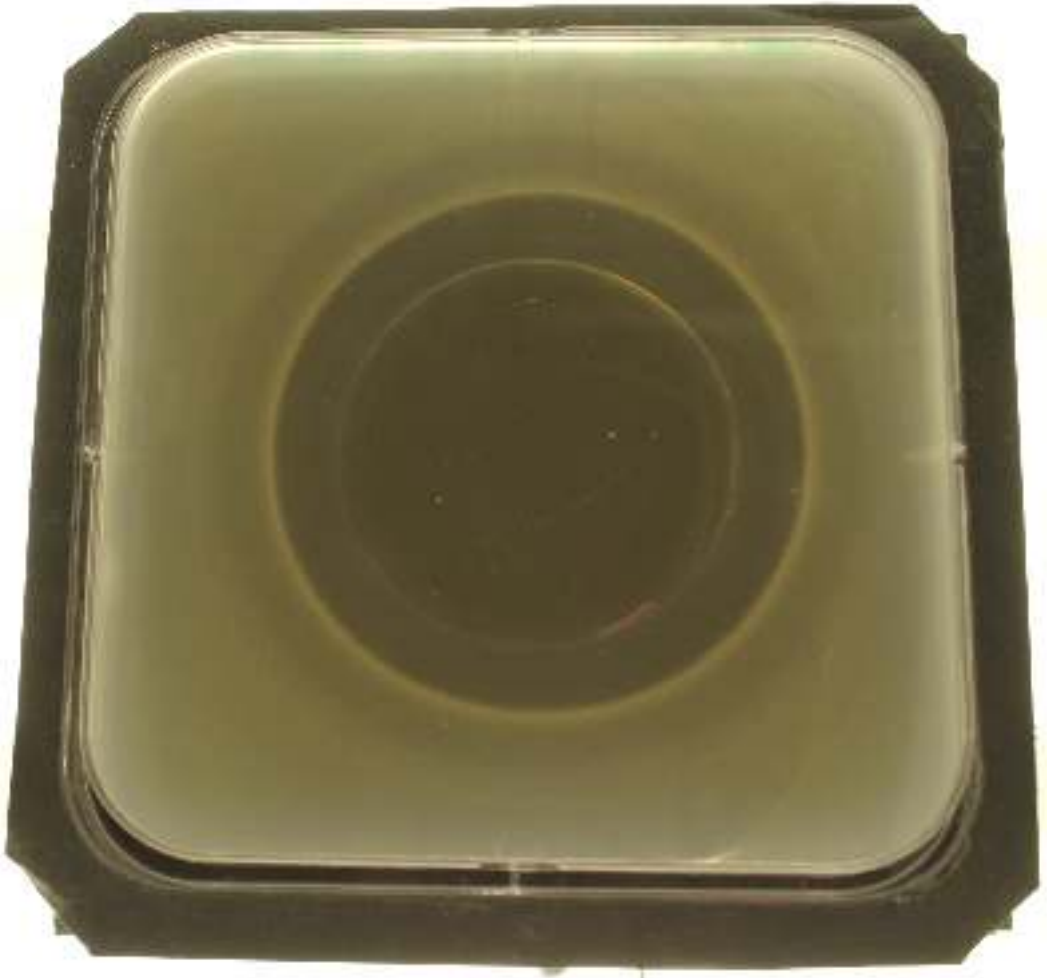
How gradients accelerate bacterial adaptation to antibiotics

Carlos Reding[+], Mark Hewlett[+], Sam Gardner, Ivana Gudelj and Robert Beardmore



Thermodynamics.

Gradients are everywhere in nature...

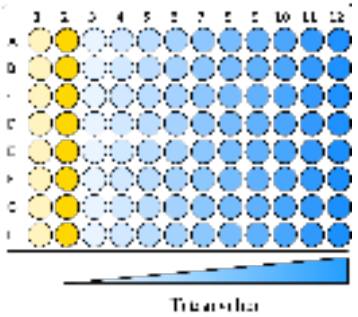
... but sometimes they are ignored.

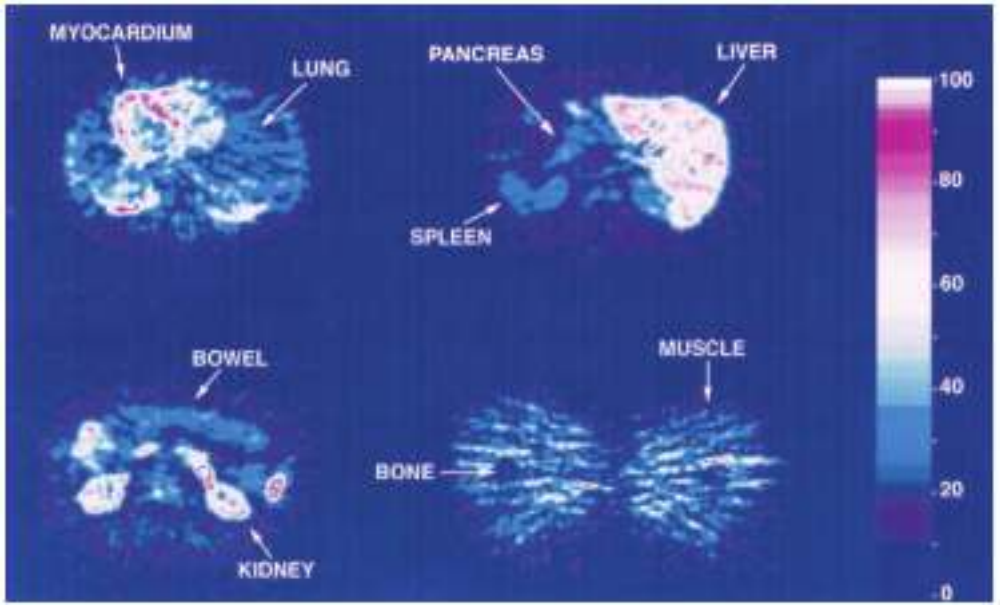
Mutational Pathway Determines Whether Drug Gradients Accelerate Evolution of Drug-Resistant Cells

Philip Greulich, Bartłomiej Waclaw, and Rosalind J. Allen

Attempts to address this problem include the characterization of mutational pathways leading to resistance [1,2], as well as theoretical [3–8] and experimental [9–11] studies of the emergence of resistance under different treatment regimens. These studies usually assume a spatially uniform drug concentration. However, in many clinical situations drug concentrations vary in space [12,13], for example, where

(2012) *Phys. Rev. Lett.*- **THEORETICAL**





Müller *et al.* (2004) *Antimicrob. Agents Chemother.*

Oops.

And resistance to antibiotics can evolve very rapidly in drug gradient

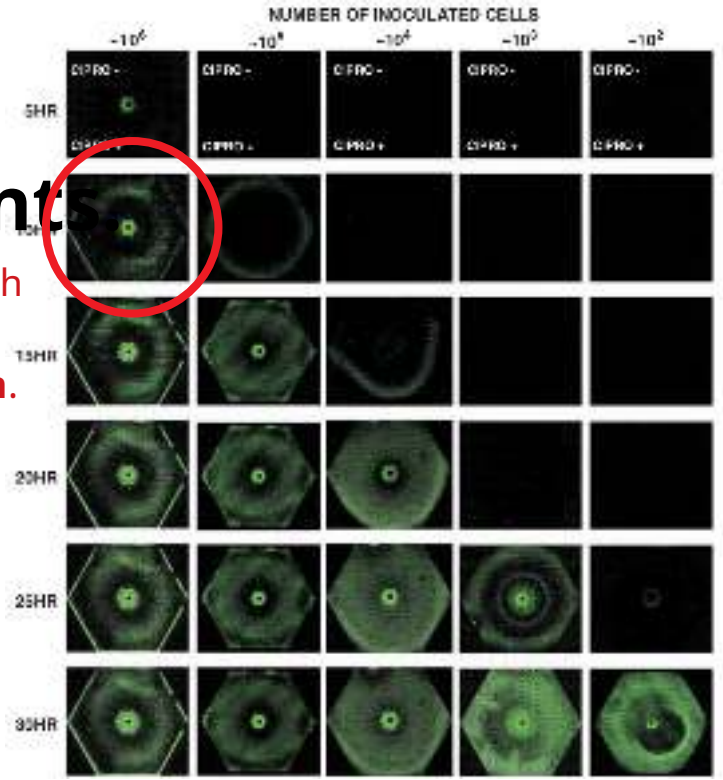
Acceleration of Emergence of Bacterial Antibiotic Resistance in Connected Microenvironments

Qiuen Zhang,[1] Guillaume Lambert,[1] David Liao,[2] Hyunsung Kim,[3] Kristelle Robin,[4] Chih-kuan Tung,[2] Nader Pourmand,[3] Robert H. Austin[1,4]

studies fail to probe how such mutations occur and spread within a population during antibiotic treatment. In particular, the importance of spatial heterogeneities and their effect on evolutionary processes during the emergence of antibiotic resistance is often overlooked. Sewall Wright

(2011) *Science.* - **EXPERIMENTAL**

Mutants isolated within 10h of exposure to the drugs, but **unknown mechanism**.



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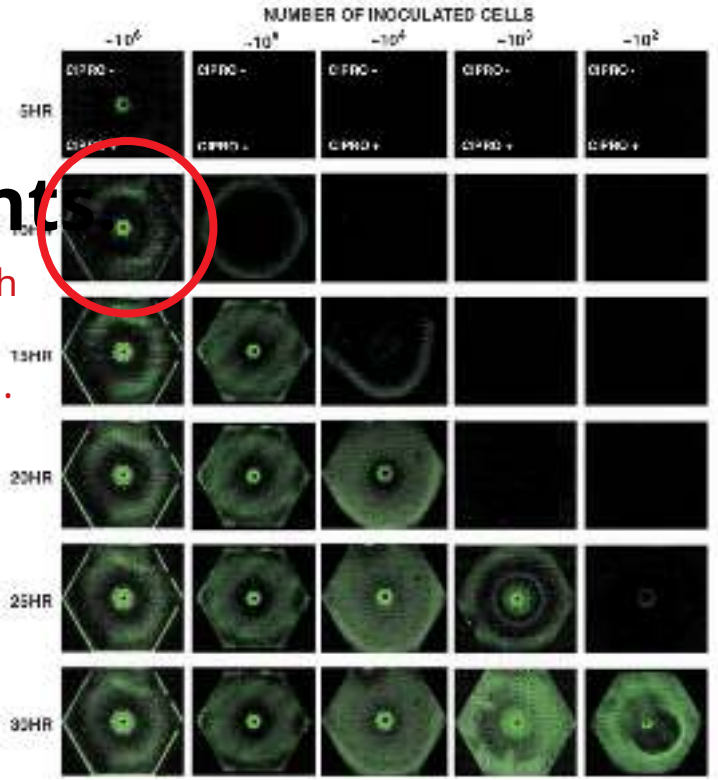

