CEPLAS
Cluster of Excellence on Plant Sciences

HEINRICH HEINE
UNIVERSITÄT DÜSSELDORF

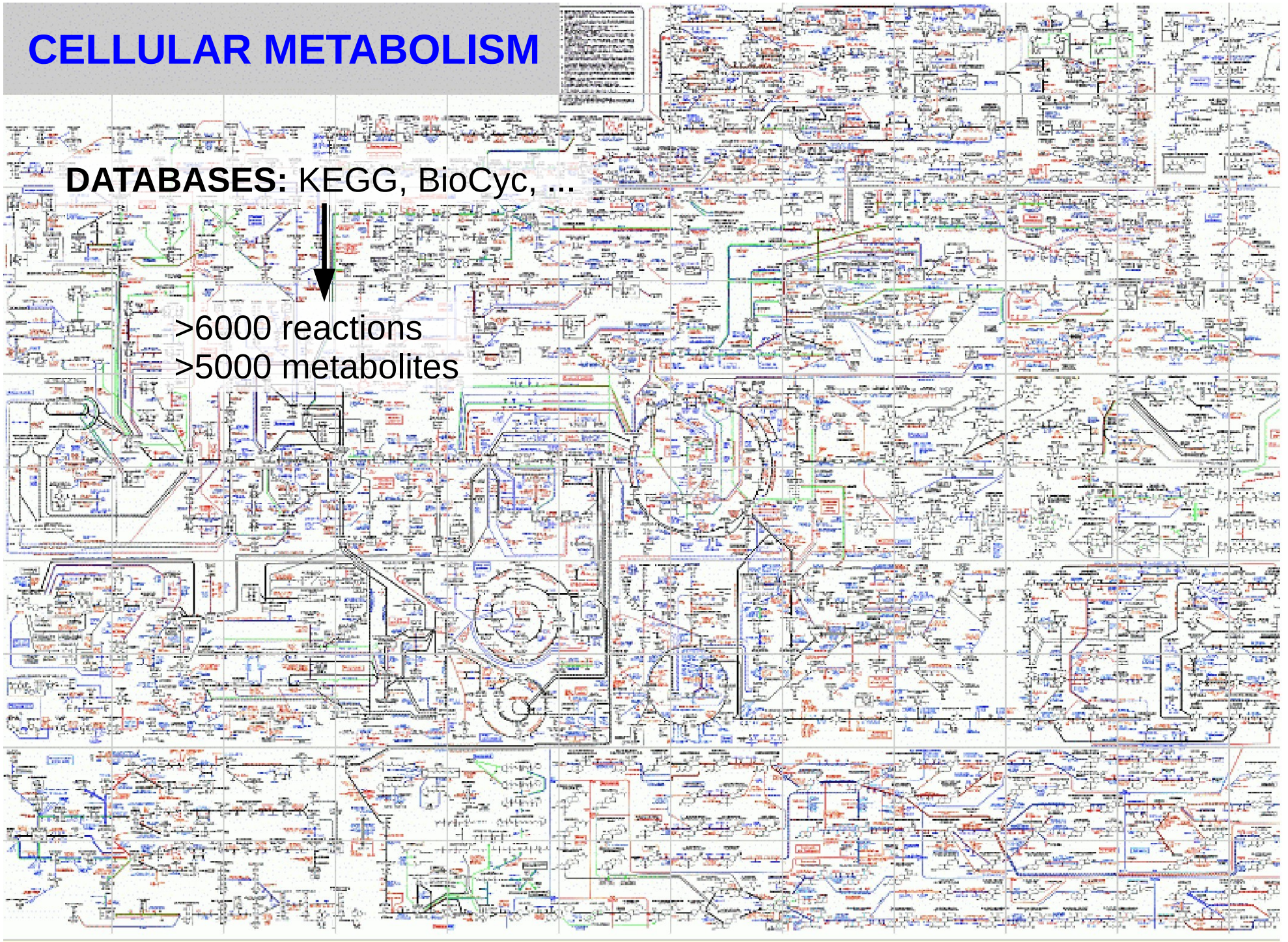
Network Expansion and Scopes

Oliver Ebenhöh

QTB Institut für Quantitative & Theoretische Biologie

17.1.2019

CELLULAR METABOLISM



DATABASES: KEGG, BioCyc, ...

>6000 reactions
>5000 metabolites

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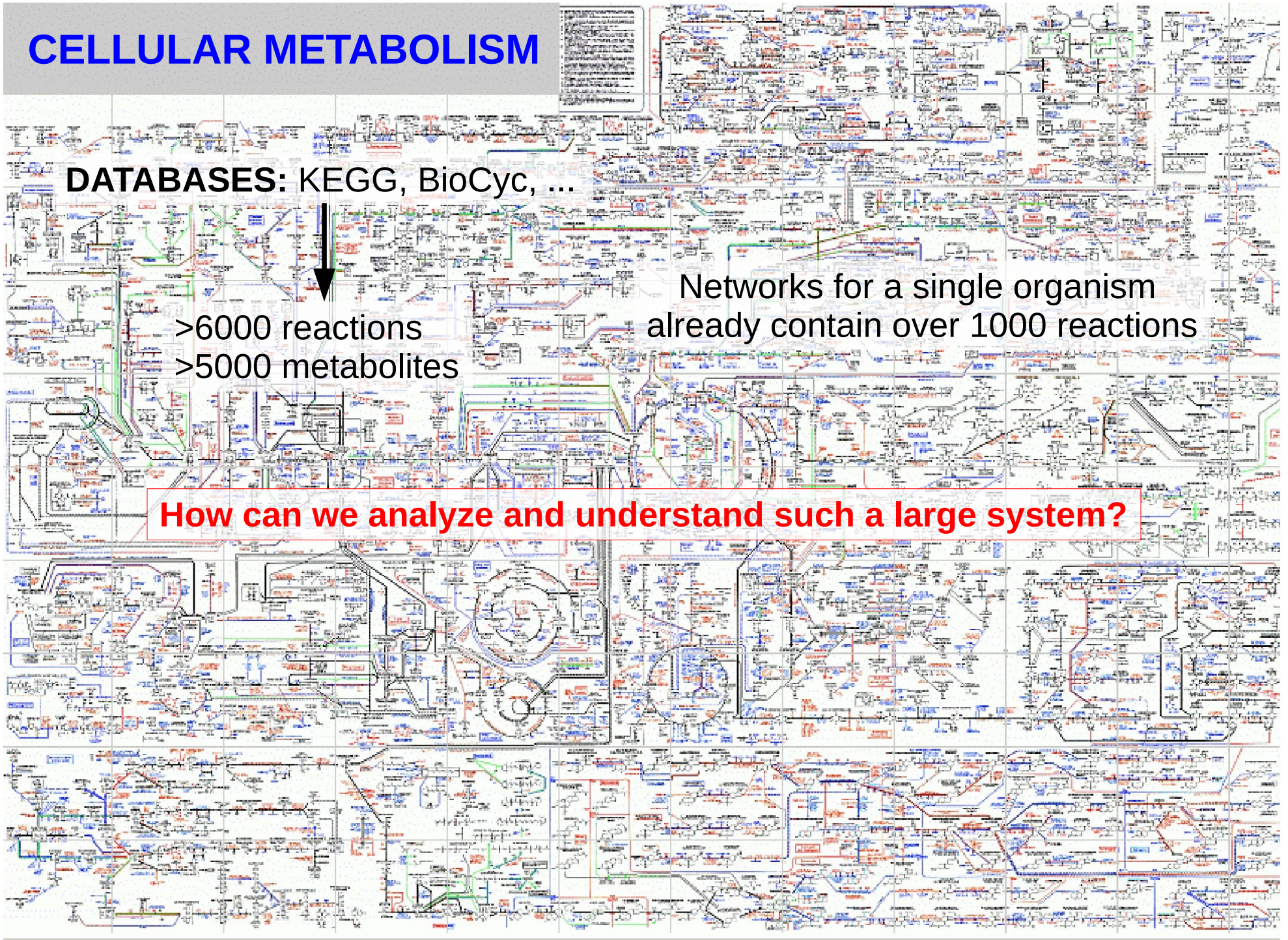

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Networks for a single organism already contain over 1000 reactions

How can we analyze and understand such a large system?

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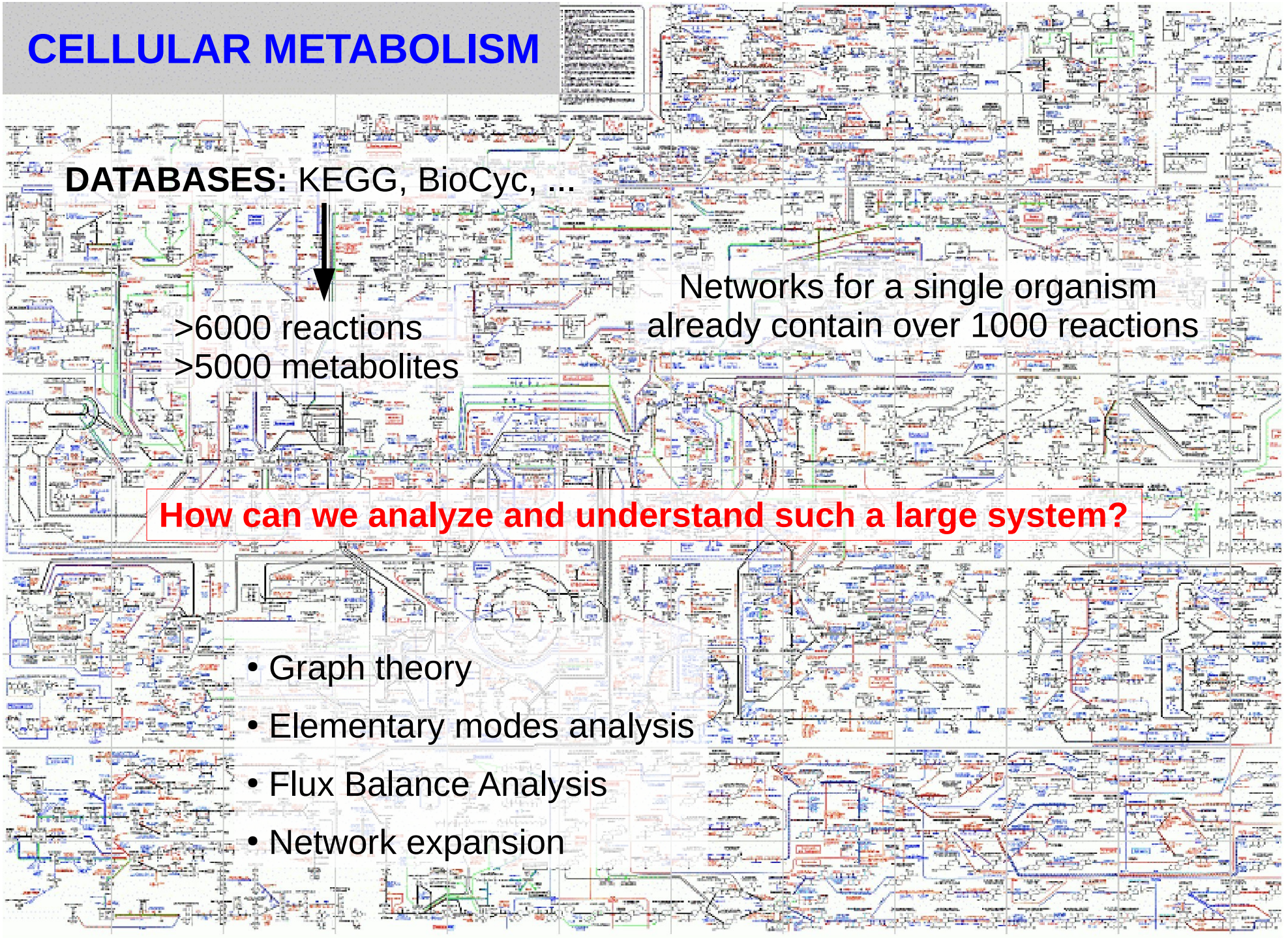

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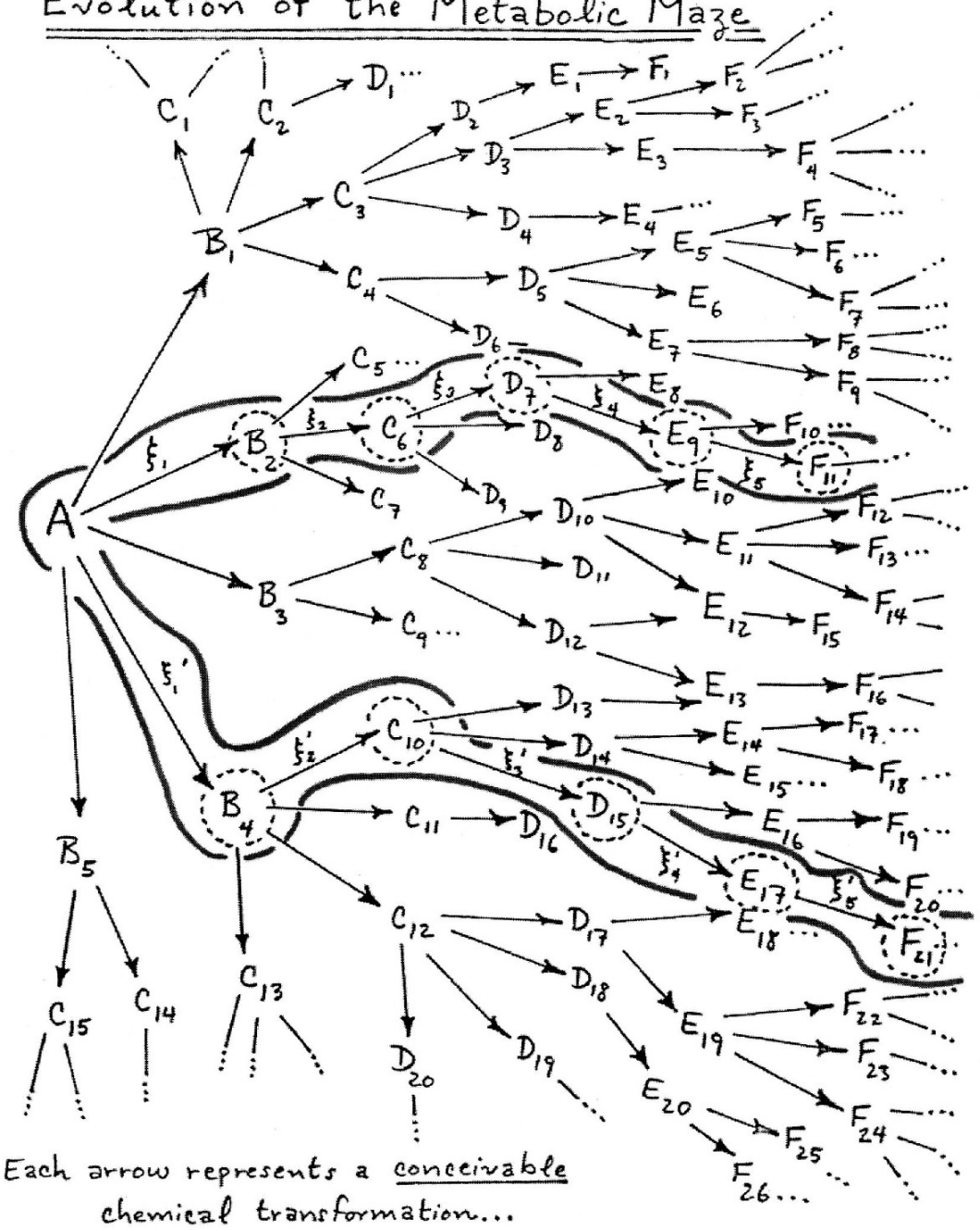
Networks for a single organism already contain over 1000 reactions

How can we analyze and understand such a large system?

- Graph theory
- Elementary modes analysis
- Flux Balance Analysis
- Network expansion

Evolution of the Metabolic Maze

Scott C. Mohr
mohr@bu.edu



Each arrow represents a conceivable chemical transformation...

Pathway Evolution

- No *fossil record* of metabolism available
- The evolutionary history must have left imprints in the present structure

Existing hypotheses on metabolic evolution

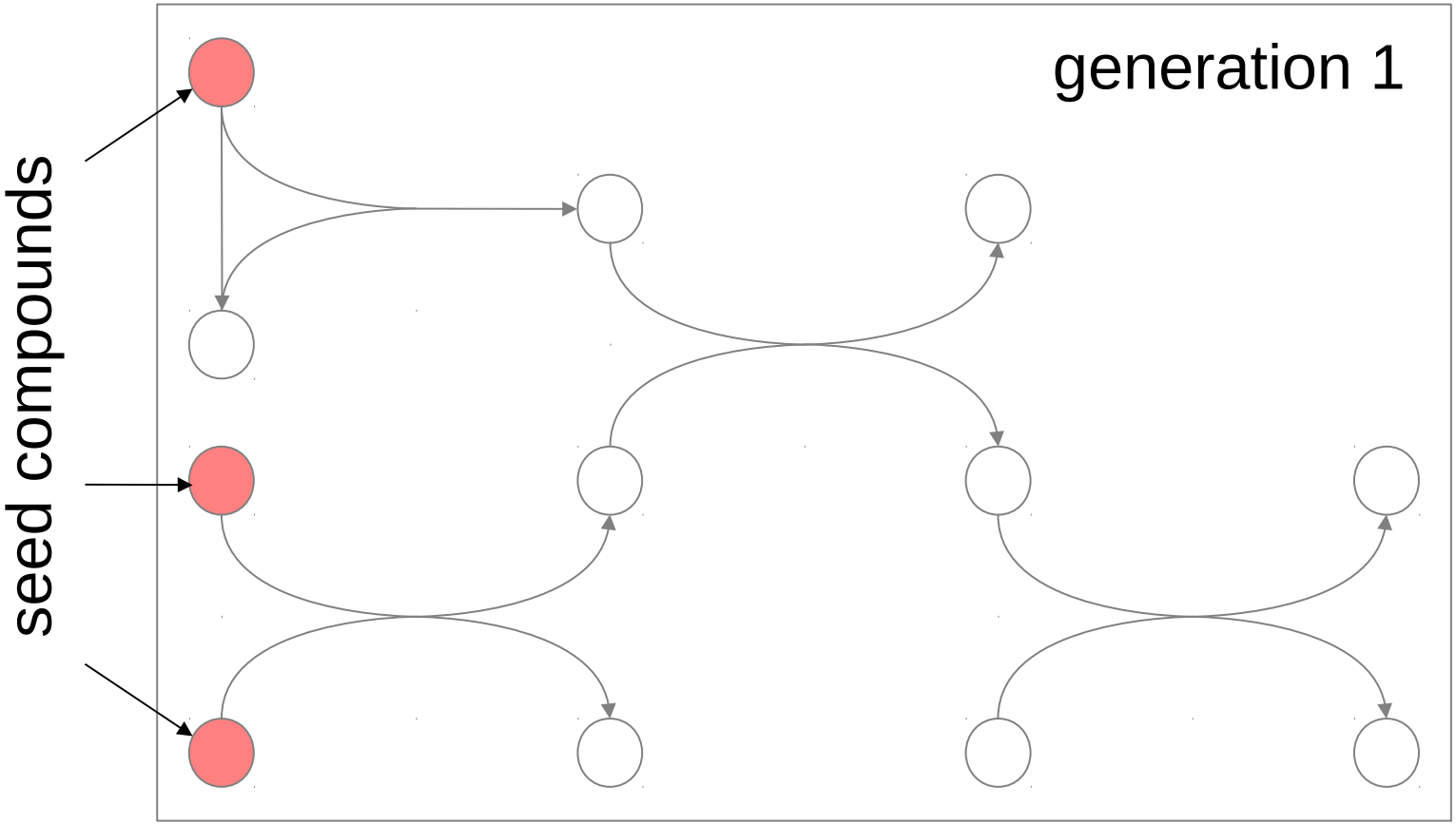
retrograde evolution

depletion of a required substrate

forward evolution

substrates in the environment trigger 'invention' of new metabolites

Concept of Network Expansion



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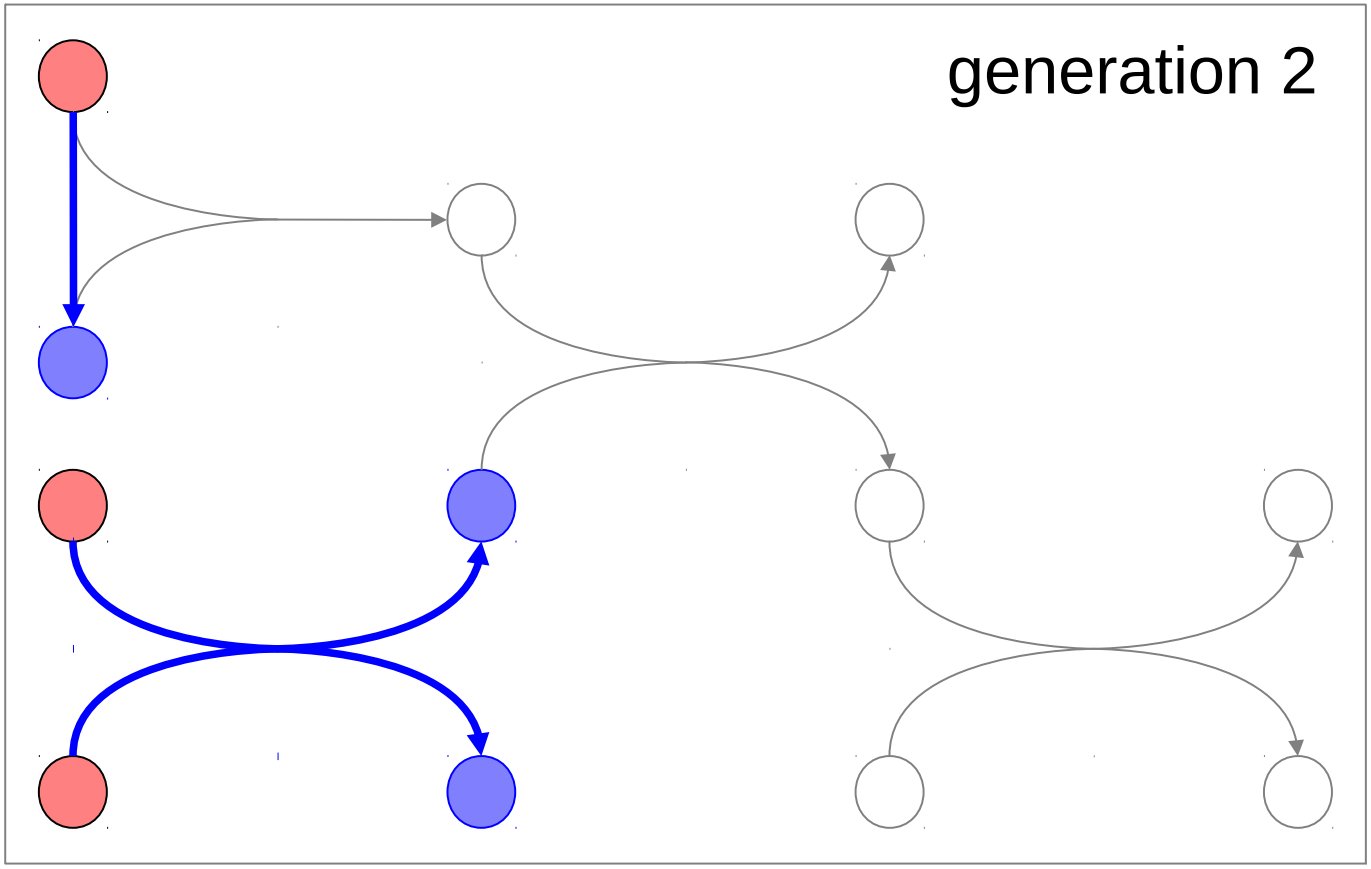

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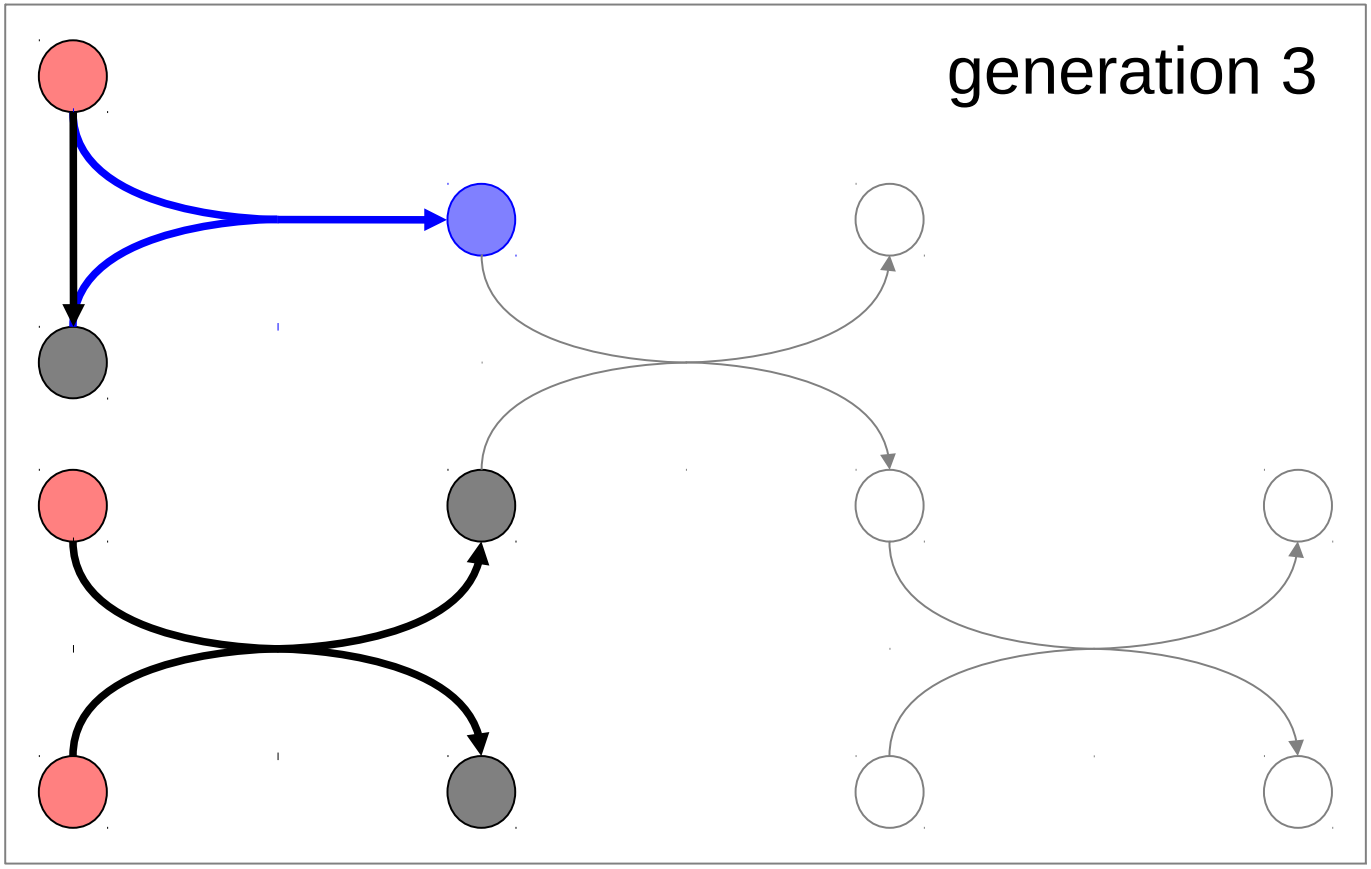

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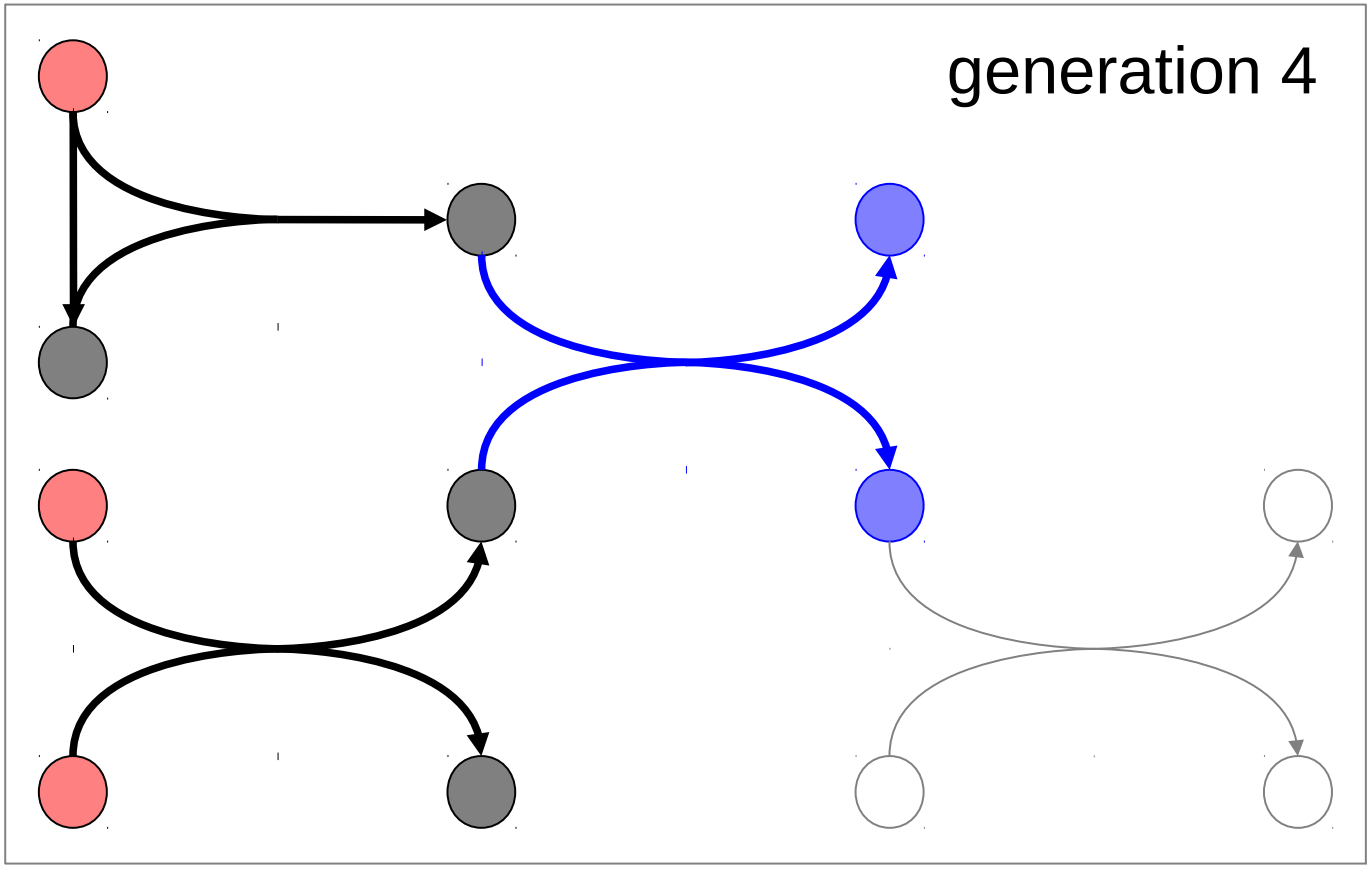

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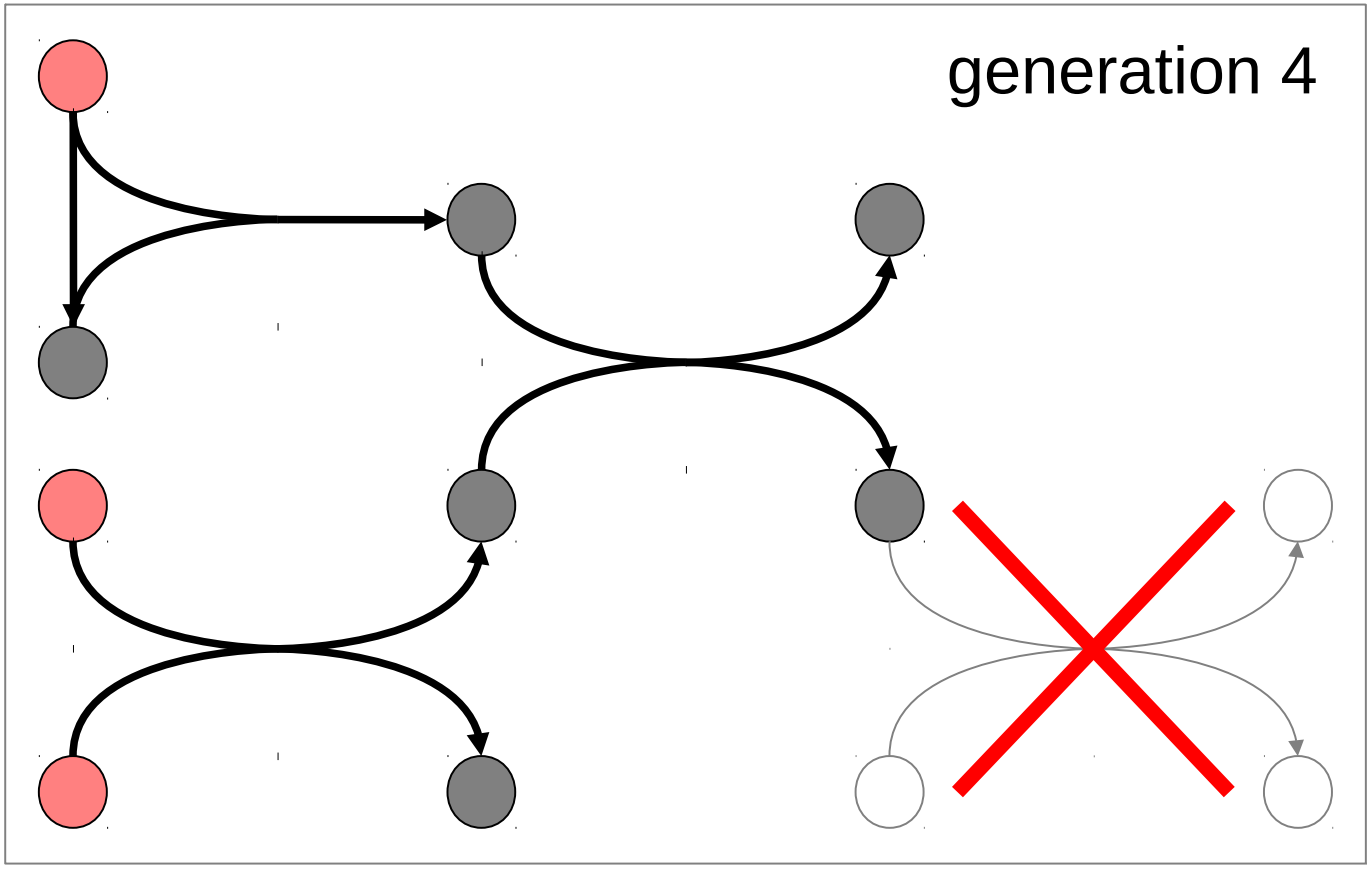
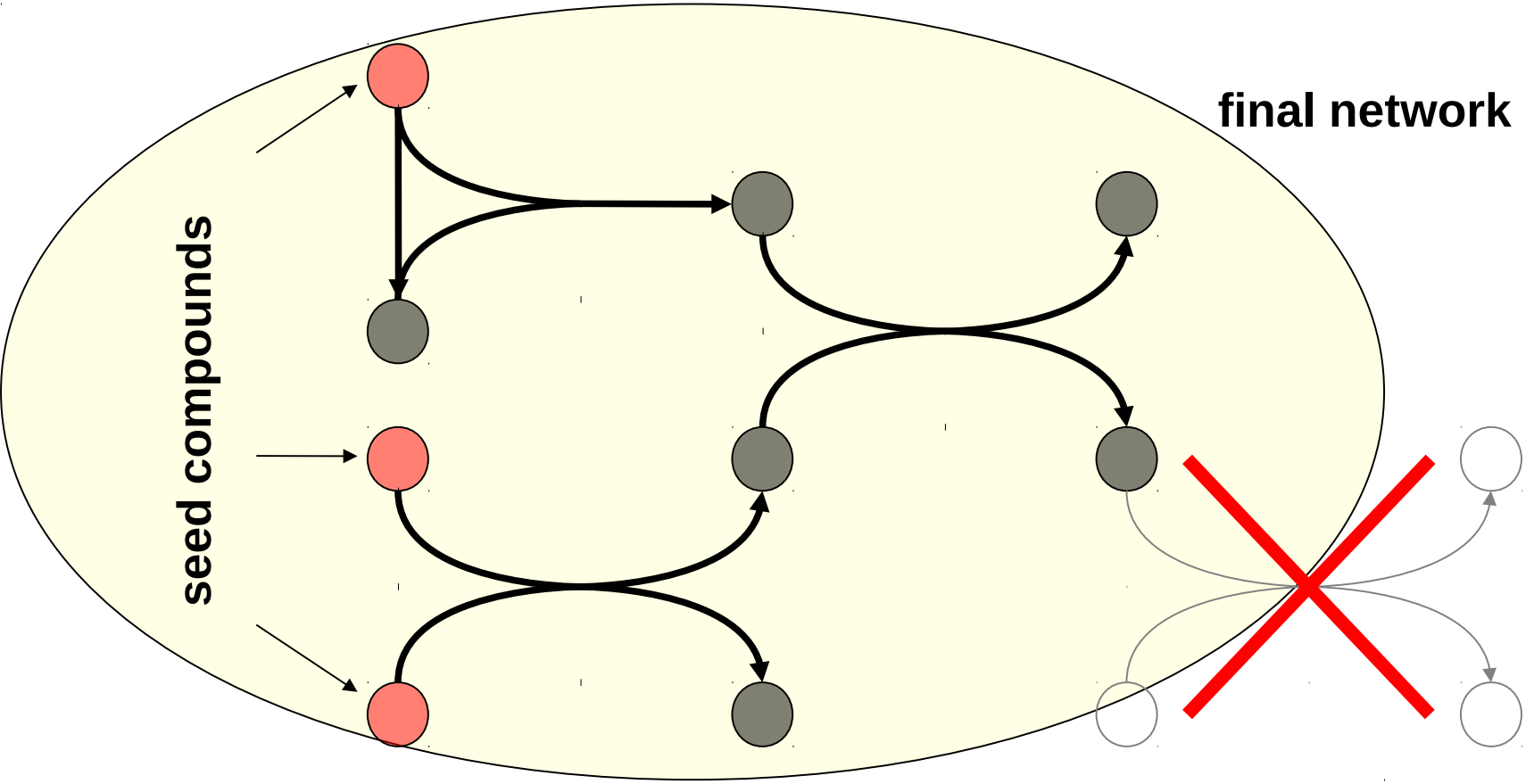


