KITP, July 2014

Single-Cell Variability of Growth in Bacteria as an Optimization Process

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Racah Institute
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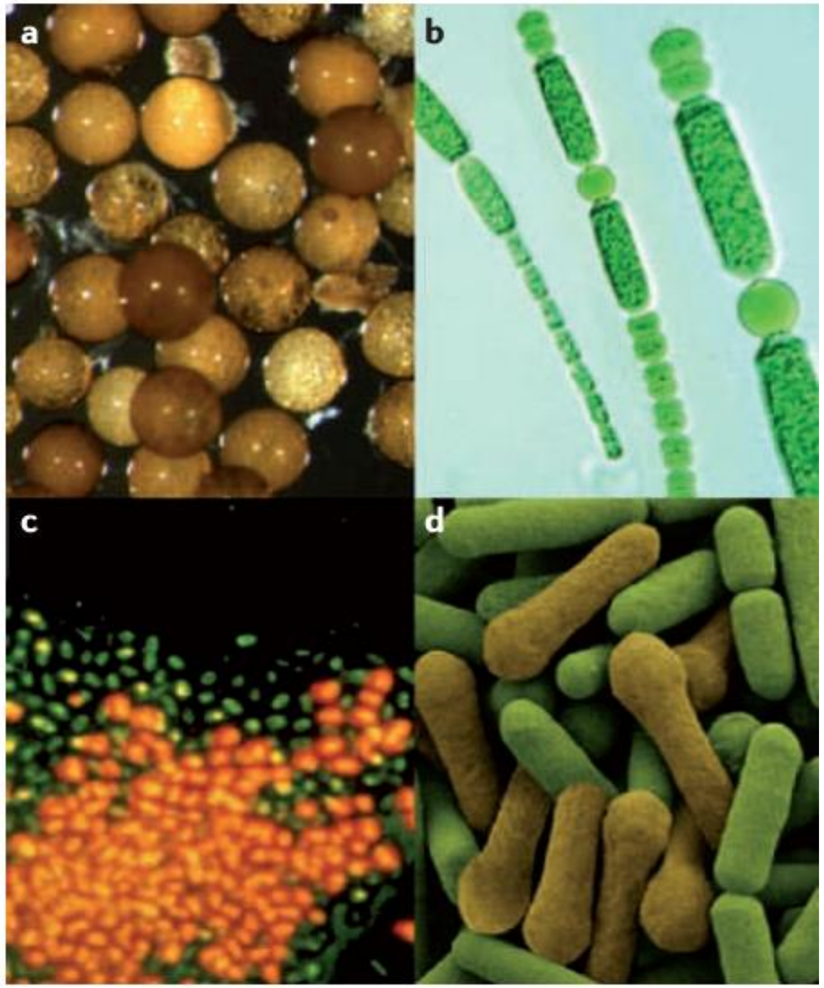
Jerusalem, Israel

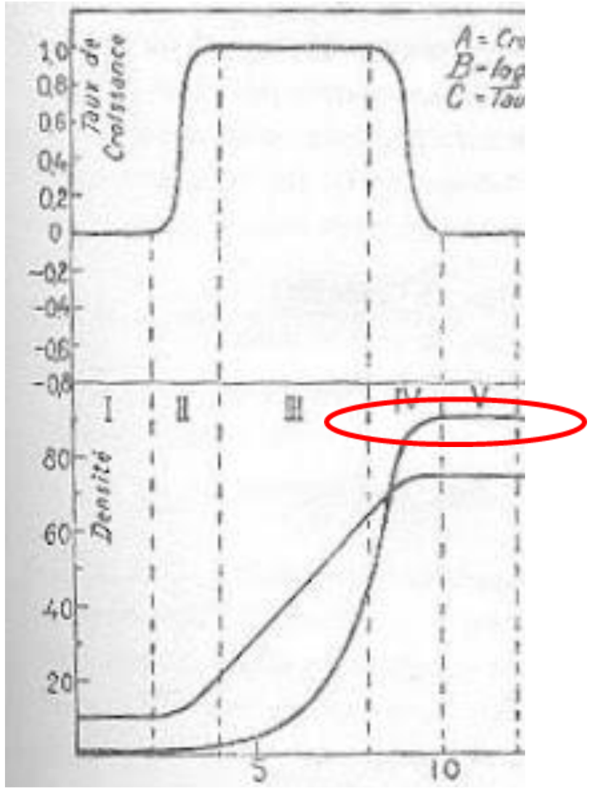
Growth arrest is the prevalent state of microorganisms in nature



Examples of microbial dormancy.

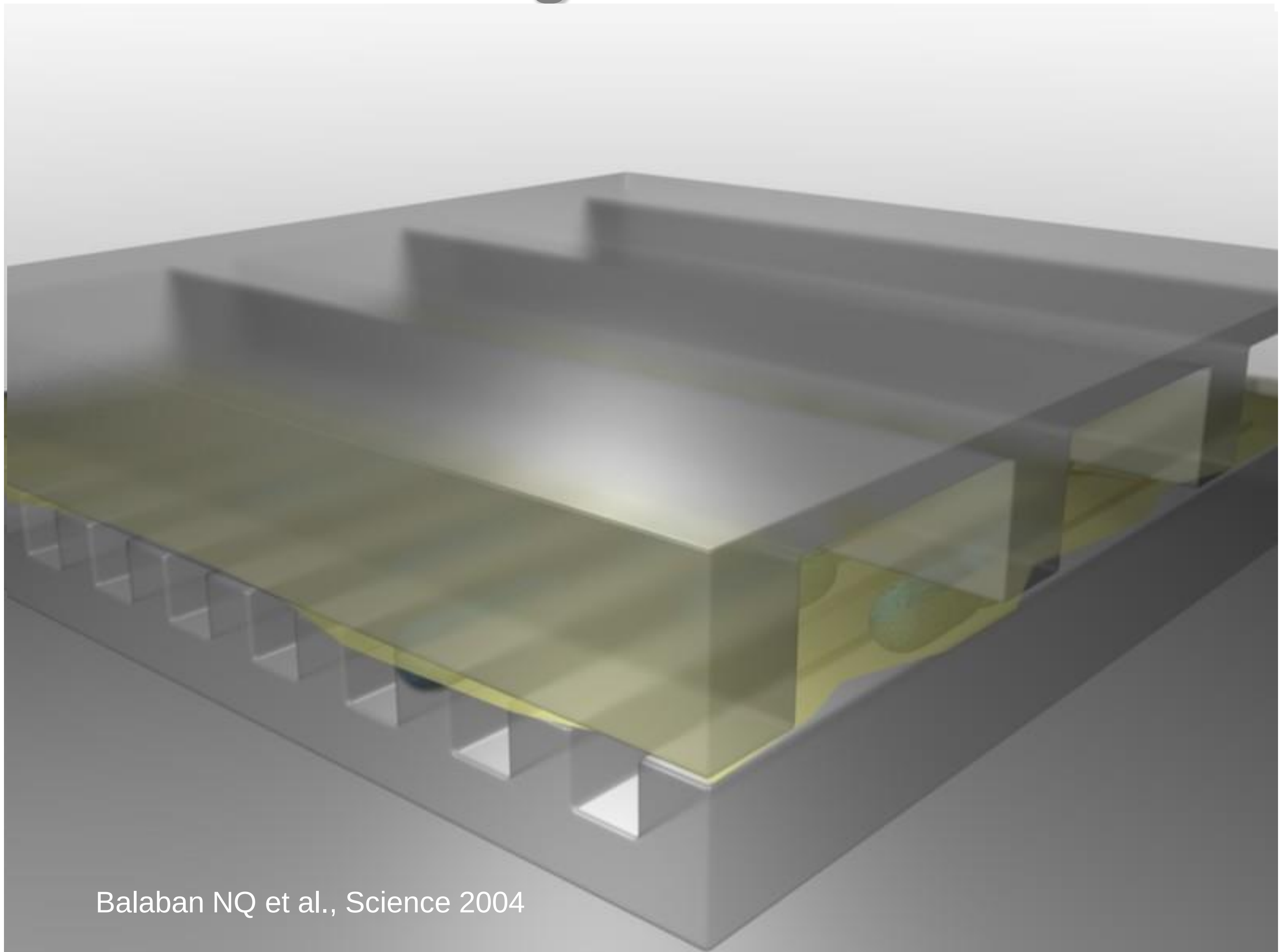
Lennon JT & Jone SE, **Nat Rev Micr** (2011)

Typical bacterial growth curve

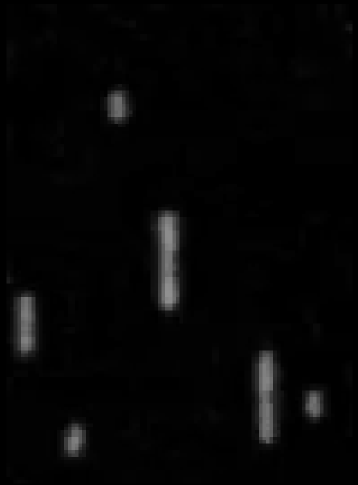


Jacques Monod, Thesis 1942

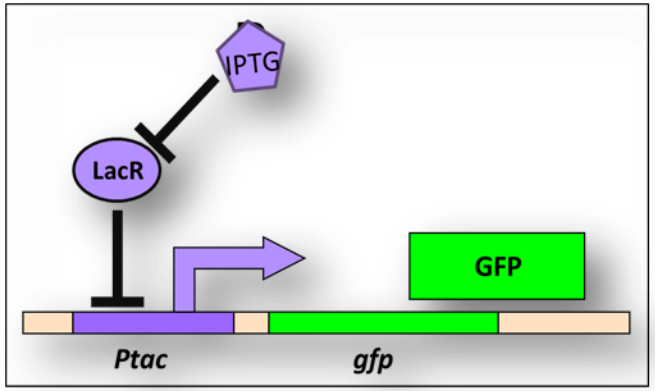
Microfluidic devices for following single-cells



Growth arrest in microfluidic devices

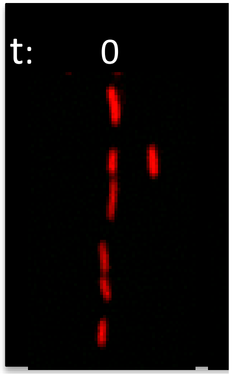


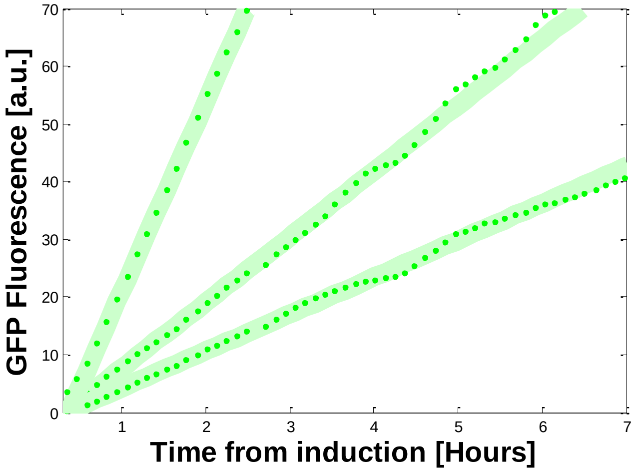
Measuring the activity of single growth arrested cells



Do stationary phase bacteria respond to induction?

