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Mathematical Determination of Competitive Feedback Inhibition Rates in Branched Metabolic Pathways

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Abstract

In this paper, we consider the problem of mathematically determining the feedback inhibition rates in multi-branched metabolic pathways. To solve the problem, we model the system with a series of nonlinear ordinary differential equations by using the law of mass action without the usual quasi-steady state assumptions. Through an equilibrium analysis, we develop formulas to calculate the feedback inhibition rates in terms of the concentrations of end-products and regulatory enzymes at equilibrium. We then prove that the linearized system of the nonlinear system at its equilibrium is exponentially stable by applying Routh's stability criterion, thus the equilibrium of the nonlinear system is locally exponentially stable. This local stability proves that the feedback inhibition rates determined by our formulas are effective in regulating the end-products. This feasibility of these feedback inhibition rates is further tested numerically using both randomly generated data and biological data.

Keywords: Feedback inhibition rate, metabolic pathway, regulation.

AMS Subject Classification: 92C45, 92C40, 93D20, 34D05

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1 Introduction

Most of the biochemical reactions of a cell are accelerated through enzymes in metabolic pathways, which are necessary for the biosynthesis of the major molecules needed in cells and organisms such as nucleotides, amino acids, sugars, and lipids. A metabolic pathway is made up of a series enzymes which take some molecular substrate and convert it into a modified molecule through a sequence of catalyzed reactions that are specifically regulated and controlled.

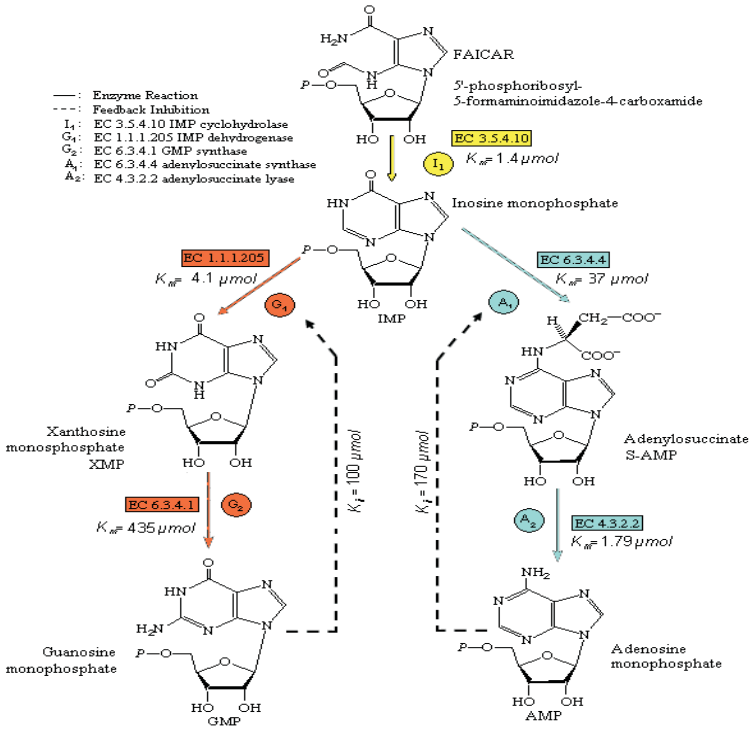

Figure 1: Biosynthetic pathway of Adenosine and Guanosine from Inosine.

If a cell or network is producing more of the end product than it needs, the end product or a byproduct may act as an inhibitor on one or more of the regulatory enzymes of the pathway. This inhibition can be caused through different process such as allosteric modification, where the affinity of the regulatory enzyme is altered, or competitive inhibition where the inhibitor or modulator inhibits active sites of the enzyme. Generally, the end product acts as an inhibitor of the first committed step in the pathway. This property enables the enzymes in metabolic pathways to have specific controls for different branches, thus being able to sustain homeostasis under dynamic controls. Note also that this process avoids unwanted intermediates in the cell. A well known example of such regulatory feedback inhibition occurs in the purine metabolism, specifically the biosynthesis of adenosine 5′-monophosphate (AMP) and guanosine 5′-monophosphate (GMP) [21, 24], as shown in Figure 1. In this metabolic pathway inosine monophosphate (IMP) is the initial metabolite and the regulatory enzymes A_1 and G_1 are the first branched steps that compete for IMP as seen in Figure 1.

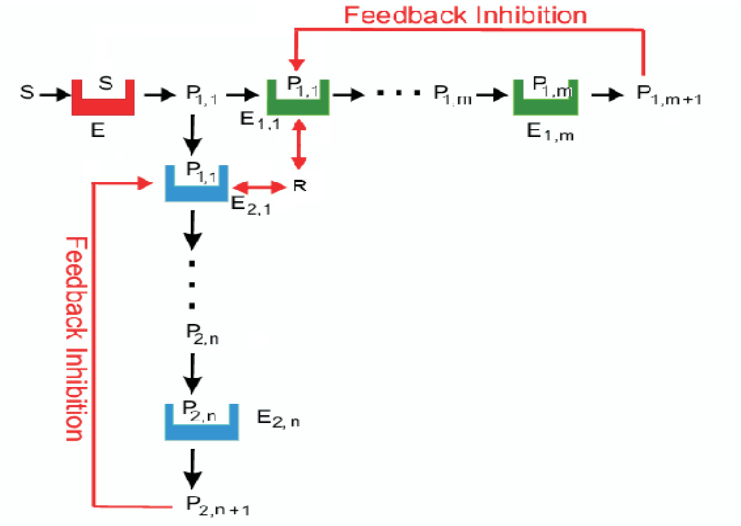

Figure 2: Feedback inhibition in a branched metabolic pathway.

To be able to mathematically analyze the feedback inhibition, we consider a generic abstract branched metabolic pathway as shown in Figure 2: an initial substrate S is catalyzed by an enzyme E to form an intermediate metabolite $P_{1,1}$, $P_{1,1}$ is catalyzed by the enzymes $E_{1,1}$ and $E_{2,1}$ to form two other intermediate metabolites $P_{1,2}$ and $P_{2,2}$, and so on. If there is an excess of the end-products $P_{1,m+1}$ and $P_{1,n+1}$ in the cell, $P_{1,m+1}$ and $P_{1,n+1}$ will inhibit the regulatory enzyme $E_{1,1}$ and $E_{2,1}$, respectively, preventing them from converting any $P_{1,1}$ to $P_{1,2}$ and $P_{2,2}$. In the real biological situations, these end-products also inhibit the enzyme E, but such inhibition is very weak and negligible. In this way, the cell keeps from synthesizing excessive amounts of the end-products, and keeps $P_{1,1}$ available for use in other pathways. This sort of inhibition, whereby a metabolic reaction is blocked by its product, is called feedback inhibition, and it is one of the most important mechanisms that regulate metabolism.

Here we have introduced a hypothetical intermediate R. We first mathematically analyzed the branched pathway without the introduction of R and found that the system has a zero eigenvalues and then it is unstable. However, in the real biological situations, the pathway system should be stable. Thus we guess that there might be a kind of communicator in the pathway, which communcates between two branches. Without this communicator, any excess product could not be reversed and used by other branches. The intermediate R could be also possible if the product of an enzyme-inhibitor complex were to suffer an appropriate structural change.

The aim of this paper is to develop a formula to calculate the feedback inhibition rates. We here consider the competitive inhibition. So the series of enzymatic reactions in a

branched metabolic pathway can be described by the following diagram

$$
\begin{array}{rcl}
S + E & \underset{k_2^0}{\overset{k_1^0}{\rightleftharpoons}} & C \xrightarrow{k_3^0} P_{1,1} + E \\
P_{1,1} + E_{1,1} & \underset{k_{12}^1}{\overset{k_{11}^1}{\rightleftharpoons}} & C_{1,1} \xrightarrow{k_{13}^1} P_{1,2} + E_{1,1} \\
P_{1,2} + E_{1,2} & \underset{k_{22}^1}{\overset{k_{21}^1}{\rightleftharpoons}} & C_{1,2} \xrightarrow{k_{23}^1} P_{1,3} + E_{1,2} \\
P_{1,3} + E_{1,3} & \underset{k_{32}^1}{\overset{k_{31}^1}{\rightleftharpoons}} & C_{1,3} \xrightarrow{k_{33}^1} P_{1,4} + E_{1,3} \\
 & \vdots & \\
P_{1,m} + E_{1,m} & \underset{k_{m2}^1}{\overset{k_{m1}^1}{\rightleftharpoons}} & C_{1,m} \xrightarrow{k_{m3}^1} P_{1,m+1} + E_{1,m} \\
P_{1,m+1} + E_{1,1} & \underset{f_1}{\overset{k_{m+1,1}^1}{\rightleftharpoons}} & W_1 \underset{k_{m+1,2}^1}{\overset{k_{m+1,3}^1}{\rightleftharpoons}} R + E_{1,1} \\
P_{1,1} + E_{2,1} & \underset{k_{12}^2}{\overset{k_{11}^2}{\rightleftharpoons}} & C_{2,1} \xrightarrow{k_{13}^2} P_{2,2} + E_{2,1} \\
P_{2,2} + E_{2,2} & \underset{k_{22}^2}{\overset{k_{21}^2}{\rightleftharpoons}} & C_{2,2} \xrightarrow{k_{23}^2} P_{2,3} + E_{2,2} \\
P_{2,3} + E_{2,3} & \underset{k_{32}^2}{\overset{k_{31}^2}{\rightleftharpoons}} & C_{2,3} \xrightarrow{k_{33}^2} P_{2,4} + E_{2,3} \\
 & \vdots & \\
P_{2,n} + E_{2,n} & \underset{k_{n2}^2}{\overset{k_{n1}}{\rightleftharpoons}} & C_{2,n} \xrightarrow{k_{n3}^2} P_{2,n+1} + E_{2,n} \\
P_{2,n+1} + E_{2,1} & \underset{f_2}{\overset{k_{n+1,1}^2}{\rightleftharpoons}} & W_2 \underset{k_{n+1,2}^2}{\overset{k_{n+1,3}^2}{\rightleftharpoons}} R + E_{2,1},
\end{array}
\tag{1.1}
$$

where S denotes an initial substrate, $E_{i,j}$ the enzymes, $P_{i,j}$ the intermediate metabolites or the final product, $C_{i,j}$ the complexes formed from $S, P_{i,j}$ and $E_{i,j}$, W_i the complex formed from the final product $P_{i,m+1}$ (or $P_{i,n+1}$) and $E_{i,1}$ (the letter W is used because the enzyme-ligand complex is a kind of waste that neutralizes the regulatory enzyme), R the intermediate metabolite produced from one branch that can be used by the other branch, and $k_{j,l}^i$ the reaction constants. The competitive feedback inhibition rates k_{ic}^1 (the subscript ic means competitive inhibition) and k_{ic}^2 are defined by

$$k_{ic}^1 = \frac{f_1}{k_{m+1,1}^1} \text{ and } k_{ic}^2 = \frac{f_2}{k_{n+1,1}^2}$$

.

To solve our problem, we model the metabolic pathway (1.1) with a system of nonlinear ordinary differential equations using the law of mass action [10, 22]. In our model, we do not

make the usual quasi-steady state assumptions employed in most enzyme catalyzed models. We then develop the following formula of computing competitive feedback inhibition rates

$$k_{ic}^1 = \frac{\bar{e}_{11}\bar{p}_1}{(E_{11}^0 - \bar{e}_{11})}, \tag{1.2}$$

$$k_{ic}^2 = \frac{\bar{e}_{21}\bar{p}_2}{(E_{11}^0 - \bar{e}_{21})}, \tag{1.3}$$

where

$$\begin{aligned}
\bar{e}_{11} &= \frac{k_{m+1,3}^1 E_{1,1}^0}{k_{m+1,3}^1 + k_{m+1,2}^1 \bar{r}}, \\
\bar{e}_{21} &= \frac{k_{n+1,3}^2 E_{2,1}^0}{k_{n+1,3}^2 + k_{n+1,2}^2 \bar{r}}, \\
\bar{p}_1 &= \frac{\bar{r} f_1 k_{m+1,2}^1}{k_{m+1,1}^1 k_{m+1,3}^1}, \\
\bar{p}_2 &= \frac{\bar{r} f_2 k_{n+1,2}^2}{k_{n+1,1}^2 k_{n+1,3}^2}.
\end{aligned}$$

and $\bar{r}$ is the positive real solution of

$$\bar{r} + \frac{k_{m+1,2}^1 E_{1,1}^0 \bar{r}}{k_{m+1,3}^1 + k_{m+1,2}^1 \bar{r}} + \frac{k_{n+1,2}^2 E_{2,1}^0 \bar{r}}{k_{n+1,3}^2 + k_{n+1,2}^2 \bar{r}} + \frac{\bar{r} f_1 k_{m+1,2}^1}{k_{m+1,1}^1 k_{m+1,3}^1} + \frac{\bar{r} f_2 k_{n+1,2}^2}{k_{n+1,1}^2 k_{n+1,3}^2} = S_0.$$

In the above equations, S^0, E_{ij}^0 denote the initial concentrations of the substrate S and the enzymes E_{ij} and the bar $^-$ denotes an equilibrium. Since the end-products and enzymes at equilibrium can be measured, this formula provide a practical method to calculate the inhibition rates.

The effects of product inhibition in unbranched metabolic pathways have been analyzed in the literature [3, 4, 5, 7, 10, 13, 14, 15, 18, 19, 22, 25, 26]. Depending on different assumptions like the quasi-steady state assumption, different mathematical models for such pathways were established, including the Michaelis-Menten system [8, 13], the Haldane equation [17], and nonlinear reaction-diffusion equations [25]. Sen and Schulz [15] showed that product inhibition may be considered an alternative mechanism to end-product inhibition by reducing the overall logarithmic gain of an unregulated pathway. Product inhibition can exert a stabilizing influence that competes with the destabilizing effect of end-product inhibition in controlling the dynamics behavior. Stantillán and Zeron [14] developed a mathematical model for the tryptophan operon and showed that product inhibition can increase the operon stability. Using the theory of cooperative and competitive systems, Sanchez [13] studied the dynamical behavior of the modified Michaelis-Menten system and derived conditions for convergence to equilibria of stable closed orbits. Instability caused by time lag was analyzed in [8]. Using the decomposition method, Sonnad and Goudar [17] presented an explicit solution to the Haldane equation as a recursive series. Effects of periodic input on the quasi-steady state assumptions were examined in [19]. Metabolic control coefficients and elasticities were calculated symbolically in [2]. Using directed graphs, the sign pattern of the control coefficients of the enzymes in abstract linear metabolic pathways was analyzed [7]. Sufficient

and necessary conditions for asymptotic stability of the steady state in general unbranched pathways with a single feedback loop were established in [3]. Strategies for representing metabolic pathways within biochemical systems theory were developed in [18, 23] and two most common strategies for generating an S-system (for synergistic and saturable systems) were clearly distinguished. However, to our knowledge, it seems that the mathematical determination of feedback inhibition rates like (1.2) and (1.3) has not yet been seen in the literature.