Concept of Scopes

Scope: set of compounds that is reached by a network expansion



The Scope describes the synthesizing capacity of the metabolic network,
if it is provided with the seed compounds

Handorf, Ebenhöh & Heinrich, JME, 2005

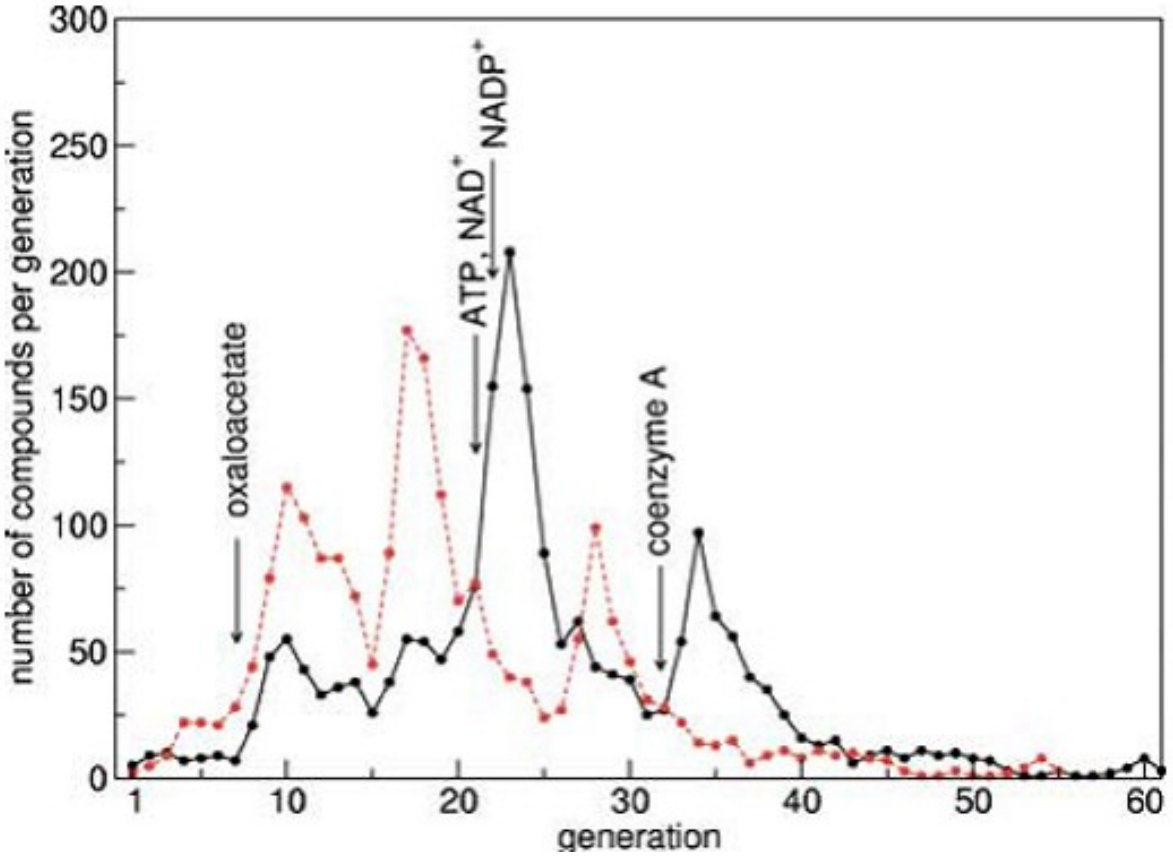
The expansion process

Initial conditions: availability of inorganic, 'prebiotic' compounds

(Martin and Russell, 2003)

carbonic acid:	H_2CO	(carbon)
methanethiol:	CH_3SH	(carbon, sulfur)
ammonia:	NH_3	(nitrogen)
pyrophosphate:	$P_2O_7^{4-}$	(phosphate)

Expansion on the complete KEGG network



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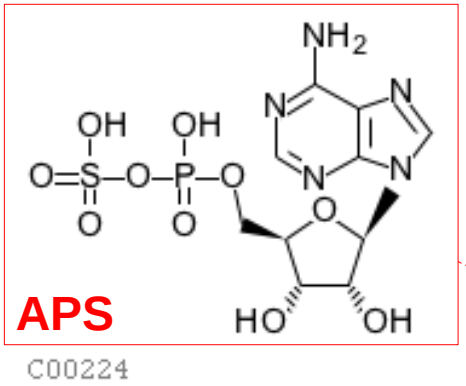

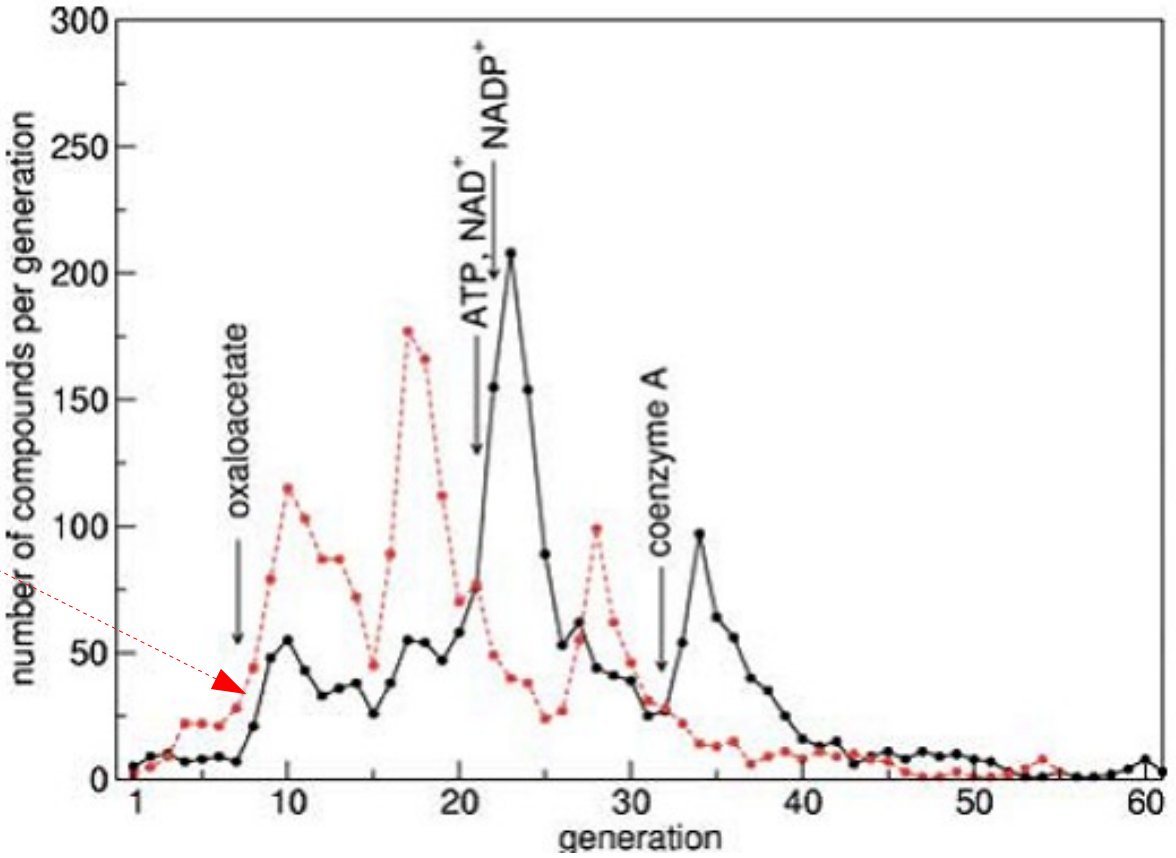

Analysis of the “global” network

A scope characterizes the
biosynthetic potential
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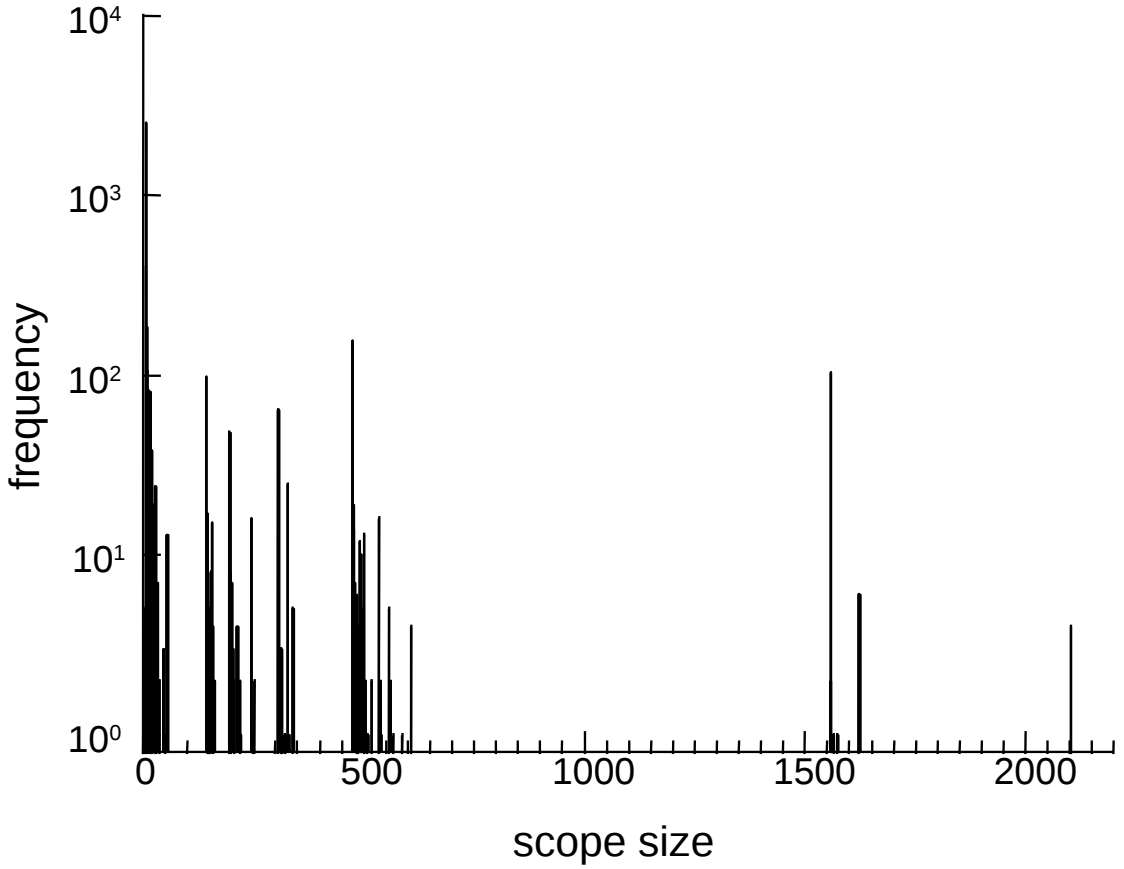
Characterize all metabolites!



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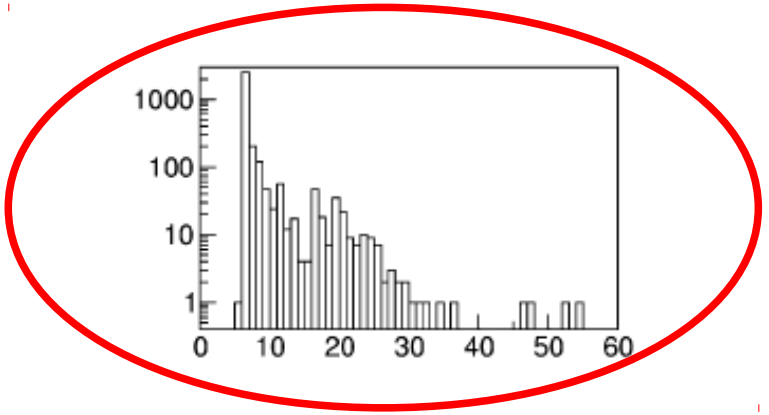
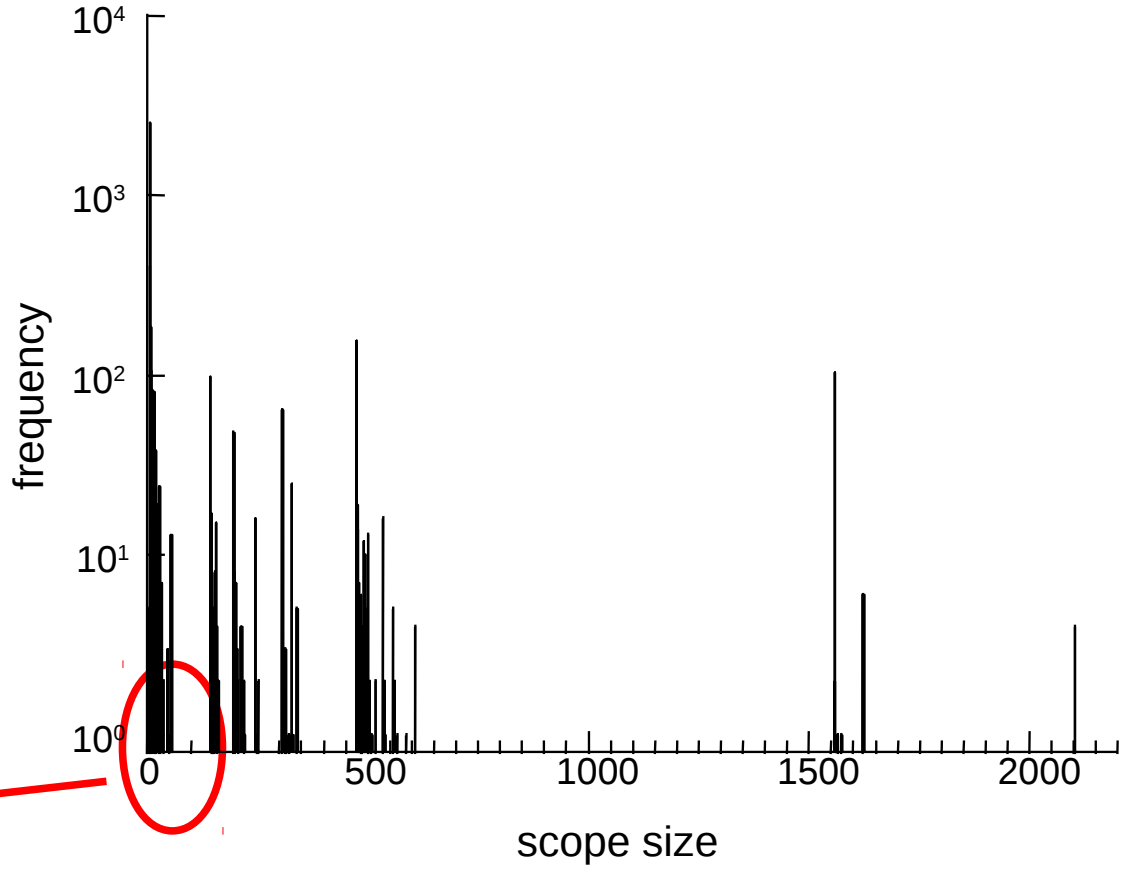
MOST METABOLITES CARRY A LOW POTENTIAL





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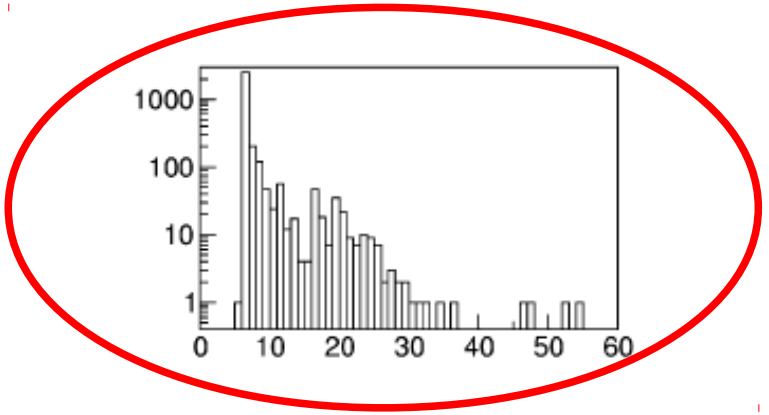

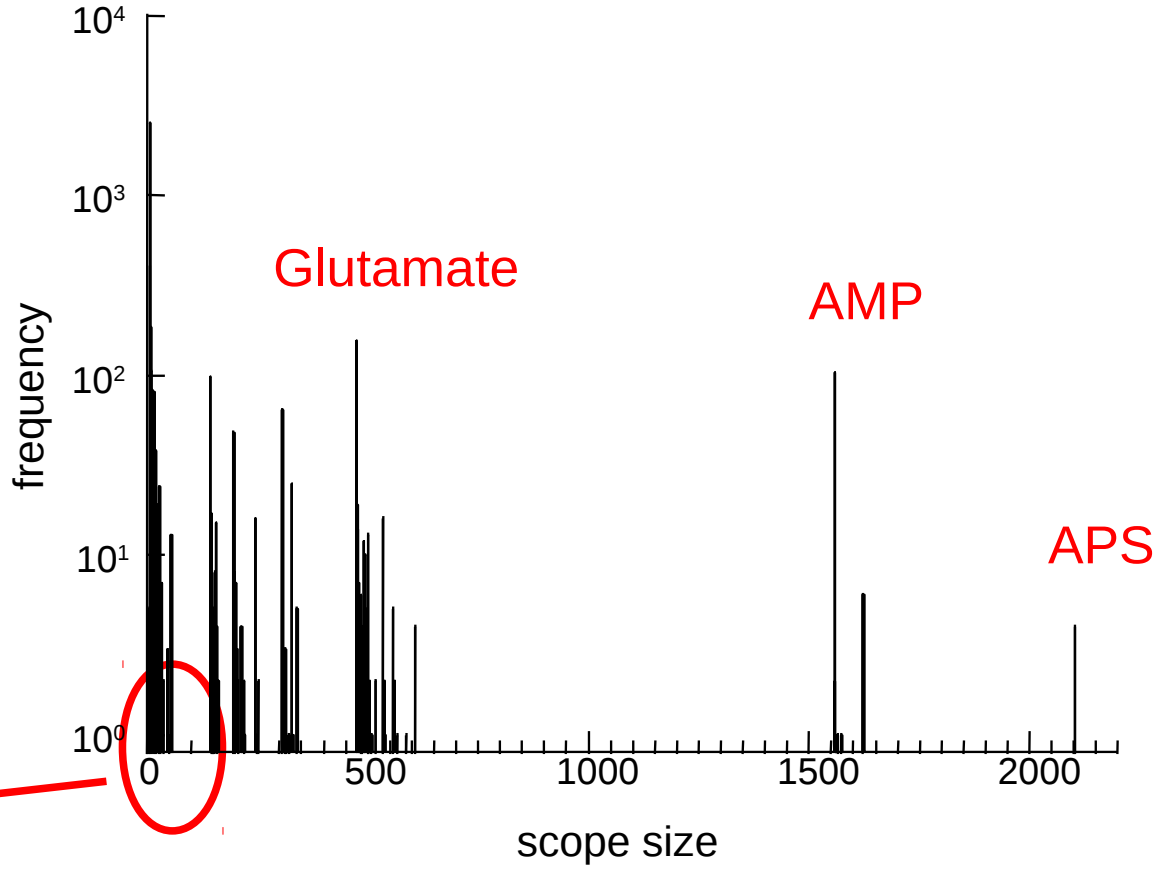

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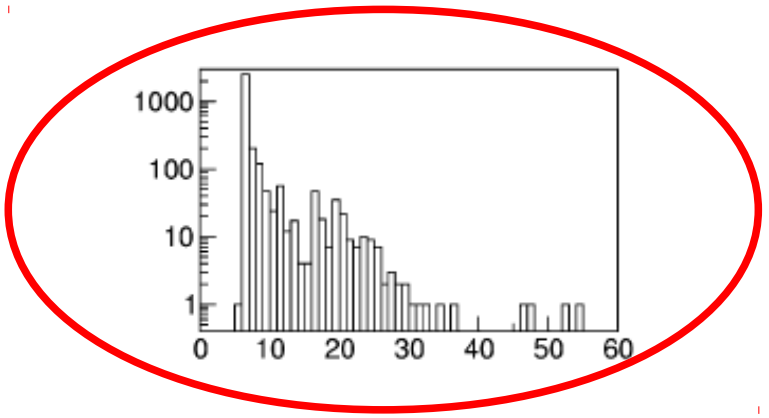
Analysis of the “global” network

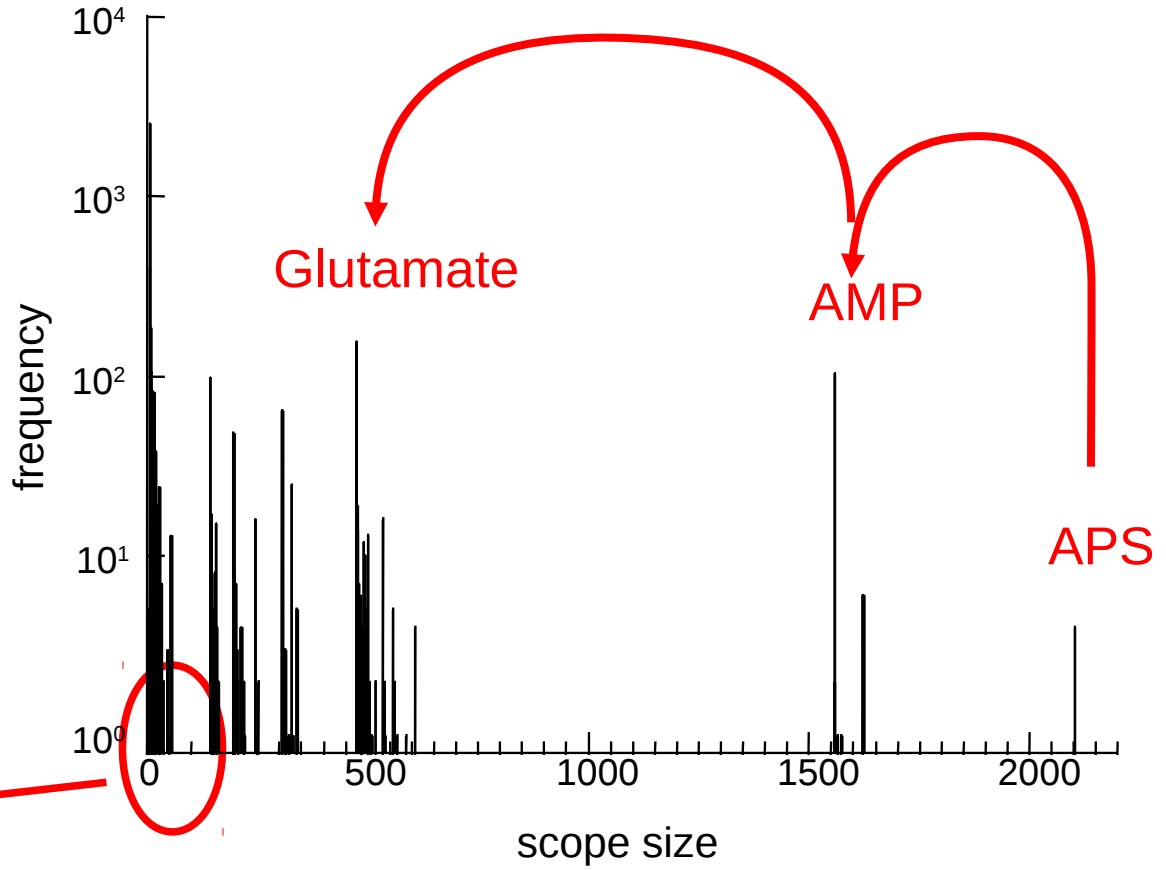
A scope characterizes the ***biosynthetic potential*** of a chemical substance

Characterise all metabolites!

MOST METABOLITES CARRY A LOW POTENTIAL

SOME SCOPES ARE INCLUDED IN OTHERS





(Handorf, Ebenhöh and Heinrich, J. Mol. Evol., 2005)

Global organisation of metabolism

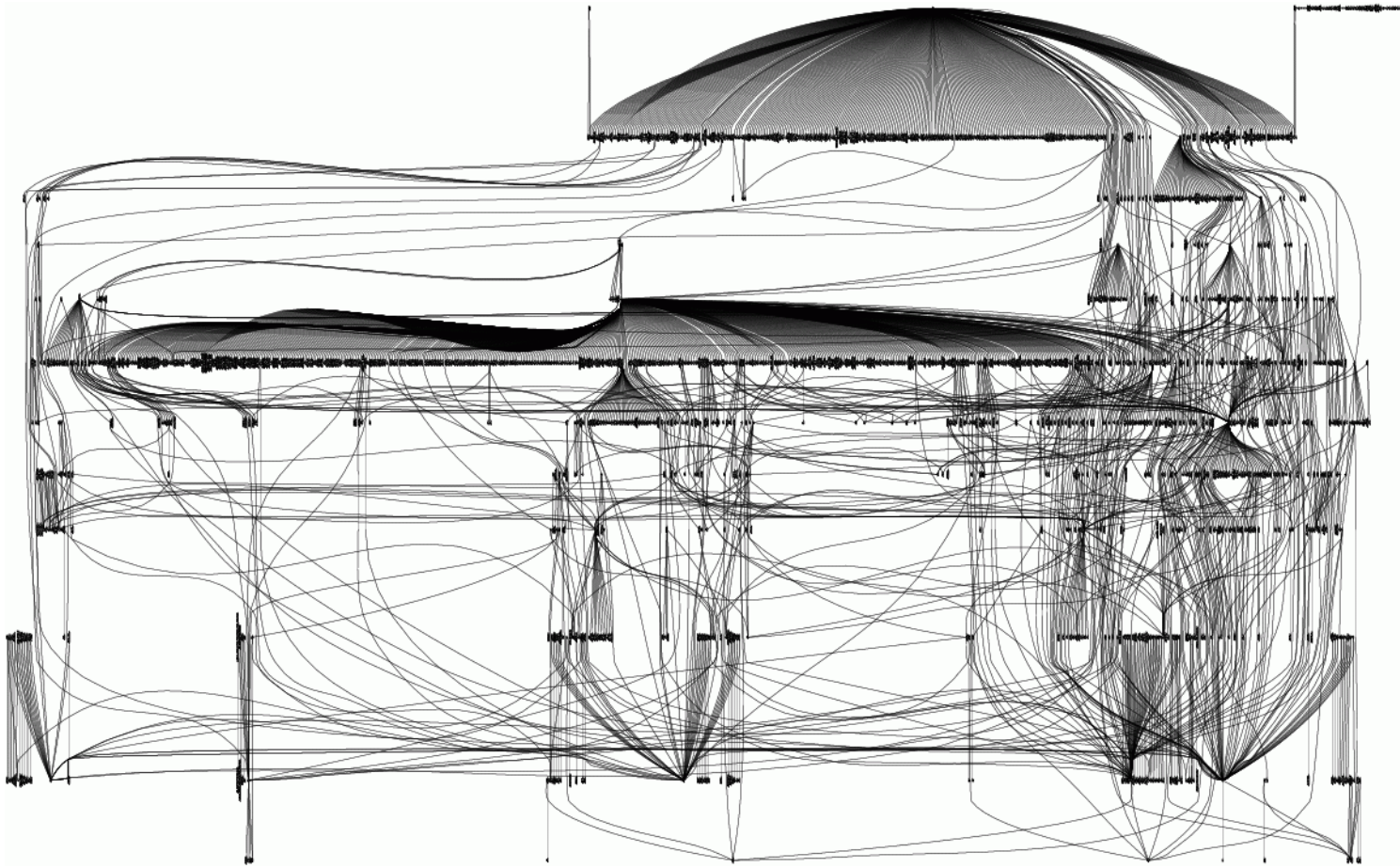
Reference network from KEGG

↓

calculate all single scopes

↓

determine all inclusion relations



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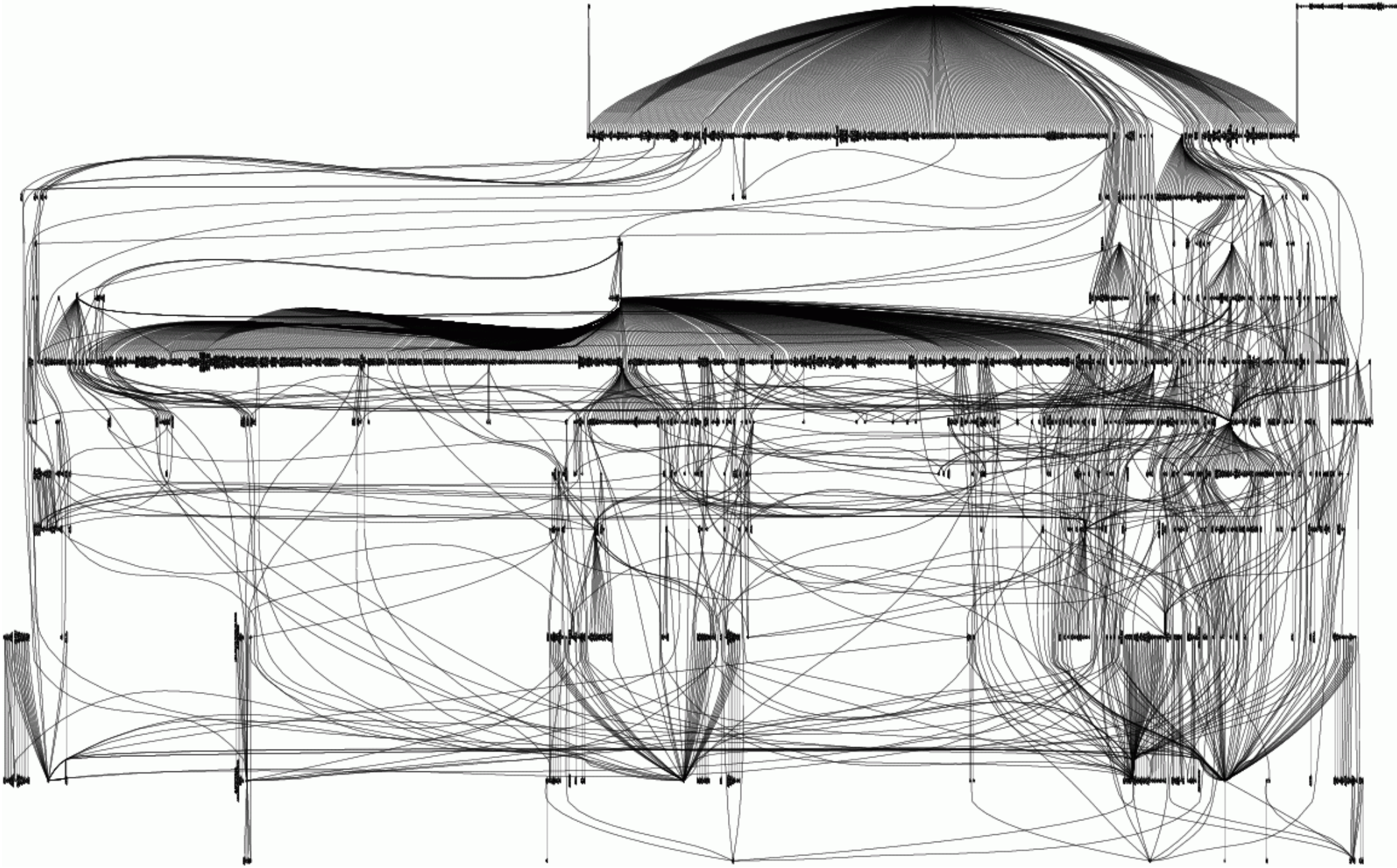

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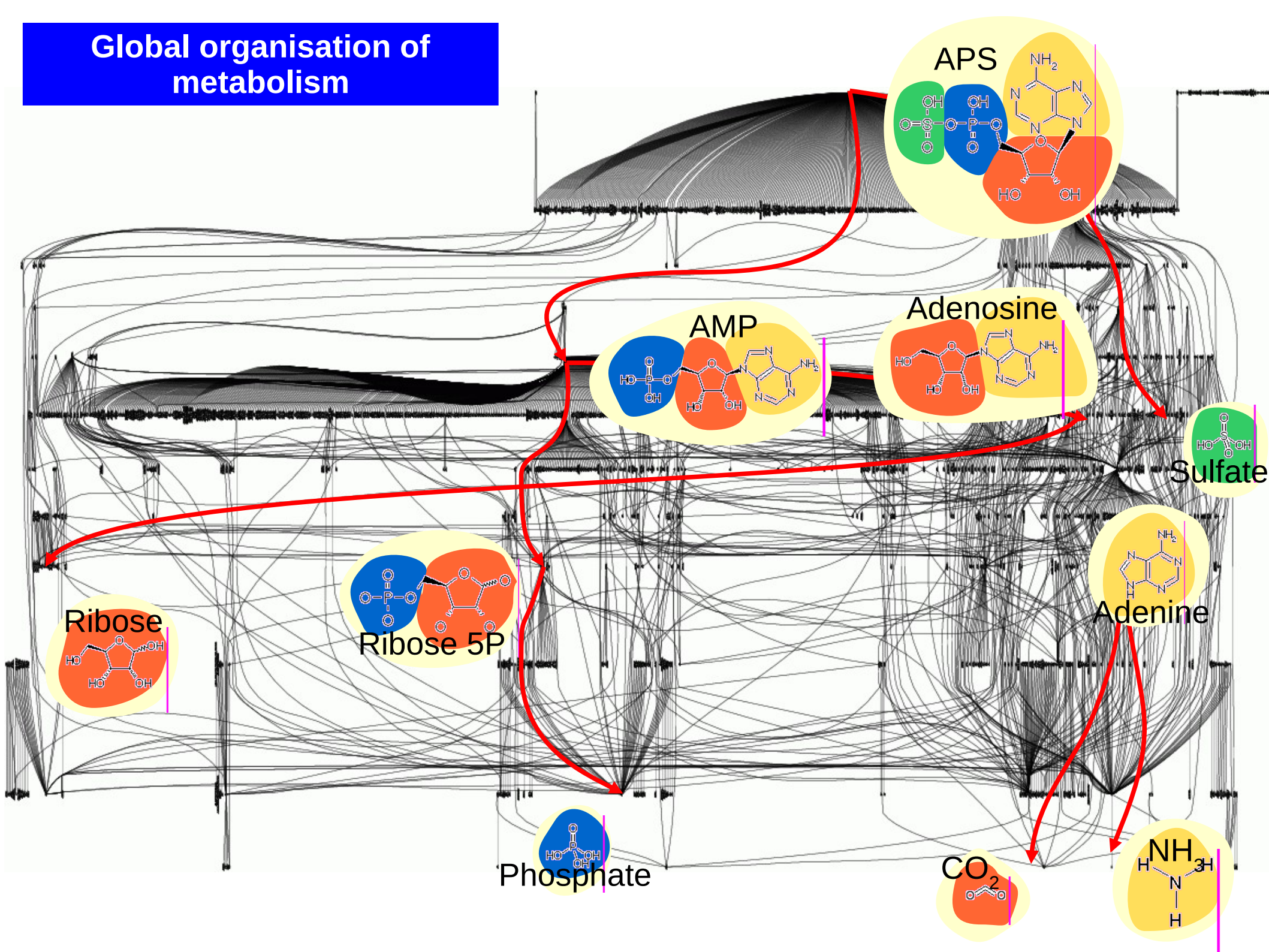

