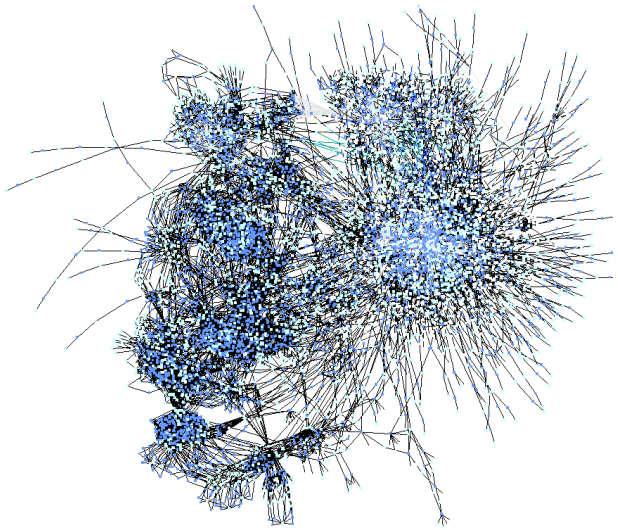
VTT TECHNICAL RESEARCH CENTRE OF FINLAND LTD



# Metabolic modelling in industrial biotechnology

Merja Oja

Guest lecture

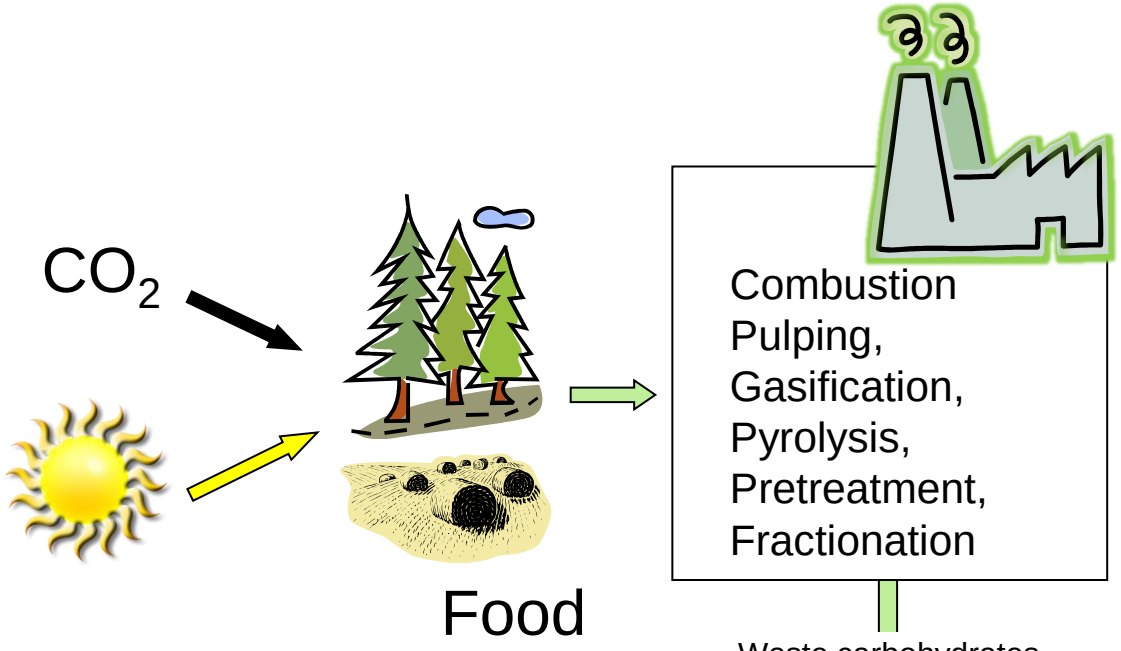
Modelling and Analysis in Bioinformatics

24.11.2015

# Outline

- Why metabolic modelling?
  - Needed in **industrial biotechnology**
  - Where production organisms are modified by **metabolic engineering**
- How metabolic modelling?
  - The metabolism of organisms can be simulated using metabolic models = **metabolic network**
  - **Constrain based methods** are used for simulation

# Industrial Biotechnology and Metabolic engineering




Current biorefinery developments

Biotechnology

# Sustainable biotechnological solutions for production of fuels and chemicals from plant biomass

BIOMASS

**SUGARS**

glucose
xylose
arabinose
galactose

engineered eukaryotic production organism

**PRODUCTS**

Biofuels,
Biopolymer
monomers

# Metabolic engineering

- The goal of an metabolic engineering project is to produce maximal amount of a product (such as bioethanol or a bioplastic precursor) in a microbial host (such as bacterium, yeast cell, plant cell)
- The metabolism of the production host is modified by adding and/or deleting genes from the genome of the organism (genetic engineering)

# Improved host for bioplastic production

**Customer:** Cargill / NatureWorks LLC

**Challenge:** To create **a new production host for lactic acid production better suited for industrial production conditions**

**Solution:** VTT engineered a yeast strain for more efficient conversion of plant materials to lactic acid. NatureWorks LLC, subsidiary of Cargill, is currently a leading producer of **plant based bioplastics**.

