Computational Support for Metabolomics: Databases, Analysis, and Visualization

Pedro Mendes

http://www.vbi.vt.edu/~mendes

Scope

Have data, want knowledge

- Definitions
- Database issues
- Data analysis
- Biochemical networks for visualization

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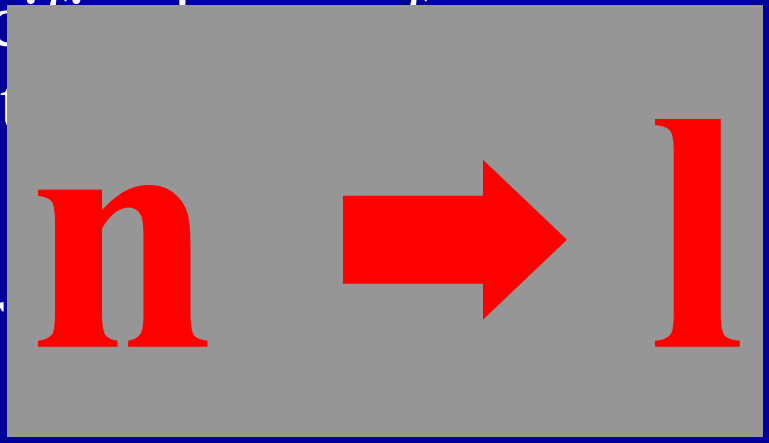
Definitions

- Metabolic profiling
  - Usually targeted to spec
    metabolites, often quant
- Metabolomics
  - Comprehensive measur
    complement of specific
- Metabolic fingerprinting
  - Collection of metabolic patterns which are used for characterization of particular states



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Definitions

- Metabonic profiling
  - Usually targeted to specific classes of metabolites, often quantitative
- Metabonomics
  - Comprehensive measurement of the metabolite complement of specific species or cell types
- Metabonic fingerprinting
  - Collection of metabolic patterns which are used for characterization of particular states

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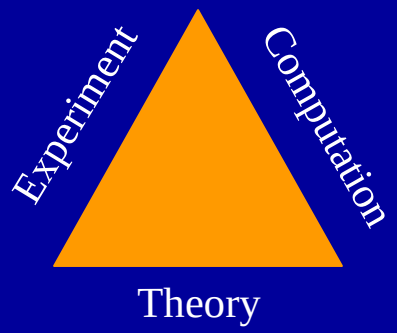
Systems Biology



- Quantitative whole-cell measurements (all mRNAs, all proteins, all sugars, etc.)
- Theoretical basis for explaining observations
- Synthesis of results through mathematical (computer) models

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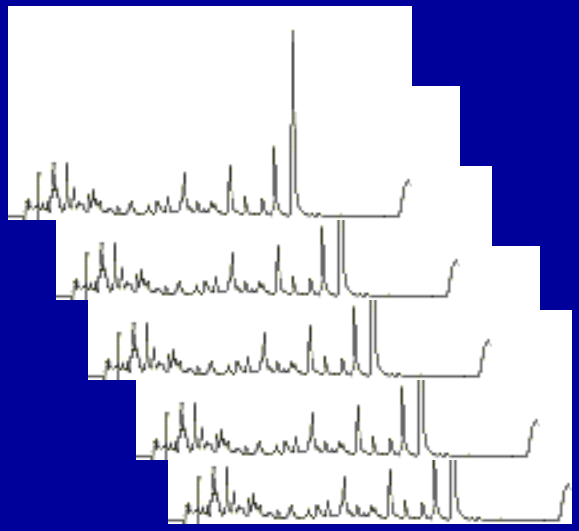
What this talk is not about…



ATP	1.277352
NADH	8.063249
NADPH	3.408609
ADP	1.554963
Orthophosphate	3.689995
CoA	8.182627
Pyrophosphate	9.292951
NH3	9.952874
S-Adenosyl-L-methionine	2.86405
AMP	9.696645
S-Adenosyl-L-homocysteine	4.708032
Pyruvate	8.779411
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Glycine	2.372851
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Succinate	4.245424
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GTP	6.890642
L-Lysine	6.186211
Glyoxylate	9.115576
L-Aspartate	9.188168
Glutathione	5.232367
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Formate	4.424477
L-Arginine	4.278822
L-Glutamine	6.067831
L-Serine	7.690047
Formaldehyde	1.427103
Thiamin diphosphate	6.424938
Alcohol	4.974474
Ascorbate	1.747568
L-Methionine	5.809962
Phosphoenolpyruvate	8.066765
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L-Phenylalanine	5.560732
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Malonyl-CoA	5.070652
Acetaldehyde	3.270844
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Sucrose	8.617488

