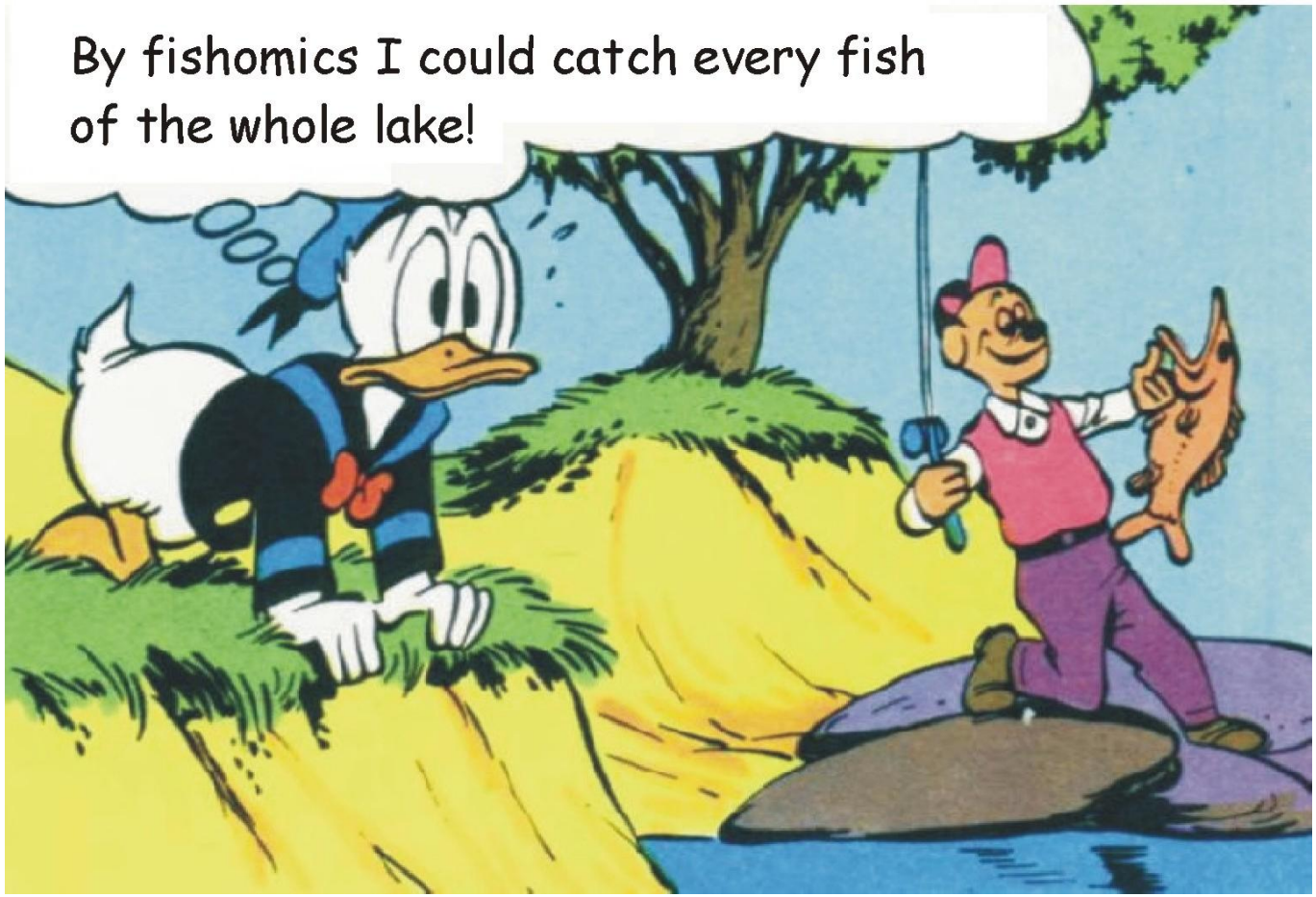
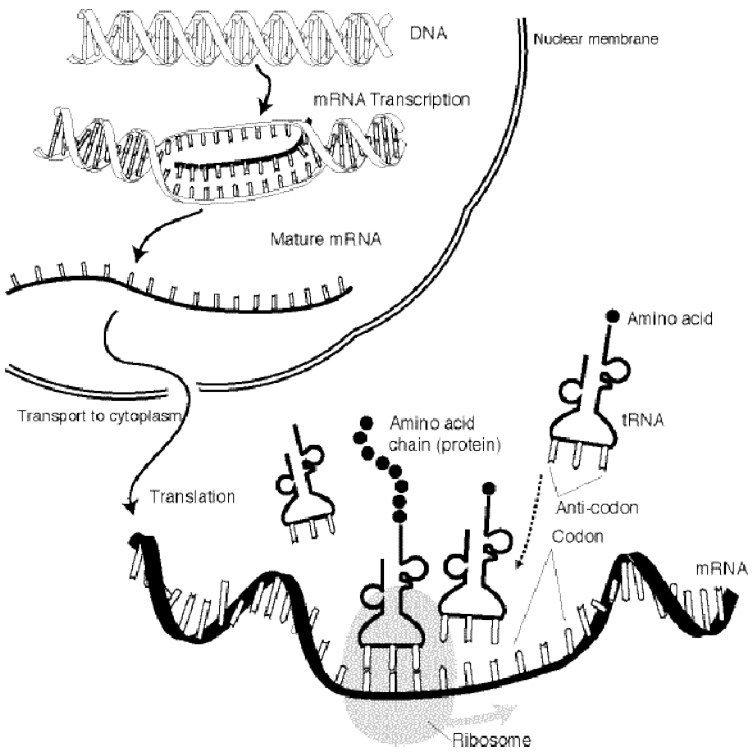
Fishomics



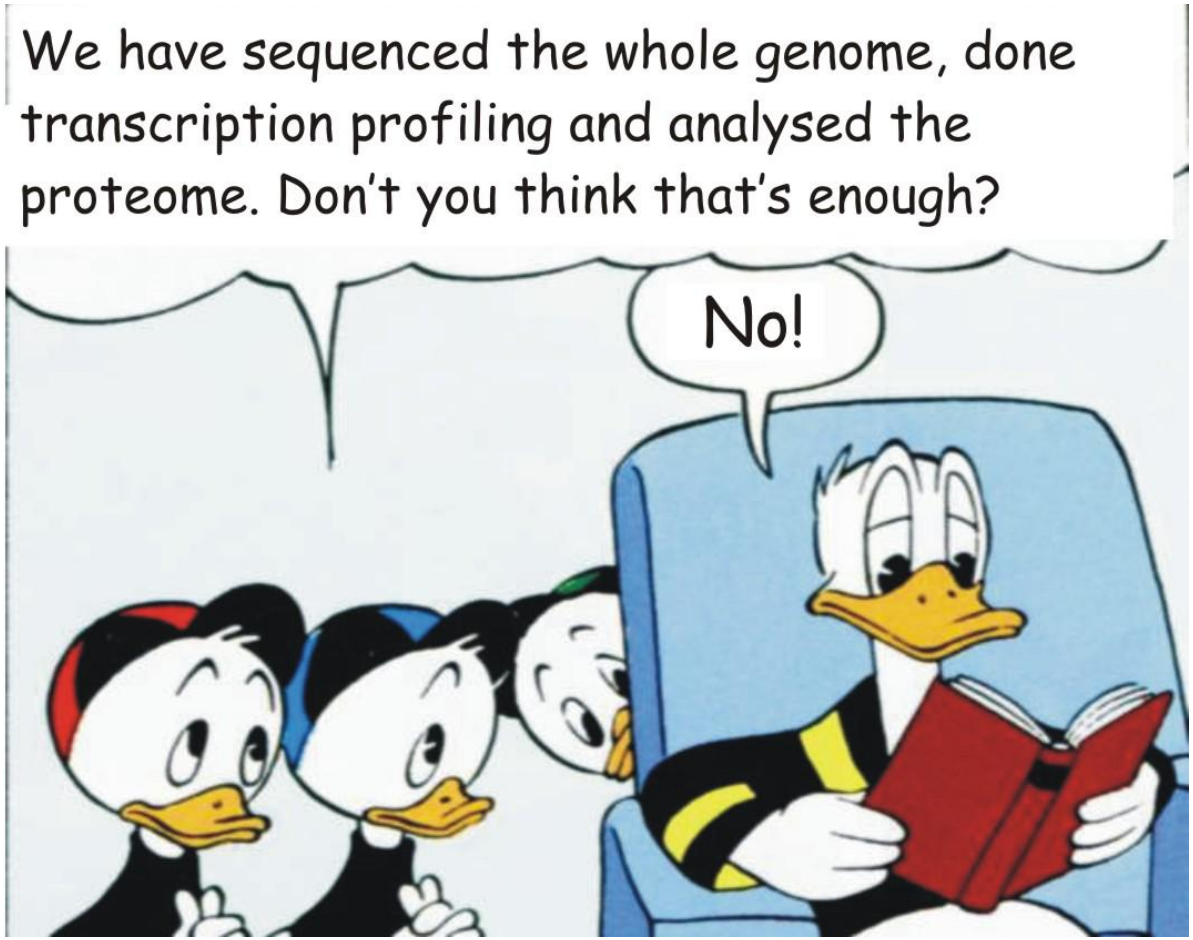


Genome

Transcriptome

Proteome

Metabolome


We have sequenced the whole genome, done transcription profiling and analysed the proteome. Don't you think that's enough?
No!

