Microbial growth control in changing environments

Theoretical and experimental study of resource allocation in *Escherichia coli*

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supervized by Prof. Johannes Geiselmann[1,2] and Dr. Hidde de Jong[2]

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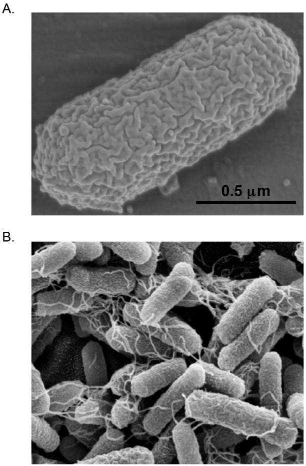
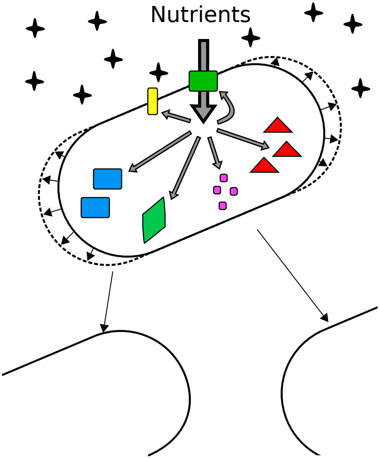
PhD Thesis Defense
March 23rd 2017

MICROBIAL GROWTH

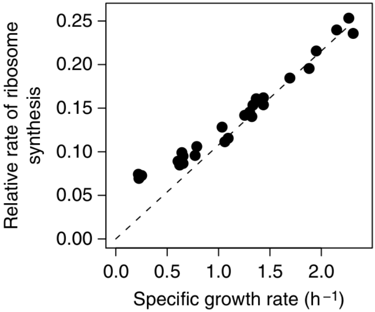




Cell composition is a resource allocation problem

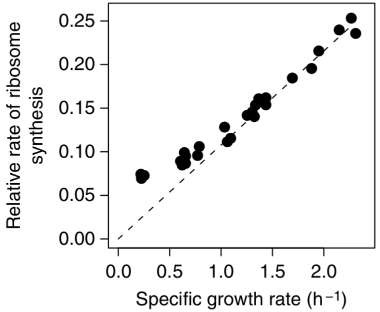
Blount, *eLife* 2015

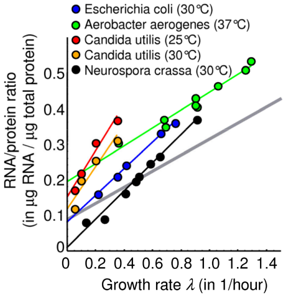
RESOURCE ALLOCATION OBEYS GROWTH LAWS



Molenaar *et al*, *Mol. Syst. Biol.* 2009

RESOURCE ALLOCATION OBEYS GROWTH LAWS

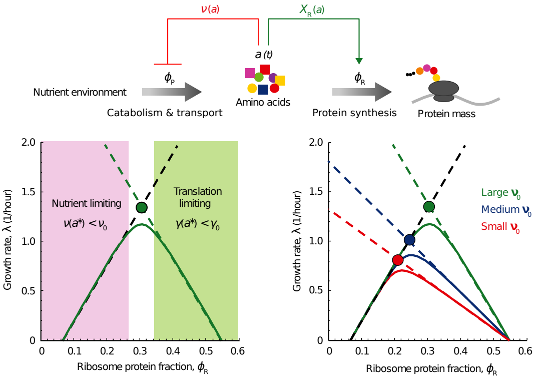




Why such regularities?

Molenaar *et al*, *Mol. Syst. Biol.* 2009
Scott *et al*, *Science* 2010

Growth laws are explained if microorganisms maximize their growth rate



Growth laws result from a balance between supply and demand of precursors

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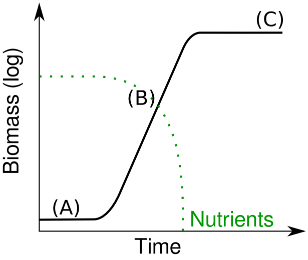
Scott *et al*, *Mol. Syst. Biol.* 2014

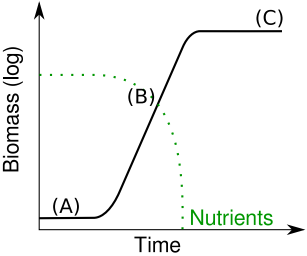
Growth laws were established at balanced growth



(B) Balanced growth

- Exponential growth $\left(\frac{dB}{dt} = \mu B\right)$
- Steady state $\left(\frac{dx}{dt} = 0\right)$
- Experimentally and theoretically convenient

Growth laws were established at balanced growth

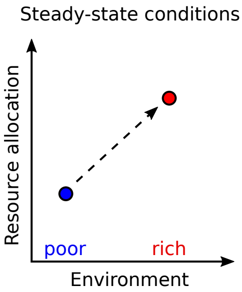


(B) Balanced growth

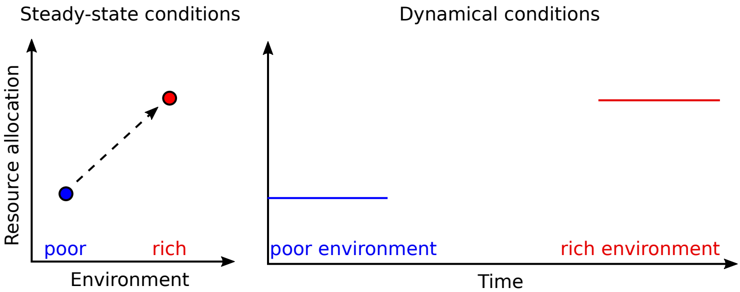
- Exponential growth $\left(\frac{dB}{dt} = \mu B\right)$
- Steady state $\left(\frac{dx}{dt} = 0\right)$
- Experimentally and theoretically convenient

Growth laws were established for laboratory conditions that are seldom encountered in nature

