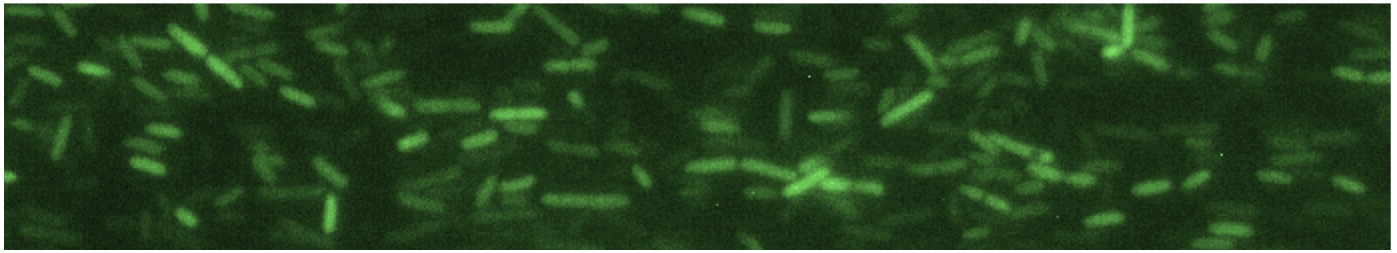
University of Pittsburgh

Individuality in Bacterial Growth Homeostasis



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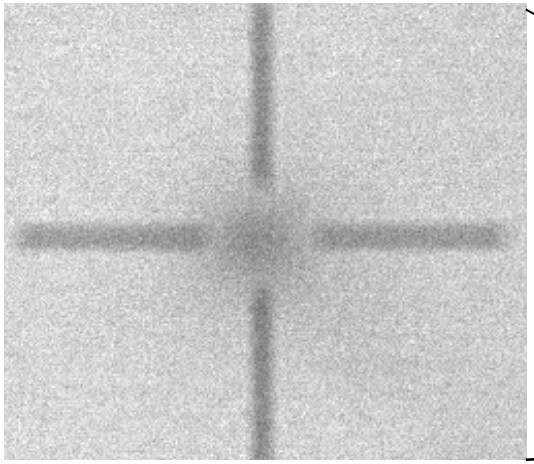
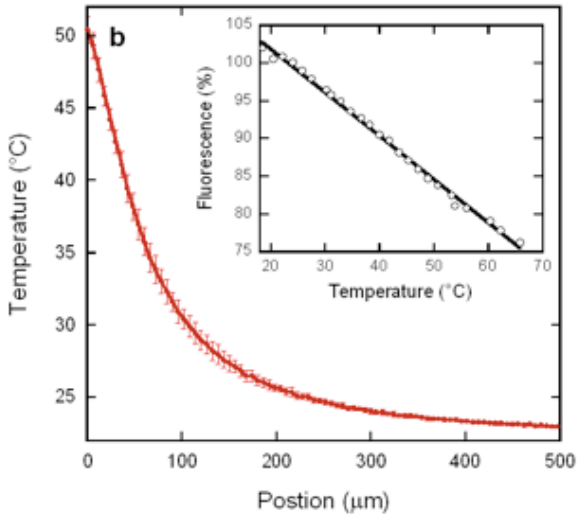
Workshop on Operation Research of Biological Systems 2018

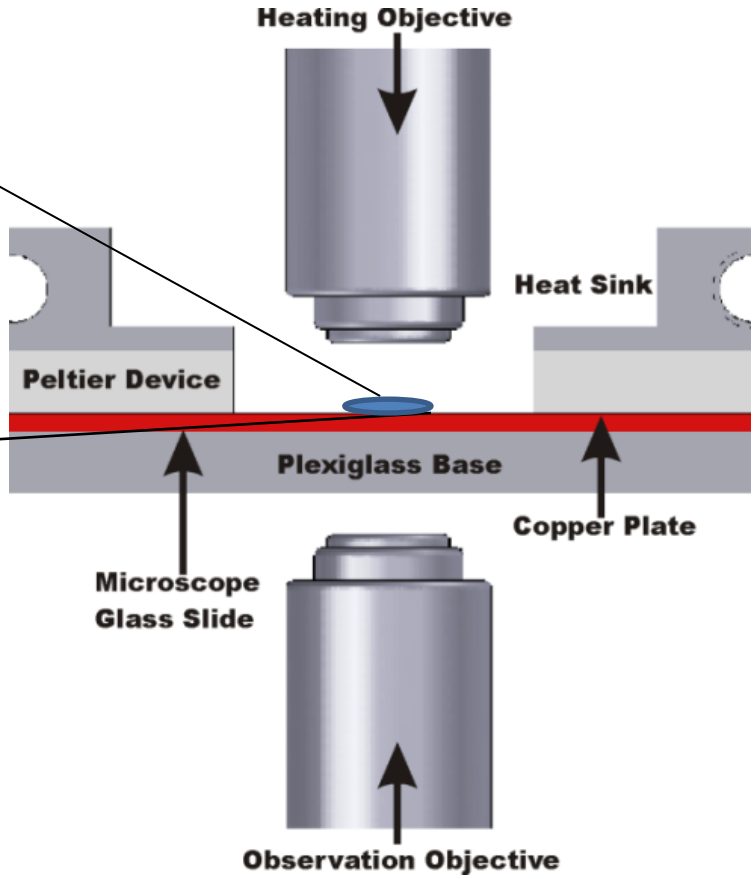
An Example of Behavior Individuality: Thermotaxis

Temperature sensitive dye

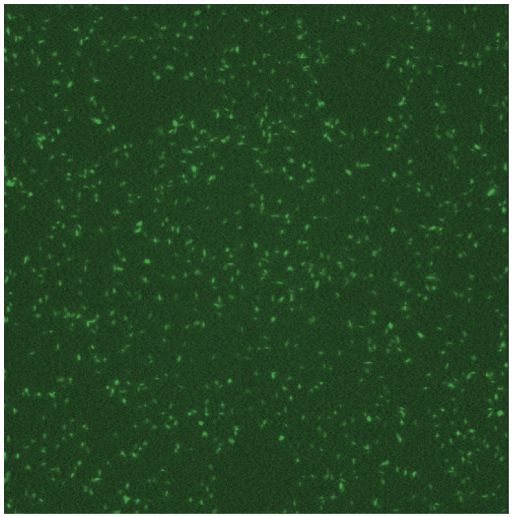


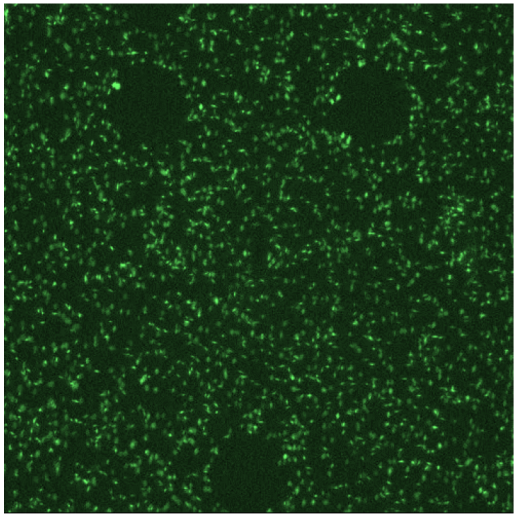


Experimental Setup

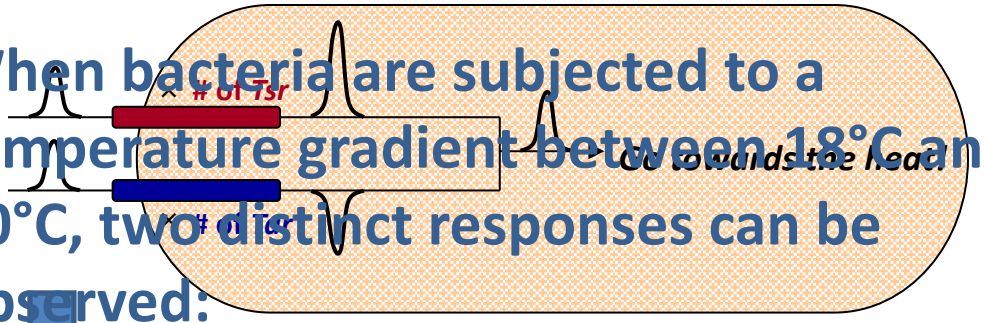


What causes this change in the response?





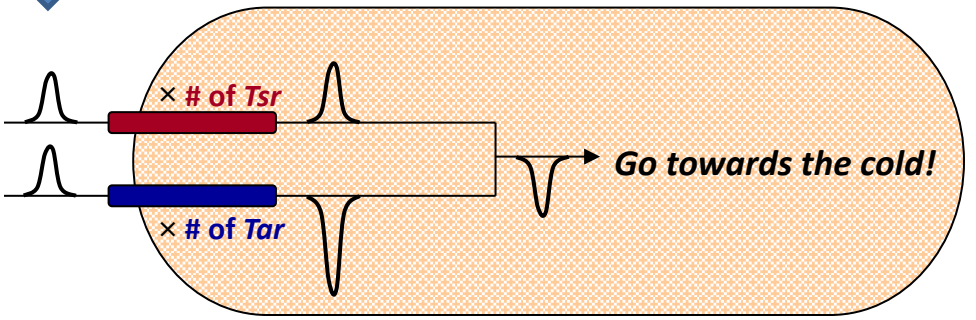
When bacteria are subjected to a temperature gradient between 18°C and 30°C, two distinct responses can be observed:



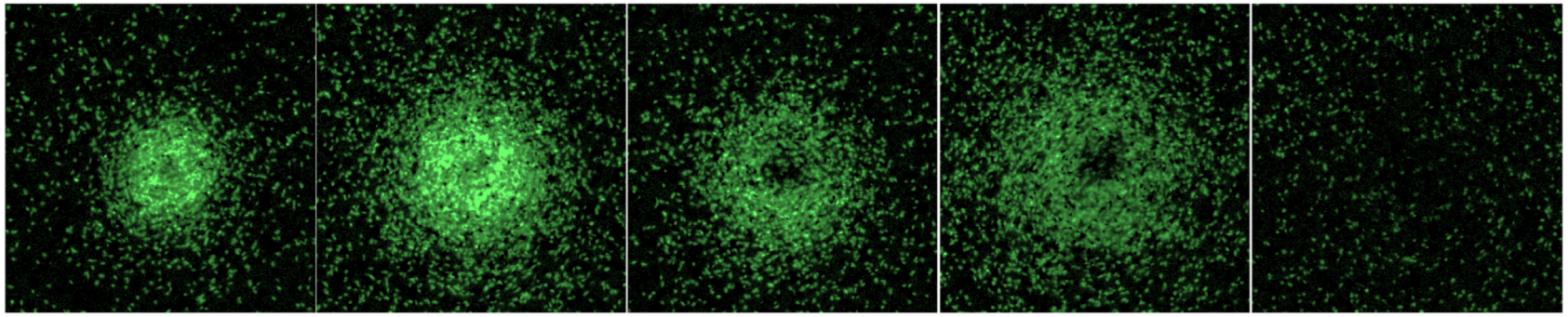
1) **Accumulation at high temperature**

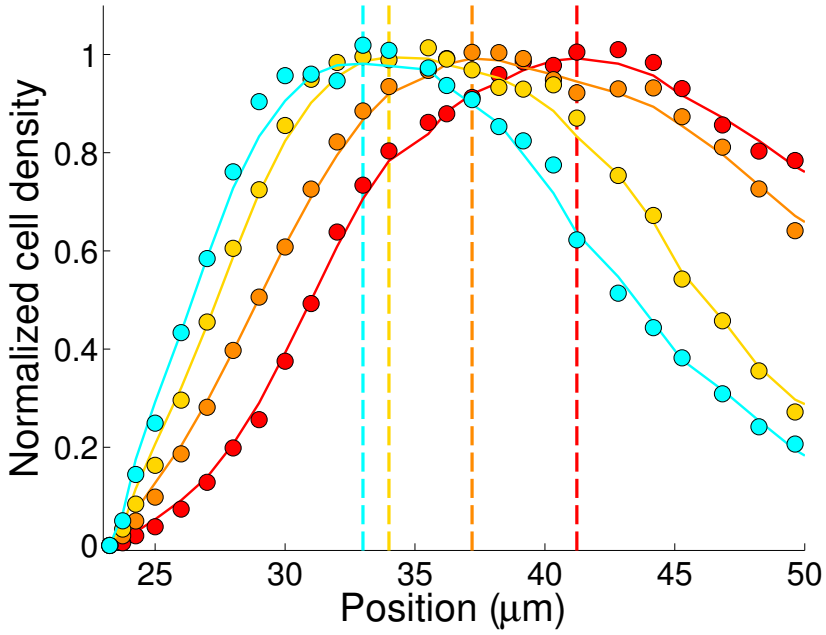
Increase in the number of the ***cryophilic*** receptors ***Tar*** relative to the number of ***thermophilic*** receptors ***Tsr*** causes a switch in the response of the bacteria to a fixed temperature gradient.

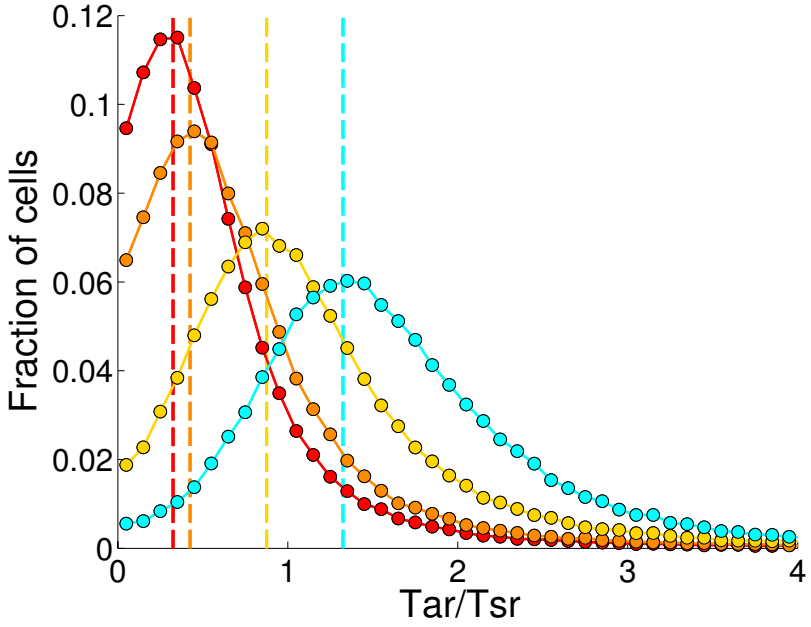
2) **Escape from high temperature**



Tar/Tsr Vs. favored temperature

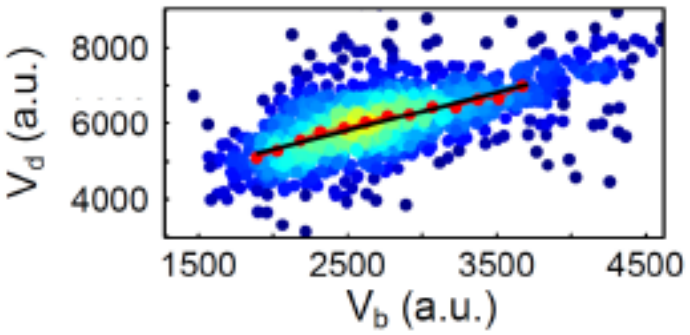




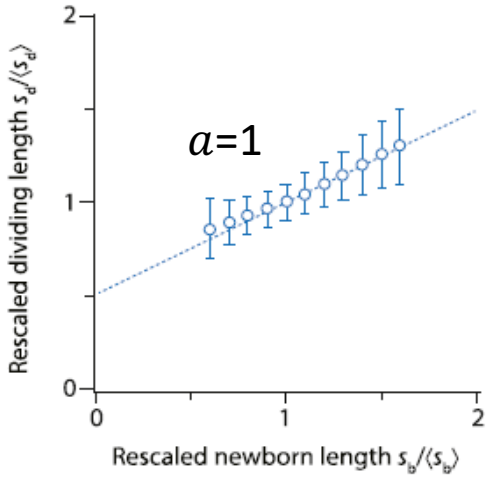


Size Homeostasis

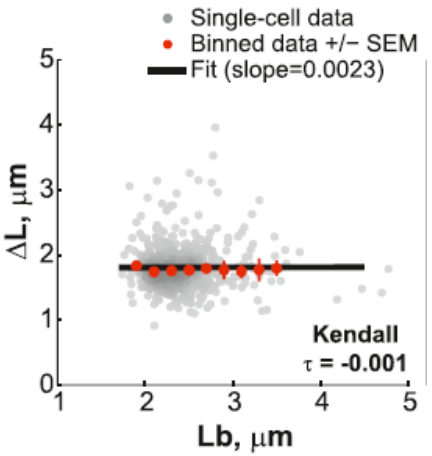
All living cells have to control their size and prevent divergence.



Soifer et al., Cur. Biol. 2016



Taheri et al., Cur. Biol. 2015



Campos et al., Cell 2014

$$V_d = V_b + \Delta$$

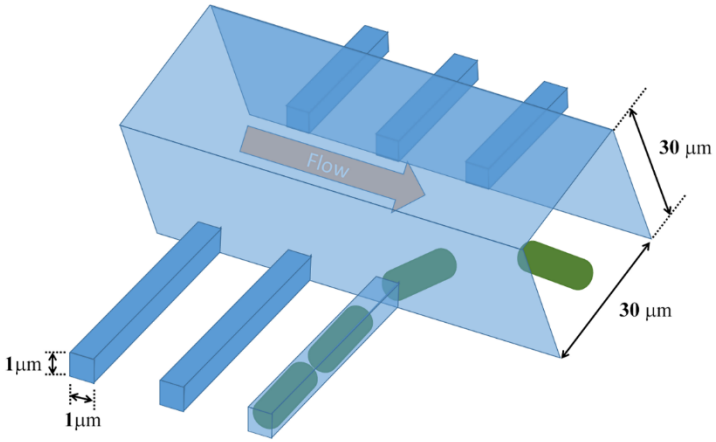
The distribution of the points is large!

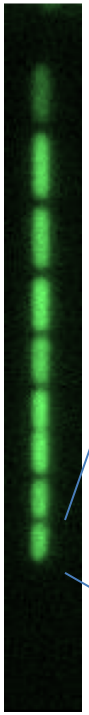
Is it noise?

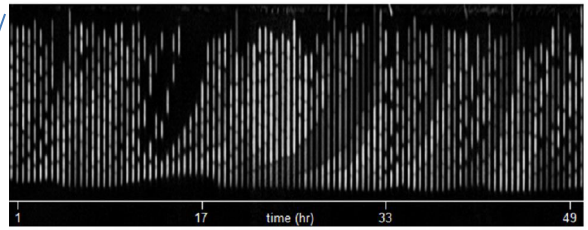
How robust is this mechanism at the single-cell level?

