

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

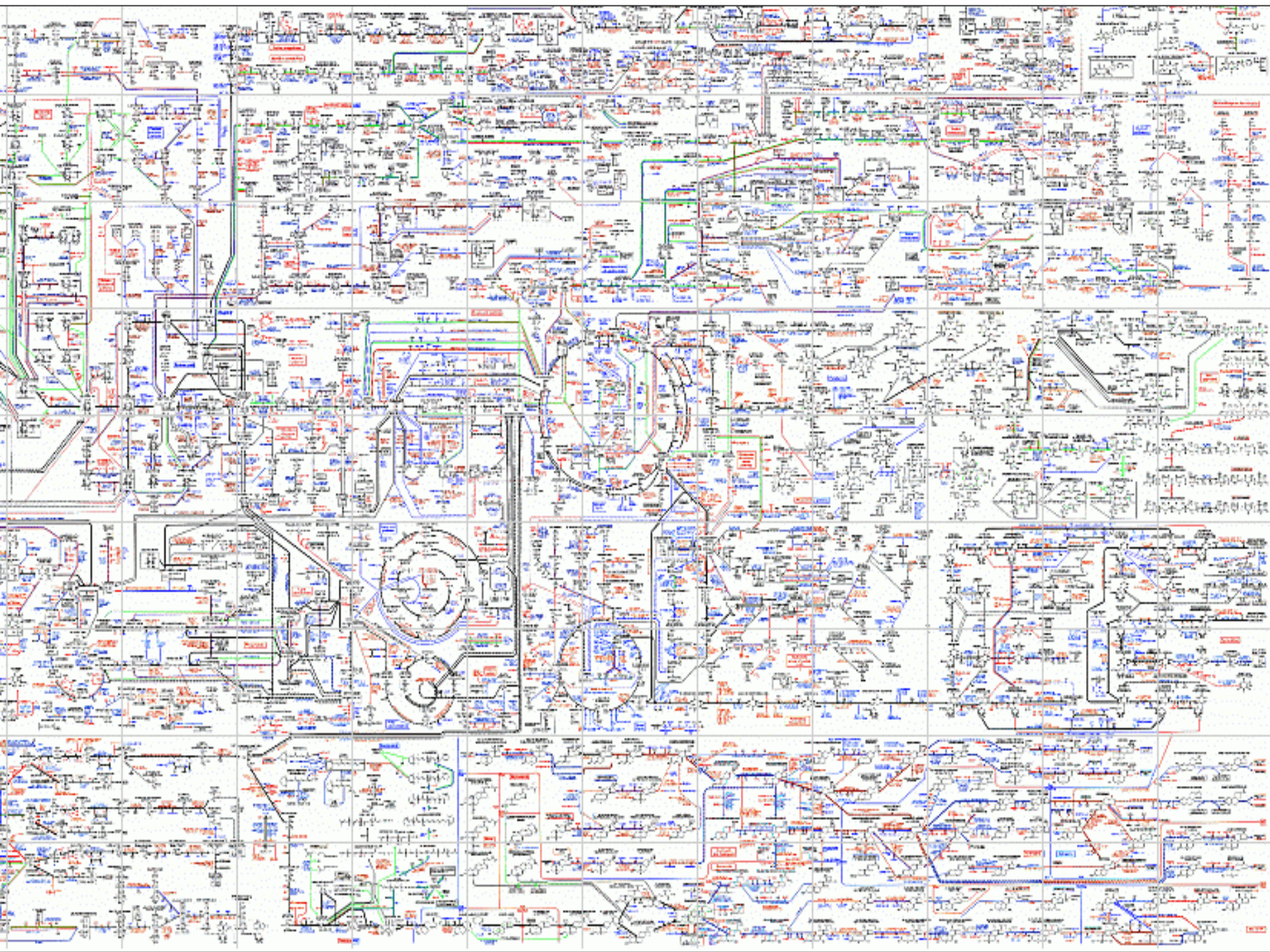
- October 17 Introduction to Metabolic Control Theory
October 24 Regulation analysis
- November 14, 28 & December 5
Morning 9am Practical on metabolic model and MCT
- December 5
Afternoon 2pm Glimpse of other useful theories