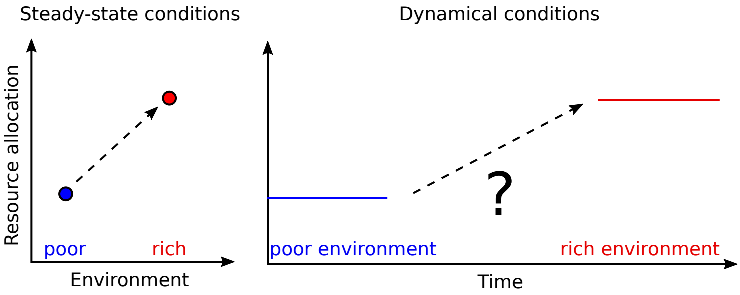
No growth laws for changing conditions



No growth laws for changing conditions



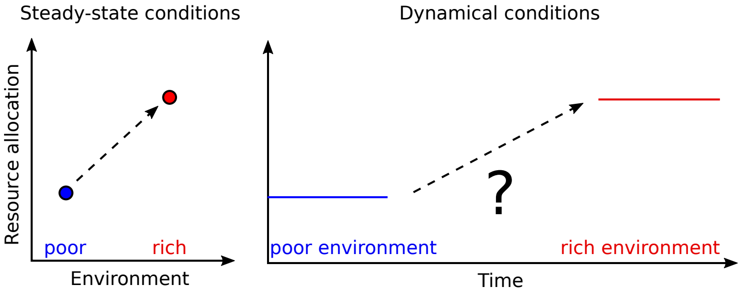
NO GROWTH LAWS FOR CHANGING CONDITIONS



Transitions are more difficult to study

PROBLEM STATEMENT

How do microorganisms dynamically reallocate their resources after a change in the environment?



Approach

Theoretical approach

What is the optimal way to dynamically allocate resources during a growth transition?

- What is the optimal resource allocation strategy?
- Can the strategy be linked to known molecular mechanisms?

Experimental approach

Do bacteria implement the theoretically optimal strategy of resource allocation?

- Measure resource allocation during a transition
- Compare with the optimal strategy

Theoretical approach

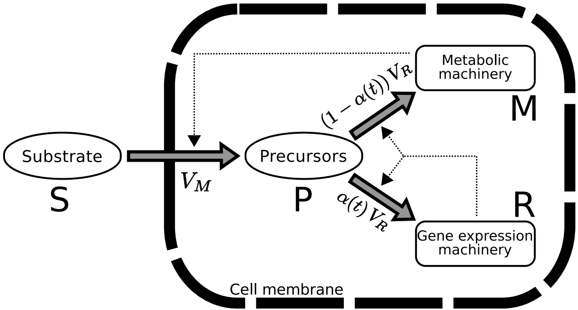
Dynamical Allocation of Cellular Resources as an Optimal Control Problem: Novel Insights into Microbial Growth Strategies

Collaborators

- Francis Mairet (Inria Sophia-Antipolis Méditerranée, project-team Biocore)
- Jean-Luc Gouzé (Inria Sophia-Antipolis Méditerranée, project-team Biocore)

Published in Giordano *et al*, *PLoS Comput Biol* 2016

SELF-REPLICATOR MODEL OF RESOURCE ALLOCATION



Two biochemical (macro)reactions:

$$\text{Metabolism:} \quad S \xrightarrow{V_M} P$$
$$\text{Macromolecule synthesis:} \quad P \xrightarrow{V_R} \alpha R + (1-\alpha)M$$

Two-dimensional dynamical system

$$\begin{aligned} \text{Precursors:} \quad \frac{dP}{dt} &= V_M - V_R \\ \text{GEM:} \quad \frac{dR}{dt} &= \alpha \cdot V_R \end{aligned}$$

Two-dimensional dynamical system

$$\begin{aligned}\text{Precursors:} \quad \frac{dp}{dt} &= v_M - v_R - \mu \cdot p \\ \text{GEM:} \quad \frac{dr}{dt} &= \alpha \cdot v_R - \mu \cdot r\end{aligned}$$

Assuming...

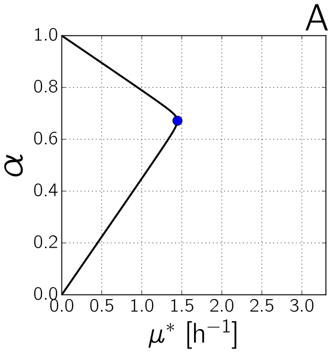
Volume: $\texttt{Vol} = \beta(M+R) \quad \Rightarrow \quad$ Growth rate: $\mu = \beta \frac{V_R}{\texttt{Vol}} = \beta v_R$

Michaelis-Menten kinetics $\quad \Rightarrow \quad v_R = \frac{k_R \cdot p}{K_R + p} \cdot r$

$$v_M = e_M \cdot (1/\beta - r)$$

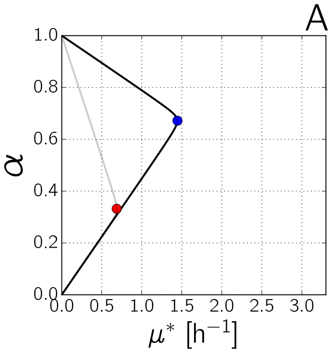
How does the cell choose the resource allocation parameter α?

