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Spectral correction for attenuating retroactivity in synthetic biology networks

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ABSTRACT
The reliability of synthetic biology systems is challenged by the undesired retroactive signals transmitted by the downstream modules to the upstream modules. These retroactive signals modify the input and output signals of the system, limiting the predictability of the system. This is viewed as similar to the loading effect on electrical, mechanical or hydraulic systems and is being studied using the typical engineering strategies. Here, we propose spectral analysis as a tool to explore and propose methods to attenuate this phenomenon. We select an existing system constituted by an input protein, which phosphorylates another protein to form the output, which further binds to another module in the downstream. The input signal with downstream connection is spectrally decomposed to four key components. We report how the dynamics of the system varies for each of the four components and its combinations. Our results show that the removal of the first harmonic component of the retroactive input signal can attenuate the retroactive effect. The biological significance and wet lab protocols for achieving this spectral correction is to be investigated further.
Keywords-Modularity, Phosphorylation, Retroactivity, Spectral analysis, Synthetic biology.

I. INTRODUCTION

Computations based on mathematical foundations have emerged as an essential component of research in biological sciences and allied areas. Technology has been integrated to biology and the objective of biotechnology has been extended to the systematic use of engineering approaches to intentionally design artificial organisms. This has catalysed the emergence of the field of synthetic biology [1]. Advancements in DNA sequencing and synthesis has enabled the field to progress significantly, leading to remarkable and diverse contributions of synthetic biologists that range from the design and production of new drugs to the production of biofuels. Engineering principles like decoupling, abstraction, standardization and modularity aid this rational construction and reliable functioning of synthetic biology systems [2]. Among these, modularity serves as a key principle that enables the decomposition of the intricate engineering systems into simpler units with defined functionalities, thereby facilitating its design and engineering process [3]. These simpler units are called as modules and are expected to retain its property when interconnected with other modules. This allows the system to possess distinctive functional or structural modules which are separable, renewable, and replaceable and can be integrated or interchanged in different ways with other systems to achieve new functionalities. Based on modularity principle, engineering of biological systems is initiated by the design and synthesis of bottom level modules in biological systems, which can then be integrated to form higher level sophisticated systems. Thus complex systems can be managed and manipulated in a simpler way. However, recent studies show that biological modules fail to exhibit this independence much like typical engineering systems, due to its context-dependent nature [4, 5, 6]. This results in several uncertainties that eventually challenges the predictable control of synthetic biology networks.

For example, when an isolated biological module is connected to another one, the signal received from the latter (downstream component) may create modifications in the dynamics of the former (upstream component) (Fig. 1). This happens as the interconnection of an upstream system with a downstream component has to compete with other biochemical reactions that constitute the upstream component. Therefore, properties of the upstream components get disrupted, which prevents the system from attaining modularity property. As a result, the output of the upstream component that is fed to the downstream component is modified. This effect on synthetic biology systems is called as retroactivity and is explained as an impedance like effect on electrical systems that changes the behaviour of an independent module when interconnected, making it dependent [7].Consequently the upstream or downstream modules lose their modularity property and exhibits an unexpected behaviour when gets integrated with each other.

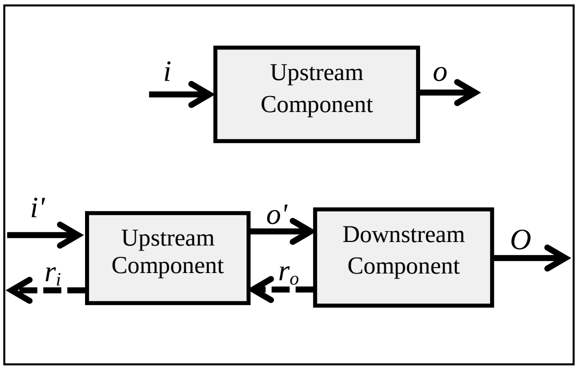

Fig. 1. Generation of retroactivity: '*i*' represents the input to the system without downstream, and '*o*' represents its output. 'r_o' represents the retroactivity to the output signal generated from the interconnection with downstream component, causing modifications in output signal, making it '*o*''. 'r_i' represents the retroactivity to the input signal causing modifications in input signal, making it '*i*''. 'r_i' and 'r_o' causes the generation of an unpredictable emergent output, '*O*' from the system.

