Stationary states of genome scale metabolic networks in continuous cell cultures

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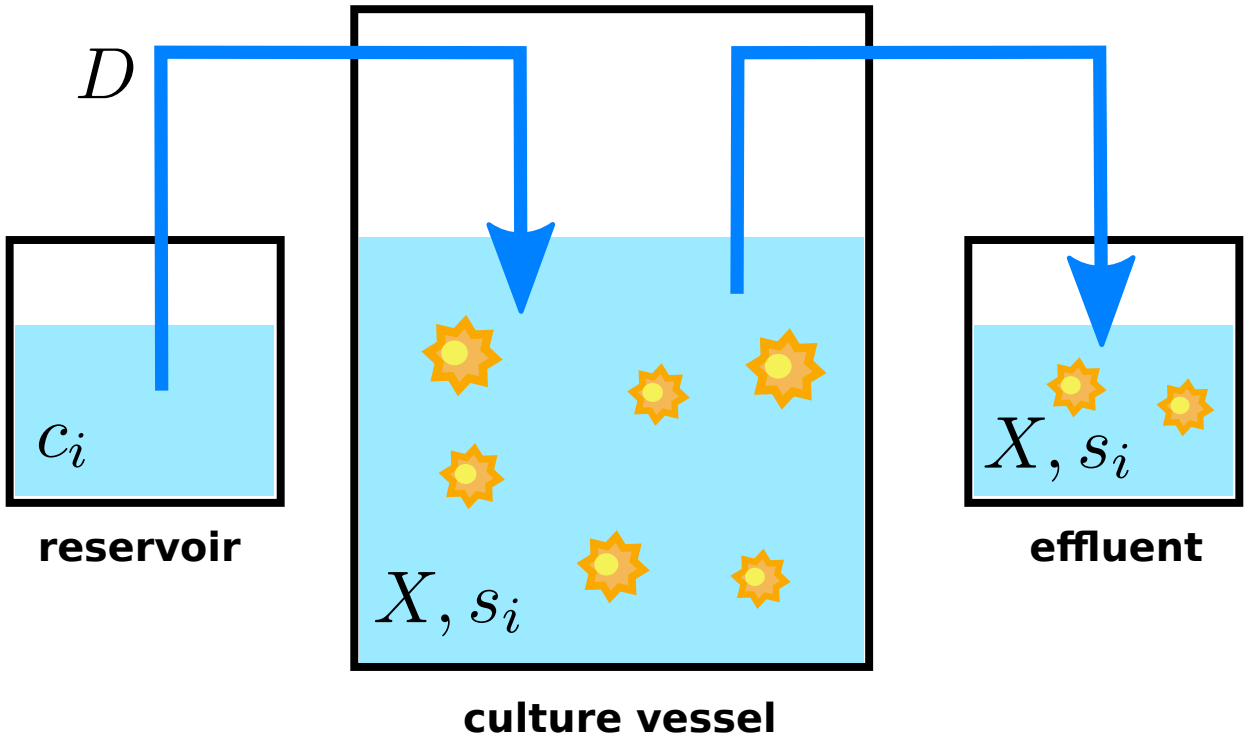
D
c_i
reservoir
X, s_i
culture vessel
X, s_i
effluent

Requirements

- It must include the internal metabolism
- It must include the chemostat
- Be computationally scalable to Genome scale metabolic networks
- Be flexible
- Toxicity
- Heterogeneity

Outline

Chemostat

$$\frac{dX}{dt} = (\mu - \sigma - D)X \tag{1}$$

$$\mu = \mu(\nu) \qquad \sigma = \sigma(s) \tag{2}$$

$$\frac{ds_i}{dt} = -u_i X - (s_i - c_i)D \tag{3}$$

The cell

$$lb_k \leq r_k \leq ub_k$$

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This is a polytope in very high dimensions

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The cell maximizes biomass production μ
Linear Programming LP

Mathematical framework

$$\frac{dX}{dt} = (\mu - \sigma - D)X$$

$$\mu = \mu(\nu) \qquad \sigma = \sigma(s)$$

$$\frac{ds_i}{dt} = -u_i X - (s_i - c_i)D$$

$$lb_k \leq r_q \leq ub_k$$

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Flux Balance

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The cell maximizes biomass production μ LP

$$u_i^*(\xi) \ldots \mu(\xi)$$

Equilibrium in metabolite's concentration

$$\frac{ds_i}{dt} = -u_i^* X - (s_i - c_i)D$$

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$$D = \mu^*(\xi) - \sigma^*(\xi)$$