Model predicts the steady-state growth laws



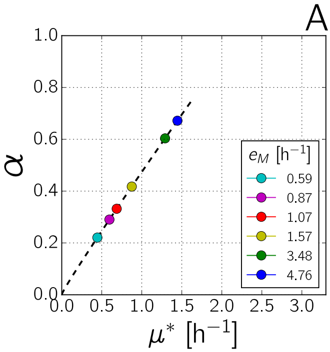
Data from Scott *et al*, *Science*, 2010

Model predicts the steady-state growth laws



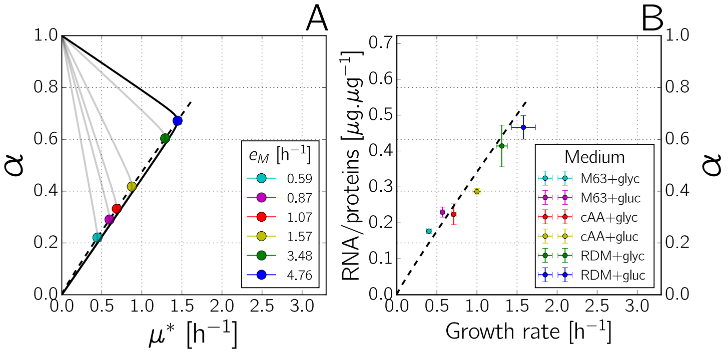
Data from Scott *et al*, *Science*, 2010

Model predicts the steady-state growth laws



Data from Scott *et al*, *Science*, 2010

MODEL PREDICTS THE STEADY-STATE GROWTH LAWS



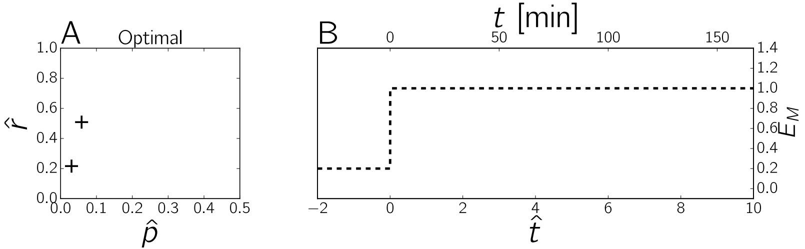
Choosing the optimal α for each environment predicts the empirical growth laws

Data from Scott *et al*, *Science*, 2010

Growth maximization during transitions

New objective: maximize biomass produced during an environmental transition

$$J(\alpha) = \int_0^\tau \mu(t, \hat{p}, \hat{r}, \alpha)\, dt$$

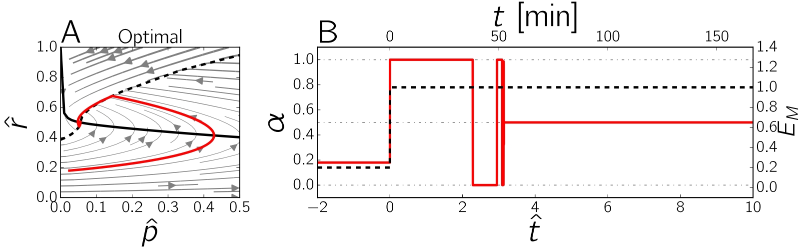


Growth maximization during transitions

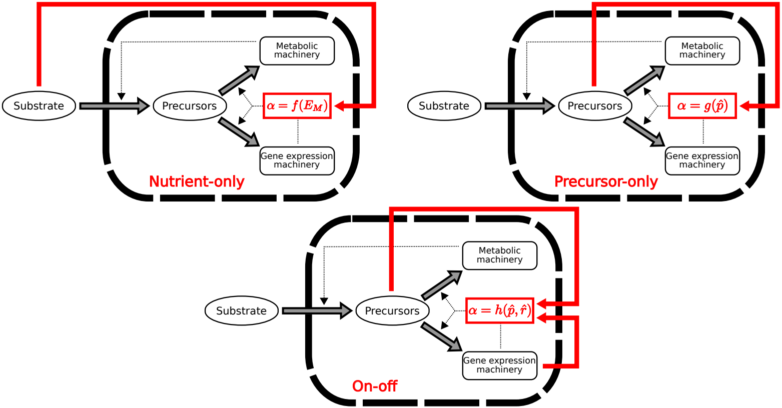
New objective: maximize biomass produced during an environmental transition

$$J(\alpha) = \int_0^\tau \mu(t, \hat{p}, \hat{r}, \alpha)\, dt$$

Optimal solution: bang-bang-singular strategy

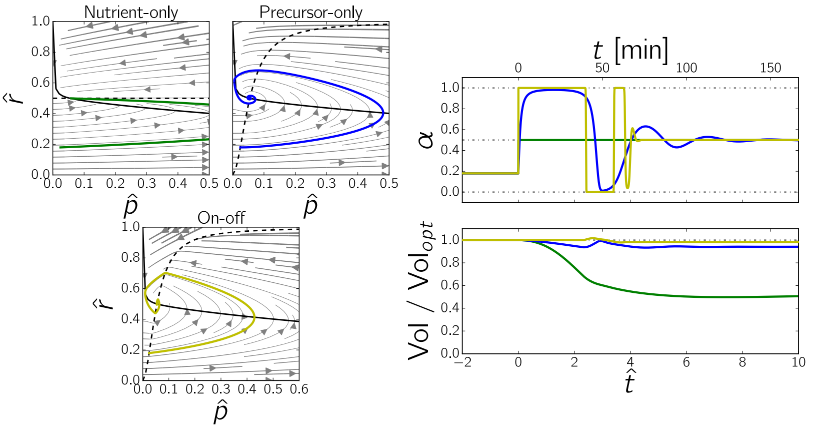


Different closed-loop control strategies for resource allocation



All closed-loop control strategies are optimal at steady state

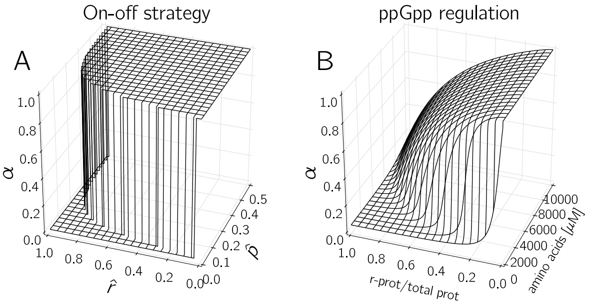
Performance of control strategies during growth transition



Equivalent strategies at steady state produce different outcomes in dynamical conditions

WHICH STRATEGY IS CLOSER TO THE ACTUAL REGULATORY MECHANISMS?

The ppGpp regulatory system in *E. coli* (Bosdriesz *et al*, 2015)...