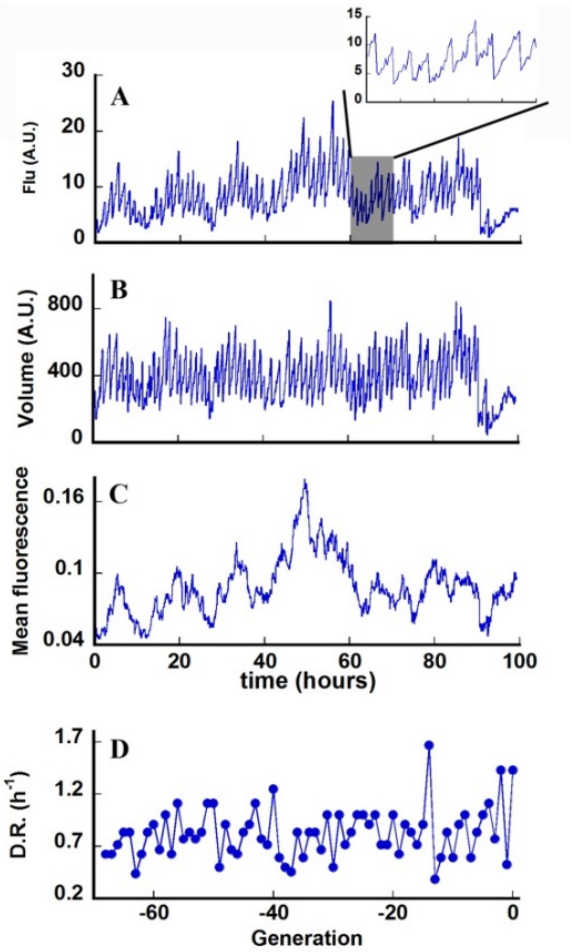
Single-cell Measurements

Micron-size traps continuously fed with nutrients by a flow through perpendicular channels



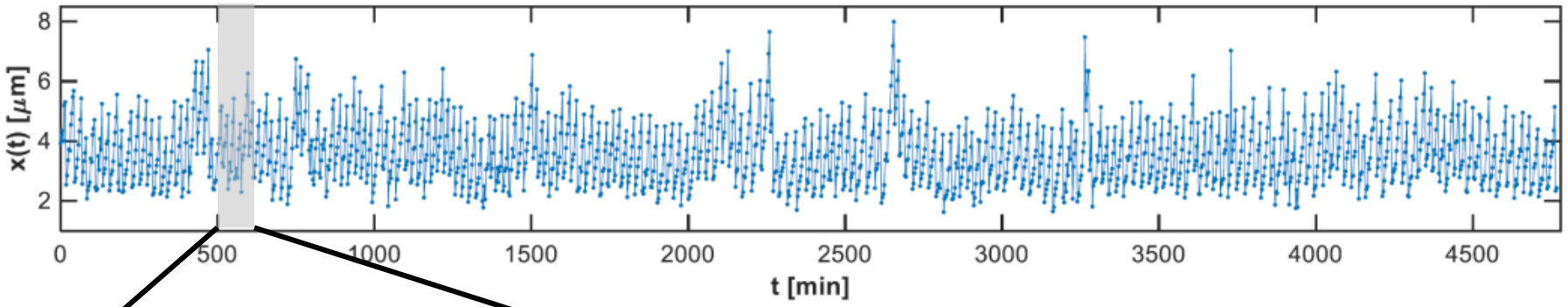


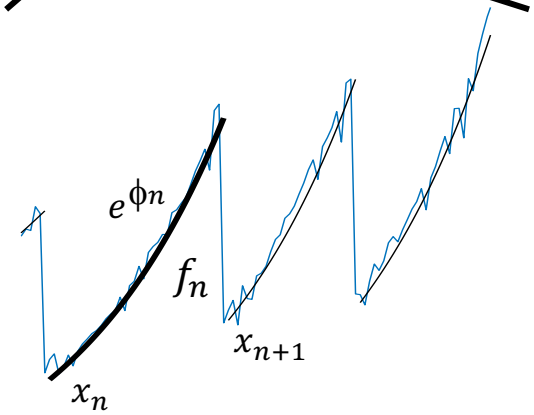




Dynamics of a single cell

Cell length of the mother cell as a function of time





Mapping

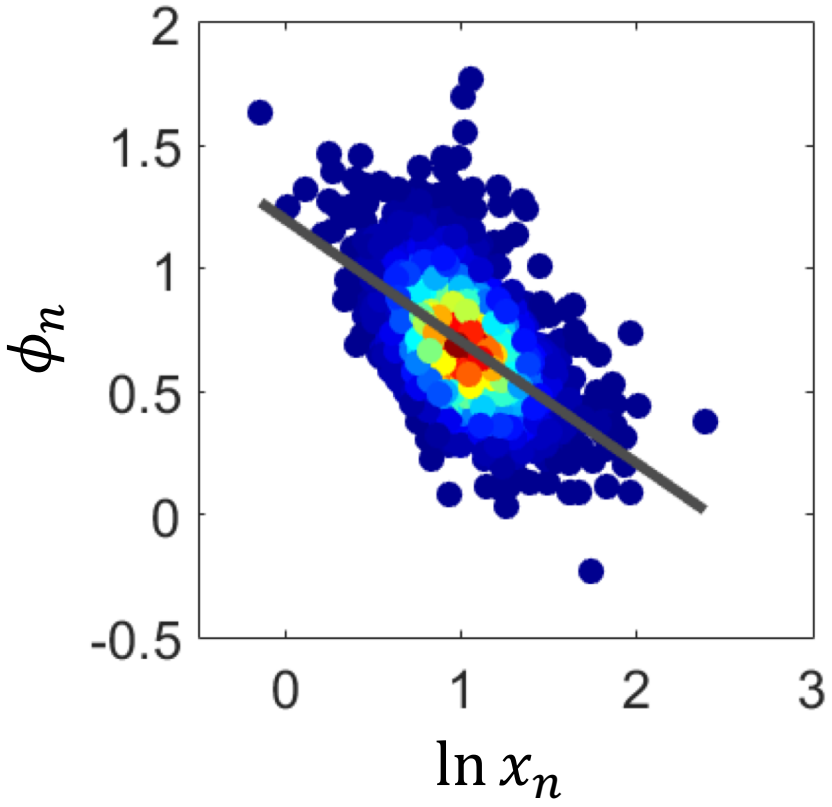
$$y_n = x_n e^{\phi_n}$$

$$x_{n+1} = f_n y_n = f_n x_n \exp(\phi_n)$$

If we assume for now that $f_n = {}^1/_2$, then for any cycle *n* as a function of the initial size x_0:

$$x_n = 2^{-n} x_0 \exp\left(\sum_{j=1}^{n} \phi_j\right)$$

The previous description of size is not stable against fluctuations if ϕ_n is random



$$\phi_n = \phi^* - \beta \ln \frac{x_n}{x^*} + \xi_n$$

$x^* = ?$ is a scaling parameter

$\phi^* + \xi_n$ is the residual accumulation exponent

$\phi^* = ?, \langle \xi_n \rangle = 0$

$\beta = 0.5$ is the feedback parameter
(the slope of the fit)

This feedback is sufficient to stabilize the cell size and prevent any divergence
For any $0 < \beta < 2$

The proposed feedback encompasses all possible models:

$$x_{n+1} = f_n x_n e^{\phi_n}$$

$$\phi_n = \phi^* - \beta \ln \frac{x_n}{x^*} + \xi_n$$

Assume: f_n=1/2, $e^{\phi^*} = 2$,

And neglect noise, then: $x_{n+1} = \frac{1}{2} x_n e^{\phi^* - \beta \ln \frac{x_n}{x^*}} = \frac{1}{2} \left[2 x_n^{(1-\beta)} x^{*\beta}\right]$

Which is exactly the form in Amir PRL 2014.

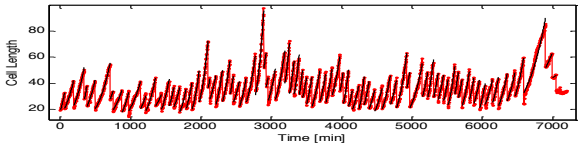
Now expand to first order
Around $\bar{x}$ $\Rightarrow$ $x_{n+1} = \frac{1}{2} \left[2(1-\beta)x_n + 2\beta x^*\right]$

And the size at division would be:

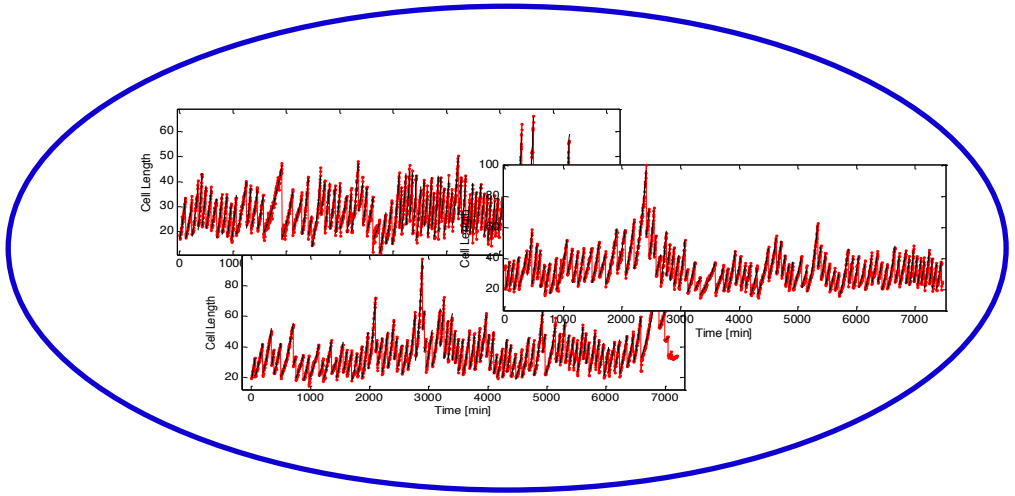
$\beta = 0$	$x_{div} = 2x_n$	Timer
$\beta = \frac{1}{2}$	$x_{div} = x_n + x^*$	Adder
$\beta = 1$	$x_{div} = 2x^*$	Sizer