The paper is organized as follows. Employing the law of mass action, we first model the pathways by a system of differential equations in Section 2. Through an equilibrium analysis, we then derive the competitive feedback inhibition rates (1.2) and (1.3) in Section 3. To show that the feedback inhibition rates are effecitve, we need to prove that the equilibrium of the system is asymptotically stable. Since we could not construct a Lyapunov function to address the global stability of the system, we instead consider its local linear stability in Section 4, showing that the real parts of all eigenvalues of the linearized system are negative using Routh's stability criterion. Finally, we present numerical examples and applications in Section 5 to further verify the feasibility of mathematically determined inhibition rates.

2 Mathematical Models

In real biological situations, concentrations of molecules in a cell may vary in different locations, and so may not be homogeneous. However, for simplicity, we here assume that the concentrations are same everywhere. Therefore, by the law of mass action [10, 22], the dynamics of the metabolic pathway (1.1) can be modeled by the following system of nonlinear ordinary differential equations

$$\begin{aligned}
\frac{ds}{dt} &= -k_1^0 es + k_2^0 c, && (2.1)\\
\frac{de}{dt} &= -k_1^0 es + (k_2^0 + k_3^0)c, && (2.2)\\
\frac{dc}{dt} &= k_1^0 es - (k_2^0 + k_3^0)c, && (2.3)\\
\frac{de_{11}}{dt} &= -k_{11}^1 e_{11} p_{11} + (k_{12}^1 + k_{13}^1)c_{11} - k_{m+1,1}^1 p_{1,m+1} e_{11} \\
&\quad + (f_1 + k_{m+1,3}^1) w_1 - k_{m+1,2}^1 r e_{11}, && (2.4)\\
\frac{dp_{11}}{dt} &= -k_{11}^1 e_{11} p_{11} + k_3^0 c + k_{12}^1 c_{11} + k_{12}^2 c_{21} - k_{11}^2 e_{21} p_{11}, && (2.5)\\
\frac{dc_{1j}}{dt} &= k_{j1}^1 e_{1j} p_{1j} - (k_{j2}^1 + k_{j3}^1) c_{1j}, \quad j = 1, \cdots, m, && (2.6)\\
\frac{de_{1j}}{dt} &= -k_{j1}^1 e_{1j} p_{1j} + (k_{j2}^1 + k_{j3}^1) c_{1j}, \quad j = 2, \cdots, m, && (2.7)\\
\frac{dp_{1j}}{dt} &= -k_{j1}^1 e_{1j} p_{1j} + k_{j2}^1 c_{1j} + k_{j-1,3}^1 c_{1,j-1}, \quad j = 2, \cdots, m, && (2.8)\\
\frac{dp_{1,m+1}}{dt} &= -k_{m+1,1}^1 e_{11} p_{1,m+1} + k_{m3}^1 c_{1,m} + f_1 w_1, && (2.9)\\
\frac{dw_1}{dt} &= k_{m+1,1}^1 e_{11} p_{1,m+1} - (f_1 + k_{m+1,3}^1) w_1 + k_{m+1,2}^1 r\, e_{11}, && (2.10)
\end{aligned}$$

$$
\begin{aligned}
\frac{de_{21}}{dt} &= -k^2_{11}e_{21}p_{11} + (k^2_{12}+k^2_{13})c_{21} - k^2_{n+1,1}p_{2,n+1}e_{21} \\
&\quad +(f_2+k^2_{n+1,3})w_2 - k^2_{n+1,2}r\ e_{21}, && (2.11)\\
\frac{dc_{21}}{dt} &= k^2_{11}e_{21}p_{11} - (k^2_{12}+k^2_{13})c_{21}, && (2.12)\\
\frac{dc_{2j}}{dt} &= k^2_{j1}e_{2j}p_{2j} - (k^2_{j2}+k^2_{j3})c_{2j}, \quad j=2,\cdots,n, && (2.13)\\
\frac{de_{2j}}{dt} &= -k^2_{j1}e_{2j}p_{2j} + (k^2_{j2}+k^2_{j3})c_{2j}, \quad j=2,\cdots,n, && (2.14)\\
\frac{dp_{2j}}{dt} &= -k^2_{j1}e_{2j}p_{2j} + k^2_{j2}c_{2j} + k^2_{j-1,3}c_{2,j-1}, \quad j=2,\cdots,n, && (2.15)\\
\frac{dp_{2,n+1}}{dt} &= -k^2_{n+1,1}e_{21}p_{2,n+1} + k^2_{n3}c_{2,n} + f_2w_2, && (2.16)\\
\frac{dw_2}{dt} &= k^2_{n+1,1}e_{21}p_{2,n+1} - (f_2+k^2_{n+1,3})w_2 + k^2_{n+1,2}r\ e_{21}, && (2.17)\\
\frac{dr}{dt} &= k^1_{m+1,3}w_1 - k^1_{m+1,2}r\ e_{11} + k^2_{n+1,3}w_2 - k^2_{n+1,2}r\ e_{21}, && (2.18)\\
s(0) &= S^0,\ e(0)=E^0,\ e_{ij}(0)=E^0_{ij},\ c_{ij}(0)=p_{ij}(0)=w_i(0)=0,\ r(0)=0, && (2.19)
\end{aligned}
$$

where $c_{i,j}, e_{i,j}, p_{i,j}, r, s, w_i$ denote the concentrations of $C_{i,j}, E_{i,j}, P_{i,j}, R, S, W_i$, respectively, and $S^0, E^0_{i,j}$ are the initial concentrations of the substrate S and the enzyme $E_{i,j}$, respectively. Adding the respective rate equations, we can readily derive that

$$
\begin{aligned}
&\frac{d}{dt}(e_{1i}+c_{1i}) = 0, \quad i=2,\cdots,m,\\
&\frac{d}{dt}(e_{2i}+c_{2i}) = 0, \quad i=2,\cdots,n,\\
&\frac{d}{dt}(e+c) = 0,\\
&\frac{d}{dt}(e_{i1}+c_{i1}+w_i) = 0, \quad i=1,2.
\end{aligned}
$$

These conservative equations are just the reflection of the enzyme conservation. In the same respect, adding the equations (2.1), (2.3), (2.10), (2.9), (2.16), (2.17), and (2.18), the right hand side is equal to $-k^0_3c + k^1_{m3}c_{1m} + k^2_{n3}c_{2n}$, which can be canceled by the right hand side of $\dfrac{d}{dt}\left(\displaystyle\sum_{i=1}^{m}(c_{1i}+p_{1i}) + \sum_{i=2}^{n}(c_{2i}+p_{2i})\right)$. Thus, we have

$$
\frac{d}{dt}\left(s + r + c + w_1 + p_{1,m+1} + w_2 + p_{2,n+1} + c_{21} + \sum_{i=1}^{m}(c_{1i}+p_{1i}) + \sum_{i=2}^{n}(c_{2i}+p_{2i})\right) = 0.
$$

This conservative equation reflects the conservation of substrate. It then follows that

$$e_{1j} + c_{1j} = E^0_{1j}, \quad j = 2, \cdots, n, \tag{2.20}$$
$$e_{2j} + c_{2j} = E^0_{2j}, \quad j = 2, \cdots, m, \tag{2.21}$$
$$e_{i1} + c_{i1} + w_i = E^0_{i1}, \quad i = 1, 2, \tag{2.22}$$
$$e + c = E^0, \tag{2.23}$$
$$s + r + c + w_1 + p_{1,m+1} + w_2 + p_{2,n+1} + c_{21} + \sum_{i=1}^{m}(c_{1i} + p_{1i}) + \sum_{i=2}^{n}(c_{2i} + p_{2i}) = S^0, \tag{2.24}$$

where E^0_{ij}, E^0, S^0 are the initial concentrations. Due to these conservative equations, the system (2.1)-(2.19) can be simplified to

$$\frac{ds}{dt} = -k^0_1 es + k^0_2 c, \tag{2.25}$$
$$\frac{de_{i1}}{dt} = -k^i_{11} e_{i1} p_{11} + (k^i_{12} + k^i_{13}) c_{i1} - k^i_{j+1,1} p_{i,j+1} e_{i1} + (f_i + k^i_{j+1,3}) w_i - k^i_{j+1,2} r\, e_{i1}, \quad i = 1, 2 \quad j = n \text{ or } m \tag{2.26}$$
$$\frac{de_{ij}}{dt} = -k^i_{j1} e_{ij} p_{ij} + (k^i_{j2} + k^i_{j3}) c_{ij}, \quad i = 1, 2 \quad j = 2, \cdots, n \text{ or } m, \tag{2.27}$$
$$\frac{dp_{11}}{dt} = -k^1_{11} e_{11} p_{11} + k^0_3 c + k^1_{12} c_{11} + k^2_{12} c_{21} - k^2_{11} e_{21} p_{11} \tag{2.28}$$
$$\frac{dp_{i2}}{dt} = -k^i_{21} e_{i2} p_{i2} + k^i_{22} c_{i2} + k^i_{1,3} c_{i,1}, \quad i = 1 \text{ or } 2, \tag{2.29}$$
$$\frac{dp_{ij}}{dt} = -k^i_{j1} e_{ij} p_{ij} + k^i_{j2} c_{ij} + k^i_{j-1,3} c_{i,j-1}, \; j = 3, \cdots, n \text{ or } m, \tag{2.30}$$
$$\frac{dp_{i,j+1}}{dt} = -k^i_{j+1,1} e_{i1} p_{i,j+1} + k^i_{j3} c_{ij} + f_i w_i, \quad i = 1, 2 \quad j = n \text{ or } m \tag{2.31}$$
$$\frac{dw_i}{dt} = k^i_{j+1,1} e_{i1} p_{i,j+1} - (f_i + k^i_{j+1,3}) w_i + k^i_{j+1,2} r\, e_{i1}, \quad i = 1, 2 \quad j = n \text{ or } m, \tag{2.32}$$
$$\frac{dr}{dt} = k^1_{m+1,3} w_1 - k^1_{m+1,2} r\, e_{11} + k^2_{n+1,3} w_2 - k^2_{n+1,2} r\, e_{21}, \tag{2.33}$$
$$s(0) = S_0, \; e_{ij}(0) = E^0_{ij}, \; p_{ij}(0) = w_i(0) = 0, r(0) = 0, \tag{2.34}$$

where c_{ij}, c, and e are given by the conservative equations (2.20) through (2.24).

3 Feedback Inhibition Rates

To determine the feedback inhibition rates k^1_{ic}, k^2_{ic}, we look at the equilibrium of the system (2.25)-(2.34), which can be found by setting all the derivatives to zero. Hereafter the bar $^-$ denotes the equilibrium state. Keeping the zero derivatives in mind, we add the equations (2.31) - (2.33) and then obtain

$$k^i_{m3}(E^0_{im} - \bar{e}_{im}) + k^i_{n3}(E^0_{in} - \bar{e}_{in}) = 0, \quad i = 1, 2.$$

Since we are considering the system in a biological situation, the enzymes should satisfies that $e_{ij} \leq E^0_{ij}$ and then at equilibrium

$$\bar{e}_{ij} = E^0_{ij}, \quad i = 1, 2,\ j = m \text{ or } n. \tag{3.1}$$

From (2.27) and (2.30), it follows that

$$k^i_{j-1,3}(E^0_{i,j-1} - \bar{e}_{i,j-1}) = k^i_{j2}(E^0_{ij} - \bar{e}_{ij}), \quad i = 1, 2,\ j = 3, \cdots, n \text{ or } m,$$

which, combining with (3.1), implies that

$$\bar{e}_{ij} = E^0_{ij}, \quad i = 1, 2,\ j = 2, \cdots, n \text{ or } m. \tag{3.2}$$

Since $\bar{e}_{ij}$ is nonzero, we can conclude from (2.27) that

$$\bar{p}_{ij} = 0, \quad i = 1, 2,\ j = 2, \cdots, n \text{ or } m. \tag{3.3}$$

Using this result, we deduce from (2.29) that

$$E^0_{i,1} - \bar{e}_{i,1} - \bar{w}_i = 0 \quad i = 1, 2. \tag{3.4}$$

Using this result and adding (2.26) to (2.32), we derive that

$$\bar{p}_{11} = 0.$$

We can take the values we have just discovered and apply them to (2.28) and obtain

$$\bar{c} = 0.$$

Since $\bar{e}$ is nonzero, it then follows from (2.25) that

$$\bar{s} = 0. \tag{3.5}$$

Finally we deduce from (2.24), (2.31) and (2.32) that

$$\begin{aligned}
-k^1_{m+1,1}\bar{e}_{1,1}\bar{p}_1 + f_1(E^0_{11} - \bar{e}_{1,1}) &= 0, && (3.6)\\
-k^2_{n+1,1}\bar{e}_{2,1}\bar{p}_2 + f_2(E^0_{21} - \bar{e}_{2,1}) &= 0, && (3.7)\\
k^1_{m+1,3}(E^0_{11} - \bar{e}_{1,1}) - k^1_{m+1,2}\bar{r}\ \bar{e}_{1,1} &= 0, && (3.8)\\
k^2_{n+1,3}(E^0_{21} - \bar{e}_{2,1}) - k^2_{n+1,2}\bar{r}\ \bar{e}_{2,1} &= 0, && (3.9)\\
\bar{r} + E^0_{11} - \bar{e}_{1,1} + E^0_{21} - \bar{e}_{2,1} + \bar{p}_1 + \bar{p}_2 &= S_0. && (3.10)
\end{aligned}$$

For simplicity, we here have set $\bar{p}_{1,m+1} = \bar{p}_1$ and $\bar{p}_{2,n+1} = \bar{p}_2$. Solving equations (3.6)–(3.9), we obtain

$$\begin{aligned}
\bar{e}_{1,1} &= \frac{k^1_{m+1,3}E^0_{1,1}}{k^1_{m+1,3} + k^1_{m+1,2}\bar{r}},\\
\bar{e}_{2,1} &= \frac{k^2_{n+1,3}E^0_{2,1}}{k^2_{n+1,3} + k^2_{n+1,2}\bar{r}},\\
\bar{p}_1 &= \frac{\bar{r}f_1k^1_{m+1,2}}{k^1_{m+1,1}k^1_{m+1,3}},\\
\bar{p}_2 &= \frac{\bar{r}f_2k^2_{n+1,2}}{k^2_{n+1,1}k^2_{n+1,3}}.
\end{aligned}$$

Substituting these equations into (3.10) gives

$$\bar{r} + \frac{k^1_{m+1,2}E^0_{1,1}\bar{r}}{k^1_{m+1,3} + k^1_{m+1,2}\bar{r}} + \frac{k^2_{n+1,2}E^0_{2,1}\bar{r}}{k^2_{n+1,3} + k^2_{n+1,2}\bar{r}} + \frac{\bar{r}f_1k^1_{m+1,2}}{k^1_{m+1,1}k^1_{m+1,3}} + \frac{\bar{r}f_2k^2_{n+1,2}}{k^2_{n+1,1}k^2_{n+1,3}} = S_0. \quad (3.11)$$

We now prove that the equation (3.11) has only one positive solution.