... is a likely candidate

WHAT WE HAVE LEARNED SO FAR

- Bang-bang resource allocation maximizes the biomass produced during a nutrient upshift
- A dynamical study uncovers differences between regulatory strategies that are equivalent at steady state
- Complex regulations are beneficial during transitions
- The ppGpp system might be an efficient way for the cell to achieve quasi-optimal resource allocation

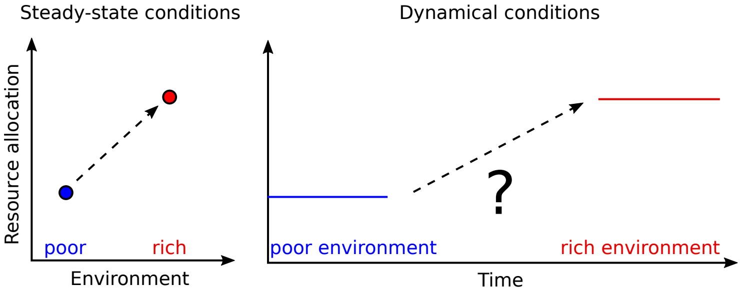
EXPERIMENTAL APPROACH

Dynamics of Resource Allocation in *E. coli* During an Acetate-Glucose Upshift

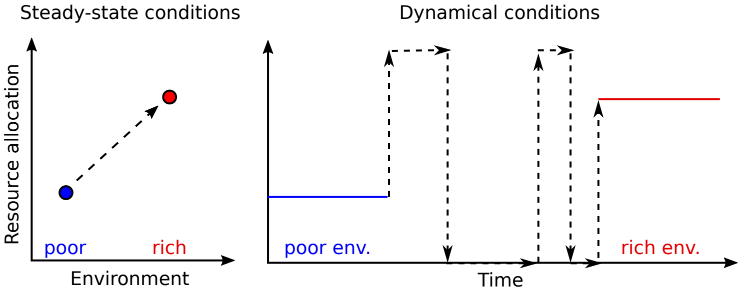
Collaborators

- Irina Mihalcescu (LIPhy, Université Grenoble Alpes, team BIOP)
- Eugenio Cinquemani (Inria Grenoble – Rhône-Alpes, project-team Ibis)

Expected optimal behavior



Expected optimal behavior

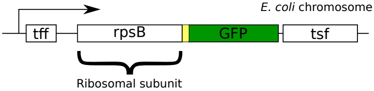


- Rapid regulatory switches
  $\rightarrow$ high temporal resolution
- Probably not that stiff
  $\rightarrow$ extended observation times
- No reason bacteria will be synchronized
  $\rightarrow$ single-cell measurements

Experimental setup

Quantification of gene expression machinery

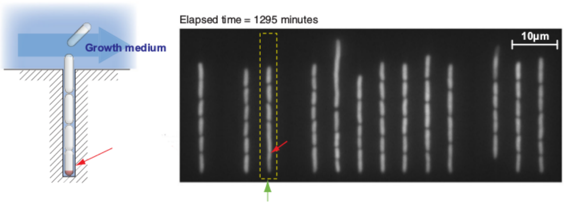
- Fluorescent labeling of the RpsB subunit of the ribosome



- Isolated on the chromosome
- Growth not affected
- Integrated into ribosomes

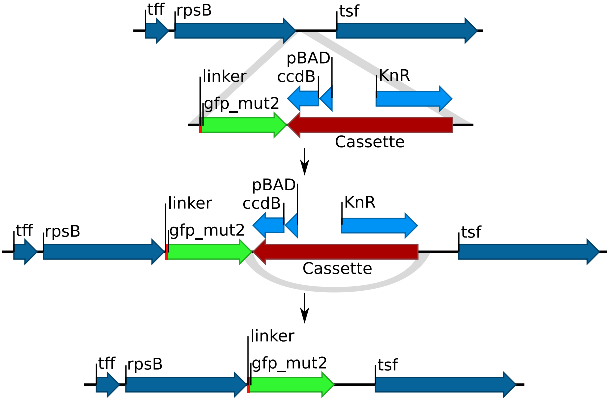
Monitoring of single-cells during growth transition

- Microscopy and microfluidics (mother machine)



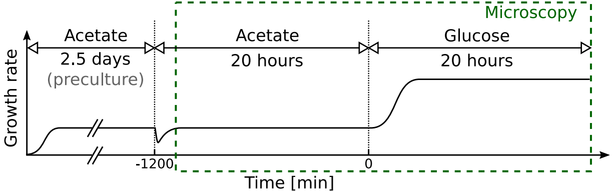
Bakshi *et al*, *Mol. Microb.* 2012; Wang *et al*, *Curr. Biol.* 2010

STRAIN CONSTRUCTION



Only *rpsB* is modified

PILOT EXPERIMENT



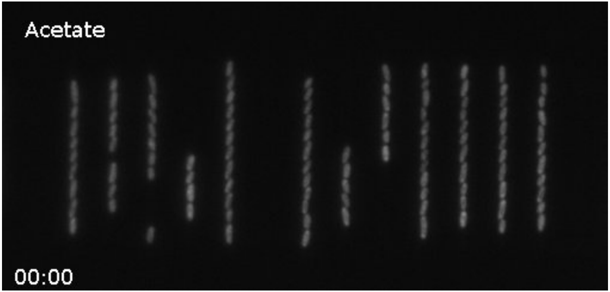
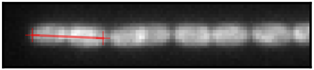


Image analysis

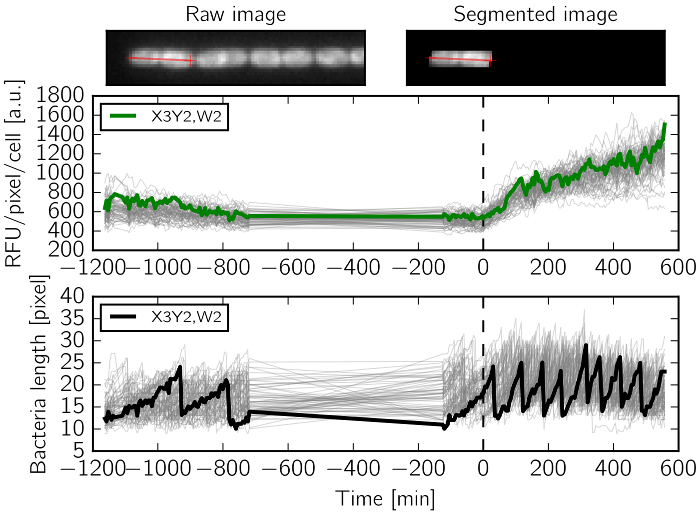
- 6 fields, 15 channels = 90 lineages (68 exploitable)
- Segmentation of the cells at the bottom of the wells only (present for the entire experiment)
- Manual segmentation (selection of the 2 poles on the fluorescence images)