Stationarity in cell's concentration

$$\frac{dX}{dt} = (\mu - \sigma - D)X$$

$$\frac{X^*}{\xi} = \mu^*(\xi) - \sigma^*(\xi)$$

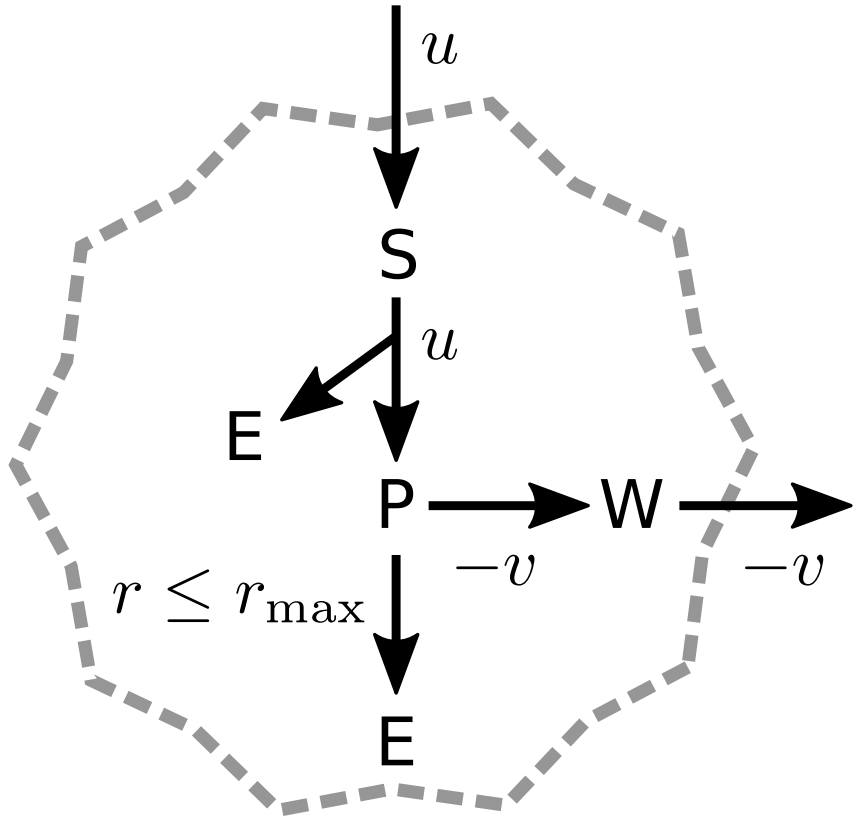
Stationarity equations

$$r_k^* \dots u_i^*(\xi) \dots \mu^*(\xi)$$

$$s_i^*(\xi) = c_i - u_i^*(\xi)\xi$$

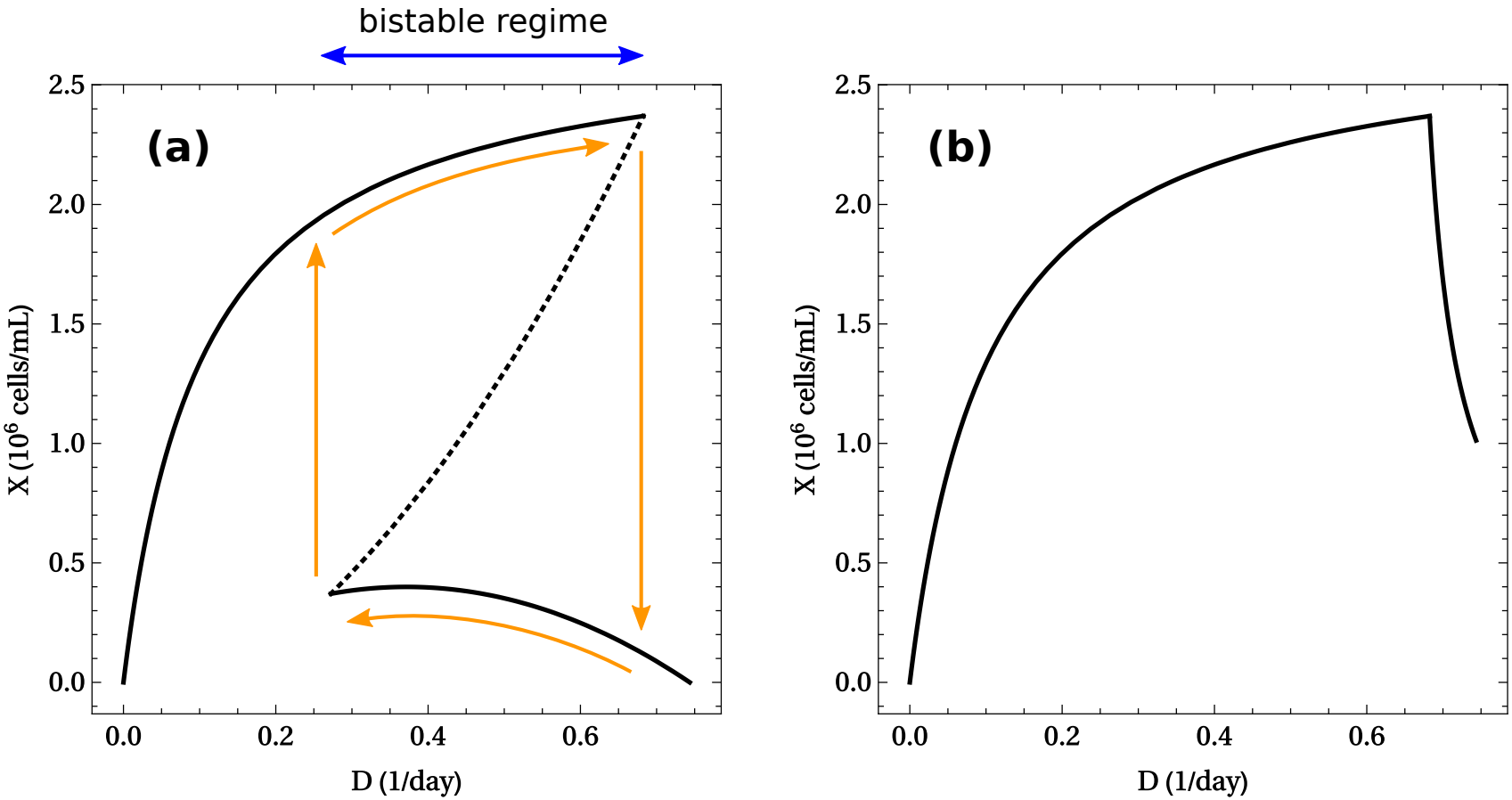
$$\frac{X^*(\xi)}{\xi} = \mu^*(\xi) - \sigma^*(\xi)$$

