Economic Principles in Cell Biology
Vienna, July 23–26, 2025

Growth Balance Analysis

Hugo Dourado

Why yet another "balance analysis"?

Growth Balance Analysis (GBA): simplified framework for nonlinear self-replicating cell models at balanced growth[1].

- **Nonlinear:** includes nonlinear kinetic rate laws.
- **Self-replicating:** metabolism + protein synthesis and dilution of **all** components.
- **Balanced growth:** constant (external and internal) concentrations in time.

A framework, not a model: find common properties to all possible models.

Mathematical simplification: allows analytical study to find fundamental principles.

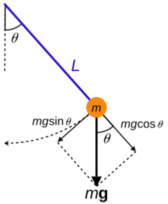
---

[1] Dourado & Lercher, An analytical theory of balanced cellular growth, *Nature Communications* 2020.

Mathematical simplification: the least number of variables and equations

Not important for linear problems, but critical for nonlinear problems!

Example: Simple pendulum



Angle θ ("generalized coordinate") completely determines the system state, no need of x,y,z.

Why looking for simplest formulation?

- Easier numerical calculations.
- Independent variables are preferable for analytical methods.
- Deeper understanding of the problem.
- Most "elegant" solution.

Balanced growth (or steady-state growth)

For a steady-state environment defined by “external” concentrations $\mathbf{a}$:

- Steady-state growth rate μ (1/h), direct measure of fitness.
- Steady-state internal concentrations $\mathbf{c}$ (g/L) of reactants (substrates, products)

$$c_i = \frac{\text{abundance of “i” (g/cell)}}{\text{volume (L/cell)}} = \text{constant}$$

Mass concentrations (not abundances) better describe cell states: i) constant, ii) reaction kinetics depend on concentrations, iii) relate to cell density (g/L).

Matching units for fluxes $\mathbf{v}$: mass per volume per time (g L^{-1} h^{-1}).

Main differences to other modeling frameworks

Density constraint: cell density[2] ρ (g/L) including **all** mass concentrations c (g/L)

$$\rho = c_\mathrm{p} + \sum_m c_m$$

where c_p is the total protein concentration, and m are all "non-protein" components[3].

Units: to match the units, we normalize $\mathbf{N}$ with the molecular weights $\mathbf{w}$ (g/mol)

$$\mathbf{N} \xrightarrow[\text{columns by } \mathbf{w}]{\text{multiply}} \mathrm{diag}(\mathbf{w})\,\mathbf{N} \xrightarrow[\sum(-)=-1,\ \sum(+)=1]{\text{scale columns, s.t.}} \mathbf{M}_\mathrm{total} \xrightarrow[\text{external rows}]{\text{exclude}} \mathbf{M}$$

$\mathbf{M}$ entries are mass fractions of reactants into $(-)$ and out $(+)$ each reaction.

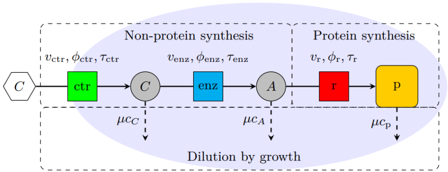
---

[2] Baldwin et al. *Archives of Microbiology* 1995, Kubitschek et al. *Journal of bacteriology* 1983, Cayley et al. *Journal of Molecular Biology* 1991

[3] Dourado et al. *PLOS Comp Bio* 2023.

GBA models: transport, protein synthesis, kinetics, dilution by growth

A) Scheme of a simple GBA model "L3"



B) Mathematical definition $(\mathbf{M}, \rho, \boldsymbol{\tau})$ of the model L3:

$$\mathbf{M} = \begin{array}{c} \\ \\ \\ \end{array}\begin{array}{ccc|c} [\text{ctr}] & [\text{enz}] & [\text{r}] & \\ 1 & -1 & 0 & (C) \\ 0 & 1 & -1 & (A) \\ 0 & 0 & 1 & (p) \end{array}$$

$$\rho = 340 \; gL^{-1}$$

$$\boldsymbol{\tau} = \left(\frac{1}{7}\left(1 + \frac{0.1}{a_C}\right), \; \frac{1}{7}\left(1 + \frac{23}{c_C}\right), \; \frac{1}{6}\left(1 + \frac{41}{c_A}\right) \right)$$

