Dynamic optimization of resource allocation in microorganisms

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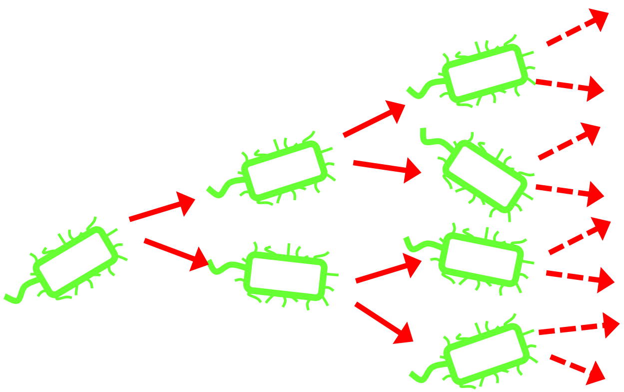
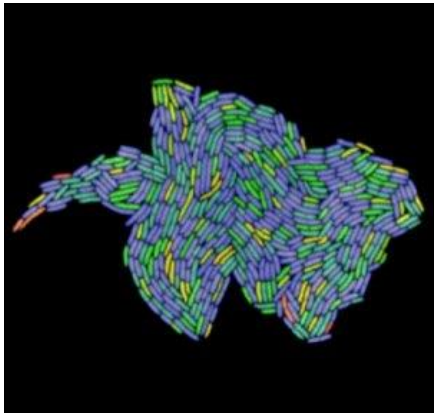
Bacterial growth

- Bacteria are unicellular organisms geared towards **growth**
  - *E. coli* cells have doubling times up to 20 min





Stewart *et al.* (2005), *PLoS Biol.*, 3(2): e45

- **Metabolism** fuels growth by production of energy and building blocks for macromolecules
  - Different **growth rates** depending on medium (carbon, nitrogen, …)

Growth and macromolecular composition

- Macromolecular composition of cell varies with growth rate
  - Quantity of DNA, RNA, protein, … per cell or per volume

TABLE 3 Parameters pertaining to the macromolecular synthesis rates in exponentially growing *E. coli* B/r as a function of growth rate at 37°C

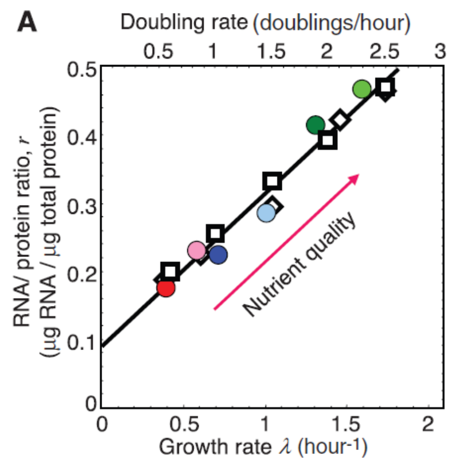
Parameter	Symbol	Units	At τ (min) and μ (doublings per h): τ, 100 μ, 0.6	τ, 60 μ, 1.0	τ, 40 μ, 1.5	τ, 30 μ, 2.0	τ, 24 μ, 2.5	Observed parameter(s)	Footnote
RNA polymerase protein/total protein	α_p	%	0.90	1.10	1.30	1.45	1.55	α_p	*a*
RNA polymerase molecules/cell	N_p	10^3 RNAP/cell	1.5	2.8	5.0	8.0	11.4	α_p, P_C	*b*
RNA polymerase activity	β_p	%	17	20	21	24	30	r_s, r_m, c_s, c_m, N_p	*c*
Active RNA polymerase per cell	N_{ap}	RNAP/cell	205	503	992	1,929	3,298		*c*
Stable RNA synthesized per total RNA synthesized	r_s/r_t	%	41	52	68	78	85	r_s/r_t	*d*
Active RNA polymerase synthesizing stable RNA	ψ_s	%	24	36	56	68	79	r_s/r_t c_s/c_m	*e*
rRNA chain elongation	c_s	Nucl./s	85	85	85	85	85	Indirect	*f*
mRNA chain elongation	c_m	Nucl./s	39	45	50	52	55	Indirect	*g*
Rate of stable RNA synthesis/cell	r_s	10^5 nucl./min/cell	3.0	9.9	29.0	66.4	132.5	R_C	*h*
Rate of mRNA synthesis/cell	r_m	10^5 nucl./min/cell	4.3	9.2	13.7	18.7	23.4	$r_s, r_s/r_t$	*i*
ppGpp concentration	ppGpp/M	pmol/OD_{460}	55	38	22	15	10	ppGpp/M	*j*
	ppGpp/P	pmol/10^{17}aa	8.5	6.6	4.2	2.9	2.0	P_M	*j*
r-Protein per total protein	α_r	%	9.0	11.4	14.8	17.5	21.1	P_M, R_M	*k*
			9	11	13.5	18.0	21.6	α_r	*l*
Ribosome activity	β_r	%	80	80	80	80	80	Indirect	*m*
Peptide chain elongation	c_p	aa residues/s	12	16	18	20	21	Indirect	*n*
Ribosomes/cell	N_r	10^3 ribosomes/cell	6.8	13.5	26.3	45.1	72.0	R_C, f_s, f_t	*o*
tRNA/cell	N_t	10^3 tRNA/cell	65	125	244	419	669	N_r, f_t	*p*
rrn genes/cell	N_{rrn}	Avg no./cell	12.4	15.1	20.0	26.9	35.9	C, D	*q*
rrn genes/genome	N_{rrn}/G	Avg no./genome	7.9	8.2	8.6	9.0	9.5	C	*r*
Initiation rate at *rrn* gene	i_{rrn}	Initiations/min/gene	4	10	23	39	58	N_r, N_{rrn}	*s*
Distance of ribosomes on mRNA	R_m/N_r	Nucl./ribosome	79	85	65	52	41	r_m, c_m, N_r	*t*