Small Network

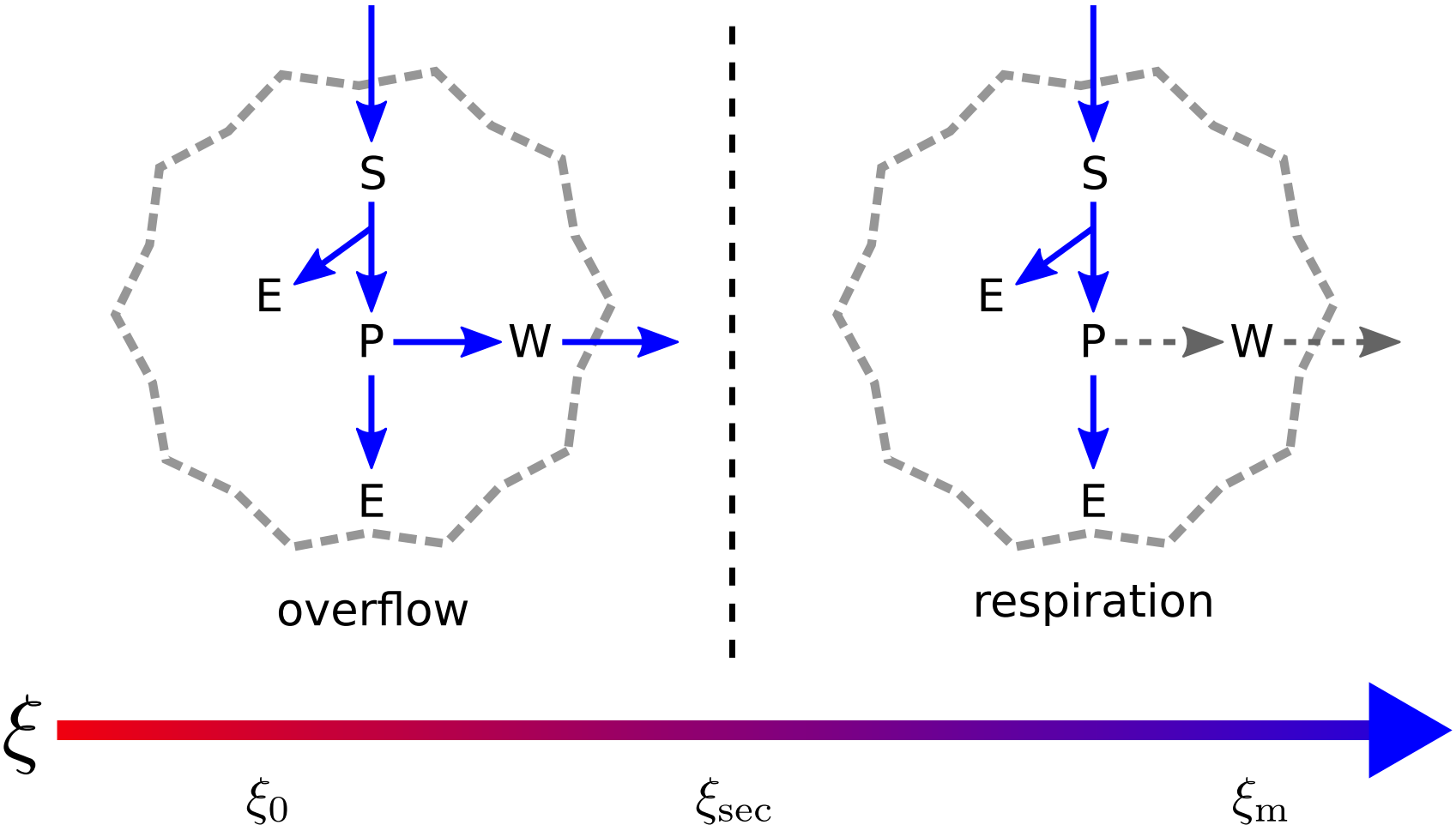


Vazquez et al.. Macromolecular crowding explains overflow metabolism in cells. Scientific Reports 6, 31007 (2016)

