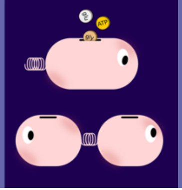

Optimization of metabolic states

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Outline

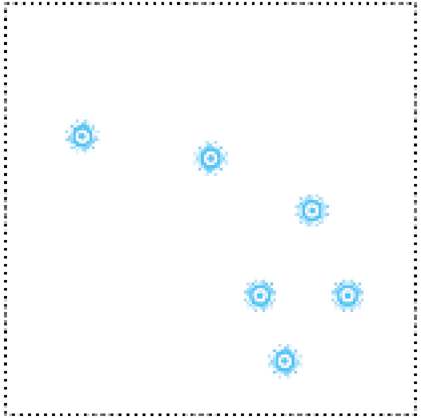
Part 1

- What are cells optimised for?
- Optimizing growth rate is a complex constrained optimization problem
- Optimal growth rate is achieved at an Elementary Flux Mode

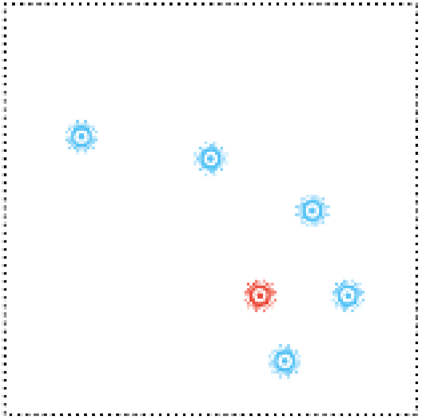
Part 2

- Algorithm to find optimal metabolic states
- Application of the algorithm to study a rate-yield trade-off

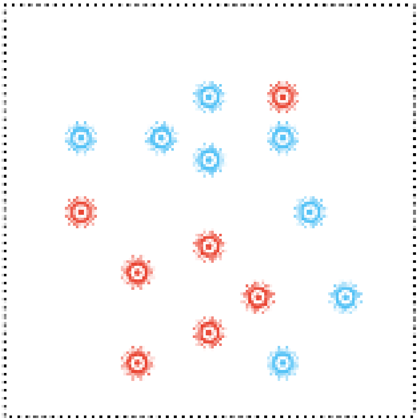
What are cells optimized for? Growth in well-mixed environment



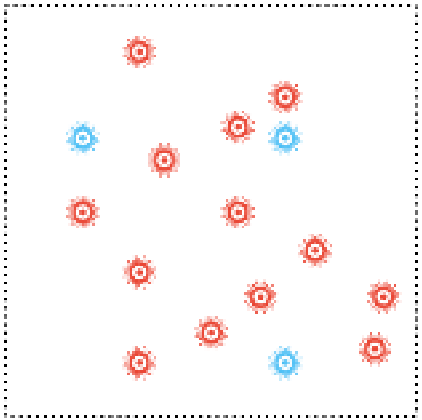
Growth in well-mixed environment



Growth in well-mixed environment



Growth in well-mixed environment: But how does a cell grow fast?



Optimisation of metabolic states

An optimal metabolic state: Maximal objective flux per enzyme investment

$$\text{Maximise} \quad \frac{\text{Objective flux}}{\text{Total enzyme}}$$

For growth rate:

Objective flux = biomass production flux

Total enzyme = enzyme in all of metabolism

(Large) constrained optimization problem

<u>Variables</u>:

Fluxes

Enzyme concentrations

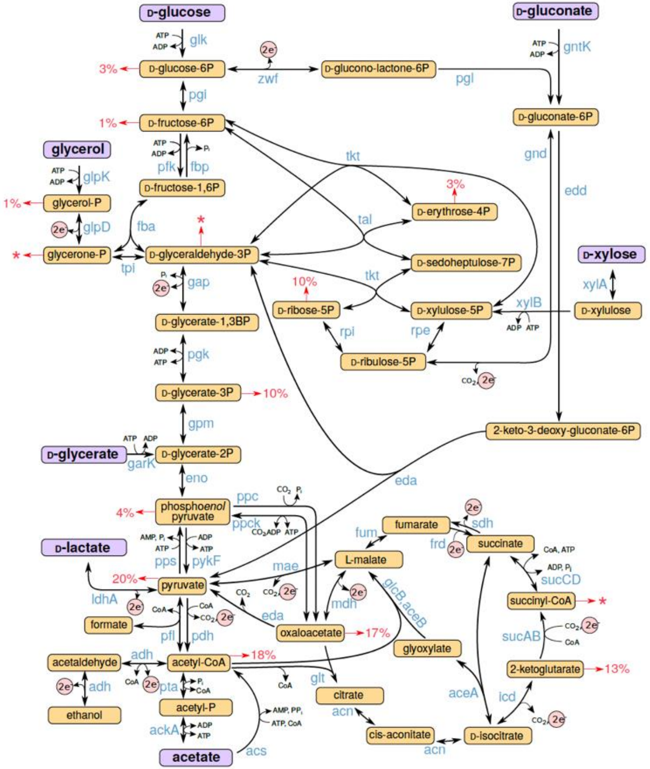
Metabolite concentrations

<u>Constraints</u>:

Steady state

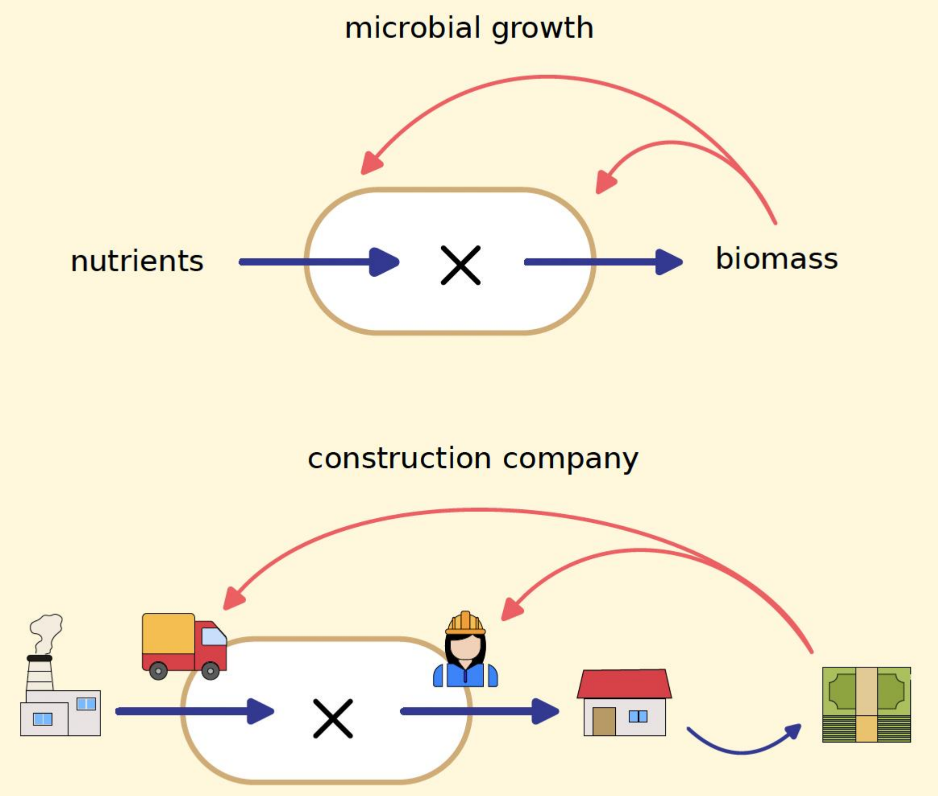
Enzyme kinetics

Can we simplify this?

