1. Metabolic Modelling in Systems and Synthetic Biology

Introduction

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C1net gas fermentation

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Designer Babies

"Within a decade or two, it may be possible to screen kids almost before conception for an enormous range of attributes, such as how tall they're likely to be, what body type they will have, their hair and eye color, what sorts of illnesses they will be naturally resistant to, and even, conceivably, their IQ and personality type." Michael D. Lemonick, Time Magazine 11 Jan 1999.

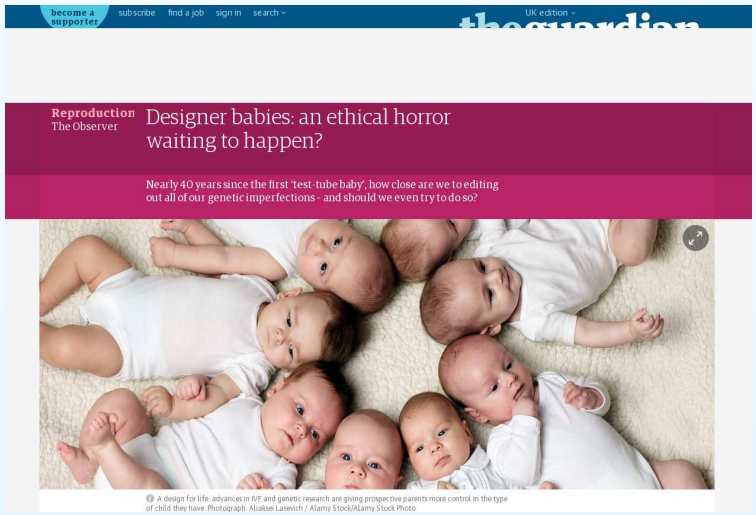
19 Years Later . . .

Still some weak argumentation, e.g. :

`https://en.wikipedia.org/wiki/Designer_baby`

A more reasoned contribution from Jan 2017:

`https://www.theguardian.com/science/2017/jan/08/desig`



Leaving aside the technical issues, there is the question of whether we can infer complex phenotypes from a genome sequence.

Simulated Life

Virtual Biochemistry

virtual liver network

A major national initiative funded by the German Federal Ministry for Education and Research

Virtual Liver Network

The Virtual Liver will be a dynamic model that represents, rather than fully replicates, human liver physiology morphology and function, integrating quantitive data from all levels of organisation.

SiC!: The Silicon Cells

A silicon cell is a precise replica of (part of) a living cell. It is based on experimentally determined rate lav
parameter values, i.e. only on data, not on fitted values or assumptions. It merely calculates the system biology implications
molecular properties that are already known. Silicon cell is not a package of software for simulations. The international silicon
program thereby differs (
cell biology that can be u
models for the purpose o
down-loading to one's ow
(http://www.genomatica.c
calculates kinetics, rather

Feature Article

The Virtual Human: Towards a Global Systems Biology of Mu Distributed Biochemical Network Models

Douglas B. Kell
School of Chemistry and The Manchester Centre for Integrative Systems Biology, The Manchester Inter Biocentre, The University of Manchester, Manchester, UK

Why Model Metabolism?

It came first!

- Metabolism is essential: it is a fundamental process of all living organisms, encompassing all the chemical conversions that convert nutrients into new cellular materials and provide energy for other processes such as movement.
- Metabolism can be useful: bread, alcoholic drinks, cheese, yoghurt, monosodium glutamate, biofuels.
- Metabolism can go wrong: single gene metabolic diseases; obesity, diabetes, heart disease, even cancer, are multi-factorial diseases with a metabolic component.
- We understand how the genes encode metabolism. But how do we exploit that understanding to predict metabolic responses?
- Biotechnological applications: for designing metabolic engineering and synthetic biology strategies.
- Another: design of effective drug therapies.
- Other cell processes yield to similar approaches: signal transduction; cell cycle; apoptosis.

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Industrial Biotechnology and Synthetic Biology

Targets for biological production of “platform chemicals” (green chemistry):

Chemical	Derivatives	Chemical	Derivatives
Succinate	1,4 butanediol	Isoprene	Synthetic rubber
3-OH propionate	Acrylate	Farnesene	
Itaconic acid	Methylmethacrylate	Glycerol	Propylene glycol
Ethanol	ethylene, biofuel	Sorbitol	
Lactate	Polylactic acid	Xylitol	
Biohydrocarbons	Biofuel	Furfural	

Choi et al, Metabolic Engineering, **28**, 223–239 (2015)

Raw materials include: lignocellulosic biomass, starch, syngas and flue gases (CO_2/CO/H_2 mixtures), methane, CO_2 and light in algae.

Design Issues in Synthetic Metabolism

- Can matter flow from inputs to the desired product?
- What is the maximum conversion efficiency obtainable?
- Can the host cell cope with the energy and redox demands of production?
- How can losses to unwanted by–products be avoided?
- How can the rate of production be accelerated?

