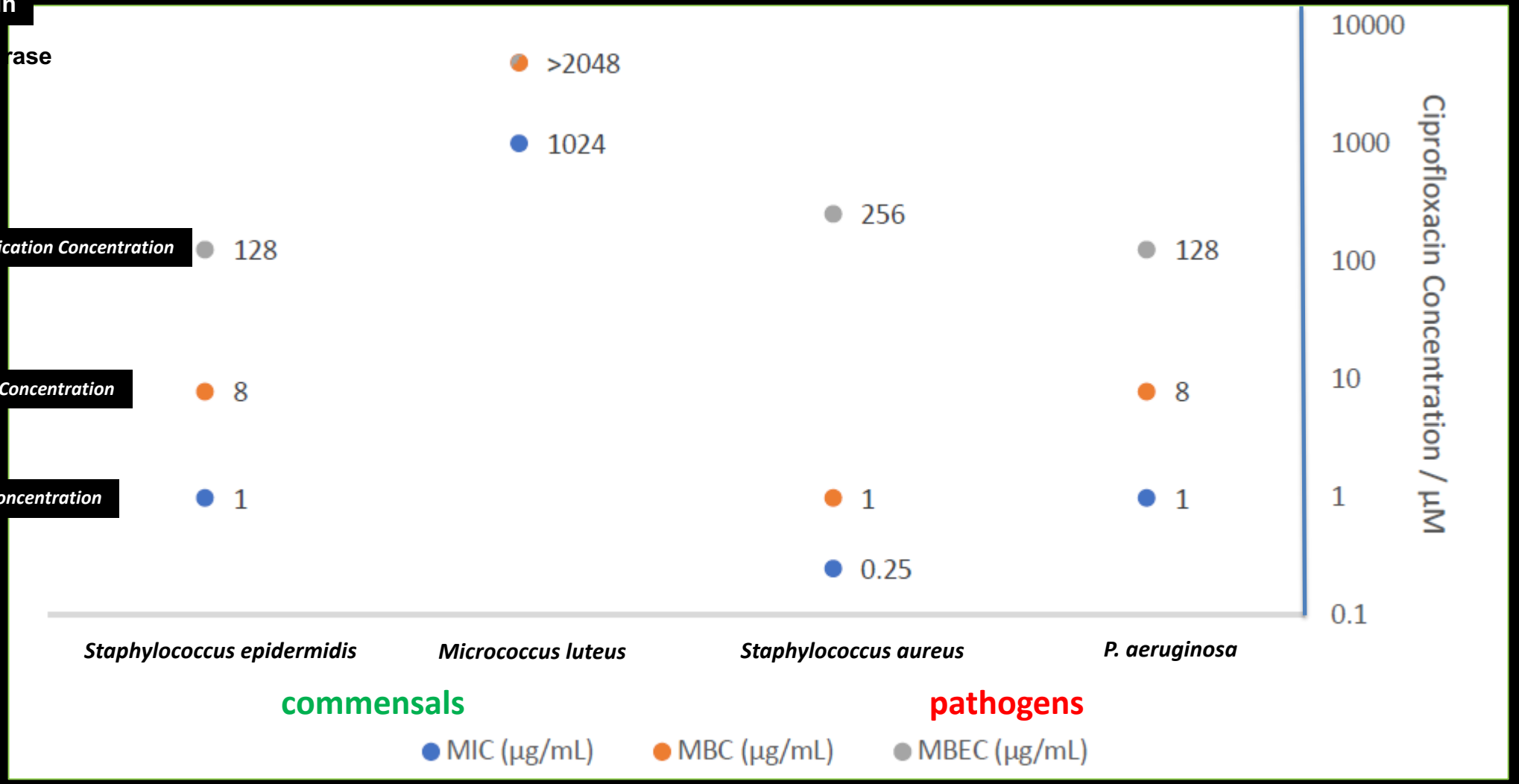


By Jenay DeCaussin - Own work, CC BY-SA 4.0, https://commons.wikimedia.org/w/index.php?curid=54059624

Antibiotics kill bacteria in biofilms less well



ciprofloxacin



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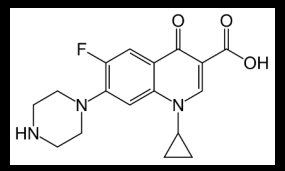


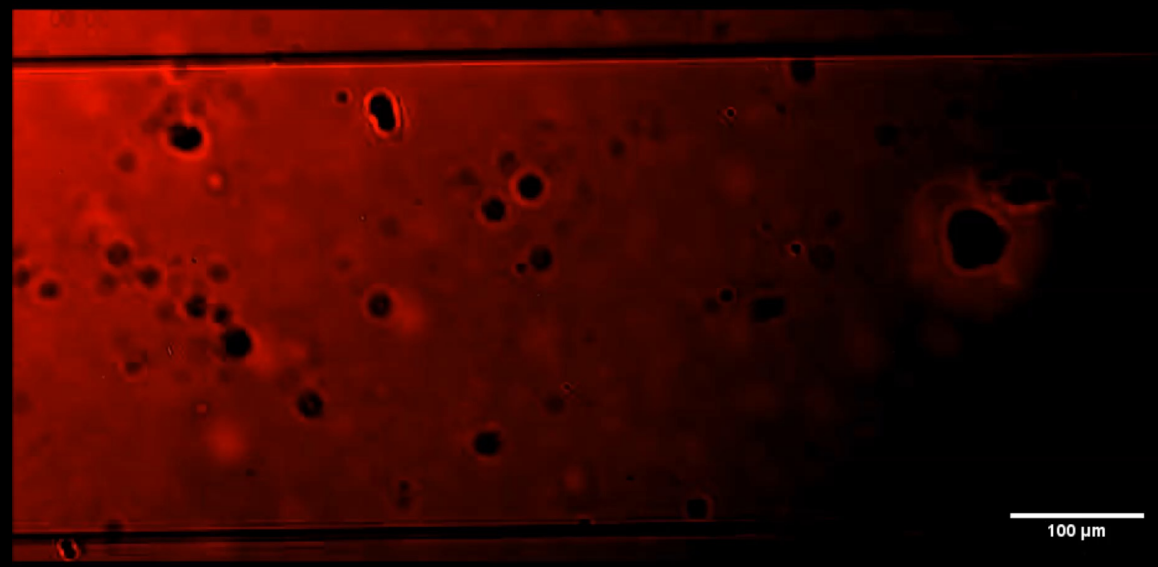
Inhibits DNA gyrase

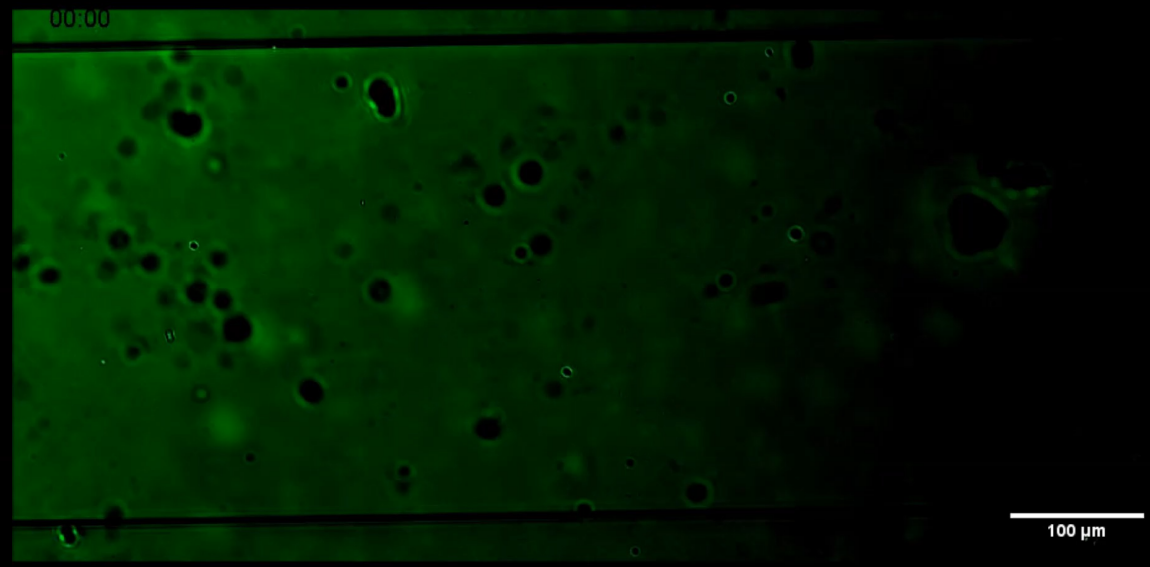
ciprofloxacin

P. aeruginosa

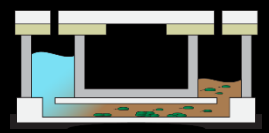
Ciprofloxacin killing of bacteria in biofilm

20 µg.ml^{-1} ciprofloxacin introduced after 16 hrs
Addition indicated by asterisk