C) Mathematical definition of a (static) medium:

$$a_C = 10 \; gL^{-1}$$

D) Cell states $(\mu, \mathbf{v}, \mathbf{c})$ must satisfy:

i) Mass conservation: $\mathbf{M}\,\mathbf{v} = \mu\mathbf{c}$

$$\underbrace{\begin{bmatrix} 1 & -1 & 0 \\ 0 & 1 & -1 \\ 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} v_{\text{ctr}} \\ v_{\text{enz}} \\ v_{\text{r}} \end{bmatrix}}_{\text{flux balance}} = \underbrace{\mu \begin{bmatrix} c_C \\ c_A \\ c_{\text{p}} \end{bmatrix}}_{\substack{\text{dilution} \\ \text{by growth}}}$$

where $\mathbf{s} :=$ column sums of $\mathbf{M} = (1, 0, 0)$

ii) Kinetic rate laws and protein sum: $\mathbf{v} \cdot \boldsymbol{\tau}(\mathbf{a}, \mathbf{c}) = c_{\text{p}}$

$$\frac{v_{\text{ctr}}}{7}\left(1 + \frac{0.1}{10}\right) + \frac{v_{\text{enz}}}{7}\left(1 + \frac{23}{c_C}\right) + \frac{v_{\text{r}}}{6}\left(1 + \frac{41}{c_A}\right) = c_{\text{p}}$$

iii) Density constraint: $\sum \mathbf{c} = \rho$

$$c_C + c_A + c_{\text{p}} = 340$$

The general GBA optimization problem

For some given GBA model $(\mathbf{M}, \boldsymbol{\tau}, \rho)$ and medium concentrations $\mathbf{a}$:

$$
\begin{array}{lll}
\underset{\mathbf{v}\in\mathbb{R}^{\mathrm{r}},\mathbf{c}\in\mathbb{R}_{+}^{\mathrm{p}}}{\text{maximize}} & \mu & \text{(Maximize growth rate)} \\
\text{subject to:} & & \\
 & \mathbf{M}\,\mathbf{v} = \mu\,\mathbf{c} & \text{(Flux balance)} \\
 & c_{\mathrm{p}} = \mathbf{v}\cdot\boldsymbol{\tau}(\mathbf{a},\mathbf{c}) & \text{(Reaction kinetics and protein sum)} \\
 & \rho = \sum \mathbf{c} & \text{(Constant cell density)}
\end{array}
$$

No alternative pathways: simplification with $\mathbf{c}$ as independent variables

1) For a square $\mathbf{M}$, there is an inverse $\mathbf{W} = \mathbf{M}^{-1}$ and

$$\mathbf{M}\,\mathbf{v} = \mu\,\mathbf{c} \quad \Rightarrow \quad \mathbf{v} = \mu\,\mathbf{W}\,\mathbf{c} \quad .$$

2) Substituting into $c_{\mathrm{p}} = \mathbf{v} \cdot \boldsymbol{\tau}(\mathbf{a}, \mathbf{c})$

$$c_{\mathrm{p}} = \mu\,(\mathbf{W}\,\mathbf{c}) \cdot \boldsymbol{\tau}(\mathbf{a}, \mathbf{c}) \quad .$$

3) Solving for μ: we get the objective function $\mu(\mathbf{c}, \mathbf{a})$

$$\boxed{\mu(\mathbf{a}, \mathbf{c}) = \frac{c_{\mathrm{p}}}{(\mathbf{W}\,\mathbf{c}) \cdot \boldsymbol{\tau}(\mathbf{a}, \mathbf{c})}} \quad .$$