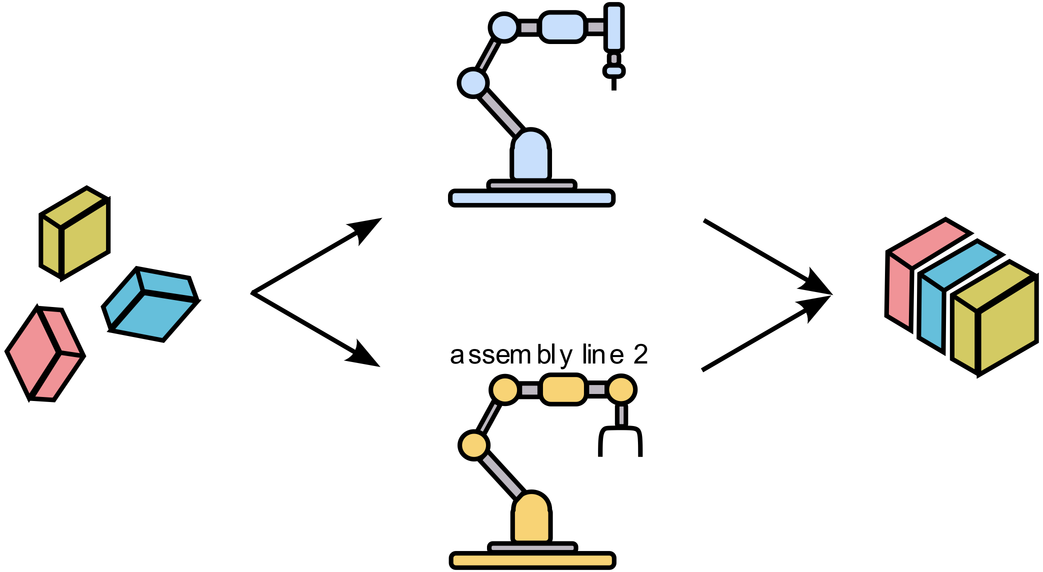


Factory analogy

We can see cells as tiny factories, so let's think about the factory and see if we can get some intuition…

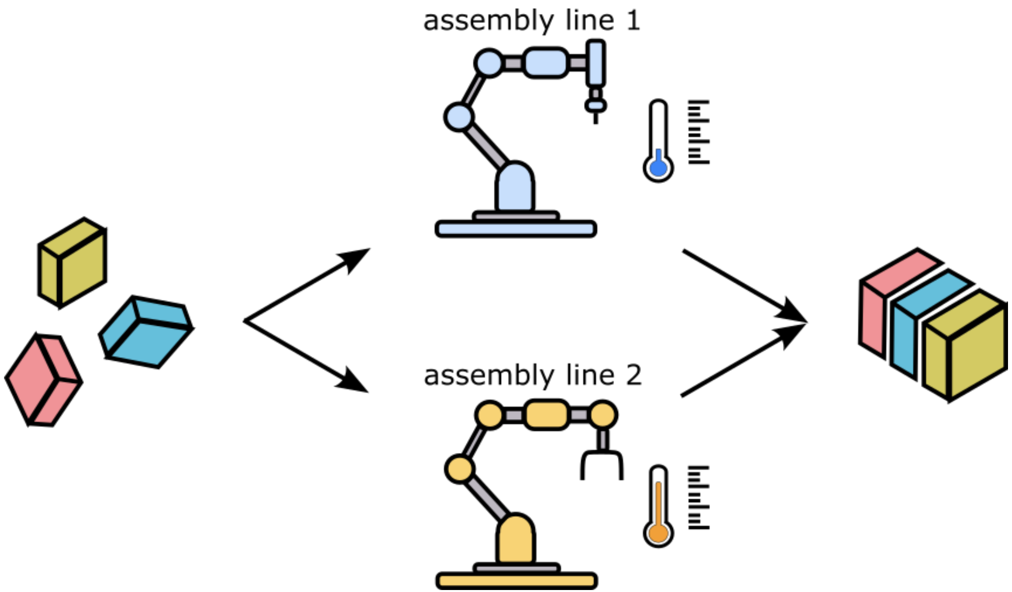


Choice between alternative assembly lines



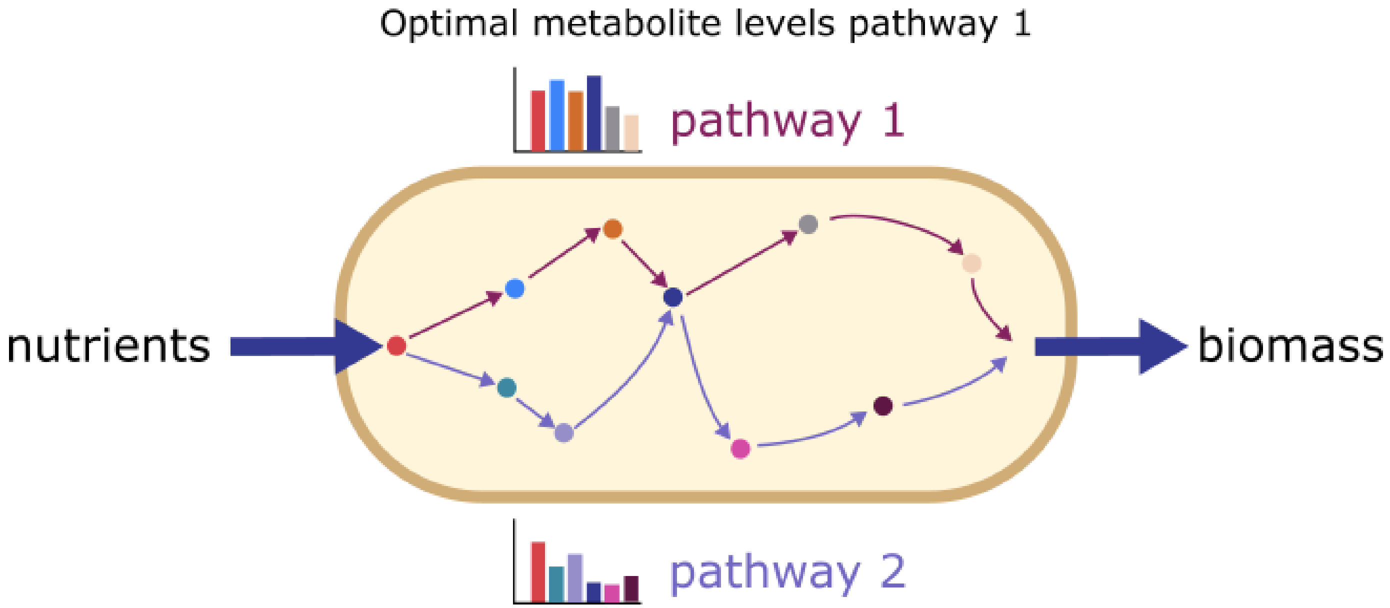
You are the manager. Do you invest in a lot of one assembly line or combine both?

Choice between alternative assembly lines



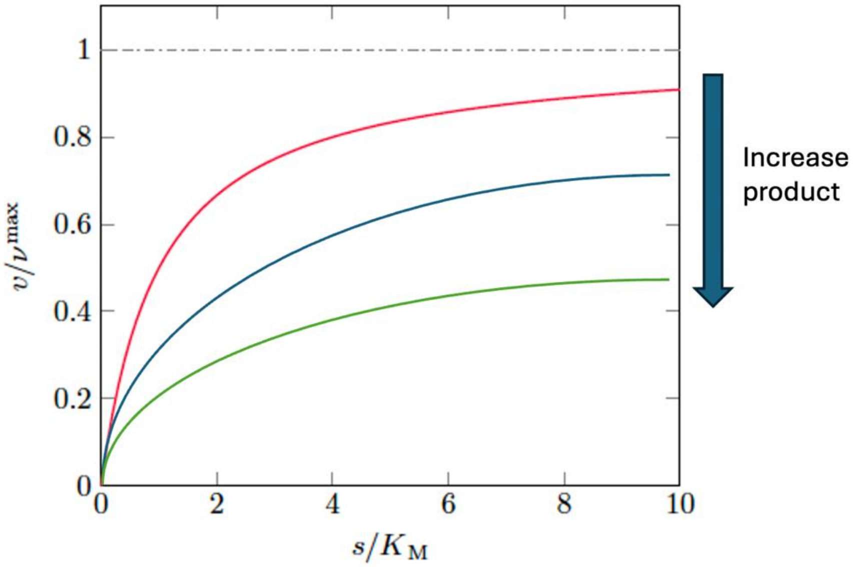
You are the manager. Do you invest in a lot of one assembly line or combine both? And at what temperature do you set the factory thermostat?

Choice between alternative metabolic pathways



You are a cell. Do you invest in a lot of one metabolic pathway or combine both? And at what levels do you keep the intermediate metabolites?