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(2011) *Science.* - **EXPERIMENTAL**

Why evolution is so fast in the presence of antibiotic gradients only studied theoretically... **why?**

On the rapidity of antibiotic resistance evolution facilitated by a concentration gradient

Rutger Hermsen[1], J. Barrett Deris, and Terence Hwa[1]

The rapid emergence of bacterial strains resistant to multiple antibiotics is posing a growing public health risk. The mechanisms underlying the rapid evolution of drug resistance are, however, poorly understood. The heterogeneity of the environments in which bacteria encounter antibiotic drugs could play an important role. E.g., in the highly compartmentalized human body, drug

(2012) *PNAS.*- **THEORETICAL**

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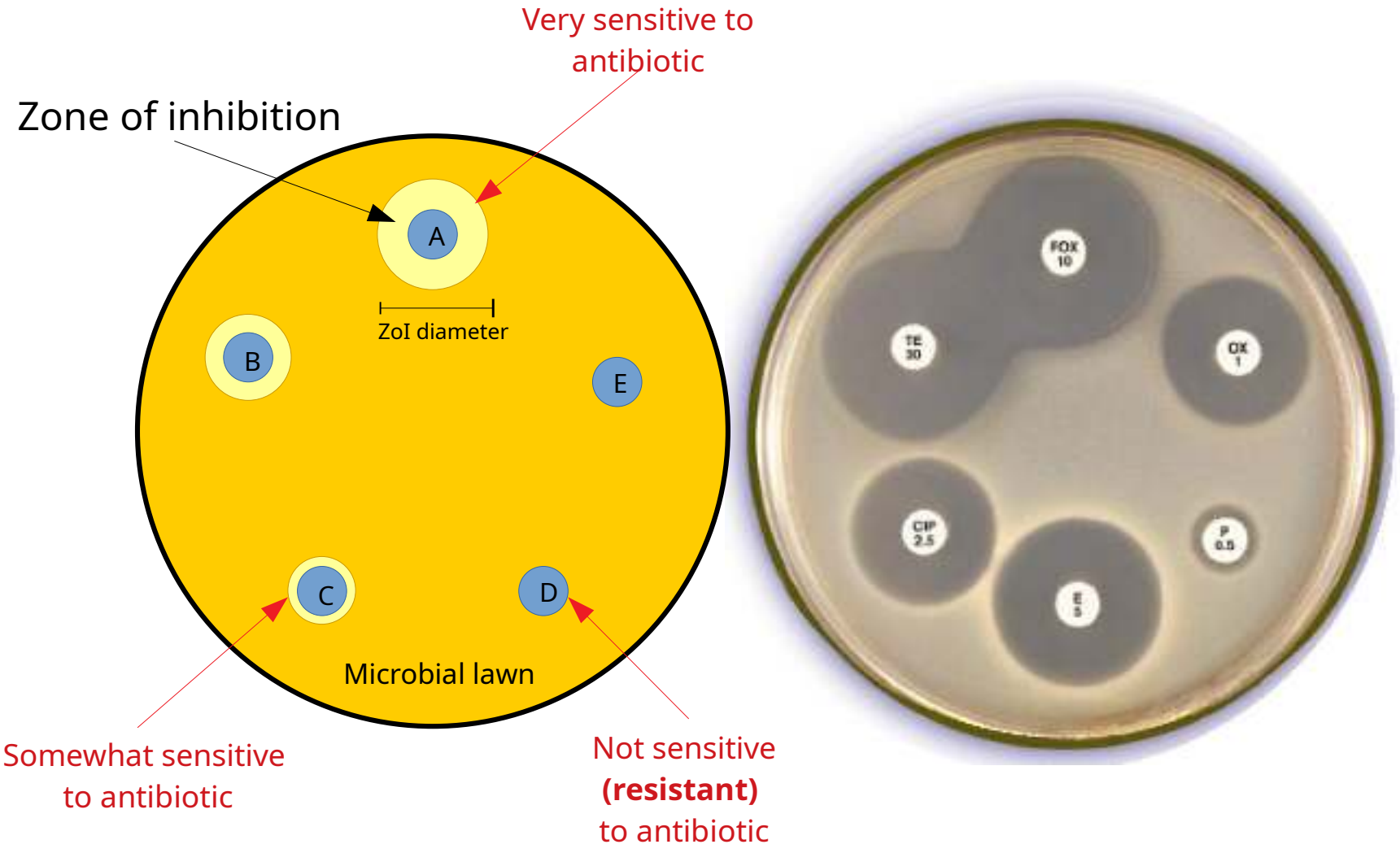
(2012) *Phys. Rev. Lett.*- **THEORETICAL**

Antibiotic sensitivity tests

Diffusion-based methods have limited use...

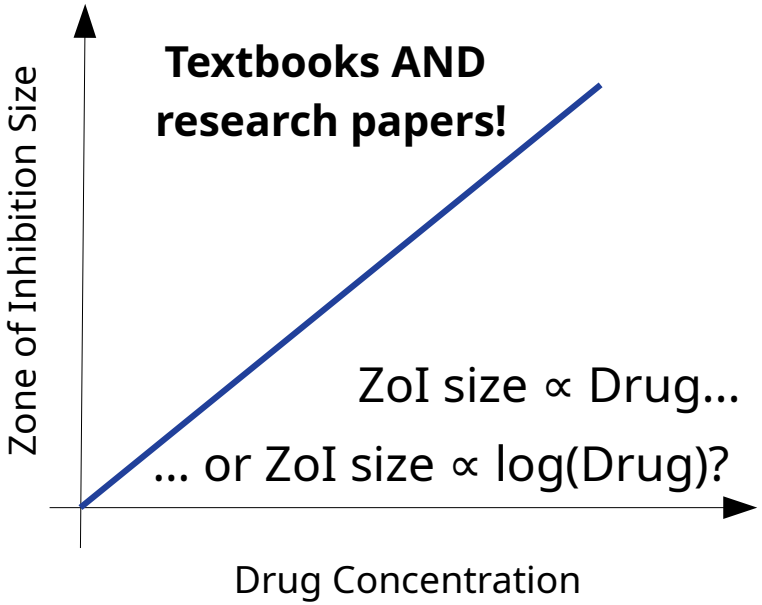


This is an **antibiogram**, one of the most common antibiotic sensitivy tests in the clinic. **Highly standardised**.

Antibiotic sensitivity tests

… difficult to translate into a specific dose.

A-level syllabus (UK)

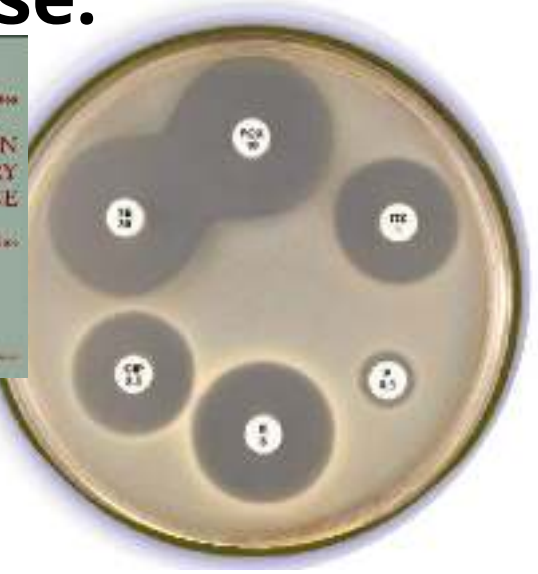


1999

2004

2012

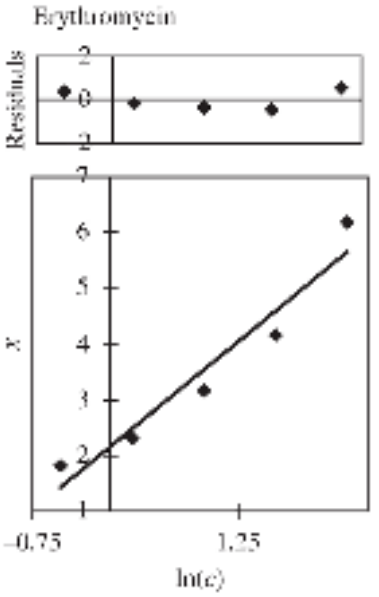
2005



How the zone of inhibition changes with antibiotic concentration is not clear (for microbiologists!), so minimum inhibitory concentrations (MICs) estimation **are insatisfactory**

Lorian (2005) Antibiotics in laboratory medicine.

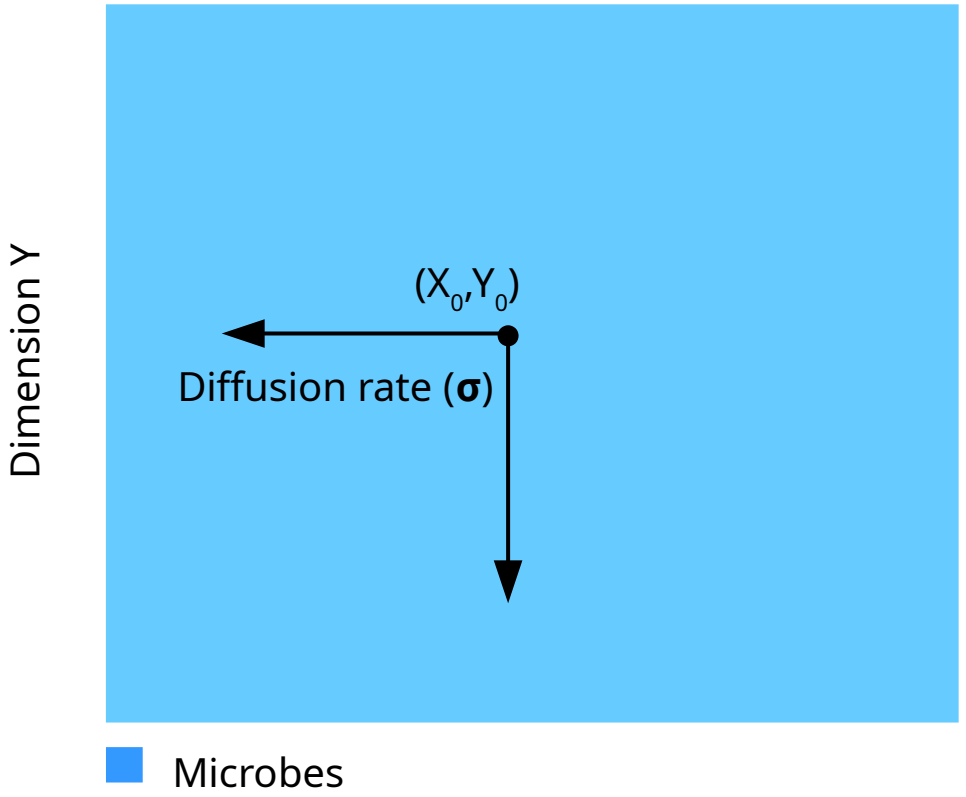
Bonev *et al.* (2009) *J. Ant. Chem.*



Bonev *et al.* (2009)

Linear diffusion theory

Mathematical antibiogram



$$A_t = \sigma(A_{xx} + A_{yy})$$

General solution:

$$A(x,y,t) = \frac{A_c}{4\pi\sigma} e^{-\frac{x^2+y^2}{4\sigma t}}$$

Diffusion identical in x and y,
so **the resulting ZoI is a circle.**

The ~~radius ***r*** of~~ a circle is defined as...

$$r = \sqrt[2]{x^2+y^2} \rightarrow r^2 = x^2+y^2$$

Rearranging here and there...

$$r = \sqrt[2]{\frac{A_c}{A_d \pi e}}$$

If diffusion occurs in three dimensions the ZoI is a **sphere.**
And the radius ***r*** of a sphere is defined as...

