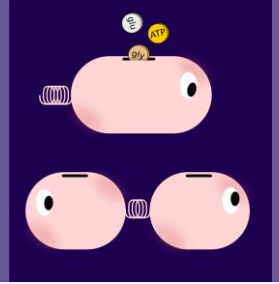
# Economic Principles in Cell Physiology

Paris, July 4-6, 2022



## Enzyme-cost efficient metabolic states

Meike Wortel, Jürgen Zanghellini

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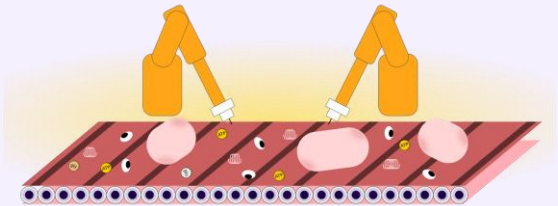

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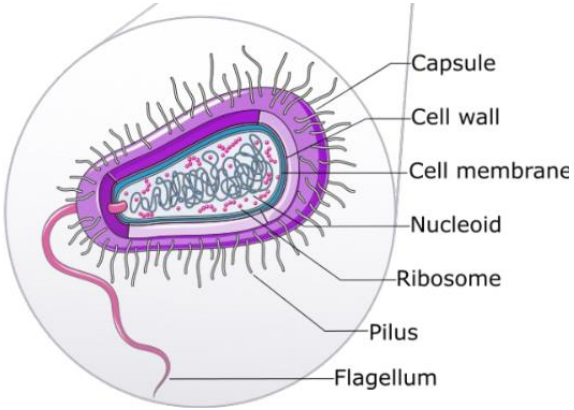
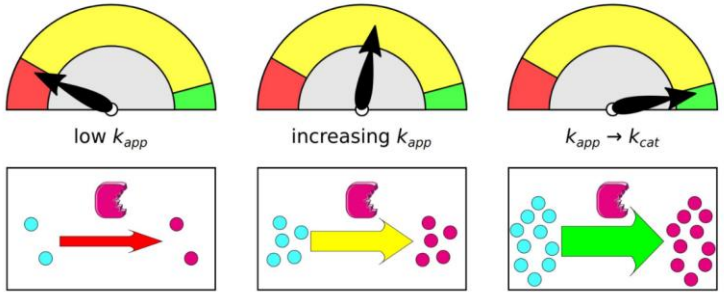
# Cells contain complex network of enzymes and metabolites



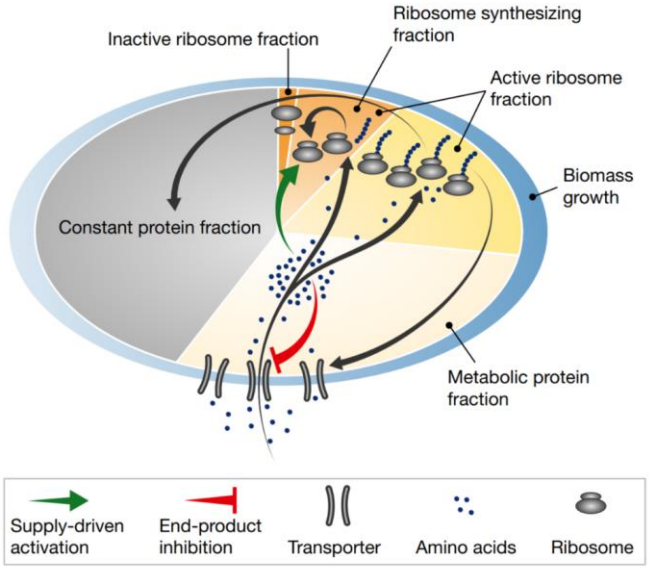

Cells contain metabolites, that are converted by enzymes to get a network of fluxes, which are influences by both enzymes and fluxes ➔ All are linked!

# Can we optimize the metabolism for growth rate?

**Main question:** what distribution of enzymes (metabolites, fluxes) minimizes enzyme cost (and thereby optimizes growth rate)

We use:

- Flux polytopes as in FBA
- Enzyme Cost Minimization from previous lecture



# Networks are large and enzyme kinetics non-linear

**Variables**

50 fluxes

50 enzymes

40 metabolites

**Parameters**

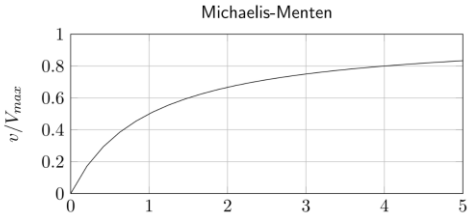
50 $k_{cat}$'s

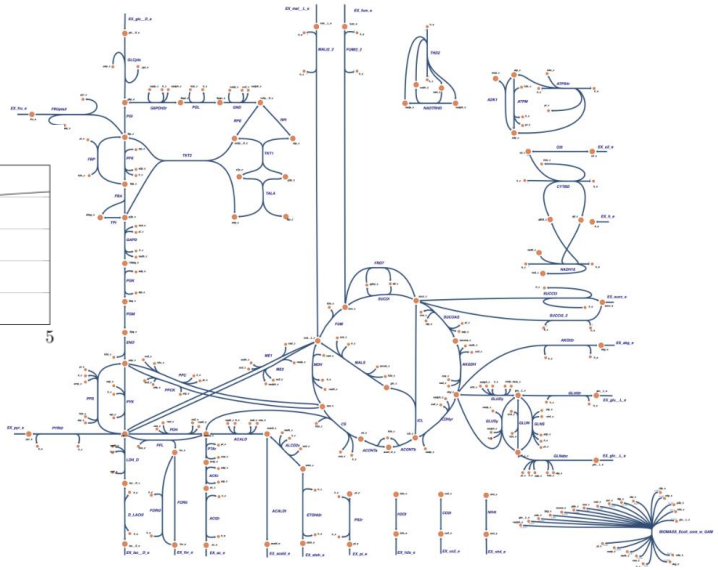
50 enzyme weights

50 Equilibrium constants

+- 150 $K_M$'s

? Allosteric regulation



**Can we simplify this problem?**

# Simplify simple example

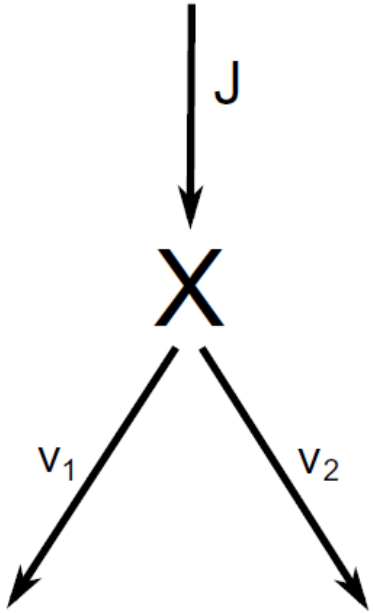
Optimize specific pathway flux:

Optimize $q_J = \frac{J}{e_1 + e_2}$

Subject to enzyme kinetics:

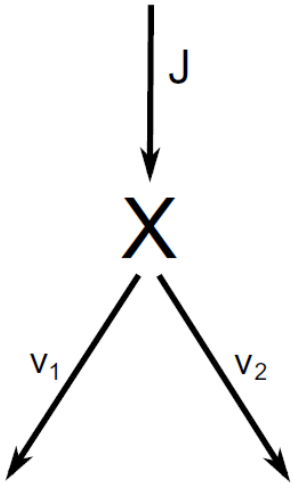
$$v_1 = e_1 \cdot k_{cat,1} \frac{X}{K_{M,1} + X}$$

$$v_2 = e_2 \cdot k_{cat,2} \frac{X}{K_{M,2} + X}$$



# Optimize the specific pathway flux

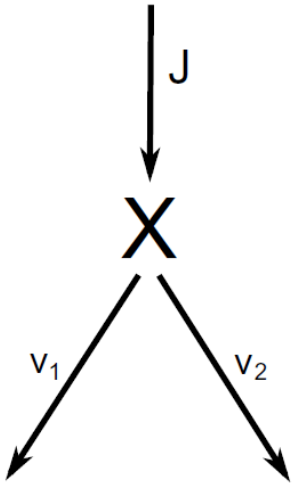
Optimize $q_J$ = $\frac{J}{e_1+e_2}$



# Optimize the specific pathway flux

Optimize $q_J = \frac{J}{e_1 + e_2}$

Approach: Set J=1 and minimize $e_1 + e_2$



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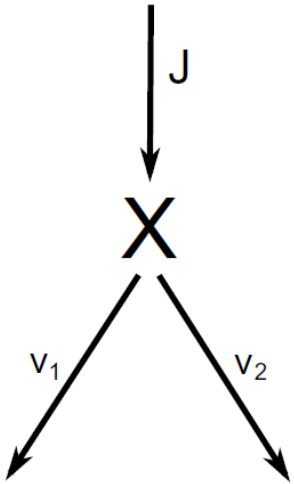


$$v_1 = e_1 \cdot k_{cat,1} \frac{X}{K_{M,1} + X} \quad \Rightarrow \quad e_1 = v_1 \cdot \frac{K_{M,1} + X}{X \cdot k_{cat,1}}$$

# Calculating the enzyme cost

$$e_1 + e_2 = v_1 \cdot \frac{K_{M,1} + X}{X \cdot k_{cat,1}} + v_2 \cdot \frac{K_{M,2} + X}{X \cdot k_{cat,2}}$$



# Calculating the enzyme cost

$$e_1 + e_2 = v_1 \cdot \frac{K_{M,1} + X}{X \cdot k_{cat,1}} + v_2 \cdot \frac{K_{M,2} + X}{X \cdot k_{cat,2}}$$

$$v_1 + v_2 = v_3 = 1 \quad v_2 = 1 - v_1$$



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$$v_1 + v_2 = v_3 = 1 \quad v_2 = 1 - v_1$$

$$e_1 + e_2 = \alpha(X) \cdot v_1 + \beta(X), \quad \text{with :}$$

$$\alpha(X) = \frac{K_{M,1} + X}{k_{cat,1} \cdot X} - \frac{K_{M,2} + X}{k_{cat,2} \cdot X}$$

$$\beta(X) = J \, \frac{K_{M,2} + X}{k_{cat,2} \cdot X}$$



# Optimal specific pathway flux for branched pathway

$$e_1 + e_2 = \alpha(X) \cdot v_1 + \beta(X), \quad \text{with :}$$

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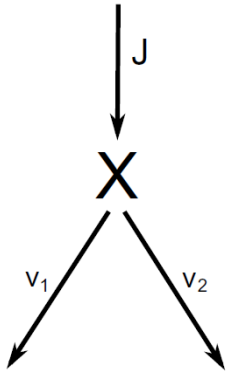
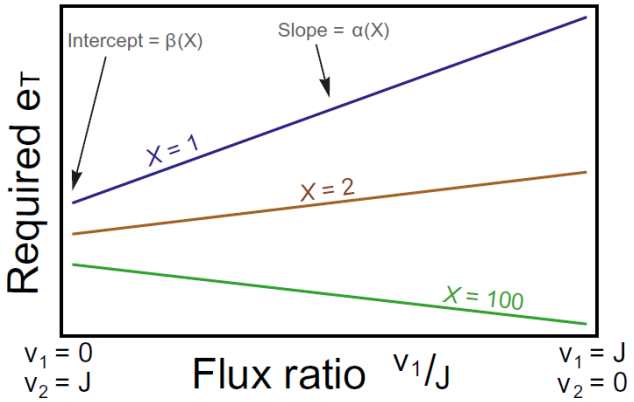
$$\beta(X) = J \, \frac{K_{M,2} + X}{k_{cat,2} \cdot X}$$



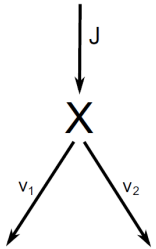

For every internal metabolite concentration, one of the branches is optimal ➔ never a combination!

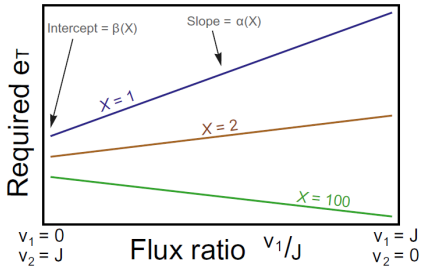
➔ We can simplify the simple problem by only comparing using either of the branches

# Optimal specific pathway flux for branched pathway

1. Fix objective flux and minimize enzymes
2. Apply steady state constraints
3. For fixed internal metabolite concentrations total enzyme depends linearly on the flux
4. Under optimal conditions, the internal metabolite has some value
5. At this value, one of the branches is optimal
6. ➔ Optimal specific pathway flux is achieved using only one branch

$$e_1 + e_2 = \alpha(X) \cdot v_1 + \beta(X)$$

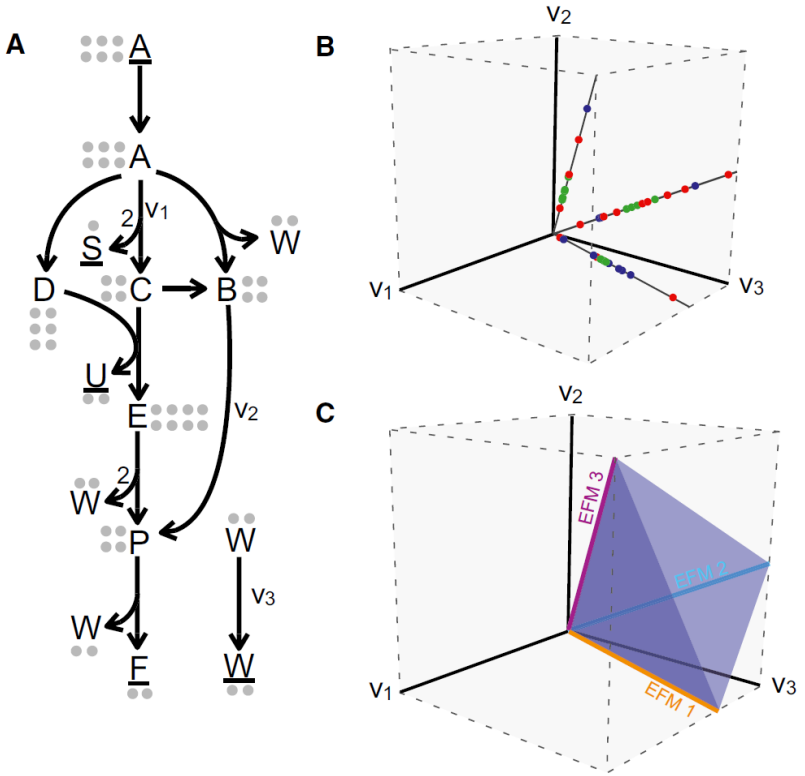



Wortel *et al.* FEBS (2014)

# Optimize the specific pathway flux for larger networks

Optimal solutions are restricted to the boundaries of the solution space.