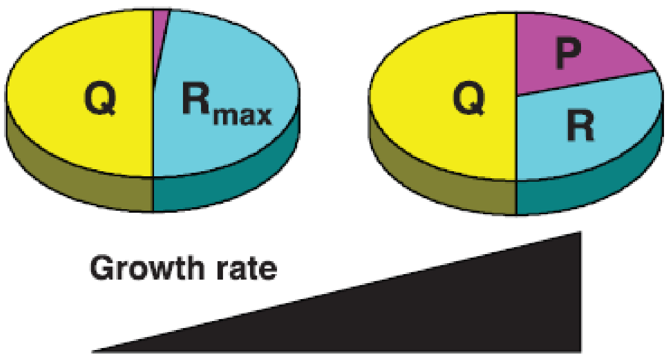
Bremer and Dennis (1996), *Escherichia Coli and Salmonella*, ASM Press, 1553-69

Growth and macromolecular composition

- Phenomenological **growth laws** capture variation of macromolecular composition with growth rate
  - Distribution of proteins over different categories

Scott *et al.* (2010), *Science*, 330(6007):1099-102





- Explanation of growth laws (implicitly or explicitly) based on **optimization principle**
  - Bacteria have evolved so as to distribute limited resources over cellular processes in order to optimize growth (biomass)

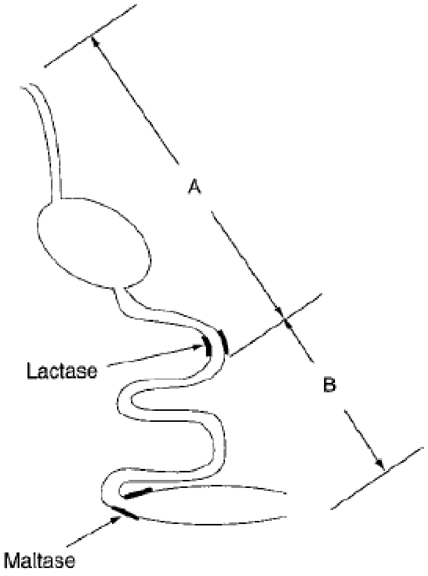
Growth and optimization

“The aim of the RESET project is to break with these classical approaches and propose a novel strategy for improving product yield and productivity.”

- Optimization: models and optimal control
- How does the cell optimize its growth?
- How can we optimize production with external inducer?
- A nice blend of biology, modelling, and mathematics…

Steady-state and dynamic optimization

- Most growth laws and data concern **steady state** (balanced growth)
  - Well-controlled and reproducible in laboratory
- However, most bacteria evolve in **dynamic** environment
  - Example: *E. coli* in human colon

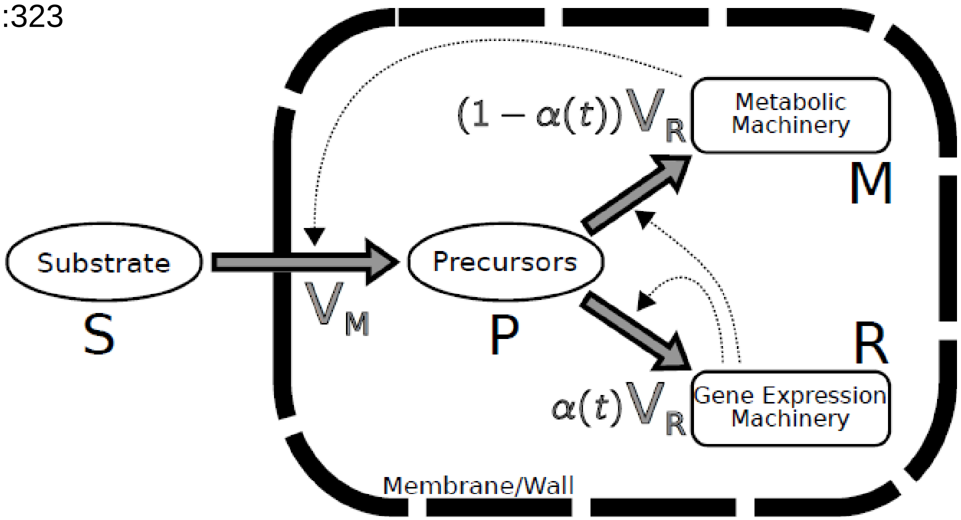


Savageau (1983), *Am. Natural.*, 122(6):732-44

Towards dynamic growth laws

- **Aim**: study optimal allocation of resources to gene expression machinery and metabolism during growth-phase transitions
  - Which allocation is optimal for sustaining maximal growth (biomass)?
- Simple model of cell: bacteria as **self-replicators**

Molenaar *et al.* (2009), *Mol. Syst. Biol.*, 5:323

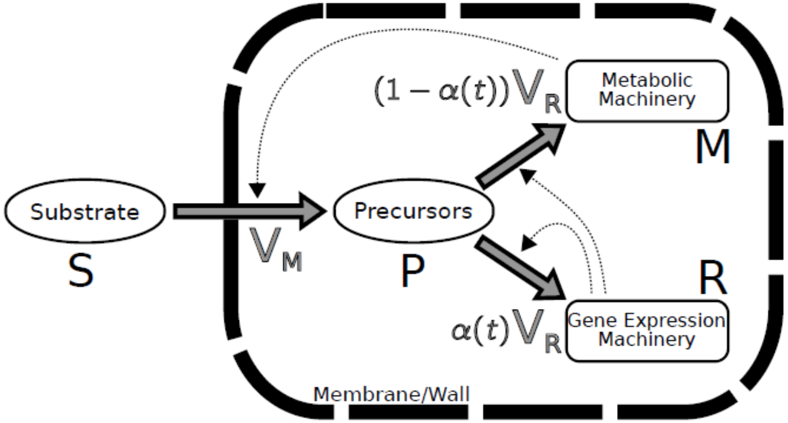


- Tools from **optimal control** theory

Self-replicator model of cell

- Reaction scheme:

$$S \xrightarrow{V_M} P$$
$$nP \xrightarrow{V_R} \alpha R + (1-\alpha) M$$



- Stochiometry model with **extensive** variables:

$$\frac{d}{dt}\begin{bmatrix} P \\ M \\ R \end{bmatrix} = \begin{bmatrix} 1 & -n \\ 0 & 1-\alpha \\ 0 & \alpha \end{bmatrix} \cdot \begin{bmatrix} V_M \\ V_R \end{bmatrix} = N \cdot V$$

