Reaction Correlation Coefficients and LPEMs

Prof Cristiana Sebu

University of Malta, Malta

More on Vectors in $\mathbf{R}^n$

Definition 1.1.3 If $\mathbf{u} = (u_1, u_2, \ldots, u_n)$ is a vector in $\mathbf{R}^n$, we define the *Euclidean norm* (or *Euclidean length*) of $\mathbf{u}$ to be

$$\|\mathbf{u}\| = \sqrt{u_1^{\,2} + u_2^{\,2} + \ldots + u_n^{\,2}}\,.$$

Definition 1.1.4 If $\mathbf{u} = (u_1, u_2, \ldots, u_n)$ and $\mathbf{v} = (v_1, v_2, \ldots, v_n)$ are vectors in $\mathbf{R}^n$, we define the *Euclidean inner product* $\mathbf{u} \cdot \mathbf{v}$ to be

$$\mathbf{u} \cdot \mathbf{v} = u_1 v_1 + u_2 v_2 + \ldots + u_n v_n\,.$$

We refer to $\mathbf{R}^n$, endowed with this inner product, as *Euclidean n-space*.

Observation 1.1.5 For each $\mathbf{u} = (u_1, u_2, \ldots, u_n)$ in $\mathbf{R}^n$, $\|\mathbf{u}\| = (\mathbf{u} \cdot \mathbf{u})^{\frac{1}{2}}$.

Definition 1.1.6 If $\mathbf{u} = (u_1, u_2, \ldots, u_n)$ and $\mathbf{v} = (v_1, v_2, \ldots, v_n)$ are vectors in $\mathbf{R}^n$, define the cosine of the angle between $\mathbf{u}$ and $\mathbf{v}$ by

$$\cos\theta = \frac{\mathbf{u} \cdot \mathbf{v}}{\|\mathbf{u}\|\|\mathbf{v}\|}.$$

Two vectors $\mathbf{u}$ and $\mathbf{v}$ in $\mathbf{R}^n$ are **orthogonal** if and only if $\mathbf{u} \cdot \mathbf{v} = 0$; that is, if and only if the angle between them is 90°.

Orthogonal Sets

Definition 1.7.1 Let $S = \{\mathbf{u}_1, \mathbf{u}_2, \ldots, \mathbf{u}_n\}$ be a set of non-zero vectors, S is an **orthogonal set** if each pair of vectors in S is orthogonal, and S is an **orthonormal set** if it is orthogonal and each vector is a unit vector.

- Orthogonal: $\mathbf{u}_i \cdot \mathbf{u}_j = 0$ if $i \neq j$
- Orthonormal: $\mathbf{u}_i \cdot \mathbf{u}_j = \begin{cases} 1 & i = j \\ 0 & i \neq j \end{cases}$

Example $S = \{\mathbf{u}_1, \mathbf{u}_2, \mathbf{u}_3\}$, where $\mathbf{u}_1 = (1,2,1)$, $\mathbf{u}_2 = (2,1,-4)$, $\mathbf{u}_3 = (3,-2,1)$, is an orthogonal set of vectors in $\mathbf{R}^3$.

Remark We can easily transform an orthogonal set of non-zero vectors into an orthonormal set as follows:

If the set $S = \{\mathbf{u}_1, \mathbf{u}_2, \ldots, \mathbf{u}_n\}$ is orthogonal, then the set $S' = \left\{ \frac{1}{\|\mathbf{u}_1\|}\mathbf{u}_1, \frac{1}{\|\mathbf{u}_2\|}\mathbf{u}_2, \ldots, \frac{1}{\|\mathbf{u}_n\|}\mathbf{u}_n \right\}$ is orthonormal.

This process of dividing each vector in an orthogonal set by its norm to produce an orthonormal set is called **normalisation**.

Exercise Normalize the set S from the previous Example.

