Metabolic modelling

Metabolic networks, reconstruction and analysis

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Computational Methods for Systems Biology

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Outline: Metabolism

- Metabolism, metabolic networks
- Metabolic reconstruction
- Flux balance analysis

A part of the lecture material has been borrowed from Juho Rousu's Metabolic modelling course!

What is metabolism?

- Metabolism (from Greek "Metabolismos" for "change", or "overthrow") is the set of chemical reactions that happen in living organisms to maintain life (Wikipedia)

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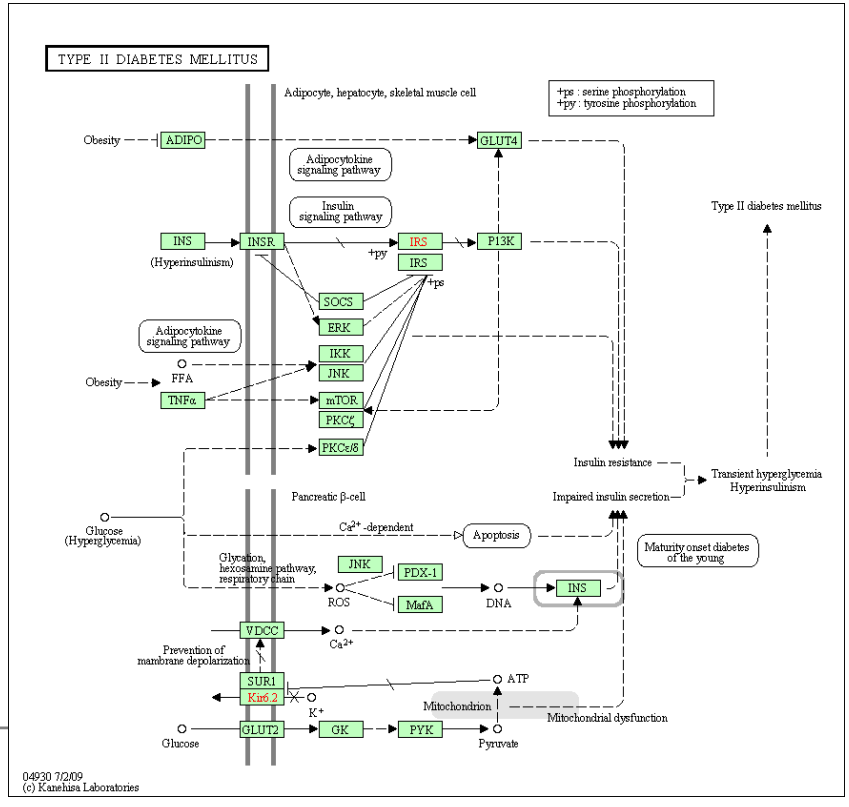
- Metabolism relates to various processes within the body that convert food and other substances into energy and other metabolic byproducts used by the body.
- Cellular subsystem that processes small molecules or *metabolites* to generate energy and building blocks for larger molecules.

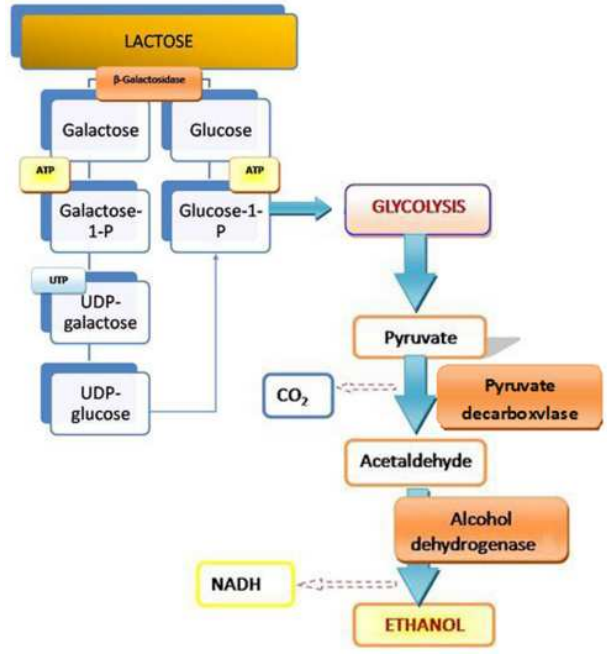
Why should we study metabolism?

- Metabolism is the “ultimate phenotype”
- Metabolic diseases (such as diabetes)
- Applications in bioengineering

Diabetes II pathway in KEGG



Lactose → Ethanol pathway, 2009.igem.org



Cellular space

- Density of biomolecules in the cell is high: plenty of interactions!
- Figure: *Escherichia coli* cross-section
  - Green: cell wall
  - Blue, purple: cytoplasmic area
  - Yellow: nucleoid region
  - White: mRNAm

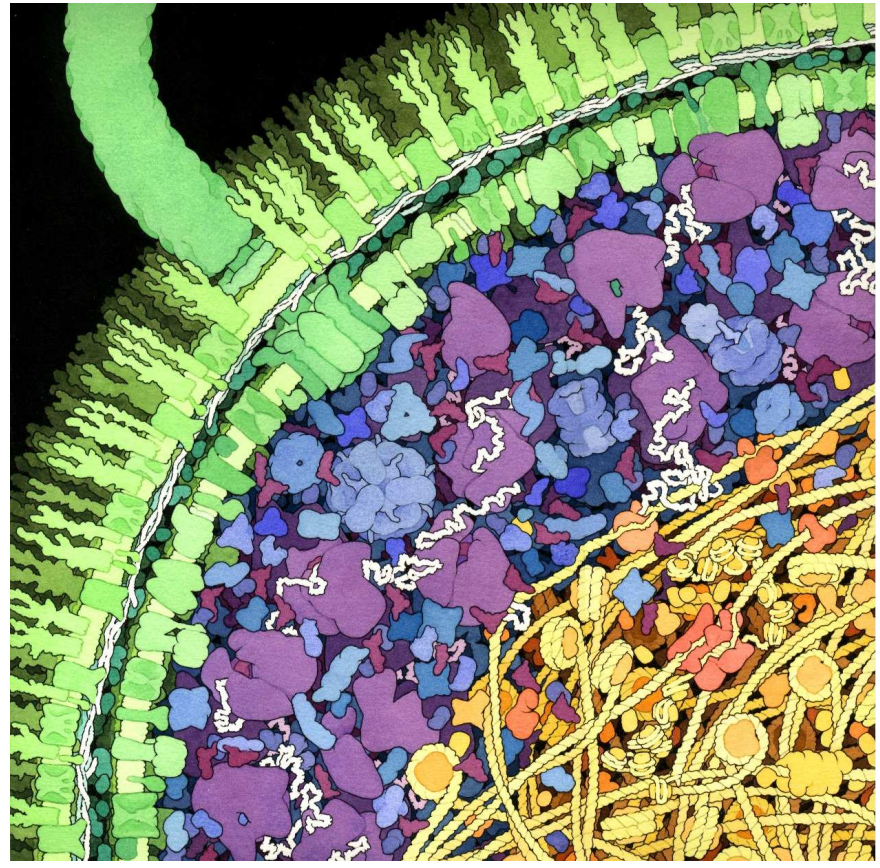
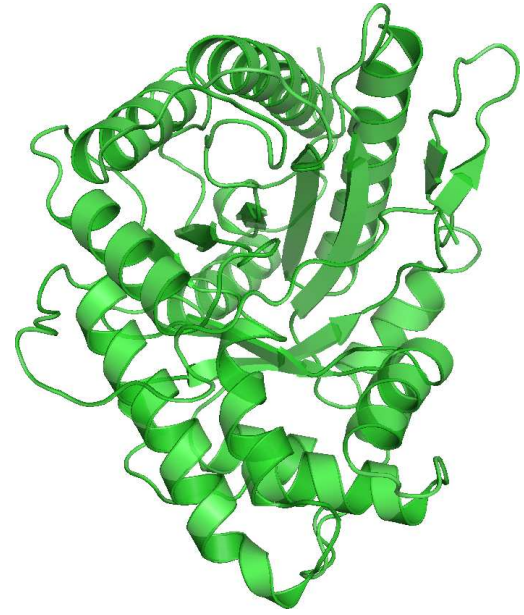


Image: David S. Goodsell

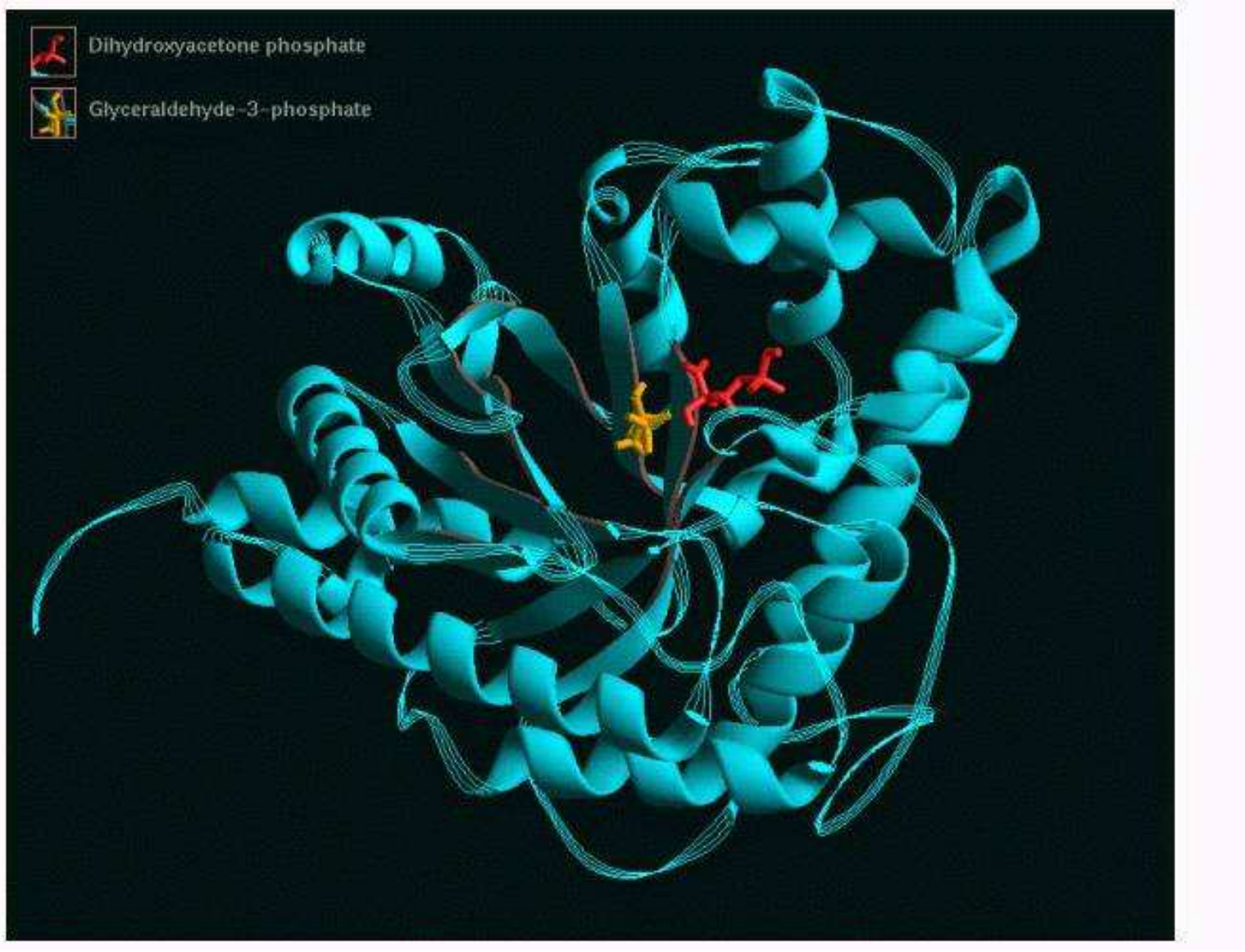
Enzymes

- Reactions catalyzed by *enzymes*
  - Example: Fructose biphosphate aldolase enzyme catalyzes reaction Fructose 1,6-biphosphate $\longrightarrow$ D-glyceraldehyde 3-phosphate + dihydroxyacetone phosphate
- Enzymes are very specific: one enzyme catalyzes typically only one reaction
- Specificity allows *regulation*



