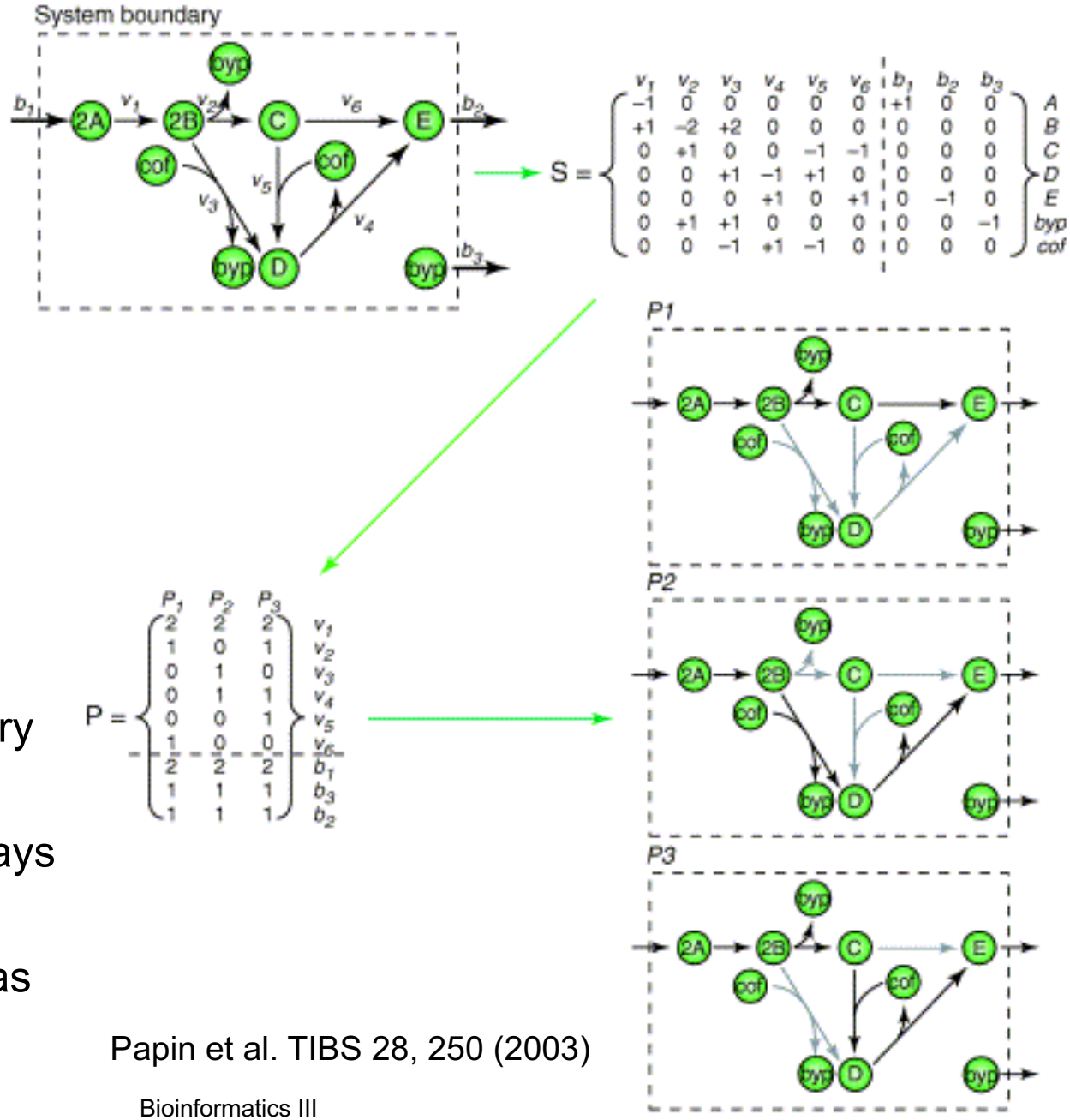
Stoichiometric matrix

Stoichiometric matrix S:
m × *n* matrix with
stochiometries of the
n reactions as columns and
participations of
m metabolites as rows.

The stochiometric matrix is an important part of the *in silico* model.

With the matrix, the methods of extreme pathway and elementary mode analyses can be used to generate a unique set of pathways P1, P2, and P3 that allow to express all steady-state fluxes as linear combinations of P1 – P3.



