

Lecture: Computational Systems Biology  
Universität des Saarlandes, SS 2012

## **05 Structural analysis**

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# Recap

- Standards
  - Systems Biology Markup Language (SBML)
  - Systems Biology Graphical Notation (SBGN)
  - Minimal information required in the annotation of models (MIRIAM)
- Software
  - COPASI, CellDesigner, etc.
- Databases
  - Pathway databases (KEGG, Reactome, ...)
  - Databases providing kinetic parameters (BRENDA, Sabio-RK)
  - Model databases (Biomodels, JWS)

# Stoichiometry (reminder)

- **Proportions** of substrate and product molecules in a reaction
- Assignment of stoichiometric coefficients is **not unique**
- Example:  $S_1 + S_2 \leftrightarrow 2 P$

Stoichiometric coefficients could be

-1, -1, 2, or

-1/2, -1/2, 1, or

1, 1, -2

# System equations / balance equations

$$\frac{dS_i}{dt} = \sum_{j=1}^r n_{ij} v_j = \mathbf{N} \mathbf{v}$$

Stoichiometric matrix  $\mathbf{N}$

rows  $\rightarrow$  chemical species

columns  $\rightarrow$  reactions

Vector of rates  $\mathbf{v}$

Direction of reaction arbitrarily assigned

Negative rate is possible ( $\rightarrow$  net flux in reverse direction)

# System representation

System is fully specified by

- Concentration values  $\mathbf{S}$
- Rates  $\mathbf{v}$  (kinetic functions)
- Parameter set  $\mathbf{p}$
- Stoichiometric matrix  $\mathbf{N}$
- In case of a steady state, we also have a vector of steady state fluxes  $\mathbf{J}$

# Information contained in $N$

- Which combination of fluxes is possible in a steady state?
- Dead ends, unbranched reaction pathways
- Conservation relations / conserved moieties

# Information contained in $\mathbf{N}$

- In a steady state (SS)

$$\frac{d\mathbf{S}}{dt} = \mathbf{N} \mathbf{v} = 0$$

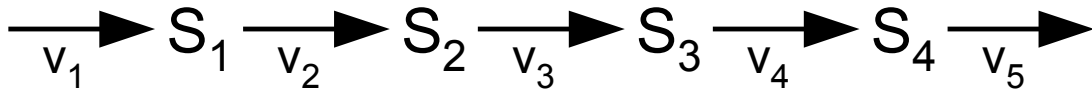
- Linear equation system for the rates in SS. Non-trivial solution when  $\text{Rank}(\mathbf{N}) < r$

# Kernel matrix $\mathbf{K}$

- Kernel matrix  $\mathbf{K}$  fulfilling  $\mathbf{N} \mathbf{K} = 0$   
shows the linear dependencies
- Choice of kernel is not unique
- Can be found using the Gauss algorithm
- It contains as columns  $r - \text{Rank}(\mathbf{N})$  basis vectors
- Every possible set of steady state fluxes  $\mathbf{J}$  is a linear combination of the columns  $\mathbf{k}_j$  of  $\mathbf{K}$



# Example



$$N = \begin{pmatrix} 1 & -1 & 0 & 0 & 0 \\ 0 & 1 & -1 & 0 & 0 \\ 0 & 0 & 1 & -1 & 0 \\ 0 & 0 & 0 & 1 & -1 \end{pmatrix}$$