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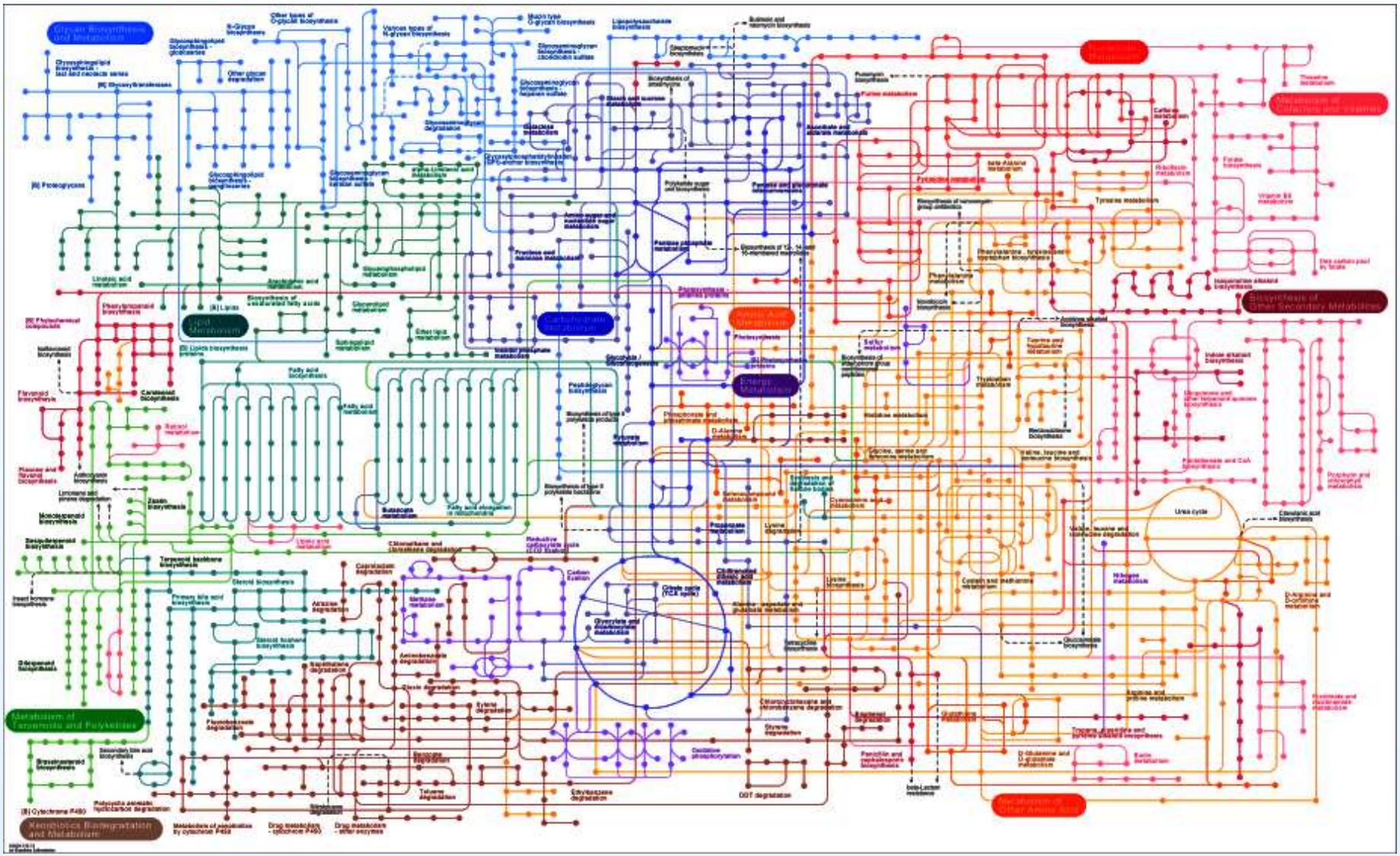
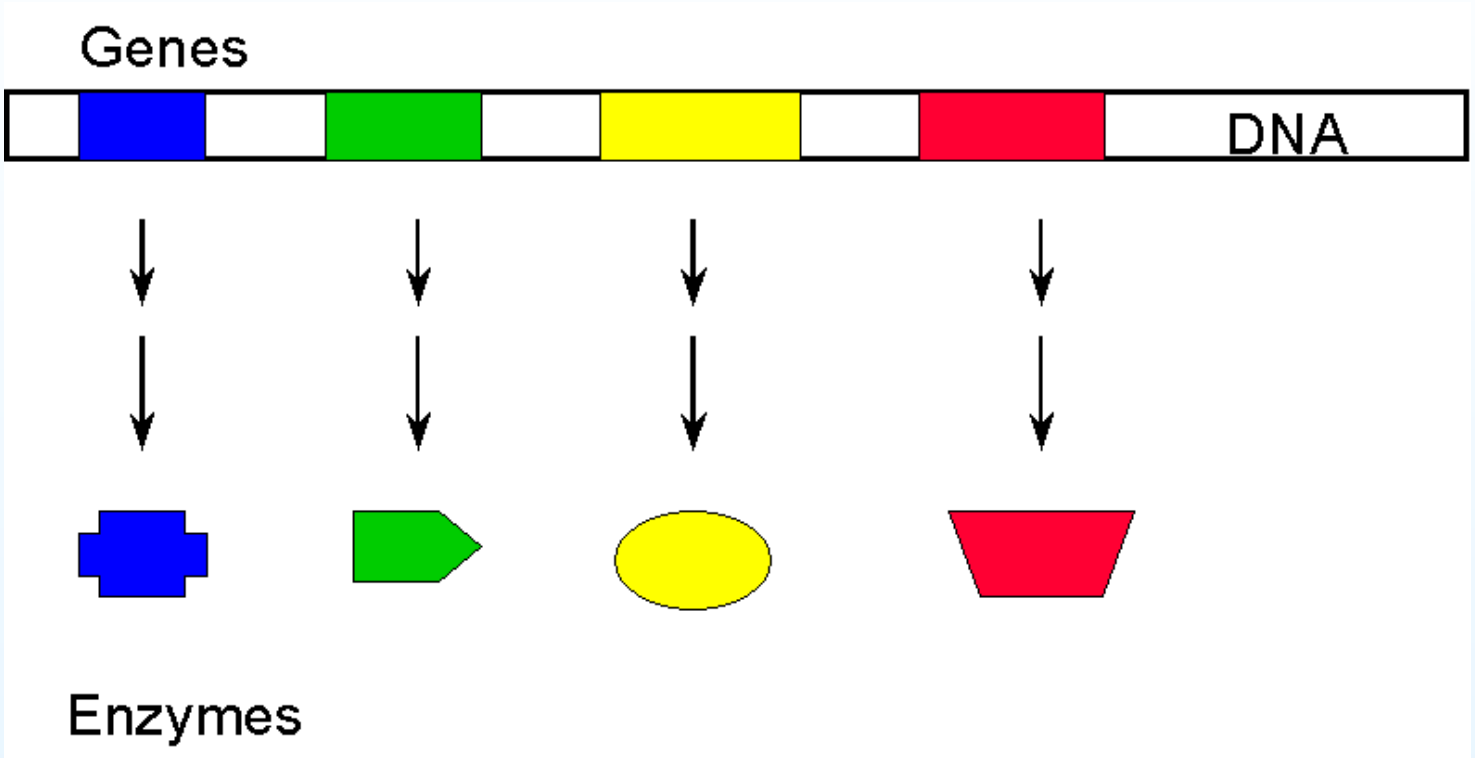
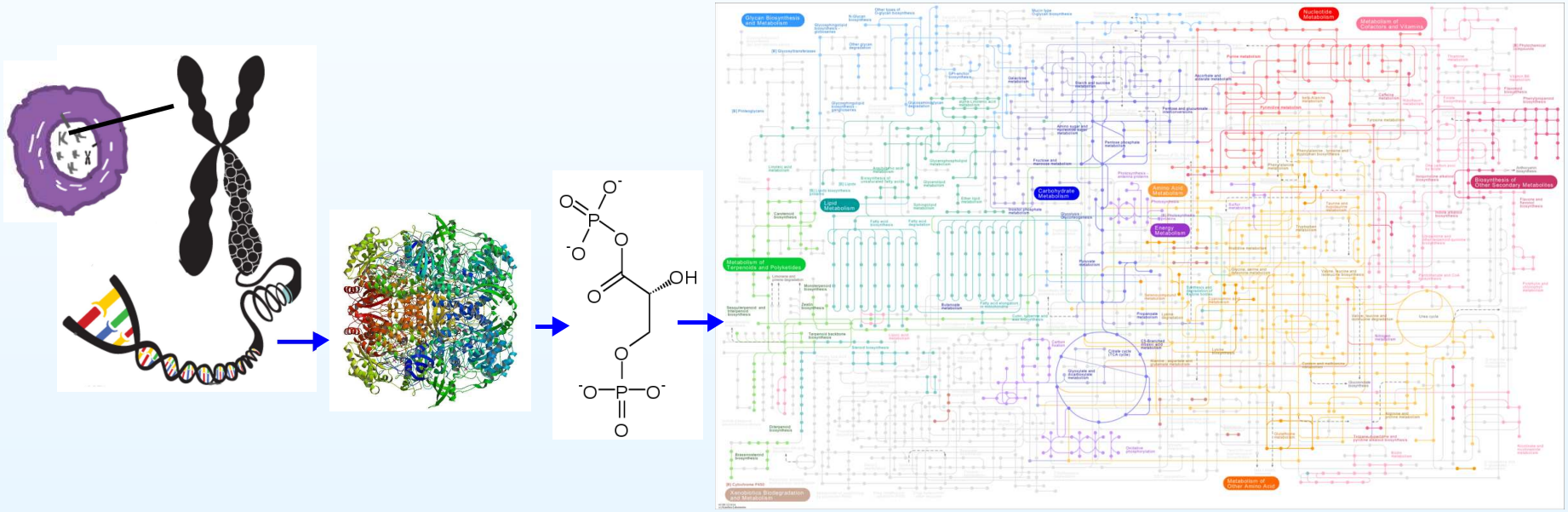
The Metabolic Network



Genes and Metabolism



Genotype to Metabolic Phenotype



Summary of Types of Metabolic Model

- Structural — needs reaction list; gives existence and number of routes; optimal stoichiometries; network flux values.

- Dynamic or Kinetic — needs full kinetic description of each enzyme/step; predicts time–courses, steady–states, sensitivity analysis or control distribution . . . Can be deterministic or stochastic.

- Sensitivity analysis / Control analysis / S–systems — needs effective kinetics near steady–state; predicts control distribution, response of steady state to perturbations.

Aims of Modelling

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- Integration of different types of data and observations in a single framework.
- Generating hypotheses for testing.
- Designing experiments *in silico*.
- Models are simplifications, and have to be designed for the specific aims.

