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ORIGINAL PAPER

# Response analysis in biochemical chain reactions with negative feedforward and feedbackward loops

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**Abstract** Finite difference equations can be used to study the responses of biochemical chain reactions at any step of the chain to an external stimulus. In this study, we developed mathematical models for two hypothetical chain reactions involving loops to study the responses in the chain as the length of the chain gets longer, so called transient and steady state responses. The first model is for a chain with a negative feedforward loop, and the second one is for a chain that has a negative feedback loop. Although both of the models have the same steady state equations and values, we showed that the chain with negative feedforward and negative feedback loops can produce significantly different behaviors. The former can bring the chain into oscillations with various periods and eventually chaos when the feedback is strong enough as the length of the reaction chain increases, whereas the latter is not capable of producing oscillations and more complicated dynamics.

**Keywords** Mathematical model · Difference equation · Chaos · Reaction kinetics · Cell signaling

## 1 Introduction

Although many intracellular regulatory networks have been extensively studied using advanced experimental techniques, it has turned out to be quite difficult to make predictions in terms of the regulation of these networks. How individual components of this cellular machine are assembled and regulated and how the behavior of metabolism as a whole are related with the properties of the individual components of this

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machine are still not completely understood. Mathematical models are extensively used to study various aspects of biological systems at large and are widely accepted as promising tools to investigate and address some of the biologically challenging questions [2–4,10,11].

In this study, we used difference equations to model two hypothetical biochemical chain reactions. These chain reactions can be thought of as signaling pathway activation at the receptor level by a stimulus and the subsequent changes in the protein levels at each step in the subsequent reactions in a pathway as responses. These stimuli undergo smaller reactions as the main reaction occurs to create new stimuli levels of different strengths and concentrations. Trzeciakowski studied steady state behaviors of linear chain reactions as a length of the chain increases [6–9]. He developed a simple nonlinear difference equation model that describes the steady state concentration of a protein at n-th step of the chain as a function of the steady state concentration of a protein in the previous step as

$$[\mathrm{S_n}] = \mathrm{f}([\mathrm{S_{n-1}}]) \tag{1.1}$$

We extended Trzeciakowski's study including two different types of negative regulatory loops: (1) negative feedforward loops and (2) negative feedback loops. Then we developed two mathematical models. The models are one-dimensional nonlinear equations with three positive adjustable parameters. Then we performed a complete steady state, stability and bifurcation analysis on our models.

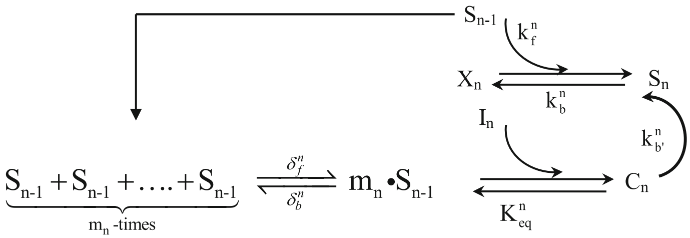
The outline of the paper is as follows. In Section 2, we describe the derivations of the models and give a complete steady state, stability and bifurcation analysis for each model. Section 3 summarizes our findings and compares these two models. The paper ends with Section 4 which includes a discussion.

## 2 Modeling chain reactions with loops and difference equations

Cellular reaction networks usually involve many different regulatory motifs including positive and negative feedback and feedforward loops. It is known that the positive feedback loops with enough nonlinearity can produce bistable behavior, and the negative feedback loop with strong enough nonlinearities can generate oscillation [5]. In this study, we focus on one step chain reactions with negative feedforward and negative feedback loops and investigate effects of such loops on the systems behavior as the length of the chain increases. One of the questions we aim to address in this study is that how these two different mechanisms are similar and how there are different in terms of transient and long term behaviors. This is particularly important if one wants to know reaction mechanisms from experimental data.

### 2.1 Chain reactions involving negative feedforward loops

We will derive a mathematical model that governs steady state concentrations of proteins in a biochemical chain reaction with negative feedforward loops at each step of the chain as the length of the chain increases. To develop the mathematical model



**Scheme 1** Schematic representation of the biochemical reactions at the n-th step of the reaction chain with a negative feedforward loop. $S_{n-1}$ is required for conversion of $X_n$ into $S_n$. $m_n$-molecules of $S_{n-1}$ come together to form a complex that interacts with $I_n$ to form another complex $C_n$, which increases conversion rate of $S_n$ into $X_n$ (the negative feedback). There is also a spontaneous conversion of $S_n$ into $X_n$ which is independent of this feedback

that governs the steady state concentration at the n-th step of the reaction chain it will be helpful to refer to Schema 1.