propidium iodide: dead



GFP: alive

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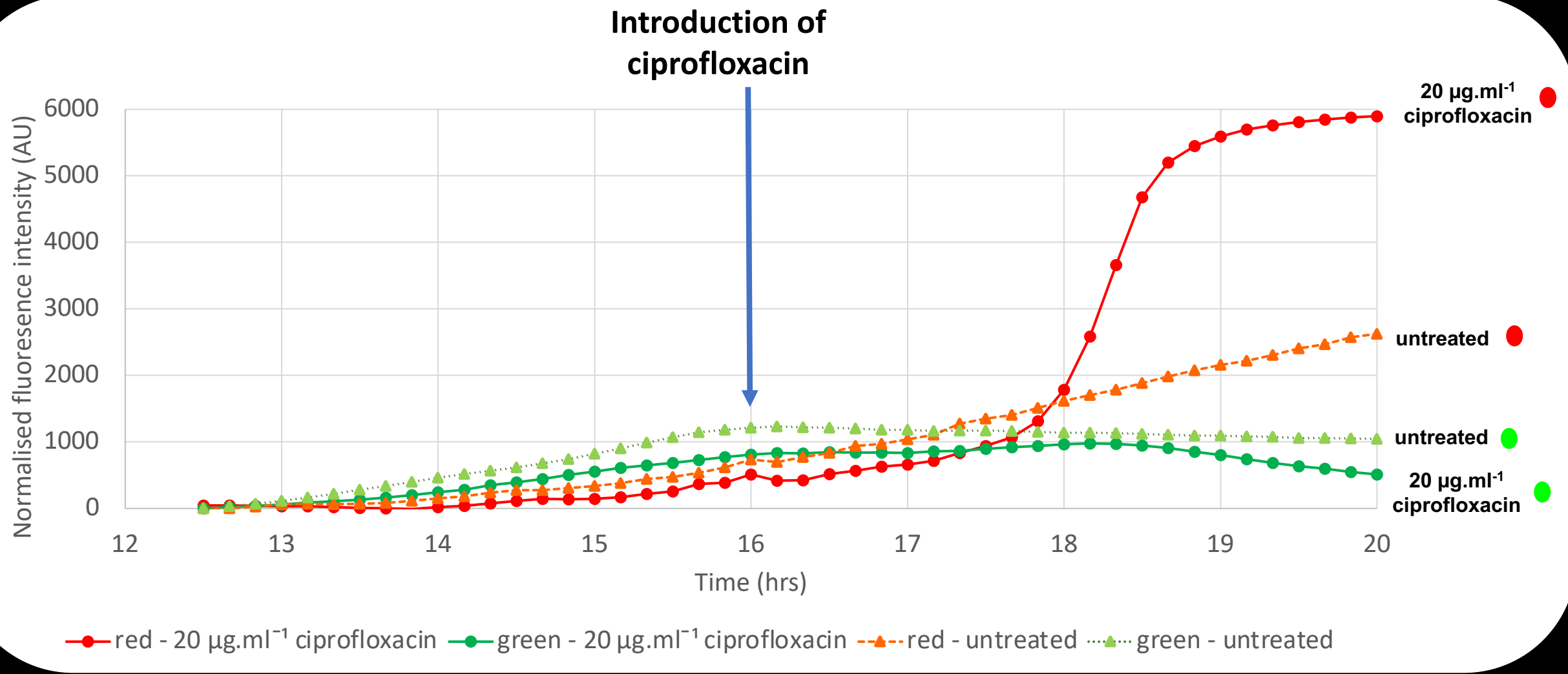
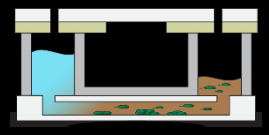
James Brown, Paul Williams

Semi-quantification of antibiotic directed bacterial killing





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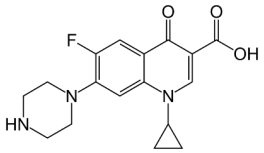
propidium iodide: dead

GFP: alive

P. aeruginosa

James Brown, Paul Williams



ciprofloxacin

Inhibits DNA gyrase

Antibiotics kill bacteria on outside of microcolonies first

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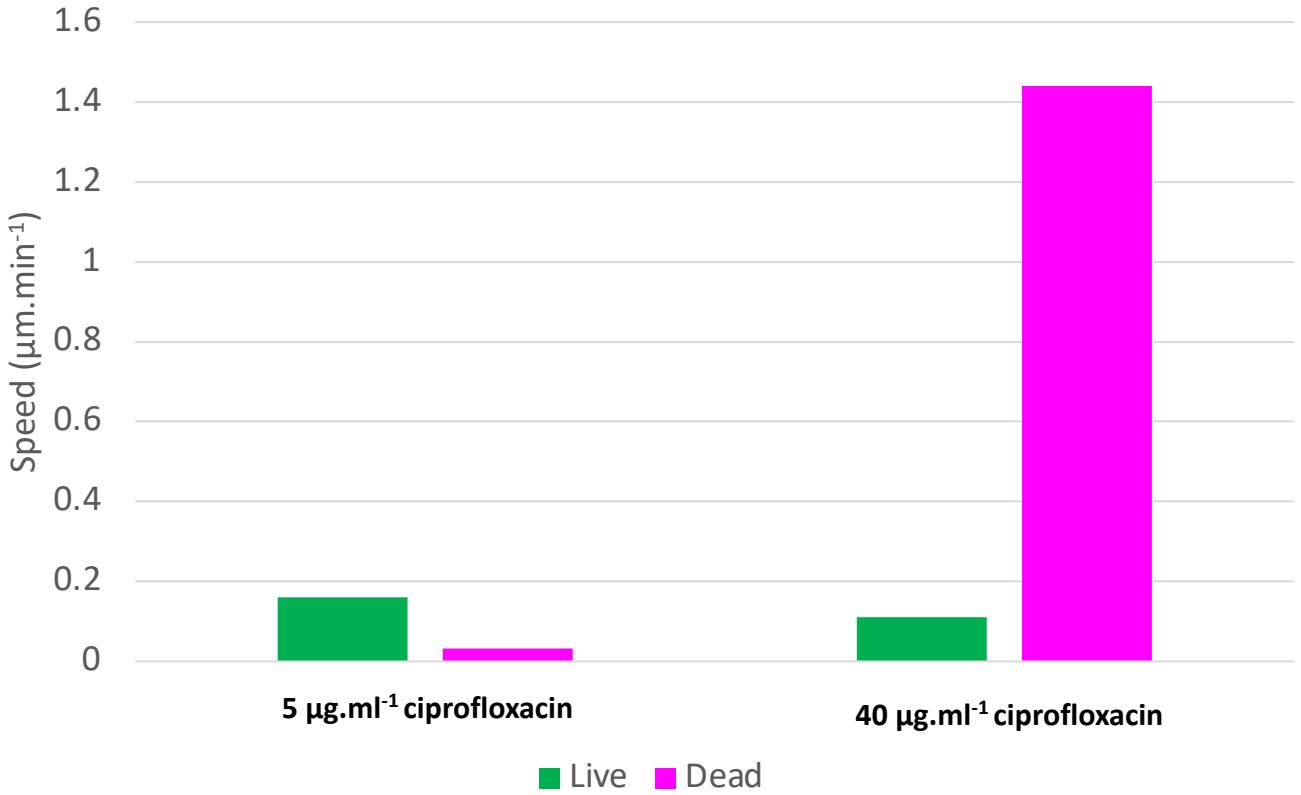
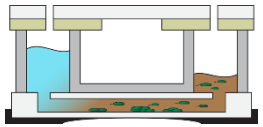
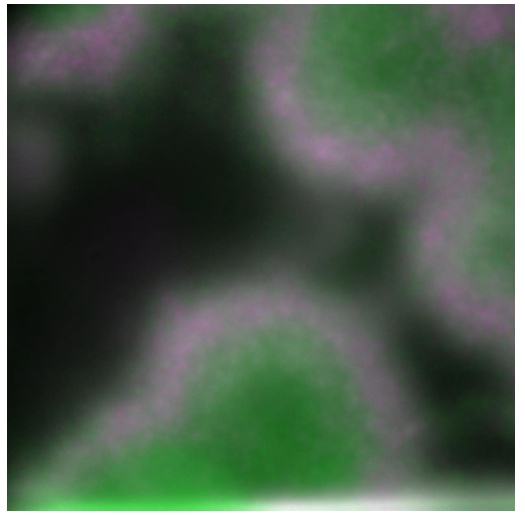
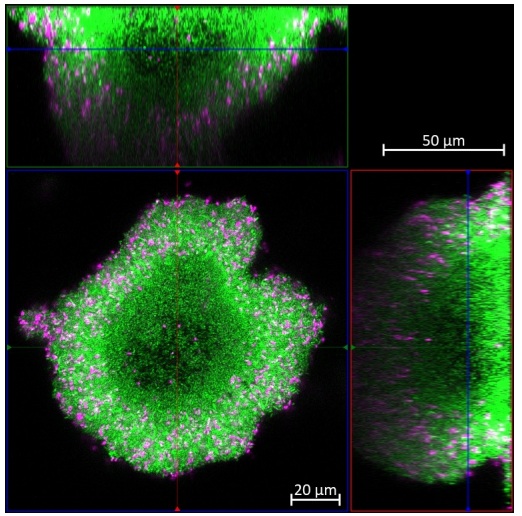


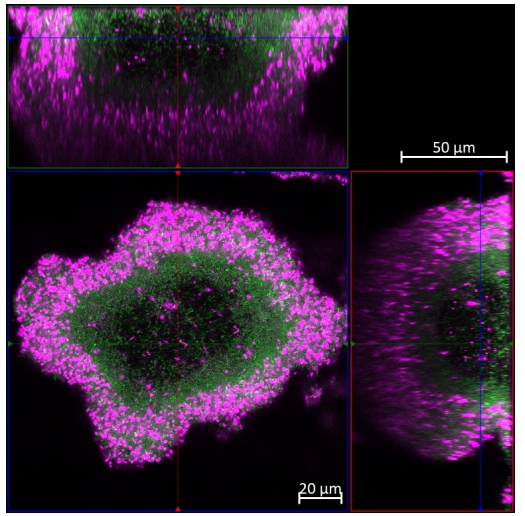


PAO1 expressing GFP (live cells; green) and stained with PI (dead cells; magenta) + 5 µg.ml^{-1} ciprofloxacin





PAO1 expressing GFP (live) and stained with PI (dead)

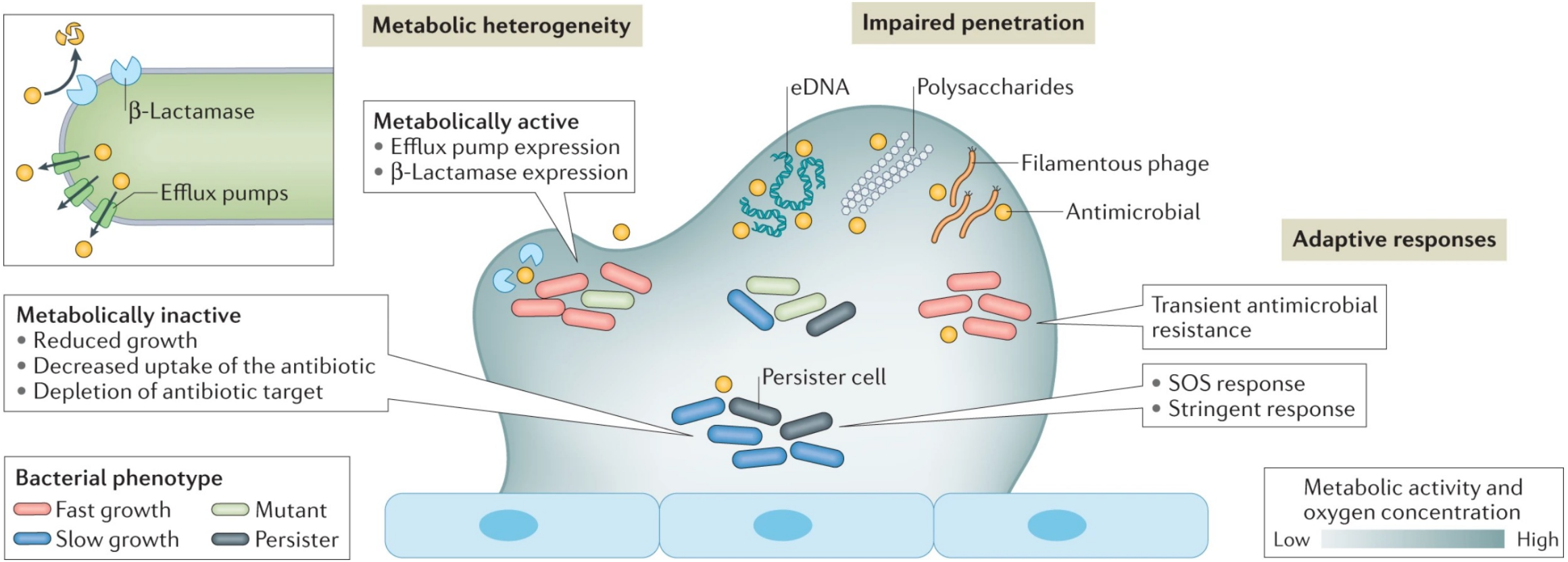


PAO1 expressing GFP (live) and stained with PI (dead) + 1 ug.ml^{-1} ciprofloxacin

