3. Exercise list. Wednesday Week 1

Exercise 3.1. Consider the binomials $b_1 = 32\,x_1x_3^3 - x_2^3x_4, b_2 = 1280\,x_1^3x_3^6 - x_2^8x_5$ and let

$$V = \{x = (x_1, \ldots, x_5) \in \mathbb{R}^5_{>0} : b_1(x) = b_2(x) = 0\}.$$

- Prove that $(1, 2, 1, 4, 5) \in V$.
- Find a monomial parametrization $\varphi\colon \mathbb{R}^3_{>0} \to V$ with exponents given by the columns of the matrix $\begin{pmatrix} 1 & 1 & 1 & 1 & 1 \\ 0 & 1 & 1 & 0 & -2 \\ 0 & 0 & 1 & 3 & 6 \end{pmatrix}$.
- Is it possible to find a monomial parametrization of V with exponents in the columns of the matrix $\begin{pmatrix} 1 & 2 & 3 & 4 & 5 \\ 0 & 1 & 2 & 3 & 4 \\ 0 & 0 & 1 & 3 & 6 \end{pmatrix}$? And with exponents in the columns of the matrix $\begin{pmatrix} 1 & 1 & 1 & 1 & 1 \\ 0 & 1 & 2 & 3 & 4 \\ 0 & 1 & 4 & 9 & 16 \end{pmatrix}$? In both cases, if it is possible, write down the parametrization.

Exercise 3.2. Given $A \in \mathbb{R}^{d\times n}$ and $c \in \mathbb{R}^n_{>0}$, denote by $a_1, \ldots, a_n$ the column vectors of A. Let $t = (t_1, \ldots, t_d) \in \mathbb{R}^d$ and

$$V = \{x \in \mathbb{R}^n_{>0} : x = (c_1 t^{a_1}, \ldots, c_n\, t^{a_n}) \text{ for some } t \in \mathbb{R}^d_{>0}\}.$$

Prove that for any vector $v \in \ker(A)$ and any $x \in V$, it holds that $x^v = c^v$.

Writing $v = v^+ - v^-$, with $v_i^+ = \max(0, v_i)$ and $v_i^- = \max(0, -v_i)$, then for any $x \in \mathbb{R}^n_{>0}$, it holds that $x^v = c^v$ if and only if x is a zero of the binomial $c^{v^-}x^{v^+} - c^{v^+}x^{v^-}$.

Exercise 3.3. Consider the following network obtained from the Shinar-Feinberg network in Exercise 2.11 by elimination of the intermediate species. This network has only 6 species.

$$\mathrm{XD} \underset{\kappa_2}{\overset{\kappa_1}{\rightleftharpoons}} \mathrm{X} \underset{\kappa_4}{\overset{\kappa_3}{\rightleftharpoons}} \mathrm{XT} \xrightarrow{\kappa_5} \mathrm{X_p} \qquad \mathrm{XT} + \mathrm{Y_p} \xrightarrow{\tau_2} \mathrm{XT} + \mathrm{Y}$$

$$\mathrm{X_p} + \mathrm{Y} \xrightarrow{\tau_1} \mathrm{X} + \mathrm{Y_p} \qquad \mathrm{XD} + \mathrm{Y_p} \xrightarrow{\tau_3} \mathrm{XD} + \mathrm{Y}$$

Recall that we denote by $x_1, x_2, x_3, x_4, x_5, x_7$ the concentrations of the species as follows:

$$x_1 = [\mathrm{XD}] \qquad x_2 = [\mathrm{X}] \qquad x_3 = [\mathrm{XT}] \qquad x_4 = [\mathrm{X}_p] \qquad x_5 = [\mathrm{Y}] \qquad x_7 = [\mathrm{Y}_p].$$

Denote the associated mass-action system by

$$\dot{x_1} = g_1, \quad \dot{x_2} = g_2, \quad \dot{x_3} = g_3, \quad \dot{x_4} = g_4, \quad \dot{x_5} = g_5, \quad \dot{x_7} = g_7.$$

In particular, $g_1 = -\kappa_1\, x_1 + \kappa_2\, x_2$, $g_3 = \kappa_3\, x_2 - (\kappa_4 + \kappa_5)\, x_3$, $g_4 = -\tau_1\, x_4x_5 + \kappa_5\, x_3$, and $g_5 = -\tau_1\, x_4x_5 + \tau_2\, x_3x_7 + \tau_3\, x_1x_7$.

