Resource allocation strategies behind rate-yield phenotypes in *E. coli*

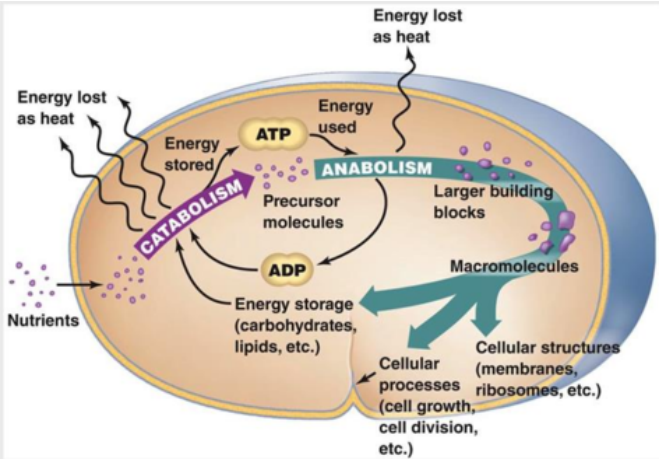
V.Baldazzi, D.Ropers, J-L.Gouzé, T.Gedeon, H.de Jong

eLife 12:e79815 (2023)

Context

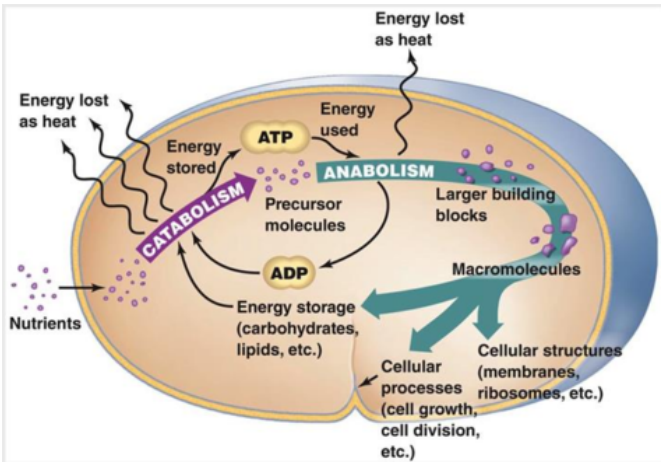
Bacterial growth involves

- the conversion of nutrients to biomass
- energy stored in nutrients transferred to energy cofactors (ATP) driving the biomass synthesis
- coupled energy and mass fluxes



Bacterial growth involves

- the conversion of nutrients to biomass
- energy stored in nutrients transferred to energy cofactors (ATP) driving the biomass synthesis
- coupled energy and mass fluxes



Two macroscopic criteria characterize microbial growth:

- **growth rate** *i.e.* the speed of conversion of nutrients into biomass
- **growth yield** *i.e.* the efficiency of the process

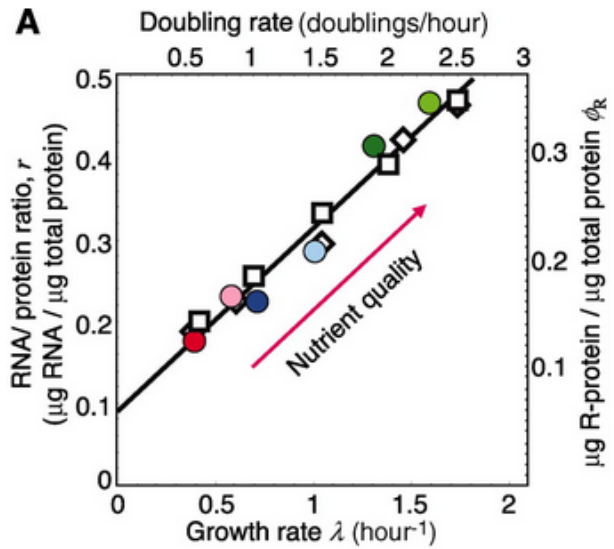
$$\frac{\text{Biomass produced}}{\text{Nutrient consumed}}$$

Bacterial growth and resource allocation

Bacterial growth has been analyzed from the perspective of proteome allocation:

- Proteins are main component of biomass
- Proteins catalyze reactions necessary for growth
- Proteome composition reflects **resource allocation strategy**

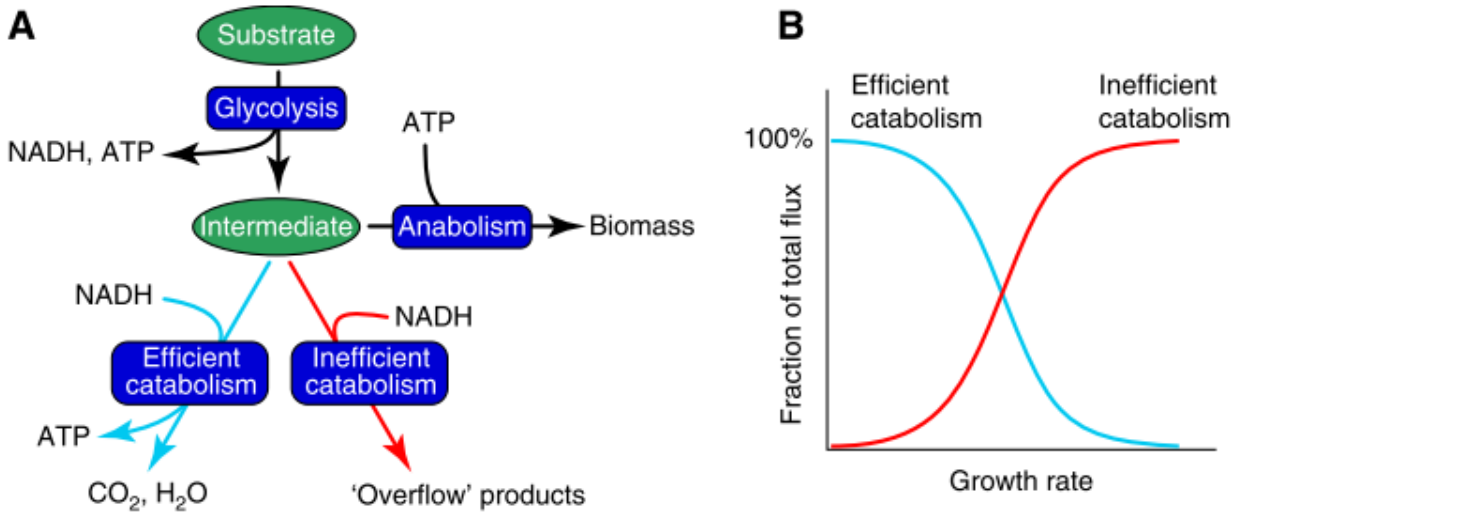
Relation between resource allocation and rate and yield.



- Linear relation between **growth rate** and ribosomal protein fraction

Scott et al. (2010)

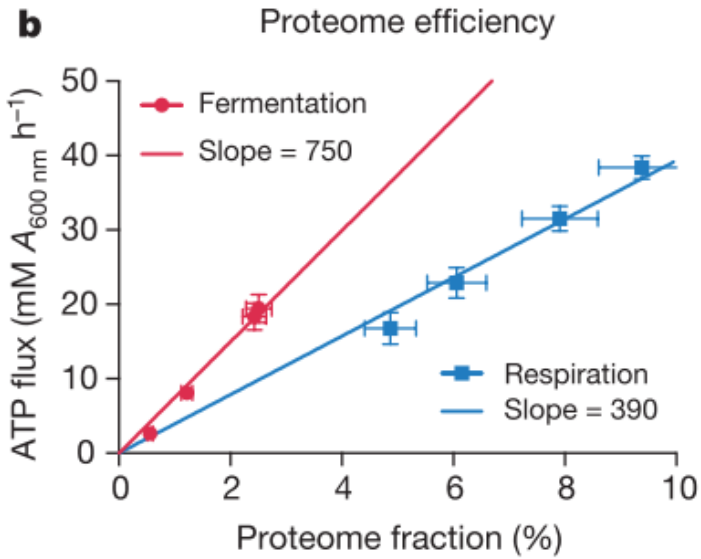
Overflow metabolism:



Molenaar et al. (2009); Basan et al. (2015)

- Switch from high-yield respiration to a low-yield fermentation for high growth rates
- Trade-off between ATP yield and investment in enzyme synthesis
  - > Gain in proteome efficiency through fermentation

Overflow metabolism:

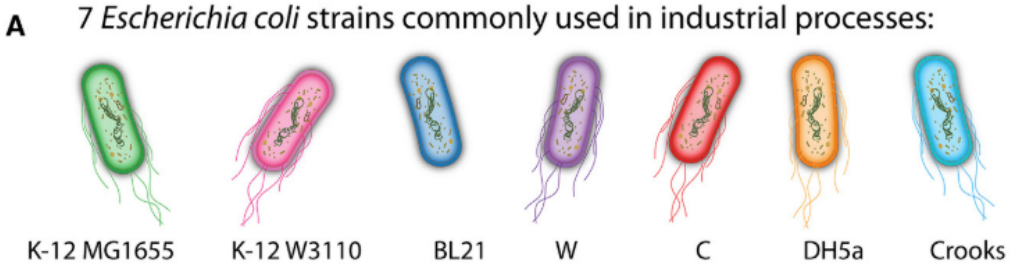


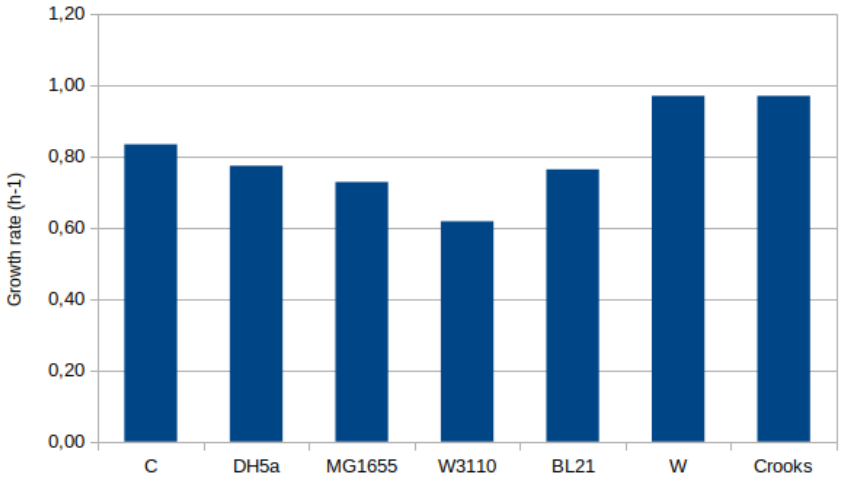
Molenaar et al. (2009); Basan et al. (2015)