Methods for metabolomics

Traditional methods

- enzymatic methods
- chromatographic methods, e.g. HPLC

System wide methods

- spectroscopic methods
  - mass spectrometroscopy (MS)
  - nuclear magnetic spectroscopy (NMR)
  - other: FT-IR, UV….
- matematical methods in data handling, statistics, modelling and simulation

Pros and cons of MS & NMR

MS

- high sensitivity
- lower cost
- different method for different compounds
- needs (internal) standards (response etc.)
- chromatographic parameters change with time
- incomplete data on labelling (but the methods are developing…)

NMR

- low sensitivity (but cold probe …)
- expensive
- un-biased
- quantitative without standards
- very repetable => exactly same spectrum even years later
- more detailed data on labelling
- non-destructive

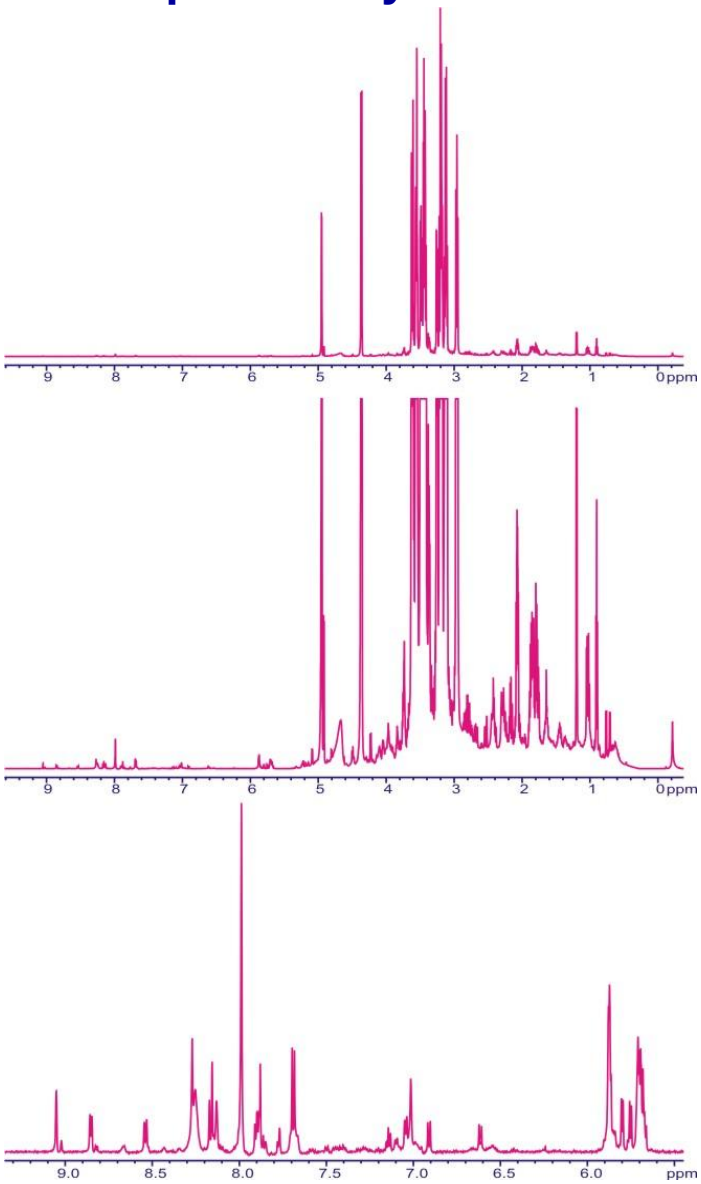
Sample preparation

- **quenching** (rapidly stopping all metabolic and other reactions)
  - liquid nitrogen
  - cold methanol
  - other methods (e.g. microwaves)

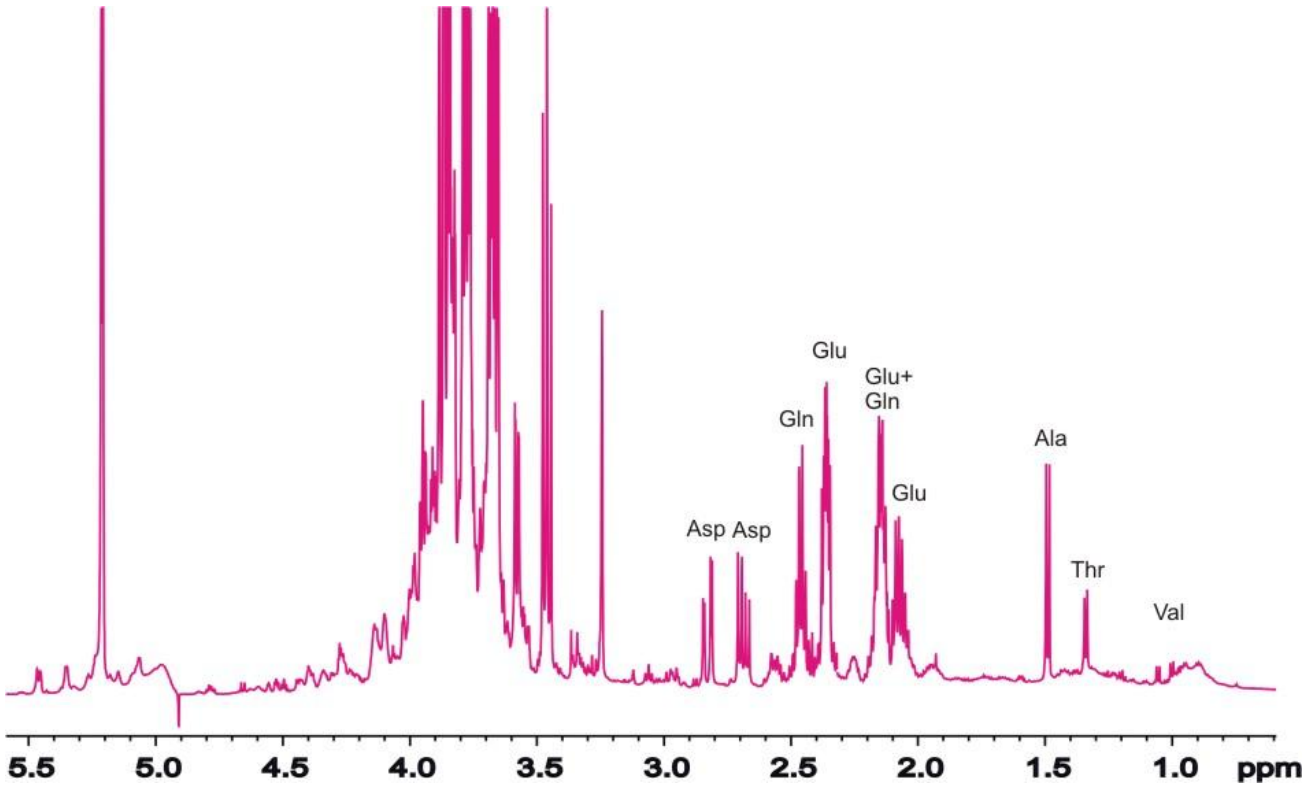
- **extraction**
  - chloroform/methanol
  - perchloric acid (PCA)
  - boiling ethanol
  - others (e.g. mechanical powdering in liq. N2)