- Show that g_2 and g_7 are $\mathbb{R}$-linear combinations of g_1, g_3, g_4, g_5 and conclude that the steady state ideal I_g satisfies $I_g = \langle g_1, g_3, g_4, g_5\rangle$.

- Prove that the ideal I_g is binomial when considered in the ring

$$\mathbb{Q}(\kappa_1, \kappa_2, \kappa_3, \kappa_4, \kappa_5, \tau_1, \tau_2, \tau_3)[x_1, x_2, x_3, x_4, x_5, x_7]$$

  but that it is *not* binomial in the ring

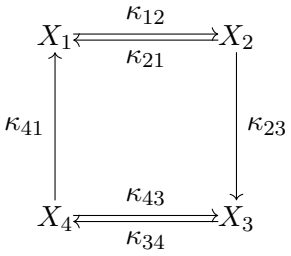
$$\mathbb{Q}[\kappa_1, \kappa_2, \kappa_3, \kappa_4, \kappa_5, \tau_1, \tau_2, \tau_3, x_1, x_2, x_3, x_4, x_5, x_7],$$

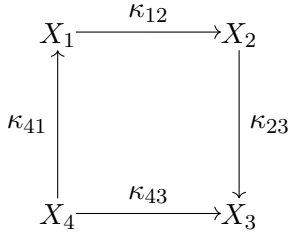
  where all parameters are considered as variables.

Remark: This last item says that to get binomial generators of I_g, the algorithm to produce a reduced Gröbner basis needs to divide by certain polynomials in the parameters. We didn't check if these denominators are polynomials which are non-vanishing when all parameters are positive. What would this fact imply if it were true?

Exercise 3.4 (Strongly connected components)**.** Recall that a digraph is *strongly connected* if every pair of nodes are part of a directed cycle; in other words, there is a directed path between the two nodes in each direction. The *strongly connected components* of a digraph are the maximal strongly connected subgraphs. Finally, the *terminal* strongly connected components are those for which there is no edge from a node in the component to another component.

Consider the following labeled two digraphs:





For each of them:

(i) Identify the strongly connected components and among those, the ones that are terminal.

(ii) Give the Laplacian matrix, after ordering the sets of nodes such that nodes in the same strongly connected component are consecutive, and the nodes in the non-terminal components are ordered before those in the terminal components. Think about the shape of the matrix.

(iii) Compute the kernel of A_κ with and without applying the Matrix-Tree Theorem.

Exercise 3.5 (Enzyme kinetics)**.** Consider the following reaction network that represents a mechanism with two substrates and two products. The reactions are as follows

$$E + A \underset{\kappa_2}{\overset{\kappa_1}{\rightleftharpoons}} EA \qquad EA + B \underset{\kappa_4}{\overset{\kappa_3}{\rightleftharpoons}} EAB \qquad EAB \underset{\kappa_6}{\overset{\kappa_5}{\rightleftharpoons}} EPQ$$

$$EPQ \underset{\kappa_8}{\overset{\kappa_7}{\rightleftharpoons}} EQ + P \qquad EQ \underset{\kappa_{10}}{\overset{\kappa_9}{\rightleftharpoons}} E + Q$$

where E is an enzyme, A, B are substrates, P, Q products, and the rest of the species are intermediate protein complexes.

Show that $U = \{E, EA, EAB, EPQ, EQ\}$ is a non-interacting set. Eliminate the concentrations of the species in U from the steady state equations as functions of the concentrations of A, B, P, Q, after using (and checking) that their sum is conserved. Use

the Matrix-Tree Theorem to this end by first constructing a suitable labeled digraph with nodes the species in U.

