Inference in Metabolic Network Models using Flux Balance Analysis

BMI/CS 776
www.biostat.wisc.edu/bmi776/
Mark Craven
craven@biostat.wisc.edu
Spring 2011

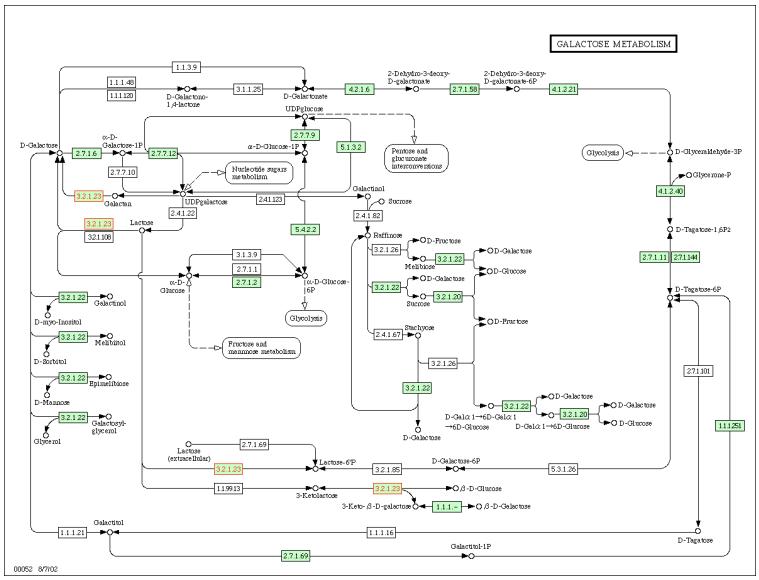
Goals for Lecture

the key concepts to understand are the following

- the FBA representation
- the role of constraints and the steady state assumption in FBA
- the role of optimization in FBA
- how dynamic behavior is simulated in FBA

Quantitative Prediction with Network Models

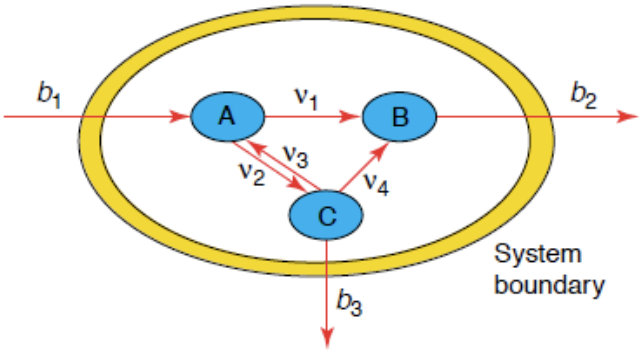
- given complete, accurate models of metabolic and regulatory networks, we could use simulations to make predictions
  - e.g. how fast will my bacteria grow if I put them in medium *M*?



Quantitative Prediction with Network Models

consider a model in which

- nodes represent metabolites
- edges represent reaction fluxes



$$\frac{dA}{dt} = -v_1 - v_2 + v_3 + b_1$$

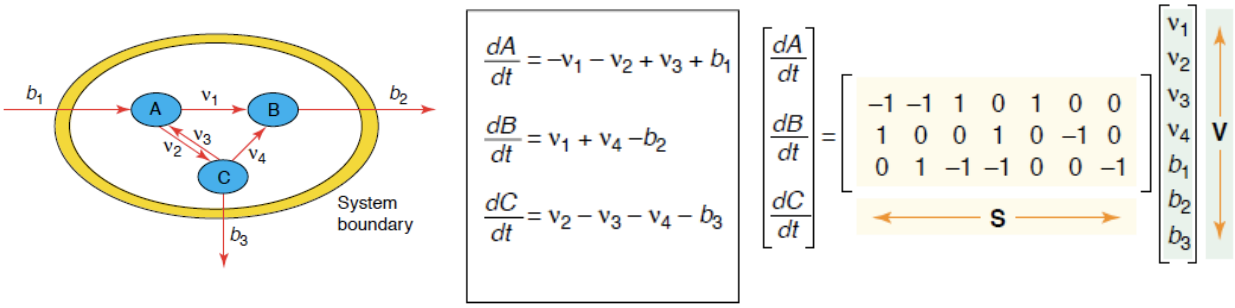
$$\frac{dB}{dt} = v_1 + v_4 - b_2$$

$$\frac{dC}{dt} = v_2 - v_3 - v_4 - b_3$$

Quantitative Prediction with Network Models

- but there are always lots of things we don't know
  - all of the metabolic reactions
  - the kinetics of most reactions
  - all of the actors/mechanisms involved in regulation
  - how the regulatory network interacts with the metabolic network
- in many cases, though, we can still make interesting predictions using *constraint-based* models
- key insight: instead of calculating exactly what a network does, narrow the range of possibilities by constraints