Toxicity is the key point



General Picture

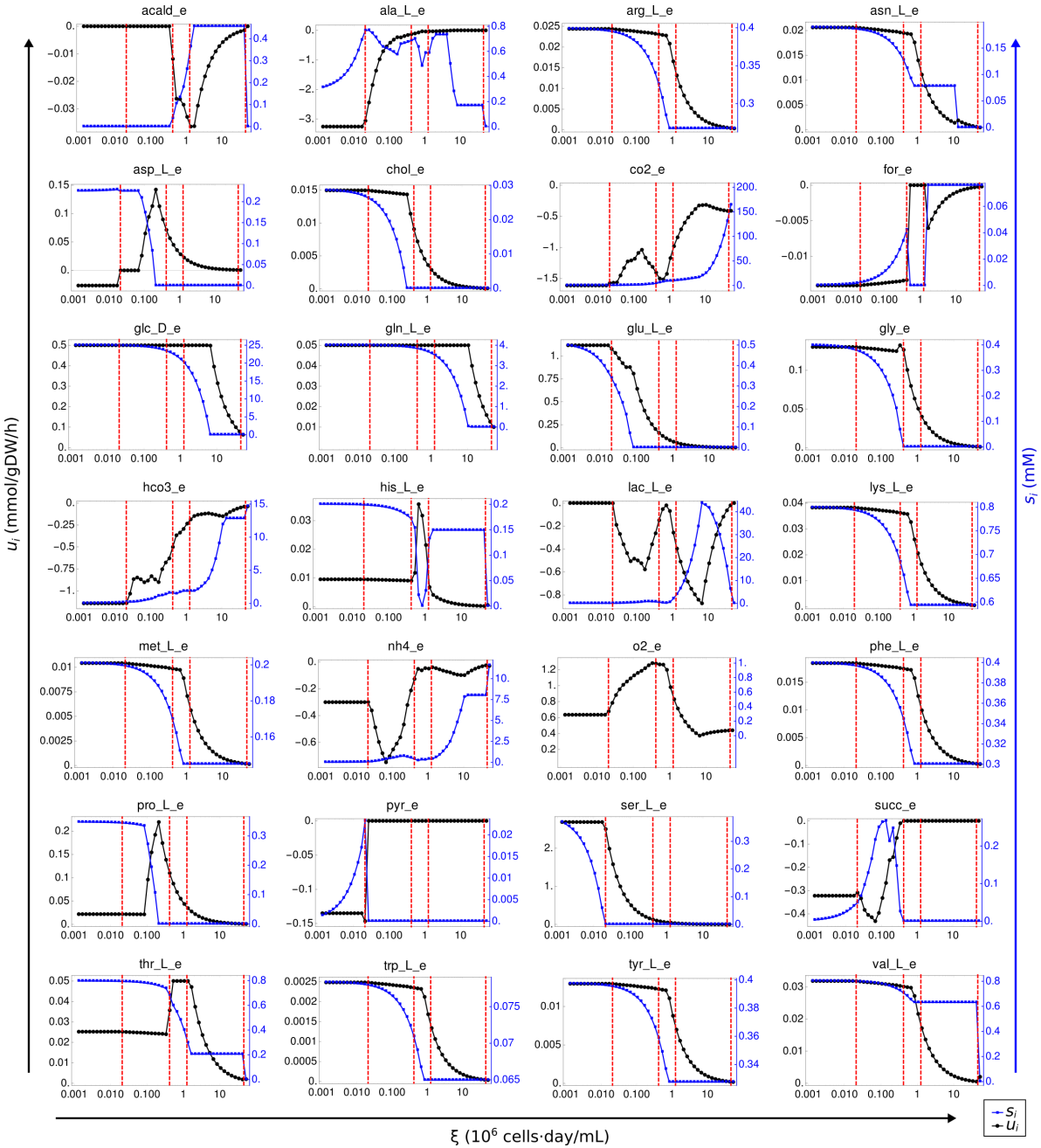


(a) Overflow. At high enough nutrient uptake the respiratory flux hit s the upper bound $r_{\max}$ and the remaining nutrients are exported as W. **(b)** Respiration. The nutrient is completely oxidized with a large energy yield. **(c)** Threshold values of ξ. ξ_0 delimits the nutrient excess regime ($\xi < \xi_0$) from the competition regime ($\xi > \xi_0$). ξ_{sec} delimits the transition between overflow metabolism ($\xi < \xi_{\text{sec}}$ and respiration ($\xi > \xi_{\text{sec}}$). Finally, maintenance demand cannot be met beyond $\xi > \xi_{\text{m}}$.