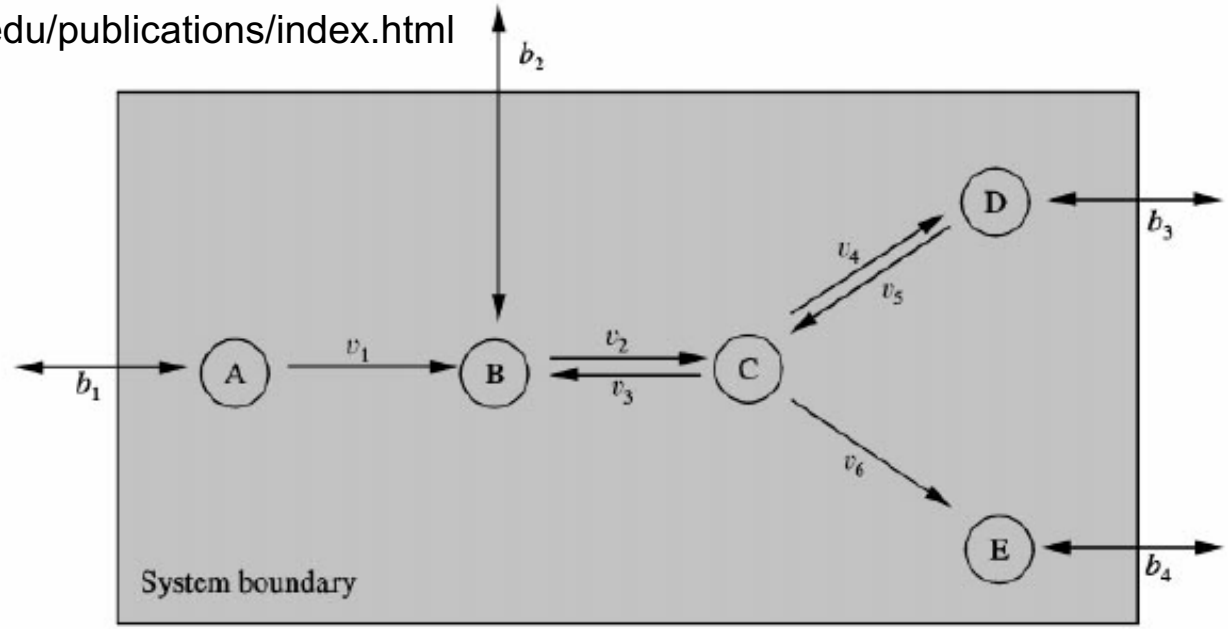
Papin et al. TIBS 28, 250 (2003)

Extreme Pathways

introduced into metabolic analysis by the lab of Bernard Palsson (Dept. of Bioengineering, UC San Diego). The publications of this lab are available at http://gcrg.ucsd.edu/publications/index.html

The extreme pathway technique is based on the stoichiometric matrix representation of metabolic networks.

All external fluxes are defined as pointing outwards.



Mass balance constraints

$$\begin{bmatrix} -1 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 \\ 1 & -1 & 1 & 0 & 0 & 0 & 0 & -1 & 0 & 0 \\ 0 & 1 & -1 & -1 & 1 & -1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & -1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & -1 \end{bmatrix} \begin{bmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \\ v_5 \\ v_6 \\ b_1 \\ b_2 \\ b_3 \\ b_4 \end{bmatrix} = \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \end{bmatrix}$$