Flux Balance Analysis



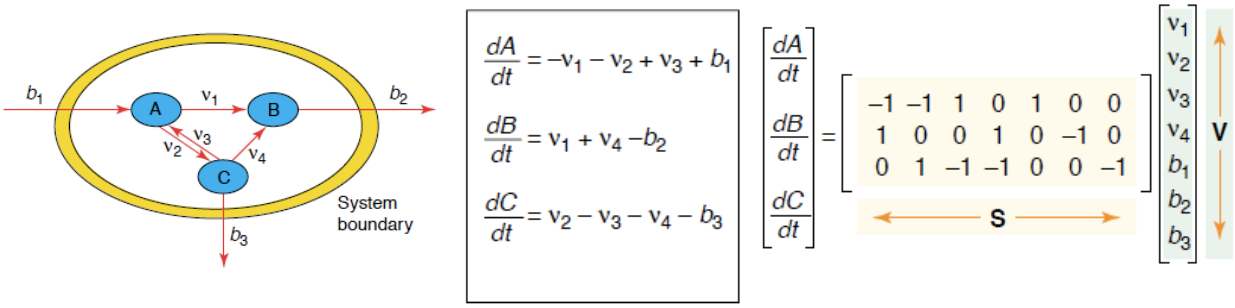
Figures from Kauffman et al., *Current Opinion in Biotechnology*, 2003.

1. metabolic reactions and metabolites (A, B, C in figure) are specified; internal fluxes (v_i) and exchange fluxes (b_i) don't have to be known
2. describe as a system of ordinary differential equations (mass balance constraints) in matrix notation: **S** is the stoichiometric matrix and **V** is the vector of fluxes

Flux Balance Analysis

3. make the *steady state mass balance* assumption: no accumulation or depletion of metabolites in the cell

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



Flux Balance Analysis

4. add known constraints; this defines a solution space for the flux-balance equations

$0 \le b_1 \le 5$
$0 \le v_1 + v_2 \le 5$
$0 \le v_1 + v_4 \le 2$
$v_3 = 0$ (irreversible reaction)

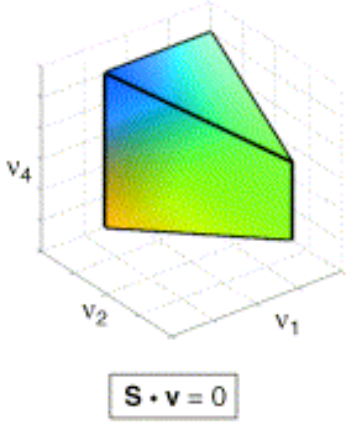


Figure from Kauffman et al., *Current Opinion in Biotechnology*, 2003.

Constraints on Cellular Functions

- *physico-chemical*: mass, energy and momentum must be conserved
- *environmental*: nutrient availability, temperature, etc.
- *topobiological*: molecules are crowded in cells and this constrains their form and function
  - e.g. bacterial DNA is about 1,000 times longer than the length of a cell; has to be tightly packed yet accessible ⇒ spatio-temporal patterns to how DNA is organized
- *regulatory*: the gene products made and their activities may be switched on and off depending on conditions

Flux Balance Analysis

5. define an objective function (e.g. maximization of biomass or ATP); find the optimal points in the solution space

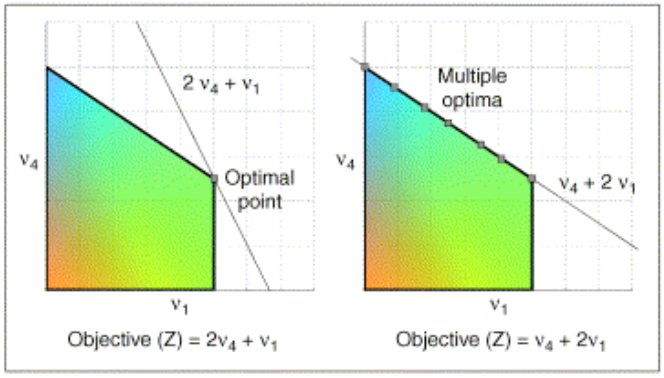


Figure from Kauffman et al., *Current Opinion in Biotechnology*, 2003.