Retroactivity is defined as an undesirable back action generated by a downstream component when it connects to an upstream component [8]. Studies on retroactivity attains significant impact as the successful engineering of predictable synthetic biology systems is being challenged by this phenomenon [9, 10]. In this paper, we propose spectral analysis as a tool to attenuate this retroactive effect based on the hypothesis that that the elimination of the key spectral components of the input signal with downstream component (here,*i*') that contribute the retroactive output signal (here, o'), makes '*o*'' closer to '*o*'.

II. METHODOLOGY

2.1. Phosphorylation System

We have chosen the phosphorylation system used by [7] as the biological mechanism for performing spectral analysis in order to attenuate retroactivity (Fig. 2). In this system, *Z* is a kinase of protein, *X*. *X* is phosphorylated when *Z* binds to it to form *Xp*. This phosphorylated *Xp*binds to the downstream component. A negative feedback on*Xp* is obtained by having a phosphatase *Y* (Enzyme that removes a phosphate group) activate the dephosphorylation of protein *Xp*. In a systemic viewpoint, we can consider *Z* as the input and *Xp* as the output of the system. It was already observed that the dynamics of both the input and output has a significant difference when connected to the downstream and when it is not connected [7].

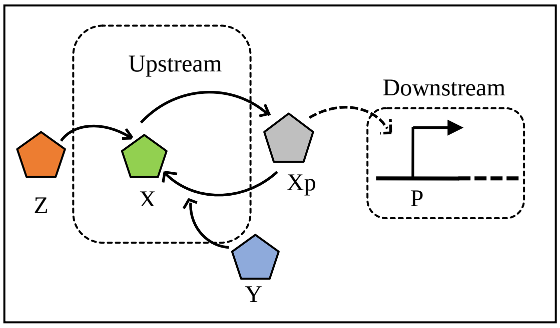

Fig. 2. Phosphorylation system

The reaction for phosphorylation can be written as:

$$X + Z \underset{\beta_2}{\overset{\beta_1}{\longleftrightarrow}} C_1 \xrightarrow{k_1} Xp + Z$$

and that for dephosphorylation can be written as:

$$Y + X_p \underset{\alpha_2}{\overset{\alpha_1}{\longleftrightarrow}} C_2 \xrightarrow{k_2} X + Y$$

whereC_1 is the protein- kinase complex and C_2 is the protein- phosphatase complex. Since *X* and *Y* are not degraded, $Y_{TOT}= Y+C_2$, $X_{TOT}=X+Xp+C_1+C_2+C$, where *C* is the complex of phosphorylated protein, *Xp* and promoter, *P*. The ordinary differential equations that model the system is given as:

$$\frac{dZ}{dt} = k(t) - \delta Z \boxed{-\beta_1.Z.X_{TOT}(1 - \frac{X_P}{X_{TOT}} - \frac{C_1}{X_{TOT}} - \frac{C_2}{X_{TOT}} \boxed{-\frac{C}{X_{TOT}}}) + (\beta_2 + k_1)C_1}$$

$$\frac{dC_1}{dt} = -(\beta_2 + k_1)C_1 + \beta_1.Z.X_{TOT}(1 - \frac{X_P}{X_{TOT}} - \frac{C_1}{X_{TOT}} - \frac{C_2}{X_{TOT}} \boxed{-\frac{C}{X_{TOT}}})$$

$$\frac{dC_2}{dt} = -(k_2 + \alpha_2)C_2 + \alpha_1.Y_{TOT}.X_P(1 - \frac{C_2}{Y_{TOT}})$$

$$\frac{dX_p}{dt} = k_1.C_1 + \alpha_2.C_2 - \alpha_1.Y_{TOT}.X_p(1 - \frac{C_2}{Y_{TOT}}) \boxed{+ k_{off}.C - k_{on}.X_p(P_{TOT} - C)}$$

$$\frac{dC}{dt} = -k_{off}.C + k_{on}.X_p(P_{TOT} - C)$$

The terms in the boxes represents the effect of retroactivity. For$\frac{dZ}{dt}$, the terms in the large box represent the retroactivity to the input. The terms in its small box and the boxes on $\frac{dC_1}{dt}$and $\frac{dX_p}{dt}$represent the retroactivity to the output (Fig. 3). The system of equations without these terms represent the system without downstream module.