James Brown, Paul Williams

P. aeruginosa

Biofilms are 1000x more resistant to antimicrobials



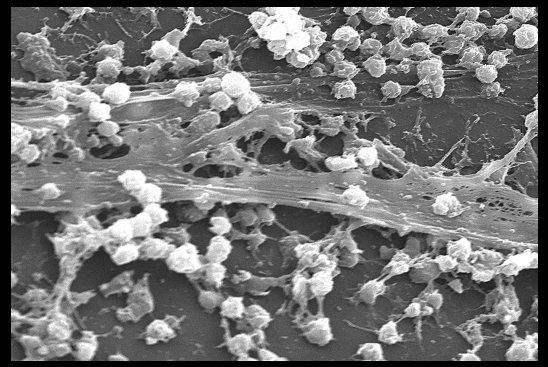
Ciofu et al. Nat Rev Micro 2022

Polymicrobial biofilms may create different barriers

Pathogen:

Staphylococcus aureus

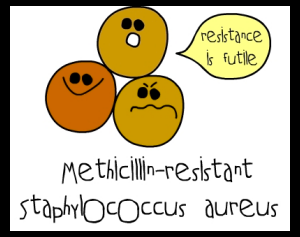
Gram positive



Staphylococcus aureus **is both a human commensal and a human pathogen.**

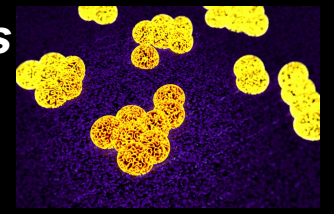
S. aureus **can form biofilms, and these are commonly associated with nosocomial infections.**

E.g. chronic wound infections, device-related infections, osteomyelitis and endocarditis.



Commensals:

- ***Staphylococcus epidermidis*****,**
  Gram positive
- ***Micrococcus luteus***
  Gram positive

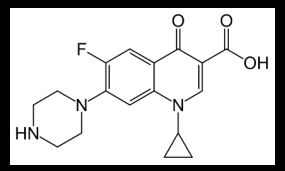


promote skin health

Perturbation of microbiota can initiate autoimmune disease

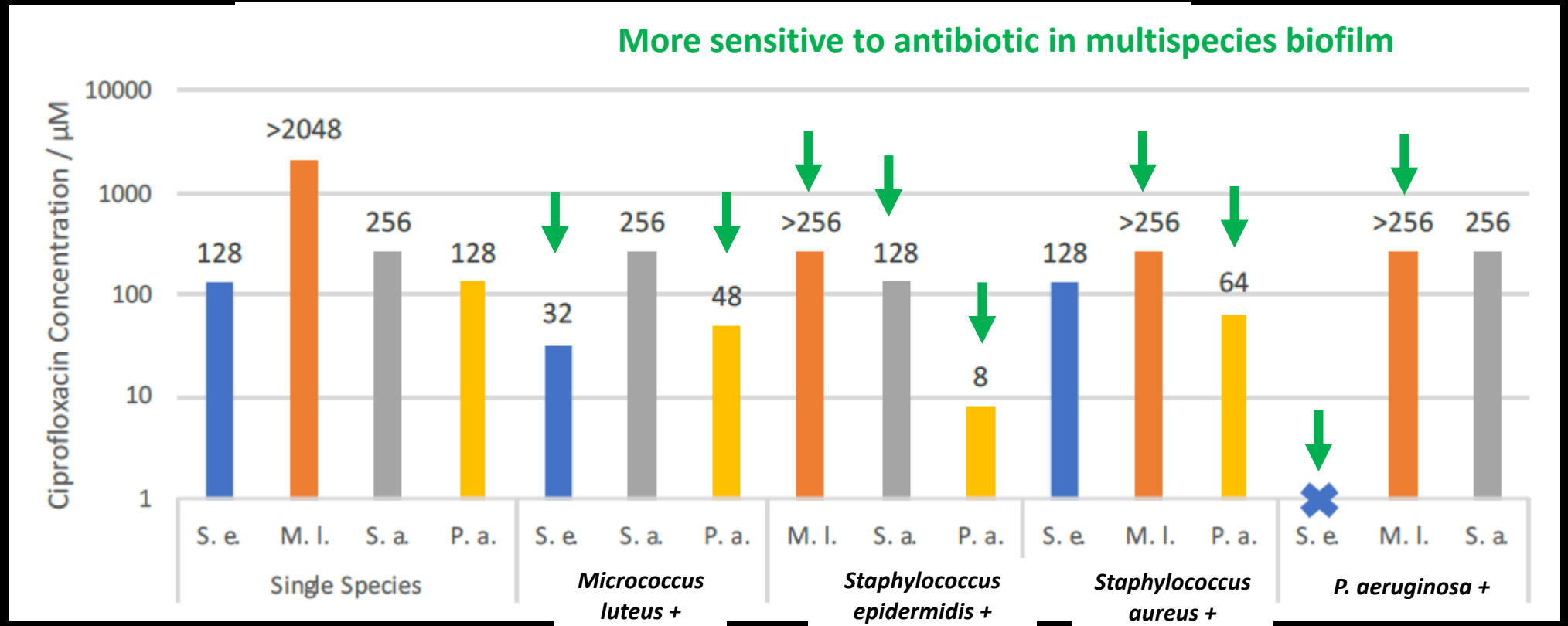
Pathogens e.g. *Staph aureus* and *P. aeruginosa* infect skin following damage e.g. by burn or trauma

Staph aureus **and** ***P. aeruginosa*** **found co-infecting CF lungs and wounds**



ciprofloxacin

If there is more than one species in the biofilm, killing by antimicrobial is altered



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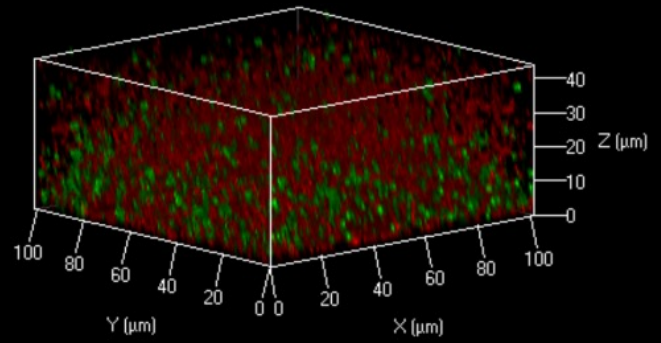
Keratinocyte monolayer below

Commensals form a layer below pathogens

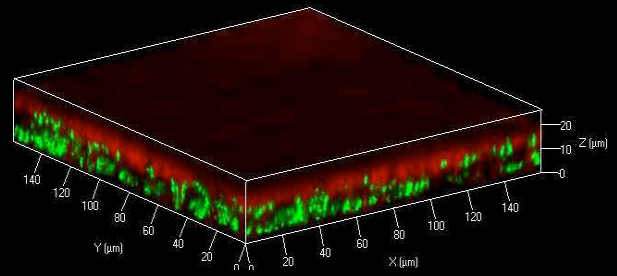
protective layer

2 Pathogens together



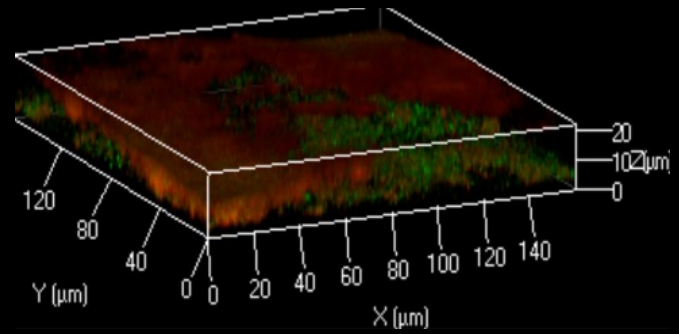
Pathogen (*P. aeruginosa*) mCherry
Pathogen (*Staph aureus*) GFP

Pathogen with commensal:



Pathogen (*P. aeruginosa*) mCherry
Commensal (*Staph epidermidis*) GFP

Pathogen >1 commensal:



Pathogen (*P. aeruginosa*)
Commensal (*Staph epidermidis*)
Commensal *Micrococcus luteus* (unlabelled)
FISH

Could agents that boost the microflora biofilm alter pathogen attack ?

Jordana-Lluch et al (20200 Frontiers in Microbiology 11:291. doi: 10.3389/fmicb.2020.00291.