**Key benefit:**

- Contributed to lowering production costs

*”Collaboration with VTT enabled Cargill to explore a larger technology space. VTT’s strong expertise in working with non-conventional microbes was very beneficial in the Cargill project.”*

- Pirkko Suominen, AVP, Director Biotechnology Development Center

**Production levels up to 92 g/l of lactic acid***

*Ref. Ilmén et al. Microbial Cell Factories 2013,12:53

# More efficient biomass-to-ethanol production

**Customer:** Mascoma Corporation

**Challenge:** Develop a novel production host for more cost-efficient lignocellulosic **ethanol production**

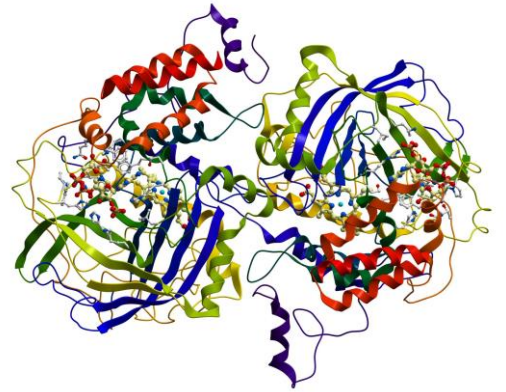
**Solution:** VTT **engineered a yeast strain capable of consolidated bioprocessing** (CBP). This significantly reduced the need for external enzymes, improving the economics of ethanol production.

**Key benefit:**

- A more cost-efficient ethanol production method

*”VTT’s deep technical knowledge and great people helped us to deliver on our project. Working with them was truly a great experience!”*

- John McBride, Sr. Director R&D, Mascoma Corp.



**50 %**

**reduction in the amount of external enzymes**

# Sustainable cosmetics from cloudberry cells

**Customer:** Lumene

**Challenge:** Enable the sustainable **production of bioactive compounds from a natural source**

**Solution:** The VTT Plant biotechnology team established a single cell culture from a rare cloudberry plant enabling the sustainable production of bioactive compounds in controlled bioreactor environment

**Key benefits:**

- Leveraging the bioactivities of Cloudberry stem cell culture enabled the creation of a new line of cosmetics

*"Achieving international growth in the cosmetics industry requires not only solid innovative product development, but also good, world class partners. VTT is a natural and preferred partner for us, because they have innovative expertise and dedication to succeed in the utilization of Arctic berries with the latest technology."*

- Tiina Isohanni, VP Innovations and Development, Lumene

**new line**

**of cosmetics from cloudberry stem cells**

# Improved biomass-to-sugar enzymes

**Customer:** AB Enzymes, Roal Ltd.

**Challenge:** Develop efficient, thermo-stable enzymes / **enzyme mixtures for the production of sugars from biomass**

**Solution:** VTT evaluated candidate enzymes, developed optimised enzyme mixtures for a range of feedstocks, and improved the properties of mixture components via protein engineering.

**Key benefits:**

- Significantly reduced the enzyme dosage necessary for total biomass hydrolysis
- Positive results opened up new market opportunities in biorefinery applications

*"With the help of VTT's expertise and competence, we have reached significant improvements in enzyme / enzyme mixture performance."*

- Jari Vehmaanperä, Global Research Director, Roal Ltd.

**Significantly reduced enzyme dosage**

# Novel bacterial strain for ethanol production

**Customer:** Deinove

**Challenge:** Unlock the potential of unique bacterial strains for ethanol production

**Solution:** VTT studied the production physiology of *Deinococcus* bacteria, analysing and characterising the hydrolytic enzymes they produced.

**Key benefits:**

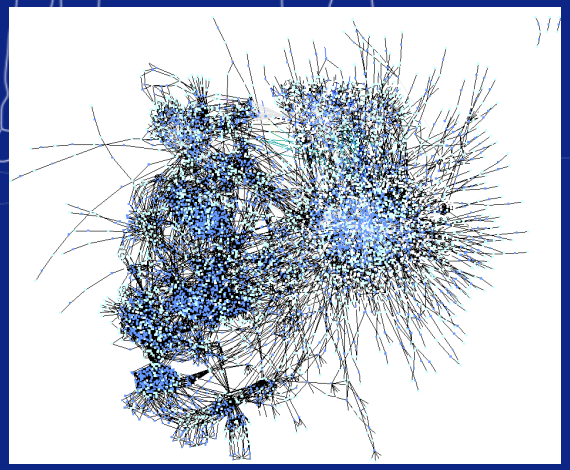
- Set the groundwork for commercialisation of the lignocellulosic ethanol production process

*"Working with VTT is a great experience, their flexibility and multi-technology competencies are unmeasurable assets that help us speed up our R&D."*