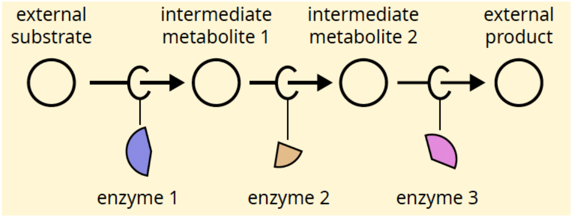
Enzymatic rates depend on substrate and product concentrations

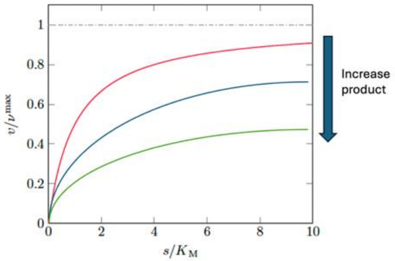


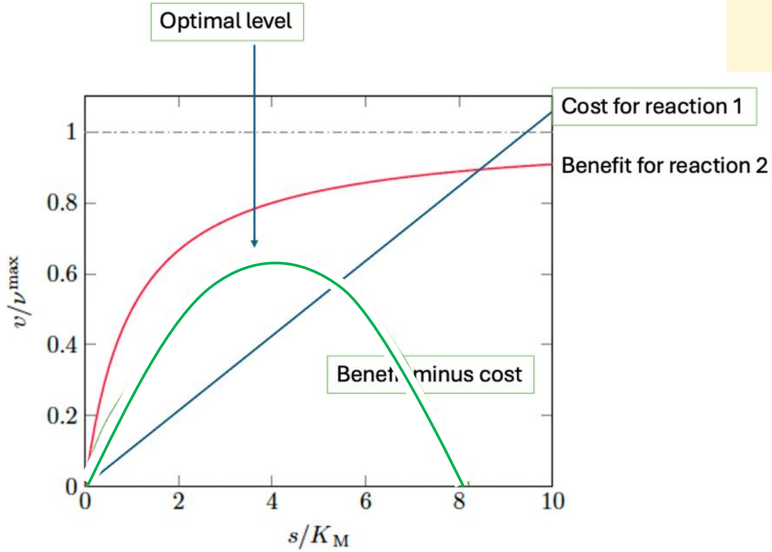
Reaction rates increase with substrate concentrations, but saturate

Reaction rates decrease with increasing product concentrations

Each pathway has optimal metabolite levels







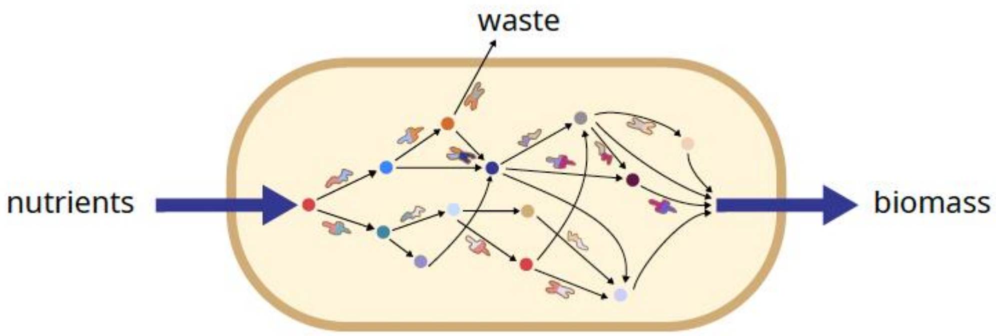
The product of one enzyme is the substrate of the next enzyme.

=> An intermediate level is optimal depending on the kinetics of the consuming and producing enzymes

Extensions to larger networks

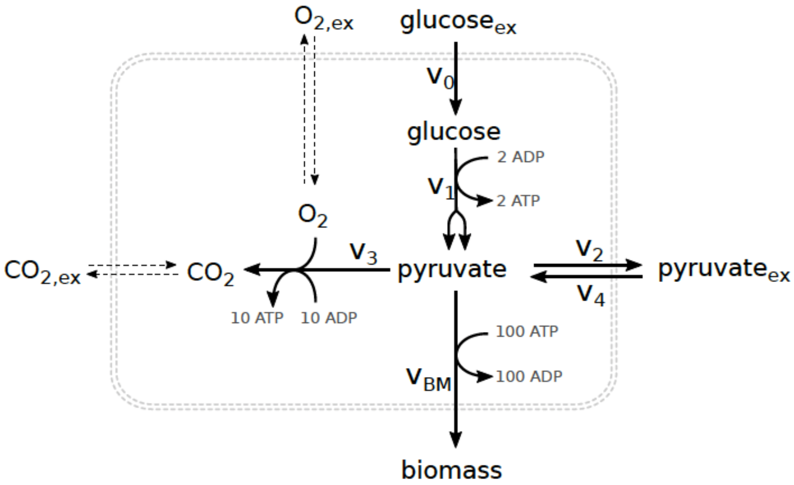
- Defining property of the pathway: Cannot omit any reaction.

- General structures that cannot omit any reaction:
- Elementary Flux Modes



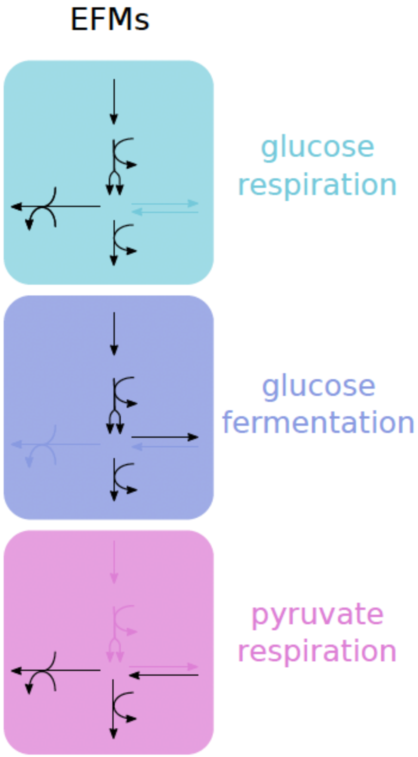
Example network

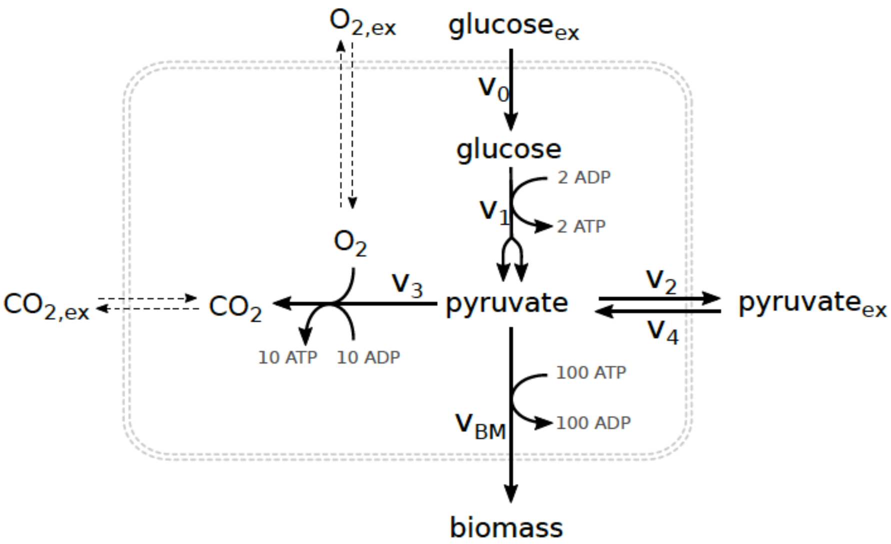
- Optimize at different external glucose concentrations (minimize total enzyme at a biomass production rate of 1)
- Every solution has associated fluxes, enzyme levels and internal metabolite levels



$[G_{ex}]$	e_{tot}	v_0	v_1	v_2	v_3	v_4	v_{BM}	e_0	e_1	e_2	e_3	e_4	e_{BM}	[G]	[P]	[ATP]	[ADP]
0.01	156.2	5	5	0	9	0	1	54.4	4.4	0	94.4	0	2.9	0.08	15.14	0.05	20.09
0.1	91.3	50	50	99	0	0	1	61.3	11.3	14.2	0	0	4.4	0.13	4.55	0.11	20.09
1	36.2	50	50	99	0	0	1	13.0	8.0	12.5	0	0	2.7	0.60	7.65	0.11	20.09

Example network





$[G_{ex}]$	e_{tot}	v_0	v_1	v_2	v_3	v_4	v_{BM}
0.01	156.2	5	5	0	9	0	1
0.1	91.3	50	50	99	0	0	1
1	36.2	50	50	99	0	0	1

Proof by contradiction

1. Assume that the negation of the statement is true
2. Show that this leads to a contradiction
3. The statement needs to be true