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Rather…

data

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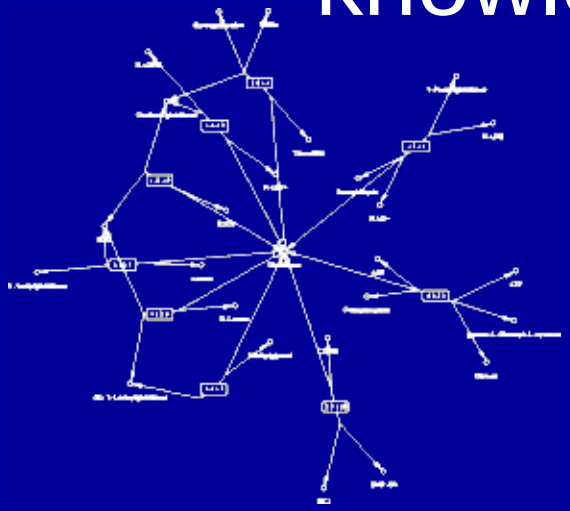
knowledge



$$\dot{A} = \frac{V_1^f \frac{S}{K_{1S}} - V_1^r \frac{A}{K_{1A}}}{1 + \frac{S}{K_{1S}} + \frac{A}{K_{1A}}} - \frac{\left(V_2^f \frac{A}{K_{2A}}\right)\left(1 - \frac{B}{S \cdot K_{2eq}}\right)\left(\frac{A}{K_{2A}} + \frac{B}{K_{2B}}\right)^{h-1}}{\left(\frac{A}{K_{2A}} + \frac{B}{K_{2B}}\right)^{h} + \frac{1 + \left(\frac{C}{K_{2C}}\right)^{h}}{1 + \alpha\left(\frac{C}{K_{2C}}\right)^{h}}}$$

$$\dot{B} = \frac{\left(V_2^f \frac{A}{K_{2A}}\right)\left(1 - \frac{B}{S \cdot K_{2eq}}\right)\left(\frac{A}{K_{2A}} + \frac{B}{K_{2B}}\right)^{h-1}}{\left(\frac{A}{K_{2A}} + \frac{B}{K_{2B}}\right)^{h} + \frac{1 + \left(\frac{C}{K_{2C}}\right)^{h}}{1 + \alpha\left(\frac{C}{K_{2C}}\right)^{h}}} - \frac{V_3^f \frac{B}{K_{3B}} - V_3^r \frac{C}{K_{3C}}}{1 + \frac{B}{K_{3B}} + \frac{C}{K_{3C}}}$$

$$\dot{C} = \frac{V_3^f \frac{B}{K_{3B}} - V_3^r \frac{C}{K_{3C}}}{1 + \frac{B}{K_{3B}} + \frac{C}{K_{3C}}} - \frac{V_4^f \frac{C}{K_{4C}} - V_4^r \frac{P}{K_{4P}}}{1 + \frac{C}{K_{4C}} + \frac{P}{K_{4P}}}$$

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Databases for Metabolomics

- Laboratory metabolic profile databases
- Species-specific metabolic profile databases
- Generic metabolic profile databases
- Qualitative metabolome databases
- Reference biochemical databases

Mendes (2002) “Emerging Bioinformatics for the Metabolome”. *Briefings in Bioinformatics* **3**, 134-145

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Lab metabolic profile DB

- Act as primary data sources
- Store data about all experimental details (i.e. metadata)
- Narrow in topic, deep in information content
- Should export data in standard formats to allow for interoperability with other DBs

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Species metabolic profile DB

- Collect all experiments published for one species
- Collect data for other types of experiments too (e.g. sequencing, microarrays)
- These are the primary point of entry for species-related information
- Examples:
  - TAIR
  - MaizeDB

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Generic metabolic profile DB

- Collects all published metabolic profiles
- Profiles must allow comparisons
- Due to size constraints, will not store much raw data, but will reference where it is (lab DB, literature)
- Few of these, preferably all mirroring the same data

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Qualitative metabolome DBs

- List all metabolites observed
- The data could be for a single organism or organized taxonomically
- This would be the equivalent to gene databases, as the list is of all potential metabolites that could be seen in an organism

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Reference biochemical DBs

- Contain reference information about biochemistry
- Many exist already: KEGG, WIT, EcoCyc, PathDB, PFMM, UM-BBD, BRENDA, SoyBase, etc.
- KEGG is perhaps the most popular, due to its nice pathway diagrams

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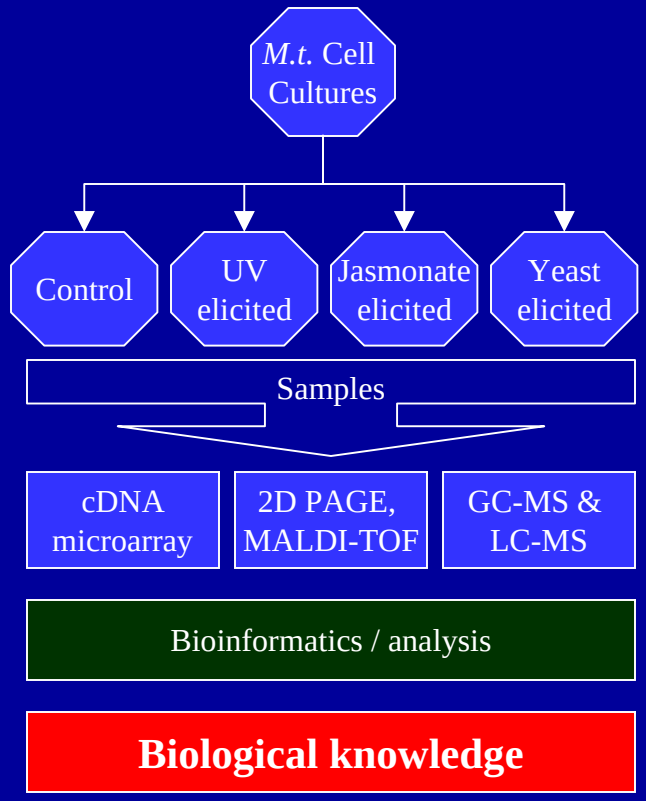
An integrated approach to functional genomics and bioinformatics in a model legume