- Jean-Paul Leonetti, VP, R&D Director, Deinove

**60 fold**

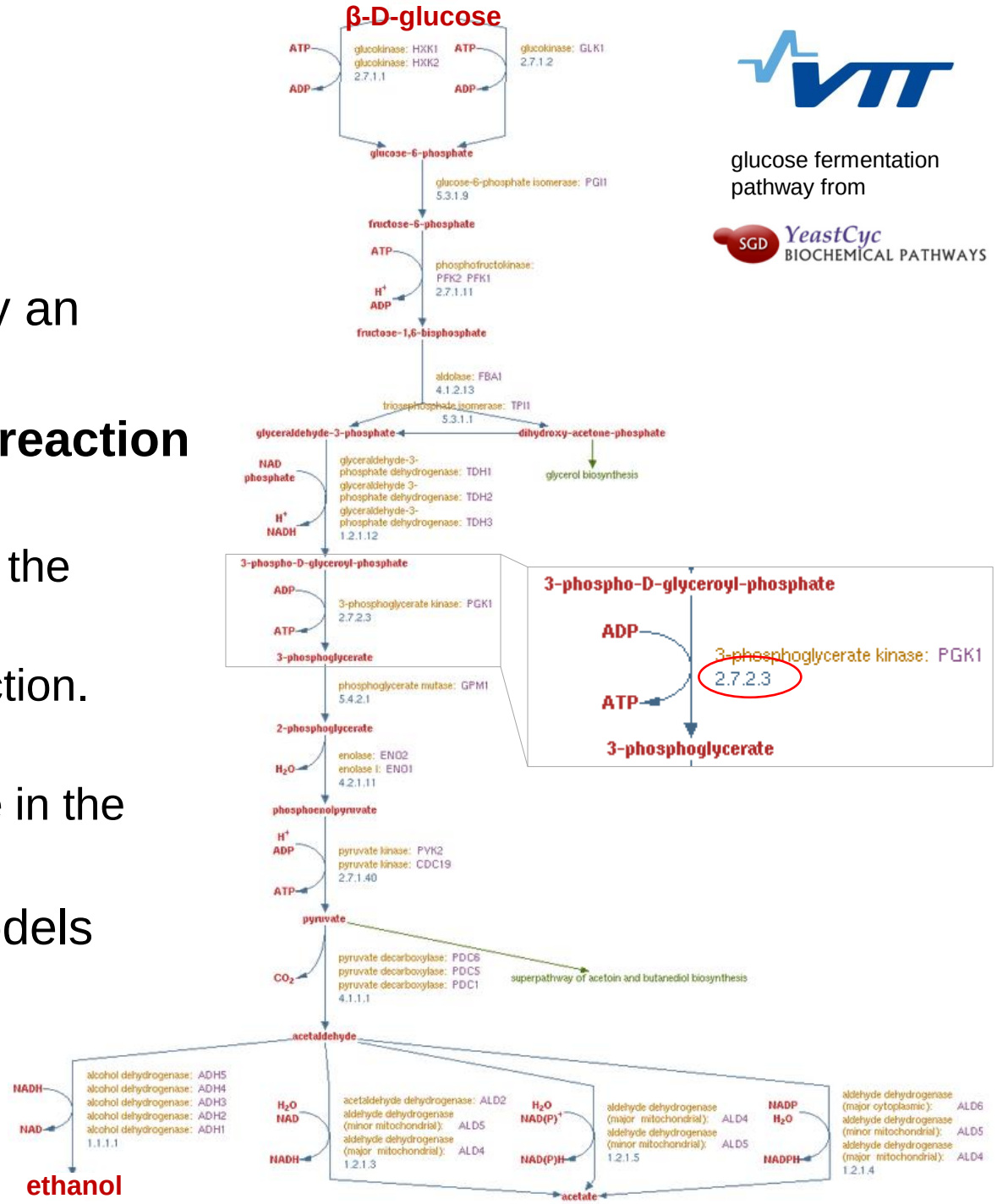
**improvement in ethanol production**



# Metabolic networks and Metabolic modelling

# Metabolic network

- Is a collection of reactions representing the biochemistry an organism could/can carry-out
- Key elements of a metabolic **reaction** are
  - **Metabolites** participating in the reaction
  - **Enzyme** catalysing the reaction. Identified by an EC number
  - **Gene(s)** coding the enzyme in the organism in question
- Whole genome metabolic models have thousands of reactions