- Volume and growth rate:

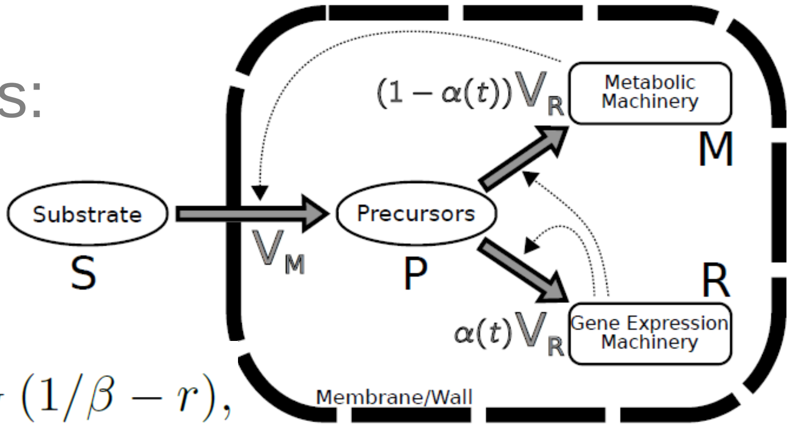
$$Vol = \beta(M + R)$$

$$\mu = \frac{1}{Vol}\frac{d\,Vol}{dt} = \frac{1}{M+R}\frac{d(M+R)}{dt}$$

Reformulated self-replicator model of cell

- Definition of intensive variables:

$$p = \frac{P}{Vol}, \quad m = \frac{M}{Vol}, \text{ and } r = \frac{R}{Vol}$$



- Kinetics:

$$v_M = k_M \frac{s}{K_M + s} m = e_M \left(1/\beta - r\right),$$

$$v_R = k_R \frac{p}{K_R + p} r,$$

$$\mu = \frac{V_R}{R + M} = \beta v_R.$$

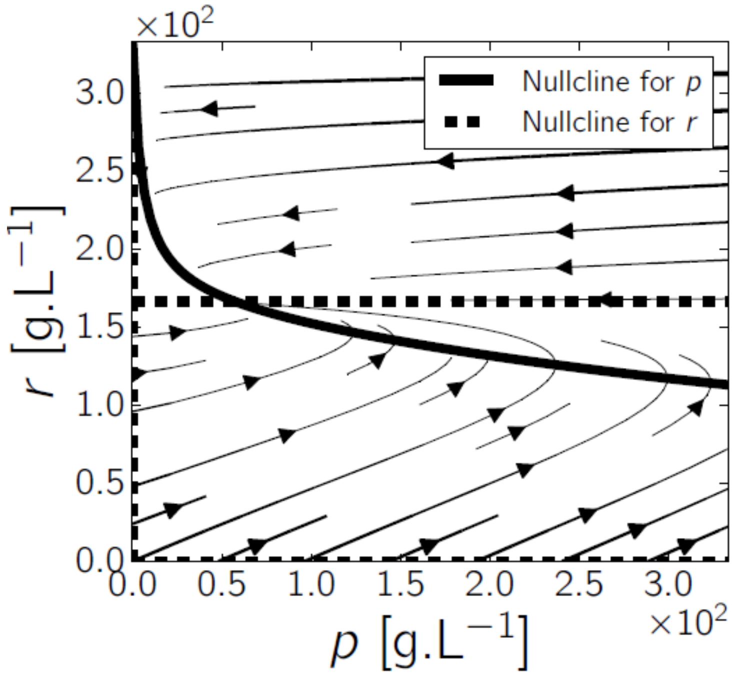
- Model with **intensive** (dimensionless) variables:

$$\begin{cases} \frac{dp}{dt} = E_M \cdot (1 - r) - (1 + p)\frac{p}{K+p} r, \\ \frac{dr}{dt} = (\alpha - r)\frac{p}{K+p} r. \end{cases}$$

Steady-state analysis of model

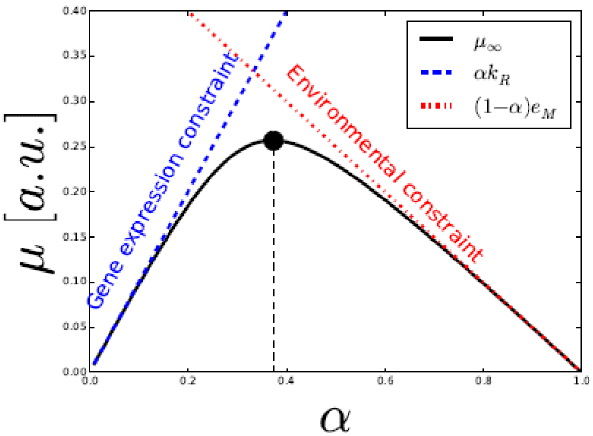
- Control parameter α determines fractional distribution of resources over metabolic and gene expression subsystems
- **Result**: for constant α, the system has a single steady state with growth rate $\mu^*(p^*, r^*, \alpha)$

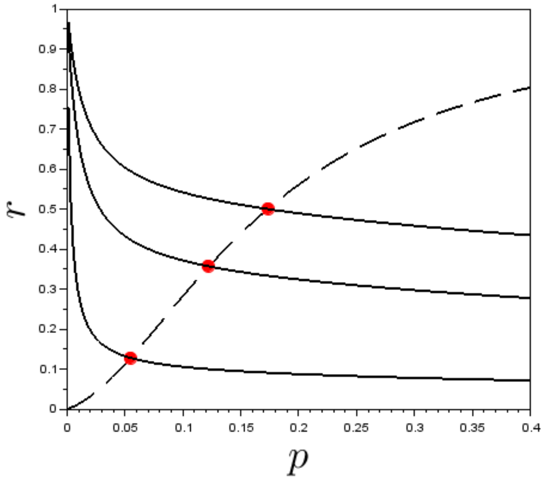
$$\begin{cases} \frac{dp}{dt} = E_M \cdot (1-r) - (1+p)\frac{p}{K+p}r, \\ \frac{dr}{dt} = (\alpha - r)\frac{p}{K+p}r. \end{cases}$$