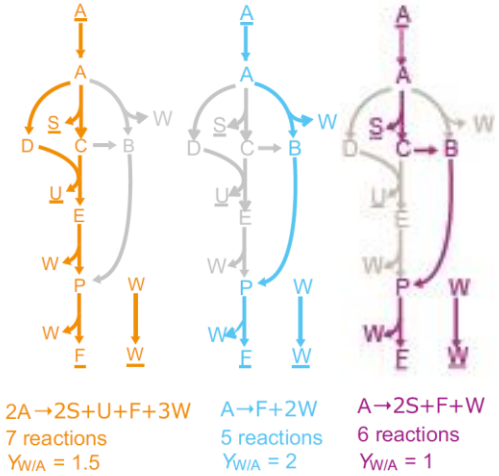

Wortel *et al.* FEBS (2014)

# Elementary Flux Modes

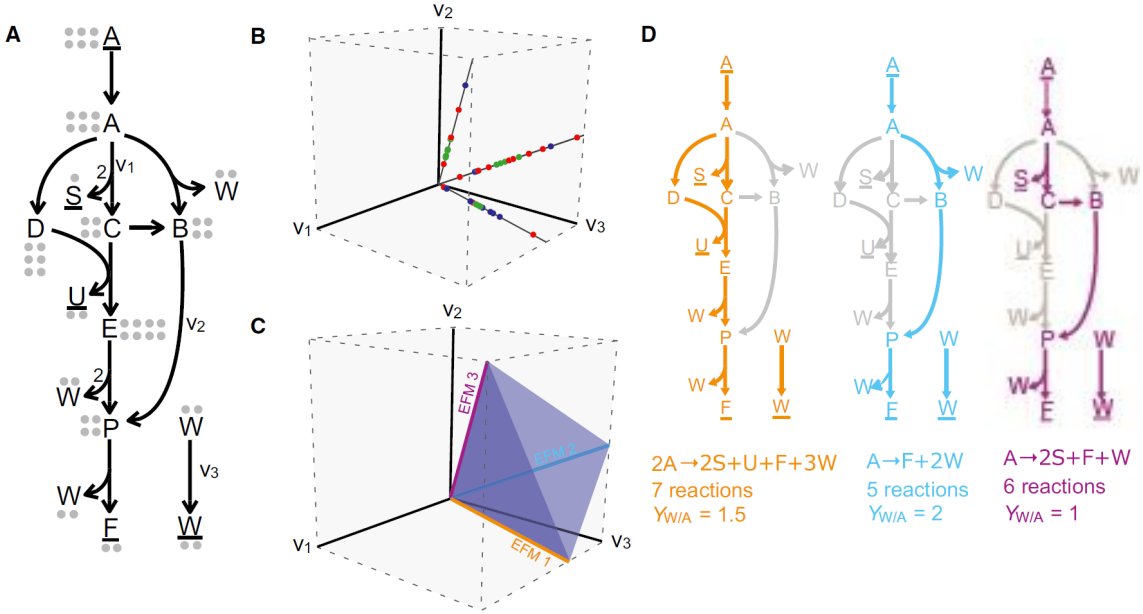
- If we know 1 flux, with the steady state conditions we know all fluxes

  or, equivalently:

- No internal fluxes can be omitted for the network to reach a steady state

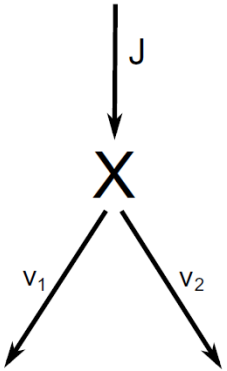


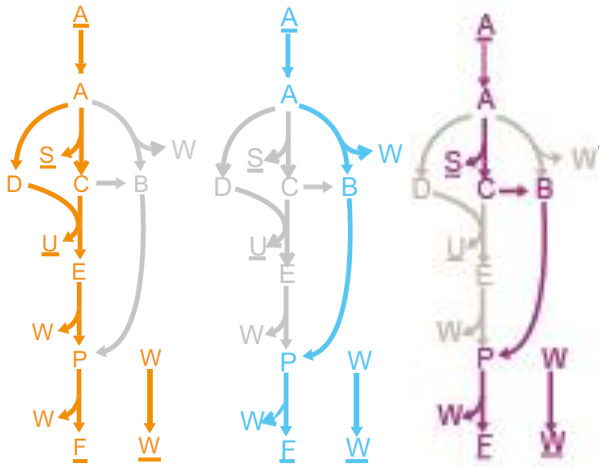
# Optimize the specific pathway flux




Different Elementary Flux Modes with different yields can be optimal

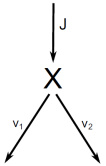
# EFMs are the ‘branches’ of the complete network

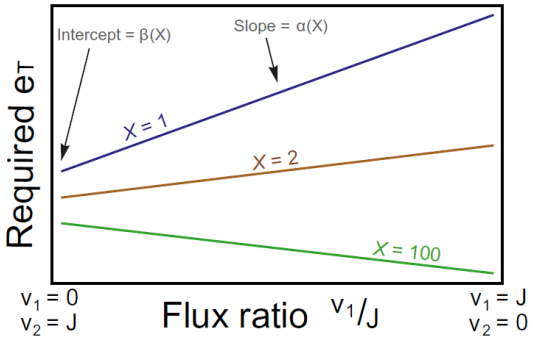


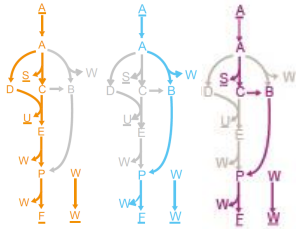



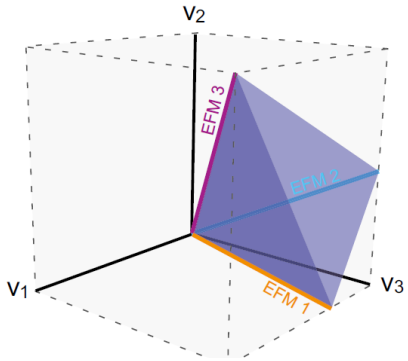

Total enzyme cost is a linear function of fluxes for fixed internal metabolite concentrations.

# EFMs are the ‘branches’ of the complete network






Total enzyme cost is a linear function of fluxes for fixed internal metabolite concentrations.

The optimum of linear functions is reached at the border of the solution space.