- **storage, preparation for measurement**
  - cold
  - method specific preparation steps

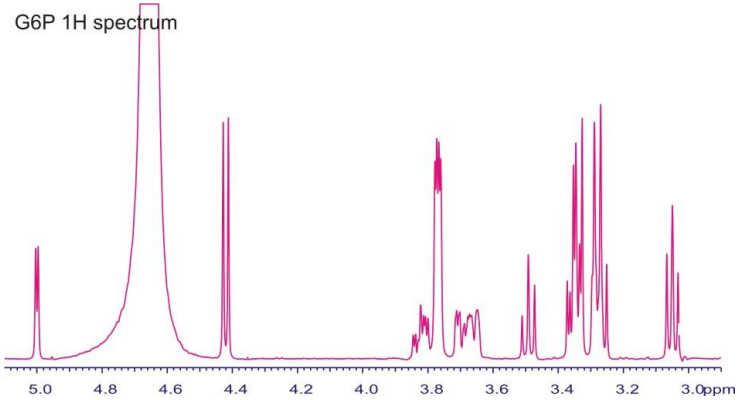
1D & 2D proton NMR spectra of yeast extract using a cold probe

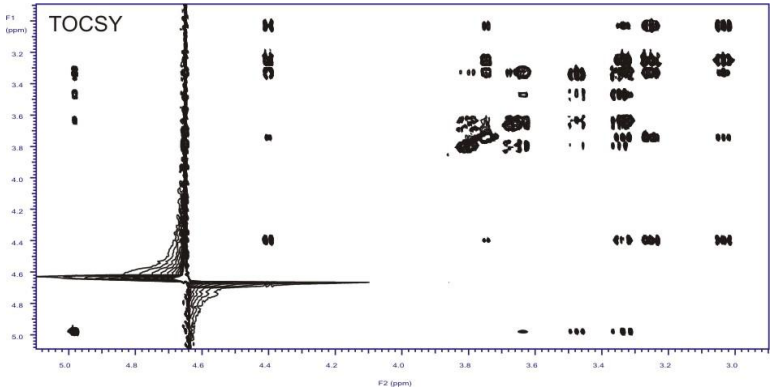


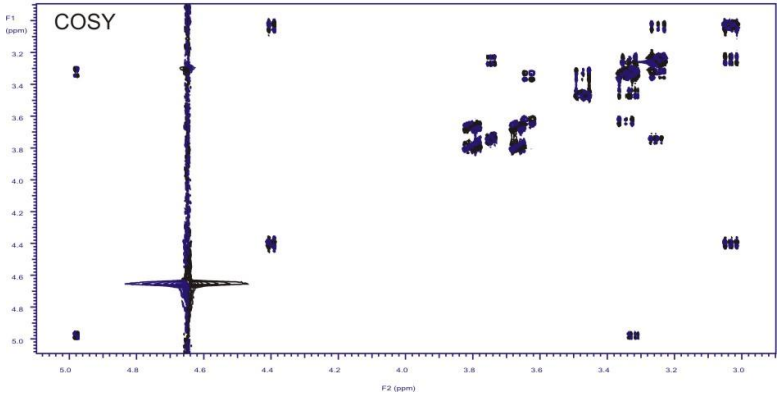
Aerobic steady steate

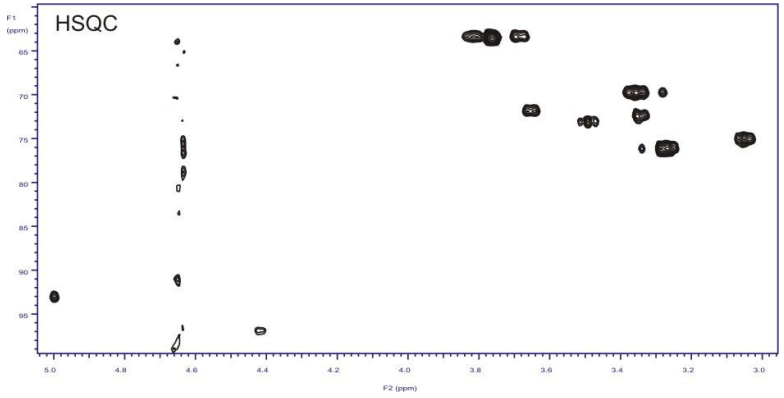


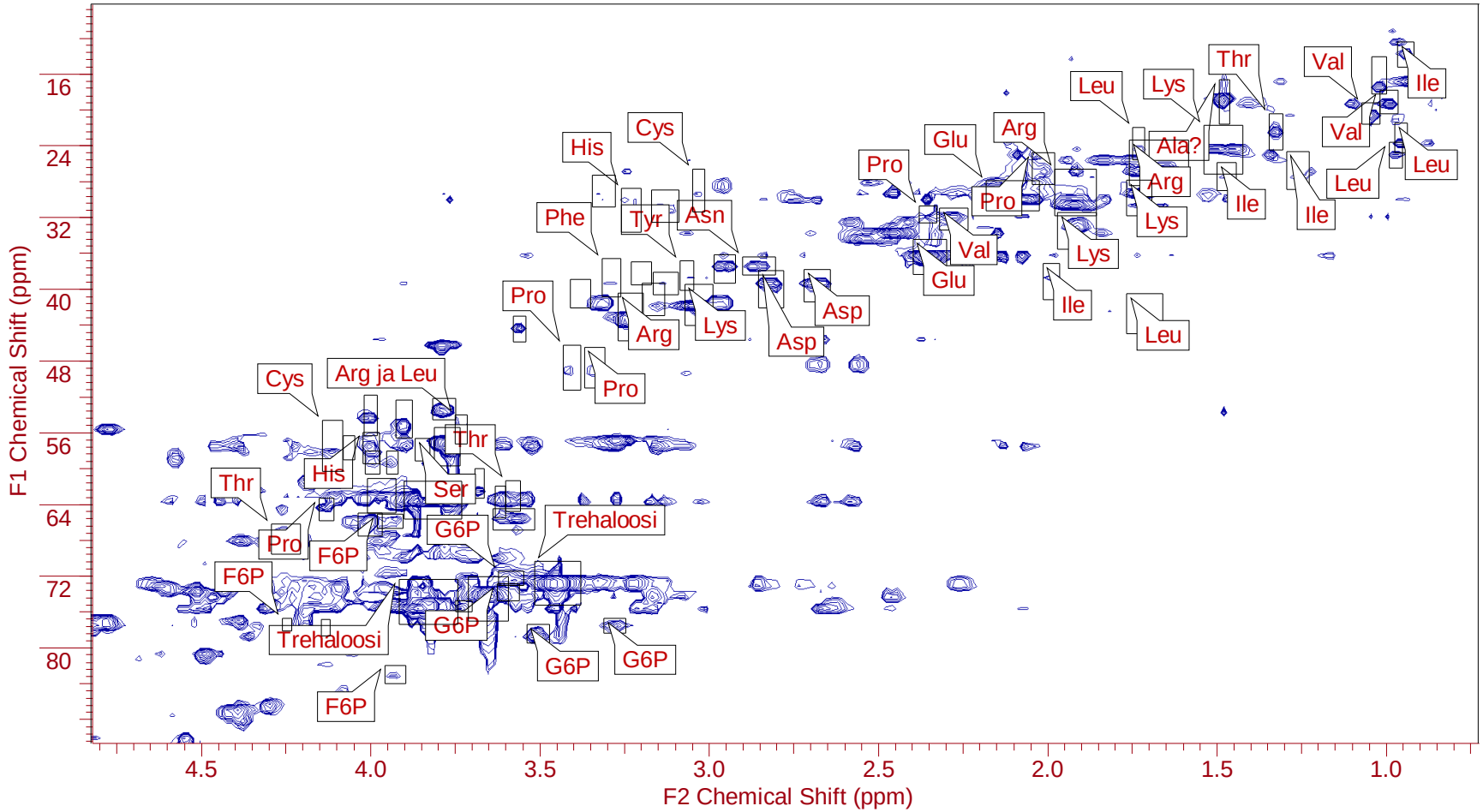
2D NMR library