Outline

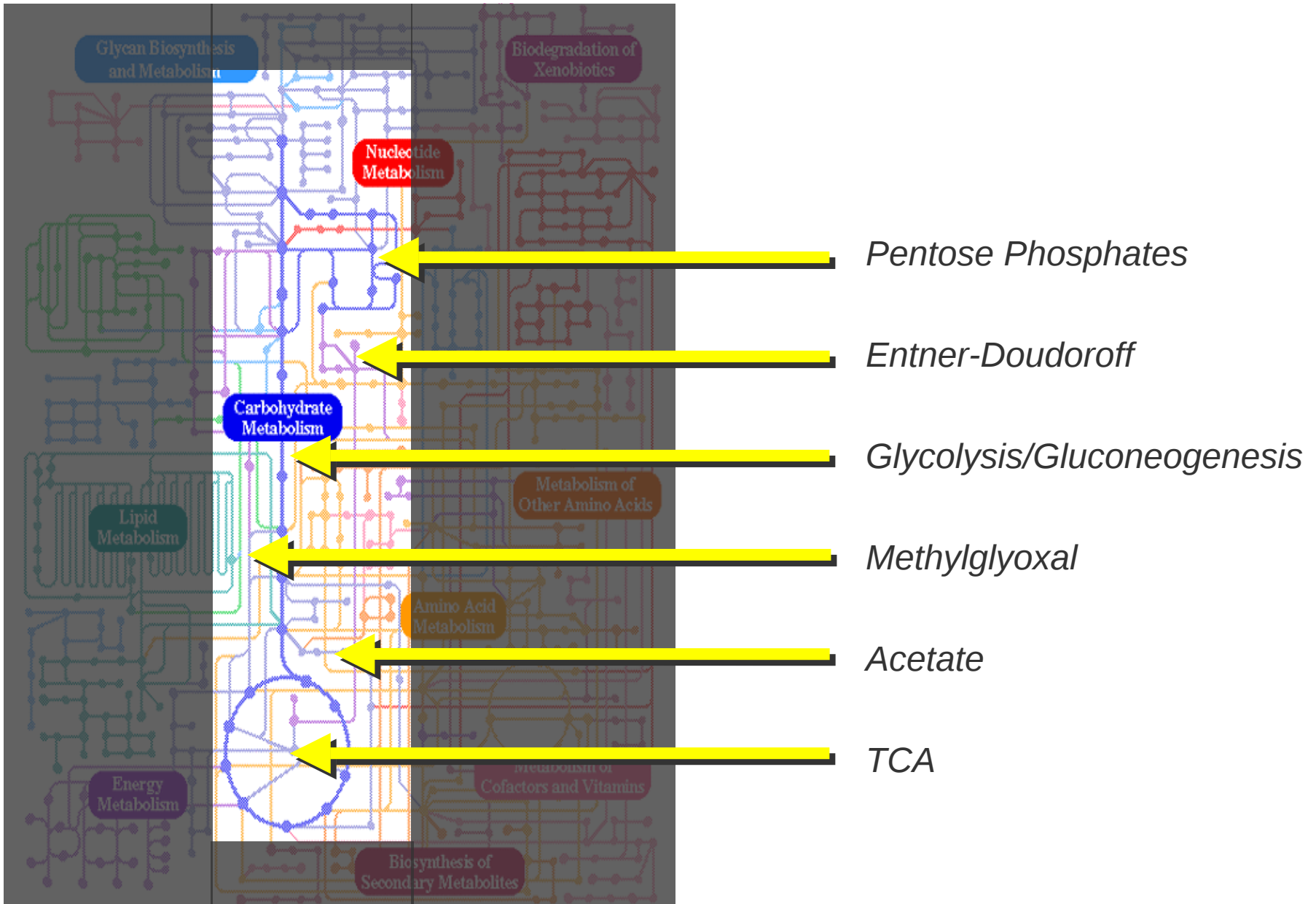
1. Introduction on metabolism
2. Methods to investigate metabolism
3. Refresher of enzyme kinetics