Lemma 3.1. *The equation (3.11) has only one positive solution.*

Proof. Consider the function

$$f(r) = r + \frac{k^1_{m+1,2}E^0_{1,1}r}{k^1_{m+1,3} + k^1_{m+1,2}r} + \frac{k^2_{n+1,2}E^0_{2,1}r}{k^2_{n+1,3} + k^2_{n+1,2}r} + \frac{rf_1k^1_{m+1,2}}{k^1_{m+1,1}k^1_{m+1,3}} + \frac{rf_2k^2_{n+1,2}}{k^2_{n+1,1}k^2_{n+1,3}} - S_0.$$

We can readily show that f is strictly increasing on $[0,\infty)$ and

$$f(0) = -S_0 < 0, \quad \lim_{r\to\infty} f(r) = \infty.$$

So by the intermediate value theorem, there exist a unique $\bar{r} > 0$ such that $f(\bar{r}) = 0$ and then the equation (3.11) has only one positive solution. □

We now summarize these results in the following theorem.

Theorem 3.1. *The system (2.25)-(2.34) has the following equilibrium state*

$$\bar{s} = 0, \quad (3.12)$$

$$\bar{p}_{ij} = 0, \quad i = 1,2,\ j = 1,\cdots,m \text{ or } n, \quad (3.13)$$

$$\bar{p}_{1,m+1} = \frac{rf_1k^1_{m+1,2}}{k^1_{m+1,1}k^1_{m+1,3}}, \quad (3.14)$$

$$\bar{p}_{2,n+1} = \frac{\bar{r}f_2k^2_{n+1,2}}{k^2_{n+1,1}k^2_{n+1,3}}, \quad (3.15)$$

$$\bar{e}_{11} = \frac{k^1_{m+1,3}E^0_{1,1}}{k^1_{m+1,3} + k^1_{m+1,2}\bar{r}}, \quad (3.16)$$

$$\bar{e}_{21} = \frac{k^2_{n+1,3}E^0_{2,1}}{k^2_{n+1,3} + k^2_{n+1,2}\bar{r}}, \quad (3.17)$$

$$\bar{e}_{ij} = E^0_{ij}, \quad i = 1,2,\ j = 2,\cdots,m \text{ or } n, \quad (3.18)$$

$$\bar{w}_i = E^0_{i1} - \bar{e}_{i1}, \quad i = 1,2, \quad (3.19)$$

where the equilibrium $\bar{r}$ is the positive real solution of (3.11). Then the feedback inhibition rates are given by

$$k^1_{ic} = \frac{\bar{e}_{11}\bar{p}_1}{(E^0_{11} - \bar{e}_{11})}, \quad (3.20)$$

$$k^2_{ic} = \frac{\bar{e}_{21}\bar{p}_2}{(E^0_{11} - \bar{e}_{21})}. \quad (3.21)$$

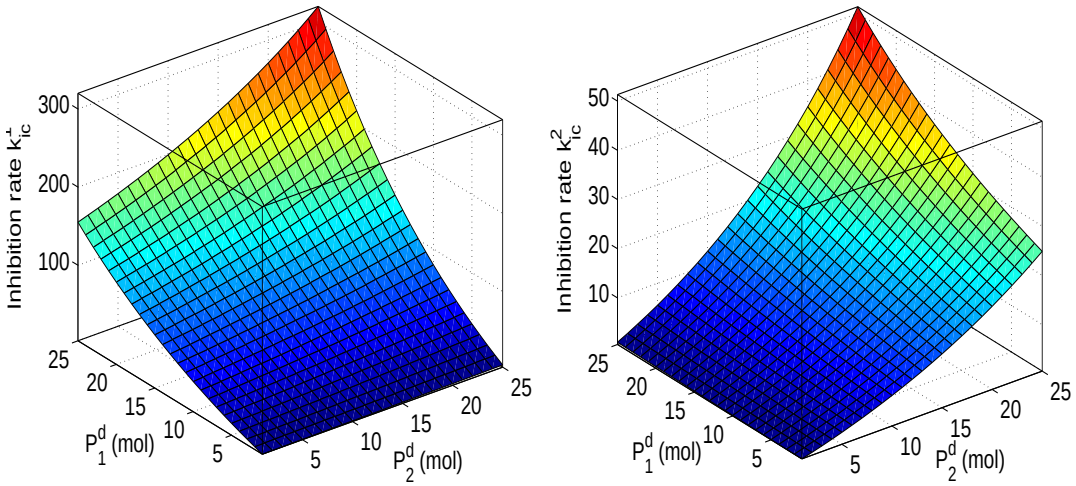

Figure 3: Inhibition rates k_{ic}^1 and k_{ic}^2 exhibit a nonlinearly increasing relation to their end-product levels.

The feedback inhibition rates k_{ic}^1 and k_{ic}^2 are plotted against the end-prodcut levels $\bar{p}_1$ and $\bar{p}_2$ in Figure 3. This figure shows that the rates increase as respective end-product level increases and they are not linearly related to the level.

Note that the feedback inhibition rates k_{ic}^1, k_{ic}^2 are independent of the intermediate metabolites, enzymes and reaction constants. Also the inhibition rate of one branch depends on the other branch through the intermediate metabolite R, which serves as a communicator between two branches.

4 Linear Stability

To show that the feedback inhibition rates k_{ic}^1, k_{ic}^2 determined mathematically through (3.20) and (3.21) are effective, we need to prove that the equilibrium (3.12)-(3.19) of the system (2.25)-(2.34) is asymptotically stable. Since we could not construct a Lyapunov function to address the global stability of the system, we consider here its local linear stability.

We note that all quantities like p_{ij} and e_{ij} are nonnegative in accord with biological situations.

The linearized system of the nonlinear system (2.25)-(2.34) at the equilibrium (3.12)-(3.19) is given by

$$\frac{d\mathbf{x}}{dt} = \mathbf{J}_{2m+2n+5}\mathbf{x}, \tag{4.1}$$

where

$$\begin{aligned}
\mathbf{x} &= (x_1, \cdots, x_{2m+2n+5})^T \\
&= (s, e_{1,1}, p_{1,1}, e_{1,2}, p_{1,2}, \cdots, e_{1,m}, p_{1,m}, p_{1,m+1}, w_1, e_{2,1}, e_{2,2}, p_{2,2}, \cdots, e_{2,n}, p_{2,n}, p_{1,n+1}, w_2, r)^T \\
&- (\bar{s}, \bar{e}_{1,1}, \bar{p}_{1,1}, \bar{e}_{1,2}, \bar{p}_{1,2}, \cdots, \bar{e}_{1,m}, \bar{p}_{1,m}, \bar{p}_{1,m+1}, \bar{w}_1, \bar{e}_{2,1}, \bar{e}_{2,2}, p_{2,2}, \cdots, \bar{e}_{2,n}, \bar{p}_{2,n}, \bar{p}_{1,n+1}, \bar{w}_2, \bar{r})^T,
\end{aligned}$$

and $\mathbf{J}_{2m+2n+5}$ denotes the Jacobian matrix

$$\mathbf{J}_{2m+2n+5} = \begin{pmatrix} \frac{\partial g_1}{\partial x_1} & \cdots & \frac{\partial g_1}{\partial x_{2m+2n+5}} \\ \vdots & \cdots & \vdots \\ \frac{\partial g_{2m+2n+5}}{\partial x_1} & \cdots & \frac{\partial g_{2m+2n+5}}{\partial x_{2m+2n+5}} \end{pmatrix}$$

at the equilibrium (3.12)-(3.19) with g_i denoting the functions of the right hand side of the system (2.25)-(2.34) corresponding to x_i. For a situation where $m = n = 2$, Jacobian matrix is equal to

$$\mathbf{J}_{13} = \begin{bmatrix} -k_1^0 E - k_2^0 & k_2^0 & -k_2^0 \\ 0 & -k_{1,2}^1 - k_{1,3}^1 - k_{3,1}^1 \bar{p}_1 - k_{3,2}^1 \bar{r} & -k_{1,1}^1 \bar{e}_{11} \\ -k_3^0 & k_3^0 - k_{1,2}^1 & -k_{1,1}^1 \bar{e}_{11} - k_3^0 - k_{1,1}^2 \bar{e}_{21} \\ 0 & 0 & 0 \\ 0 & -k_{1,3}^1 & 0 \\ 0 & -k_{3,1}^1 \bar{p}_1 & 0 \\ 0 & k_{3,1}^1 \bar{p}_1 + k_{3,2}^1 \bar{r} & 0 \\ 0 & 0 & -k_{1,1}^2 \bar{e}_{21} \\ 0 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & -k_{3,2}^1 \bar{r} & 0 \end{bmatrix}$$

$$
\begin{array}{ccc}
k_2^0 & -k_2^0 & -k_2^0 \\
0 & 0 & -k_{3,1}^1 \bar{e}_{11} \\
k_3^0 & -k_3^0 & -k_3^0 \\
-k_{2,2}^1 - k_{2,3}^1 & -k_{2,1}^1 E_{1,2}^0 & 0 \\
-k_{2,2}^1 & -k_{2,1}^1 E_{1,2}^0 & 0 \\
-k_{2,3}^1 & 0 & -k_{3,1}^1 \bar{e}_{11} \\
0 & 0 & k_{3,1}^1 \bar{e}_{11} \\
0 & 0 & 0 \\
0 & 0 & 0 \\
0 & 0 & 0 \\
0 & 0 & 0 \\
0 & 0 & 0 \\
0 & 0 & 0
\end{array}
$$

$$
\begin{array}{ccc}
0 & k_2^0 & k_2^0 \\
-k_{1,2}^1 - k_{1,3}^1 + f_1 + k_{3,3}^1 & 0 & 0 \\
-k_{1,2}^1 & k_3^0 - k_{1,2}^2 & k_3^0 \\
0 & 0 & 0 \\
-k_{1,3}^1 & 0 & 0 \\
f_1 & 0 & 0 \\
-f_1 - k_{3,3}^1 & 0 & 0 \\
0 & -k_{1,2}^2 - k_{1,3}^2 - k_{3,1}^2 \bar{p}_2 - k_{3,2}^2 \bar{r} & 0 \\
0 & 0 & -k_{2,2}^2 - k_{2,3}^2 \\
0 & -k_{1,3}^2 & -k_{2,2}^2 \\
0 & -k_{3,1}^2 \bar{p}_2 & -k_{2,3}^2 \\
0 & k_{3,1}^2 \bar{p}_2 + k_{3,2}^2 \bar{r} & 0 \\
k_{3,3}^1 & -k_{3,2}^2 \bar{r} & 0
\end{array}
$$

$$\left[\begin{array}{cccc}
-k_2^0 & -k_2^0 & 0 & -k_2^0 \\
0 & 0 & 0 & -k_{3,2}^1 \bar{e}_{11} \\
-k_3^0 & -k_3^0 & -k_{1,2}^2 & -k_3^0 \\
0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 \\
0 & 0 & 0 & k_{3,2}^1 \bar{e}_{11} \\
0 & -k_{3,1}^2 \bar{e}_{21} & -k_{1,2}^2 - k_{1,3}^2 + f_2 + k_{3,3}^2 & -k_{3,2}^2 \bar{e}_{21} \\
-k_{2,1}^2 E_{2,2}^0 & 0 & 0 & 0 \\
-k_{2,1}^2 E_{2,2}^0 & 0 & -k_{1,3}^2 & 0 \\
0 & -k_{3,1}^2 \bar{e}_{21} & f_2 & 0 \\
0 & k_{3,1}^2 \bar{e}_{21} & -f_2 - k_{3,3}^2 & k_{3,2}^2 \bar{e}_{21} \\
0 & 0 & k_{3,3}^2 & -k1_{3,2} \bar{e}_{11} - k_{3,2}^2 \bar{e}_{21}
\end{array}\right],$$

where $E = E^0 + E_{1,1}^0 + E_{2,1}^0 + \bar{r} + \bar{p}_1 + \bar{p}_2 - \bar{e}_{11} - \bar{e}_{21} - S^0 > 0$ because of (2.24).

Theorem 4.1. *(i) For $m, n \leq 2$, the characteristic polynomials of the Jacobian matrix $\mathbf{J}_{2m+2n+5}$ are equal to*

$$\begin{aligned}
\det(\lambda \mathbf{I} - \mathbf{J}_{2m+2n+5}) &= P_q(\lambda) \times P_c(\lambda) \times (\lambda^2 + (k_2^0 + k_3^0 + k_1^0 E)\lambda + k_3^0 k_1^0 E) \\
&\quad \times \prod_{i=2}^{m} \left[\lambda^2 + (k_{i,2}^1 + k_{i,3}^1 + E_{1,i}^0 k_{i,1}^1)\lambda + E_{1,i}^0 k_{i,1}^1 k_{i,3}^1\right] \\
&\quad \times \prod_{i=2}^{n} \left[\lambda^2 + (k_{i,2}^2 + k_{i,3}^2 + E_{2,i}^0 k_{i,1}^2)\lambda + E_{2,i}^0 k_{i,1}^2 k_{i,3}^2\right], \quad (4.2)
\end{aligned}$$

where

$$\begin{aligned}
P_c(\lambda) &= \lambda^3 + \left(k_{1,3}^2 + k_{1,2}^2 + k_{1,2}^1 + k_{1,3}^1 + k_{1,1}^1 \bar{e}_{11} + k_{1,1}^2 \bar{e}_{21}\right) \lambda^2 \\
&\quad + (k_{1,1}^1 \bar{e}_{11} k_{1,3}^1 + k_{1,3}^2 k_{1,2}^1 + k_{1,1}^2 \bar{e}_{21} k_{1,3}^1 + k_{1,3}^2 k_{1,1}^1 \bar{e}_{11} + k_{1,2}^2 k_{1,1}^1 \bar{e}_{11} \\
&\quad + k_{1,2}^2 k_{1,2}^1 + k_{1,3}^2 k_{1,3}^1 + k_{1,3}^2 k_{1,1}^2 \bar{e}_{21} + k_{1,1}^2 \bar{e}_{21} k_{1,2}^1 + k_{1,2}^2 k_{1,3}^1)\lambda \\
&\quad + k_{1,3}^2 k_{1,1}^2 \bar{e}_{21} k_{1,2}^1 + k_{1,3}^2 k_{1,1}^1 \bar{e}_{11} k_{1,3}^1 + k_{1,2}^2 k_{1,1}^1 \bar{e}_{11} k_{1,3}^1 + k_{1,3}^2 k_{1,1}^2 \bar{e}_{21} k_{1,3}^1, \quad (4.3)
\end{aligned}$$

