1 Simple linear chain reactions
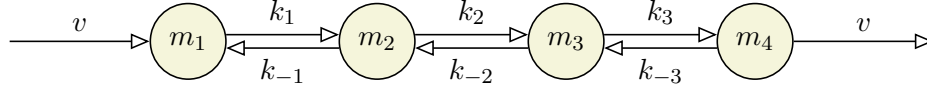

For every two successive metabolites:

$$k_i m_i - k_{-i} m_{i+1} = v \quad \Rightarrow \quad m_{i+1} = \frac{k_i}{k_{-i}} m_i - \frac{v}{k_{-i}} \geq 0$$

$$\Rightarrow m_i \geq \frac{v}{k_i} \qquad \forall i$$

Solving all concentration equations:

$$
\begin{aligned}
m_{i+1} &= \left(\frac{k_0}{k_{-0}} \cdot \frac{k_1}{k_{-1}} \cdot \cdots \cdot \frac{k_i}{k_{-i}} \right) m_0 \\
&\quad - \left(\frac{k_i}{k_{-i}} \cdots \frac{k_1}{k_{-1}} \frac{1}{k_{-0}} + \cdots + \frac{k_i}{k_{-i}} \frac{1}{k_{-(i-1)}} + \frac{1}{k_{-i}} \right) v \\
&= A_i m_0 - B_i v \geq 0
\end{aligned}
\tag{1}
$$

$$\Rightarrow v \leq m_0 . \min_i \left\{ \frac{A_i}{B_i} \right\} \tag{2}$$

Note: the only degree of freedom (else than flux v) is m_0. If it is set and the above constraint for v is added, all concentrations m_i's would be set in favor of conciliate the model (*i.e.*, kinetic equations force model to nothing more than the above upper bound for v).

Note: if two concentrations in the chain (or one more than m_0) were defined, then the equations wouldn't remain trivial and v and other concentrations would be definite.

2 Orphan kinetic equation
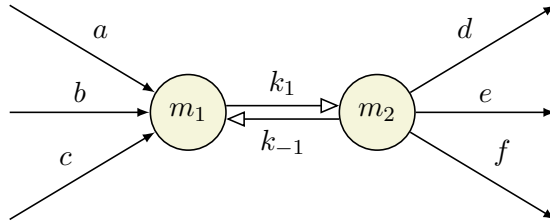
In a case like above, in which kinetic equations for a to f (all for m_1 and m_2 except the one between them) are missed, containing kinetic equation for the reaction between m_1 and m_2 has no effect:

$$k_1 m_1 - k_{-1} m_2 = v$$

$$\Rightarrow m_2 = \frac{k_1}{k_{-1}} m_1 - \frac{v}{k_{-1}}$$

No matter what would be the FBA result (v), as m_2 would be set by the equation above, according to some arbitrary value for m_1 just satisfying $m_1 \geq \frac{v}{k_1}$ (or starting with arbitrary $m_2 \geq 0$ and setting $m_1 = \frac{k_{-1}}{k_1} m_2 + \frac{v}{k_1}$). Therefore not any orphan kinetic equation helps at all.

Note: Aggregating two above conclusions, kinetic equations for orphan chains wouldn't help too (as all m_i's would be determined by equation 1 in which the first concentration, m_0, would get some arbitrary value but big enough to satisfy 2).
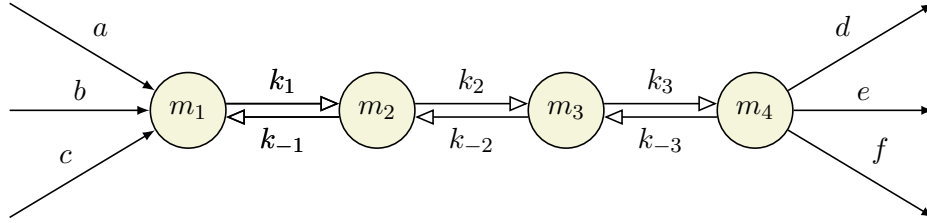