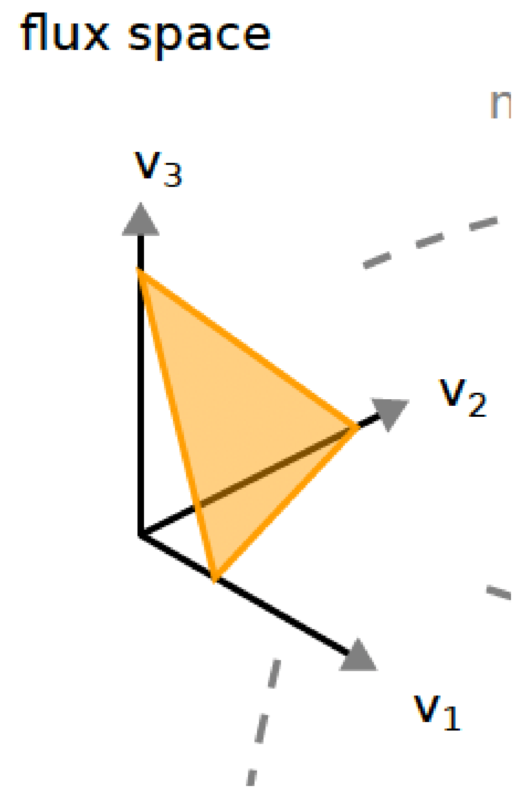
Defining the problem

An optimal metabolic state:

Maximal objective flux per enzyme investment

$$\text{Maximize} \quad \frac{\text{Objective flux}}{\text{Total enzyme}}$$

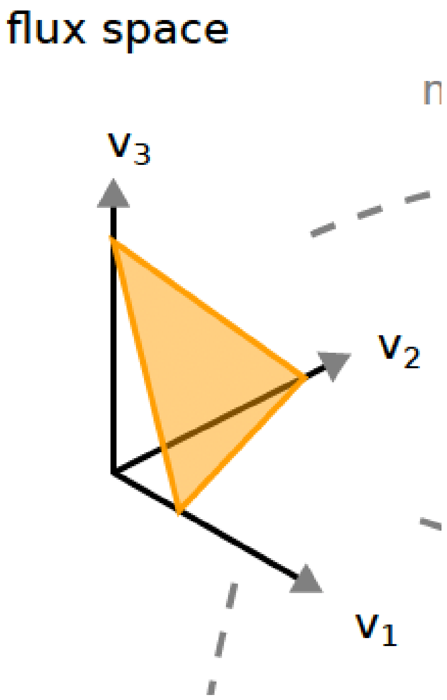
We fix the objective flux (at any value, e.g. 1) and minimize the total enzyme



Minimizing the total enzyme

Flux depends on enzyme in a complicated way:

$$v = \underbrace{e \cdot k_{\text{cat}}{}^{+}}_{V_{\text{max}}} \cdot \underbrace{\left(1 - e^{\frac{\Delta_r G'}{RT}}\right)}_{\eta^{\text{for}}} \cdot \underbrace{\frac{\frac{s}{K_{\text{S}}}}{1 + \frac{p}{K_{\text{P}}} + \frac{s}{K_{\text{S}}}}}_{\eta^{\text{sat}}}$$



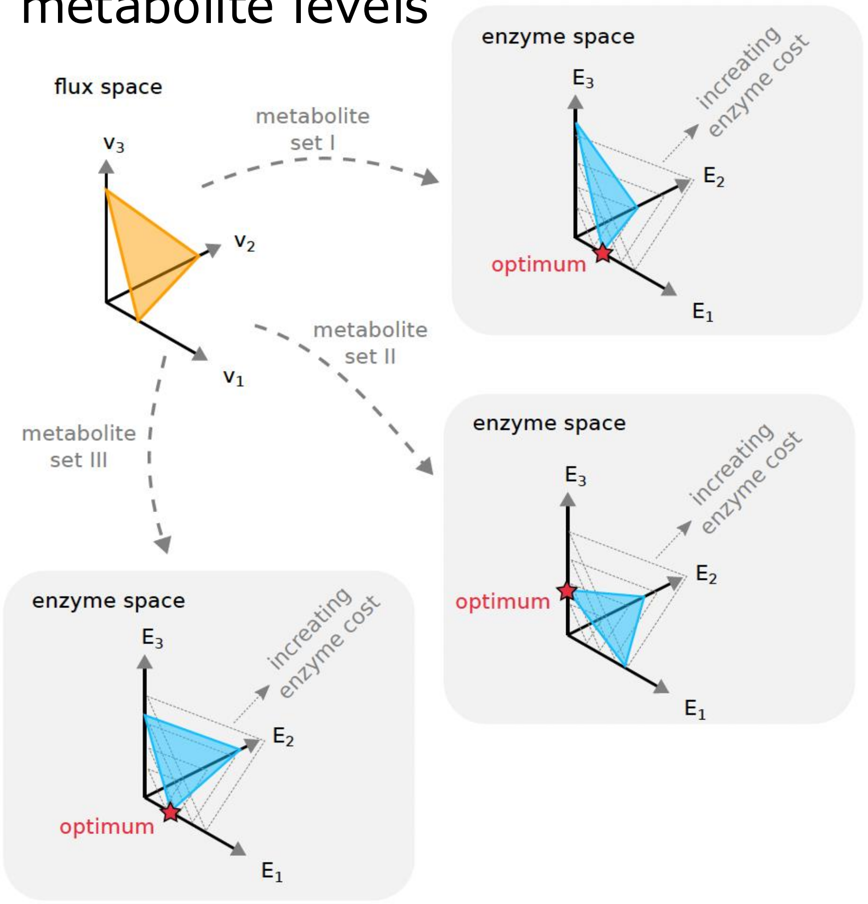
Converting to enzyme space for fixed metabolite levels

Flux depends on enzyme in a complicated way:

$$v = \underbrace{e \cdot k_{\mathrm{cat}}{}^{+}}_{V_{\max}} \cdot \underbrace{\left(1 - e^{\frac{\Delta_r G'}{RT}}\right)}_{\eta^{\mathrm{for}}} \cdot \underbrace{\frac{\frac{s}{K_{\mathrm{S}}}}{1 + \frac{p}{K_{\mathrm{P}}} + \frac{s}{K_{\mathrm{S}}}}}_{\eta^{\mathrm{sat}}}$$

But when we fix the metabolites:

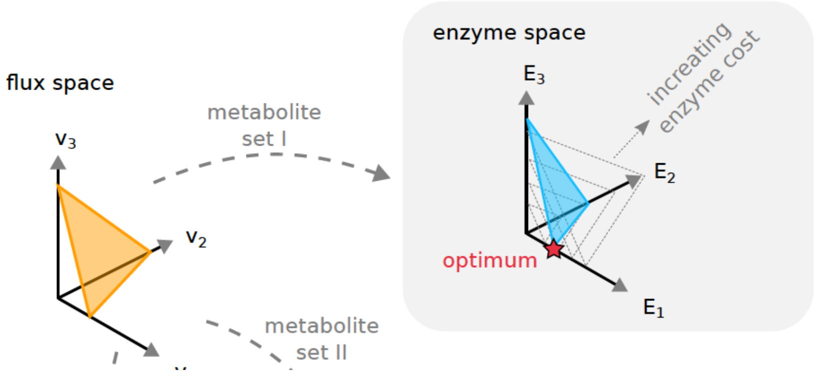
$$v_i = e_i \cdot \kappa_i$$



1. Assume that the negation of the statement is true

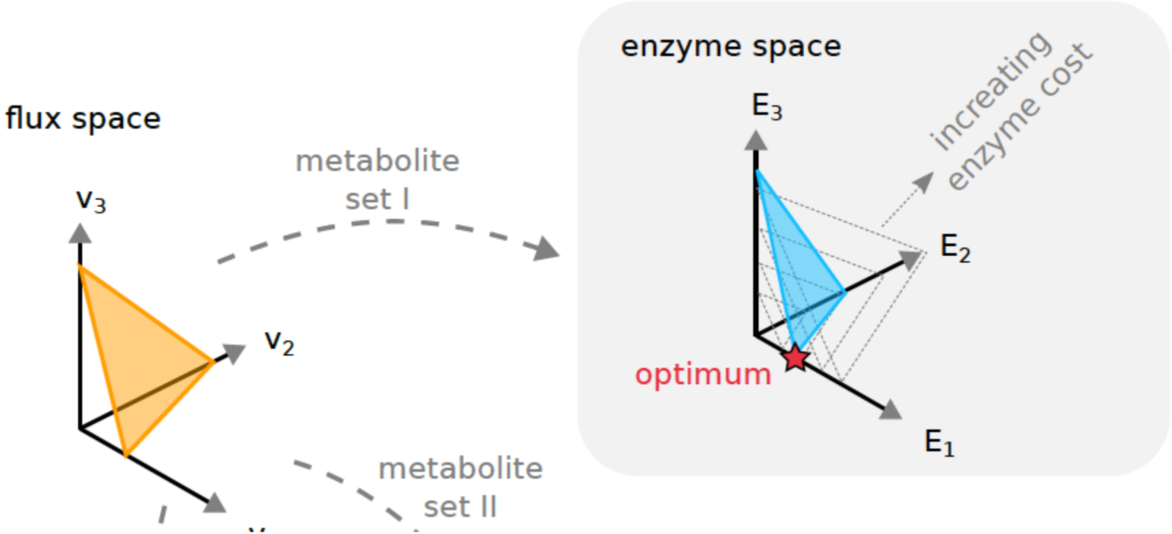
What if the optimal state is not an EFM?