$$(\mathbf{S} \cdot \mathbf{v} = \mathbf{0})$$

Internal flux constraints

$$v_j \geq 0, \quad j = 1,\ldots,6$$

Exchange flux constraints

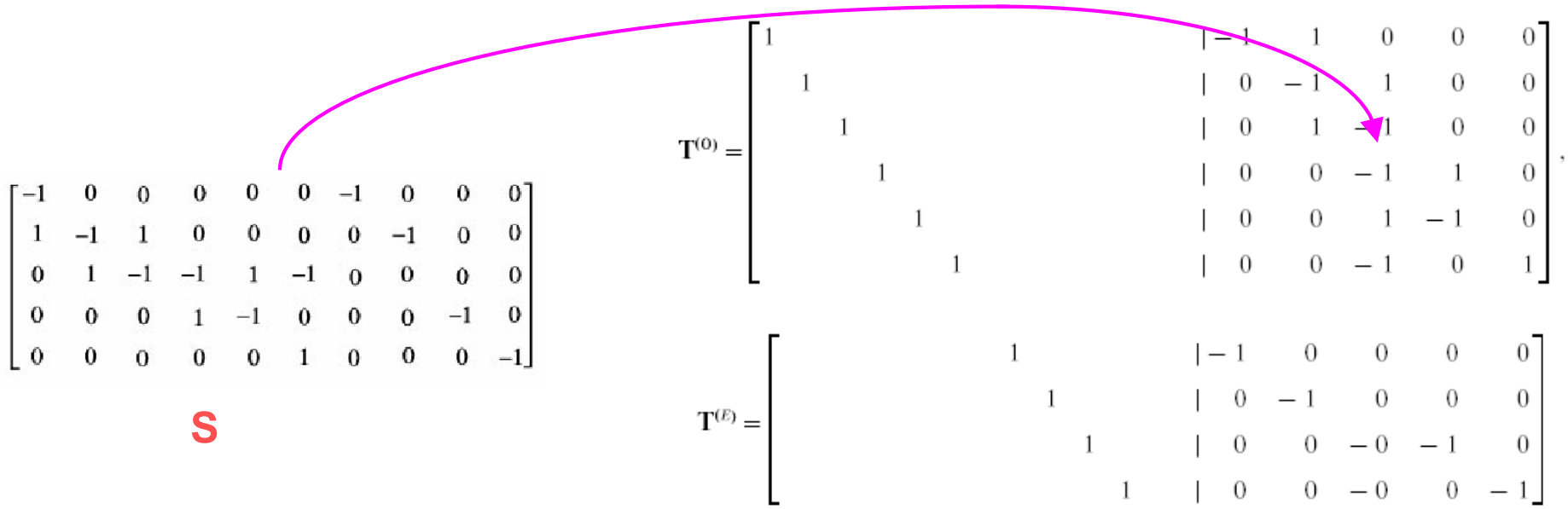
$$-\infty \leq b_j \leq +\infty, \quad j = 1,\ldots,4$$

Schilling, Letscher, Palsson, J. theor. Biol. 203, 229 (2000)

Extreme Pathways – algorithm - setup

The algorithm to determine the set of extreme pathways for a reaction network follows the pinciples of algorithms for finding the extremal rays/ generating vectors of convex polyhedral cones.

Combine $n \times n$ identity matrix (**I**) with the transpose of the stoichiometric matrix $\mathbf{S}^T$. **I** serves for bookkeeping.



Schilling, Letscher, Palsson,
J. theor. Biol. 203, 229 (2000)

separate internal and external fluxes

Examine constraints on each of the exchange fluxes as given by

$$\alpha_j \leq b_j \leq \beta_j$$

If the exchange flux is constrained to be positive $\rightarrow$ do nothing.

If the exchange flux is constrained to be negative $\rightarrow$ multiply the corresponding row of the initial matrix by -1.

If the exchange flux is unconstrained $\rightarrow$ move the entire row to a temporary matrix $\mathbf{T}^{(E)}$.

This completes the first tableau $\mathbf{T}^{(0)}$.

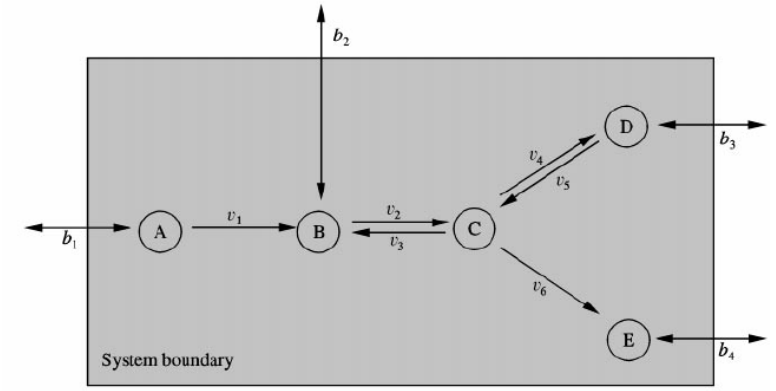
Schilling, Letscher, Palsson,
J. theor. Biol. 203, 229 (2000)

idea of algorithm

(1) Identify all metabolites that do not have an unconstrained exchange flux associated with them.