4) The only constraint left:

$$\boxed{\rho = \sum \mathbf{c}} \quad .$$

The GBA problem with no alternative pathways: analytical solution

Reformulated problem: for some given GBA model $(\mathbf{M}, \boldsymbol{\tau}, \rho)$ and medium $\mathbf{a}$

$$\underset{\mathbf{c} \in \mathbb{R}^{\mathrm{p}}_{+}}{\text{maximize}} \qquad \mu(\mathbf{c}, \mathbf{a}) = \frac{c_{\mathrm{p}}}{(\mathbf{W}\,\mathbf{c}) \cdot \boldsymbol{\tau}(\mathbf{a}, \mathbf{c})}$$

subject to:

$$\sum \mathbf{c} = \rho \quad .$$

Analytical conditions for optimal states: using Lagrange multipliers, we find

$$\boxed{\mu\,(\mathbf{W}\,\mathbf{c}) \cdot \frac{\partial \boldsymbol{\tau}}{\partial c_m} + \mu\,\boldsymbol{\tau} \cdot (\mathbf{W}_m - \mathbf{W}_{\mathrm{p}}) + 1 = 0 \quad \forall\ m} \tag{1}$$

We got: # algebraic equations = # variables (solvable).

Cell economics: the costs and benefits of reactants

Substituting $\mathbf{v} = \mu\, \mathbf{W}\, \mathbf{c}$ into the solution (1)

$$\frac{c_\mathrm{p}}{\mu}\frac{\partial \mu}{\partial c_m} = -\sum_j v_j \frac{\partial \tau^j}{\partial c_m} + \mu \sum_j \tau_j \left(W^j_\mathrm{p} - W^j_m\right) - 1 = 0 \quad \forall\, m$$

Economics analogy: costs and benefits (in terms of protein allocation)

(marginal) reactant value = local benefit + global benefit + density cost (= 0 if optimal)

Protein is the underlying "currency"

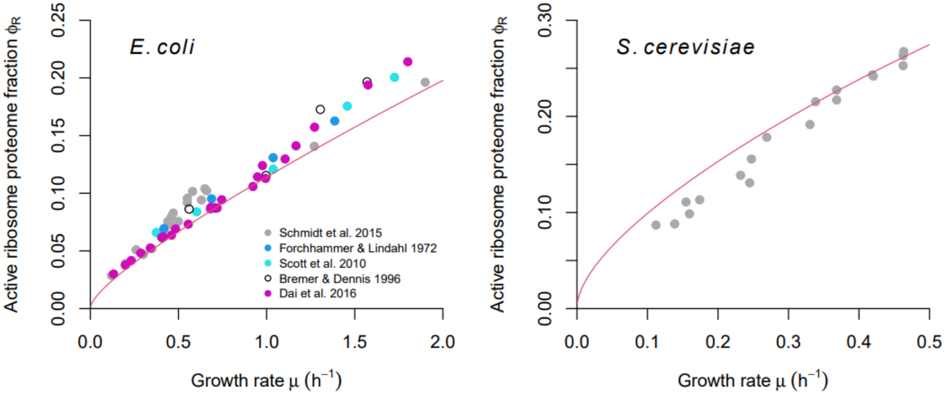
$$-\sum_j v_j \frac{\partial \tau^j}{\partial c_m} = -\sum_j \left(\frac{\partial p_j}{\partial c_m}\right)_{\mathbf{v}=const.}, \text{ and } \mu \sum_j \tau_j \left(W^j_\mathrm{p} - W^j_m\right) = -\sum_j \left(\frac{\partial p_j}{\partial c_m}\right)_{\boldsymbol{\tau},\, \mu=const.} \approx 0.03^{a}$$

---

[a] Dourado, Quantitative principles of optimal cellular resource allocation, *PhD Thesis* 2020.

Comparison to data: *E. coli* and yeast ribosome proteome fraction ϕ_{r} vs. μ

All data available, *in vivo* data close to the predicted optimality[4] (red lines, no fitting).



---

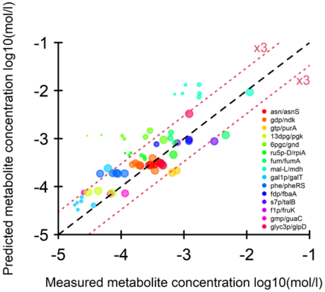
[4] Dourado & Lercher, An analytical theory of balanced cellular growth, *Nature Communications* 2020.