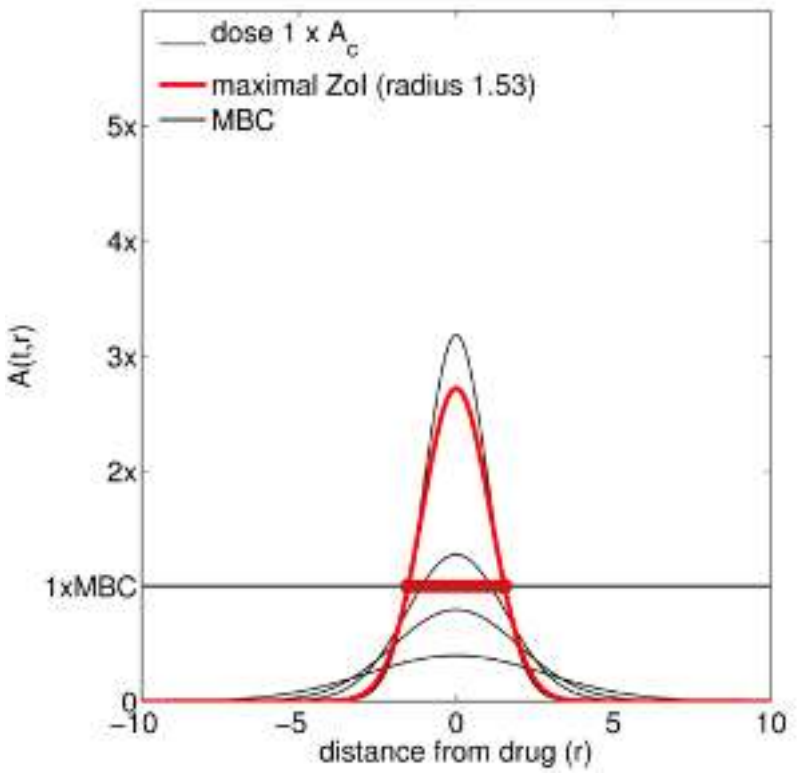
$$r = \sqrt[3]{x^2+y^2+z^2} \rightarrow r^3 = x^2+y^2+z^2$$

$$r = \sqrt[3]{\frac{A_c}{A_d \pi e}}$$

Reding-Roman *et al.*, *in prep.*

What maths have to say.

Mathematical antibiogram



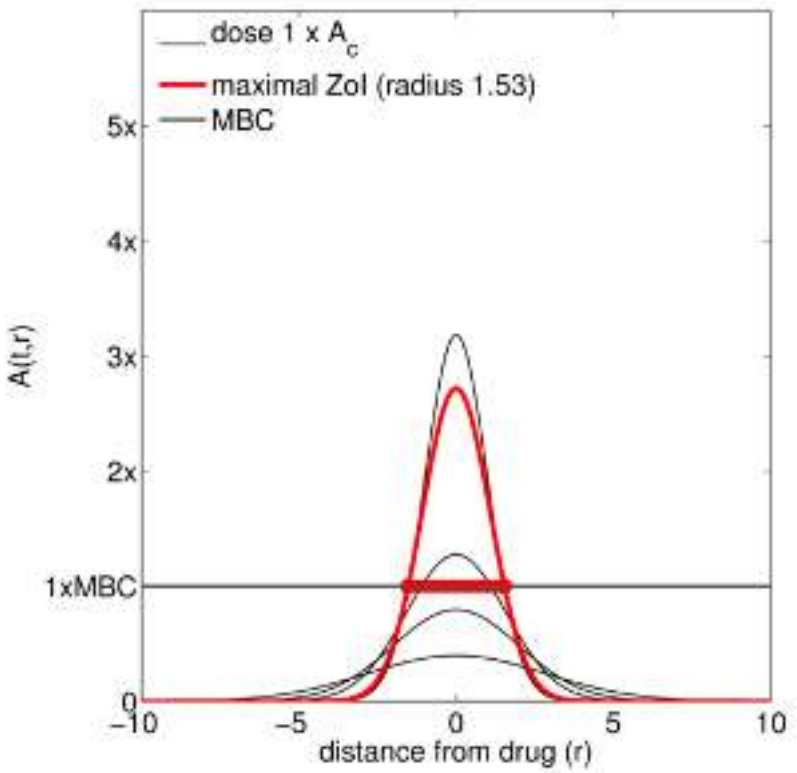
Radius of 1.53

Reding-Roman *et al.*, *in prep.*

What maths have to say.

Mathematical antibiogram

A_c here = 2x A_c on the left



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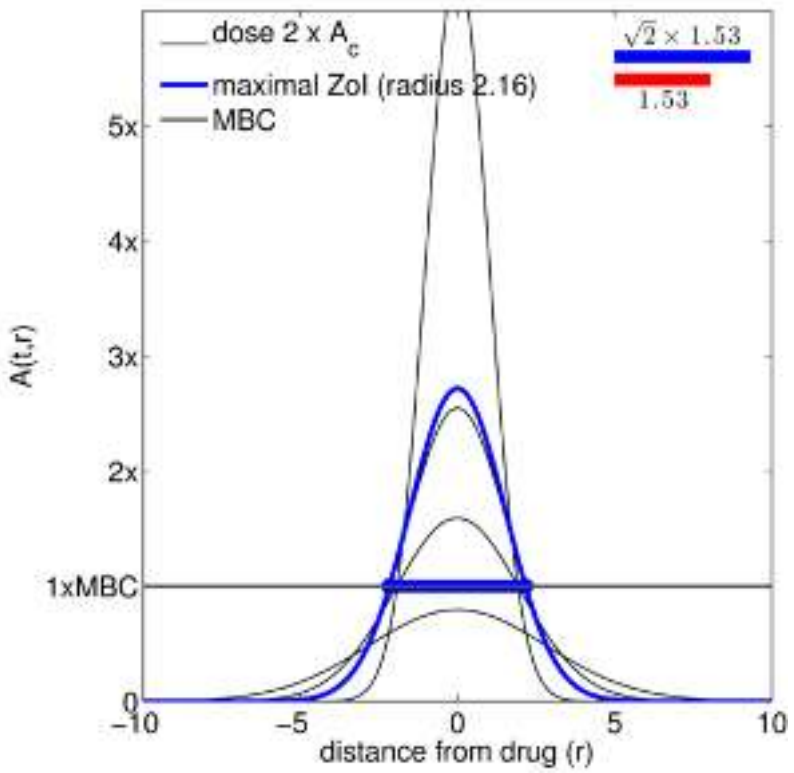


Radius just ~40% wider

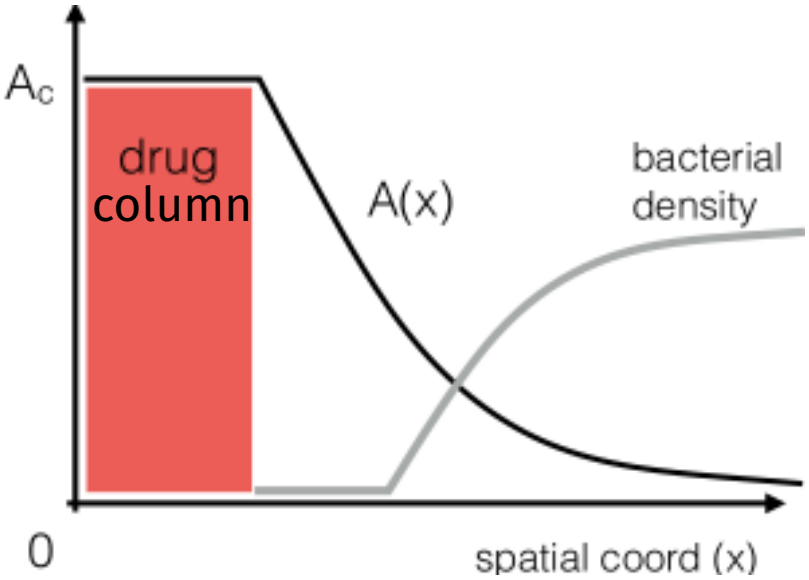
Only when 4x A_c the radius should double

(8x if 3D)

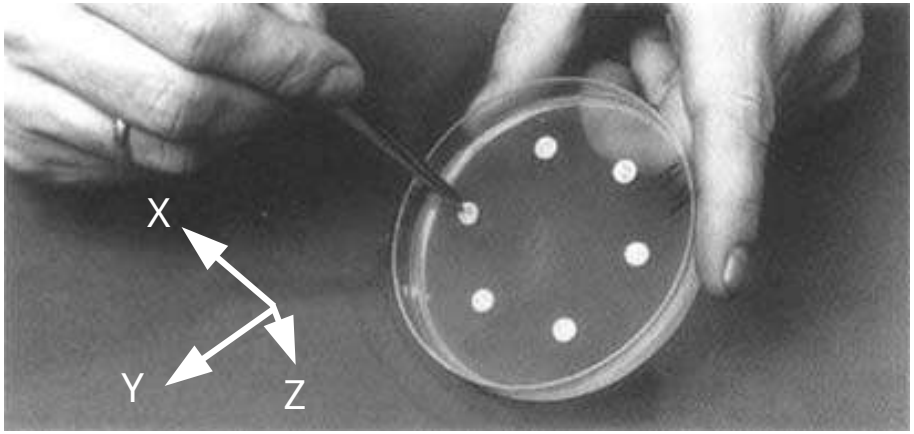
Reding-Roman *et al.*, *in prep.*

Reality check.

What does the data say?

