Introduction on metabolism & refresher in enzymology

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General objectives of the course

- Understand the general behaviour of metabolic systems
- Ability to model their dynamics
- Express how kinetic enzyme properties affect metabolite concentrations and fluxes
- Express how networks respond to changes in environment
- Examine how experimental data may be used to identify a metabolic model
- Interpret these behaviours in terms of biological regulation
- Generalize to signal transduction networks

Course prerequisites

- Knowledge of enzyme kinetics
- Linear algebra
  - Matrix rank analysis, diagonalization, etc…
  - Familiarity with a mathematical package such as Scilab, Maple, R or Matlab
- Dynamical systems
  - Jacobian
  - Stability analysis

Course schedule

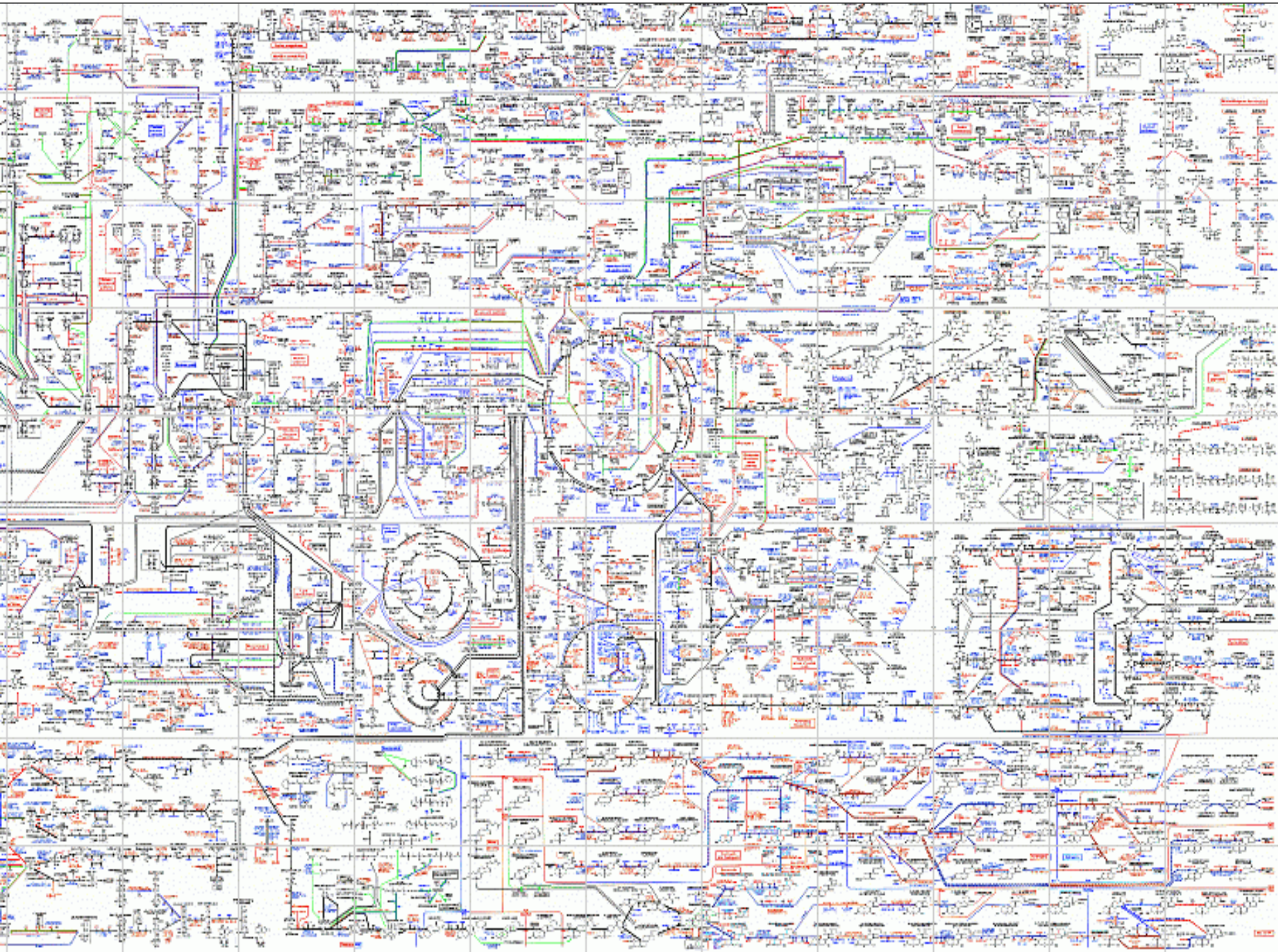
- November 2 Introduction to Metabolic Control Theory
  November 30 Regulation analysis
- November 23, December 7 & 14
  Morning 9am Practical on metabolic model and MCT
  Afternoon 2pm Theory