(Approximate) Enzyme-substrate optimality (without k_{cat})

Considering the Michaelis-Menten kinetics and no global benefit:

$$-\sum_j v_j \frac{\partial \tau^j}{\partial c_m} + \cancelto{0}{\mu \sum_j \tau_j \left(W_{\text{p}}^j - W_m^j\right)} - 1 = 0 \quad \Rightarrow \quad \boxed{p_j = c_m \left(1 + \frac{c_m}{K_j^m}\right)}$$

E. coli enzymes and substrates are close to this optimality[5]



---

[5] Dourado et al. On the optimality of the enzyme–substrate relationship in bacteria, *PLOS Biology* 2021.

“Growth Control Analysis”: holistic view of the growing cell

Metabolic Control Analysis (MCA): perturbations on metabolism (open system).

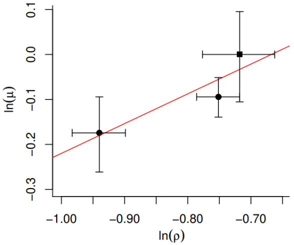
Growth Control Analysis (GCA): perturbations self-replicating system (closed system), all is connected ⇒ analytical expressions[6].

- Growth Control Coefficients: change in μ by perturbing one concentration c_i.
- Growth Adaptation Coefficients A: change in <u>optimal</u> μ^* by changing parameters.

E.g.: changing in the density ρ

$$A_\rho = \frac{\rho}{\mu^*}\frac{\mathrm{d}\mu^*}{\mathrm{d}\rho} = \frac{\rho}{c_\mathrm{p}}\left(1 - \mu \sum_j \tau_j W_\mathrm{p}^j\right)$$

Comparing to *E. coli* data[7] →



---

[6] Dourado & Lercher, *Nature Communications* 2020, [7] Cayley et. al, *Biophys. J* 2000

The general GBA problem: formulation on $\mathbf{q}$

Problem simplification on new (adimensional) independent variables:

$$\mathbf{q} := \frac{\mathbf{v}}{\mu\,\rho}$$

We get the optimality condition[7] for each reaction j ($s_j :=$ sum of column M_j)

$$M^{\mathrm{p}}_j - \mu\,\tau_j - \mathbf{v}\cdot\frac{\partial\boldsymbol{\tau}}{\partial\mathbf{c}}\mathbf{M}_j + s_j\,\mathbf{v}\cdot\frac{\partial\boldsymbol{\tau}}{\partial\mathbf{c}}\,\mathbf{c}/\rho = 0$$

Cell economics: the protein costs and benefits of each reaction

$$\underbrace{\text{production benefit}}_{\text{(protein production)}} + \underbrace{\text{local cost}}_{\text{(protein in } j\text{)}} + \underbrace{\text{local benefit}}_{\text{(local saturation)}} + \underbrace{\text{transport benefit}}_{\text{(global saturation)}} \ (= 0 \text{ if opt.})$$

---

[7] Dourado et al. Mathematical properties of optimal fluxes in cellular reaction networks at balanced growth, *PLOS Comp Biol* 2023.

Grow Control Analysis: Grow Adaptation Coefficient for k_{cat}

We can show from first principles (using the Envelope Theorem)[8] that:

$$A_{k^j_{\mathrm{cat}}} = \frac{k^j_{\mathrm{cat}}}{\mu^*} \frac{\mathrm{d}\mu^*}{\mathrm{d}k^j_{\mathrm{cat}}} = \phi_j$$

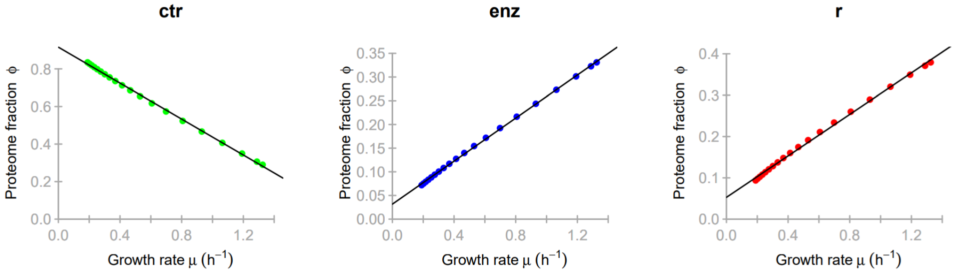
Proportional change in μ^* is exactly the same as proportion of protein allocated to j.