Aldolase (PDB 4ALD)

Fructose biphosphate aldolase



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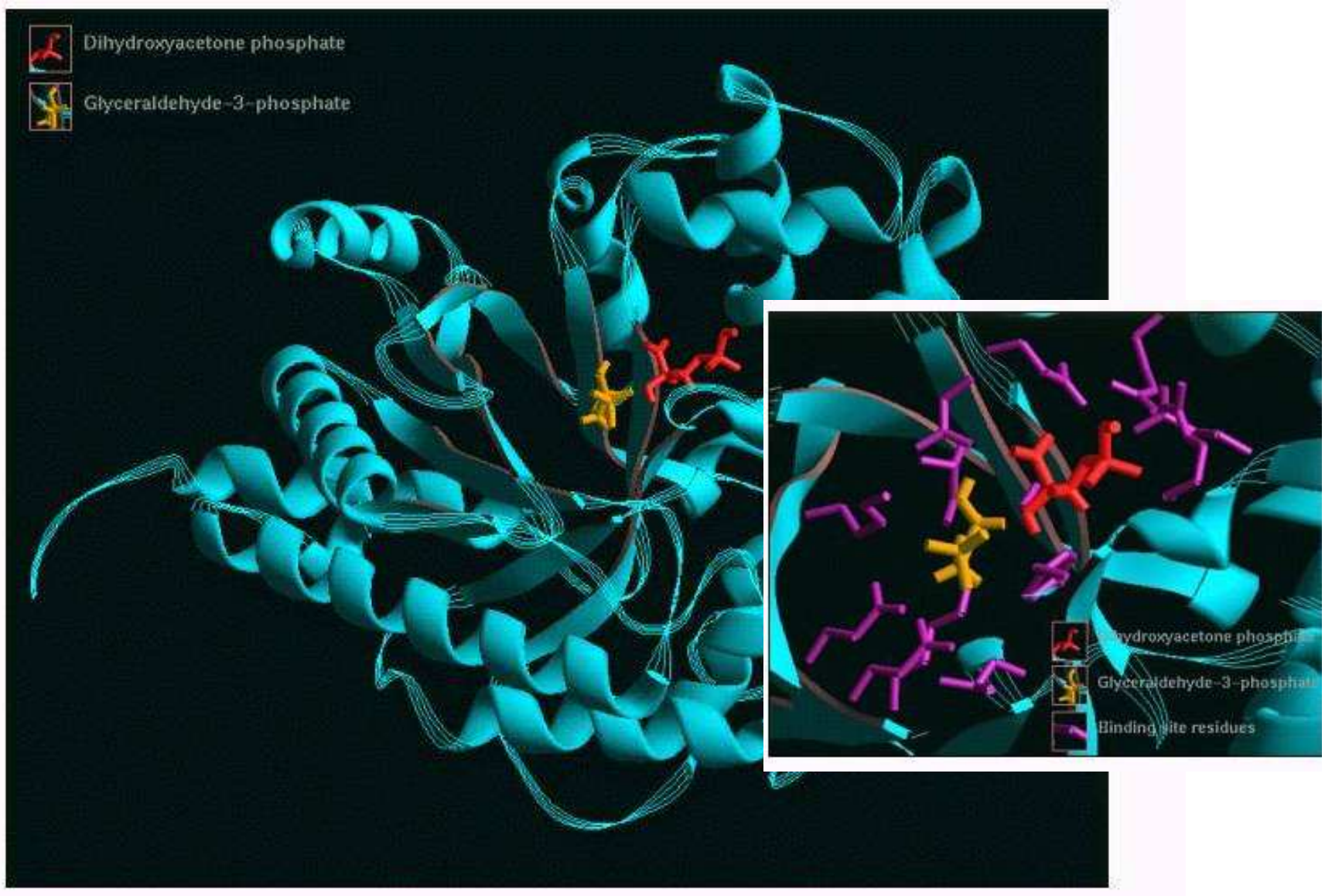
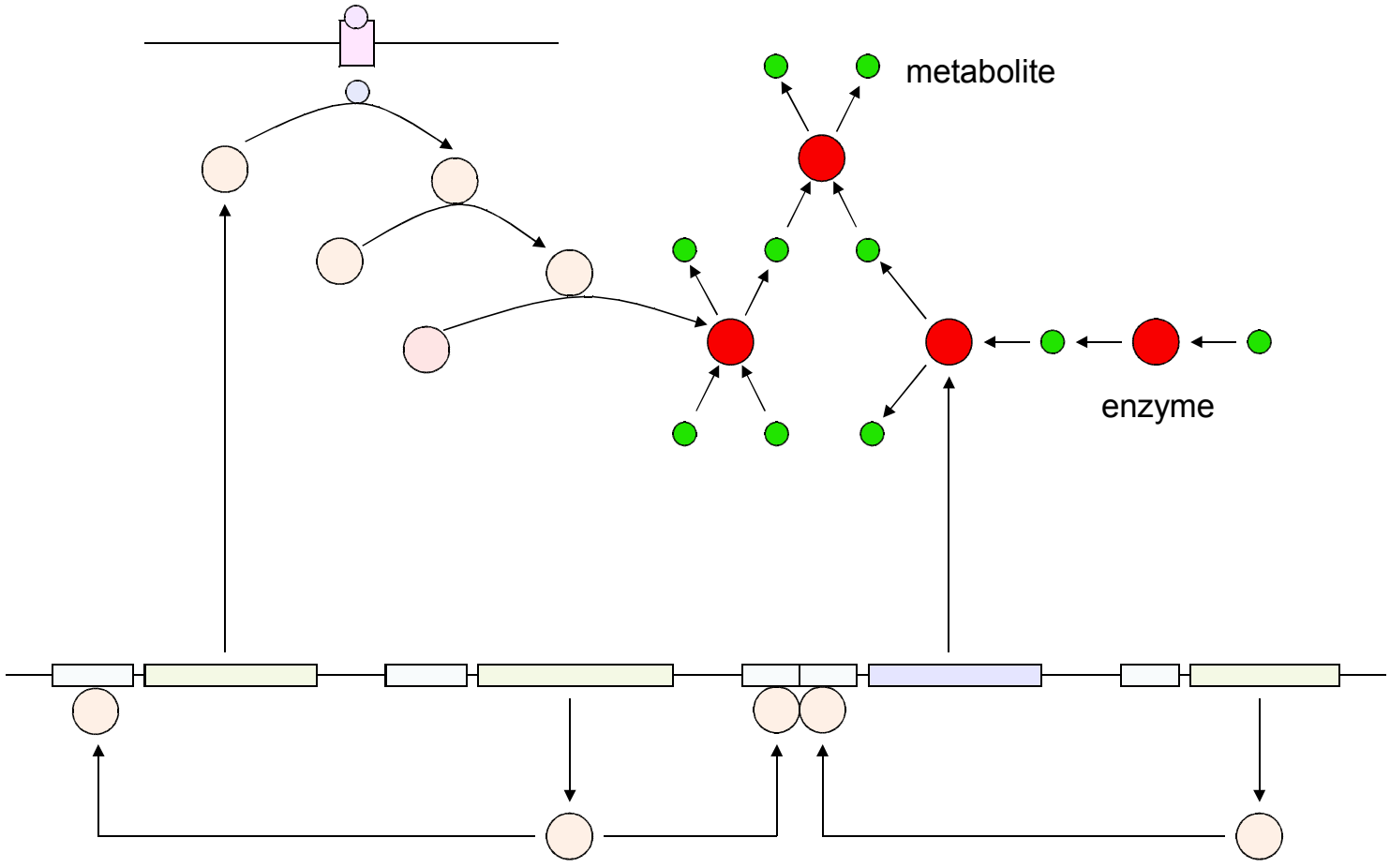
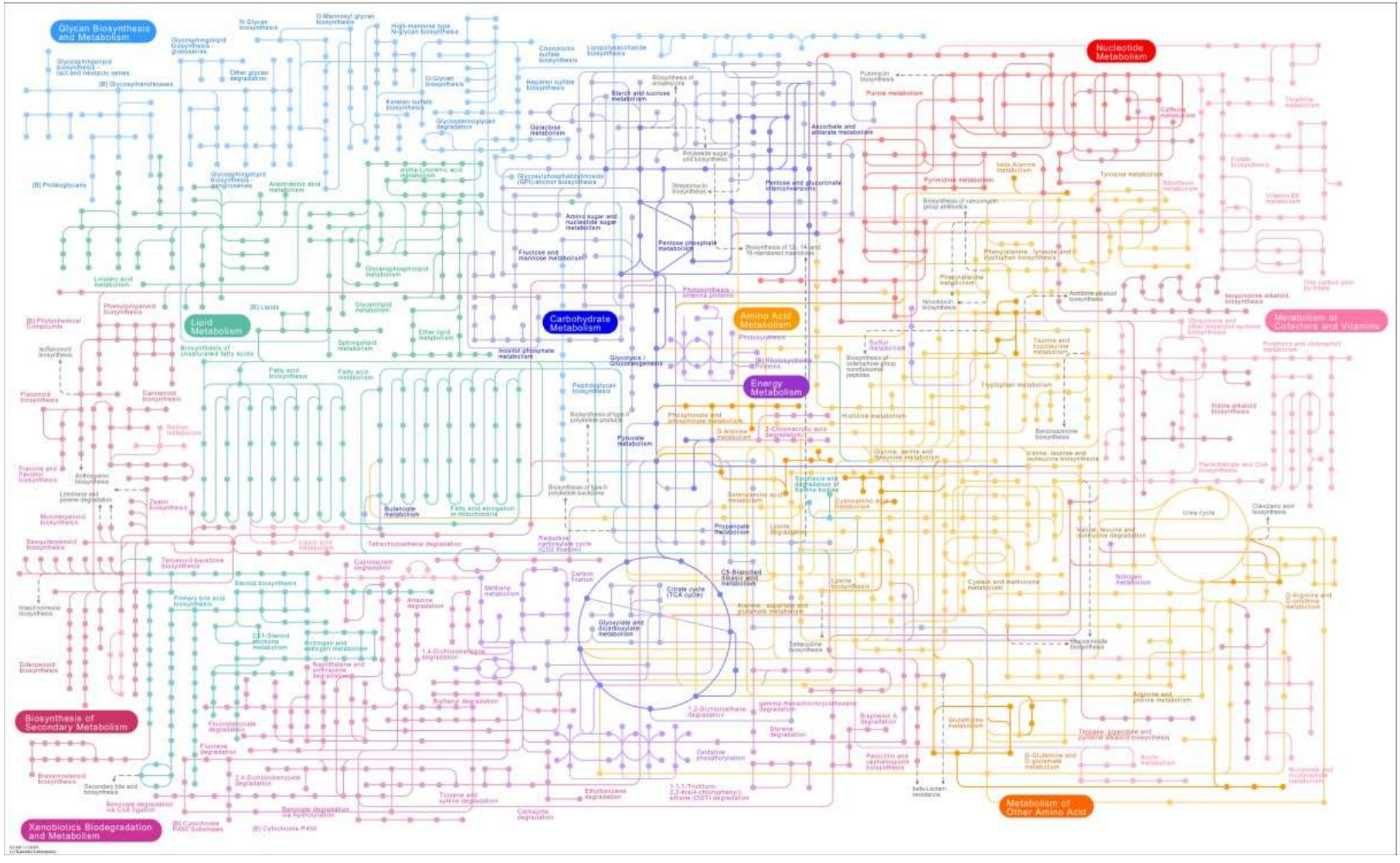