Outline

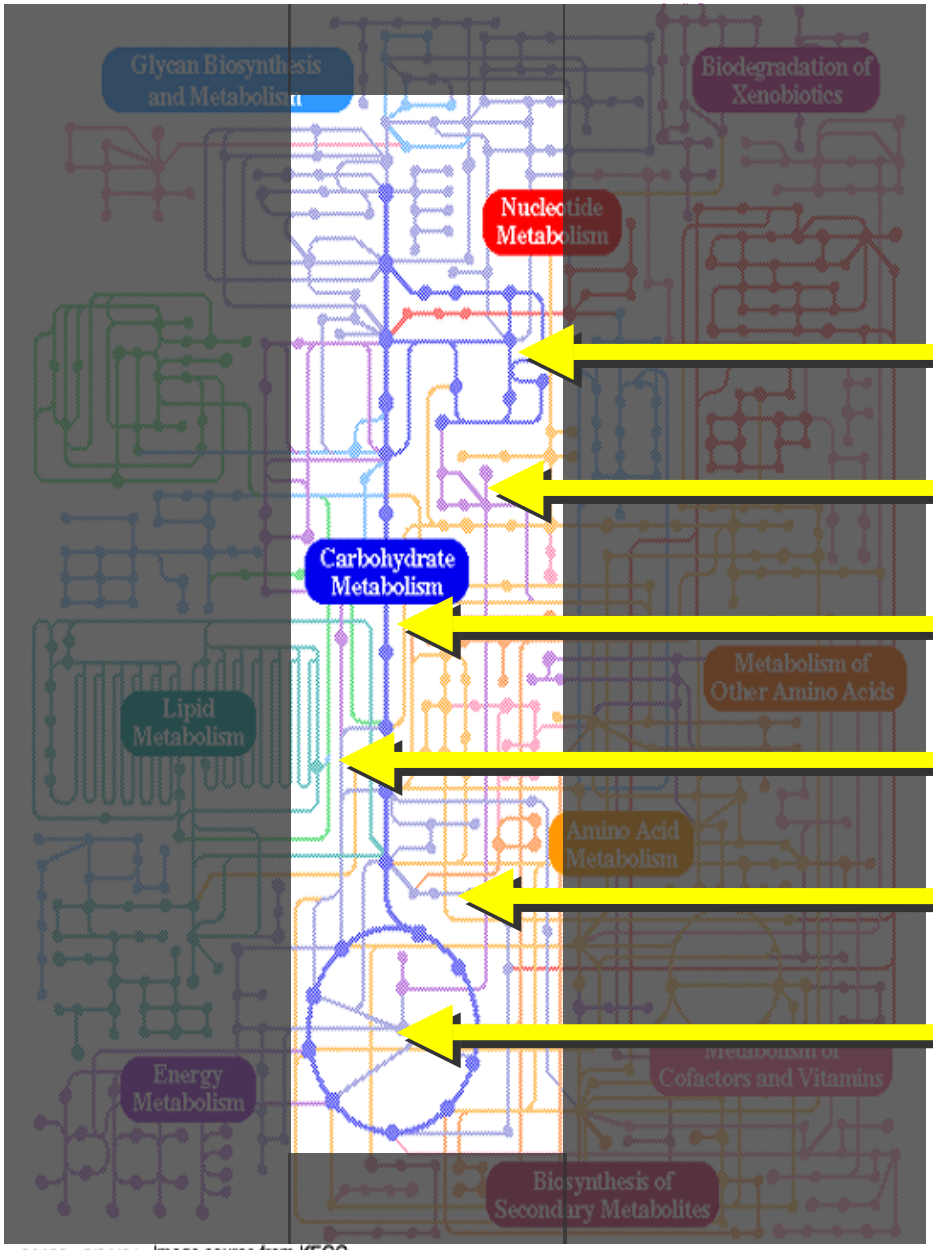
1. Introduction on metabolism
2. Methods to investigate metabolism
3. Refresher of enzyme kinetics

1. What is metabolism?

- Life’s chemical factory
  - Typically several hundred reactions involving small molecules
- Balances
  - Nutrients and outputs
  - Energy
  - Redox…
- Fast turnover
- Almost always catalyzed by enzymes