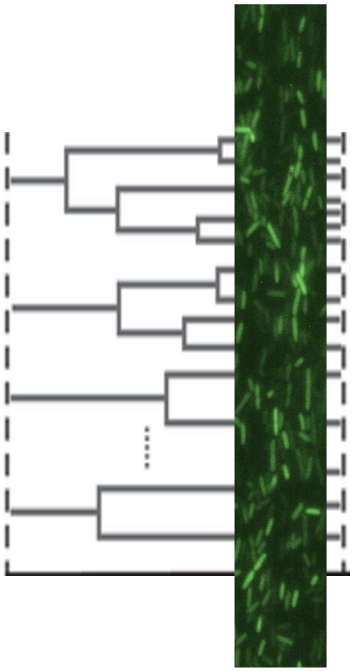
Statistical Ensembles

Temporal single-cell trace



Ensemble of lineages



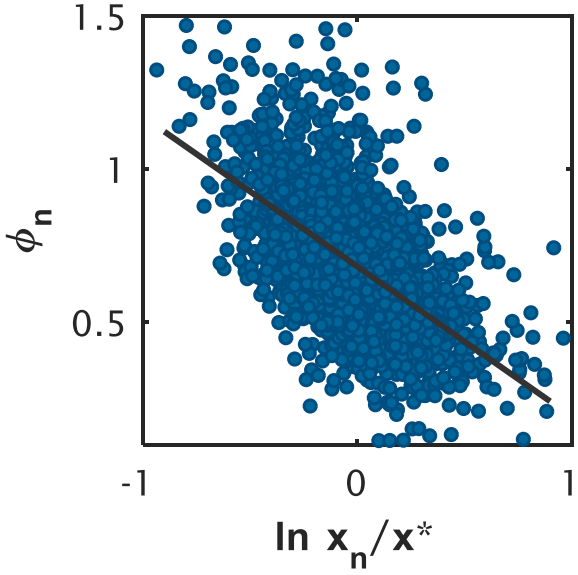


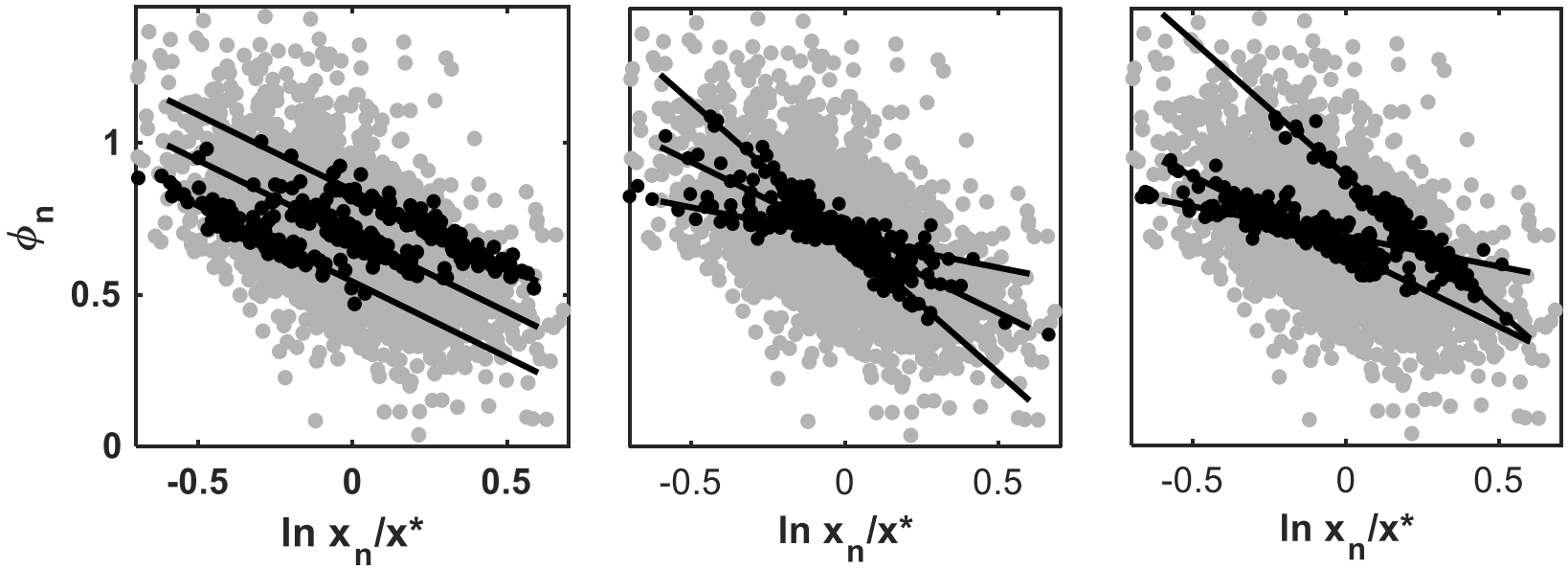
Population snapshot

What is the cause of the wide distribution?

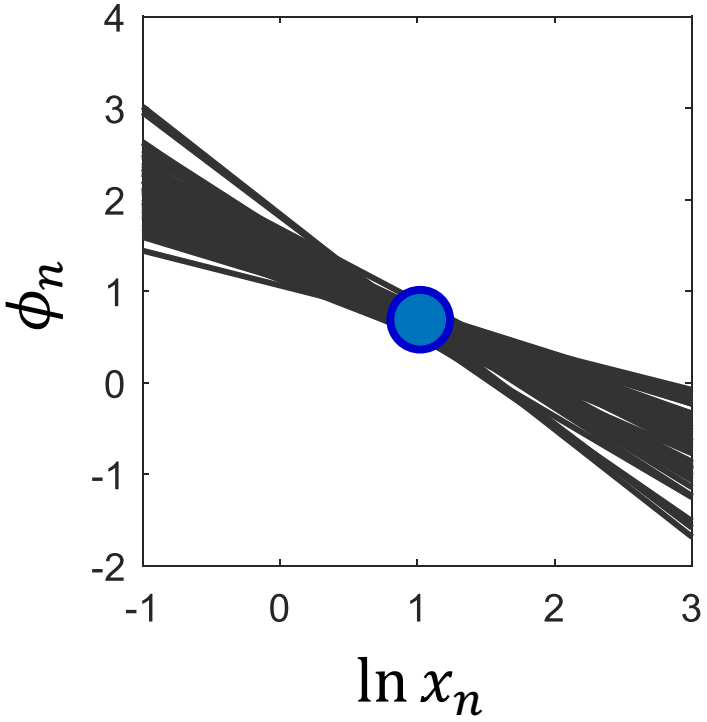
Correlation scatter-plots are very noisy

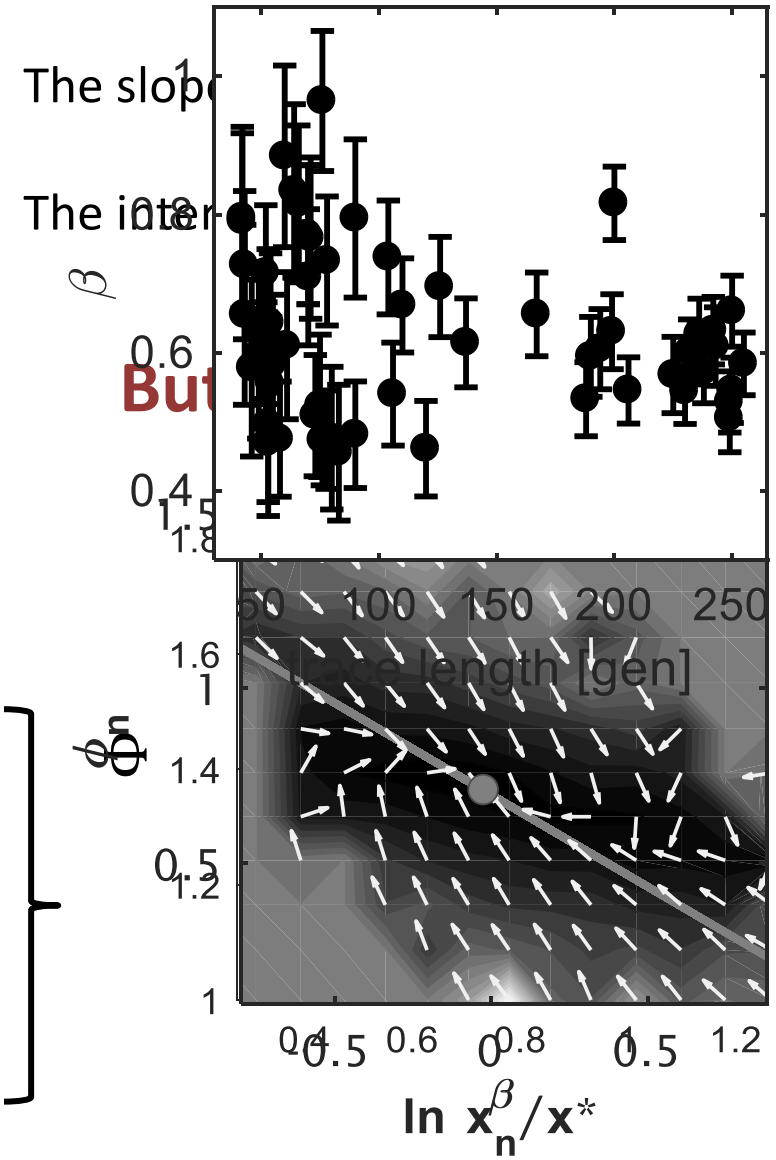
With long enough traces, we can disentangle the single-cell correlations





