Lecture #6:

Flux balance analysis

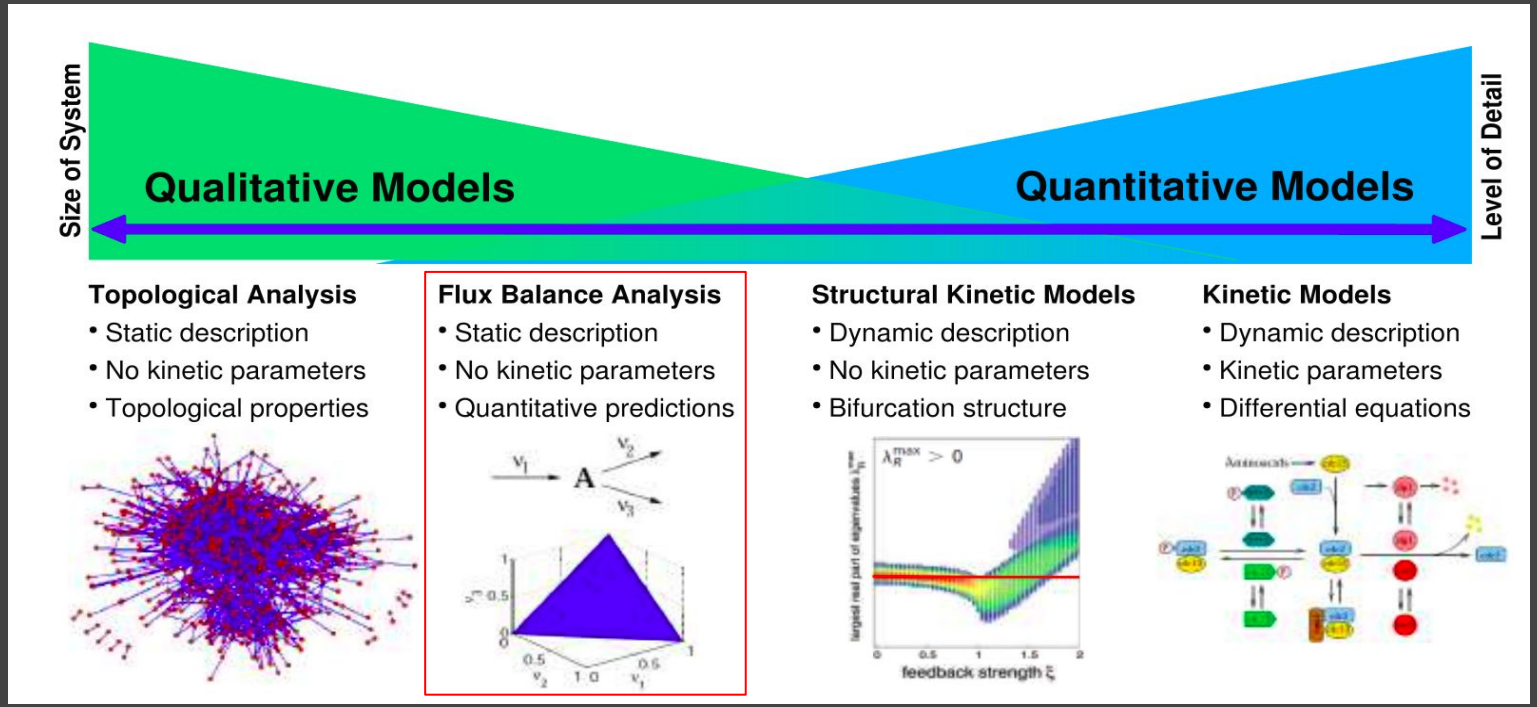
May, 4

The most common types of biological networks

- Protein-protein interaction networks (PPI)
- Gene/transcriptional regulatory networks
- Cell signalling networks
- ***Metabolic networks***
- Etc...

Metabolic models sorted by its level of detailing

- Each type of ***metabolic network*** is just a component of some specific ***metabolism modelling approach***. Thus, they might be very different.



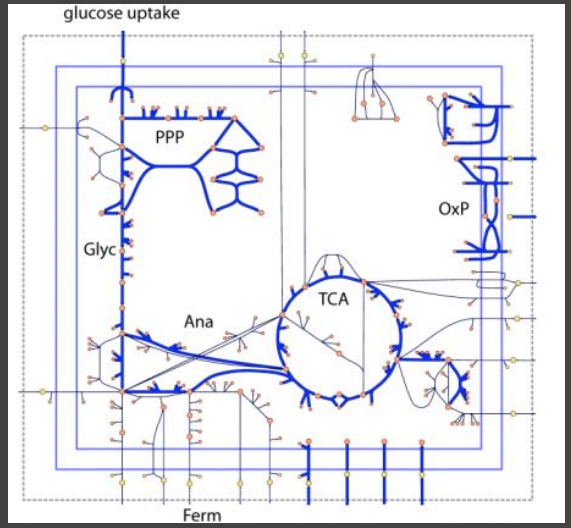
What is FBA?

