Technische Universität Braunschweig

Plant Biotechnology and Bioinformatics

Metabolic flux & modeling

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Availability of slides

- All materials are freely available (CC BY) - after the lectures:
  - StudIP: Lecture: Grundlagen der Biochemie und Bioinformatik der Pflanzen (Bio-MB 09)
  - GitHub: https://github.com/bpucker/teaching

- Questions: Feel free to ask at any time

- Feedback, comments, or questions: b.pucker[a]tu-bs.de

Metabolism

- Metabolism:
  - Biochemical modification of chemical compounds in living organisms
  - Enzymatic transformation of organic molecules in cells
  - All processes handling substances in an organism

- Metabolism requires input (substrates) and generated output (products)

- Enzymes catalyze the reactions (facilitation)

- Examples:
  - gene expression
  - biosynthesis
  - signaling

Plant metabolites

- Extremely diverse specialized metabolism
- Many metabolites are restricted to one or a few species
- 200,000-1,000,000 metabolites (estimated)
- Major groups:
  - Phenylpropanoids
  - Benzenoids
  - Flavonoids
  - Terpenes
  - N-containing compounds

Maeda & Fernie, 2021: 10.1146/annurev-arplant-080620-031054
Fernie & Tohge, 2017: 10.1146/annurev-genet-120116-024640
Wang et al., 2019: 10.1016/j.molp.2019.06.001

Specialized plant metabolites as drug candidates

- Morphine (pain)
- Taxol (cancer)
- Withanolides (cancer)
- Camptothecin (cancer)

https://commons.wikimedia.org/wiki/File:Camptotheca_acuminata_HK.jpg CC BY-SA 3.0

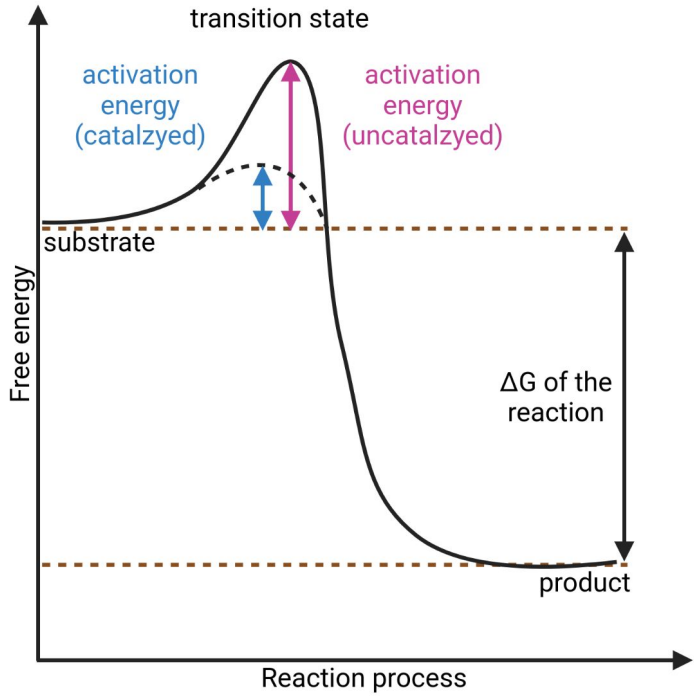
Specialized plant metabolites for stress adaptation

- Anthocyanins as high light, drought, and salt stress response
- Flavonols as UV response
- Proanthocyanidins against herbivores, fungi, bacteria

Urtica dioica
(Lena Fürstenberg)