In this hypothetical system, we assume that the production rate of $S_n$ at the n-th step of the chain is proportional to the concentration of the product at the previous step ($S_{n-1}$) with the proportionality constant $k_f^n$. The backward rate of the reaction at the n-th step of the chain is the sum of two rates, that is $\left(k_b^n + k_{b'}^n[C_n]\right)[S_{n-1}]$. Here $k_b^n$ is the rate constant for the spontaneous conversion of $S_n$ into $X_n$. The contribution of the feedforward loop is to increase the conversion rate of $S_n$ into $X_n$ by $k_{b'}^n[C_n]$. We assume that $m_n$ molecules of the product of the previous step come together to form a complex and these interactions are at equilibrium,

$$\left[m_n \cdot S_{n-1}\right] = \frac{[S_{n-1}]^{m_n}}{\Gamma_{eq}^n}, \tag{1.2}$$

where $\Gamma_{eq}^n$ is given by $\Gamma_{eq}^n = \frac{\delta_b^n}{\delta_f^n}$. Then this complex interacts with a protein $I_n$ to form another complex $C_n$. Our model also assumes that $I_n$ is in abundance, which allows us to treat it as a parameter, rather than a variable.

At equilibrium, the formation of the protein $C_n$ at n-th step is given by

$$[C_n] = \frac{[m_n \cdot S_{n-1}][I_n]}{K_{eq}^n} \tag{1.3}$$

Here $K_{eq}^n$ is the dissociation constant for this reaction. The smaller this constant is the faster the $S_{n-1}$ binds to $I_n$. As in [7], our model assumes that $[m_n \cdot S_{n-1}]$ is negligible compared to $[X_n]$ and $[S_n]$ and the total $S_n$ concentration at every step remains constant over time. This allows us to write down the following linear equation:

$$[X_n] + [S_n] = S_{tot}^n \tag{1.4}$$

According to mass action kinetics, the differential equation that governs the temporal change of the $S_n$ concentration becomes

$$\frac{d[S_n]}{dt} = k_f^n[X_n][S_{n-1}] - \left(k_b^n + k_{b'}^n[C_n]\right)[S_n] \tag{1.5}$$

Since we are interested in the steady state behaviors of the products it is reasonable to write

$$\frac{d[S_n]}{dt} = 0 \tag{1.6}$$

After substituting Eqs. (1.2) and (1.3) into Eq. (1.5) and solving the resultant equation under Eq. (1.6) for $[X_n]$ we get

$$[X_n] = \frac{\left(k_b^n + \frac{k_{b'}^n[I_n][S_{n-1}]^{m_n}}{\Gamma_{eq}^n K_{eq}^n}\right)[S_n]}{k_f^n[S_{n-1}]} \tag{1.7}$$

Then plugging Eq. (1.7) into Eq. (1.4) and solving it for $[S_n]$ gives us the steady state concentration of the product at the n-th step of the chain in terms of $\left[S_{n-1}\right]$ as

$$\frac{[S_n]}{S_{tot}^n} = \frac{k_f^n\Gamma_{eq}^n K_{eq}^n[S_{n-1}]}{k_b^n\Gamma_{eq}^n K_{eq}^n + k_f^n\Gamma_{eq}^n K_{eq}^n[S_{n-1}] + k_{b'}^n[S_{n-1}]^{m_n}[I_n]} \tag{1.8}$$

From now on, we will assume that at every step in the reaction $S_{tot}^n = S_{tot}$, $K_{eq}^n = K_{eq}$, $\Gamma_{eq}^n = \Gamma_{eq}$, $k_f^n = k_f$, $k_b^n = k_b$, $k_{b'}^n = k_{b'}$ $[I_i] = [I]$, $m_n = m$ and $m \geq 1$ is an integer. Without loss of generality, we will assume that the highest value for $\frac{[S_n]}{S_{tot}^n}$ is 1. If we define $\alpha_1 = \frac{k_b}{k_f}$ and $\alpha_2 = \frac{k_{b'}[I]}{k_f K_{eq}\Gamma_{eq}}$ then Eq. (1.8) becomes a non-linear first order difference equation with three positive parameters $\alpha_1, \alpha_2$ and m as follows:

$$[S_n] = \frac{[S_{n-1}]}{\alpha_1 + [S_{n-1}] + \alpha_2[S_{n-1}]^m} \tag{1.9}$$

which describes the steady state concentration of the protein at the n-th step of the chain in terms of that of the protein in the previous chain.

### *2.1.1 Steady state analysis*

Suppose that $[S^*]$ represents the concentration of the protein at the reaction step n at which this concentration is also equal to that of the product in the previous reaction; that is to say, $[S^*] = f([S^*])$. We name this value of [S] as the steady state. At the steady state, Eq. (1.9) becomes

$$[S^*] = \frac{[S^*]}{\alpha_1 + [S^*] + \alpha_2[S^*]^m} \tag{1.10}$$

It can be seen from this equation that $[S^*] = 0$ is a trivial solution and zero steady state for any values of the parameters $\alpha_1, \alpha_2$ and m. The possible nonzero steady state values come from the solution of

$$\alpha_1 + [S^*] + \alpha_2[S^*]^m = 1 \tag{1.11}$$

which is the value that the function g crosses [S]-axis where

$$g([S]) = \alpha_2[S]^m + [S] + \alpha_1 - 1 \tag{1.12}$$

g is a polynomial function with degree m and it is continues everywhere on the real number line.