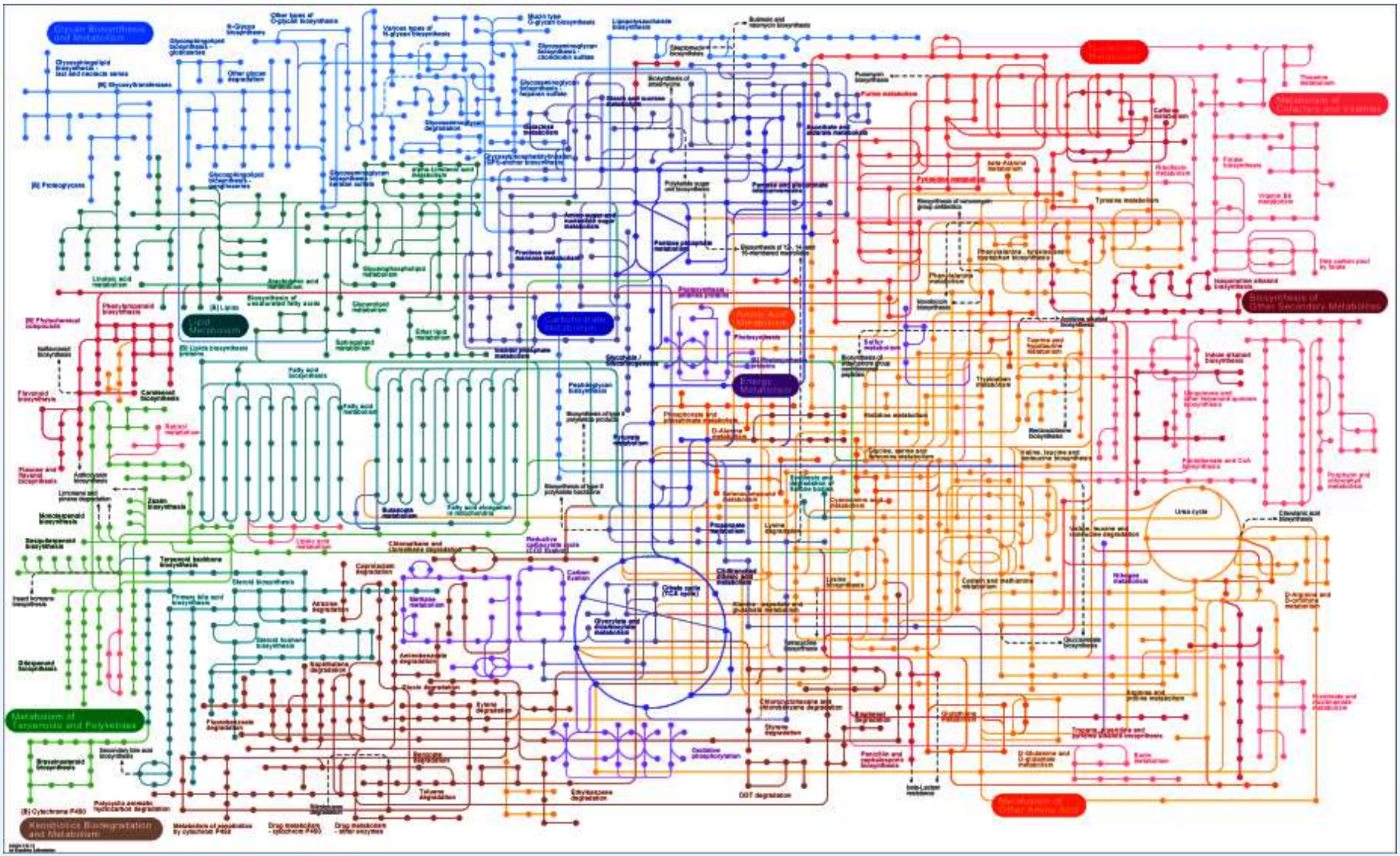
Outline of the Week

- **Today**. Theory: Introduction and mathematical representation of metabolism. Practical: Installing ScrumPy and using Python
- **Tuesday**. Theory: the main conceptual tools for structural modelling of metabolism and their application:
  - Null space analysis and enzyme subsets
  - Elementary modes

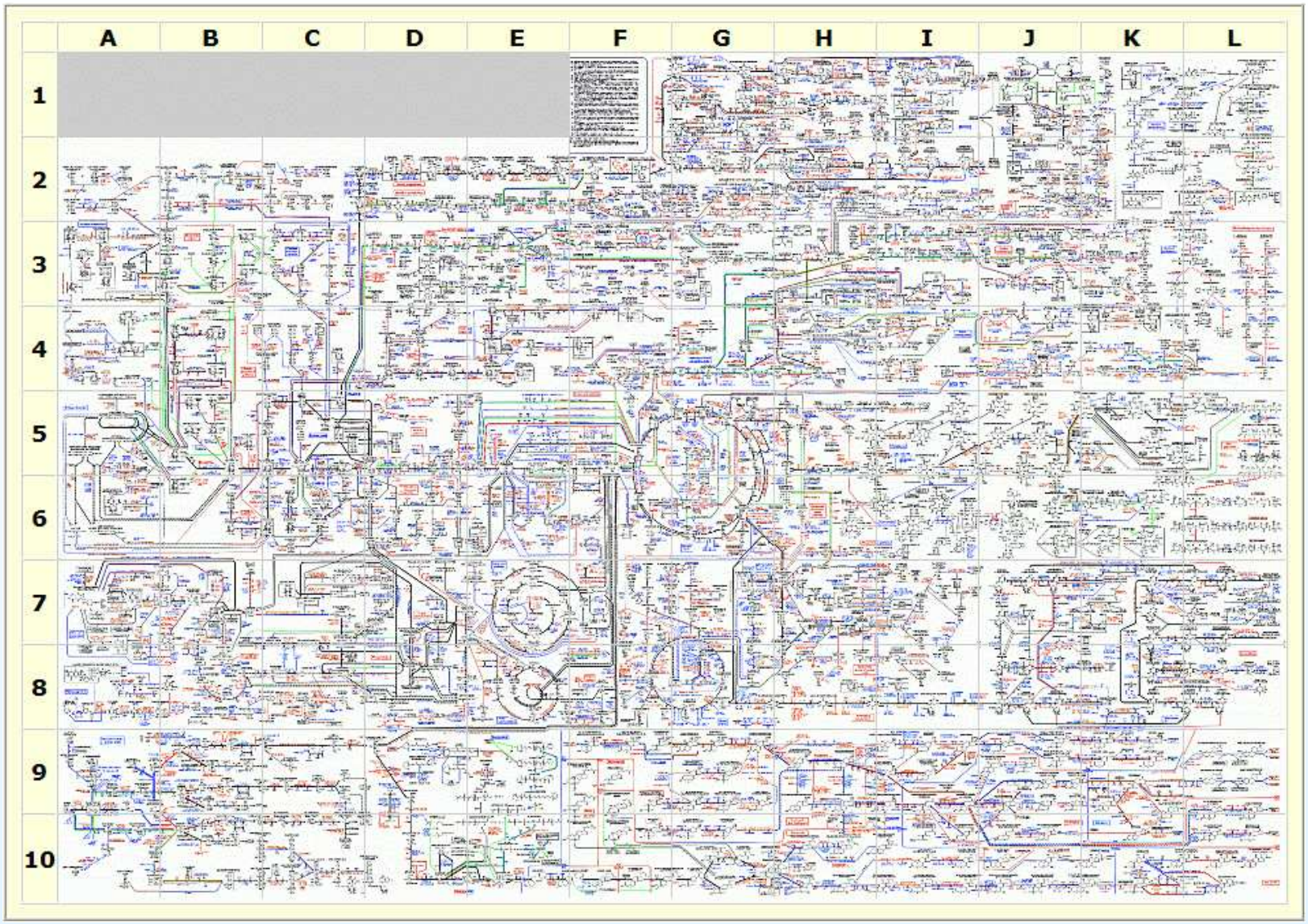
  Practical: introduction to modelling with ScrumPy
- **Wednesday**. Theory: Linear programming (aka Flux Balance Analysis); building genome scale metabolic models. Practical: more model analysis with ScrumPy; constructing a model from databases; Free half–afternoon; Conference dinner
- **Thursday**. Theory: applications of genome-scale modelling. Practical: FBA with ScrumPy;
- **Friday**. Practical: FBA of a genome scale model. Final questions and answers.

Model Formulation

The Metabolic Network

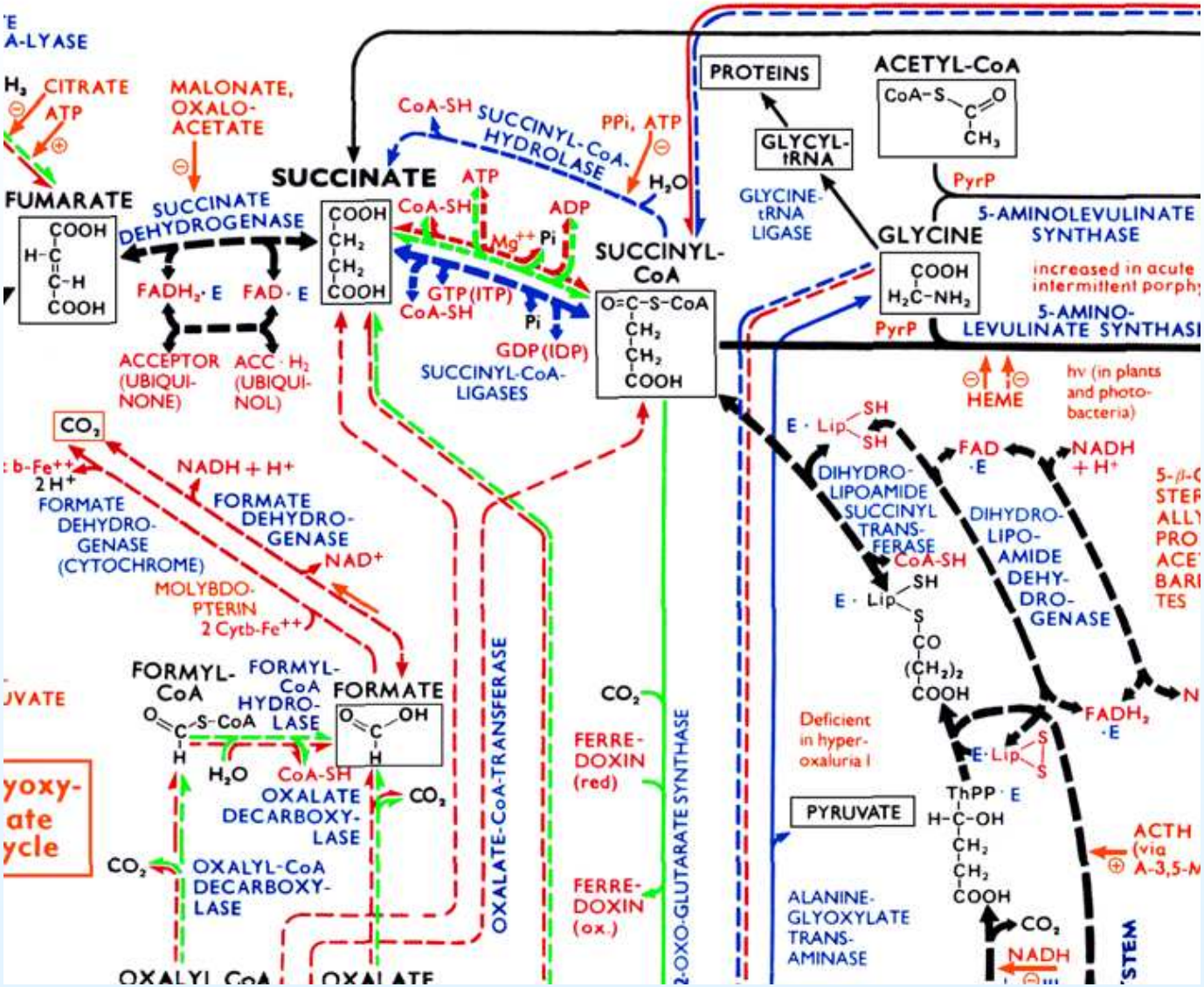


The Metabolic Network — More Detail



From Expasy Biochemical Pathways: http://www.expasy.ch/cgi-bin/search-biochem-index