$r = 5$  reactions

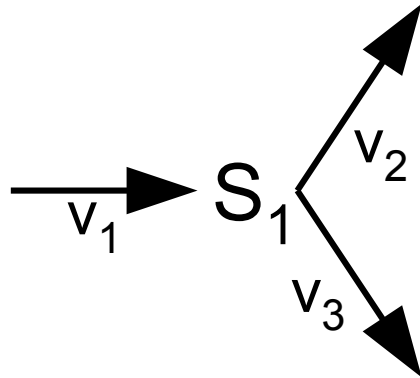
$$\text{Rank}(N) = 4$$

Therefore, kernel matrix  
must contain  $1 = 5 - 4$   
basis vectors

$$k = (1 \ 1 \ 1 \ 1 \ 1)^T$$

*In SS flux through all reactions must be equal*

# Example



$$N = (1 \quad -1 \quad -1)$$

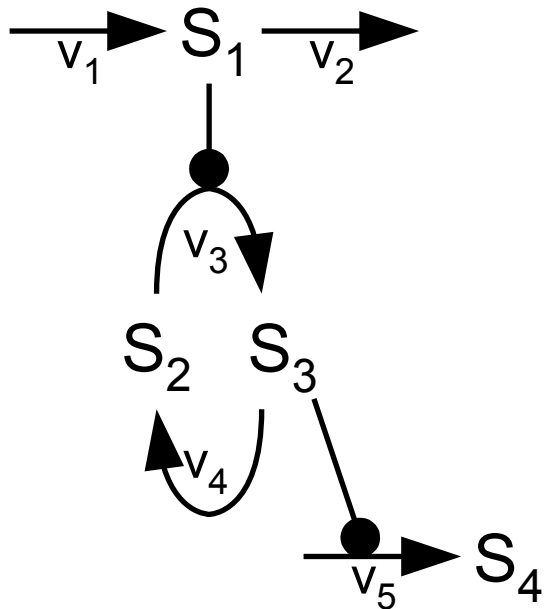
$$K = (\mathbf{k}_1 \quad \mathbf{k}_2) = \begin{pmatrix} 1 & 1 \\ 1 & 0 \\ 0 & 1 \end{pmatrix}$$

$r = 3$  reactions

$$\text{Rank}(N) = 1$$

Therefore, kernel matrix  
must contain  $2 = 3 - 1$   
basis vectors

For SS fluxes  $J = \alpha_1 \cdot \mathbf{k}_1 + \alpha \cdot \mathbf{k}_2$  holds



# Example

$$N = \begin{pmatrix} 1 & -1 & 0 & 0 & 0 \\ 0 & 0 & -1 & 1 & 0 \\ 0 & 0 & 1 & -1 & 0 \\ 0 & 0 & 0 & 0 & 1 \end{pmatrix}$$

$r = 5$  reactions

$\text{Rank}(N) = 3$

Therefore, kernel matrix  
must contain  $2 = 5 - 3$   
basis vectors

$$\mathbf{k}_1 = (1 \quad 1 \quad 0 \quad 0 \quad 0)^T$$

$$\mathbf{k}_2 = (0 \quad 0 \quad 1 \quad 1 \quad 0)^T$$

*In SS production and degradation of  $S_1$  are balanced ( $J_1 = J_2$ ), fluxes through cycle must be equal ( $J_3 = J_4$ ), and  $J_5$  must be zero*

# Kernel of $N$

- **Equilibrium reactions:** zero entries in each basis vector  
→ net flow always zero in SS
- **Unbranched path:** same entries for a set of rows in all basis vectors  
→ net rate of corresponding reactions is always the same in a SS