$$
\begin{aligned}
P_q(\lambda) \;=\;& \lambda^4 + (k_{2,3}^2 + k_{2,1}^1 P_1^d + f_1 + k_{2,2}^2 \bar{r} + k_{2,3}^1 + \bar{e}_{21} k_{2,2}^2 + k_{2,1}^1 \bar{e}_{11} \\
&+ f_2 + \bar{e}_{21} k_{2,1}^2 + k_{2,2}^1 \bar{e}_{11} + P_2^d k_{2,1}^2 + k_{2,2}^1 \bar{r})\lambda^3 + (\bar{e}_{21} k_{2,1}^2 \bar{r} k_{2,2}^1 + \bar{e}_{21} \bar{e}_{11} k_{2,2}^2 k_{2,1}^1 \\
&+ P_1^d k_{2,1}^1 k_{2,2}^2 \bar{r} + k_{2,2}^2 \bar{r} k_{2,2}^1 \bar{e}_{21} + k_{2,2}^2 \bar{r} k_{2,2}^1 \bar{e}_{11} + \bar{e}_{21} k_{2,1}^2 k_{2,2}^2 \bar{r} + f_1 P_2^d k_{2,1}^2 \\
&+ \bar{e}_{11} f_2 k_{2,2}^1 + k_{2,2}^2 \bar{r}^2 k_{2,2}^1 + \bar{e}_{21} k_{2,2}^2 k_{2,3}^1 + \bar{e}_{21} \bar{e}_{11} k_{2,1}^2 k_{2,2}^1 + \bar{e}_{21} \bar{e}_{21} k_{2,2}^2 k_{2,1}^2 \\
&+ k_{2,2}^1 f_1 \bar{e}_{11} + k_{2,2}^1 k_{2,1}^1 \bar{e}_{11} \bar{e}_{11} + k_{2,1}^1 \bar{e}_{11} k_{2,3}^1 + k_{2,3}^1 P_2^d k_{2,1}^2 + P_1^d k_{2,1}^1 f_2 \\
&+ \bar{e}_{21} k_{2,2}^2 f_1 + \bar{e}_{11} k_{2,3}^2 k_{2,1}^1 + P_1^d k_{2,1}^1 k_{2,3}^2 + f_2 \bar{r} k_{2,2}^1 + k_{2,3}^2 \bar{r} k_{2,2}^1 + \bar{e}_{21} f_1 k_{2,1}^2 \\
&+ \bar{e}_{21} k_{2,3}^1 k_{2,1}^2 + \bar{e}_{21} k_{2,2}^2 f_2 + \bar{e}_{11} f_2 k_{2,1}^1 + k_{2,3}^1 k_{2,2}^2 \bar{r} + \bar{e}_{21} k_{2,3}^2 k_{2,1}^2 + \bar{e}_{11} k_{2,3}^2 k_{2,2}^1 \\
&+ P_1^d k_{2,1}^1 P_2^d k_{2,1}^2 + \bar{e}_{11} k_{2,1}^1 P_2^d k_{2,1}^2 + \bar{e}_{11} P_2^d k_{2,1}^2 k_{2,2}^1 + \bar{e}_{21} P_1^d k_{2,1}^1 k_{2,1}^2 + P_2^d k_{2,1}^2 \bar{r} k_{2,2}^1 \\
&+ \bar{e}_{21} \bar{e}_{11} k_{2,1}^2 k_{2,1}^1 + \bar{e}_{21} k_{2,2}^2 P_2^d k_{2,1}^2 + f_1 k_{2,2}^2 \bar{r} + k_{2,3}^2 f_1 + f_2 k_{2,3}^1 + f_2 f_1 + k_{2,3}^2 k_{2,3}^1 \\
&+ \bar{e}_{21} P_1^d k_{2,1}^1 k_{2,2}^2 + k_{2,2}^1 \bar{r} k_{2,1}^1 \bar{e}_{11} + k_{2,2}^1 \bar{e}_{11} k_{2,1}^1 P_1^d + \bar{e}_{11} k_{2,1}^1 k_{2,2}^2 \bar{r})\lambda^2 \\
&+ (\bar{e}_{21} \bar{e}_{11} k_{2,3}^1 k_{2,3}^2 k_{2,1}^1 + \bar{e}_{11} f_2 k_{2,1}^1 \bar{r} k_{2,2}^1 \\
&+ \bar{e}_{11} f_2 k_{2,3}^1 k_{2,1}^1 + \bar{e}_{21} k_{2,2}^2 f_1 P_2^d k_{2,1}^2 + k_{2,2}^2 k_{2,1}^1 \bar{r} k_{2,2}^1 \bar{e}_{11} \bar{e}_{11} \\
&+ \bar{e}_{21} \bar{e}_{11} k_{2,1}^2 k_{2,1}^1 k_{2,2}^2 \bar{r} + \bar{e}_{21} \bar{e}_{11} k_{2,1}^2 k_{2,1}^1 \bar{r} k_{2,2}^1 + \bar{e}_{11} P_1^d k_{2,1}^1 P_2^d k_{2,1}^2 k_{2,2}^1 \\
&+ \bar{e}_{11} P_1^d k_{2,1}^1 f_2 k_{2,2}^1 + \bar{e}_{21} P_1^d k_{2,1}^1 k_{2,2}^2 f_2 + \bar{e}_{21} \bar{e}_{11} k_{2,2}^2 k_{2,3}^1 k_{2,1}^1 + \bar{e}_{11} \bar{e}_{11} k_{2,3}^2 k_{2,1}^1 k_{2,2}^1 \\
&+ \bar{e}_{21} \bar{e}_{11} k_{2,3}^2 k_{2,1}^2 k_{2,1}^1 + \bar{e}_{21} \bar{e}_{11} P_1^d k_{2,1}^1 k_{2,1}^2 k_{2,2}^1 + \bar{e}_{11} k_{2,1}^1 P_2^d k_{2,1}^2 \bar{r} k_{2,2}^1 + \bar{e}_{21} \bar{e}_{21} k_{2,2}^2 f_1 k_{2,1}^2 \\
&+ \bar{e}_{11} f_2 f_1 k_{2,2}^1 + \bar{e}_{21} k_{2,3}^2 k_{2,2}^2 k_{2,1}^1 \bar{r} k_{2,2}^1 \bar{e}_{21} \bar{e}_{11} + \bar{e}_{21} k_{2,3}^2 k_{2,3}^1 k_{2,1}^2 + \bar{e}_{11} k_{2,3}^1 k_{2,1}^1 P_2^d k_{2,1}^2 \\
&+ \bar{e}_{21} \bar{e}_{11} k_{2,2}^2 k_{2,1}^1 P_2^d k_{2,1}^2 + \bar{e}_{21} k_{2,2}^2 f_2 f_1 + \bar{e}_{11} k_{2,1}^1 k_{2,2}^2 \bar{r}^2 k_{2,2}^1 + \bar{e}_{11} \bar{e}_{11} k_{2,1}^1 P_2^d k_{2,1}^2 k_{2,2}^1 \\
&+ \bar{e}_{11} f_1 k_{2,2}^2 \bar{r} k^1 2, 2 + \bar{e}_{21} k_{2,1}^2 k_{2,2}^2 \bar{r}^2 k_{2,2}^1 + \bar{e}_{11} \bar{e}_{11} \bar{e}_{21} k_{2,1}^2 k^1 2, 2 \bar{e}_{21} \bar{e}_{11} + \bar{e}_{11} k_{2,3}^1 k_{2,1}^1 k_{2,2}^2 \bar{r} \\
&+ \bar{e}_{21} k_{2,3}^1 k_{2,2}^2 P_2^d k_{2,1}^2 + \bar{e}_{11} P_1^d k_{2,1}^1 k_{2,3}^2 k_{2,2}^1 + \bar{e}_{21} \bar{e}_{11} f_1 k_{2,1}^2 k_{2,2}^1 + \bar{e}_{11} k_{2,3}^2 k_{2,3}^1 k_{2,1}^1 \\
&+ \bar{e}_{21} k_{2,3}^2 f_1 k_{2,1}^2 + \bar{e}_{11} \bar{e}_{21} \bar{e}_{21} k_{2,2}^2 k_{2,1}^2 k_{2,1}^1 + \bar{e}_{21} \bar{e}_{21} k_{2,2}^2 k_{2,3}^1 k_{2,1}^2 + \bar{e}_{21} k_{2,1}^2 P_1^d k_{2,1}^1 \bar{r} k_{2,2}^2 \\
&+ \bar{e}_{11} \bar{e}_{11} f_2 k_{2,1}^1 k_{2,2}^1 + \bar{e}_{21} \bar{e}_{21} P_1^d k_{2,1}^1 k_{2,2}^2 k_{2,1}^2 + \bar{e}_{21} k_{2,3}^1 k_{2,1}^2 k_{2,2}^2 \bar{r} + \bar{e}_{21} k_{2,2}^2 P_2^d k_{2,1}^2 \bar{r} k_{2,1}^1 \\
&+ \bar{e}_{11} k_{2,3}^2 f_1 k_{2,2}^1 + \bar{e}_{11} f_1 P_2^d k_{2,1}^2 k_{2,2}^1 + \bar{e}_{21} \bar{e}_{11} k_{2,3}^2 k_{2,1}^2 k_{2,2}^1 + \bar{e}_{21} k_{2,2}^2 f_2 \bar{r} k_{2,2}^1 \\
&+ \bar{e}_{11} k_{2,3}^2 k_{2,1}^1 \bar{r} k_{2,2}^1 + \bar{e}_{21} \bar{e}_{11} k_{2,2}^2 f_2 k_{2,1}^1 + \bar{e}_{11} P_1^d k_{2,1}^1 k_{2,2}^2 \bar{r} k_{2,2}^1 + \bar{e}_{21} k_{2,2}^2 f_2 k_{2,3}^1 \\
&+ \bar{e}_{21} f_1 k_{2,1}^2 k_{2,2}^2 \bar{r} + \bar{e}_{21} P_1^d k_{2,1}^1 k_{2,3}^2 k_{2,1}^2) \lambda \\
&+ \bar{e}_{11} \bar{e}_{21} \bar{e}_{21} k_{2,2}^2 k_{2,3}^1 k_{2,1}^2 k_{2,1}^1 + \bar{e}_{21} \bar{e}_{11} k_{2,3}^1 k_{2,1}^2 k_{2,1}^1 k_{2,2}^2 \bar{r} \\
&+ k_{2,2}^2 k_{2,1}^2 k_{2,1}^1 \bar{r} k_{2,2}^1 \bar{e}_{11} \bar{e}_{11} \bar{e}_{21} + \bar{e}_{21} \bar{e}_{11} k_{2,3}^2 k_{2,1}^2 k_{2,1}^1 \bar{r} k_{2,2}^1 + \bar{e}_{21} \bar{e}_{11} k_{2,3}^1 k_{2,2}^2 k_{2,1}^1 P_2^d k_{2,1}^2 \\
&+ \bar{e}_{21} \bar{e}_{11} k_{2,2}^2 k_{2,1}^1 P_2^d k_{2,1}^2 \bar{r} k_{2,2}^1 + \bar{e}_{11} \bar{e}_{11} \bar{e}_{21} k_{2,3}^2 k_{2,1}^2 k_{2,1}^1 k_{2,2}^1 + \bar{e}_{21} \bar{e}_{11} k_{2,3}^2 f_1 k_{2,1}^2 k_{2,2}^1 \\
&+ k_{2,2}^2 k_{2,1}^2 k_{2,1}^1 \bar{r} k_{2,2}^1 \bar{e}_{11} \bar{e}_{21} \bar{e}_{21} + \bar{e}_{21} \bar{e}_{11} k_{2,2}^2 f_2 k_{2,3}^1 k_{2,1}^1 + \bar{e}_{21} \bar{e}_{11} k_{2,1}^2 k_{2,1}^1 k_{2,2}^2 \bar{r}^2 k_{2,2}^1 \\
&+ \bar{e}_{21} \bar{e}_{11} k_{2,3}^2 k_{2,3}^1 k_{2,1}^2 k_{2,1}^1 + \bar{e}_{21} \bar{e}_{11} f_1 k_{2,1}^2 k_{2,2}^2 \bar{r} k_{2,2}^1 + \bar{e}_{21} \bar{e}_{11} P_1^d k_{2,1}^1 k_{2,3}^2 k_{2,1}^2 k_{2,2}^1 \\
&+ \bar{e}_{21} \bar{e}_{11} f_2 k_{2,2}^2 k_{2,1}^1 \bar{r} k_{2,2}^1 + \bar{e}_{21} \bar{e}_{11} P_1^d k_{2,1}^1 k_{2,1}^2 k_{2,2}^2 \bar{r} k_{2,2}^1. \qquad (4.4)
\end{aligned}
$$

(ii) For $m, n \leq 2$, the real parts of all eigenvalues of the Jacobian matrix $\mathbf{J}_{2m+2n+5}$ are negative. Hence the system (2.25)-(2.34) is locally exponentially stable.

Proof. (i) The characteristic polynomial (4.2) is verified by using Maple software. The Maple codes for this are available upon request.

(ii) Since it is easy to see that all the quadratic polynomials have real negative roots, it suffices to show that the real parts of the roots of the cubic and quartic polynomials (4.3) and (4.4) are negative. These cubic and quartic equations are both solvable exactly, but the size of their solutions are huge and it is difficult to tell whether their real parts are negative. So we use Routh's stability criterion [12].

All the symbolic computations below were done using the Maple software. The Maple codes are available upon request.

The Routh's array for the cubic polynomial is as follows:

$$\begin{array}{ll} \lambda^3: & 1, \quad a_2 \\ \lambda^2: & a_1, \quad a_3 \\ \lambda^1: & b_1 \\ \lambda^0: & c_1 \end{array}$$