3 Tree Network

We should be careful about the structure "tree", as a true whole tree never really appears in metabolic network. The reason behind, is that if any metabolite is a leaf of a tree, it would be blocked and consequently plays no rule in the metabolic network. In the case of exchange reactions, in real there are two hidden "blank" metabolite known as source and sink (in the familiar reaction forms of $[Blank] \rightarrow A$ or $B \rightarrow [Blank]$):
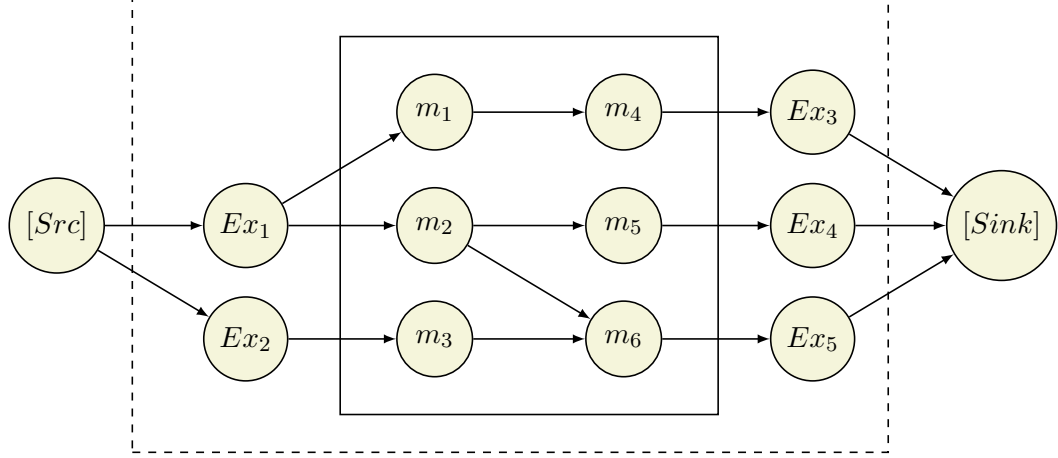
As shown in the picture above, gathering out-exchange reactions (Ex_4, Ex_5 and Ex_6) to $[Sink]$, forces the network to have loops and therefore not to be a tree, which could be mistakenly considered as a tree if only the bounded system would be taken into account.

The more accurate case to consider, is the "orphan tree" within the metabolic network, which also contains the cases in which all kinetic equations are included in a metabolic network structured in tree when excluding "Blank" reactions (like the dashed-bounded network in the picture above). Before running into it, two building blocks of trees are discussed first.

3.1 Multi-Input Multi-Output Structure
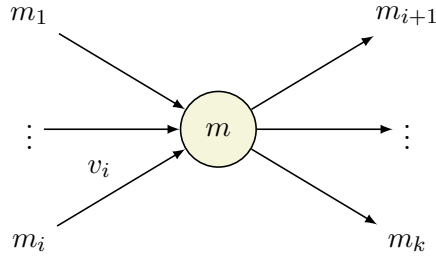
This is the case when a metabolite is not related to the rest of the network by a simple chain (*i.e.*, $k > 2$). There will be following k equations for this metabolite (note that the directions of the arrows in the above picture are not meaningful in two-sided kinetic equations):

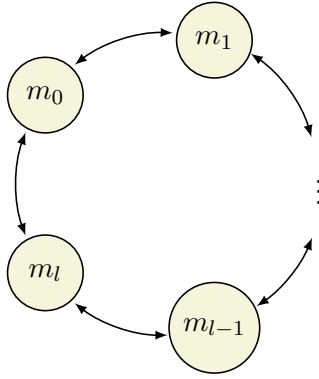$$k_i m_i - k_{-i} m = v_i \qquad \forall i \in \{1, \cdots, k\}$$

3

In which the standard direction of all reactions is considered to be in favor of producing m.

3.2 Orphan Tree

T.B.C.

4 Cycle

In the case of cycle, the kinetic + stoichiometric equations fully determines the fluxes and the concentrations!