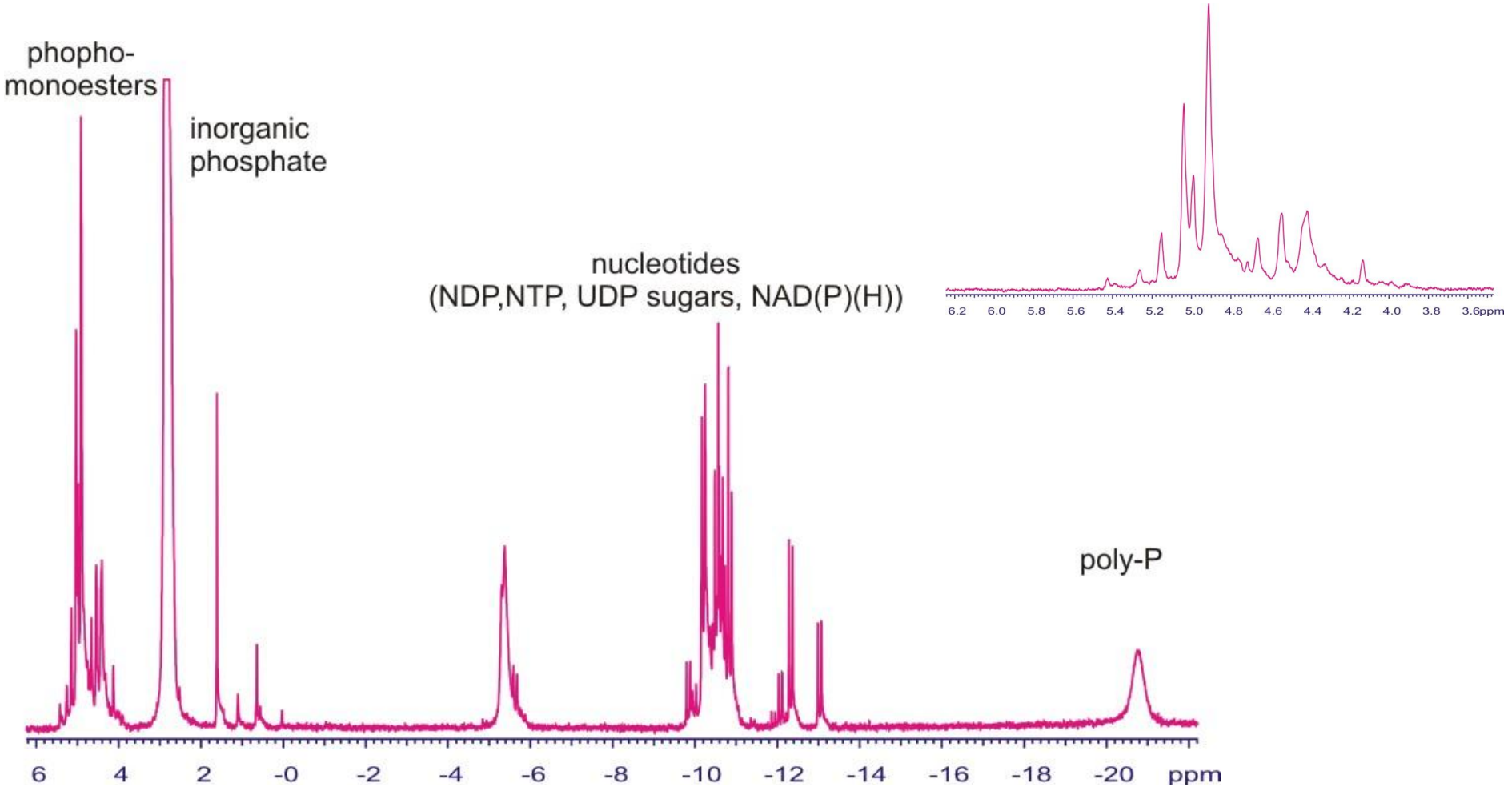






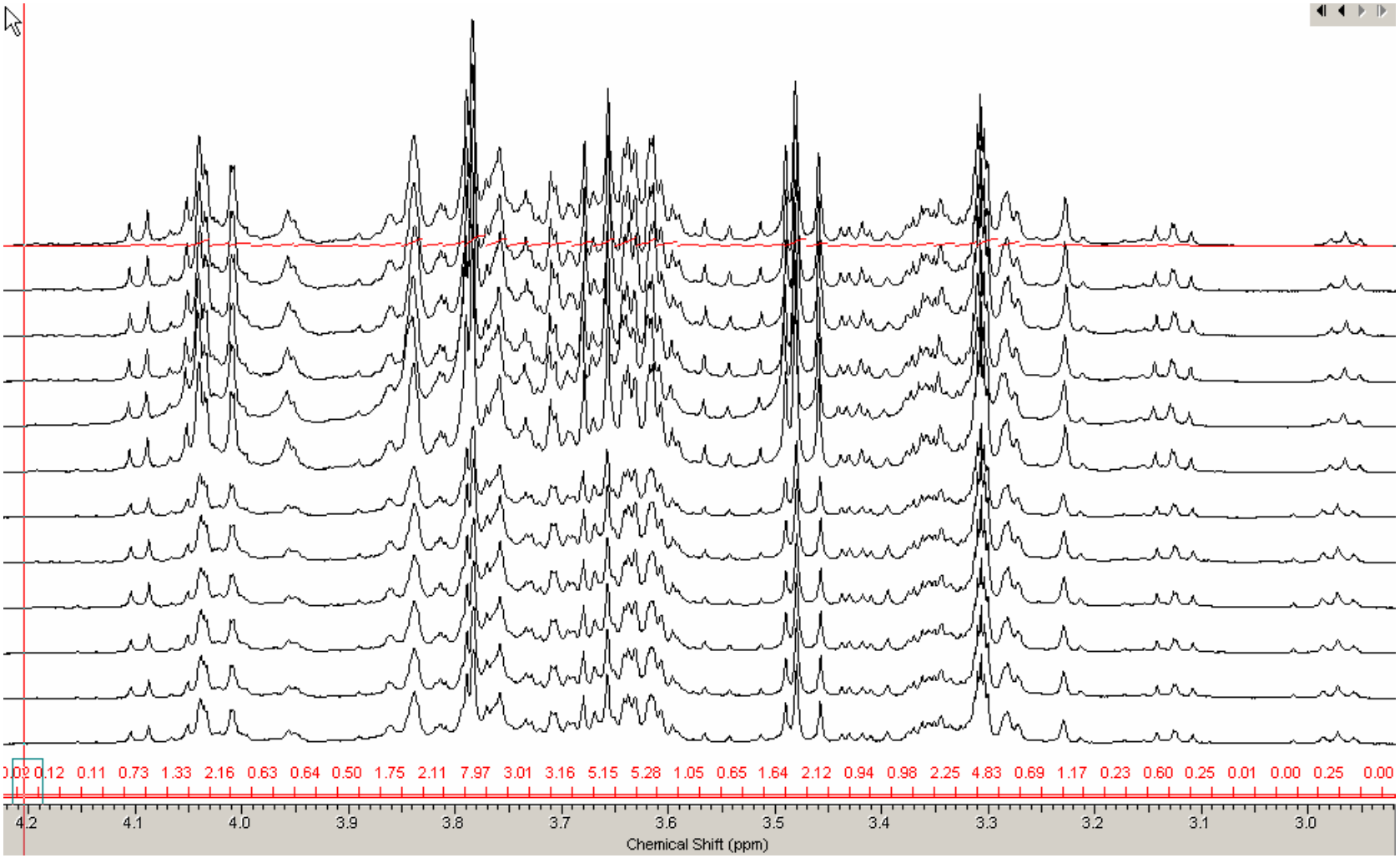

F1 Chemical Shift (ppm)
16
24
32
40
48
56
64
72
80
F2 Chemical Shift (ppm)
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
Cys
Arg ja Leu
Thr
His
Thr
Ser
G6P
Trehaloosi
Pro
F6P
F6P
G6P
Trehaloosi
G6P
G6P
F6P
His
Cys
Phe
Tyr
Asn
Pro
Arg
Lys
Asp
Asp
Pro
Pro
Glu
Arg
Leu
Lys
Thr
Val
Ile
Ala?
Arg
Pro
Val
Glu
Lys
Ile
Leu
Lys
Ile
Ile
Val
Leu
Leu