where

$$\begin{aligned}
a_1 &= k^1_{1,3}+k^2_{1,3}+k^2_{1,2}+k^1_{1,1}\bar{e}_{11}+k^2_{1,1}\bar{e}_{21}+k^1_{1,2},\\
a_2 &= k^2_{1,3}k^1_{1,3}+k^2_{1,3}k^2_{1,1}\bar{e}_{21}+k^2_{1,1}\bar{e}_{21}k^1_{1,2}+k^2_{1,2}k^1_{1,3}+k^2_{1,3}k^1_{1,2}+k^2_{1,1}\bar{e}_{21}k^1_{1,3}\\
&\quad +k^2_{1,3}k^1_{1,1}\bar{e}_{11}+k^2_{1,2}k^1_{1,1}\bar{e}_{11}+k^2_{1,2}k^1_{1,2}+k^1_{1,1}\bar{e}_{11}k^1_{1,3},\\
a_3 &= k^2_{1,3}k^2_{1,1}\bar{e}_{21}k^1_{1,2}+k^2_{1,3}k^2_{1,1}\bar{e}_{21}k^1_{1,3}+k^2_{1,3}k^1_{1,1}\bar{e}_{11}k^1_{1,3}+k^2_{1,2}k^1_{1,1}\bar{e}_{11}k^1_{1,3},\\
b_1 &= \frac{a_1a_2-a_3}{a_1}\\
&= =(2k^2_{1,3}k^2_{1,1}\bar{e}_{21}k^1_{1,2}+2k^2_{1,3}k^2_{1,1}\bar{e}_{21}k^1_{1,3}+2k^2_{1,3}k^1_{1,1}\bar{e}_{11}k^1_{1,3}\\
&\quad +2k^2_{1,2}k^1_{1,1}\bar{e}_{11}k^1_{1,3}+2k^2_{1,2}k^1_{1,3}k^2_{1,1}\bar{e}_{21}+2k^2_{1,3}k^1_{1,2}k^1_{1,1}\bar{e}_{11}\\
&\quad +k^2_{1,3}k^2_{1,1}\bar{e}_{21}k^2_{1,2}+2k^2_{1,1}\bar{e}_{21}k^1_{1,2}k^1_{1,3}+2k^2_{1,1}\bar{e}_{21}k^1_{1,2}k^2_{1,2}\\
&\quad +2k^2_{1,3}k^1_{1,1}\bar{e}_{11}k^2_{1,2}+2k^2_{1,2}k^1_{1,1}\bar{e}_{11}k^1_{1,2}+k^1_{1,1}\bar{e}_{11}k^1_{1,3}k^1_{1,2}\\
&\quad +k^2_{1,3}k^1_{1,3}k^1_{1,3}+k^2_{1,3}k^2_{1,3}k^1_{1,3}+k^2_{1,2}k^1_{1,3}k^1_{1,3}+k^2_{1,2}k^2_{1,2}k^1_{1,3}+k^2_{1,3}k^2_{1,3}k^2_{1,1}\bar{e}_{21}\\
&\quad +k^2_{1,3}k^2_{1,1}k^2_{1,1}\bar{e}_{21}\bar{e}_{21}+k^2_{1,1}k^2_{1,1}\bar{e}_{21}\bar{e}_{21}k^1_{1,2}+k^2_{1,1}\bar{e}_{21}k^1_{1,2}k^1_{1,2}+k^2_{1,1}\bar{e}_{21}k^1_{1,3}k^1_{1,3}\\
&\quad +k^2_{1,1}k^2_{1,1}\bar{e}_{21}\bar{e}_{21}k^1_{1,3}+k^2_{1,1}k^2_{1,1}k^1_{1,1}\bar{e}_{11}+k^2_{1,3}k^1_{1,1}k^1_{1,1}\bar{e}_{11}\bar{e}_{11}+k^2_{1,2}k^2_{1,2}k^1_{1,1}\bar{e}_{11}\\
&\quad +k^2_{1,2}k^1_{1,1}k^1_{1,1}\bar{e}_{11}\bar{e}_{11}+k^1_{1,1}\bar{e}_{11}k^1_{1,3}k^1_{1,3}+k^1_{1,1}k^1_{1,1}\bar{e}_{11}\bar{e}_{11}k^1_{1,3}+2k^2_{1,3}k^1_{1,3}k^2_{1,2}\\
&\quad +2k^2_{1,3}k^1_{1,3}k^1_{1,2}+2k^2_{1,2}k^1_{1,3}k^1_{1,2}+2k^2_{1,3}k^1_{1,2}k^2_{1,2}\\
&\quad +2k^2_{1,3}k^2_{1,1}\bar{e}_{21}k^1_{1,1}\bar{e}_{11}+k^2_{1,1}\bar{e}_{21}k^1_{1,2}k^1_{1,1}\bar{e}_{11}+k^2_{1,3}k^2_{1,3}k^1_{1,2}\\
&\quad +k^2_{1,3}k^1_{1,2}k^1_{1,2}+k^2_{1,2}k^2_{1,2}k^1_{1,2}+k^2_{1,2}k^1_{1,2}k^1_{1,2}+2k^2_{1,1}\bar{e}_{21}k^1_{1,3}k^1_{1,1}\bar{e}_{11}\\
&\quad +k^2_{1,2}k^1_{1,1}\bar{e}_{11}k^2_{1,1}\bar{e}_{21})/(k^1_{1,3}+k^2_{1,3}+k^2_{1,2}+k^1_{1,1}\bar{e}_{11}+k^2_{1,1}\bar{e}_{21}+k^1_{1,2}),\\
c_1 &= \frac{a_3b_1-a_1*0}{b_1}=a_3.
\end{aligned}$$

Since all terms in the above expressions are positive, the first column of Routh's array is all positive and then all the real part of the root of the cubic polynomial are negative.

Routh's array for the quratic polynomial is given by

$$\begin{array}{lll} \lambda^4: & 1, & a_2, \quad a_4 \\ \lambda^3: & a_1, & a_3 \\ \lambda^2: & b_1, & b_2 \\ \lambda^1: & c_1, & c_2 \\ \lambda^0: & d_1, & d_2 \end{array}$$

where

$$\begin{aligned}
a_1 &= \bar{e}_{21}k^2_{2,1} + k^1_{2,1}\bar{e}_{11} + k^2_{2,3} + f_2 + k^1_{2,3} + f_1 \\
&\quad +\bar{e}_{21}k^2_{2,2} + k^1_{2,2}\bar{e}_{11} + k^1_{2,2}\bar{r} + k^2_{2,2}\bar{r} + k^1_{2,1}P^d_1 + P^d_2k^2_{2,1}, \\
a_2 &= k^1_{2,3}P^d_2k^2_{2,1} + \bar{e}_{21}k^2_{2,2}f_1 + \bar{e}_{21}k^2_{2,2}f_2 + f_1k^2_{2,2}\bar{r} + f_2\bar{r}k^1_{2,2} + \bar{e}_{11}f_2k^1_{2,1} \\
&\quad +P^d_1k^1_{2,1}k^2_{2,3} + k^2_{2,2}\bar{r}^2k^1_{2,2} + k^1_{2,1}\bar{e}_{11}k^1_{2,3} + \bar{e}_{21}k^1_{2,3}k^2_{2,1} + k^1_{2,3}k^2_{2,2}\bar{r} + \bar{e}_{11}k^2_{2,3}k^1_{2,2} \\
&\quad +P^d_1k^1_{2,1}f_2 + \bar{e}_{21}f_1k^2_{2,1} + k^1_{2,2}k^1_{2,1}\bar{e}_{11}\bar{e}_{11} + k^1_{2,2}f_1\bar{e}_{11} + \bar{e}_{21}\bar{e}_{21}k^2_{2,2}k^2_{2,1} + f_1P^d_2k^2_{2,1} \\
&\quad +\bar{e}_{11}k^2_{2,3}k^1_{2,1} + \bar{e}_{11}f_2k^1_{2,2} + k^2_{2,3}\bar{r}k^1_{2,2} + \bar{e}_{21}k^2_{2,3}k^2_{2,1} + \bar{e}_{21}k^2_{2,2}k^1_{2,3} + f_2k^1_{2,3} \\
&\quad +k^2_{2,3}k^1_{2,3} + \bar{e}_{21}P^d_1k^1_{2,1}k^2_{2,2} + f_2f_1 + P^d_1k^1_{2,1}k^2_{2,2}\bar{r} + \bar{e}_{21}\bar{e}_{11}k^2_{2,1}k^1_{2,1} + k^1_{2,2}\bar{r}k^1_{2,1}\bar{e}_{11} \\
&\quad +\bar{e}_{11}k^1_{2,1}P^d_2k^2_{2,1} + P^d_2k^2_{2,1}\bar{r}k^1_{2,2} + \bar{e}_{11}k^1_{2,1}k^2_{2,2}\bar{r} + \bar{e}_{21}k^2_{2,2}P^d_2k^2_{2,1} + P^d_1k^1_{2,1}P^d_2k^2_{2,1} \\
&\quad +\bar{e}_{21}P^d_1k^1_{2,1}k^2_{2,1} + k^1_{2,2}\bar{e}_{11}k^1_{2,1}P^d_1 + \bar{e}_{21}k^2_{2,1}k^2_{2,2}\bar{r} + k^2_{2,2}\bar{r}k^1_{2,2}\bar{e}_{21} + k^2_{2,2}\bar{r}k^1_{2,2}\bar{e}_{11} \\
&\quad +k^2_{2,3}f_1 + \bar{e}_{11}P^d_2k^2_{2,1}k^1_{2,2} + \bar{e}_{21}\bar{e}_{11}k^2_{2,2}k^1_{2,1} + \bar{e}_{21}k^2_{2,1}\bar{r}k^1_{2,2} + \bar{e}_{21}\bar{e}_{11}k^2_{2,1}k^1_{2,2}, \\
a_3 &= \bar{e}_{11}k^2_{2,3}k^1_{2,3}k^1_{2,1} + \bar{e}_{11}\bar{e}_{11}f_2k^1_{2,1}k^1_{2,2} + \bar{e}_{11}k^2_{2,3}f_1k^1_{2,2} + \bar{e}_{11}\bar{e}_{11}k^1_{2,1}P^d_2k^2_{2,1}k^1_{2,2} \\
&\quad +\bar{e}_{11}k^1_{2,3}k^1_{2,1}P^d_2k^2_{2,1} + \bar{e}_{11}\bar{e}_{11}\bar{e}_{21}k^2_{2,1}k^1_{2,1}k^1_{2,2} + \bar{e}_{21}k^2_{2,2}f_1P^d_2k^2_{2,1} + \bar{e}_{21}\bar{e}_{11}k^1_{2,3}k^2_{2,1}k^1_{2,1} \\
&\quad +\bar{e}_{21}\bar{e}_{11}k^2_{2,1}k^1_{2,1}\bar{r}k^1_{2,2} + \bar{e}_{21}k^1_{2,3}k^2_{2,1}k^2_{2,2}\bar{r} + \bar{e}_{21}k^1_{2,3}k^2_{2,2}P^d_2k^2_{2,1} + \bar{e}_{21}k^2_{2,1}k^2_{2,2}\bar{r}^2k^1_{2,2} \\
&\quad +\bar{e}_{21}\bar{e}_{11}k^2_{2,3}k^2_{2,1}k^1_{2,1} + k^2_{2,2}k^1_{2,1}\bar{r}k^1_{2,2}\bar{e}_{21}\bar{e}_{11} + \bar{e}_{11}P^d_1k^1_{2,1}k^2_{2,3}k^1_{2,2} + \bar{e}_{21}\bar{e}_{11}f_1k^2_{2,1}k^1_{2,2} \\
&\quad +\bar{e}_{21}\bar{e}_{11}k^2_{2,1}k^1_{2,1}k^2_{2,2}\bar{r} + \bar{e}_{21}k^2_{2,1}P^d_1k^1_{2,1}\bar{r}k^2_{2,2} + \bar{e}_{21}\bar{e}_{11}k^2_{2,3}k^2_{2,1}k^1_{2,2} + \bar{e}_{21}\bar{e}_{21}P^d_1k^1_{2,1}k^2_{2,2}k^2_{2,1} \\
&\quad +\bar{e}_{21}\bar{e}_{21}k^2_{2,2}f_1k^2_{2,1} + \bar{e}_{21}k^2_{2,3}k^1_{2,3}k^2_{2,1} + \bar{e}_{11}f_2k^1_{2,3}k^1_{2,1} + \bar{e}_{21}\bar{e}_{21}k^2_{2,2}k^1_{2,3}k^2_{2,1} + \bar{e}_{21}k^2_{2,2}f_2k^1_{2,3} \\
&\quad +\bar{e}_{21}k^2_{2,3}f_1k^2_{2,1} + \bar{e}_{21}k^2_{2,2}f_2f_1 + \bar{e}_{21}k^2_{2,2}P^d_2k^2_{2,1}\bar{r}k^1_{2,2} + k^2_{2,1}k^2_{2,2}\bar{r}k^1_{2,2}\bar{e}_{21}\bar{e}_{21} + \bar{e}_{21}\bar{e}_{11}k^2_{2,2}f_2k^1_{2,1} \\
&\quad +\bar{e}_{11}P^d_1k^1_{2,1}P^d_2k^2_{2,1}k^1_{2,2} + \bar{e}_{21}P^d_1k^1_{2,1}k^2_{2,2}f_2 + \bar{e}_{11}k^1_{2,1}P^d_2k^2_{2,1}\bar{r}k^1_{2,2} + \bar{e}_{11}k^1_{2,1}k^2_{2,2}\bar{r}^2k^1_{2,2} \\
&\quad +\bar{e}_{11}P^d_1k^1_{2,1}f_2k^1_{2,2} + \bar{e}_{11}P^d_1k^1_{2,1}k^2_{2,2}\bar{r}k^1_{2,2} + \bar{e}_{11}k^1_{2,3}k^1_{2,1}k^2_{2,2}\bar{r} + \bar{e}_{21}f_1k^2_{2,1}k^2_{2,2}\bar{r} + \bar{e}_{11}f_2k^1_{2,1}\bar{r}k^1_{2,2} \\
&\quad +k^2_{2,1}k^2_{2,2}\bar{r}k^1_{2,2}\bar{e}_{21}\bar{e}_{11} + \bar{e}_{11}\bar{e}_{21}\bar{e}_{21}k^2_{2,2}k^2_{2,1}k^1_{2,1} + \bar{e}_{21}\bar{e}_{11}k^2_{2,2}k^1_{2,1}P^d_2k^2_{2,1} + \bar{e}_{11}f_1P^d_2k^2_{2,1}k^1_{2,2} \\
&\quad +\bar{e}_{21}\bar{e}_{11}P^d_1k^1_{2,1}k^2_{2,1}k^1_{2,2} + \bar{e}_{21}P^d_1k^1_{2,1}k^2_{2,3}k^2_{2,1} + \bar{e}_{21}k^2_{2,2}f_2\bar{r}k^1_{2,2} + k^2_{2,2}k^1_{2,1}\bar{r}k^1_{2,2}\bar{e}_{11}\bar{e}_{11} \\
&\quad +\bar{e}_{11}k^2_{2,3}k^1_{2,1}\bar{r}k^1_{2,2} + \bar{e}_{11}f_1k^2_{2,2}\bar{r}k^1_{2,2} + \bar{e}_{21}\bar{e}_{11}k^2_{2,2}k^1_{2,3}k^1_{2,1} + \bar{e}_{21}P^d_1k^1_{2,1}k^2_{2,2}P^d_2k^2_{2,1} \\
&\quad +\bar{e}_{21}k^2_{2,3}k^2_{2,1}\bar{r}k^1_{2,2} + \bar{e}_{11}f_2f_1k^1_{2,2} + \bar{e}_{11}\bar{e}_{11}k^2_{2,3}k^1_{2,1}k^1_{2,2},
\end{aligned}$$