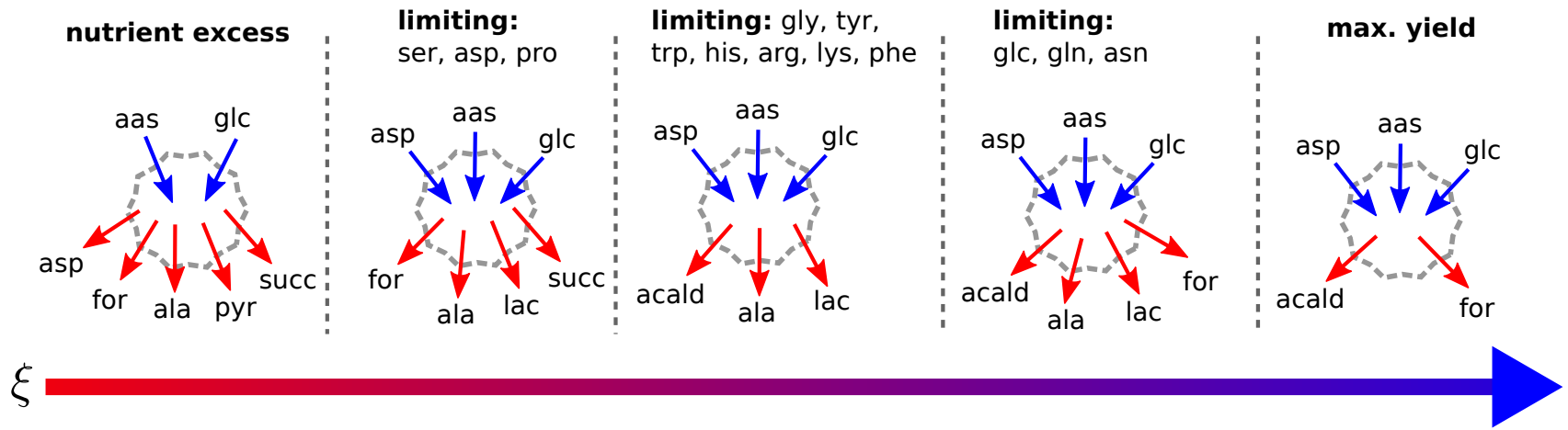
Genome Scale: CHO-K1 line

- 6663 reactions
- $V_{glc} = 0.5 mmol/\text{gDW/h}$
- $V_i = .1 V_{glc}$

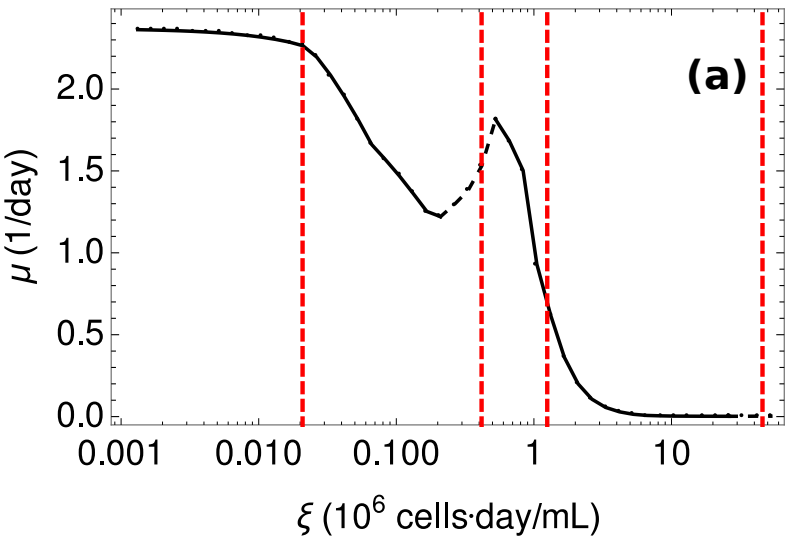
Metabolite uptakes and concentration

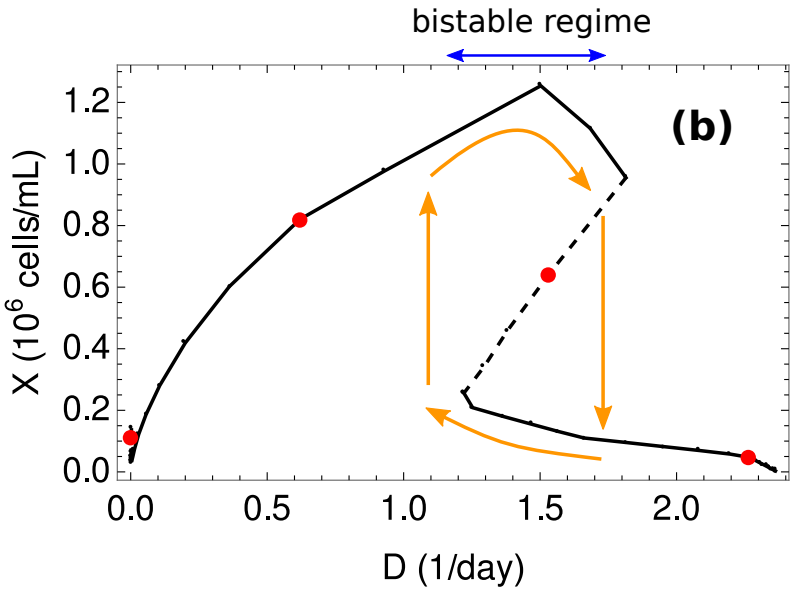


General picture of the transitions



Steady state and bifurcation





J. Fernandez-de-Cossio Diaz, K. León and **R. M.**, *Characterizing stationary states of genome scale metabolic networks in continuous culture, PLOS Computational Biology.* **13** *(11): e1005835 (2017)*

Constraints

$$\sum_k S_{ik} r_k - e_i - y_i \mu + u_i = 0$$

$$lb_k \leq r_q \leq ub_k$$

$$-L_i \leq u_i \leq min\{V_i, c_i \frac{D}{X}\}$$

$$\sum_i r_i < K$$

We must explore this polytope

Stationarity: Dealing with the heterogeneity

$$\frac{d\vec{X}}{dt} = (\mu - \sigma - D)\vec{X}$$

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$$\textit{Effetive Growth rate} = \mu(\nu) - \sigma(s) = \overline{D}$$

Maximum Entropy Principle

$$\textit{If } s \textit{ is fixed, } \mu(\nu) - \sigma(s^*) = D$$

Maximum Entropy Principle

$$P_{s^*}(\nu) \sim e^{\beta(\mu(\nu)-\sigma(s^*))}$$

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$$s_i = c_i - \frac{1}{D}\sum_a u_i^a$$