Steady-state analysis of model

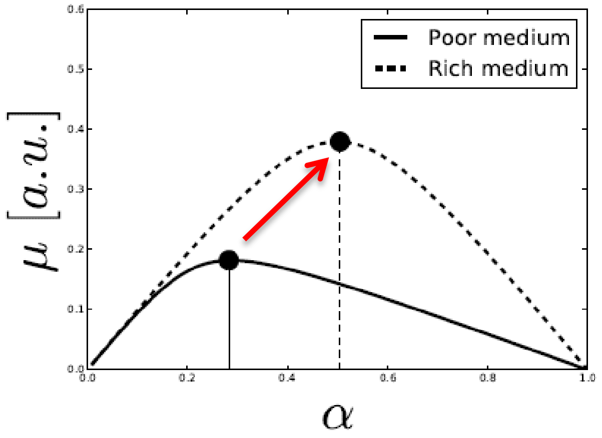
- **Result**: system admits single maximum growth rate for value $\alpha = \alpha_{opt} \in [0, 1]$
    - Maximum varies with medium quality, represented by parameter e_M





Dynamic optimal control problem

- Bacterial cell has to reallocate resources after change in environment to reach optimal growth rate (change α)



- What is the **best dynamic resource allocation strategy**?
- **Optimal control problem** for biomass

$$\max_{\alpha \in \mathcal{U}} J(\alpha) := \int_0^{+\infty} \mu(p, r, \alpha, t) dt$$

with set of admissible controls: $\mathcal{U} = \{\alpha : \mathbb{R}^+ \rightarrow [0, 1]\}$

Pontryagin Maximum Principe

$$H := \lambda_p E_M(1-r) - \frac{p}{K+p} r \left[\lambda_p(1+p) + \lambda_r r + \lambda_0\right] + \alpha\lambda_r \frac{p}{K+p} r.$$

$$\dot{\lambda}_p = \frac{K}{(K+p)^2} r \left[\lambda_p(1+p) + \lambda_r(r-\alpha) + \lambda_0\right] + \frac{p}{K+p} r \lambda_p,$$

$$\dot{\lambda}_r = \lambda_p E_M + \frac{p}{K+p}\left[\lambda_p(1+p) + \lambda_r(2r-\alpha) + \lambda_0\right].$$

The maximization condition is given by:

$$\alpha(t) \in \operatorname{argmax}_{v\in[0,1]} H(x(t), \lambda(t), \lambda_0, v),$$
$$\text{a.e. } t \in [0, +\infty).$$

The switching function:

$$\phi := \lambda_r \tfrac{p}{K+p} r$$

$$\begin{cases} \alpha = 1 \iff \phi > 0, \\ \alpha = 0 \iff \phi < 0. \end{cases}$$

Characterization of singular arcs

- The singular arc corresponds to the optimal steady-state:

$$\phi(t) = \dot{\phi}(t) = 0, \forall t \in [t_1, t_2] \quad \Longrightarrow \quad (\hat{p}(t), \hat{r}(t)) = (\hat{p}^\star_{opt}, \hat{r}^\star_{opt})$$

- Kelley condition:

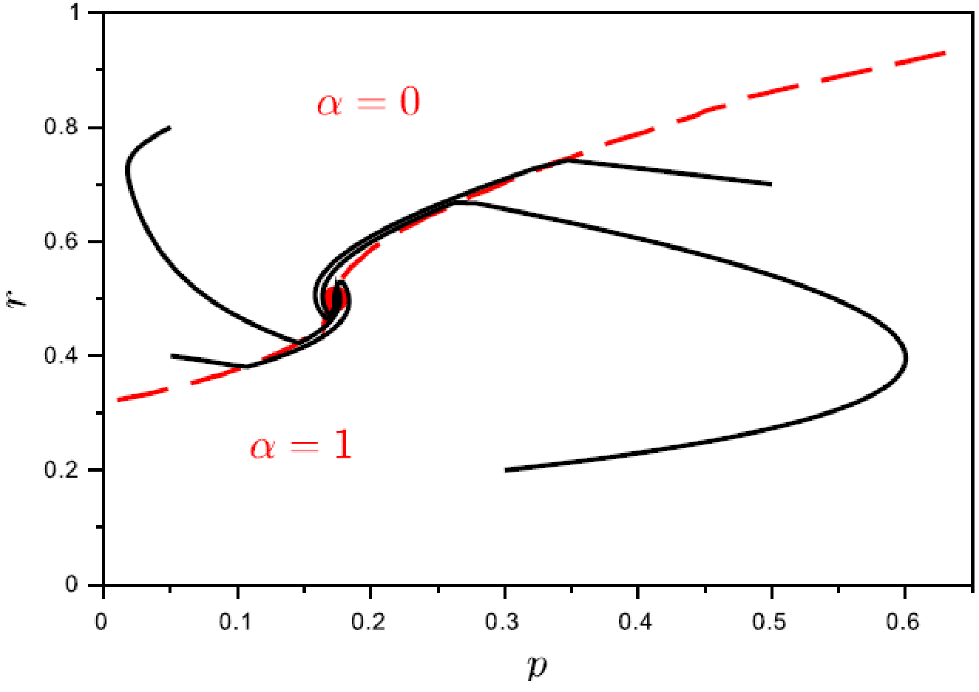
$$(-1)^q \frac{\partial}{\partial \alpha} \frac{d^{2q}}{dt^{2q}} \phi(t) < 0 \quad \text{for } q = 2 \quad \Longrightarrow$$