Different feedback strength





The slope: $\ln x^* \rightarrow x^* = 2.7\ \mu m$

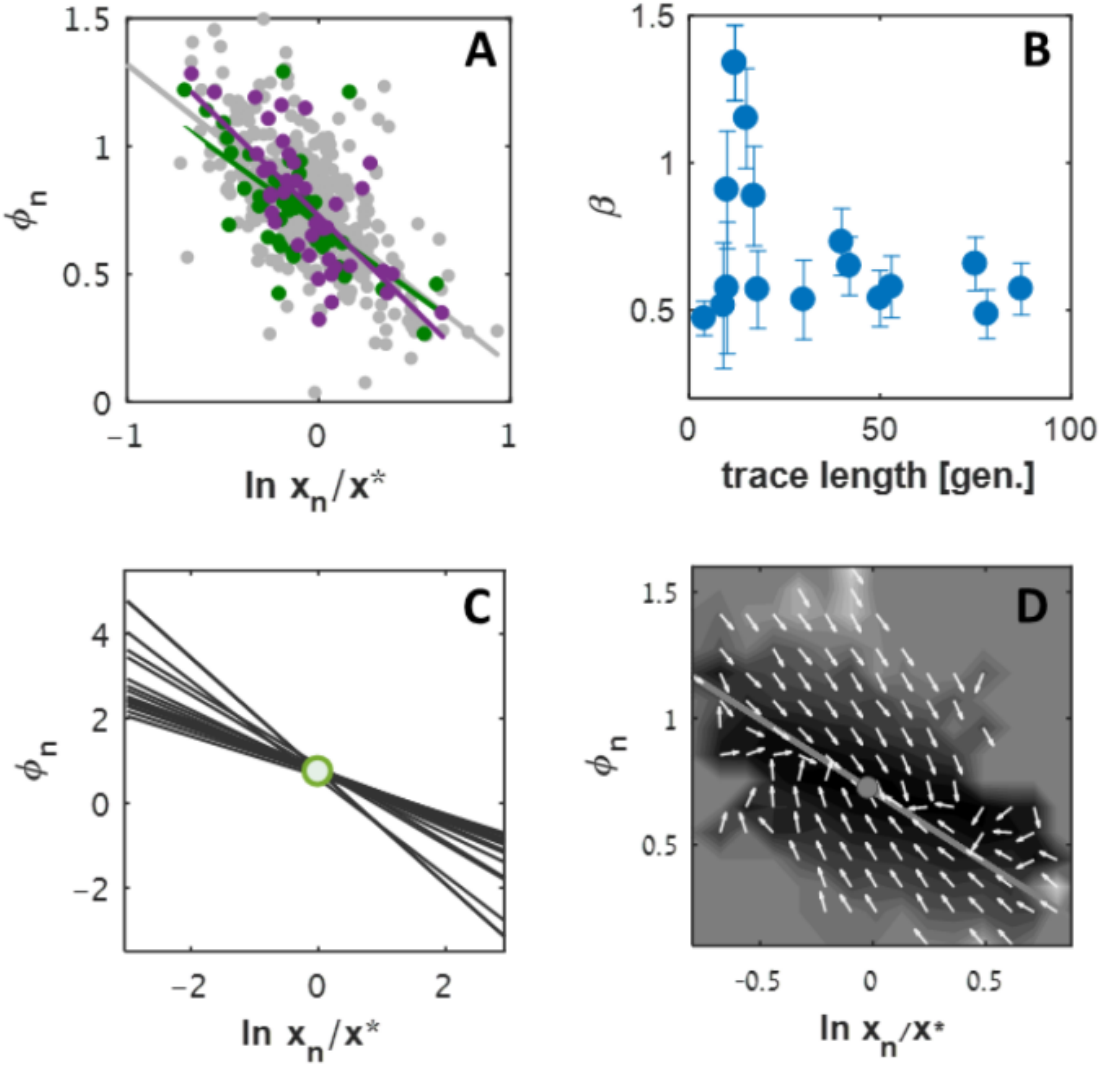
Which is the average cell-length at the start of the cell-cycle

The intercept: $\boldsymbol{\phi^* = \ln 2}$

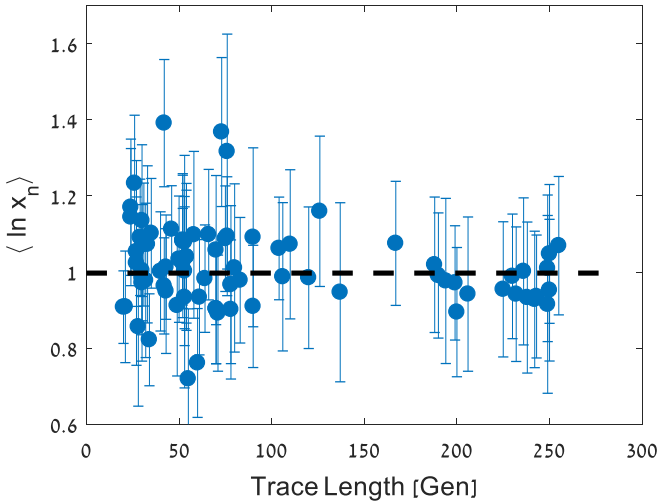
Different Medium, Same behavior

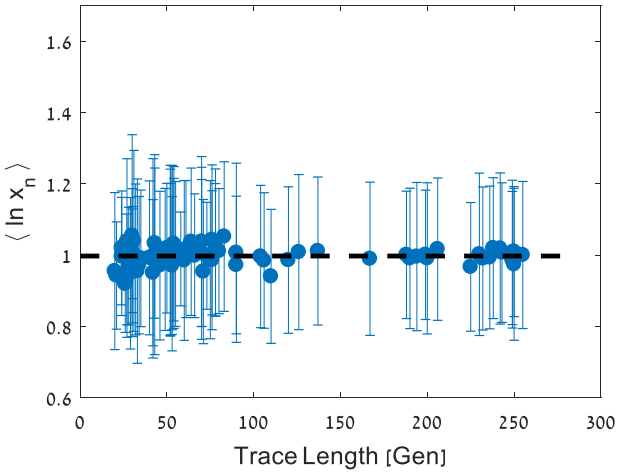
The same behavior is observed in M9 medium supplemented with Lactose.

The strength of the feedback is variable among cells, yet the attractor of cell size dynamics is the same for all cells.



Different Size due to Slow Dynamics

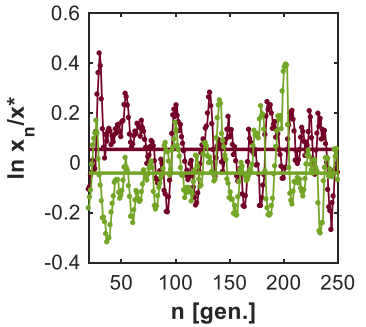


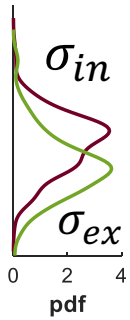


Due to the fact that the average of the division ratio changes from cell to cell, we see differences in the cell-size:

$$\overline{\ln x_n} = \ln x^* + \frac{\phi^* + \overline{\ln f_n}}{\beta}$$

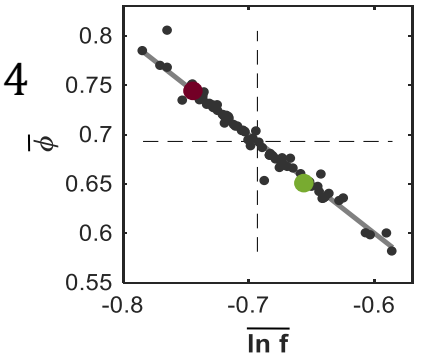
This could be due to slow dynamics, which would mean that the division ratio changes slowly and therefore there isn't enough statistics during the lifetime of the cell.