# Formal description of the optimization problem

$$\min_{\mathbf{x},\mathbf{e}} \left\{ \underbrace{\sum_{i=1}^{r} e_i}_{\text{objective}} \;\middle|\; \underbrace{\underbrace{\mathbf{N}\cdot\mathbf{v}=\mathbf{0}}_{\text{steady state}},\ \underbrace{\forall i : v_i = e_i f_i(\mathbf{x})}_{\text{enzyme kinetics}},\ \underbrace{\forall i : e_i \geq 0}_{\text{positive enzyme concentrations}},\ \underbrace{v_r = 1}_{\text{objective flux}}}_{\text{constraints}} \right\}$$



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constraints

Replace enzymes by rates using enzyme kinetics

$$\min_{\mathbf{x},\mathbf{v}} \left\{ \sum_{i=1}^{r} \frac{v_\mathrm{i}}{f_\mathrm{i}(\mathbf{x})} \middle| \mathbf{N}\cdot\mathbf{v}=\mathbf{0}, \forall i : \frac{v_\mathrm{i}}{f_\mathrm{i}(\mathbf{x})} \geq 0, v_\mathrm{r} = 1 \right\}$$



# Formal description of the optimization problem

$$\min_{\mathbf{x},\mathbf{e}} \Big\{ \underbrace{\sum_{i=1}^{r} e_i}_{\text{objective}} \;\Big|\; \underbrace{\underbrace{\mathbf{N}\cdot\mathbf{v}=\mathbf{0}}_{\text{steady state}},\; \underbrace{\forall i : v_i = e_i f_i(\mathbf{x})}_{\text{enzyme kinetics}},\; \underbrace{\forall i : e_i \geq 0}_{\text{positive enzyme concentrations}},\; \underbrace{v_r = 1}_{\text{objective flux}}}_{\text{constraints}} \Big\}$$

Replace enzymes by rates using enzyme kinetics

$$\min_{\mathbf{x},\mathbf{v}} \left\{ \sum_{i=1}^{r} \frac{v_i}{f_i(\mathbf{x})} \,\middle|\, \mathbf{N}\cdot\mathbf{v}=\mathbf{0}, \forall i : \frac{v_i}{f_i(\mathbf{x})} \geq 0, v_r = 1 \right\}$$

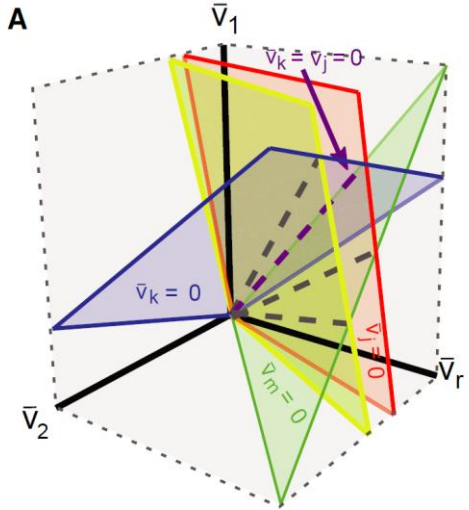
Set metabolites to their optimal concentrations ($x_o$)

$$c_i = 1/f_i(\mathbf{x_o})$$

$$\min_{\mathbf{v}} \left\{ \sum_{i=1}^{r} c_i v_i \,\middle|\, \mathbf{N}\mathbf{v}=\mathbf{0}, \forall i : v_i c_i \geq 0, v_r = 1 \right\}$$



# Why are Elementary Flux Modes the boundaries of the solution space?

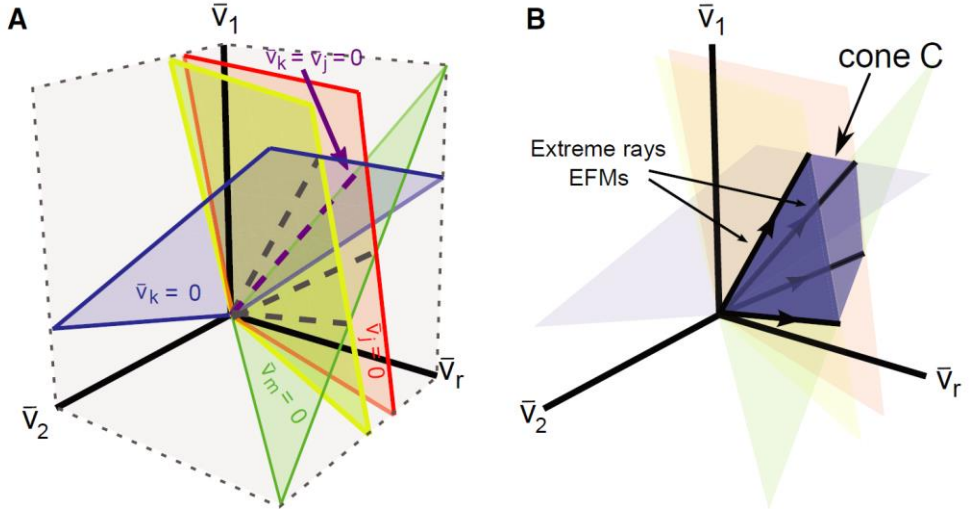



Solution space with steady state constrains added.

Rays are EFMs because they do not intersect any additional planes.

Wortel *et al.* FEBS (2014)

# Why are Elementary Flux Modes the boundaries of the solution space?

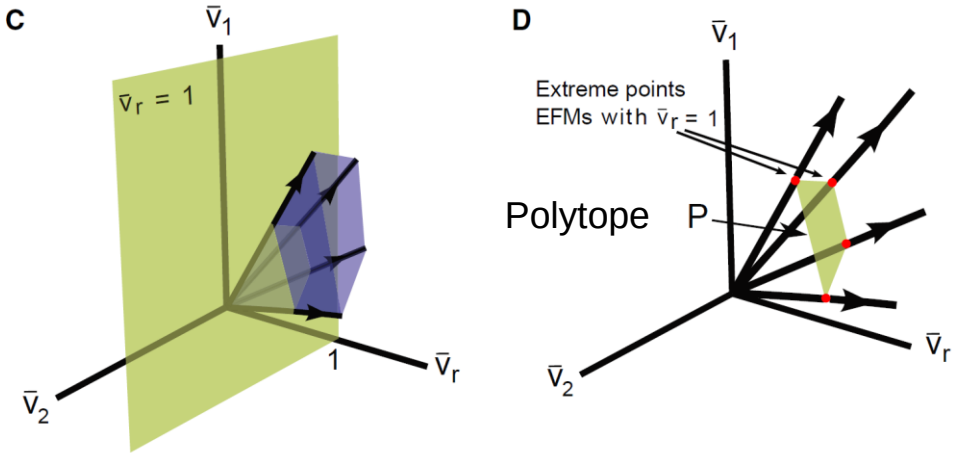



$$\min_{\bar{v}} \left\{ \sum_{i=1}^{\cdot} c_i \bar{v}_i \,\middle|\, \underbrace{\bar{\mathbf{N}}\bar{\mathbf{v}} = \mathbf{0}, \bar{\mathbf{v}} \geq 0}_{\text{C, cone, a convex set}} , \underbrace{v_r = 1}_{\text{hyperplane}} \right\}$$

Solution space with steady state constrains added.

Take all the reactions to be positive.

# Why are Elementary Flux Modes the boundaries of the solution space?




$$P = C \cap \{\mathbf{v} | v_r = 1\}$$

Fix the objective flux.

We end up with a space where the extremes are EFMs.