**Theorem 1** *When $\alpha_1 - 1 < 0$, the function g in Eq. (1.12) crosses positive part of the [S]-axis at least once for any positive integer value of m. Furthermore, if $m = 1$ then this positive intersection point is always less than one and it is a nonzero steady state for this model given by Eq. (1.9).*

*Proof* Since g is a continuous function, $\lim_{[S]\to\infty} g([S]) \to \infty$ and $g(0) = \alpha_1 - 1 < 0$, according to the Intermediate Value Theorem there has to be a positive $[S^*]$ such that $g([S^*]) = 0$. Since the number of sign changes in the coefficient of the polynomial g is 1, then Descartes' rule of sign changes theorem assures us that this is the only positive root of $g([S]) = 0$. When $m = 1$, $[S] = \frac{1-\alpha_1}{\alpha_2+1} < 1$. This completes the proof.

**Theorem 2** *The function g in Eq. (1.12) can cross the positive part of the [S]-axis one time at most for any positive integer value of m.*

*Proof* Let's assume that $g([S]) = 0$ has two distinct positive solutions $[S_1]$ and $[S_2]$. That is to say

$$g'[S] = 0 \tag{1.13}$$

has to have one positive solution. But $g'([S]) = m[S]^{m-1} + 1 = 0$, and since m is a positive integer Eq. (1.13) has no positive solution and this is a contradiction. Therefore $g([S]) = 0$ has at most one positive solution, and this completes the proof.

*2.1.2 Stability analysis*

In this section, we investigate the local stability and bifurcation analysis of the model with a negative feedforward loop given in Eq. (1.9), which is a nonlinear first-order difference equation with three parameters. Let's assume $[S^*]$ is one of the steady state solutions of Eq. (1.9). If

$$\left| \left.\frac{df([S])}{d[S]}\right|_{[S]=[S^*]} \right| < 1 \tag{1.14}$$

then [S*] is a locally stable steady state. If

$$\left| \frac{df([S])}{d[S]} \bigg|_{[S]=[S^*]} \right| > 1 \tag{1.15}$$

then this steady state is locally unstable. If

$$\left| \frac{df([S])}{d[S]} \bigg|_{[S]=[S^*]} \right| = 1 \tag{1.16}$$

then [S*] could be a locally stable or unstable steady state that requires further analysis. Furthermore, it is also possible that [S] = [S*] can be a bifurcation point in which qualitative behaviors of the solution of Eq. (1.9) can change. We pay special attention to the solution of Eq. (1.16) in the parameters space to investigate any sort of bifurcations in this paper.

The right hand side of Eq. (1.9) is a continuous and smooth function of [S] and its derivative with respect to [S] becomes

$$\frac{df([S])}{d[S]} = \frac{\alpha_1 + (1-m)\alpha_2[S]^m}{(\alpha_1 + [S] + \alpha_2[S]^m)^2} \tag{1.17}$$

At $[S^*] = 0$, this derivative becomes $\frac{df([S])}{d[S]}\Big|_{[S^*]=0} = 1/\alpha_1$ which is stable whenever $|1/\alpha_1| < 1$ and this steady state becomes unstable when $|1/\alpha_1| > 1$. This derivative can also be equal to 1 when $\alpha_1 = 1$ which means that for this specific value of $\alpha_1$, $[S^*] = 0$ could be stable, unstable or a bifurcation point. When we differentiate Eq. (1.17) one more time with respect to [S], we get

$$\frac{d^2f([S])}{d[S]^2} = \frac{\left((1-m)m\alpha_2[S]^{m-1}\right)(\alpha_1+[S]+\alpha_2[S]^m)^2 - 2\left(\alpha_1+(1-m)\alpha_2[S]^m\right)(\alpha_1+[S]+\alpha_2[S]^m)(1+\alpha_2 m[S]^{m-1})}{(\alpha_1+[S]+\alpha_2[S]^m)^4} \tag{1.18}$$

This derivative at $[S^*] = 0$ becomes $\frac{d^2f([S])}{d[S]^2} = \Big|_{[S^*]=0} = \frac{-2}{\alpha_1^2} \neq 0$ for any positive values of $\alpha_1$. This guarantees that $[S^*] = 0$ is an unstable steady state when $\alpha_1 = 1$ [1].

If we look at the value of Eq. (1.17) at the nonzero-steady state [S*] given by Eq. (1.11) which is only possible when $\alpha_1 - 1 < 0$ and check if

$$\frac{df([S])}{d[S]}\bigg|_{[S]=[S^*]} = 1 \tag{1.19}$$

has a positive solution, we see that in fact $[S^*] = \frac{m(1-\alpha_1)}{m-1} > 0$ is a solution of Eq. (1.19)and it can be made less than one when $1 - m\alpha_1 < 0$. Therefore, the derivative given in Eq. (1.17) can be 1 for a set of positive parameters and this point could be a bifurcation point. When we look at the second derivative given by Eq. (1.18) at this nonzero nonzero-steady state, we get

$$\left.\frac{d^2f([S])}{d[S]^2}\right|_{[S]=[S^*]} = -(m+1)\alpha_2 m[S]^{m-1} - 2 \neq 0 \tag{1.20}$$

which means that this steady state is always unstable and can't be a bifurcation point.
When we look at solution of

$$\left.\frac{df([S])}{d[S]}\right|_{[S]=[S^*]} = -1 \tag{1.21}$$

at nonzero steady state given by Eq. (1.11), we see that this equation can have a positive solution for $m > 1$ as

$$\alpha_1 + (1-m)\alpha_2[S^*]^m + 1 = 0 \Rightarrow [S^*] = \sqrt[m]{\frac{1+\alpha_1}{(m-1)\alpha_2}} \tag{1.22}$$

and this solution can be made less than 1 by increasing the parameter $\alpha_2$ which can be a bifurcation point for this system. In order to decide if $[S] = [S^*]$ is a bifurcation point for a set of parameters, it is possible to look at the Schwarzian derivative which includes the second and the third derivatives of the right hand side of the function in Eq. (1.9) and check its sign to decide stability of the steady state. That may give some answers but this derivative is not easy to study analytically. We, instead, prefer to study this case numerically.