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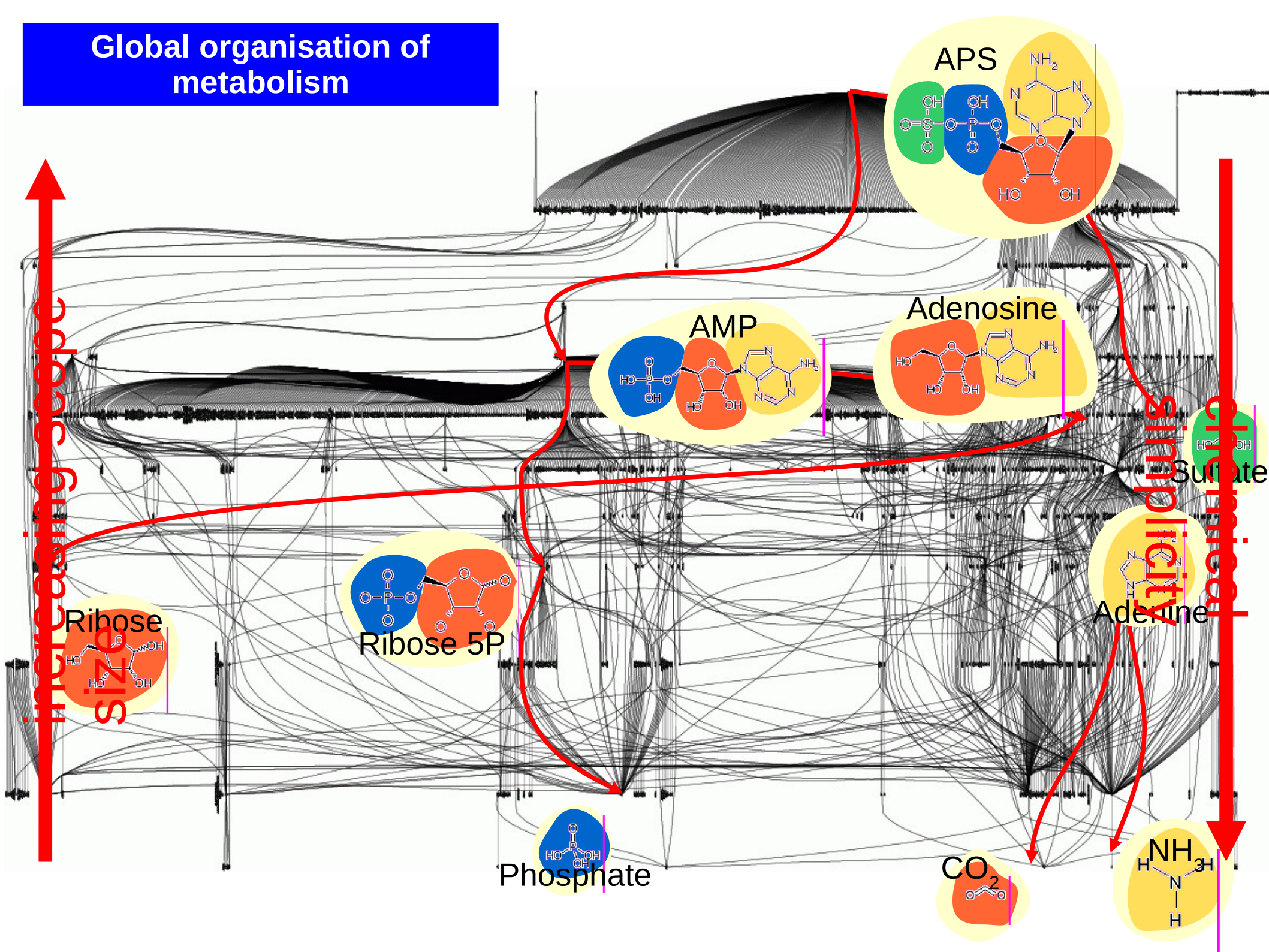
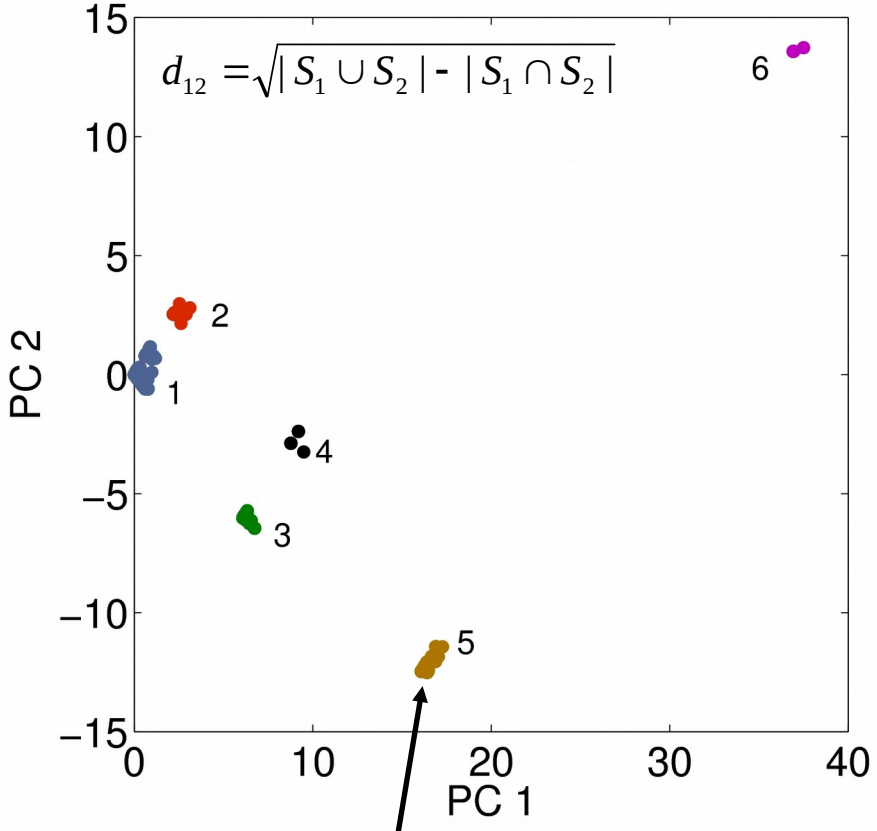


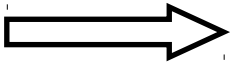
Similarity of biosynthetic potentials

Many metabolites carry ***similar*** biosynthetic potentials

Groups with similar potentials can be identified by ***clustering analysis***



metabolites with similar biosynthetic potentials



"consensus scope"
(typical biosynthetic potential)

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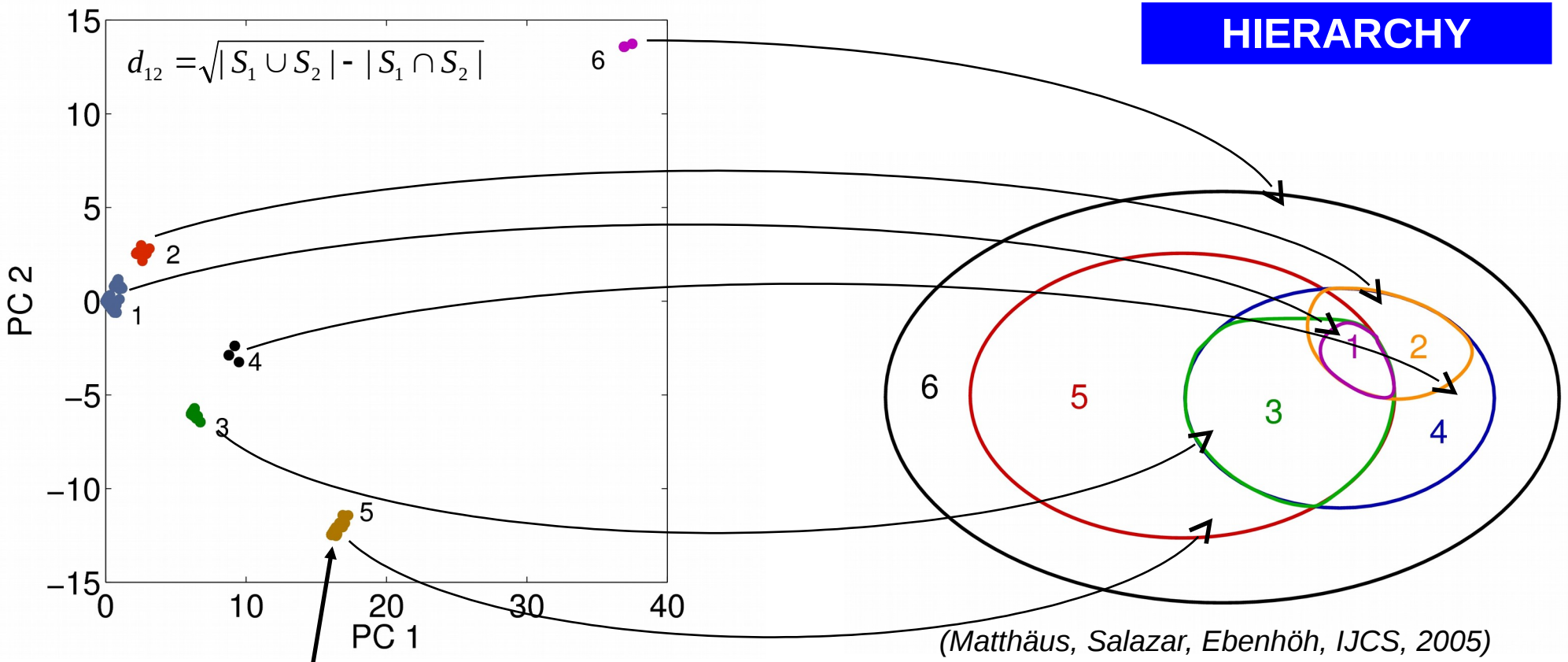

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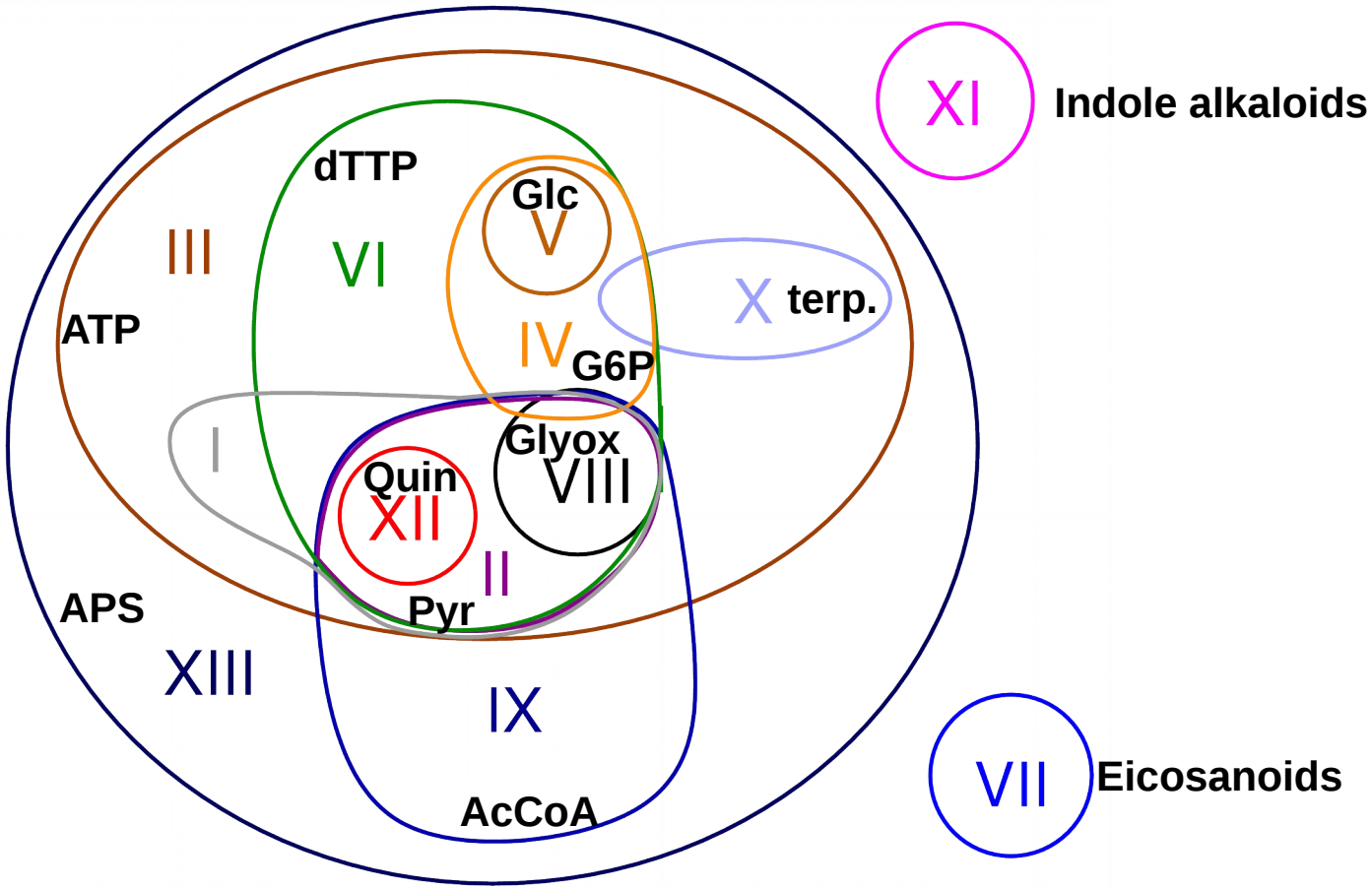
higher resultion with

$$d_{12} = 1 - \frac{|S_1 \cap S_2|}{|S_1 \cup S_2|}$$

HIERARCHY



(Matthäus, Salazar and Ebenhöh, PLoS Comp Biol, 2008)

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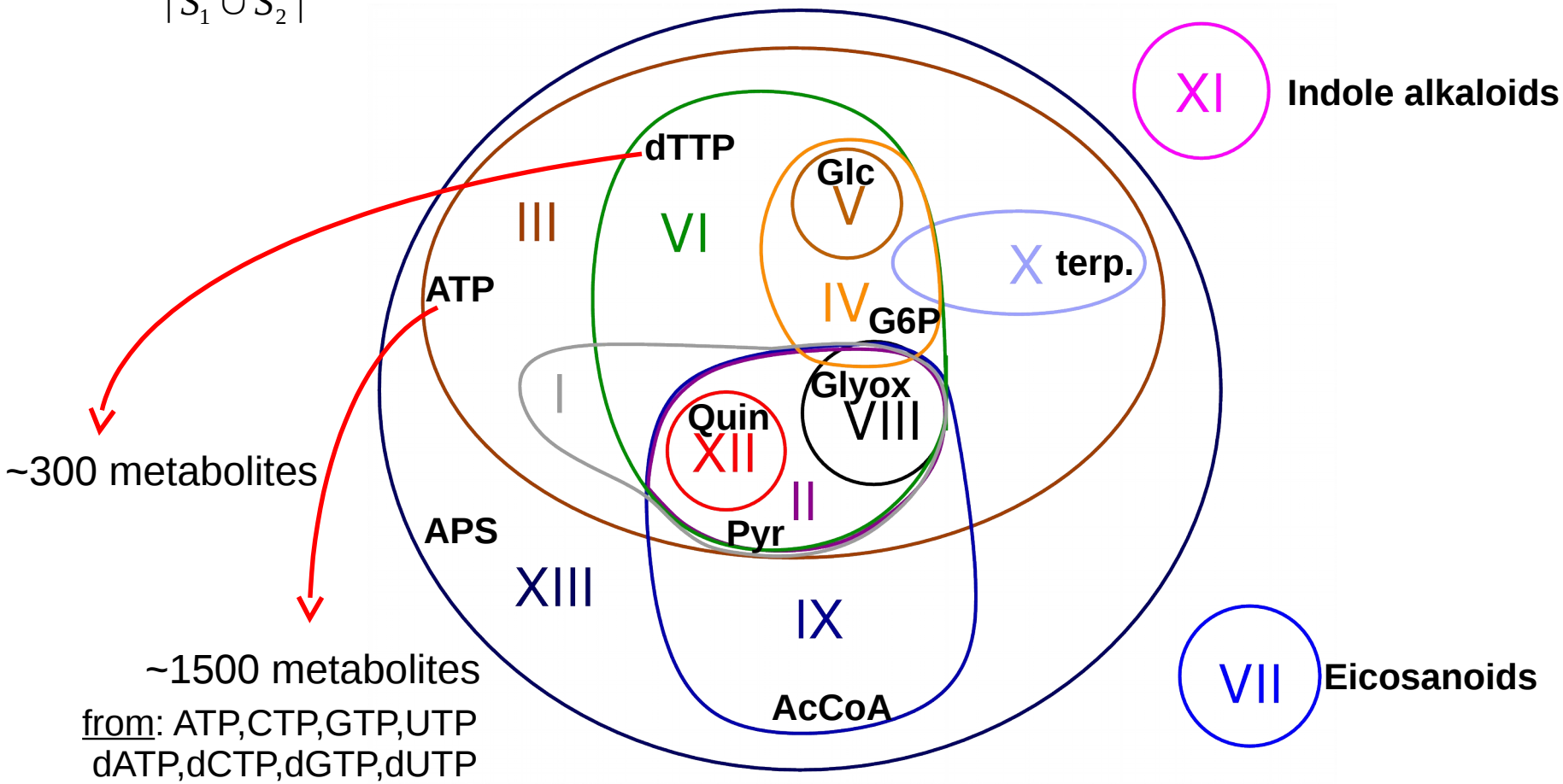

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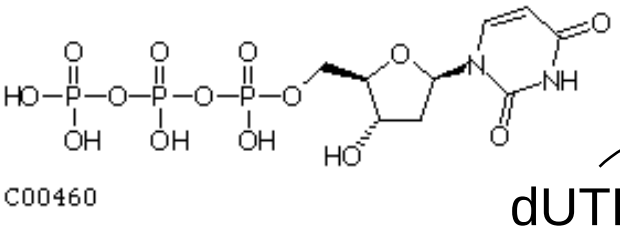
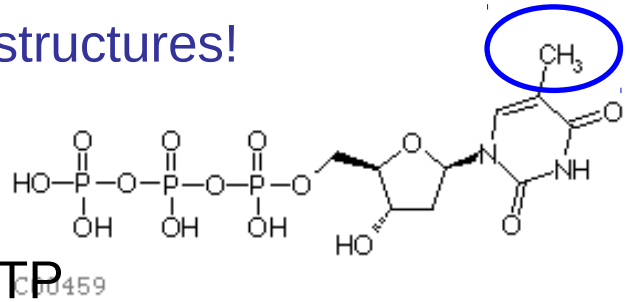
Separation of biosynthetic potentials

almost identical chemical structures!



dUTP



dTTP

produces 1500 metabolites

produces 300 metabolites

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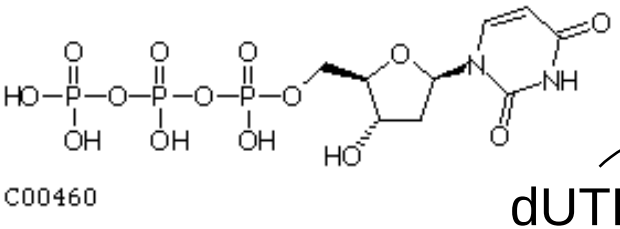

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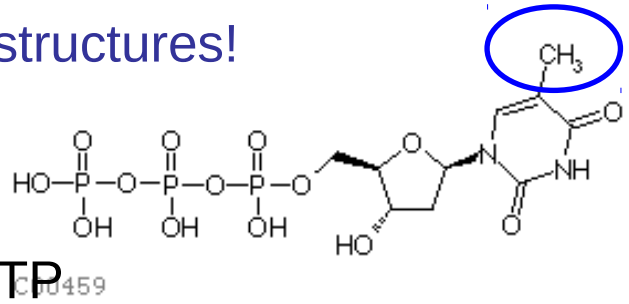

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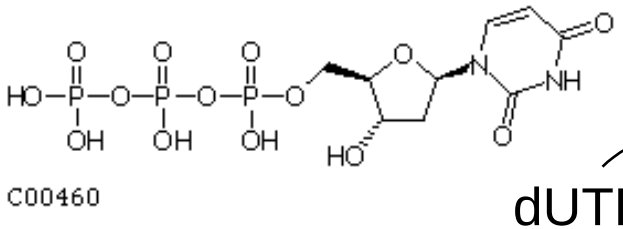

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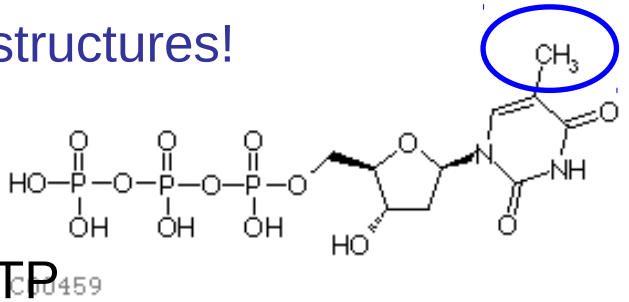

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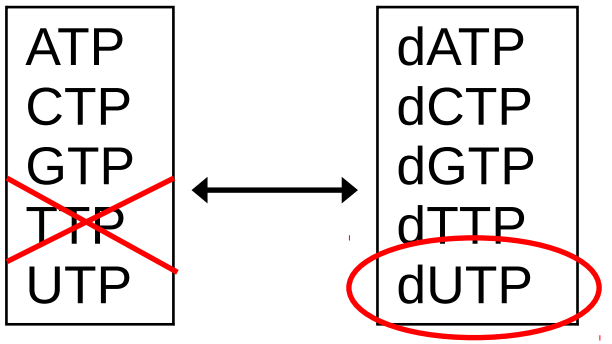
Explanation



does not exist!

should be low!

RNA

DNA

The chemical complexity alone does not determine the biosynthetic potential!

Single Organisms

Producibility in the flux language

What are the ***biosynthetic capabilities*** of a network?

Let *U* denote the set of available nutrient metabolites.

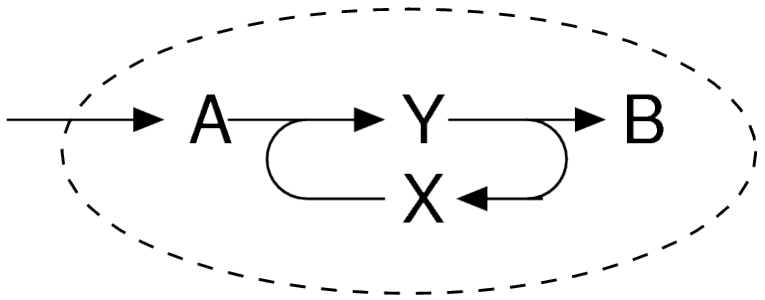
A metabolite is ***producible*** from the nutrients *U* if there exists a flux solution such that

- its own concentration increases
- only nutrients are consumed
- all others are at least balanced

Metabolite *k* is producible if $\exists v: [Sv]_k > 0 \ \wedge \ [Sv]_i \geqslant 0 \ \ \forall \ \ i \notin U$

Let $P(U)$ denote the set of all metabolites producible from nutrients *U*

Growth and Dilution: Toy models



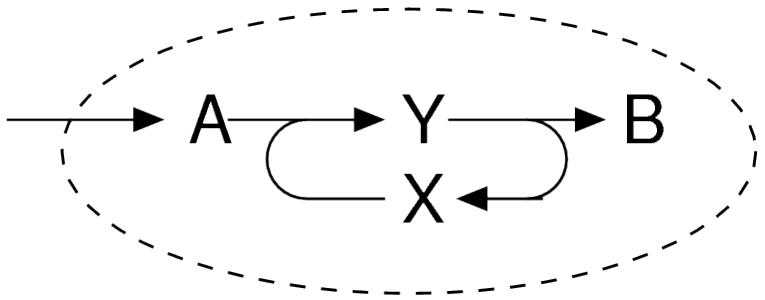
$U = \{A\}$ $P = \{B\}$

X and *Y* not producible from *A*!

What if the cell is growing? ⟹ Dilution! $\Rightarrow X, Y \rightarrow 0$

B is not producible under growth!

Growth and Dilution: Toy models



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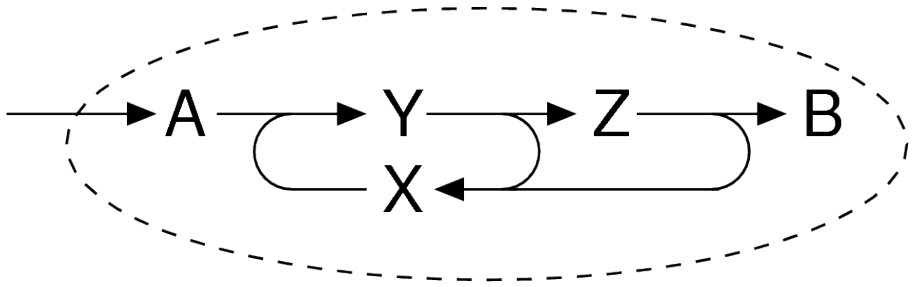

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Sustainability

A metabolite is ***sustainable*** from nutrients *U* if there exists a flux solution such that

- its own concentration increases
- only nutrients are consumed
- all other required intermediates are sustainable

Let *U* denote the set of available nutrient metabolites.

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Recursive definition of sustainable metabolites:

Let $P_0 = P(U)$

Define forbidden set of reactions: $F_n = \{ j \mid \exists\ i \notin P_n\ :\ S_{ij} < 0 \}$

$$P_{n+1} = \{ k \mid \exists v:\ v_j = 0\ \ \forall\ \ j \in F_n\ \wedge\ [Sv]_k > 0\ \wedge\ [Sv]_i \geqslant 0\ \ \forall\ \ i \notin U \}$$

Let $S(U)$ denote the set of all metabolites sustainable from U, defined by

$$S(U) = \lim_{n \to \infty} P_n$$

Takes a long time to compute!

Relating scopes to flux models

Let $\Sigma(U)$ denote the scope of U

It can simply be shown that $\Sigma(U) \subseteq S(U) \subseteq P(U)$

Numerical experiment for the network of *E.coli* (Reed et al., 2003)

determine $\Sigma(U)$ and $S(U)$

for all sets $U = \{k, H_2O\}$

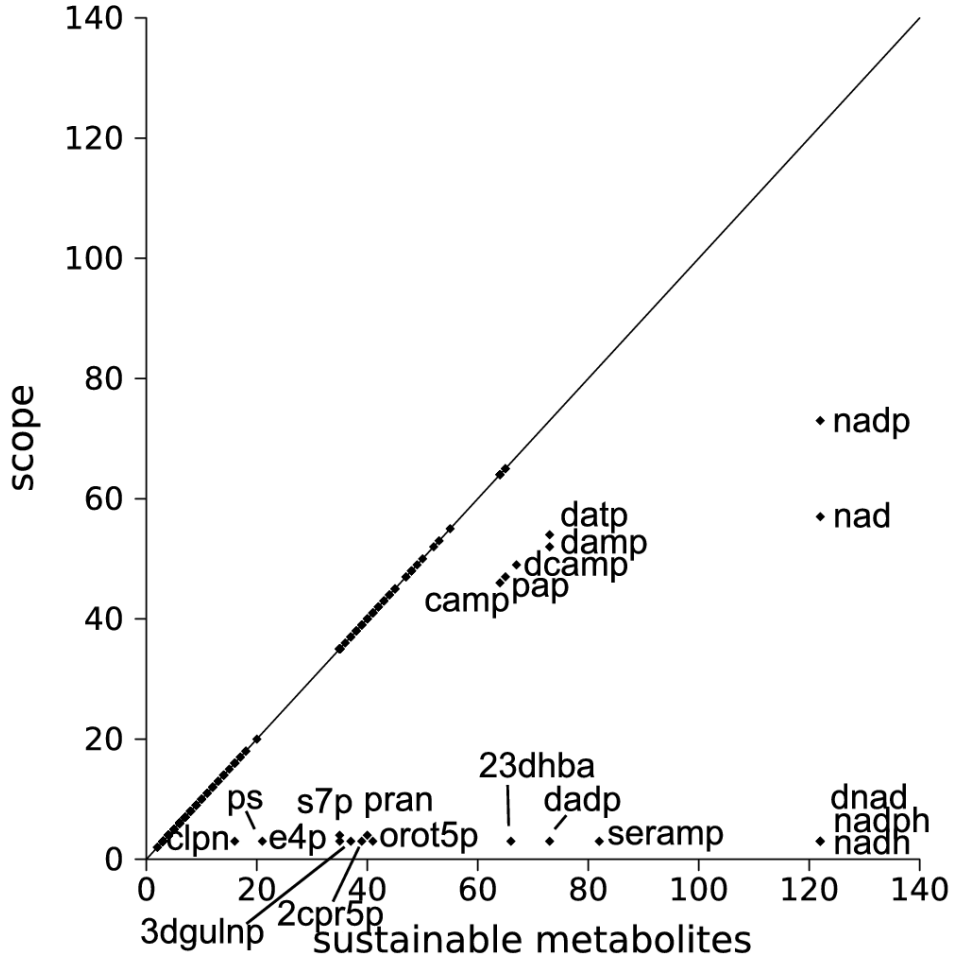
Observation:

for most metabolites:

$$\Sigma(U) = S(U)$$

→ Agreement even better for more complex sets U

(Kruse and Ebenhöh, GI 2008)



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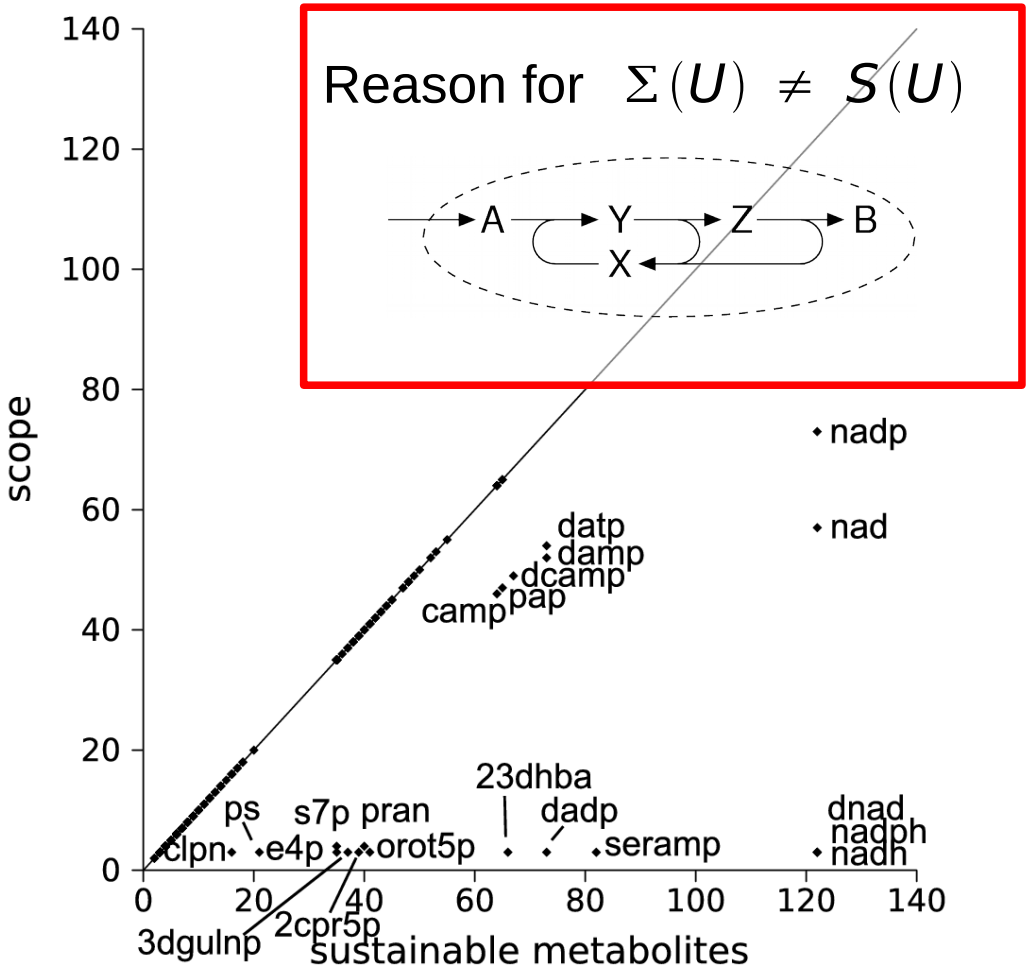
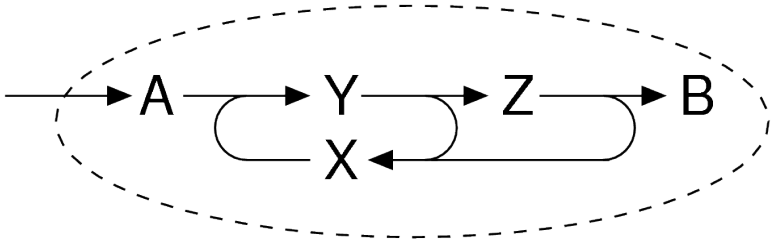
→ Agreement even better for more complex sets U

(Kruse and Ebenhöh, GI 2008)

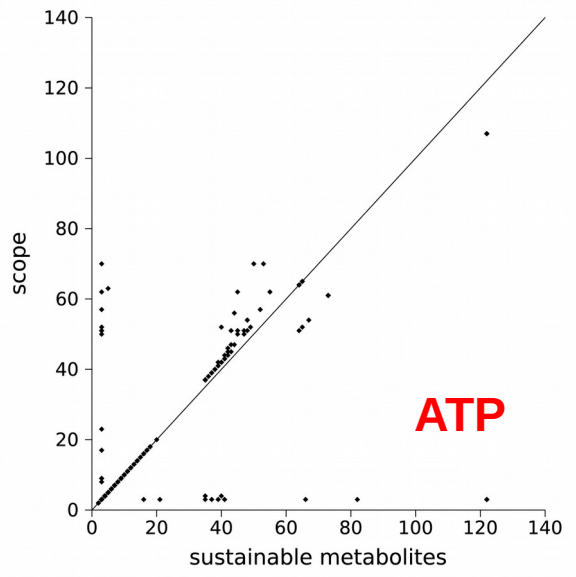


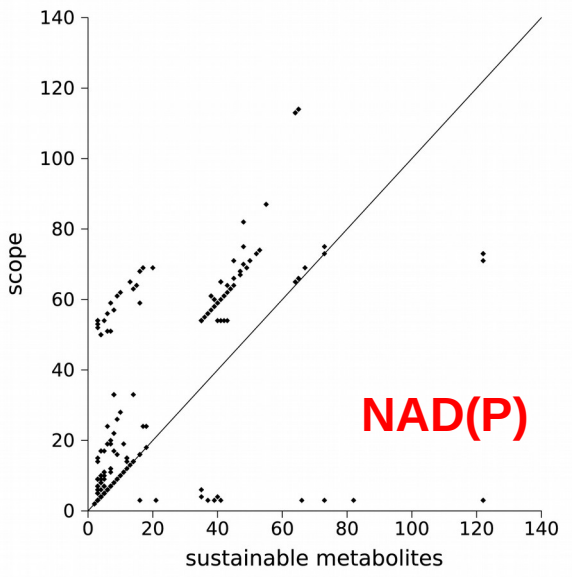
The role of cofactors

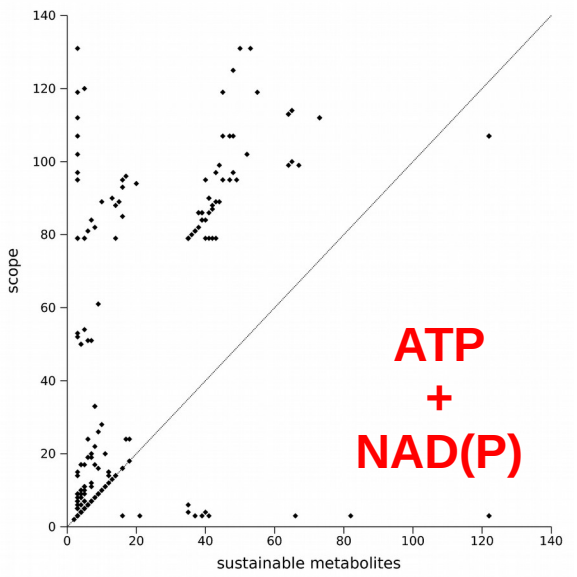
Common cofactors (ATP/NADH) are of the type