Gram-Schmidt Orthogonalization Process

Suppose the set of vectors $\{\mathbf{v}_1, \mathbf{v}_2, \ldots, \mathbf{v}_n\}$ is a basis, an **orthogonal** basis $\{\mathbf{w}_1, \mathbf{w}_2, \ldots, \mathbf{w}_n\}$ can be obtained as follows:

$$\mathbf{w}_1 = \mathbf{v}_1$$

$$\mathbf{w}_2 = \mathbf{v}_2 - \frac{\mathbf{v}_2 \cdot \mathbf{w}_1}{\mathbf{w}_1 \cdot \mathbf{w}_1}\mathbf{w}_1$$

$$\mathbf{w}_3 = \mathbf{v}_3 - \frac{\mathbf{v}_3 \cdot \mathbf{w}_1}{\mathbf{w}_1 \cdot \mathbf{w}_1}\mathbf{w}_1 - \frac{\mathbf{v}_3 \cdot \mathbf{w}_2}{\mathbf{w}_2 \cdot \mathbf{w}_2}\mathbf{w}_2$$

.....

$$\mathbf{w}_n = \mathbf{v}_n - \frac{\mathbf{v}_n \cdot \mathbf{w}_1}{\mathbf{w}_1 \cdot \mathbf{w}_1}\mathbf{w}_1 - \frac{\mathbf{v}_n \cdot \mathbf{w}_2}{\mathbf{w}_2 \cdot \mathbf{w}_2}\mathbf{w}_2 - \ldots - \frac{\mathbf{v}_n \cdot \mathbf{w}_{n-1}}{\mathbf{w}_{n-1} \cdot \mathbf{w}_{n-1}}\mathbf{w}_{n-1}.$$

Example Apply the Gram-Schmidt Orthogonalization Process to orthogonalize and then orthonormalize the set of vectors $\{(0,1,1,0,0),(1,1,0,0,1)\}$.

Example Apply the Gram-Schmidt Orthogonalization Process to orthogonalize and then orthonormalize the set of vectors $\{(1,0,-1),(2,-1,0),(1,2,1)\}$.

Matrix multiplication

If A is an $m \times r$ matrix and B is $r \times n$ matrix then the product AB is the $m \times n$ matrix.

$$AB = \begin{pmatrix} a_{11} & a_{12} & \cdots & a_{1r} \\ a_{21} & a_{22} & \cdots & a_{2r} \\ \vdots & \vdots & & \vdots \\ a_{i1} & a_{i2} & \cdots & a_{ir} \\ \vdots & \vdots & & \vdots \\ a_{m1} & a_{m2} & \cdots & a_{mr} \end{pmatrix} \begin{pmatrix} b_{11} & b_{12} & \cdots & b_{1j} & \cdots & b_{1n} \\ b_{21} & b_{22} & \cdots & b_{2j} & \cdots & b_{2n} \\ \vdots & \vdots & & \vdots & & \vdots \\ b_{r1} & b_{r2} & \cdots & b_{rj} & \cdots & b_{rn} \end{pmatrix}$$

$$(AB)_{ij} = a_{i1}b_{1j} + a_{i2}b_{2j} + a_{i3}b_{3j} + \cdots a_{ir}b_{rj} = \sum_{k=1}^{r} a_{ik}b_{kj}$$

Example Consider the matrices $A = \begin{pmatrix} 3 & 0 \\ -1 & 2 \\ 1 & 1 \end{pmatrix}$, $B = \begin{pmatrix} 4 & -1 \\ 0 & 2 \end{pmatrix}$, $C = \begin{pmatrix} 1 & 4 & 2 \\ 3 & 1 & 5 \end{pmatrix}$.

Compute AB, AC, CA and BC.