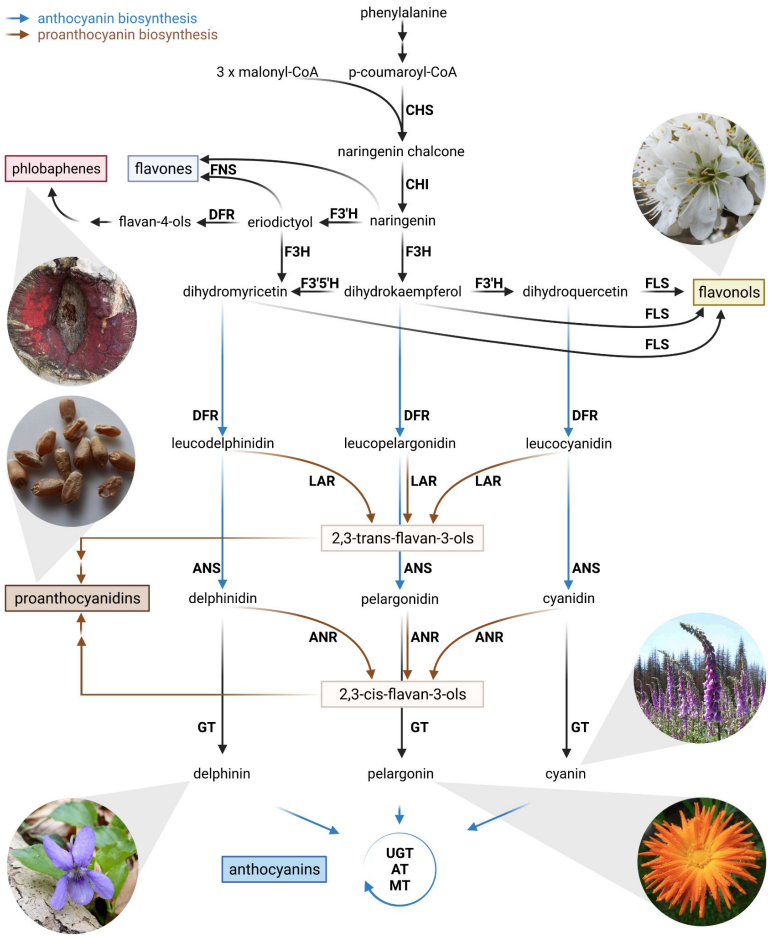
Enzymes

- Bind substrates and facilitate conversion
- Enzymes do not change direction of reaction
- Enzymatic activity depends on:
  - pH
  - Temperature
  - Substrate concentration
  - Product concentration



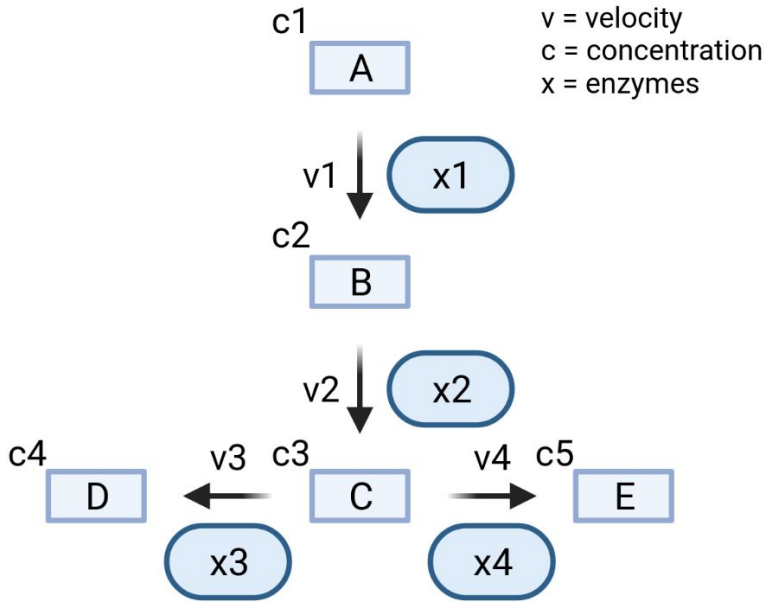
Metabolic networks

- Metabolism of plants can be divided into reactions that form pathways/networks
- Many steps/pathways are still unknown resulting in gaps (12k different flavonoids)
- Metabolism is regulated by external factors e.g. environmental conditions
- Metabolism can be described by a metabolic model



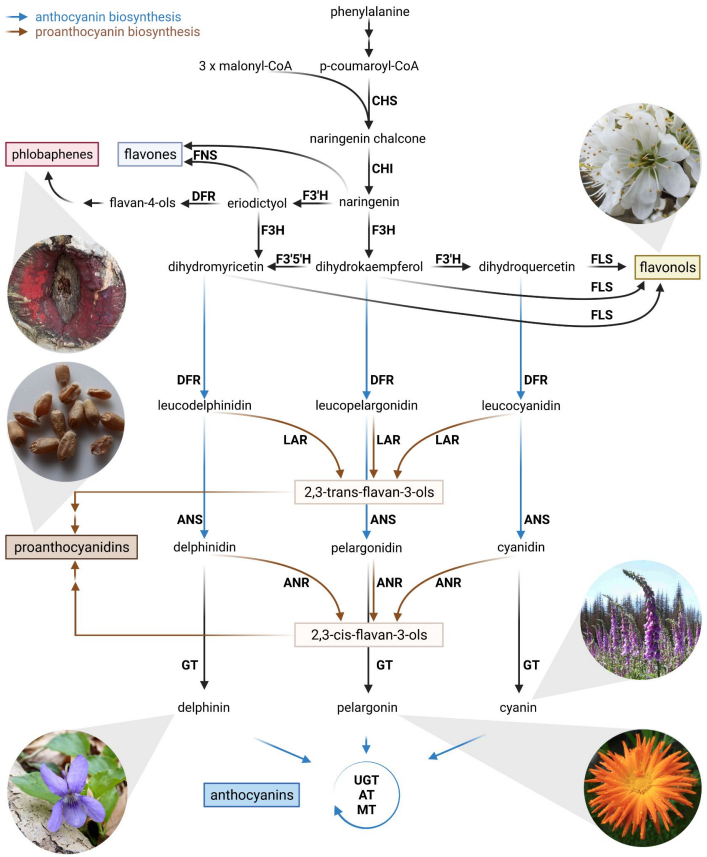
What is a metabolic model?