The Metabolic Network — Zoom in



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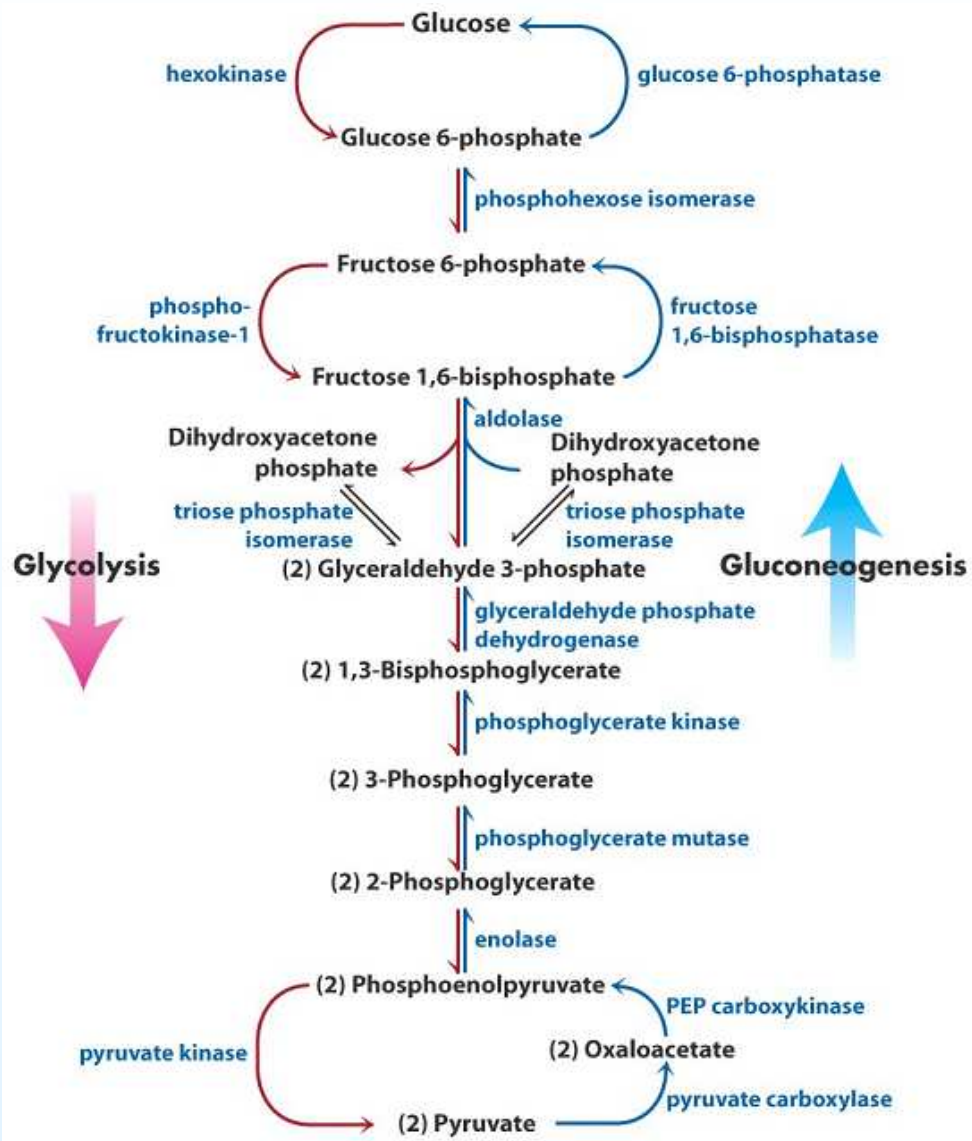
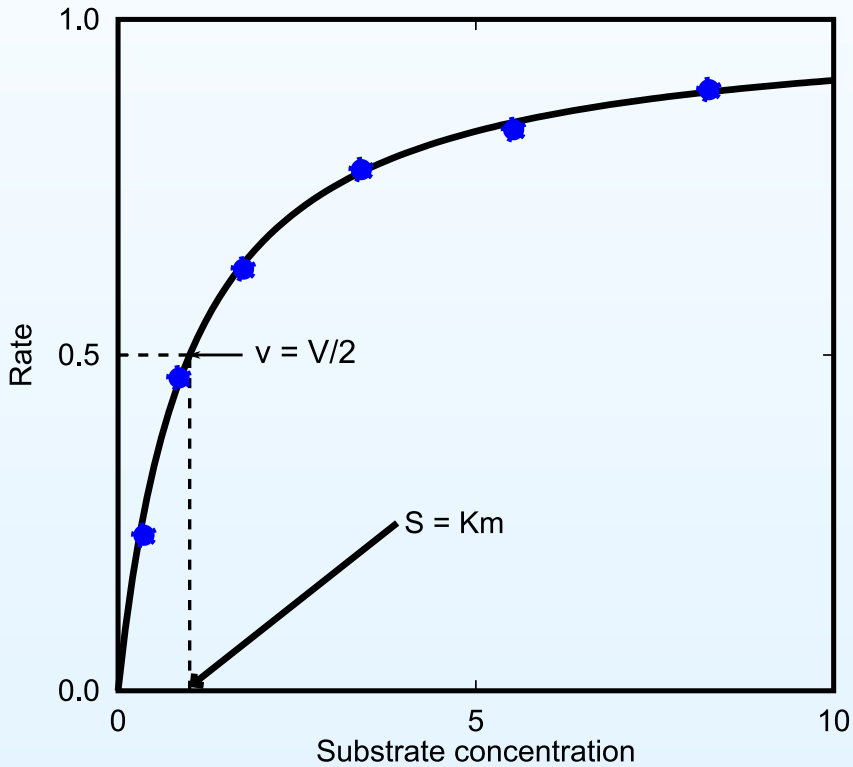
A Metabolic 'Pathway'



From Nelson & Cox, Lehninbger's Biochemistry, 4th ed.

Michaelis–Menten Enzyme Kinetics

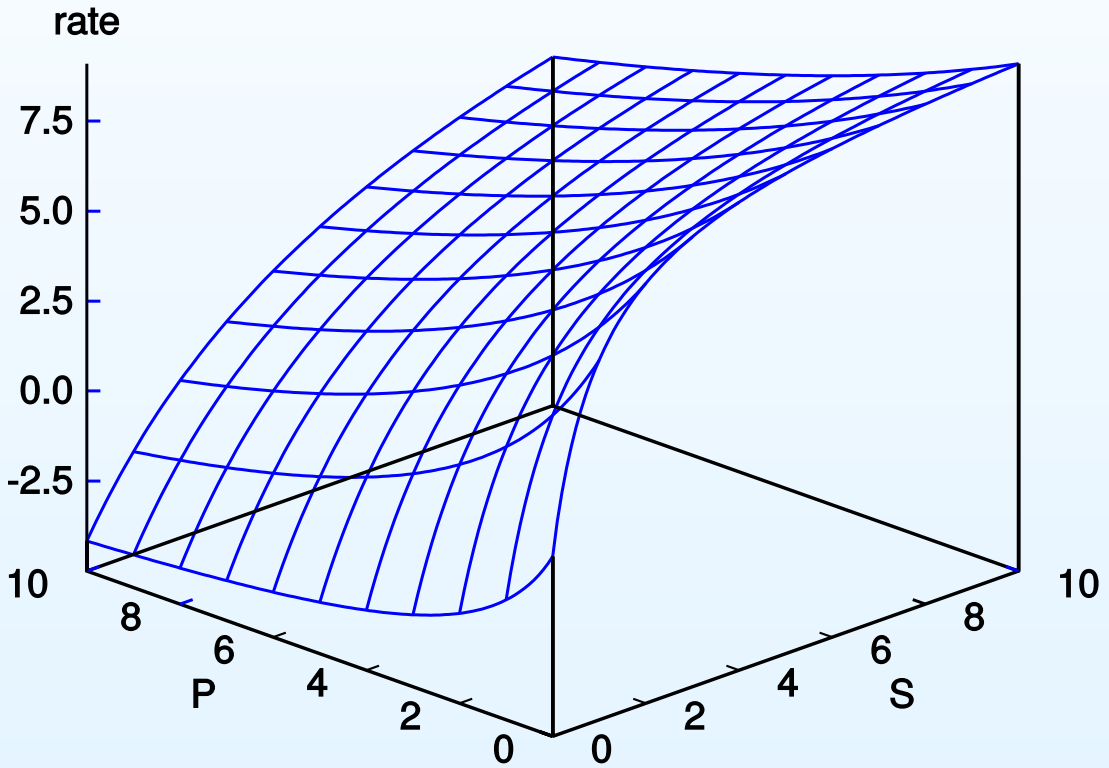
$$v = \frac{SV}{S + K_{\mathrm{m}}} \quad \text{or} \quad v = f(S)$$



The K_{m} and V have arbitarily been set to 1, where V is the *limiting rate* (or maximum velocity, V_{m}) and K_{m} is the *Michaelis constant*.