Metabolism: an overview

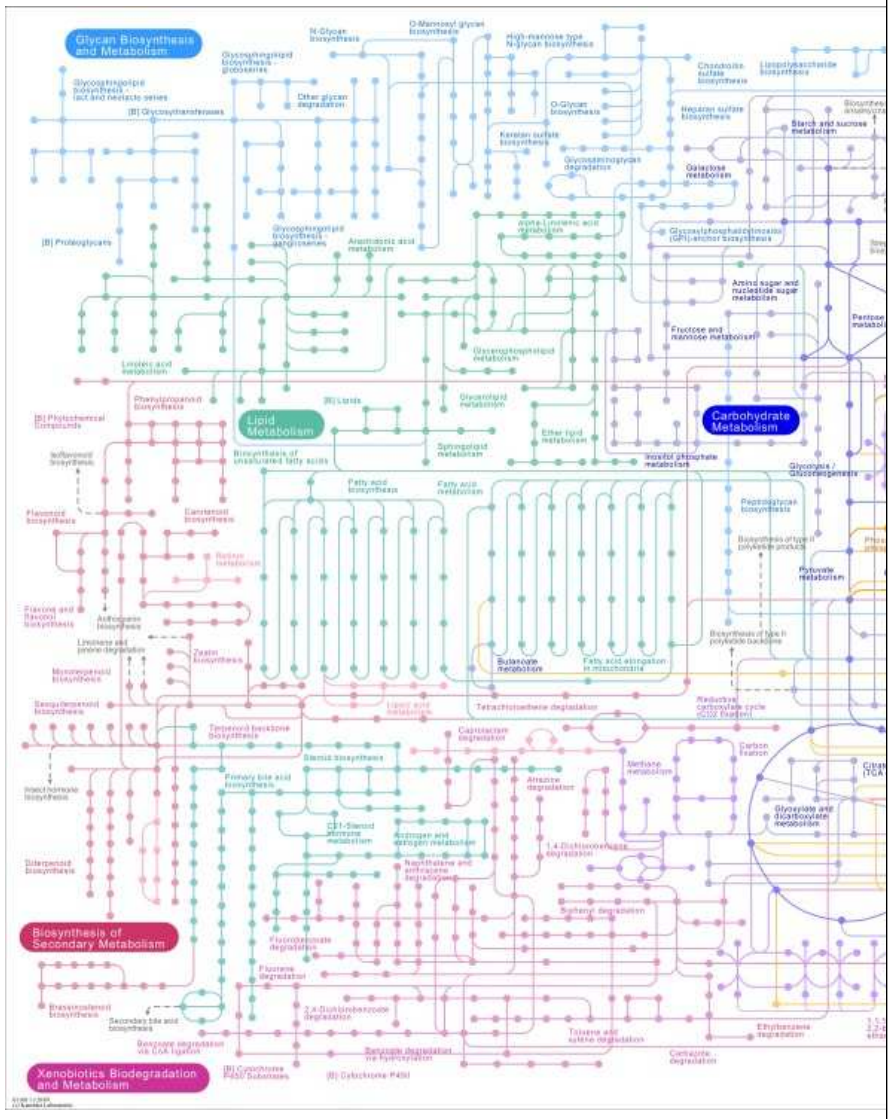


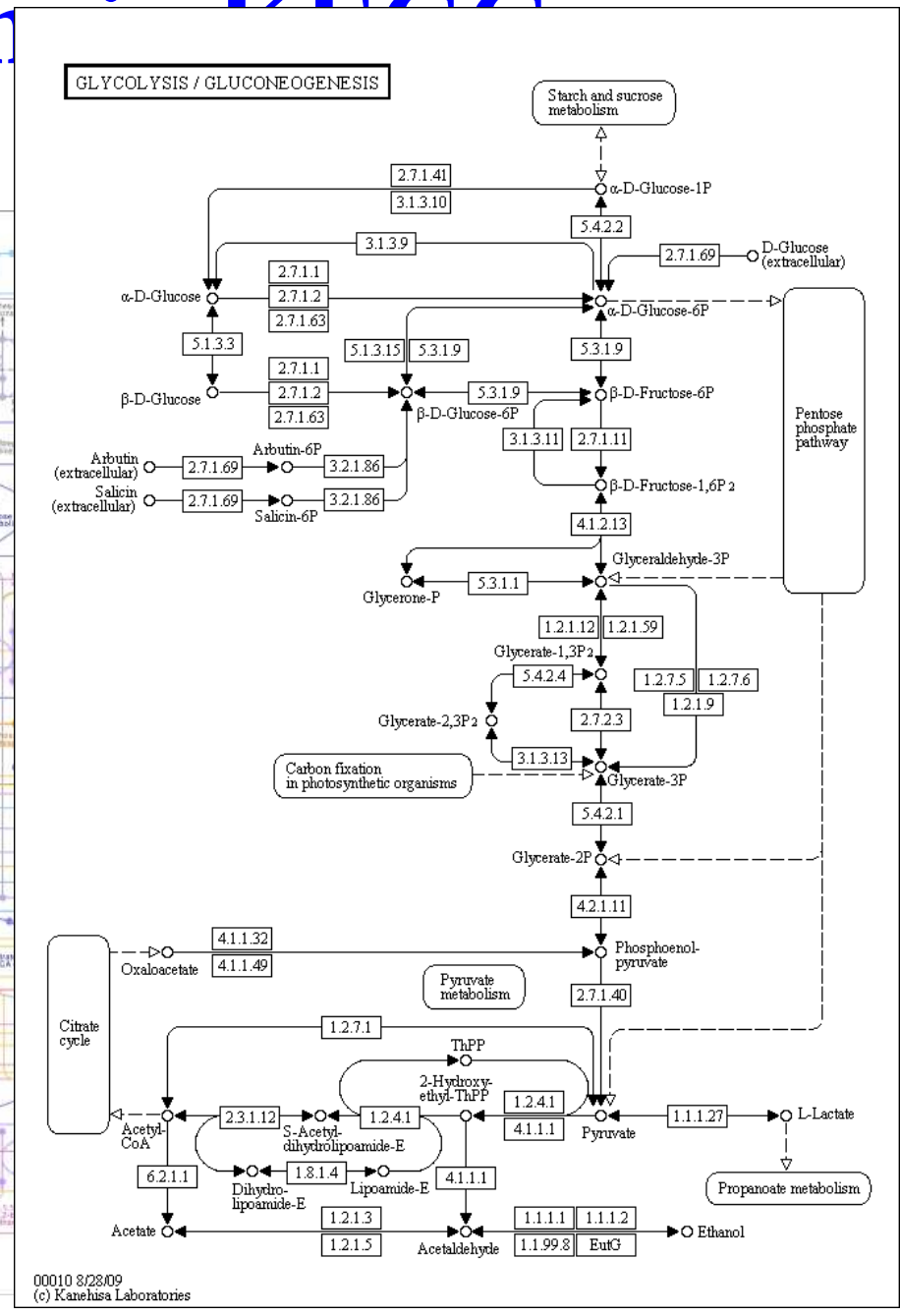
Metabolism in KEGG



KEGG Pathway overview: 8049 reactions (27 Nov 2009)

Metabolism in KEGG





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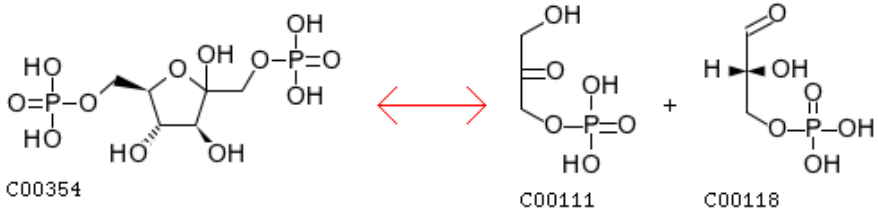