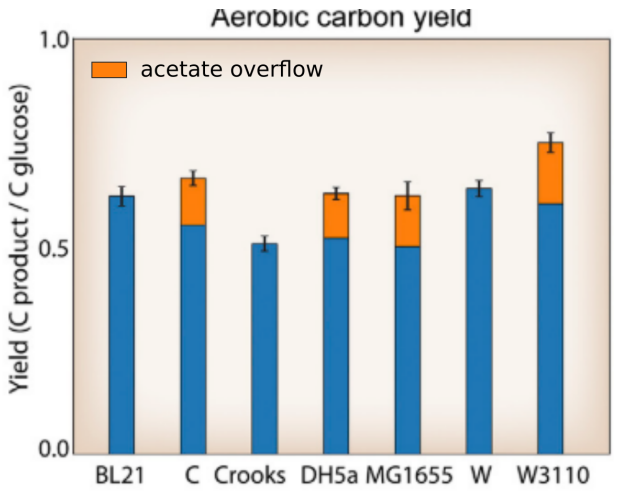
- Switch from high-yield respiration to a low-yield fermentation for high growth rates
- Trade-off between ATP yield and investment in enzyme synthesis
  - > Gain in proteome efficiency through fermentation

Phenotypic variability in bacterial growth

In same conditions, different *E. coli* strains show large variations in growth rate and yield







Monk et al. (2016)

Questions

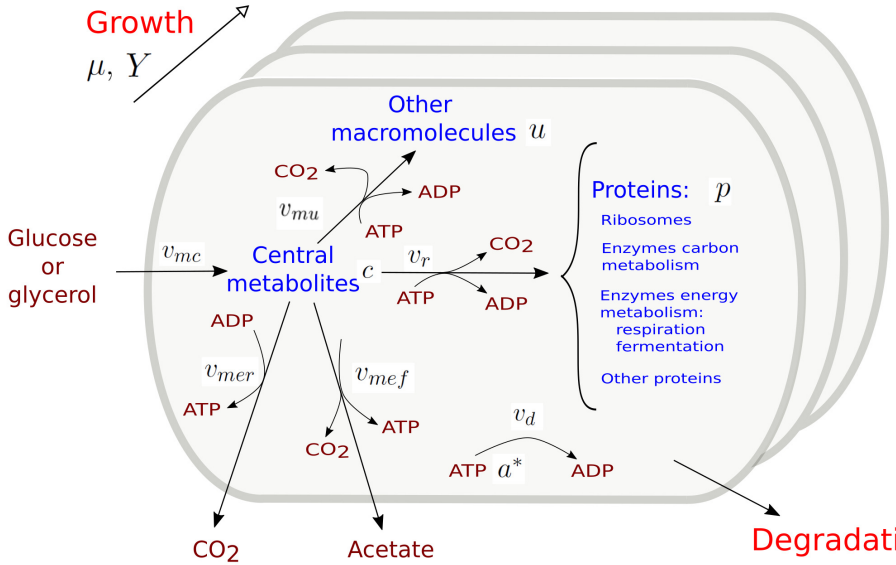
- What is the range of rate-yield phenotypes predicted by changes in proteome composition?
- Does the predicted range correspond to observed rate-yield variability?

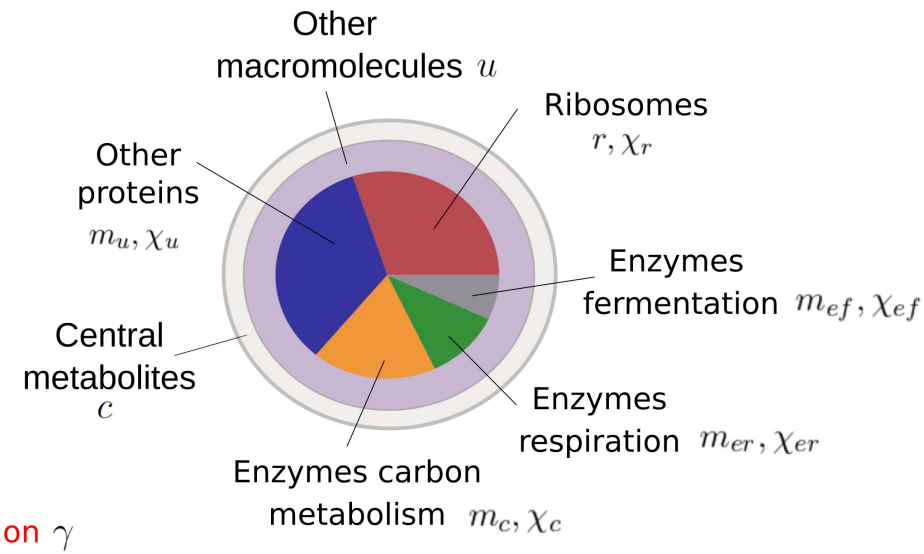
Approach

Develop a coarse-grained model of **coupled energy and mass fluxes** in microorganisms, based on minimal assumptions

Baldazzi et al. (2023)

Model definition





- Macroscopic reactions for carbon uptake and metabolism, biomass synthesis, ATP production
- Biomass $B = \beta$ (Proteins + Other macromolecules + Metabolites)
- 5 allocation parameters $\chi_u, \chi_r, \chi_c, \chi_{er}, \chi_{ef}$ define the resource allocation strategy of the cell ($\sum_i \chi_i = 1$)

Model equations

Carbon balance:

$$\frac{dc}{dt} = v_{mc} - v_{mer} - \rho_{mef}\, v_{mef} - \rho_{ru}\left(v_r + v_{mu}\right) - \left(\mu + \gamma\right) c,$$

$$\frac{du}{dt} = v_{mu} - \left(\mu + \gamma\right) u,$$

$$\frac{dr}{dt} = \chi_r\, v_r - \left(\mu + \gamma\right) r,$$

$$\frac{dm_u}{dt} = \chi_u\, v_r - \left(\mu + \gamma\right) m_u,$$

$$\frac{dm_c}{dt} = \chi_c\, v_r - \left(\mu + \gamma\right) m_c,$$

$$\frac{dm_{er}}{dt} = \chi_{er}\, v_r - \left(\mu + \gamma\right) m_{er},$$

$$\frac{dm_{ef}}{dt} = \chi_{ef}\, v_r - \left(\mu + \gamma\right) m_{ef}.$$

Energy balance:

$$\frac{da^*}{dt} = n_{mer}\, v_{mer} + n_{mef}\, v_{mef} - n_r\, v_r - n_{mu}\, v_{mu} - v_d$$

Assumptions

Total biomass concentration

- $1/\beta = (m_c + m_{er} + m_{ef} + r + m_u + u + c)$

Assumptions

Total biomass concentration

- $1/\beta = (m_c + m_{er} + m_{ef} + r + m_u + u + c)$

Total concentration of energy co-factors

- $a + a^* = a_0$ by some undefined mechanisms

Assumptions

Total biomass concentration

- $1/\beta = (m_c + m_{er} + m_{ef} + r + m_u + u + c)$

Total concentration of energy co-factors

- $a + a^* = a_0$ by some undefined mechanisms

Rates

- Michaelis-Menten kinetics to define the reaction rates

$$v_r(r, c, a^*) = r\, k_r\, \frac{c}{c + K_r}\, \frac{a^*}{a^* + K_{ar}},$$

- Constant external substrate concentration S

$$v_{mc}(m_c) = m_c\, k_{mc}\, \frac{S}{S + K_S} = m_c\, e_s$$

Model calibration

We used different datasets for *E.coli* BW2115 on minimal medium:

- Metabolite concentrations from Bennet et al. (2009), Gerosa et al. (2015)
- Protein concentrations from Schmidt et al. 2016
- Metabolic fluxes from van Rijsewijk and al. (2011), Gerosa et al. (2015)

1. Use literature information to estimate the total biomass density, the total protein and metabolites concentrations
2. Use proteomics and metabolomics data to estimate the concentrations of the different constituents distinguished in the model, based on **proportions**
3. Derive rate parameters using the fluxes and concentrations

Different carbon sources: glucose, glycerol
Different culture conditions: batch, chemostat

Sampling of possible allocation strategies

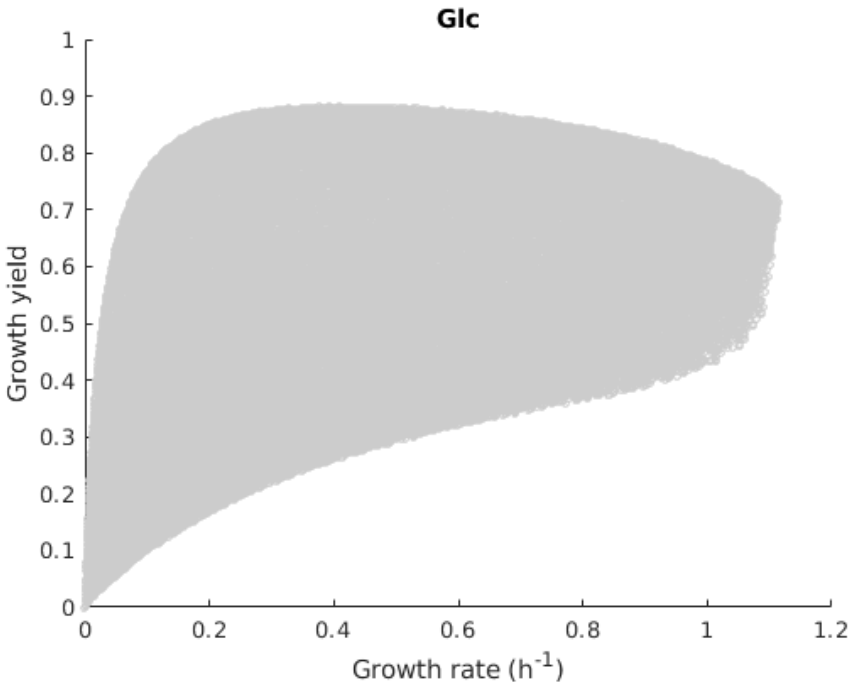
1. **Random sampling** of $(\chi_u, \chi_r, \chi_c, \chi_{er}, \chi_{ef})$ values
2. Numerical simulation and steady-state computation (variables, fluxes)
3. Plot of steady-state growth rate (μ) vs yield (Y):