31P spectrum of yeast extract

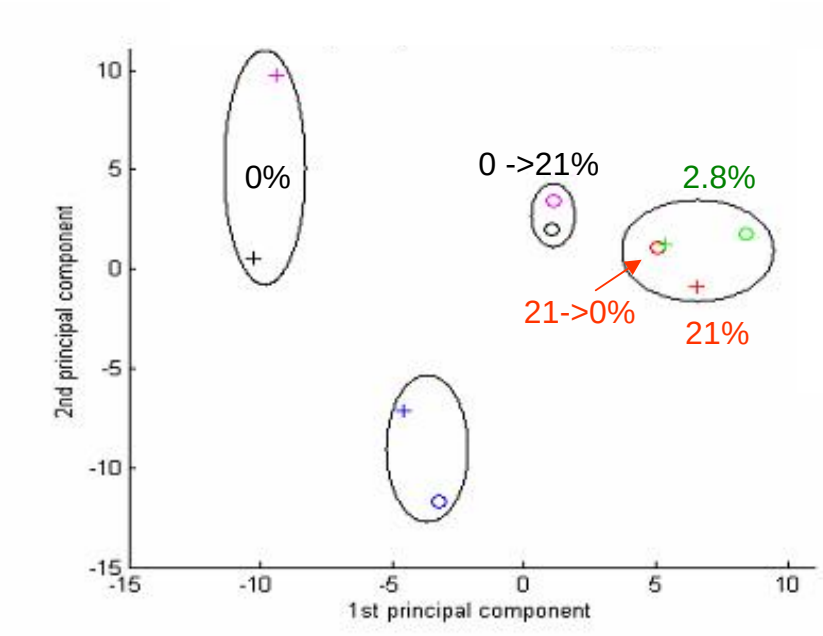


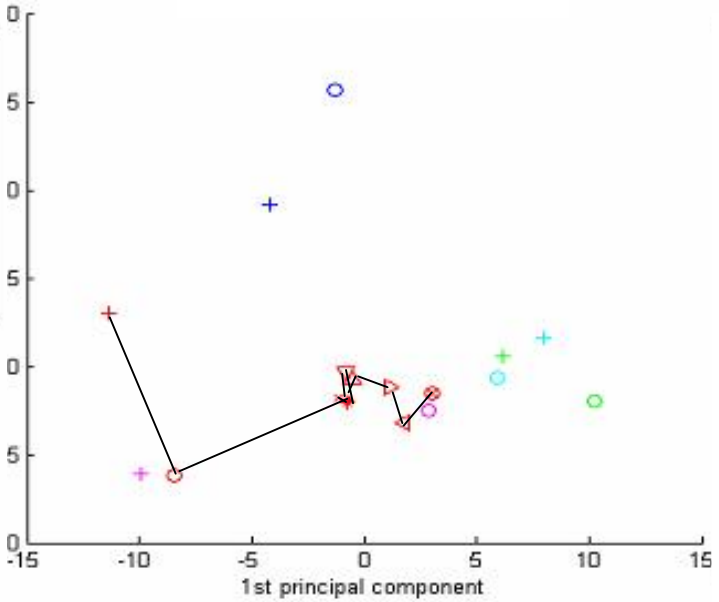
Intra-cellular metabolites measured by LC-MS(-MS)

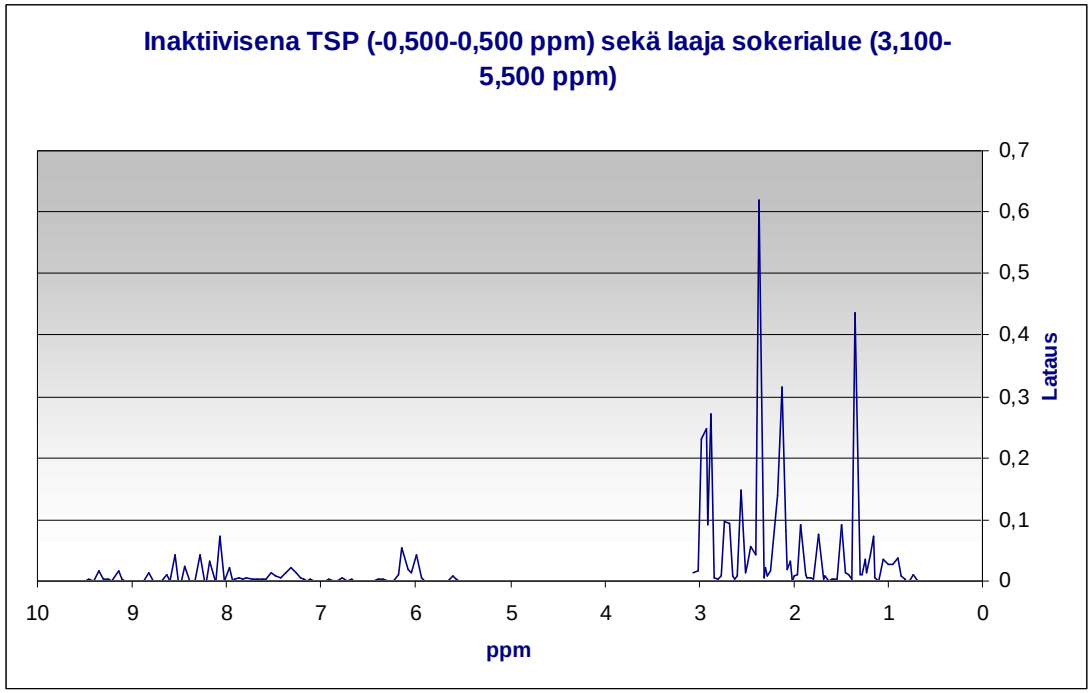
- D-Glucose 6-phosphate
- D-Fructose 6-phosphate
- D-Mannose 6-phosphate
- D-Glucose 1-phosphate
- Trehalose 6-phosphate
- D-Fructose 1,6-bisphosphate
- Succinate
- Fumarate
- Malate
- alpha-Ketoglutaric acid
- Oxaloacetate
- Citrate
- Isocitrate
- Glyoxylate
- Pyruvate
- D-Glycerate 3-phosphate
- Phosphoenolpyruvate
- D-Ribulose 5-phosphate
- 6-Phospho-D-gluconate
- E4P
- ATP, ADP, AMP and other nucleotides


0.02 0.12 0.11 0.73 1.33 2.16 0.63 0.64 0.50 1.75 2.11 7.97 3.01 3.16 5.15 5.28 1.05 0.65 1.64 2.12 0.94 0.98 2.25 4.83 0.69 1.17 0.23 0.60 0.25 0.01 0.00 0.25 0.00
4.2 4.1 4.0 3.9 3.8 3.7 3.6 3.5 3.4 3.3 3.2 3.1 3.0
Chemical Shift (ppm)

Principal component analysis of NMR spectra

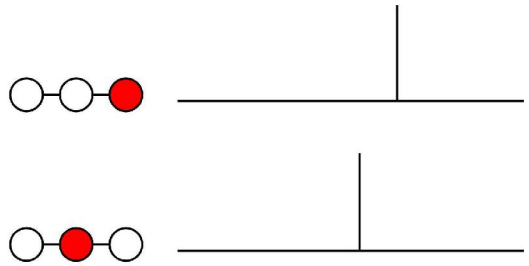




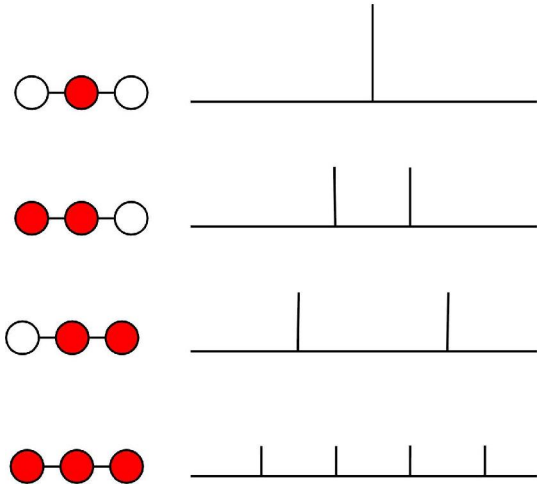

Inaktiivisena TSP (-0,500-0,500 ppm) sekä laaja sokerialue (3,100-5,500 ppm)
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
Lataus
10
9
8
7
6
5
4
3
2
1
0
ppm