Remark. The elimination of enzymes and intermediates performed here via the Matrix-Tree Theorem is the old King–Altman method, developed by King and Altman for calculating the rate function of an enzyme (Cornish-Bowden, 1995, Section 5.3). Linear elimination gives a formalization of this method widely used in enzyme kinetics.

Exercise 3.6 (Phosphorylation cycles)**.** In this exercise we consider a well-known and studied family of phosphorylation networks comprising a substrate S that can be phosphorylated in n-sites in an ordered manner. The substrate admits $n+1$ phosphoforms, where S_0 denotes the unphosphorylated form and S_i, $i = 1, \dots, n$, denotes the form where the first i sites are phosphorylated. Phosphorylation is catalyzed by a kinase E and dephosphorylation by a phosphatase F, and these processes follow a Michaelis-Menten mechanism.

The network is as follows:

$$\begin{aligned} E + S_0 \underset{\kappa_2}{\overset{\kappa_1}{\rightleftharpoons}} Y_1 \xrightarrow{\kappa_3} E + S_1 \cdots \longrightarrow E + S_i \underset{\kappa_{6i+2}}{\overset{\kappa_{6i+1}}{\rightleftharpoons}} Y_{i+1} \xrightarrow{\kappa_{6i+3}} E + S_{i+1} \cdots \\ \cdots \longrightarrow E + S_{n-1} \underset{\kappa_{6n-4}}{\overset{\kappa_{6n-5}}{\rightleftharpoons}} Y_n \xrightarrow{\kappa_{6n-3}} E + S_n \\ F + S_n \underset{\kappa_{6n-1}}{\overset{\kappa_{6n-2}}{\rightleftharpoons}} Z_n \xrightarrow{\kappa_{6n}} F + S_{n-1} \cdots \longrightarrow F + S_{i+1} \underset{\kappa_{6i+5}}{\overset{\kappa_{6i+4}}{\rightleftharpoons}} Z_{i+1} \xrightarrow{\kappa_{6i+6}} F + S_i \cdots \\ \cdots \longrightarrow F + S_1 \underset{\kappa_5}{\overset{\kappa_4}{\rightleftharpoons}} Z_1 \xrightarrow{\kappa_6} F + S_0. \end{aligned}$$

It has $6n$ reactions and $3n + 3$ species. In this exercise you will show basic properties of these networks, and in particular show that the positive steady state variety admits a rational parametrization.

Part I. To start with, we study the network for $n = 1$:

$$\begin{aligned} E + S_0 \underset{\kappa_2}{\overset{\kappa_1}{\rightleftharpoons}} Y_1 \xrightarrow{\kappa_3} E + S_1 \\ F + S_1 \underset{\kappa_5}{\overset{\kappa_4}{\rightleftharpoons}} Z_1 \xrightarrow{\kappa_6} F + S_0. \end{aligned}$$

To ease the notation, we let $X_1 = E, X_2 = F, X_3 = S_0, X_4 = S_1$.

(i) Write the mass-action system associated with the network and find the dimension of the stoichiometric subspace.

(ii) Show that $x_1 + y_1$, $x_2 + z_1$ and $x_3 + x_4 + y_1 + z_1$ are conservation laws, but that $x_4 + y_1 + z_1$ is not a conservation law.

(iii) Show that $U = \{X_4, Y_1, Z_1\}$ is a non-interacting set. Give the labeled digraph G_U such that the system $\dot{x}_4 = 0, \dot{y}_1 = 0, \dot{z}_1 = 0$ corresponds to the first 3 rows of a system of the form

$$A_\kappa \begin{pmatrix} x_4 \\ y_1 \\ z_1 \\ 1 \end{pmatrix} = \begin{pmatrix} 0 \\ 0 \\ 0 \\ 0 \end{pmatrix},$$

for A_κ the Laplacian matrix of G_U. Use the Matrix-Tree Theorem to solve this linear system.

(iv) Conclude that the positive steady state variety admits a rational parametrization in x_1, x_2, x_3, and that the set of positive steady states in a stoichiometric compatibility class can be described by means of three polynomial equations in x_1, x_2, x_3.