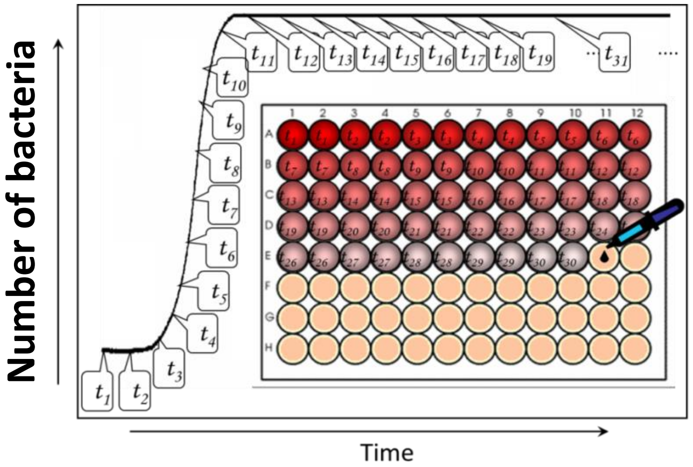
Fluorescence induction of growth arrested cells in microfluidic devices





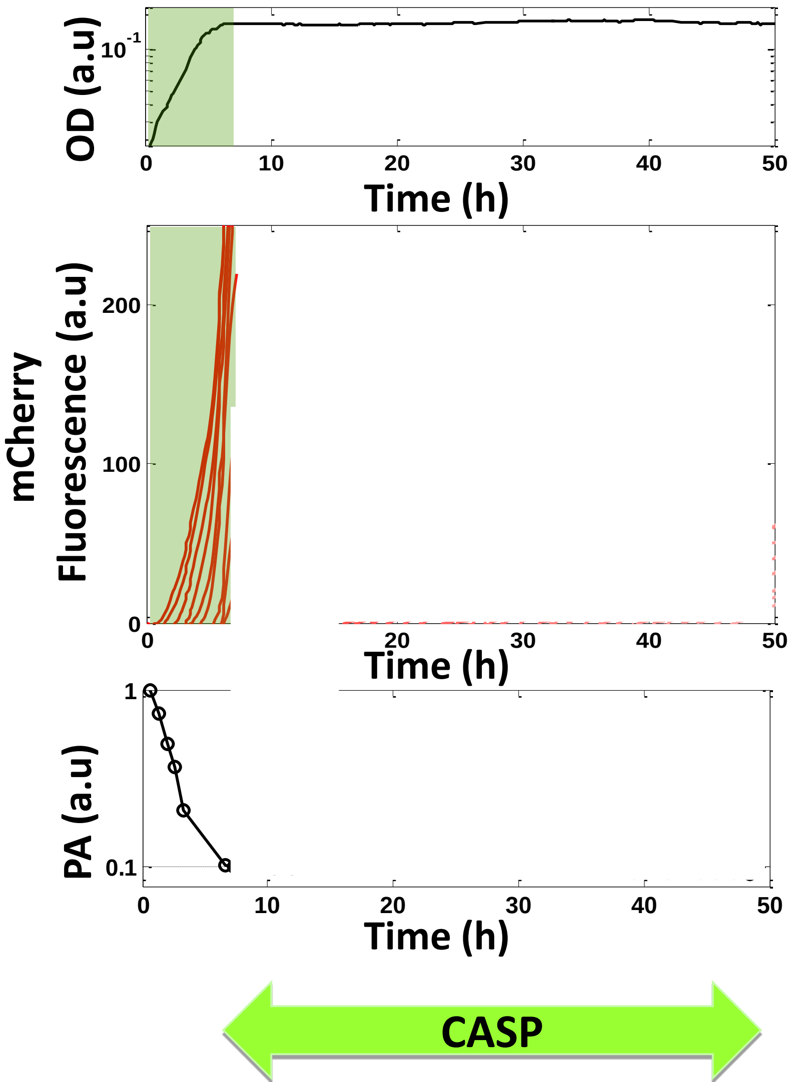
CASP : Constant Activity Stationary Phase

CASP: Constant Activity Stationary Phase in batch cultures

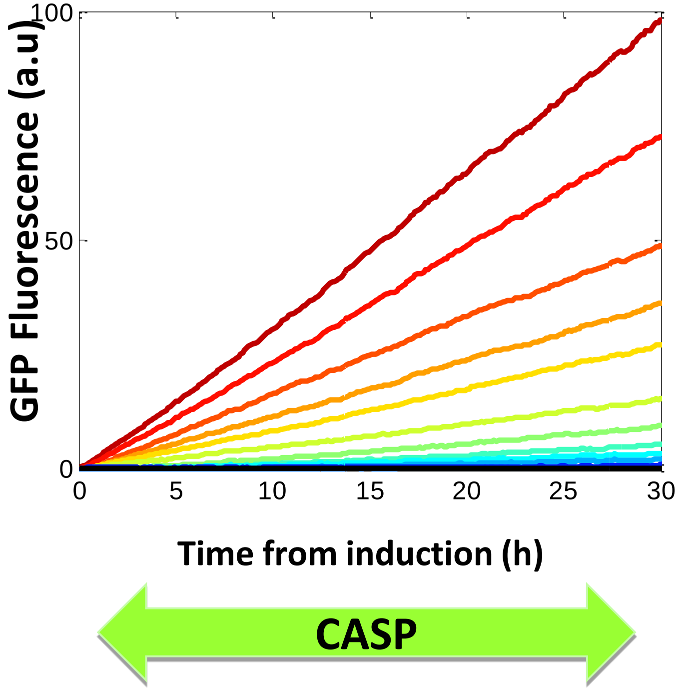


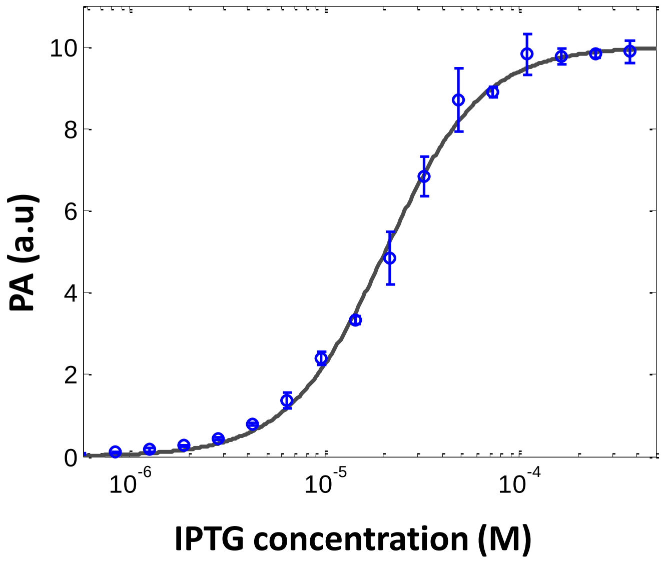
At CASP, cells produce fluorescence at a constant rate for several days, despite the absence of growth