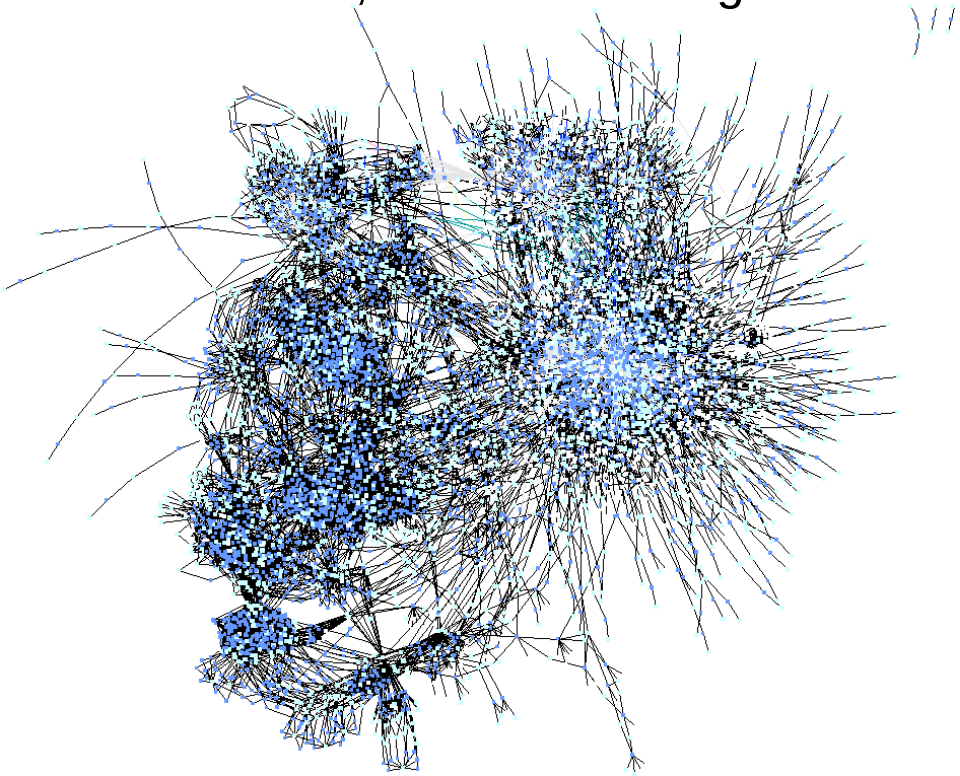


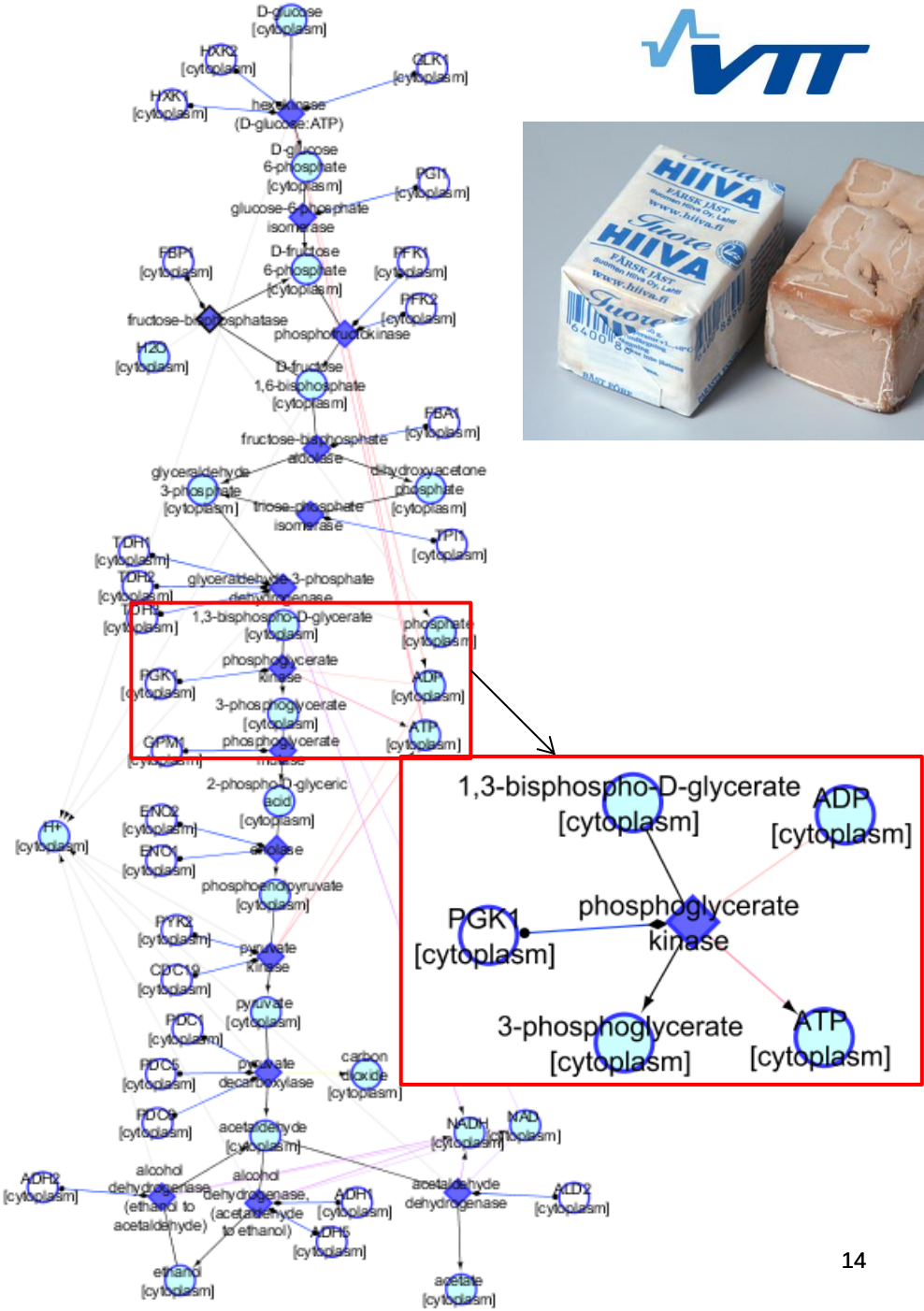

glucose fermentation pathway from

SGD YeastCyc BIOCHEMICAL PATHWAYS

# Example of a whole genome metabolic model: Baker's yeast *S. cerevisiae*

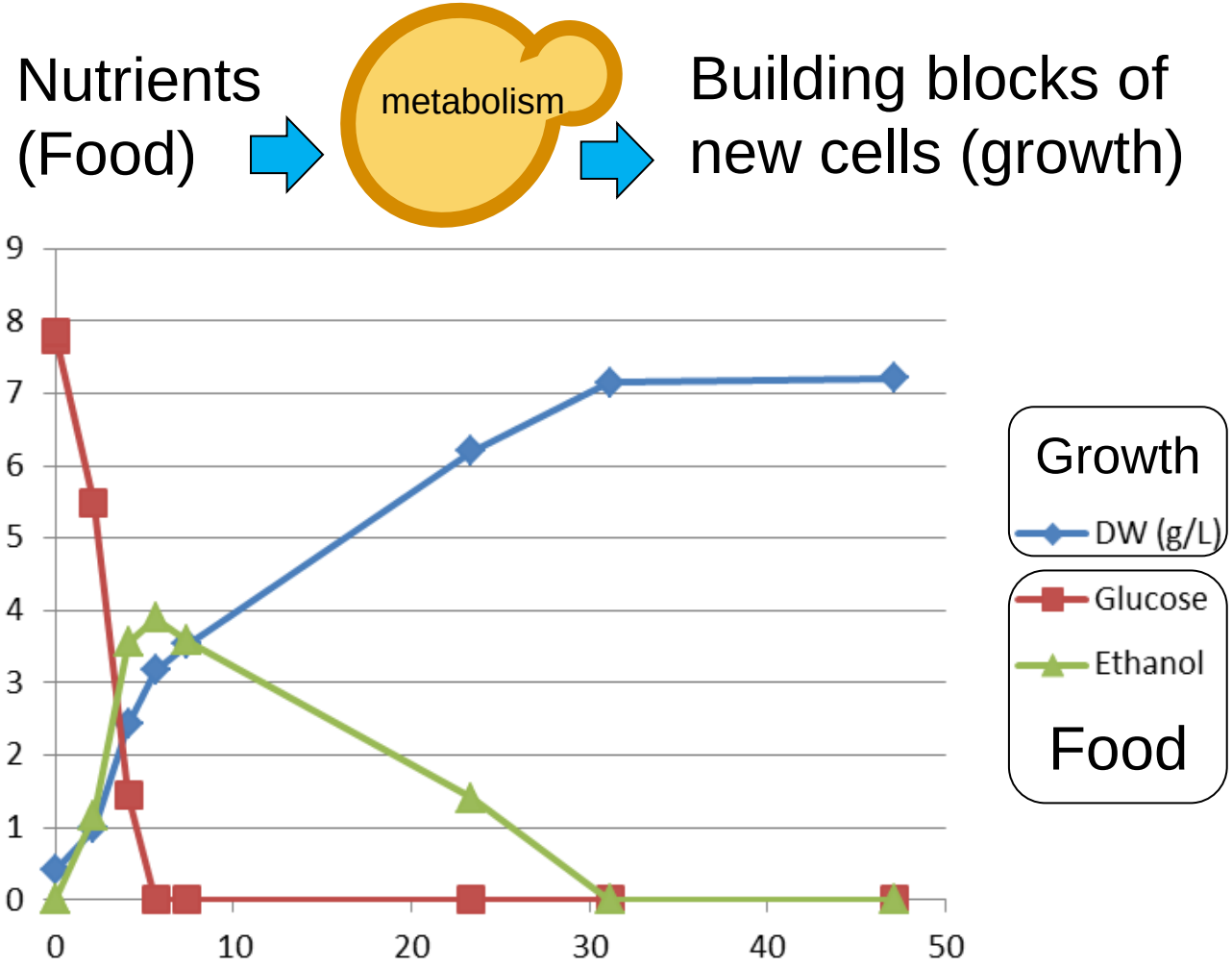
- *S. cerevisiae* model v7.5 has
  - 3494 reactions
  - 2221 metabolites
  - 909 genes
- In this network representation nodes are either reactions, metabolites or genes