- The state has associated enzyme and metabolite concentrations and fluxes
- We fix only the metabolite levels
- And do the conversion to enzyme space



2. Show that this leads to a contradiction

But in the enzyme space, minimal total enzyme is achieved at an extreme ray, and that is an Elementary Flux Mode!



3. The statement needs to be true

Metabolic states that optimize a specific flux are Elementary Flux Modes!

Summary of the first part

The **optimization problem** for optimal metabolic states is:

$$\text{maximize} \quad \frac{\text{Objective flux}}{\text{Total enzyme}}$$

The flux distribution that satisfies this objective is an **Elementary Flux Mode**

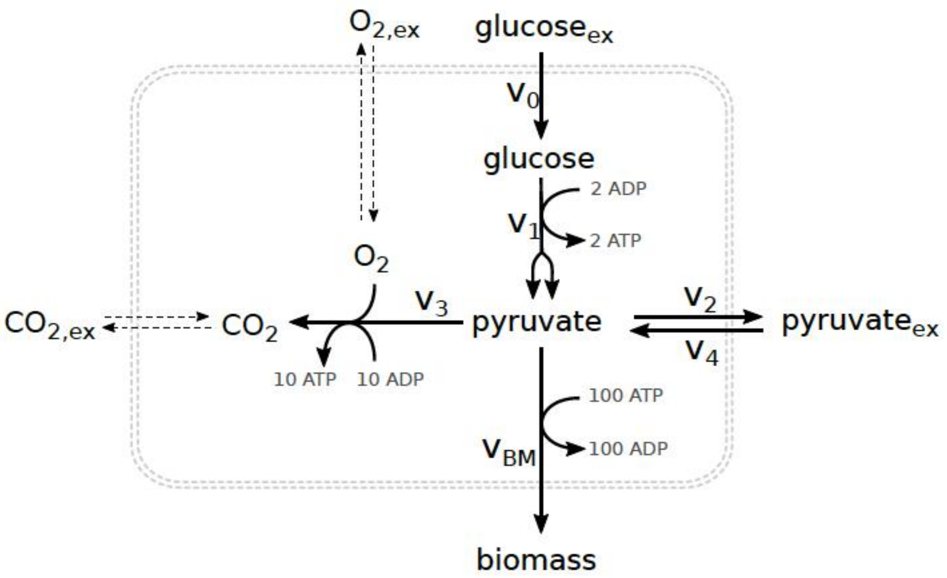
But how can we use this to understand how cells grow fast?

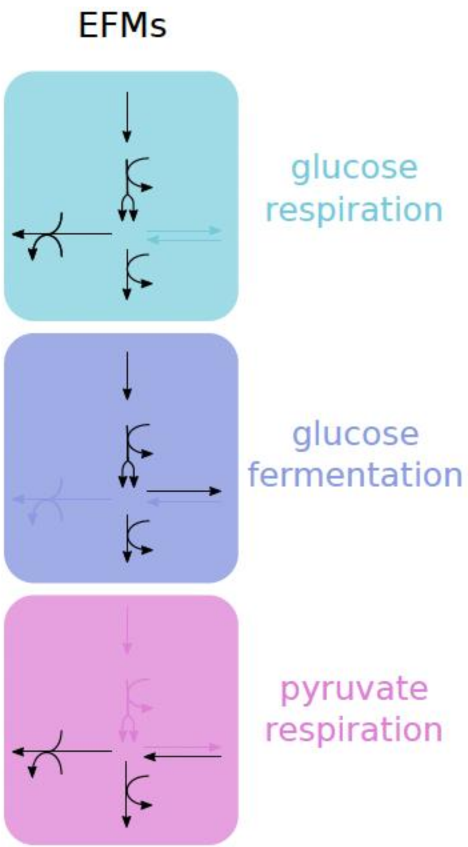
Constructive algorithm to find the optimal state

1. Enumerate all Elementary Flux Modes
2. Calculate the maximal specific flux for every EFM using enzyme cost minimization
3. Compare the EFMs and choose the best one

We call this ”Enzyme Flux Cost Minimization”

Using the algorithm for the example network





$[G_{ex}]$	e_{tot}	v_0	v_1	v_2	v_3	v_4	v_{BM}	e_0	e_1	e_2	e_3	e_4	e_{BM}	[G]	[P]	[ATP]	[ADP]
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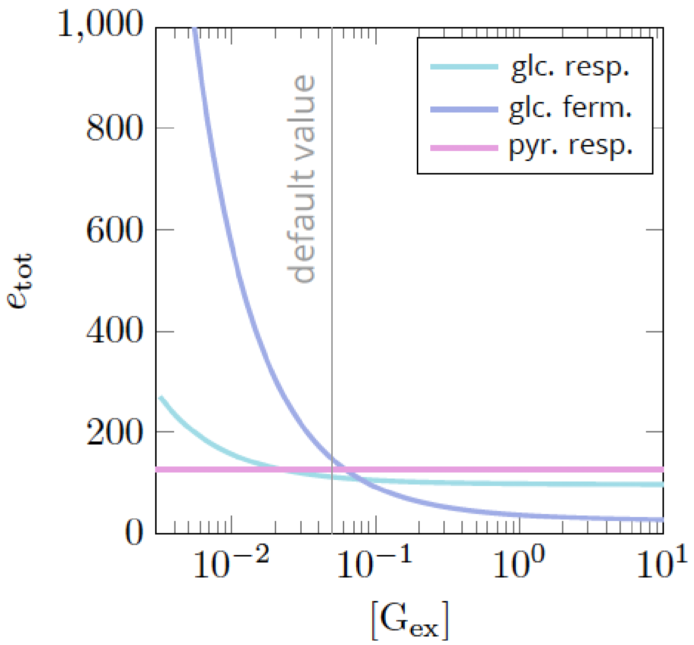
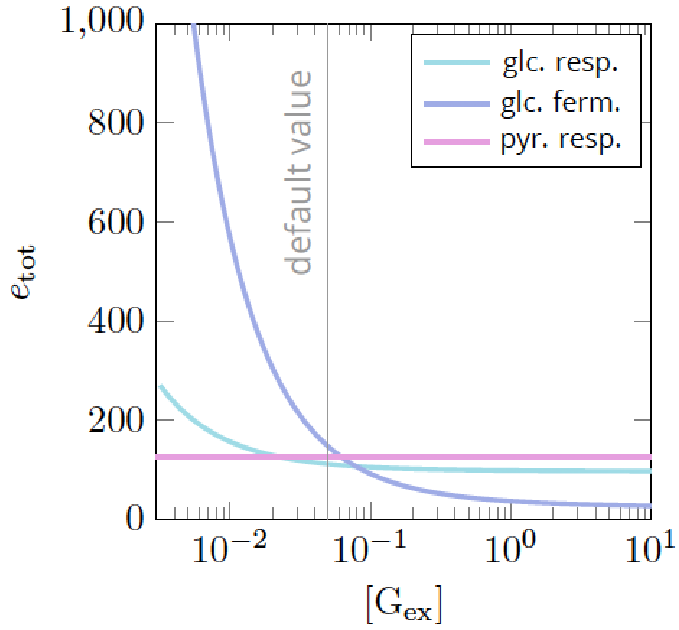
1	36.2	50	50	99	0	0	1	13.0	8.0	12.5	0	0	2.7	0.60	7.65	0.11	20.09