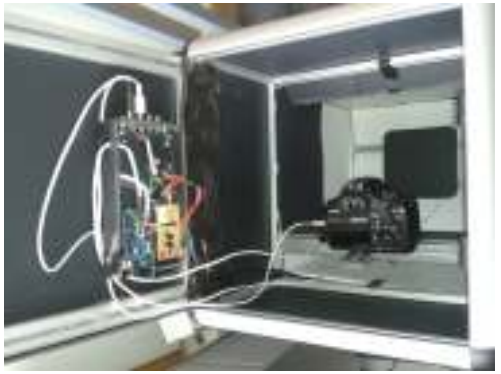


We designed a protocol that mimics diffusion strictly in 2D...
(Maths easier to check)



Added difficulty: Z has different size! Not easy to look beneath an agar plate...

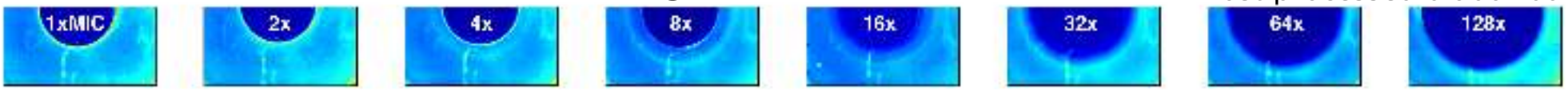
...and built a device to read them: the **biobox**.



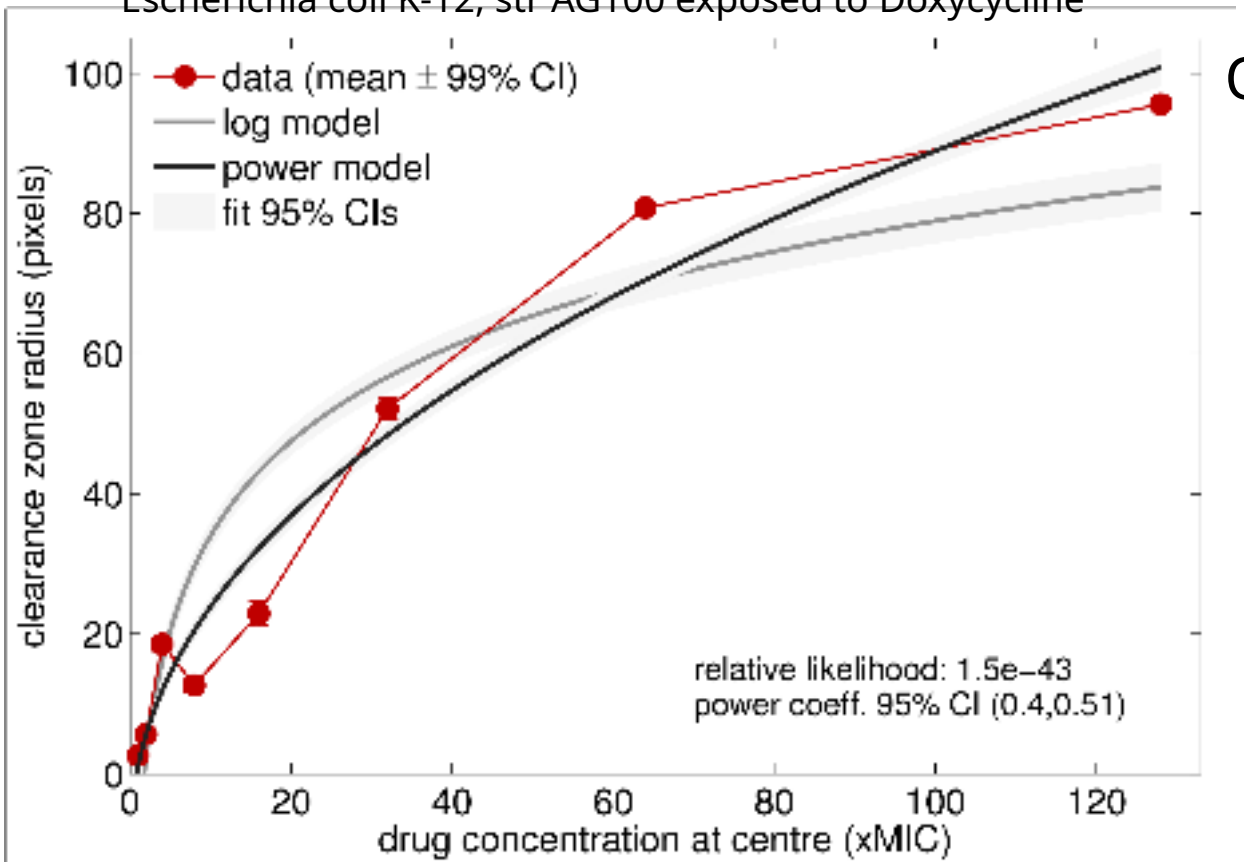
Reality check.

What does the data say?

Post-processed biobox data.



Escherichia coli K-12, str AG100 exposed to Doxycycline



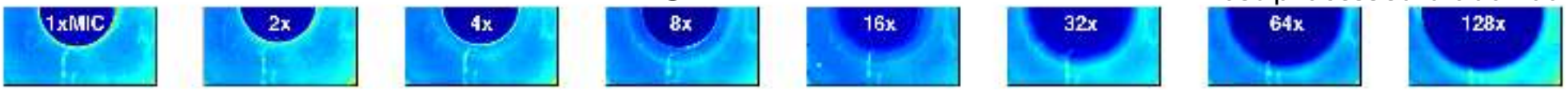
Okay, your diffusion “theory” seems to work. **Now, what?**

Reding-Roman *et al.*, *in prep.*

Reality check.

What does the data say?

Post-processed biobox data.



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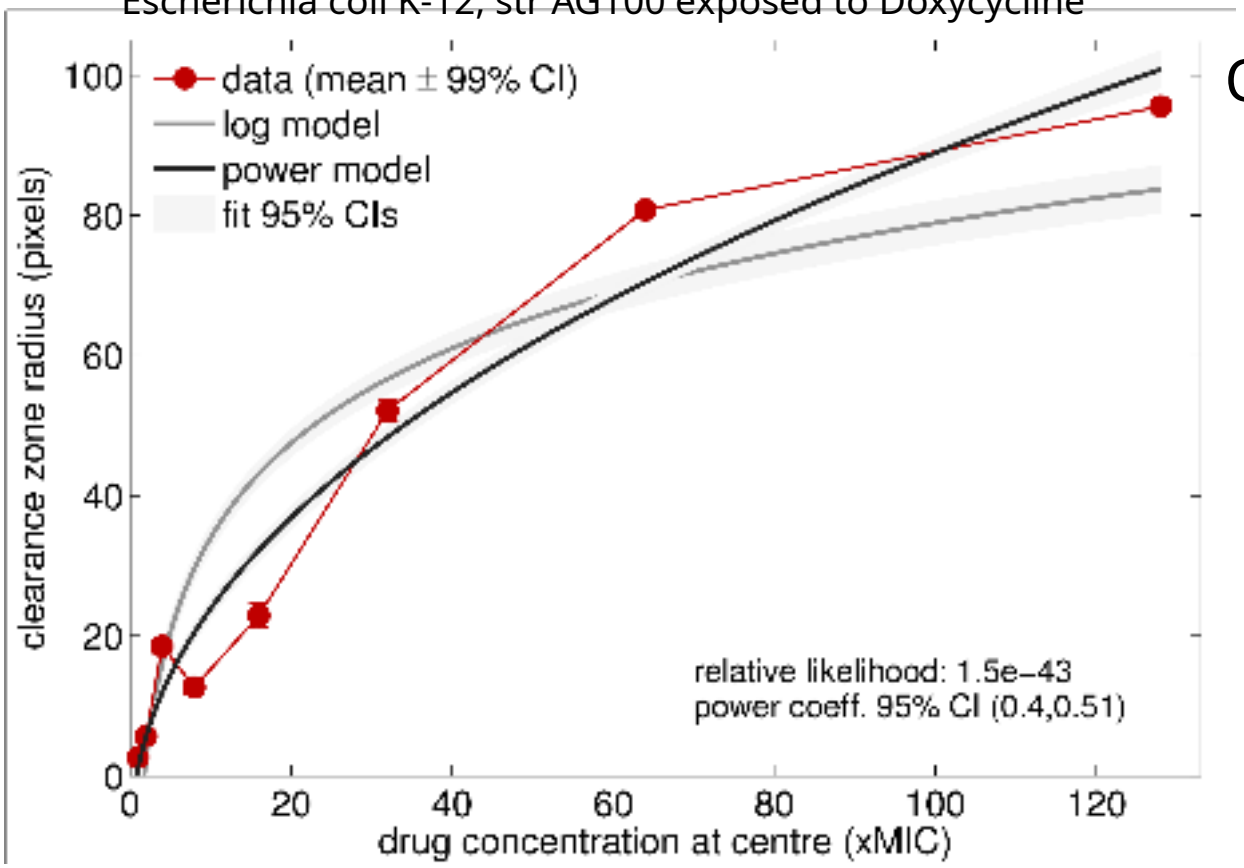

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Now we apply it to Monod’s growth law, and see what happens…

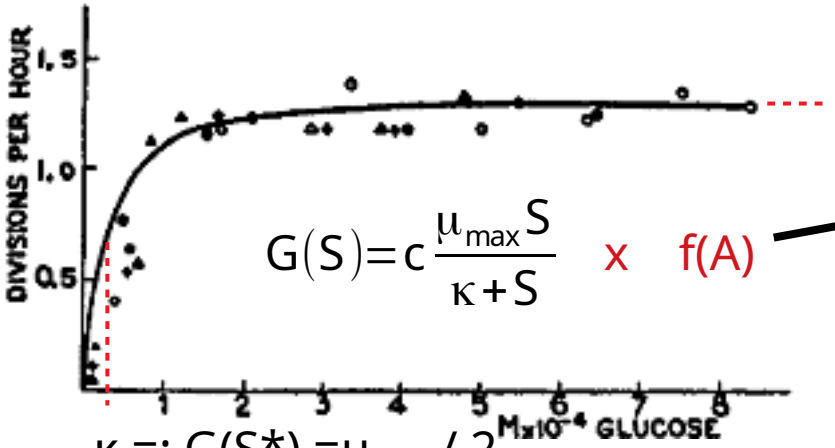
Reding-Roman *et al.*, *in prep.*

Changing the law.