The total number of such metabolites is denoted by μ.

The example system contains only one such metabolite, namely C (μ = 1).



What is the **main idea** of this step?

- We want to find balanced extreme pathways that don‘t change the concentrations of metabolites when flux flows through (input fluxes are channelled to products not to accumulation of intermediates).
- The stochiometrix matrix describes the coupling of each reaction to the concentration of metabolites X.
- Now we need to balance combinations of reactions that leave concentrations unchanged. Pathways applied to metabolites should not change their concentrations $\rightarrow$ the matrix entries need to be brought to 0.

Schilling, Letscher, Palsson,
J. theor. Biol. 203, 229 (2000)

keep pathways that do not change concentrations of internal metabolites

(2) Begin forming the new matrix $\mathbf{T}^{(i)}$ by copying all rows from $\mathbf{T}^{(i-1)}$ which already contain a zero in the column of $\mathbf{S}^T$ that corresponds to the first metabolite identified in step 1, denoted by index *C*. (Here 3rd column of $\mathbf{S}^T$.)

$T^{(0)}$ =

						A	B	**C**	D	E
1						-1	1	**0**	0	0
	1					0	-1	1	0	0
		1				0	1	-1	0	0
			1			0	0	-1	1	0
				1		0	0	1	-1	0
					1	0	0	-1	0	1

$T^{(1)}$ =

1						-1	1	**0**	0	0

+

Schilling, Letscher, Palsson, J. theor. Biol. 203, 229 (2000)

balance combinations of other pathways

(3) Of the remaining rows in $\mathbf{T}^{(i-1)}$ add together all possible **combinations of rows** which contain values of the opposite sign in column C, such that the addition produces a zero in this column.

$T^{(0)}$ =

1						-1	1	**0**	0	0
	1					0	-1	1	0	0
		1				0	1	-1	0	0
			1			0	0	-1	1	0
				1		0	0	1	-1	0
					1	0	0	-1	0	1

$T^{(1)}$ =

1	0	0	0	0	0	-1	1	0	0	0
0	1	1	0	0	0	0	0	**0**	0	0
0	1	0	1	0	0	0	-1	**0**	1	0
0	1	0	0	0	1	0	-1	**0**	0	1
0	0	1	0	1	0	0	1	**0**	-1	0
0	0	0	1	1	0	0	0	**0**	0	0
0	0	0	0	1	1	0	0	**0**	-1	1
1	2	3	4	5	6	7	8	9	10	11

Schilling, et al.
JTB 203, 229

remove “non-orthogonal” pathways

(4) For all rows added to $\mathbf{T}^{(i)}$ in steps 2 and 3 check that no row exists that is a non-negative combination of any other rows in $\mathbf{T}^{(i)}$.

One method for this works as follows:
let *A(i)* = set of column indices *j* for which the elements of row *i* = 0.

For the example above
A(1) = {2,3,4,5,6,9,10,11}
A(2) = {1,4,5,6,7,8,9,10,11}
A(3) = {1,3,5,6,7,9,11}
A(4) = {1,3,4,5,7,9,10}
A(5) = {1,2,4,6,7,9,11}
A(6) = {1,2,3,6,7,8,9,10,11}
A(7) = {1,2,3,4,7,8,9}

Then check to determine if there exists another row *(h)* for which *A(i)* is a subset of A*(h)*.

If $A(i) \subseteq A(h),\ i \neq h$
where
$A(i) = \{ j : T_{i,j} = 0,\ 1 \leq j \leq (n+m) \}$
then row *i* must be eliminated from $\mathbf{T}^{(i)}$

Schilling et al.
JTB 203, 229

repeat steps for all internal metabolites

(5) With the formation of $\mathbf{T}^{(i)}$ complete steps 2 – 4 for all of the metabolites that do not have an unconstrained exchange flux operating on the metabolite, incrementing *i* by one up to μ. The final tableau will be $T^{(\mu)}$.

Note that the number of rows in $T^{(\mu)}$ will be equal to *k*, the number of extreme pathways.