Maximum Entropy Principle

$$P_{s^*}(\nu) \sim e^{\beta(\mu(\nu)-\sigma(s^*))}$$

$$s_i^* = c_i - \frac{X}{D}\int_{\Pi} u_i(\nu) P_{s^*}(\nu) d\nu$$

In short

$$D = \frac{X}{\xi} = \frac{\int_{\Pi} d\nu \left[\mu(\nu) - \sigma(s^*)\right] e^{\beta(\mu(\nu)-\sigma(s^*))}}{\int_{\Pi} d\nu e^{\beta(\mu(\nu)-\sigma(s^*))}}$$

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$$s_i^* = c_i - \xi \int_{\Pi} u_i(\nu) P_{s^*}(\nu) d\nu$$

Homogeneous vs Heterogeneous Chemostat

$$D = \frac{X}{\xi} = \langle \mu(\nu) - \sigma(s^*) \rangle_{P_{s^*}}$$

$$s_i^* = c_i - \xi \int_{\Pi} u_i(\nu) P_{s^*}(\nu) d\nu$$

$$\frac{X^*(\xi)}{\xi} = \mu^*(\xi) - \sigma^*(\xi)$$

$$s_i^*(\xi) = c_i - \xi u_i^*(\xi)$$

Summarizing

$$\sum_k S_{ik} r_k - e_i - y_i \mu + u_i = 0$$

$$lb_k \leq r_q \leq ub_k$$

$$-L_i \leq u_i \leq min\{V_i, c_i \frac{D}{X}\}$$

$$\sum_i r_i < K$$

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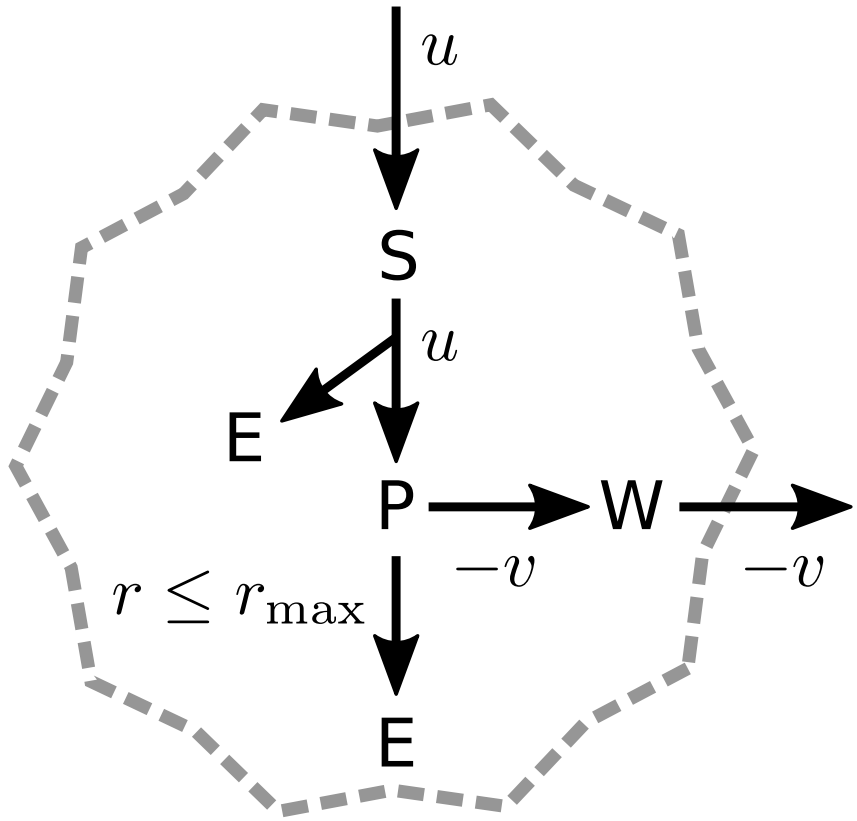
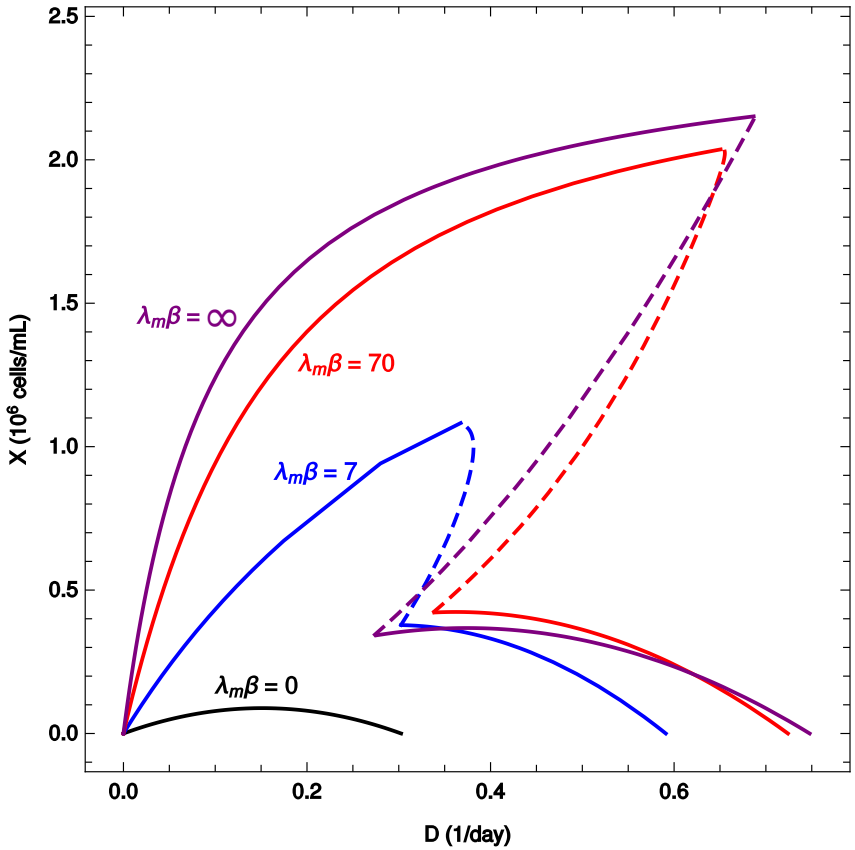
Small Network again