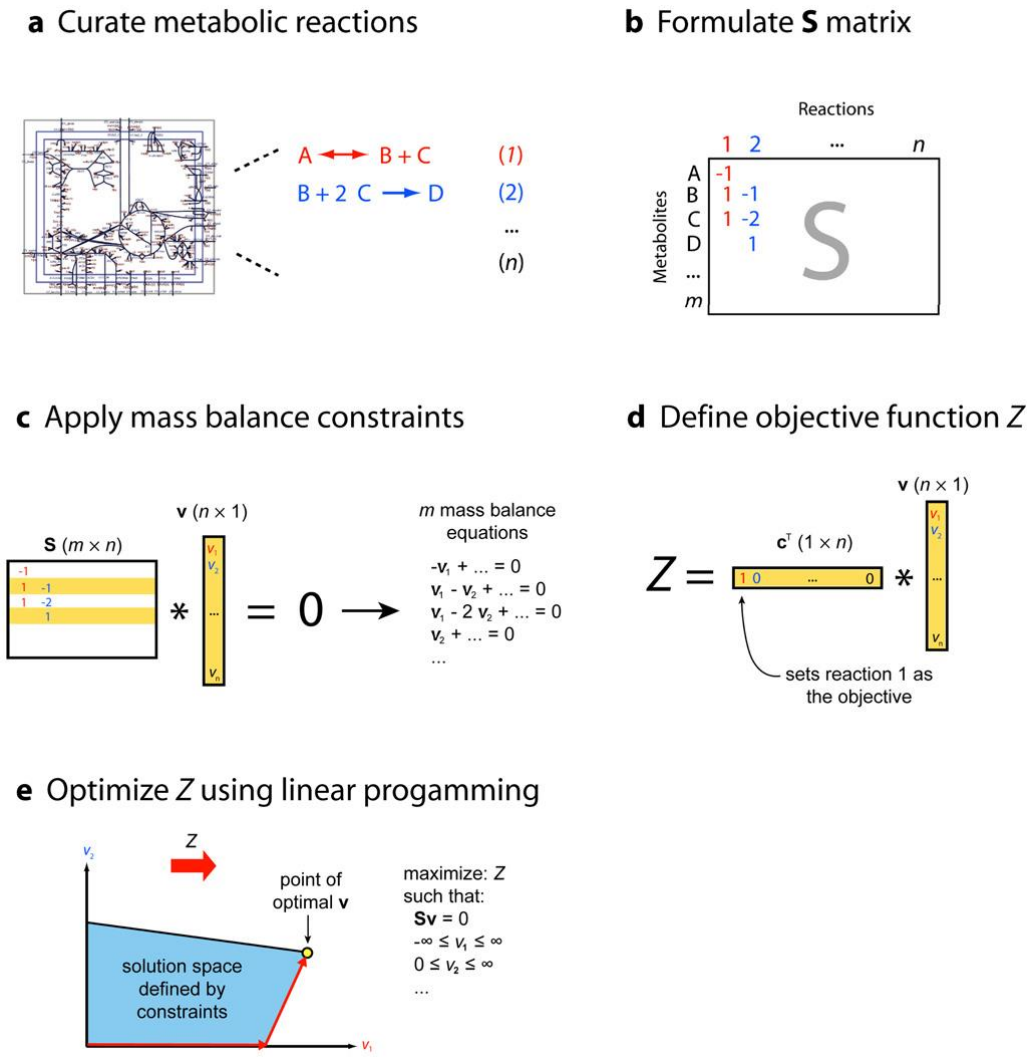
# Metabolic modelling is used to model cell behaviour

- Metabolic modelling estimates the flow of metabolites through the metabolic network. Modelling is used to predict the growth rate of an organism or the rate of production of a biotechnologically important metabolite.