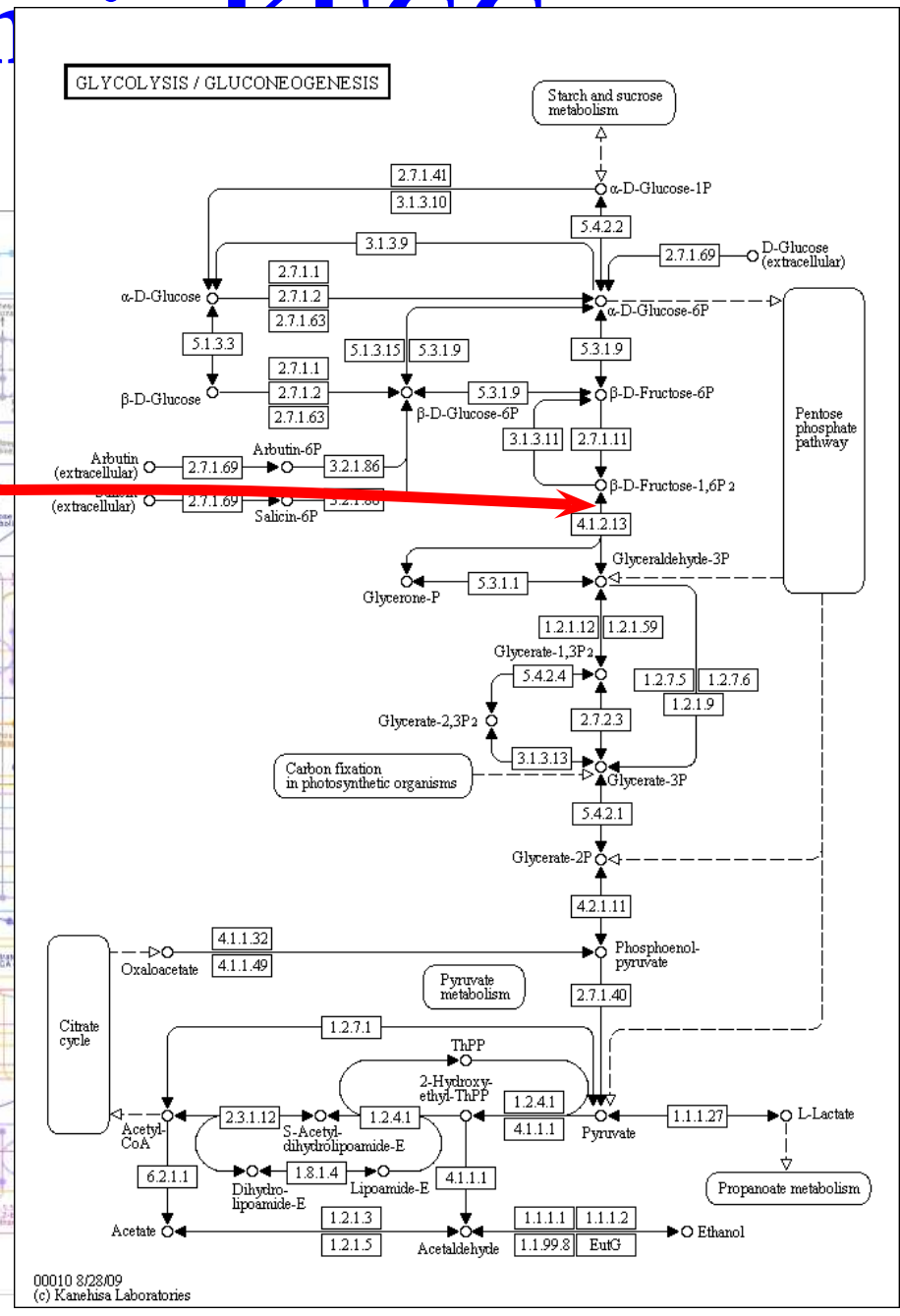

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Metabolic networks

- Metabolic network is a graph model of metabolism
- Different flavors: bipartite graphs, substrate graphs, enzyme graphs
- Bipartite graphs:
  - Nodes: reactions, metabolites
  - Edges: consumer/producer relationships between reactions and metabolites
  - Edge labels can be used to encode *stoichiometry*

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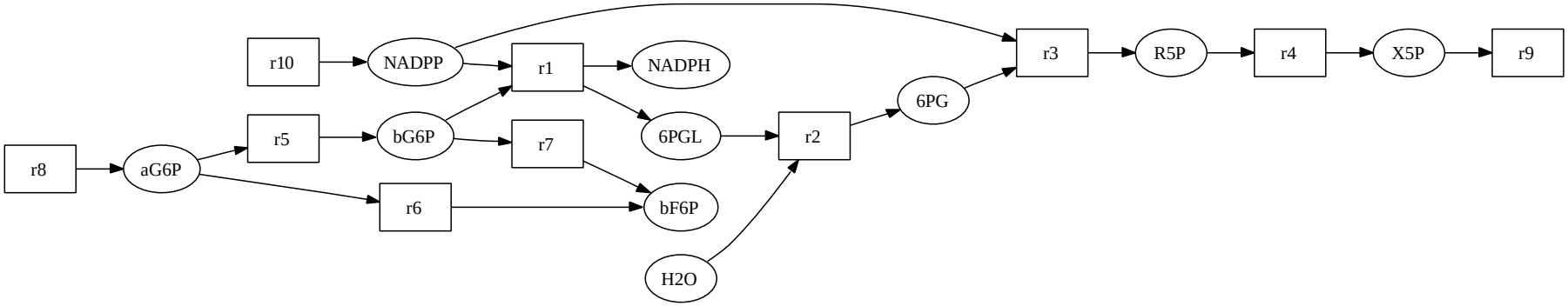


Stoichiometric matrix

- The stoichiometric coefficient s_{ij} of metabolite i in reaction j specifies the number of metabolites produced or consumed in a single reaction step
  - $s_{ij} > 0$: reaction produces metabolite
  - $s_{ij} < 0$: reaction consumes metabolite
  - $s_{ij} = 0$: metabolite does not participate in reaction
- Example reaction: 2 $m_1 \rightarrow m_2$ + m_3
  Coefficients: $s_{1,1} = -2$, $s_{2,1} = s_{3,1} = 1$
- Coefficients comprise a *stoichiometric matrix* $S = (s_{ij})$.

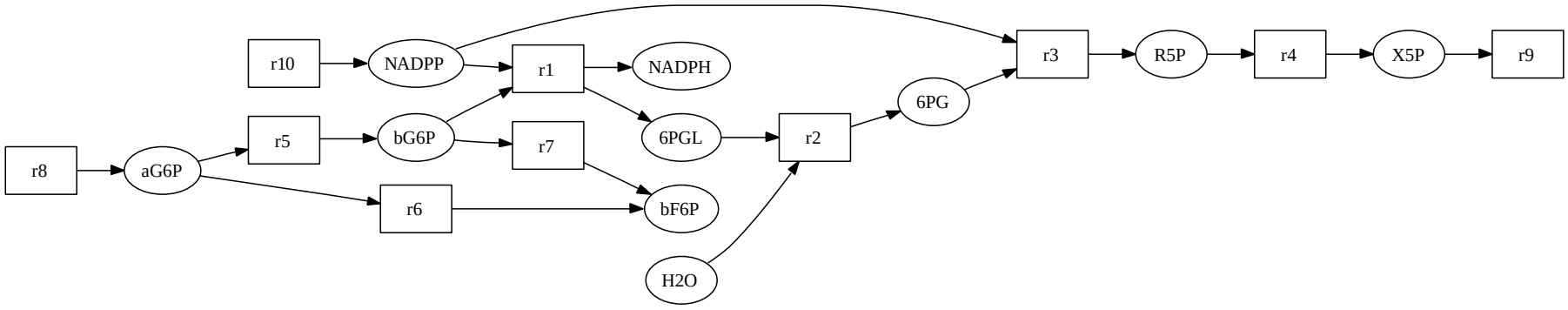
Systems equations

- Rate of concentration changes determined by the set of *systems equations*:

$$\frac{dx_i}{dt} = \sum_j s_{ij} v_j,$$

  - x_i: concentration of metabolite i
  - s_{ij}: stoichiometric coefficient
  - v_j: rate of reaction j

Stoichiometric matrix: example

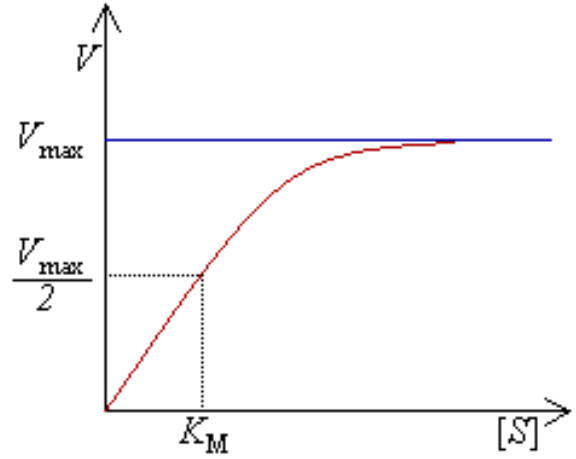


	r_1	r_2	r_3	r_4	r_5	r_6	r_7	r_8	r_9	r_{10}	r_{11}	r_{12}
βG6P	-1	0	0	0	1	0	-1	0	0	0	0	0
αG6P	0	0	0	0	-1	-1	0	1	0	0	0	0
βF6P	0	0	0	0	0	1	1	0	0	0	0	0
6PGL	1	-1	0	0	0	0	0	0	0	0	0	0
6PG	0	1	-1	0	0	0	0	0	0	0	0	0
R5P	0	0	1	-1	0	0	0	0	0	0	0	0
X5P	0	0	0	1	0	0	0	0	-1	0	0	0
$NADP^+$	-1	0	-1	0	0	0	0	0	0	1	0	0
NADPH	1	0	1	0	0	0	0	0	0	0	1	0
H_2O	0	-1	0	0	0	0	0	0	0	0	0	1

Modelling metabolism:kinetic models

- Dynamic behaviour: how metabolite and enzyme concentrations change over time $\rightarrow$ Kinetic models
  - Detailed models for individual enzymes
- For simple enzymes, the Michaelis-Menten equation describes the reaction rate v adequately:

$$v = \frac{v_{max}[S]}{K_M + [S]},$$



where v_{max} is the maximum reaction rate, $[S]$ is the substrate concentration and K_M is the Michaelis constant.