Spatially-extended Monod

Jacques Monod

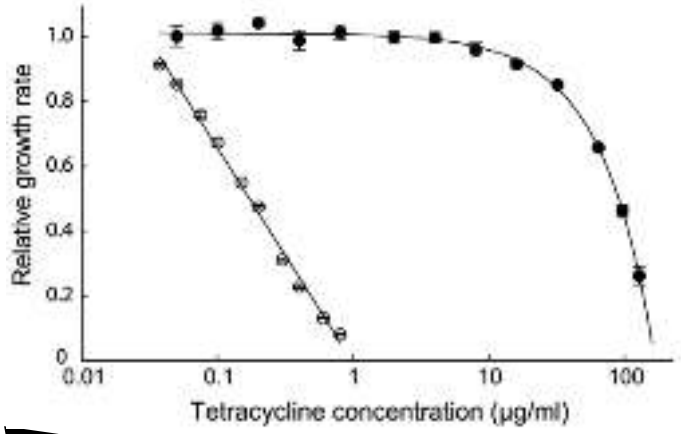
Monod (1949) *Ann. Rev.*



μ_{max}

$$G(S) = c \frac{\mu_{max} S}{\kappa + S} \quad x \quad f(A)$$

$\kappa =: G(S^*) = \mu_{max} / 2$



Gullberg *et al.* (2011) *PloS Pathogens.*

Antibiotics reduce growth rate...

Reding-Roman *et al.*, *in prep.*

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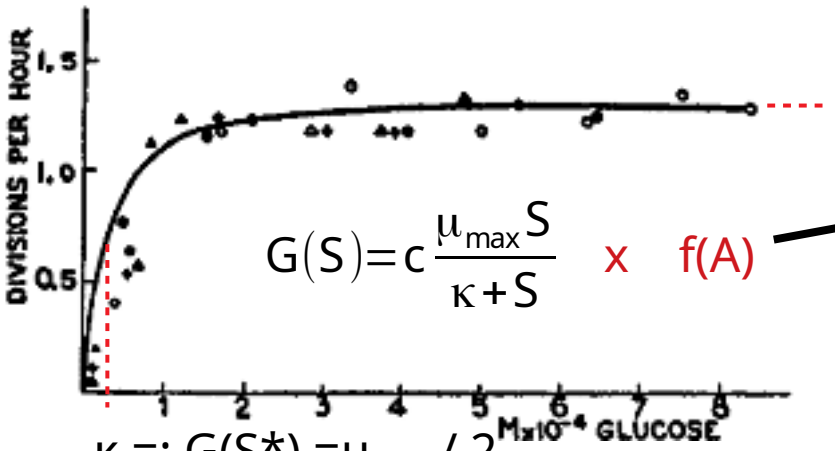

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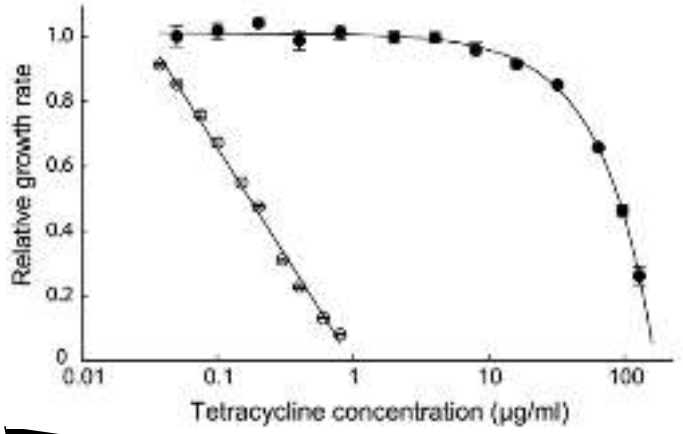

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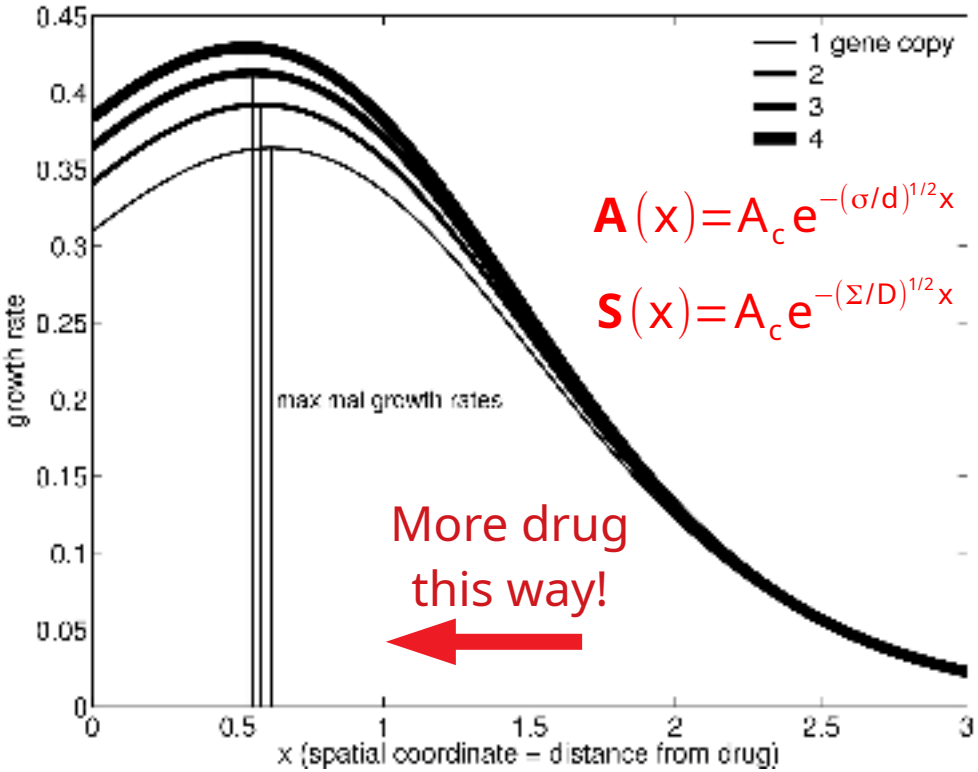
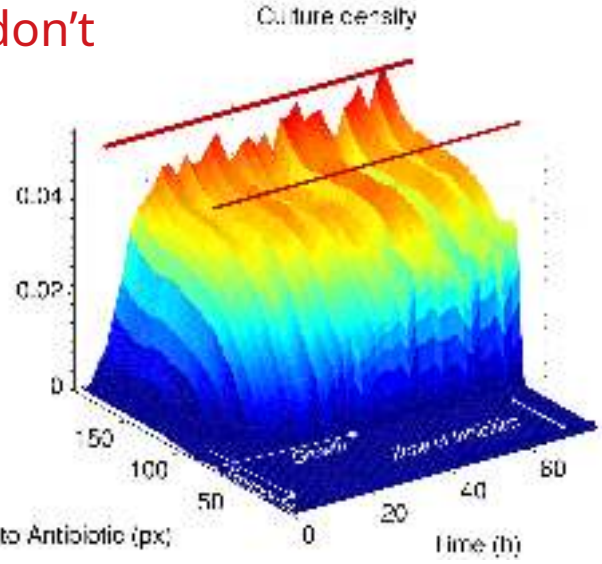


$$A(x) = A_c e^{-(\sigma/d)^{1/2} x}$$

$$S(x) = A_c e^{-(\Sigma/D)^{1/2} x}$$

More drug this way!

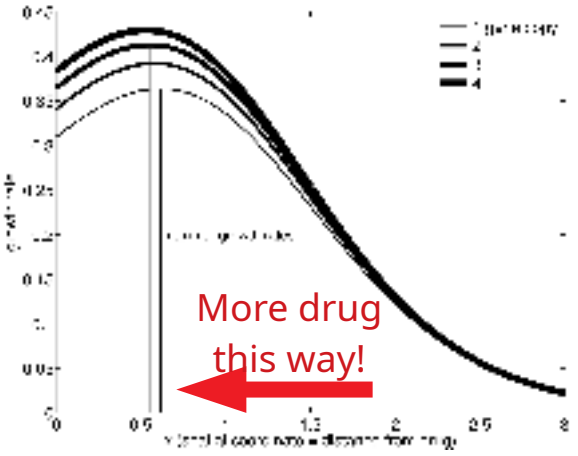
...as long as they don't diffuse!