# Flux balance analysis (FBA)

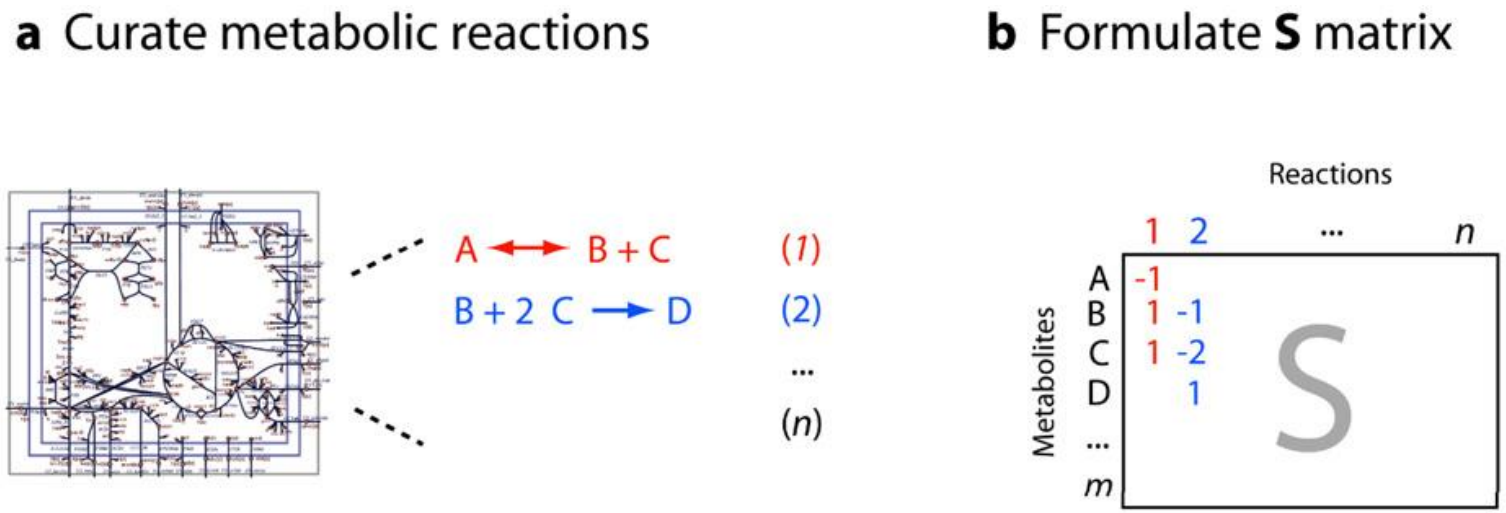
- Flux balance analysis is a technique used to solve what are the **fluxes** inside the cell
- The flux distribution explains which metabolic reactions are active and how much active
- Analysis the flux distribution allows understanding of cell behaviour




http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3108565/

# Formulation of a FBA problem

- Representation of the metabolic network as stoichiometric matrix **S**
- Stoichiometric coefficients of each reaction (one column of the **S** matrix) are the constraints on the flow of metabolites through the network.

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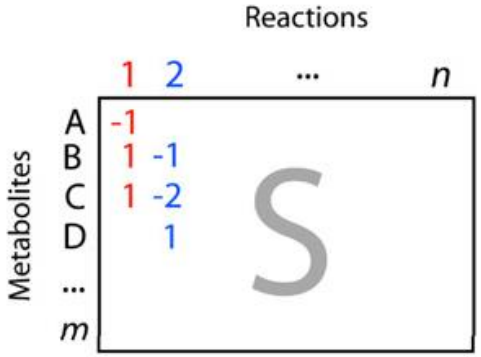
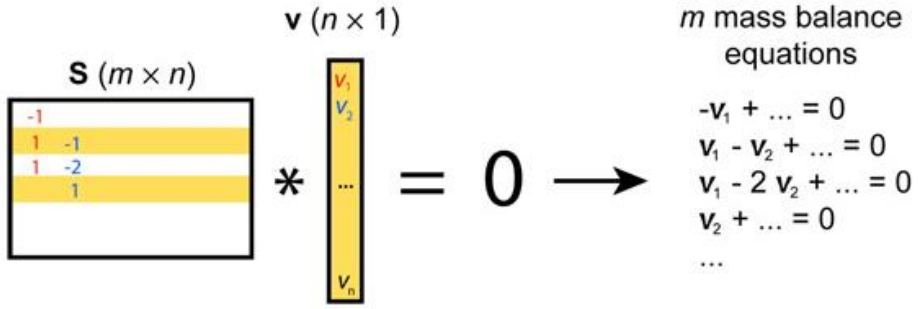
# Formulation of a FBA problem

- Flux vector **v** (length *n*) represents the flux through each of the reactions in the network.
- The concentrations of all metabolites are represented by the vector **x**, with length *m*.
- Typically in FBA a steady state is assumed. This means that the fluxes will remain constant and metabolite concentrations do not change. Using this assumption we define **S v =** d**x/**dt **= 0**