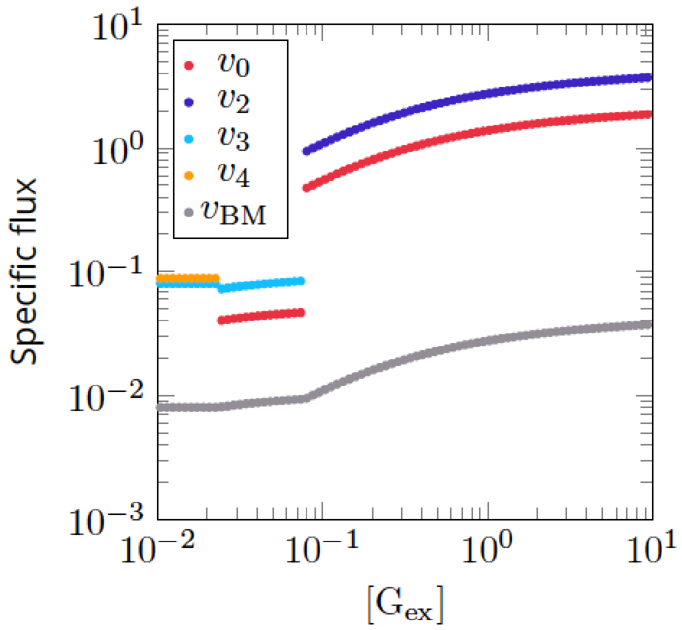
Optimizing at a range of external substrate concentrations



Minimizing the total enzyme at a biomass flux of 1 for a range of external glucose.

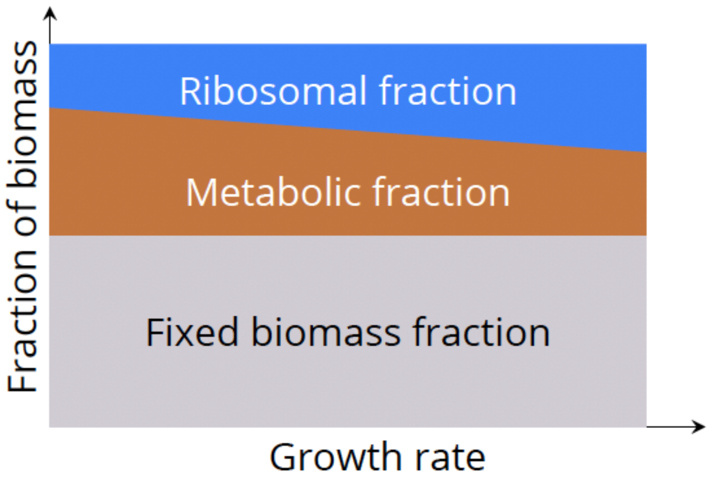
Fluxes change discontinuously as function of the substrate

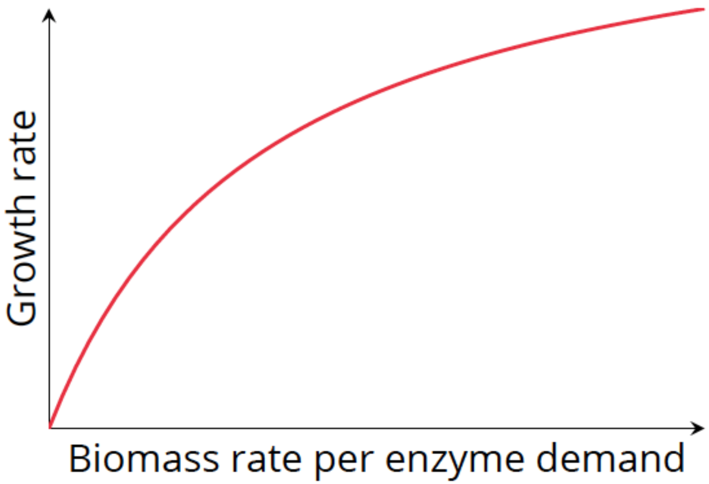




Shift from respiration to fermentation at high glucose concentrations

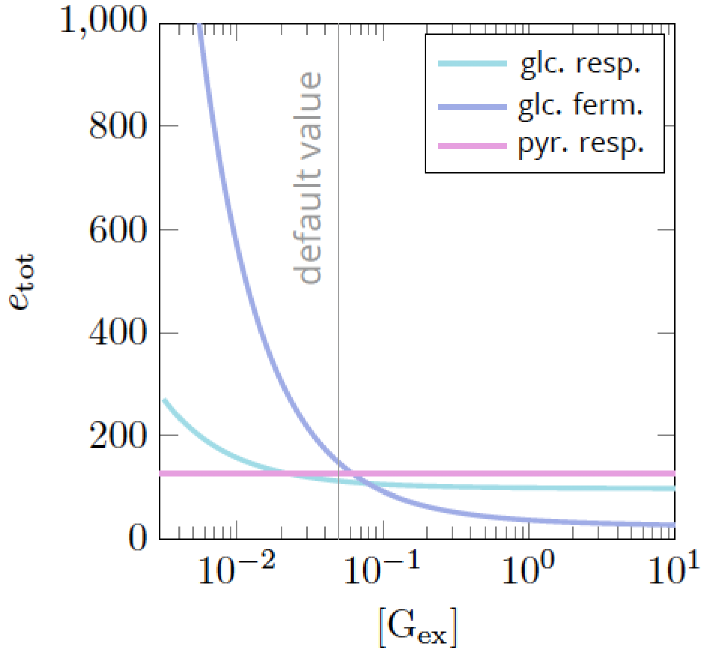
Cells do not consist only of metabolic enzymes

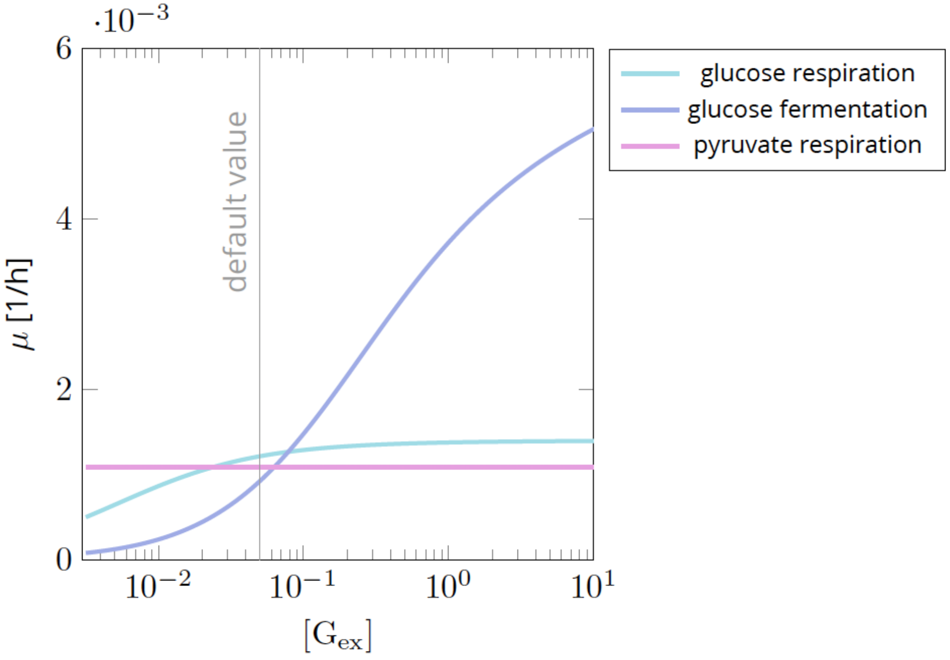




Conversion form specific biomass flux to growth rate is monotonous => Optimum is the same!

Conversion to growth rate for the toy model





Same results but different axis.

Trade-off between growth rate and yield?

Yield = biomass per substrate

Growth rate = biomass per time

Correlated or trade-off?