Kinetic models

- Require a lot of data to specify
  - 10-20 parameter models for more complex enzymes
- Limited to small to medium-scale models

Spatial modelling

- “Bag-of-enzymes”
  - all molecules (metabolites and enzymes) in one “bag”
  - all interactions potentially allowed
- Compartmentalized models
- Models of spatial molecule distributions

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Compartments

- Metabolic models of eukaryotic cells are divided into *compartments*
  - Cytosol
  - Mitochondria
  - Nucleus
  - ...and others
- Extracellular space can be thought as a “compartment” too
- Metabolites carried across compartment borders by *transport reactions*

Modelling metabolism: steady-state models

- *Steady-state assumption*: internal metabolite concentrations are constant over time, $\frac{d\mathbf{x}}{dt} = 0$
- External (exchange) metabolites not constrained

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$$\sum_j s_{ij} v_j = S\mathbf{v} = \mathbf{0}$$

- *Is this assumption meaningful? Think of questions we can ask under the assumption!*

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- Steady-state reaction rate (*flux*) v_i
- Holds in certain conditions, for example in chemostat cultivations

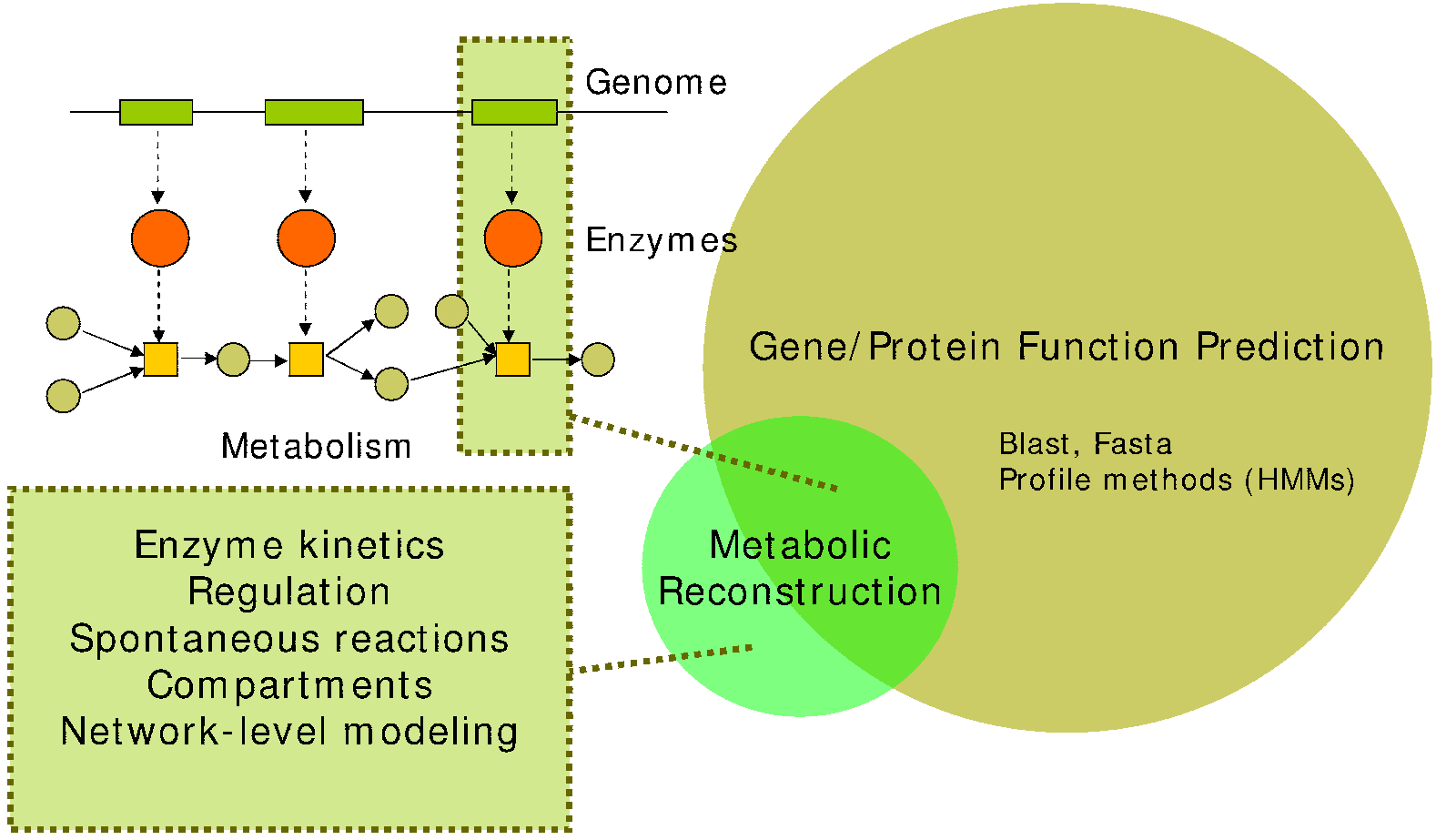
Outline: Metabolic reconstruction

- Metabolism, metabolic networks
- Metabolic reconstruction
- Flux balance analysis

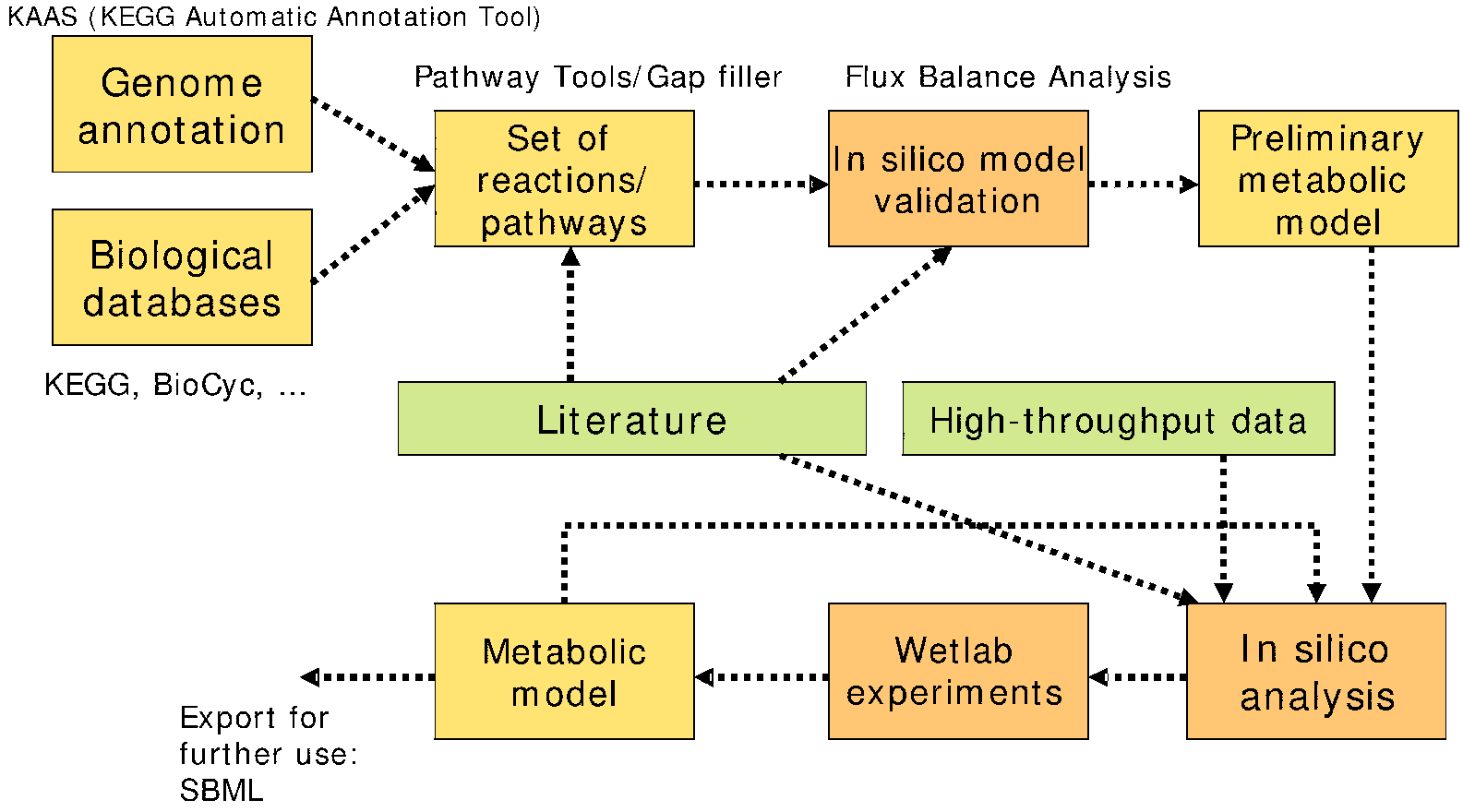
Metabolic reconstruction

- Reconstruction problem: *infer the metabolic network from sequenced genome*
- Determine genes coding for enzymes and assemble metabolic network?
  - *Subproblem of genome annotation?*

Metabolic reconstruction



Reconstruction process



Read more: Puchałka et al., Genome-Scale Reconstruction and Analysis of the Pseudomonas putida KT2440 Metabolic Network Facilitates Applications in Biotechnology. PLoS Computational Biology 2008.

Data sources for reconstruction

- Biochemistry
  - Enzyme assays: measure enzymatic activity
- Genomics
  - Annotation of open reading frames
- Physiology
  - Measure cellular inputs (growth media) and outputs
  - Biomass composition

Resources

- Databases
  - KEGG
  - BioCyc
- Ontologies
  - Enzyme Classification (EC)
  - Gene Ontology
- Software
  - Pathway Tools
  - KEGG Automatic Annotation Server (KAAS)
  - MetaSHARK, MetaTIGER
  - IdentiCS
  - RAST

Annotating sequences

1. Find genes in sequenced genome (available software)
   - GLIMMER (microbes)
   - GlimmerM (eukaryotes, considers intron/exon structure)
   - GENSCAN (human)
2. Assign a function to each gene
   - BLAST, FASTA against a database of annotated sequences (e.g., UniProt)
   - Profile-based methods (HMMs, see InterProScan for a unified interface for different methods)
   - Protein complexes, isozymes

Assembling the metabolic network

- In principle: for each gene with annotated enzymatic function(s), add reaction(s) to network (gene-protein-reaction associations)

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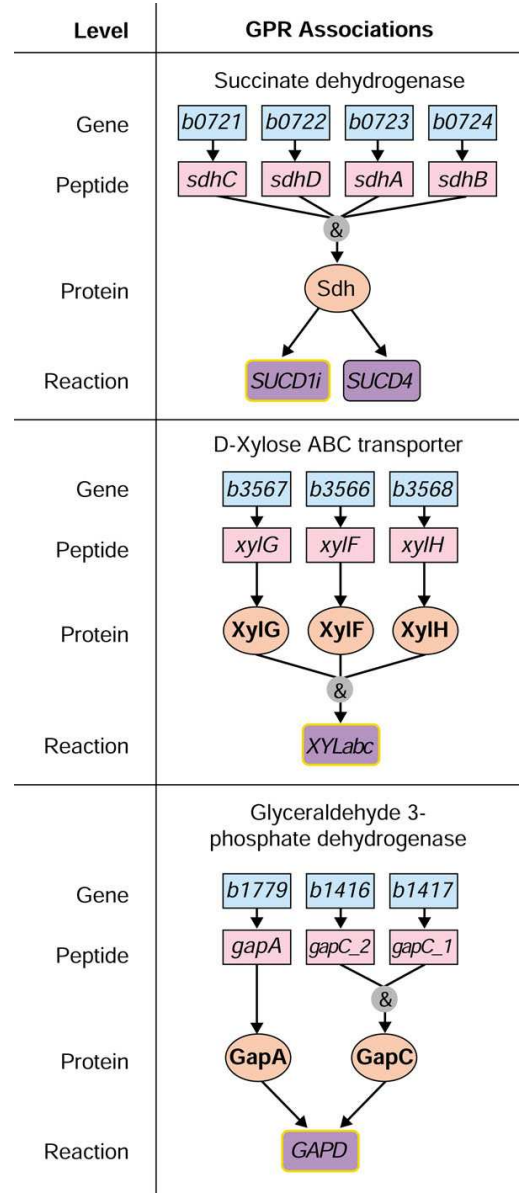
- Multiple peptides may form a single protein (top)
- Proteins may form complexes (middle)
- Different genes may encode isozymes (bottom)