$$\mu = \frac{1}{B}\frac{dB}{dt}$$

$$Y = \frac{1}{\beta}\frac{\mu}{v_{mc}}$$

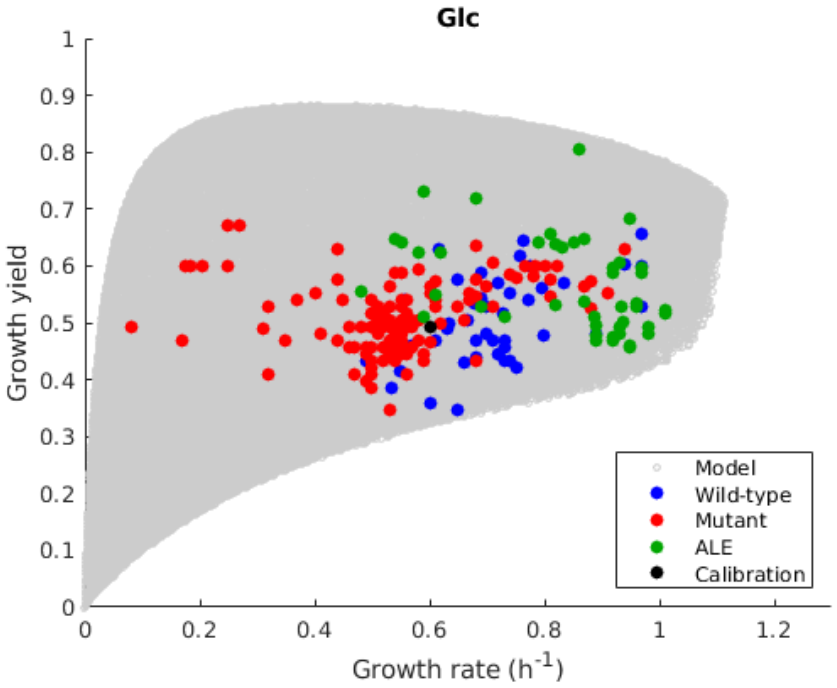
- No optimisation!
- Fixed environment

Predicted vs observed rate-yield phenotypes

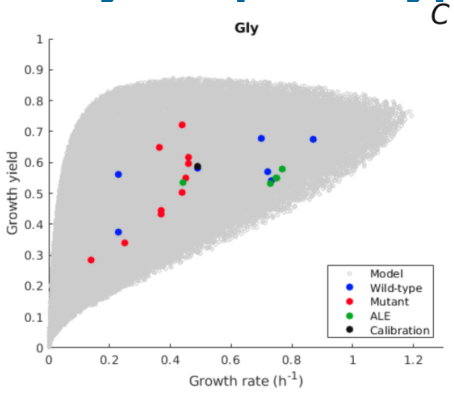


- Maximum yield increase at low growth rates: lower burden of the non-growth-associated maintenance costs
- Trade-off between rate and maximum yield at higher growth rates

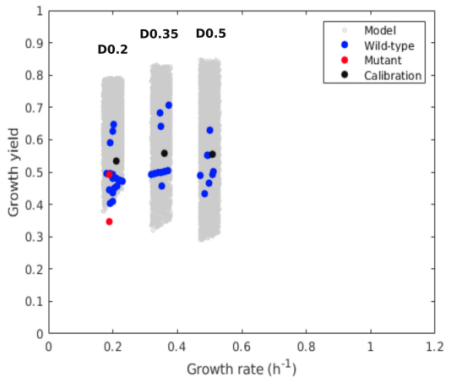
Predicted vs observed rate-yield phenotypes



C

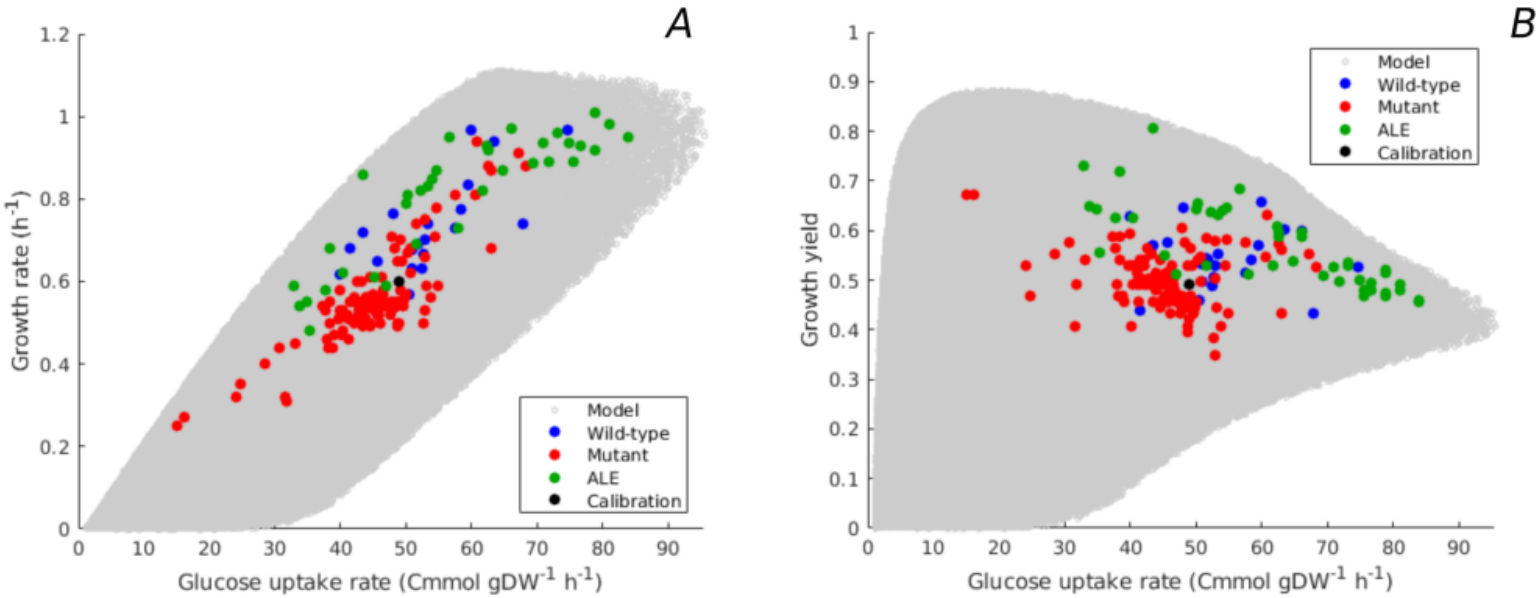


D



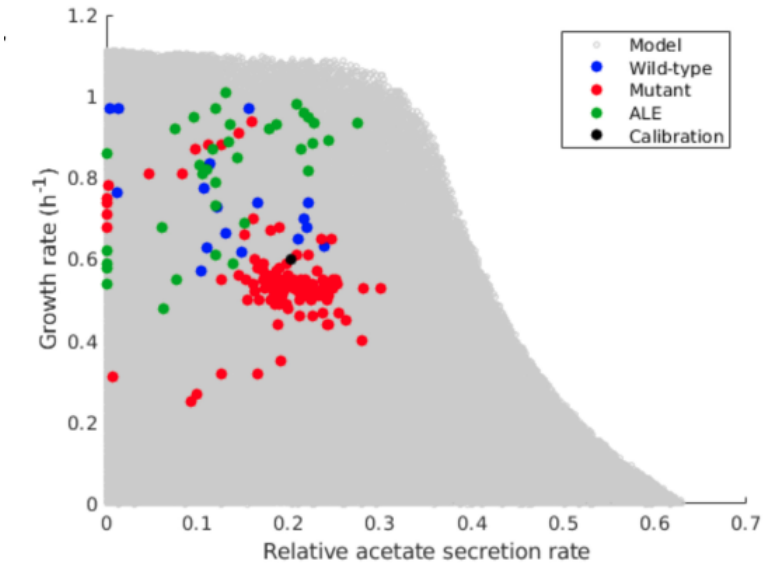
- Maximum yield increase at low growth rates: lower burden of the non-growth-associated maintenance costs
- Trade-off between rate and maximum yield at higher growth rates
- Very good agreement with data from different *E. coli* strains

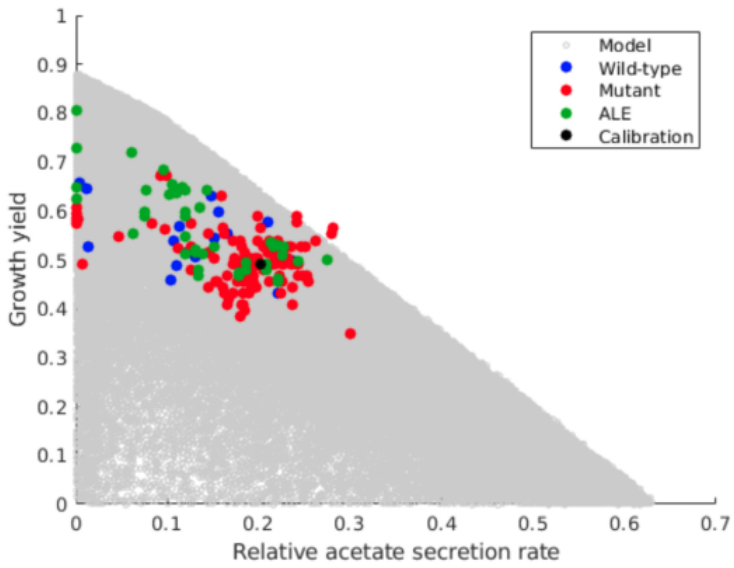
Predicted vs observed fluxes



- Correlation between growth rate and glucose uptake
- Additional trade-off between maximum growth yield and glucose uptake (*Cheng et al. (2019)*)