Central C metabolism subnetwork



Pentose Phosphates

Entner-Doudoroff

Glycolysis/Gluconeogenesis

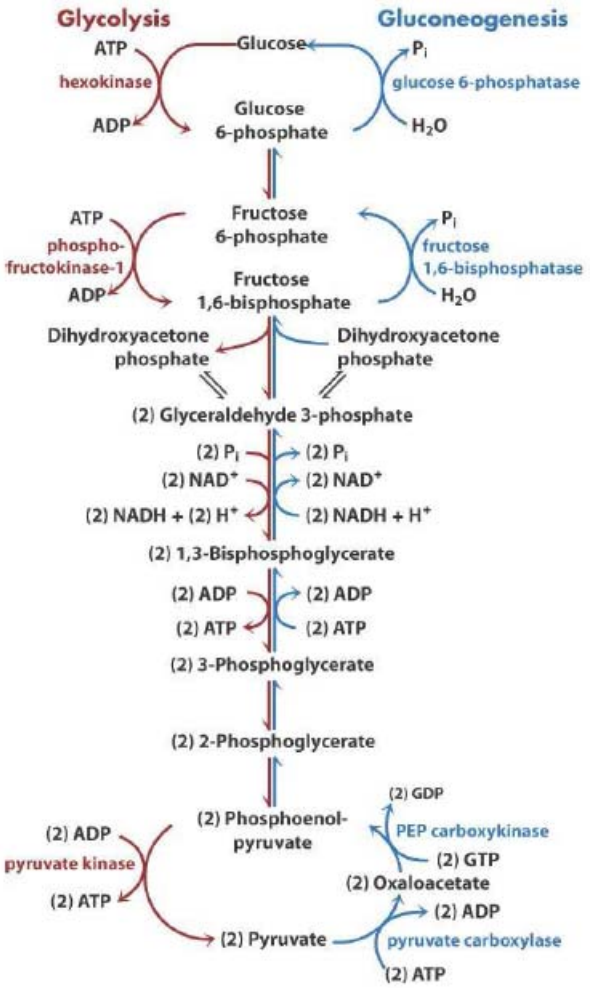
Methylglyoxal

Acetate

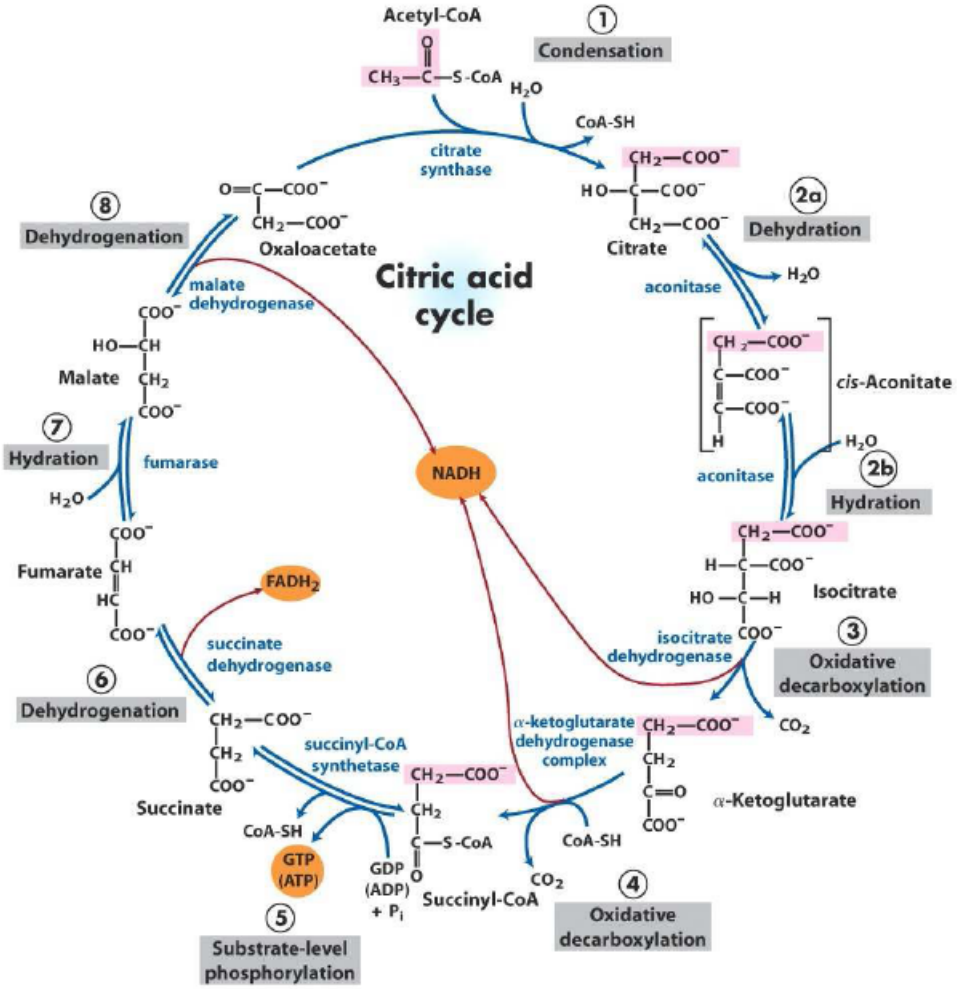
TCA

01100 5/31/04 Image source from KEGG

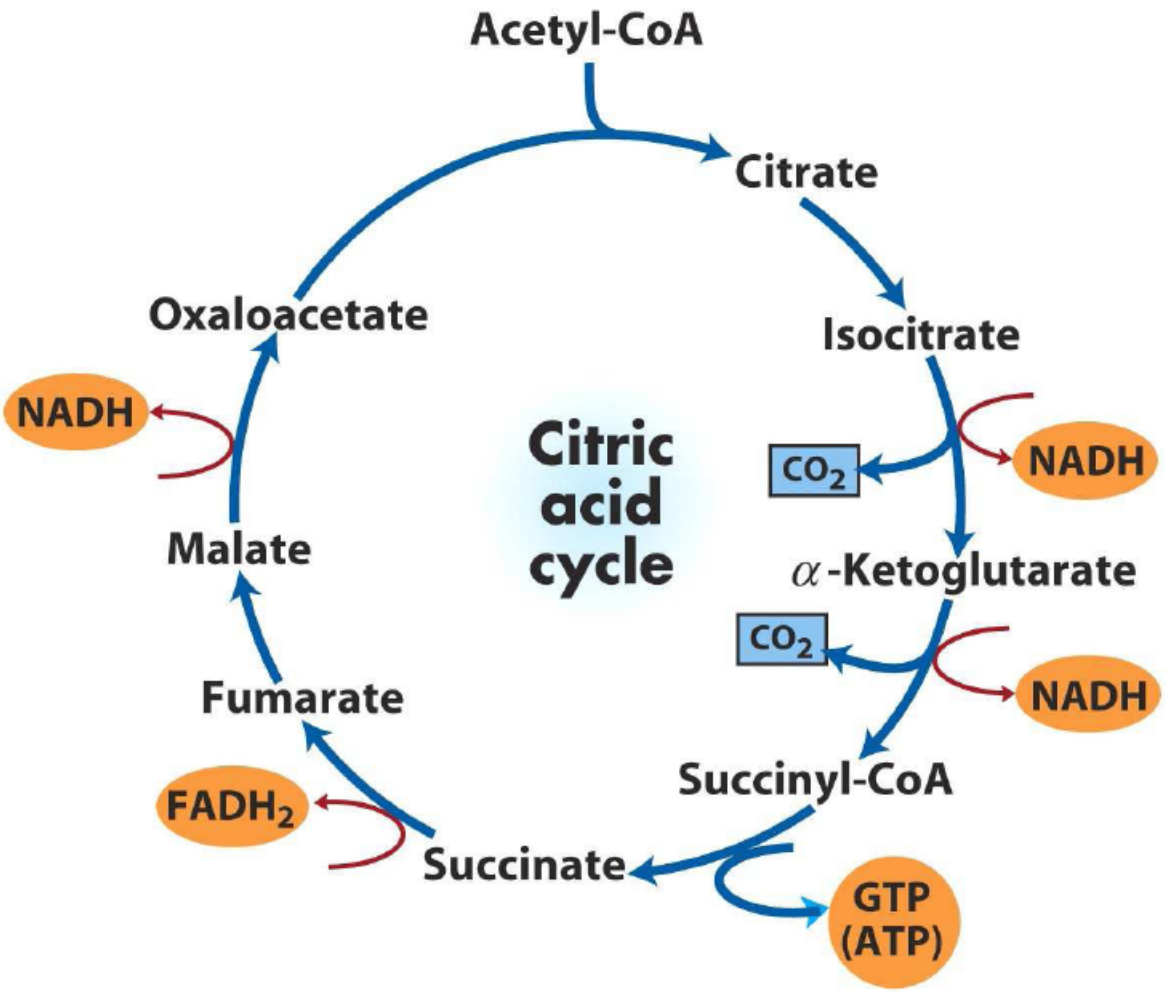
Glycolysis / gluconeogenesis



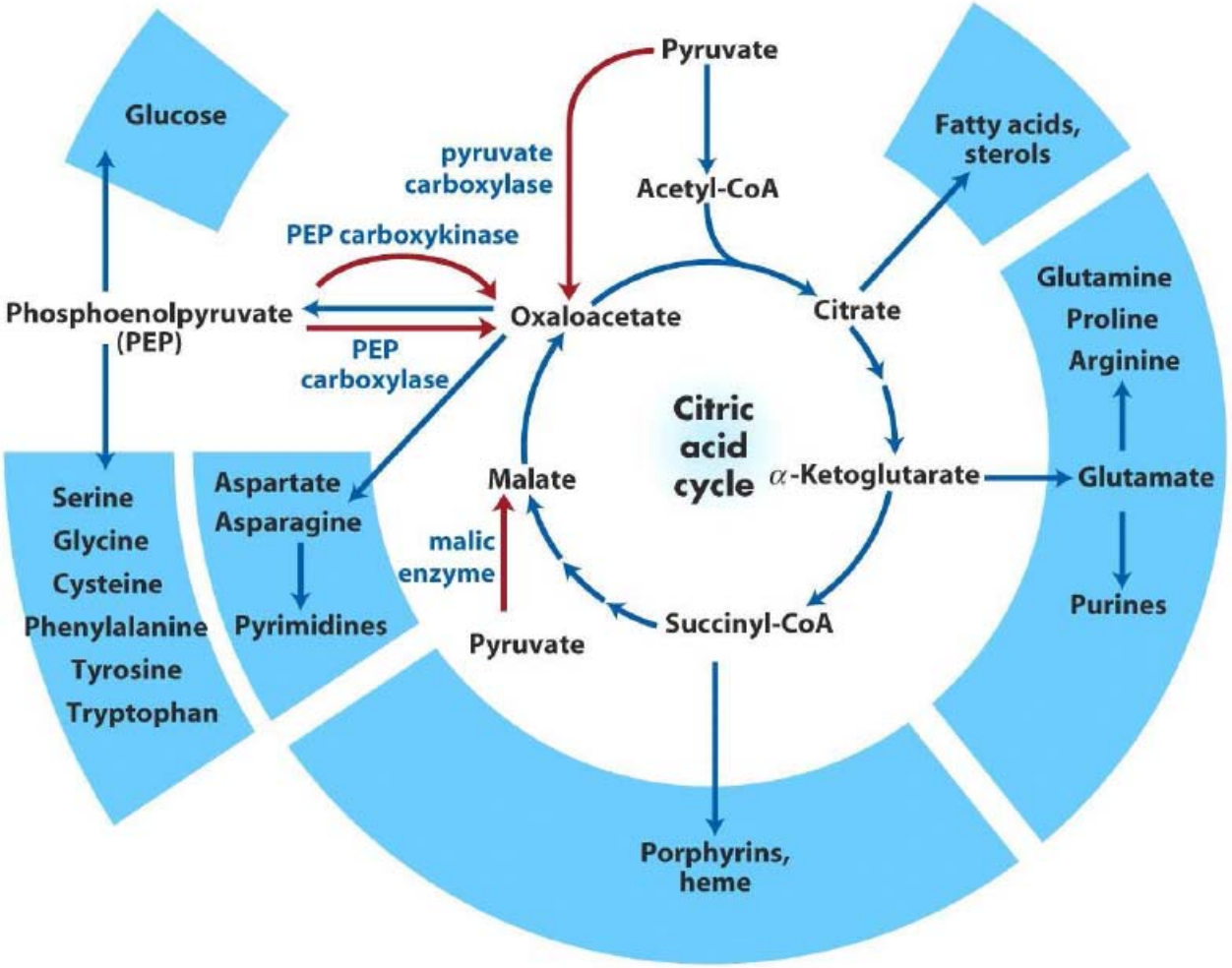
TCA cycle


Acetyl-CoA
1
Condensation
$CH_3—C—S\text{-}CoA$