Raw image



Segmented image

Results of the image analysis



Reconstruction of the growth rate and resource allocation

Dynamical system

$$\begin{aligned} \dot{r}(t) &= \mu(t) \cdot \frac{\alpha(t)}{\beta} - \mu(t) \cdot r(t), && (1) \\ \dot{V}(t) &= \mu(t) \cdot V(t), && (2) \end{aligned}$$

with initial conditions $r(0) = r_0$, $V(0) = V_0$

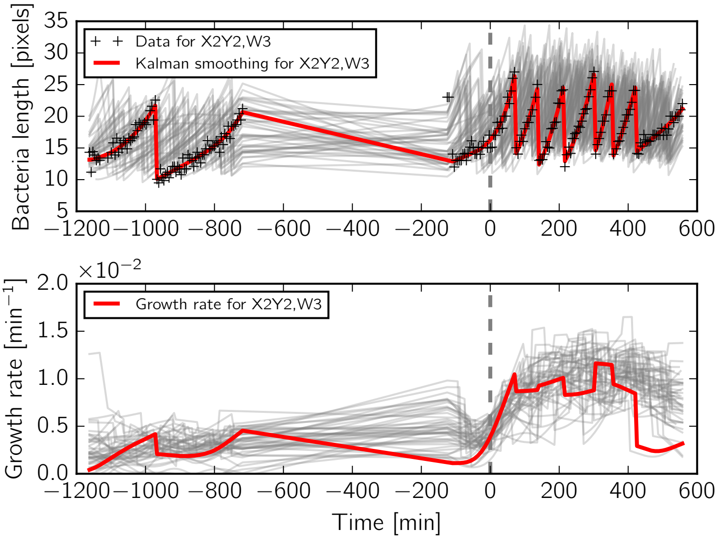
Measurement model

$$\begin{aligned} L(t_k) &= \lambda \cdot V(t_k) + \epsilon_k, && (3) \\ F(t_k) &= \gamma \cdot r(t_k) + \eta_k, && (4) \end{aligned}$$

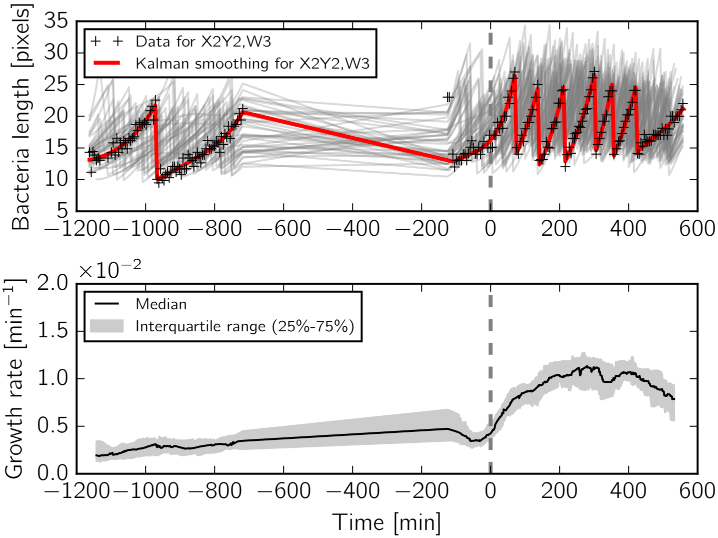
at each time-point t_k, $0 \leq k \leq N-1$

Problem: reconstructing $\gamma\alpha(\cdot)/\beta$ and $\mu(\cdot)$ from measurements $\{F(t_0), ..., F(t_{N-1})\}$ and $\{L(t_0), ..., L(t_{N-1})\}$

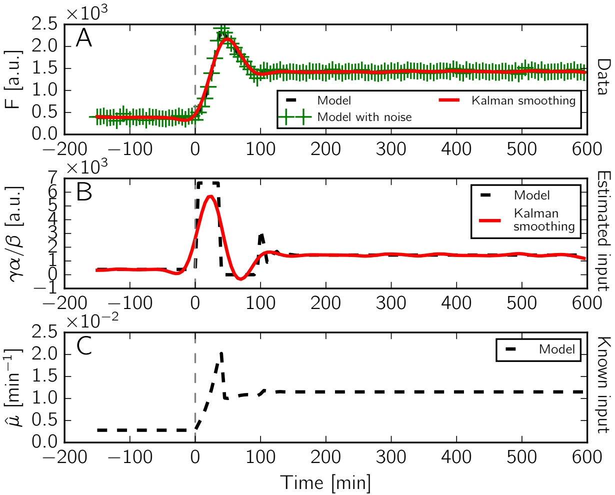
Results of the growth-rate reconstruction



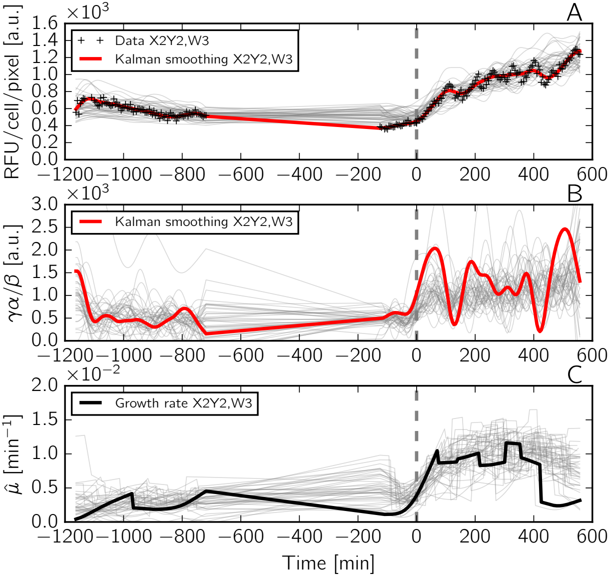
Results of the growth-rate reconstruction