Mendes, Dixon, Sumner, May, Weller, Smith

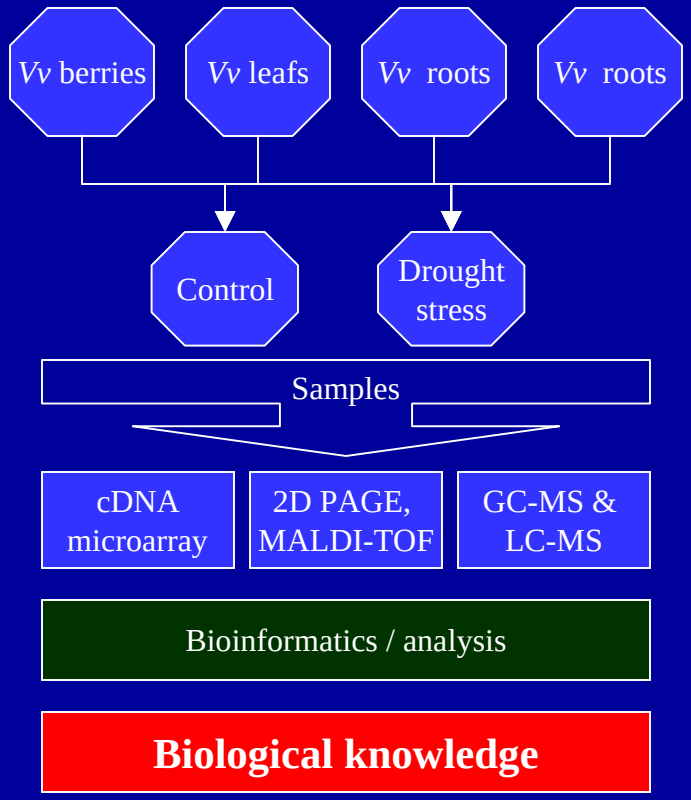


- Focus on the isoflavonoid pathway
- The data set contains information relevant to other processes too
- Quantification is important
- The ultimate aim is to derive causal relationships

http://medicago.vbi.vt.edu

Integrative Functional Genomic Resource Development in *Vitis vinifera*: Abiotic Stress and Wine Quality.

Cramer, Cushman, Mendes, Schooley



- Focus on flavor and stress compounds
- The data set contains information relevant to other processes too
- Quantification is important
- The ultimate aim is to derive causal relationships

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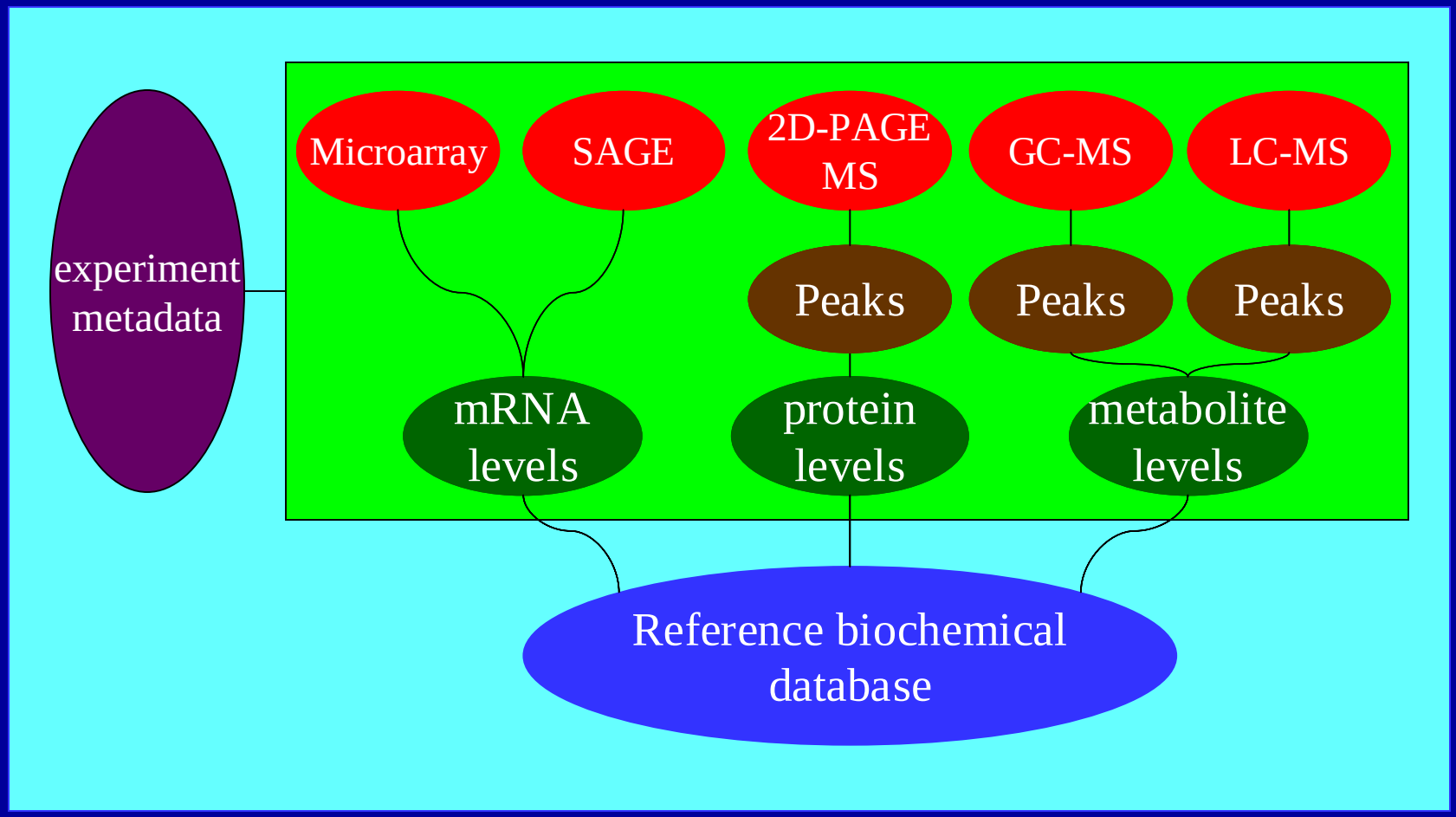
DOME, a Database for Functional Genomics