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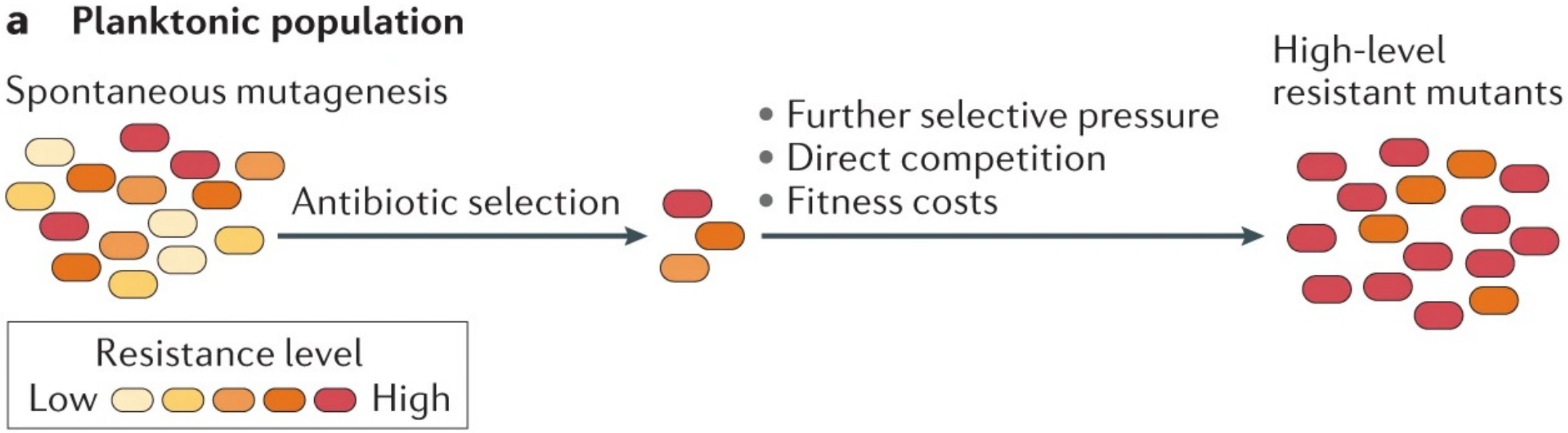
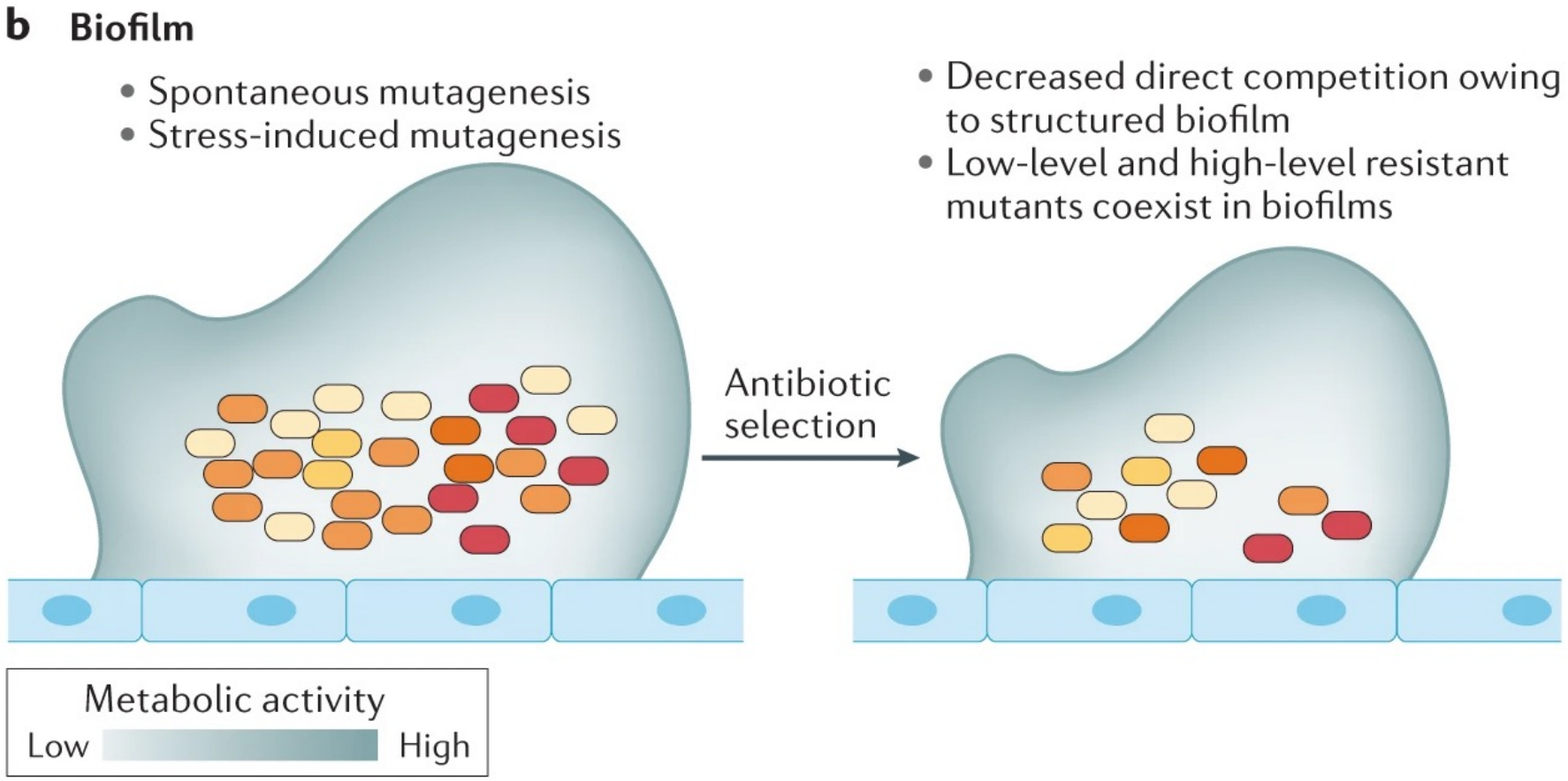
Elena Jordana-Lluch,
Paul Williams

Traditionally, AMR studied using free-living microbes



Ciofu et al. Nat Rev Micro 2022

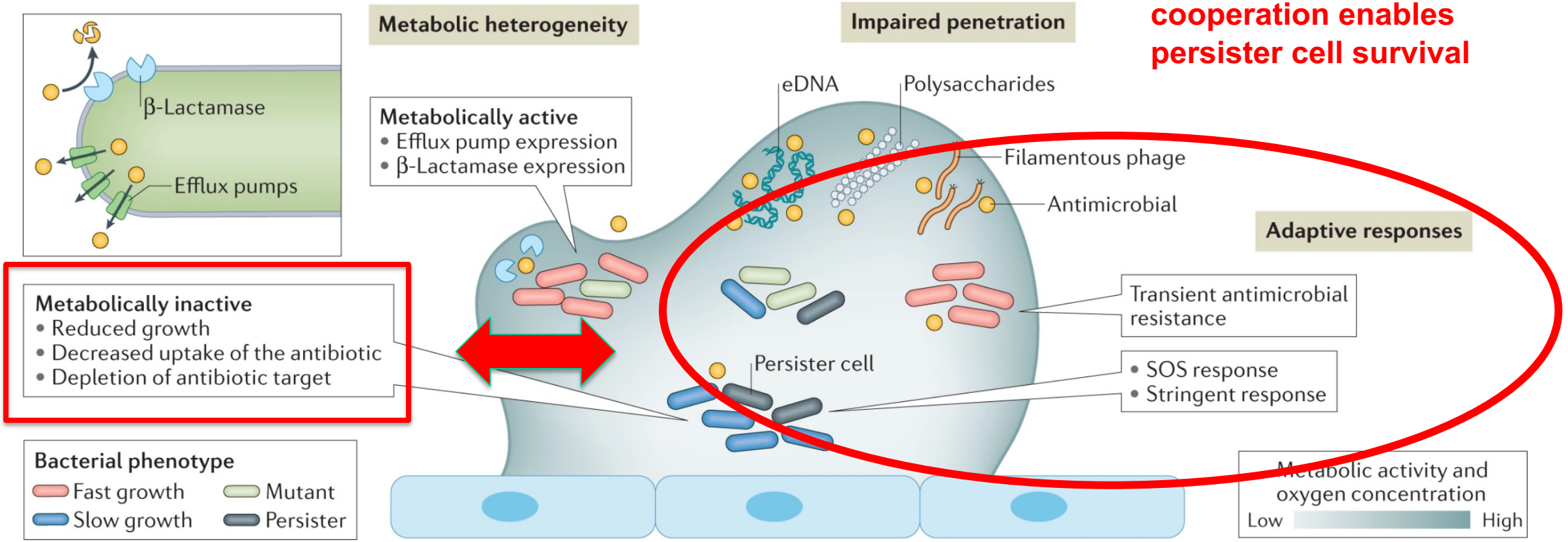
Selection pressures for AMR are different in a biofilm