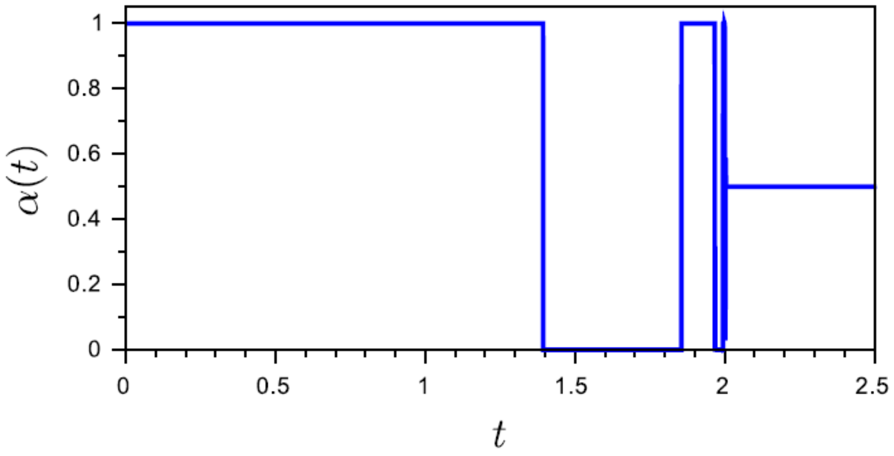
Chattering arc
(Fuller's phenomena)

- Optimal strategy: Turnpike?

Optimal strategy

- Numerical solutions by a direct method (bocop)

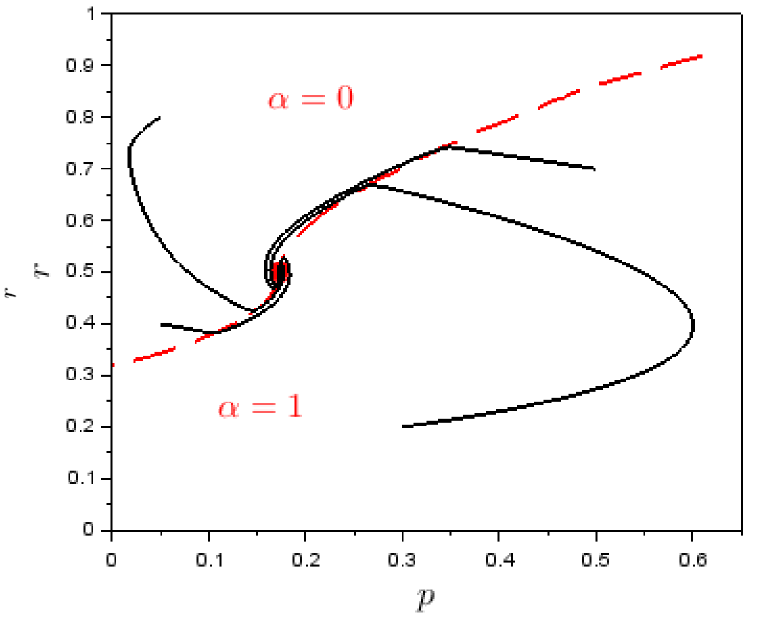


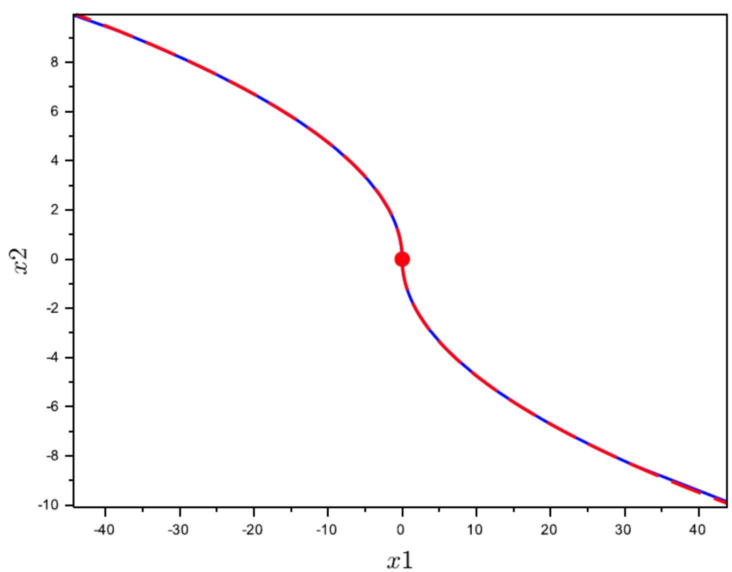


How to compute the switching curve ?

- The tangent of the switching curve at (p_{opt},r_{opt}) is vertical.
- Backward integration starting from (p_{opt},$r_{opt}+\varepsilon$)
- Validation on Fuller's problem:

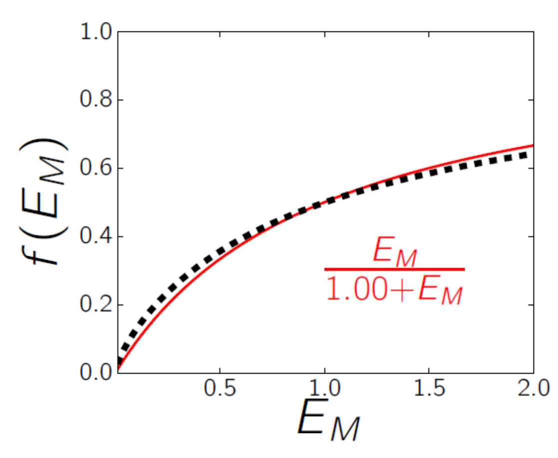
minimize $\int_0^T x_1^2(t)\,dt$ given $\left\{\begin{array}{rcl}\dot{x}_1 & = & x_2,\\ \dot{x}_2 & = & u,\end{array}\right.$