H_2O
CoA-SH
citrate synthase
$CH_2—COO^-$
$HO—C—COO^-$
$CH_2—COO^-$
Citrate
2a
Dehydration
aconitase
H_2O
$CH_2—COO^-$
$C—COO^-$
$C—COO^-$
H
cis-Aconitate
H_2O
aconitase
2b
Hydration
$CH_2—COO^-$
$H—C—COO^-$
$HO—C—H$
COO^-
Isocitrate
isocitrate dehydrogenase
3
Oxidative decarboxylation
CO_2
α-ketoglutarate dehydrogenase complex
$CH_2—COO^-$
CH_2
$C=O$
COO^-
α-Ketoglutarate
CoA-SH
CO_2
4
Oxidative decarboxylation
$CH_2—COO^-$
CH_2
$C—S\text{-}CoA$
O
Succinyl-CoA
succinyl-CoA synthetase
CoA-SH
GTP (ATP)
GDP (ADP) + P_i
5
Substrate-level phosphorylation
$CH_2—COO^-$
CH_2
COO^-
Succinate
succinate dehydrogenase
$FADH_2$
6
Dehydrogenation
COO^-
CH
HC
COO^-
Fumarate
fumarase
H_2O
7
Hydration
COO^-
$HO—CH$
CH_2
COO^-
Malate
malate dehydrogenase
8
Dehydrogenation
$O=C—COO^-$
$CH_2—COO^-$
Oxaloacetate
Citric acid cycle
NADH