The Reversible M–M Eqn.

$$v_{net} = \frac{(V_{\mathrm{f}}/K_{\mathrm{m,S}})\,(S - P/K_{\mathrm{eq}})}{1 + S/K_{\mathrm{m,S}} + P/K_{\mathrm{m,P}}} \quad \text{or} \quad v = f(S, P)$$



Simultaneous dependence of enzyme rate on both substrate and product. The parameters have been set to: $K_{\mathrm{m,S}}$ =1; $V_{\mathrm{m,f}}$ = 10; $K_{\mathrm{m,P}} = 2$, and $K_{\mathrm{eq}} = 4$.

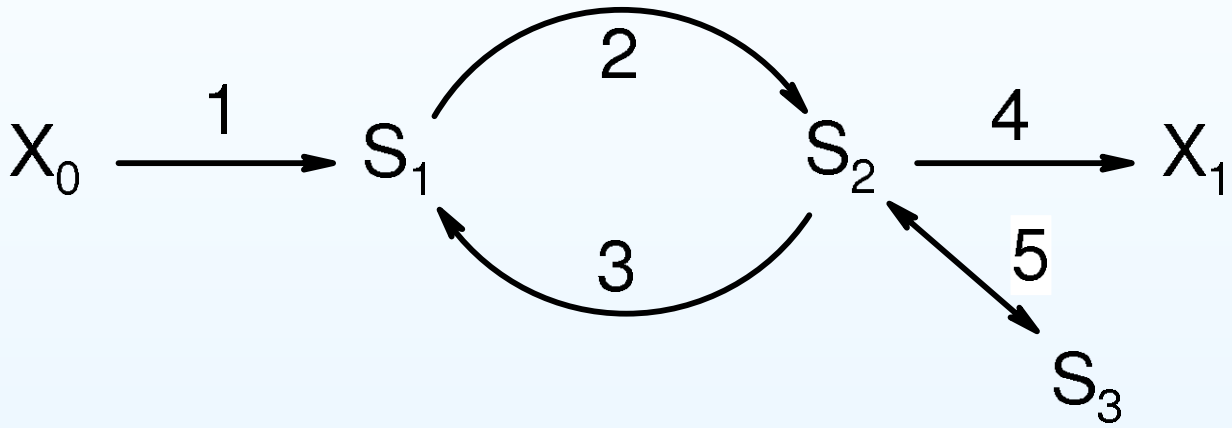
Steady state

In a metabolic network there is a flow of matter from the *source* to the *sink*. At steady state, the concentrations of the intermediates remain constant because their rates of formation exactly equal their rates of degradation. The flow through the pathway also remains constant.

If there are very slow changes in the concentrations of metabolites, or the pathway flux, because of slow changes in the source or sink, the pathway may be regarded as being in *quasi steady state* provided the time scale of the changes is very much longer than the time taken by the pathway to approach steady state.

Reaction Network to Mathematical Object

Consider a simple metabolic network, e.g.:



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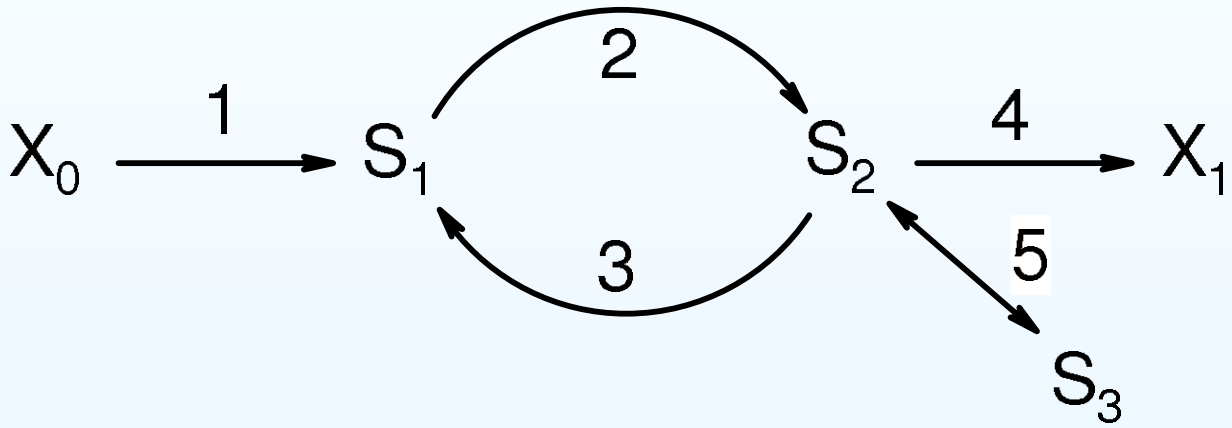

r1: X_0 -> S_1 ~

r2: S_1 -> S_2 ~

r3: S_2 -> S_1 ~

r4: S_2 -> X_1 ~

r5: S_2 <> S_3 ~

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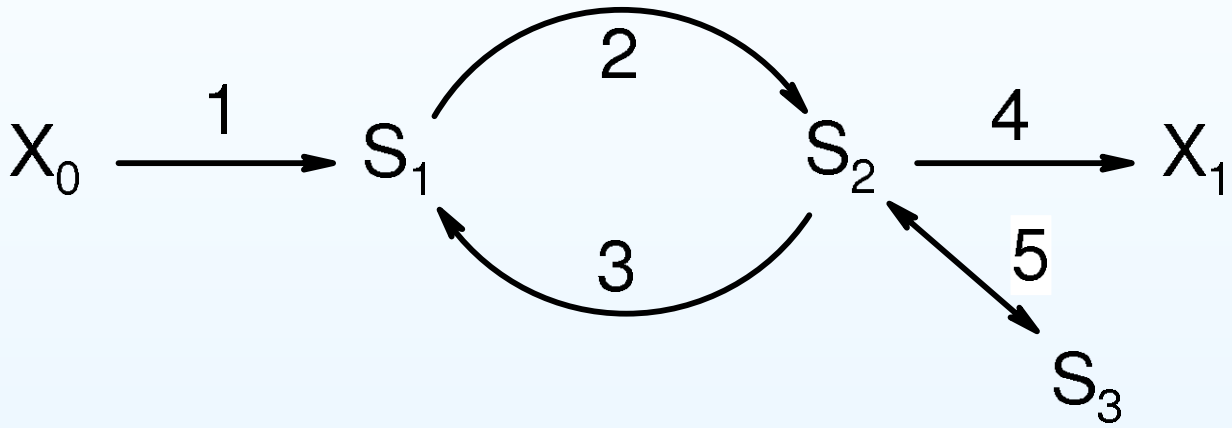

r1: X_0 -> S_1 $\sim$
r2: S_1 -> S_2 $\sim$
r3: S_2 -> S_1 $\sim$
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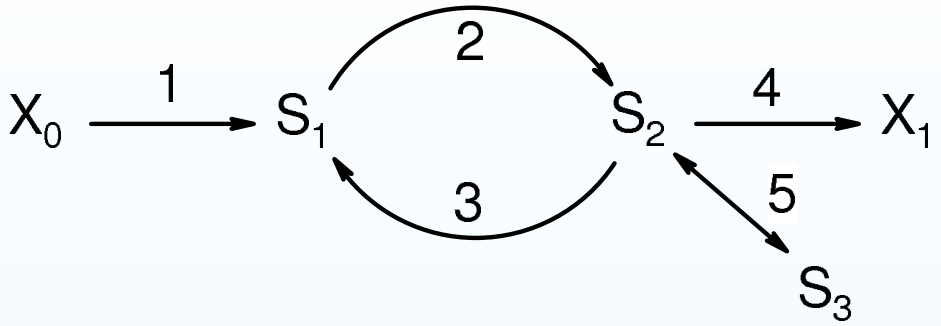
r5: S_2 <> S_3 $\sim$

	$r1$	$r2$	$r3$	$r4$	$r5$
S_1	1	−1	1	0	0
S_2	0	1	−1	−1	−1
S_3	0	0	0	0	1

Kinetics of the Metabolites



By inspection of the diagram:

$$\begin{aligned}
\frac{dS_1}{dt} &= v_1 - v_2 + v_3 \\
\frac{dS_2}{dt} &= v_2 - v_3 - v_4 - v_5 \\
\frac{dS_3}{dt} &= v_5
\end{aligned}$$

How can we generalize this?

Separation of Structure and Kinetics

The rate at which the substrate concentrations are changing is given by $\mathbf{N}.\mathbf{v}$, where $\mathbf{N}$ is the stoichiometry matrix, and $\mathbf{v}$ is a vector of enzyme kinetic functions. So for our substrate cycle network:

$$\begin{bmatrix} \frac{dS_1}{dt} \\ \frac{dS_2}{dt} \\ \frac{dS_3}{dt} \end{bmatrix} = \begin{bmatrix} 1 & -1 & 1 & 0 & 0 \\ 0 & 1 & -1 & -1 & -1 \\ 0 & 0 & 0 & 0 & 1 \end{bmatrix} \cdot \begin{bmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \\ v_5 \end{bmatrix}$$

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where each v_i is the rate function for enzyme i, depending on the variable metabolites and the parameters $V_{\mathrm{m,i}}$, $K_{\mathrm{m,i}}$ etc, as $f_i(\mathbf{S})$.