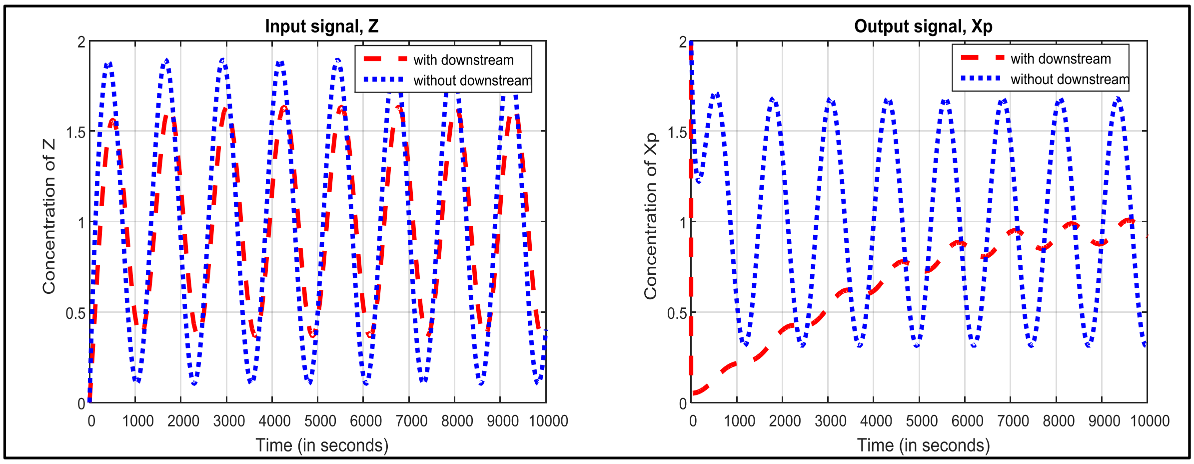

Fig. 3. Depiction of retroactivity in phosphorylation system: (a) Input signal with downstream module and without downstream module (b) Output signal with downstream module and without downstream module. Difference in the signals in both cases indicates the presence of retroactivity.

2.2. Spectral Analysis

We decomposed the input signal, Z into its spectral components when the system is connected with the downstream module and identified the key components that contribute the signal (Fig. 4 and TABLE 1). Spectral decomposition enables to represent a time domain signal based on its constituting spectral (frequency) components so that the characteristics of the signal can be analysed. Here, from the spectral components of Z, four key components with high peaks are selected to analyze its effect in the output signal.

Now the Simulink model of the given phosphorylation system with downstream module is modified so that each of those key components and its possible combinations are subtracted from the input signal. Thus we analyse the effect of each of these selected spectral components in the output signal and investigate in which case the output signal exhibits more similar behaviour to the output signal generated without downstream component. A sample model that simulate the system with input signal without Components 2,3 and 4,i.e., possessing Component 1 only is given in Fig. 5. Results are discussed in section 3.

TABLE 1. Key spectral components of input signal Z with downstream module

Component	Amplitude	Frequency
Component 1	0.1577	0.0008
Component 2	0.4225	0.00096
Component 3	0.04471	0.001361
Component 4	0.02738	0.001842