---

[8] Dourado et al. Mathematical properties of optimal fluxes in cellular reaction networks at balanced growth, *PLOS Comp Biol* 2023.

Simplified mathematical formulation also facilitates numerical solutions

Model L3 on different media ($\approx 0.1\,s$):



Genome-scale GBA models are feasible: 10 reactions $\approx 1\,s$, 100 reactions $\approx 1\,min$.

Computational tools for GBA

Numerical implementation in R (including also dynamical simulations):

`https://github.com/HDourado/Growth_Mechanics`

Online tool: Cell growth simulator

`https://cellgrowthsim.com/`

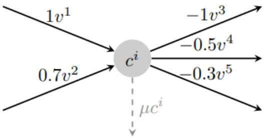
Summary

- **GBA: self-replicating cell models on independent variables, easier to study.**
- **Analytical conditions for optimal balanced growth (fundamental principles).**
- **Experimental indications that cells do implement near optimal strategies.**
- **Proteins emerge as the “currency” in cell economics from first principles.**

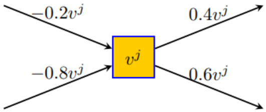
(soon chapter in the EPCB book)

Constraints on GBA

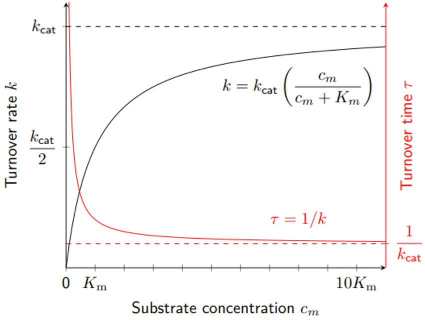
A) Flux balance for each reactant “i”



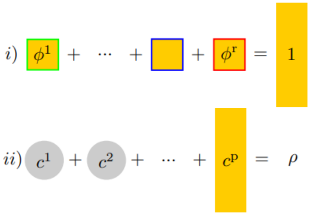
B) Mass balance within each reaction “j”



C) Kinetics: $v = p \cdot k(\mathbf{c}, \mathbf{x})$ or $p = v \cdot \tau(\mathbf{c}, \mathbf{x})$



D) Density constraints



Michaelis-Menten kinetics with activation

Based on "Convenience kinetics"[9], we define the Michaelis-Menten kinetics with activation, corresponding "activation constants" $\mathbf{A}$

$$\tau_j = \frac{1}{k_{\text{cat}}^j} \prod_m \left(1 + \frac{A_j^m}{c_m}\right) \left(1 + \frac{K_j^m}{c_m}\right) \prod_n \left(1 + \frac{K_j^n}{a^n}\right)$$

---

[9] Liebermeister & Klipp, Bringing metabolic networks to life: convenience rate law and thermodynamic constraints, 2006.

Optimal substrate mass concentration = free enzyme mass concentration

The optimal mass concentration balance for minimal ρ :

$$c_m = \frac{p^j\, K_m^j}{K_m^j + c_m} \quad .$$

But this corresponds exactly to the free enzyme mass concentration

$$p^j_{\text{free}} := p^j - p^j \left(\frac{c_m}{c_m + K_m^j} \right) = \frac{p^j\, K_m^j}{K_m^j + c_m} \quad .$$

Thus[10],

$$c_m = p^j_{\text{free}} \quad .$$

---

[10] Dourado et al. On the optimality of the enzyme–substrate relationship in bacteria, *PLOS Biology* 2021