- Simplified illustration of a metabolism based on genome sequence annotation
- Simplification can cause differences between prediction and reality
- Enzymes, substrate, intermediates, and products are displayed
- Metabolic networks are influenced by genetic regulation (often ignored)



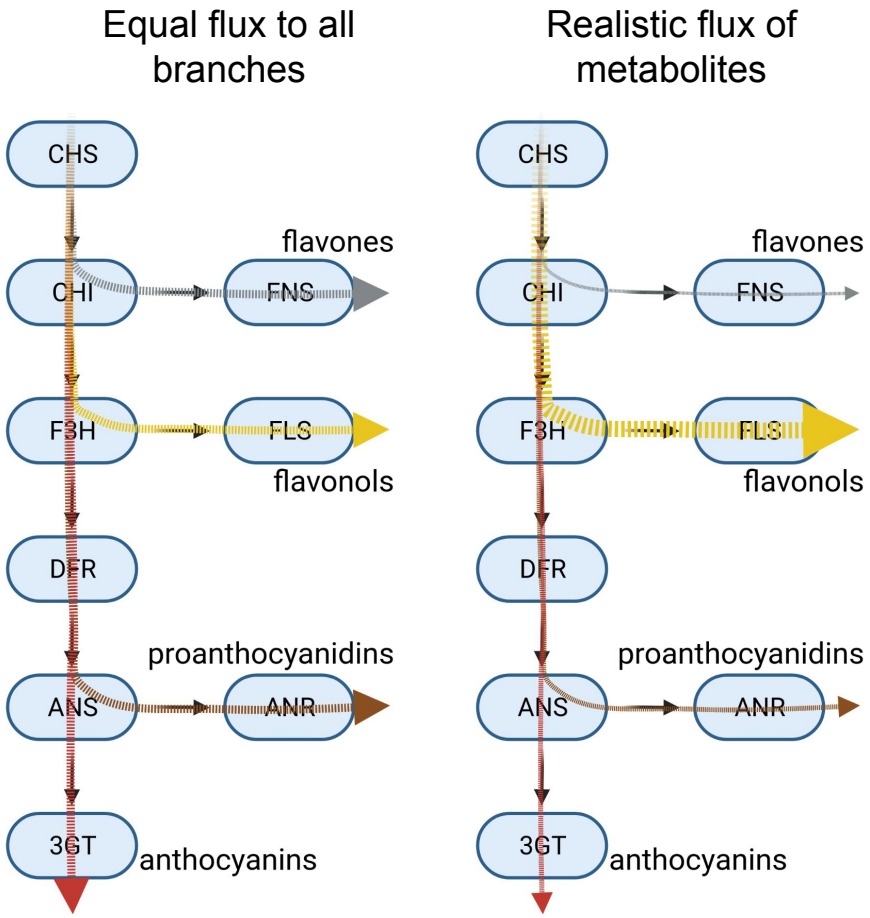
What is the purpose of metabolic models?

- Understand (complex) biological systems
  - Example: flux of metabolites into different branches of flavonoid biosynthesis
  - Example: diseases involving multiple factors
- Identification of targets for engineering
  - Example: find bottleneck in the flavonol biosynthesis
- Prediction of behaviour of biological systems
  - Example: what is the consequence of a FLAVONOL SYNTHASE (*FLS*) knock-out