# Formal description of the optimization problem

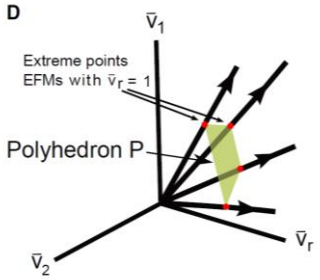
$$\min_{\mathbf{x},\mathbf{e}} \left\{ \underbrace{\sum_{i=1}^{r} e_i}_{\text{objective}} \;\middle|\; \underbrace{\mathbf{N}\cdot\mathbf{v}=\mathbf{0}}_{\text{steady state}},\ \underbrace{\forall i : v_i = e_i f_i(\mathbf{x})}_{\text{enzyme kinetics}},\ \underbrace{\forall i : e_i \geq 0}_{\text{positive enzyme concentrations}},\ \underbrace{v_r = 1}_{\text{objective flux}} \right\}$$

constraints

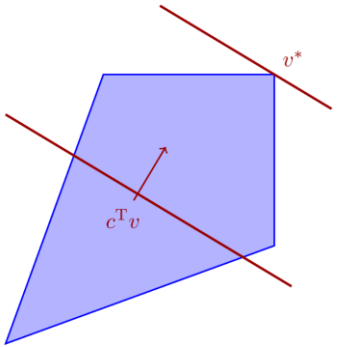
Can be simplified to (for fixed metabolite concentrations): $\min_{\mathbf{v}} \left\{ \sum_{i=1}^{r} c_i v_i \,\middle|\, \mathbf{N}\mathbf{v}=\mathbf{0}, \forall i : v_i c_i \geq 0, v_r = 1 \right\}$

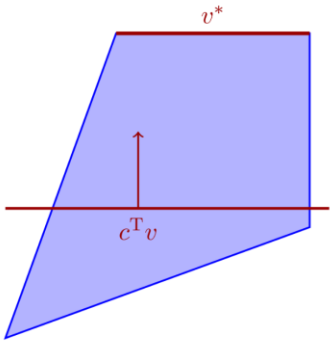
Minimize a linear function: $\sum_{i=1}^{\bar{r}} c_i \bar{v}_i$

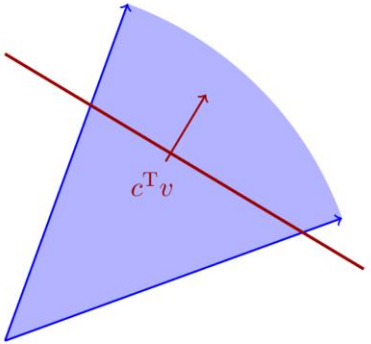
Over a space where the EFMs are the extreme points:



# Compare with FBA




Extreme points are EFMs
In FBA usually not

Adding kinetics
➔ non-uniqueness does not occur

Minimizing enzymes
➔ unboundedness does not occur

Finding the objective (optimal metabolite levels) is nonlinear ⇔ FBA is linear

# Enzyme-Flux Cost Minimization (EFCM)

Procedure:

1. Network description (kinetics, enzyme costs, etc.)
2. Find EFMs
3. Optimize each EFM using ECM
4. Compare EFMs

Limitations:

- size
- parameter availability



# Applications of EFCM: Trade-off between growth rate and yield

Low-yield strategies are often observed (Crabtree effect, Warburg effect)
In toy models a trade-off between growth rate and yield

Experimental verification has lead to mixed results

Does this still hold for a large interconnected network?

➔ Calculate the growth rate for larger networks

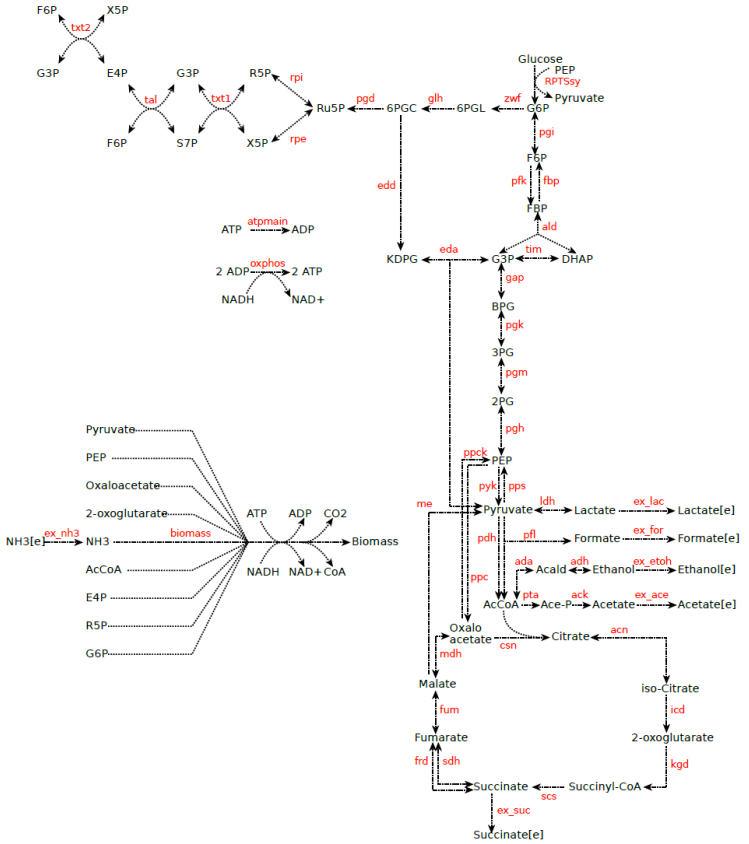


# Applications of EFCM

Procedure:

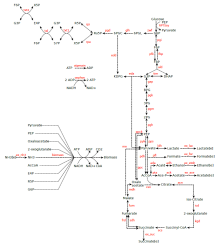
1. Network description (kinetics, enzyme costs, etc.)

- Databases (Brenda, eQuilibrator)
- Parameter balancing
- Molecular weights




Wortel *et al.* Plos Comp (2018)

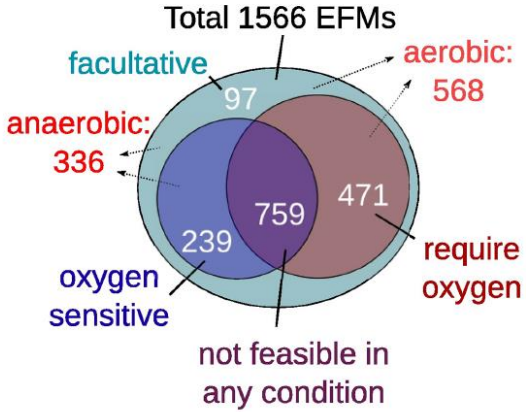
# Applications of EFCM



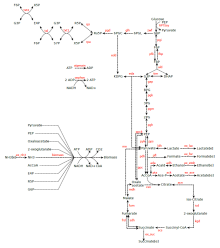
Procedure:

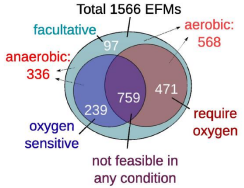
1. Network description (kinetics, enzyme costs, etc.)
2. Find EFMs

- EFMtool



# Applications of EFCM






Procedure:

1. Network description (kinetics, enzyme costs, etc.)
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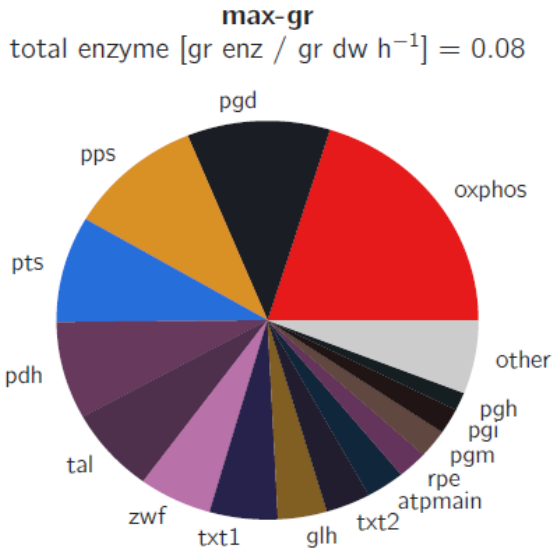
3. Optimize each EFM using ECM