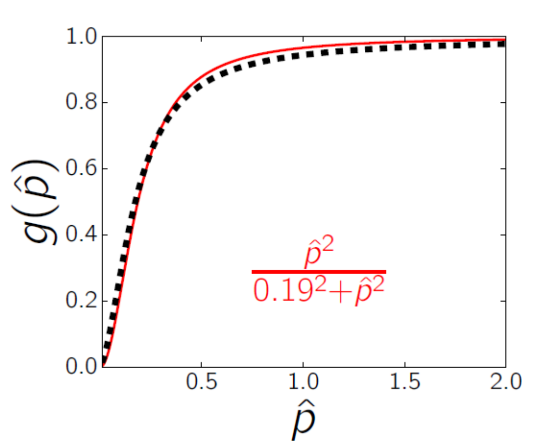


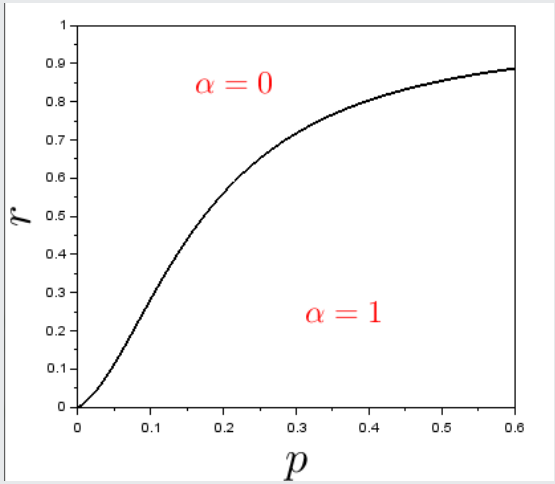


Simple feedback control strategies

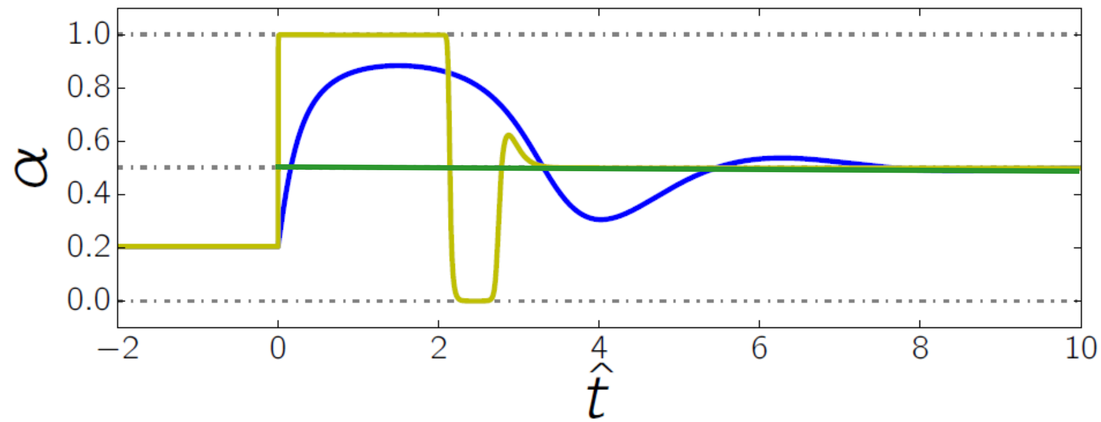
Substrate	Precursor	Switch
$\alpha = f(E_M)$	$\alpha = g(\hat{p})$	$\alpha = \begin{cases} 0, & \text{if } \hat{r} > g(\hat{p}) \\ 1, & \text{if } \hat{r} < g(\hat{p}) \end{cases}$