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The steady state is a set of non–linear simultaneous equations that can be solved for the steady state values of $\mathbf{S}$.

Steady State Structural Modelling

Any metabolic network at steady state satisfies the relationship $\mathbf{N}.\mathbf{v} = \mathbf{0}$, where $\mathbf{N}$ is the stoichiometry matrix, exemplified by our model network:

$$\begin{matrix} S_1 \\ S_2 \\ S_3 \end{matrix} \begin{bmatrix} 1 & -1 & 1 & 0 & 0 \\ 0 & 1 & -1 & -1 & -1 \\ 0 & 0 & 0 & 0 & 1 \end{bmatrix} \cdot \begin{bmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \\ v_5 \end{bmatrix} = \begin{bmatrix} 0 \\ 0 \\ 0 \end{bmatrix}$$

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The equation is linear, but under–determined. Though solutions are not unique, they distinguish between **feasible** and **non–feasible** states of the network.

Formal Representation - Structure

Structural Analysis: Null Space Vectors

Any observed set of velocities at steady state will be a linear combination of a set of vectors $\mathbf{K}$ referred to as a basis for the null space of the stoichiometry matrix. In this case:

$$\mathbf{K} = \begin{bmatrix} 1 & 0 \\ 1 & 1 \\ 0 & 1 \\ 1 & 0 \\ 0 & 0 \end{bmatrix}$$

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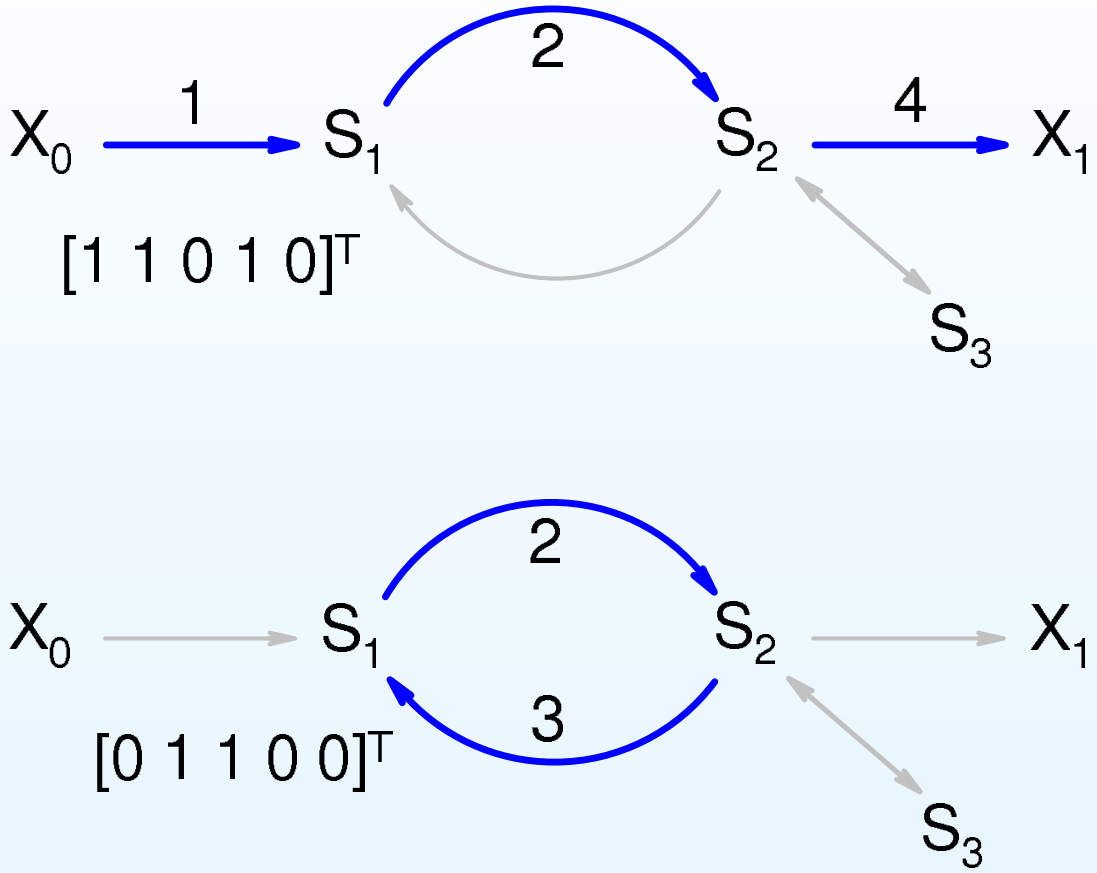
The null space can be computed from the stoichiometry matrix using standard algorithms.

Null Space Vectors as Pathways



$[1\ \ 1\ \ 0\ \ 1\ 0]^T$ and $[0\ \ 1\ \ 1\ \ 0\ 0]^T$

The Null Space and Pathway Fluxes

Any feasible set of velocities at steady state is a linear combination of these null space vectors, e.g.:

$$\mathbf{K} = \begin{bmatrix} 1 & 0 \\ 1 & 1 \\ 0 & 1 \\ 1 & 0 \\ 0 & 0 \end{bmatrix} \begin{matrix} \\ \\ \\ \leftarrow \text{subset} \\ \leftarrow \text{dead} \end{matrix}$$

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and:

$$\begin{bmatrix} 1 & 0 \\ 1 & 1 \\ 0 & 1 \\ 1 & 0 \\ 0 & 0 \end{bmatrix} \cdot \begin{bmatrix} a \\ b \end{bmatrix} = \begin{bmatrix} a \\ a+b \\ b \\ a \\ 0 \end{bmatrix} = \begin{bmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \\ v_5 \end{bmatrix}$$

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and:

$$\begin{bmatrix} 1 & 0 \\ 1 & 1 \\ 0 & 1 \\ 1 & 0 \\ 0 & 0 \end{bmatrix} \cdot \begin{bmatrix} a \\ b \end{bmatrix} = \begin{bmatrix} a \\ a+b \\ b \\ a \\ 0 \end{bmatrix} = \begin{bmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \\ v_5 \end{bmatrix}$$

The Null Space and Pathway Fluxes

Any feasible set of velocities at steady state is a linear combination of these null space vectors, e.g.:

$$\mathbf{K} = \begin{bmatrix} 1 & 0 \\ 1 & 1 \\ 0 & 1 \\ 1 & 0 \\ 0 & 0 \end{bmatrix} \begin{matrix} \leftarrow \text{subset} \\ \\ \\ \leftarrow \text{subset} \\ \leftarrow \text{dead} \end{matrix}$$