Schilling et al.
JTB 203, 229

balance external fluxes

(6) Next we append $\mathbf{T}^{(E)}$ to the bottom of $\mathbf{T}^{(\mu)}$. (In the example here $\mu = 1$.)
This results in the following tableau:

$T^{(1/E)} =$

1										-1	1	0	0	0
	1	1								0	0	0	0	0
	1		1							0	-1	0	1	0
	1				1					0	-1	0	1	0
		1		1						0	1	0	-1	0
			1	1						0	0	0	0	0
				1	1					0	0	0	-1	1
						1				-1	0	0	0	0
							1			0	-1	0	0	0
								1		0	0	0	-1	0
									1	0	0	0	0	-1

Schilling et al.
JTB 203, 229

balance external fluxes

(7) Starting in the *n+1* column (or the first non-zero column on the right side), if $T_{i,(n+1)} \neq 0$ then add the corresponding non-zero row from $\mathbf{T}^{(E)}$ to row *i* so as to produce 0 in the *n+1*-th column.

This is done by simply multiplying the corresponding row in $\mathbf{T}^{(E)}$ by $T_{i,(n+1)}$ and adding this row to row *i* .

Repeat this procedure for each of the rows in the upper portion of the tableau so as to create zeros in the entire upper portion of the *(n+1)* column.

When finished, remove the row in $\mathbf{T}^{(E)}$ corresponding to the exchange flux for the metabolite just balanced.

Schilling et al.
JTB 203, 229

balance external fluxes

(8) Follow the same procedure as in step (7) for each of the columns on the right side of the tableau containing non-zero entries.

(In our example we need to perform step (7) for every column except the middle column of the right side which correponds to metabolite C.)

The final tableau $\mathbf{T}^{(final)}$ will contain the transpose of the matrix **P** containing the extreme pathways in place of the original identity matrix.

Schilling et al.
JTB 203, 229

pathway matrix

$\mathbf{T}^{(final)} =$

1						-1	1			0	0	0	0	0	0
	1	1								0	0	0	0	0	0
	1		1				-1	1		0	0	0	0	0	0
	1				1		-1		1	0	0	0	0	0	0
		1		1			1	-1		0	0	0	0	0	0
			1	1						0	0	0	0	0	0
				1	1			-1	1	0	0	0	0	0	0

$\mathbf{P}^{T} =$

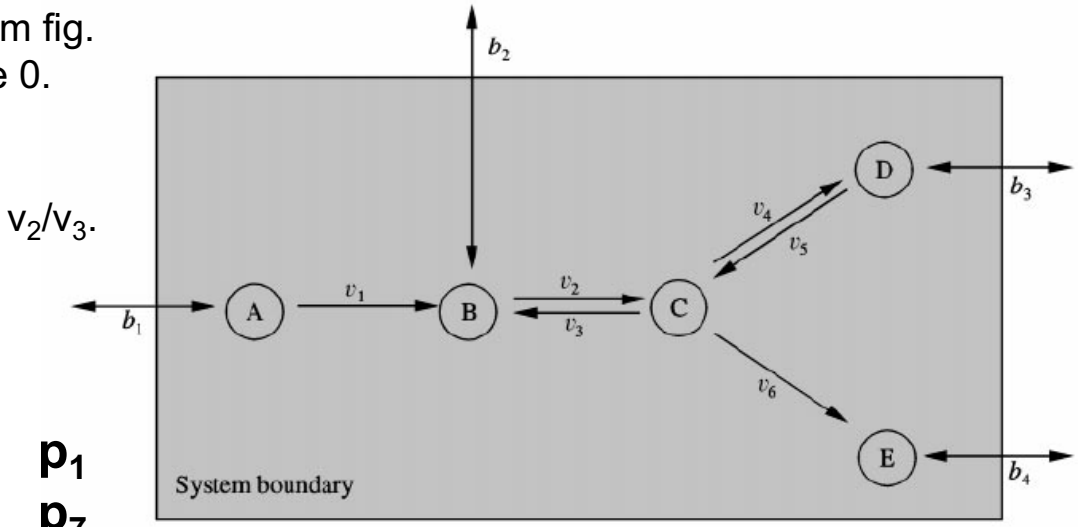
v_1	v_2	v_3	v_4	v_5	v_6	b_1	b_2	b_3	b_4	
1	0	0	0	0	0	-1	1	0	0	$\mathbf{p_1}$
0	1	1	0	0	0	0	0	0	0	$\mathbf{p_7}$
0	1	0	1	0	0	0	-1	1	0	$\mathbf{p_3}$
0	1	0	0	0	1	0	-1	0	1	$\mathbf{p_2}$
0	0	1	0	1	0	0	1	-1	0	$\mathbf{p_4}$
0	0	0	1	1	0	0	0	0	0	$\mathbf{p_6}$
0	0	0	0	1	1	0	0	-1	1	$\mathbf{p_5}$