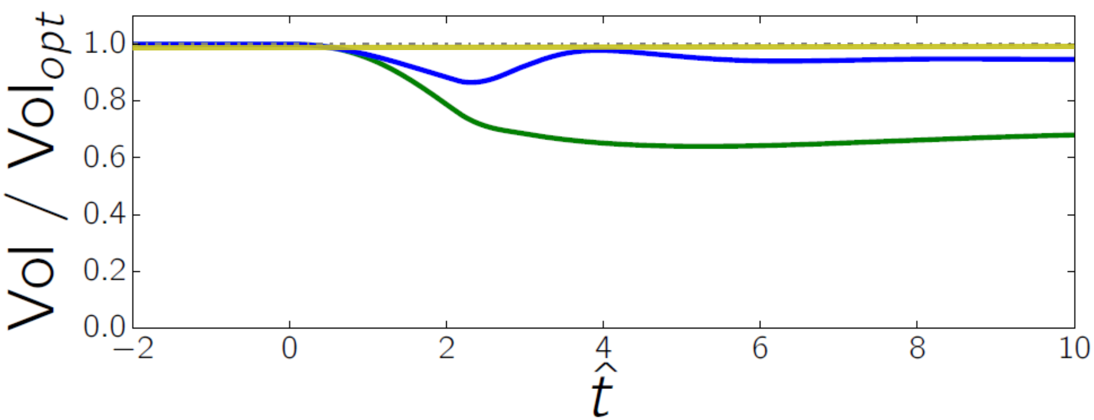


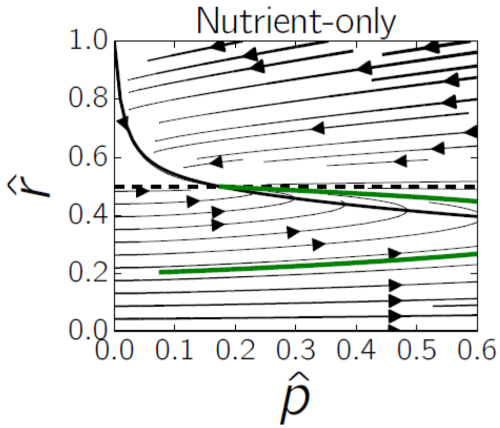


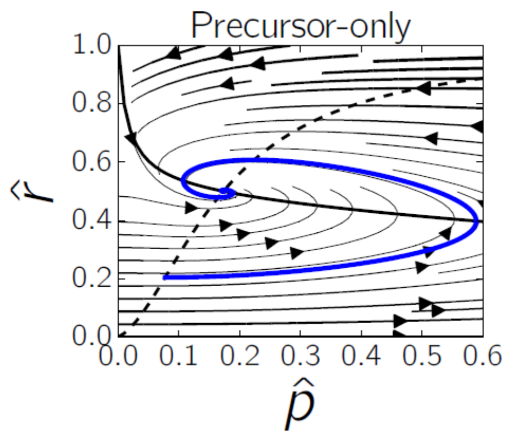


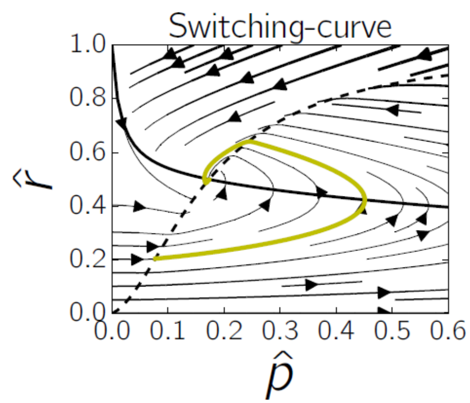
Comparison of control strategies





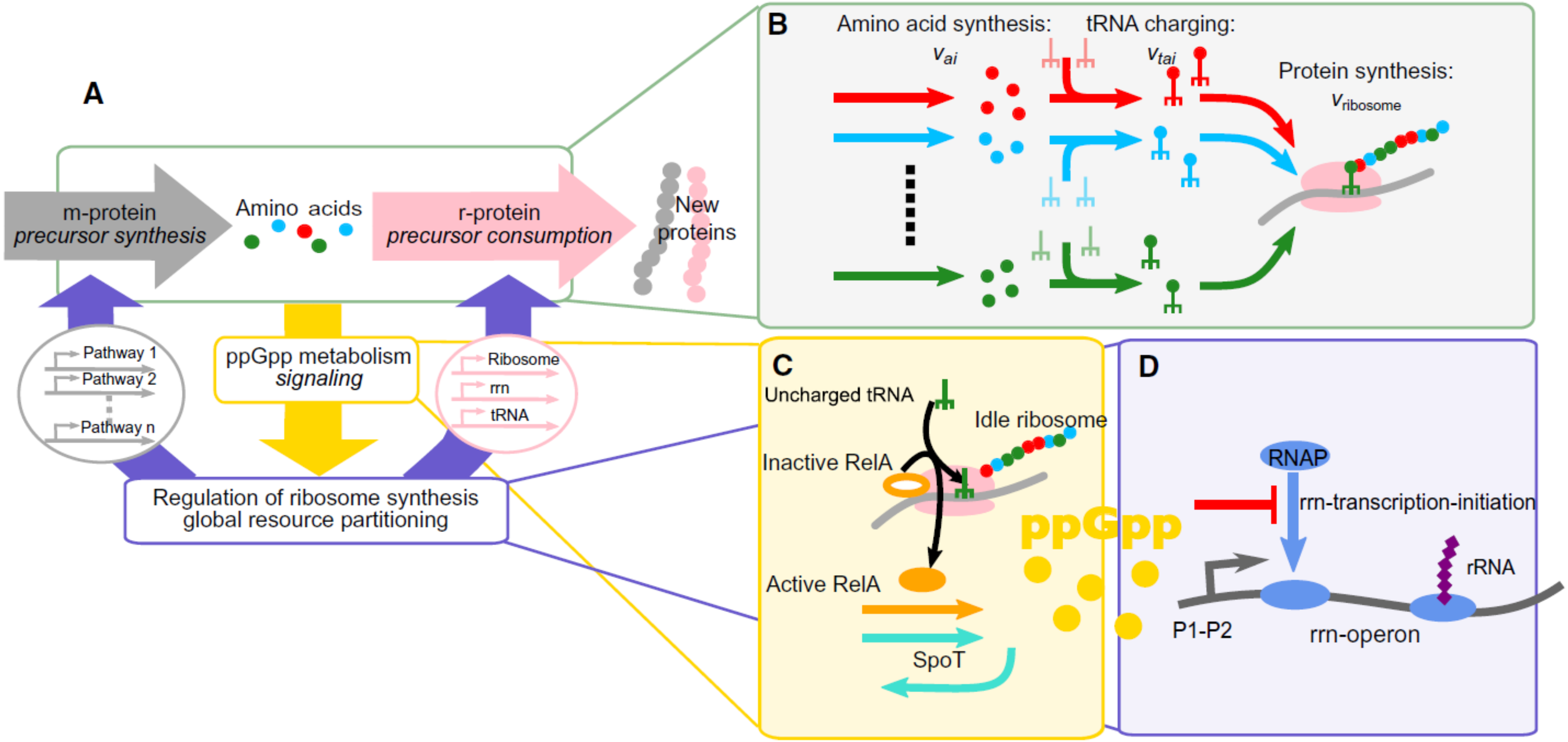






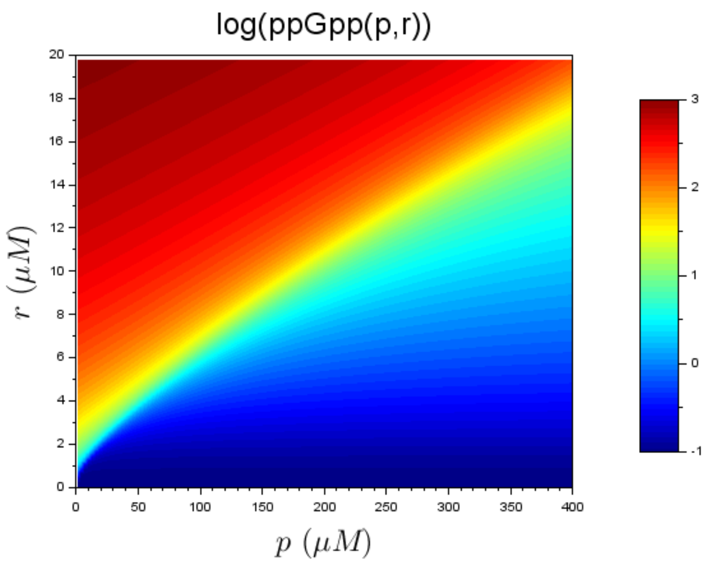
Biological implementation?