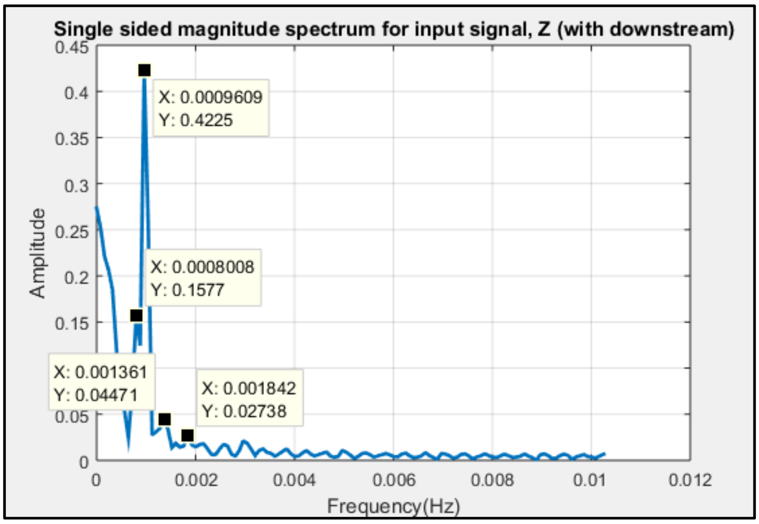

Fig. 4. Spectrum of input signal Z, with downstream module

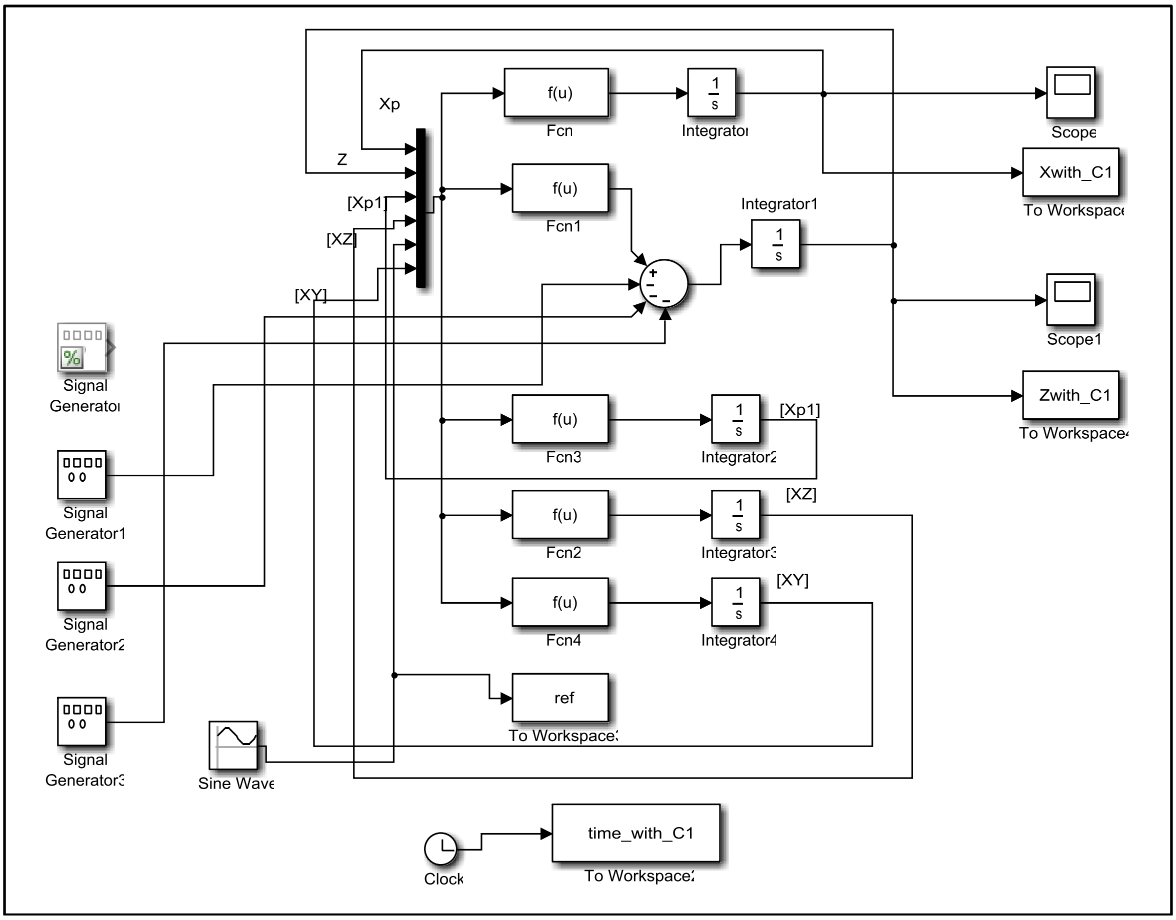

Fig. 5. Simulink model of phosphorylation system that removes Components 2, 3 and 4 from the input signal