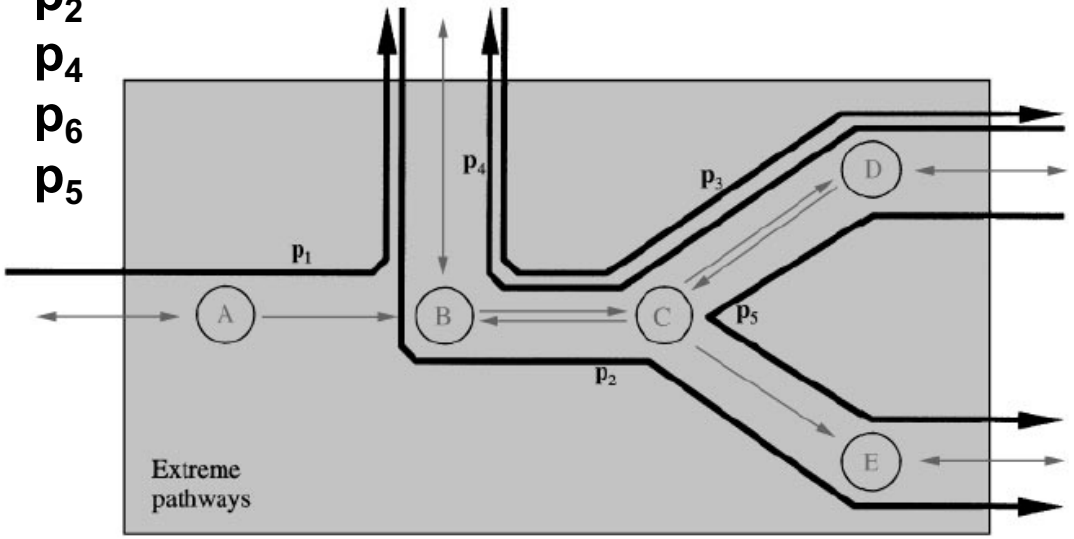
Schilling et al.
JTB 203, 229

Extreme Pathways for model system

2 pathways p_6 and p_7 are not shown in the bottom fig. because all exchange fluxes with the exterior are 0. Such pathways have no net overall effect on the functional capabilities of the network.
They belong to the cycling of reactions v_4/v_5 and v_2/v_3.

v_1	v_2	v_3	v_4	v_5	v_6	b_1	b_2	b_3	b_4	
1	0	0	0	0	0	-1	1	0	0	p_1
0	1	1	0	0	0	0	0	0	0	p_7
0	1	0	1	0	0	0	-1	1	0	p_3
0	1	0	0	0	1	0	-1	0	1	p_2
0	0	1	0	1	0	0	1	-1	0	p_4
0	0	0	1	1	0	0	0	0	0	p_6
0	0	0	0	1	1	0	0	-1	1	p_5