6. analyze the system behavior under different conditions: varying constraints, adding or removing reactions etc.

Determining Optimal States

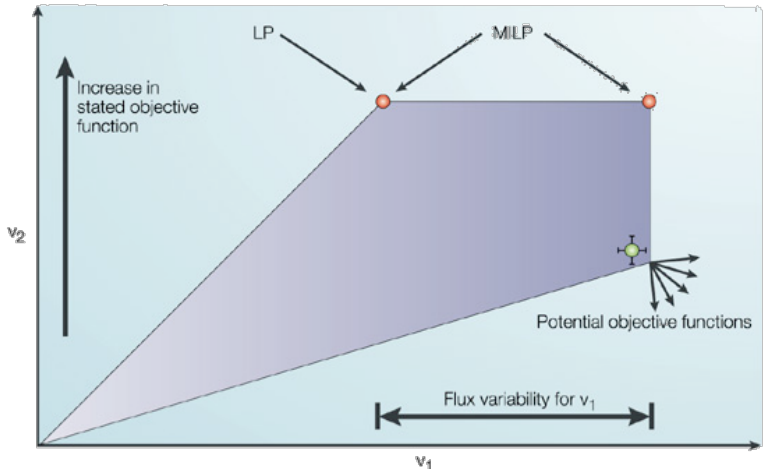
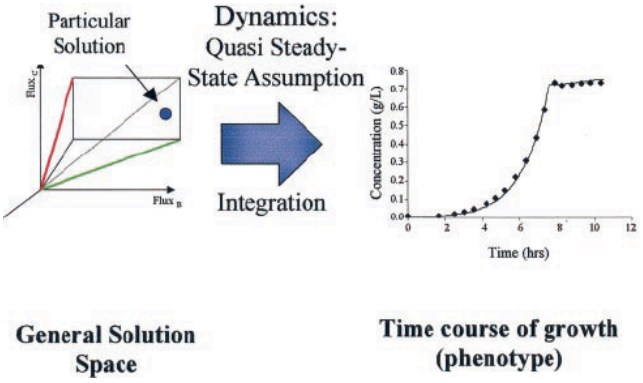


Figure from Price et al., *Nature Reviews Microbiology*, 2004.

- given an objective function, we can find one optimal state with *linear programming* (LP), or all optimal states with *mixed-integer LP*
- given an experimental measurement of fluxes, can calculate potential objective functions that would lead towards that state

Simulating Dynamic Behavior



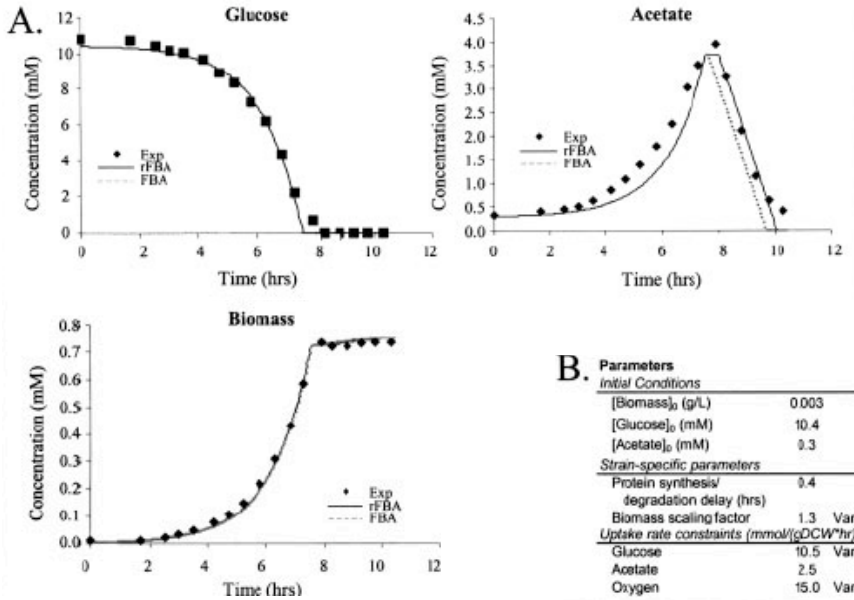
- The "core" FBA method assumes the cell is at steady state, so how can we simulate dynamic behavior, like growth curves?