$$
\begin{aligned}
a_4 &= \bar{e}_{11}\bar{e}_{21}\bar{e}_{21}k^2_{2,2}k^1_{2,3}k^2_{2,1}k^1_{2,1} + \bar{e}_{21}\bar{e}_{11}f_2k^2_{2,2}k^1_{2,1}\bar{r}k^1_{2,2} + \bar{e}_{21}\bar{e}_{11}k^2_{2,2}k^1_{2,1}P^d_2k^2_{2,1}\bar{r}k^1_{2,2}\\
&+k^2_{2,2}k^2_{2,1}k^1_{2,1}\bar{r}k^1_{2,2}\bar{e}_{11}\bar{e}_{21}\bar{e}_{21} + \bar{e}_{21}\bar{e}_{11}k^2_{2,3}f_1k^2_{2,1}k^1_{2,2} + \bar{e}_{21}\bar{e}_{11}k^2_{2,3}k^1_{2,3}k^2_{2,1}k^1_{2,1}\\
&+k^2_{2,2}k^2_{2,1}k^1_{2,1}\bar{r}k^1_{2,2}\bar{e}_{11}\bar{e}_{11}\bar{e}_{21} + \bar{e}_{21}\bar{e}_{11}P^d_1k^1_{2,1}k^2_{2,3}k^2_{2,1}k^1_{2,2} + \bar{e}_{21}\bar{e}_{11}k^2_{2,3}k^2_{2,1}k^1_{2,1}\bar{r}k^1_{2,2}\\
&+\bar{e}_{21}\bar{e}_{11}k^1_{2,3}k^2_{2,1}k^1_{2,1}k^2_{2,2}\bar{r} + \bar{e}_{11}\bar{e}_{11}\bar{e}_{21}k^2_{2,3}k^2_{2,1}k^1_{2,1}k^1_{2,2} + \bar{e}_{21}\bar{e}_{11}k^2_{2,1}k^1_{2,1}k^2_{2,2}\bar{r}^2k^1_{2,2}\\
&+\bar{e}_{21}\bar{e}_{11}P^d_1k^1_{2,1}k^2_{2,1}k^2_{2,2}\bar{r}k^1_{2,2} + \bar{e}_{21}\bar{e}_{11}f_1k^2_{2,1}k^2_{2,2}\bar{r}k^1_{2,2} + \bar{e}_{21}\bar{e}_{11}k^2_{2,2}f_2k^1_{2,3}k^1_{2,1}\\
&+\bar{e}_{21}\bar{e}_{11}k^1_{2,3}k^2_{2,2}k^1_{2,1}P^d_2k^2_{2,1},\\
b_1a_1 &= a_1a_2 - a_3\\
&= 2\,\bar{e}_{21}\,\bar{e}_{11}\,k^1_{2,3}k^2_{2,1}k1_{2,1} + 2\,\bar{e}_{21}\,\bar{e}_{11}\,k^2_{2,1}k^1_{2,1}\bar{r}k1_{2,2} + 2\,\bar{e}_{11}\,P^d_1\,k^1_{2,1}k^2_{2,2}\bar{r}k^1_{2,2} + 2\,\bar{e}_{11}\,f_1k^2_{2,2}\bar{r}k^1_{2,2}\\
&+2\,\bar{e}_{21}\,\bar{e}_{11}\,k^2_{2,2}k^1_{2,3}k^1_{2,1} + 2\,\bar{e}_{21}\,P^d_1\,k^1_{2,1}k^2_{2,2}P^d_2\,k^2_{2,1} + 2\,\bar{e}^2_{21}k^2_{2,2}f_1k^2_{2,1} + 2\,\bar{e}_{11}\,f_2k^1_{2,3}k^1_{2,1}\\
&+2\,\bar{e}_{11}\,f_2f_1k^1_{2,2} + 2\,\bar{e}^2_{11}k^2_{2,3}k^1_{2,1}k^1_{2,2} + 2\,\bar{e}_{11}\,k^2_{2,3}f_1k^1_{2,2} + 2\,\bar{e}_{21}\,k^2_{2,2}f_2k^1_{2,3} + 2\,\bar{e}^2_{11}f_2k^1_{2,1}k^1_{2,2}\\
&+2\,\bar{e}_{21}\,k^2_{2,2}f_2f_1 + 2\,\bar{e}_{11}\,k^2_{2,3}k^1_{2,3}k^1_{2,1} + 2\,\bar{e}_{21}\,k^2_{2,3}f_1k^2_{2,1} + 2\,\bar{e}^2_{21}k^2_{2,2}k^1_{2,3}k^2_{2,1}\\
&+2\,\bar{e}_{21}\,k^2_{2,3}k^1_{2,3}k^2_{2,1} + 2\,P^d_2\,k^2_{2,1}f_2f_1 + 2\,P^d_2\,k^2_{2,1}k^2_{2,3}k^1_{2,3} + 2\,P^d_2\,k^2_{2,1}f_2k^1_{2,3} + 2\,P^d_2\,k^2_{2,1}k^2_{2,3}f_1\\
&+P^d_2\,{k^2_{2,1}}^2\bar{e}_{21}\,k^2_{2,3} + 2\,P^d_2\,{k^2_{2,1}}^2\bar{e}_{21}\,f_1 + 2\,P^d_2\,{k^2_{2,1}}^2\bar{e}_{21}\,k^1_{2,3} + 2\,P^d_2\,{k^2_{2,1}}^2\bar{e}^2_{21}k^2_{2,2} + {P^d_2}^2{k^2_{2,1}}^2\bar{e}_{11}\,k1_{2,1}\\
&+{P^d_2}^2{k^2_{2,1}}^2P^d_1\,k^1_{2,1} + {P^d_2}^2{k^2_{2,1}}^2\bar{e}_{11}\,k^1_{2,2} + {P^d_2}^2{k^2_{2,1}}^2\bar{e}_{21}\,k^2_{2,2} + {P^d_2}^2{k^2_{2,1}}^2\bar{r}k^1_{2,2} + {k^1_{2,2}}^2\bar{r}^2k^2_{2,2}\bar{e}_{21}\\
&+{k^1_{2,2}}^2\bar{r}^2\bar{e}_{21}\,k^2_{2,1} + 2\,k^1_{2,2}\bar{r}k^2_{2,3}k^1_{2,3} + 2\,k^1_{2,2}\bar{r}f_2k^1_{2,3} + 2\,{k^1_{2,2}}^2\bar{r}\bar{e}_{11}\,k^2_{2,3} + 2\,{k^1_{2,2}}^2\bar{r}\bar{e}_{11}\,f_2\\
&+2\,k^1_{2,2}\bar{r}^2k^1_{2,3}k^2_{2,2} + {k1_{2,2}}^2\bar{r}f_1\bar{e}_{11} + 2\,{k^1_{2,2}}^2\bar{r}k1_{2,1}\bar{e}^2_{11} + 2\,{k^1_{2,2}}^2\bar{r}^2k^2_{2,2}\bar{e}_{11} + {k^1_{2,2}}^2\bar{r}^2k^1_{2,1}\bar{e}_{11}\\
&+{k1_{2,2}}^2\bar{r}^2P^d_2\,k^2_{2,1} + {k^1_{2,1}}^2\bar{e}^2_{11}k^2_{2,2}\bar{r} + 2\,{k^1_{2,1}}^2\bar{e}_{11}\,P^d_1\,f_2 + 2\,{k^1_{2,1}}^2\bar{e}_{11}\,P^d_1\,k2_{2,3}\\
&+2\,\bar{e}_{11}\,P^d_1\,k^1_{2,1}P^d_2\,k^2_{2,1}k^1_{2,2} + 2\,\bar{e}_{21}\,k^2_{2,2}f_2\bar{r}k^1_{2,2} + 2\,\bar{e}_{21}\,P^d_1\,k^1_{2,1}k^2_{2,2}f_2 + 2\,\bar{e}_{21}\,\bar{e}_{11}\,f_1k^2_{2,1}k^1_{2,2}\\
&+2\,\bar{e}_{11}\,k^1_{2,1}P^d_2\,k^2_{2,1}\bar{r}k^1_{2,2} + 2\,\bar{e}_{11}\,P^d_1\,k^1_{2,1}f_2k^1_{2,2} + 2\,\bar{e}_{21}\,k^2_{2,2}f_1P^d_2\,k^2_{2,1} + 2\,\bar{e}^2_{11}k^1_{2,1}P^d_2\,k^2_{2,1}k^1_{2,2}\\
&+2\,\bar{e}_{21}\,\bar{e}_{11}\,k^2_{2,1}k^1_{2,1}k^2_{2,2}\bar{r} + 2\,\bar{e}_{21}\,k^2_{2,1}P^d_1\,k^1_{2,1}\bar{r}k^2_{2,2} + 2\,\bar{e}_{21}\,\bar{e}_{11}\,k^2_{2,3}k^2_{2,1}k^1_{2,2}\\
&+2\,\bar{e}^2_{21}P^d_1\,k^1_{2,1}k^2_{2,2}k2_{2,1} + 2\,\bar{e}_{21}\,k^2_{2,2}P^d_2\,k^2_{2,1}\bar{r}k1_{2,2} + 2\,k^2_{2,1}k^2_{2,2}\bar{r}k^1_{2,2}\bar{e}^2_{21} + 2\,\bar{e}_{21}\,\bar{e}_{11}\,k^2_{2,2}k^1_{2,1}P^d_2\,k^2_{2,1}\\
&+2\,\bar{e}_{11}\,f_1P^d_2\,k^2_{2,1}k^1_{2,2} + 2\,\bar{e}_{21}\,\bar{e}_{11}\,P^d_1\,k^1_{2,1}k2_{2,1}k^1_{2,2} + 2\,\bar{e}_{21}\,P^d_1\,k^1_{2,1}k^2_{2,3}k^2_{2,1} + 2\,k^2_{2,2}k^1_{2,1}\bar{r}k^1_{2,2}\bar{e}^2_{11}\\
&+2\,k^2_{2,1}k^2_{2,2}\bar{r}k1_{2,2}\bar{e}_{21}\,\bar{e}_{11} + 2\,\bar{e}_{21}\,\bar{e}_{11}\,k^2_{2,3}k^2_{2,1}k^1_{2,1} + 2\,k^2_{2,2}k^1_{2,1}\bar{r}k^1_{2,2}\bar{e}_{21}\,\bar{e}_{11} + 2\,\bar{e}_{11}\,\bar{e}^2_{21}k^2_{2,2}k^2_{2,1}k^1_{2,1}\\
&+2\,\bar{e}_{21}\,k^1_{2,3}k^2_{2,1}k^2_{2,2}\bar{r} + 2\,\bar{e}_{21}\,f_1k^2_{2,1}k^2_{2,2}\bar{r} + 2\,\bar{e}_{11}\,f_2k^1_{2,1}\bar{r}k^1_{2,2} + 2\,\bar{e}_{21}\,\bar{e}_{11}\,k^2_{2,2}f_2k^1_{2,1}\\
&+2\,\bar{e}_{11}\,k^1_{2,1}k^2_{2,2}\bar{r}^2k^1_{2,2} + 2\,\bar{e}_{21}\,k^2_{2,3}k^2_{2,1}\bar{r}k^1_{2,2} + 2\,\bar{e}_{11}\,k^2_{2,3}k^1_{2,1}\bar{r}k^1_{2,2} + 2\,\bar{e}_{11}\,k1_{2,3}k^1_{2,1}k^2_{2,2}\bar{r}\\
&+2\,\bar{e}_{21}\,k^1_{2,3}k^2_{2,2}P^d_2\,k^2_{2,1} + 2\,\bar{e}_{21}\,k^2_{2,1}k^2_{2,2}\bar{r}^2k^1_{2,2} + 2\,\bar{e}_{11}\,k^1_{2,3}k^1_{2,1}P^d_2\,k^2_{2,1} + 2\,\bar{e}^2_{11}\bar{e}_{21}\,k^2_{2,1}k^1_{2,1}k^1_{2,2}\\
&+2\,\bar{e}_{11}\,P^d_1\,k^1_{2,1}k^2_{2,3}k^1_{2,2} + f_2{f_1}^2 + k^2_{2,3}{f_1}^2 + {f_2}^2f_1 + {f_2}^2k^1_{2,3} + 2\,f_2k^2_{2,3}k^1_{2,3} + {f_1}^2P^d_2\,k^2_{2,1} + \bar{e}_{21}\,k^2_{2,2}{f_1}^2\\
&+{f_1}^2k^2_{2,2}\bar{r} + \bar{e}_{21}\,{f_1}^2k^2_{2,1} + k^1_{2,2}{f_1}^2\bar{e}_{11} + {P^d_2}^2{k^2_{2,1}}^2f_1 + {P^d_2}^2{k^2_{2,1}}^2k^1_{2,3} + {k^1_{2,2}}^2\bar{r}^2k^2_{2,3} + {k^1_{2,2}}^2\bar{r}^2f_2\\
&+{k^1_{2,2}}^2\bar{r}^3k^2_{2,2} + \bar{e}_{21}\,k^2_{2,2}{f_2}^2 + P^d_1\,k^1_{2,1}{f_2}^2 + \bar{e}_{11}\,{f_2}^2k^1_{2,2} + \bar{e}_{11}\,{f_2}^2k^1_{2,1} + {f_2}^2\bar{r}k^1_{2,2} + {k^1_{2,1}}^2\bar{e}^2_{11}k^2_{2,3}\\
&+{k^1_{2,1}}^2\bar{e}^2_{11}f_2 + {k^1_{2,1}}^2\bar{e}^3_{11}k^1_{2,2} + {k^1_{2,1}}^2\bar{e}^2_{11}k^1_{2,3} + {k^1_{2,1}}^2P^{d^2}_1f_2 + {k^1_{2,1}}^2P^{d^2}_1k^2_{2,3} + {k1_{2,3}}^2k^2_{2,2}\bar{r}\\
&+\bar{e}_{21}\,{k^1_{2,3}}^2k^2_{2,1} + {k^1_{2,3}}^2P^d_2\,k^2_{2,1} + k1_{2,1}\bar{e}_{11}\,{k^1_{2,3}}^2 + \bar{e}_{21}\,k^2_{2,2}{k^1_{2,3}}^2 + {k^1_{2,2}}^2\bar{e}^2_{11}k2_{2,3} + {k^1_{2,2}}^2\bar{e}^2_{11}f_2\\
&+{k^1_{2,2}}^2\bar{e}^2_{11}f_1 + {k^1_{2,2}}^2\bar{e}^3_{11}k^1_{2,1} + {k^2_{2,2}}^2\bar{r}^2f_1 + {k^2_{2,2}}^2\bar{r}^2k^1_{2,3} + {k^2_{2,2}}^2\bar{r}^3k^1_{2,2} + \bar{e}_{11}\,{k^2_{2,3}}^2k^1_{2,2} + \bar{e}_{11}\,{k^2_{2,3}}^2k^1_{2,1}
\end{aligned}
$$