Effect of the heterogeneity



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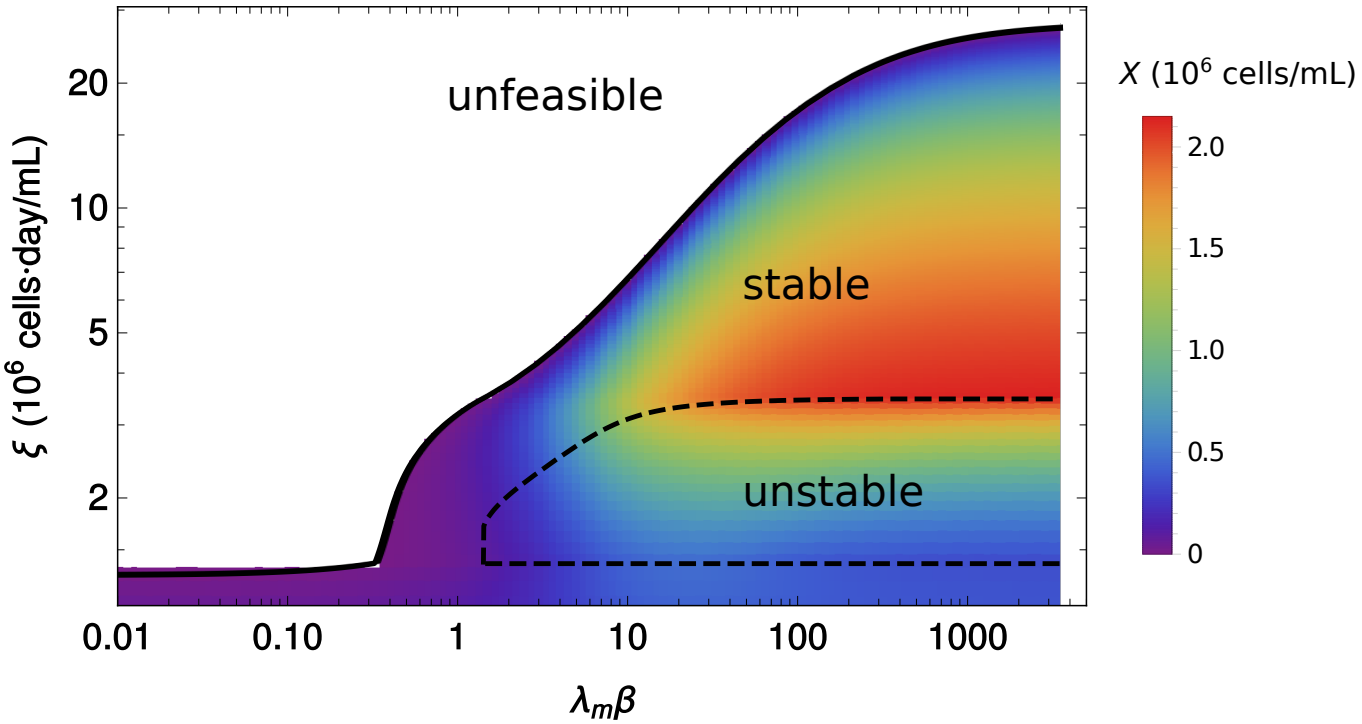