Transpose of a matrix

If A is an $m \times n$ matrix, then its **transpose** A^T is an $n \times m$ matrix given by interchanging the rows and columns of A.

$$\left(a_{ij}\right)_{m\times n}^{T} = \left(a_{ji}\right)_{n\times m}$$

Example 7 Compute the transpose of the following matrices

$$B = \begin{pmatrix} 2 & 3 \\ 1 & 4 \\ 5 & 6 \end{pmatrix}, \quad C = \begin{pmatrix} 1 & 3 & 5 \end{pmatrix}, \quad D = \begin{pmatrix} 3 & 5 & -2 \\ 5 & 4 & 1 \\ -2 & 1 & 7 \end{pmatrix} \quad \text{and} \quad E = \begin{pmatrix} 4 \end{pmatrix}.$$

Orthogonalization of vectors via Gaussian elimination

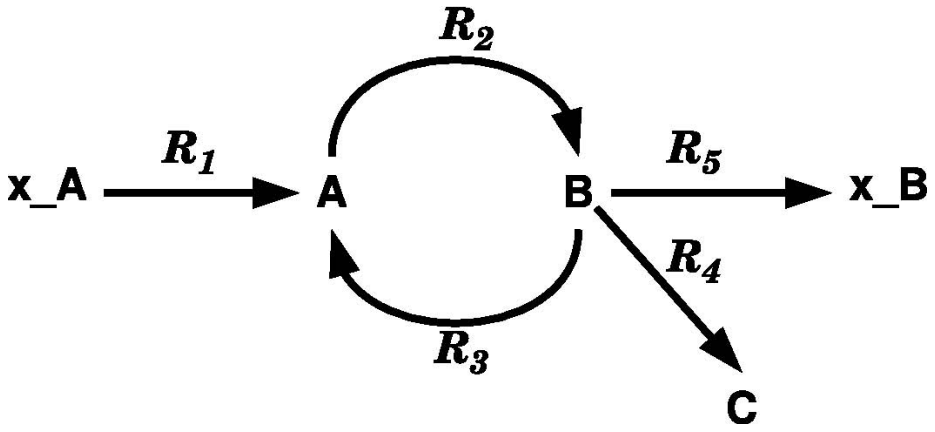
If the set of vectors $\{\mathbf{v}_1, \mathbf{v}_2, \ldots, \mathbf{v}_n\}$ are written as rows of a matrix A, then applying Gaussian elimination to the augmented matrix $(AA^T|A)$ will produce the orthogonalized vectors in place of A.

However, the matrix $(AA^T|A)$ must be brought to row echelon form, using only the elementary row operations of **adding a scalar multiple of one row to another** or **multiplying a row by a scalar**.

Definition 4.2 The **reaction correlation coefficient**, ϕ_{ij}, is the cosine of $\theta_{ij}^{\mathbf{K}}$, the angle between rows i and j of the kernel matrix $\mathbf{K}$, i.e.

$$\phi_{ij} = \cos\left(\theta_{ij}^{\mathbf{K}}\right).$$

Observation 4.3 $-1 \leq \phi_{ij} \leq 1$ or $-\frac{\pi}{2} \leq \theta_{ij}^{\mathbf{K}} \leq \frac{\pi}{2}$. Thus, the minimal possible difference between reactions is $\theta_{ij}^{\mathbf{K}} = 0$ (reaction vectors are parallel) and maximum absolute value $\theta_{ij}^{\mathbf{K}} = \frac{\pi}{2}$ (reaction vectors are orthogonal).



Example 4.4 Reactions 1 and 5 are members of the same subset.

$$\mathbf{v} = \begin{pmatrix} R_1 \\ R_2 \\ R_3 \\ R_4 \\ R_5 \end{pmatrix} = w_1 \begin{pmatrix} 0 \\ 1 \\ 1 \\ 0 \\ 0 \end{pmatrix} + w_2 \begin{pmatrix} 1 \\ 1 \\ 0 \\ 0 \\ 1 \end{pmatrix} = \begin{pmatrix} 0 & 1 \\ 1 & 1 \\ 1 & 0 \\ 0 & 0 \\ 0 & 1 \end{pmatrix} \begin{pmatrix} w_1 \\ w_2 \end{pmatrix} = \mathbf{Kw} = \begin{pmatrix} 0w_1 + 1w_2 \\ 1w_1 + 1w_2 \\ 1w_1 + 0w_2 \\ 0w_1 + 0w_2 \\ 0w_1 + 1w_2 \end{pmatrix} \begin{matrix} \leftarrow \text{ subset} \\ \\ \\ \leftarrow \text{ dead} \\ \leftarrow \text{ subset} \end{matrix}$$

$$\mathbf{K} = \begin{pmatrix} 0 & 1 \\ 1 & 1 \\ 1 & 0 \\ 0 & 0 \\ 0 & 1 \end{pmatrix} \quad \text{and} \quad \phi_{15} = \phi_{51} = \cos\left(\theta_{15}^{\mathbf{K}}\right) = \frac{(0,1)\cdot(0,1)}{\left\|(0,1)\right\|^2} = 1 \quad \Rightarrow \quad \theta_{15}^{\mathbf{K}} = 0$$

(reaction vectors are parallel).