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\begin{aligned}
&+\bar{e}_{21}\,{k2_{2,3}^{2}}^{2}k2_{2,1}^{2}+{k2_{2,3}^{2}}^{2}\bar{r}k1_{2,2}^{1}+2\,P_{\mathit{2}}^{d}\,k2_{2,1}^{2}\bar{e}_{11}\,k1_{2,1}^{1}k2_{2,2}^{2}\bar{r}+2\,P_{\mathit{2}}^{d}\,{k2_{2,1}^{2}}^{2}\bar{e}_{21}\,\bar{r}k1_{2,2}^{1}\\
&+2\,P_{\mathit{2}}^{d}\,k2_{2,1}^{2}\bar{e}_{11}\,k2_{2,3}^{2}k1_{2,2}^{1}+2\,P_{\mathit{2}}^{d}\,k2_{2,1}^{2}\bar{e}_{11}\,k2_{2,3}^{2}k1_{2,1}^{1}+2\,P_{\mathit{2}}^{d}\,k2_{2,1}^{2}\bar{e}_{21}\,k2_{2,2}^{2}f_{2}+2\,P_{\mathit{2}}^{d}\,k2_{2,1}^{2}P_{\mathit{1}}^{d}\,k1_{2,1}^{1}f_{2}\\
&+2\,P_{\mathit{2}}^{d}\,k2_{2,1}^{2}f_{1}k2_{2,2}^{2}\bar{r}+2\,P_{\mathit{2}}^{d}\,k2_{2,1}^{2}k2_{2,3}^{2}\bar{r}k1_{2,2}^{1}+2\,P_{\mathit{2}}^{d}\,k2_{2,1}^{2}\bar{e}_{11}\,f_{2}k1_{2,2}^{1}+2\,P_{\mathit{2}}^{d}\,k2_{2,1}^{2}\bar{e}_{11}\,f_{2}k1_{2,1}^{1}\\
&+2\,P_{\mathit{2}}^{d}\,k2_{2,1}^{2}k1_{2,3}^{1}k2_{2,2}^{2}\bar{r}+2\,P_{\mathit{2}}^{d}\,k2_{2,1}^{2}f_{2}\bar{r}k1_{2,2}^{1}+2\,P_{\mathit{2}}^{d}\,k2_{2,1}^{2}k2_{2,2}^{2}\bar{r}^{2}k1_{2,2}^{1}+2\,P_{\mathit{2}}^{d}\,k2_{2,1}^{2}P_{\mathit{1}}^{d}\,k1_{2,1}^{1}k2_{2,3}^{2}\\
&+P_{\mathit{2}}^{d}\,{k2_{2,1}^{2}}^{2}\bar{e}_{21}\,k2_{2,2}^{2}\bar{r}+2\,P_{\mathit{2}}^{d}\,{k2_{2,1}^{2}}^{2}\bar{e}_{21}\,P_{\mathit{1}}^{d}\,k1_{2,1}^{1}+2\,P_{\mathit{2}}^{d}\,{k2_{2,1}^{2}}^{2}\bar{e}_{21}\,\bar{e}_{11}\,k1_{2,2}^{1}+2\,P_{\mathit{2}}^{d}\,k2_{2,1}^{2}k2_{2,2}^{2}\bar{r}k1_{2,2}^{1}\bar{e}_{11}\\
&+2\,P_{\mathit{2}}^{d}\,{k2_{2,1}^{2}}^{2}\bar{e}_{21}\,\bar{e}_{11}\,k1_{2,1}^{1}+2\,P_{\mathit{2}}^{d}\,k2_{2,1}^{2}P_{\mathit{1}}^{d}\,k1_{2,1}^{1}k2_{2,2}^{2}\bar{r}+2\,k1_{2,2}^{1}\bar{r}P_{\mathit{1}}^{d}\,k1_{2,1}^{1}f_{2}+2\,k1_{2,2}^{1}\bar{r}\bar{e}_{21}\,k1_{2,3}k2_{2,1}^{2}\\
&+2\,k1_{2,2}^{1}\bar{r}k1_{2,3}^{1}P_{\mathit{2}}^{d}\,k2_{2,1}^{2}+2\,k1_{2,2}^{1}\bar{r}k1_{2,1}^{1}\bar{e}_{11}\,k1_{2,3}^{1}+2\,k1_{2,2}^{1}\bar{r}P_{\mathit{1}}^{d}\,k1_{2,1}^{1}k2_{2,3}^{2}+2\,k1_{2,2}^{1}\bar{r}\bar{e}_{21}\,k2_{2,2}^{2}k1_{2,3}^{1}\\
&+2\,k1_{2,2}^{1}\bar{r}\bar{e}_{21}\,P_{\mathit{1}}^{d}\,k1_{2,1}^{1}k2_{2,1}^{2}+2\,k1_{2,2}^{1}{}^{2}\bar{r}\bar{e}_{21}\,\bar{e}_{11}\,k2_{2,1}^{2}+2\,k1_{2,2}{}^{2}\bar{r}\bar{e}_{21}\,P_{\mathit{1}}^{d}\,k1_{2,1}^{1}k2_{2,2}^{2}+2\,k1_{2,2}^{1}\bar{r}P_{\mathit{1}}^{d}\,k1_{2,1}^{1}P_{\mathit{2}}^{d}\,k2_{2,1}^{2}\\
&+2\,k1_{2,2}^{1}{}^{2}\bar{r}\bar{e}_{11}\,P_{\mathit{2}}^{d}\,k2_{2,1}^{2}+k1_{2,2}{}^{2}\bar{r}\bar{e}_{11}\,k1_{2,1}^{1}P_{\mathit{1}}^{d}+2\,k1_{2,2}^{1}\bar{r}^{2}P_{\mathit{1}}^{d}\,k1_{2,1}^{1}k2_{2,2}^{2}+k2_{2,3}^{2}{k1_{2,3}^{1}}^{2}+f_{2}{k1_{2,3}^{1}}^{2}+{k2_{2,3}^{2}}^{2}k1_{2,3}^{1}\\
&+{k2_{2,3}^{2}}^{2}f_{1}+{k1_{2,1}^{1}}^{2}\bar{e}_{11}^{2}P_{\mathit{2}}^{d}\,k2_{2,1}^{2}+{k1_{2,1}^{1}}^{2}\bar{e}_{\mathit{11}}^{2}\bar{e}_{21}\,k2_{2,1}^{2}+{k1_{2,1}^{1}}^{2}\bar{e}_{11}^{2}k1_{2,2}^{1}\bar{r}+{k1_{2,1}^{1}}^{2}\bar{e}_{11}^{2}\bar{e}_{21}\,k\mathit{2}_{2,2}\\
&+2\,{k1_{2,1}^{1}}^{2}\bar{e}_{11}^{2}k1_{2,2}^{1}P_{\mathit{1}}^{d}+2\,k1_{2,1}^{1}P_{\mathit{1}}^{d}\,k2_{2,3}^{2}k1_{2,3}^{1}+2\,k1_{2,1}^{1}P_{\mathit{1}}^{d}\,f_{2}k1_{2,3}^{1}+{k1_{2,1}^{1}}^{2}P_{\mathit{1}}^{d}\,\bar{e}_{11}\,k1_{2,3}^{1}+{k1_{2,1}^{1}}^{2}P_{\mathit{1}}^{d^{2}}\bar{e}_{21}\,k2_{2,1}^{2}\\
&+{k1_{2,1}^{1}}^{2}P_{\mathit{1}}^{d^{2}}\bar{e}_{21}\,k2_{2,2}^{2}+{k1_{2,1}^{1}}^{2}P_{\mathit{1}}^{d^{2}}P_{\mathit{2}}^{d}\,k2_{2,1}^{2}+{k1_{2,1}^{1}}^{2}P_{\mathit{1}}^{d^{2}}k1_{2,2}^{1}\bar{e}_{11}+{k1_{2,1}^{1}}^{2}P_{\mathit{1}}^{d^{2}}k2_{2,2}^{2}\bar{r}+{k1_{2,2}^{1}}^{2}\bar{e}_{11}^{2}\bar{e}_{21}\,k2_{2,1}^{2}\\
&+{k1_{2,2}^{1}}^{2}\bar{e}_{11}^{2}k2_{2,2}^{2}\bar{r}+{k1_{2,2}^{1}}^{2}\bar{e}_{11}^{2}P_{\mathit{2}}^{d}\,k2_{2,1}^{2}+{k1_{2,2}^{1}}^{2}\bar{e}_{11}^{2}k1_{2,1}^{1}P_{\mathit{1}}^{d}+{k2_{2,2}^{2}}^{2}\bar{r}^{2}\bar{e}_{11}\,k1_{2,1}^{1}+2\,{k2_{2,2}^{2}}^{2}\bar{r}^{2}k1_{2,2}^{1}\bar{e}_{21}\\
&+2\,k2_{2,2}^{2}\bar{r}k2_{2,3}^{2}k1_{2,3}^{1}+2\,k2_{2,2}^{2}\bar{r}k2_{2,3}^{2}f_{1}+{k2_{2,2}^{2}}^{2}\bar{r}\bar{e}_{21}\,f_{2}+2\,{k2_{2,2}^{2}}^{2}\bar{r}\bar{e}_{21}\,f_{1}+2\,k2_{2,2}^{2}\bar{r}^{2}k2_{2,3}^{2}k1_{2,2}^{1}\\
&+2\,{k2_{2,2}^{2}}^{2}\bar{r}\bar{e}_{21}^{2}k2_{2,1}^{2}+2\,{k2_{2,2}^{2}}^{2}\bar{r}\bar{e}_{21}\,k1_{2,3}^{1}+{k2_{2,2}^{2}}^{2}\bar{r}^{2}\bar{e}_{21}\,k2_{2,1}^{2}+k\mathit{2}_{2,2}{}^{2}\bar{r}^{2}k1_{2,2}^{1}\bar{e}_{11}\\
&+{k2_{2,2}^{2}}^{2}\bar{r}^{2}P_{\mathit{1}}^{d}\,k1_{2,1}^{1}+\bar{e}_{21}^{2}{k2_{2,1}^{2}}^{2}\bar{r}k1_{2,2}^{1}+\bar{e}_{21}^{2}{k2_{2,1}^{2}}^{2}k2_{2,2}^{2}\bar{r}+\bar{e}_{21}^{2}{k2_{2,1}^{2}}^{2}P_{\mathit{1}}^{d}\,k1_{2,1}^{1}+\bar{e}_{21}^{2}{k2_{2,1}^{2}}^{2}\bar{e}_{11}\,k1_{2,2}^{1}\\
&+\bar{e}_{21}^{2}{k2_{2,1}^{2}}^{2}\bar{e}_{11}\,k1_{2,1}^{1}+\bar{e}_{21}^{2}{k2_{2,2}^{2}}^{2}\bar{r}k1_{2,2}^{1}+\bar{e}_{21}^{2}{k2_{2,2}^{2}}^{2}P_{\mathit{1}}^{d}\,k1_{2,1}^{1}+\bar{e}_{21}^{2}{k2_{2,2}^{2}}^{2}P_{\mathit{2}}^{d}\,k2_{2,1}^{2}+\bar{e}_{21}^{2}{k2_{2,2}^{2}}^{2}\bar{e}_{11}\,k1_{2,1}^{1}\\
&+2\,f_{1}\bar{e}_{11}\,k2_{2,3}^{2}k1_{2,1}^{1}+2\,{k1_{2,1}^{1}}^{2}\bar{e}_{11}\,\bar{e}_{21}\,P_{\mathit{1}}^{d}\,k2_{2,1}^{2}+2\,{k1_{2,1}^{1}}^{2}\bar{e}_{11}\,\bar{e}_{21}\,P_{\mathit{1}}^{d}\,k2_{2,2}^{2}+2\,{k1_{2,1}^{1}}^{2}\bar{e}_{11}\,P_{\mathit{1}}^{d}\,P_{\mathit{2}}^{d}\,k2_{2,1}^{2}\\
&+2\,{k1_{2,1}^{1}}^{2}\bar{e}_{11}\,P_{\mathit{1}}^{d}\,k2_{2,2}^{2}\bar{r}+2\,k1_{2,1}^{1}P_{\mathit{1}}^{d}\,k1_{2,3}^{1}k2_{2,2}^{2}\bar{r}+2\,k1_{2,1}P_{\mathit{1}}^{d}\,\bar{e}_{21}\,k1_{2,3}^{1}k2_{2,1}^{2}+2\,k1_{2,1}^{1}P_{\mathit{1}}^{d}\,k1_{2,3}^{1}P_{\mathit{2}}^{d}\,k2_{2,1}^{2}\\
&+2\,k1_{2,1}^{1}P_{\mathit{1}}^{d}\,\bar{e}_{21}\,k2_{2,2}^{2}k1_{2,3}^{1}+k1_{2,1}^{1}{}^{2}P_{\mathit{1}}^{d}\,k1_{2,2}^{1}\bar{r}\bar{e}_{11}+k1_{2,2}^{1}{}^{2}\bar{e}_{11}\,k2_{2,2}^{2}\bar{r}\bar{e}_{21}\,f_{2}+2\,k2_{2,2}^{2}\bar{e}_{\mathit{11}}\,\bar{e}_{21}\,k2_{2,2}^{2}f_{2}\\
&+2\,k1_{2,2}^{1}\bar{e}_{11}\,\bar{e}_{21}\,k2_{2,2}^{2}f_{1}+2\,k1_{2,2}^{1}\bar{e}_{11}\,\bar{e}_{21}^{2}k2_{2,2}^{2}k2_{2,1}^{2}+k1_{2,2}^{1}\bar{e}_{11}\,\bar{e}_{21}\,k2_{2,2}^{2}k1_{2,3}^{1}+2\,k1_{2,2}^{1}\bar{e}_{11}\,\bar{e}_{21}\,P_{\mathit{1}}^{d}\,k1_{2,1}^{1}k2_{2,2}^{2}\\
&+2\,k1_{2,2}^{1}\bar{e}_{11}\,\bar{e}_{21}\,k2_{2,2}^{2}P_{\mathit{2}}^{d}\,k2_{2,1}^{2}+2\,k1_{2,2}^{1}\bar{e}_{11}^{2}\bar{e}_{21}\,k2_{2,2}^{2}k1_{2,1}^{1}+2\,k2_{2,2}^{2}\bar{r}\bar{e}_{11}\,k2_{2,3}^{2}k1_{2,2}^{1}+2\,k2_{2,2}^{2}\bar{r}\bar{e}_{11}\,k2_{2,3}^{2}k1_{2,1}^{1}\\
&+2\,k2_{2,2}^{2}\bar{r}\bar{e}_{21}\,k2_{2,3}^{2}k2_{2,1}^{2}+2\,k2_{2,2}^{2}\bar{r}P_{\mathit{1}}^{d}\,k1_{2,1}^{1}k2_{2,3}^{2}+2\,{k2_{2,2}^{2}}^{2}\bar{r}\bar{e}_{21}\,P_{\mathit{1}}^{d}\,k1_{2,1}^{1}+k2_{2,2}^{2}{}^{2}\bar{r}\bar{e}_{21}\,P_{\mathit{2}}^{d}\,k2_{2,1}^{2}\\
&+2\,{k2_{2,2}^{2}}^{2}\bar{r}\bar{e}_{21}\,\bar{e}_{\mathit{11}}\,k2_{2,1}^{1}+P_{\mathit{1}}^{d}\,k2_{2,1}^{1}{k2_{2,3}^{2}}^{2}+\bar{e}_{21}^{2}{k2_{2,1}^{2}}^{2}k2_{2,3}^{2}+\bar{e}_{21}^{2}{k2_{2,1}^{2}}^{2}f_{1}+\bar{e}_{21}^{2}{k2_{2,1}^{2}}^{2}k1_{2,3}+\bar{e}_{21}^{3}{k2_{2,1}^{2}}^{2}k2_{2,2}^{2}\\
&+\bar{e}_{21}^{2}{k2_{2,2}^{2}}^{2}f_{2}+\bar{e}_{21}^{2}{k2_{2,2}^{2}}^{2}f_{1}+\bar{e}_{21}^{3}{k2_{2,2}^{2}}^{2}k2_{2,1}^{2}+\bar{e}_{21}^{2}{k2_{2,2}^{2}}^{2}k1_{2,3}^{1}+2\,f_{1}k\mathit{2}_{2,3}k1_{2,3}^{1}+2\,f_{1}f_{2}k1_{2,3}^{1}\\
&+2\,f_{2}k2_{2,3}^{2}f_{1}+\bar{e}_{21}\,k2_{2,2}^{2}\bar{e}_{11}\,k2_{2,3}^{2}k1_{2,2}^{1}+\bar{e}_{21}\,{k2_{2,2}^{2}}^{2}\bar{r}k1_{2,2}^{1}\bar{e}_{11}+2\,f_{1}\bar{e}_{11}\,k1_{2,1}^{1}k2_{2,2}^{2}\bar{r}+2\,f_{1}k2_{2,2}^{2}\bar{r}k1_{2,2}^{1}\bar{e}_{21}\\
&+2\,f_{1}\bar{e}_{21}\,k\mathit{2}_{2,1}\bar{r}k1_{2,2}^{1}+2\,f_{1}P_{\mathit{1}}^{d}\,k1_{2,1}^{1}f_{2}+2\,f_{1}k2_{2,3}^{2}\bar{r}k1_{2,2}^{1}+2\,f_{1}\bar{e}_{11}\,f_{2}k1_{2,1}^{1}+2\,f_{1}k1_{2,3}^{1}k2_{2,2}^{2}\bar{r}\\
&+2\,f_{1}\bar{e}_{21}\,k1_{2,3}^{1}k2_{2,1}^{2}+2\,f_{1}f_{2}\bar{r}k1_{2,2}^{1}+2\,f_{1}k1_{2,2}^{1}k1_{2,1}^{1}\bar{e}_{11}^{2}+2\,f_{1}k1_{2,3}^{1}P_{\mathit{2}}^{d}\,k2_{2,1}^{2}+f_{1}k1_{2,1}^{1}\bar{e}_{11}\,k1_{2,3}^{1}\\
&+2\,f_{1}k2_{2,2}^{2}\bar{r}^{2}k1_{2,2}^{1}+2\,f_{1}P_{\mathit{1}}^{d}\,k1_{2,1}^{1}k2_{2,3}^{2}+2\,f_{1}\bar{e}_{21}\,k2_{2,2}^{2}k1_{2,3}^{1}+2\,f_{1}\bar{e}_{11}\,k2_{2,3}^{2}k1_{2,2}^{1}+2\,f_{1}\bar{e}_{11}\,k2_{2,3}^{2}k1_{2,1}^{1}\\
&+2\,f_{2}f_{1}k2_{2,2}^{2}\bar{r}+f_{2}\bar{e}_{21}\,k2_{2,3}^{2}k2_{2,1}^{2}+2\,f_{2}k2_{2,3}^{2}\bar{r}k1_{2,2}+2\,f_{2}\bar{e}_{21}\,f_{1}k2_{2,1}^{2}+2\,f_{2}k1_{2,3}^{1}k2_{2,2}^{2}\bar{r}+2\,f_{2}\bar{e}_{21}\,k1_{2,3}^{1}k2_{2,1}^{2}\\
&+2\,f_{2}\bar{e}_{21}^{2}k2_{2,2}^{2}k2_{2,1}^{2}+2\,f_{2}k2_{2,2}^{2}\bar{r}^{2}k1_{2,2}^{1}+2\,f_{2}P_{\mathit{1}}^{d}\,k1_{2,1}k2_{2,3}^{2}+2\,k1_{2,3}^{1}\bar{e}_{11}\,k2_{2,3}^{2}k1_{2,2}^{1}+2\,k1_{2,3}^{1}\bar{e}_{11}\,f_{2}k1_{2,2}^{1}\\
&+k1_{2,3}^{1}k1_{2,2}^{1}f_{1}\bar{e}_{11}+2\,k1_{2,3}^{1}k1_{2,2}^{1}k1_{2,1}^{1}\bar{e}_{11}^{2}+k2_{2,3}^{2}\bar{e}_{21}\,k2_{2,2}^{2}f_{2}+2\,k2_{2,3}^{2}\bar{e}_{21}\,k2_{2,2}^{2}f_{1}+2\,k2_{2,3}^{2}\bar{e}_{21}^{2}k2_{2,2}^{2}k2_{2,1}^{2}\\
&+2\,k2_{2,3}^{2}\bar{e}_{21}\,k2_{2,2}^{2}k1_{2,3}^{1}+2\,f_{1}\bar{e}_{11}\,k2_{2,1}^{1}P_{\mathit{2}}^{d}\,k2_{2,1}^{2}+2\,f_{1}\bar{e}_{21}\,P_{\mathit{1}}^{d}\,k1_{2,1}^{1}k2_{2,1}^{2}+2\,f_{1}\bar{e}_{21}\,\bar{e}_{11}\,k2_{2,1}^{1}k2_{2,1}^{1}\\
&+2\,f_{1}\bar{e}_{21}\,P_{\mathit{1}}^{d}\,k1_{2,1}^{1}k2_{2,2}^{2}+2\,f_{1}P_{\mathit{1}}^{d}\,k1_{2,1}P_{\mathit{2}}^{d}\,k2_{2,1}^{2}+19f_{1}k1_{2,2}^{1}\bar{r}k1_{2,1}\bar{e}_{11}+2\,f_{1}P_{\mathit{2}}^{d}\,k2_{2,1}^{2}\bar{r}k1_{2,2}^{1}
\end{aligned}
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\begin{aligned}
&+2\,f_1\bar{e}_{21}\,\bar{e}_{11}\,k^2_{2,2}k^1_{2,1}+2\,f_1k^1_{2,2}\bar{e}_{11}\,k^1_{2,1}P^d_1+2\,f_1P^d_1\,k^1_{2,1}k^2_{2,2}\bar{r}+2\,f_2\bar{e}_{11}\,k1_{2,1}k^2_{2,2}\bar{r}\\
&+2\,f_2\bar{e}_{21}\,k^2_{2,1}\bar{r}k^1_{2,2}+f_2\bar{e}_{21}\,k^2_{2,1}k^2_{2,2}\bar{r}+2\,f_2\bar{e}_{21}\,P^d_1\,k^1_{2,1}k^2_{2,1}+2\,f_2\bar{e}_{21}\,\bar{e}_{11}\,k^2_{2,1}k^1_{2,2}\\
&+2\,f_2k^2_{2,2}\bar{r}k^1_{2,2}\bar{e}_{11}+2\,f_2\bar{e}_{21}\,\bar{e}_{11}\,k2_{2,1}k^1_{2,1}+2\,f_2P^d_1\,k^1_{2,1}k2_{2,2}\bar{r}+2\,k^1_{2,3}\bar{e}_{21}\,\bar{e}_{11}\,k^2_{2,1}k^1_{2,2}\\
&+2\,k^1_{2,3}k^2_{2,2}\bar{r}k^1_{2,2}\bar{e}_{11}+2\,k^1_{2,3}\bar{e}_{11}\,P^d_2\,k^2_{2,1}k^1_{2,2}+k^1_{2,3}k^1_{2,2}\bar{e}_{11}\,k^1_{2,1}P^d_1+2\,k^2_{2,3}k^2_{2,2}\bar{r}k^1_{2,2}\bar{e}_{21}\\
&+2\,k^2_{2,3}\bar{e}_{21}\,P^d_1\,k^1_{2,1}k^2_{2,2}+k^2_{2,3}\bar{e}_{21}\,k^2_{2,2}P^d_2\,k^2_{2,1}+2\,k^2_{2,3}\bar{e}_{21}\,\bar{e}_{11}\,k^2_{2,2}k^1_{2,1}>0, \qquad (4.5)
\end{aligned}
$$