# (Ir-)reversibility

Some reactions can be considered as being (practically) irreversible

→ no change in stoichiometric matrix  $\mathbf{N}$

But, kernel matrix  $\mathbf{K}$  will be restricted by this

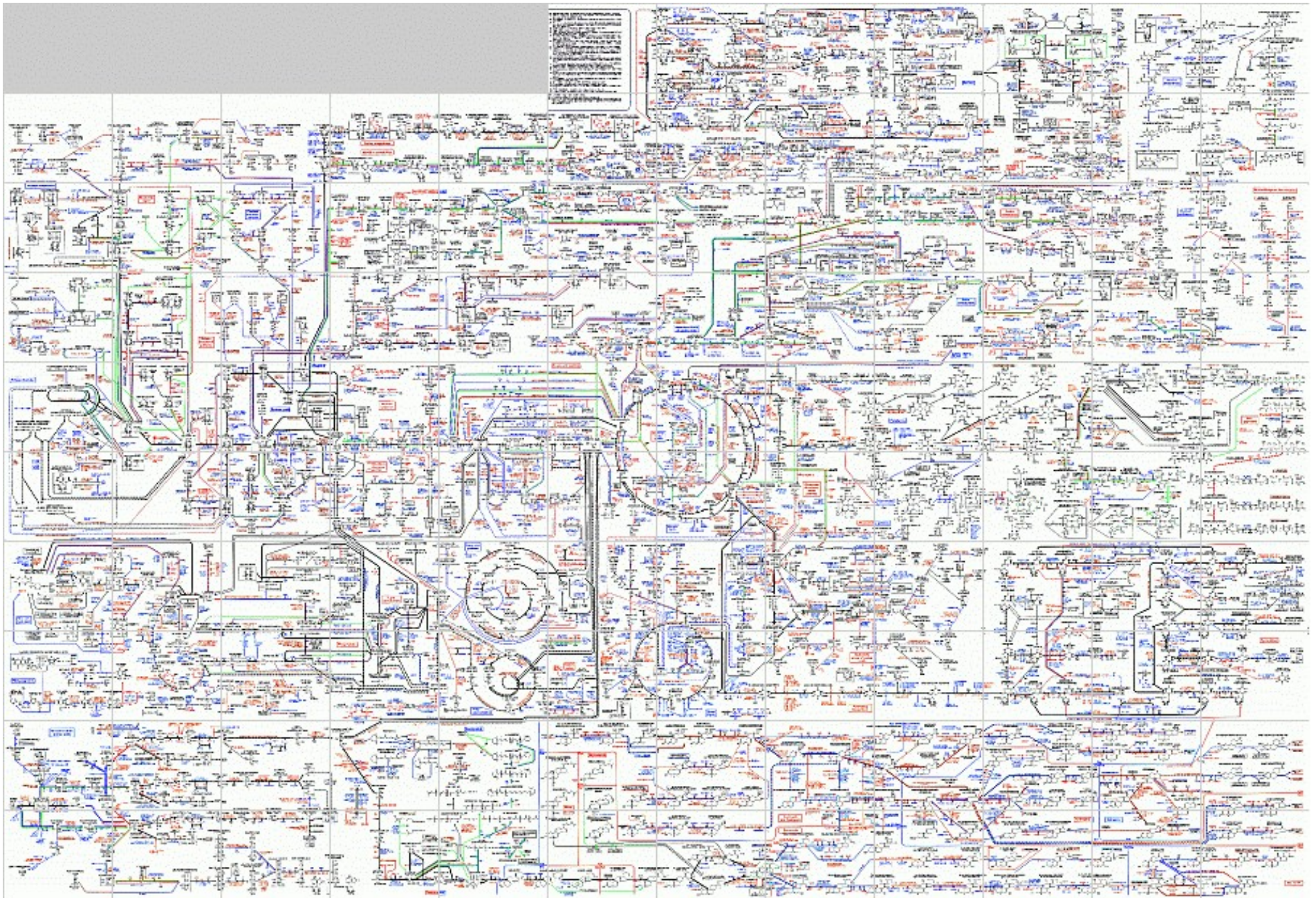
→ Some values may not become negative (or positive)

# Pathways

*"Set of subsequent reactions that are linked by common metabolites"*

Examples: glycolysis, amino acid synthesis pathways, ...

# Pathways?



# Pathways

- Difficult to separate looking at the complete **network** of reactions
- Which direct routes connect external metabolites or go in cycles?  
→ "flux modes"



# Elementary flux modes

- **Minimal** sets of reactions that allow steady state dynamics
- All steady state fluxes are linear combinations of elementary flux modes
- Calculation only requires structural information (stoichiometry matrix)