- Nonlinear optimization



# Applications of EFCM

Procedure:

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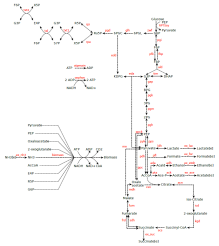
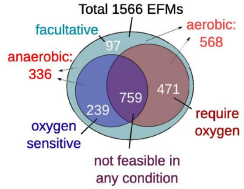
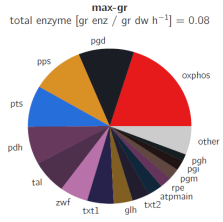
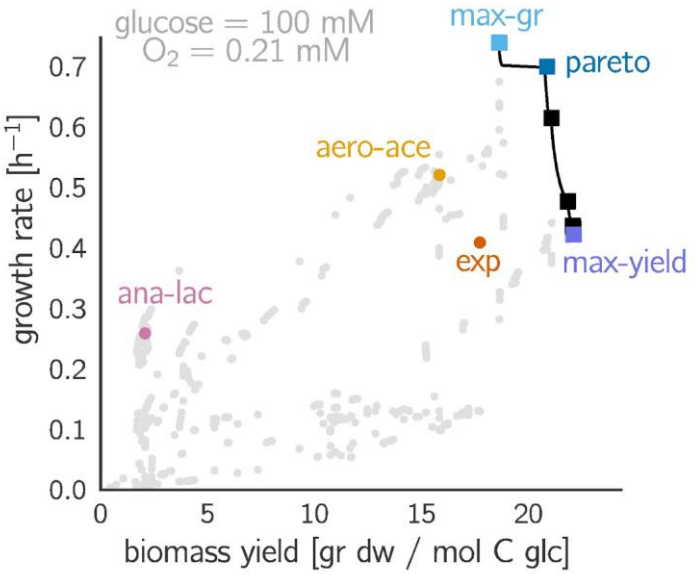
4. Compare EFMs

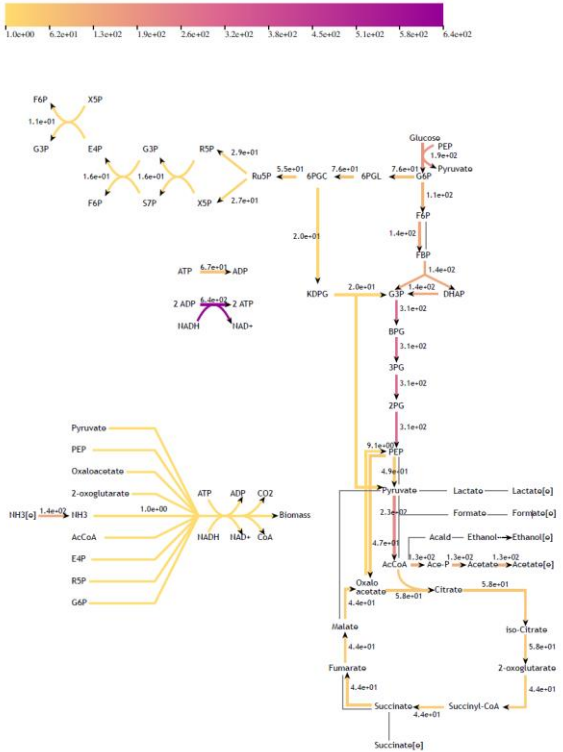




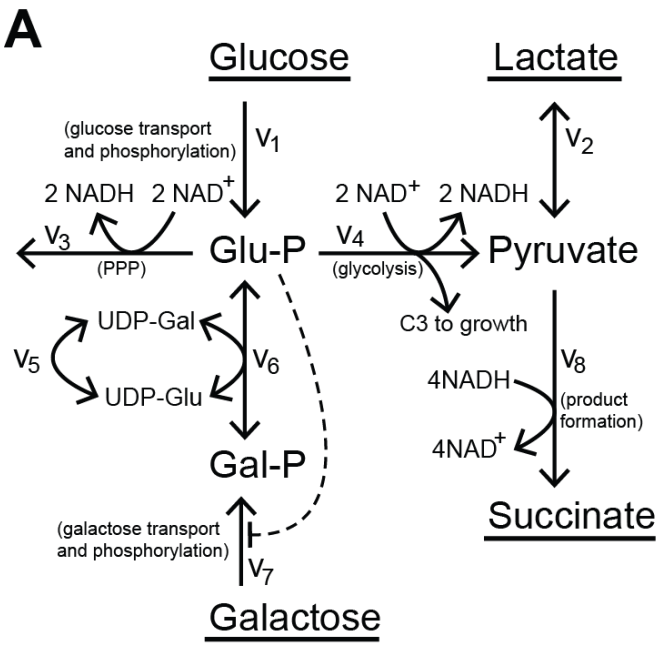



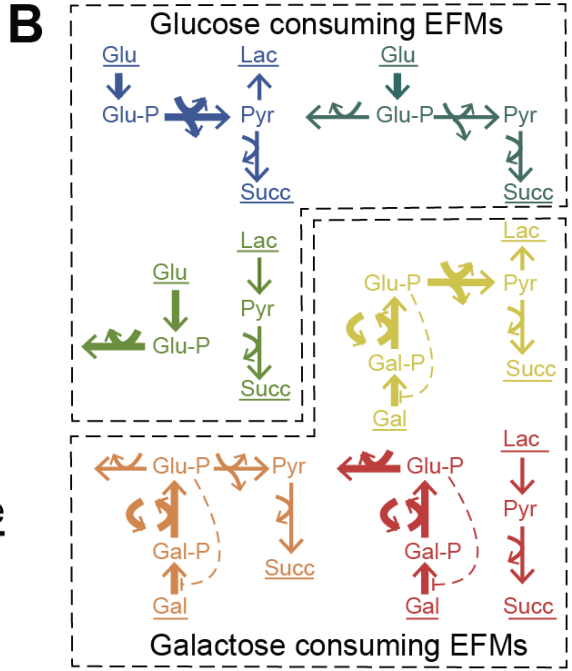



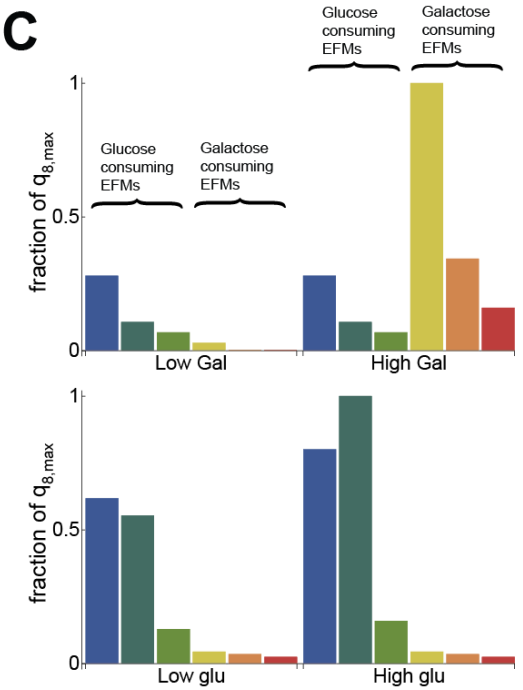
# EFMs are not always ‘simple’ flux patterns