Ciofu et al. Nat Rev Micro 2022

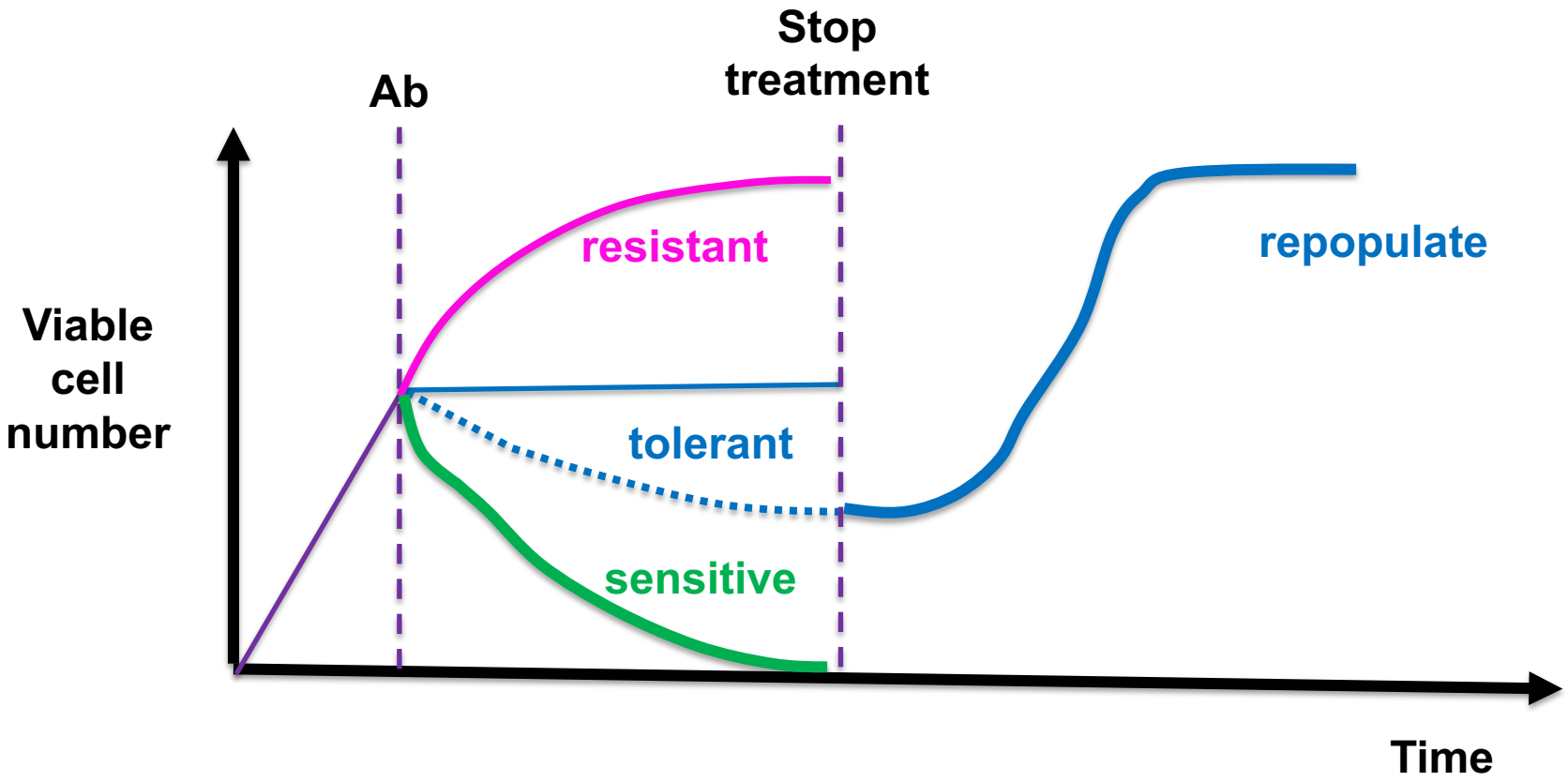
Biofilms are stressful environments

Biofilm population cooperation enables persister cell survival



Ciofu et al. Nat Rev Micro 2022

Antimicrobial tolerance



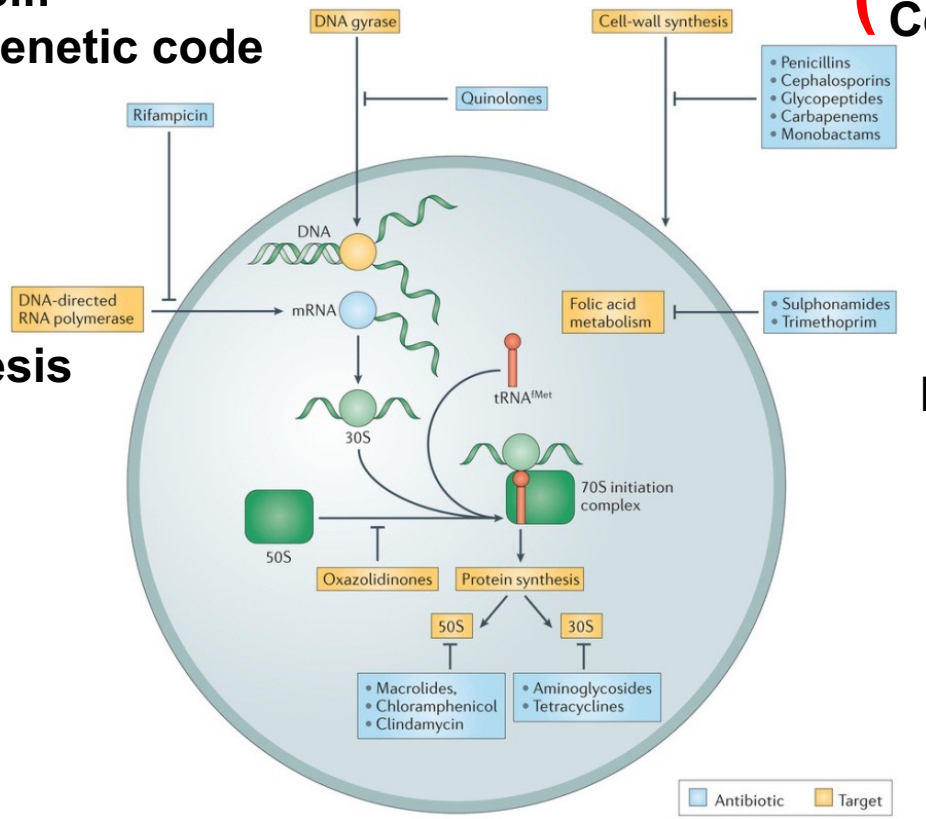
Antibiotics target active cells

Inside cell:
maintenance of genetic code

(Cell wall/membrane structure)

Inside cell:
Protein building [illegible]nesis

Inside cell:
Metabolism

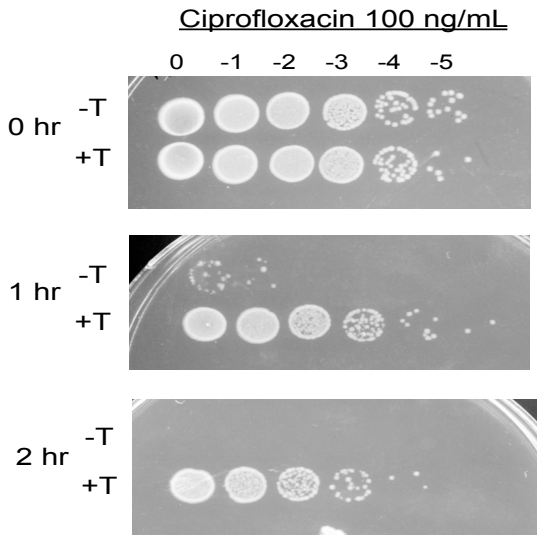


persister cell tolerance

Kim Lewis *Nature Reviews Drug Discovery* **12**, 371–387 (2013)

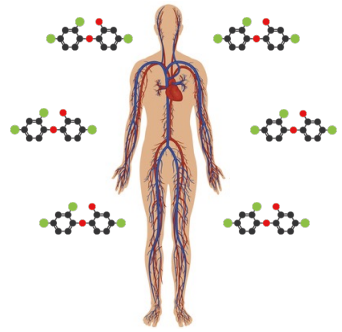
Biocides can trigger antibiotic tolerance in free-living bacteria

E. coli



Westfall et al. (2019)

Triclosan



- Triclosan: biocide widely used biocide in both domestic and healthcare products.
- Accumulates in environment and human body.
  - Low levels isolated in human urine, blood, nasal secretions.
- Triclosan is a fatty acid synthesis inhibitor.

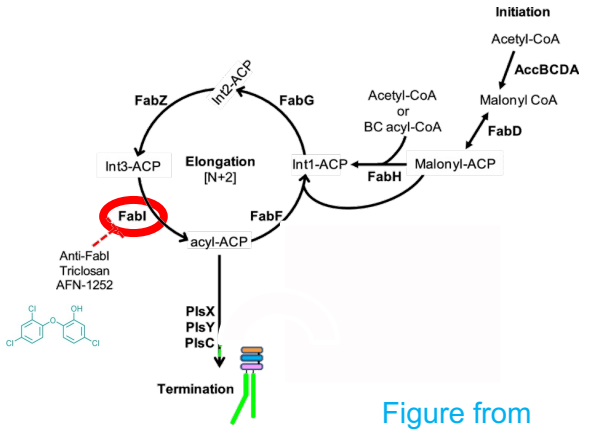
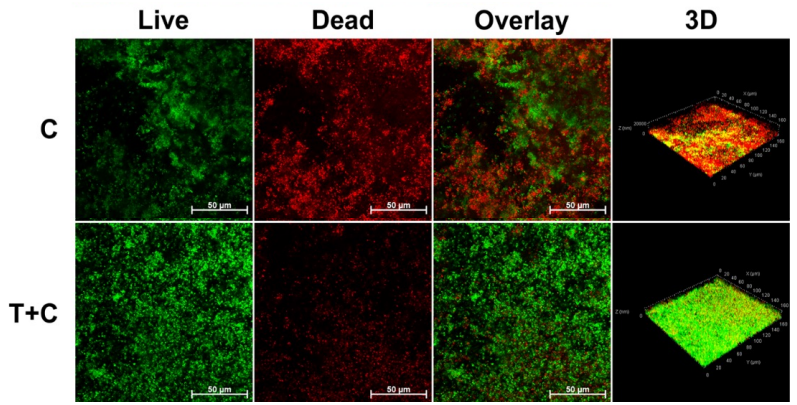


Figure from Morvan et al. (2017)

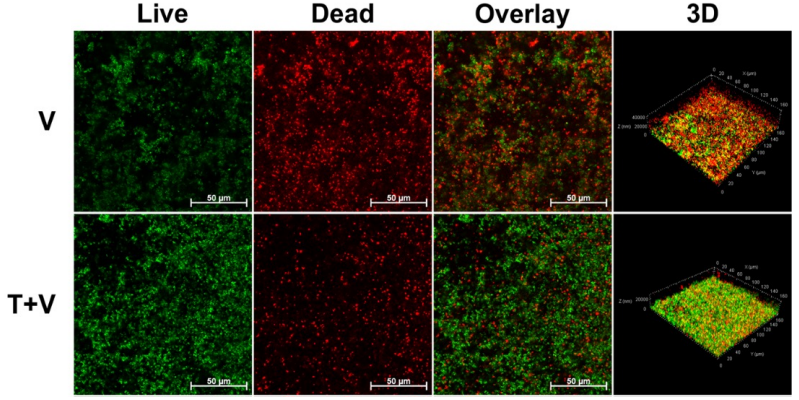
Biocides can induce antibiotic tolerance in biofilms