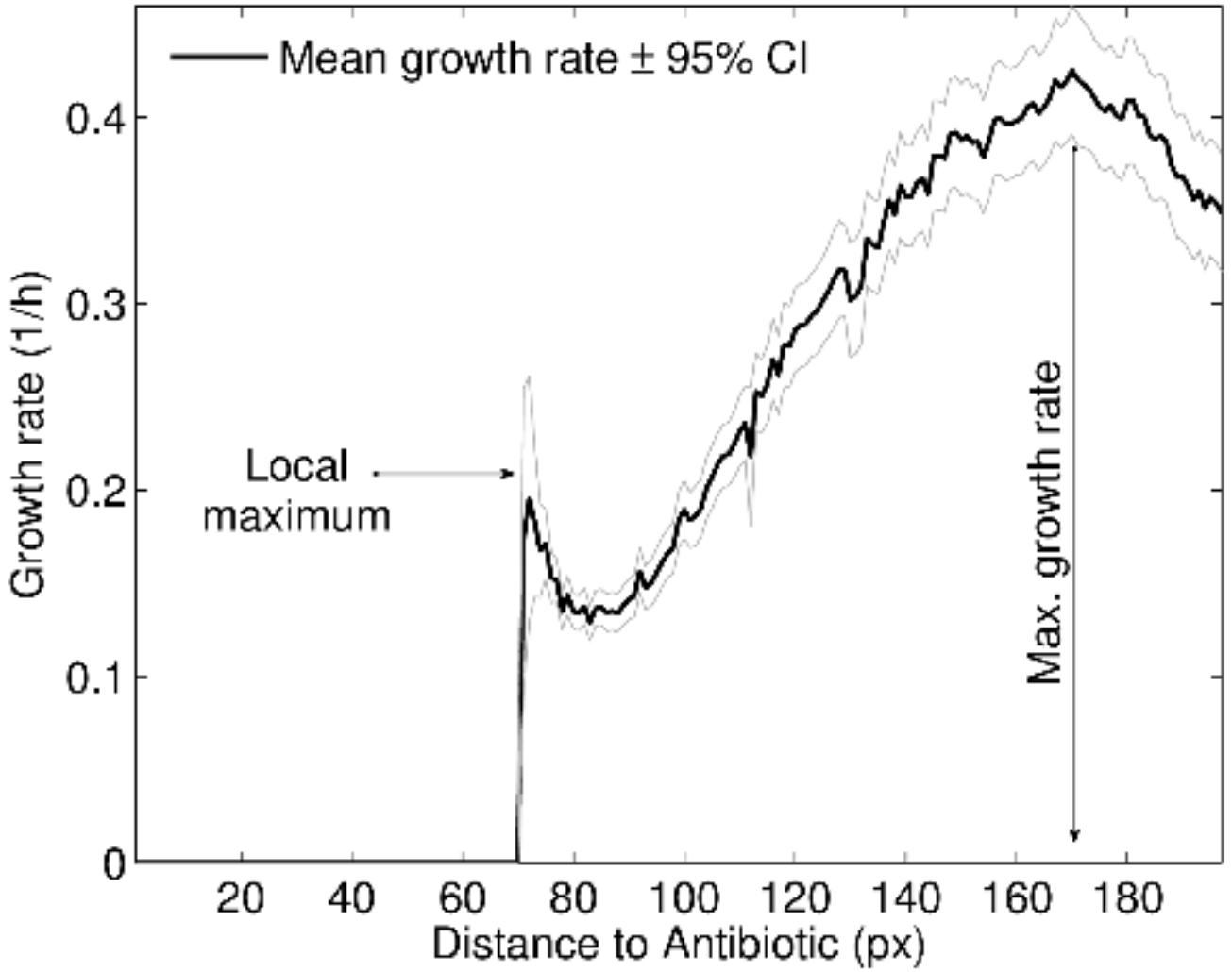


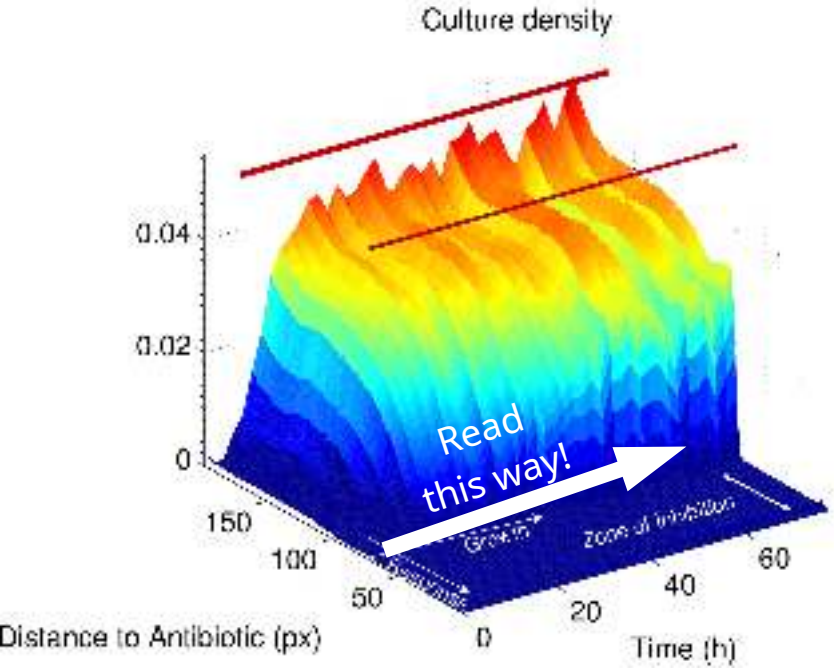
We check this out in the biobox.

Reding-Roman *et al.*, *in prep.*

Bacterial growth rate maximised in gradients



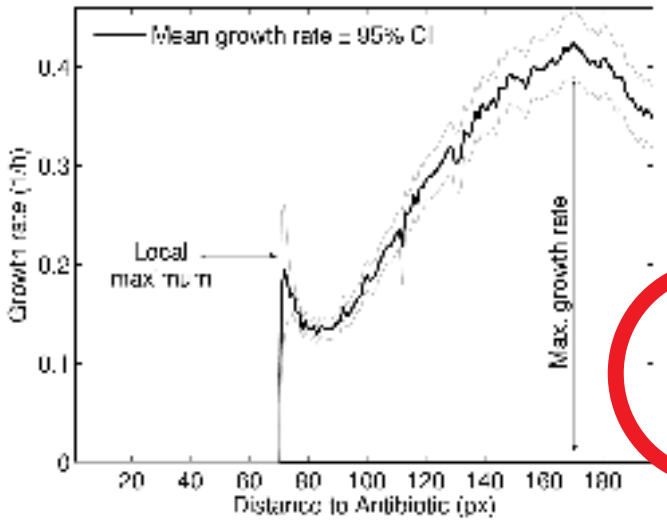


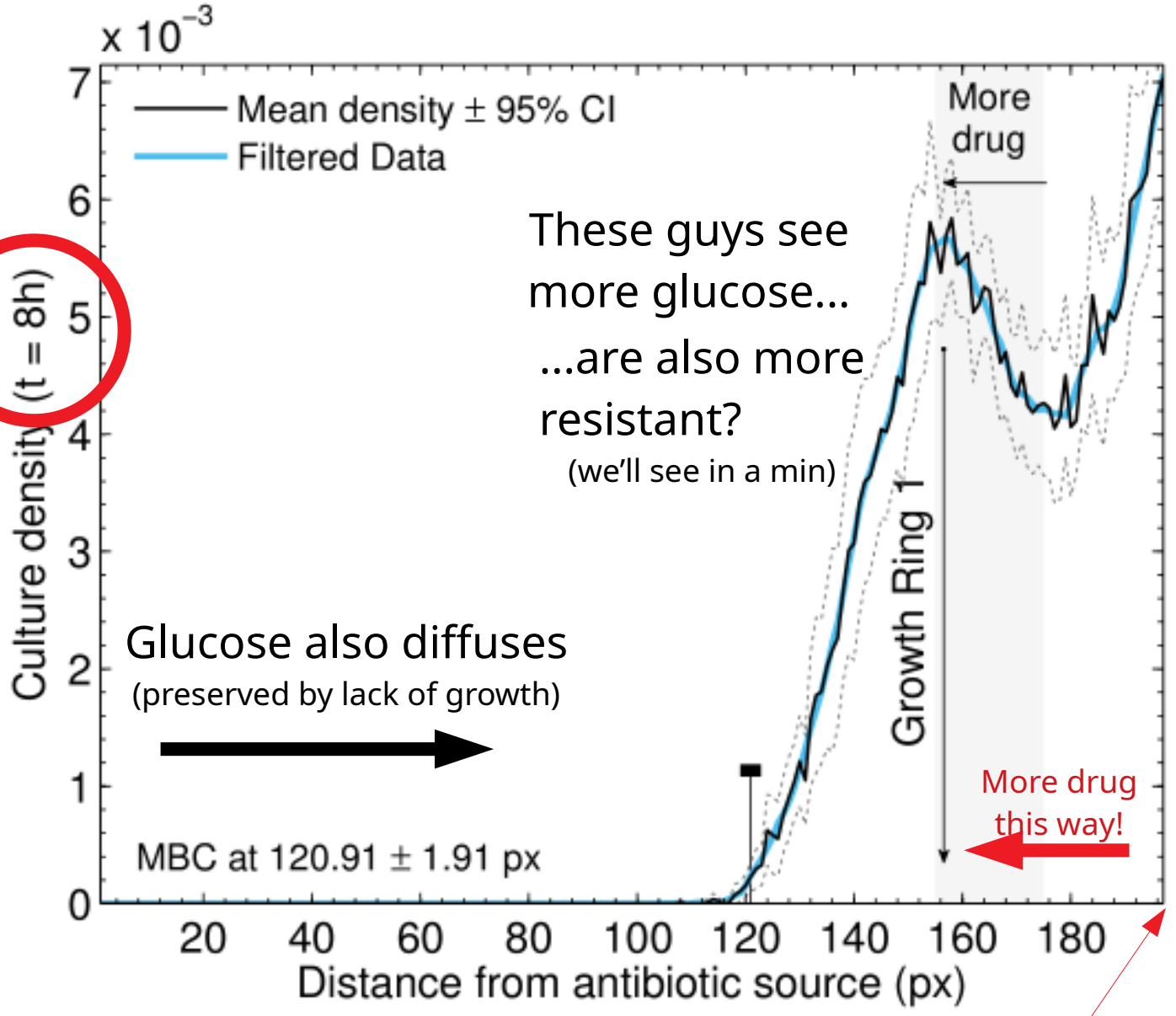


Fitting logistic to image data.

8h? These things are used for weeks!

Bacterial growth rate maximised in gradients





Reding-Roman *et al.*, *in prep.*

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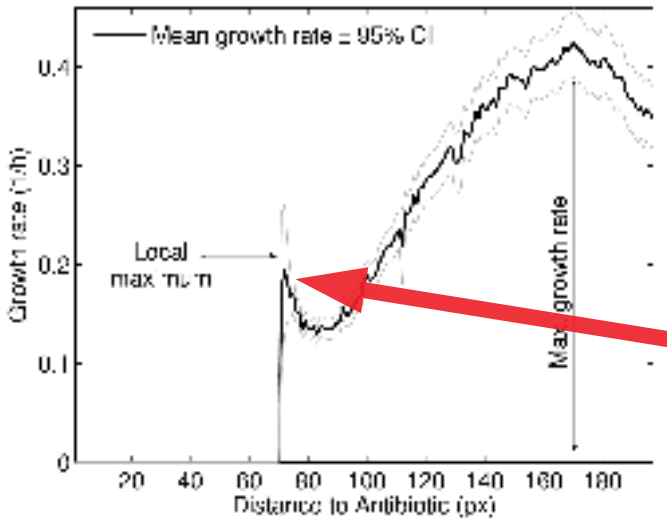
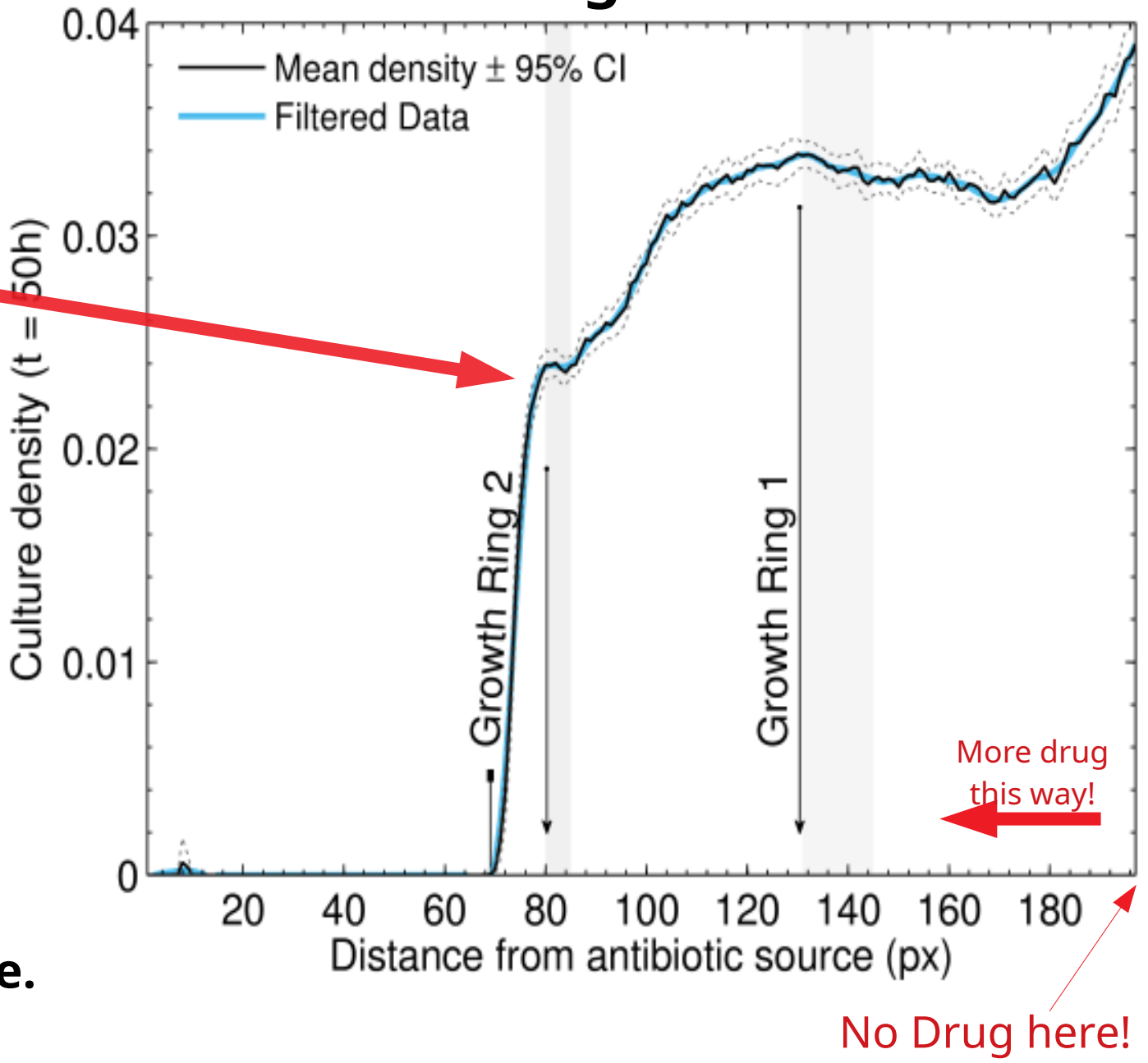