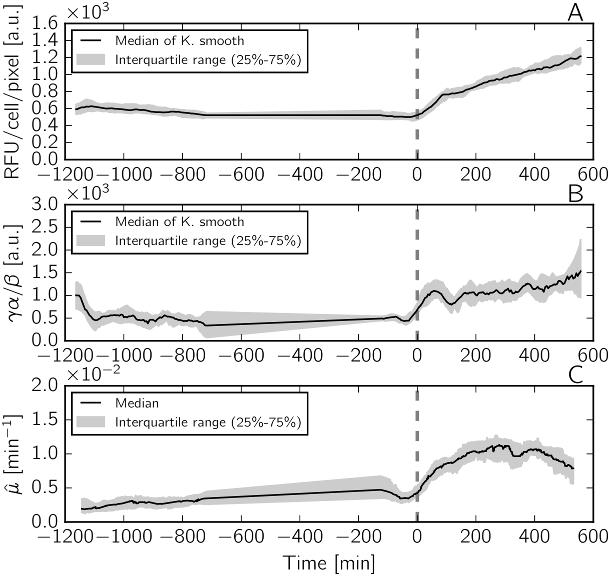
Reconstruction of resource allocation on synthetic data



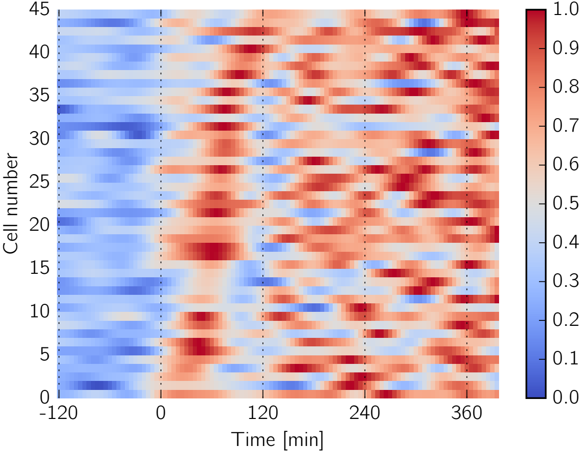
RESULTS OF THE RECONSTRUCTION OF $\alpha(\cdot)$



RESULTS OF THE RECONSTRUCTION OF $\alpha(\cdot)$



HEATMAP OF THE $\alpha(\cdot)$ RECONSTRUCTION



The first oscillation in the resource allocation profile is conserved in all cells

Still a lot to do...

We showed that:

- Dynamical resource allocation can be reconstructed via ribosome tagging and live imaging
- Kalman smoothing is convenient for such a reconstruction
- Oscillatory features are visible, but need to be confirmed

Further work should focus on:

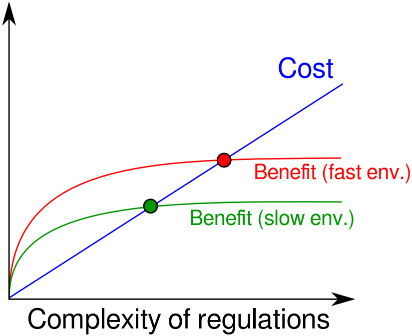
- Long steady states before and after the upshift
  - $\rightarrow$ crucial for calibrating the reconstruction algorithm
- More cells
  - $\rightarrow$ for statistics, but automatic image analysis needed
- Other environmental changes, cross-validation, etc.

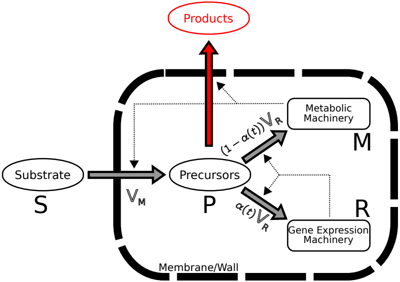
CONCLUSION

- Simple models are valuable for understanding fundamental principles of microbial growth
- Bang-bang regulatory scheme maximize biomass in dynamical conditions
- Complex regulation is only beneficial for unbalanced growth
- Known mechanisms of ribosome synthesis regulation (ppGpp) suggest bang-bang resource allocation during transitions
- Difficult to confirm experimentally, but preliminary results are encouraging

Perspective

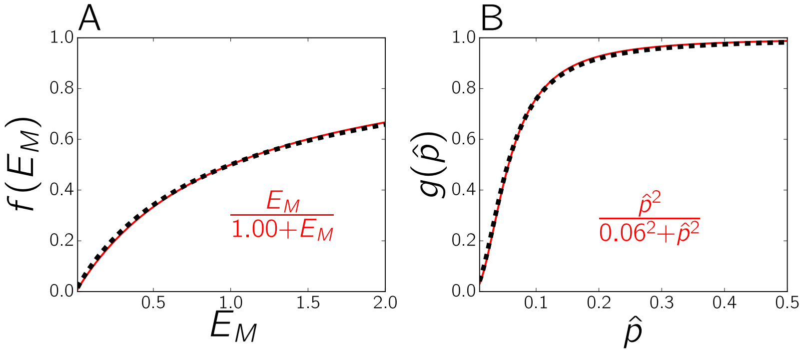
- Is there a fundamental relationship between the dynamics of the environment and the complexity of regulations?
- Can we apply this approach to maximize industrial production yields?