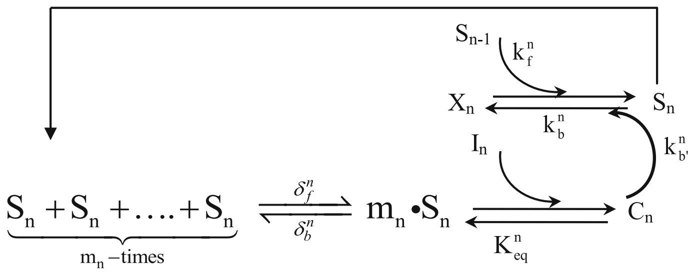
## 2.2 Chain reactions involving negative feedback loops

In this section, we will derive a mathematical model that governs steady state concentrations of proteins in a biochemical chain reaction with negative feedback loops at each step of the chain as the length of the chain increases. In this scenario, m-molecules of $S_n$ not $S_{n-1}$ are catalyzing the backward reaction as seen in Schema 2.

The derivation of the mathematical model is very similar to the one with negative feedforward loop and becomes

$$[S_n] = \frac{[S_{n-1}]}{\alpha_1 + [S_{n-1}] + \alpha_2[S_n]^m} \tag{1.23}$$

which is a first order nonlinear differential equation in implicit form with three parameters.




**Scheme 2** Schematic representation of biochemical reactions at n-th step of the reaction chain with a negative feedback loop. $S_{n-1}$ is required for the conversion of $X_n$ into $S_n$. $m_n$-molecules of $S_n$ comes together to form a complex that interacts with $I_n$ to form another complex $C_n$, which increases the conversion rate of $S_n$ into $X_n$ (the negative feedback). There is also a spontaneous conversion of $S_n$ into $X_n$ which is independent of this feedback

### *2.2.1 Steady state analysis*

The steady state analysis of the negative feedback model is that same as the negative feedforward model, as they share the same steady state equation.

### *2.2.2 Stability analysis*

In order to study the stability of the fixed points we need to implicitly differentiate the Eq. (1.23) assuming that $S_{n-1}$ is an independent variable and $S_n$ depends on $S_{n-1}$ to get $\frac{d[S_n]}{d[S_{n-1}]}$ as

$$\frac{d[S_n]}{d[S_{n-1}]} = \frac{1-[S_n]}{\alpha_1 + [S_{n-1}] + \alpha_2(m+1)[S_n]^m} \tag{1.24}$$

At $[S^*] = 0$, $\left.\frac{d[S_n]}{d[S_{n-1}]}\right|_{[S^*]=0} = \frac{1}{\alpha_1}$ which makes $[S^*] = 0$ a stable steady state when $|1/\alpha_1| < 1$ and unstable when $|1/\alpha_1| < 1$. This derivative becomes 1 when $\alpha_1 = 1$. Once again, at this specific value of $\alpha_1$, this system could be stable, unstable or this point could be a bifurcation point.

$$\frac{d^2[S_n]}{d[S_{n-1}]^2} = \frac{-\frac{d[S_n]}{d[S_{n-1}]}\left(\alpha_1+[S_{n-1}]+\alpha_2(m+1)\,[S_n]^m\right)-\left(1+\alpha_2(m+1)m\,[S_n]^{m-1}\,\frac{d[S_n]}{d[S_{n-1}]}\right)(1-[S_n])}{\left(\alpha_1+\left[S_{n-1}\right]+\alpha_2(m+1)\,[S_n]^m\right)^2} \tag{1.25}$$

At $[S^*] = 0$, this derivative becomes

$$\left.\frac{d^2[S_n]}{d[S_{n-1}]^2}\right|_{[S^*]=0} = \frac{-\left.\frac{d[S_n]}{d[S_{n-1}]}\right|_{[S^*]=0}\alpha_1 - 1}{(\alpha_1)^2} = \frac{-2}{\alpha_1^2} \neq 0$$

for any positive values of $\alpha_1$. As in previous section, $[S^*] = 0$ is an unstable steady state when $\alpha_1 = 1$.