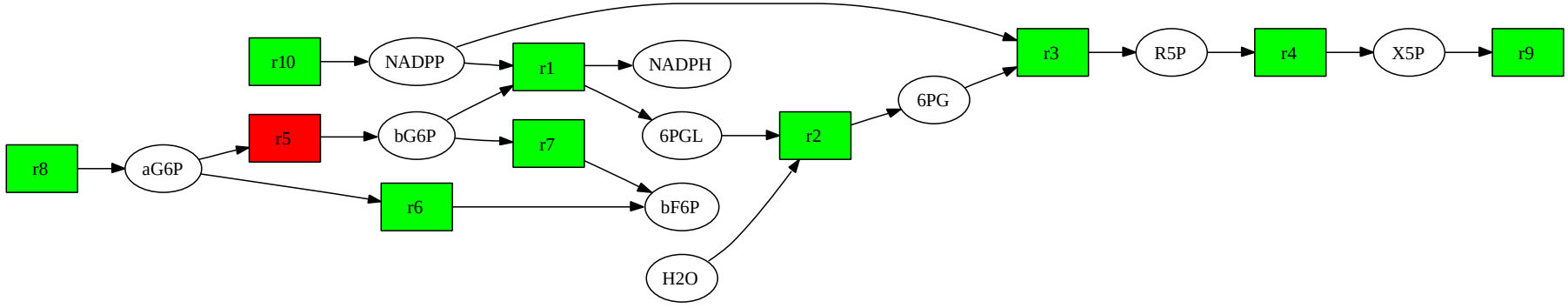
Reed *et al.*, Genome

Gaps in metabolic networks

- Assembled network often contains so-called *gaps*
- Informally: gap is a reaction
  - “missing” from the network...
  - ...required to perform some function.
- A large amount of manual work is required to fix networks
- Recently, computational methods have been developed to fix network consistency problems

Gaps in metabolic networks

May carry steady-state flux – Blocked – Gap



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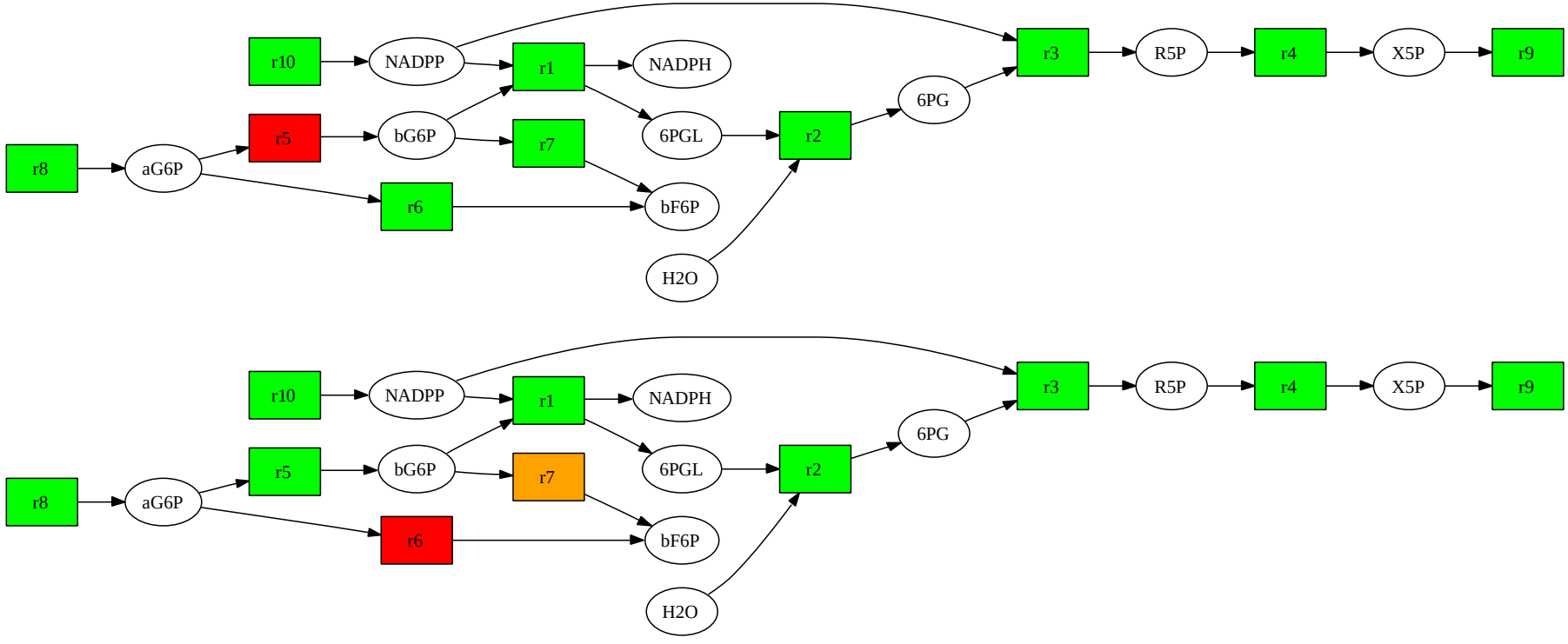

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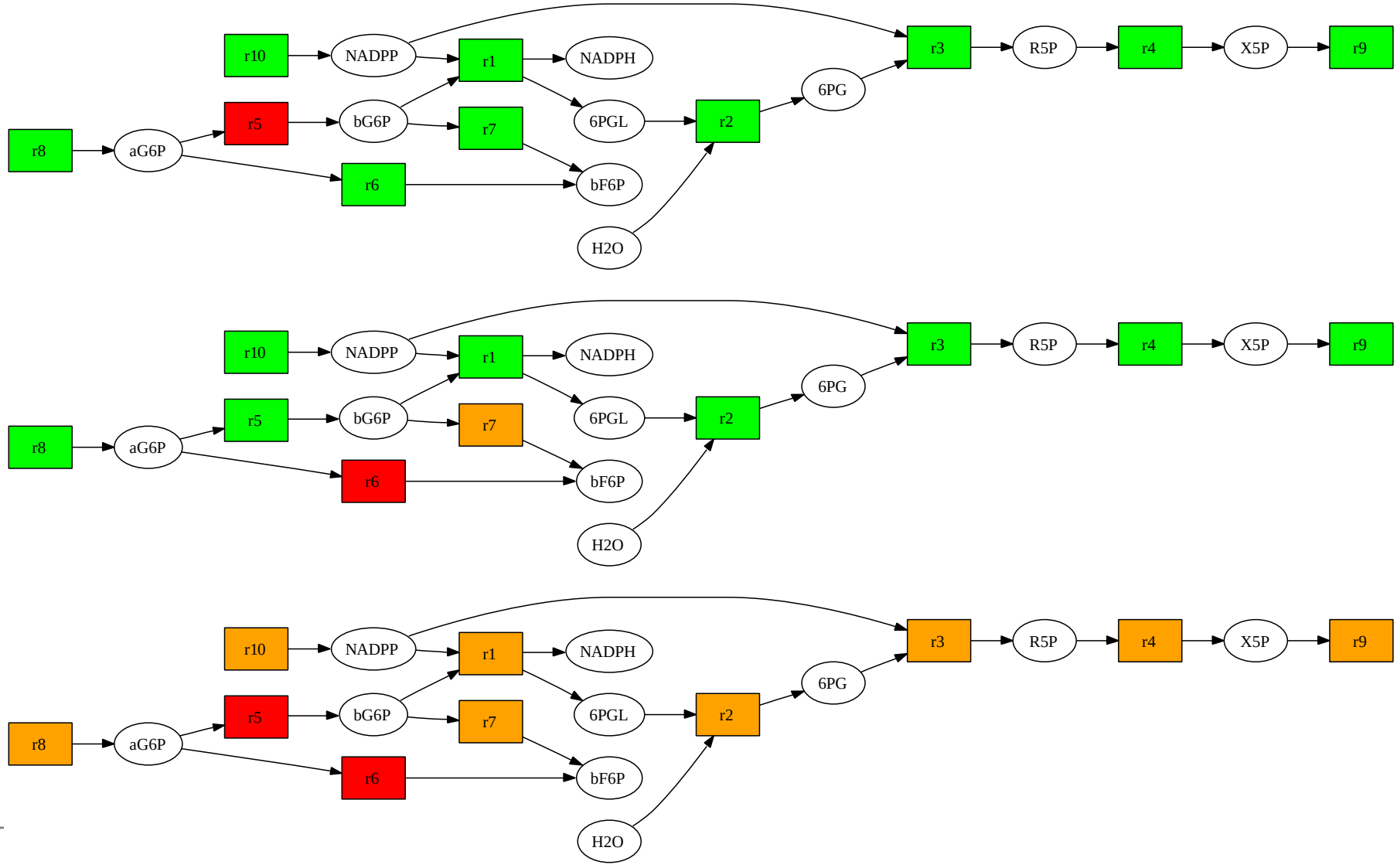


In silico validation of metabolic models

- Reconstructed genome-scale metabolic networks are very large: hundreds or thousands of reactions and metabolites
- Manual curation is often necessary
- Amount of manual work needed can be reduced with computational methods
- Aims to provide a good basis for further analysis and experiments
- Does not remove the need for experimental verification

Outline: Flux balance analysis

- Metabolism, metabolic networks
- Metabolic reconstruction
- Flux balance analysis

Flux Balance Analysis: preliminaries

- Recall that in a steady state, metabolite concentrations are constant over time,

$$\frac{dx_i}{dt} = \sum_{j=1}^{r} s_{ij} v_j = 0, \ \texttt{for}\ i = 1, \ldots, n.$$