1. What is metabolism?

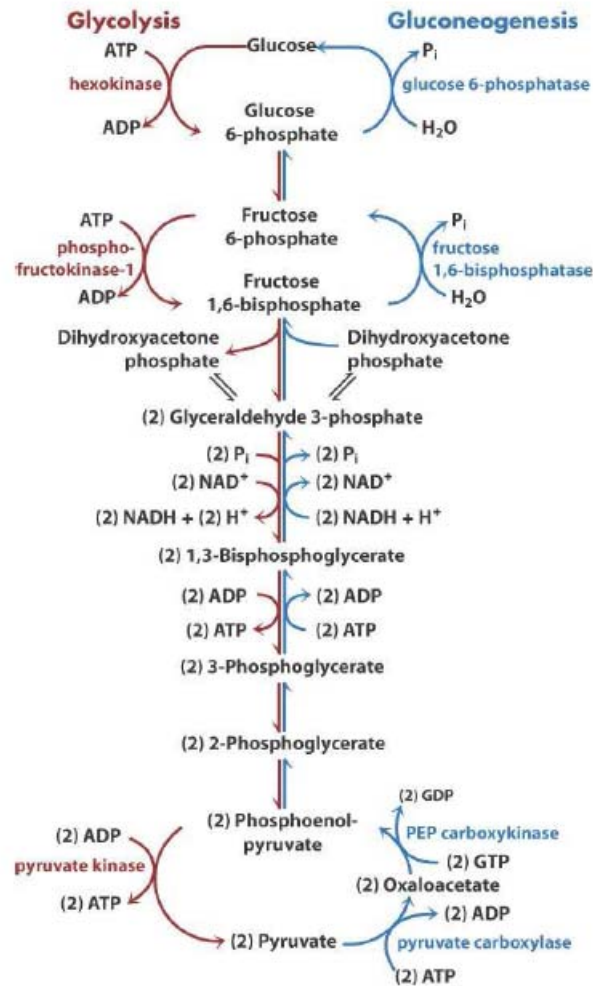
- Life's chemical factory
 - Typically several hundred reactions involving small molecules
- Balances
 - Nutrients and outputs
 - Energy
 - Redox...
- Fast turnover
- Almost always catalyzed by enzymes