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Metadata

- To allow merging data of several types, it is essential that the database captures the data about the experiments.
- In DOME, experimental designs are represented in a finely structured classification.
- The database is general enough that it can store different kinds of experiments, such as time series or steady state experiments.
- The experimental metadata is what allows one to initially combine microarray, proteomic and metabolite profile samples.

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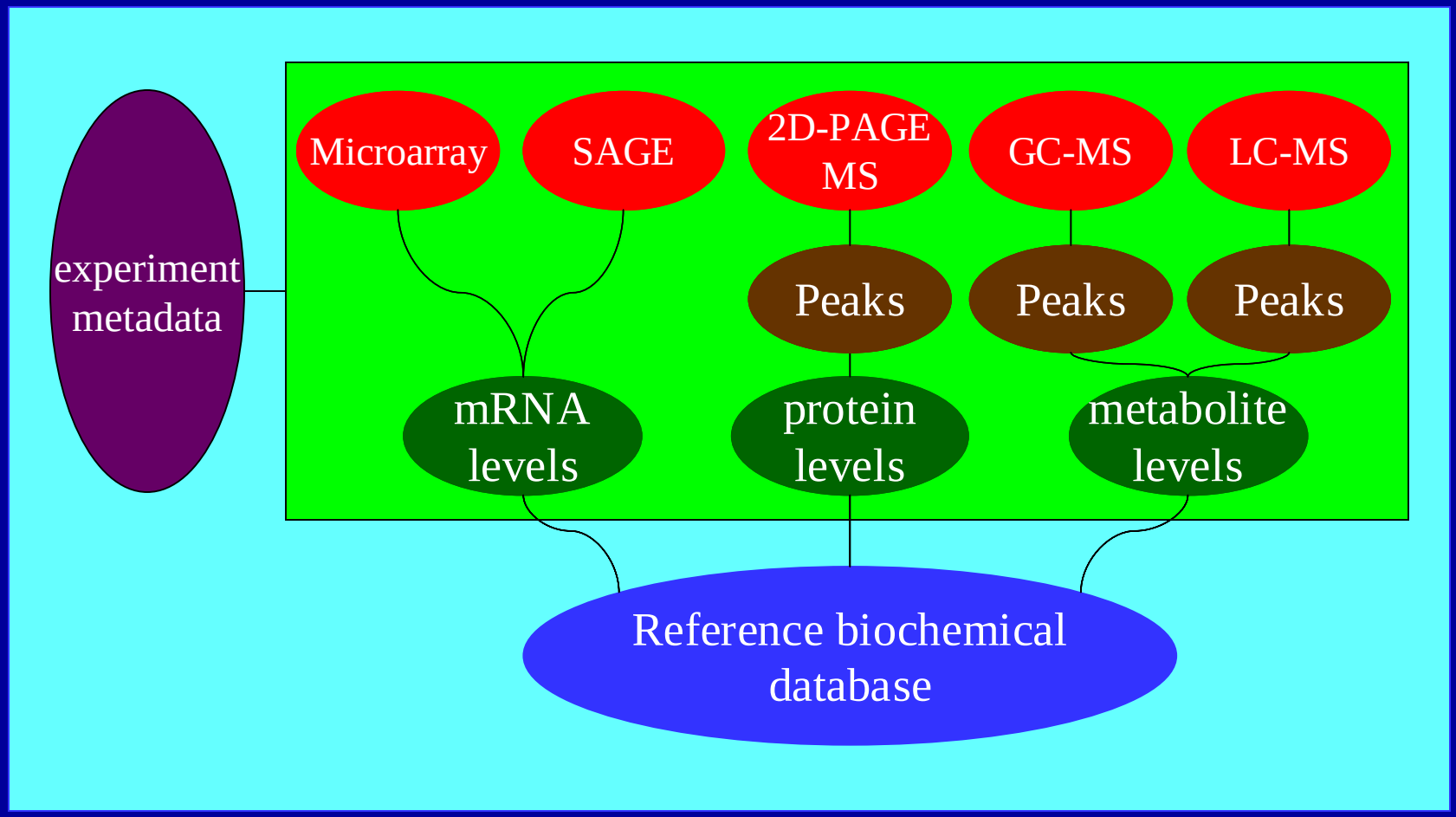
DOME, a Database for Functional Genomics



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Desired properties of reference DBs

- All metabolites should be specific
  - no "an alcohol“, “acyl-CoA”, “amino acids”, etc.
- Enzymes should be single-entities
  - include all isoenzymes, etc.
- It is preferable to have fewer data if these are more reliable!
- Facts should be substantiated:
  - References
  - Classify evidence
- None of the existing reference DBs comply with all the requirements listed here

Wittig, U. & De Beuckelaer, A. (2001), *Brief. Bioinformatics* **2**, 126-142.