When we look at Eq. (1.24) at a non-zero steady state given by Eq. (1.11) when $\alpha_1 - 1 < 0$ and check if

$$\left.\frac{d[S_n]}{d[S_{n-1}]}\right|_{[S]=[S^*]} = 1 \tag{1.26}$$

has a positive solution, we see that $[S^*] = \frac{m(1-\alpha_1)}{m-1}$ is a solution of Eq. (1.26) and this can be less than 1 when $m > \alpha_1^{-1}$. The second derivative at this non-zero steady state is

$$\begin{aligned}\left.\frac{d^2[S_n]}{d[S_{n-1}]^2}\right|_{[S]=[S^*]} &= \frac{-1\left(\alpha_1 + [S^*] + \alpha_2(m+1)[S^*]^m\right) - \left(1 + \alpha_2(m+1)m[S^*]^{m-1}\right)(1-[S^*])}{(\alpha_1 + [S^*] + \alpha_2(m+1)[S^*]^m)^2} \\ &= \frac{-1}{1+\alpha_2 m[S^*]^m} - \frac{\left(1+\alpha_2(m+1)m[S^*]^{m-1}\right)}{(1-[S^*])\left(1+\alpha_2 m[S^*]^m\right)^2} < 0\end{aligned}$$

This says that this steady state is in fact also unstable.

As can be seen from Eq. (1.24), $\left.\frac{d[S_n]}{d[S_{n-1}]}\right|_{[S]=[S^*]} > 0$ for any $[S^*] < 1$. $\left.\frac{df([S])}{d[S]}\right|_{[S]=[S^*]} = -1$ has no solution and we can't talk about any types of bifurcations of the system with negative feedback loop in this case.

## 3 Results

We studied two models with two different types of negative feedback loops. The first model had a negative feedforward loop and the second one had a negative feedback loop. The negative feedforward loop model showed much more complicated behavior compared to the one with a negative backward loop. In Fig. 1, the one parameter bifurcation diagram for the model with negative feedforward loop modeled by Eq. (1.9) is given when $m = 3$ for various values of $\alpha_2$. In this plot, the dotted line shows unstable steady states and the solid lines represent stable steady states. As seen in this plot, $[S^*] = 0$ is always a steady state. When $\alpha_1 > 1$ there is only one stable steady state. When $\alpha_1 < 1$, there are two steady states; the one at $[S^*] = 0$ is unstable, and the non-zero steady state is stable when $\alpha_2 = 0$, 2 or 10. When $\alpha_2 = 40$ this branch loses

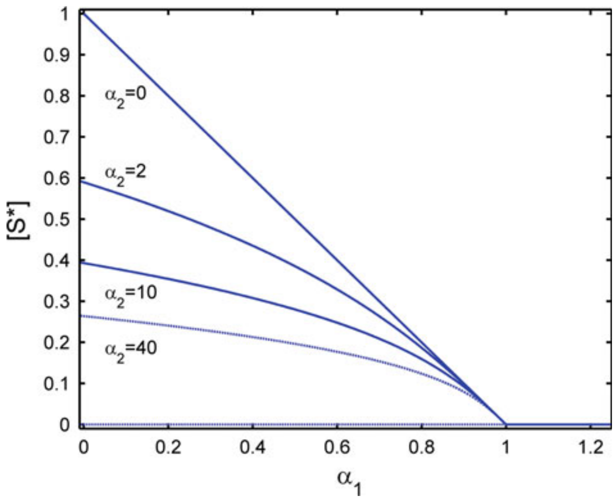



**Fig. 1** A one parameter bifurcation diagram with stable and unstable steady states for the system with negative feedforward loop modeled by Eq. (1.9) when $m = 3$ for various values of $\alpha_2$. *Dotted lines* show unstable steady states and the *solid lines* represent stable steady states. The details are given in the text

its stability and the system oscillates with period 2 (see Fig. 2). The negative effect of the feedback is obvious in this plot. Since $\alpha_2$ is defined as $\alpha_2 = k_{b'}^n[I_n]/k_f^n K_{eq}^n$, increasing this parameter means making the negative feedback stronger, producing smaller steady state response $[S^*]$. Since the model with negative feedback loop modeled by Eq. (1.23) has the same steady state equation with this model, it has similar bifurcation diagram. If we were to plot this diagram for the model in Eq. (1.23), everything would be same except the branch when $\alpha_2 = 40$ would be a solid line since we proved in Sect. 2.2.2 that this model does not oscillate and can only be stable or unstable for a given set of parameters.

We proved that at least three molecules in the previous reaction are required to form a complex and then negatively feed forward the next reaction in the chain in order to have the whole system oscillate. In Fig. 2, we numerically solved the negative feedforward loop model equation Eq. (1.9) for different values of $\alpha_2$ and truncated the transient part and only plotted the long term behavior of the model to generate this one parameter bifurcation diagram numerically. The parameter values in this plot are set to be $m = 3$ and $\alpha_1 = 0.1$. As seen in the figure, an increase in $\alpha_2$ value decreases the responses at later steps of the reaction chain until a threshold value of $\alpha_2$. However, after passing that threshold value, which is about $\alpha_2 = 15$, the responses at later steps start to oscillate with period 2.

We numerically studied the negative feedforward model for higher values of m. In Fig. 3, we plotted the long term response of the chain as the strength of the negative feedback $\alpha_2$ increases when $m = 8$ and $\alpha_1 = 0.1$. For this plot, we solved Eq. (1.9) numerically for $n = 50{,}000$ times and discarded the transient part and kept the cycles with a maximum period of 5000 for increasing values $\alpha_2$. This figure shows that for this value of m, the system undergoes period doubling bifurcation and then it finally becomes chaotic (See Fig. 7).