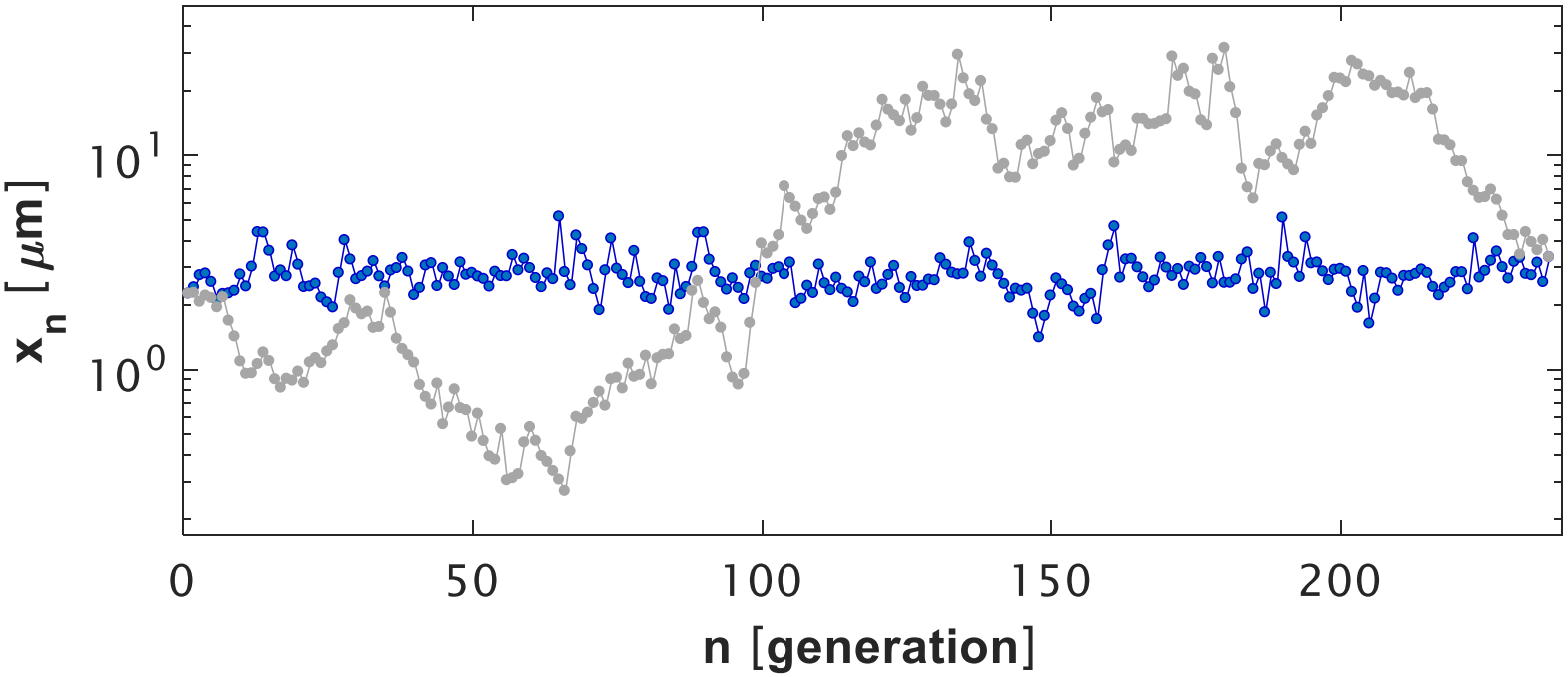


$\sigma_{in} = 0.26$

$\sigma_{ex} = 0.14$

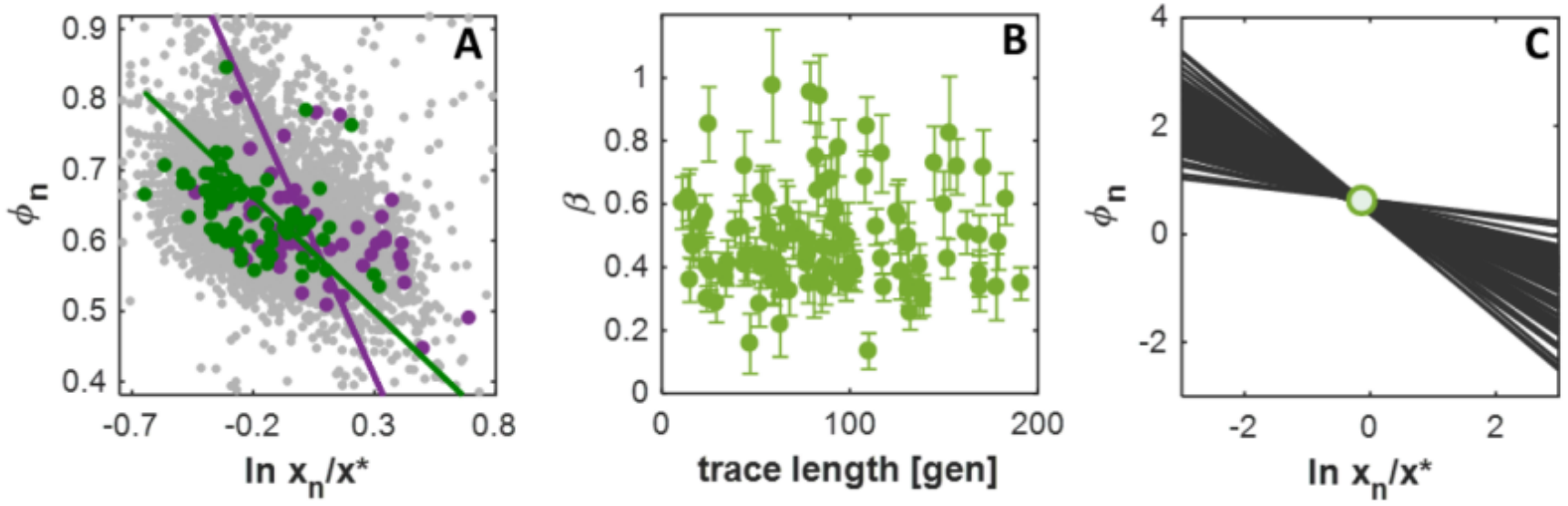


Order Matters



When the order of parameters is shuffled, the cell length can diverge for extended periods of time.

Other Data Exhibit Similar behavior

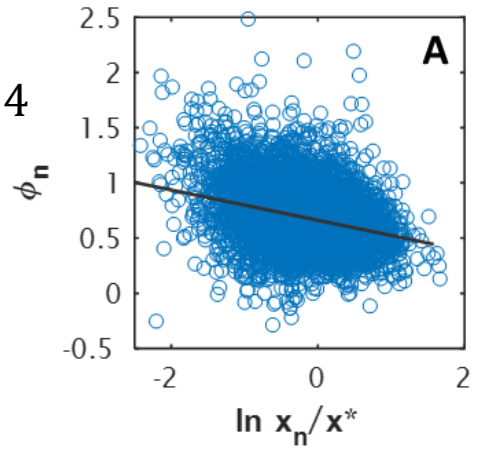


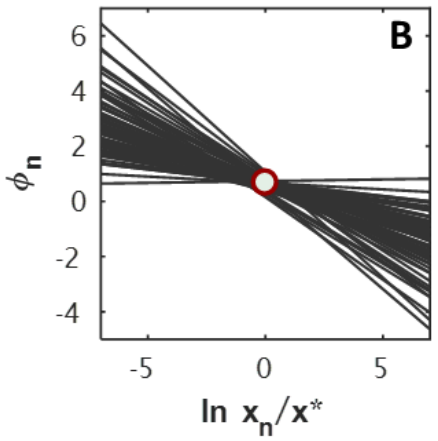
Wang P, et al. (2010) Robust growth of Escherichia coli. *Curr Biol* 20(12):1099–103.

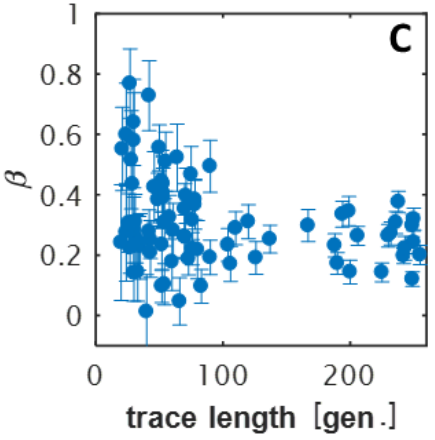
Can we extract a mechanism from this Data?

λ-Pr in LB

$\langle \beta \rangle = 0.14$

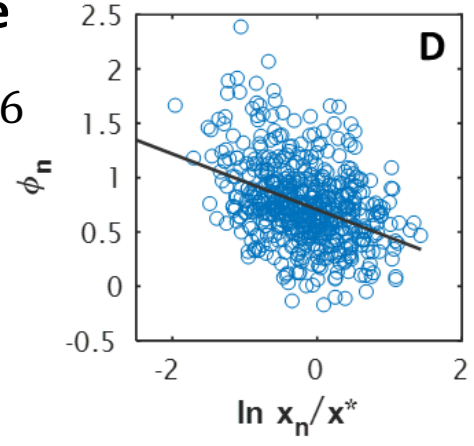


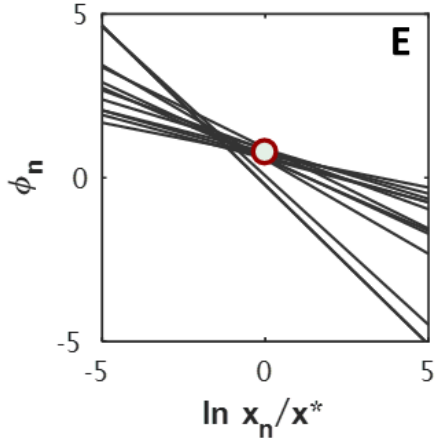


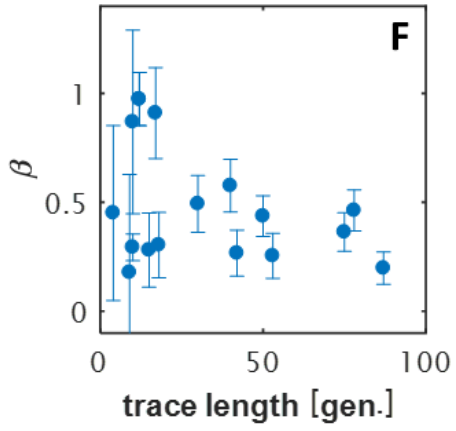


Lac-Pr in Lactose

$\langle \beta \rangle = 0.26$





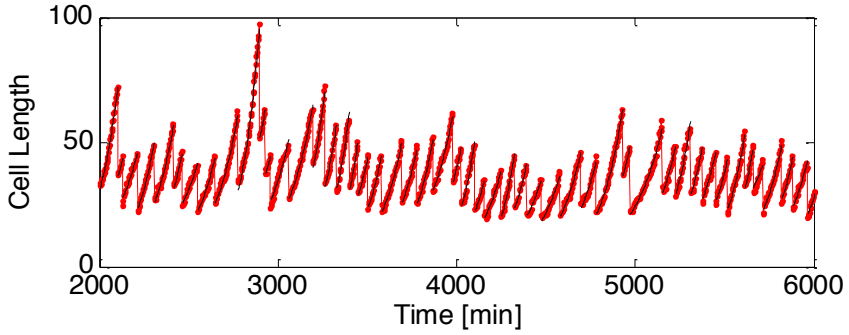


$\beta \neq 1/2$

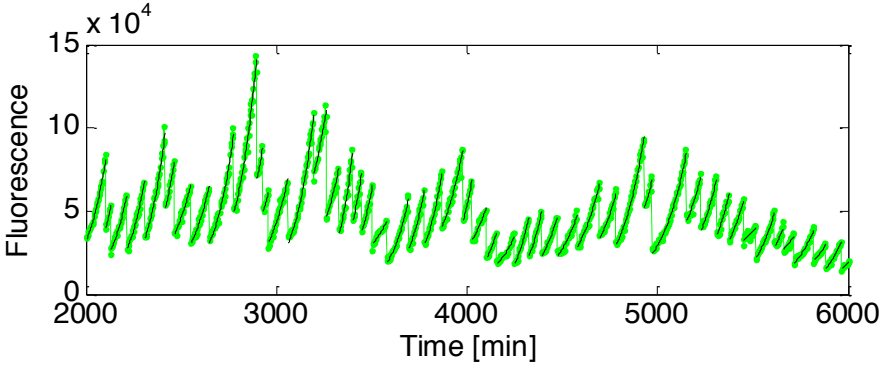
Proteins do not exhibit adder behavior ($\beta \neq {}^1/_2$) even on average.
Single-cell trace behavior is similar: variable β, common pivot point.