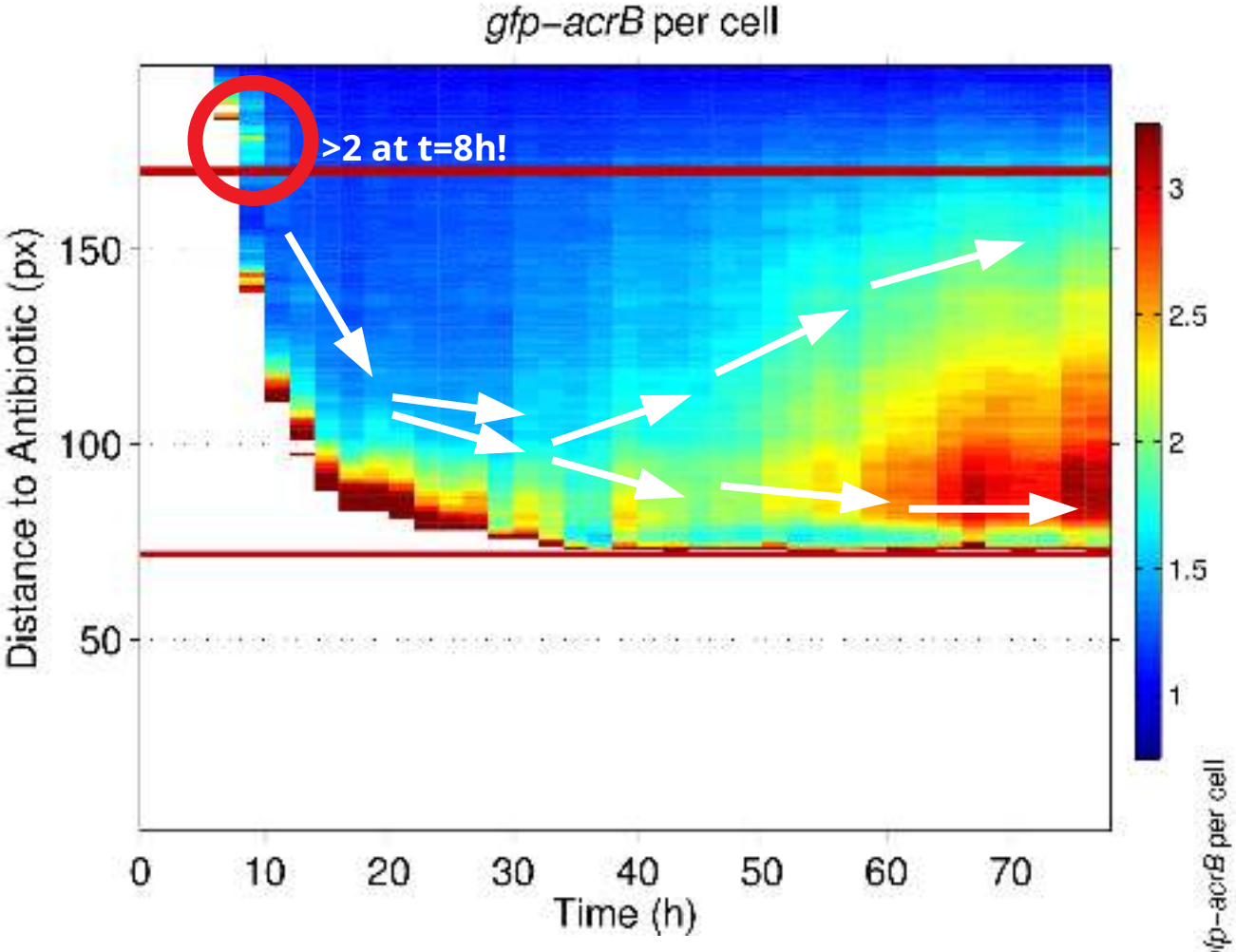
If the exposure to the drug persists, more rings develop:

2 after 50h of exposure.

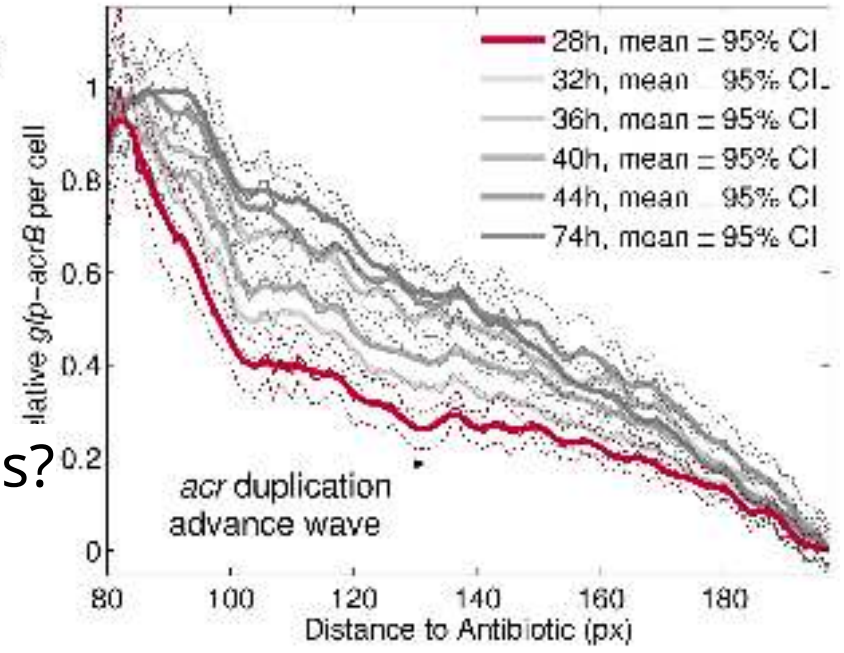
Reding-Roman *et al.*, *in prep.*

Model based on increase in resistance gene copy number.

Tracking AcrAB-TolC abundance with the biobox



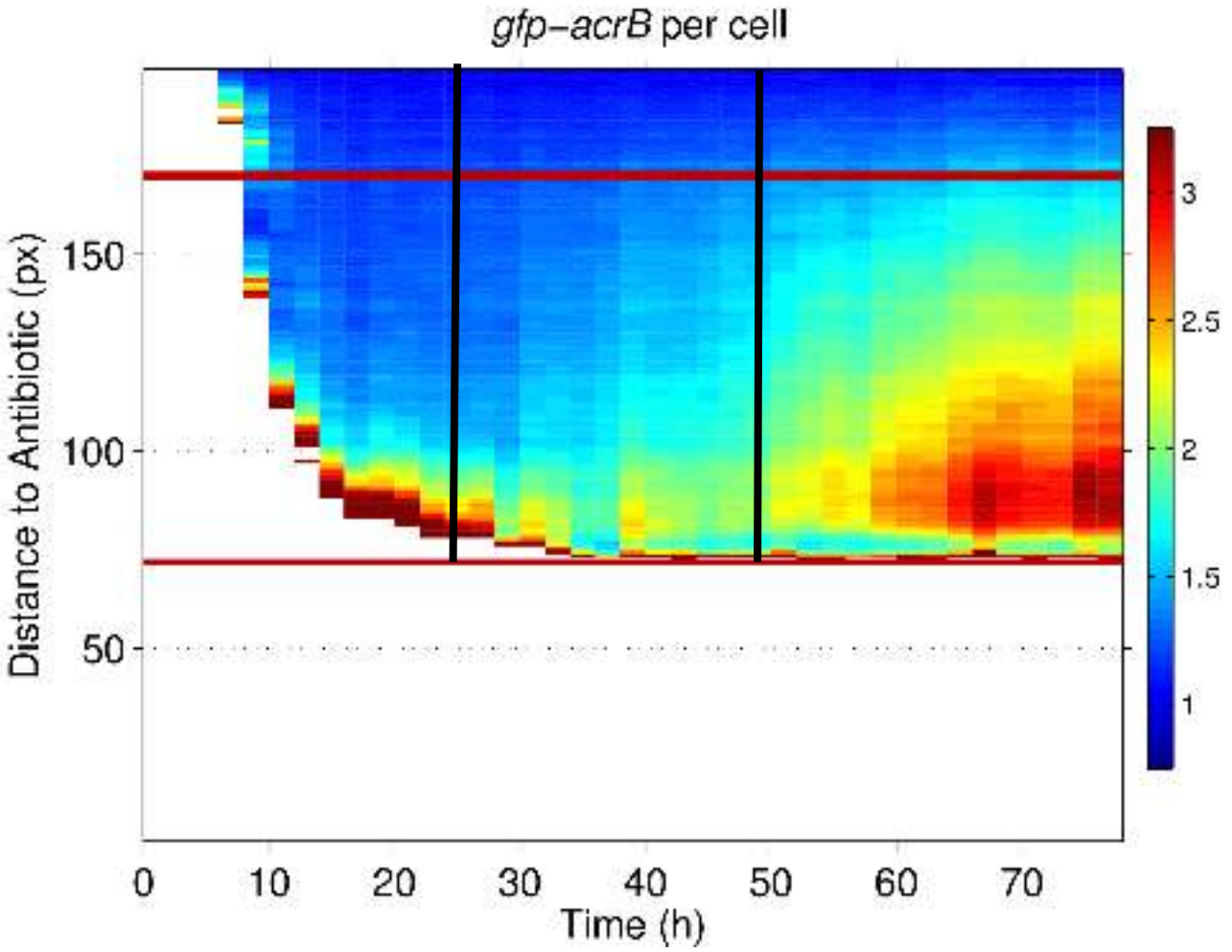
Fisher predicted this... in 1937!