Drawback: $\mathbf{K}$ is, in most cases, not unique and depends upon both the algorithm used for its calculation and the initial row and column order of $\mathbf{N}$.

The angles between the row vectors of any $\mathbf{K}$ for a given $\mathbf{N}$ are unique, provided $\mathbf{K}$ is orthogonal, that is $\mathbf{K}^T\mathbf{K} = \mathbf{I}$, in which case $\mathbf{K}$ represents an orthonormal basis of the null-space of $\mathbf{N}$.

Theorem 4.5. Let A and B be orthogonal matrices with equal dimensions, then

$$\cos\left(\theta_{ij}^{\mathbf{A}}\right) = \cos\left(\theta_{ij}^{\mathbf{B}}\right)$$

for any rows i and j.*

* M. G. Poolman, C. Sebu, M. K. Pidcock and D. A. Fell, *Modular decomposition of metabolic systems via null space analysis*, Journal of Theoretical Biology 249(4): 691-705, (2007).

Observation 4.6 ϕ_{ij} is Pearson's correlation coefficient between the fluxes carried by reactions i and j for all possible steady states of the system.*

For a pair of reactions, i and j, with corresponding rows in $\mathbf{K}$, $\mathbf{K}_i$ and $\mathbf{K}_j$:

$$\phi_{ij} = \cos\left(\theta_{ij}^{\mathbf{K}}\right) = \frac{\mathbf{K}_i \cdot \mathbf{K}_j}{\|\mathbf{K}_i\|\|\mathbf{K}_j\|} = \frac{\mathbf{K}_i \mathbf{K}_j^T}{\sqrt{\mathbf{K}_i \mathbf{K}_i^T}\sqrt{\mathbf{K}_j \mathbf{K}_j^T}}.$$

$\phi_{ij} = \pm 1$: $\mathbf{K}_i$ and $\mathbf{K}_j$ are parallel, and thus carry steady-state flux in a fixed ratio. Hence, the reactions are members of the same subset.

$\phi_{ij} = 0$: $\mathbf{K}_i$ and $\mathbf{K}_j$ are orthogonal $\Longrightarrow$ reactions i and j are in stoichiometrically disconnected subsystems (no correlation between fluxes).

* M. G. Poolman, C. Sebu, M. K. Pidcock and D. A. Fell, *Modular decomposition of metabolic systems via null space analysis*, Journal of Theoretical Biology 249(4): 691-705, (2007).

Remark $\left\{\left(0,\frac{1}{\sqrt{2}},\frac{1}{\sqrt{2}},0,0\right),\left(\sqrt{\frac{2}{5}},\frac{1}{\sqrt{10}},-\frac{1}{\sqrt{10}},0,\sqrt{\frac{2}{5}}\right)\right\}$ is an orthonormal basis for $\mathbf{N}$ .The following kernel matrix is orthogonal

$$\mathbf{K}=\begin{pmatrix} & \\ 0 & \sqrt{\frac{2}{5}} \\ \frac{1}{\sqrt{2}} & \frac{1}{\sqrt{10}} \\ \frac{1}{\sqrt{2}} & -\frac{1}{\sqrt{10}} \\ 0 & 0 \\ & \\ 0 & \sqrt{\frac{2}{5}} \end{pmatrix} \text{ and } \phi_{15}=\phi_{51}=\cos\left(\theta_{15}^{\mathbf{K}}\right)=\frac{\left(0,\sqrt{\frac{2}{5}}\right)\cdot\left(0,\sqrt{\frac{2}{5}}\right)}{\left\|\left(0,\sqrt{\frac{2}{5}}\right)\right\|^2}=1 .$$

Significance of the reaction correlation coefficient ϕ

Spans the continuum of possible correlations between any pair of reaction fluxes, from being completely dependent to completely independent.