Gefen O. *et al.*, PNAS *(2014)*

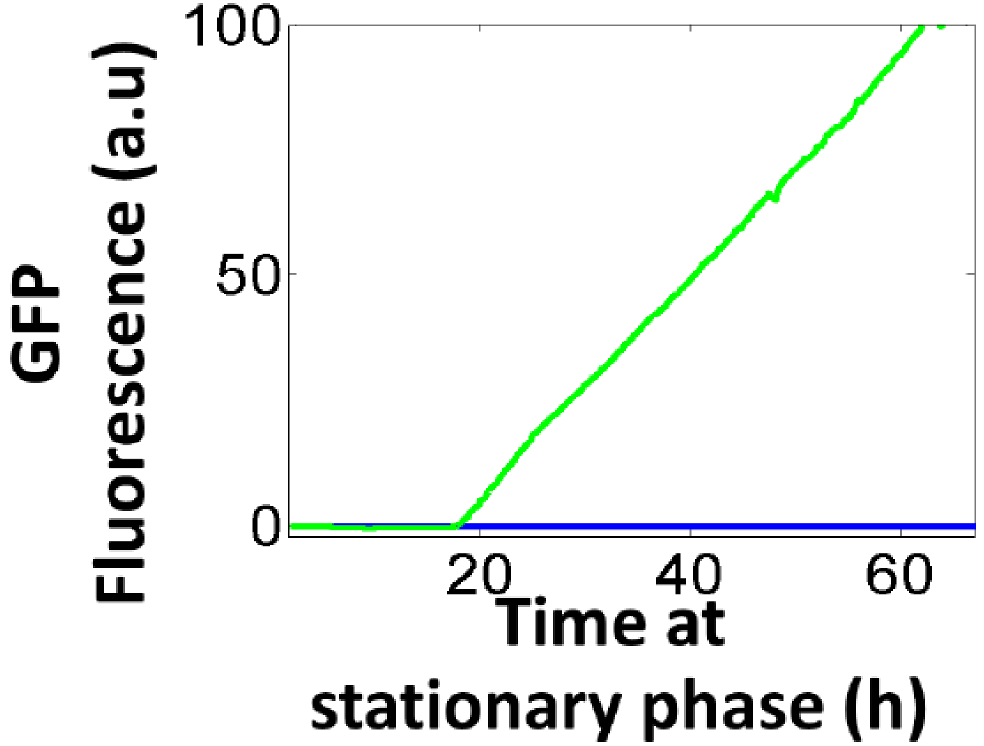


Precise promoter activity measurements at CASP

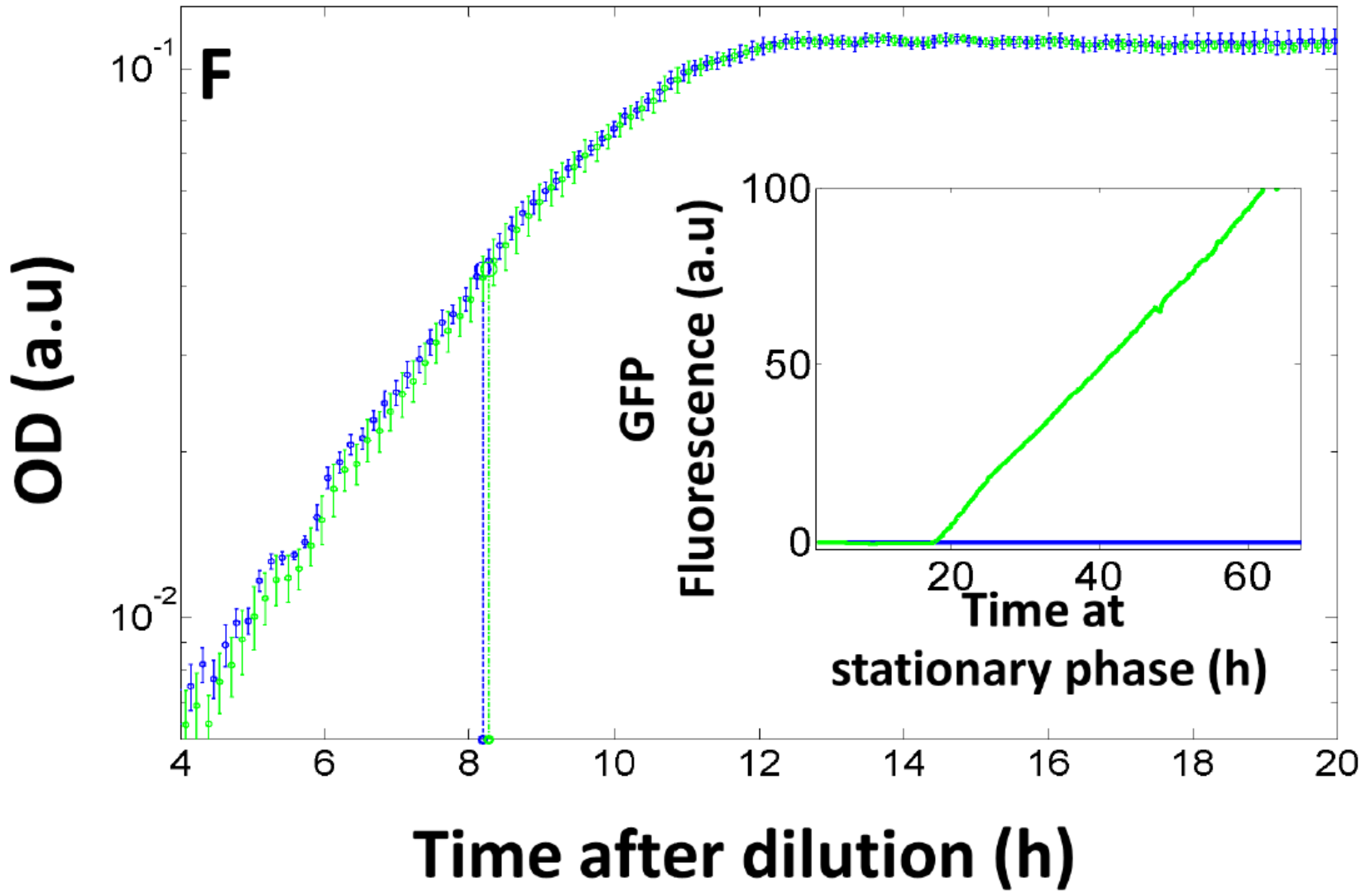




Fitness cost of protein production at CASP

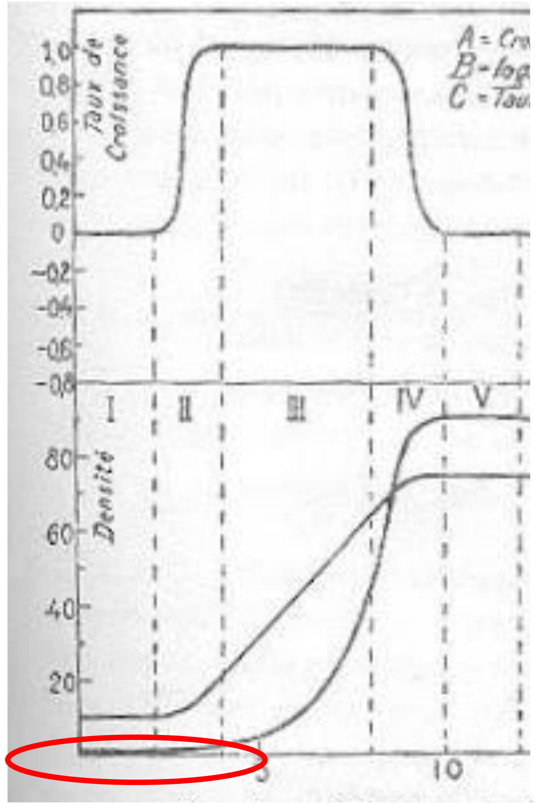


No detectable cost of protein production at CASP



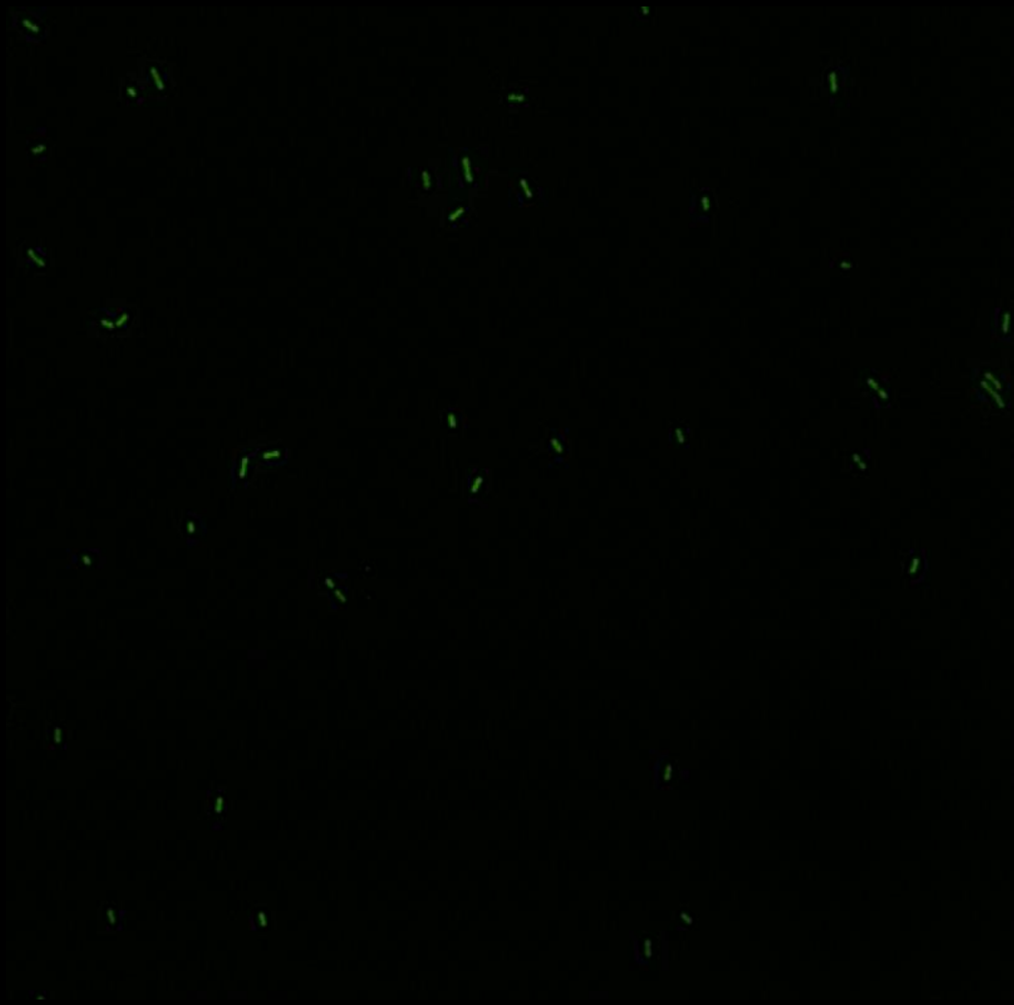

DriversEdGuru.com

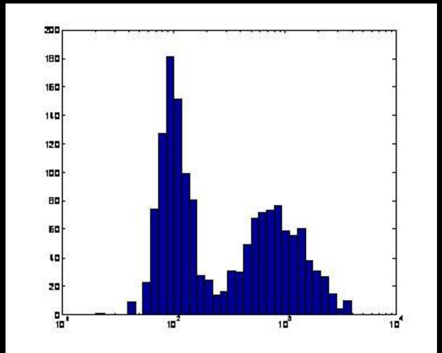
Typical bacterial growth curve



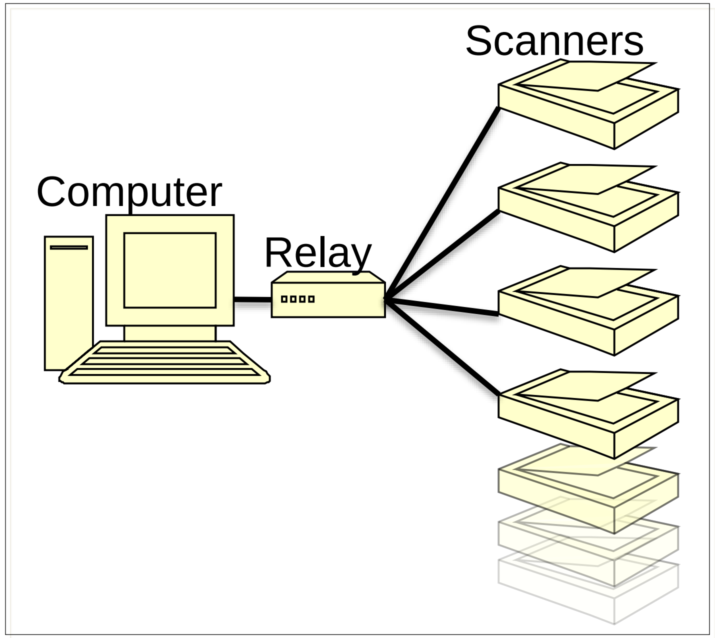
Jacques Monod, Thesis 1942

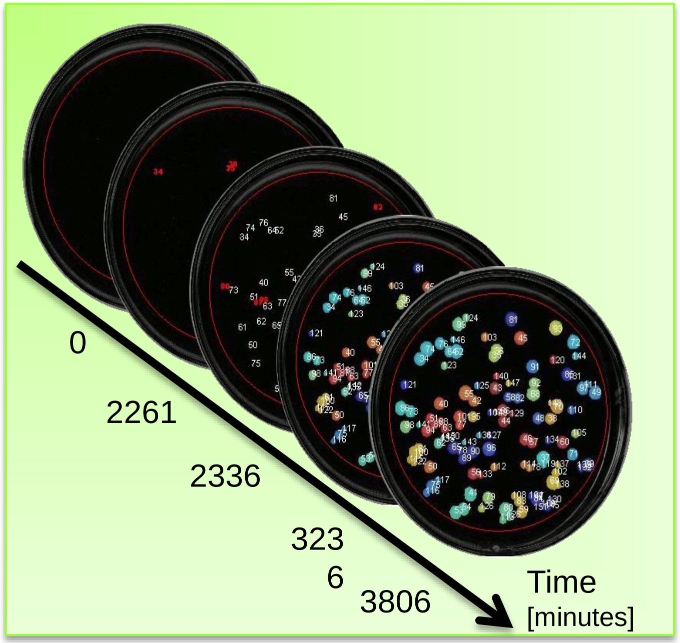
Growth arrest during the lag phase

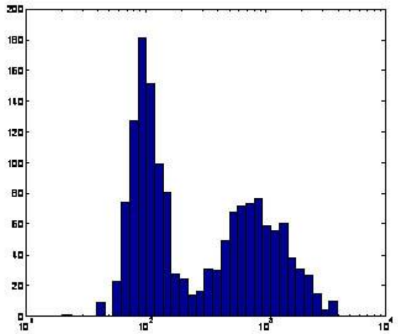




ScanLag: High throughput assay for lag variability quantification



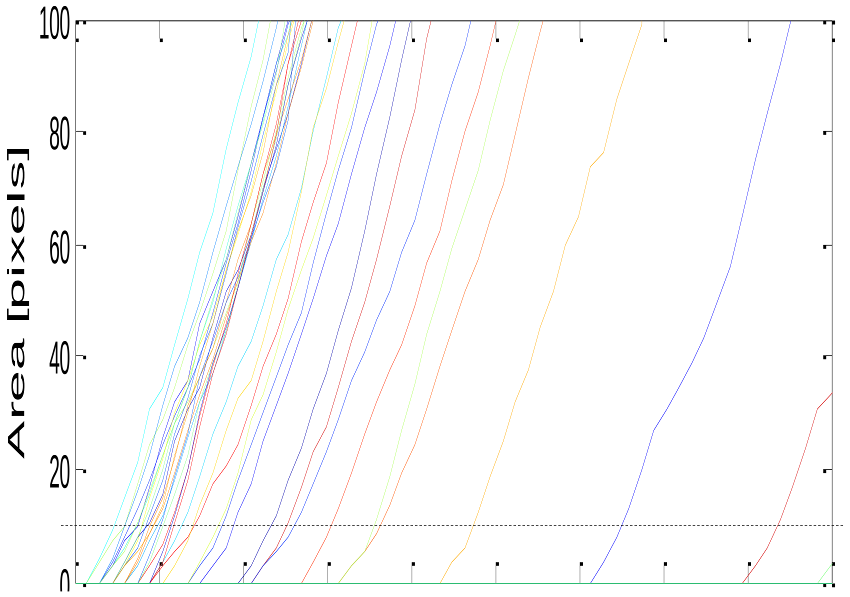


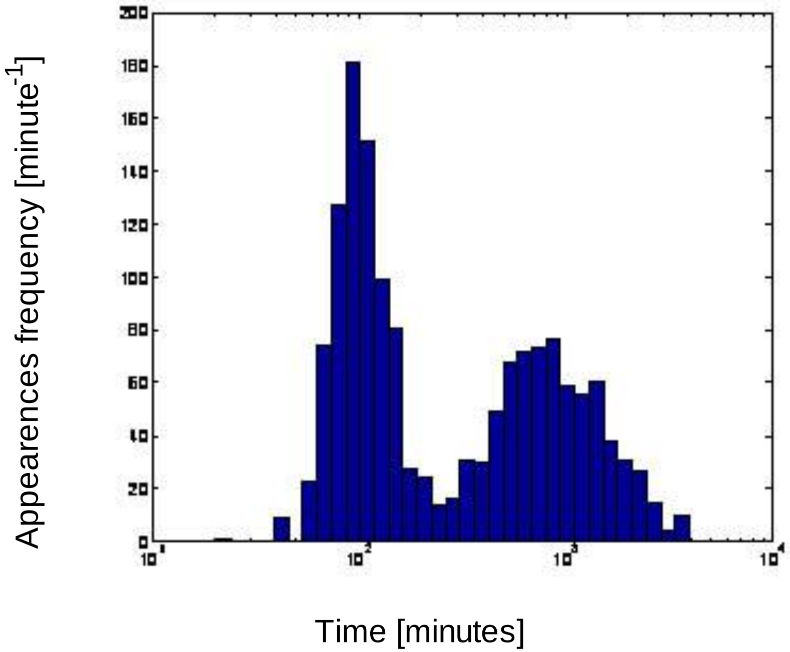


Irit Levin-Reisman *et al.* Nature Methods (2010)

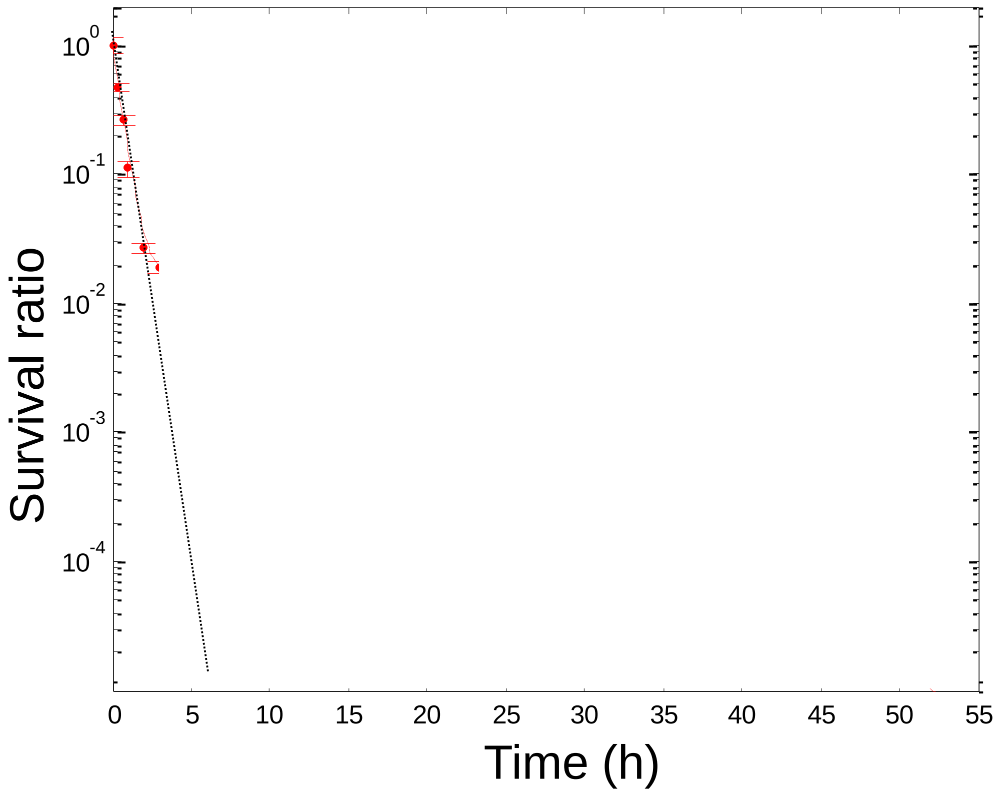
Irit Levin-Reisman *et al.* Jove (*in press*)

Appearance distribution can reflect growth arrest distribution





Persistence to antibiotics