Benefits of ^{13}C labeling & NMR

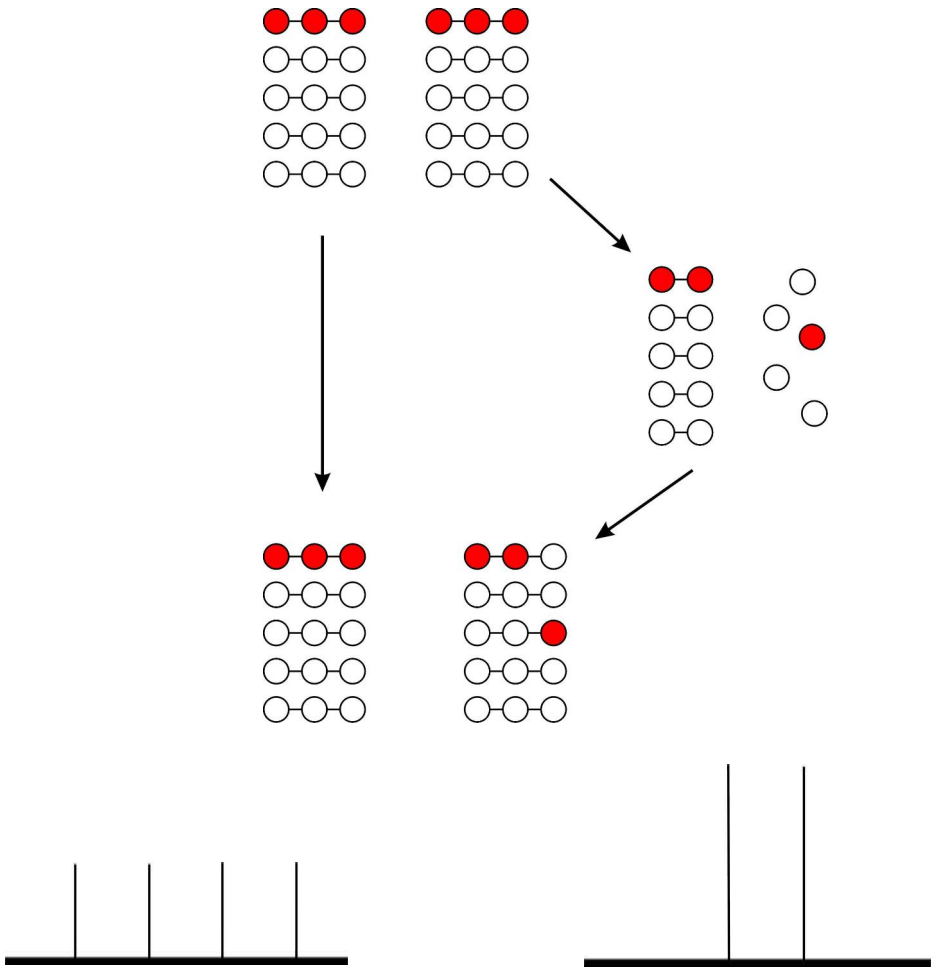
- position sensitive



- isotopomer sensitive



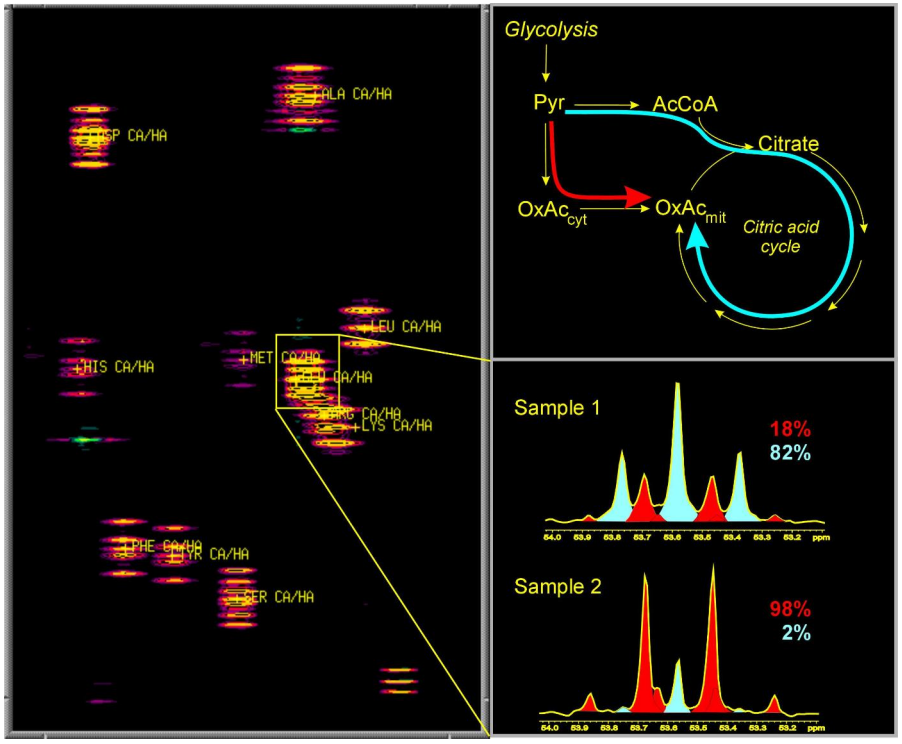
Uniformly labeled carbon source (~10%), METAFoR