⇨ We add cofactors to the seed
(ATP does not have to be produced to be used as a cofactor)







We tend to overestimate the 'true' biosynthetic capacity (under constant growth)

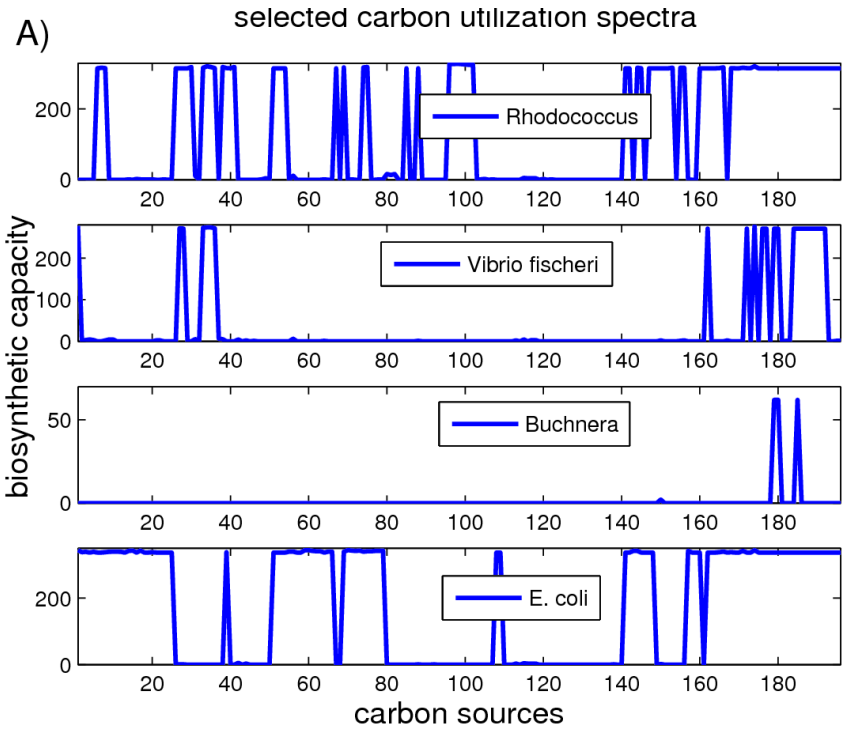
But that's OK to give a meaning to “The scope of glucose”

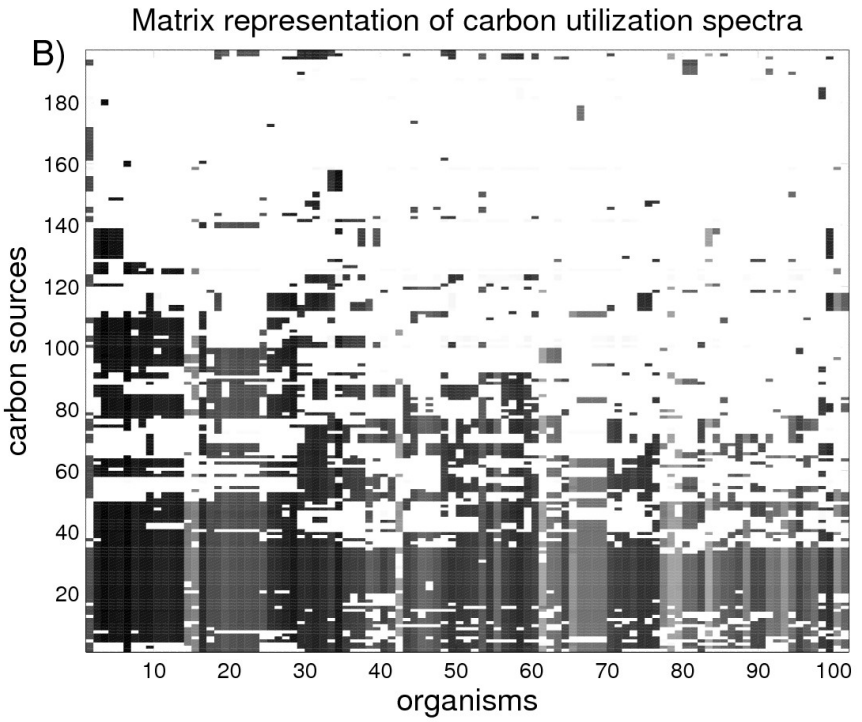
Single organisms

Investigate biosynthetic capacities of organisms on various carbon sources:

- 447 organism specific networks (KEGG)
- 200 carbon sources

CARBON UTILIZATION SPECTRA





Single organisms

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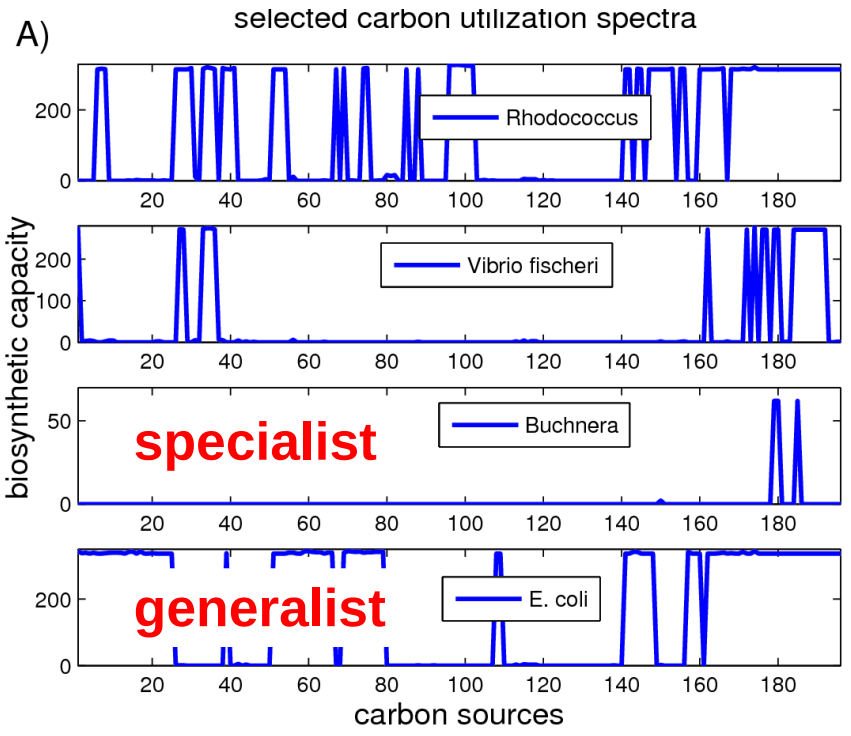
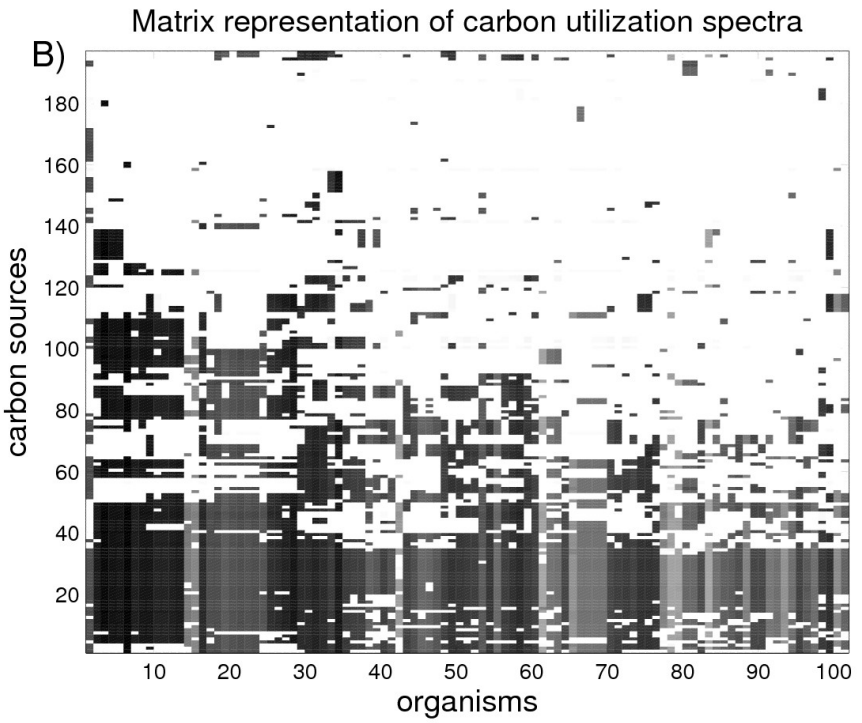
CARBON UTILIZATION SPECTRA

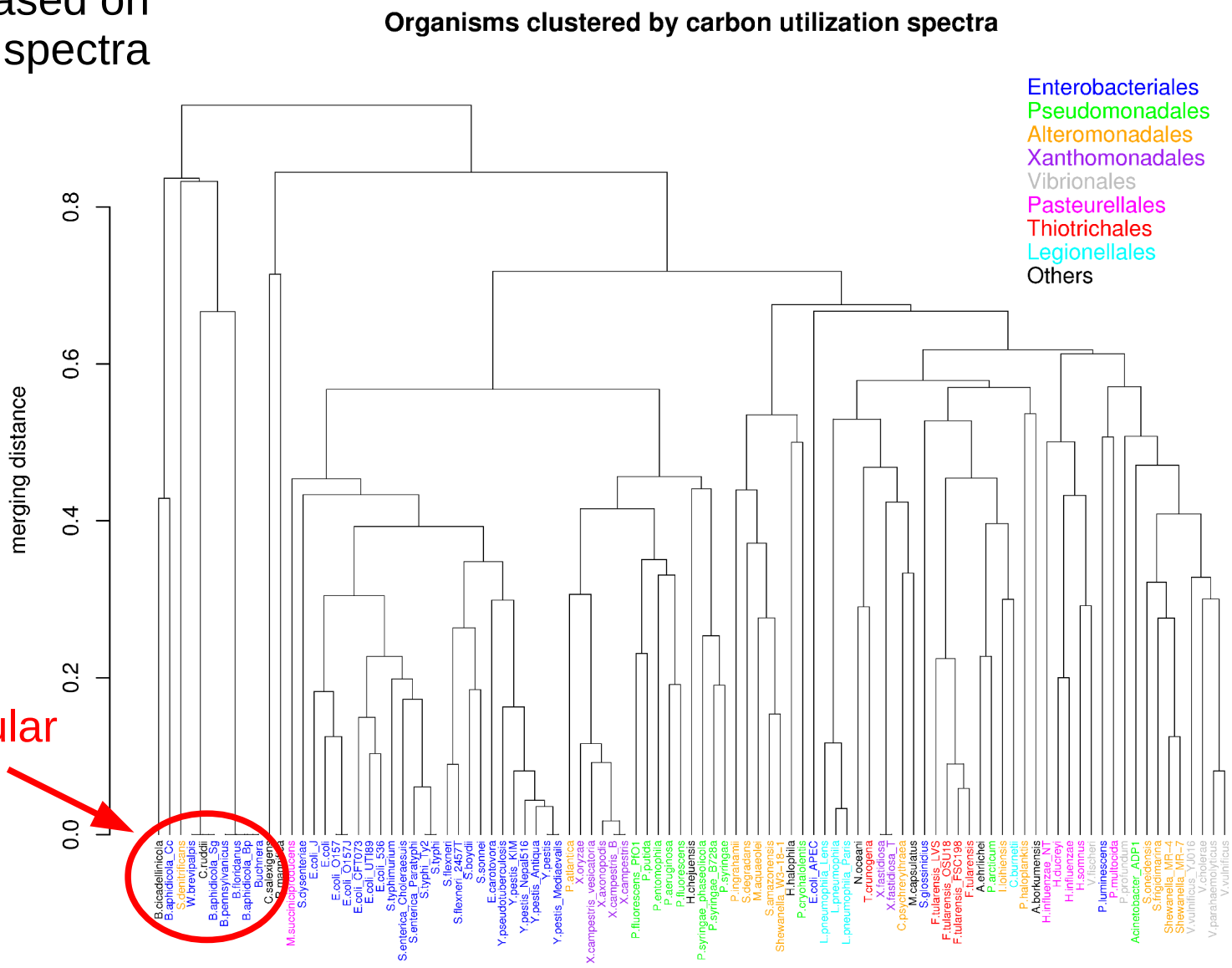




It is in principle possible to distinguish between generalists and specialists

Single organisms

A phenetic tree based on carbon utilization spectra



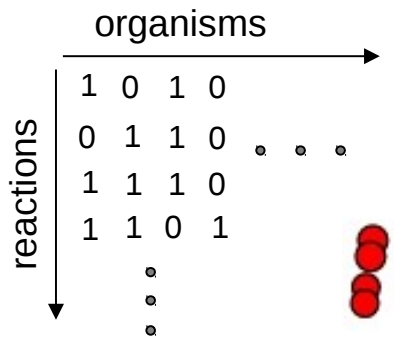
many intracellular parasites

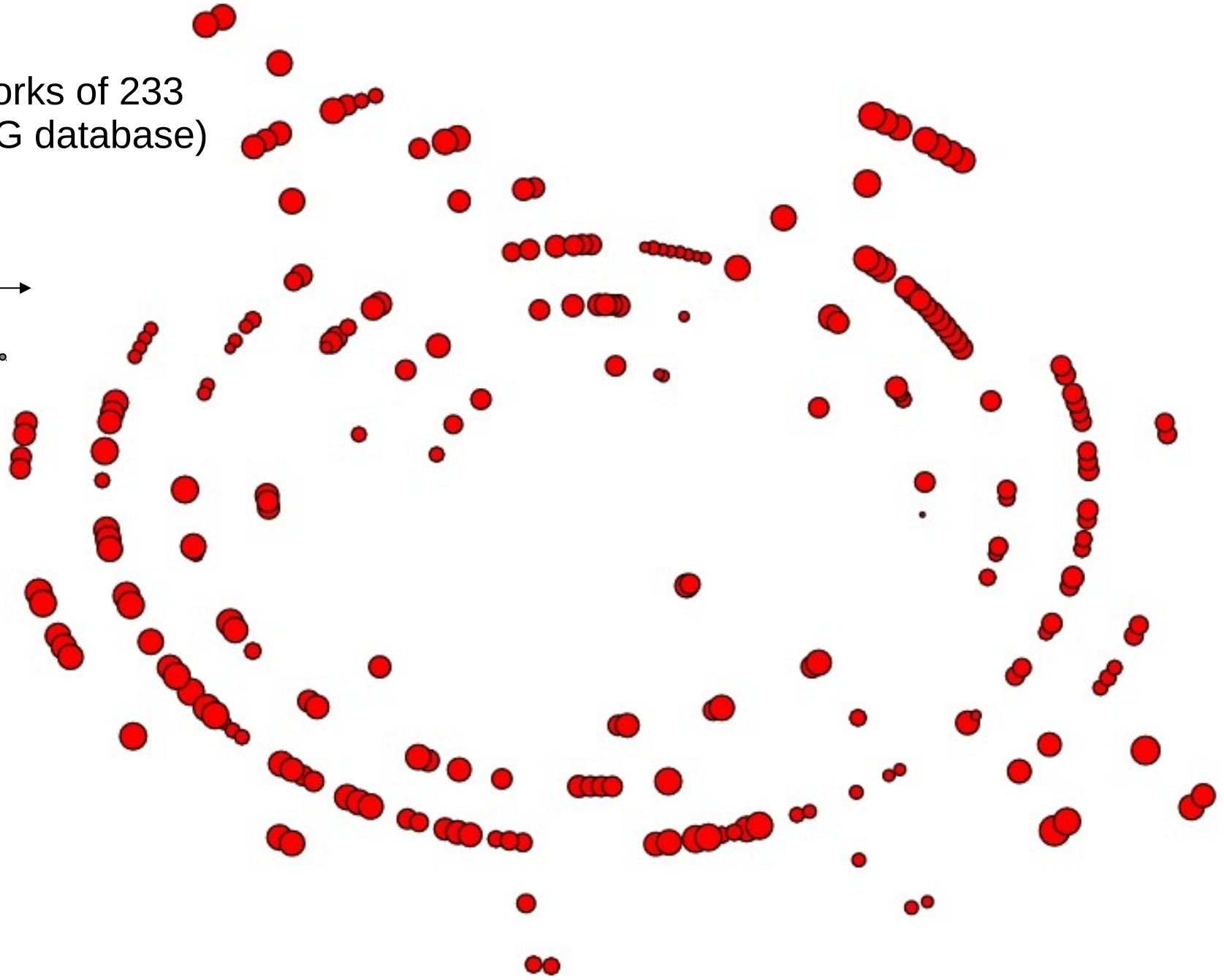
⇨ Classification of organisms by 'lifestyle'?

(Ebenhöh and Handorf, EURASIP, 2009)

Single organisms

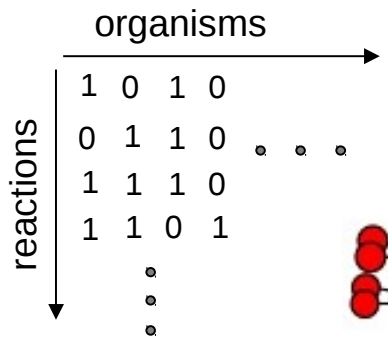
Metabolic networks of 233 organisms (KEGG database)

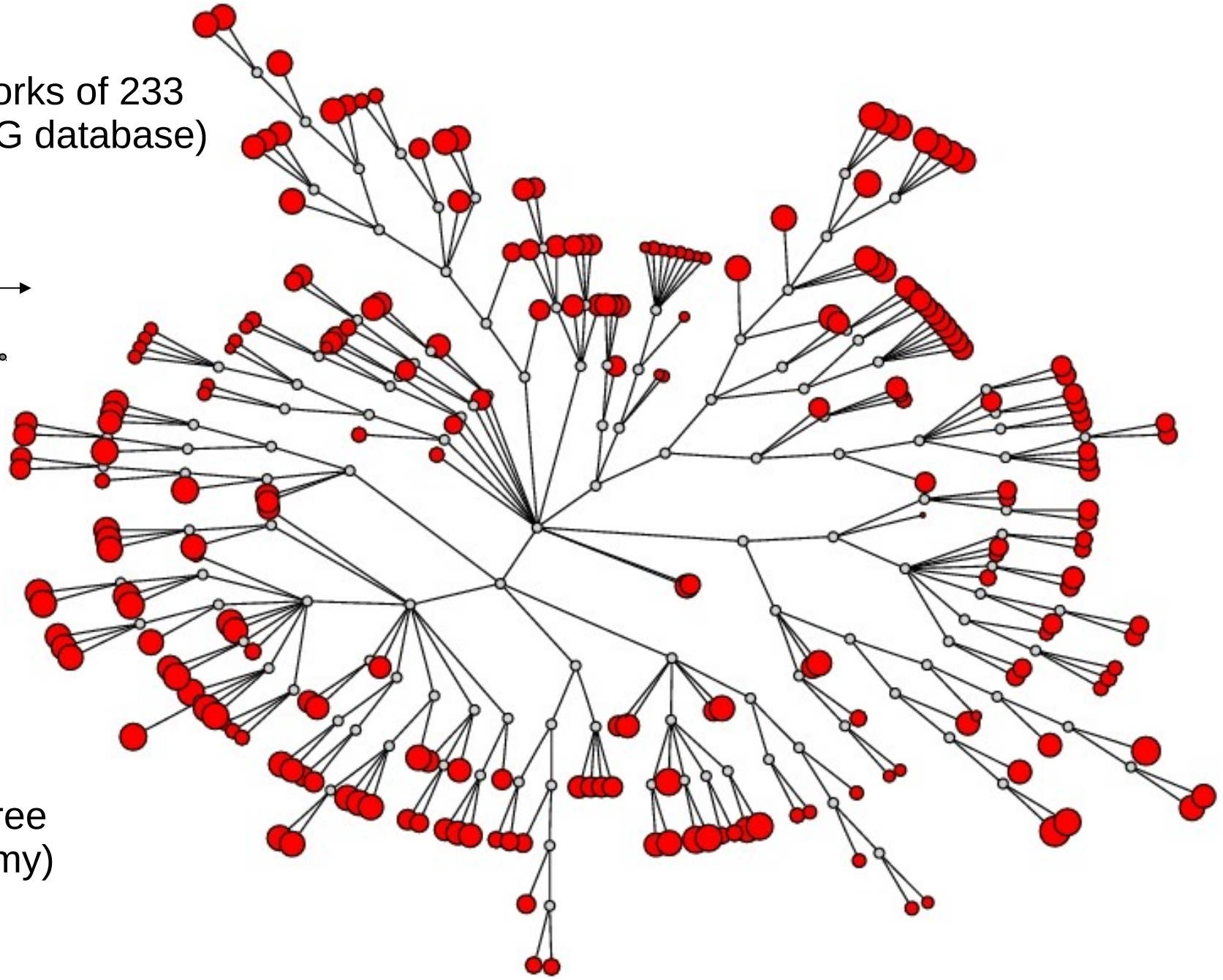




Single organisms

Metabolic networks of 233 organisms (KEGG database)

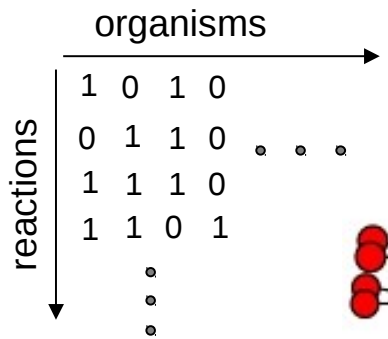




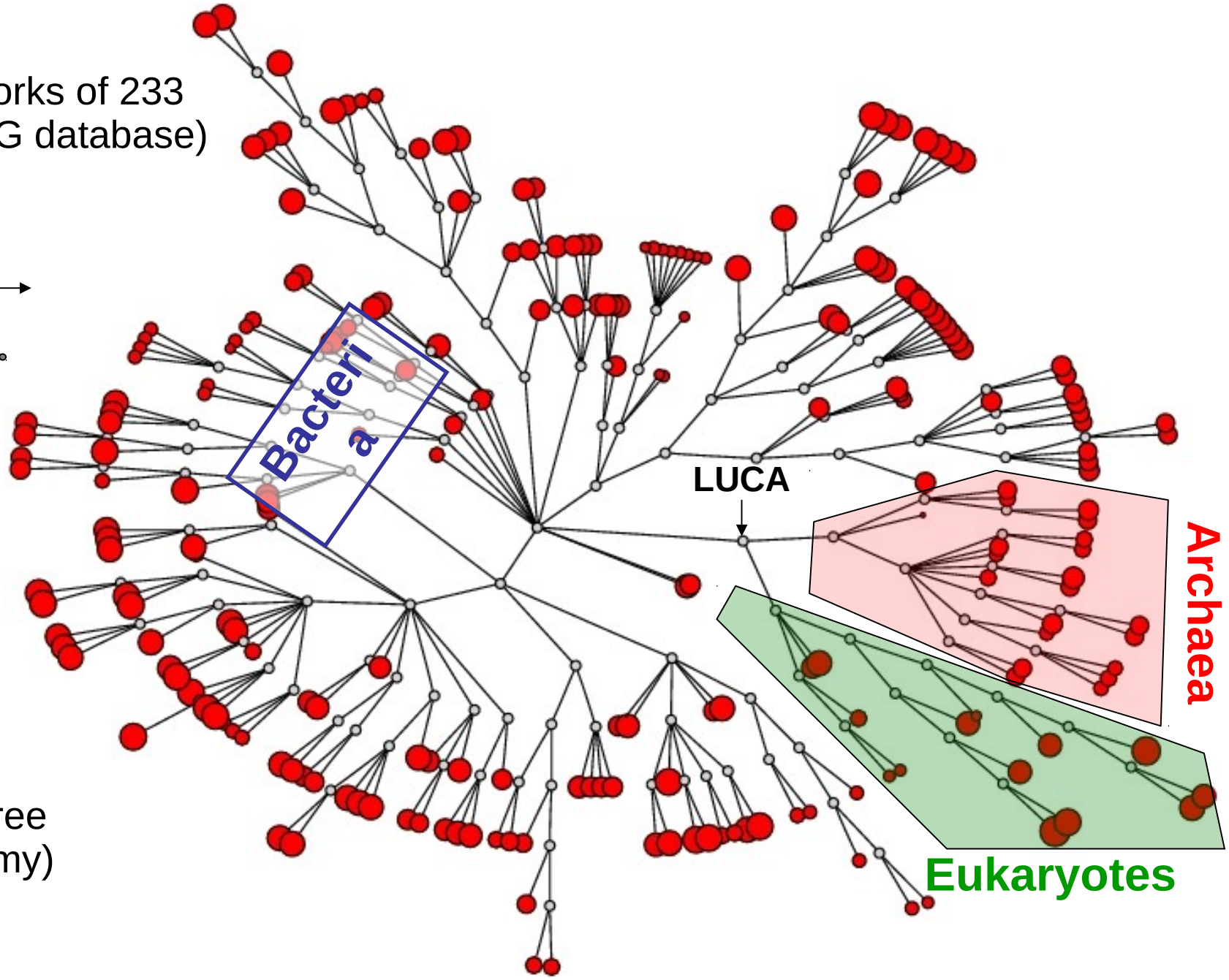
Evolutionary tree (NCBI Taxonomy)

The tree of life

Metabolic networks of 233 organisms (KEGG database)



Evolutionary tree (NCBI Taxonomy)



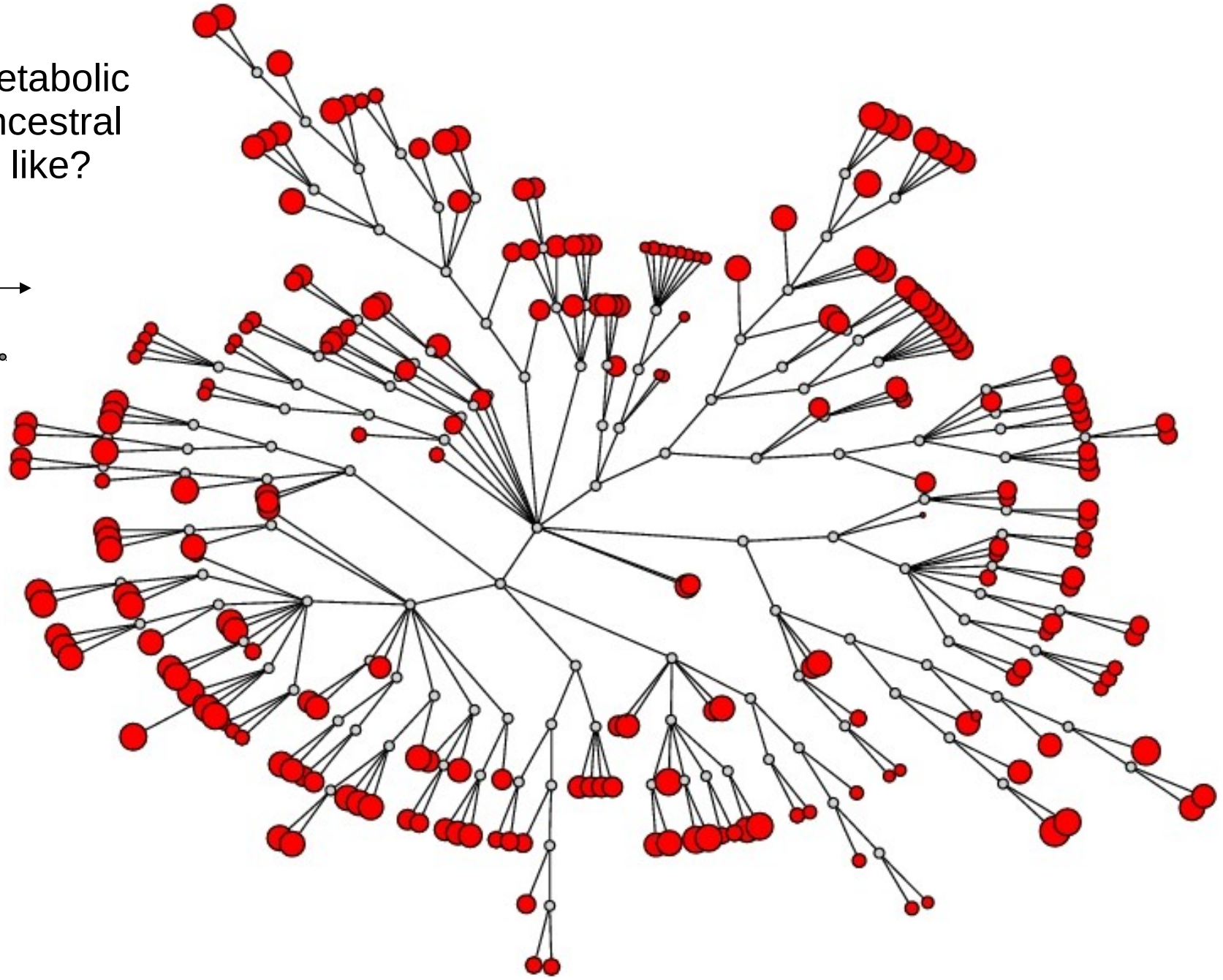
Ancestral networks

How did the metabolic networks of ancestral species look like?

organisms

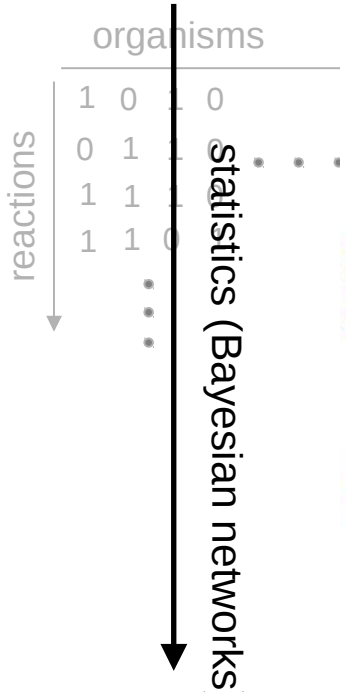
reactions

1	0	1	0	
0	1	1	0	. . .
1	1	1	0	
1	1	0	1	
⋮				

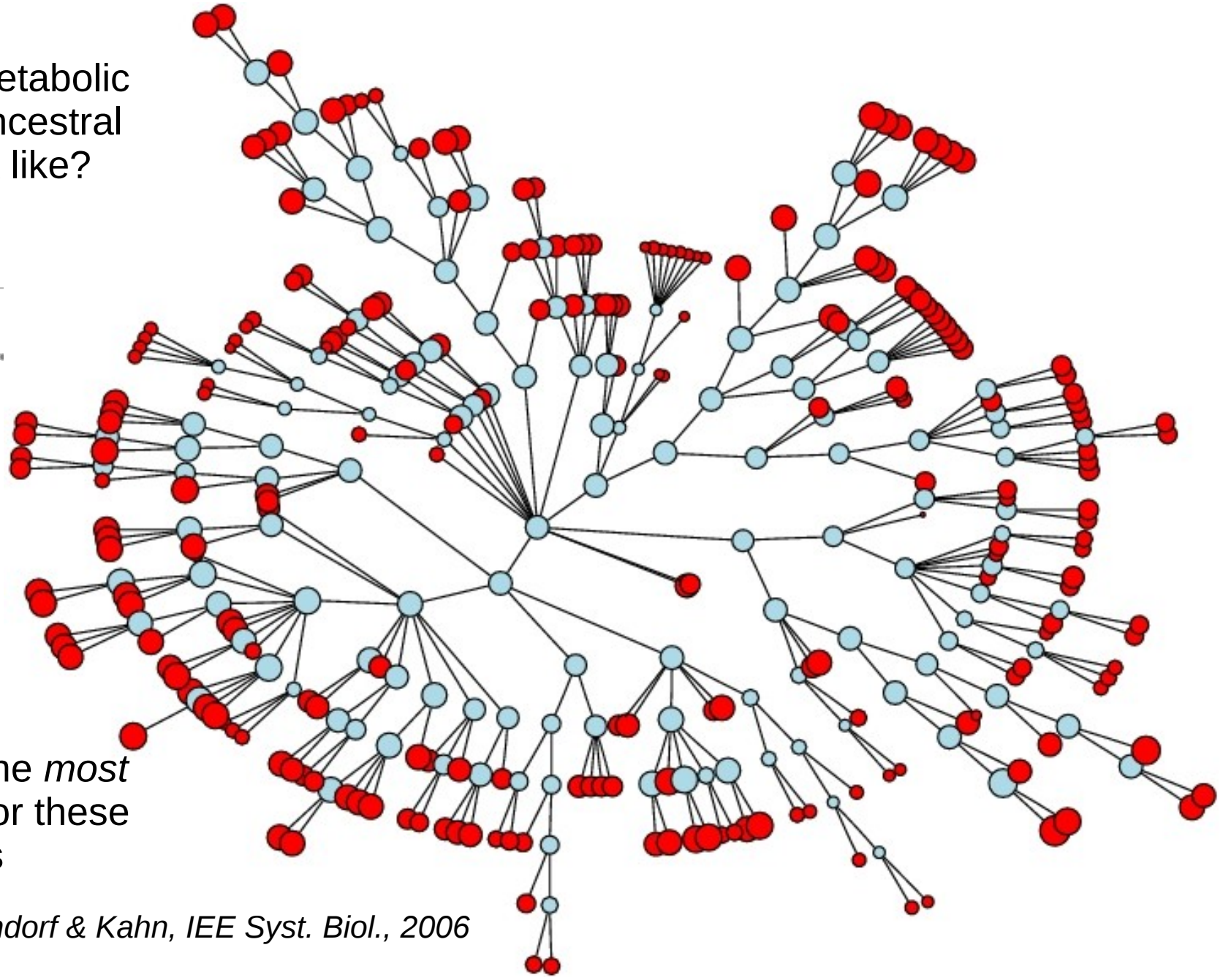


Reconstruction of ancestral networks

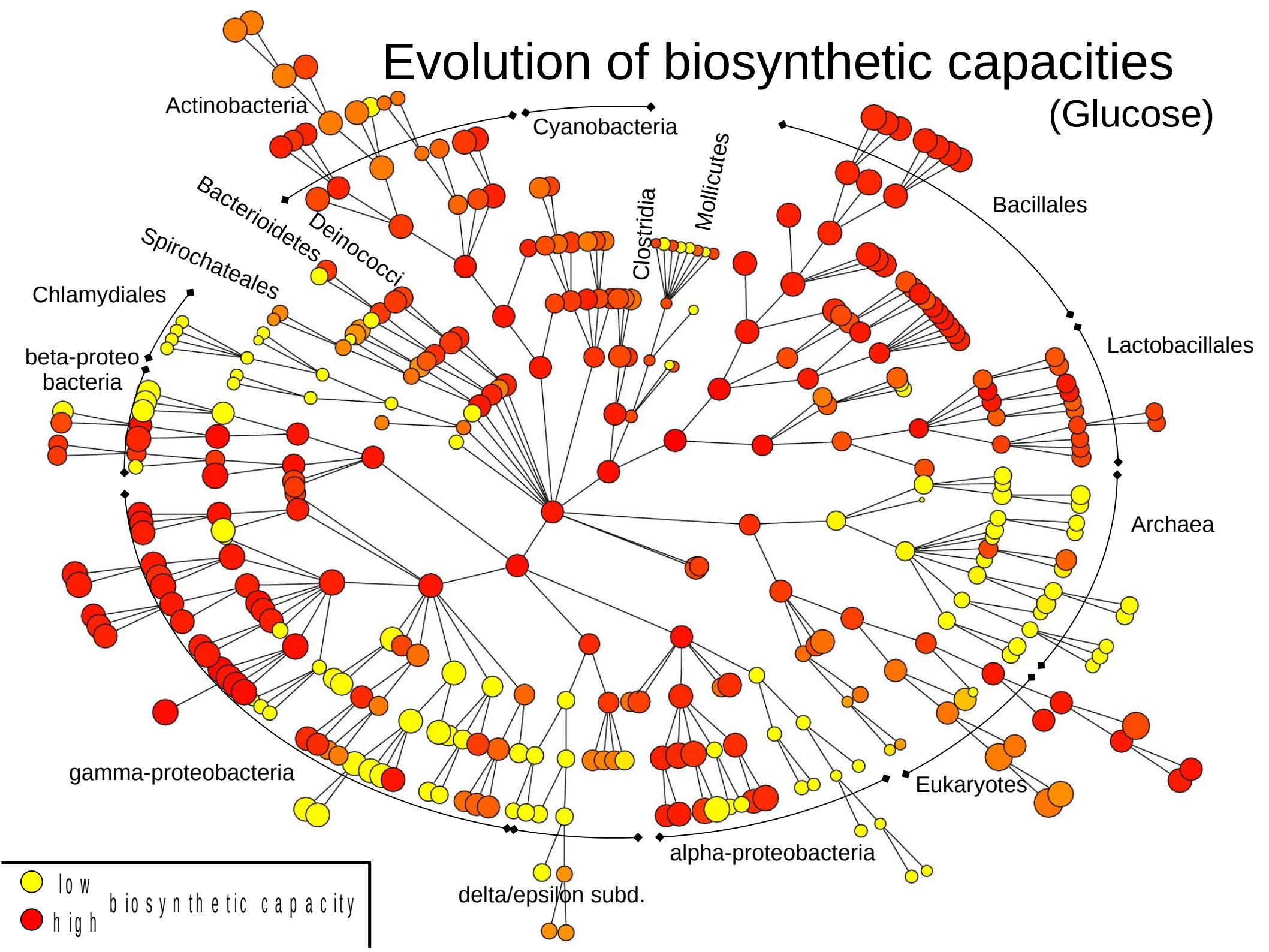
How did the metabolic networks of ancestral species look like?