**b** Formulate **S** matrix



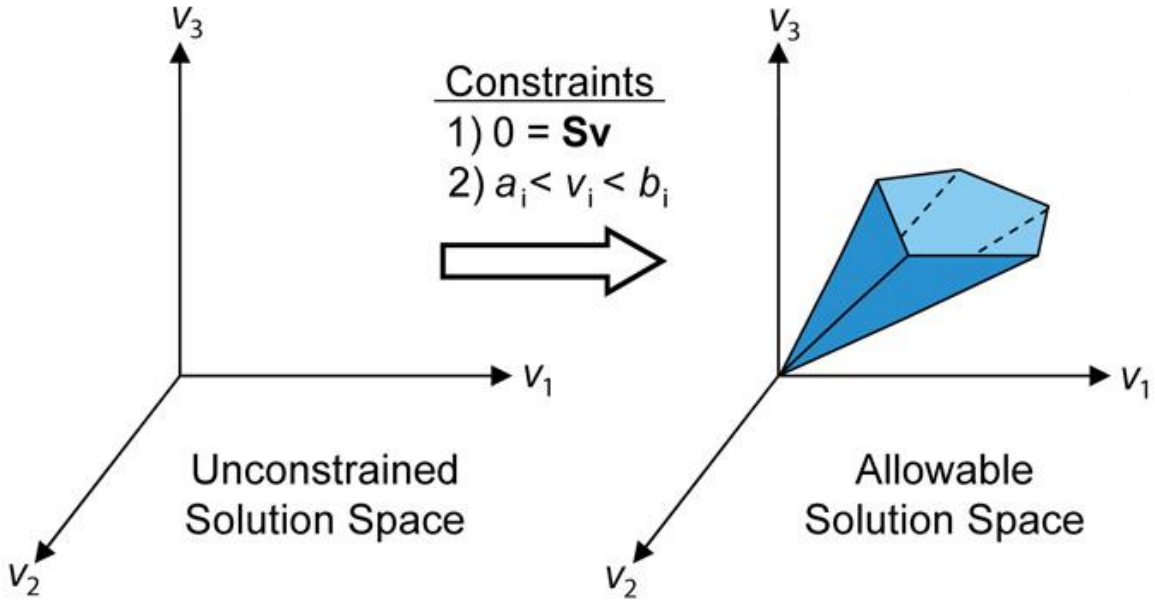

**c** Apply mass balance constraints




http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3108565/

# Formulation of a FBA problem

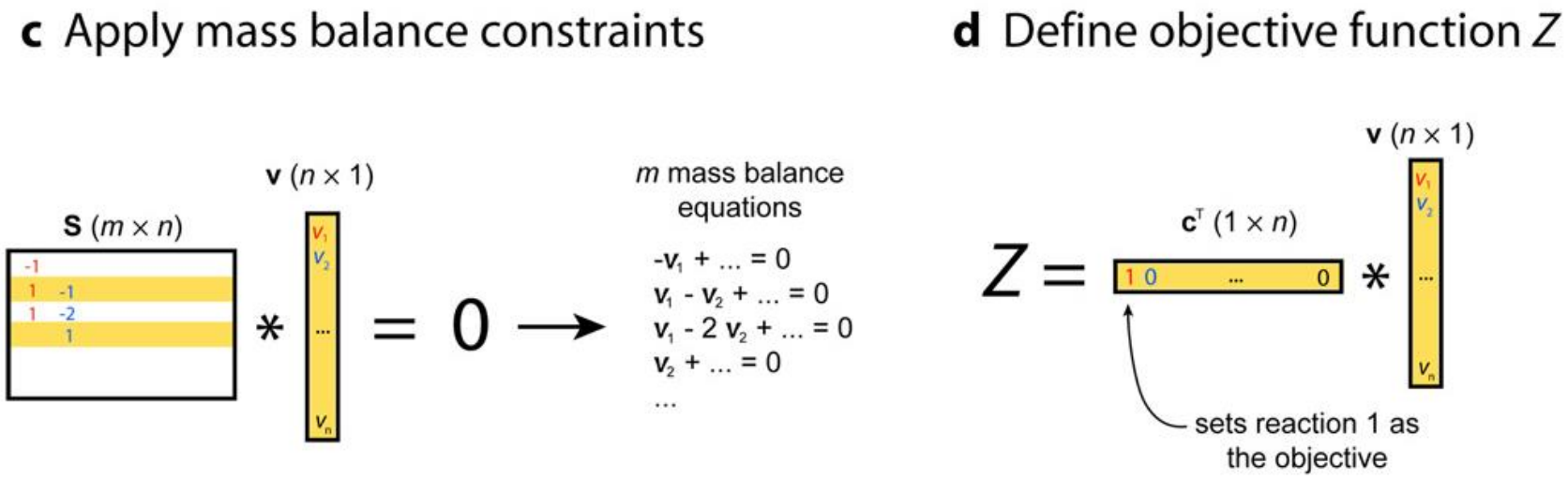
- In any realistic large-scale metabolic model, there are more reactions than there are compounds ($n > m$). In other words, there are more unknown variables than equations, so there is no unique solution to the system of equations, **S v = 0**




http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3108565/

# Formulation of a FBA problem

- The **S v=0** constraints define a range of solutions, but it is still possible to identify and analyze single points within the solution space.
  - which point corresponds to the maximum growth rate?
  - or to maximum ATP (=energy) production of an organism?
- FBA seeks to maximize or minimize an objective function $Z = \mathbf{c}^T\mathbf{v}$



# Formulation of a FBA problem

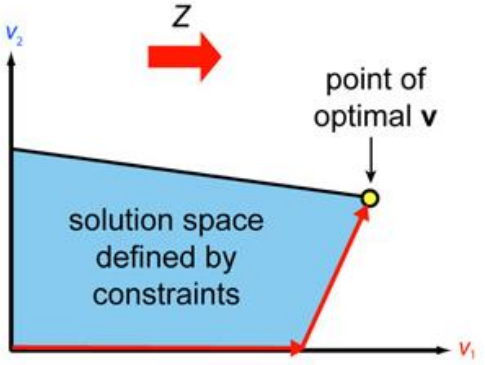
- Solve the problem $\max Z = \mathbf{c}^T\mathbf{v}$