Stoichiometric model can be given as

$$\mathbf{S} = [S_{II}\ S_{IE}]$$

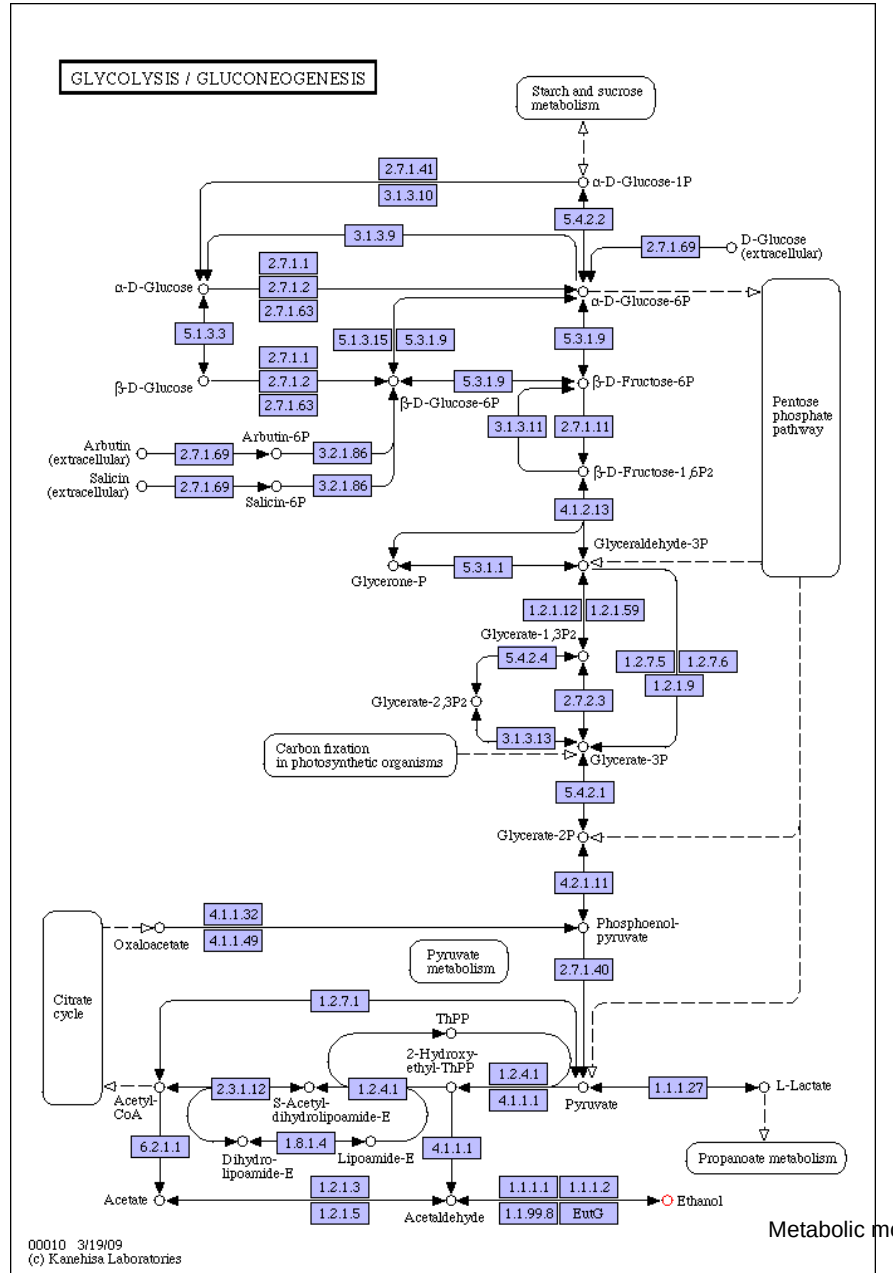
where S_{II} describes internal metabolites - internal reactions, and S_{IE} internal metabolites - exchange reactions.

Flux Balance Analysis (FBA)

- FBA is a framework for investigating the theoretical capabilities of a stoichiometric metabolic model $\mathbf{S}$
- Analysis is constrained by
  1. Steady state assumption $\mathbf{S}v = 0$
  2. Thermodynamic constraints: (ir)reversibility of reactions
  3. Limited reaction rates of enzymes: $V_{min} \leq v \leq V_{max}$
- Note that constraints (2) can be included in V_{min} and V_{max}.

Flux Balance Analysis (FBA)

- In FBA, we are interested in determining the theoretical maximum (minimum) *yield* of some metabolite, given model
- For instance, we may be interested in finding how efficiently yeast is able to convert sugar into ethanol
- Figure: glycolysis in KEGG



Flux Balance Analysis (FBA)

- FBA has applications both in metabolic engineering and metabolic reconstruction
- Metabolic engineering: find out possible reactions (pathways) to insert or delete
- Metabolic reconstruction: validate the reconstruction given observed metabolic phenotype

Formulating an FBA problem

- We formulate an FBA problem by specifying parameters c in the optimization function Z,

$$Z = \sum_{i=1}^{r} c_i v_i.$$

- Examples:
  - Set $c_i = 1$ if reaction i produces "target" metabolite, and $c_i = 0$ otherwise
  - Growth function: maximize production of biomass constituents
  - Energy: maximize ATP (net) production

Solving an FBA problem

- Given a model $\mathbf{S}$, we then seek to find the maximum of Z while respecting the FBA constraints,

(1) $$\max_v Z = \max_v \sum_{i=1}^{r} c_i v_i \quad \texttt{such that}$$

(2) $$\mathbf{S}v = 0$$

(3) $$V_{min} \leq v \leq V_{max}$$

- (We could also replace $\max$ with $\min$.)
- This is a *linear program*, having a linear objective function and linear constraints

Solving a linear program

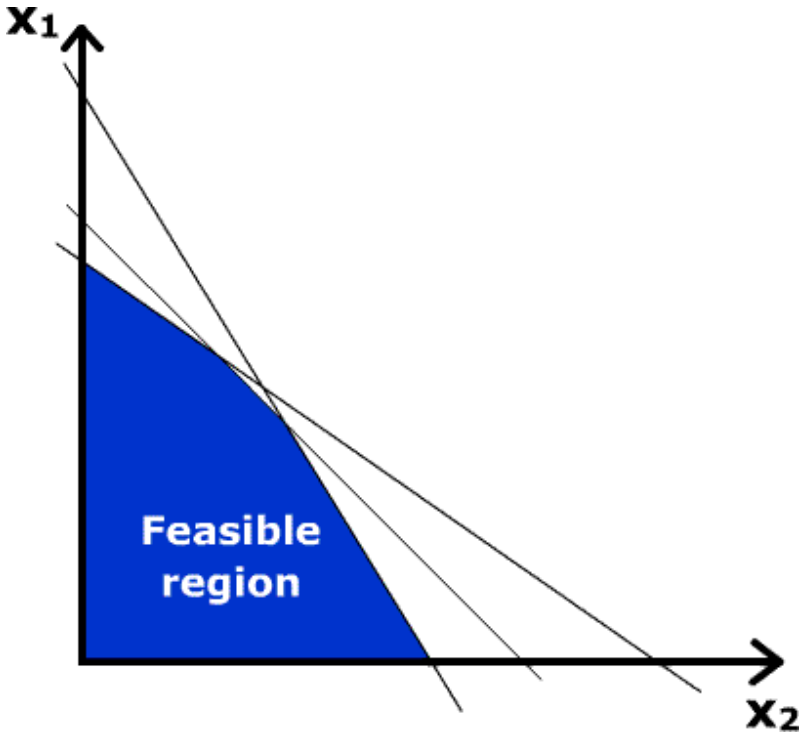
- General linear program formulation:

$$\max_{x_i} \sum_i c_i x_i \quad \texttt{such that}$$

$$Ax \leq b$$

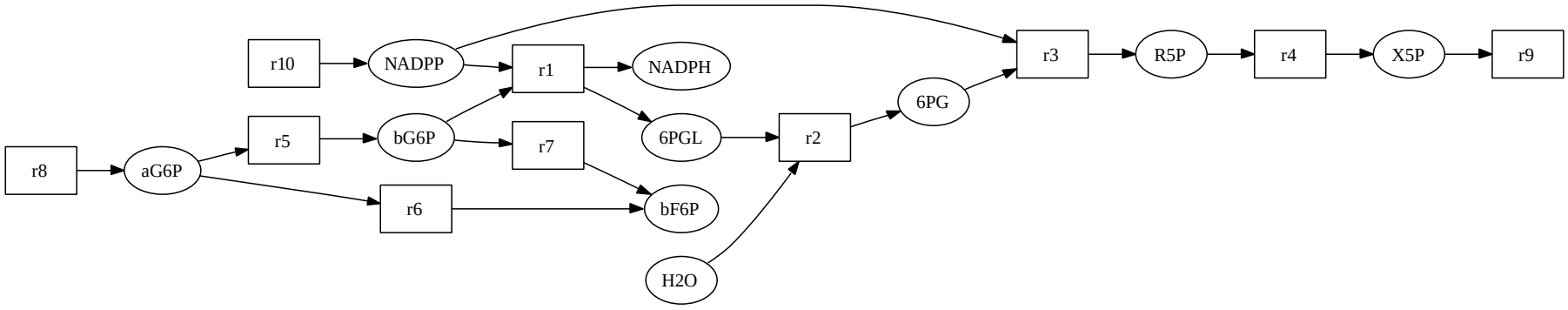
- Algorithms: simplex (worst-case exponential time), interior point methods (polynomial)
- Matlab solver: linprog (Statistical Toolbox)
- Many solvers around, efficiency with (very) large models varies

Linear programs



- Linear constraints define a convex polyhedron (*feasible region*)
- If the feasible region is empty, the problem is *infeasible*.
- Unbounded feasible region (in direction of objective function): no optimal solution
- Given a linear objective function, where can you find the maximum value?

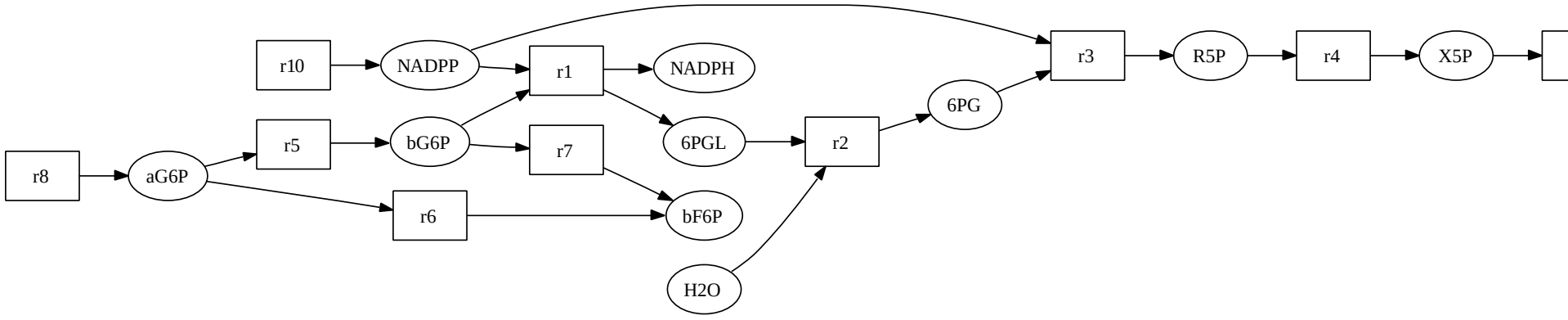
Flux Balance Analysis: example



- Let's take our running example...
- Unconstrained uptake (exchange) reactions for $NADP^+$ (r_{10}), NADPH and H_2O (not drawn)
- Constrained uptake for αG6P, $0 \leq v_8 \leq 1$
- Objective: production of X5P (v_9)

$$c = (0, 0, 0, 0, 0, 0, 0, 0, 1, 0, 0, 0)$$

Flux Balance Analysis: example



	r_1	r_2	r_3	r_4	r_5	r_6	r_7	r_8	r_9	r_{10}	r_{11}	r_{12}
βG6P	-1	0	0	0	1	0	-1	0	0	0	0	0
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6PG	0	1	-1	0	0	0	0	0	0	0	0	0
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NADPH	1	0	1	0	0	0	0	0	0	0	1	0
H_2O	0	-1	0	0	0	0	0	0	0	0	0	1