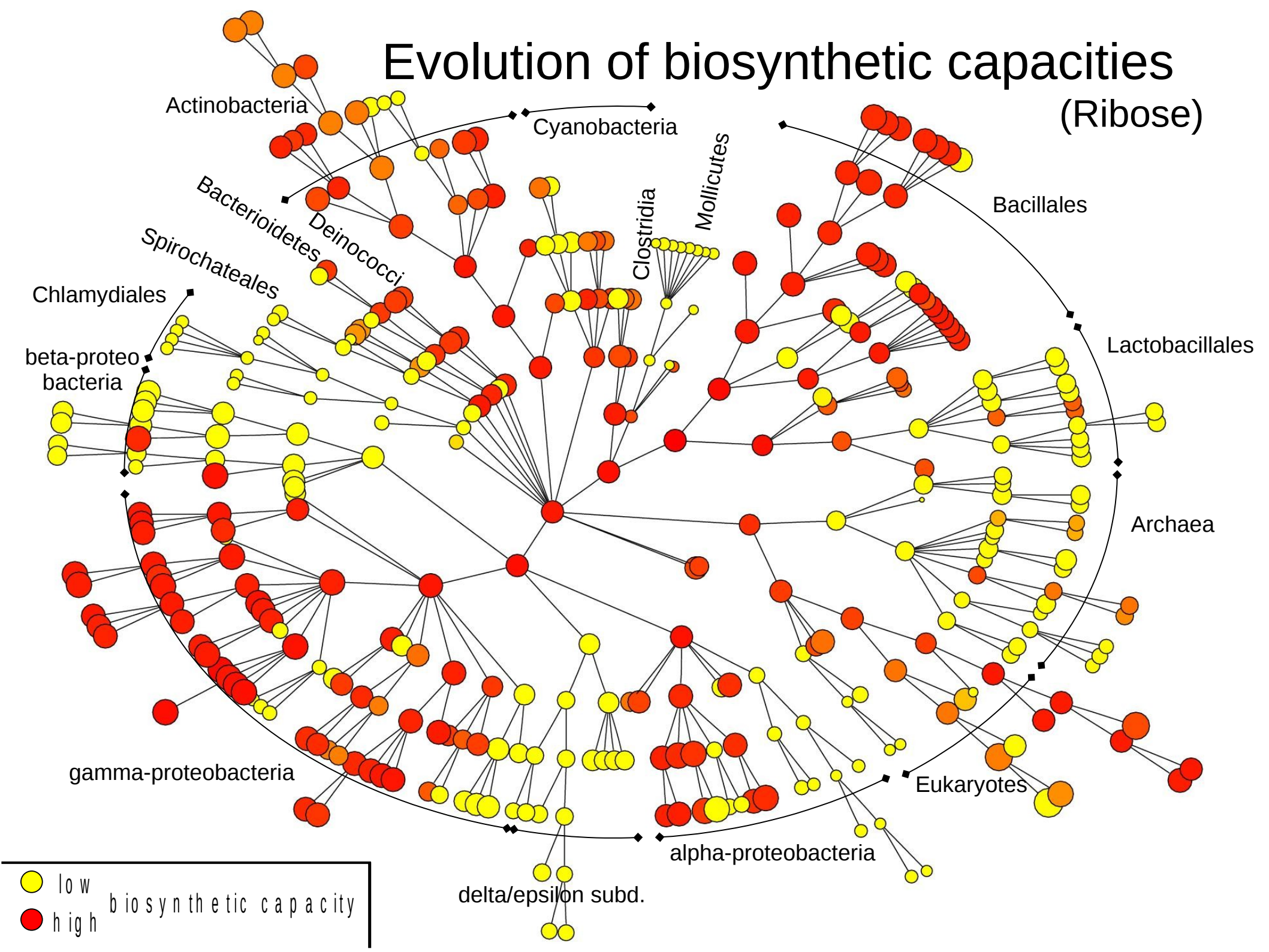


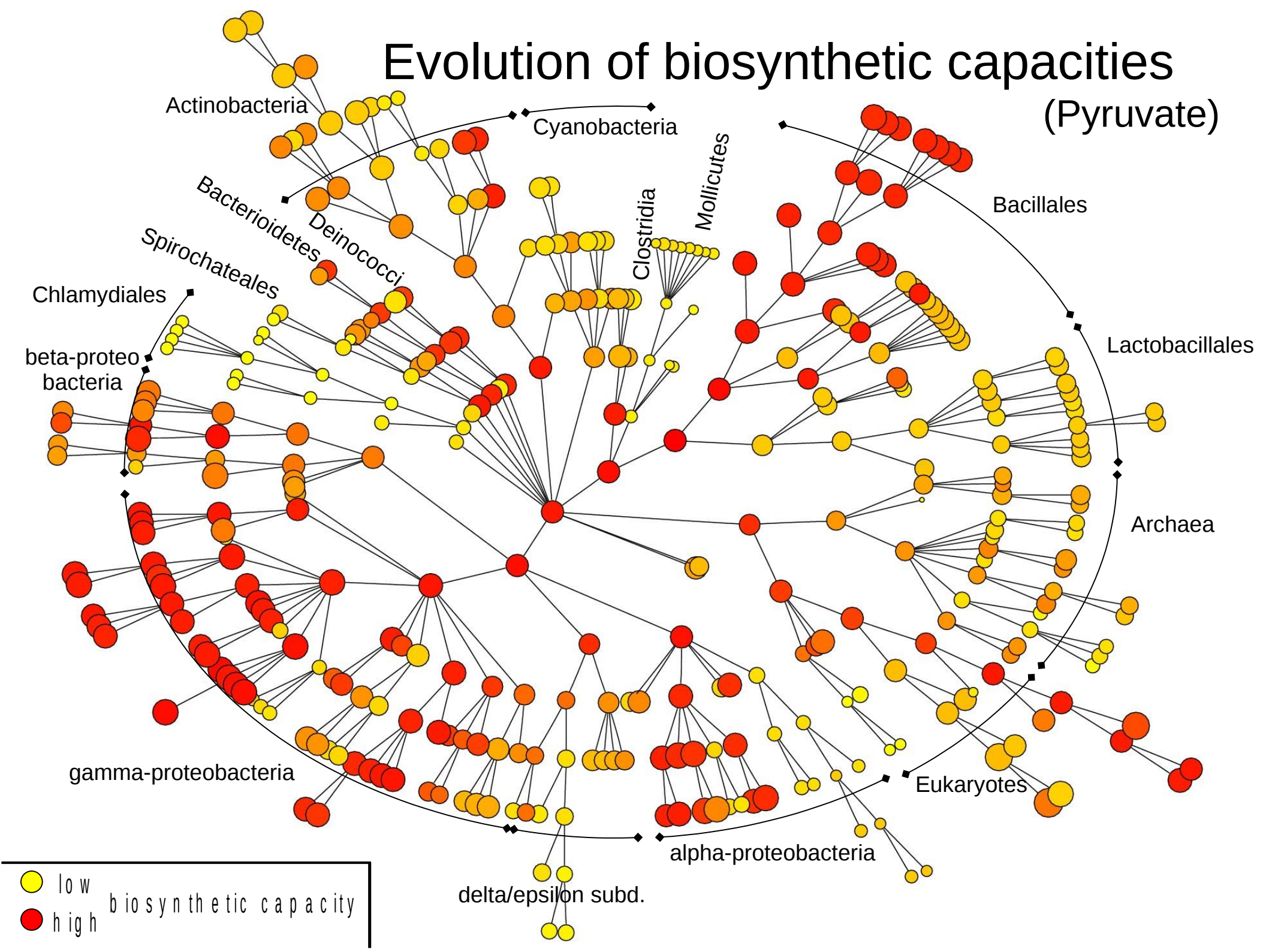
We calculated the *most likely scenario* for these networks



Ebenhöh, Handorf & Kahn, IEE Syst. Biol., 2006

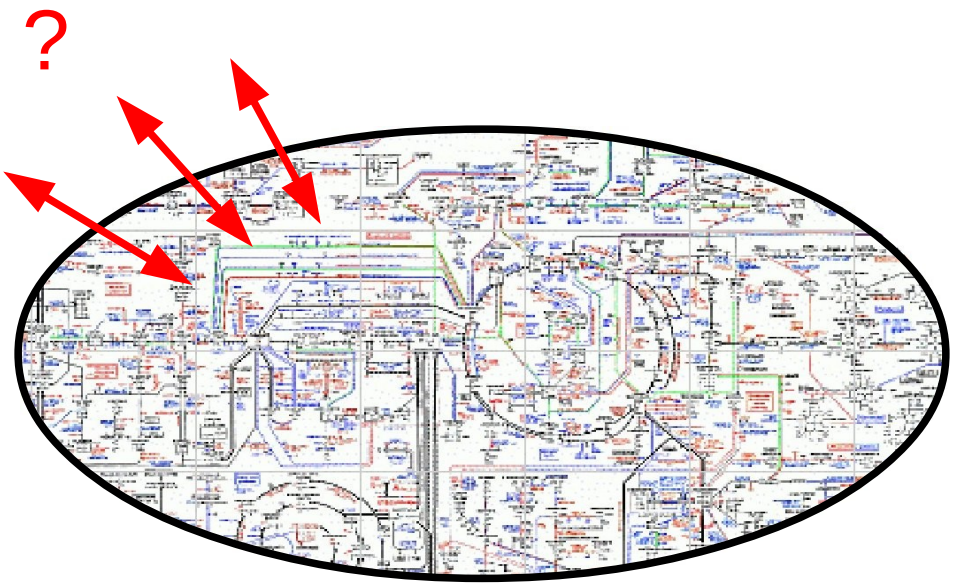

Evolution of biosynthetic capacities
(Glucose)
Actinobacteria
Cyanobacteria
Bacterioidetes
Deinococci
Spirochateales
Clostridia
Mollicutes
Bacillales
Chlamydiales
beta-proteo
bacteria
Lactobacillales
Archaea
gamma-proteobacteria
Eukaryotes
alpha-proteobacteria
delta/epsilon subd.
low
high
biosynthetic capacity


Evolution of biosynthetic capacities
(Ribose)
Actinobacteria
Cyanobacteria
Bacterioidetes
Deinococci
Spirochateales
Chlamydiales
Clostridia
Mollicutes
Bacillales
Lactobacillales
beta-proteo
bacteria
Archaea
gamma-proteobacteria
Eukaryotes
alpha-proteobacteria
delta/epsilon subd.
low
high
biosynthetic capacity


Evolution of biosynthetic capacities
(Pyruvate)
Actinobacteria
Cyanobacteria
Bacterioidetes
Deinococci
Spirochateales
Chlamydiales
Clostridia
Mollicutes
Bacillales
Lactobacillales
beta-proteo
bacteria
Archaea
gamma-proteobacteria
Eukaryotes
alpha-proteobacteria
delta/epsilon subd.
low
high
biosynthetic capacity

The inverse problem

Networks are relatively easy to obtain (e.g. from KEGG)

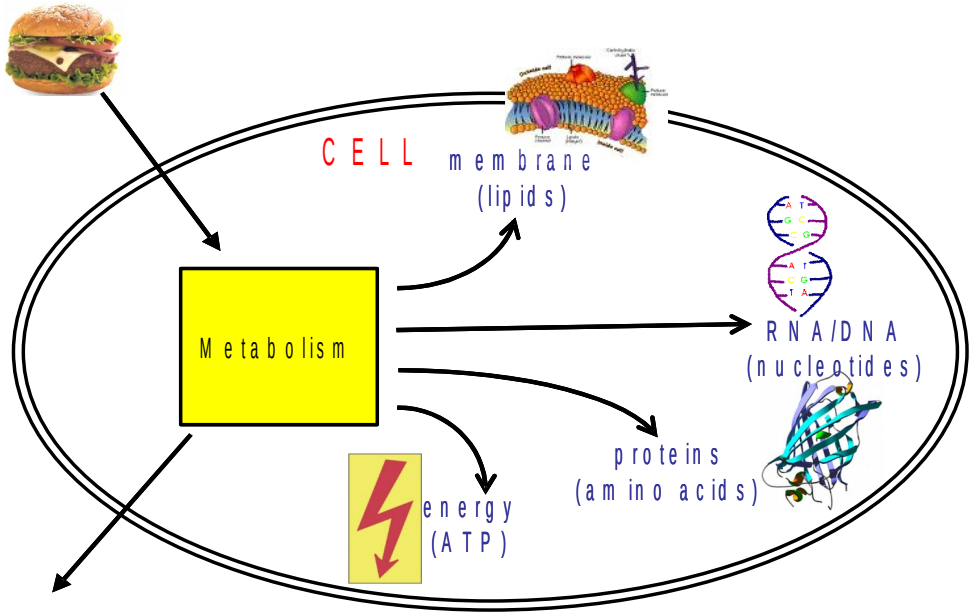


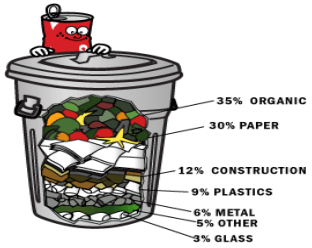
... but information of transport processes across the cellular membrane is often poorly characterized!

CAN WE PREDICT NUTRIENT MEDIA FROM THE NETWORK STRUCTURE?

Inferring nutrient requirements

Biological knowledge

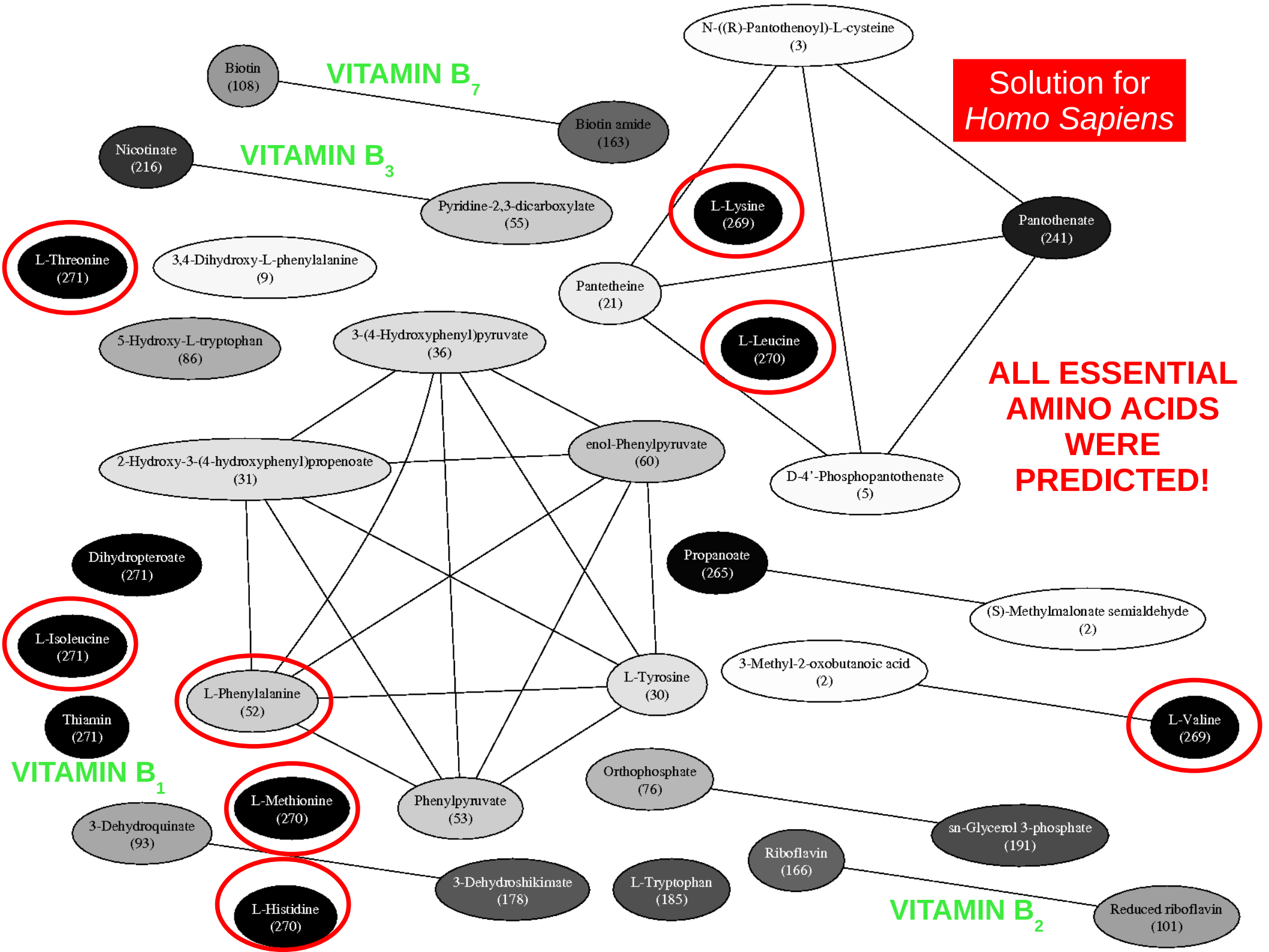


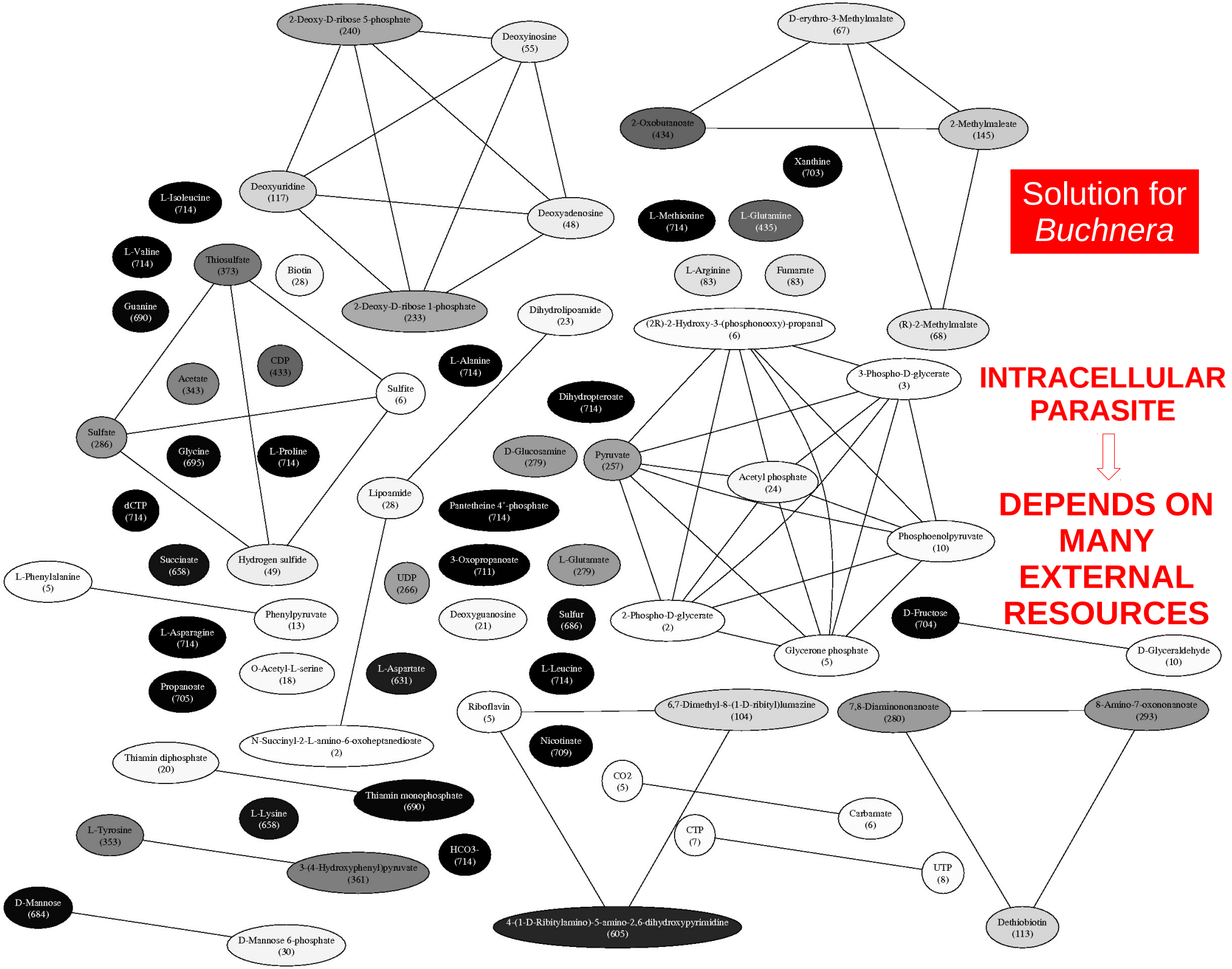


Mathematics

Every network must be able to produce precursors:

- amino acids
- nucleotides
- lipids
- energy
- etc...

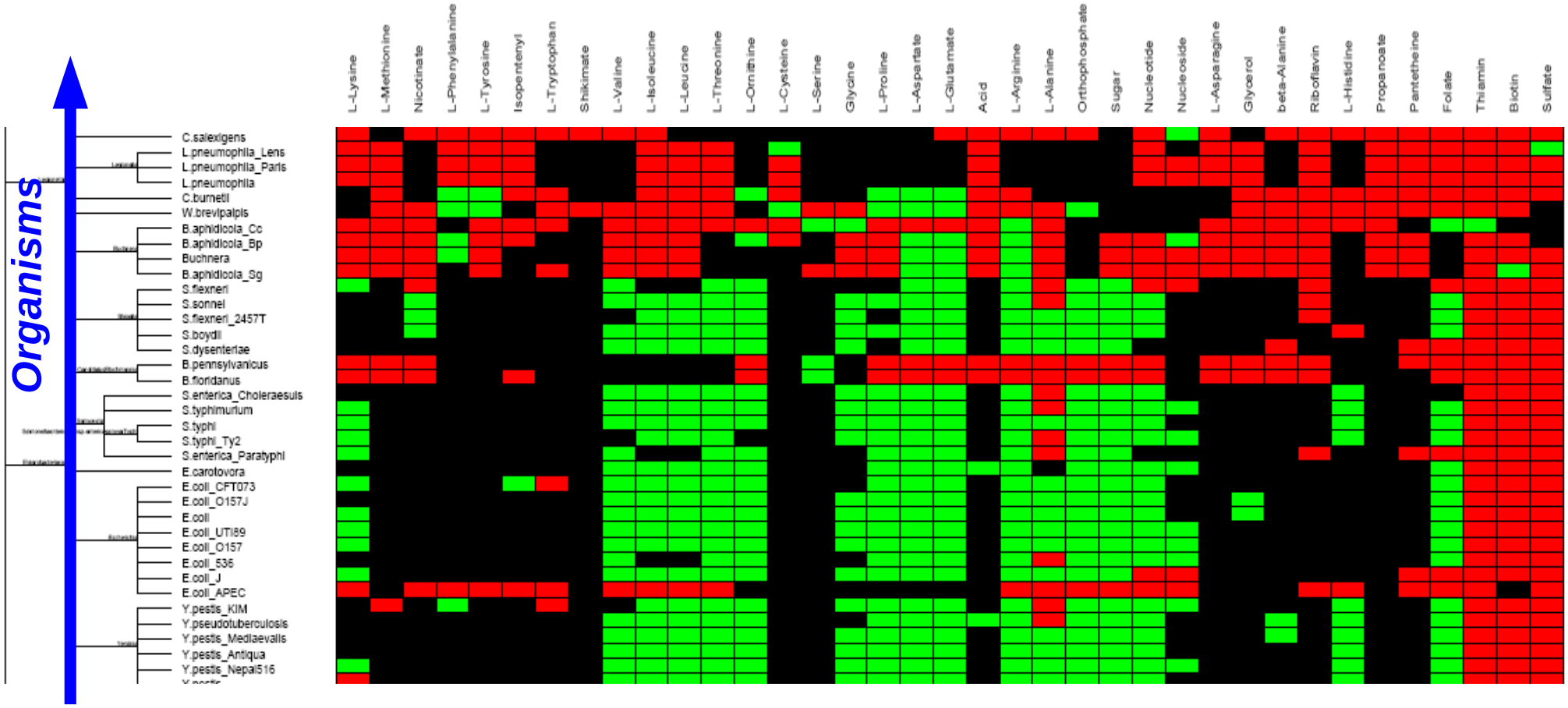

Solution for
Homo Sapiens
VITAMIN B7
Biotin
(108)
Biotin amide
(163)
VITAMIN B3
Nicotinate
(216)
Pyridine-2,3-dicarboxylate
(55)
N-((R)-Pantothenoyl)-L-cysteine
(3)
L-Lysine
(269)
Pantothenate
(241)
L-Threonine
(271)
3,4-Dihydroxy-L-phenylalanine
(9)
Pantetheine
(21)
L-Leucine
(270)
5-Hydroxy-L-tryptophan
(86)
3-(4-Hydroxyphenyl)pyruvate
(36)
ALL ESSENTIAL
AMINO ACIDS
WERE
PREDICTED!
2-Hydroxy-3-(4-hydroxyphenyl)propenoate
(31)
enol-Phenylpyruvate
(60)
D-4'-Phosphopantothenate
(5)
Dihydropteroate
(271)
Propanoate
(265)
(S)-Methylmalonate semialdehyde
(2)
L-Isoleucine
(271)
3-Methyl-2-oxobutanoic acid
(2)
L-Phenylalanine
(52)
L-Tyrosine
(30)
L-Valine
(269)
Thiamin
(271)
VITAMIN B1
Orthophosphate
(76)
L-Methionine
(270)
Phenylpyruvate
(53)
3-Dehydroquinate
(93)
sn-Glycerol 3-phosphate
(191)
Riboflavin
(166)
3-Dehydroshikimate
(178)
L-Tryptophan
(185)
L-Histidine
(270)
VITAMIN B2
Reduced riboflavin
(101)


2-Deoxy-D-ribose 5-phosphate (240)
Deoxyinosine (55)
Deoxyuridine (117)
Deoxyadenosine (48)
2-Deoxy-D-ribose 1-phosphate (233)
L-Isoleucine (714)
L-Valine (714)
Guanine (690)
Thiosulfate (373)
Biotin (28)
CDP (433)
Acetate (343)
Sulfite (6)
Sulfate (286)
Glycine (695)
L-Proline (714)
dCTP (714)
Succinate (658)
Hydrogen sulfide (49)
L-Phenylalanine (5)
Phenylpyruvate (13)
L-Asparagine (714)
O-Acetyl-L-serine (18)
Propanoate (705)
L-Alanine (714)
Dihydrolipoamide (23)
Lipoamide (28)
UDP (266)
L-Aspartate (631)
N-Succinyl-2-L-amino-6-oxoheptanedioate (2)
Thiamin diphosphate (20)
Thiamin monophosphate (690)
L-Lysine (658)
L-Tyrosine (353)
3-(4-Hydroxyphenyl)pyruvate (361)
HCO3- (714)
D-Mannose (684)
D-Mannose 6-phosphate (30)
D-erythro-3-Methylmalate (67)
2-Oxobutanoate (434)
2-Methylmaleate (145)
Xanthine (703)
L-Methionine (714)
L-Glutamine (435)
L-Arginine (83)
Fumarate (83)
(2R)-2-Hydroxy-3-(phosphonooxy)-propanal (6)
(R)-2-Methylmalate (68)
3-Phospho-D-glycerate (3)
Dihydropteroate (714)
D-Glucosamine (279)
Pyruvate (257)
Acetyl phosphate (24)
Pantetheine 4'-phosphate (714)
3-Oxopropanoate (711)
L-Glutamate (279)
Deoxyguanosine (21)
Sulfur (686)
Phosphoenolpyruvate (10)
2-Phospho-D-glycerate (2)
Glycerone phosphate (5)
D-Fructose (704)
D-Glyceraldehyde (10)
L-Leucine (714)
Riboflavin (5)
6,7-Dimethyl-8-(1-D-ribityl)lumazine (104)
7,8-Diaminononanoate (280)
8-Amino-7-oxononanoate (293)
Nicotinate (709)
CO2 (5)
Carbamate (6)
CTP (7)
UTP (8)
4-(1-D-Ribitylamino)-5-amino-2,6-dihydroxypyrimidine (605)
Dethiobiotin (113)
Solution for Buchnera
INTRACELLULAR PARASITE
DEPENDS ON MANY EXTERNAL RESOURCES

Global resource types

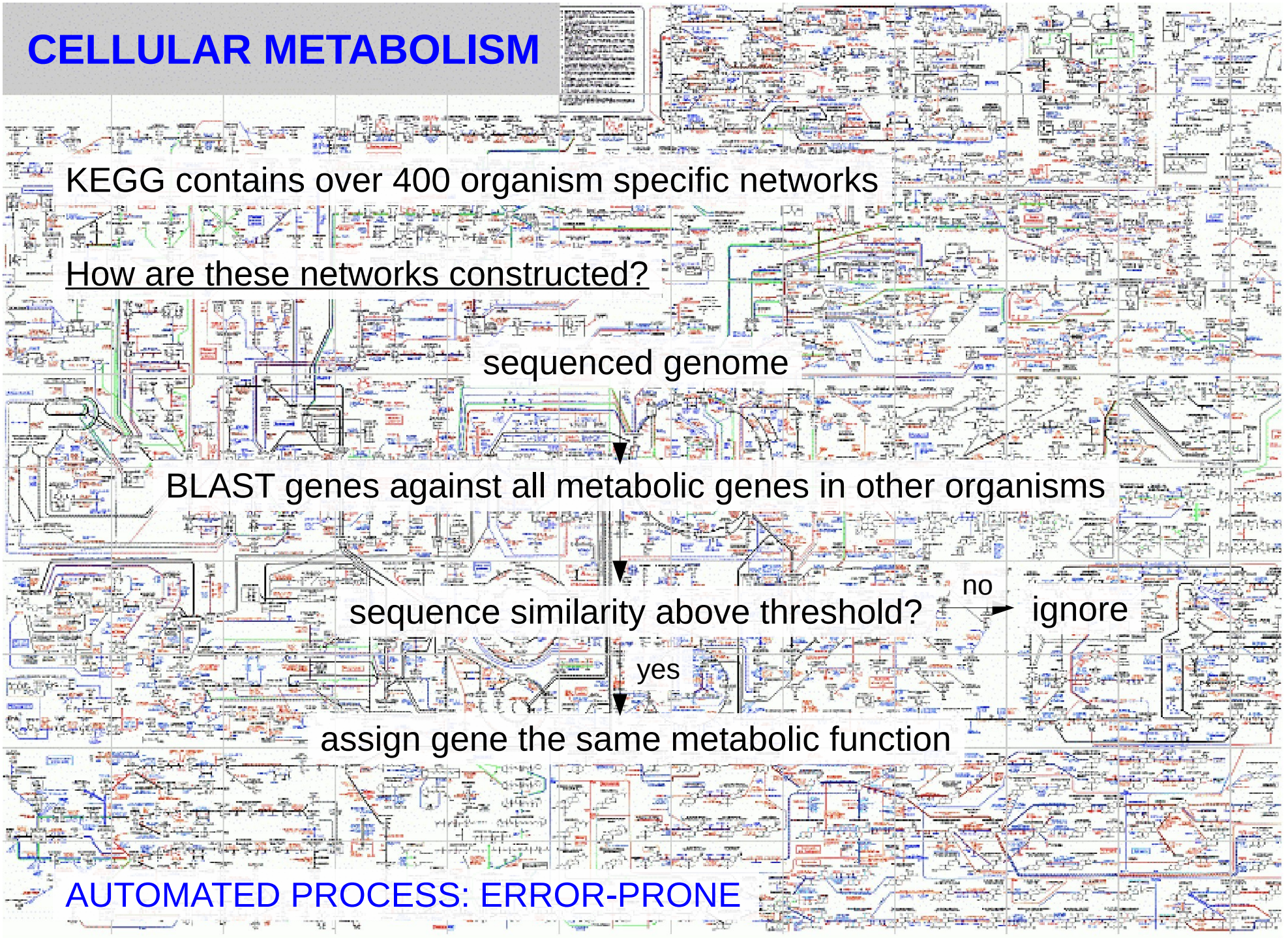
The comparison of the results for 400 organisms allows to define

36 global resource types



Handorf, Christian, Ebenhöh & Kahn, JTB, 2008

CELLULAR METABOLISM



KEGG contains over 400 organism specific networks

How are these networks constructed?

sequenced genome

BLAST genes against all metabolic genes in other organisms

sequence similarity above threshold?