# Which EFM is optimal is condition dependent

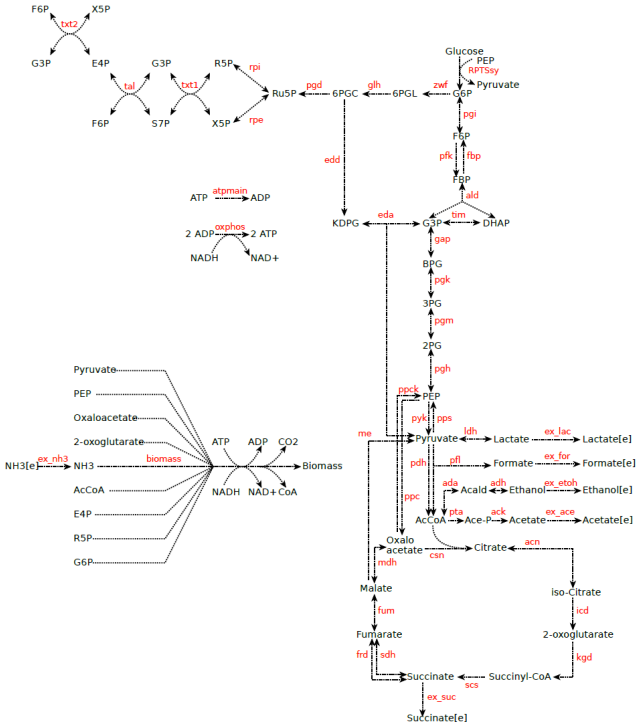


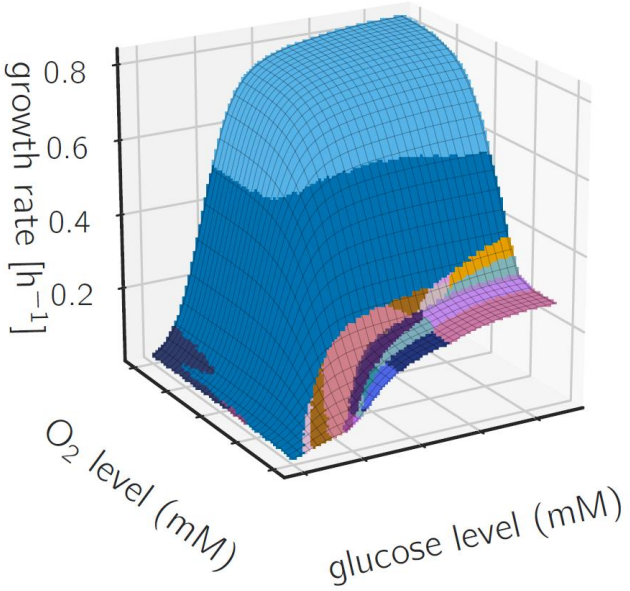




Wortel *et al.* FEBS (2014)

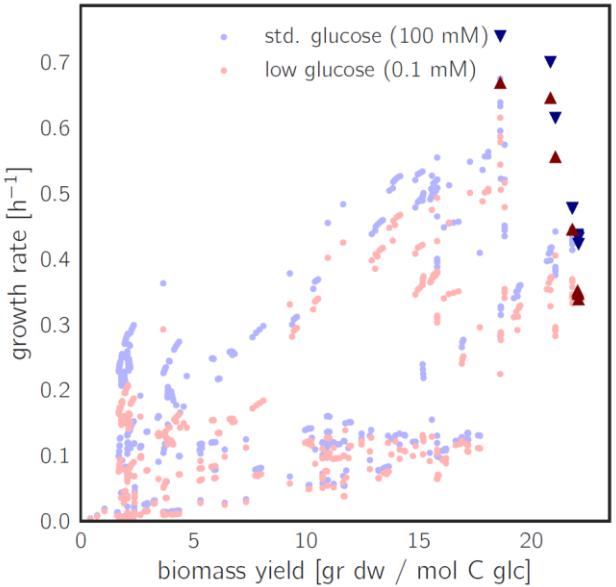
# Which EFM is optimal is condition dependent

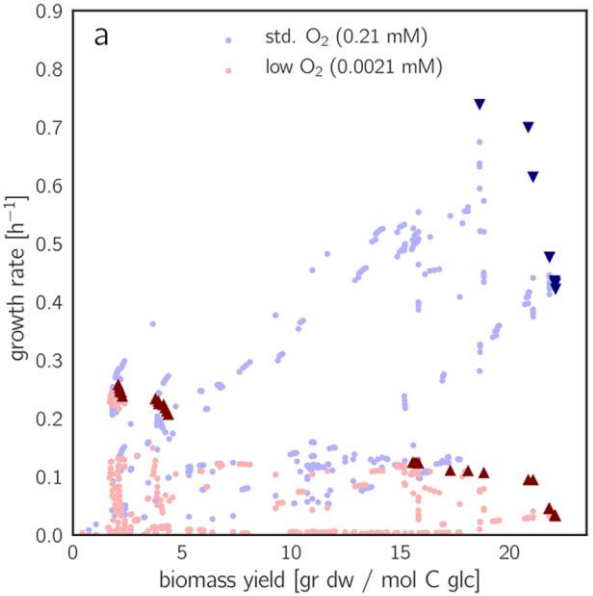


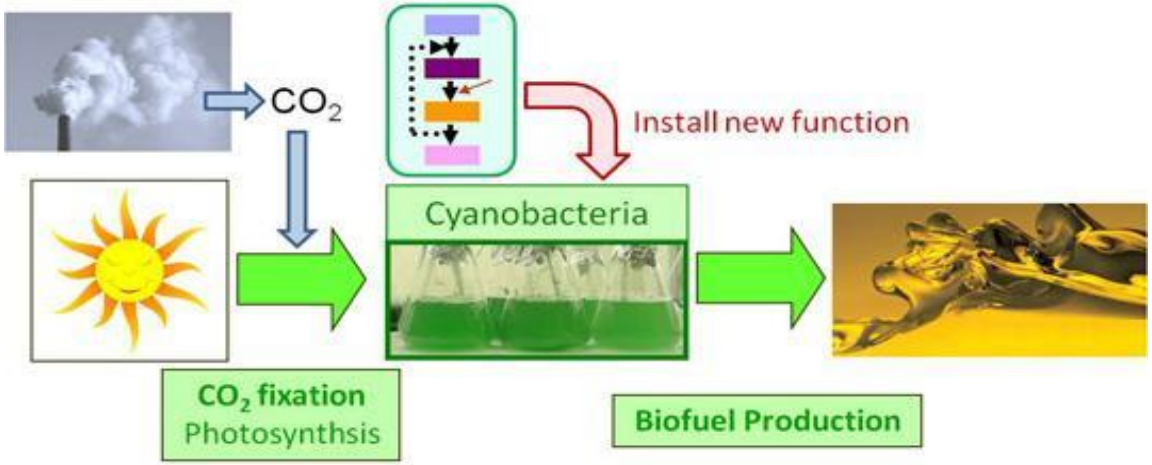

Wortel *et al.* Plos Comp (2018)