TCA cycle


Acetyl-CoA
Citrate
Isocitrate
NADH
CO_2
α-Ketoglutarate
CO_2
NADH
Succinyl-CoA
GTP (ATP)
Succinate
$FADH_2$
Fumarate
Malate
NADH
Oxaloacetate
Citric acid cycle

Anaplerosis



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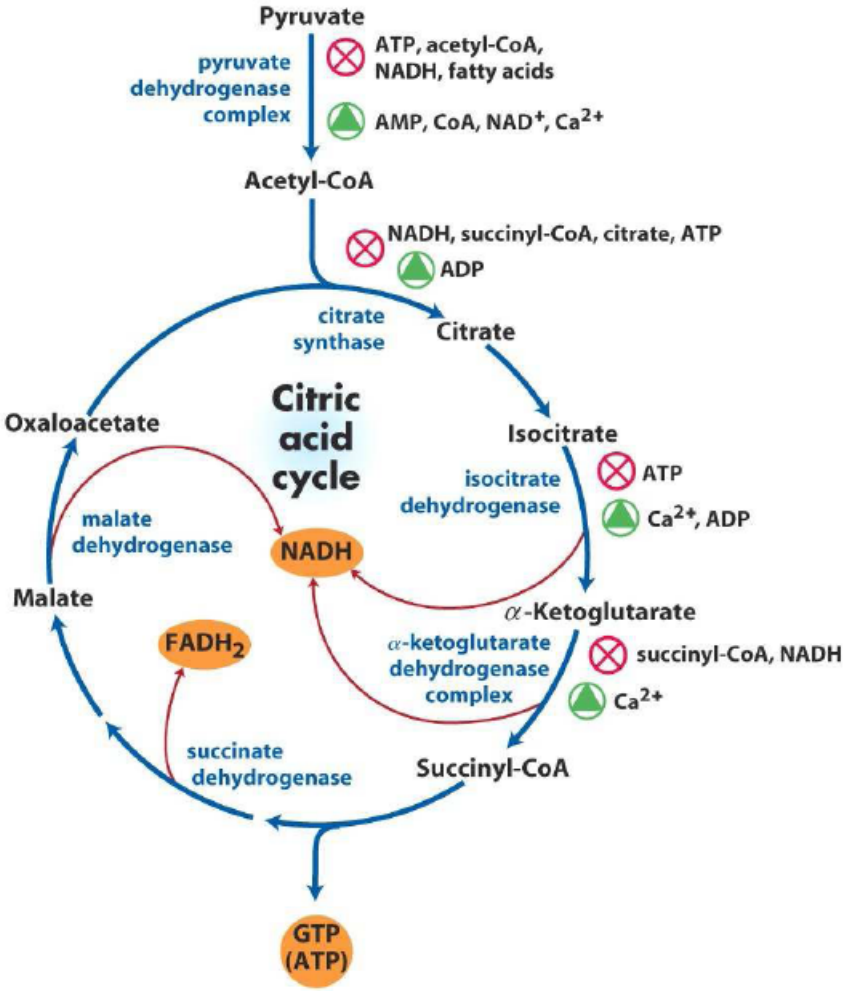
Regulation



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2. Methods to investigate metabolism

- Metabolomics: metabolite identification and quantitation
- Fluxomics
- Analytical tools based on
  - Nuclear Magnetic Resonance (NMR)
  - Mass spectrometry (MS)
  - Liquid chromatography (LC)