S. aureus

Ciprofloxacin

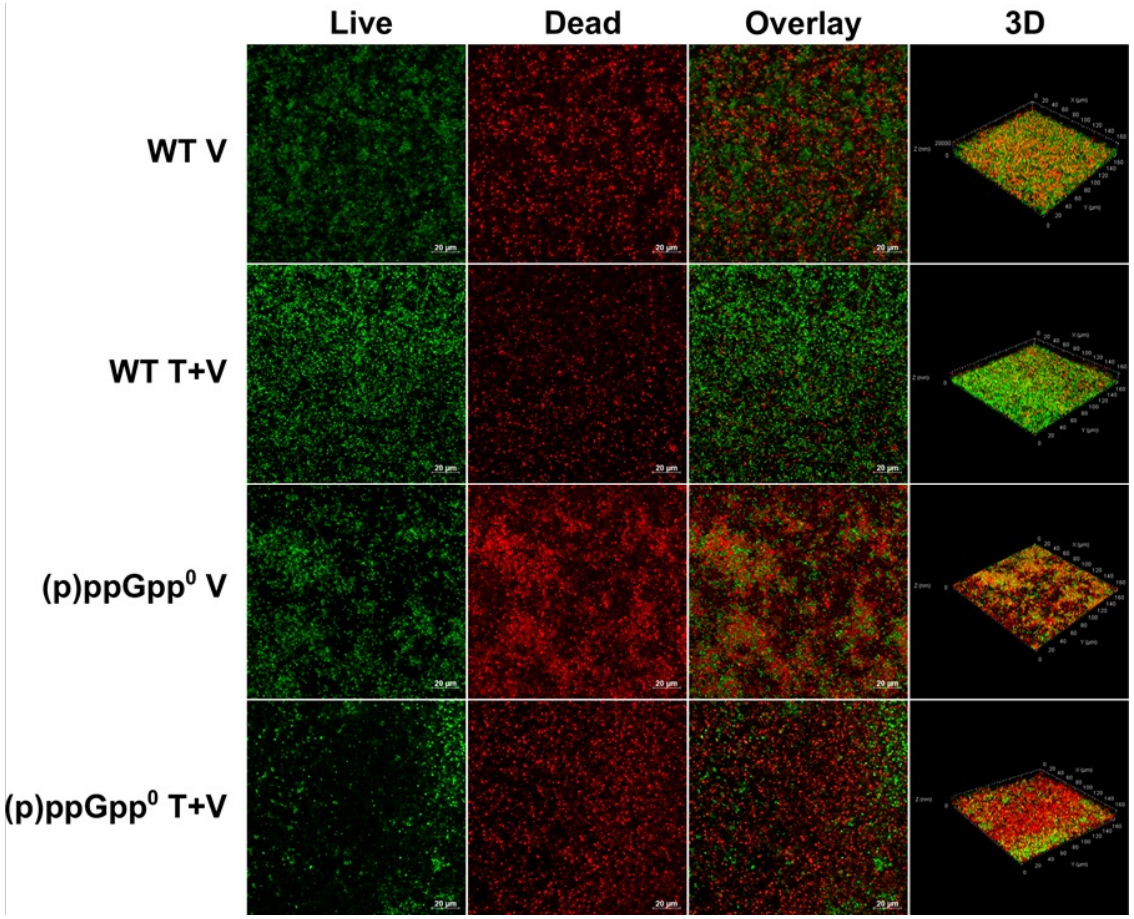


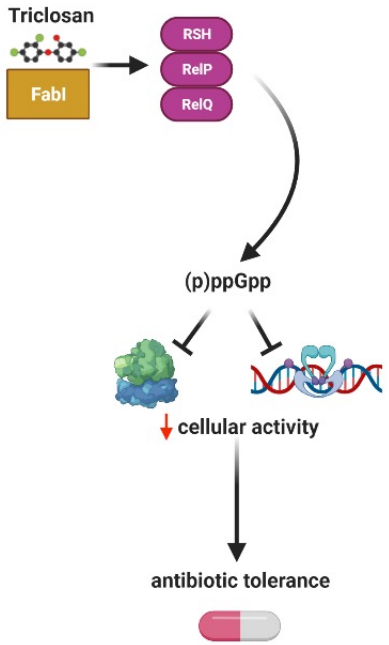
Vancomycin



Functional stringent response linked to antibiotic tolerance in biofilms

S. aureus

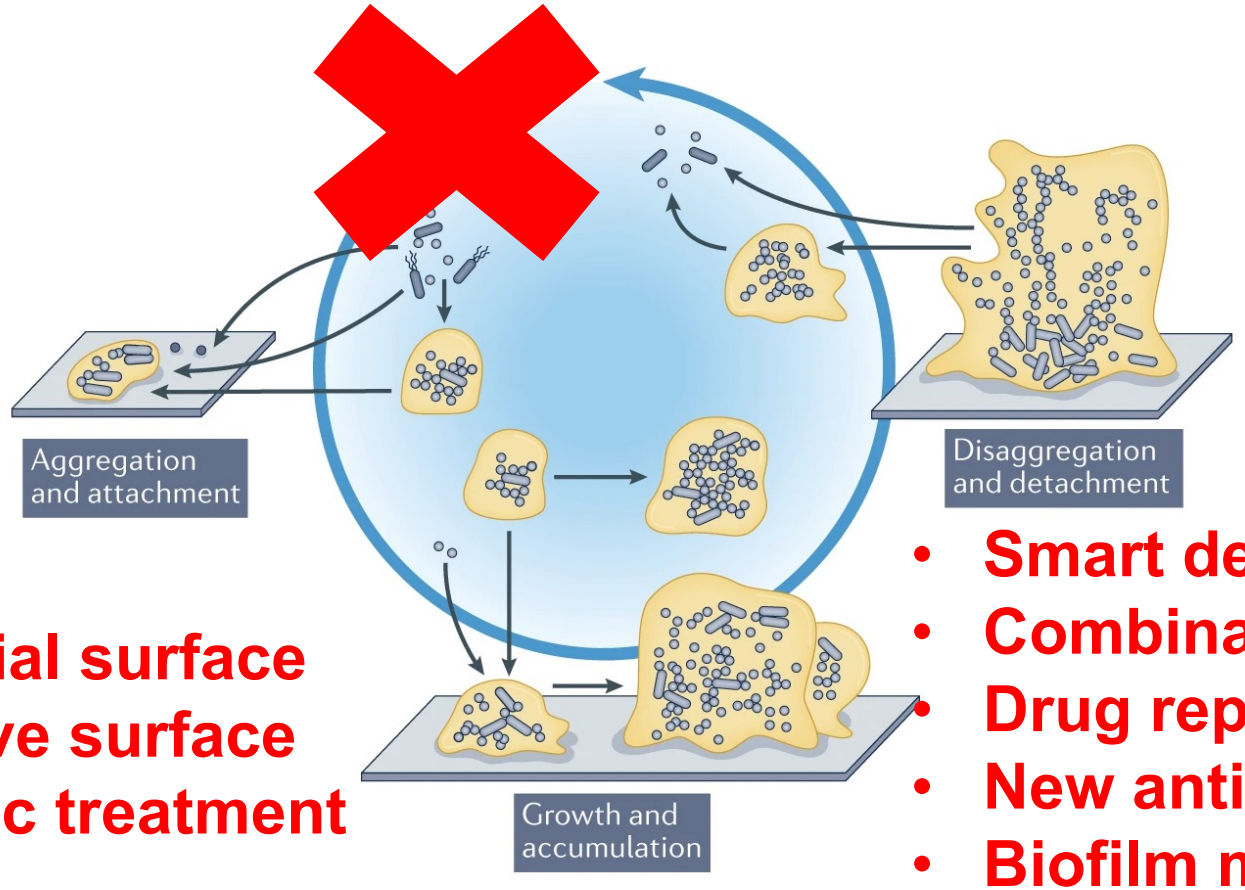




(p)ppGpp^0 mutant strain (Δ*rsh*$_{syn}$, Δ*relP*, Δ*relQ*).

Dean Walsh, Christiane Wolz

Any ways to break the biofilm cycle?



- Antimicrobial surface
- Antiadhesive surface
- Prophylactic treatment

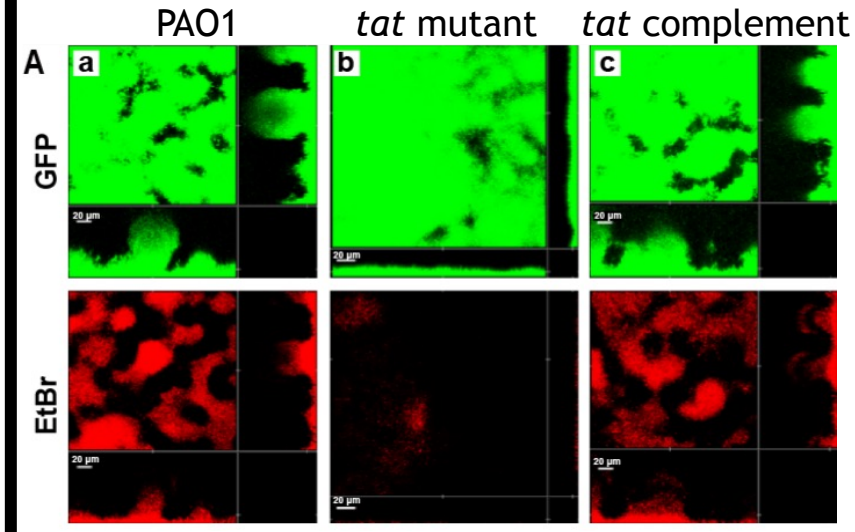
- Smart delivery of current antibiotics
- Combination treatment
- Drug repurposing
- New antibiotics (vs persisters)
- Biofilm matrix targeting
- Stimulate immune clearance

Sauer et al. Nat Rev Micro 2022

Tat mutants create thin biofilms

P. aeruginosa mutants in the Tat pathway release less eDNA

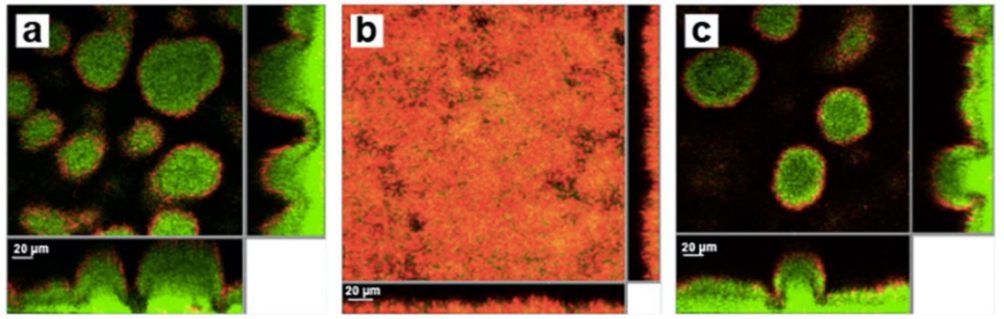
Biofilms containing live cells (green fluorescence) and eDNA (red fluorescence)



Live cells (GFP)/eDNA (EtBr)

The thin, flat biofilms were more sensitive to tobramycin.