# Flux modes

- Definition

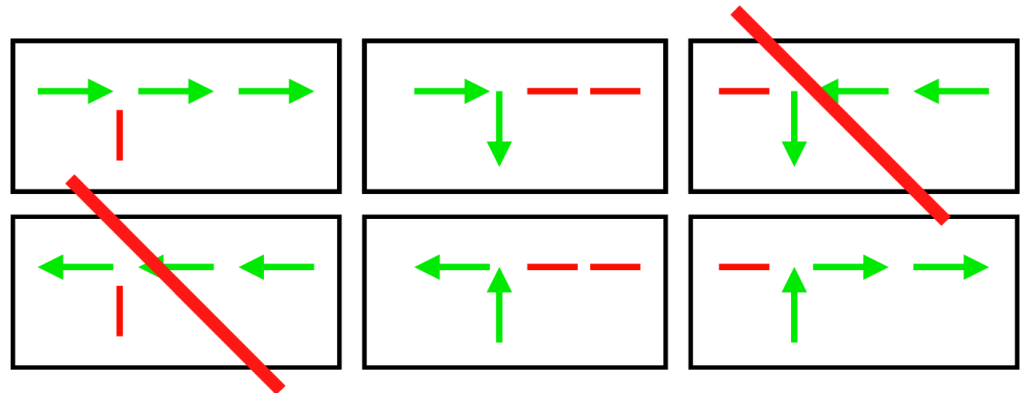
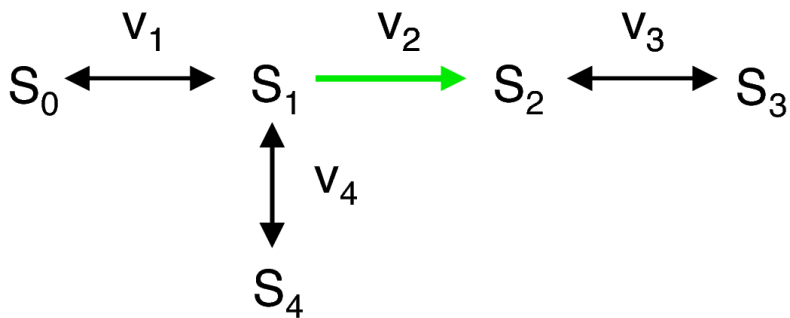
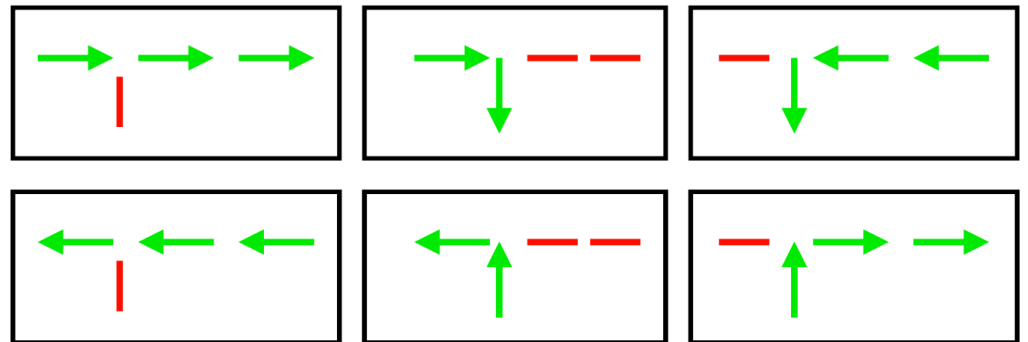
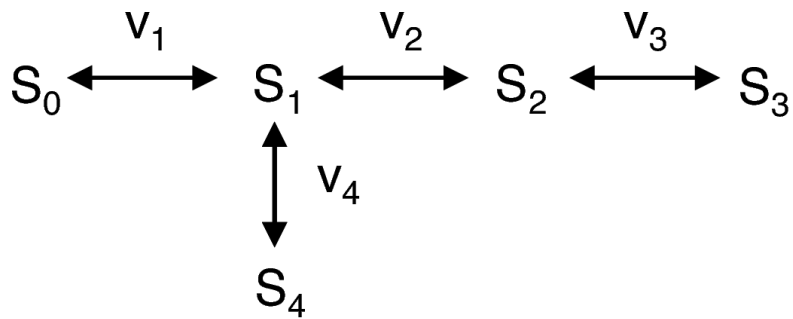
$$M = \{ \mathbf{v} \in R^r \mid \mathbf{v} = \lambda \cdot \hat{\mathbf{v}}, \lambda > 0 \}$$

with  $\hat{\mathbf{v}}$  an  $r$ -dimensional vector (not the null vector) such that

- it corresponds to a steady state
  - all sign restrictions (irreversible reactions) are fulfilled
- 
- Can be calculated using COPASI or specialised software, such as Metatool

# Examples

## Elementary Flux Modes



# Flux modes

- A flux mode  $M$  comprising  $v$  is called reversible if the set  $M'$  comprising  $-v$  is also a flux mode
- A flux mode is an **Elementary flux mode** (EFM) if it uses a minimal set of reactions and cannot be further decomposed
- An elementary flux mode can be interpreted as a minimal set of enzymes that could operate at steady state (with all irreversible reactions used in the appropriate direction)
- Number of EFMs  $\geq$  number of basis vectors of the null space