Thank you

Control strategies can be approximated by biologically relevant functions



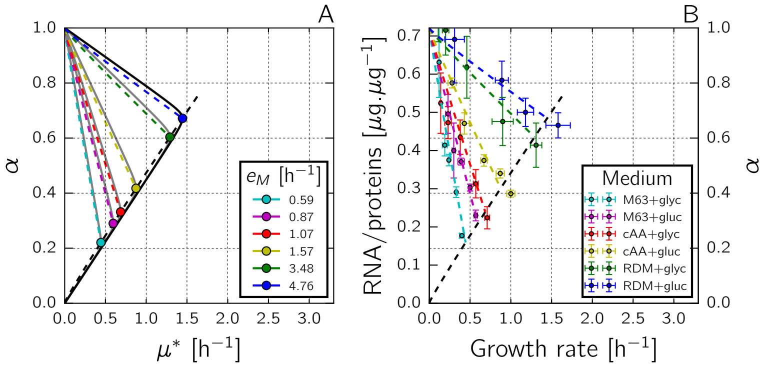
$$f(E_M) = \frac{E_M + \sqrt{K\,E_M}}{E_M + 2\sqrt{K\,E_M} + 1} \qquad g(\hat{p}) = \frac{\hat{p}}{\hat{p} + \frac{K}{K+\hat{p}}(1+\hat{p})}$$

The on-off strategy

$$\alpha = h(\hat{p}, \hat{r}) = \begin{cases} 0, \text{ if } \hat{r} > g(\hat{p}), \\ 1, \text{ if } \hat{r} < g(\hat{p}), \\ \alpha^*_{opt}, \text{ if } (\hat{p}, \hat{r}) = (\hat{p}^*_{opt}, \hat{r}^*_{opt}). \end{cases}$$

$$\text{with } \; g(\hat{p}) = \frac{\hat{p}}{\hat{p} + \frac{K}{K+\hat{p}}(1+\hat{p})}$$

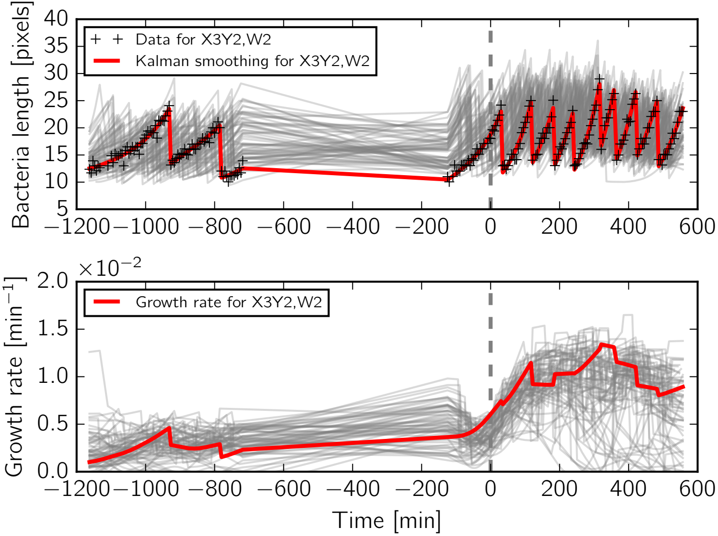
Model predicts the steady-state growth laws



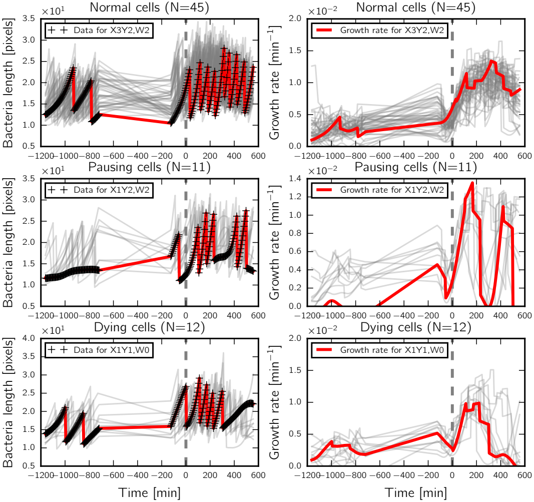
Choosing the optimal α for each environment predicts the empirical growth laws

From data in Scott *et al*, Science, 2010

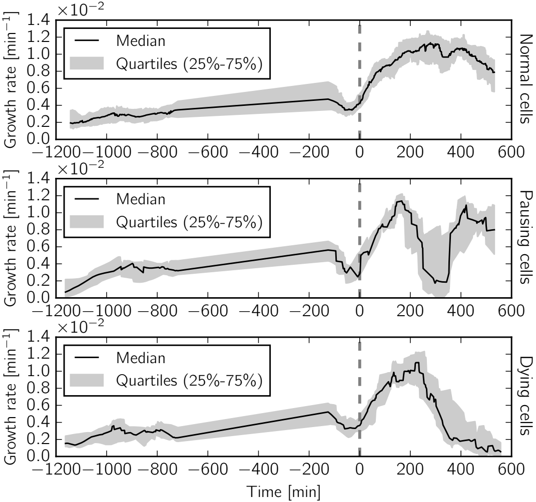
Results of the growth-rate reconstruction (68 cells)



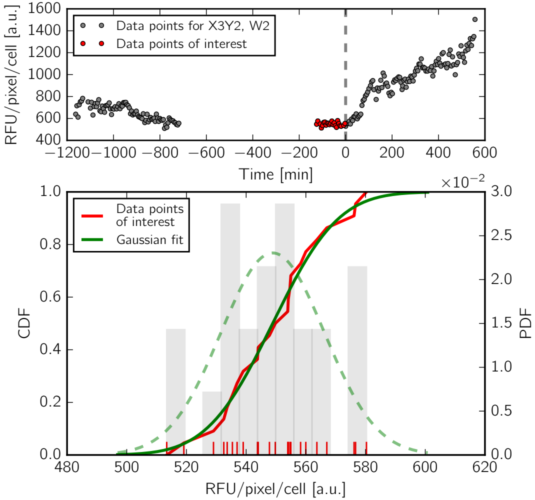
CELL CATEGORIES IDENTIFIED IN THE ANALYSIS