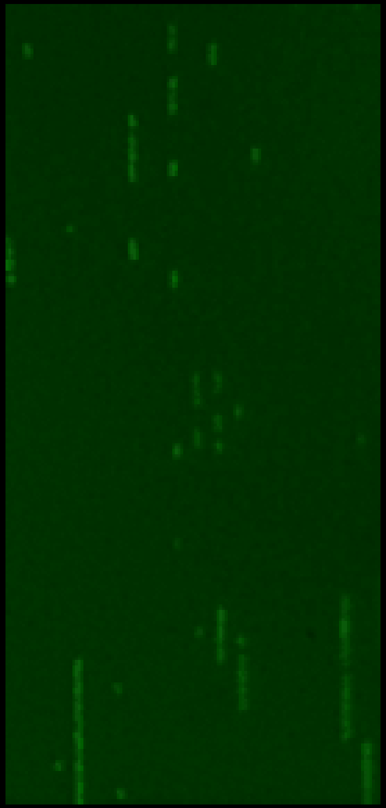


Bigger, The Lancet 1944

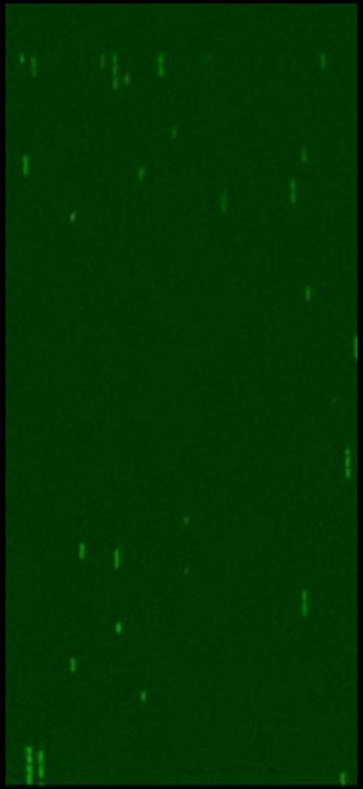
$$N(t) = N_0(\alpha e^{-\mu_1 t} + (1-\alpha) e^{-\mu_2 t})$$

Balaban Curr Opi Gen & Dev 2011

Direct observation of the single cell response to atibiotics

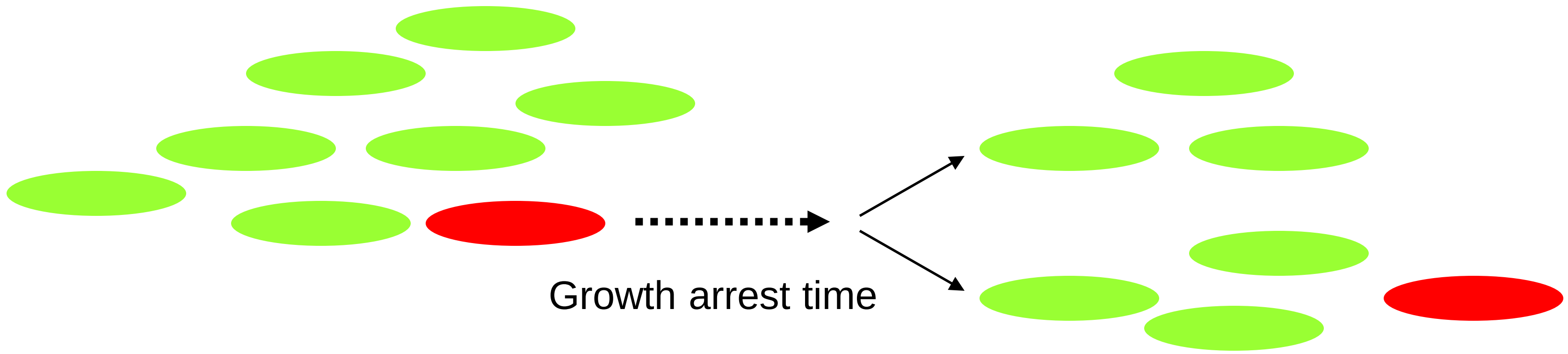


Persistence is not due to a mutation



High persistence is due to a sub-population of growth arrested bacteria

Extended lag variability as a bet-hedging strategy



Main Questions

- Molecular level: What controls the duration of the lag?

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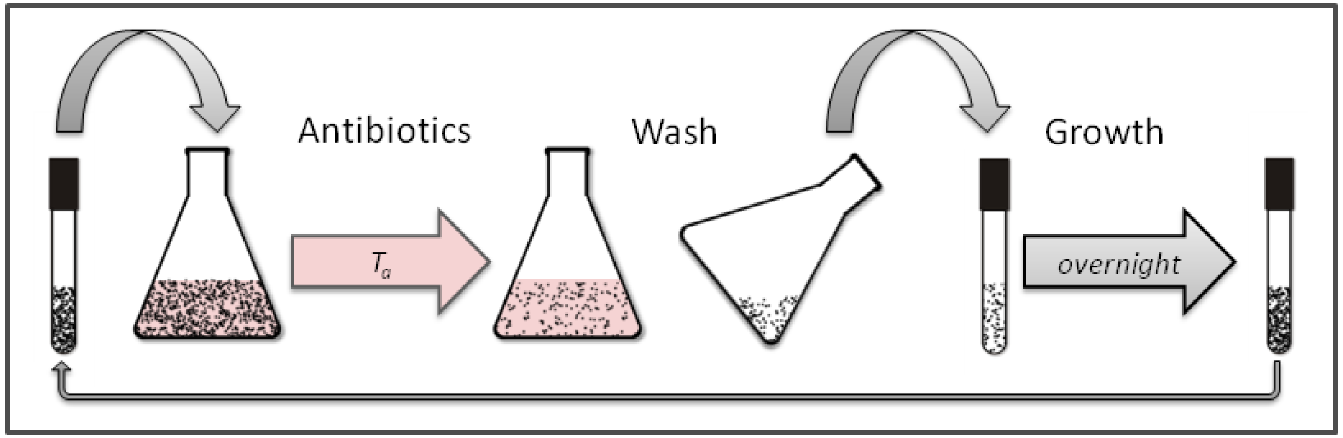
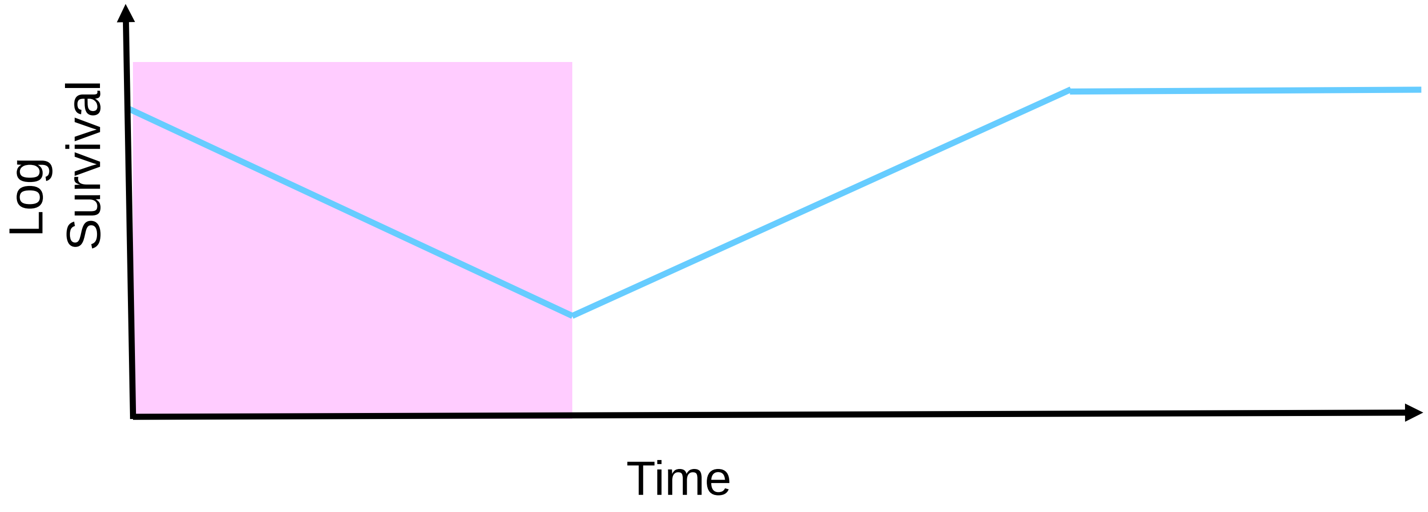
- Molecular level: What controls the duration of the lag?
- Cell level: Is growth arrest at lag similar to stationary phase arrest?
- Population level: How can growth arrested and growing cells co-exist?
- Evolutionary level: Is lag extension a strategy selected by evolution or the by-product of defective cell-cycle?