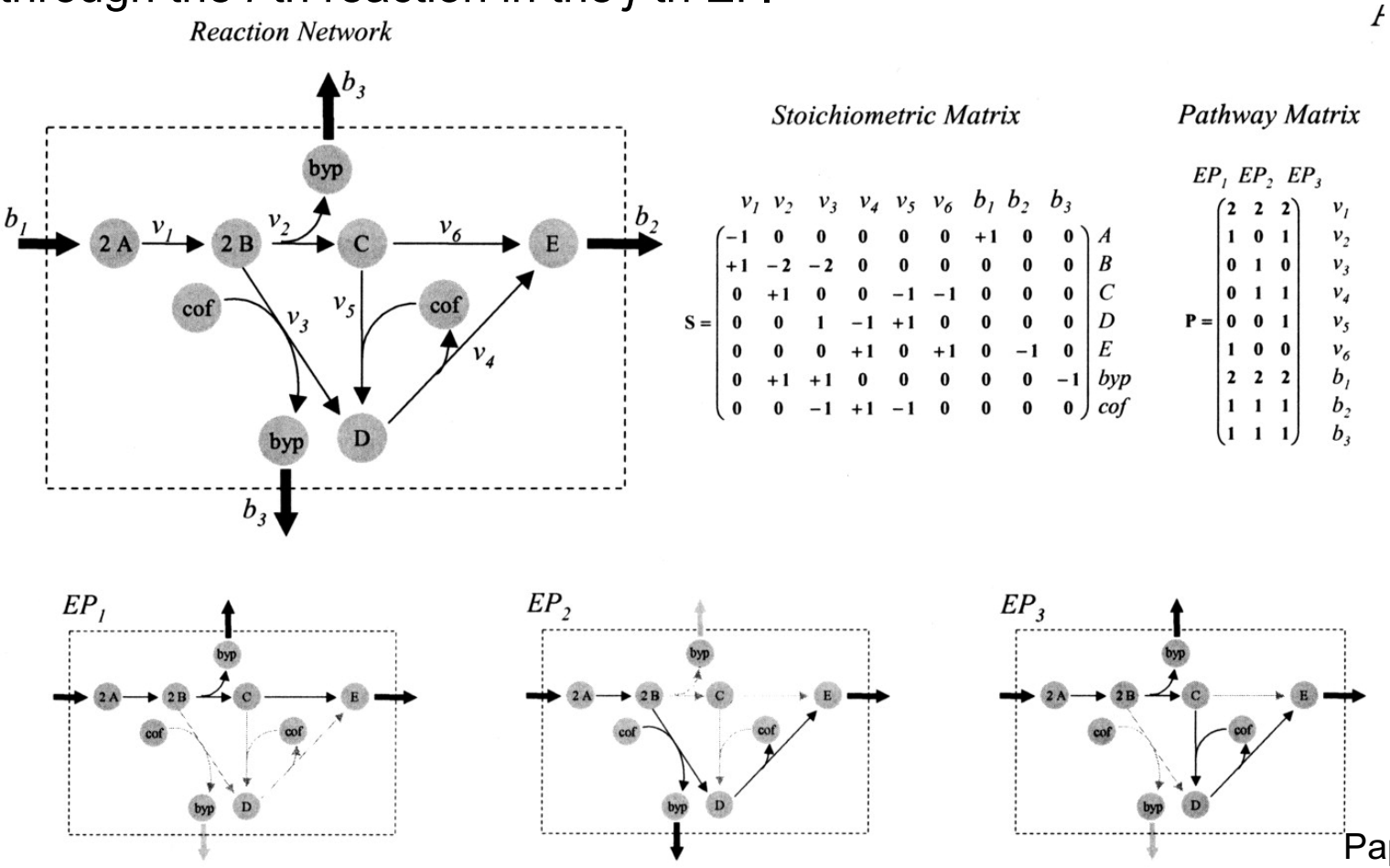


Schilling et al.
JTB 203, 229

How reactions appear in pathway matrix

In the matrix **P** of extreme pathways, each column is an EP and each row corresponds to a reaction in the network.

The numerical value of the *i,j*-th element corresponds to the relative flux level through the *i*-th reaction in the *j*-th EP.



Papin, Price, Palsson, Genome Res. 12, 1889 (2002)

Properties of pathway matrix

After normalizing **P** to a matrix with entries 0 or 1,
the symmetric **Pathway Length Matrix $\mathbf{P}_{LM}$** can be calculated:

$$\mathbf{P}_{LM} = \mathbf{P}^T \cdot \mathbf{P}$$

where the values along the diagonal correspond to the length of the EPs.