III. RESULTS

The output signal, *Xp* after removing each of the four components and its combinations from the input signal, *Z*,is given in Fig. 5, Fig. 6 and Fig. 7. The first signal (first legend) in each plot in all figures represents the original output signal of the system with downstream module. The second signal (second legend)is the original output signal of the system when downstream module is not connected. The third signal (third legend) represents the output signal when each of the four components and its possible combinations are removed from the input signal. Fig. 6 shows the case 1 where each of the four components is removed from the input signal, Z. Fig. 7 represents the case 2 where combinations of three components are removed from the input signal. Fig. 8 depicts the third case where combinations of two components are removed from the input signal.

Analysis of case 1 gives us an indication that removal of Components 1 and 2 makes the output signal closer to the one without downstream within which removal of component 1 is exhibiting a more related behavior. In case 2, removal of combinations of Components- 1, 2, and 4 and 1, 2, and 3 gives very different output signal. Meanwhile removing the combination of Components-2, 3 and 4 and 1, 3 and 4 makes the output signal closer to the one without downstream. Among them the latter has more significant similarity with the compared signal.

In case 3, Fig. 8 (a) shows that when only the Components 1 and 2 are present, the output signal is almost similar to the one with downstream which means that retroactivity is still there. In Fig. 8 (b) and 8 (c) where combinations of Components 2, 4 and 2, 3 are removed resp., the signal is getting closer at the final time points. Comparatively, in Fig. 8 (d) and 8 (e), the signals show more correlation that too from the initial time points. Here Components 1, 4 and 1, 3 are removed resp. In the final plot (Fig. 8 (f)) where Components 1 and 2 are removed, is not exhibiting much similarity. We also look into the case when all the four components are present i.e. the signal as it is and when all four components are removed (Fig. 9).