Equations for balance growth states: model L3

1) Original problem: Implicit constraints on μ, involving $v_1, v_2, v_3, c_1, c_2, c_3, a_1$ (6 variables, 5 equations)

$$\begin{aligned} v_1 - v_2 &= \mu\, c_1 \\ v_2 - v_3 &= \mu\, c_2 \qquad \textbf{(mass conservation)} \\ v_3 &= \mu\, c_3 \end{aligned}$$

$$\frac{v_1}{7}\left(1+\frac{1}{a_1}\right)+\frac{v_2}{7}\left(1+\frac{23}{c_1}\right)+\frac{v_3}{6}\left(1+\frac{41}{c_2}\right)=c_3 \qquad \textbf{(kinetics and protein sum)}$$

$$c_1 + c_2 + c_3 = 340 \qquad \textbf{(constant cell density)}$$

2) GBA: Explicit constraint on $\mu(c_1, c_2, a_1)$ (using $c_3 = 340 - c_1 - c_2$)

$$\mu(c_1,c_2,a_1)=\frac{340-c_1-c_2}{\frac{1}{7}\left(1+\frac{1}{a_1}\right)+\frac{340-c_1}{7\cdot 340}\left(1+\frac{23}{c_1}\right)+\frac{340-c_1-c_2}{6\cdot 340}\left(1+\frac{41}{c_2}\right)} \qquad \textbf{(constrained growth rate)}$$

3) Analytical conditions for optimal balanced growth state (system of algebraic equations)

$$\mu\,\frac{23}{7}\,\frac{(340-c_1)}{(c_1)^2}+\mu\left[\frac{1}{7}\left(1+\frac{23}{c_1}\right)+\frac{1}{6}\left(1+\frac{41}{c_2}\right)\right]-1=0 \qquad (m=1)$$

$$\mu\,\frac{40\,(340-c_1-c_2)}{6\,(c_2)^2}+\mu\left[\frac{1}{5}\left(1+\frac{41}{c_2}\right)\right]-1=0 \qquad (m=2)$$

The general GBA problem: formulation on $\mathbf{q}$

General formulation on $\mathbf{q}$ in few steps

Substituting $\mathbf{v} = \mu\,\rho\,\mathbf{q}$ into $\mathbf{M}\,\mathbf{v} = \mu\,\mathbf{c}$

$$\rho\,\mathbf{M}\,\mathbf{q} = \mathbf{c} \quad \text{(independent of } \mu\text{)}.$$

Substituting $\mathbf{c} = \rho\,\mathbf{M}\,\mathbf{q}$ into $c_{\mathrm{p}} = \mathbf{v}\cdot\boldsymbol{\tau}(\mathbf{a},\mathbf{c})$

$$M_{\mathrm{r}}^{\mathrm{p}} q_{\mathrm{r}} = \mu\,\mathbf{q}\cdot\boldsymbol{\tau}(\rho\,\mathbf{M}\,\mathbf{q},\mathbf{a})$$

Solving for μ:

$$\boxed{\mu(\mathbf{q},\mathbf{a}) = \frac{M_{\mathrm{r}}^{\mathrm{p}} q_{\mathrm{r}}}{\mathbf{q}\cdot\boldsymbol{\tau}(\rho\,\mathbf{M}\,\mathbf{q},\mathbf{a})}}$$

The density constraint:

$$\rho = \sum \mathbf{c} \quad \Leftrightarrow \quad \boxed{\mathbf{s}\cdot\mathbf{q} = 1}$$

The general GBA problem: analytical “solution”

Reformulated problem: for some given model $(\mathbf{M}, \boldsymbol{\tau}, \rho)$ and environment $\mathbf{a}$

$$\underset{\mathbf{q}\in\mathbb{R}^{\mathrm{r}}}{\text{maximize}} \qquad \mu(\mathbf{q},\mathbf{a}) = \frac{M^{\mathrm{p}}_{\mathrm{r}}\, q_{\mathrm{r}}}{\mathbf{q}\cdot\boldsymbol{\tau}(\rho\,\mathbf{M}\,\mathbf{q},\mathbf{a})}$$

subject to:

$$\mathbf{s}\cdot\mathbf{q} = 1$$

$$\mathbf{q}\odot\boldsymbol{\tau}(\rho\,\mathbf{M}\,\mathbf{q},\mathbf{a}) \geq \mathbf{0} \quad .$$