Can be regarded as a quantitative generalisation of the qualitative concept of the reaction subset.

LPEMs

LPEMs[**] is a novel algorithm which uses Linear Programming (LP) to decompose a flux vector, $\mathbf{v}$, into a linear combination of EMs, without requiring the calculation of the entire set of EMs of the network.

EMs consist of the smallest non-decomposable pathways within a metabolic system, that can combine to form every possible steady-state flux.

Decomposing flux measurements obtained from experiments or simulations into such a set of constituents allows for their interpretation as a weighted sum of pathways (each with associated external input and output), thus revealing how potential routes within the network can contribute to the observed overall system behaviour, as well as providing the means to assign relative flux values to EMs.

[**] Y. Said, D. Singh, C. Sebu and M. Poolman, *A Novel Algorithm to Calculate Elementary Modes: Analysis of Campylobacter jejuni Metabolism*, Biosystems **234**, (2023). https://doi.org/10.1016/j.biosystems.2023.105047.

Most of methods previously developed rely on having calculated a complete set of EMs a priori, which is not computationally practical for large metabolic models.

The Algorithm

Let $\mathbf{v} \in \mathbb{R}^r$ be the flux vector to be decomposed into EMs (r is the number of reactions).

Output is a matrix, $\mathbf{E}$, whose column vectors, e_i, consist of EMs, such that:

$$\mathbf{v} = \sum_{i=1}^{t} e_i,$$

where t is the number of EMs.

EMs are not normalised in this instance, i.e. the magnitude of each e_i reflects the contribution of that EM to $\mathbf{v}$.

Prior to starting the decomposition, $\mathbf{v}$ is ensured to be at steady-state. If not, $\mathbf{v}$ is approximated to the closest vector that satisfies $\mathbf{Nv} = \mathbf{0}$, and the excess is saved as an error vector.

Then, the algorithm proceeds to iteratively obtain EMs, whilst simultaneously eliminating components of the flux vector (starting from the smallest first).

At each iteration, the following LP problem is used to obtain a solution, $\mathbf{v}'$, that has specific properties that depend on $\mathbf{v}$:

$$\mathcal{F}(\mathbf{v}, \mathbf{N}) = \text{argmin} \sum_{i=1}^{r} |v_i'|$$

$$\text{subject to} \begin{cases} \mathbf{Nv}' = \mathbf{0}, \\ v'_{\min} = v_{\min}, \\ |v_i'| \leq |v_i|, \quad \text{sign}(v_i') = \text{sign}(v_i), \text{for all } i \in \{1, 2, \ldots r\}. \end{cases}$$

Once an LP solution is found, the Rank Test is applied to determine whether $\mathbf{v}'$ is an EM (Step 4 in Algorithm 1).

If the solution is not an EM, it is decomposed into constituent EMs by creating a submatrix matrix of $\mathbf{N}$ that contains only the reactions present within $\mathbf{v}'$ and enumerating its EMs. A set of fluxes are assigned to the EMs by solving

$$\mathbf{E}\mathbf{w} = \mathbf{v}',$$

where $\mathbf{w}$ is a vector of flux weightings assigned to each EM[1].

The one or more obtained EMs are saved, and the loop restarts using the modified flux vector $\mathbf{v}$:

$$\mathbf{v} - \mathbf{v}' \rightarrow \mathbf{v}.$$

[1] M. G. Poolman, K. V. Venkatesh, M. K. Pidcock, and D. A. Fell. *A method for the determination of flux in elementary modes, and its application to Lactobacillus rhamnosus*. Biotechnology and Bioengineering, 88(5):601–612, 2004.

$$\begin{pmatrix} v_1 \\ v_2 \\ \vdots \\ v_{\min} \\ \vdots \\ v_r \end{pmatrix} - \begin{pmatrix} v'_1 \\ v'_2 \\ \vdots \\ v'_{\min} \\ \vdots \\ v'_r \end{pmatrix} = \begin{pmatrix} v_1 - v'_1 \\ v_2 - v'_2 \\ \vdots \\ 0 \\ \vdots \\ v_r - v'_r \end{pmatrix}$$

The loop continues to iteratively append EMs to $\mathbf{E}$ whilst simultaneously eliminating components of the flux vector $\mathbf{v}$, until $\mathbf{v} = \mathbf{0}$.