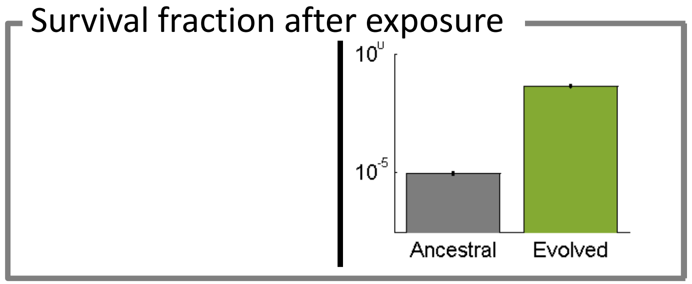
Protocol of evolution under cyclic antibiotic exposure



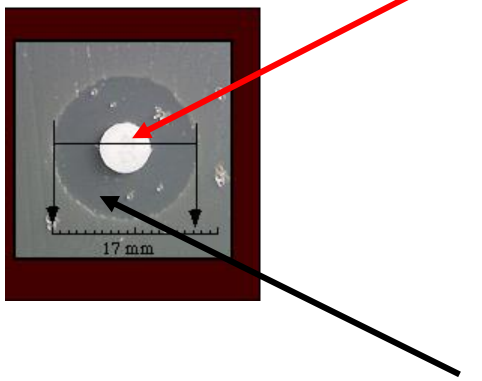


Ofer Fridman

The evolved populations survive extensive antibiotic treatment

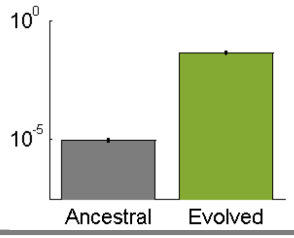


Failure of antibiotic treatments is typically attributed to resistance



Evolution of tolerance

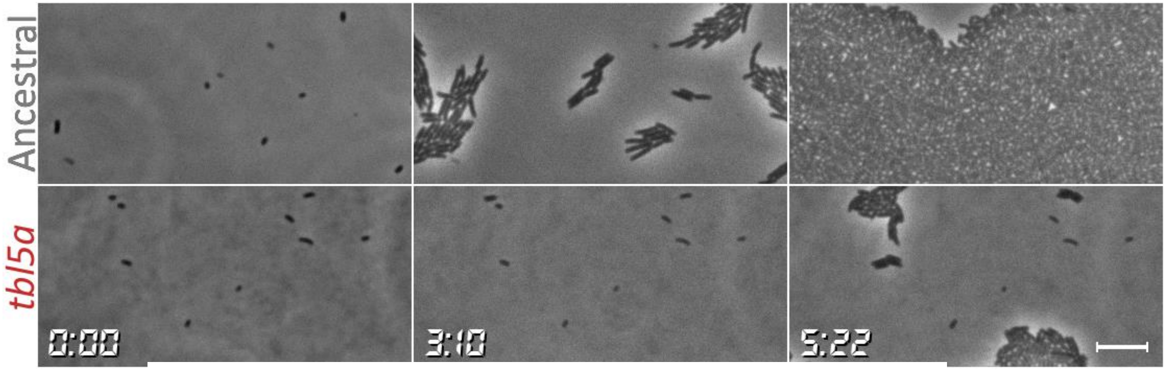
Survival fraction after exposure

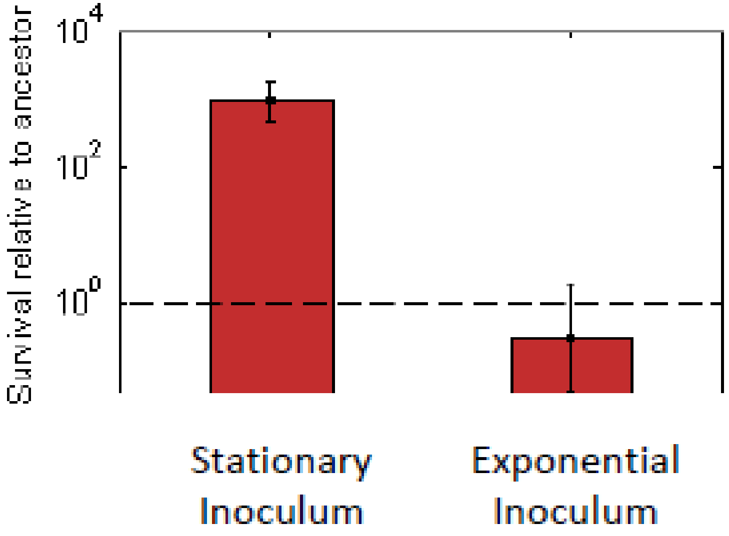


No difference in sensitivity to the antibiotic

No difference in growth rate

Tolerance is due to an extension of the lag time





Evolution of tolerance

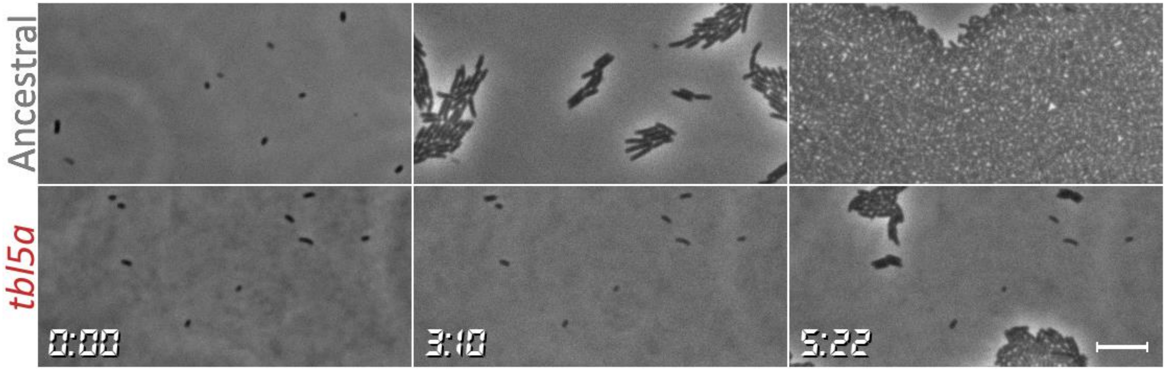
T_a=duration of intermittent antibiotic treatment

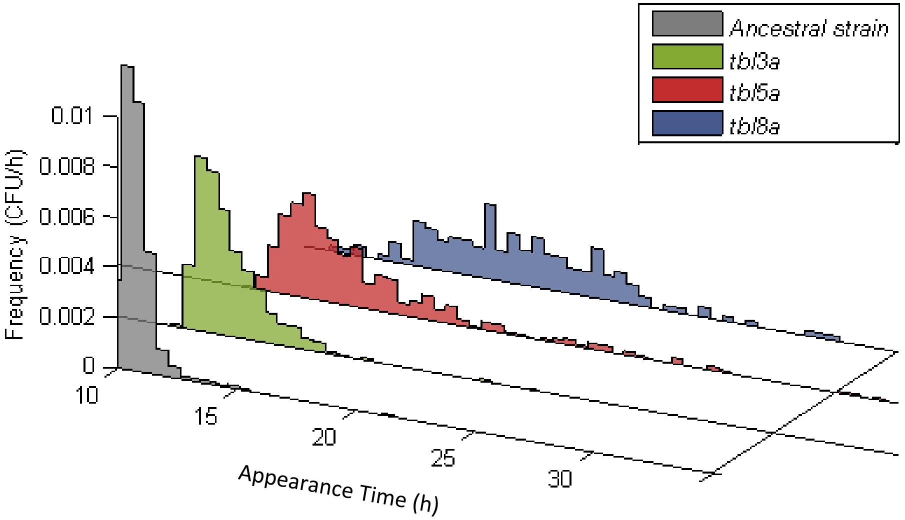
T_a=3h T_a=5h T_a=8h

No difference in sensitivity to the antibiotic

No difference in growth rate

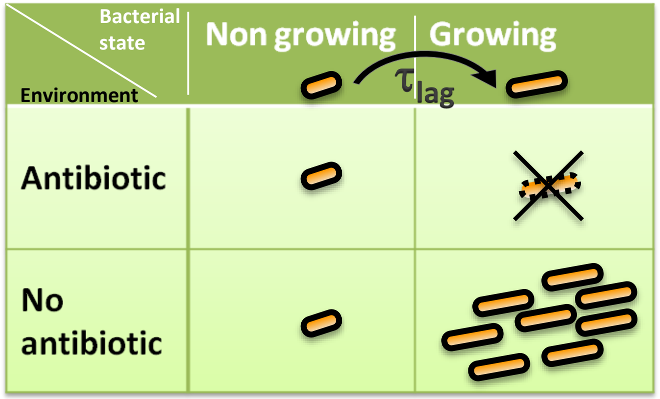
Tolerance is due to an extension of the lag time