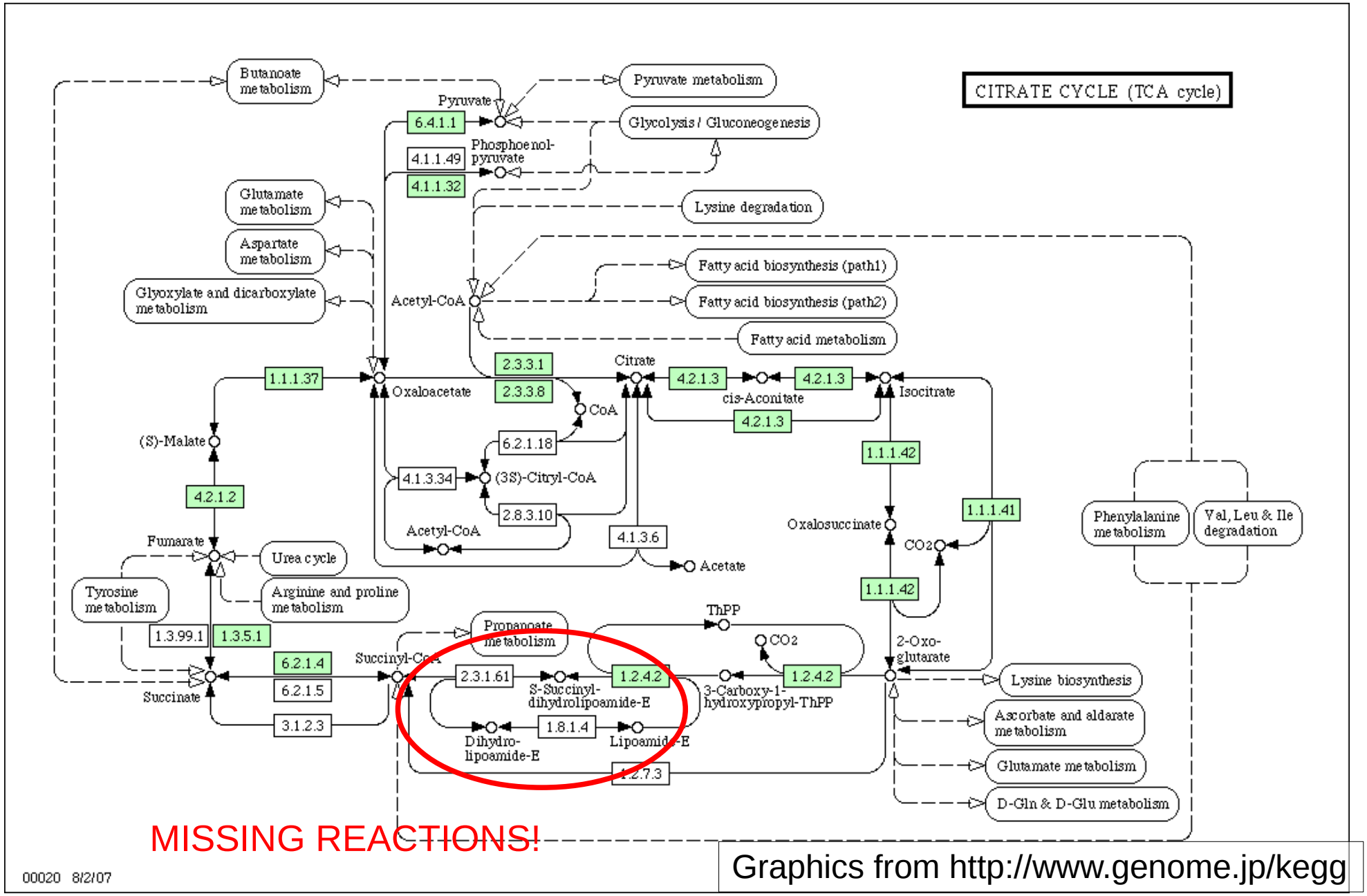
no → ignore

yes

assign gene the same metabolic function

AUTOMATED PROCESS: ERROR-PRONE

Closing gaps in metabolism

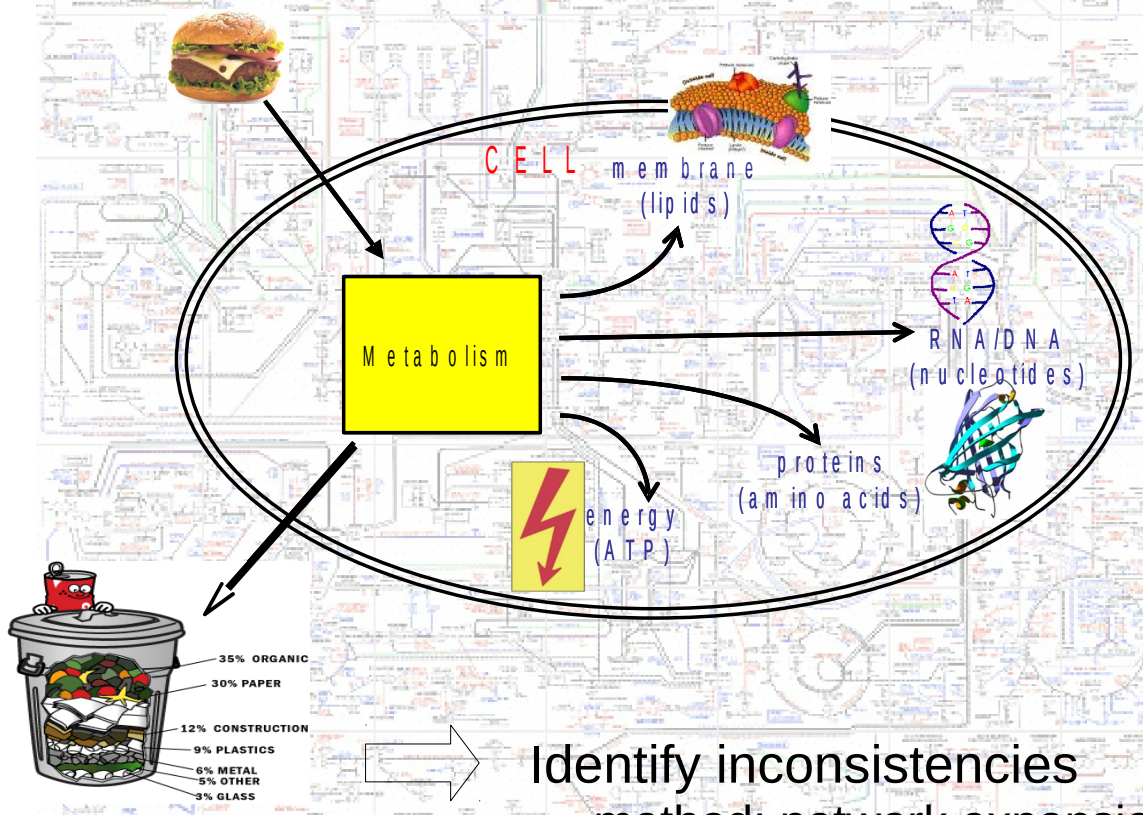


MISSING REACTIONS!

Graphics from http://www.genome.jp/kegg

Combining expert knowledge with mathematics

Expert knowledge



Mathematics

Plausibility check:

Test whether the network can produce from experimentally verified growth media

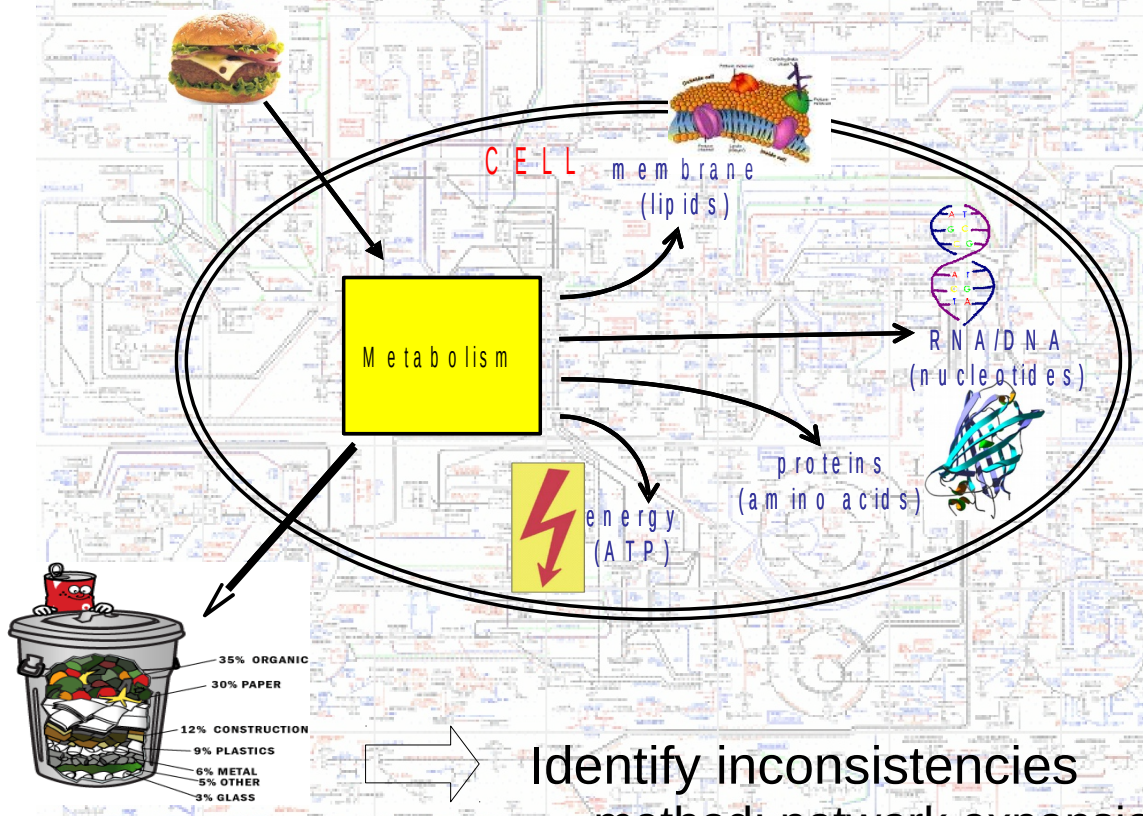
- amino acids
- nucleotides
- lipids
- energy
- other experimentally observed metabolites

Identify inconsistencies

method: network expansion (Handorf et. al, 2005)

Combining expert knowledge with mathematics

Expert knowledge



Mathematics

Plausibility check:

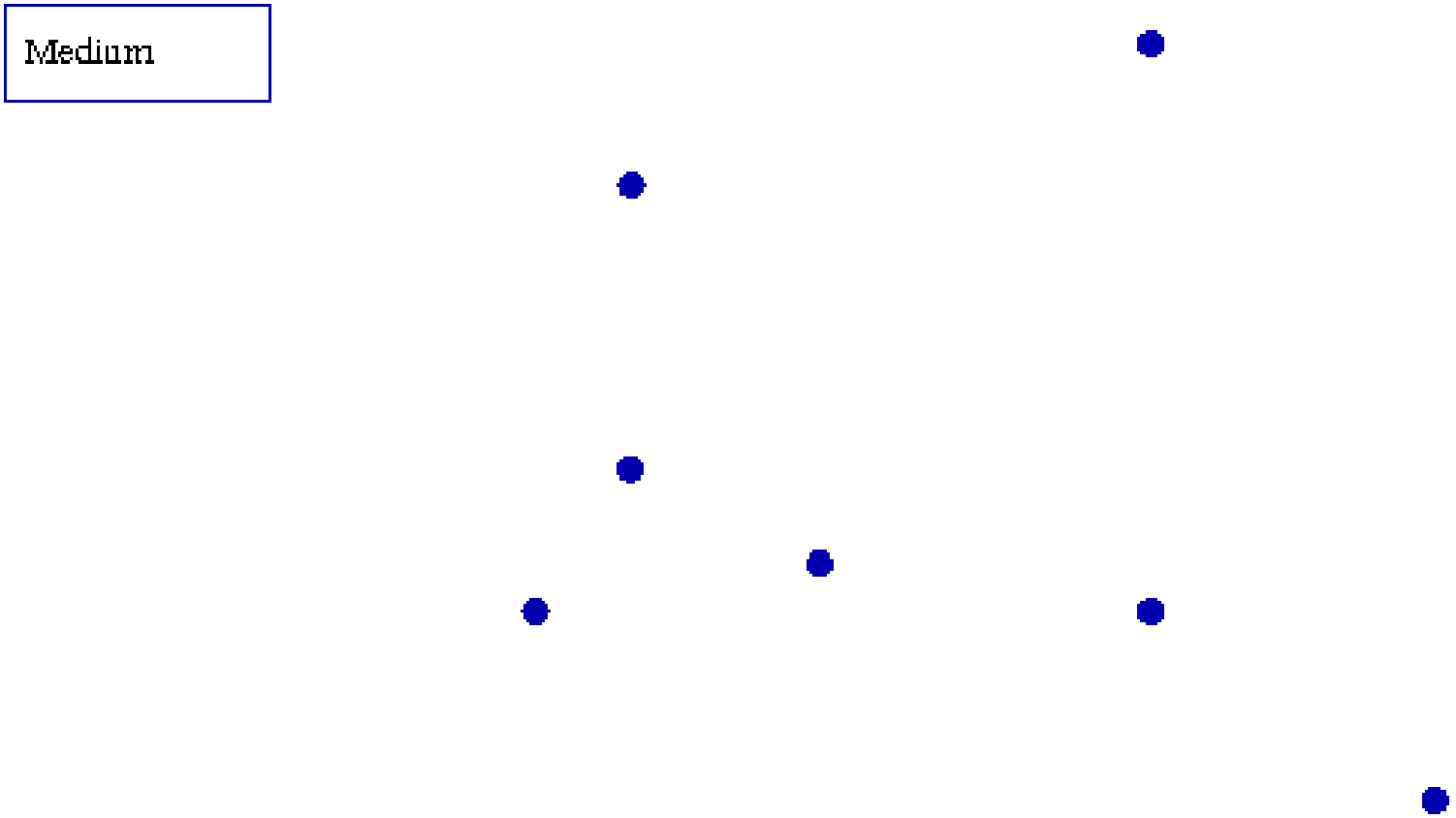
Test whether the network can produce from experimentally verified growth media

- amino acids
- nucleotides
- lipids
- energy
- other experimentally observed metabolites

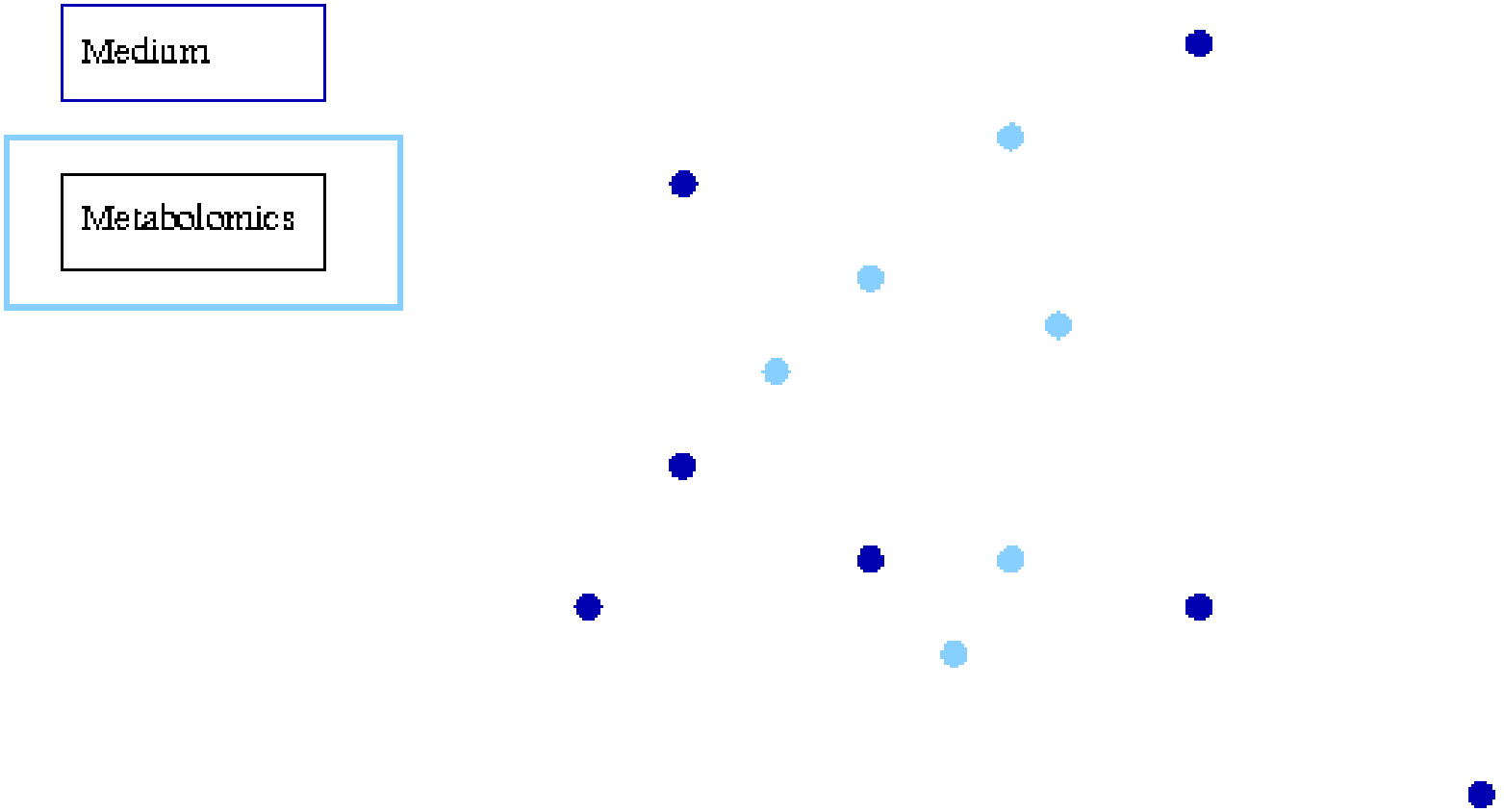
⇨ Identify inconsistencies
method: network expansion (Handorf et. al, 2005)

⇨ Fix networks

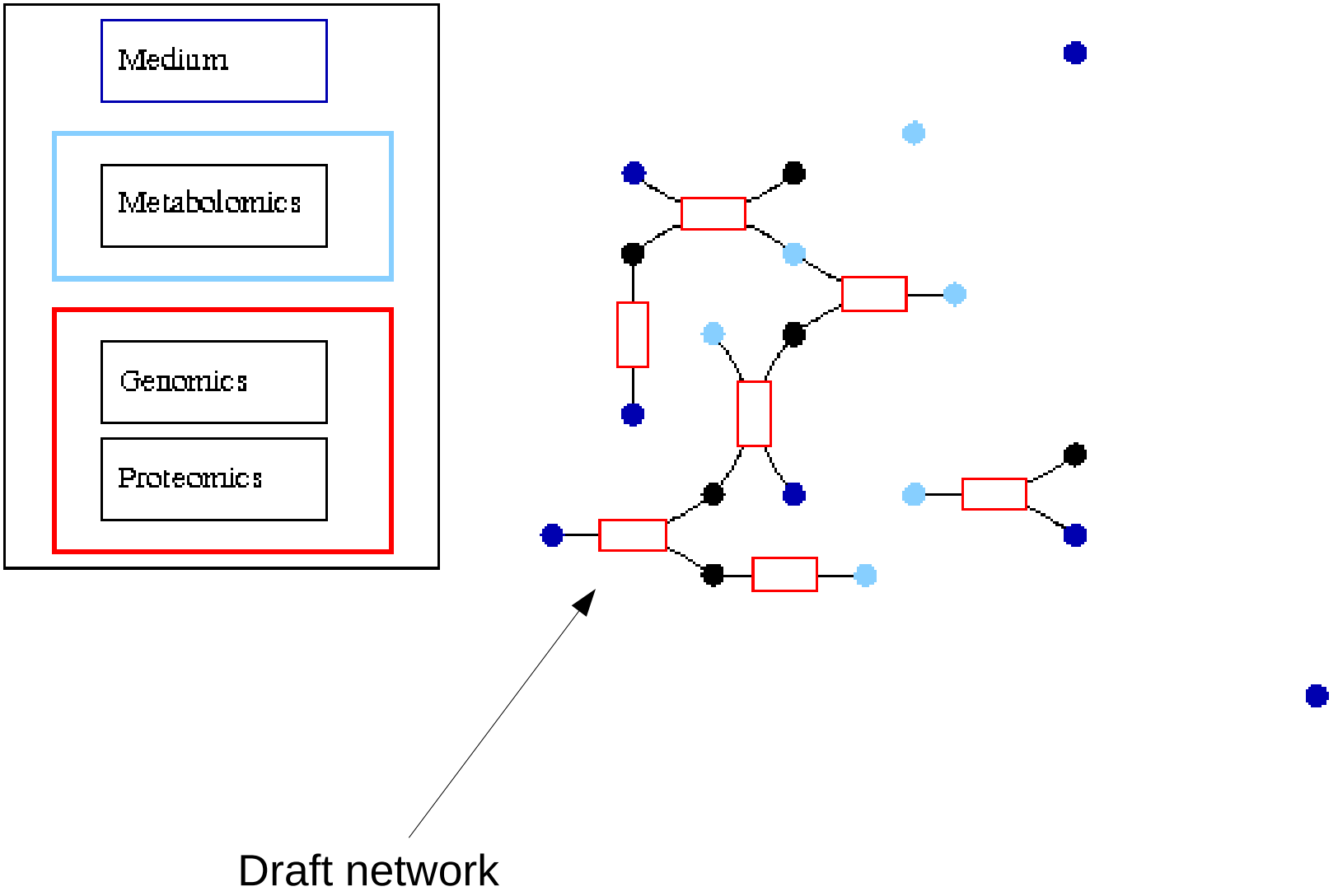
Filling the gaps



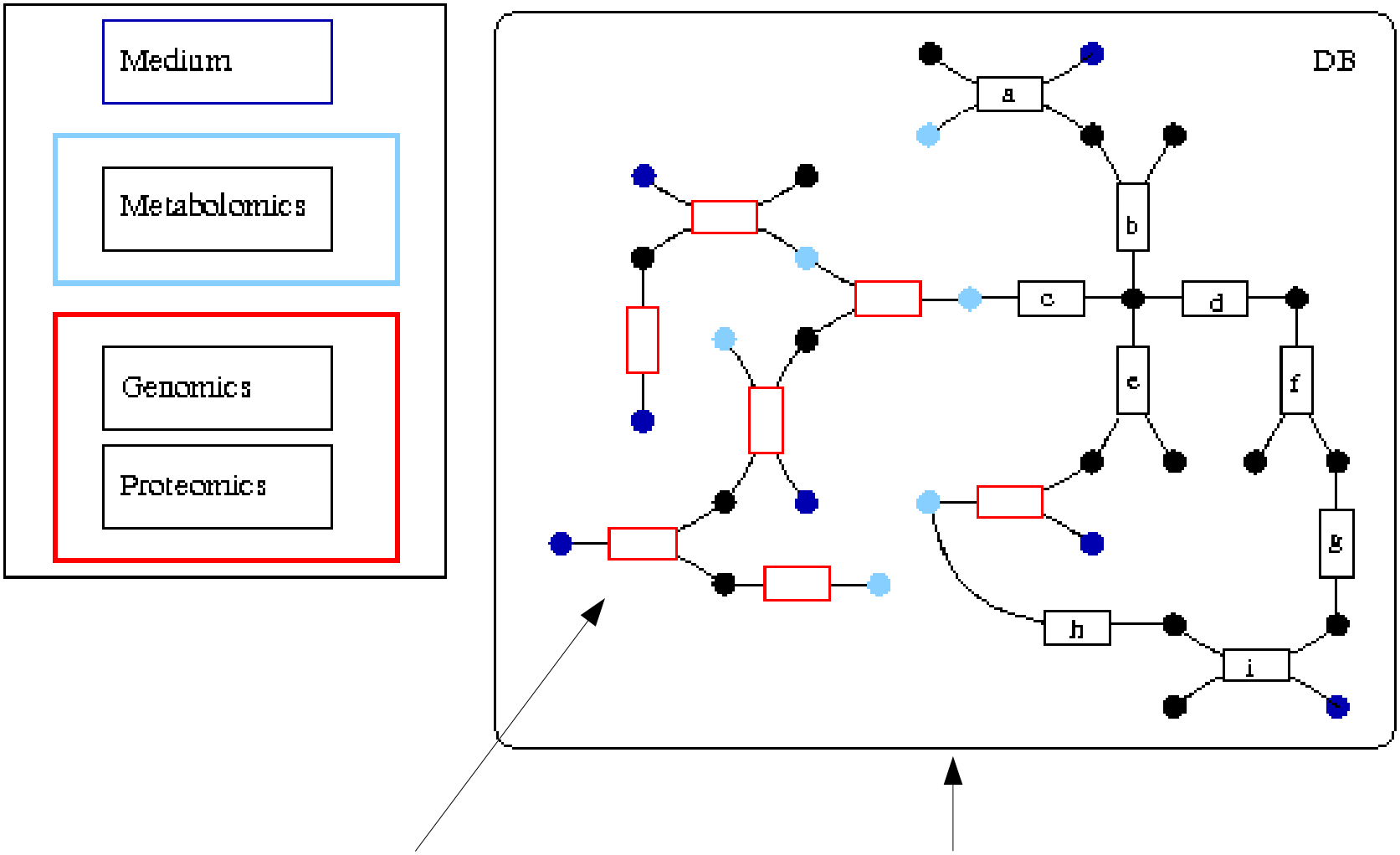
Filling the gaps



Filling the gaps

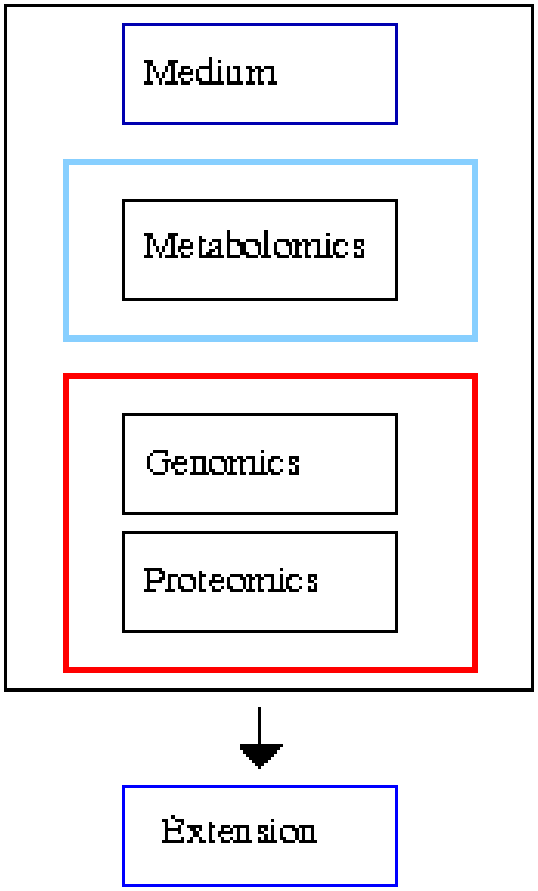


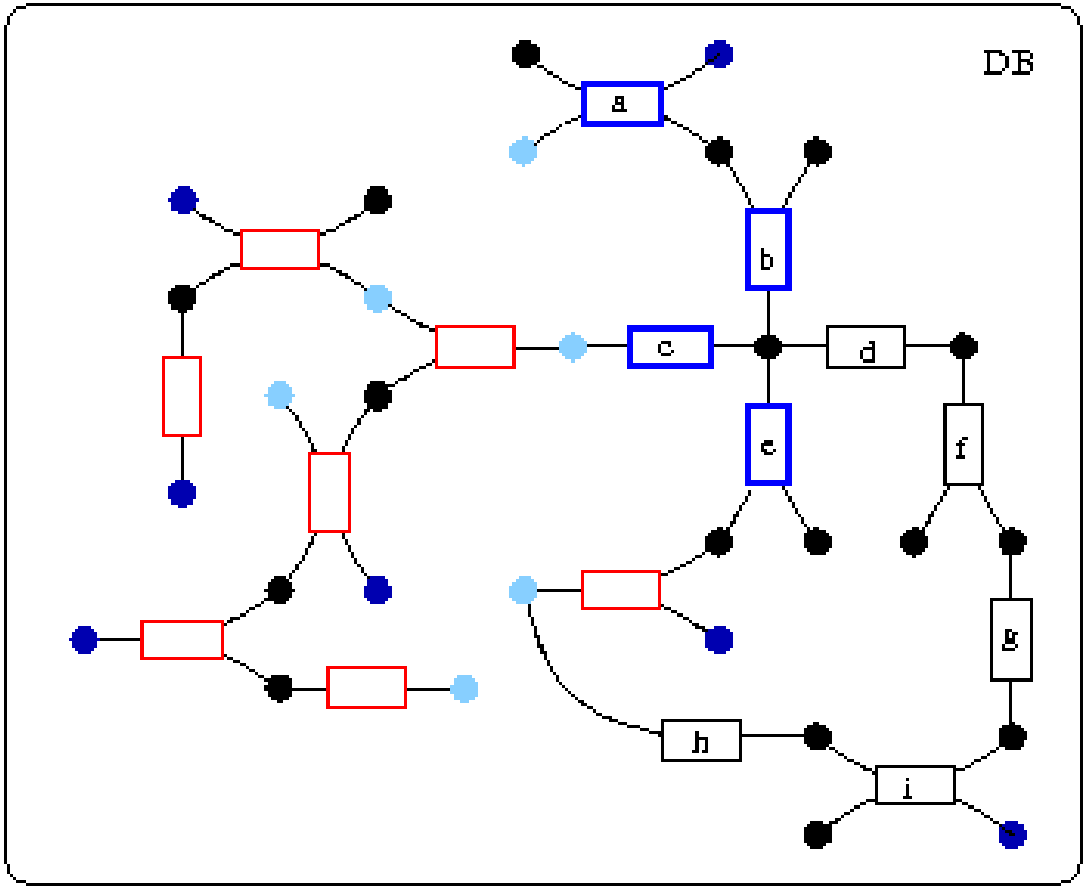
Filling the gaps



Draft network embedded in larger network (from database)

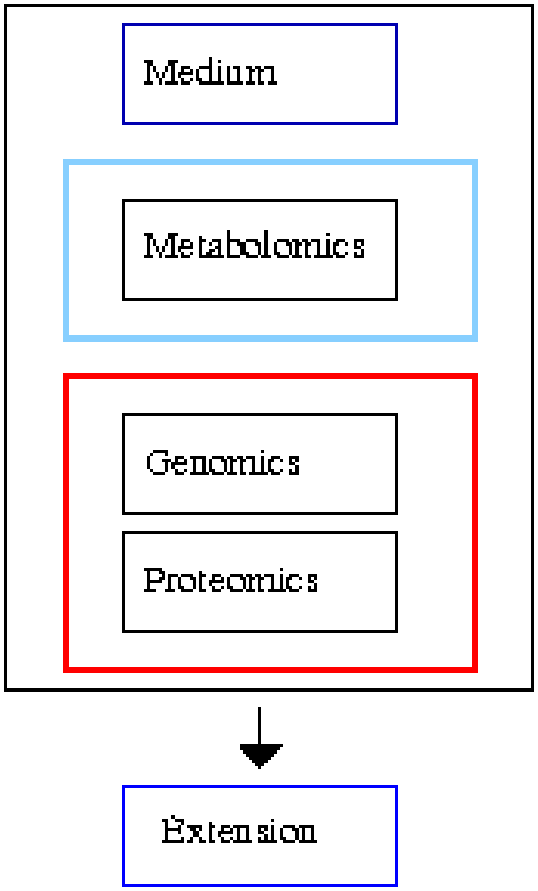
Filling the gaps

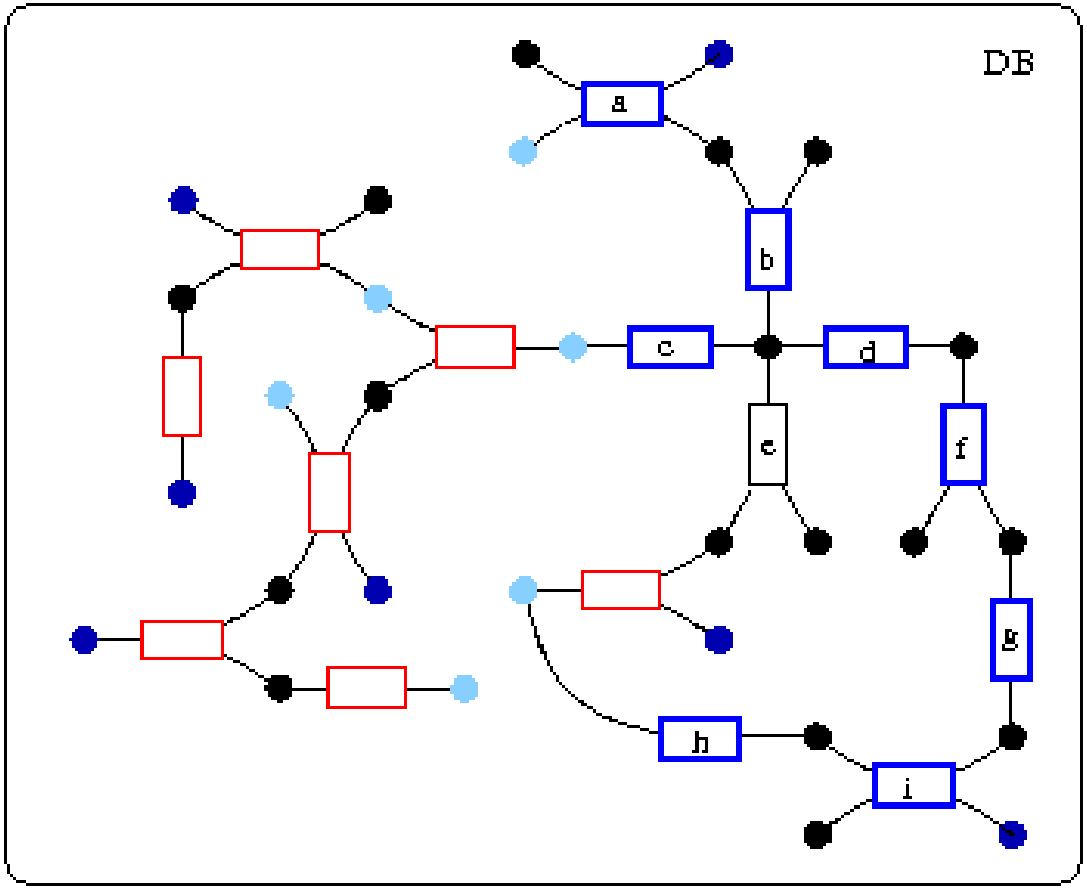




Solution 1: minimal extension with 4 reactions

Filling the gaps

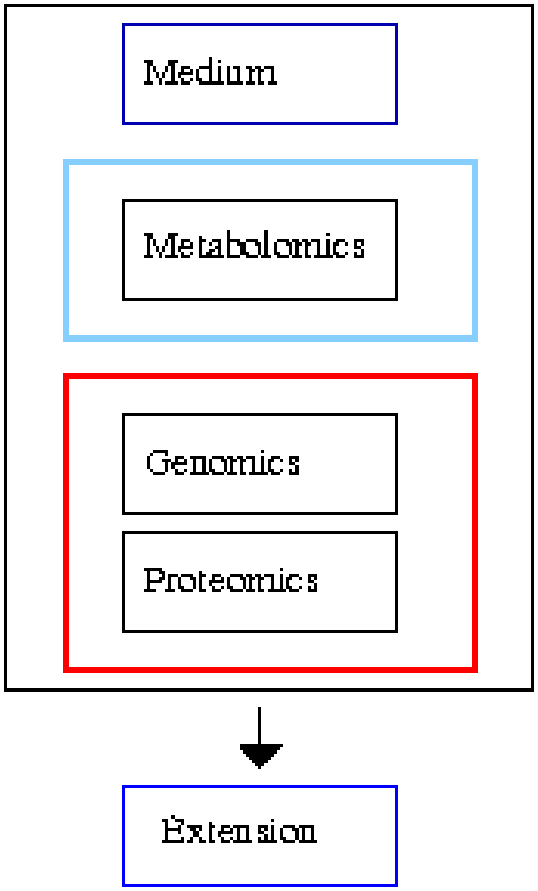


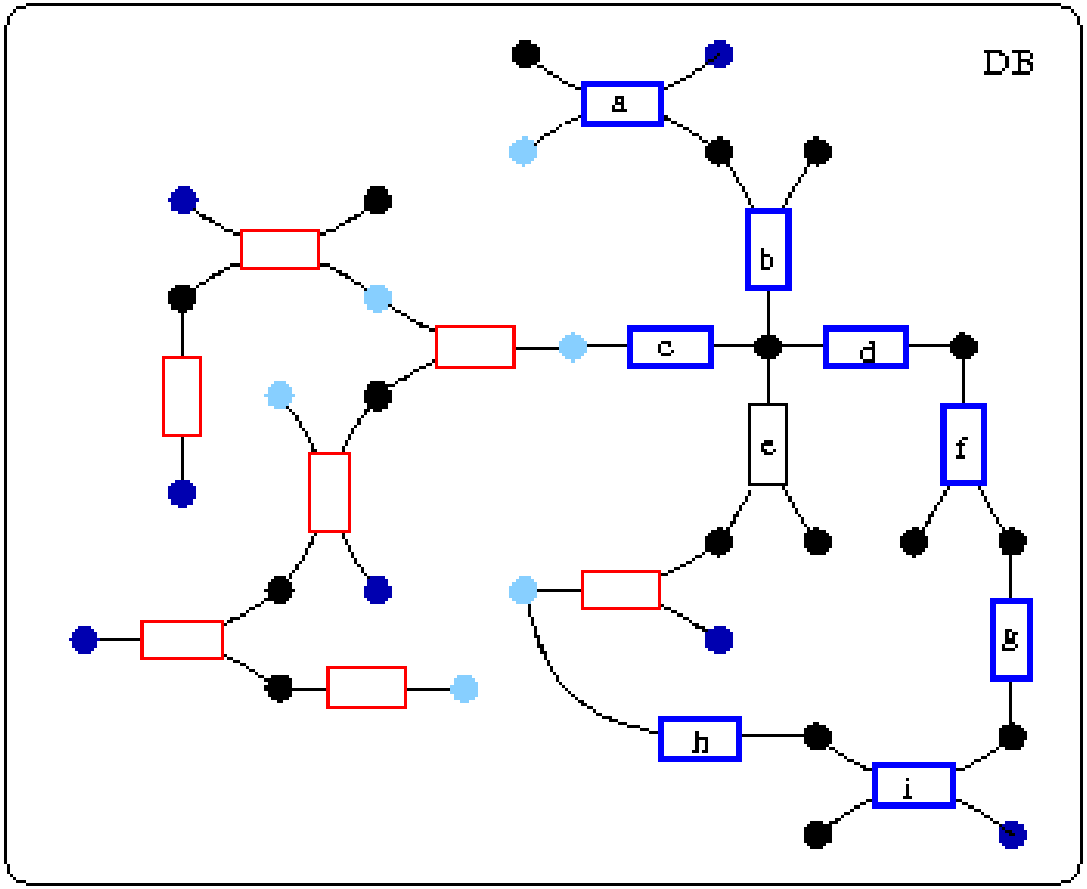


Solution 1: minimal extension with 4 reactions

Solution 2: minimal extension with 8 reactions

Filling the gaps





- greedy algorithm (traversing all reactions)
- depends on the order of the reaction lists

Solution 1: minimal extension with 4 reactions

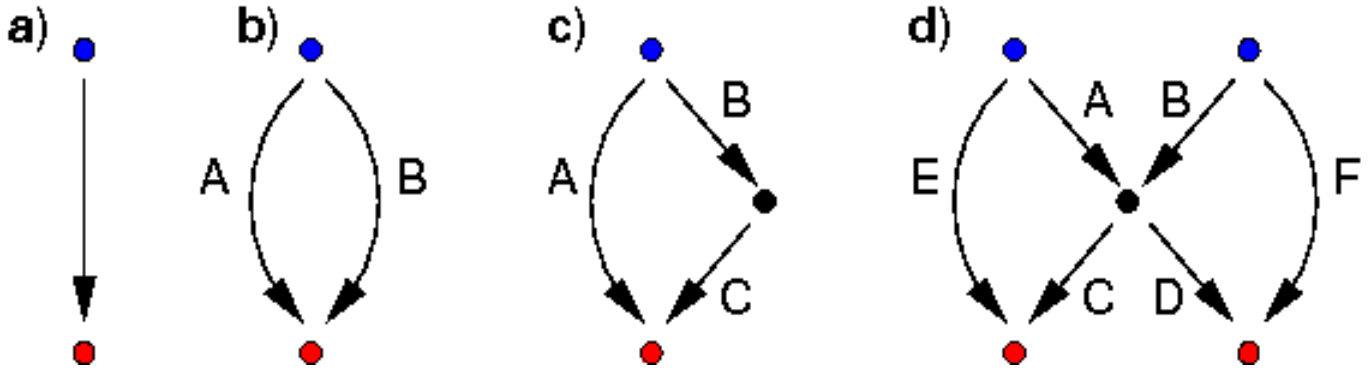
Solution 2: minimal extension with 8 reactions

Simple scenarios

nutrients →

possible missing reactions

observed →



	a)	b)	c)	d)
Minimal solutions:	A	A B	A B+C	A+C+D A+C+F A+D+E B+C+D B+C+F B+D+E E+F

In a realistic case there will be a multitude of solutions!