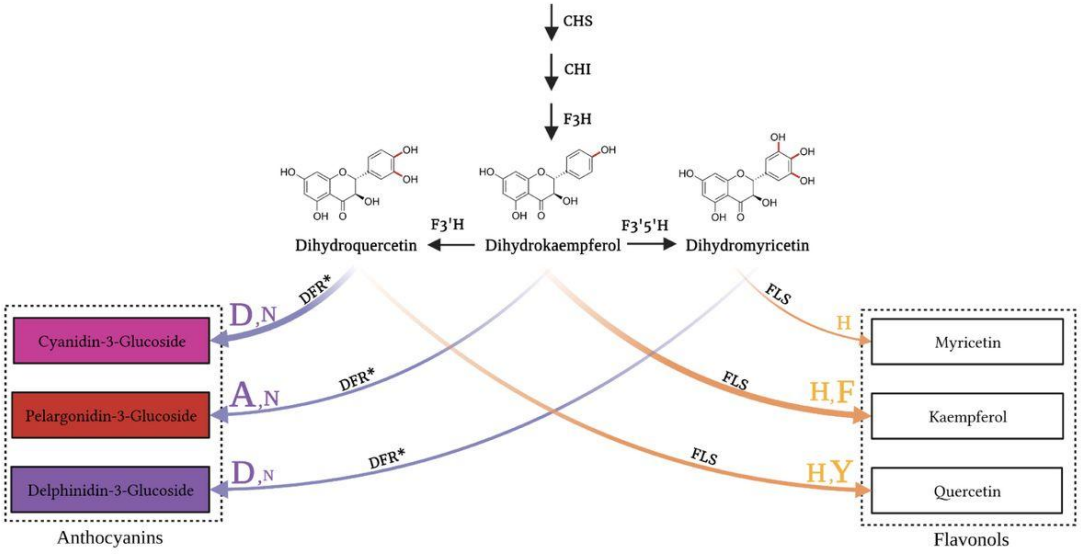
Competing branches

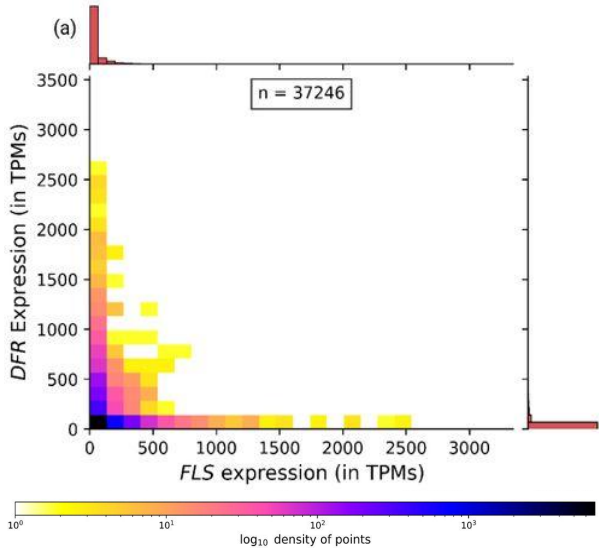
- Different branches of a pathway can compete for substrate
- Activity of different branches can vary between conditions/tissues
- Metabolic modeling can be applied to optimize flux through pathway



EXAMPLE: *FLS* vs. *DFR* competition

- FLS and DFR are competing for the same substrate
- Hydroxylation pattern preferences control flux into branches
- Gene expression differences control activity of branches





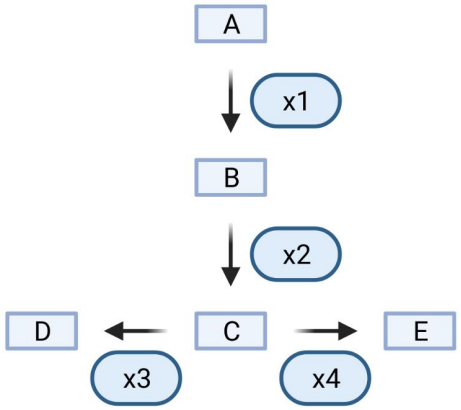
Choudhary & Pucker, 2023: 10.1101/2023.11.05.565693

What is needed for a metabolic model?

1. Knowledge about metabolic network (connections of metabolites)
2. Metabolite concentrations
3. Enzyme properties that determine reactions

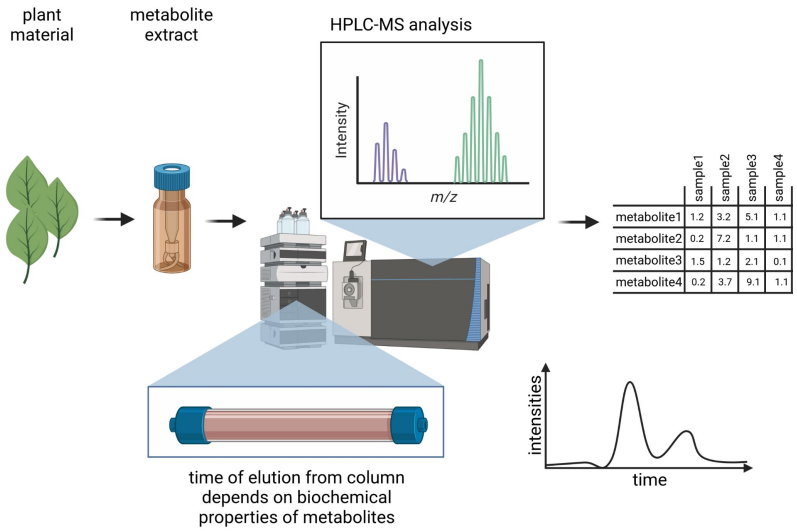
(1) Knowledge about metabolic networks

- Pathway databases
  - KEGG
  - MetaCyc
- Information from the literature
  - PubMed
  - GoogleScholar
- Knock-out experiments to understand pathway topology