Correlated: at same uptake, higher yield is higher growth

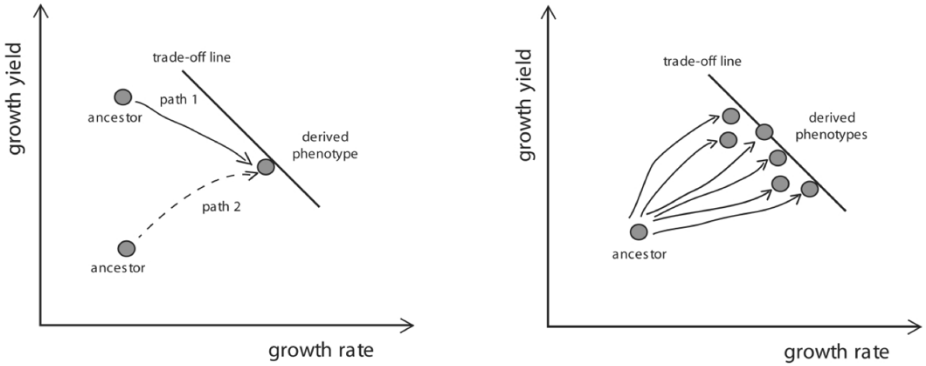
Trade-off: driving force can increase yield or rate

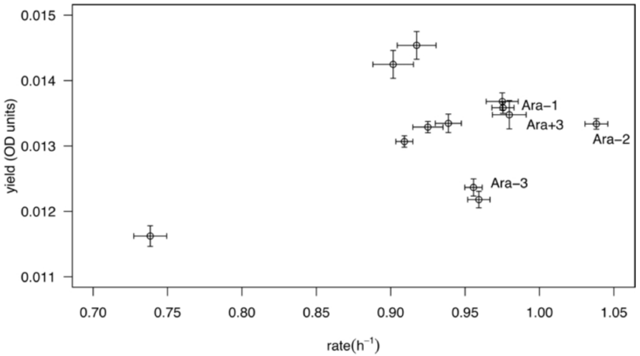
Trade-off between growth rate and yield? Empirical results

Yield = biomass per substrate

Growth rate = biomass per time

Correlated or trade-off?

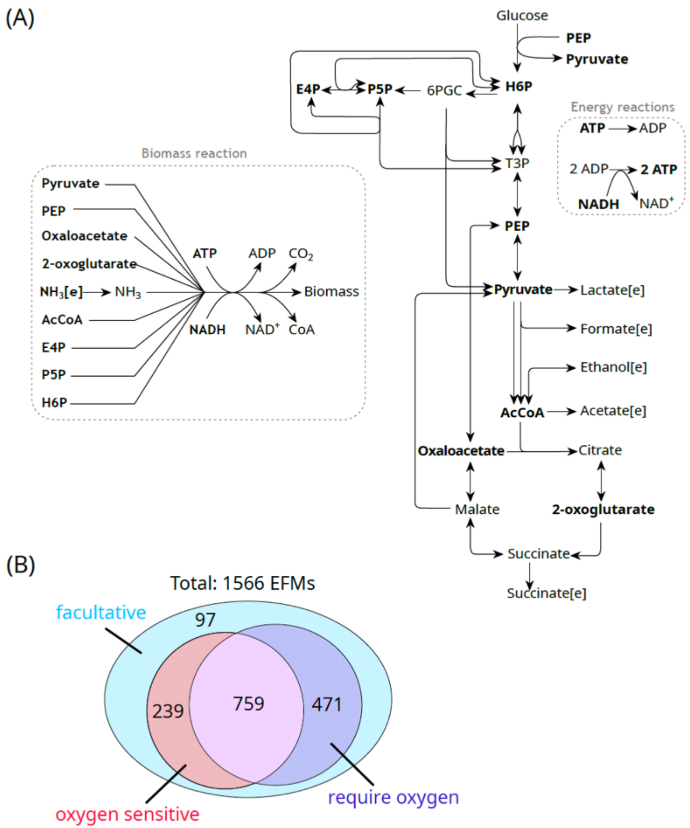




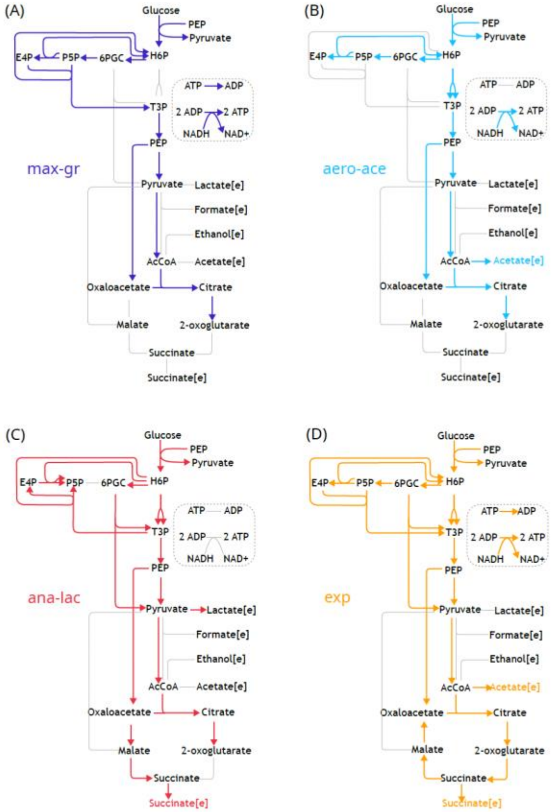
Novak er al. Am. Nat. (2006)

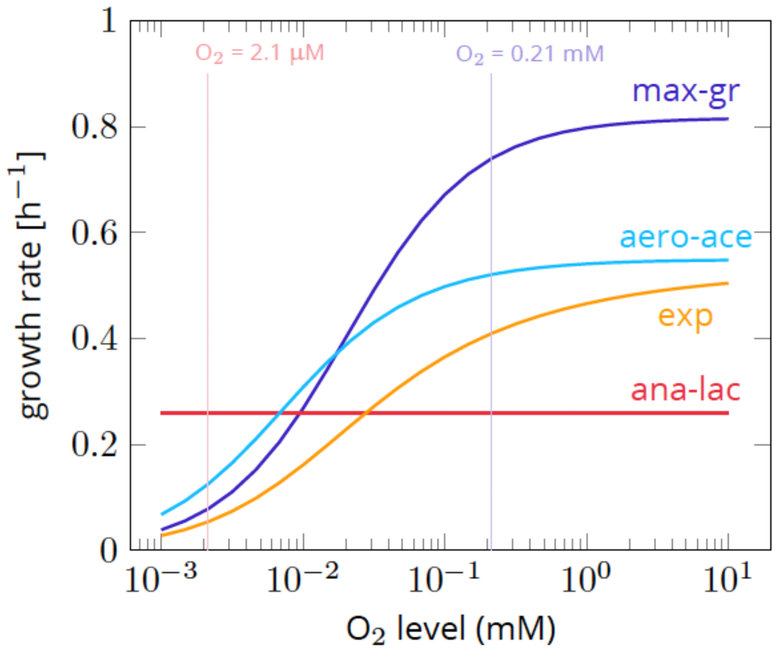
Application to *Escherichia coli* central carbon metabolism

Step 1: Enumerate the EFMs



Step 2: Compare the EFMs at different conditions

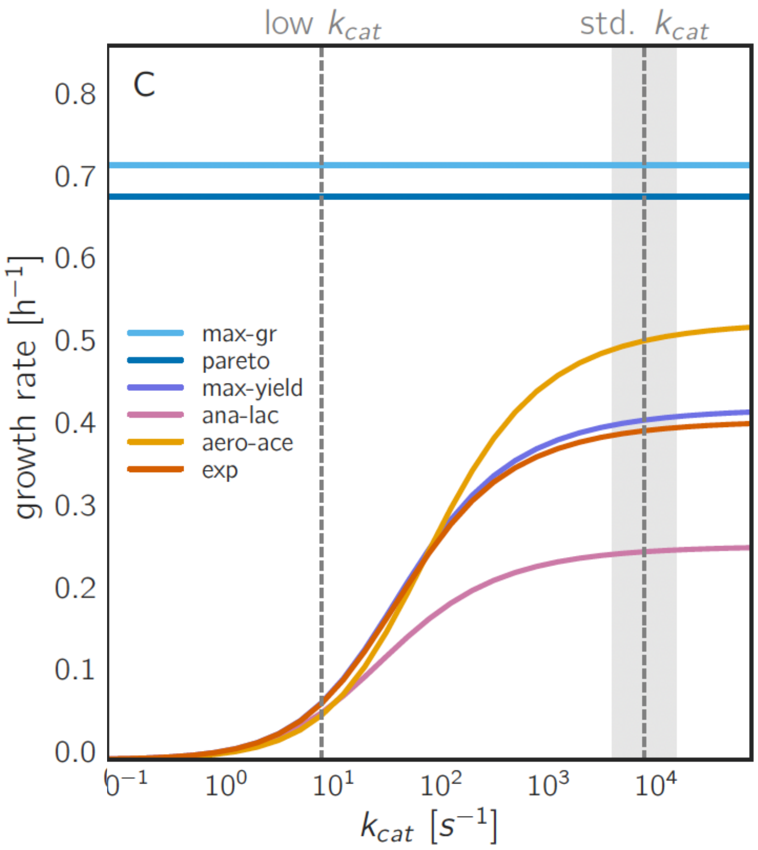




Changing a kinetic parameter

Changing the catalytic efficiency of a single reaction affects the efficiency of EFMs.