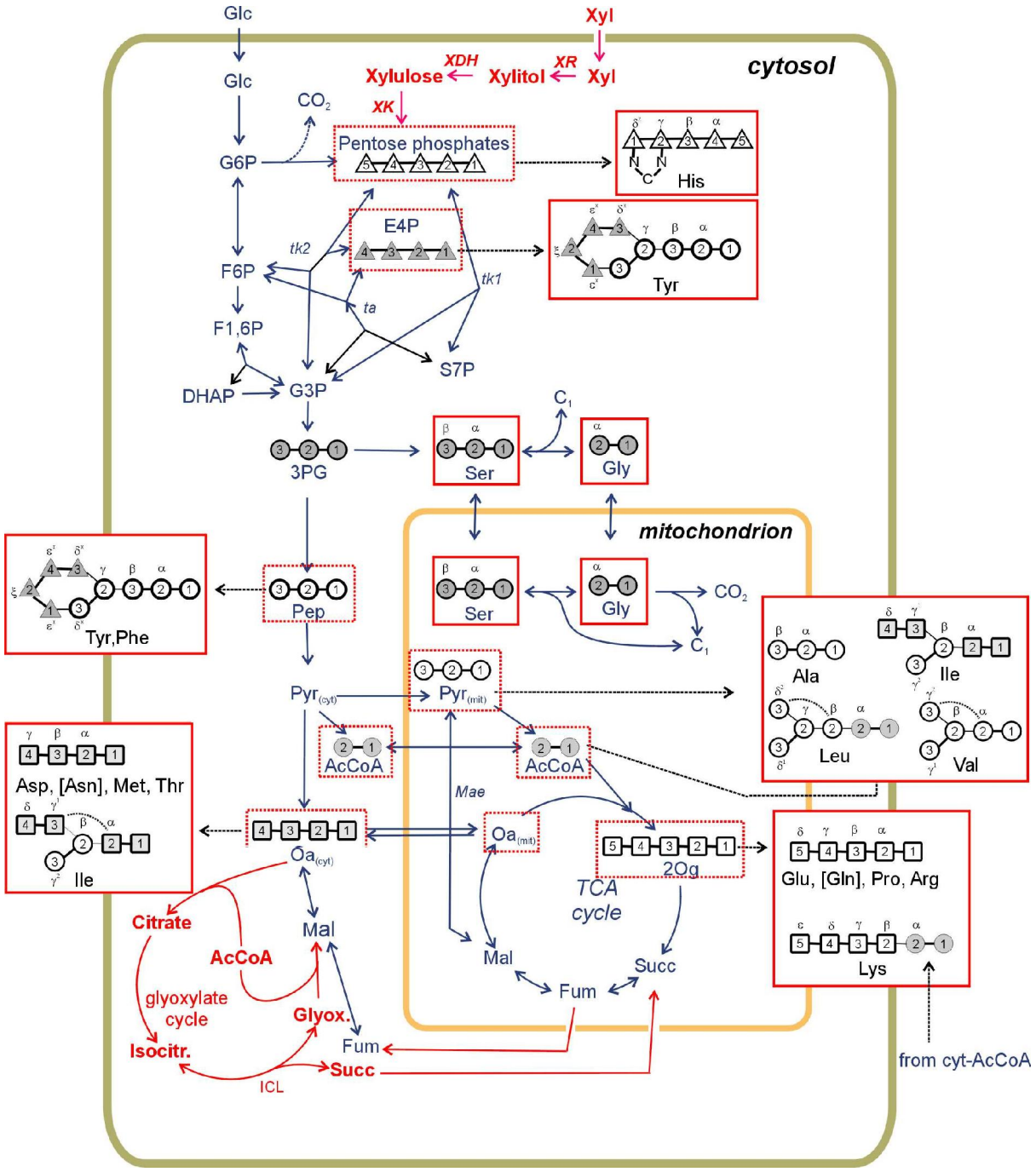


Metabolic flux ratio (METAFoR) profiling

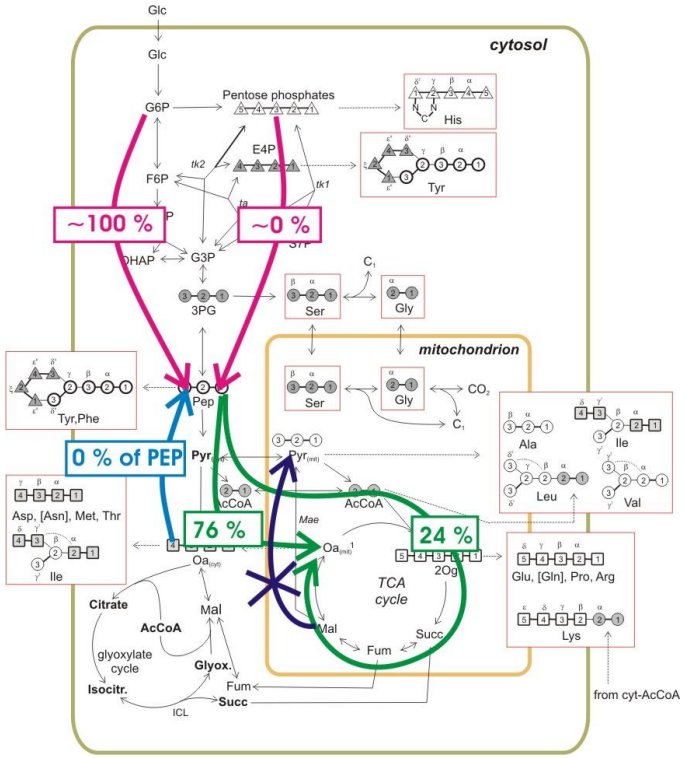
- about 10% of the carbon source uniformly ^{13}C labeled
- harvest cells, hydrolyze with 6M HCl
- remove acid, disolve in D_2O
- acquire 2D $^{1}H,^{13}C$ HSQC spectrum
- obtain the flux ratios from cross peak fine structures
- FCAL (R. Glaser)

- alternatively: use cell extracts and NMR/MS analysis

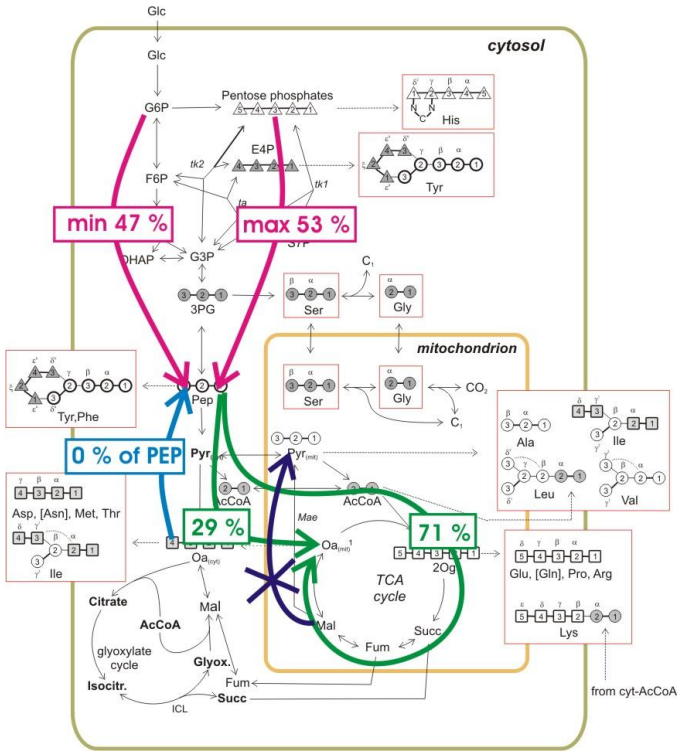



Glc
Xyl
cytosol
Glc
XDH
Xylulose
Xylitol
XR
Xyl
CO2
XK
Pentose phosphates
G6P
His
E4P
tk2
tk1
ta
F6P
Tyr
F1,6P
S7P
DHAP
G3P
C1
3PG
Ser
Gly
mitochondrion
Tyr,Phe
Pep
Ser
Gly
CO2
C1
Ala
Ile
Pyr(cyt)
Pyr(mit)
Leu
Val
AcCoA
AcCoA
Asp, [Asn], Met, Thr
Mae
Oa(mit)
Oa(cyt)
2Og
Glu, [Gln], Pro, Arg
Ile
TCA
cycle
Citrate
Mal
AcCoA
Mal
Succ
glyoxylate
cycle
Glyox.
Fum
Fum
Isocitr.
Succ
ICL
Lys
from cyt-AcCoA