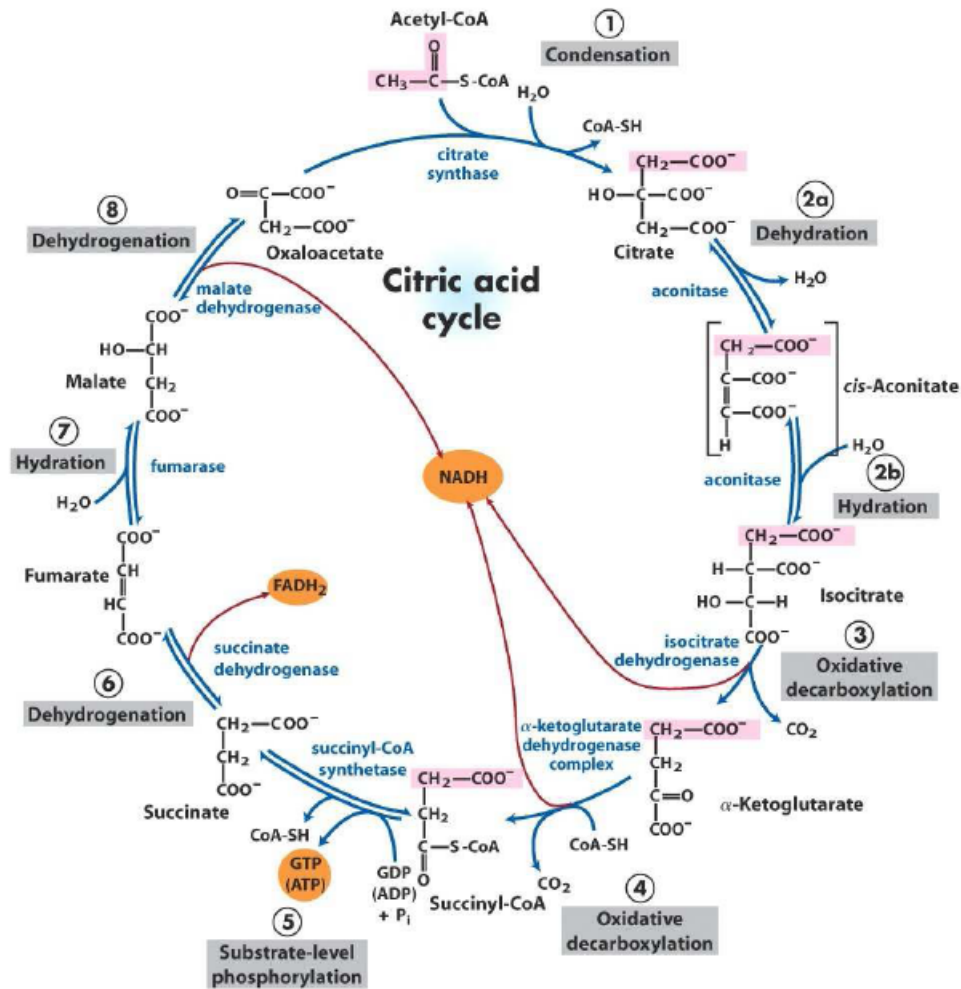
Central C metabolism subnetwork



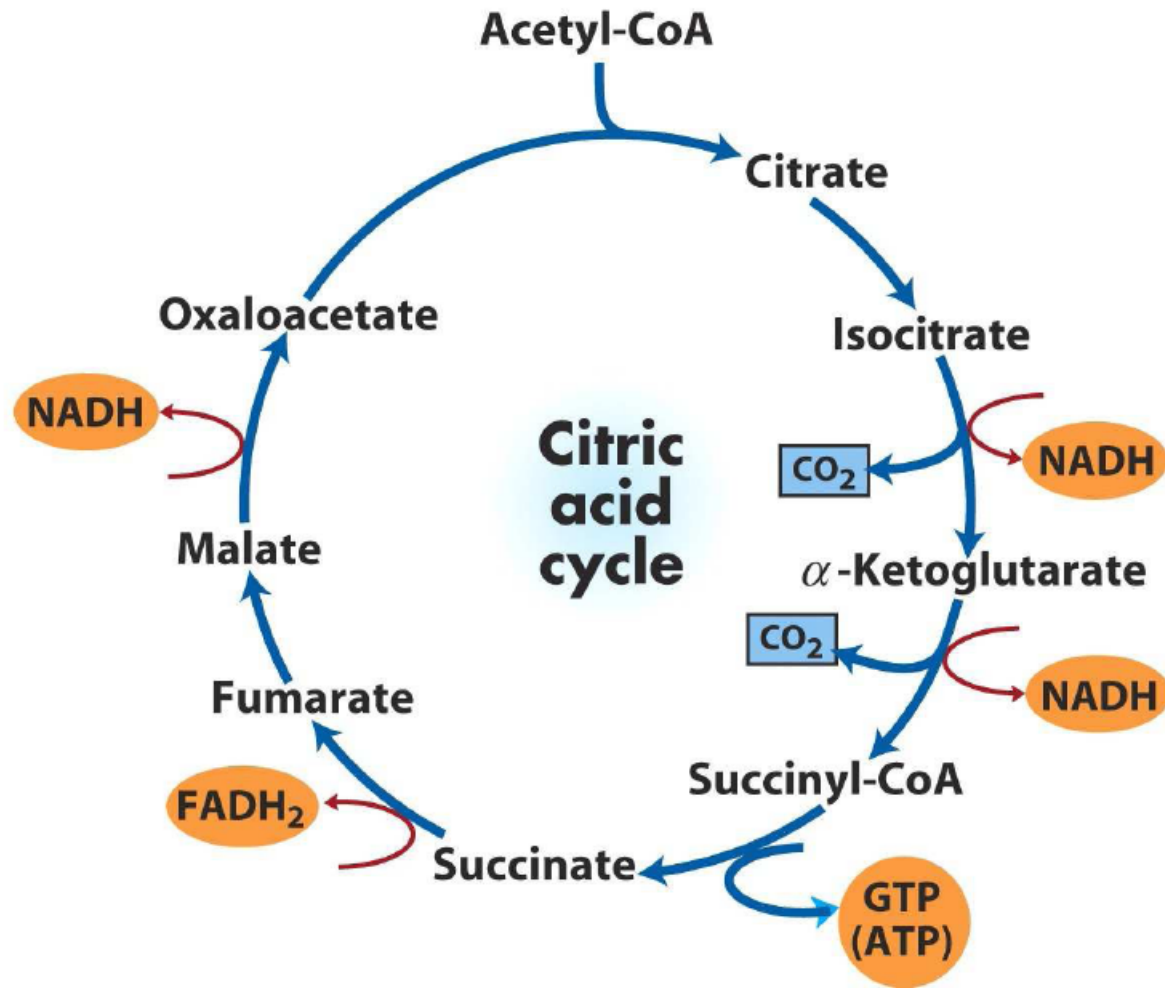
Glycolysis / gluconeogenesis



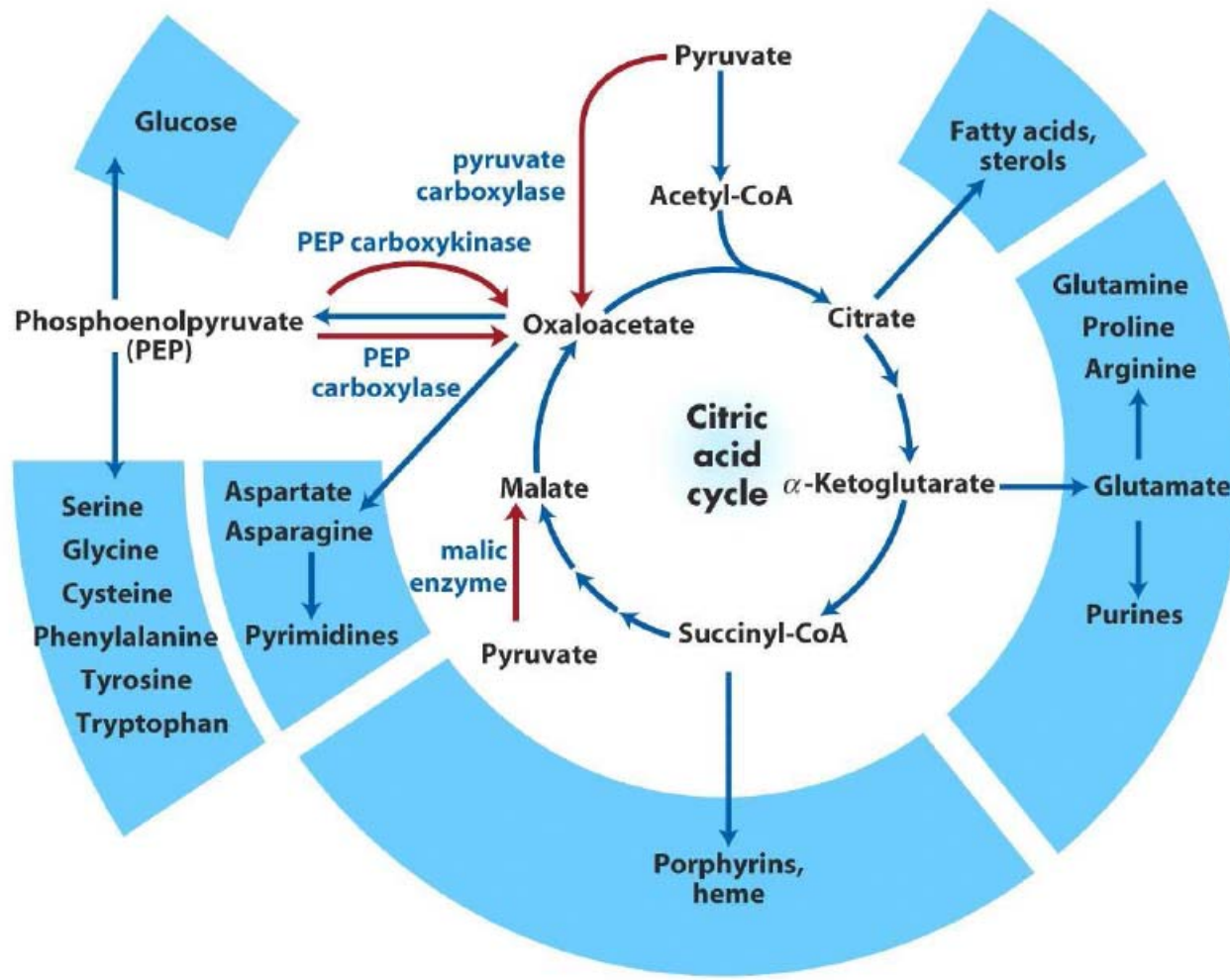
TCA cycle



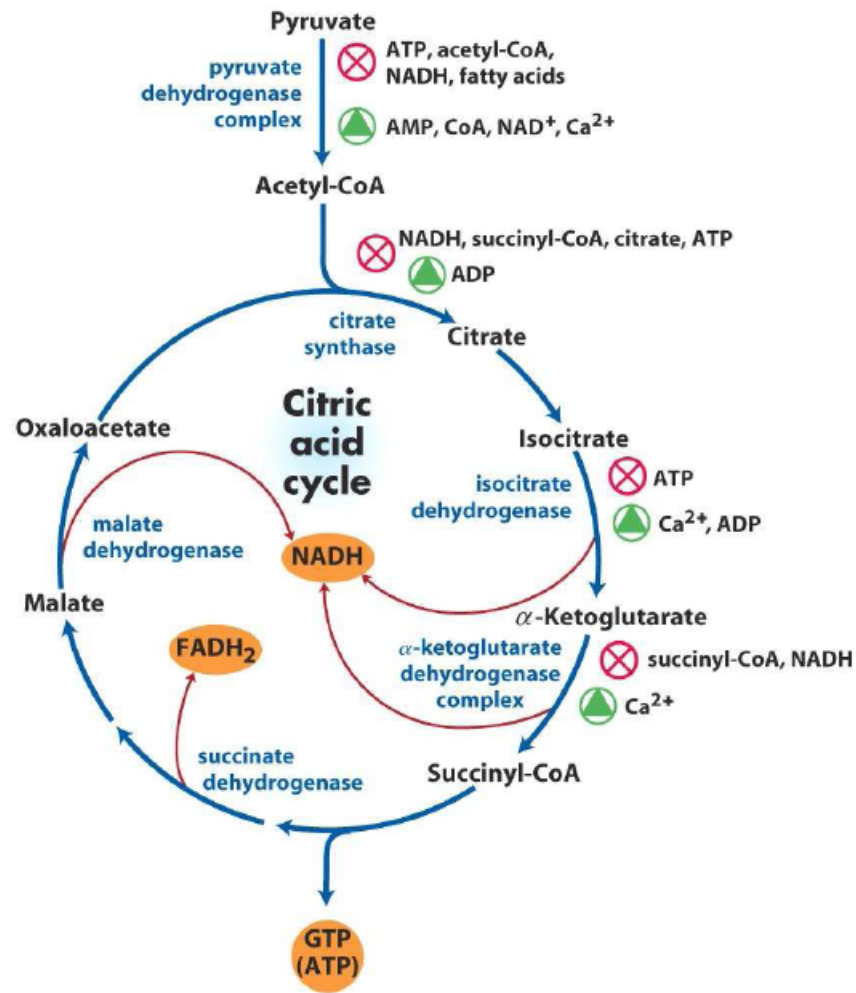