HOW CAN WE IDENTIFY THE CORRECT SOLUTION?

Case study

Test method on well investigated organism: *E. coli*

Stoichiometric model
from EcoCyc database

↓

Mimick draft networks by
artificially removing reactions

↓

Draft networks unable to
produce essential precursors

↓

Calculate extensions

↓

Compare to originally
removed reactions

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400 draft networks
(100 each with 20,50,100,200 reactions removed)

↓

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↓

Calculate extensions

100 extensions
for all 400 cases

↓

Compare to originally
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Determine prediction quality for all 40000 extensions

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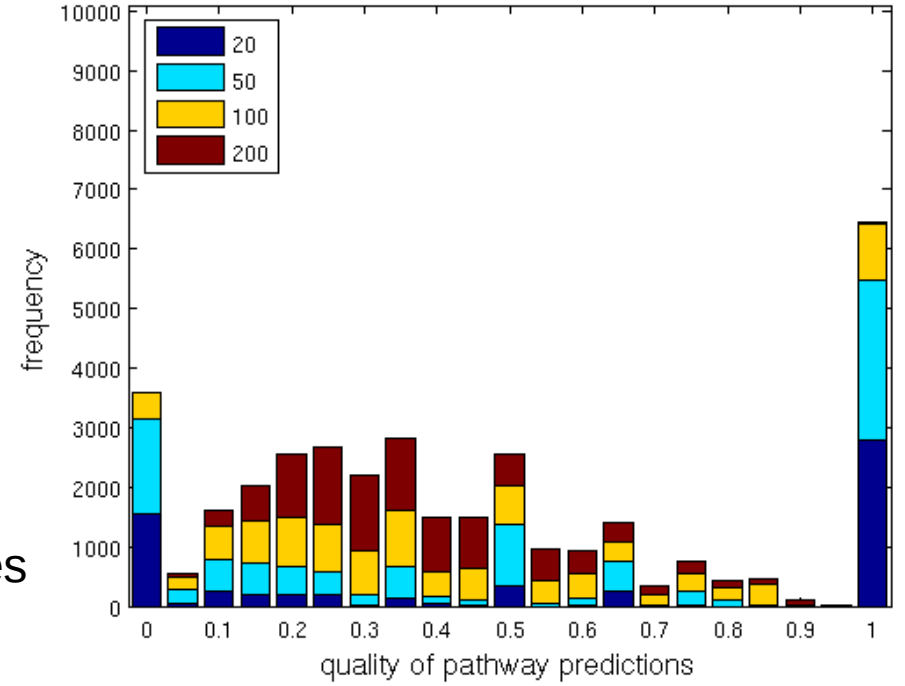
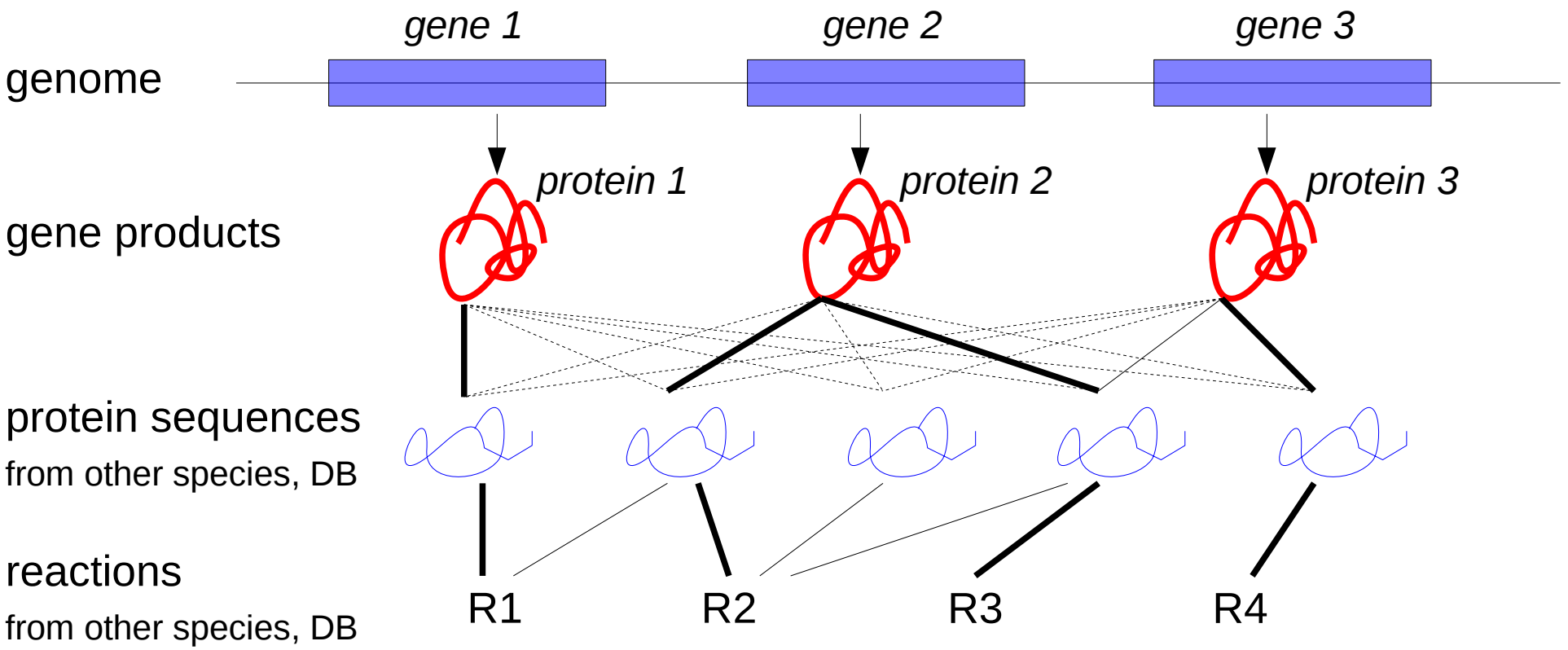


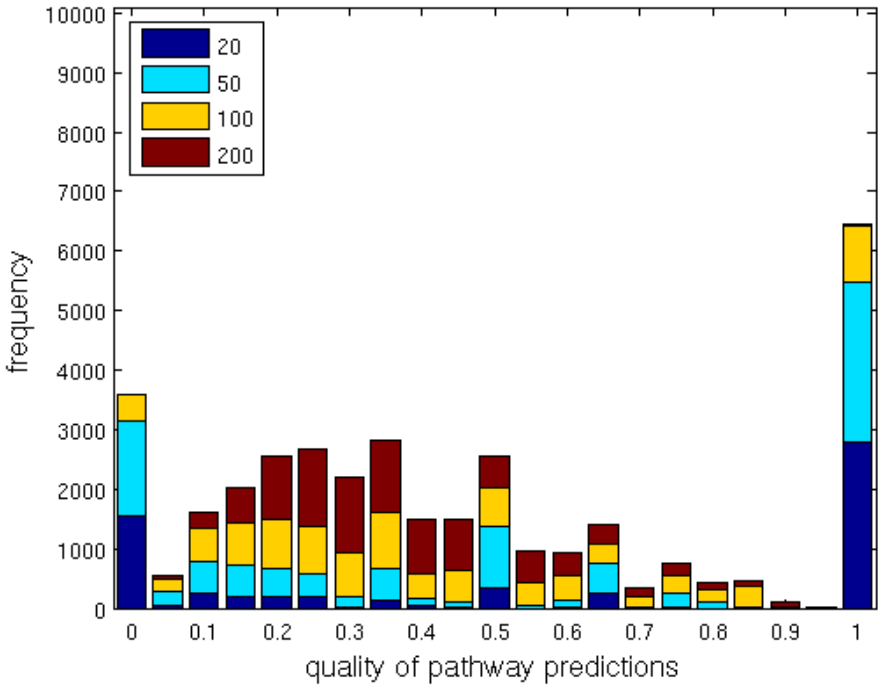
Including genomic information



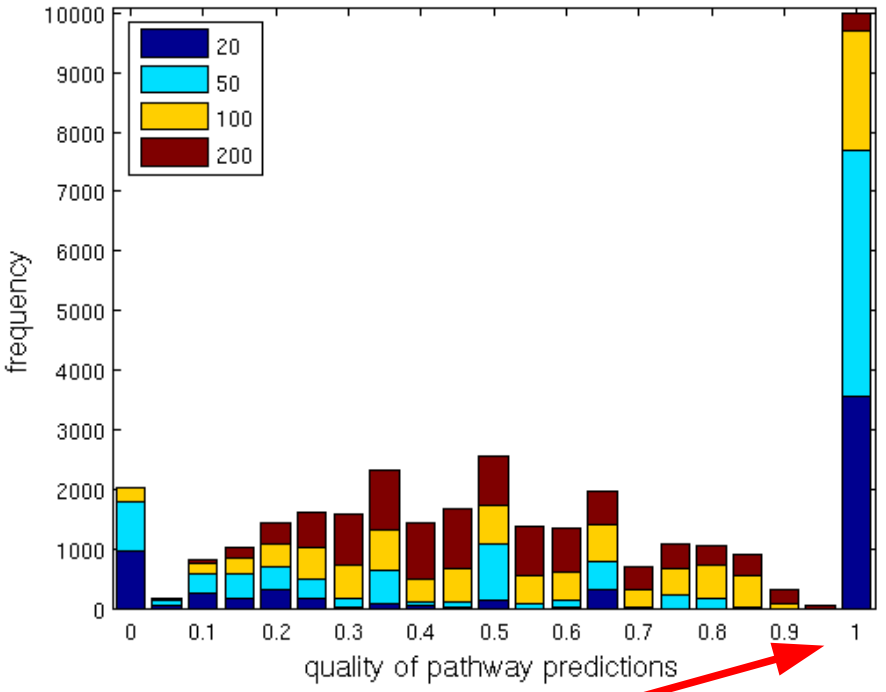
- find best fits and assign scores according to sequence homology
- extensions: preferentially include reactions with good score

Sequence information improves predictions

Fully randomized lists

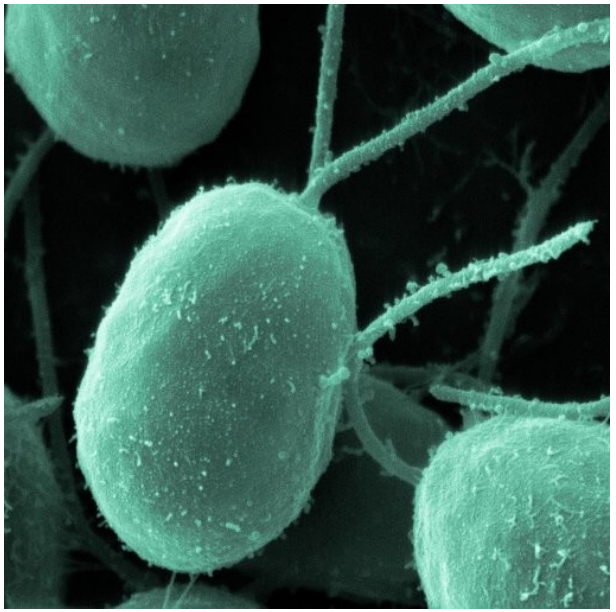


Partly randomized lists
(including sequence information)



fraction of good predictions is considerably improved!

The real world: *Chlamydomonas reinhardtii*



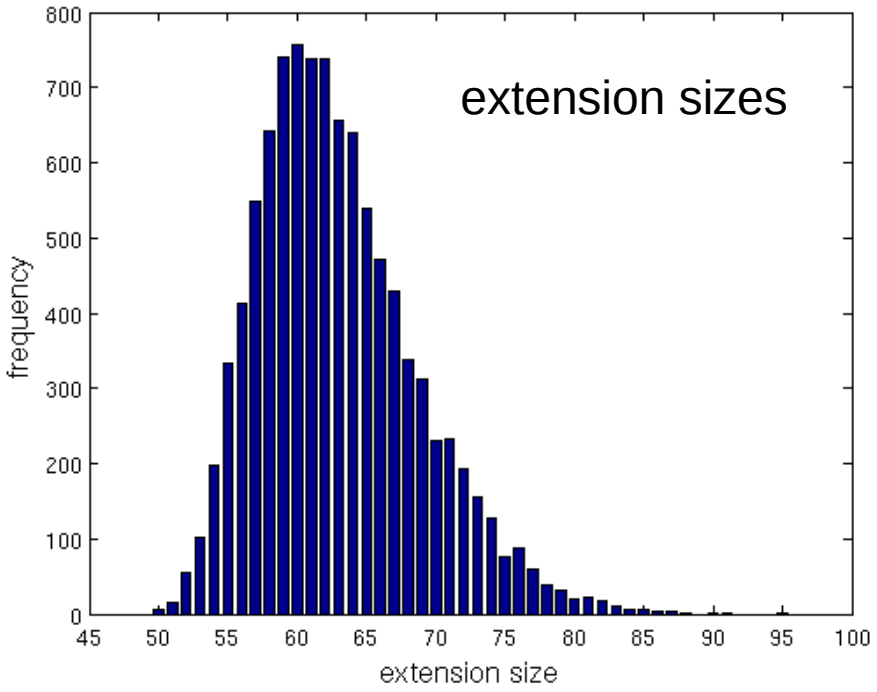
Model organism of the *Go*FORSYS research consortium (***photosynthesis and growth*** **-** http://www.goforsys.de)

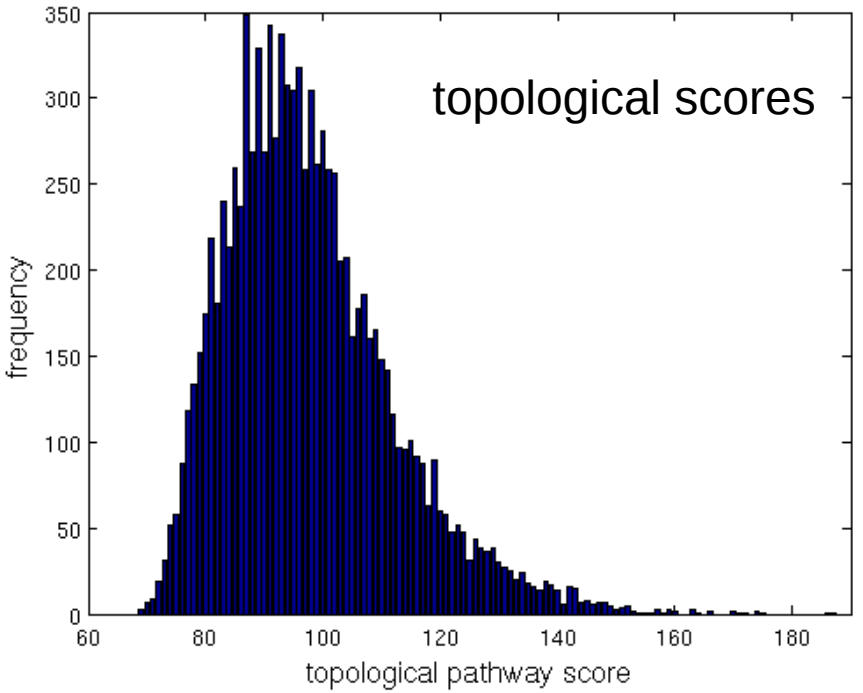
- 15143 genes (JGI)
- 2213 functional annotated genes in KEGG
- 1258 biochemical reactions (Patrick May)
- 159 measured metabolites (Stefan Kempa)
- 30 not producible by draft network

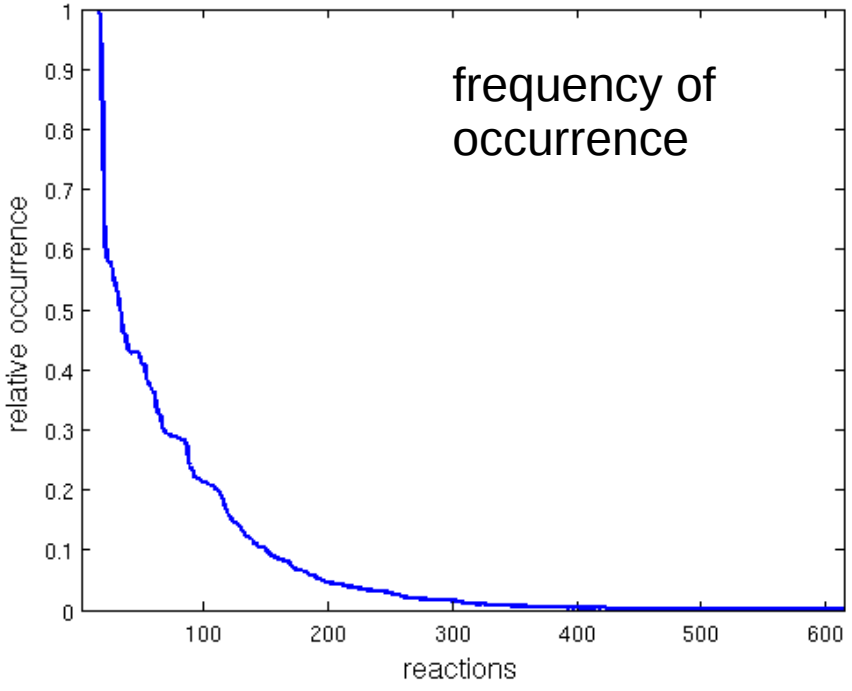
615 distinct reactions in 10000 calculated minimal extensions

May et al., *Genetics* (2008) Chlamydomonas special issue

Extension results for Chlamy







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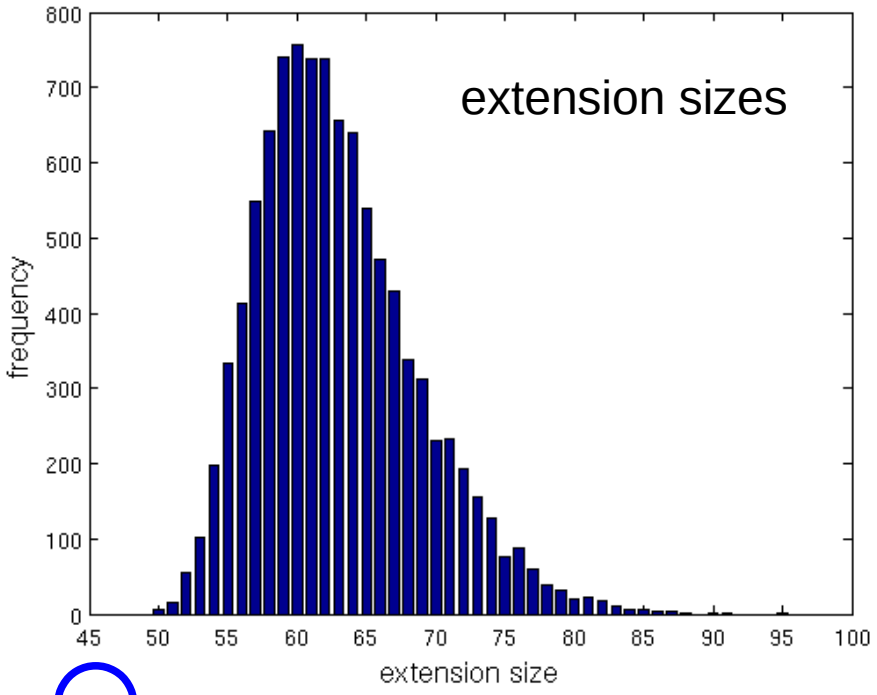
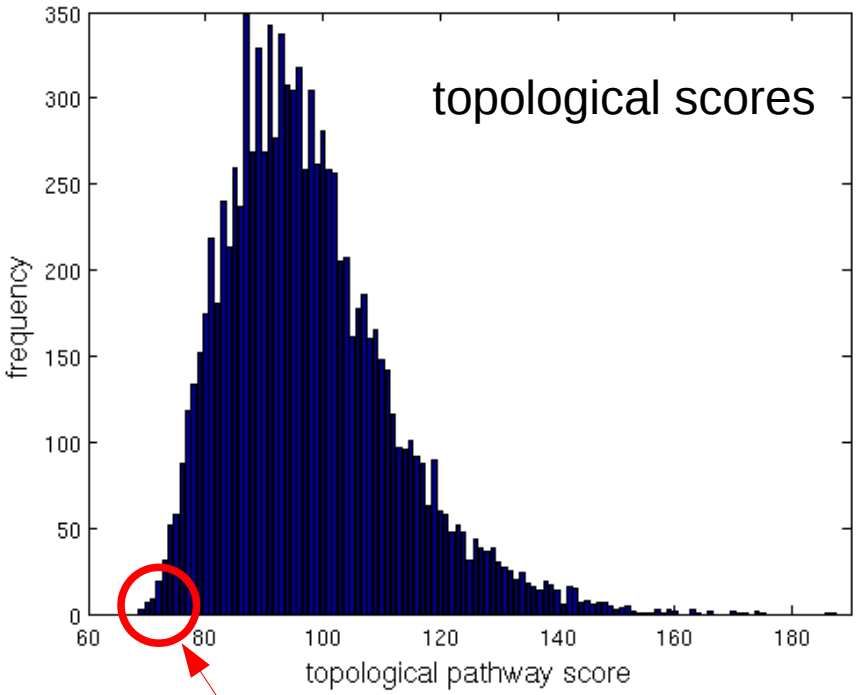
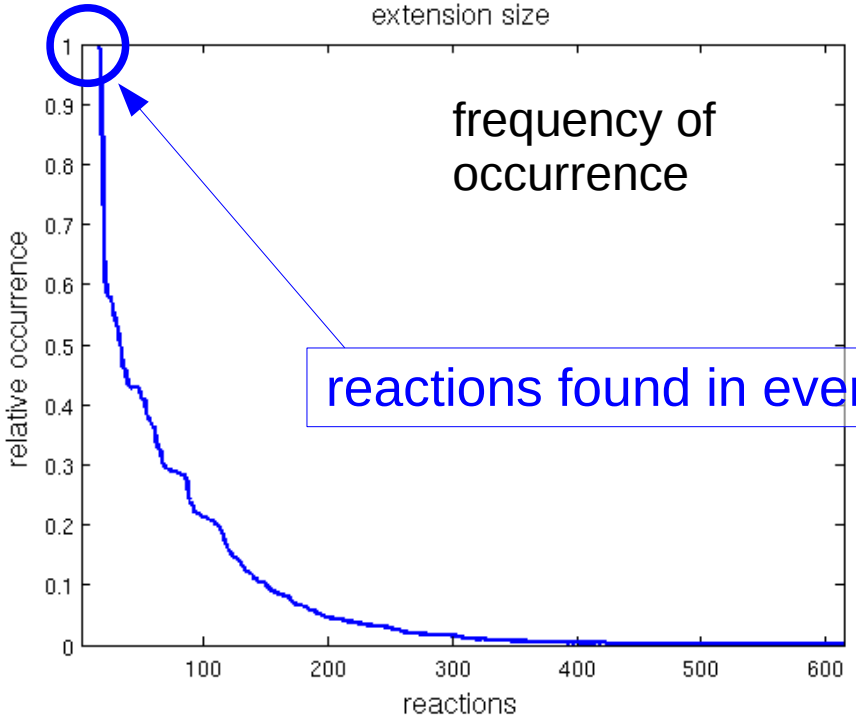




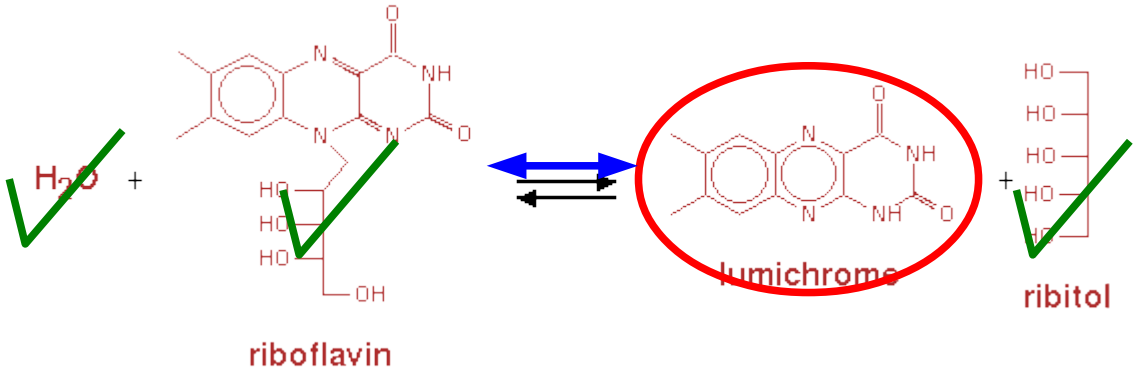
probably good pathway predictions



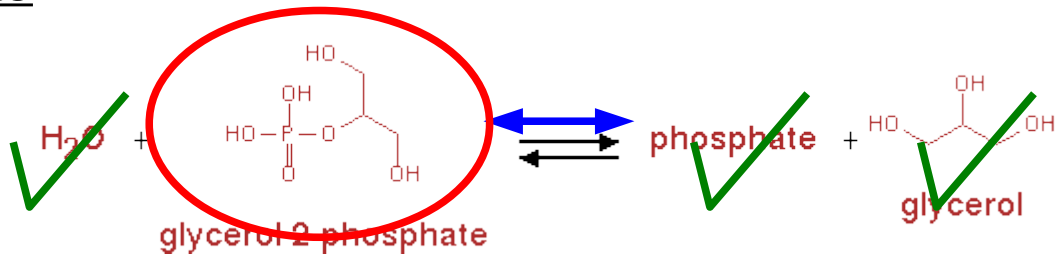
reactions found in every extension (15)

Some specific examples

lumichrome



glycerol 2-phosphate



experimentally observed metabolite

metabolites/reactions annotated in Chlamy

predicted reactions

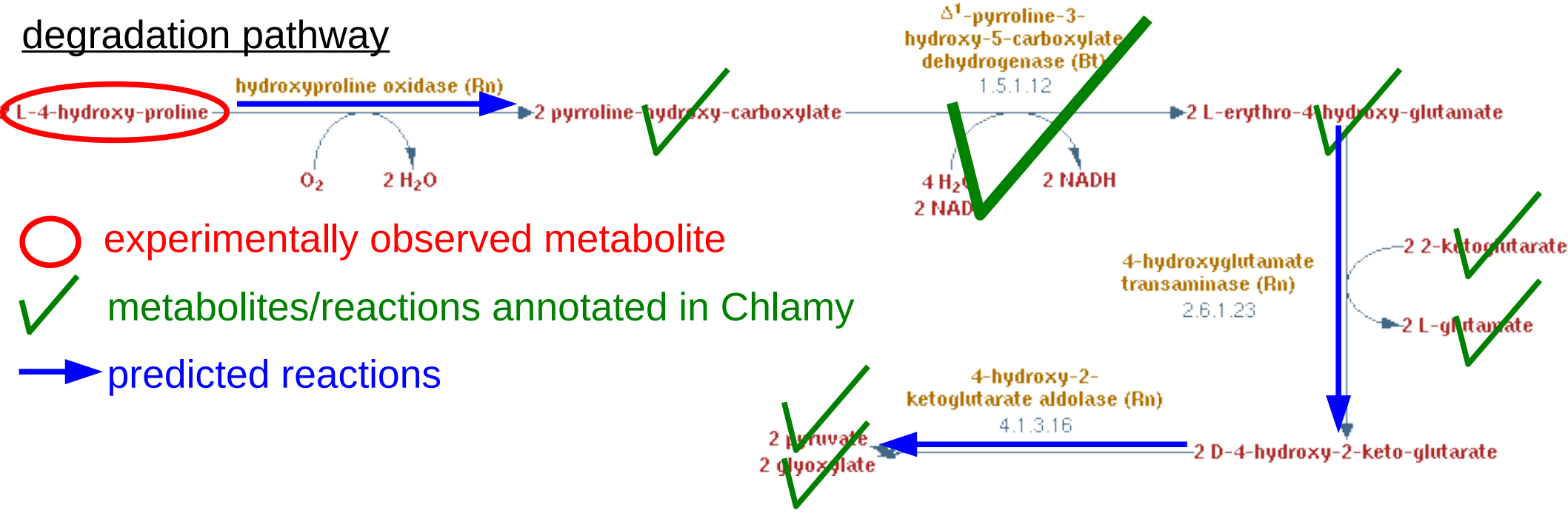
graphics from www.metacyc.org

Completion of a pathway

L-4-hydroxyproline

- in animals: important structural component of collagen
- in plants: found in some glycoproteins and cell wall proteins

degradation pathway



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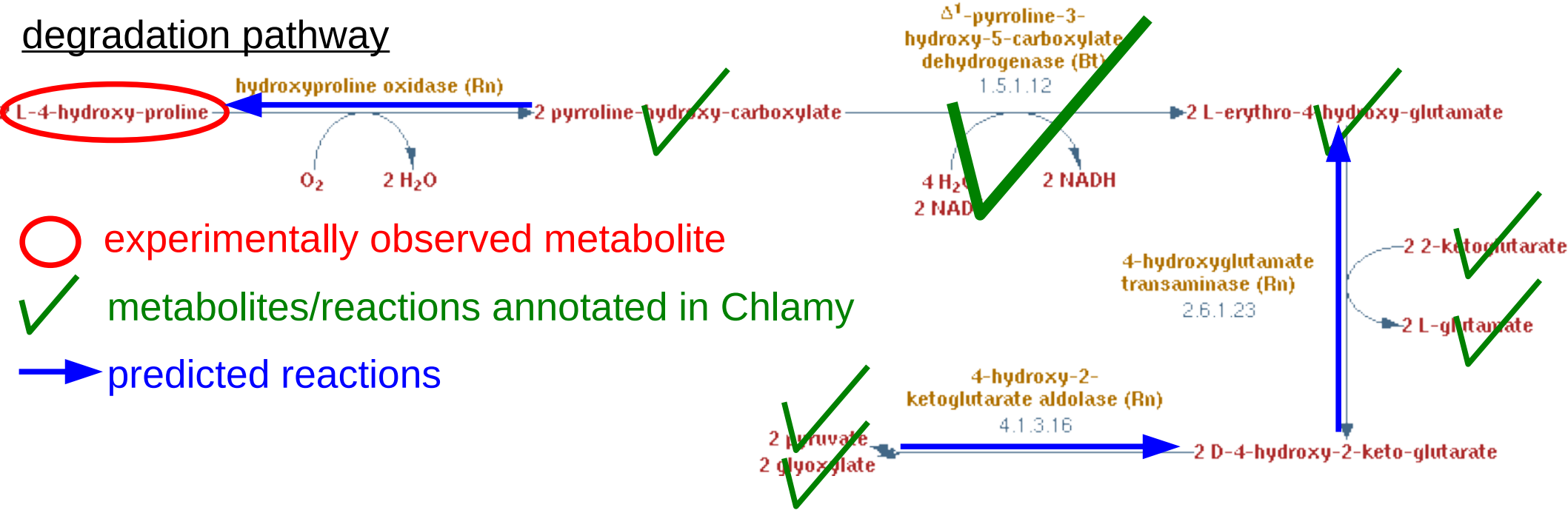