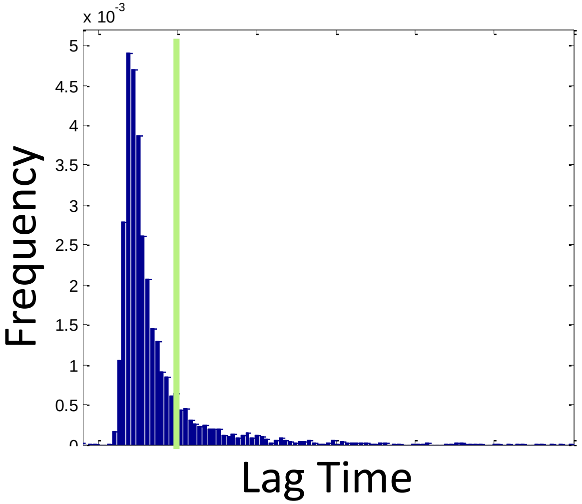
Fridman O., et al. Nature 2014

The evolved lag time as an optimization process

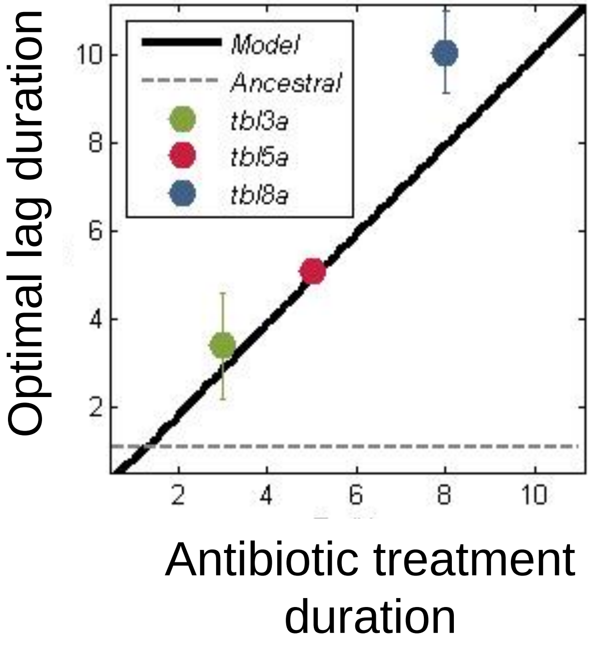


$$\dot{L} = -\frac{L}{\tau_{lag}}$$

$$\dot{G} = \alpha \frac{G}{\tau_{grow}} + \frac{L}{\tau_{lag}}$$

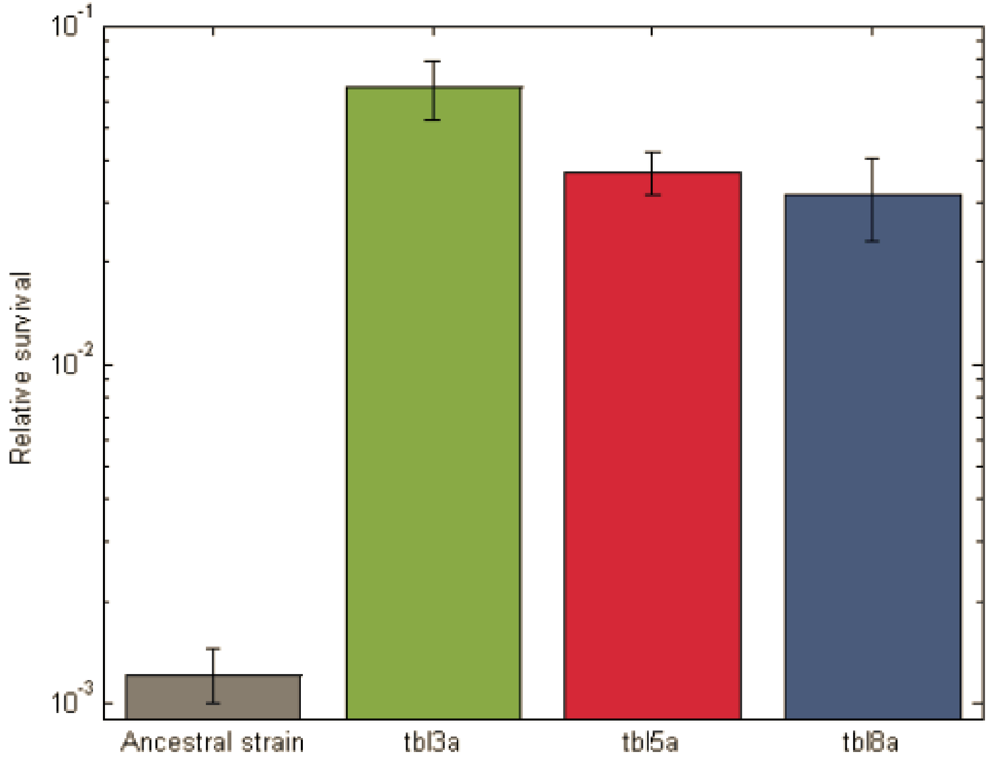


The evolved lag time is optimized to the duration of the antibiotic exposure



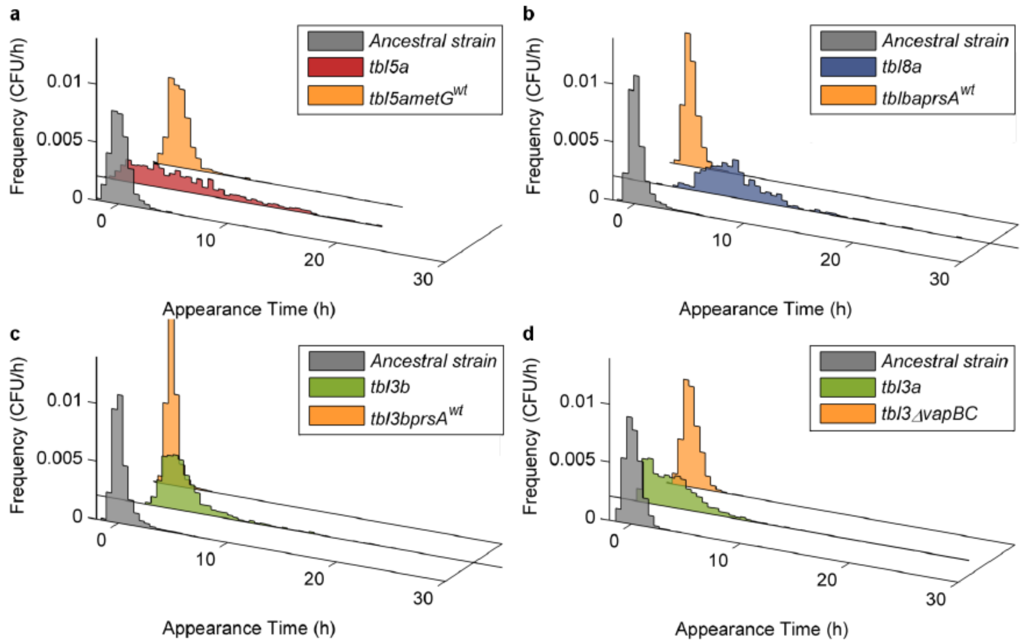
The tolerance-by-lag strains have adapted to the duration of the treatment, not to its precise chemical nature

Strains evolved under cell-wall targeting antibiotic are also tolerant to DNA gyrase inhibitors

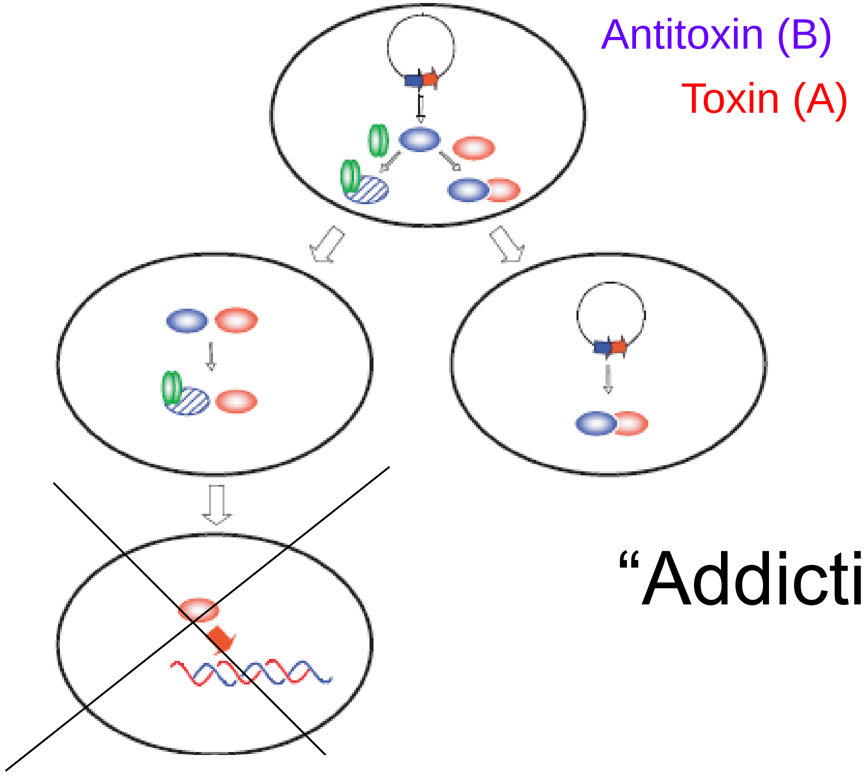


Tolerance-by-lag genes (*tbl*)

- Toxin-antitoxin module (vapBC-like)
- Methionyl-tRNA synthetase (metG)
- Ribose-phosphate diphosphokinase (prsA)



Toxin-Antitoxin Modules on Plasmids



“Addiction” module

Hayes, F. Science 301, p.1492 (2003)

Toxin-Antitoxin Chromosomal Modules (Aizenman E et al. PNAS 1996)

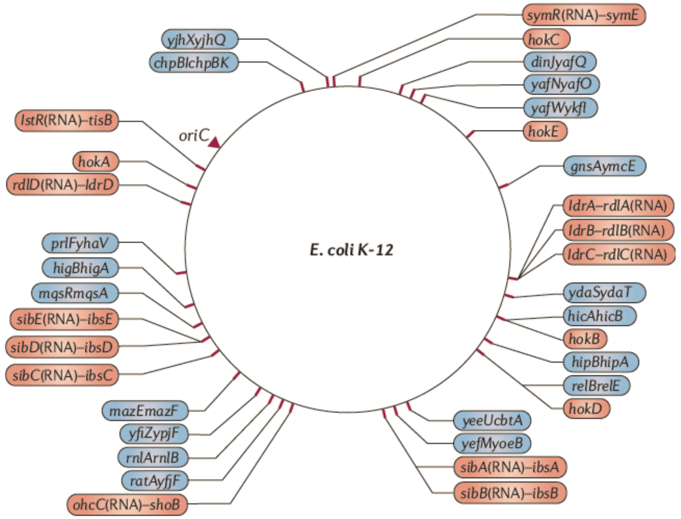
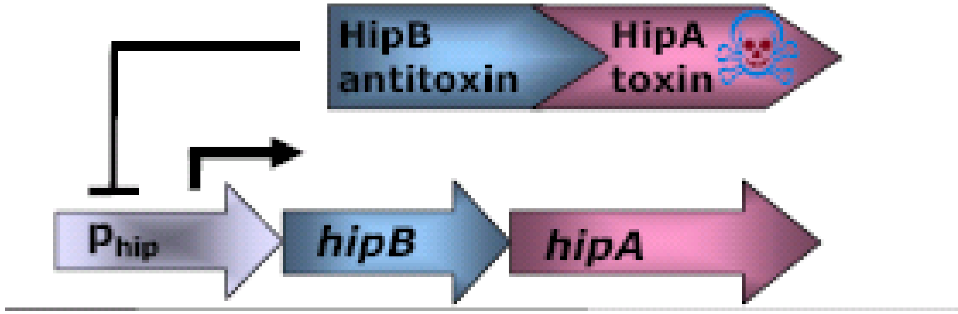


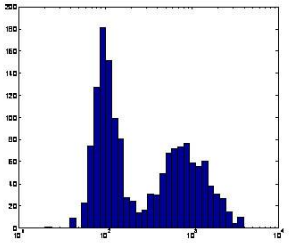
Figure 2 | **Genomic map of the toxin–antitoxin modules in *Escherichia coli*.**