Quasi Steady-State Simulations

- the time constants that describe metabolic transients are fast (milliseconds to tens of seconds)
- the time constants associated with transcriptional regulation (minutes) and cell growth (hours) are slow
- *quasi steady-state assumption*: behavior inside cell is in steady-state during short time intervals
- can do simulations by iteratively
  - changing representation of external environment (e.g. glucose levels)
  - doing steady-state FBA calculations

Quasi Steady-State Example

A.



B.

Parameters		
Initial Conditions		
$[Biomass]_0$ (g/L)	0.003	Estimated
$[Glucose]_0$ (mM)	10.4	Estimated
$[Acetate]_0$ (mM)	0.3	Estimated
Strain-specific parameters		
Protein synthesis/ degradation delay (hrs)	0.4	Estimated
Biomass scaling factor	1.3	Varma and Palsson, 1994
*Uptake rate constraints (mmol/(gDCW*hr))*		
Glucose	10.5	Varma and Palsson, 1994
Acetate	2.5	Estimated
Oxygen	15.0	Varma and Palsson, 1994

Incorporating Regulatory Constraints

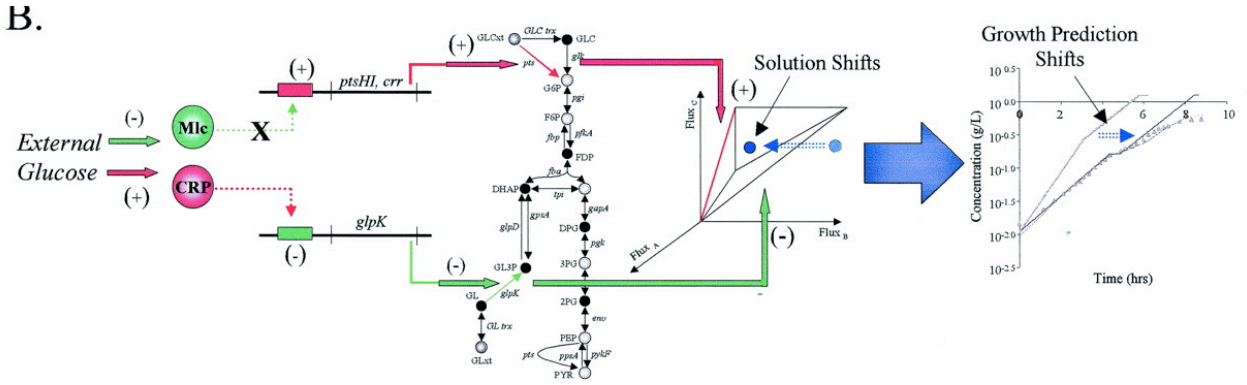
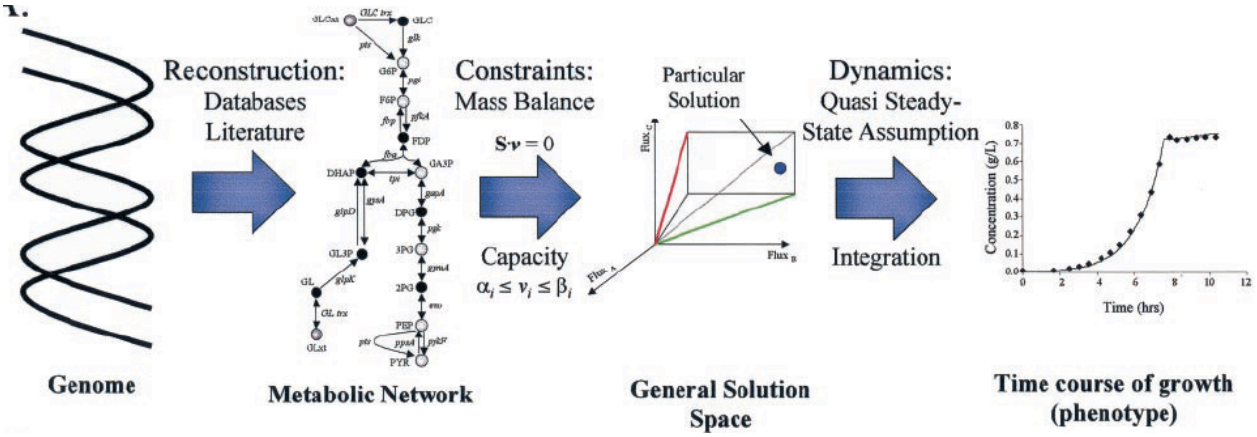


Figure from Covert & Palsson., *Journal of Biological Chemistry*, 2002.