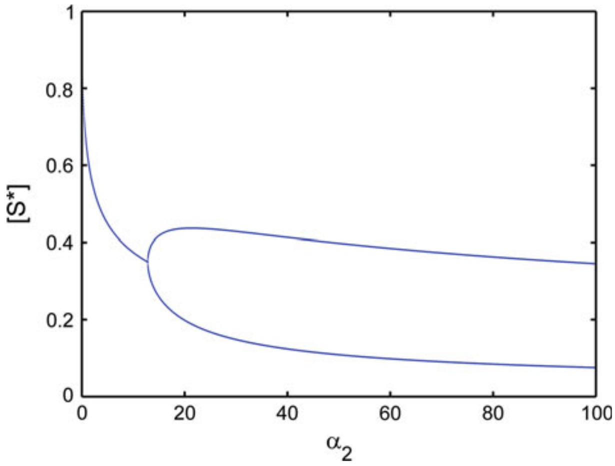



**Fig. 2** A one parameter bifurcation diagram with a period two oscillation produced for the system with negative feedforward loop modeled by Eq. (1.9). The parameter values are m=3 and $\alpha_1 = 0.1$. As seen in the figure, after passing a threshold value of $\alpha_2 = 15$ the responses at later steps start to oscillate with period 2

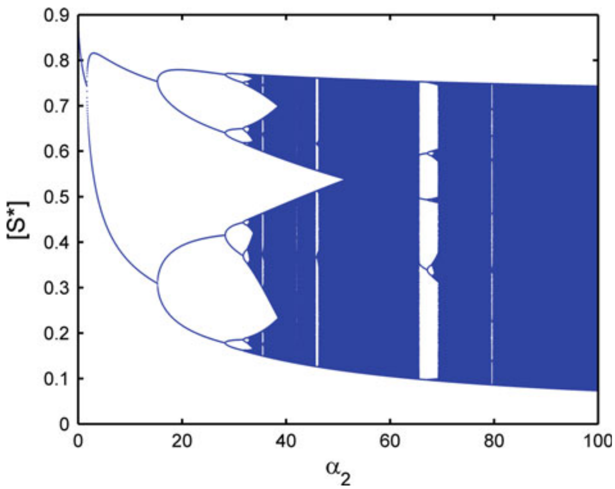



**Fig. 3** Period doubling bifurcation and chaos arising in the system with negative feedforward loop modeled by Eq. (1.9) when $m = 8$ and $\alpha_1 = 0.1$. This figure shows that higher m values can produce rich behaviors including stable steady states, limit cycles with different periods and even chaos

Figure 4 shows how the negative feedback plays a role in the system behavior when $m = 8$, $\alpha_1 = 0.1$ for various values of $\alpha_2$ in the negative feedforward model. The plot with the triangle marker is for a no negative feedback case ($\alpha_2 = 0$); in this case the system has a stable steady state and this steady state value is the highest. The plot with diamonds has a weak negative feedback strength ($\alpha_2 = 1$) and has a lower steady state value. The plot with circles has a stronger feedback strength ($\alpha_2 = 20$) and has a stable period solution with period 4. The plot with squares has the strongest negative

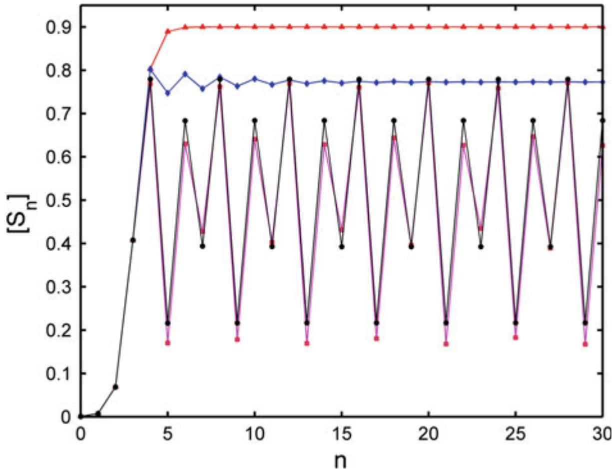



**Fig. 4** This is an example of the model simulation that shows how the negative feedback plays role on the system behavior and cause rich transient and long term changes including oscillations with various periods when m $= 8$, $\alpha_1 = 0.1$ for various values of $\alpha_2$. For the details, see the text