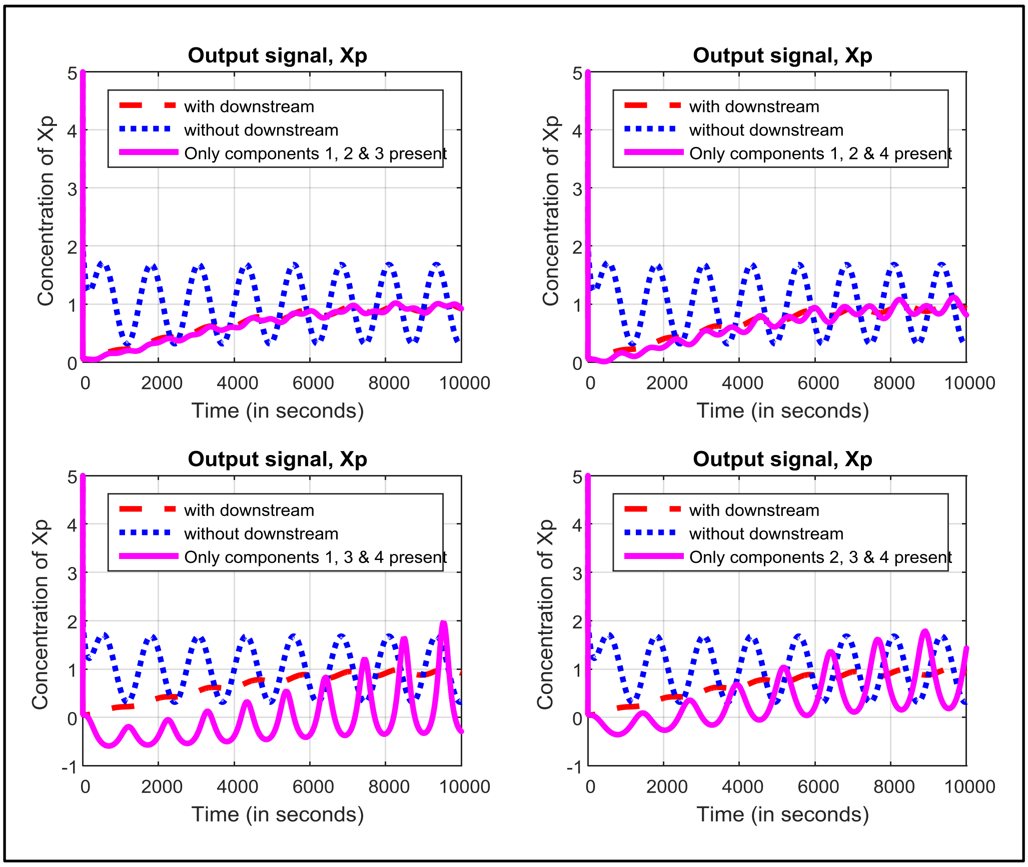

Fig. 6. Case 1: Effect in output signal on removing each of the four components from the input signal

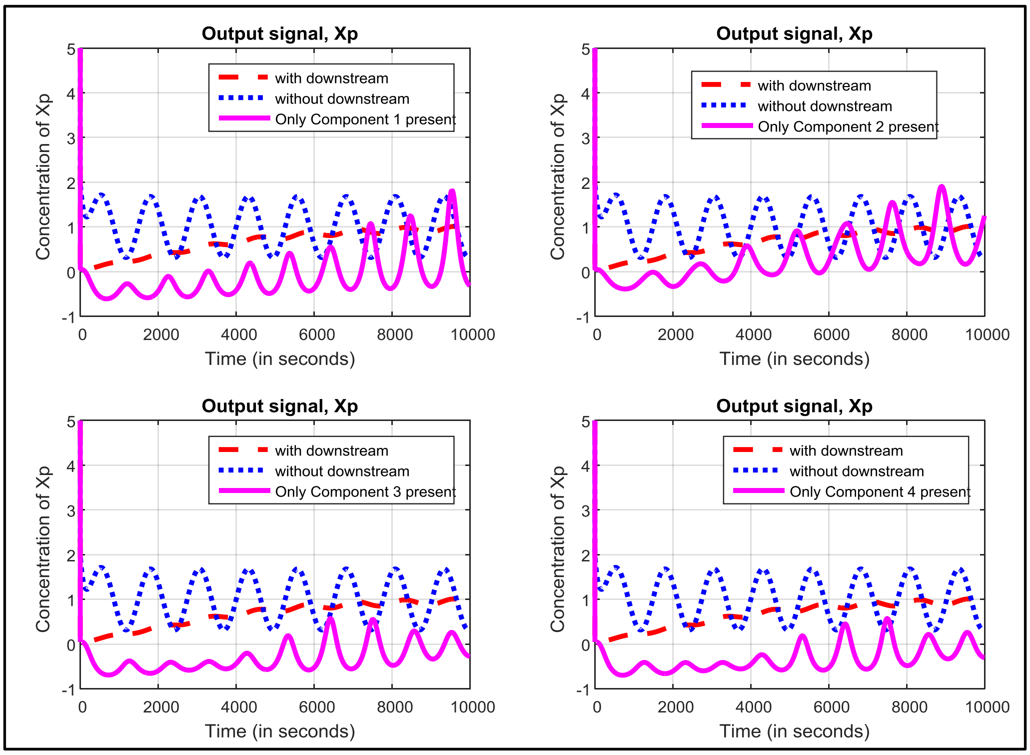

Fig. 7. Case 2: Effect in output signal on removing combinations of three components from the input signal