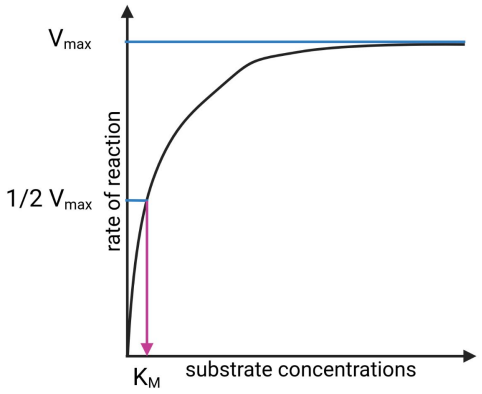
(2) Metabolite concentrations

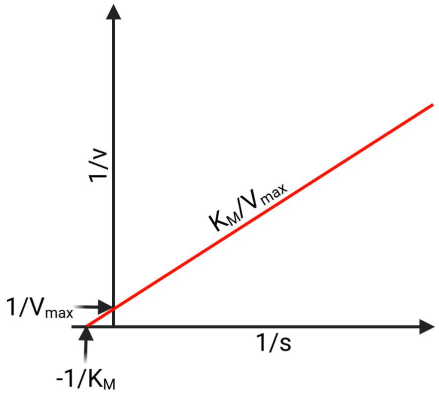
- Metabolite concentrations can be taken from the literature
- HPLC allows to quantify metabolite concentrations in a sample
- Simulation of a concentration range if no information are available

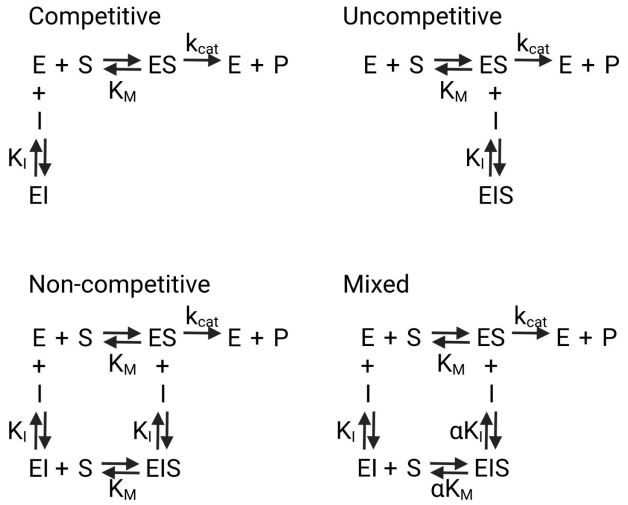


(3) Enzyme properties

- K_M = substrate affinity
- V_{max} = reaction speed
- K_I = affinity for inhibitor
- enzyme abundance ($[E]_T$) = transcription, transcript stability, translation efficiency, protein stability
- $k_{cat} = V_{max} / [E]_T$







Summary of data sources

Database/ Resource	Scope				
	Enzymes	Genes	Reactions	Pathways	Metabolites
KEGG	x	x	x	x	x
BioCyc	x	x	x	x	x
MetaCyc	x		x	x	x
ENZYME	x		x		x
BRENDA	x		x		x
PubMed	x	x	x	x	x
Google Scholar	x	x	x	x	x

The language of systems biology

- SBML = Systems Biology Markup Language
- Similar to HTML, but for biology
- Formal description of biological processes