Metabolomics

Metabolites

LC-NMR

NMR

- Complex mixtures
- Identification / Structure

LC-NMR

- Targetted
- Suitable for high throughput

LC-MS

IC-MS

MS/MS

LC-MS/MS

Lipids, sugars, organic acids, aminoacids, coA esters…

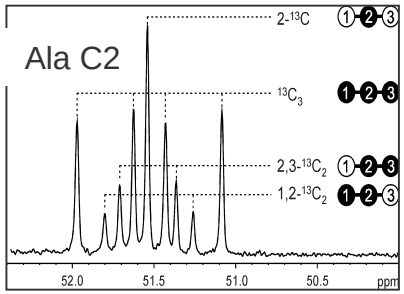
IC-MS/MS

Sugar phosphates, nucleotides, organic acids…

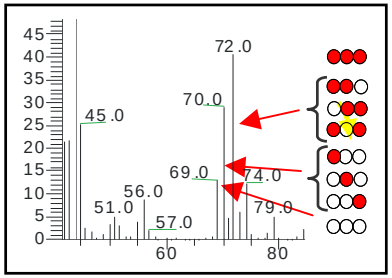
Flux measurements

[U-^{13}C]-glucose

Biomass Metabolites

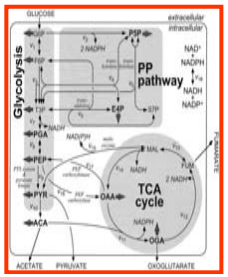


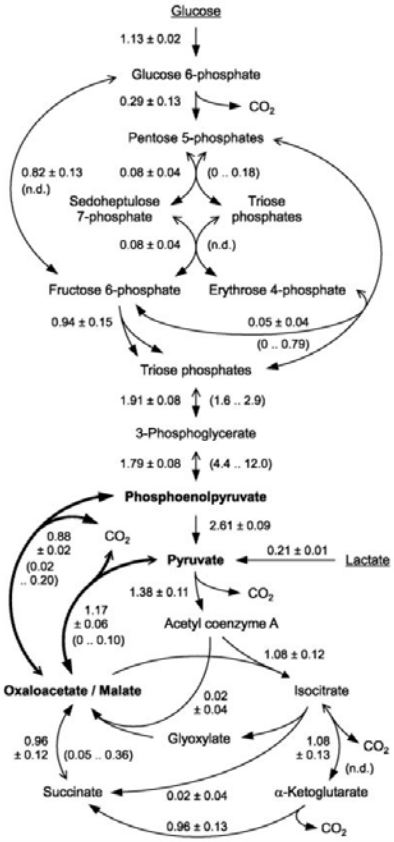
NMR: position isotopomers



MS: mass isotopomers (GC-MS or LC-MS)

Metabolic network





Mapping of fluxes

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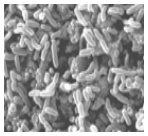
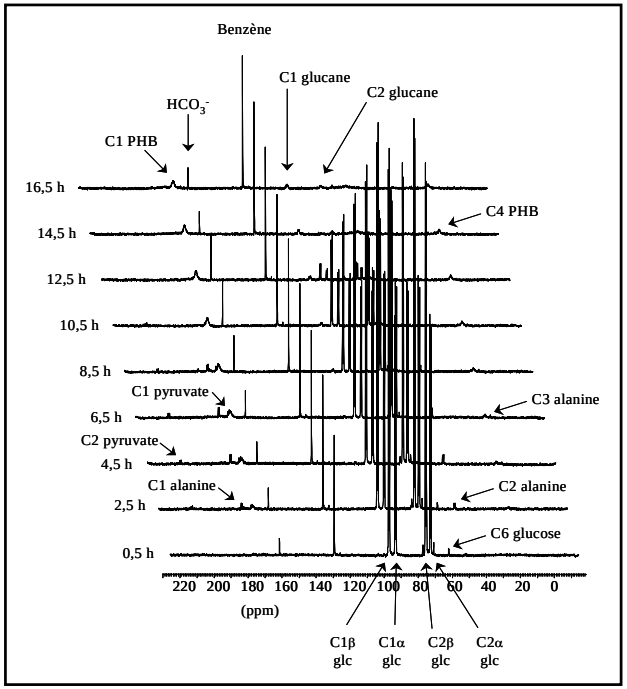
In vivo NMR



(cells, tissues, organs)

(physiological conditions)



^{13}C

Carbon distribution
In vivo dynamics

^{31}P

Energy metabolism
pH, compartmentation
etc.

^{15}N

Nitrogen metabolism
N/C metabolic coupling

3. Enzyme kinetics: Michaelis-Menten

Mass action kinetics:

$$v_1 = k_1 E.S - k_{-1} ES$$

$$v_2 = k_2 ES$$

Quasi steady-state:

$$v_1 = v_2 = v$$

$$E + ES = E_0$$

Michaelis-Menten

$$v = E_0 \frac{k_E S}{1 + \frac{S}{K_m}}$$ reaction rate $(M.s^{-1})$

$$K_m = \frac{k_{-1} + k_{cat}}{k_1}$$ Michaelis constant (M)