The elimination of components starting by the smallest first is based on the assumption that the smallest component is the most likely to contribute to the least amount of EMs.

The algorithm is implemented in Python and is publicly accessible as part of release 3 of ScrumPy3.

Algorithm 1 Decomposing $\mathbf{v}$ into a set of EMs, $\mathbf{E}$

while $|\mathbf{v}| > \mathbf{0}$ **do**
  $v_{\min}$ = minimum value in $\mathbf{v}$
  $\mathbf{v}' = \mathcal{F}(\mathbf{v}, \mathbf{N})$ given by Equation (3.2)
  if $\mathbf{v}'$ is not an EM **then**
    decompose $\mathbf{v}'$ into a set of EMs
    append these EMs to $\mathbf{E}$
  else
    append $\mathbf{v}'$ to $\mathbf{E}$
  end if
  $\mathbf{v} - \mathbf{v}' \rightarrow \mathbf{v}$
end while.

Mathematical considerations

Let $S(\mathbf{v})$ denote the set of indices of the non-zero elements of $\mathbf{v}$, i.e.

$$S(\mathbf{v}) = \{i \in \mathbb{N} | \, 1 \leq i \leq r \text{ and } v_i \neq 0\}.$$

and $\mathbf{N}_s$ denote a sub-matrix of $\mathbf{N}$ that contains only the reactions in $S(\mathbf{v})$.

- *The Rank Test (Step 4 in Algorithm 1)*: The vector, $\mathbf{v}$, is an EM if $\mathbf{N}_s$ has a nullspace of dimension one, i.e. $\dim\big(\mathrm{Ker}(\mathbf{N}_s)\big) = 1$ [2].
- *Decomposing $\boldsymbol{v}$ into EMs (Step 5 in Algorithm 1)*: The EMs of the sub-model generated by $\mathbf{N}_s$ is a subset of the EMs of the original model. There exists a minimal decomposition of $\mathbf{v}$ into at most $\dim\big(\mathrm{Ker}(\mathbf{N}_s)\big)$ EMs.
- *Step 10 in Algorithm 1*: If $\mathbf{v}$ and $\mathbf{v}'$ are two steady-state flux vectors, then $\mathbf{v} - \mathbf{v}'$ is a steady-state flux vector of the system.

[2] J. Gagneur and S. Klamt. *Computation of elementary modes: A unifying framework and the new binary approach*. BMC Bioinformatics, 5:1–21, 2004.