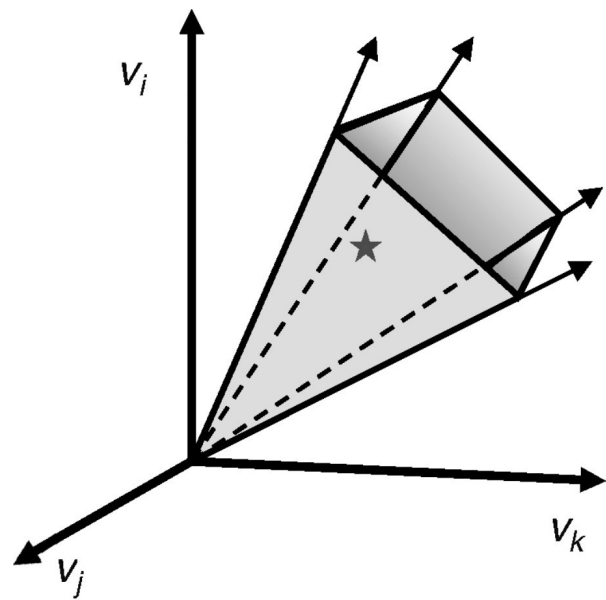
# Convex flux cone

Split up all reactions into forward and backward direction → uni-directional representation

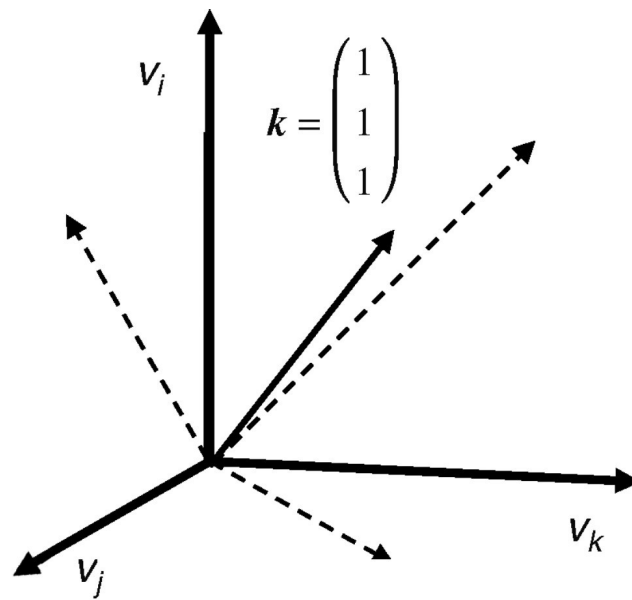
$SS \rightarrow$  flux vector is element of null space of  $N$  spanned by kernel matrix  $K$ . Rows of  $K$  can be interpreted as hyperplanes in flux space. The intersection of these hyperplanes forms the null space

Convex cone in flux space formed by basis vectors and considering irreversibility

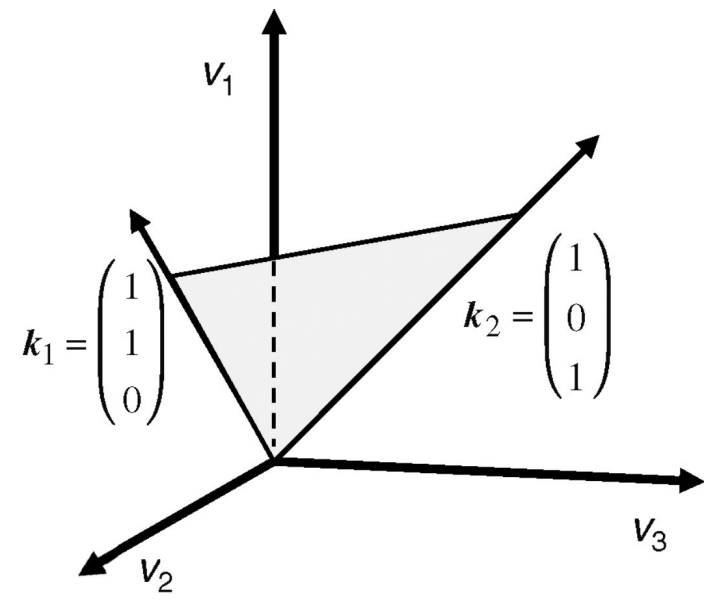
# Convex flux cone



(a)



(b)



(c)

# Stoichiometric analyses

**Elementary flux mode analysis** (and the related concept of **extreme pathways**) can be used:

- to understand the range of metabolic pathways in a network
- to test a set of enzymes for the production of a desired product
- to detect nonredundant pathways
- to analyse enzyme deficiencies
- in an application called **Flux balance analysis (FBA)** to find optimal flux distributions