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B-Net, a reference biochemical database

- To provide a reference for our metabolomic analyses and visualization
- Species specific
- Stores facts recovered from the literature
- Original metabolite data from KEGG, NIST and TAIR, reactions from EC, followed by draconian curation
- Also serves as a qualitative metabolome database

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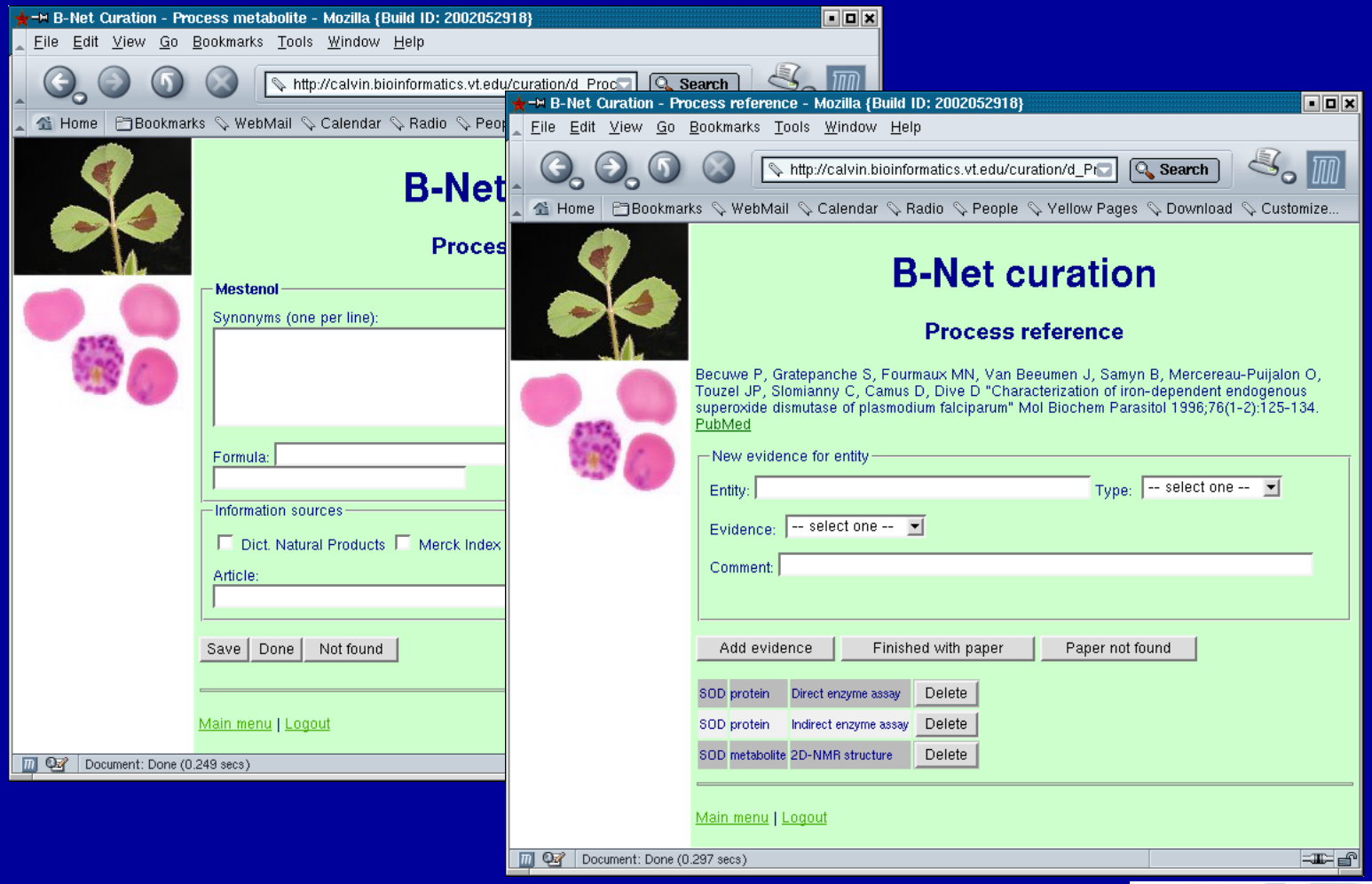
Computer-assisted curation



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Algorithms for Data Analysis

- Unsupervised methods (looking for patterns)
  - Clustering, PCA
  - Self-organizing maps
- Supervised methods (calibration)
  - Nonlinear regression
  - Feed-forward neural networks
  - Genetic algorithms
- System identification (reverse engineering)
  - Bayesian belief networks
  - Metabolic control analysis
  - Nonlinear dynamics

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Metabolic networks for visualization and data integration

- Metabolic diagrams can be used to visualize metabolomic data together with gene expression or proteomics
- Useful to display data from one sample or to compare two samples
- KEGG has nice diagrams that can be used for this purpose