Flux balance analysis (FBA) – is a method that uses a linear programming technique for simulating metabolism in genome-scale metabolic models in order to predict the phenotypic responses.

- FBA is the leading method to simulate and manipulate cellular growth *in silico* (https://doi.org/10.1186/1471-2105-1-1, https://doi.org/10.1111/tpj.14707, https://doi.org/10.1371/journal.pcbi.1005728).
- Simulations performed using FBA are computationally inexpensive and can calculate steady-state metabolic fluxes for large models (over 2000 reactions) in a few seconds.
- But for reliable results of FBA simulations you need a very accurate model and some *a priori* knowledge about environmental and intracellular predetermined conditions.

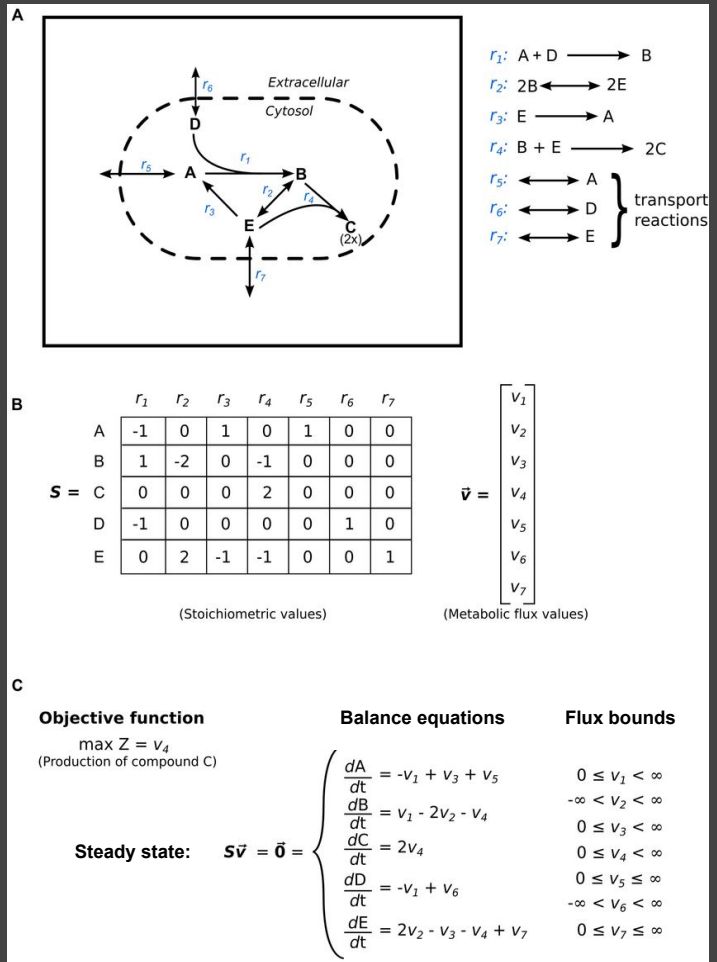
What we are dealing with in *flux* balance analysis?

- FBA model contains a **list of reactions**: adp[m] + pi[m] + 4 h[c] ⇄ atp[m] + h2o[m] + 3 h[m].
- Model for FBA implies **steady state** (we can measure the "*depth*" of the flow but not its "*speed*").
- Fluxes through some reactions are **restricted**: e.g., glc uptake is no more than X.
- We can set the **objective function** by ourselves: e.g., MΦ should produce itaconate and NO.