TCA cycle



Anaplerosis



Regulation



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



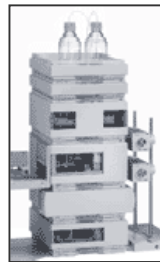
LC-NMR

NMR

- Complex mixtures
- Identification / Structure

LC-NMR

- Targetted
- Suitable for high throughput



LC-MS



IC-MS



MS/MS

LC-MS/MS

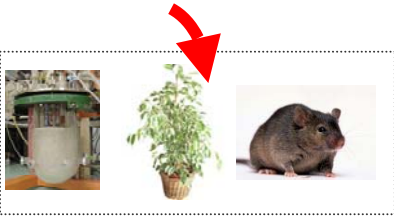
Lipids, sugars, organic acids, aminoacids, coA esters...

IC-MS/MS

Sugar phosphates, nucleotides, organic acids...

Flux measurements

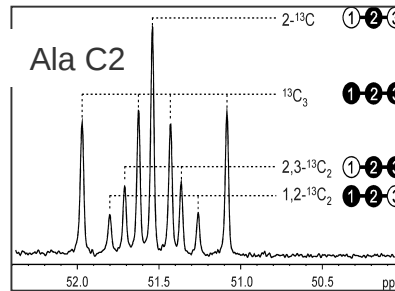
[U-¹³C]-glucose



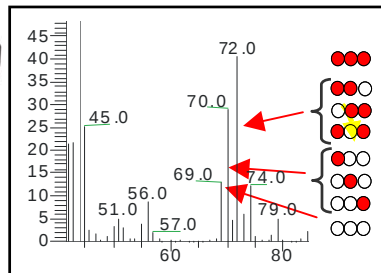
Biomass
Metabolites



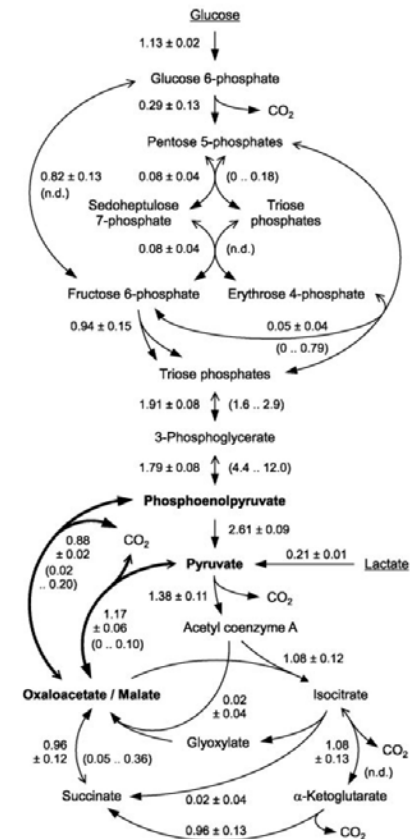
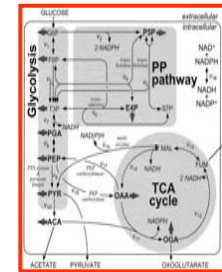
NMR: position isotopomers