# Trade-off between growth rate and yield is condition dependent



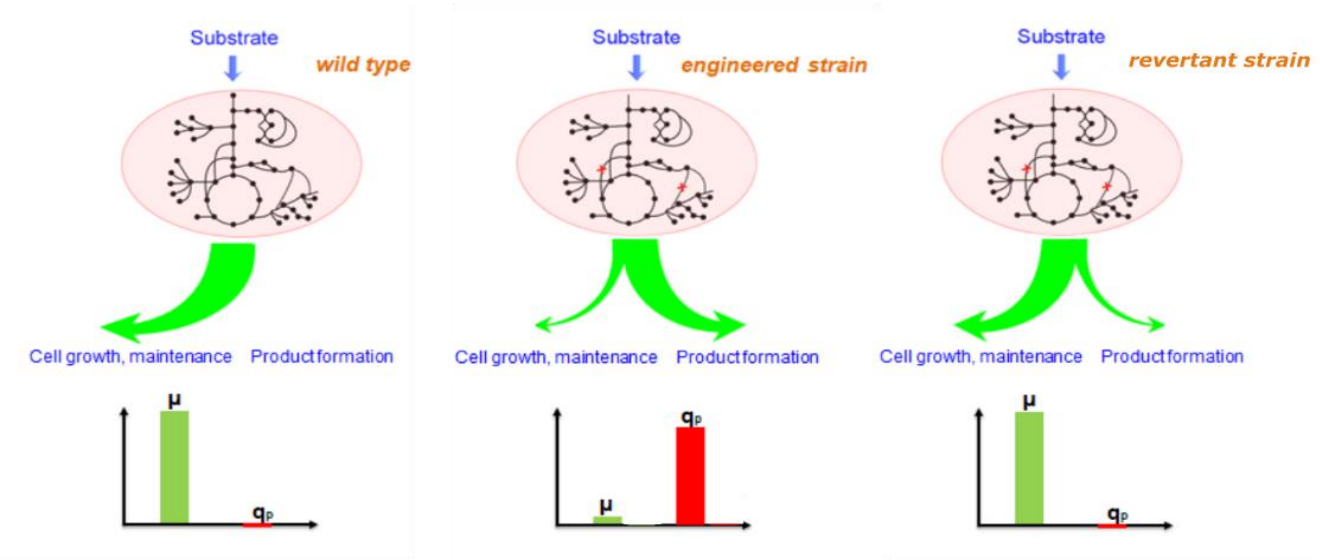
# Applications of EFCM: Growth-coupled production




Engineering organisms for sustainable production

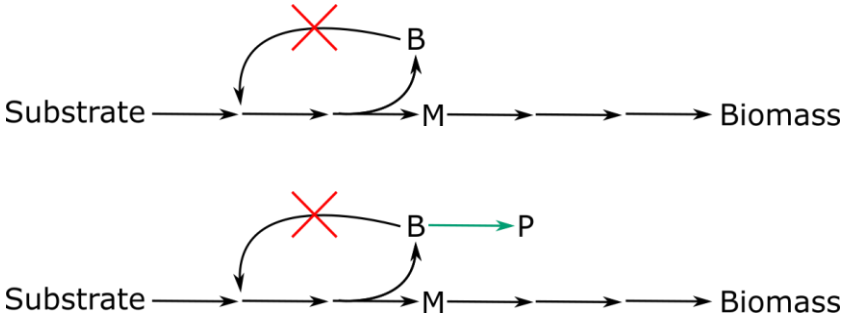
Guillaume, Orlova *et al.* in prep

# Applications of EFCM: Growth-coupled production




Product formation is lost because it does not align with cell growth rate

# Applications of EFCM: Growth-coupled production

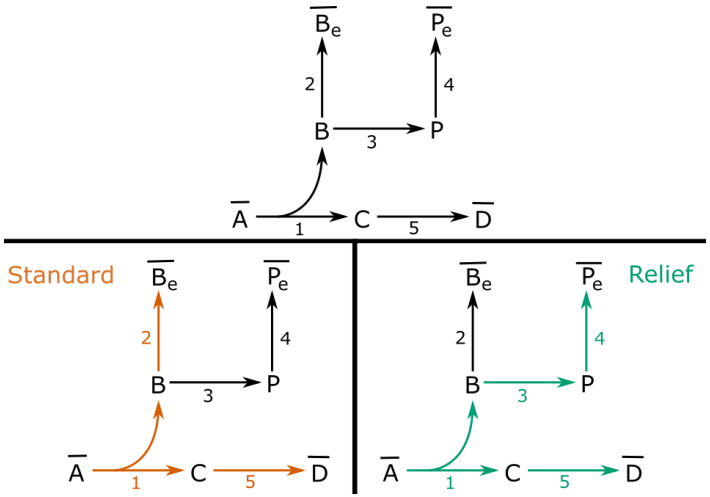


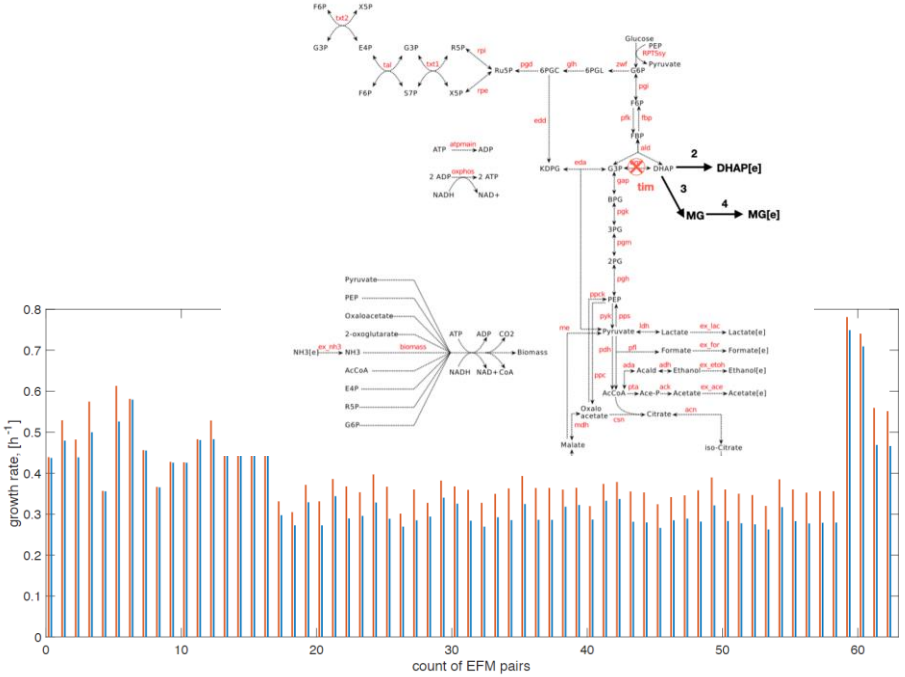

Solutions it one or two-step growth-coupled production

Two-step increases the possible products

EFCM can predict when two-step production is growth beneficial

# Applications of EFCM: Growth-coupled production




Two-step production can be beneficial when it is a ‘cheaper’ way to remove the product

Guillaume, Orlova *et al.* in prep

# Conclusions

- Optimizing complete metabolism includes enzymes, fluxes and metabolites that are all linked
- Enzyme-cost efficient state are reached at Elementary Flux Modes
  - Reduction of variables to n (n = #EFMs) problems with only the metabolites as variables
- Applications include trade-offs between growth rate and yield, condition dependent effects, and the growth effects of engineered strains