$$
b_2 = \frac{a_1a_4}{a_1}=a_4>0, \qquad (4.6)
$$

$$
c_1 = \frac{b_1a_3-a_1b_2}{b_1}>0, \qquad (4.7)
$$

$$
d_1 = \frac{b_2c_1-b_1*0}{c_1}=b_2=a_4>0. \qquad (4.8)
$$

Because the expression of c_1 is over 200 pages long, it is impractical to include it here. Using the Maple software, we checked a number of times and found that all terms in the expression are positive. Therefore the first column of Routh's array is all positive and then all the real parts of the roots of the quartic polynomial are negative. □

Although we could not prove that the characteristic polynomial (4.2) is true for generic m and n, we guess so since we verified it for a number of combinations of m and n with $m, n \leq 3$ using the Maple software. For large m and n, it takes long long time (weeks or months) for the Maple to compute the polynomial.

5 Numerical Examples and Applications

We now further numerically test that the feedback inhibition rates k^1_{ic} and k^2_{ic} determined mathematically through (3.20) and (3.21) are working effectively in regulating products. We first use random data with no biological relevance. So we take the initial enzyme $E^0 = 400$, all the other subsequent initial enzymes $E^0_{i,j} = 1000$, the initial substrate $S^0 = 50$, the reaction constants $k^0 = [0.000002,\ 0.0000045,\ 0.000023]$,

$$
\mathbf{k^1} = \begin{pmatrix}
0.00001 & 0.03 & 0.05 \\
0.000003 & 0.02 & 0.03 \\
0.0006 & 0.005 & 0.007 \\
0.00004 & 0.002 & 0.008 \\
0.000013 & 0.0012 & 0.005 \\
0.000026 & 0.00345 & 0.0057 \\
0.000201 & 0.0803 & 0.00125 \\
0.000013 & 0.0012 & 0.005 \\
0.0000043 & 0.0302 & 0.00013
\end{pmatrix}
$$

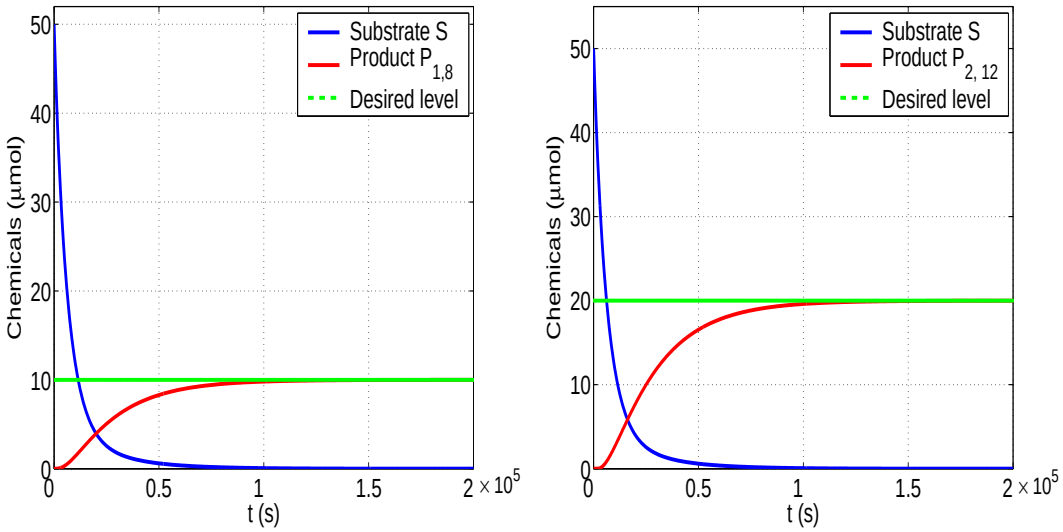

Figure 4: Regulation of products by competitive feedback inhibition.

$$\mathbf{k^2} = \begin{pmatrix} 0.00001 & 0.03 & 0.05 \\ 0.000003 & 0.02 & 0.03 \\ 0.0006 & 0.005 & 0.007 \\ 0.00004 & 0.002 & 0.008 \\ 0.000013 & 0.0012 & 0.005 \\ 0.000026 & 0.00345 & 0.0057 \\ 0.000201 & 0.0803 & 0.00125 \\ 0.000013 & 0.0012 & 0.005 \\ 0.0000043 & 0.00302 & 0.0013 \\ 0.000036 & 0.0065 & 0.0097 \\ 0.0000021 & 0.00603 & 0.00805 \\ 0.000026 & 0.00345 & 0.0057 \\ 0.00000467 & 0.000572 & 0.05763 \\ 0.0456 & 0.0575 & 0.0000397 \end{pmatrix}.$$

Using the `ode45` from MATLAB, we numerically solve the system (2.25)-(2.34) and plot these numerical solutions in Figure 4. This figure clearly indicates that the end-products are regulated to the given levels 10 and 20, respectively. The feedback inhibition causes the regulatory enzymes $E_{1,1}$ and $E_{2,1}$ to stop producing excess amounts of the products, which is how the products converge to the desired concentrations.

We next consider a well known example of regulatory feedback inhibition which occurs in the Purine Metabolism, specifically the biosynthesis of adenosine 5′-monophosphate (AMP) and guanosine 5′-monophosphate (GMP) [21, 24]. In this metabolic pathway inosine monophosphate (IMP) is the initial metabolite and the regulatory enzymes A_1 and G_1 are the first branched steps that compete for IMP as seen in Figure 1.

The Purine Biosynthesis metabolic pathway serves as crucial role in DNA and RNA synthesis, intermediates in biosynthetic reactions, energy storage and metabolic regulators. Enzymes pertaining purine biosynthetic pathways have been linked to noteworthy disorders

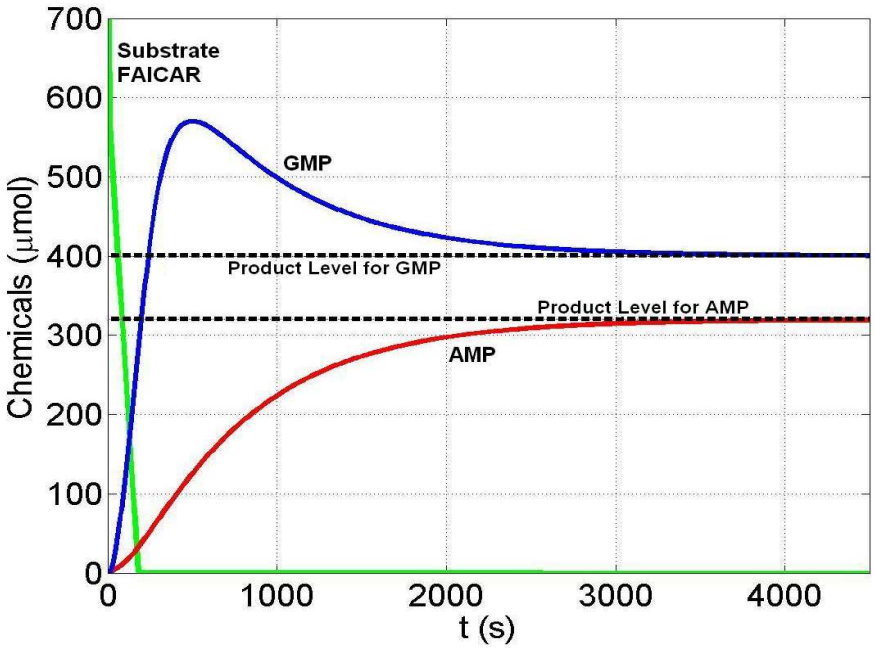

Figure 5: Regulation of IMP and AMP to desire product levels

such as Down's syndrome, Lesh-Nyhan syndrome[20], immunodeficiencies [11] and cancer[20].

For simplification purposes, we have excluded secondary regulatory controls such as ATP, GDP and GTP inhibitive concentrations[24], as well as the other mixed inhibitions within the metabolic network. We assume that the system is in appropriate ph, temperature, and other conditions so that all enzyme activity can be depicted through their respective Michaelis-Menten Constants $k^i_{m,j}$ shown in Figure 1, which are defined by

$$k^i_{m,j} = \frac{k^i_{j,2} + k^i_{j,3}}{k^i_{j,1}}, \quad \text{where } i = 1, 2 \text{ and } j = 1...m \text{ or } n \tag{5.1}$$

Although Michaelis-Menten Constants $k^i_{m,j}$ are available from experiments, the reaction constants $k^i_{j,2}, k^i_{j,3}, k^i_{j,1}$ are usually difficult to be determined in experiments. So we randomly generate the constants $k^i_{j,2}, k^i_{j,3}$ and then get $k^i_{j,1}$ through the equation (5.1). With these reaction constants and the inhibition rates determined mathematically through (3.20) and (3.21), we use the MATLAB to numerically solve the system (2.25)-(2.34) again. Figure 5 shows that the products are successfully regulated to the given levels $400\mu mol$ for GMP and $320\mu mol$ for AMP, respectively.

The numeric analysis of these simplified metabolic examples reveals that the feedback inhibition rates determined mathematically through (3.20) and (3.21) can be utilized for better understanding of competitive feedback inhibition of products in cell metabolism.

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