$$k_E = \frac{k_{cat}}{K_m}$$ catalytic efficiency $(M^{-1}.s^{-1})$

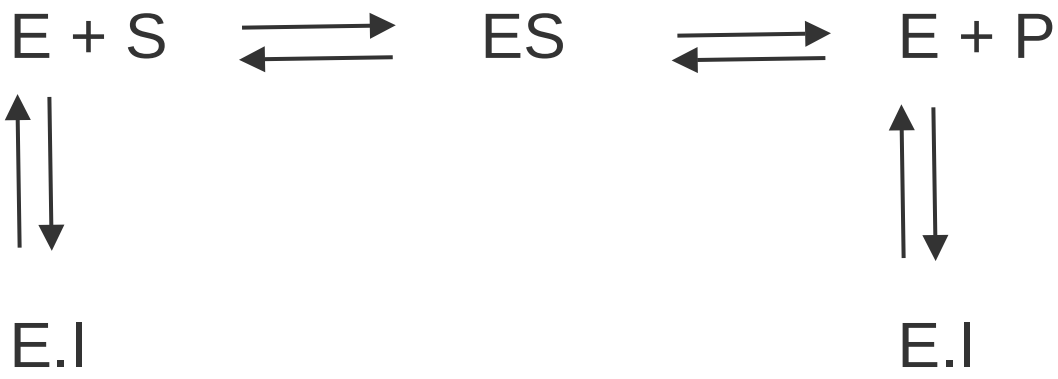
k_{cat} is the maximal turnover rate (s^{-1})

Reversible Michaelis-Menten

$$v = E_0 \frac{k_+ S - k_- P}{1 + \frac{S}{K_S} + \frac{P}{K_P}}$$

This is the default expression for kinetic modelling,
even when $k_- = 0$,
because it also accounts for competitive product inhibition.

Competitive inhibition



$$v = E_0 \frac{k_+ S - k_- P}{1 + \frac{S}{K_S} + \frac{P}{K_P} + \frac{I}{K_{Ic}}}$$

Other inhibitions

- Uncompetitive (more effective at high substrate concentration)

$$v = E_0 \frac{k_+ S - k_- P}{1 + \left(\frac{S}{K_S} + \frac{P}{K_P} \right) \left(1 + \frac{I}{K_{Iu}} \right)}$$

- Mixed

$$v = E_0 \frac{k_+ S - k_- P}{1 + \left(\frac{S}{K_S} + \frac{P}{K_P} \right) \left(1 + \frac{I}{K_{Iu}} \right) + \frac{I}{K_{Ic}}}$$

Multiple substrates and products

If substrates and products bind independently and in random order:

$$v = E_0 \frac{k_{cat}^{+} \prod_i \frac{S_i}{K_{S_i}} - k_{cat}^{-} \prod_j \frac{P_j}{K_{P_j}}}{\prod_i \left(1 + \frac{S_i}{K_{S_i}}\right) + \prod_j \left(1 + \frac{P_j}{K_{P_j}}\right) - 1}$$

‘Convenience kinetics’

Liebermeister & Klipp, 2006, *Theoret. Biol. Med. Mod.* **3**:41

Haldane relationships

Equilibrium constraint:

$$K_{eq} = \frac{k_{cat}^{+}}{k_{cat}^{-}} \frac{\prod_{j} K_{P_j}}{\prod_{i} K_{S_i}}$$

Cooperativity

Hill equation:

$$v = E_0 \frac{k_{cat}(S / K_{0.5})^h}{1 + (S / K_{0.5})^h}$$

h is the Hill coefficient. Typically : $0.5 < h < 4$

This equation is purely empirical
(actually it is wrong for $S << K_{0.5}$)

$K_{0.5}$ is a phenomenological constant (not a K_m)

2-site cooperative binding

Adair equation

$$v = 2E_0 k_{cat} \frac{S/K_1 + S^2/K_1K_2}{1 + 2S/K_1 + S^2/K_1K_2}$$

realistically captures site dependencies

Saturation

$$E + ES = E_0$$

$$\frac{E.S}{ES} = \frac{k_{-1}}{k_1} = K_d$$

$$Y = \frac{ES}{E_0} = \frac{S / K_d}{1 + S / K_d}$$ saturation coefficient

Further reading

- *Understanding the Control of Metabolism*, by David Fell
  Portland Press, London, 1997
- *Fundamentals of Enzyme Kinetics*, by Athel Cornish-Bowden
  Portland Press, London, 2004

For the practical course

- The practical course will rely heavily on the theoretical course
- Familiarize yourself with the COPASI modeling environment http://www.copasi.org
  - COPASI handbook
- Refresh your course in linear algebra
- Be prepared to use your favourite mathematical package such as Scilab, Maple, R or Matlab
- You will be evaluated on the practical course