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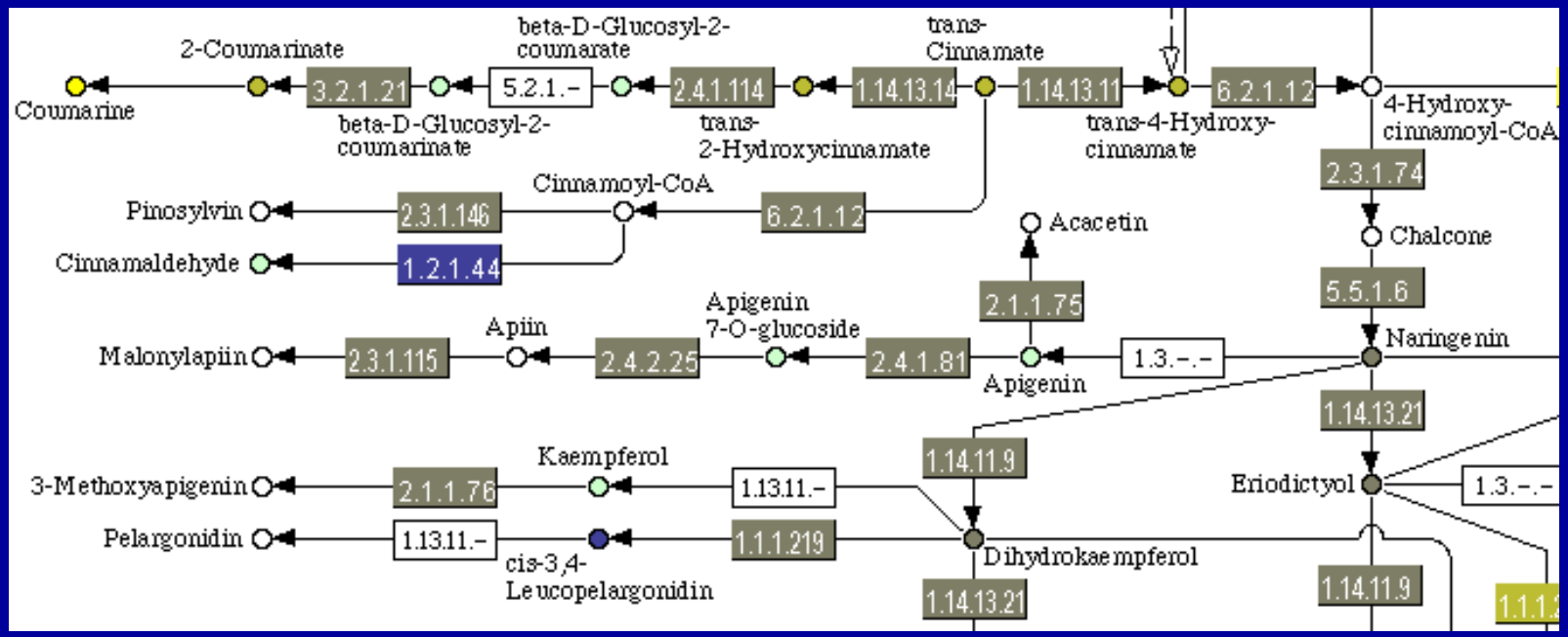
Data integration through metabolic networks



- KEGG maps do not include all side reactions
- Not all biochemistry can be reduced to a template

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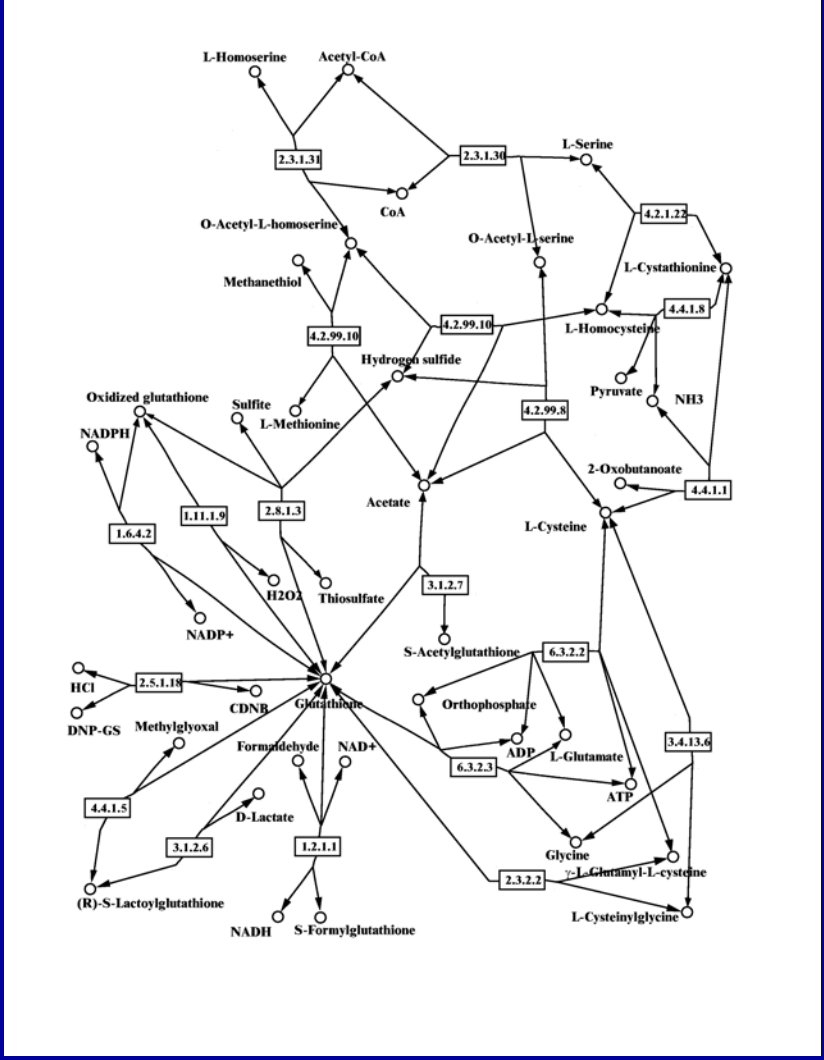
Known glutathione neighborhood in *S. cerevisiae*