# Conservation relations

- If a substance is neither added to/produced nor removed/degraded from the reaction system its total concentration stays constant.  
Example: enzyme concentration in Michaelis-Menten reaction mechanism
- This also holds if the substance builds complexes with other compounds or for (conserved) subparts of molecules (moieties)



# Conservation relations

Consider matrix  $\mathbf{G}$  with

$$\mathbf{G} \mathbf{N} = 0$$

then

$$\mathbf{G} \dot{\mathbf{S}} = \mathbf{G} \mathbf{N} \mathbf{v} = 0$$

Integrating leads to

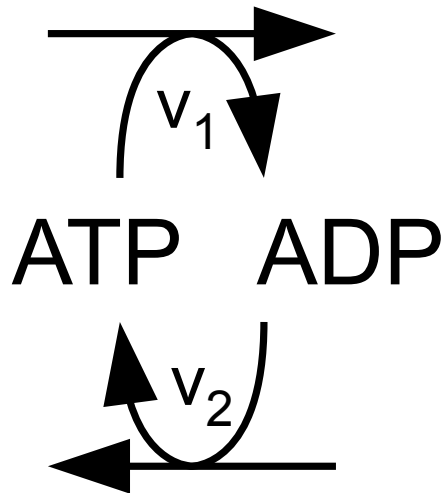
$$\mathbf{G} \mathbf{S} = \text{constant}$$

# Conservation relations

- Number of independent rows of  $\mathbf{G}$  equals  $n - \text{Rank}(\mathbf{N})$  with  $n$  the number of chemical species in the model
- $\mathbf{G}^T$  is the kernel matrix of  $\mathbf{N}^T$   
→ Conservation relations correspond to the null space of  $\mathbf{N}^T$
- It can be found using the Gauss algorithm
- $\mathbf{G}$  is not unique, but every linear combination of its rows is again a valid solution

# Example

$$\mathbf{S} = (ATP \ ADP)^T$$



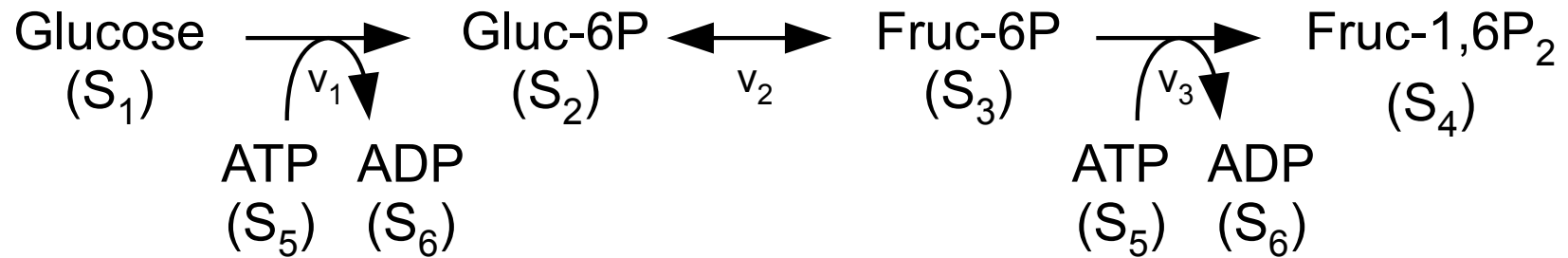
$$\mathbf{N} = \begin{pmatrix} -1 & 1 \\ 1 & -1 \end{pmatrix}$$

$$\mathbf{G} = (1 \ 1)$$

$ATP + ADP = constant$

The constant is dependent on the initial condition!

# Example



$$N^T = \begin{pmatrix} -1 & 1 & 0 & 0 & -1 & 1 \\ 0 & -1 & 1 & 0 & 0 & 0 \\ 0 & 0 & -1 & 1 & -1 & 1 \end{pmatrix} \quad G = \begin{pmatrix} 2 & 1 & 1 & 0 & 0 & 1 \\ 0 & 0 & 0 & 0 & 1 & 1 \\ 1 & 1 & 1 & 1 & 0 & 0 \end{pmatrix} = \begin{pmatrix} g_1 \\ g_2 \\ g_3 \end{pmatrix}$$