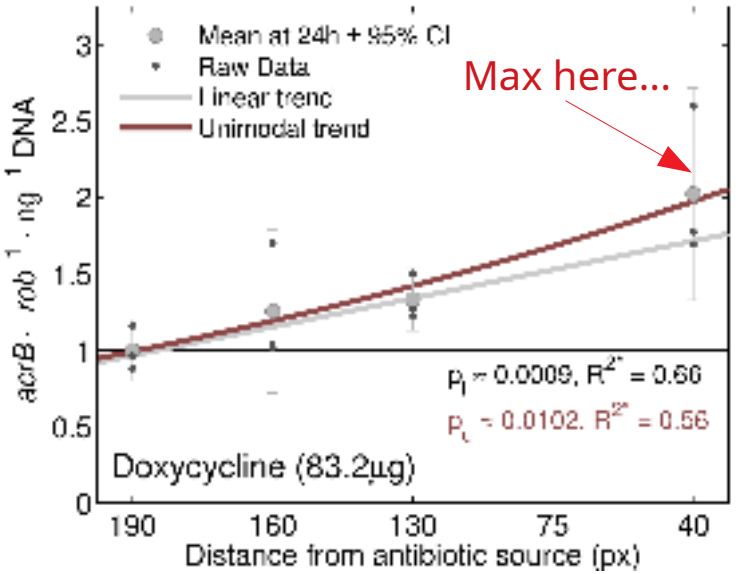
Is the travelling wave of AcrAB-TolC caused by expression change or mutations?

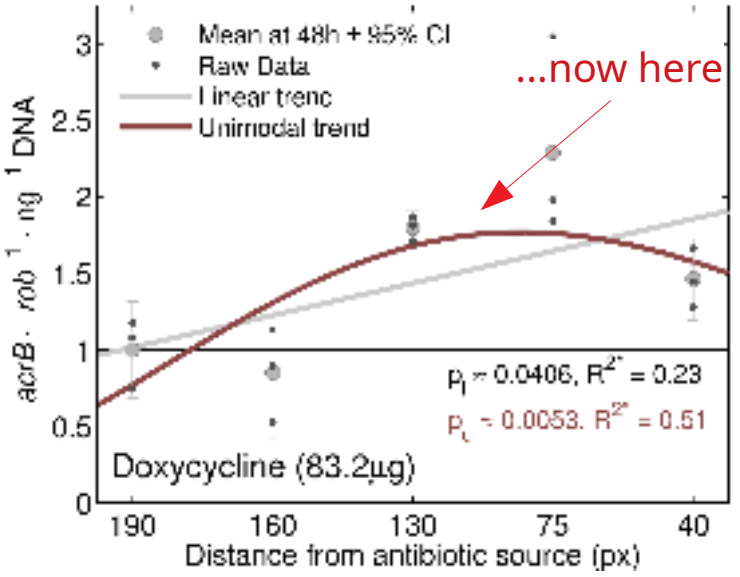
Model based on increase in resistance gene copy number.

Tracking AcrAB-TolC abundance with the biobox



(needed to see the rings to sample!)

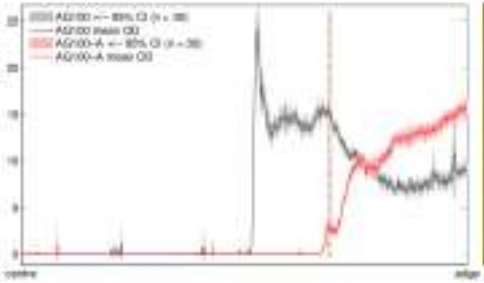


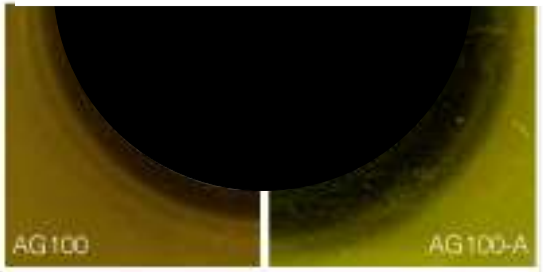


Reding-Roman *et al.*, *in prep.*

Summary

1) The mathematical antibiogram says that microbes only see twice as much drug is four (or eight) times higher.





2) Antibiotics help microbes grow faster in gradients by creating *reservoirs* of carbon. Those growing closer to the drug access more food!

3) And if they become resistant, they gain access to even more food! So there's an incentive to be resistant.

4) Resistance by increasing copies of *acr* operon (within a day).

5) Spread of resistance consistent with 80yrs old theory.

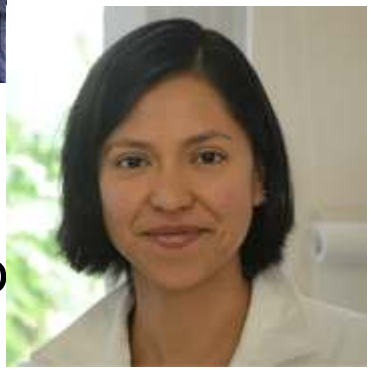
6) Rings **mostly ignored!** Google for antibiogram pictures and see...

EPSRC
Engineering and Physical Sciences Research Council

Prof. Rob Beardmore and Prof. Ivana Gudelj
(funding, help w/ maths)

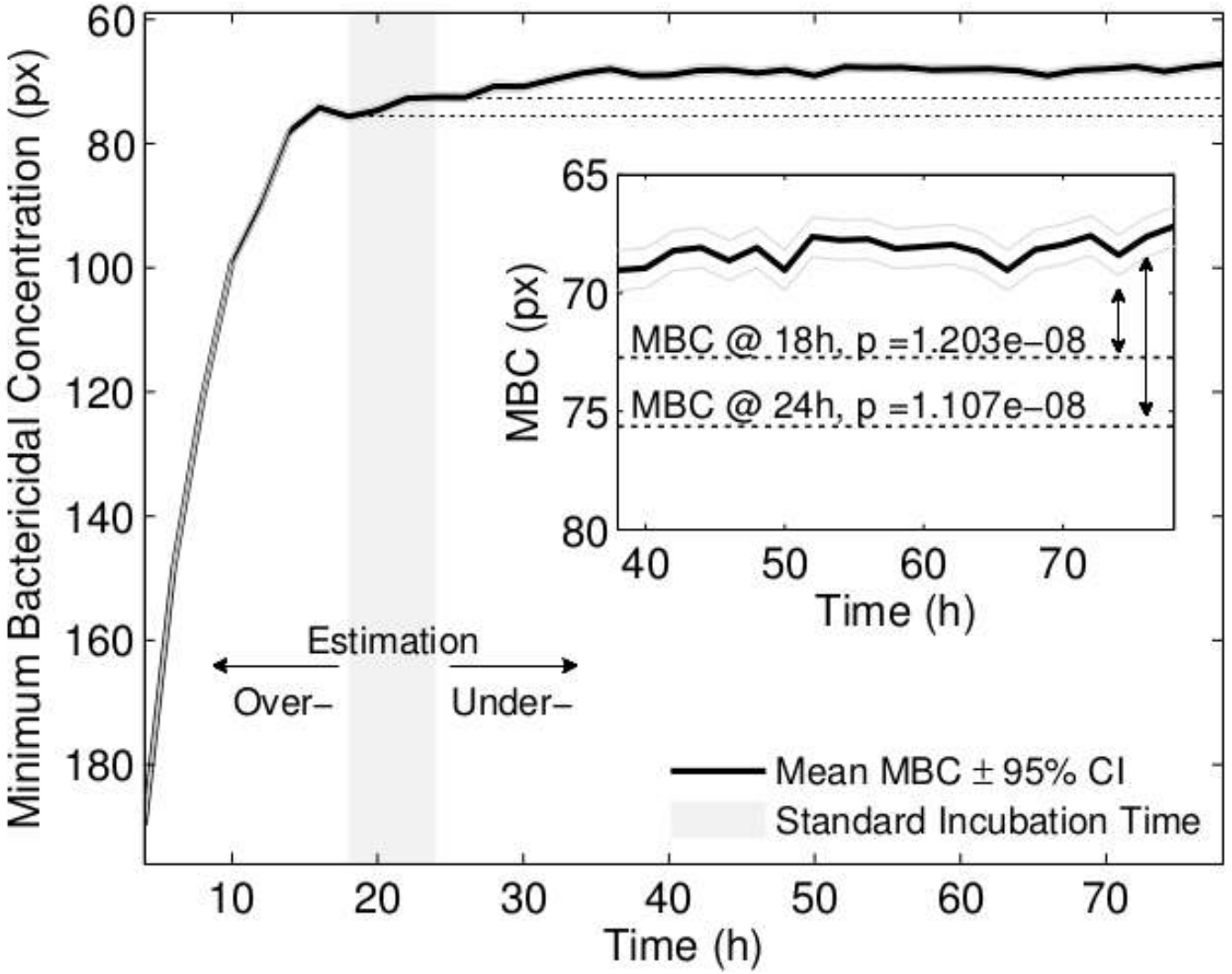
People involved in the biobox one way or another...

Dr Rafael Pena-Miller (UNAM, Mexi



Dr Ayari Fuentes-Hernandez (UNAM, Mexico

Dr Michael Sieber (Max Plank Institute)


Minimum Bactericidal Concentration (px)
60
80
100
120
140
160
180
10
20
30
40
50
60
70
Time (h)
Estimation
Over–
Under–
Mean MBC ± 95% CI
Standard Incubation Time
MBC (px)
65
70
75
80
40
50
60
70
Time (h)
MBC @ 18h, p =1.203e–08
MBC @ 24h, p =1.107e–08