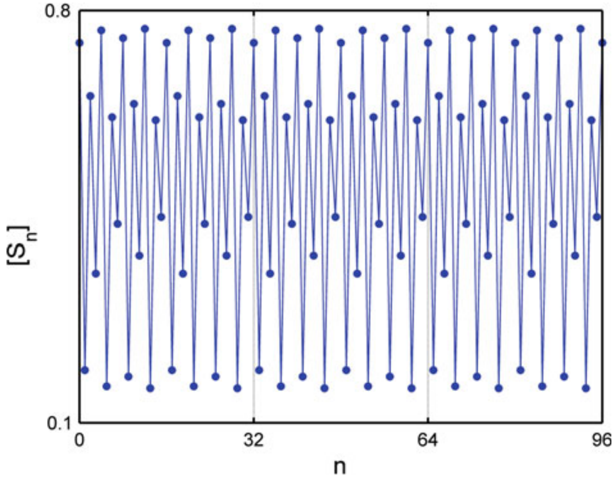



**Fig. 5** This is an example of the model output when m $= 8$, $\alpha_1 = 0.1$ and $\alpha_2 = 32.5$. In this simulation, the period of the cycle is 32

feedback ($\alpha_2 = 30$) and has a stable periodic solution with period 8. For all these simulations, the initial starting point is chosen as $S(0) = 7.44 \times 10^{-4}$. As we keep increasing the $\alpha_2$ value, the period becomes very sensitive to the $\alpha_2$ value and the model can generate a solution with much a longer period. Figure 5 shows the periodic solution with period 32 when $\alpha_2 = 32.5$.

In Fig. 6, we plotted the effect of the negative feed forward loop on the system behavior when there is only one stable steady state. In this simulation $\alpha_1 = 0.8$ and m $= 8$ and $\alpha_2$ values vary. The plot with the triangle marker is for the case where there

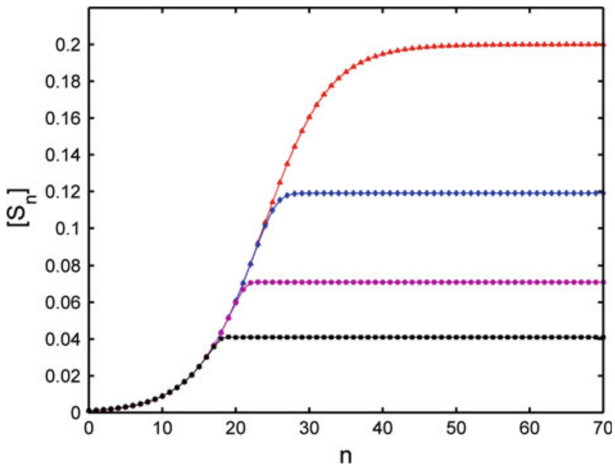



**Fig. 6** This shows the effect of the negative feed forward loop on the system behavior when there is only one stable steady state. In this simulation $\alpha_1 = 0.8$ and m = 8 and $\alpha_2$ values vary. The details are given in the text

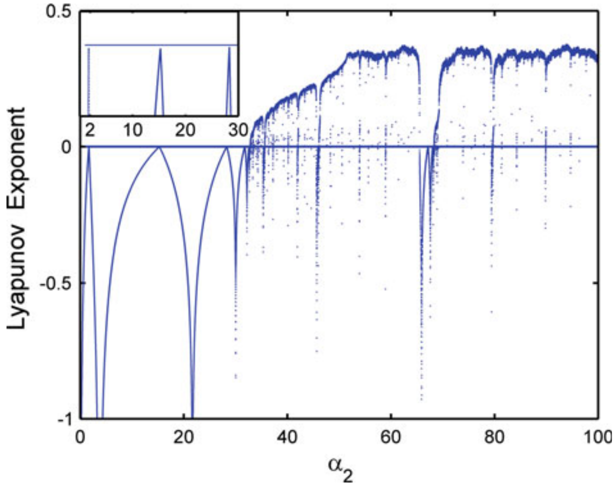



**Fig. 7** The Lyapunov exponent for the model given by Eq. (1.9). The parameter values are m = 8, $\alpha_1 = 0.1$ and the initial starting point for the simulation is $S(0) = 7.44 \times 10^{-2}$. See the text for the details

is no negative feedback ($\alpha_2 = 0$). In this case, the system has the highest steady state value. The plot with diamonds has the weakest negative feedback ($\alpha_2 = 20 \times 10^3$) and has a lower stable steady state value, the plot with circles has stronger negative feedback ($\alpha_2 = 20 \times 10^7$) and the plot with square markers has the strongest negative feedback ($\alpha_2 = 20 \times 10^9$). For all these simulations, the initial starting point is chosen as $S(0) = 10^{-3}$.

Figure 7 shows the Lyapunov exponent for the model given by Eq. (1.9) for an initial point of $S(0) = 7.44 \times 10^{-2}$. The parameter values are m = 8 and $\alpha_1 = 0.1$ and $\alpha_2$ varies between 0 and 100. As seen in this figure, the system can have positive

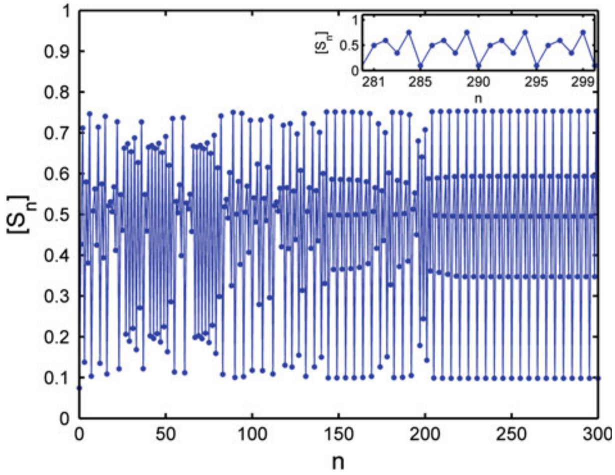



**Fig. 8** Periodic behavior arising back for larger values of $\alpha_2$ in Eq. (1.9). In this simulation, we set m $= 8, \alpha_1 = 0.1$ and $\alpha_2 = 66$ then we run the simulation long enough to converge to that stable periodic orbit with length 5. As seen in this plot, there is a very irregular behavior until n is about 200 then the system starts to converge to the cycle. The inset box has the transient part of the simulation is truncated and shows the converged cycle. In this simulation the initial point was set to be $S(0) = 7.44 \times 10^{-2}$