The multi-dimensional phenotype

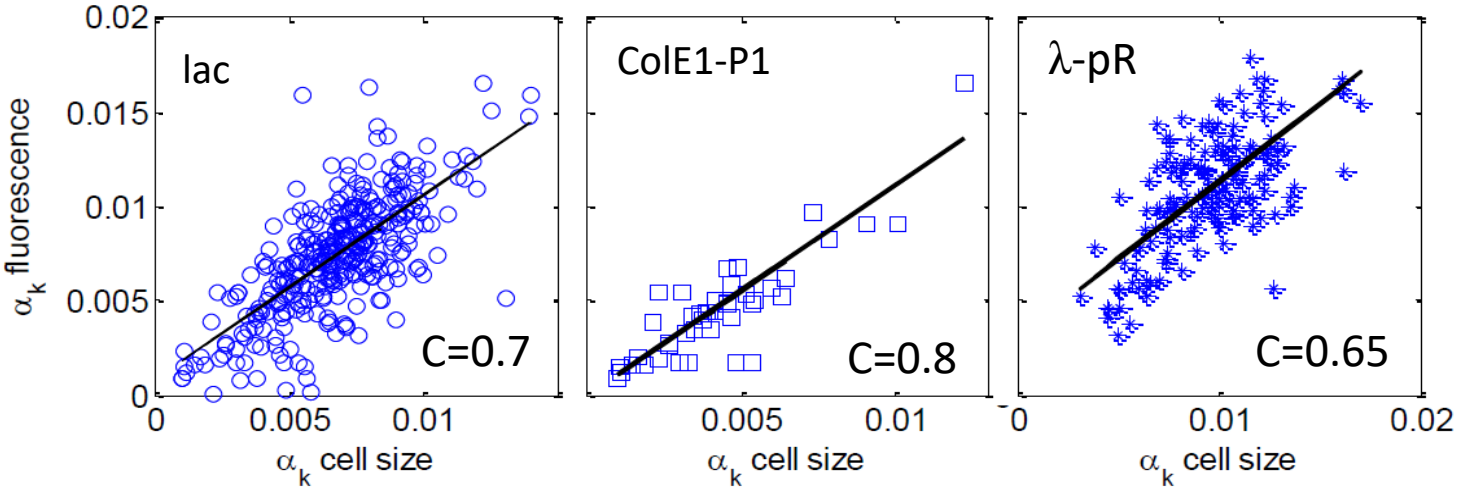
Cell Size



Protein content

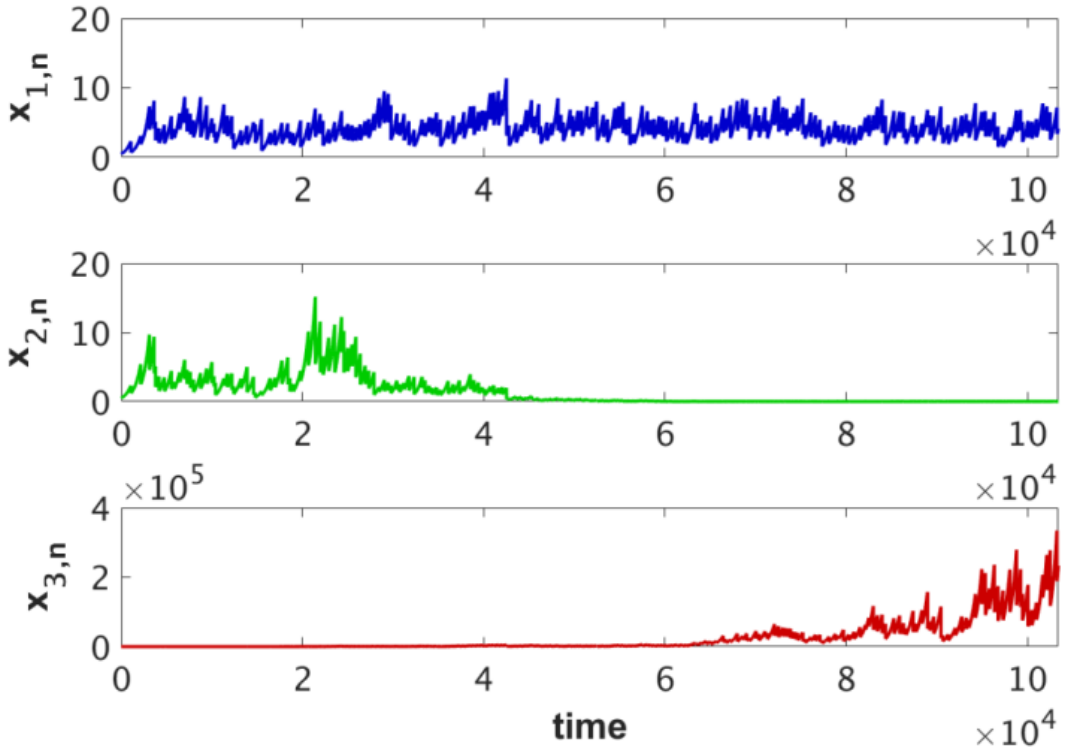


Protein expression is strongly correlated to cell-size



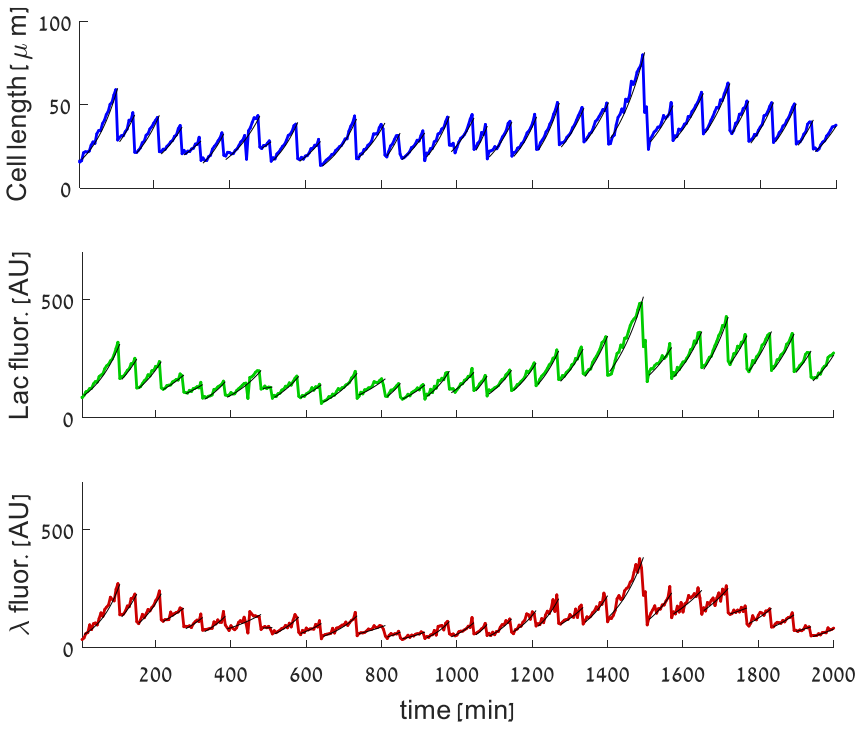
Stability of Exponential Growth under the control of another

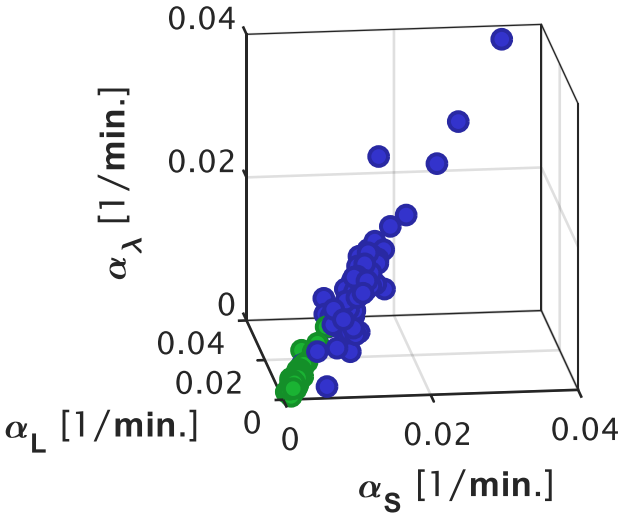
These traces of three components out of 50 were generated under the assumption that x_1 controls cell division, and that the other components are enslaved to the first, namely, components are assumed to grow with the same exponential rates, up to noise. It is seen that components that do not control cell division are not stable and can either decay to zero, as in the case of x_2, or diverge, as in the case of x_3.



3 components measured simultaneously

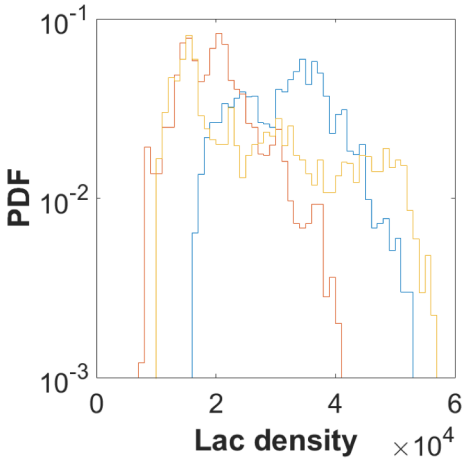
Correlations are strong along individual cycles

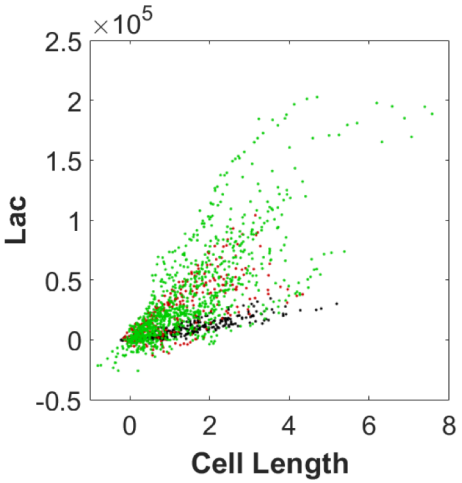




Protein amount and cell size are correlated but not dependent

Susman et al, PNAS (2018)





Multi-component phenotype model

This can we explain also the exponential growth of protein?