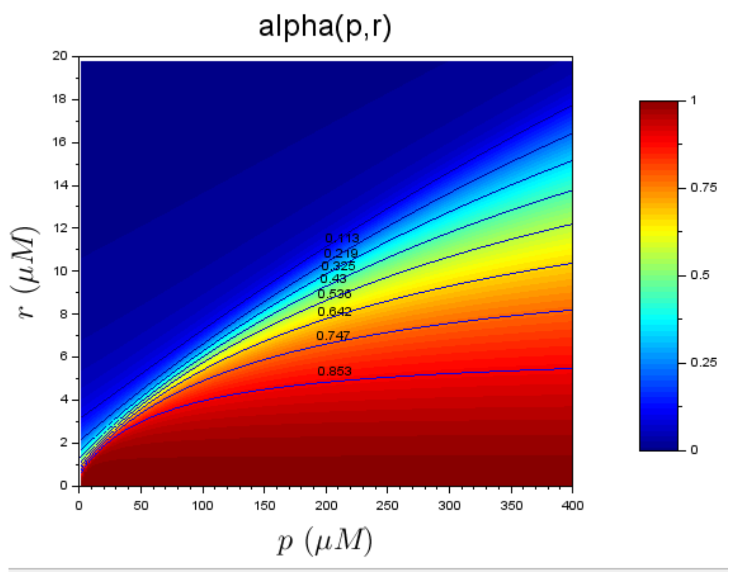
- Regulation of ressource allocation via ppGpp (Bosdriesz et al., 2015)



Biological implementation?

- Regulation of resource allocation via ppGpp (Bosdriesz et al., 2015)
- Quasi steady-state approximation:
  – Fast variables: ppGpp, tRNA





Conclusions and perspectives

- Study of resource allocation in bacteria from first principles
  - Self-replicator model derived from two macro-reactions and some common assumptions on reaction kinetics

- Optimal strategy: turnpike with chattering

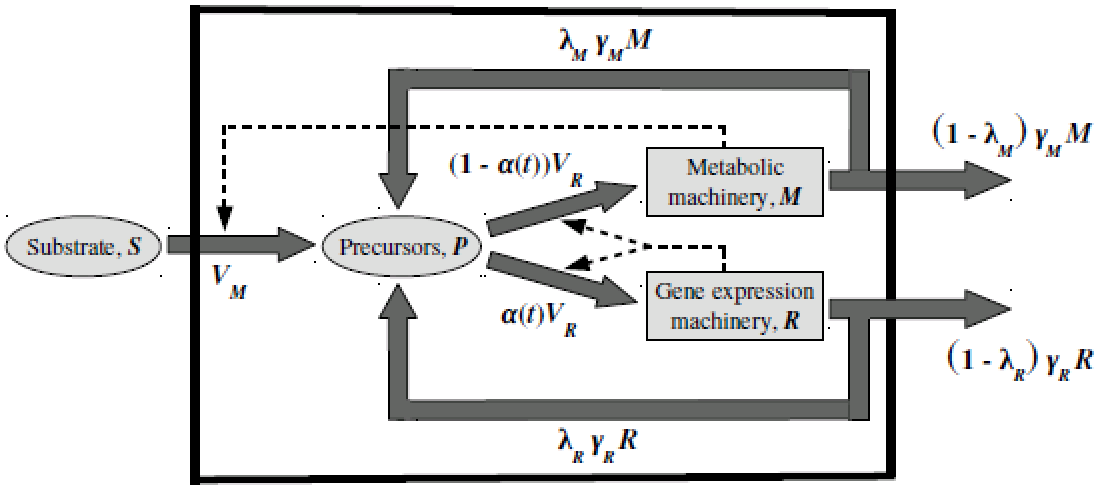
- Near-optimal strategy: switch depending on the imbalance between precursors and ribosomes

- Implementation via ppGpp

- Experimental test of control strategy using fluorescent reporters

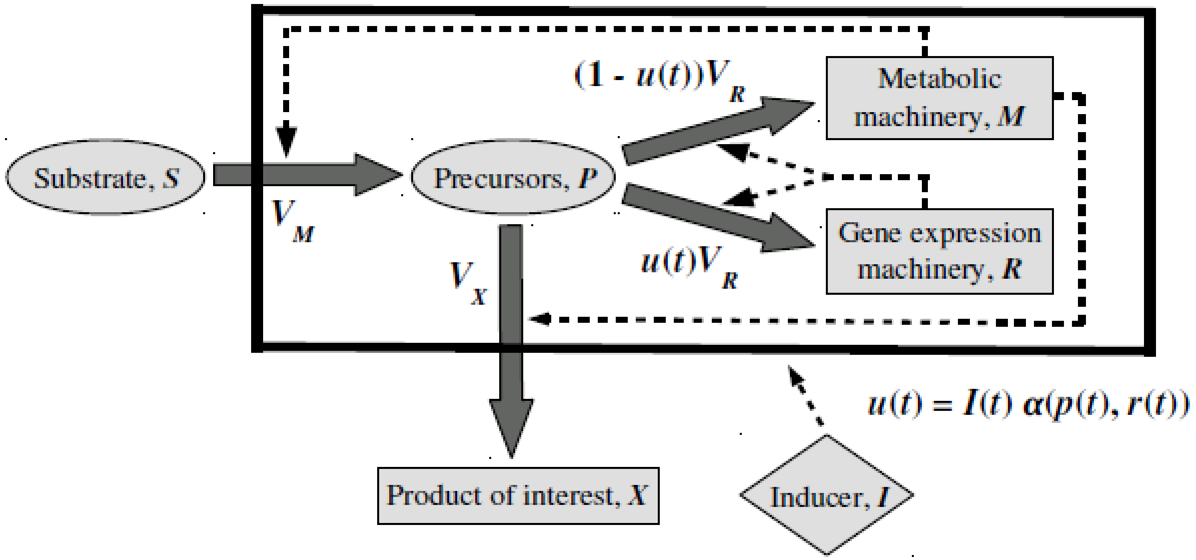
Conclusions and perspectives

- Some generalizations: degradation and recycling
- Similar results (paper submitted to OCAM)



Conclusions and perspectives

- Some generalizations: maximum of production with an inducer (Reset)
- Growth or production?
- Limited amount of substrate? ANR Maximic, new…



Collaborators (RESET)

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