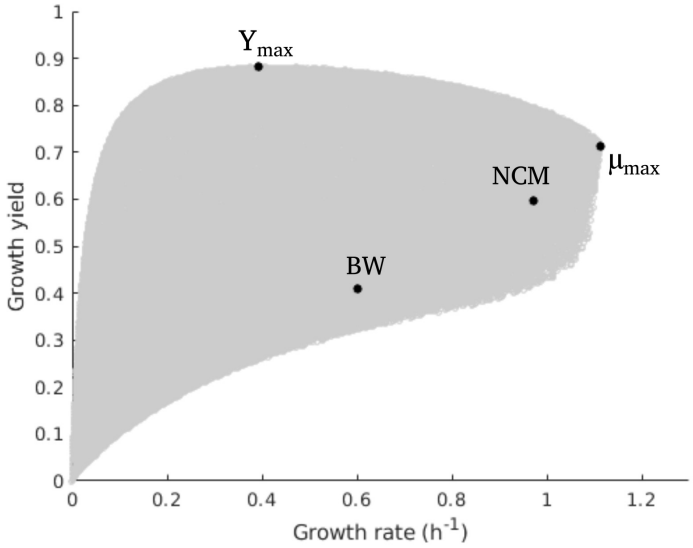
Predicted vs observed fluxes

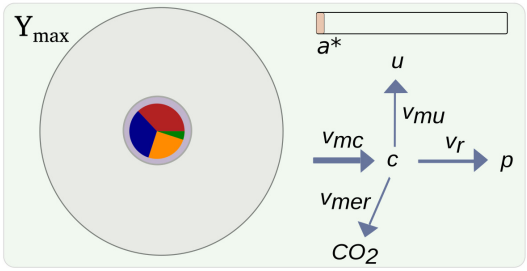


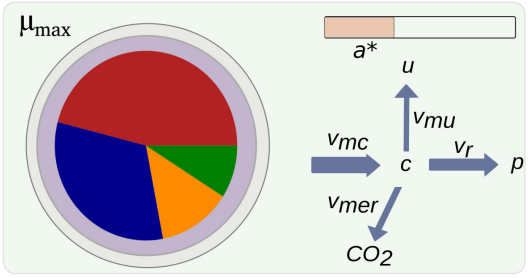


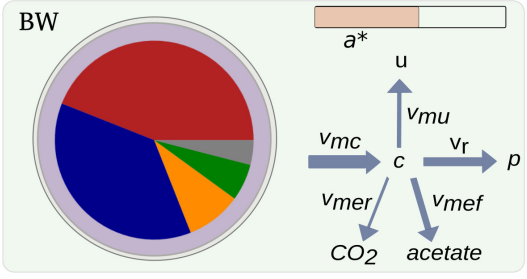
- Trade-off between acetate secretion rate and growth yield
  - > maximum growth yield requires respiration
- No relation between growth rate and acetate secretion rate
  - > high growth rates are possible for a wide range of ATP production modes
- Existence of wild-type and ALE (Artificial Lab Evolution) strains with high growth rate and no fermentation

Opening the black box...



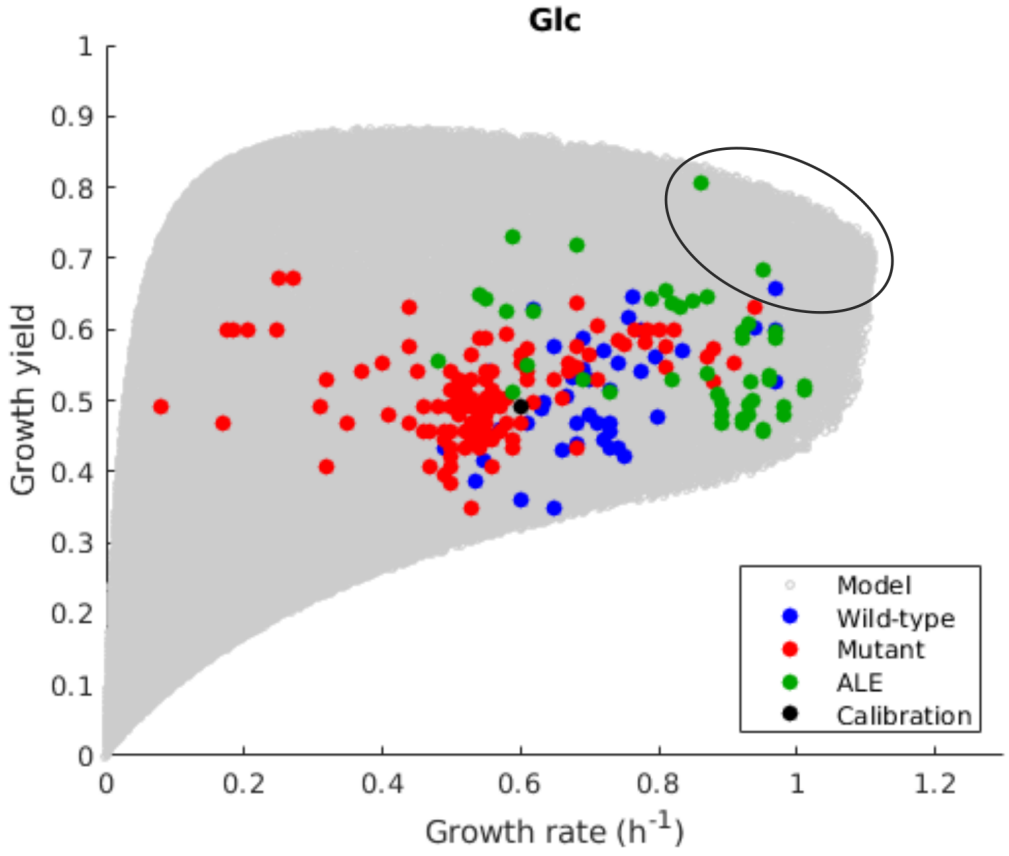




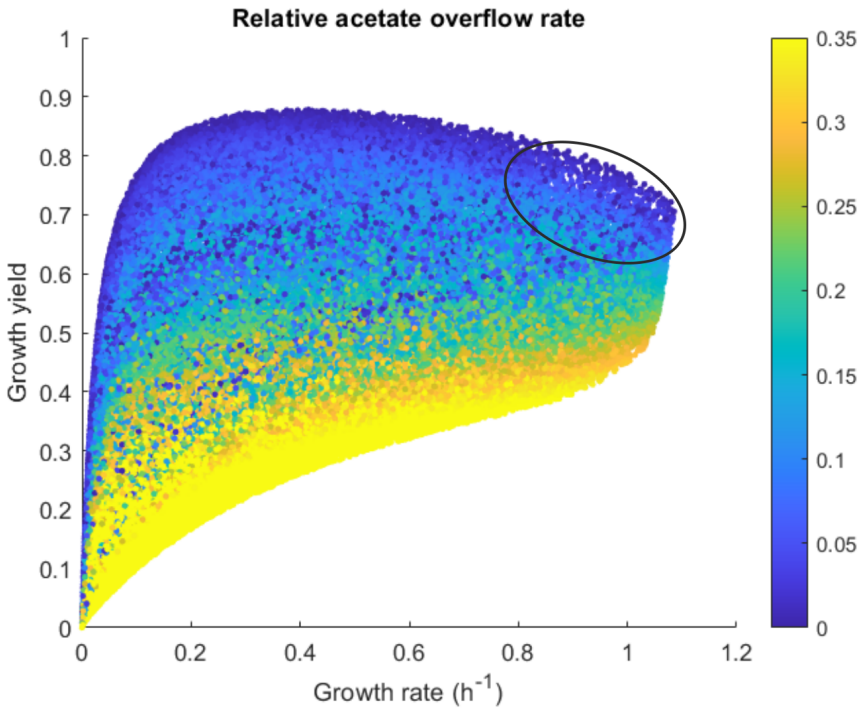


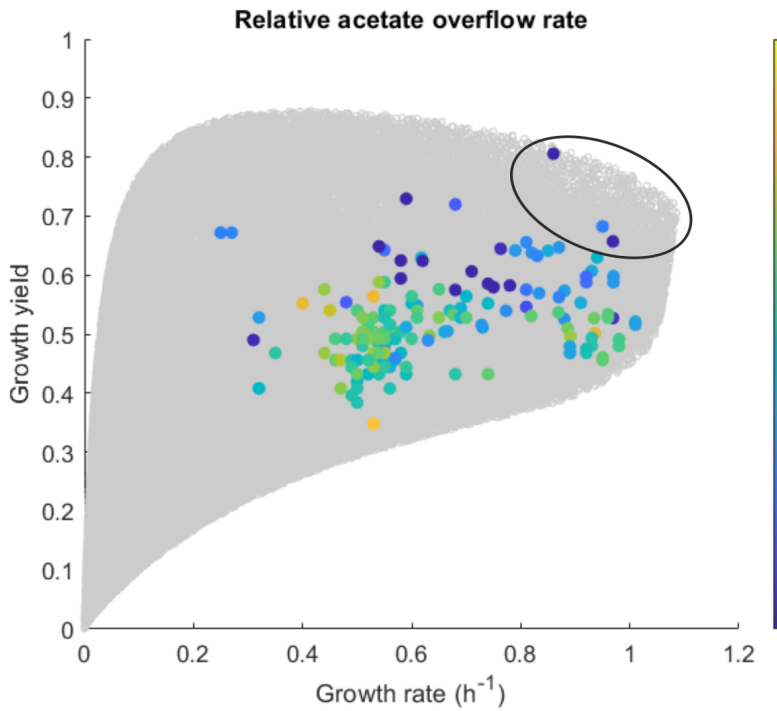
- Model allows to connect rate-yield phenotypes to the underlying resource allocation strategies
- Tight and complex relation between resource allocation, biomass composition and fluxes

Focus on a specific rate-yield phenotype: **high rate and high yield**



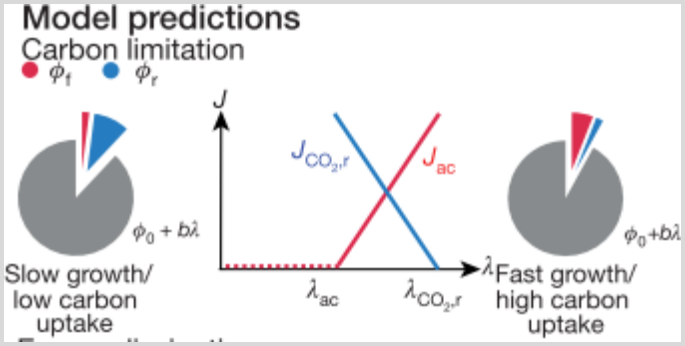
Focus on a specific rate-yield phenotype: **high rate and high yield**





Accepted view

- Fast growth needs a larger ribosomal fraction
- Switch from respiration to fermentation allows to *free* proteome resources for growth
  - > Fermentation requires less protein than respiration



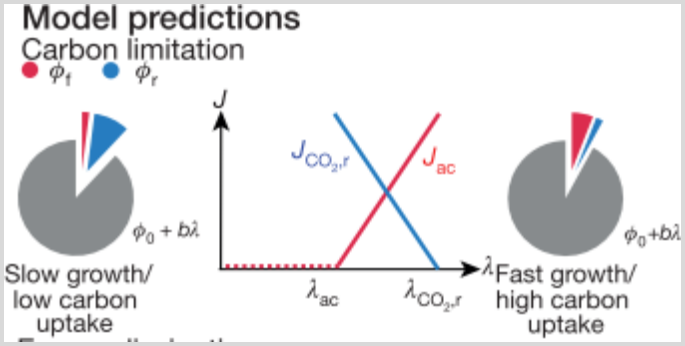
Basan et al. (2015)

$\Rightarrow$ Overflow metabolism is required for fast growth

Strategies for fast and efficient growth

Accepted view

- Fast growth needs a larger ribosomal fraction
- Switch from respiration to fermentation allows to *free* proteome resources for growth
  - > Fermentation requires less protein than respiration



Basan et al. (2015)

$\Rightarrow$ Overflow metabolism is required for fast growth

Which resource allocation strategies can lead to **fast and efficient** growth?