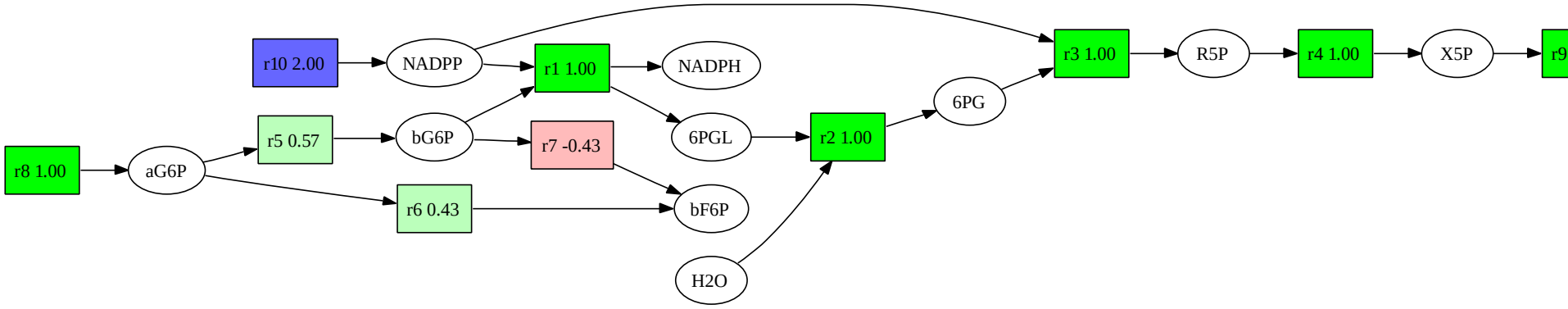
Flux Balance Analysis: example

- Solve the linear program

$$\begin{aligned} \max_v \sum_i^r c_i v_i &= \max v_9 && \text{subject to} \\ \sum_{i=1}^r s_{ij} v_i &= 0 && \text{for all } j = 1, \ldots, 10 \\ 0 \leq v_8 &\leq 1 \end{aligned}$$

- Hint: Matlab's linprog offers nice convenience functions for specifying equality constraints and bounds

Flux Balance Analysis: example



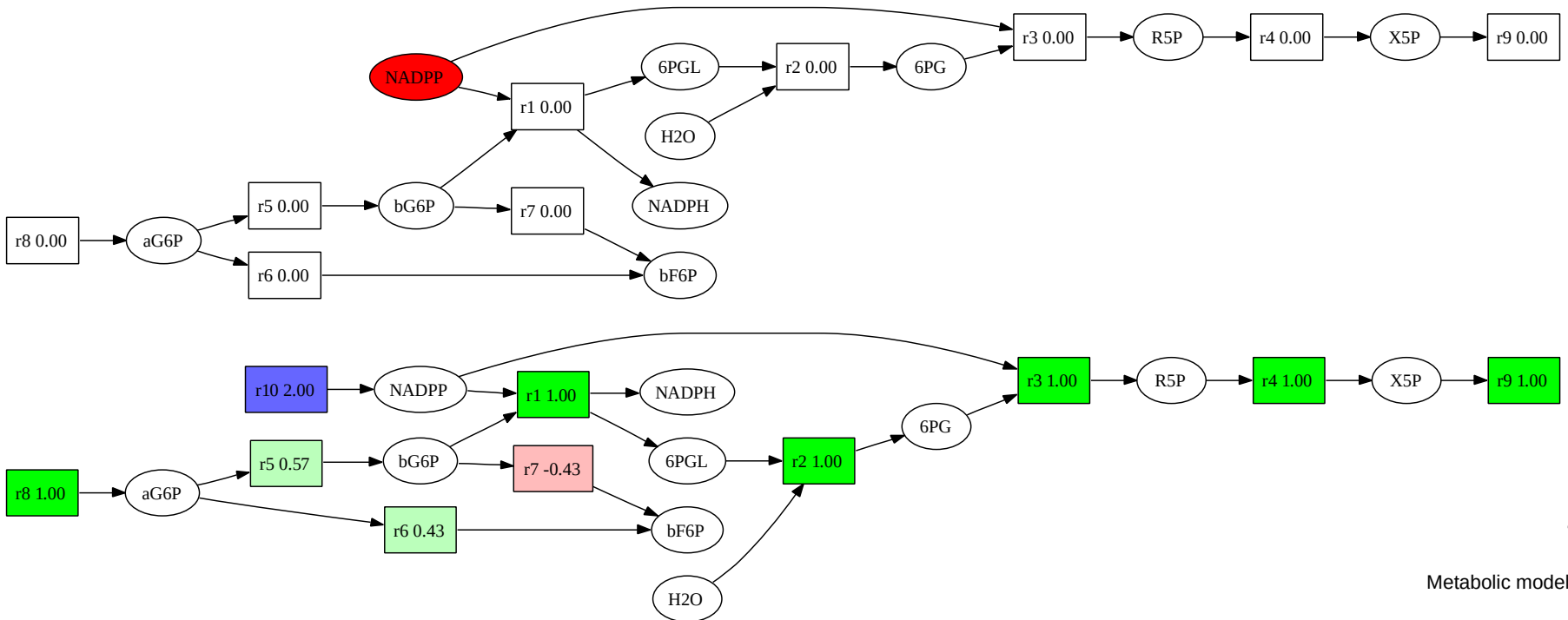
- Figure gives one possible solution (flux assignment v)
- Reaction r_7 (red) operates in backward direction
- Uptake of NADP$^+$ $v_{10} = 2v_8 = 2$
- How many solutions (different flux assignments) are there for this problem?

FBA validation of a reconstruction

- Check if it is possible to produce metabolites that the organism is known to produce
  - Maximize production of each such metabolite at time
  - Make sure max. production is above zero
- To check biomass production (growth), add a reaction to the model with stoichiometry corresponding to biomass composition

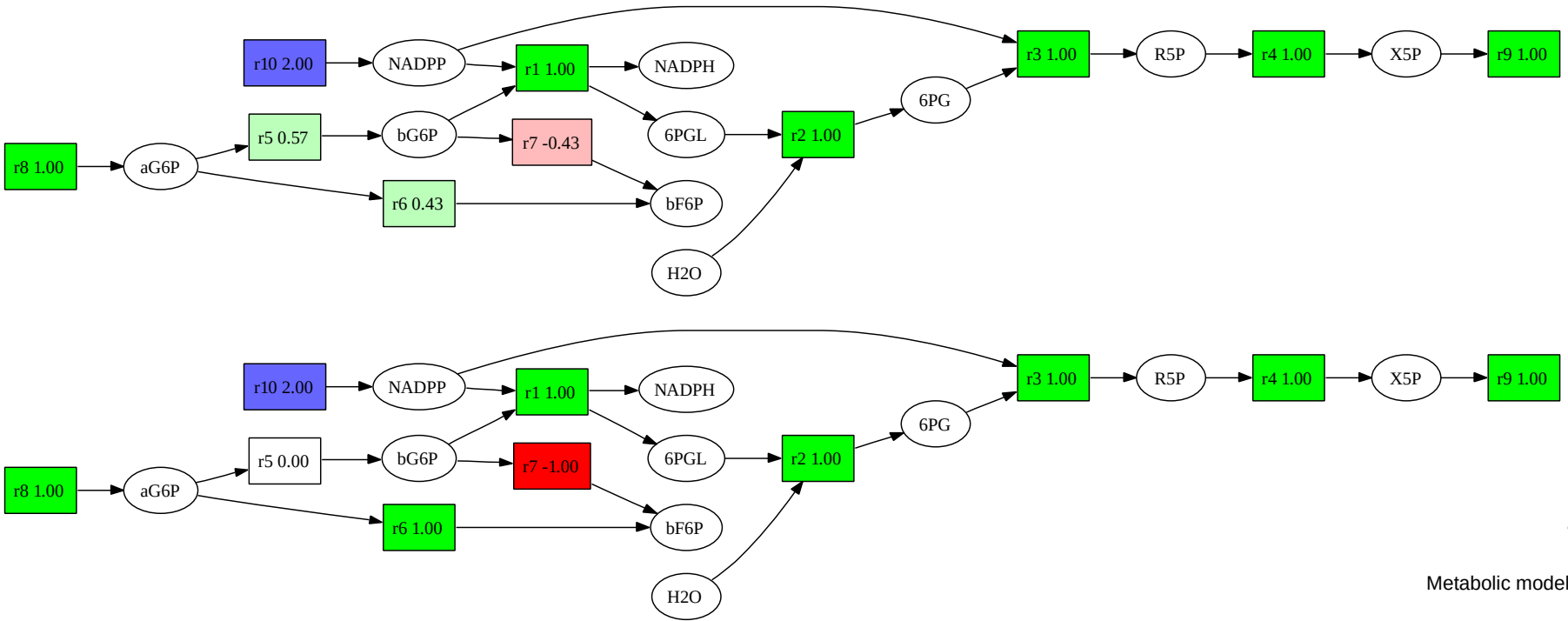
FBA validation of a reconstruction

- If a maximum yield of some metabolite is lower than measured → missing pathway
- Iterative process: find metabolite that cannot be produced, fix the problem by changing the model, try again



FBA validation of a reconstruction

- FBA gives the maximum flux given stoichiometry only, i.e., not constrained by regulation or kinetics
- In particular, assignment of internal fluxes on alternative pathways can be arbitrary (of course subject to problem constraints)



Further reading

- Metabolic modelling: course material
- M. Durot, P.-Y. Bourguignon, and V. Schachter: Genome-scale models of bacterial metabolism: ... FEMS Microbiol Rev. 33:164-190, 2009.
- N. C. Duarte *et. al*: Global reconstruction of the human metabolic network based on genomic and bibliomic data. PNAS 104(6), 2007.
- V. Lacroix, L. Cottret, P. Thebault and M.-F. Sagot: An introduction to metabolic networks and their structural analysis. IEEE Transactions on Computational Biology and Bioinformatics 5(4), 2008.
- E. Pitkänen, A. Rantanen, J. Rousu and E. Ukkonen: A computational method for reconstructing gapless metabolic netwo Proceedings of the BIRD'08, 2008.