Glucose, aerobic batch

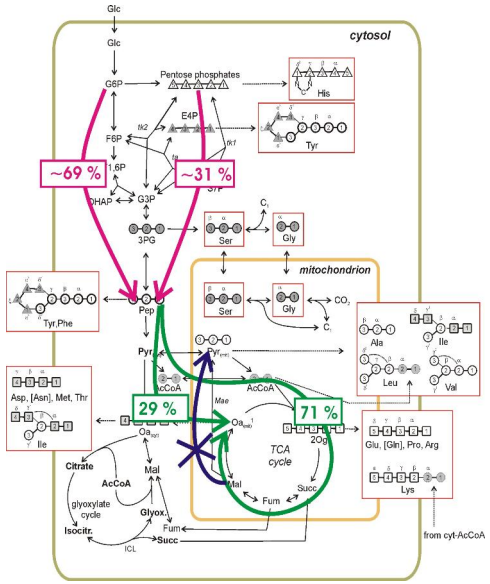


Glucose, aerobic chemostat

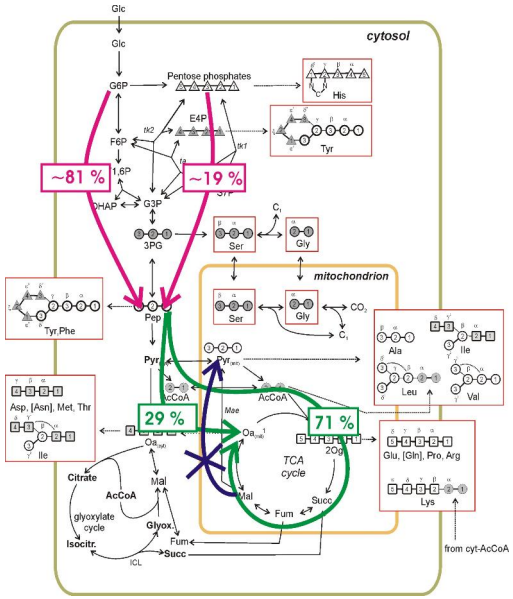


Metabolic flux ratios under steady state

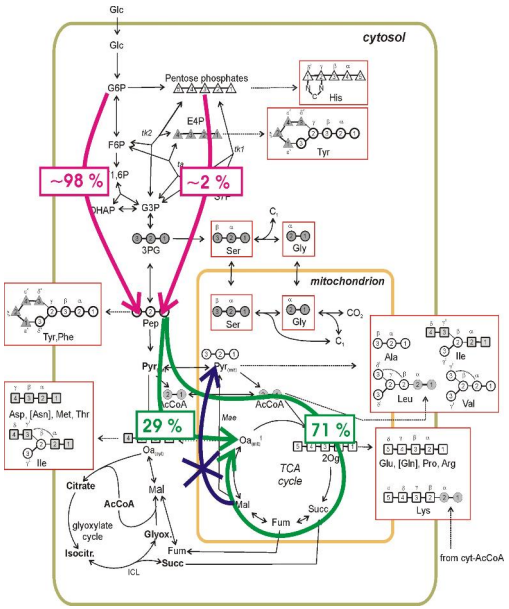
aerobic



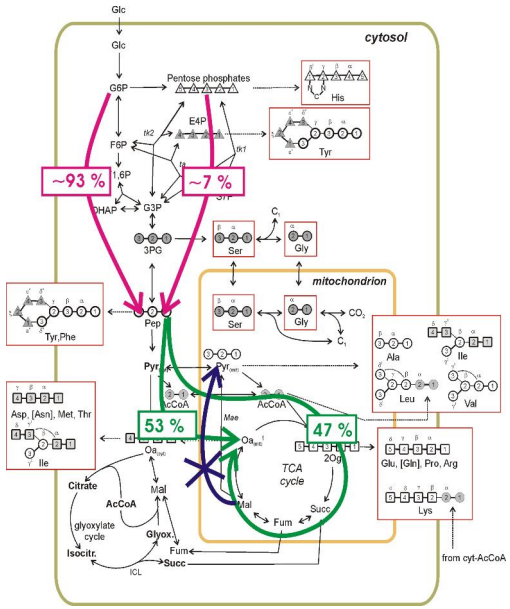
2.8% O_2



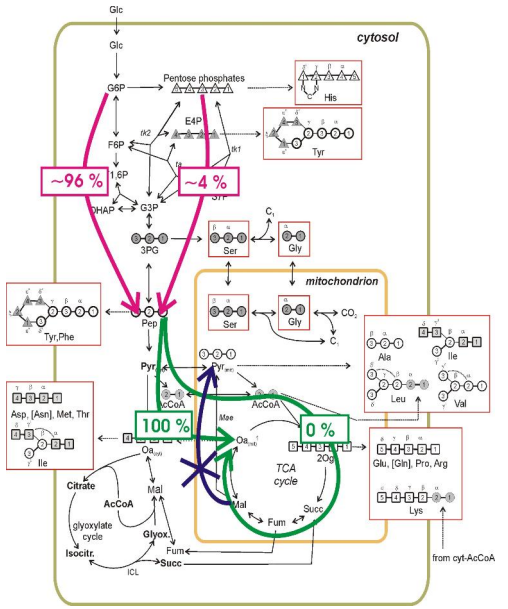
1.0% O_2



0.5% O_2

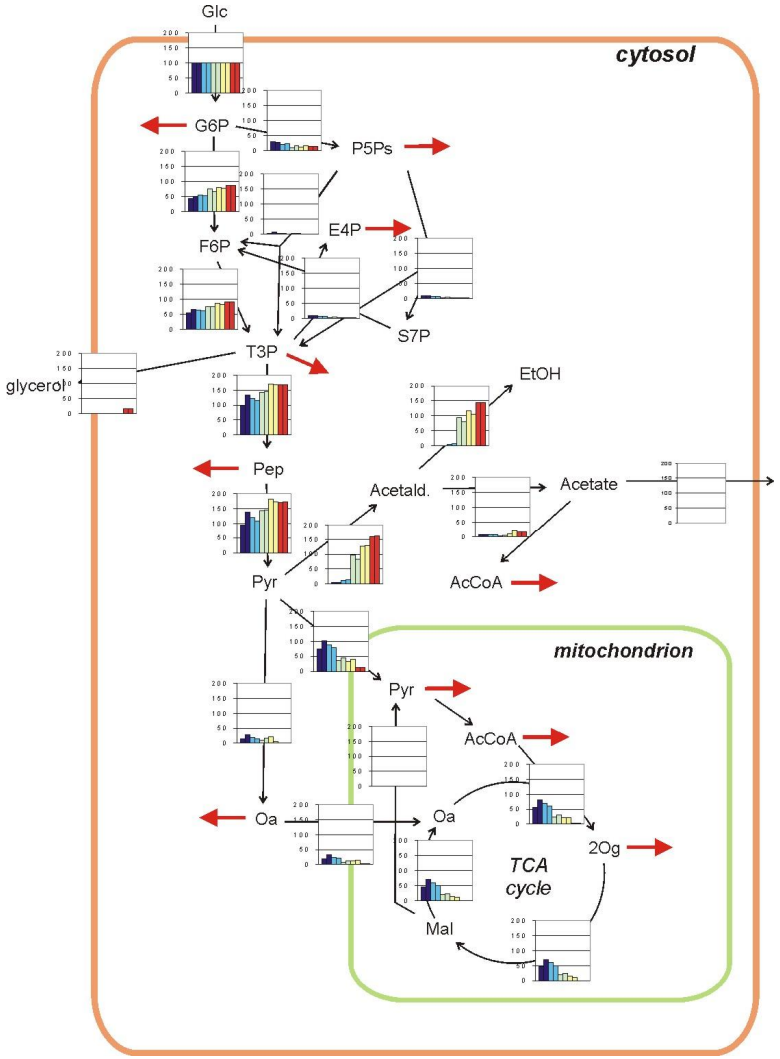


anaerobic

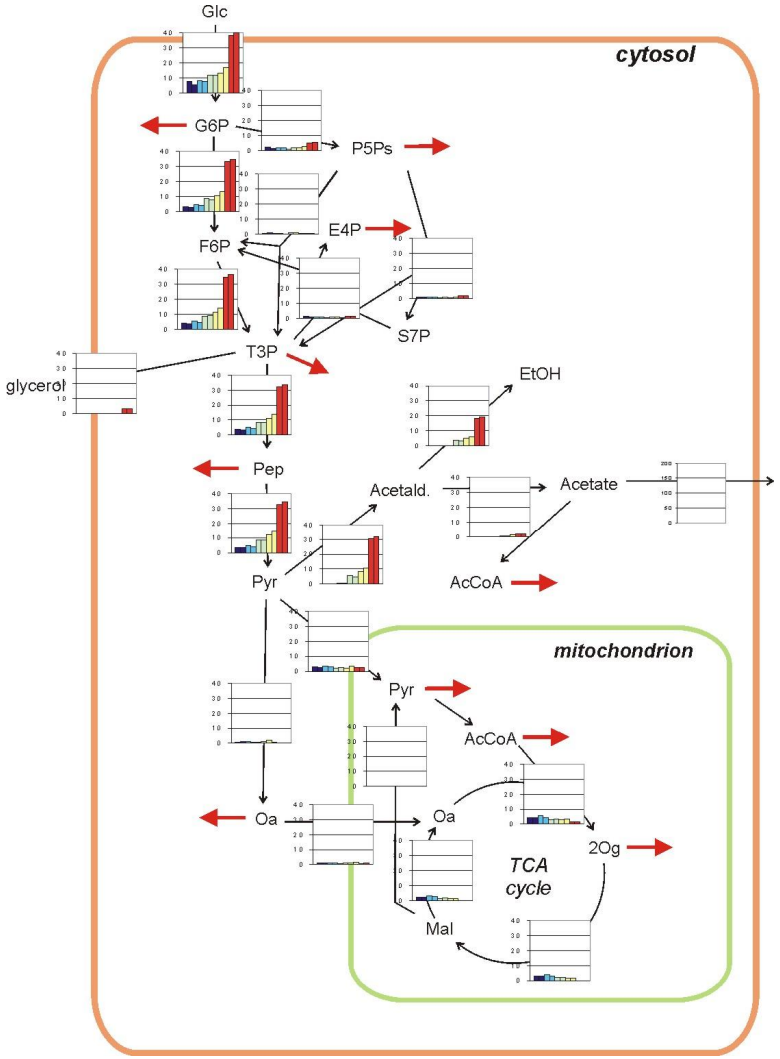


Results of ^{13}C MFA

Fluxes normalised to glucose uptake (100)



Fluxes as C-mmol g^{-1}(DW) h^{-1}



Simulated ^{1}H NMR spectra of the eight ^{13}C isotopomers of serine