Yoshihiro Yamaguchi and Masayori Inouye

Nature Reviews Microbiology, 2011

Toxin-antitoxin modules control of lag time duration

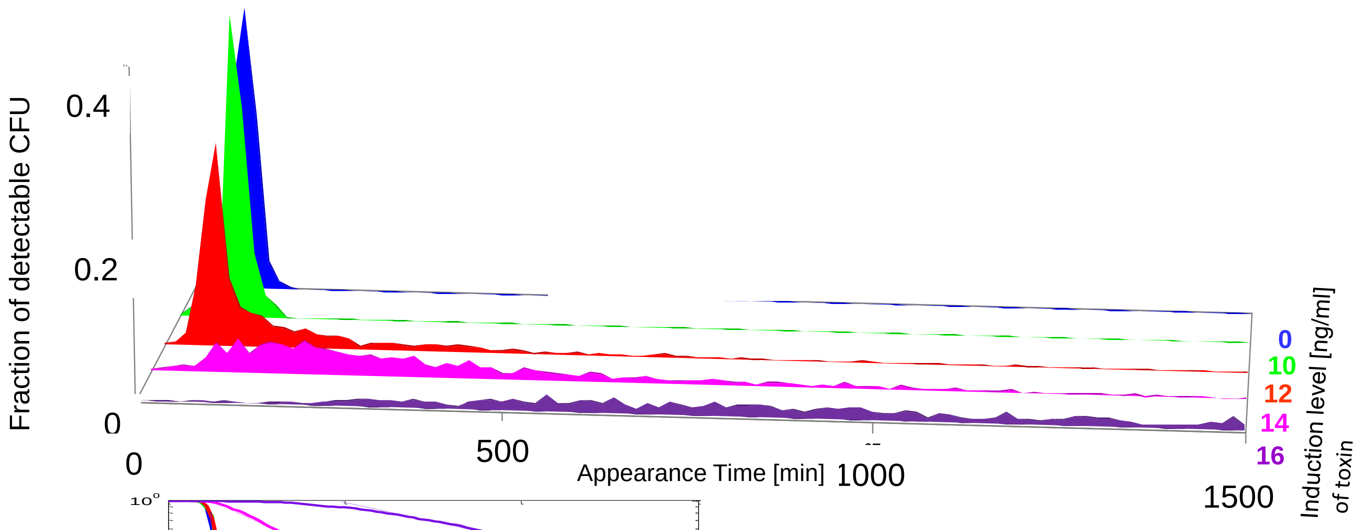


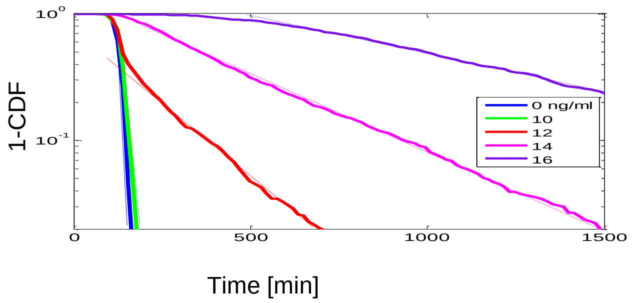


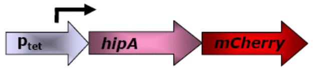
•**Mutations in *hipA* lead to high persistence**
(Harris Moyed *J Bac*, 1983, 1988, 1991,1994;
Korch *et al Mol Mic* 2003)

The *hip* (*hi*gh *p*ersistence toxin-antitoxin module)

The level of induction of the toxin determines the growth arrest duration

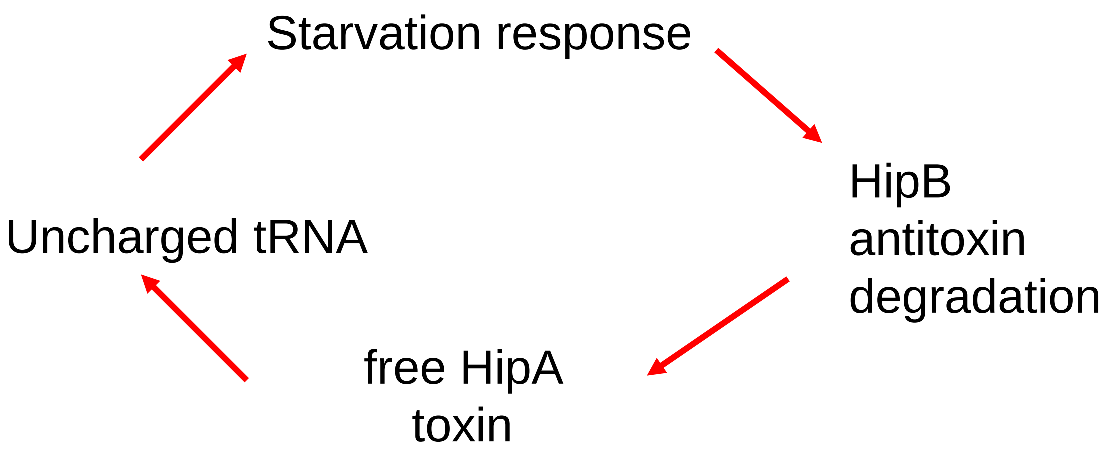






Eitan Rotem

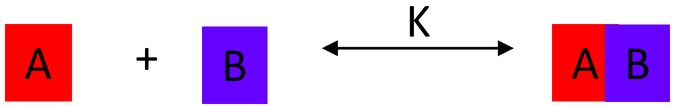
HipA toxin induces a long lag by phosphorylating GltX, the glutamyl tRNA synthetase



Kaspy I., *et al,* (*Nature Communication, 2013*)
Collaboration with Gad Glaser

The HipA toxin extends the lag by mimicking starvation

Threshold amplification of noise



A=toxin B=antitoxin

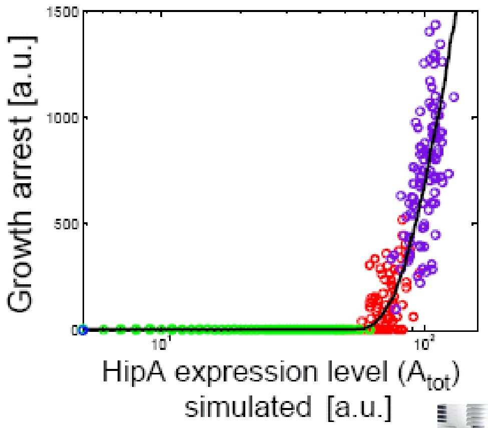
$$\frac{dA}{dt} = \alpha - k_+ A \cdot B + k\_AB - \mu A$$

$$\frac{dB}{dt} = \beta - k_+ A \cdot B + k\_AB - \mu^* B$$

$$\frac{dAB}{dt} = k_+ A \cdot B - k\_AB - \mu AB$$

(Lenz DL et al., 2004; Levine E. et al., 2007; Buchler NE, 2008)

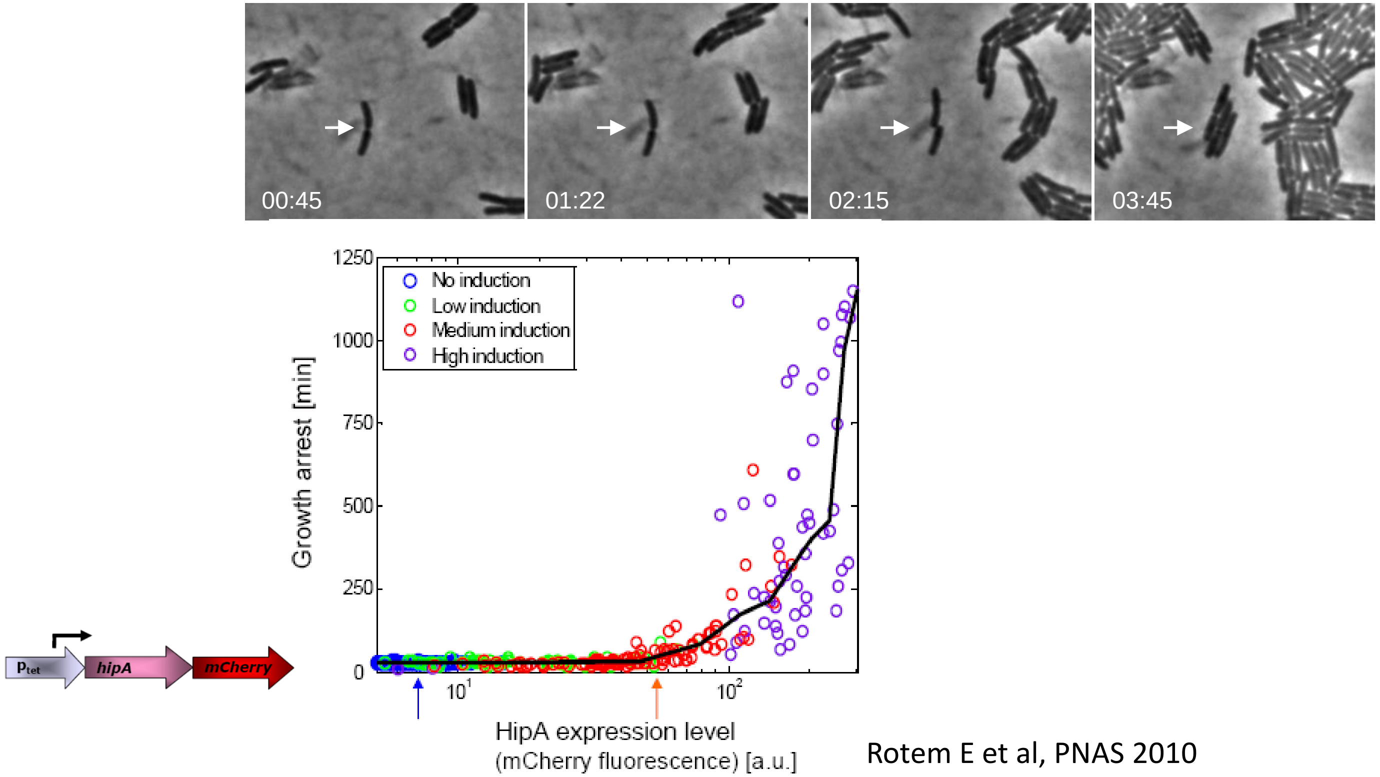
Stochastic simulations of growth arrest



Adiel Loinger

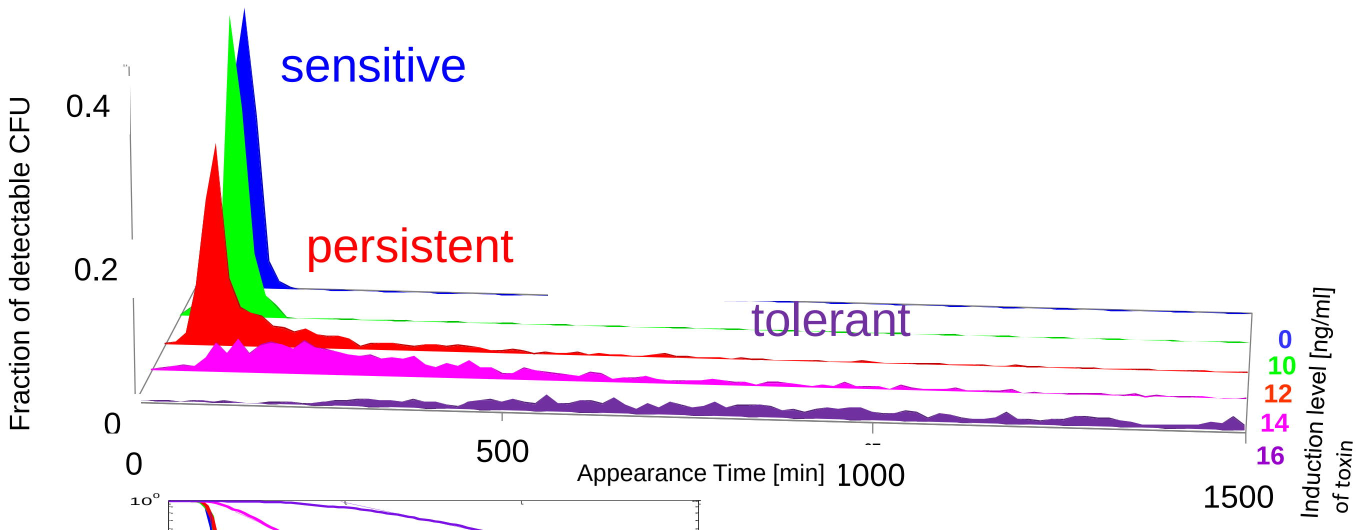
In collaboration with O. Biham

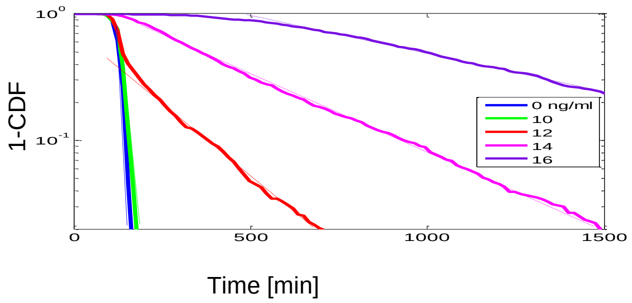
From molecular noise to phenotypic variability of growth at the single-cell level