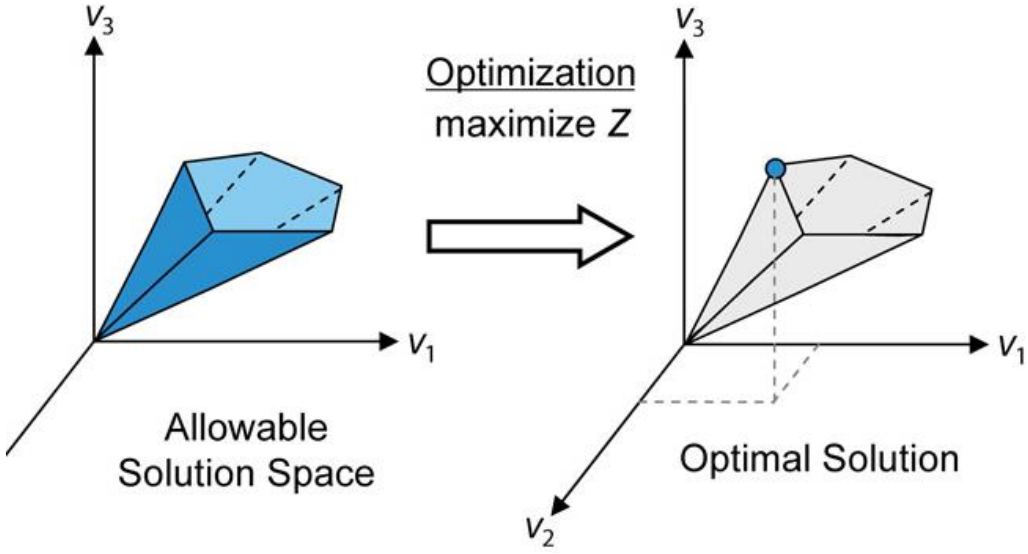
$$\mathbf{S}\,\mathbf{v} = \mathbf{0}$$

$$a_i < v_i < b_i$$

- In FBA this set of linear equations are solved using linear programming

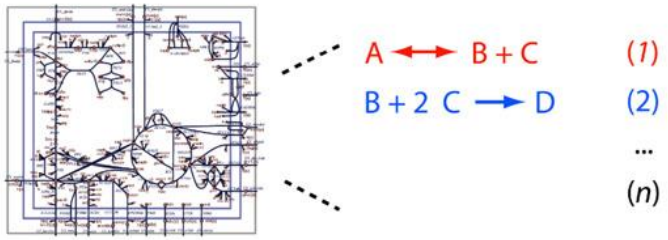
**e** Optimize *Z* using linear progamming



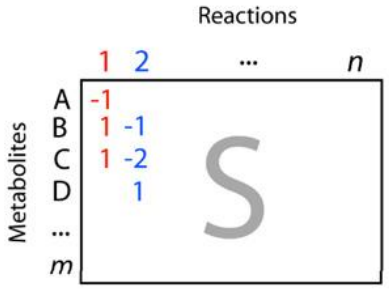



# FBA

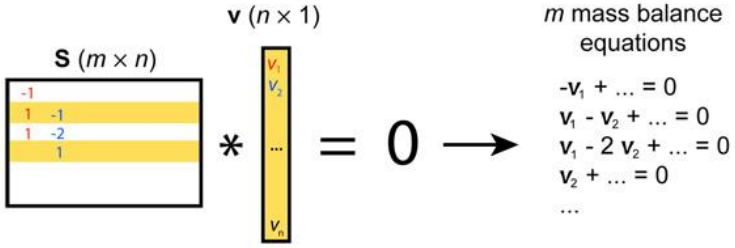
**a** Curate metabolic reactions



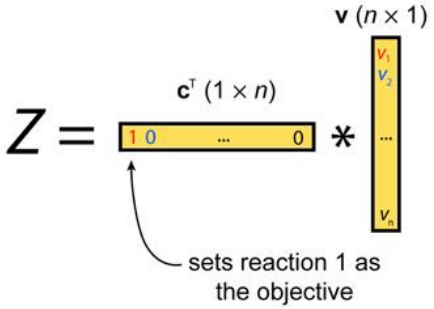

**b** Formulate **S** matrix



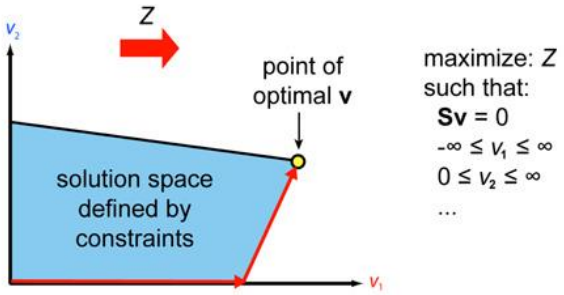

**c** Apply mass balance constraints




**d** Define objective function *Z*




**e** Optimize *Z* using linear progamming





Orth, J. D., Thiele, I., & Palsson, B. Ø. (2010). What is flux balance analysis? *Nat Biotechnol*, *28*(3), 245–248. doi:10.1038/nbt.1614.What http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3108565/

# FBA allows simulation

- Using the FBA framework different scenarios can be tested *in silico* before tedious laboratory experiments
  - Effect of different growth conditions
  - Effect of genetic modifications, i.e. deletion of gene(s)
- Analysis of the flux distributions allows understanding of
  - Which pathways are activated for production of the given target metabolite?
  - How is the cell generating energy (ATP) in the given conditions?
  - Are there problems in cofactor balancing, should more oxygen be given?

# Additional analyses

- Alternate optimal solutions can be identified through flux variability analysis (FVA).
  - FBA is used to maximize and minimize every reaction in a network at a time
- FBA derived constraint based methods can be used to propose optimal genetic engineering strategies in metabolic engineering project
  - gene deletion(s)
    - OptKnock [Burgard, et al. 2003]
    - OptGene [Patil et al. 2005]
  - gene additions / deletions
    - OptStrain [Pharkya, et al. 2004]
  - gene overexpressions / known down
    - OptForce [Ranganathan, 2010]

# Reading

- Orth, J. D., Thiele, I., & Palsson, B. Ø. (2010). What is flux balance analysis? *Nat Biotechnol*, *28*(3), 245–248. doi:10.1038/nbt.1614.What
  - http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3108565/

- If you are interested in metabolic engineering:
  - Nielsen, J., & Jewett, M. C. (2008). Impact of systems biology on metabolic engineering of Saccharomyces cerevisiae. *FEMS Yeast Research*, *8*(1), 122–31. doi:10.1111/j.1567-1364.2007.00302.x

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