- Two objectives: sensitivity analysis and selection on enzyme properties

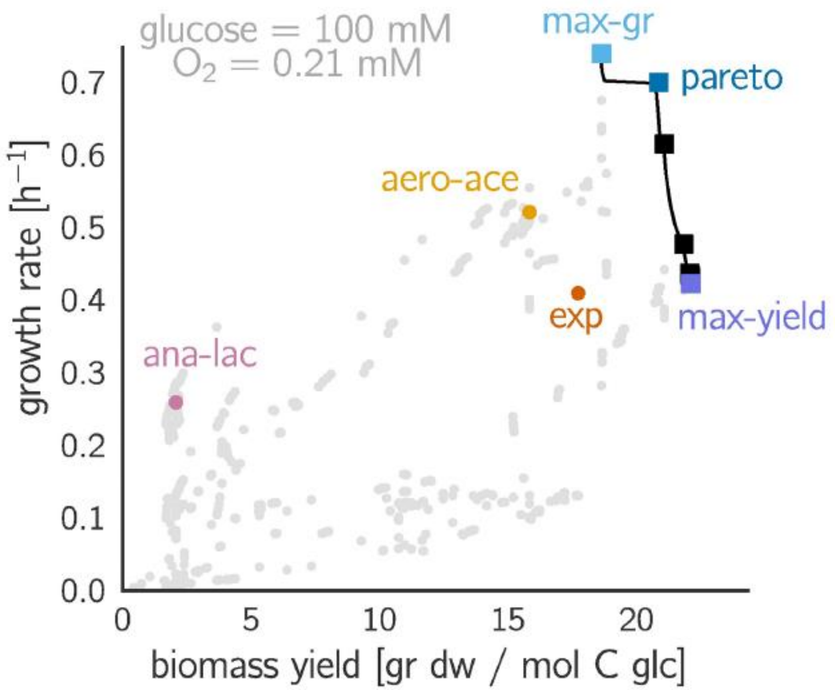


Only a slight trade-off between growth rate and yield

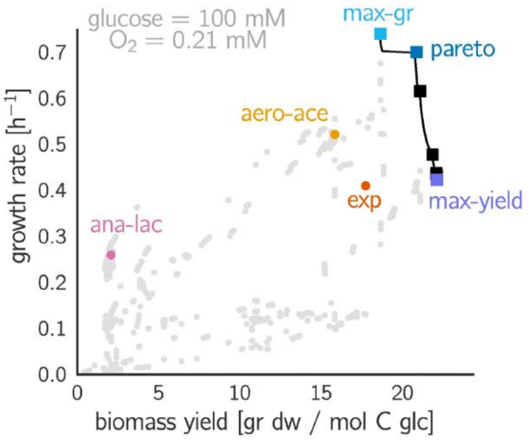
Every dot is an EFM

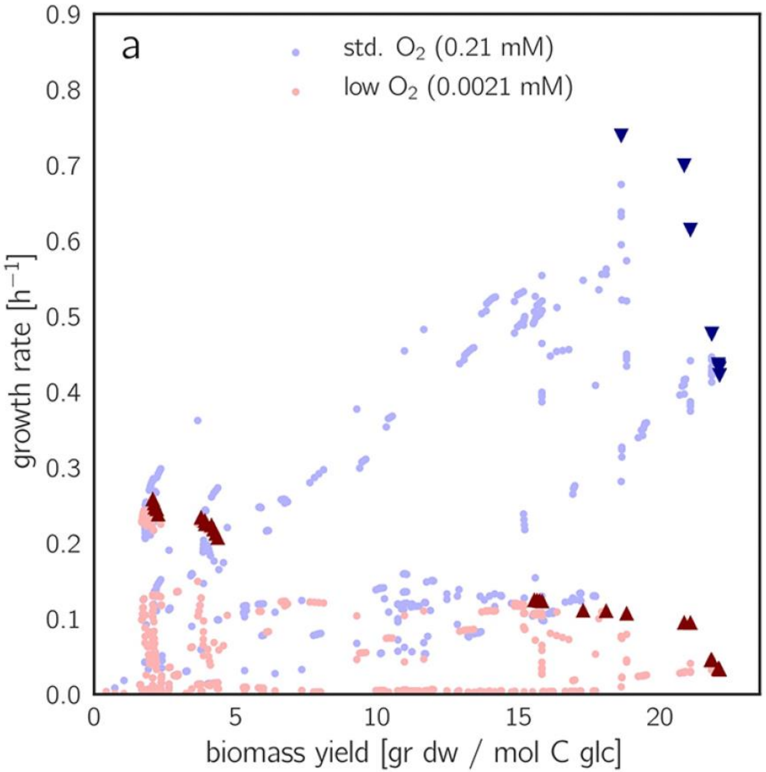
Most EFMs are not optimal for either growth rate of yield

The optimal EFMs form a small Pareto front (high growth rate only comes at a small yield decrease)



Trade-off between growth rate and yield is condition dependent





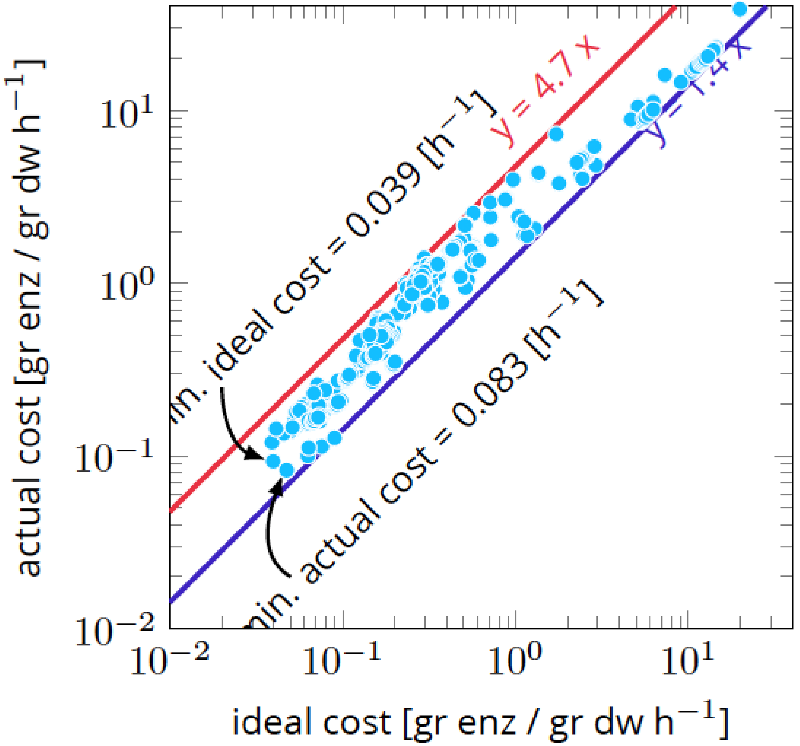
At low oxygen there is a large Pareto front!

How much does it matter to include the kinetics?

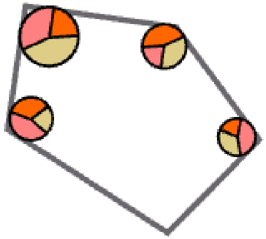
Even with the EFMs, Enzyme Flux Cost Minimization is much more computationally expensive than FBA

Is it worth doing?

Depends on your question!



Take home messages optimisation of metabolic states



- Optimization for growth is biomass flux / total enzyme
- Optimal states are Elementary Flux Modes
- We can use this to find the optimal states
- Trade-off between yield and rate is condition dependent

Don’t forget the feedback!

Acknowledgements:

Authors: Andreas Kremling, Wolfram Liebermeister, Elad Noor

Reviewers/discussions/comments: Jürgen Zanghellini, David S. Tourigny, Hugo Dourado, Stefan Müller, Coen Berns

Image credits: Michela Pauletti, Elad Noor