Part II. We now proceed to study the network for general n. (You might want to study the network for $n = 2$ first, to gain more insight). We let $X_1 = E, X_2 = F, X_i = S_{i-3}$ for $i = 3, \ldots, n+3$.

(i) Write the mass-action system associated with the network and show that the dimension of the stoichiometric subspace is $3n$.

(ii) Show that $x_1 + y_1 + \cdots + y_n$, $x_2 + z_1 + \cdots + z_n$ and $x_3 + \cdots + x_{n+3} + y_1 + \cdots + y_n + z_1 + \cdots + z_n$ are conservation laws. What do these quantities correspond to biochemically speaking? Is the network conservative?

(iii) Show that $U = \{X_4, \ldots, X_{n+3}, Y_1, \ldots, Y_n, Z_1, \ldots, Z_n\}$ is a non-interacting set. Give the labeled digraph G_U such that the system $\dot{x}_i = 0$ for $i = 4, \ldots, n+3$, $\dot{y}_i = 0, \dot{z}_i = 0$, for $i = 1, \ldots, n$ can be solved from a system of the form

$$A_\kappa \, \xi = 0,$$

for A_κ the Laplacian matrix of G_U and $\xi = (x_4, \ldots, x_{n+3}, y_1, \ldots, y_n, z_1, \ldots, z_n, 1)$. Use the Matrix-Tree Theorem to solve this linear system.

(iv) Conclude that the positive steady state variety admits a rational parametrization in x_1, x_2, x_3, and that the set of positive steady states in a stoichiometric compatibility class can be described by means of three polynomial equations in x_1, x_2, x_3.

Remark. You should have obtained that the parametrization is monomial!

Exercise 3.7 (PTM networks)**.** In [Thomson M, Gunawardena J (2009) The rational parameterization theorem for multisite post-translational modification systems. J Theor Biol 261:626–636], the authors introduced a class of networks called *Post-Translational Modification (PTM) Networks*. Post-translational modifications are common mechanisms in cell signaling and include, for instance, phosphorylation and methylation. The networks in this class have the peculiarity to always admit a description of the steady state variety by means of linear elimination. The networks studied in Exercise 3.6 are examples of PTM networks. In this exercise you will learn about these networks.

The set of species of a PTM network can be written as the disjoint union of three sets:

$$\mathrm{Enz} = \{E_1, \ldots, E_L\}, \quad \mathrm{Sub} = \{S_1, \ldots, S_N\}, \quad \mathrm{Int} = \{Y_1, \ldots, Y_P\},$$

called respectively sets of enzymes, substrates and intermediates. The reactions that are allowed are of the following form:

$$E_i + S_j \to Y_\ell, \qquad Y_\ell \to E_i + S_j, \qquad Y_i \to Y_j,$$

for some indices i, j, ℓ. You might recognise these reactions as the building blocks of the Michaelis-Menten mechanism, but we now also allow for reactions between intermediate species. We say that two species are *linked* if they are part of complexes in the same

connected component of the reaction network. It is additionally assumed that the set of intermediates is partitioned

$$\mathrm{Int} = \mathrm{Int}_1 \sqcup \ldots \sqcup \mathrm{Int}_L,$$

such that two intermediates Y_i, Y_j belong to the same subset if they are linked, and such that all intermediates in the subset Int_i are linked to exactly the enzyme E_i, $i = 1, \ldots, L$. We assume that all species take part of at least one reaction.

Let us consider the first set Int_1, and assume intermediates are ordered such that $\mathrm{Int}_1 = \{Y_1, \ldots, Y_\ell\}$.

(i) Show that $e_1 + \sum_{j=1}^{\ell} y_j$ is conserved but $\sum_{j=1}^{\ell} y_j$ is not.

(ii) Under what conditions do the linear equations

$$e_1 + \sum_{j=1}^{\ell} y_j = T_1, \qquad \dot{y}_j = 0, \quad j = 1, \ldots, \ell,$$

have a unique positive solution in $e_1, y_1, \ldots, y_\ell$? (Think of linear elimination and the Matrix-Tree theorem).

(iii) Think about whether one could eliminate substrates and intermediates instead of enzymes and intermediates.