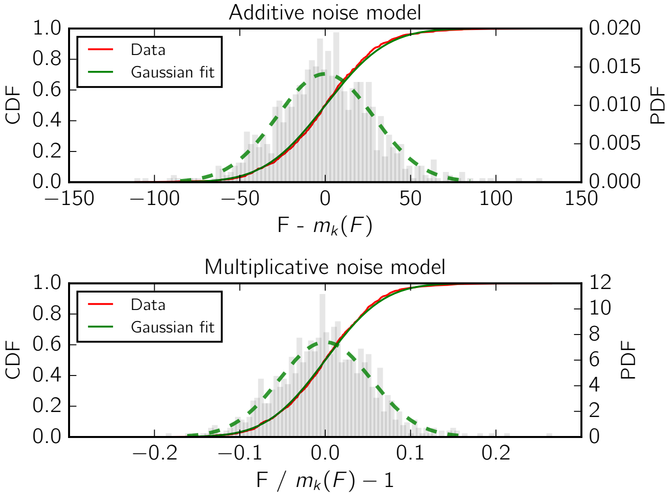
Robust statistics for the cell categories (growth rate)



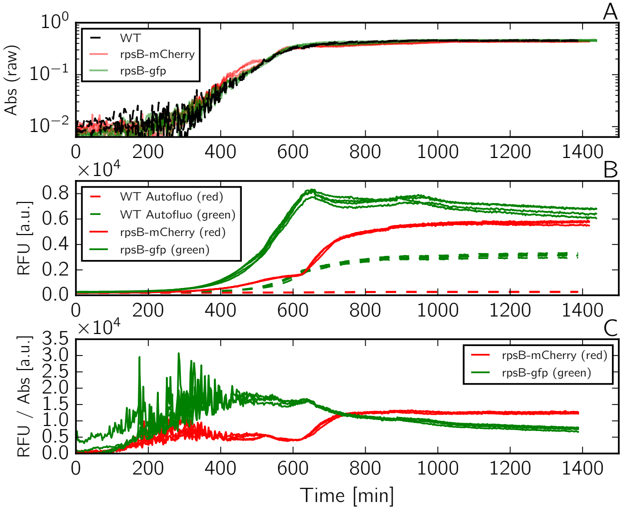
Noise estimation (1/2)



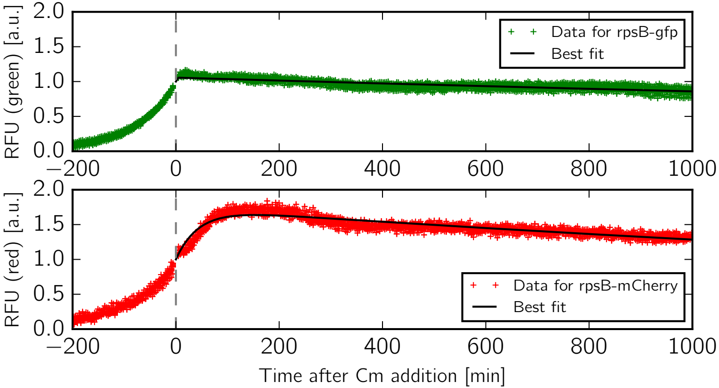
Noise estimation (2/2)



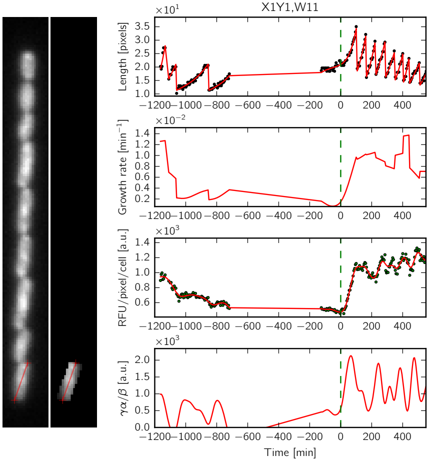
No growth difference between WT and rpsB-tagged strains



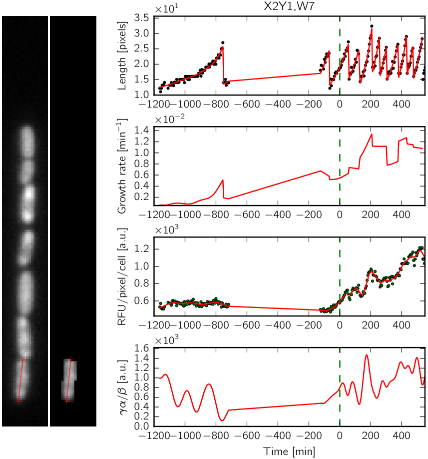
Maturation/Degradation



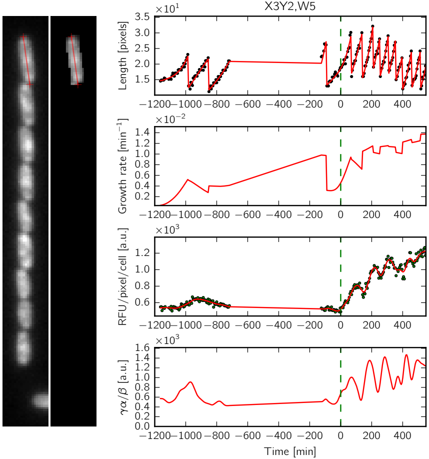
COMPLETE ANALYSIS CELL 1



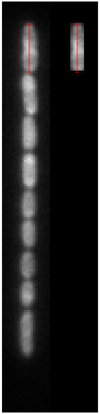
Complete analysis cell 2

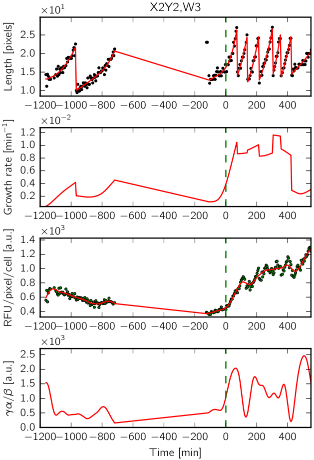


Complete analysis cell 3



Complete analysis cell 4





Complete analysis cell 5