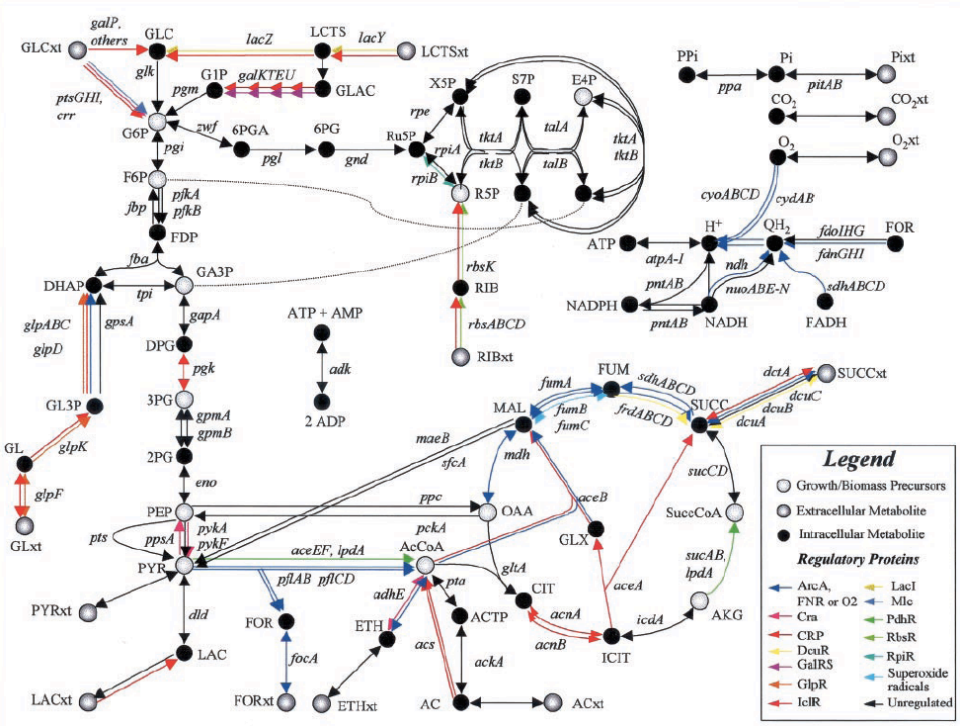
- we can ask how the optimal solution changes when we introduce regulatory constraints
- e.g. the presence of external glucose causes
  - Mlc to stop repressing a glucose transporting operon
  - CRP to repress a glycerol kinase gene