$g_2$ : conservation of *ATP + ADP*

$g_3$ : conservation of sugars

$g_1$ : with  $g_4 = -g_1 + 3 \cdot g_2 + 2 \cdot g_3 = (0 \ 1 \ 1 \ 2 \ 3 \ 2)$

→ conservation of phosphate groups

# Reducing the system

- Idea: Eliminate linearly dependent ODEs (species) and replace them by algebraic equations
- First, reorder

$$\mathbf{N} = \begin{pmatrix} \mathbf{N}_R \\ \mathbf{N}' \end{pmatrix} = \mathbf{L} \mathbf{N}_R = \begin{pmatrix} \mathbf{I}_{Rank(\mathbf{N})} \\ \mathbf{L}' \end{pmatrix} \mathbf{N}_R$$

$\mathbf{L}$  is called  
"link matrix"

- Then

$$\dot{\mathbf{S}} = \begin{pmatrix} \dot{\mathbf{S}}_{indep} \\ \dot{\mathbf{S}}_{dep} \end{pmatrix} = \begin{pmatrix} \mathbf{I}_{Rank(\mathbf{N})} \\ \mathbf{L}' \end{pmatrix} \mathbf{N}_R \mathbf{v}$$

with

$$\dot{\mathbf{S}}_{dep} = \mathbf{L}' \cdot \dot{\mathbf{S}}_{indep}$$

# Reducing the system (cont.)

- Integration leads to

$$\mathbf{S}_{dep} = \mathbf{L}' \cdot \mathbf{S}_{indep} + constant$$

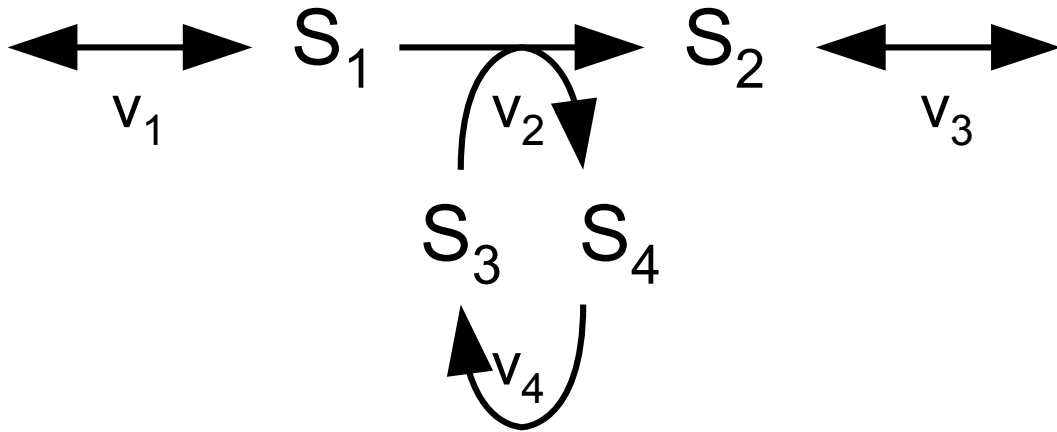
- This holds during the entire time course. Therefore, we can replace the full system by the reduced/simplified system

$$\dot{\mathbf{S}}_{indep} = \mathbf{N} \mathbf{v}$$

supplemented with the set of algebraic equations above

- Reduced system plus algebraic equations to express the dependent species is equivalent to the full system.

# Example



$$N = \begin{pmatrix} 1 & -1 & 0 & 0 \\ 0 & 1 & -1 & 0 \\ 0 & -1 & 0 & 1 \\ 0 & 1 & 0 & -1 \end{pmatrix}$$

$$N_R = \begin{pmatrix} 1 & -1 & 0 & 0 \\ 0 & 1 & -1 & 0 \\ 0 & -1 & 0 & 1 \end{pmatrix}$$

$$L = \begin{pmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \\ 0 & 0 & -1 \end{pmatrix}$$

$$L' = (0 \quad 0 \quad -1)$$

$$G = (0 \quad 0 \quad 1 \quad 1)$$

Reduced system:

$$\dot{S}_1 = v_1 - v_2$$

$$\dot{S}_2 = v_2 - v_3$$

$$\dot{S}_3 = v_4 - v_2$$

$$S_4 = \text{constant} - S_3$$

# Conservation relations (summary)

Linear combinations of species whose overall concentration stays constant

Might not be obvious from visual inspection of the network diagram only

Important for reducing the system