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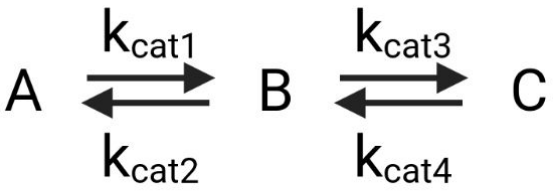
</sbml>
```

Novere, 2006: 10.1186/1471-2202-7-S1-S11

Modeling metabolic pathways

- Different methods for modeling metabolic networks:
  - Ordinary differential equation (ODE) systems
    - Each compound concentration in the system is described by a differential equation
    - System needs to be solved to understand a pathway
  - Petri nets
    - System with token that are passed through the system
    - Metabolic reactions are represented by transitions
    - Named after Carl Adam Petri

Differential equation system



$$\frac{d[A]}{dt} = \frac{k_{cat2} * [B]}{K_{M2} + [B]} - \frac{k_{cat1} * [A]}{K_{M1} + [A]}$$

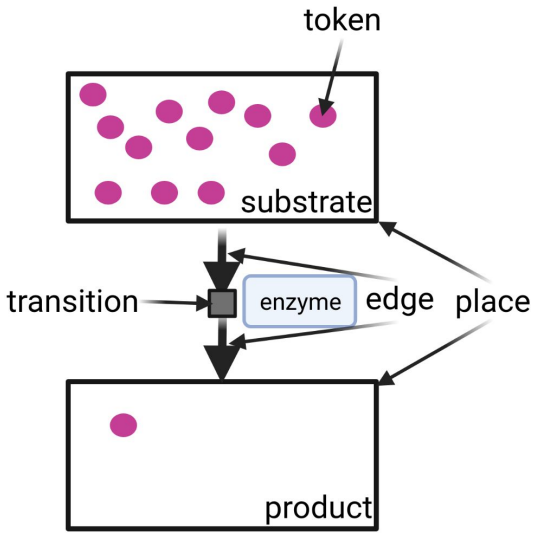
$$\frac{d[B]}{dt} = \frac{k_{cat1} * [A]}{K_{M1} + [A]} + \frac{k_{cat4} * [C]}{K_{M4} + [C]} - \frac{k_{cat2} * [B]}{K_{M2} + [B]} - \frac{k_{cat3} * [B]}{K_{M3} + [B]}$$

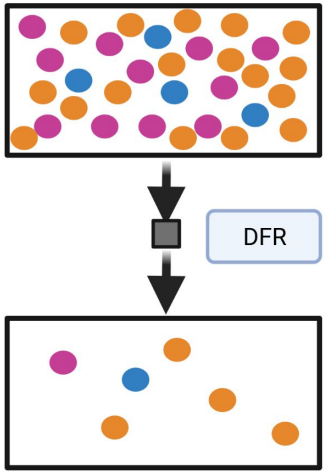
$$\frac{d[C]}{dt} = \frac{k_{cat3} * [B]}{K_{M3} + [B]} - \frac{k_{cat4} * [C]}{K_{M4} + [C]}$$

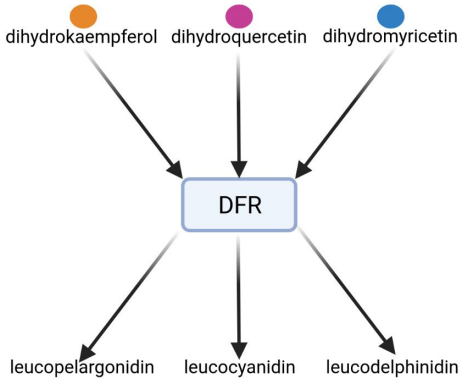
Mogilner et al., 2006: 10.1016/j.devcel.2006.08.004

Petri nets

- Metabolites are represented by transitions
- Reactions are represented by edges