A Case Study: Predicting *E. Coli* Growth



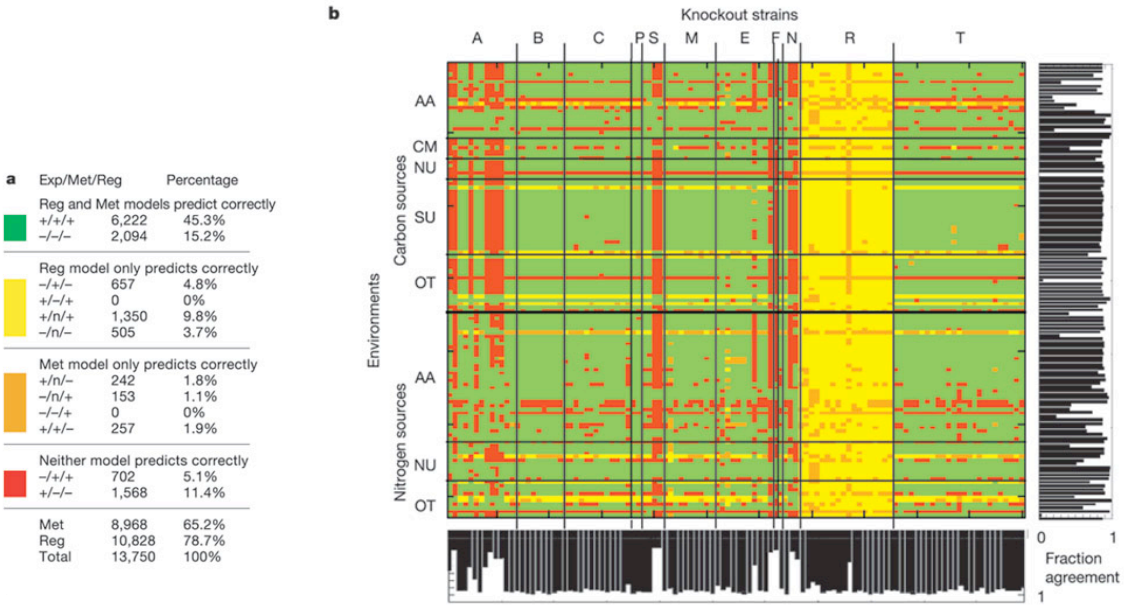
- E. coli model accounts for 906 metabolic genes
- 104 regulatory genes (regulating expression of 479 metabolic genes)

Part of the Model

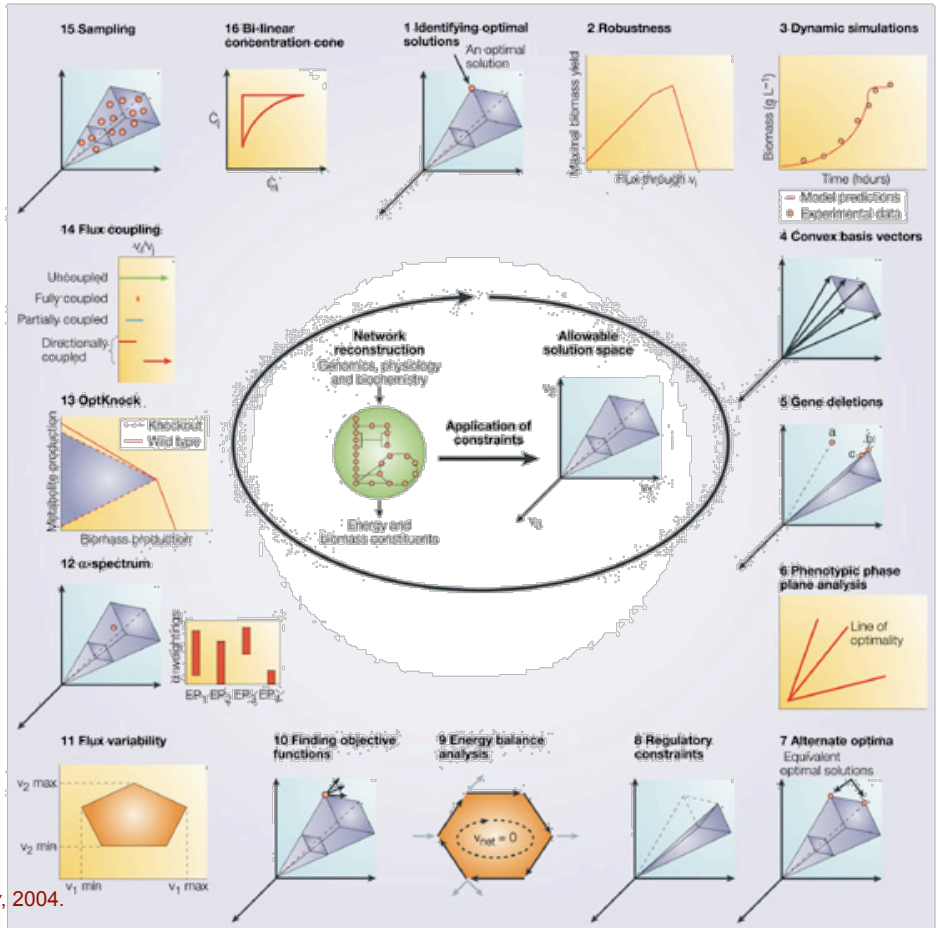


Predicting Growth Phenotypes

- model predicts growth for various knockout strains/ environments
- compare predictions to experimentally measured growth



More FBA Analyses



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