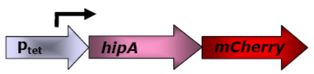


Rotem E et al, PNAS 2010

The level of induction of the toxin determines the growth arrest duration

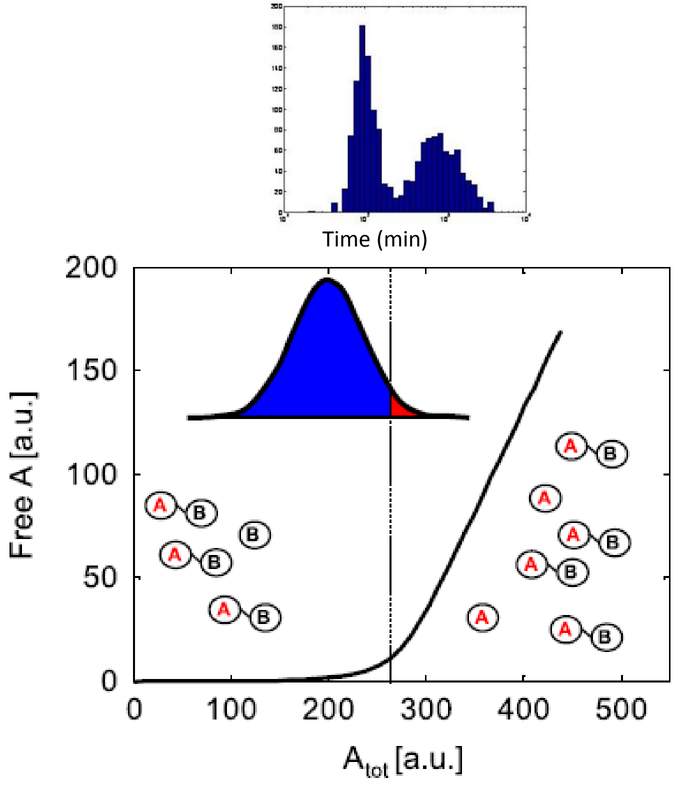




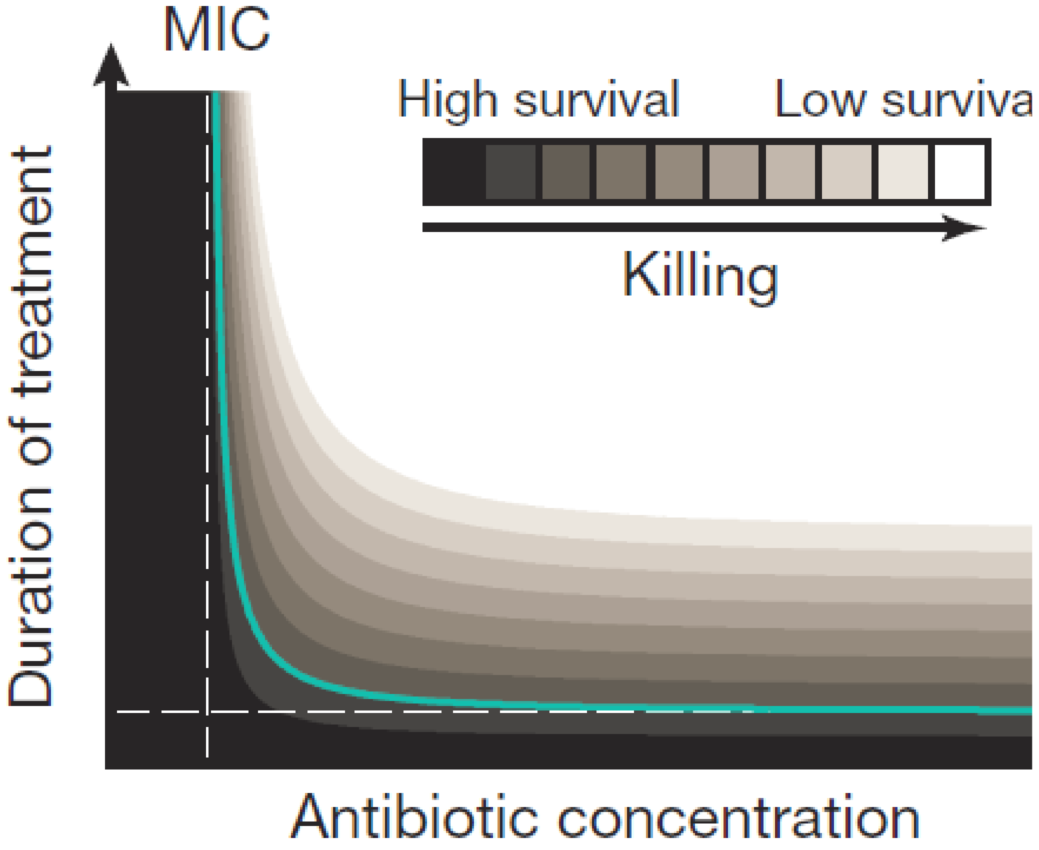


Eitan Rotem

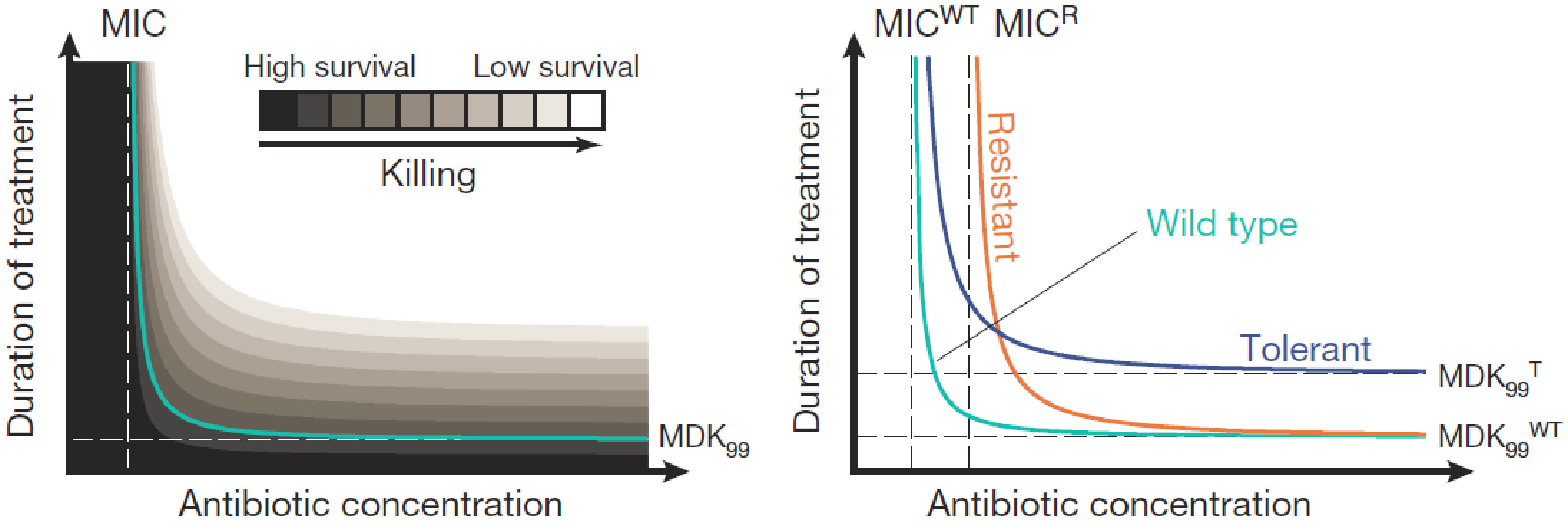
Difference between large numbers leads to amplification of noise



MIC and MDK: a global framework to define resistance, tolerance and persistence

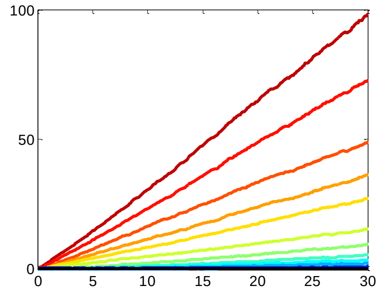


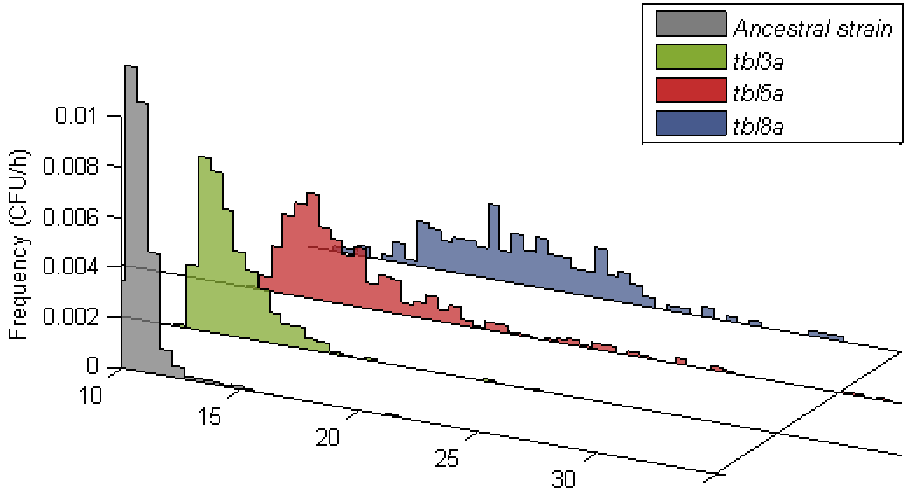
MIC and MDK


MIC
High survival
Low survival
Killing
Duration of treatment
MDK99
Antibiotic concentration
MICWT MICR
Resistant
Wild type
Tolerant
MDK99T
MDK99WT
Duration of treatment
Antibiotic concentration

Summary

- **CASP**: **C**onstant **A**ctivity **S**tationary **P**hase enables quantitative measurements on non growing cells
- Toxin-antitoxin modules are ideally suited to control tolerance and persistence by mimicking starvation
- Growth arrest can evolve to match the time scale of the stress
  - Understand *tbl* mutations
  - Search for similar effects in patients treated with cyclic exposure to antibiotics





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Orit Gefen
Eitan Rotem
Sivan Pearl
Ira Ronin
Ofer Fridman
Irit Levin-Reisman
Eliq Oster
Noga Weiss
Amir Goldberg

Broad Institute

Noam Shoresh

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