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

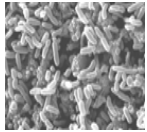


Metabolic network

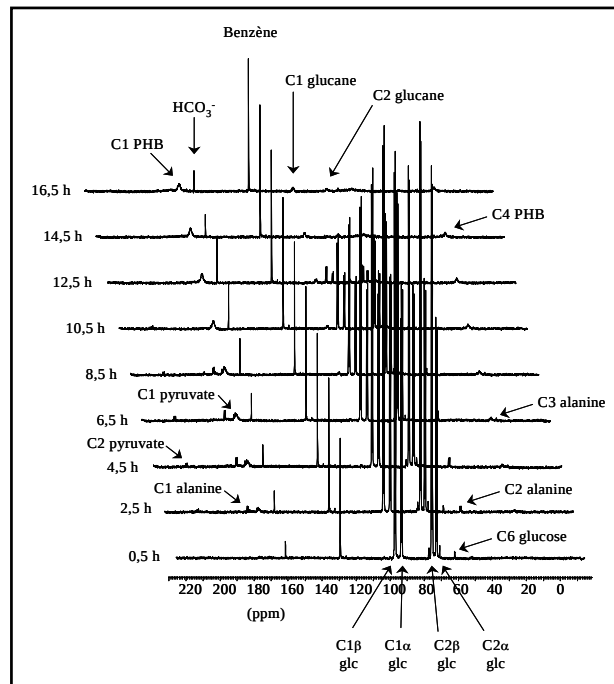


Mapping of fluxes

In vivo NMR



(cells, tissues, organs)



^{13}C

Carbon distribution
In vivo dynamics

^{31}P

Energy metabolism
pH, compartmentation
etc.

^{15}N

Nitrogen metabolism
N/C metabolic coupling

(physiological conditions)

3. Enzyme kinetics: Michaelis-Menten



Mass action kinetics:

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

$$v_2 = k_2 ES$$

Quasi steady-state:

$$v_1 = v_2 = v$$

$$E + ES = E_0$$

Michaelis-Menten



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

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

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

$$k_{cat} \quad \text{is the maximal turnover rate (s}^{-1}\text{)}$$

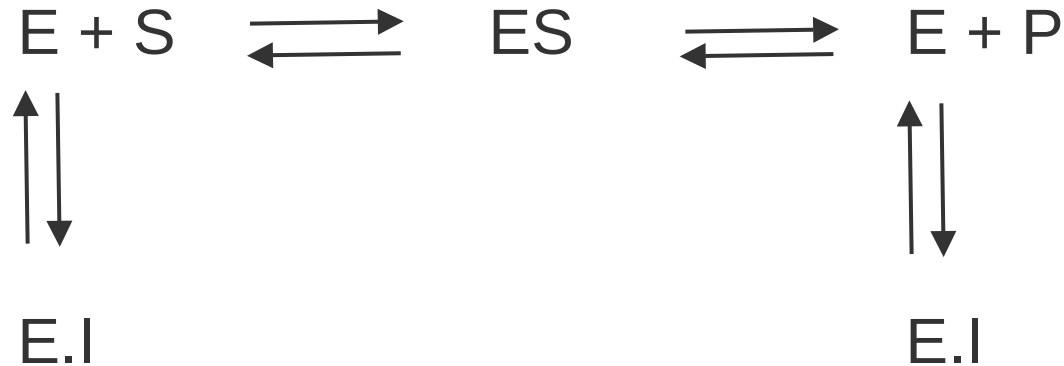
Reversible Michaelis-Menten



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

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 $S \ll K_{0.5}$)

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

2-site cooperative binding

Adair equation

$$v = 2E_0k_{cat} \frac{S / K_1 + S^2 / K_1K_2}{1 + 2S / K_1 + S^2 / K_1K_2}$$

realistically captures site dependencies

Saturation



$$E + ES = E_0$$

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

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

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