Analytical conditions for optimal states: using KKT conditions, we find

$$\boxed{\left(M^{\mathrm{p}}_{j} - \mu\,\tau_j - \mu\,\mathbf{q}\cdot\frac{\partial\boldsymbol{\tau}}{\partial q^{j}} + s_j\mu\,\mathbf{q}\cdot\frac{\partial\boldsymbol{\tau}}{\partial\mathbf{q}}\,\mathbf{q}\right) q_j = 0 \quad \forall\ j} \tag{2}$$

Using $\mathrm{s}\cdot\mathbf{q} = 1$: # algebraic equations = # variables (solvable)..

Equations for balance growth states: model L3

1) Original problem: Implicit constraints on μ, involving $v_1, v_2, v_3, c_1, c_2, c_3, a_1$ (6 variables, 5 equations)

$$\begin{aligned} v_1 - v_2 &= \mu\, c_1 \\ v_2 - v_3 &= \mu\, c_2 \qquad\qquad \textbf{(mass conservation)} \\ v_3 &= \mu\, c_3 \\ \frac{v_1}{7}\left(1+\frac{1}{a_1}\right)+\frac{v_2}{7}\left(1+\frac{23}{c_1}\right)+\frac{v_3}{6}\left(1+\frac{41}{c_2}\right) &= c_3 \qquad\qquad \textbf{(kinetics and protein sum)} \\ c_1 + c_2 + c_3 &= 340 \qquad\qquad \textbf{(constant cell density)} \end{aligned}$$

2) GBA: Explicit constraint on $\mu(q_2, q_3, a_1)$ (from the density constraint $q_1 = 1$)

$$\mu(q_2,q_3,a_1) = \frac{q_3}{\frac{1}{7}\left(1+\frac{1}{a_1}\right)+\frac{q_2}{7}\left(1+\frac{23}{340(1-q_2)}\right)+\frac{q_3}{6}\left(1+\frac{41}{340(q_2-q_3)}\right)} \qquad \textbf{(constrained growth rate)}$$

3) Analytical conditions for optimal balanced growth state (system of algebraic equations)

$$\frac{1}{7}\left(1+\frac{23}{340(1-q_2)}\right)+\frac{23q_2}{7\left[340(1-q_2)\right]^2}-\frac{41q_3}{6\left[340(q_2-q_3)\right]^2}=0 \qquad (j=2)$$

$$1-\mu\frac{1}{6}\left(1+\frac{41}{340(q_2-q_3)}\right)-\mu\,\frac{41q_3}{6\left[340(q_2-q_3)\right]^2}=0 \qquad (j=3)$$

The dynamic generalization: fitness optimization

For some given model $(\mathbf{M}, \boldsymbol{\tau}, \rho)$ **and dynamic medium** $\mathbf{a}(t)$**:**

$$\underset{\mathbf{v}(t),\, \mathbf{c}(t)}{\text{maximize}} \quad \int_0^T \mu \, \mathrm{d}t \qquad \text{(Maximize fitness)}$$

subject to:

$$\begin{aligned}
&\mathbf{M}\,\mathbf{v} = \mu\,\mathbf{c} + \dot{\mathbf{c}} && \text{(Mass conservation)}\\
&c_\mathrm{p} = \mathbf{v} \cdot \boldsymbol{\tau}(\mathbf{a}, \mathbf{c}) && \text{(Reaction kinetics and protein sum)}\\
&\rho = \sum \mathbf{c} && \text{(Constant cell density)}
\end{aligned}$$

Main trick for analytical "solution": define the "generalized fluxes" $\mathbf{q}$ such that

$$\rho\,\mathbf{M}\,\mathbf{q} = \mathbf{c} \quad ,$$

then reformulate the problem on $\dot{\mathbf{q}}, \mathbf{q}, \mathbf{a}$, and solve Euler-Lagrange equations.