Tobramycin-treated biofilms showing live cells (green fluorescence) and dead cells (red fluorescence)



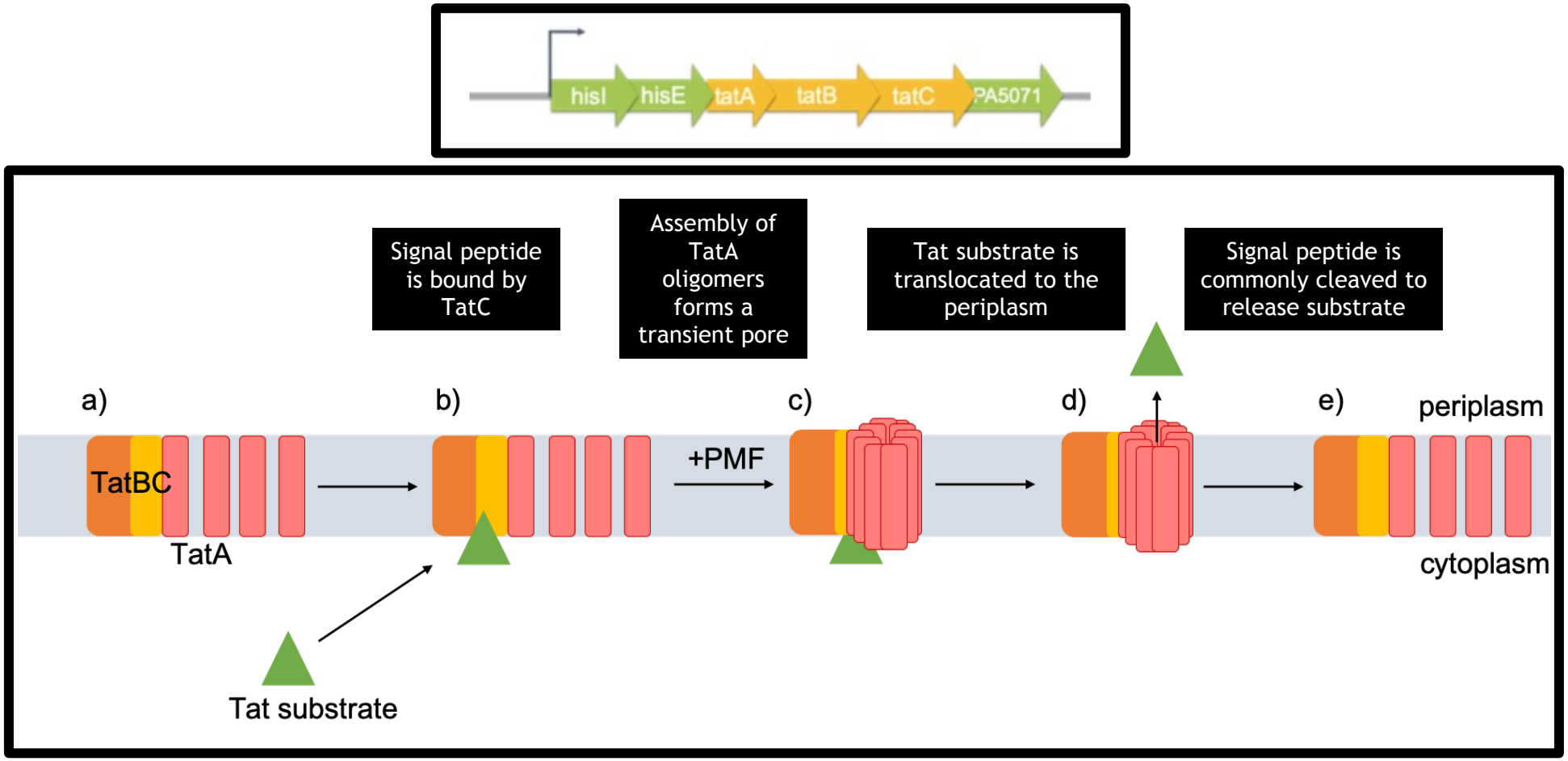
Live cells (GFP)/Dead cells (PI)

Frances Smith, Paul Williams, Tim Tolker Neilson

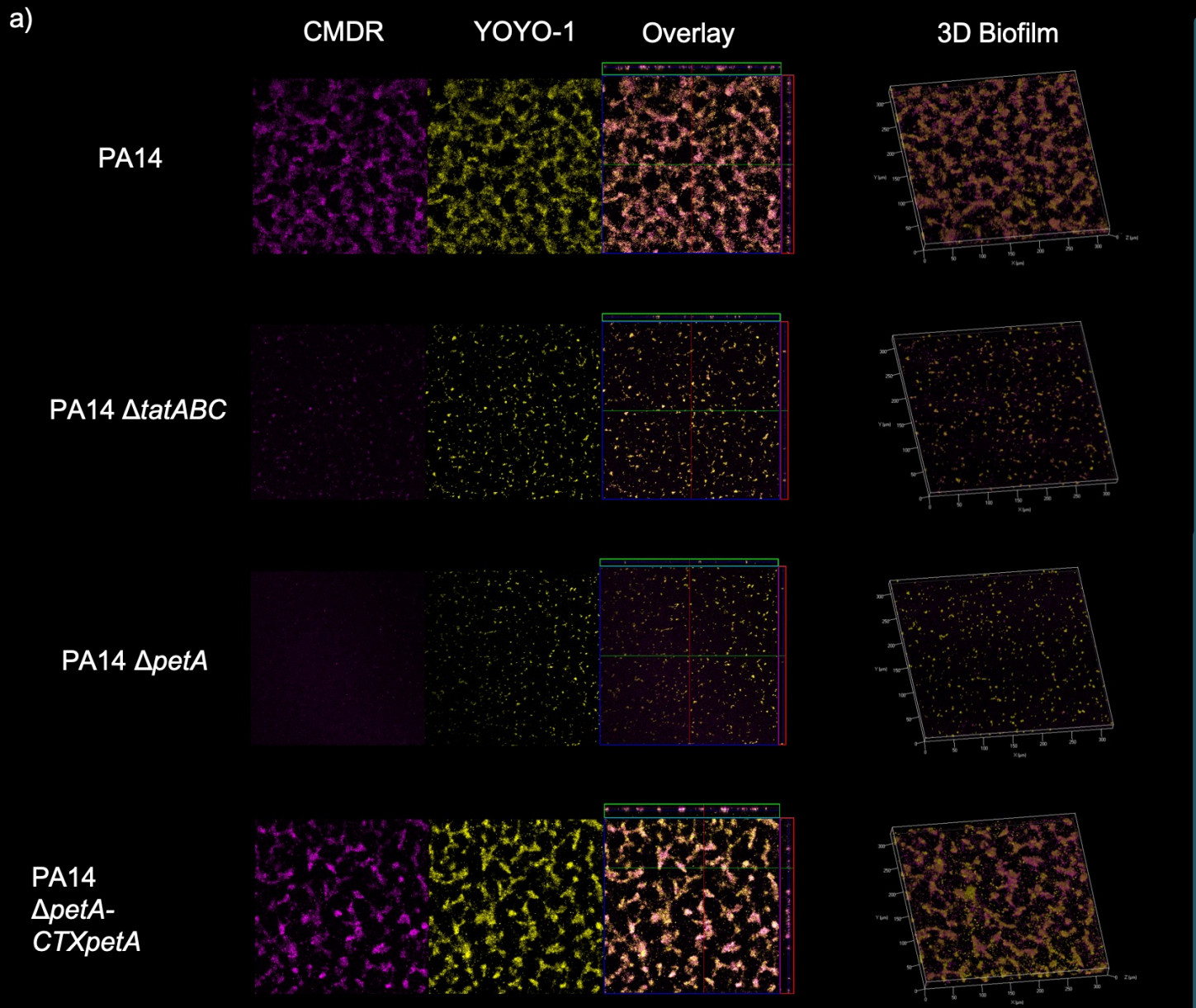
Soh et al. PlLoS Pathogen 2021

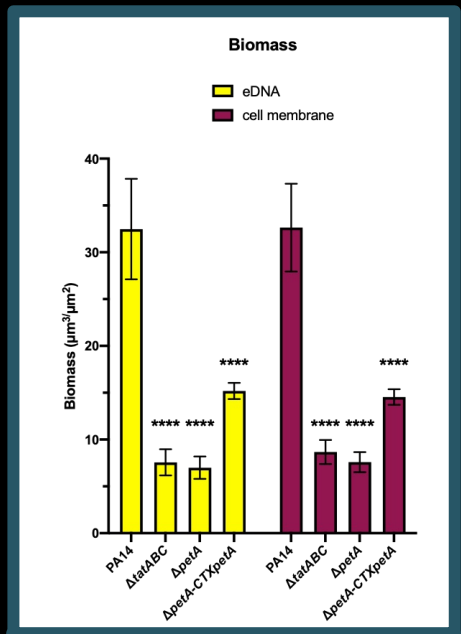
The Twin Arginine Translocation (Tat)

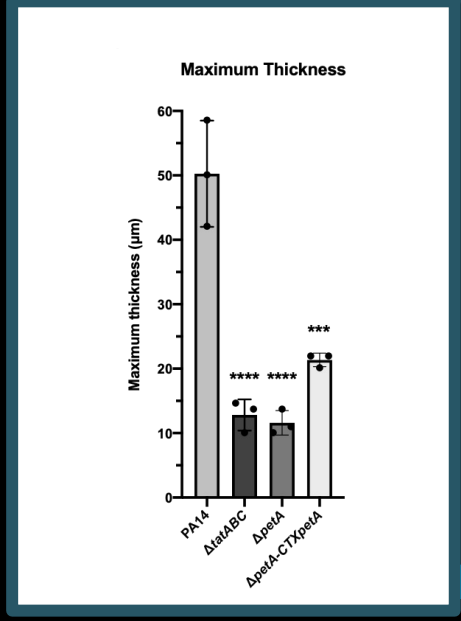
- localized to the inner membrane
- moves fully-folded proteins into the periplasm.
- 34 known substrates of the Tat system in *P. aeruginosa*
- Range of functions including phosphate acquisition (phospholipase C), iron acquisition (pyoverdin maturation enzymes), anaerobic and aerobic respiration, motility and copper resistance.