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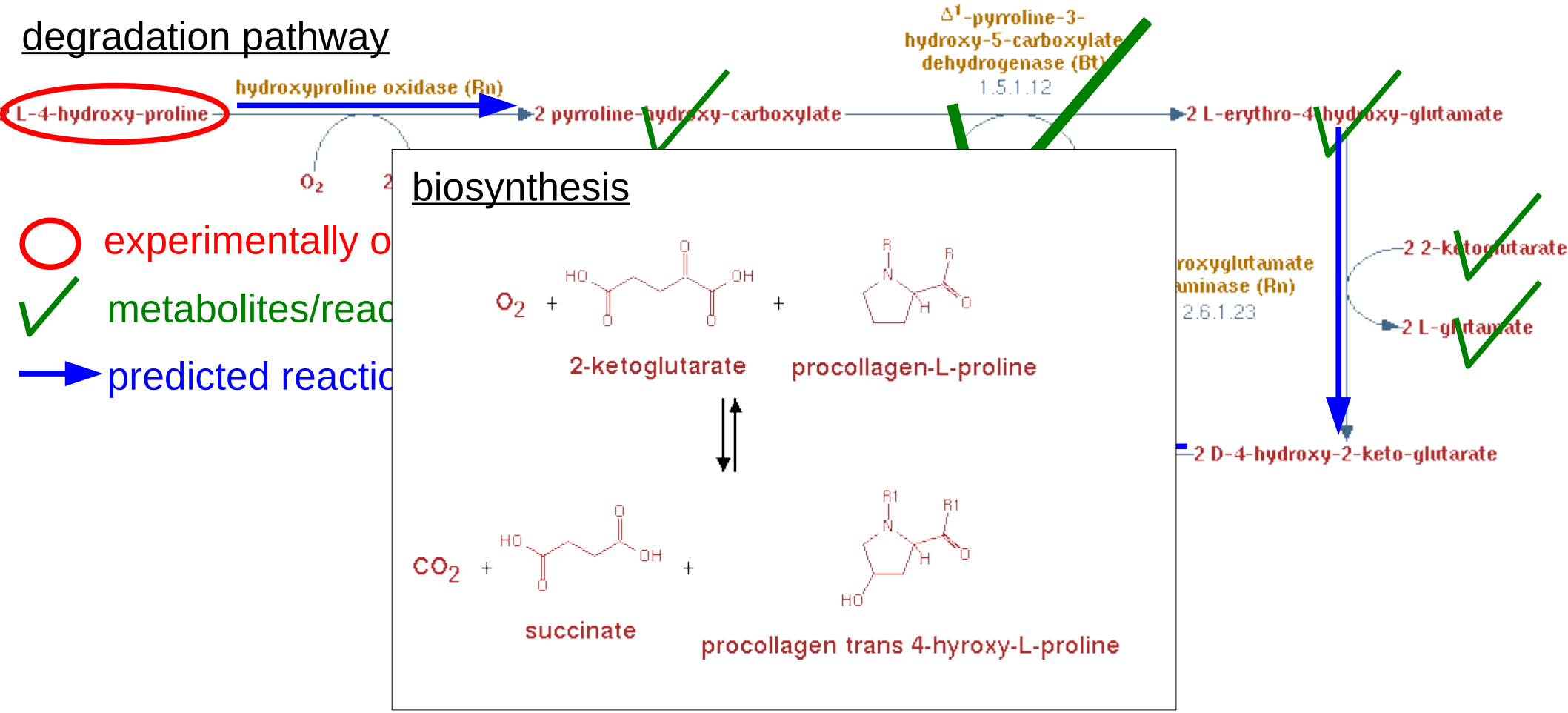
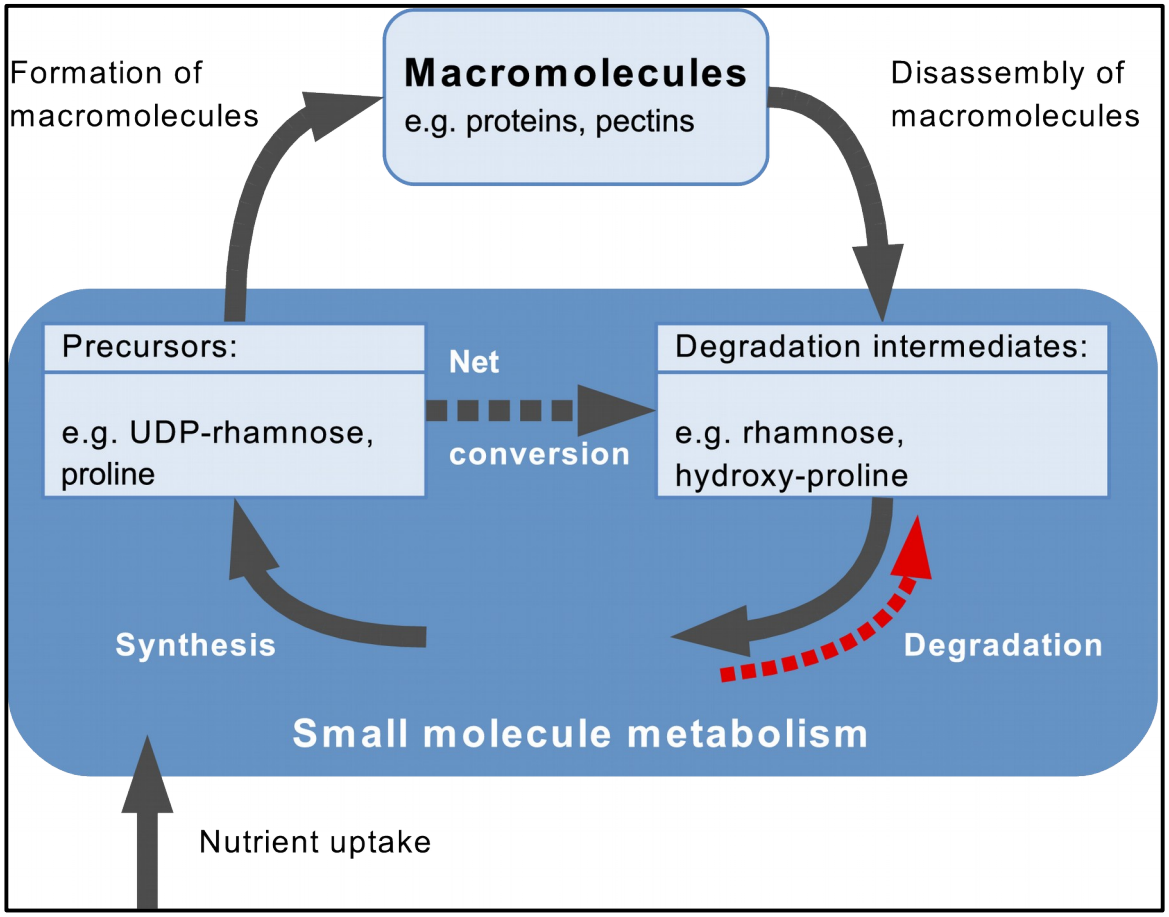


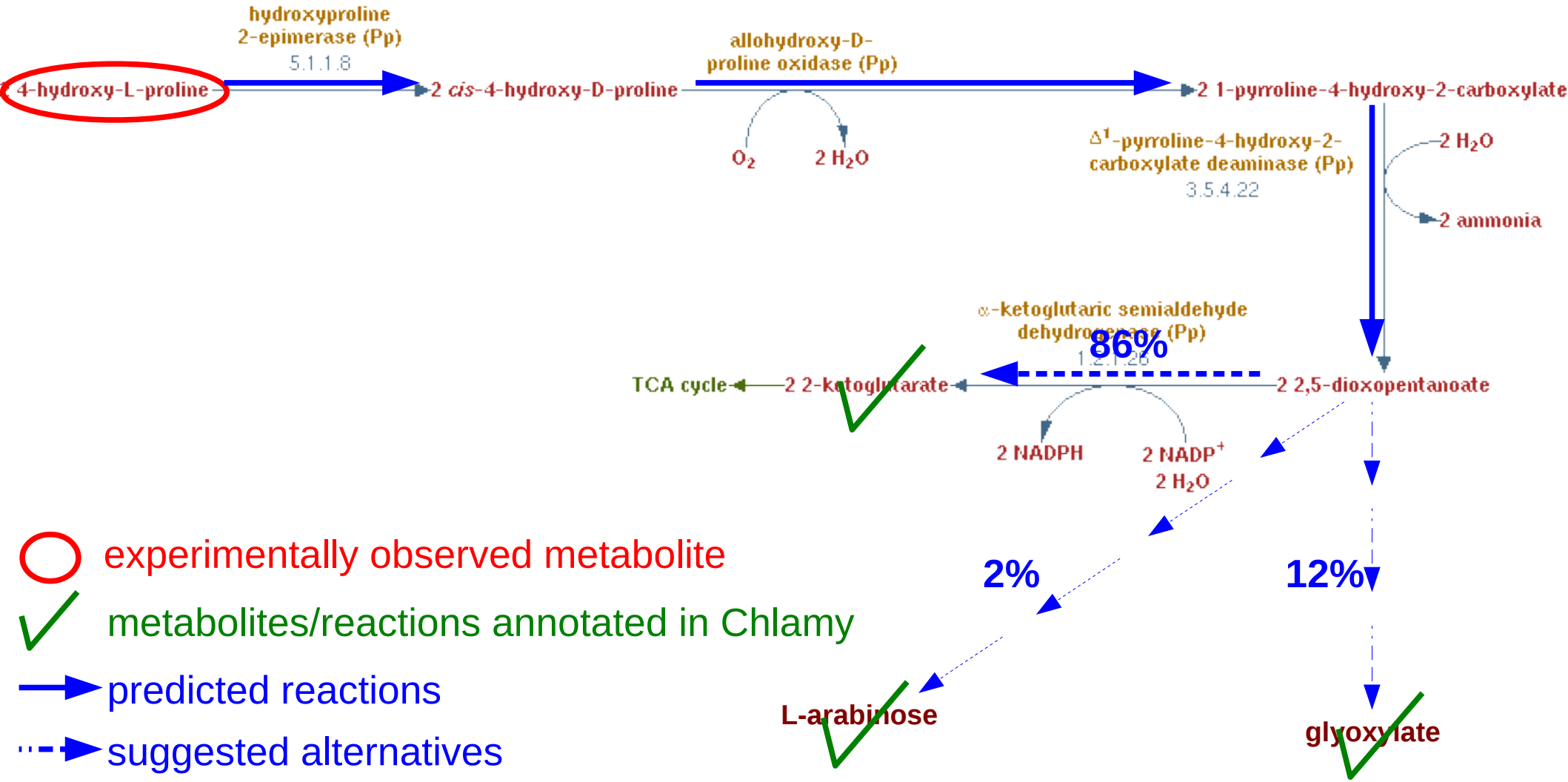
graphics from www.metacyc.org

Where are the limits of metabolism?



Prediction of alternative routes

4-hydroxy-L-proline



experimentally observed metabolite

metabolites/reactions annotated in Chlamy

predicted reactions

suggested alternatives

graphics from www.metacyc.org

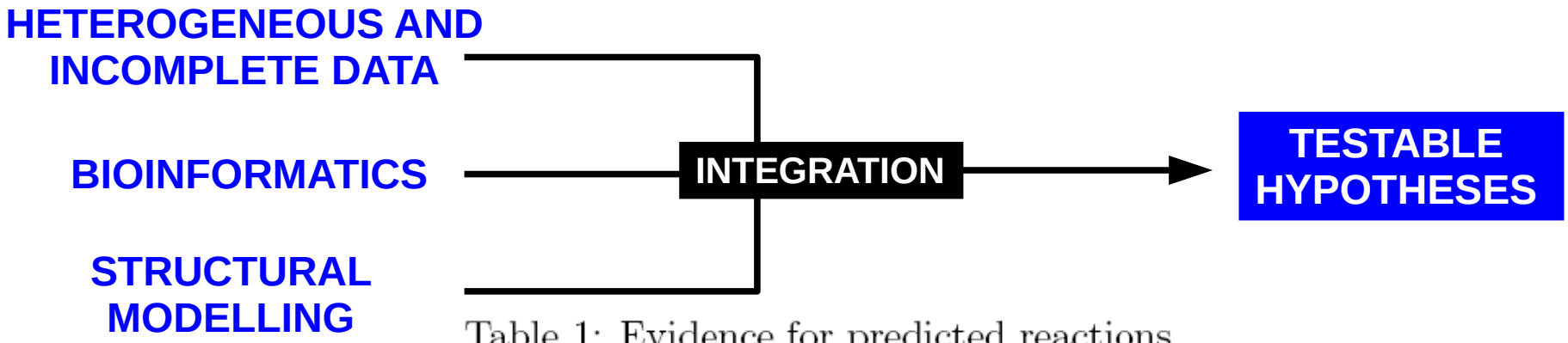


Table 1: Evidence for predicted reactions.

Target	Reaction/ EC number	Evidence	Comment
Ergosterol	1.14.99.7	+	Blast hit (136985) against human (ERG1)
	1.1.1.270	+	Blast hit (191061) against human (DHB7)
	1.3.1.70	+	orthologs (196516, 126431) to yeast (ERG24)
	1.3.1.71	+/−	Blast hit (196516) against yeast (ERG4)
	1.14.13.70	+	ortholog (196411) to Arabidopsis (AT1G11680)
	1.14.13.72	+	orthologs (142288,186886) to human (NP_006736.1)
	C-8 sterol isomerase	−	Blast hit (160258) against Arabidopsis (AT1G20050)
			but more likely C-8,7 sterol isomerase (5.3.3.5)
	5.3.3.5	+	ortholog (160258) to Arabidopsis (AT1G20050)
	C-22 sterol desaturase	+	ortholog (196874) to yeast (ERG5)
Lumichrome	3.5.99.1	−	no hit
N-acetyl-L-phenylalanine	2.3.1.53	−	no sequences available
L-rhamnose	5.3.1.14	−	no hit
	2.7.1.5	−	no hit
	4.1.2.19	−	no hit
	2.7.7.64	+	ortholog (32796) to Arabidopsis (AT5G52560)
	3.1.3.23	+/−	Blast hit (196269) to *E. coli* (SUPH)
Hydroxyproline	hydroxyproline oxidase	+	ortholog (146649) to Arabidopsis (AT3G30775)
	2.6.1.23	−	maybe 2.6.1.1
	4.1.3.16	−	no sequences available
Phenylacetaldehyde	4.1.1.43	+	ortholog (135197) to yeast PDC5
	4.1.1.53	+	Blast hit (40158) to *Solanum lycopersicum* AADC1A

(Christian et al., Mol BioSystems, 2009)

Organisms and their environment

No organism lives in complete isolation

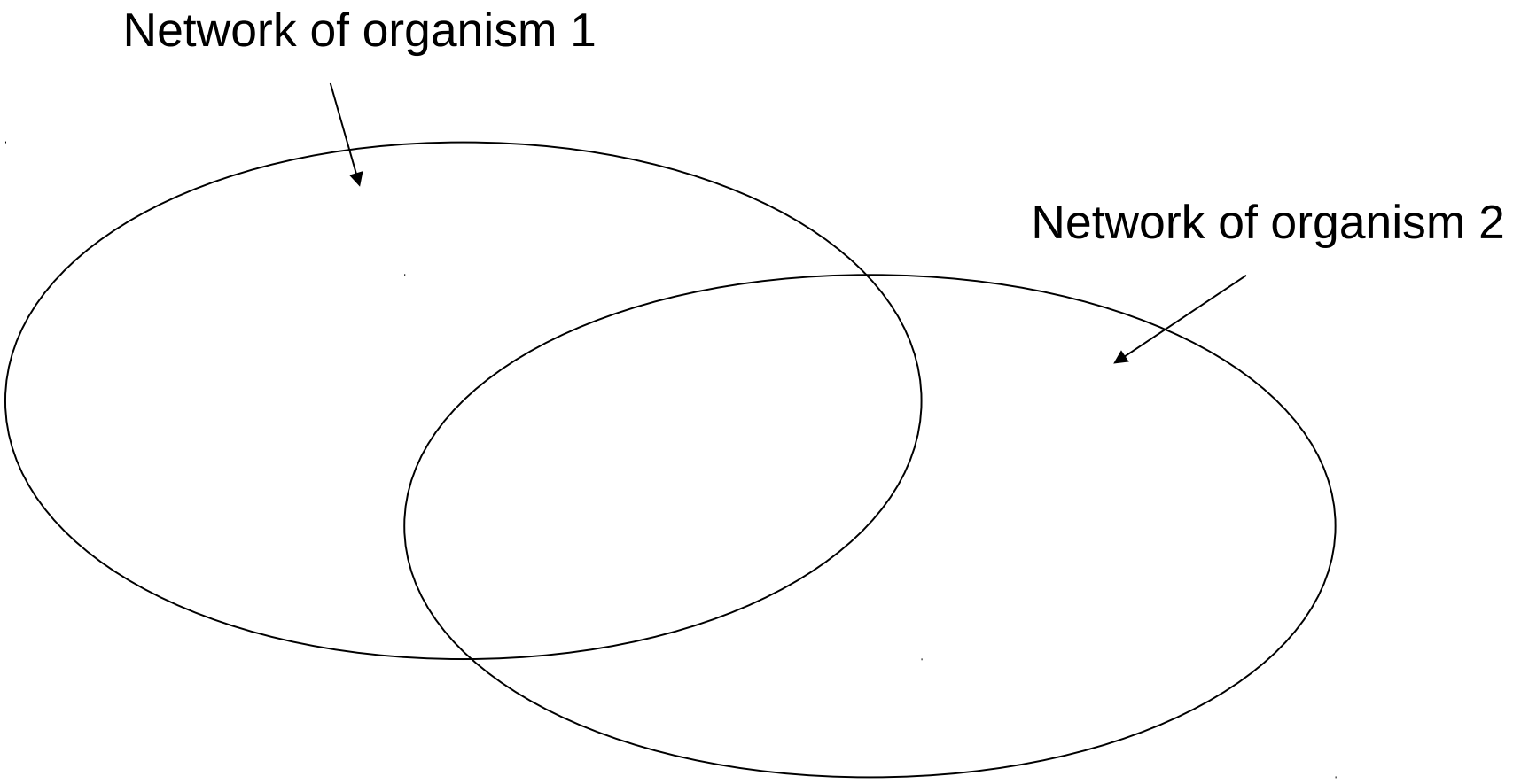
Organisms shape the environment (e.g. by excreted products)

Organisms are themselves part of the environment of others (ecosystem)

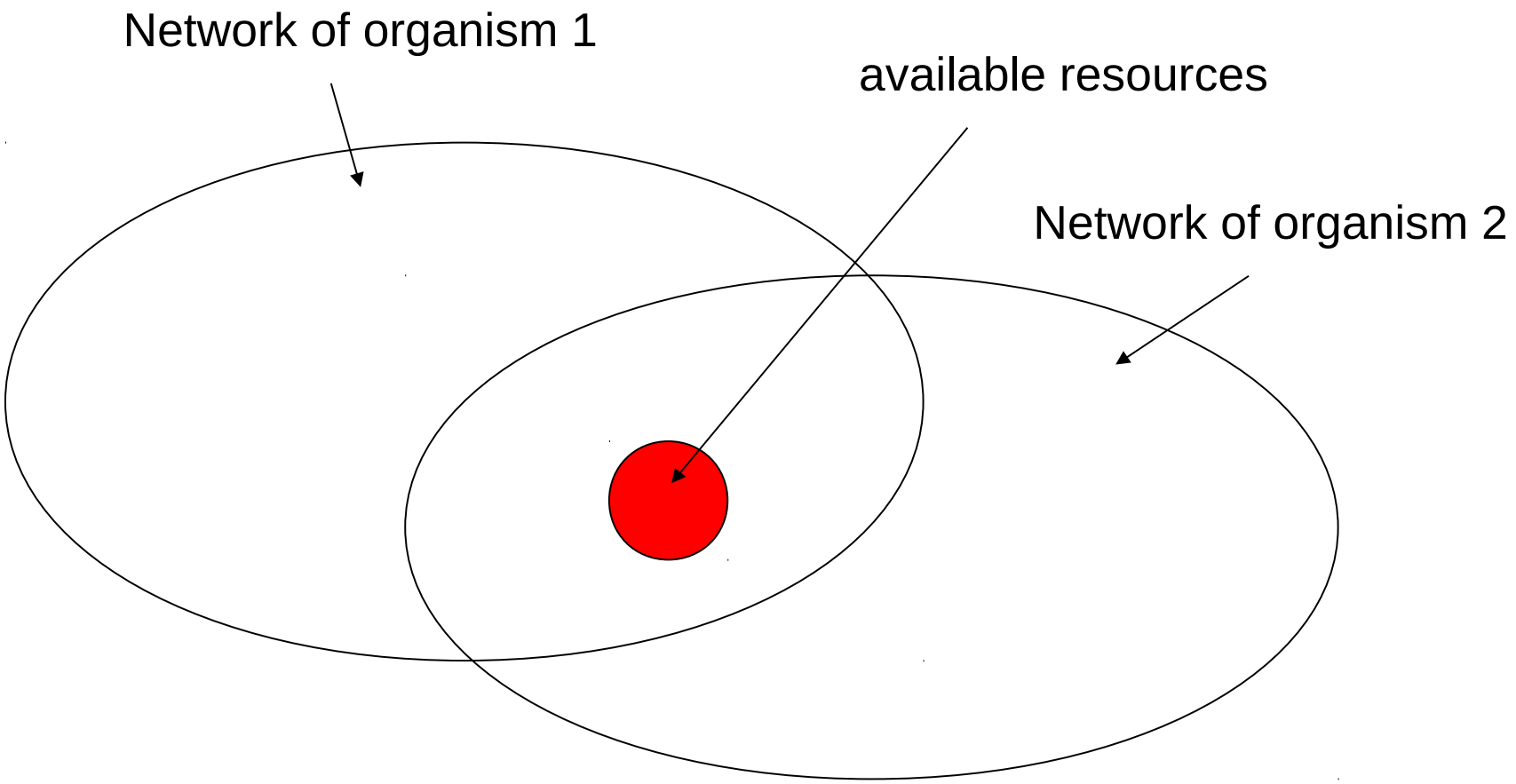
<u>Interaction on the level of metabolic networks</u>

- Biodegradation
  involves many microorganisms, requires the special metabolic capabilities
- Symbiosis
  e.g. plants (fabacaea) and Rhizobia (nitrogen fixing bacteria)
- Parasitism
  e.g. Wolbachia live inside insect cells

Metabolic synergy



Metabolic synergy



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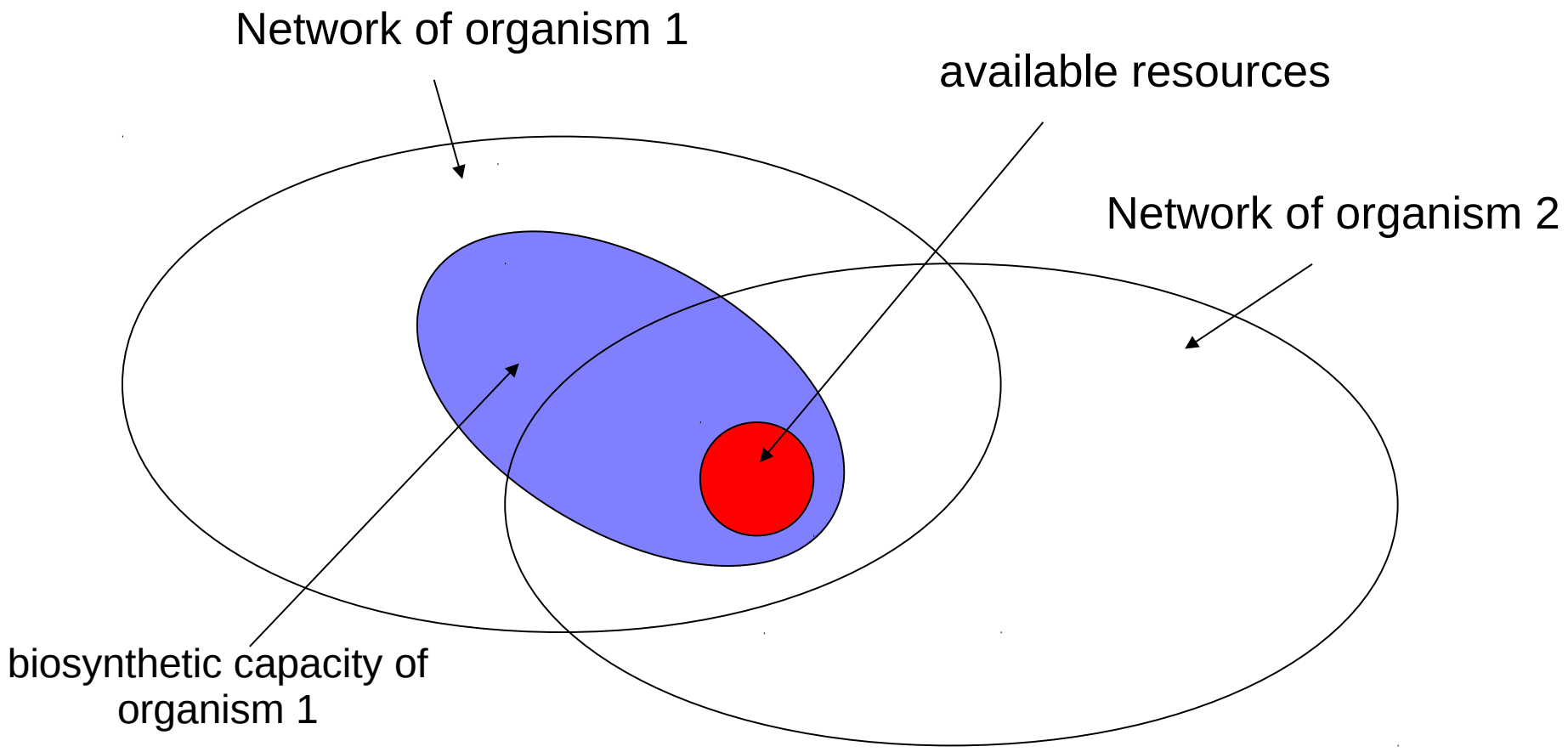

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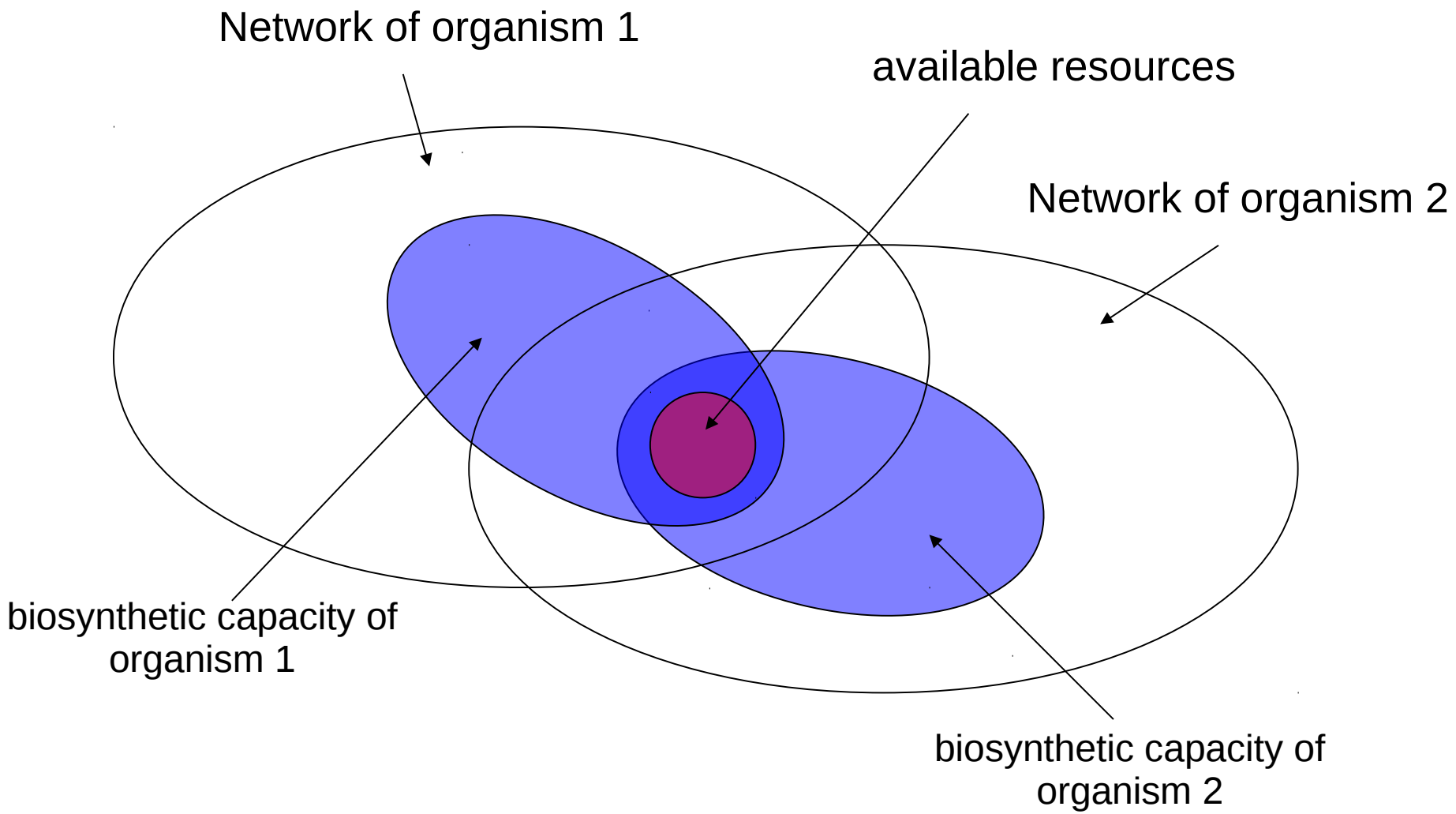

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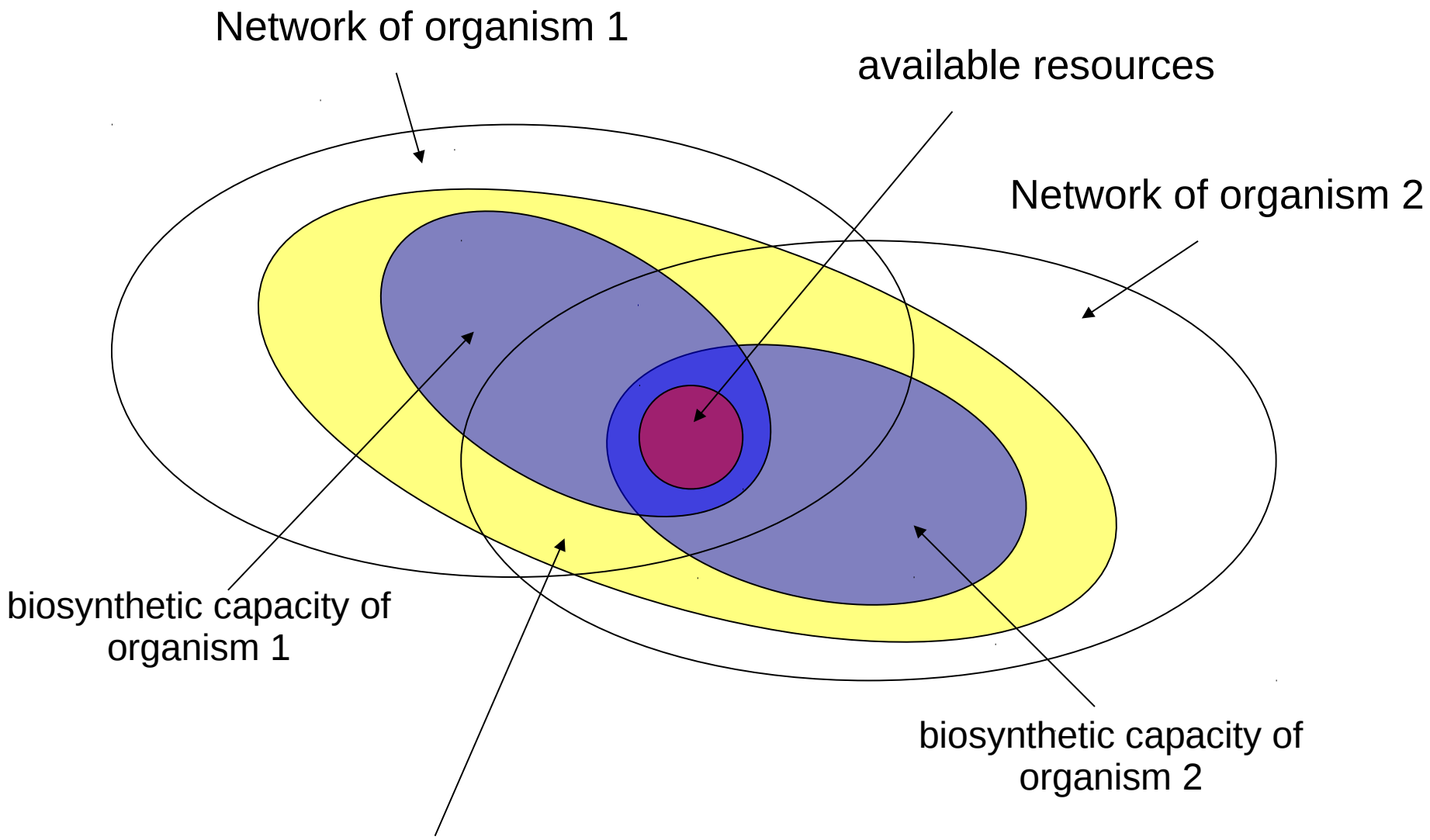

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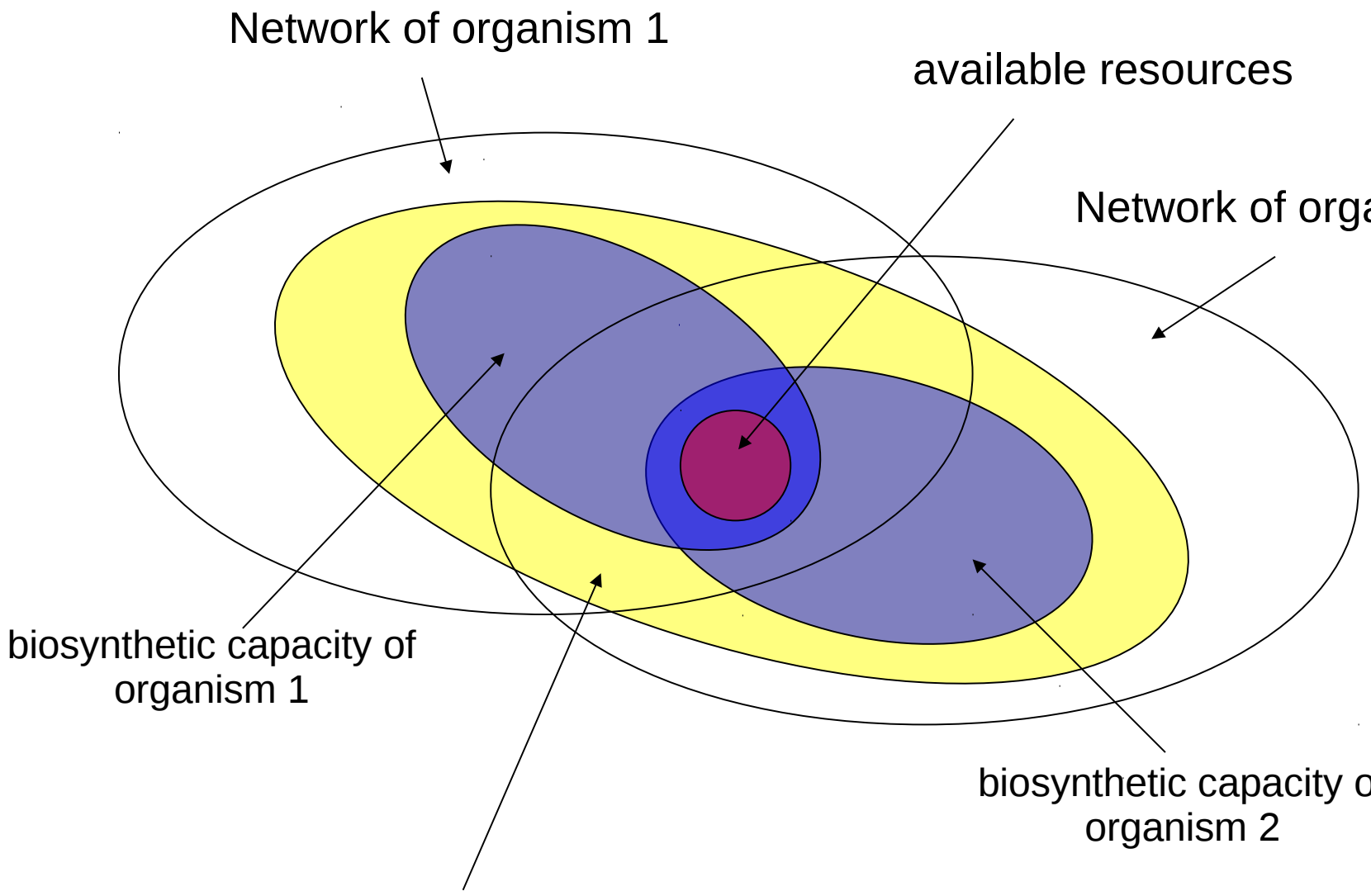
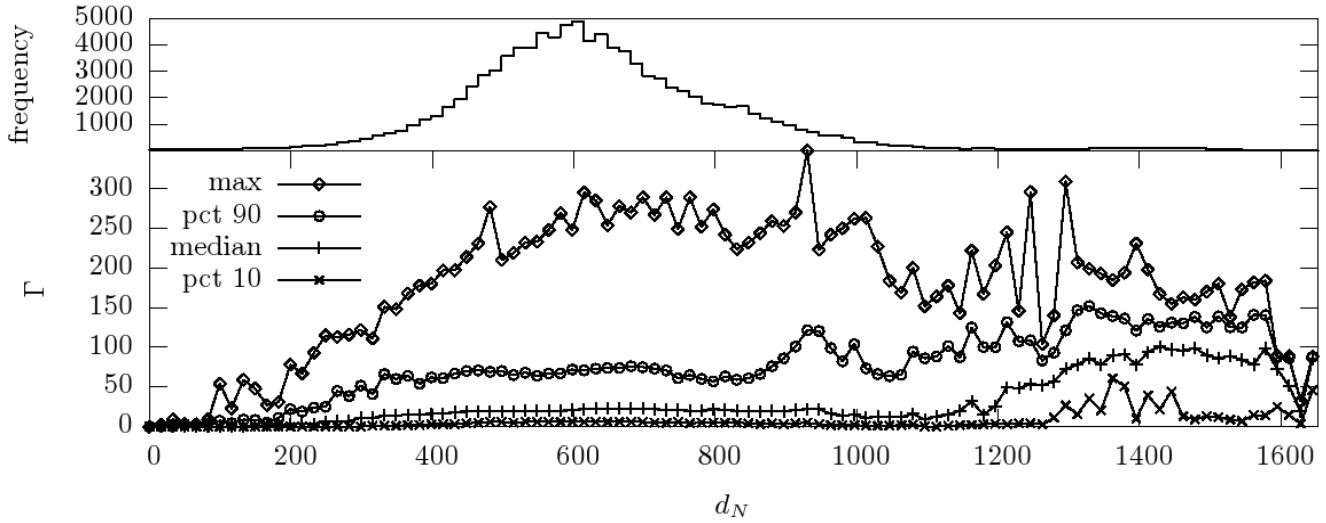


Biosynthetic capacity for the joined network is larger than the sum of the single capacities! → Synergy

Synergy vs. network dissimilarity

Statistics...
Which pairs are best suited to yield synergetic effects?



(Christian, Handorf and Ebenhöh, 2007)

A simplistic view with lots of space for improvement:

- Transport processes
- Quantification of negative effects (FBA)
- ...

⇨ Investigate specific examples

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