Having stoichiometry equations, state that a single flux v is mounted on the circle. For $l + 1$ concentrations, $l + 1$ kinetic equations exist. As m_0 to m_l are like a chain, by 1 we have $m_l = A_{l-1}m_0 - B_{l-1}v$. Also the reactions between m_0 and m_l states that $k_l m_l - k_{-l}m_0 = v$. Therefore we have:

$$k_l A_{l-1} m_0 - k_l B_{l-1} v = k_{-l} m_0 + v$$

$$\Rightarrow m_0 = \frac{1 + k_l B_{l-1}}{k_l A_{l-1} - k_{-l}} v = \frac{B_l}{A_l - 1} v \tag{3}$$

$$\text{or } v = \frac{A_l - 1}{B_l} m_0 \tag{4}$$

And then all concentrations would be determined with respect to v. If any concentration like m_0 is definite, then v would take the value defined by 4 and all variables would be founded.

Note: as our equations are linear, superposition is held. It means if v_1 and v_2 are two flux vectors satisfying stoichiometric and kinetic equations corresponding to concentration vectors m_1 and m_2, also $\lambda_1 v_1 + \lambda_2 v_2$ (if is non-negative element-wise) and $\lambda_1 m_1 + \lambda_2 m_2$ satisfy those equations too.

# 5 Simplified Formulation for a Network of Single-metabolite Reactions

Consider our metabolic network to be a graph and not a hyper-graph (*i.e.*, all reactions have a single substrate a single product), containing n reactions with fluxes v and $|m|$ metabolites with concentrations m. Steady-state condition could be modeled by knowing the stoichiometry matrix $S_{m \times n}$ as follows:

$$Sv = \begin{bmatrix} s_{11} & s_{12} & \cdots & s_{1n} \\ s_{21} & s_{22} & \cdots & s_{2n} \\ \vdots & \vdots & \ddots & \vdots \\ s_{m1} & s_{m2} & \cdots & s_{mn} \end{bmatrix} \begin{bmatrix} v_1 \\ v_2 \\ \vdots \\ v_n \end{bmatrix} = \vec{0}_m \tag{5}$$

Besides, kinetic equations could be modeled by a $n \times |m|$ kinetic matrix K as follows:

$$Km = \begin{bmatrix} k_{11} & k_{12} & \cdots & k_{1m} \\ k_{21} & k_{22} & \cdots & k_{2m} \\ \vdots & \vdots & \ddots & \vdots \\ k_{n1} & k_{n2} & \cdots & k_{nm} \end{bmatrix} \begin{bmatrix} m_1 \\ m_2 \\ \vdots \\ m_m \end{bmatrix} = \begin{bmatrix} v_1 \\ v_2 \\ \vdots \\ v_n \end{bmatrix} = v \tag{6}$$

In which for the j'th row of the K we have $v_j = k'_j m = k_1^j m_{j1} - k_{-1}^j m_{j2}$, denoting the kinetic equation for the flux v_j as depicted in the picture bellow:
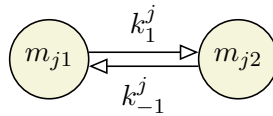
(Note that if we are containing external reactions in $Sv = 0$, we should handle the $[Blank]$ metabolite separately.)

Combining these two equations, we would get a single and almost fully determined system of linear equations:

$$(SK)_{|m| \times |m|} m = \begin{bmatrix} s_{11} & s_{12} & \cdots & s_{1n} \\ s_{21} & s_{22} & \cdots & s_{2n} \\ \vdots & \vdots & \ddots & \vdots \\ s_{m1} & s_{m2} & \cdots & s_{mn} \end{bmatrix} \begin{bmatrix} k_{11} & k_{12} & \cdots & k_{1m} \\ k_{21} & k_{22} & \cdots & k_{2m} \\ \vdots & \vdots & \ddots & \vdots \\ k_{n1} & k_{n2} & \cdots & k_{nm} \end{bmatrix} \begin{bmatrix} m_1 \\ m_2 \\ \vdots \\ m_m \end{bmatrix} = \vec{0}_m \tag{7}$$

And to have a fully determined system, it would be enough to assign a value to exchange reactions, or equivalently assign a concentration to external

metabolites (and v_{EX} should be replaced with $\vec{0}_m$ in 7).
Each column of SK would be like bellow:

$$(SK)_{i,l} = \sum_{j=1}^{n} s_{i,j} k_{j,l}$$

$$= \begin{cases} -\sum_j s_{i,j} k_j^i & i = l \\ s_{i,l \to i} k_{l \to i} & i \neq l \end{cases} \tag{8}$$