Central metabolic pathways

Mathematical basis of FBA



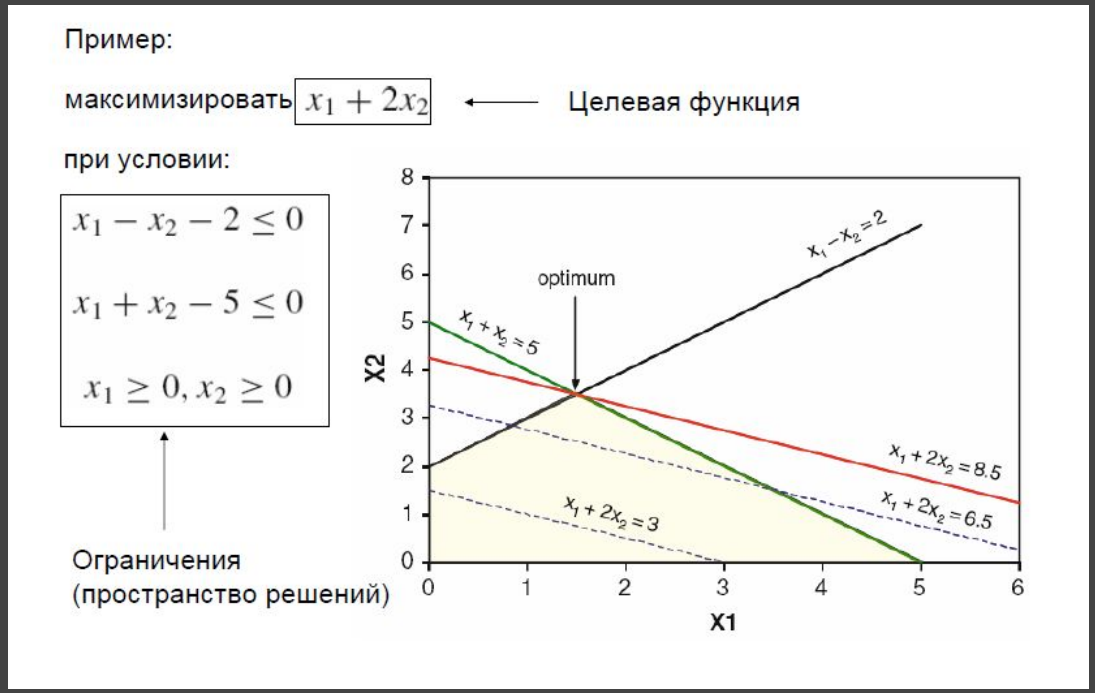
S =	r_1	r_2	r_3	r_4	r_5	r_6	r_7
A	-1	0	1	0	1	0	0
B	1	-2	0	-1	0	0	0
C	0	0	0	2	0	0	0
D	-1	0	0	0	0	1	0
E	0	2	-1	-1	0	0	1

- In equation for steady state [Sv = 0] we have *m* metabolites and *r* reactions:
  - $m < r$
- Mathematically, this means an undefined system: not enough balance equations to determine flows, the system of equations can have an infinite number of solutions.
- For the solution we use the linear programming technique.

Solving linear programming problem to find the optimal solution (geometric representation)

A special solver will do it for you:

- GLPK (free)
- CPLEX (commercial)
- Gurobi (commercial)
- …



Summing up

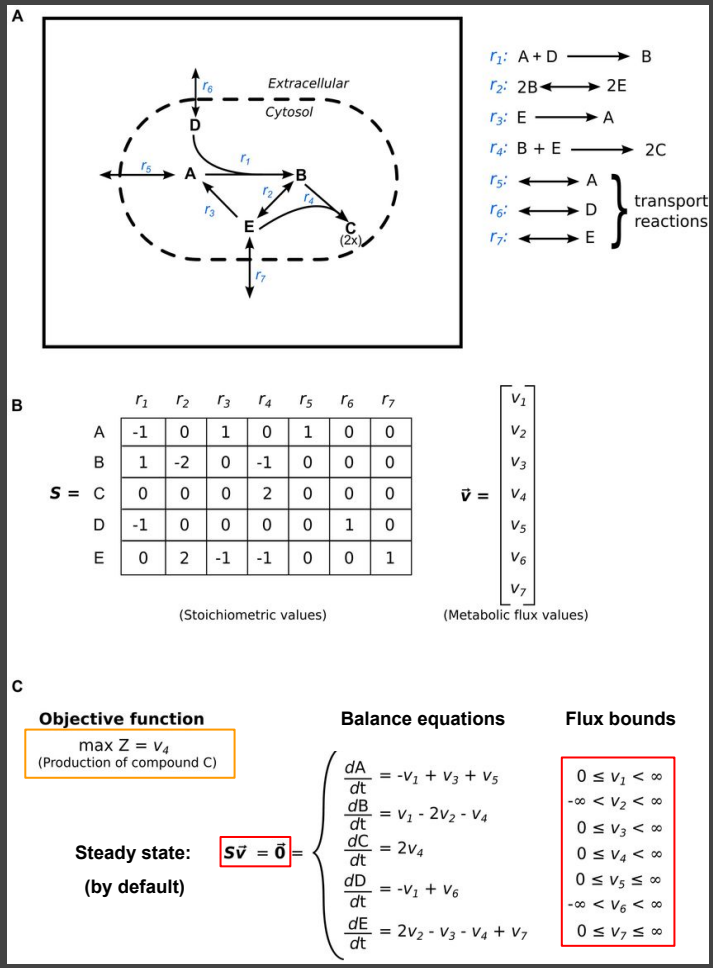
FBA steps:

1. Create/find an appropriate model network
2. Define a solution space

$$Nv = 0$$
$$\alpha_i \le v_i \le \beta_i$$

3. Define an objective function

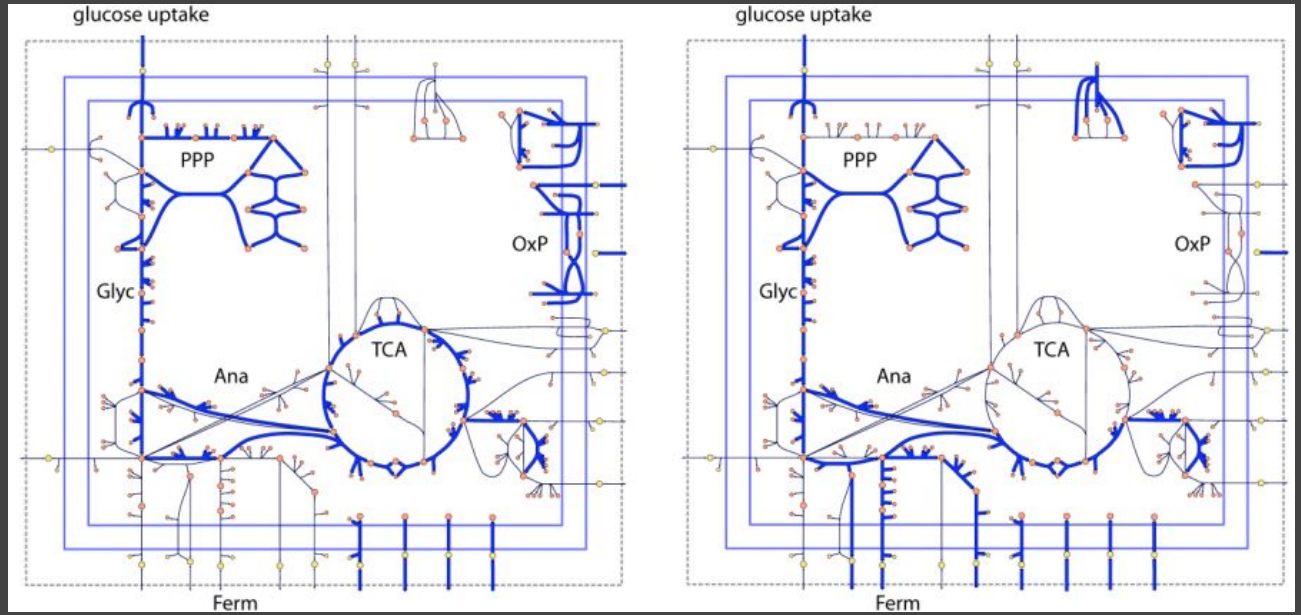
$$z = \sum_j c_{ij} v_j$$



Example of fluxes distributions with the same objective function but in different solution spaces

OF = growth
(aerobic conditions)

OF = growth
(anaerobic conditions)



The range of the tasks that we can solve by FBA

What we might be interested in:

- Survival prediction
- Cell growth rates
- Fluxes redirection discovery (to understand mechanisms of metabolic processes)
- …

How we can investigate these questions:

- Changing cell growth objective function (changing a set of reactions contributing to cell growth)
- Testing growth media to discover its minimal content (nutrient uptake rates) or limiting nutrients
- Turn off some reactions (gene knockout simulation)
- Maximize production of some metabolite (e.g., itaconate in immune cells)
- …

Interactive FBA: Escher

For the practical part let's use real MΦ-specific model designed by Bordbar et al (2012)

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Model-driven multi-omic data analysis elucidates metabolic immunomodulators of macrophage activation

Aarash Bordbar[1,4], Monica L Mo[1,4], Ernesto S Nakayasu[2], Alexandra C Schrimpe-Rutledge[2], Young-Mo Kim[2], Thomas O Metz[2], Marcus B Jones[3], Bryan C Frank[2], Richard D Smith[2], Scott N Peterson[3], Daniel R Hyduke[1], Joshua N Adkins[2] and Bernhard O Palsson[1,*]

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In this paper the RAW 264.7 metabolic model was constructed based on transcriptomic and proteomic data, and validated for its quantitative accuracy in the prediction of growth rate, ATP, and nitric oxide production.

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