The Tat substrate Δ*petA* mutation affects biofilm growth too







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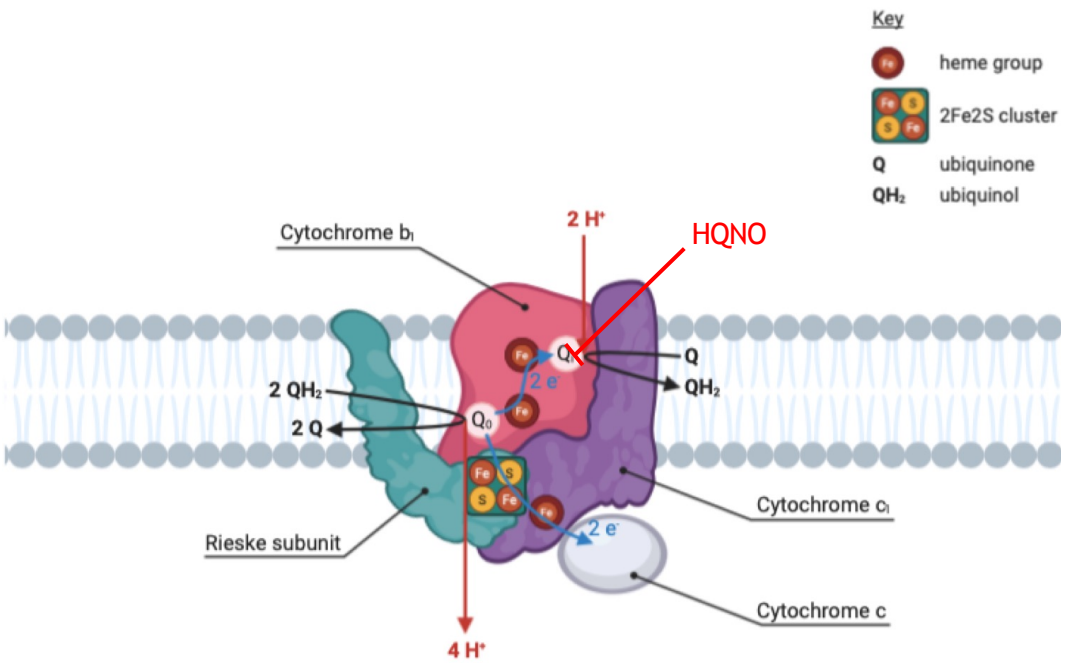
Soh et al. PlLoS Pathogen 2021

Frances Smith

PetA is the Rieske subunit of cytochrome bc_1 complex



1. Rieske mutants are unable undergo HQNO autopoisoning at Qi site

- Cannot undergo programmed cell death, which is required for eDNA release

2. The electron transport chain is disrupted

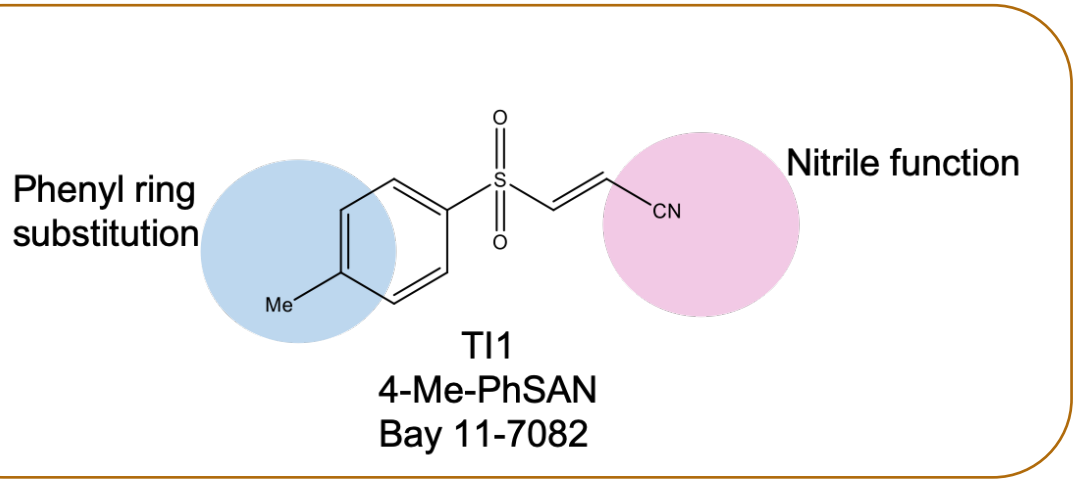
- ATP within cell reduced in both Δ*tatABC* and Δ*petA* mutants
- Cell may have increased aromatic ring catabolism and carbon utilisation to generate energy, and reduced energy-expensive processes such as secondary metabolite biosynthesis

Soh et al. PlLoS Pathogen 2021

Frances Smith

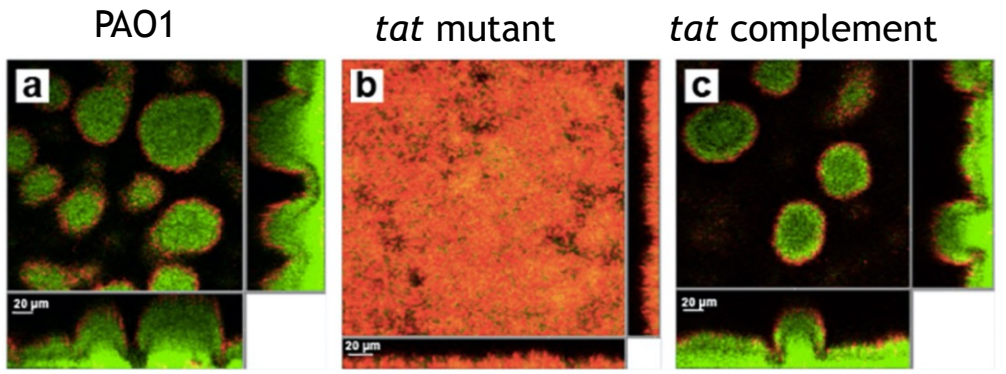
Could Tat inhibitors augment antibiotic sensitivity?

Tat inhibitor Bay 11-7082



Thin, flat biofilms of tat mutants are more sensitive to tobramycin.

Tobramycin-treated biofilms showing live cells (green fluorescence) and dead cells (red fluorescence)

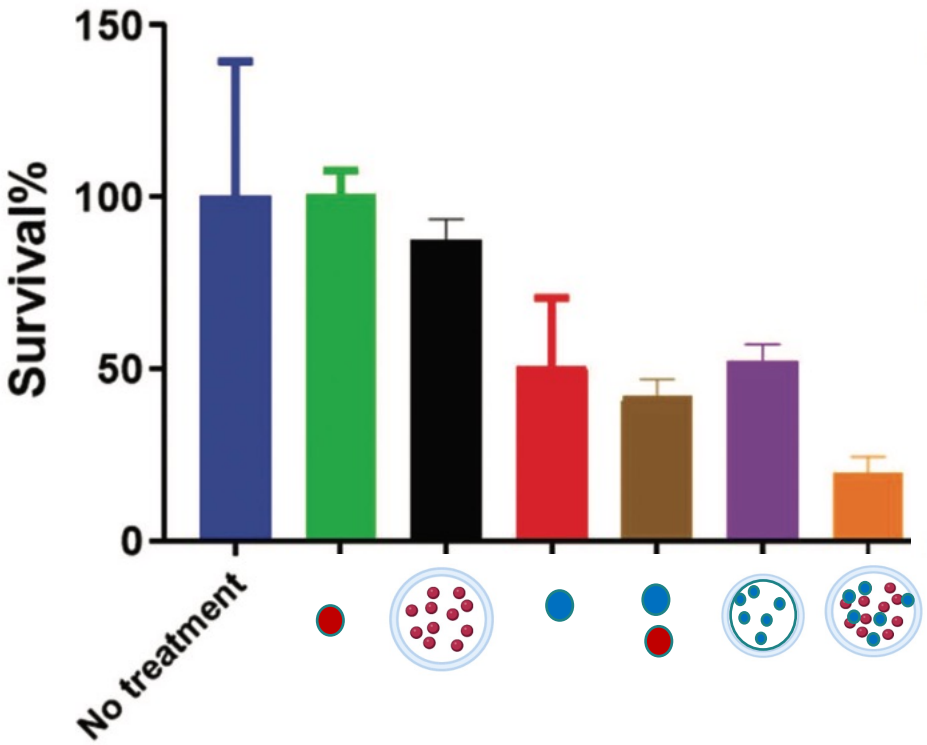


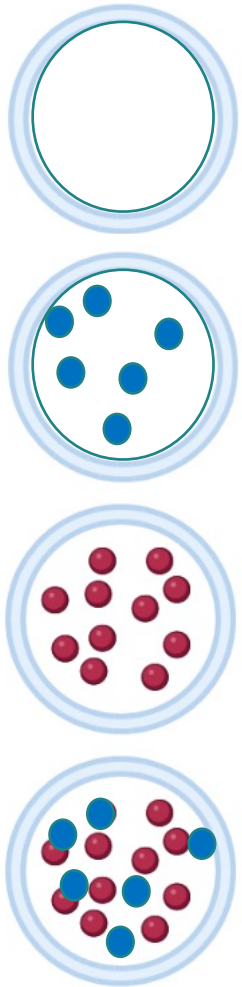
Live cells (GFP)/Dead cells (PI)

Frances Smith

Nanoparticle delivery of anti-biofilm and antibiotics combination is effective

Biofilm





Empty versicle

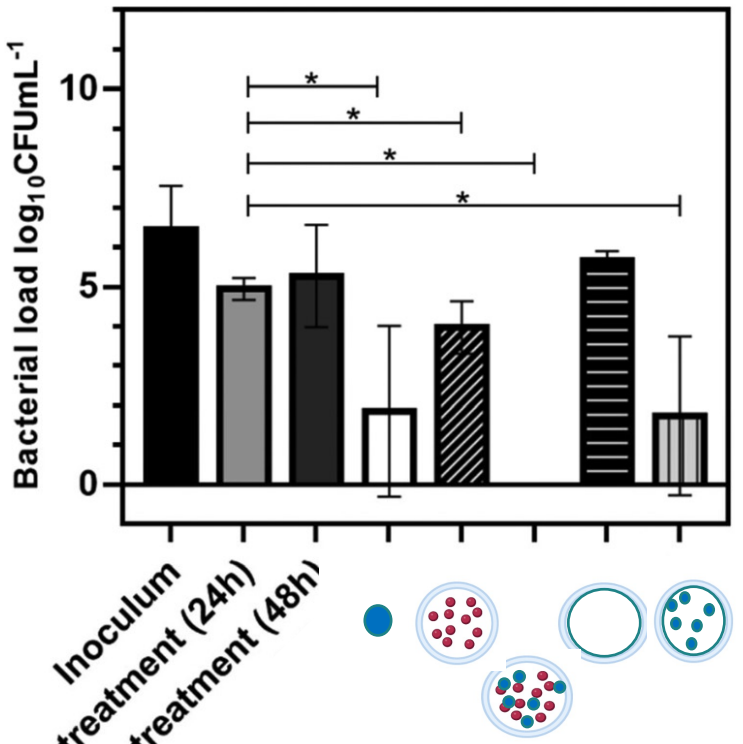
ciprofloxacin

anti-biofilm QSI

Both

BioRender.com

Porcine skin infection model



Singh *et al*. 2019

Summary: finding and targeting Biofilm weak spots to improve antimicrobial options and curb rising AMR

- **Biofilms are less sensitive to our current antimicrobials**
- **Rising AMR remains an issue**

- **The location of actively growing/metabolising bacteria in biofilms can be mapped to plot potential antimicrobial targets**
- **Antimicrobial penetration and killing can be tracked over time to identify the location of barriers including persisters**
- **Complex polymicrobial communities alter sensitivity to antimicrobials**
- **Mechanisms of antimicrobial tolerance in biofilms are emerging**
- **There is scope for novel antimicrobial targets that weaken biofilms**

Thanks to:

Nottingham

Birte Hollmann
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