- All metabolites but H2O are included
- Each metabolite appears only once
- Reactions are included if:
  - Reaction observed directly
  - Gene for enzyme detected in genome

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Small World Inside Metabolic Networks

Rank by degree	connectivity	Rank by distance	Importance no.
Glutamate	51	Glutamate	2.46
Pyruvate	29	Pyruvate	2.59
CoA	29	CoA	2.69
2-oxoglutarate	27	Glutamine	2.77
Glutamine	22	Acetyl CoA	2.86
Aspartate	20	Oxoisovalerate	2.88
Acetyl CoA	17	Aspartate	2.91
Phosphoribosyl PP	16	2-Oxoglutarate	2.99
Tetrahydrofolate	15	Phosphoribosyl PP	3.10
Succinate	14	Anthranilate	3.10
3-Phosphoglycerate	13	Chorismate	3.13
Serine	13	Valine	3.14
Oxoisovalerate	12	3-Phosphoglycerate	3.15

- Wagner & Fell (2001), *Proc. R. Soc. Lond. B* **268**, 1803-1810.

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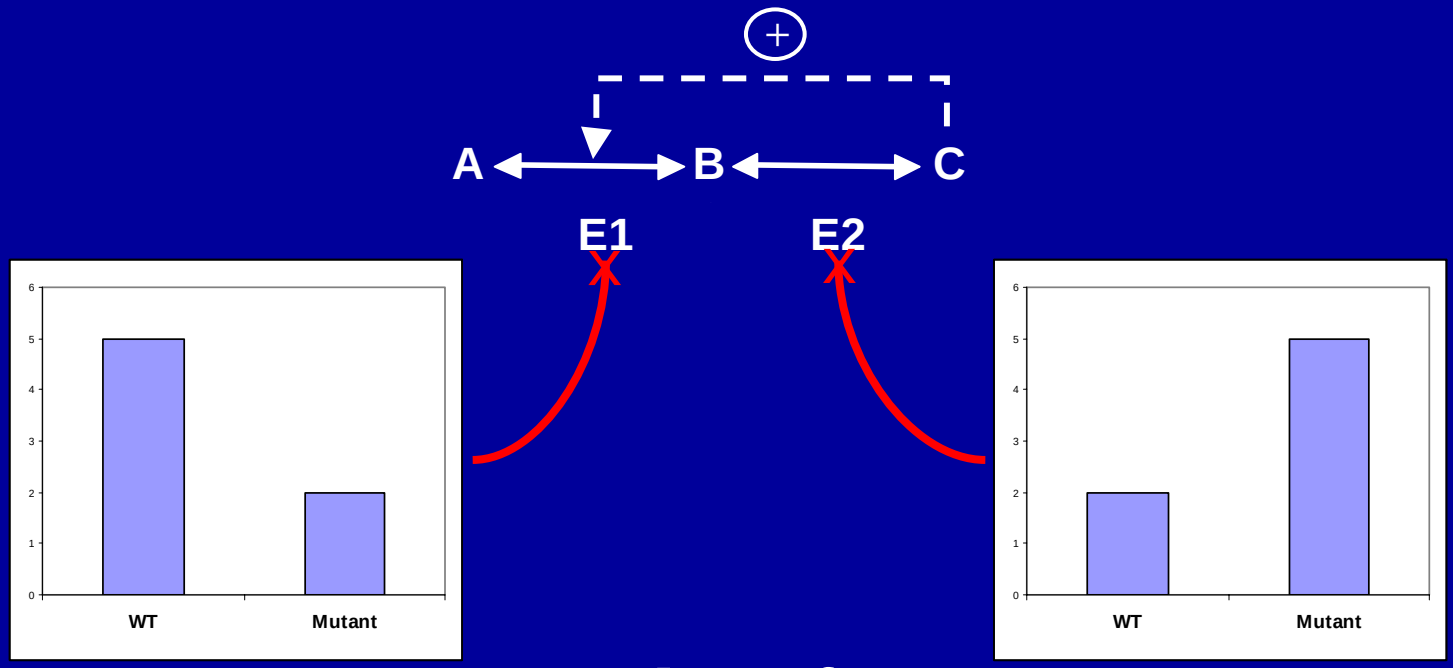
Beware of simplistic ad-hoc interpretations…



But what if…?

- Chance, B., Holmes, W., Higgins, J. & Connelly, C. M. (1958) Localization of interaction sites in multi-component transfer systems: theorems derived from analogues., *Nature. 162*, 1190-1193.
- Heinrich, R. & Rapoport, T. A. (1974) A linear steady-state treatment of enzymatic chains. Critique of the crossover theorem and a general procedure to identify interaction sites with an effector., *Eur. J. Biochem. 42*, 97-105.