Pathway Length

$$\mathbf{P} = \begin{pmatrix} 2 & 2 & 2 \\ 1 & 0 & 1 \\ 0 & 1 & 0 \\ 0 & 1 & 1 \\ 0 & 0 & 1 \\ 1 & 0 & 0 \\ 2 & 2 & 2 \\ 1 & 1 & 1 \\ 1 & 1 & 1 \end{pmatrix} \rightarrow \tilde{\mathbf{P}} = \begin{pmatrix} 1 & 1 & 1 \\ 1 & 0 & 1 \\ 0 & 1 & 0 \\ 0 & 1 & 1 \\ 0 & 0 & 1 \\ 1 & 0 & 0 \\ 1 & 1 & 1 \\ 1 & 1 & 1 \\ 1 & 1 & 1 \end{pmatrix} \rightarrow \tilde{\mathbf{P}}^T \bullet \tilde{\mathbf{P}} = \begin{array}{c} \begin{matrix} EP_1 & EP_2 & EP_3 \end{matrix} \\ \begin{pmatrix} 6 & 4 & \textcircled{5} \\ & 6 & 5 \\ & & 7 \end{pmatrix} \end{array} \begin{matrix} EP_1 \\ EP_2 \\ EP_3 \end{matrix}$$

Comments:

1) The lengths of EP_1, EP_2,and EP_3 are 6, 6, and 7, respectively, the highlighted diagonal elements of the final matrix.

2) EP_2 and EP_3 have a shared length of 5 (indicated by the circle). As seen in the schematics above, they share reactions v_1, v_4, b_1, b_2, and b_3.

The off-diagonal terms of $\mathbf{P}_{LM}$ are the number of reactions that a pair of extreme pathways have in common.

Papin, Price, Palsson, Genome Res. 12, 1889 (2002)

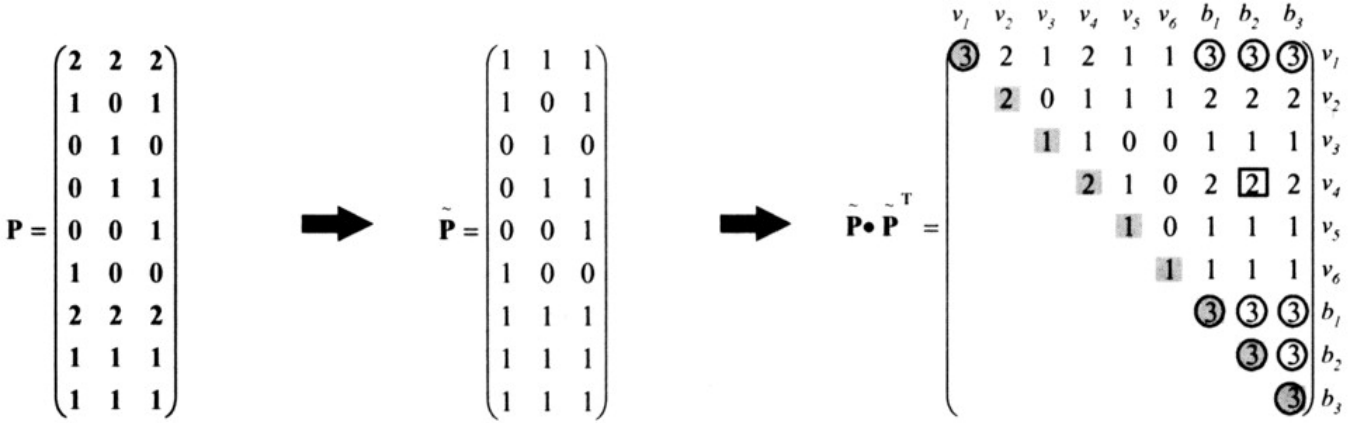
Properties of pathway matrix

One can also compute a **reaction participation matrix $\mathbf{P}_{PM}$** from **P**:

$$\mathbf{P}_{PM} = \mathbf{P} \cdot \mathbf{P}^T$$

where the diagonal correspond to the number of pathways in which the given reaction participates.

Reaction Participation



C

Comments:

1) The number of extreme pathways in which each reaction participates is indicated in the diagonal elements, as highlighted in the final matrix. These can then be expressed as a percentage of the total number of extreme pathways. For example, reaction v_1 has a participation value of 3. Since there are 3 extreme pathways, this can be expressed as 100% reaction participation.

2) The off diagonal terms can indicate correlated groups of reactions. Reactions v_1, b_1, b_2, and b_3 participate in 3 pathways. They also have a shared participation of 3, meaning they act as a correlated group (indicated by circles).

Papin, Price, Palsson, Genome Res. 12, 1889 (2002)