Strategies for fast and efficient growth

Fast and efficient growth rate is supported by:

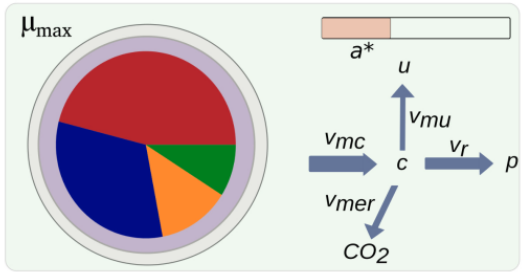
- increased nutrient uptake
- increased protein synthesis

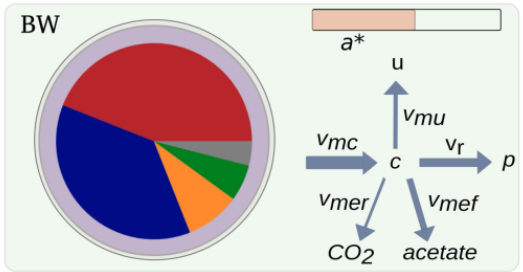
but...

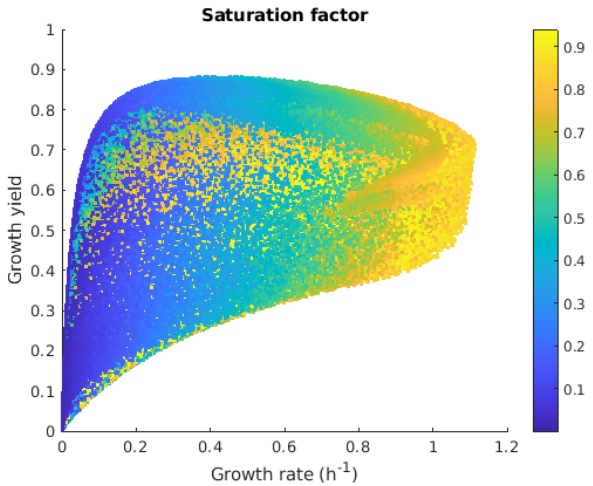
- total protein (ribosome) concentration is lower
- ATP concentration is reduced

$\Rightarrow$ Increased **enzyme saturation** thanks to increased **metabolite concentration**

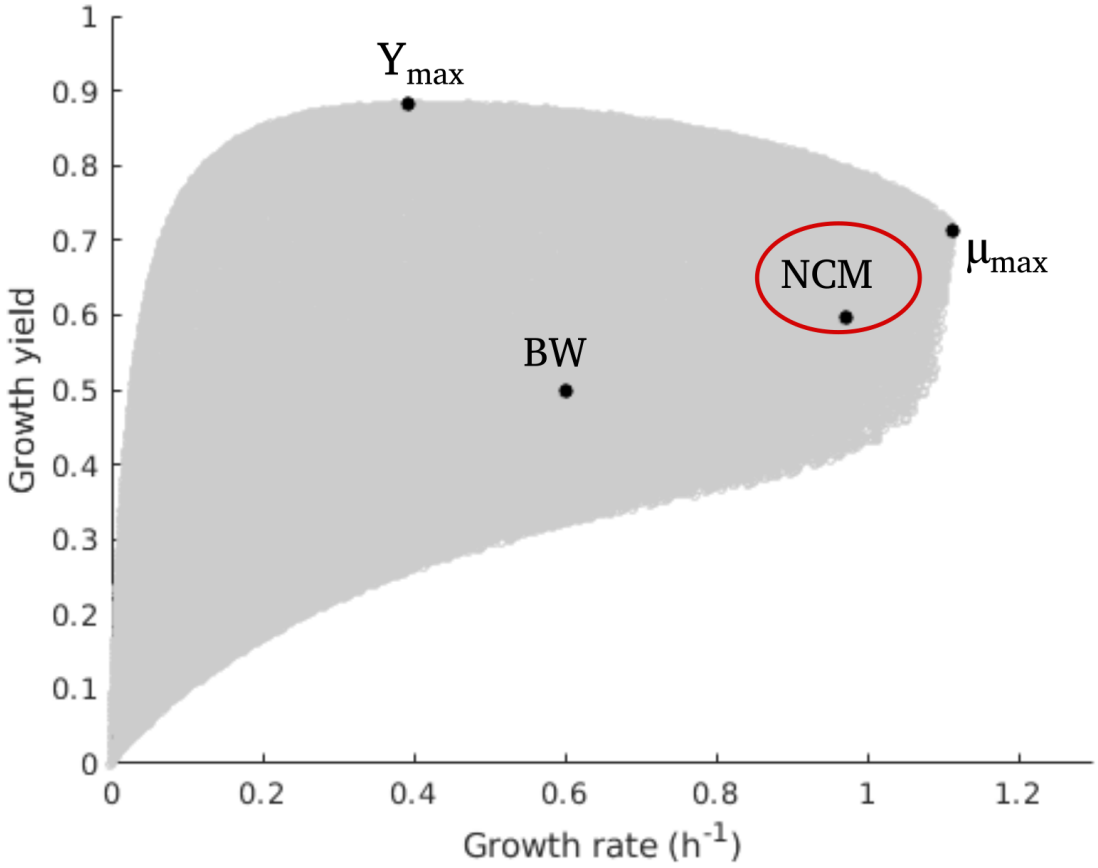
$$v_r = k_r\, r \frac{c}{c + K_r} \frac{a^*}{a^* + K_{ar}}$$





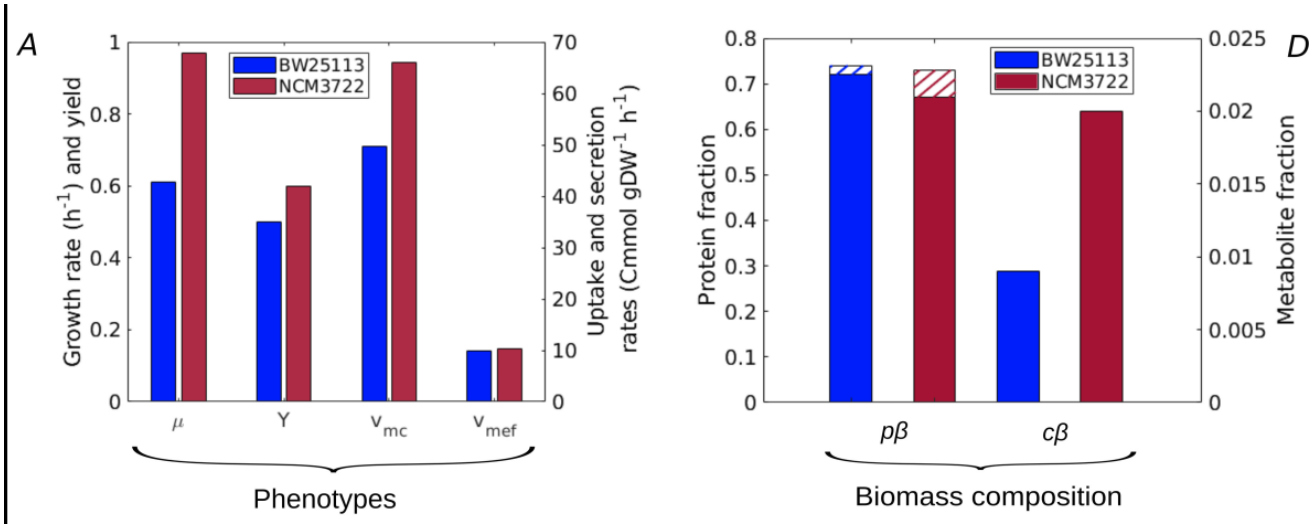


How these results compare to known observations?



How these results compare to known observations?

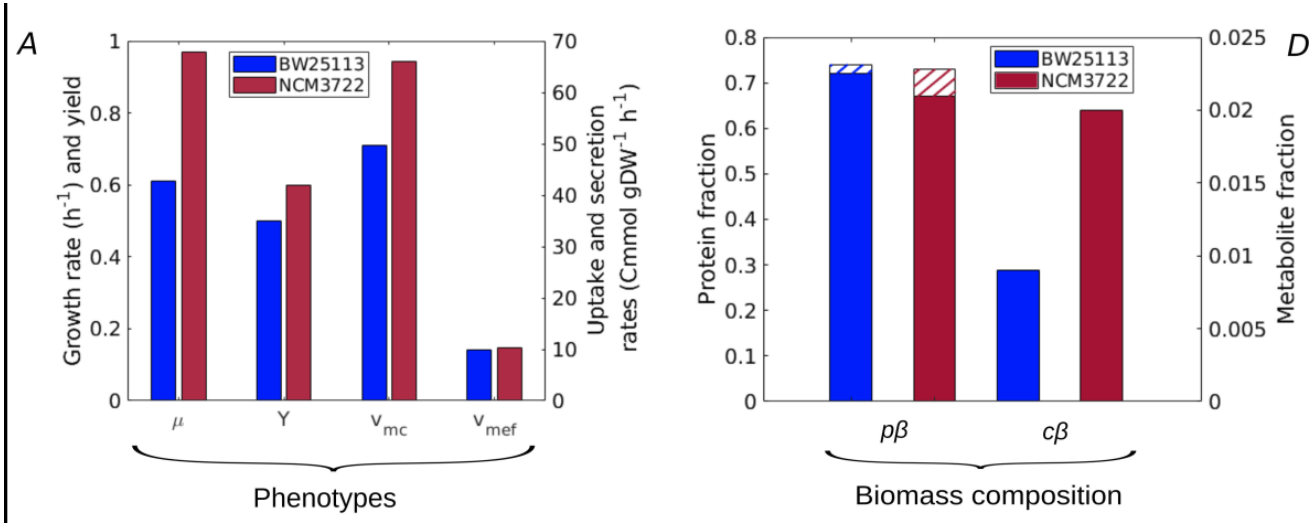
NCM vs BW phenotype:



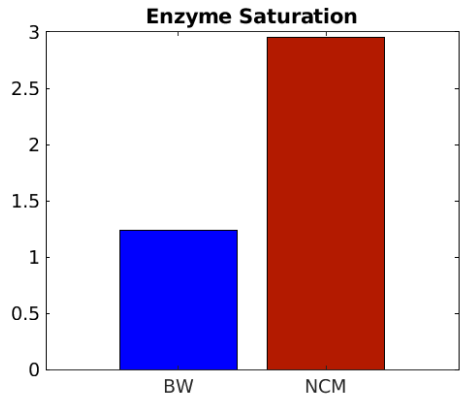
- Increased uptake rate v_{mc}
- Increased metabolite fraction

How these results compare to known observations?