- Tokens move along the edges between transitions
- Colored tokens can be used to represent different substrates of the same enzyme





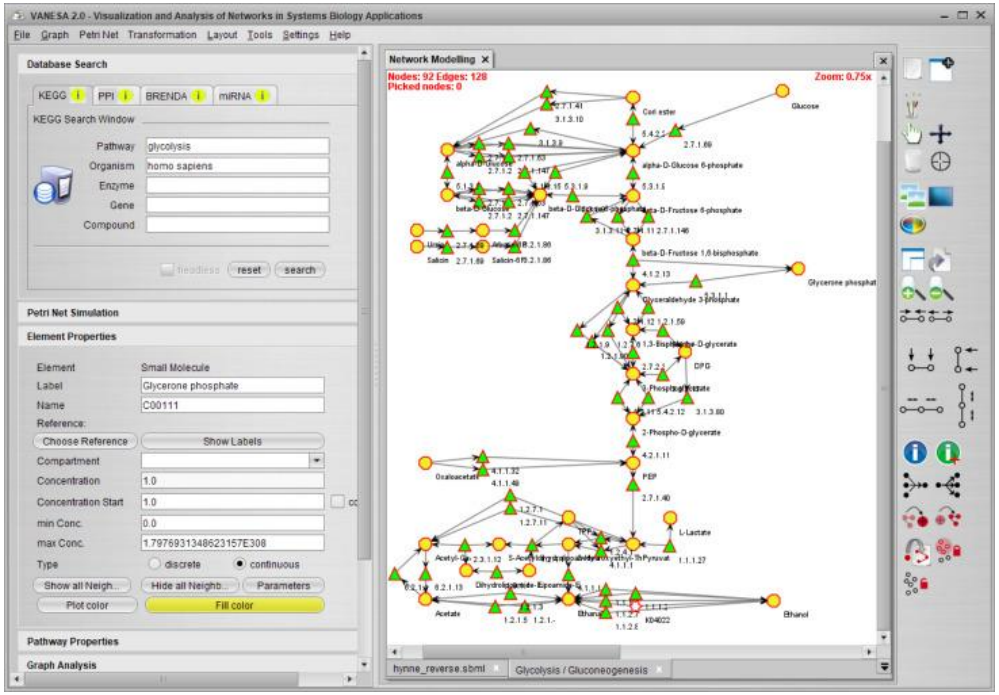


Tools

- VANESA: Petri net-based tool for modeling
- CellDesigner: differential equation system-based modeling
- Cytoscape: visualization of systems biology data sets
- bio.tools: overview of additional tools (https://bio.tools/)

VANESA (Petri nets)

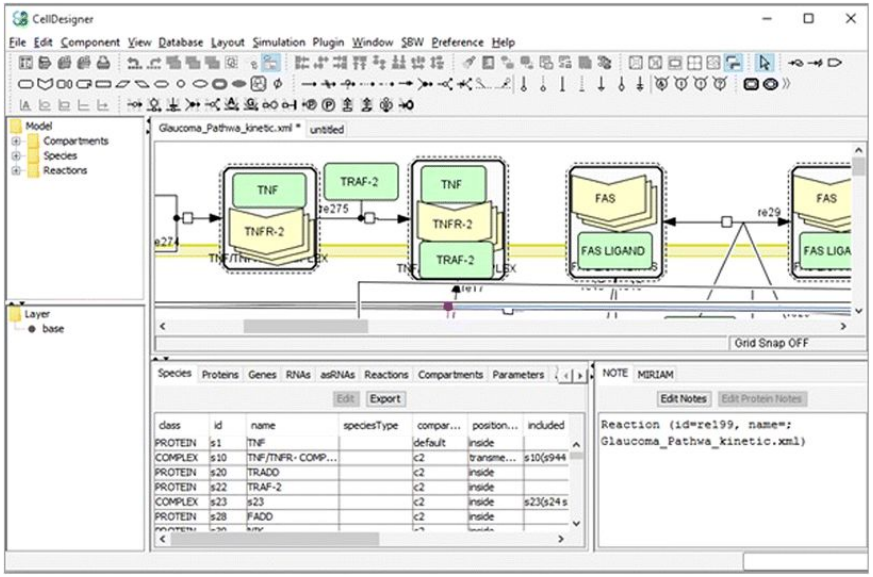
- Open source tool for metabolic simulation based on petri nets
- Graphical user interface for construction of model
- Visualization of model and results
- Modeling based on input values



https://cbrinkrolf.github.io/VANESA/
Brinkrolf et al., 2014: biecoll-jib-2014-239
Brinkrolf et al., 2018: 10.1515/jib-2018-0018
Brinkrolf et al., 2021: 10.1016/j.biosystems.2021.104531

CellDesigner

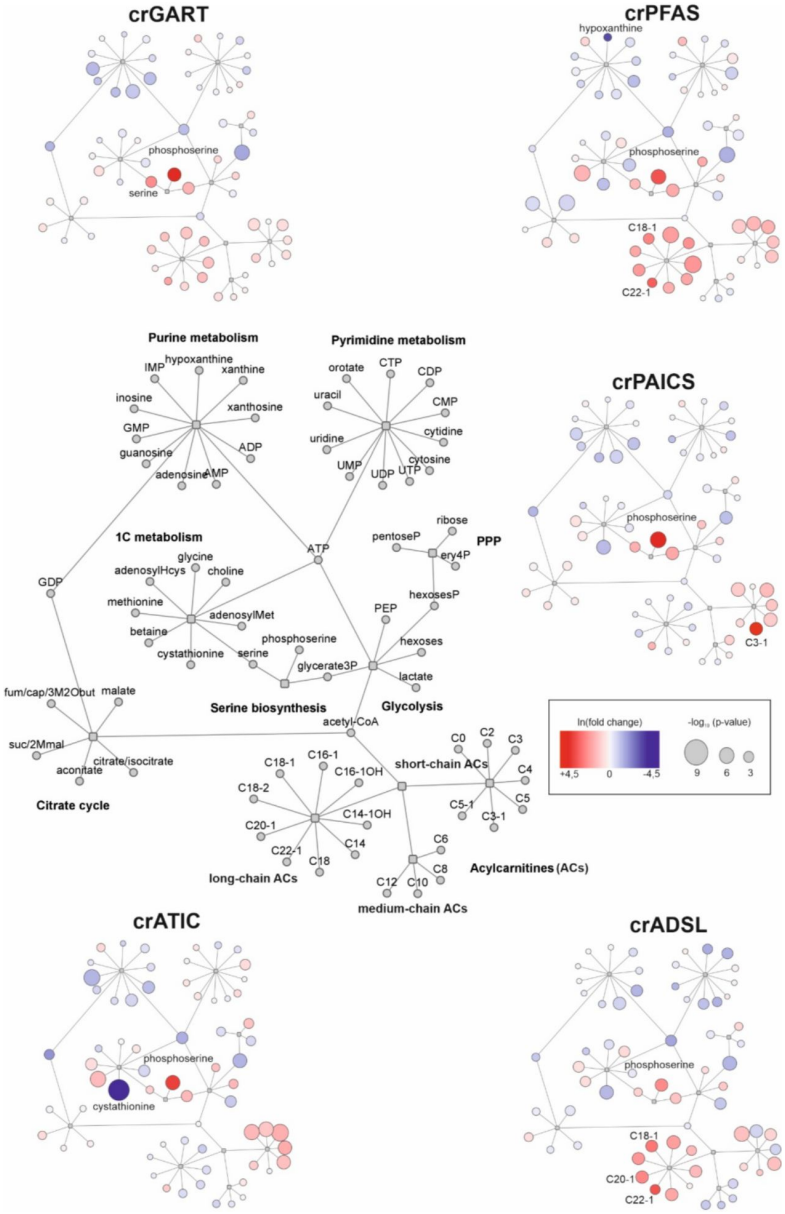
- Software for the modeling of biochemical networks
- Graphical user interface facilitates use by biologists
- Available for Windows, Mac, Linux: https://www.celldesigner.org/
- SBML files are visualized and modified



Funahashi et al., 2003: 10.1016/S1478-5382(03)02370-9

Cytoscape

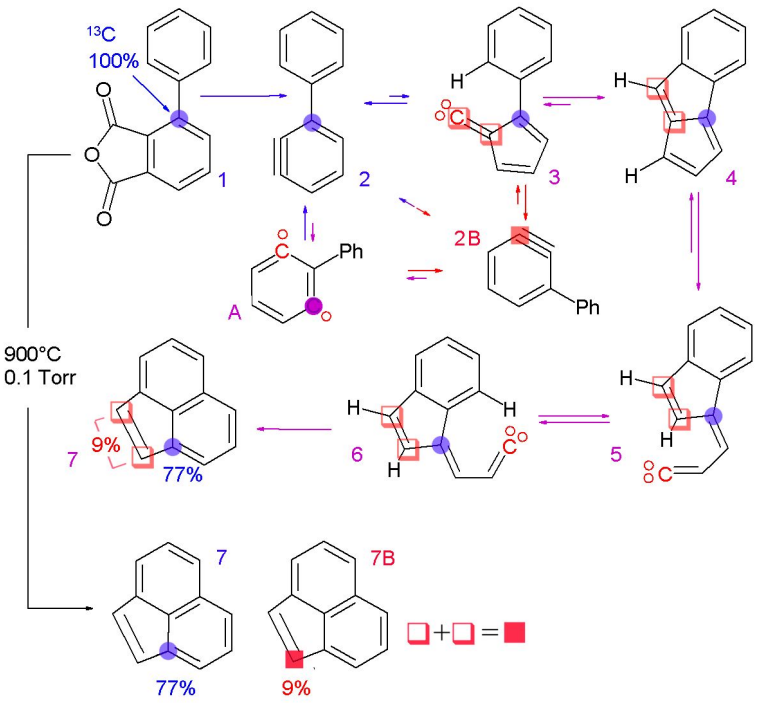
- Open source tool for network data integration, analysis, and visualization
- Graphical user interface makes application easy
- Co-expression networks can be visualized
- Transcriptional/metabolic up- and down-regulation can be displayed



Shannon et al., 2003: 10.1101/gr.1239303
Madrova et al., 2022: 10.3390/metabo12030241

Data sources: MetaboLights, Isotope labeling

- MetaboLights provides details about detected metabolites
- Isotope labeling of substrates can reveal reaction mechanisms
- Flux into different pathways can be measured based on isotope distribution



https://en.wikipedia.org/wiki/Isotopic_labeling#/media/File:Benzyneconversioncolouredlabels.png

Summary

- Enzyme kinetics
- Metabolic networks
- Systems biology / modeling

Time for questions!

Questions

1. What are important groups of specialized metabolites in plants?
2. What is the influence of enzymes on reactions?
3. What are the objectives of metabolic modeling?
4. Which tools are available to visualize metabolic networks?
5. Which information is required to build a metabolic model?
6. Which enzyme properties are important in the context of metabolic modeling?
7. You observe a red pigment in a novel plant species. How do you identify what this pigment is?