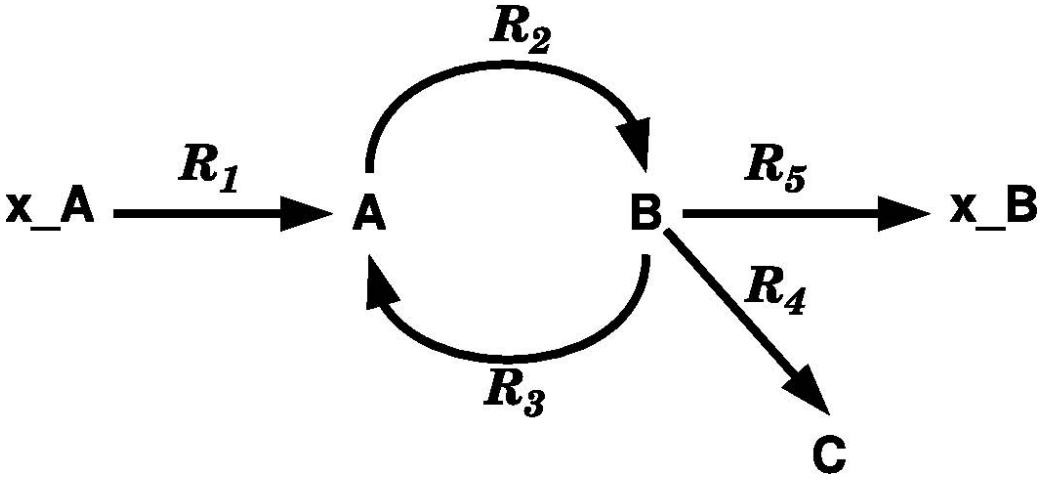
Rank of a matrix and dimension theorem

Definition The rank of a matrix A, written $\mathrm{rank}\left(A\right)$, is equal to the number of nonzero rows (i.e. rows with nonzero pivot entries) in a row-echelon form of A.

Theorem If A is an $m \times n$ matrix, then

$$\mathrm{rank}(\mathbf{A}) + \dim\big(\mathrm{Ker}(\mathbf{A})\big) = n.$$

Example



$$\begin{pmatrix} \frac{dA}{dt} \\ \frac{dB}{dt} \\ \frac{dC}{dt} \end{pmatrix} = \underbrace{\begin{pmatrix} 1 & -1 & 1 & 0 & 0 \\ 0 & 1 & -1 & -1 & -1 \\ 0 & 0 & 0 & 1 & 0 \end{pmatrix}}_{\mathbf{N}} \underbrace{\begin{pmatrix} R_1 \\ R_2 \\ R_3 \\ R_4 \\ R_5 \end{pmatrix}}_{\mathbf{v}} = \begin{pmatrix} 0 \\ 0 \\ 0 \end{pmatrix}$$

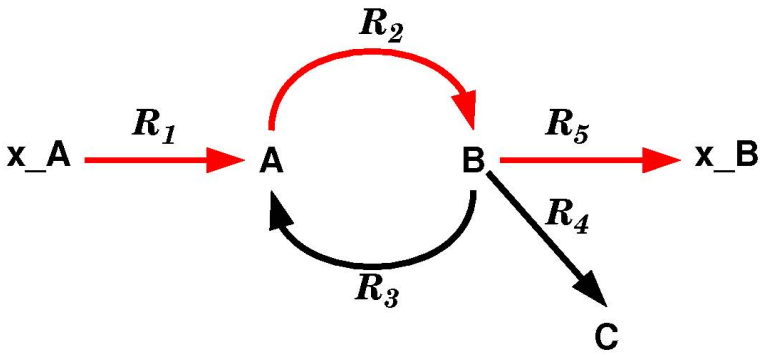
$$\mathbf{N}\mathbf{v} = \mathbf{0}$$

$$\mathbf{v} \in \mathrm{Ker}(\mathbf{N})$$

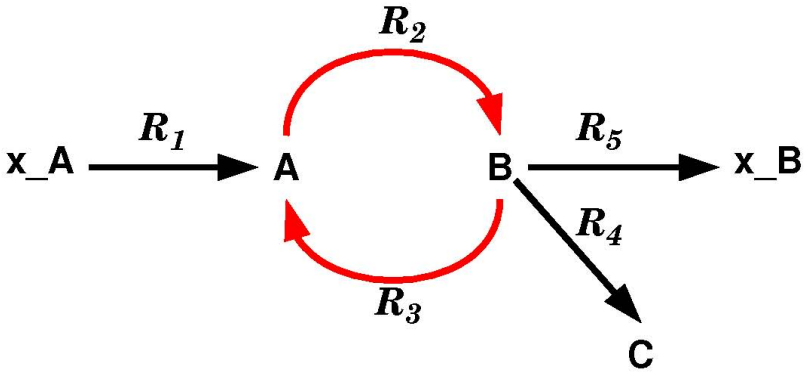
$\{(0,1,1,0,0),(1,1,0,0,1)\}$ is a basis for $\mathrm{Ker}(\mathbf{N})$.

Elementary modes

1. $(1,1,0,0,1)$



2. $(0,1,1,0,0)$



Non Elementary Modes