Lyapunov exponents when $\alpha_2 > 30$ which is a clear indication of a chaotic behavior for a deterministic system such as Eq. (1.9). We studied this system for different initial starting points and the model produced very similar results. However, there are still negative Lyapunov exponents for larger values of $\alpha_2$ that says the model is not sensitive to the initial starting points for this particular parameter settings.

In Fig. 8, we plotted the returning periodic behavior for larger values of $\alpha_2$ in Eq. (1.9). As can be seen in Fig. 7, there is a range for $\alpha_2$ around $\alpha_2 = 66$ for which the Lyapunov exponent is less than zero that is an indication of regular behavior. It is also visible in Fig. 3 which has a clear window at about $\alpha_2 = 66$, that Eq. (1.9) has a periodic solution with period 5. In this simulation, we set m $= 8, \alpha_1 = 0.1$ and $\alpha_2 = 66$ then we run the simulation long enough to converge to the stable periodic orbit with length 5. As seen in this plot, there is a very irregular behavior until n is about 200 then the system starts to converge to the cycle. The inset box has the transient part of the simulation truncated and shows the converged cycle. In this simulation the initial point was set to be $S(0) = 7.44 \times 10^{-2}$.

Figure 9 shows the effect of negative feedback loop modeled by Eq. (1.23) on the system behavior when there is only one stable steady state. In this simulation all the parameters are same as the parameters that are used to produce Fig. 6 including the initial starting point. This plot is produced by solving Eq. (1.23) implicitly. Since this equation can have at most one positive solution, we wrote a code that numerically picked the positive solution of such an equation to iterate it. The plot with the triangle markers is for the case where there is no negative feedback ($\alpha_2 = 0$). In this case, the system has the highest steady state value. The plot with diamonds has the weakest negative feedback strength ($\alpha_2 = 60$) and has a lower stable steady state value, the plot with circles has a stronger negative feedback strength ($\alpha_2 = 300$) which gives

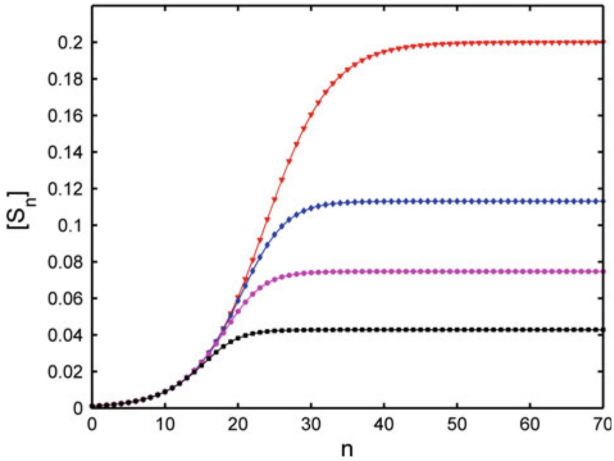



**Fig. 9** This shows the effect of the negative feedback loop on the system behavior when there is only one stable steady state. In this simulation all the parameters are the same as the parameters that are used to produce Fig. 6 including the initial starting point. This plot is produced by solving Eq. (1.23) implicitly. The comparison of this simulation and the similar simulation produced for the negative feedforward loop is given in the results section

an even lesser steady state value, and the plot with square markers has the strongest negative feedback strength ($\alpha_2 = 2000$) which produces the lowest steady state value. It is interesting to see this plot compared with the plot in Fig. 6 because in order to get about same response level we have to increase $\alpha_2$ much less compared to the model with negative feedforward loop case.

## 4 Discussions

The two models have one very significant difference. The negative feedforward model has the possibility of oscillating and even displaying chaotic behavior if a high enough number of $S_{n-1}$ molecules bound together in the secondary complex. With the negative feedback model there is no possibility of either oscillation or chaos, no matter how high the m value is. Oscillation requires at least three $S_{n-1}$ molecules bound in the secondary complex, and the $\alpha_2$ value, which determines the rate at which the secondary complex enters and affects the model, must be high. If $m > 3$ then the system can both display oscillations with various periods and undergoes period doubling bifurcation as $\alpha_2$ increases, and eventually becomes chaotic when $\alpha_2$ is large enough in the feedforward loop.

As can be seen in Figs. 6 and 9, these two mechanisms could require completely different levels of feedback strengths to get quantitatively comparable responses.

We also would like to emphasize that even though our models in this study are overly simplified, assuming that rate constants at each reaction step after receptor activation are equal, a more realistic model for a chain reaction with the negative feedforward loops should still produce rich transient and long term behaviors which have to be achieved if one or more of our assumptions are relaxed.

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