NCM vs BW phenotype:



- Increased uptake rate v_{mc}
- Increased metabolite fraction



What about the strategy found by *Basan et al.*?

Our model reduces to the one of Basan *et al.* when assuming

- The concentrations of metabolites, ATP and other macromolecules are **constant**
- Biomass = Total proteins mass

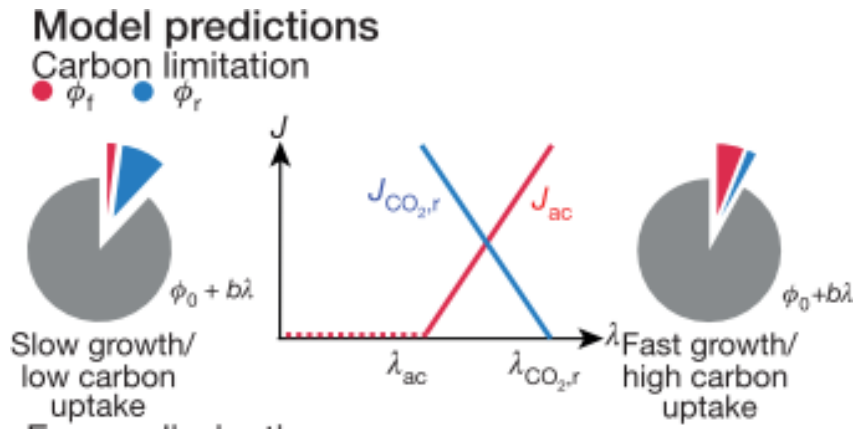
Under these hypotheses:

$$v_r = k_r\, r \frac{c}{c + K_r} \frac{a^*}{a^* + K_{ar}} = k'_r\, r,$$

$$v_{mer} = k'_{mer}\, m_{er},$$

$$v_{mef} = k'_{mef}\, m_{ef} \ldots$$

The trade-off between enzymes and metabolites is no longer possible!



What about the strategy found by *Basan et al.*?

Our model reduces to the one of Basan *et al.* when assuming

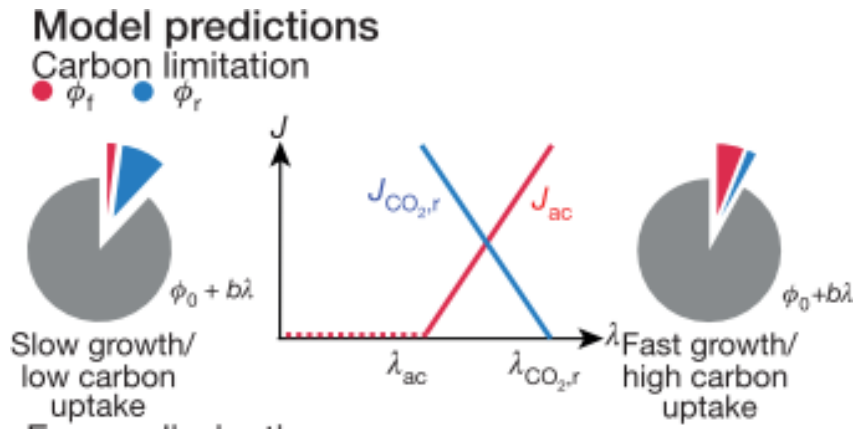
- The concentrations of metabolites, ATP and other macromolecules are **constant**
- Biomass = Total proteins mass

Under these hypotheses:

$$v_r = k_r\, r \frac{c}{c + K_r} \frac{a^*}{a^* + K_{ar}} = k'_r\, r,$$

$$v_{mer} = k'_{mer}\, m_{er},$$

$$v_{mef} = k'_{mef}\, m_{ef} \ldots$$



The trade-off between enzymes and metabolites is no longer possible!

Our model allows to account for alternative resource allocation strategies

Conclusions and take-home messages

- Coarse-grained model of coupled mass and energy fluxes based on minimal assumptions
- Model can reproduce the observed variability in *E. coli* rate-yield phenotypes
  - > resource (protein) allocation strategy as a major determinant

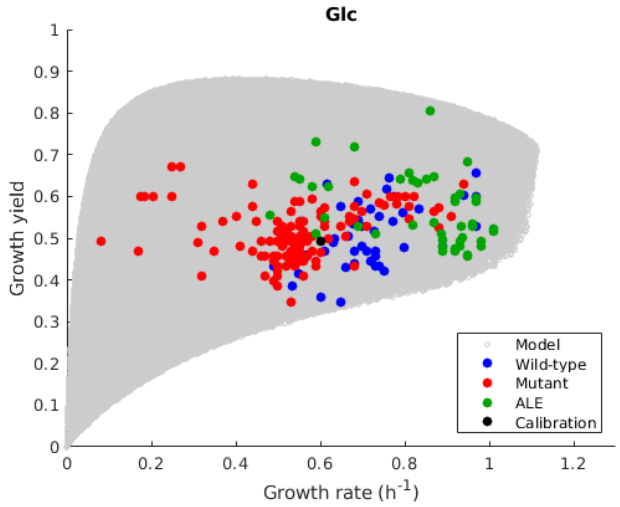
Model helps to better understand cell functioning and capabilities:

- Common lab *E.coli* strains are not optimal for growth on a single substrate
- Fermentation is not required for high growth rates
  - > some evolved strains only use respiration
- Strategies for fast and efficient growth can be achieved by enzyme saturation
  - > importance to account for metabolites in biomass composition

Perspectives

From the experimental side:

- Better characterize interesting ALE strains
- Explore the (low-rate)-high yield zone
- Apply to other organisms



From the theoretical side:

- Mathematical analysis of rate-yield trade-off (Pareto front)
- Dynamics of resource allocation
  - > regulatory function for χs as a function of concentrations
  - > explanation of strain evolution towards fast and efficient growth

Open PhD position at Inria-INRAE

Multi-omics data integration for the analysis of microbial community dynamics in plant leaves

collab. Simon Labarthe (Inria Bordeaux, France)

Thank you for your attention!

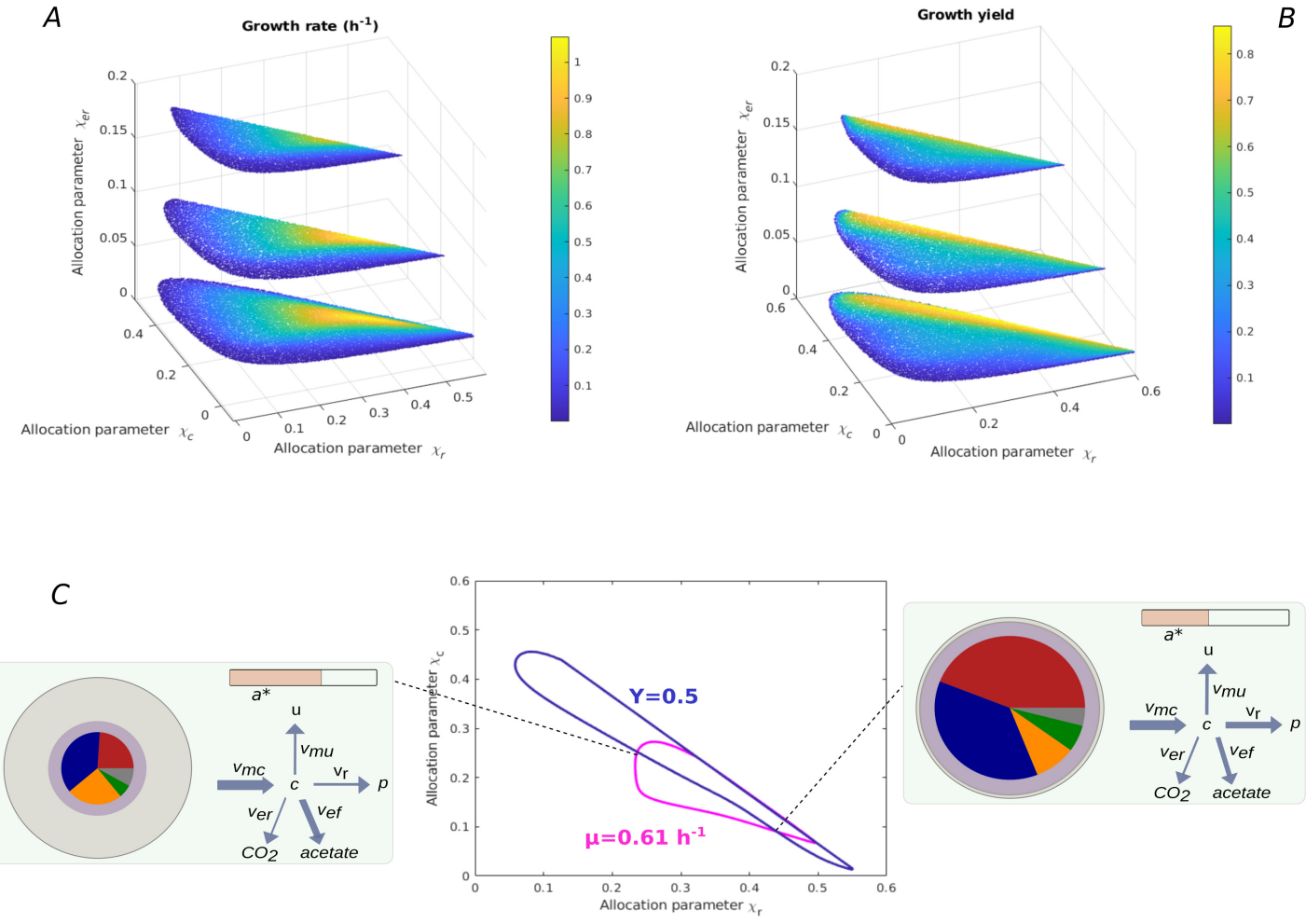
Any questions?