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Exploring the space

$$\prod$$

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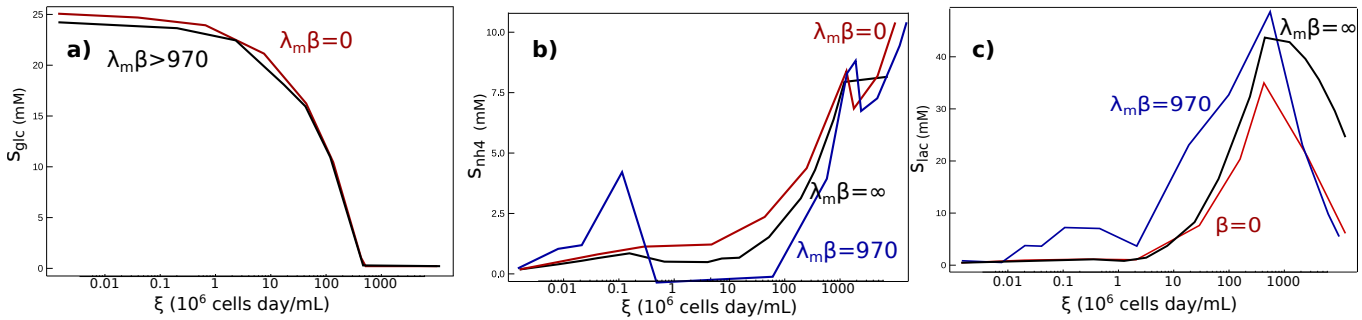
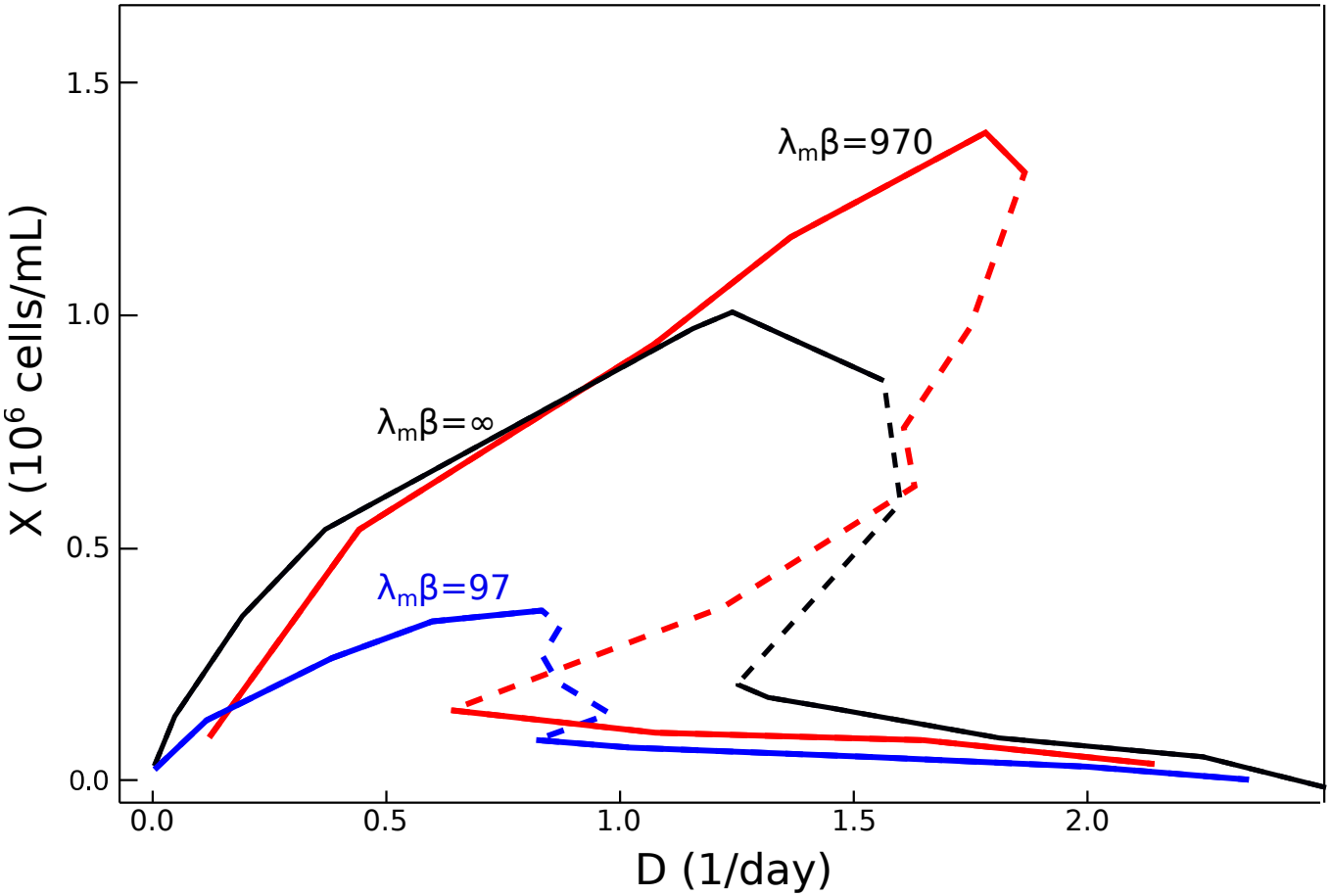
For $\beta = \infty$: Expectation Propagation *Alfredo Braunstein, Anna Paola Muntoni, Andrea Pagnani, An analytic approximation of the feasible space of metabolic networks, Nat. Comm.* **8**, *14915 (2017)*

Here generalized for finite β

Genome Scale Metabolic Networks



Genome Scale Metabolic Networks



J.

Fernandez-de-Cossio Diaz, and **R. M.**, *Maximum Entropy and Population Heterogeneity in continuos cell cultures*,

arXiv:1807.04218

Conclusions

- We developed a mathematical framework to determine the stationary states in a chemostat
- The presence of toxic waste:
  - drives the appareance of many stationary states
  - makes relevant the history of the system
- We provided a scheme to estimate the metabolic flux distribution of an heterogeneous culture in a chemostat
- The presence of heterogeneity in the culture
  - changes the concentration of metabolites
  - allows stationary states with a larger number of cells
- Everything is computationally tractable in Genome Scale metabolic networks

Collaborators and acknowledgments

- Jorge Fernández de Cossio. Centre for Molecular Immunology-CIM and Physics Faculty, UH. Cuba
- Kalet León. Centre for Molecular Immunology-CIM. Cuba

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- Daniele de Martino. IST, Viena. Austria
- Ernesto Chico. Centre for Molecular Immunology. Cuba