When we measure any specific cellular variable, such as protein content, we are probing the result of the complex interactions in the cell. When we measure several of them, an effective interaction between them will be observed. This interaction can be viewed as an effective description of their participation in the large dynamical system that is the cell, and which may contain many other hidden variables:

$$\frac{d}{dt}\vec{x}\,(t) = K\vec{x}\,(t)$$

***K* is the interactions matrix which we assume to be constant**

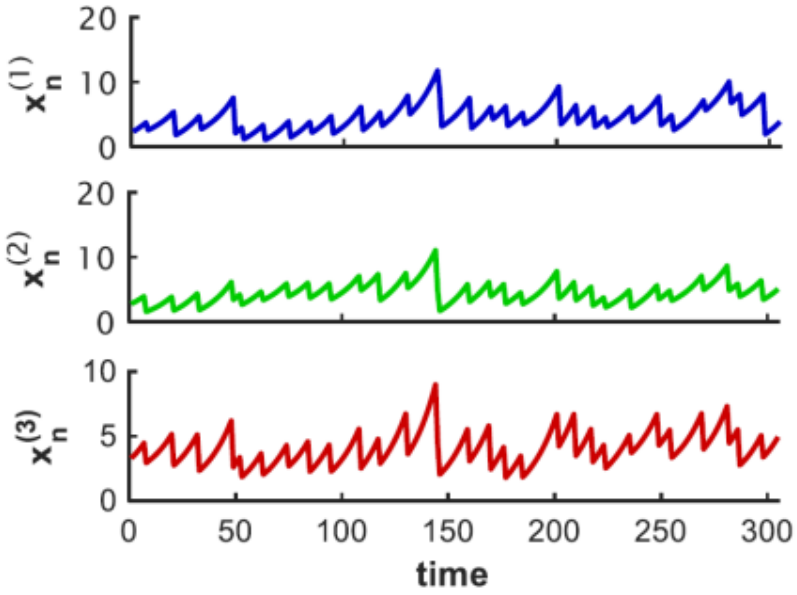
$$\phi_n^{(i)} = \phi^* - \beta_i \ln \frac{x_n^{(i)}}{x^{*(i)}} + \xi_n$$

The feedback can be on one or more components

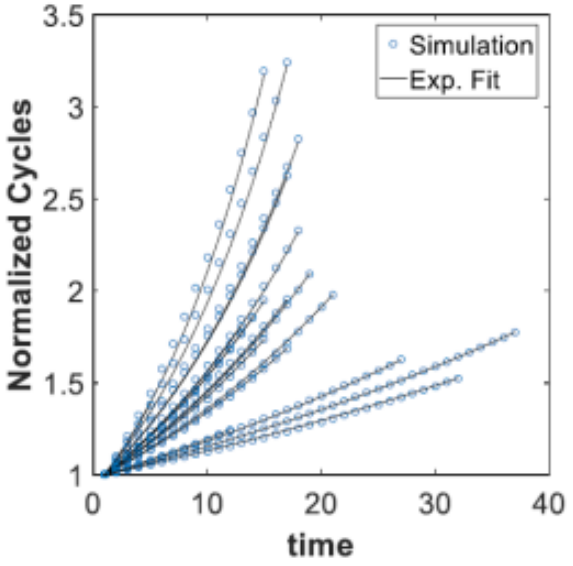
The solution within the cell cycle:

$$\vec{x}(t) = \sum_{i=1}^{N} c^{(i)} e^{\lambda_i t} v_i \qquad 0 < t < T$$

Where: $c = V^{-1}\vec{x}(0)$

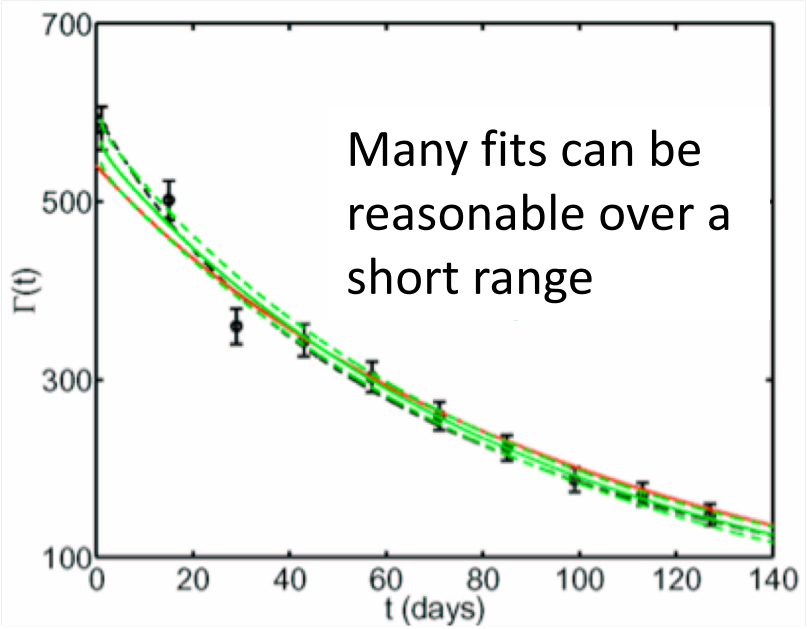


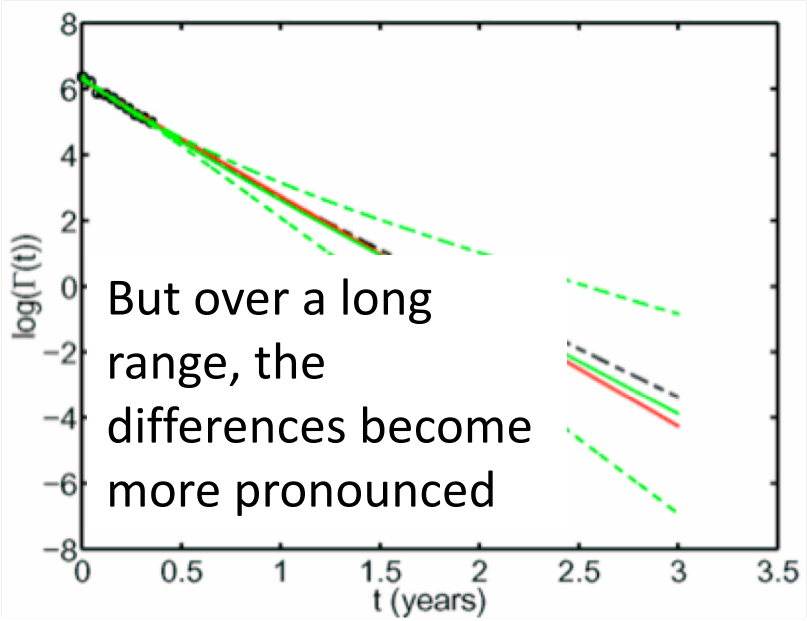
Due to the limited dynamic range, each cell cycle can be fit with a simple function with single effective exponent.



Effect of Limited Dynamics Range

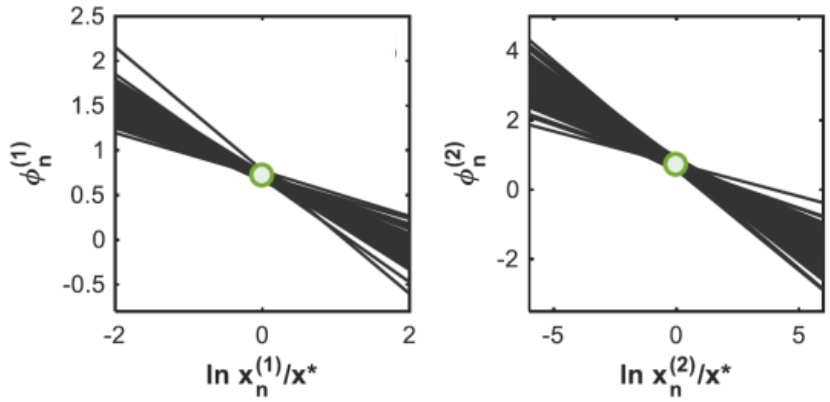
Example of radioactive decay of three elements.
A mixture of three elements will produce a Geiger counter signal which theoretically will be $y(A,\gamma,t) = A_1 e^{-\gamma_1 t} + A_2 e^{-\gamma_2 t} + A_3 e^{-\gamma_3 t}$



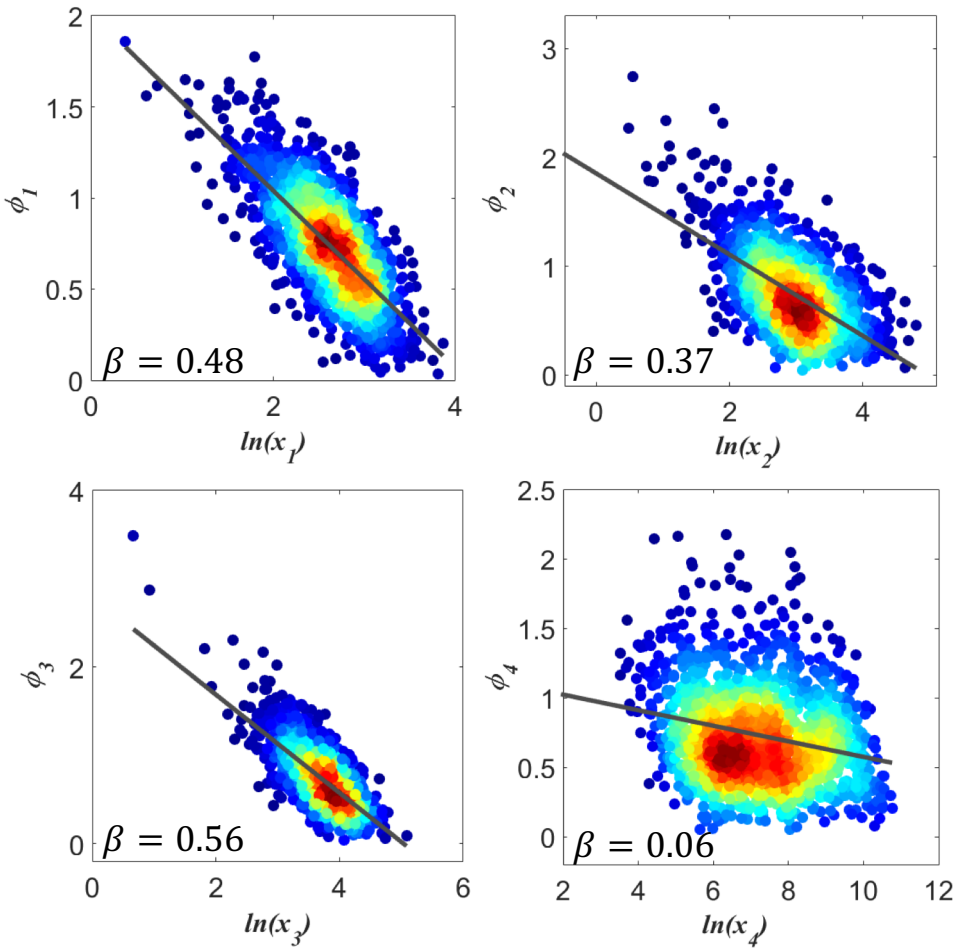


James Sethna

The feedback is variable and can be induced



And its strength does not reflect the control mechanism



Conclusions:

- **Cellular growth dynamics point to a feedback mechanism with a universal attractor to stabilize the growth**
- **The feedback strength however varies among cells**
- **This feedback appears in all measured properties of the cell, therefore, it doesn't necessarily point to the control mechanism**
- **The Data we have so far is not sufficient to reverse engineer the cell division control mechanism**

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