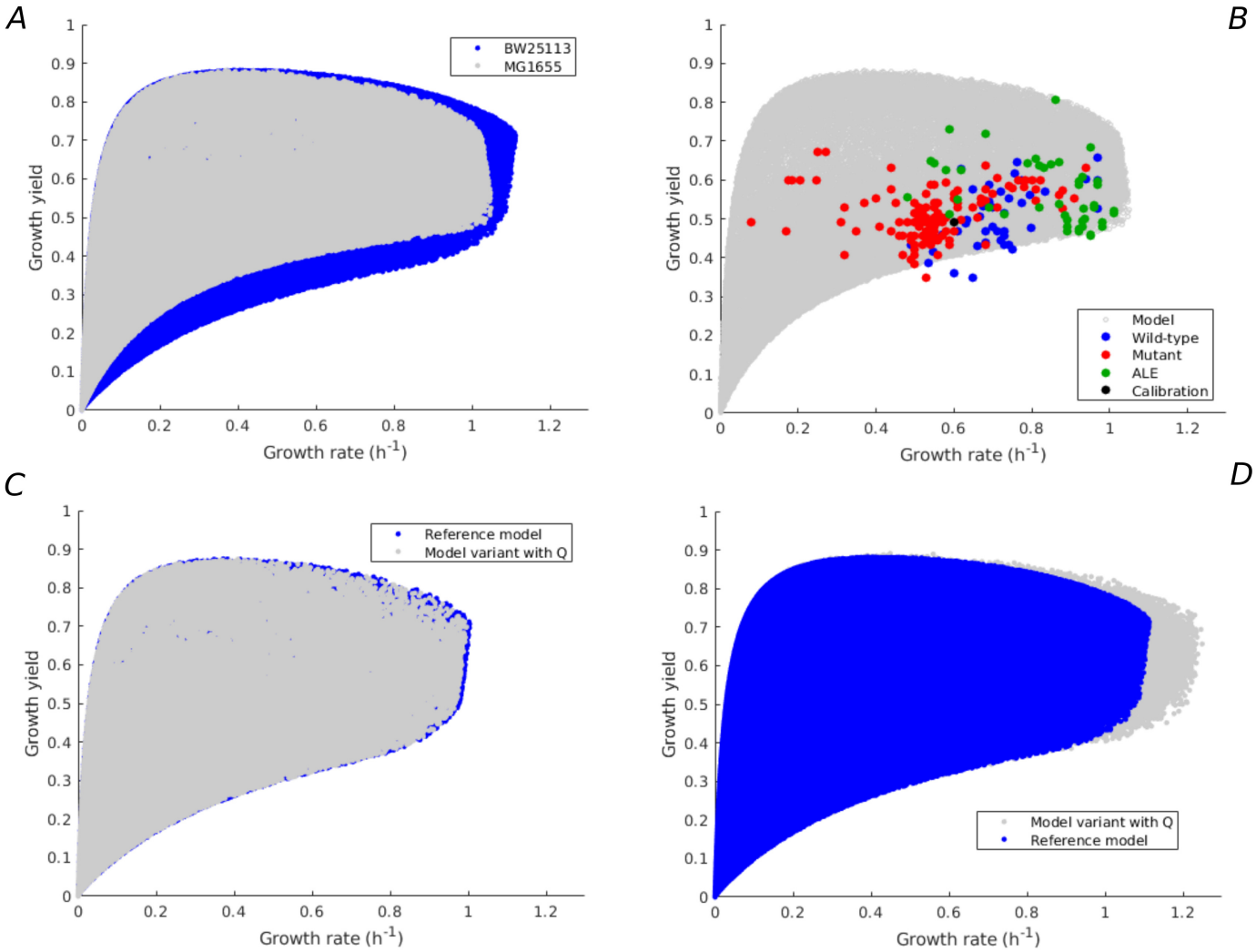
Inria INRAE

Rate and yield expression

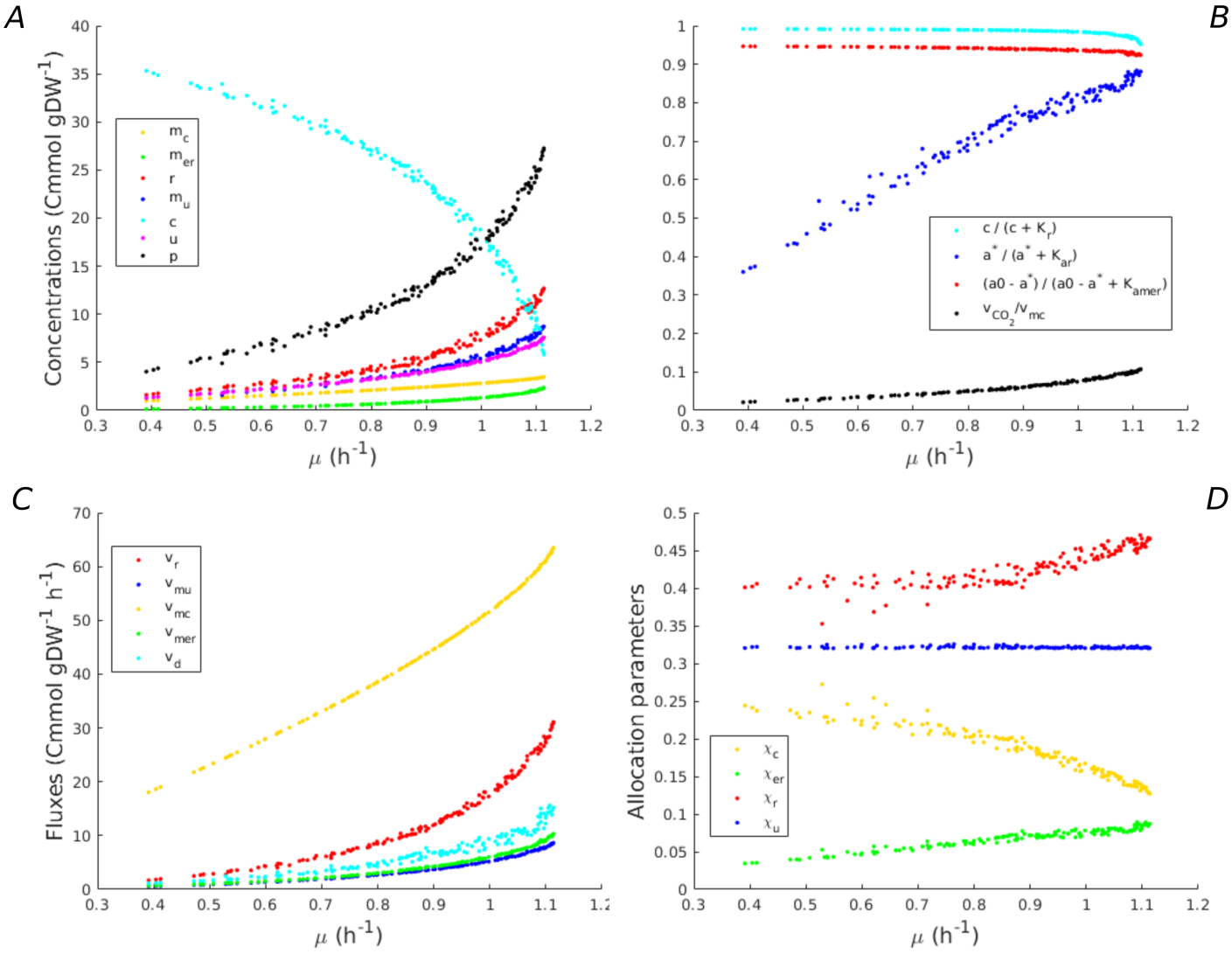
$$\begin{aligned}\mu =& \frac{1}{B}\frac{dB}{dt} = \beta\,\frac{1}{B}\,\frac{d(M_u + R + M_c + M_{er} + M_{ef} + C + U)}{dt} \\ =& \beta\left(v_{mc} - v_{mer} - \rho_{mef}\,v_{mef} - (\rho_{ru} - 1)\,(v_r + v_{mu})\right) - \gamma\end{aligned}$$

$$Y = \frac{1}{\beta}\,\frac{\mu}{v_{mc}} = \frac{v_{mc} - v_{mer} - \rho_{mef}\,v_{mef} - (\rho_{ru} - 1)\,(v_r + v_{mu}) - \gamma/\beta}{v_{mc}}$$


A
Growth rate (h-1)
Allocation parameter χer
Allocation parameter χc
Allocation parameter χr
B
Growth yield
Allocation parameter χer
Allocation parameter χc
Allocation parameter χr
C
a*
u
vmu
vmc
c
vr
p
ver
vef
CO2
acetate
Y=0.5
μ=0.61 h-1
Allocation parameter χc
Allocation parameter χr


A
BW25113
MG1655
Growth yield
Growth rate (h-1)
B
Model
Wild-type
Mutant
ALE
Calibration
Growth yield
Growth rate (h-1)
C
Reference model
Model variant with Q
Growth yield
Growth rate (h-1)
D
Model variant with Q
Reference model
Growth yield
Growth rate (h-1)

Pareto front



Parameter estimations: assumptions

ATP yield coefficients set from literature data

ATP cost for biomass synthesis: theoretical values from literature + correction for energy spilling

Affinity Constants following Bennet et al. (2009) we assumed that

- reactions involved in central carbon metabolism approximately work in the linear regime i.e. Michaelis-Menten constant close to substrate concentration (K_m values from Dourado et al. (2001))
- reactions involving ATP/NAD+ cofactors are saturated ($K_m >>$ ATP/NAD+ concentrations
- reactions involving ADP/NADH work in a linear regime, with K_m values close to their cofactor value

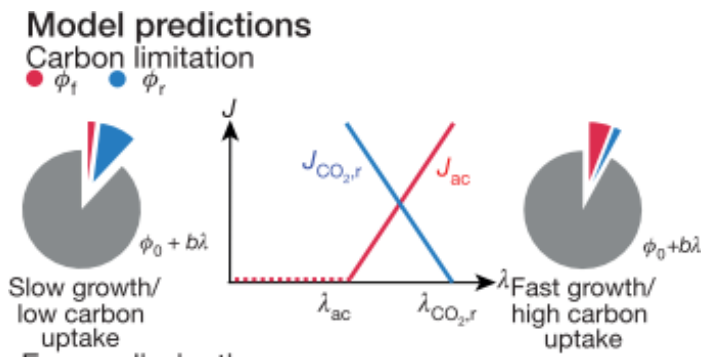
Comparison to Basan model

Our model can be simplified to the one of Basan's using the following assumptions:

1. Assume that concentration of carbon metabolites and other macromolecules are constant and negligible with respect to proteins
   - > Biomass $\approx$ Proteins
   - > Linear rates $v = kE$
2. Neglect energy dissipation v_d
3. $(\mu + \gamma)\, c$ small

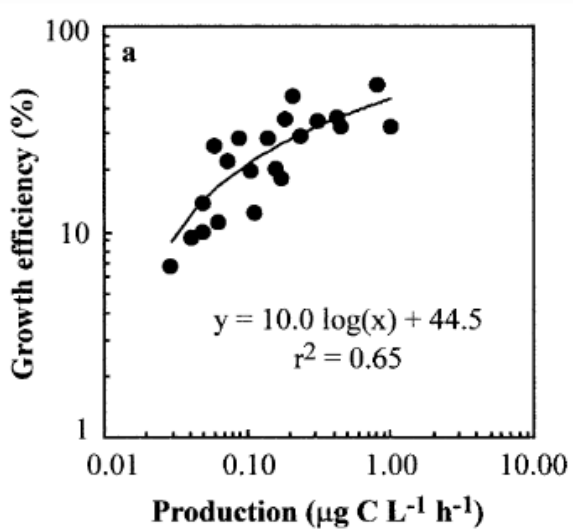
$$\Downarrow$$

At high μ the fraction of fermentation protein increases thanks to their lower protein cost ($n_{mef}\, k_{mef} > n_{mer}\, k_{mer}$).