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Metabolite neighborhoods

- Metabolic pathways are an artificial concept derived from historical developments
- Biochemical network is highly interconnected
- Difficult to visualize the whole network
- Metabolite neighborhood is the set of reactions that are connected to a center metabolite, plus all other metabolites that are part of those reactions

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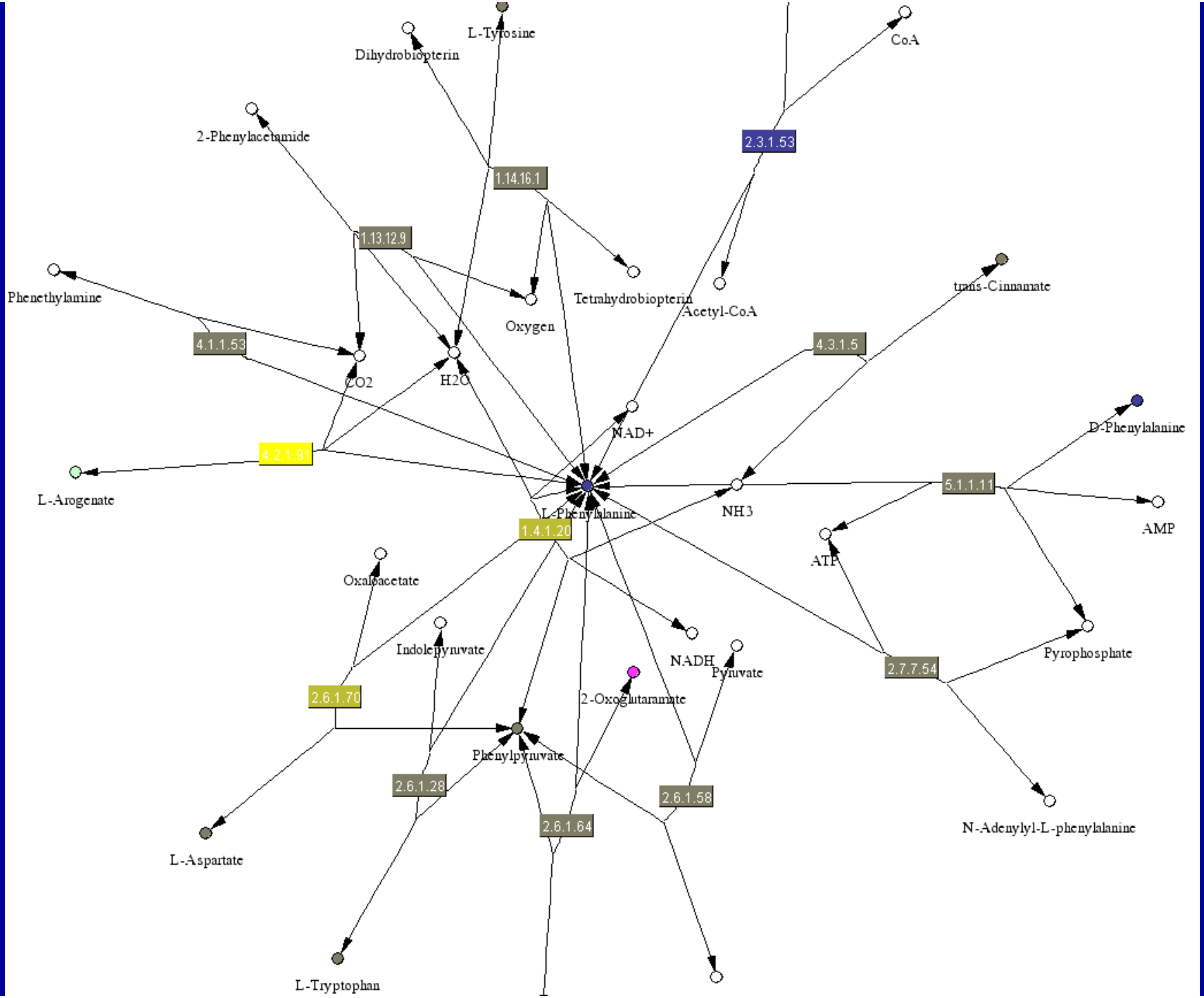
L-Tyrosine
Dihydrobiopterin
CoA
2-Phenylacetamide
2.3.1.53
1.14.16.1
1.13.12.9
Phenethylamine
Tetrahydrobiopterin
Acetyl-CoA
trans-Cinnamate
Oxygen
4.1.1.53
4.3.1.5
CO2
H2O
NAD+
D-Phenylalanine
4.2.1.91
L-Arogenate
L-Phenylalanine
NH3
5.1.1.11
AMP
1.4.1.20
ATP
Oxaloacetate
Indolepyruvate
NADH
Pyruvate
Pyrophosphate
2.7.7.54
2.6.1.70
2-Oxoglutaramate
Phenylpyruvate
2.6.1.28
2.6.1.58
2.6.1.64
L-Aspartate
N-Adenylyl-L-phenylalanine
L-Tryptophan

Concentration *versus* Flux

$$J_x(t) = \frac{dx}{dt} \approx \frac{x_t - x_{t-\tau}}{\tau}$$

- Flux is no more than the time derivative of concentration
- But flux is independent of concentration:

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- Mudita Singhal
- M. Kulkarni
- Liang Xu
- Jessica Caldwell
- Robin Oakes
- Fernina Taliaferro
- Anh Tran
- Olga Brazhnik
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- Rohan Luktuke

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- Johns-Hopkins: Sullivan

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