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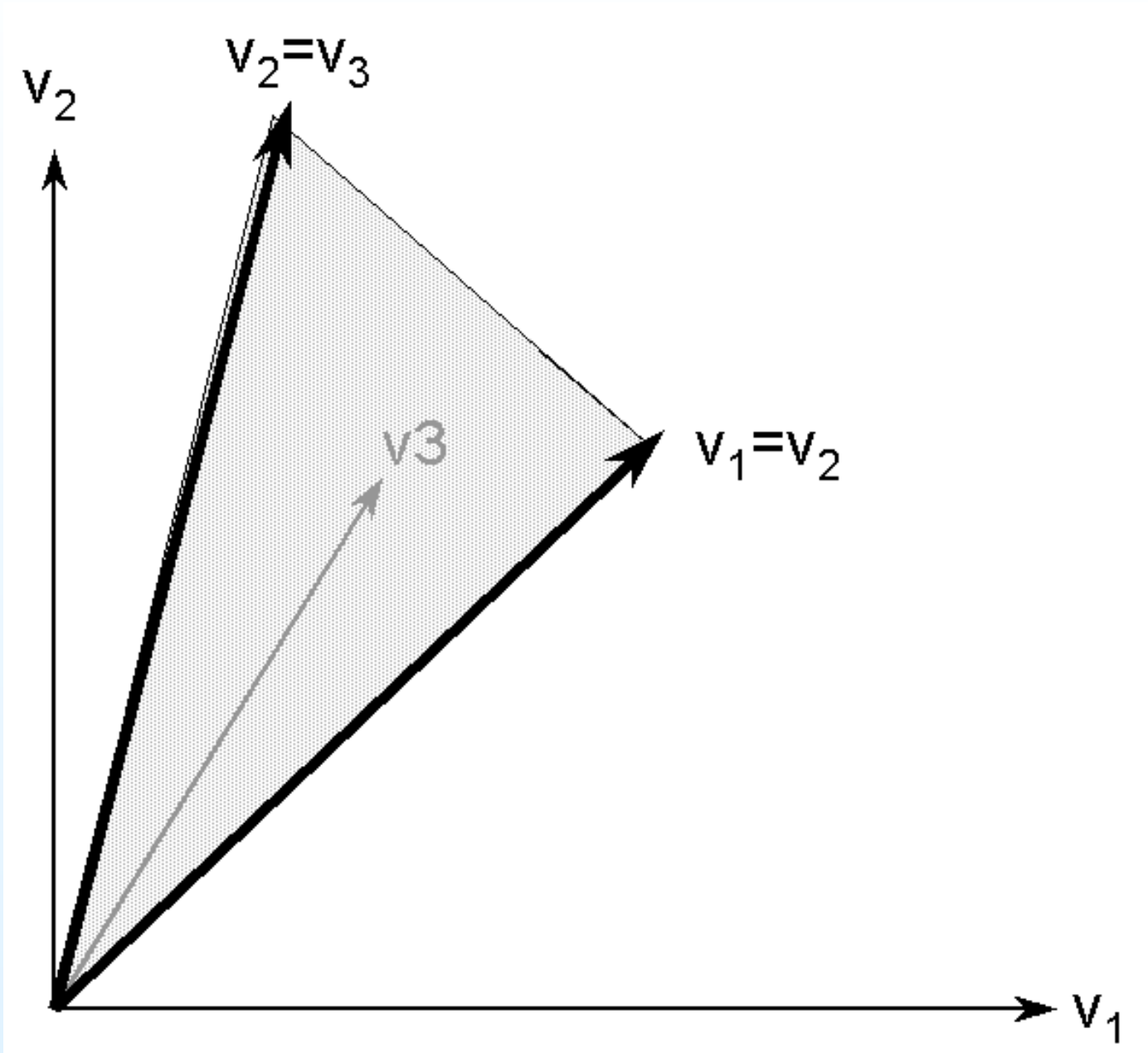
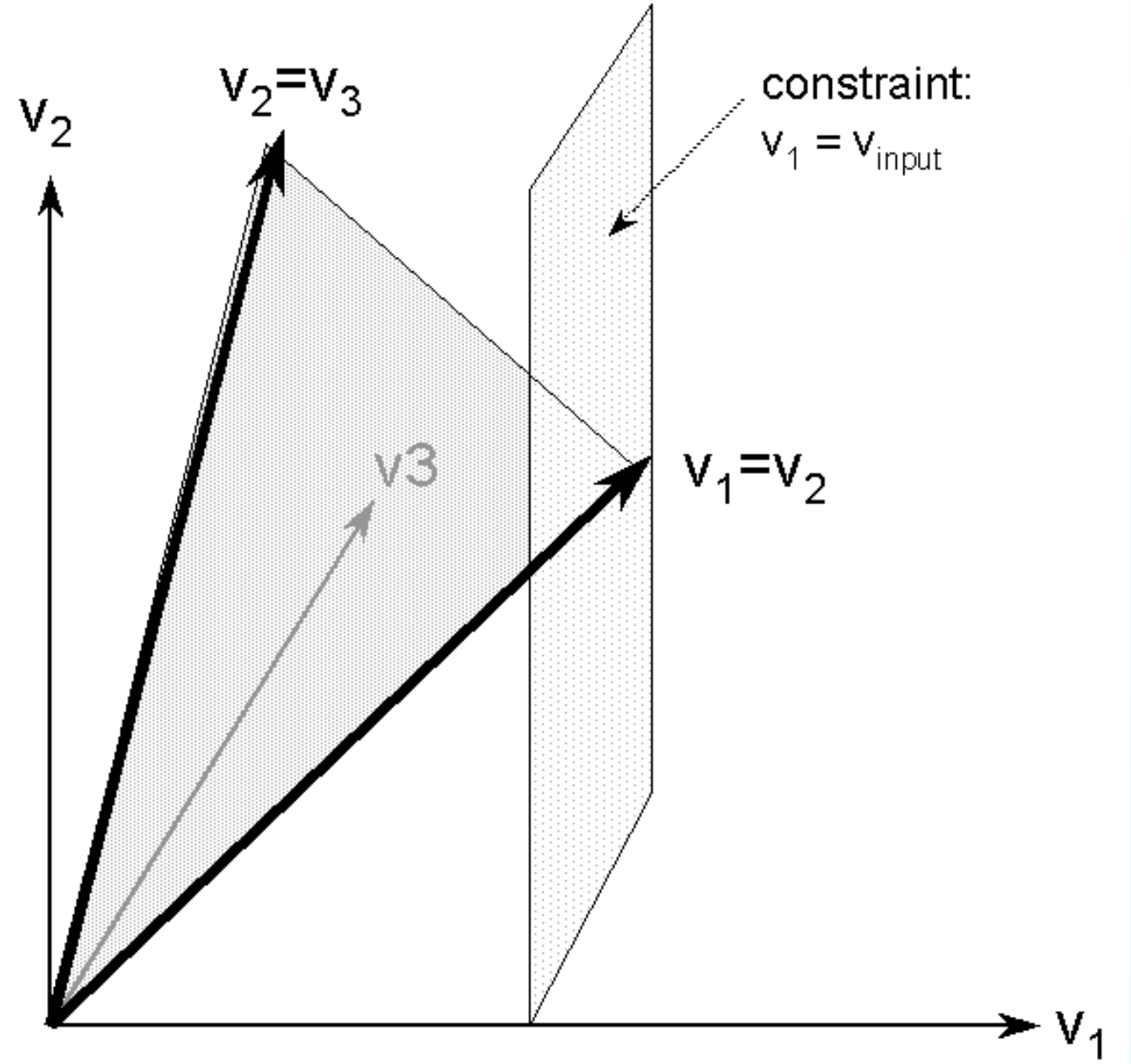
- Subsets can be identified from the null space, which can be rapidly calculated even for a genome–scale metabolic model.

Null Space - Geometrical Interpretation



Linear Programming Solution —1



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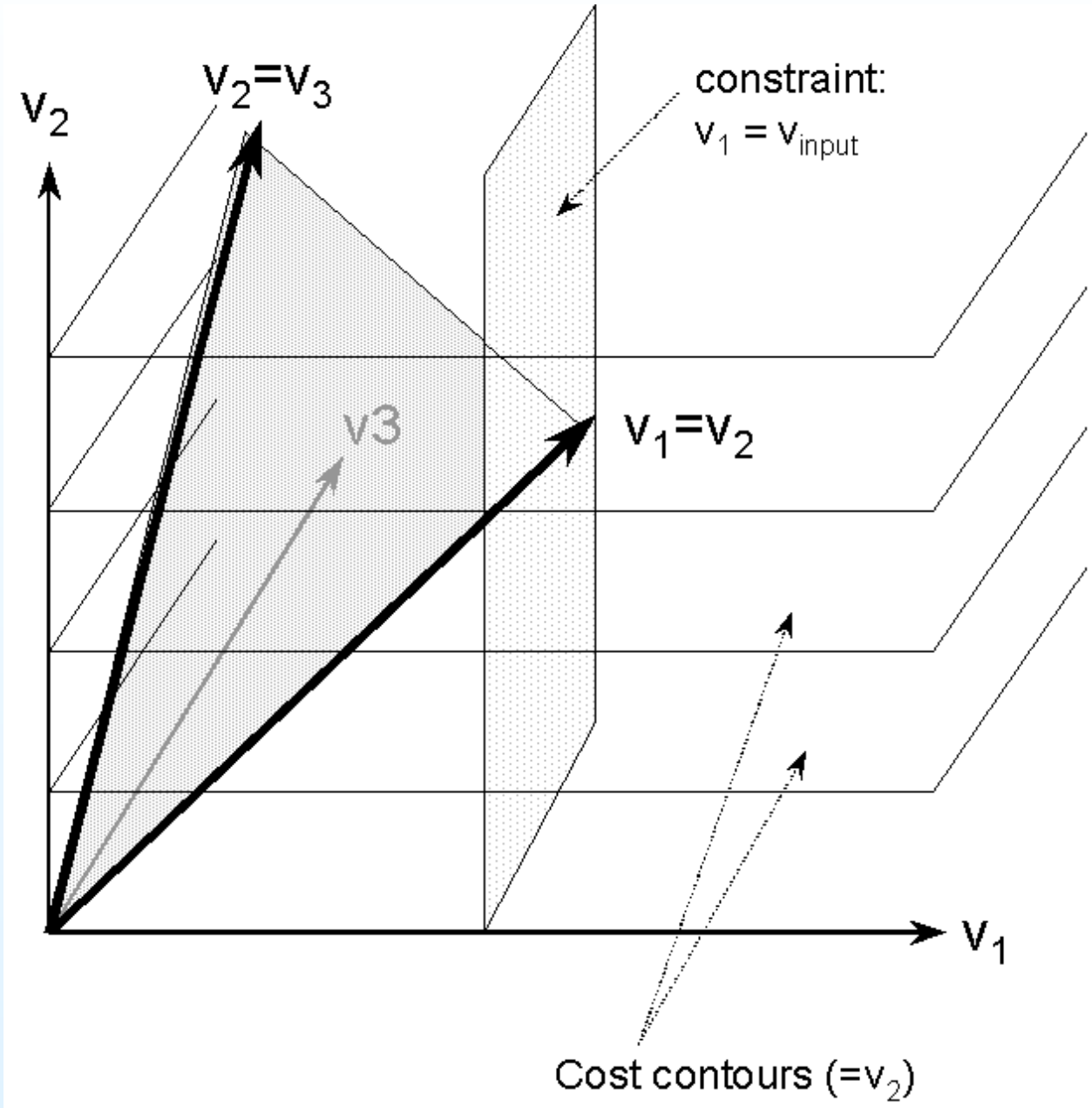

Advantages of Structural Analysis

- Knowledge is more complete for network structure than for enzyme kinetics.
- Structural analysis involves simple linear equations; dynamic analysis involves non–linear enzyme kinetic functions.
- The network structure places limitations that constrain the network dynamics, irrespective of the kinetics, e.g.:
  - Whether viable routes exist from nutrients to stated metabolic products;
  - Whether some routes remain after deletion (knock–out mutation) of the steps catalysed by a particular enzyme;
  - What the maximum obtainable conversion yield is for formation of any metabolite from a given set of sources, and
- Structural models underlie kinetic models, and other techniques such as Metabolic Flux Analysis and Metabolic Control Analysis.