The relation between growth and yield is complex:

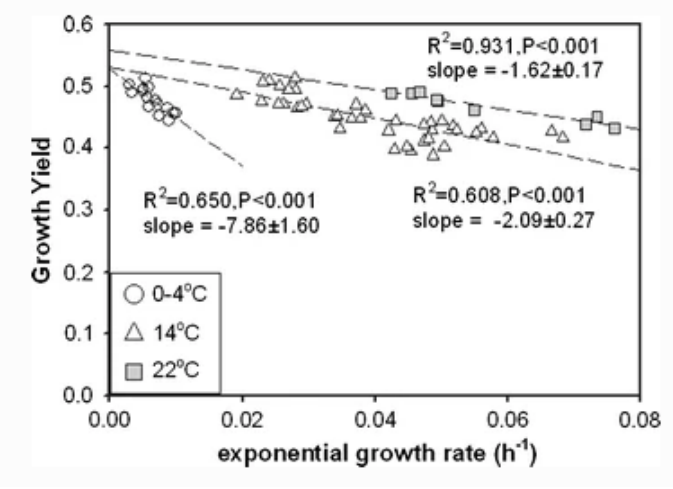
- A **positive relation** has been observed in nutrient-limited aquatic ecosystems *Smith & Praie (2004)*

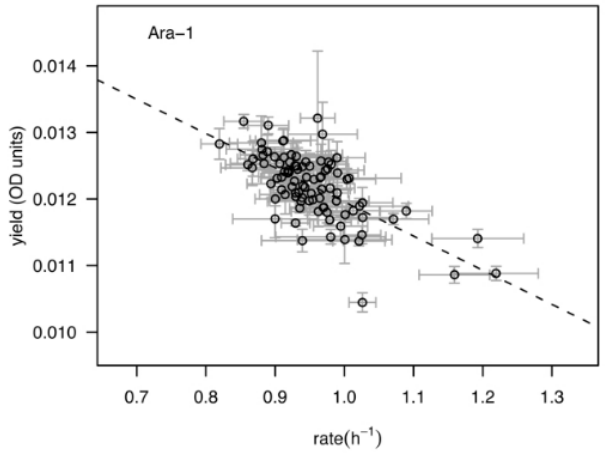


The relation between growth and yield is complex:

- A **positive relation** has been observed in nutrient-limited aquatic ecosystems *Smith & Praie (2004)*
- A **trade-off** has been observed in natural and laboratory communities

  Lipson (2009), Novak et al. (2006)





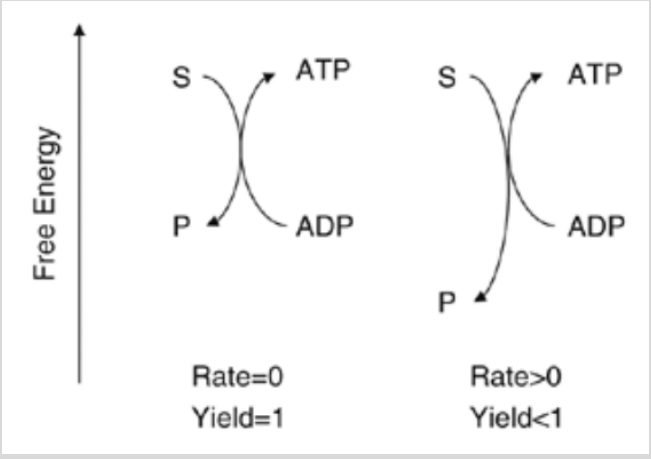
Trade-off between growth rate and yield

At the reaction level:

Thermodynamic trade-off

- Rate is proportional to ΔG
- Maximal yield for $\Delta G = 0$

McLean (2008)

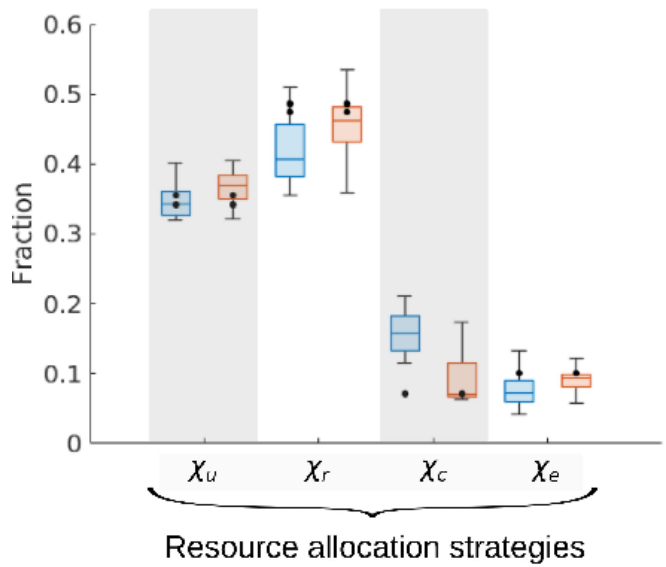


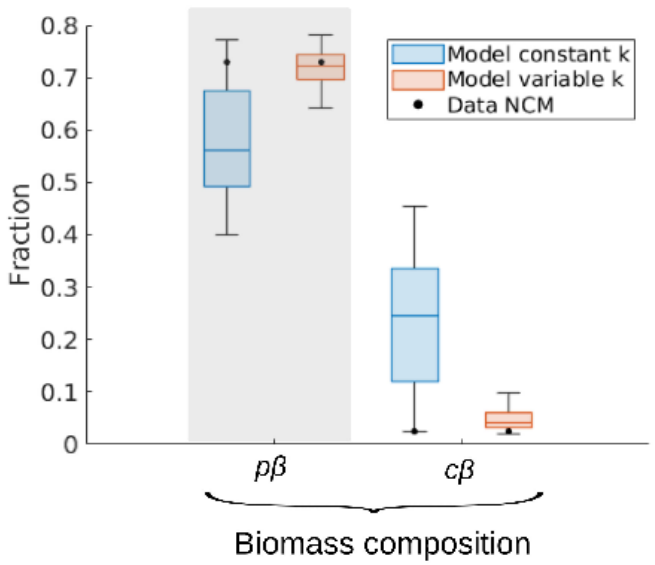
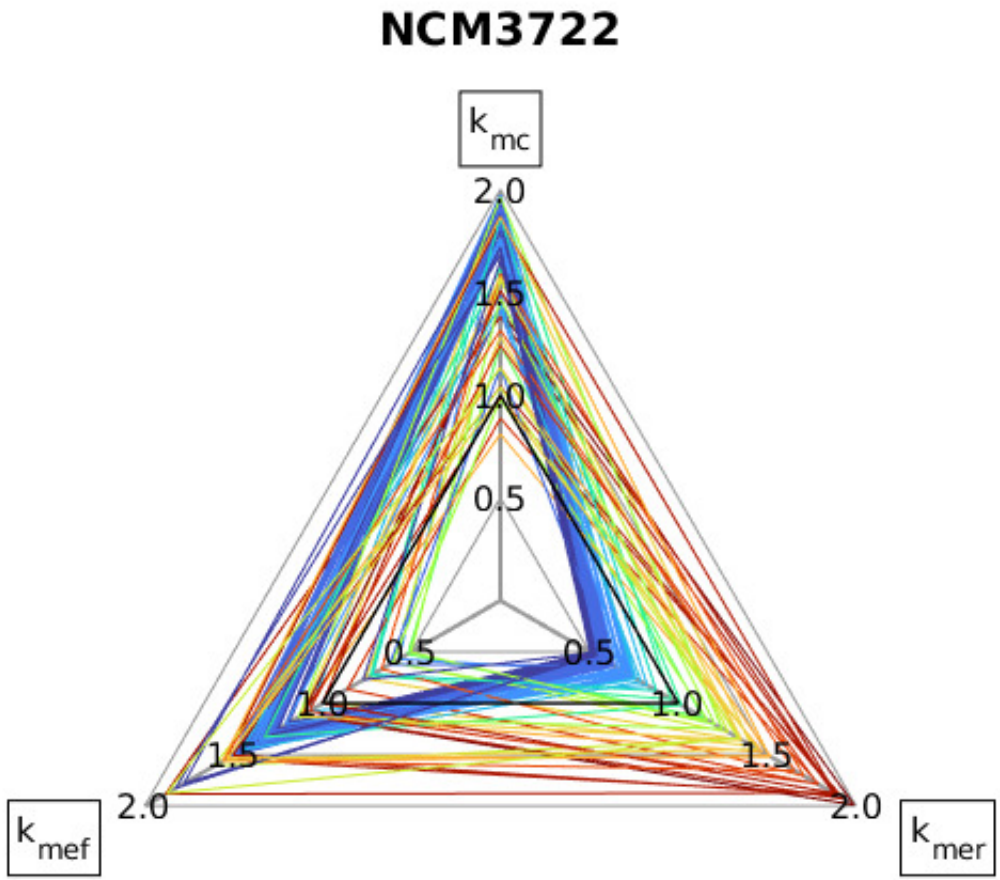
At the system level

- ATP-producing pathways with different ATP yield
- catabolism-anabolism imbalance
  - > energy-spilling reactions

Model predictions for NCM-like phenotype

- Look for resources allocation strategies giving the expected (μ, Y, v_{mc}, v_{mef}) for NCM strain
- Comparison to NCM proteomic data
  1. constant metabolic rates
  2. variable metabolic rates : k_{mc}, k_{mer}, k_{mef}





NCM predictions