Structural Analysis Methods

- Null space vectors *Fell, Palsson et al*
- Computer construction of transformation routes *Serriotsis & Bailey; Mavrovouniotis et al*
- Graph analysis techniques *various*
- Elementary modes *Schuster et al*
- Convex basis / Extreme pathways *Palsson et al*
- Reaction (enzyme) subsets
- Linear programming - single optimal route *Small & Fell, Palsson et al*. Became Flux Balance Analysis and gave rise to genome–scale metabolic modelling.

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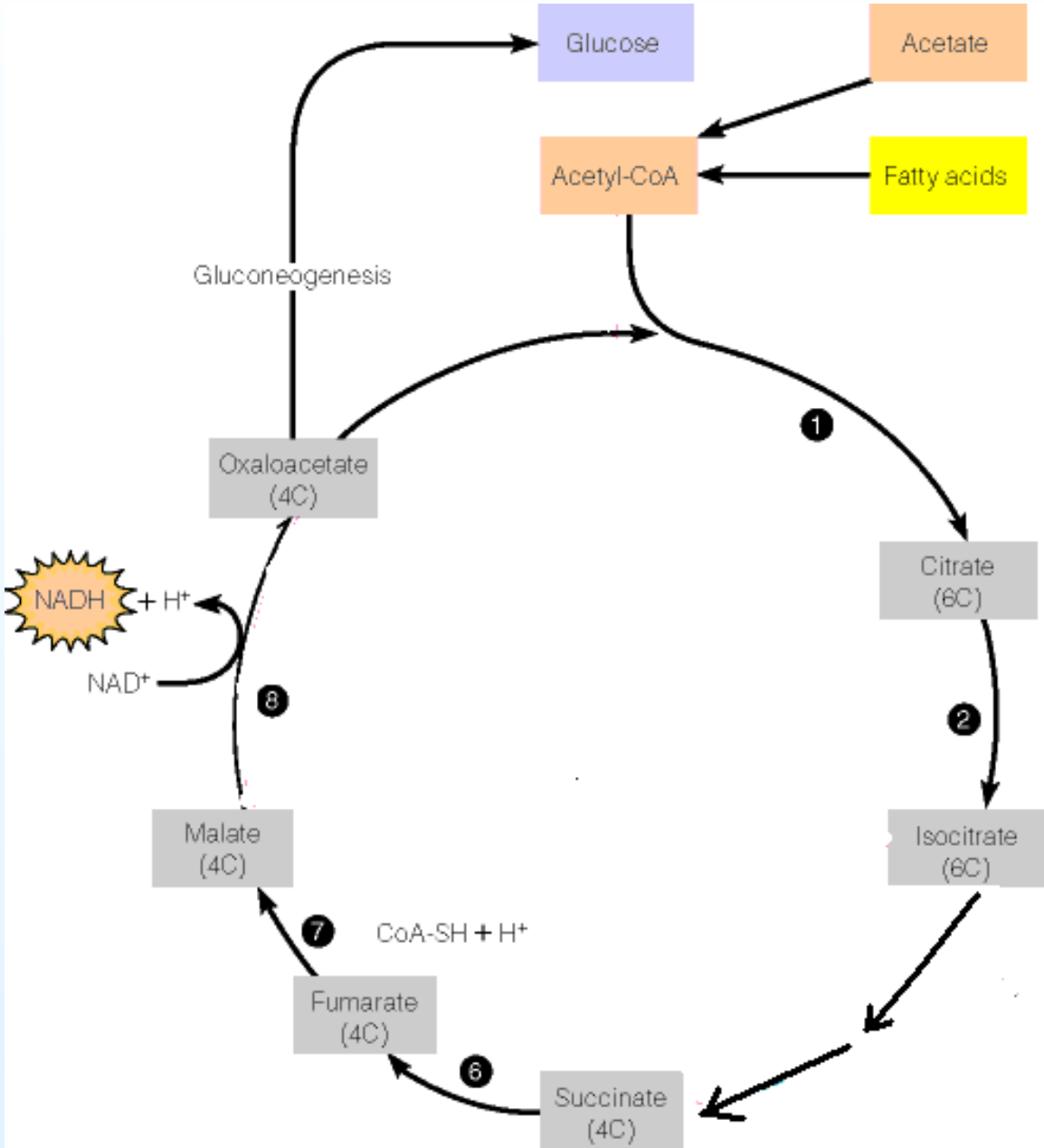

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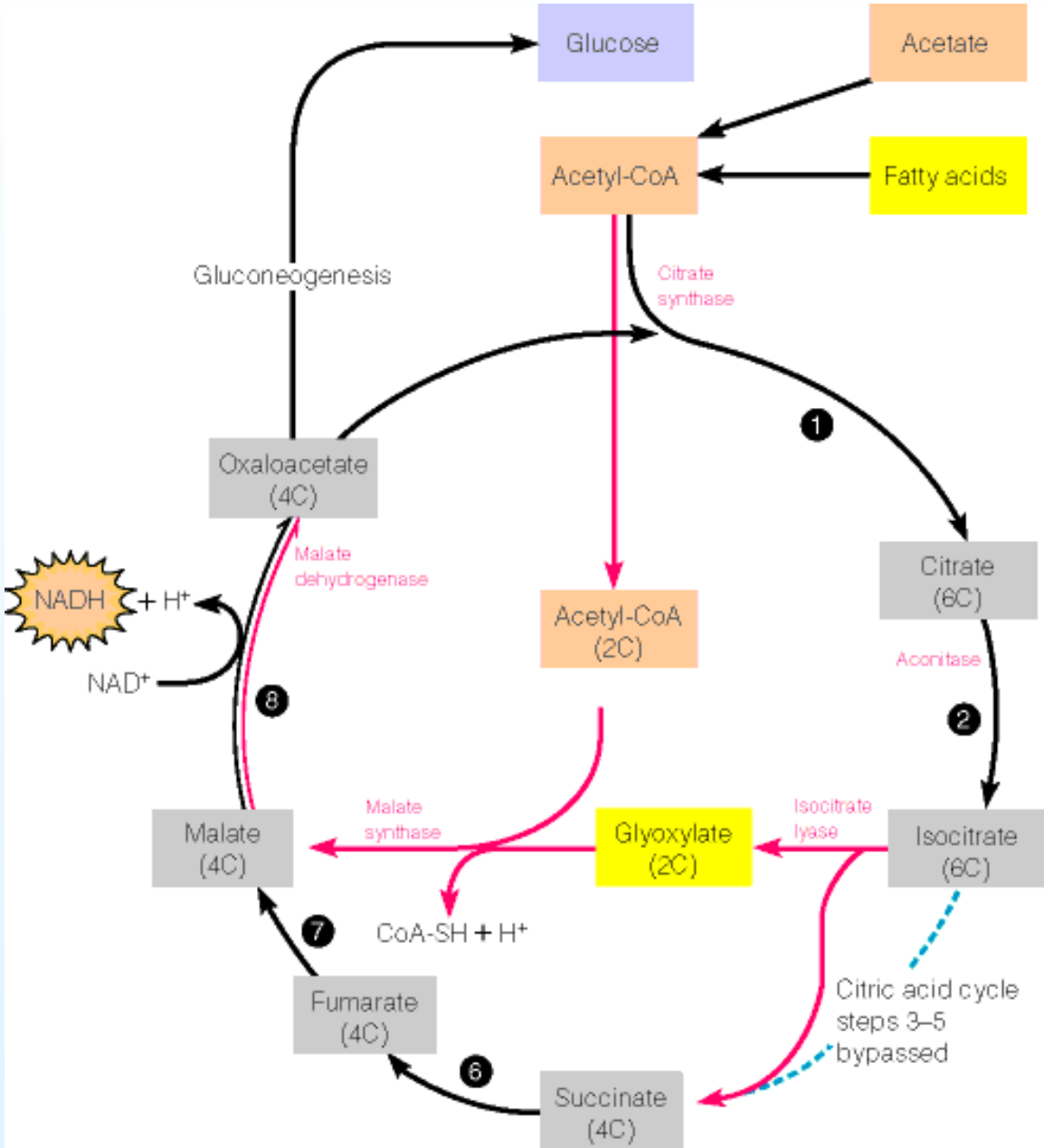

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- Structural modelling investigates the implications of these network constraints.