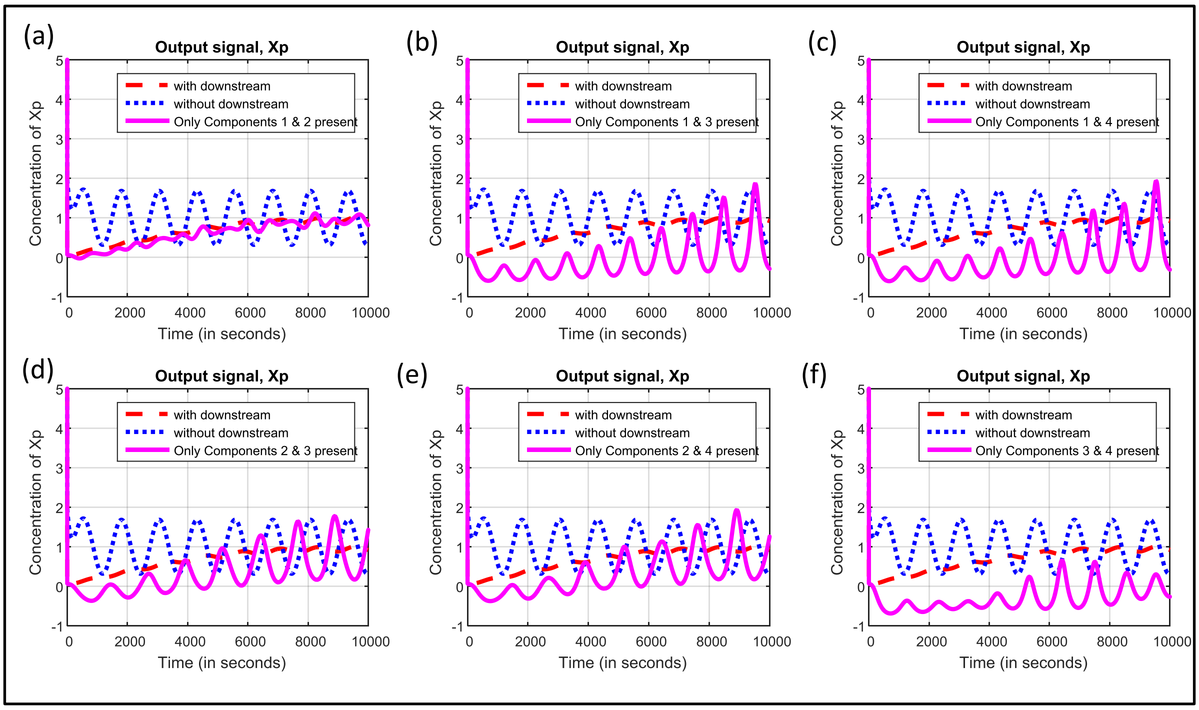

Fig. 8. Case 3: Effect in output signal on removing combinations of two components from the input signal

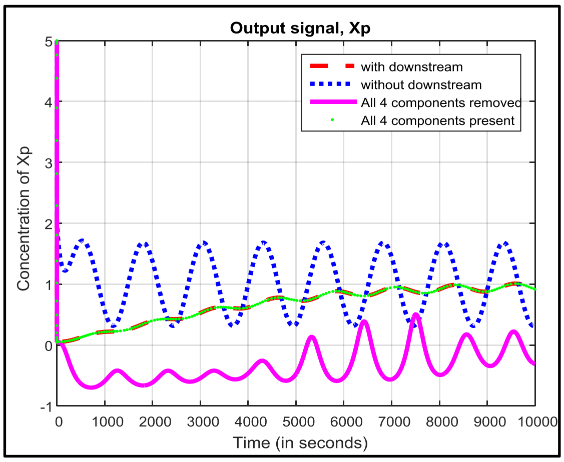

Fig. 9. Effect in output signal when all components are removed from the input signal

IV. CONCLUSIONS

From our study, is it clear that Component 1 (first harmonic) has a significant role in generating the retroactivity effect to the output when the upstream component is connected with the downstream component.This validates our hypothesis that removal of Component 1 from the input signal of the system connected with downstream can make it closer to the input signal when downstream component is not connected. Among the four selected components, component 1 possess the smallest frequency too. Hence we reach the conclusion that in the phosphorylation system we considered here, the first spectral component in the input signal with downstream is contributing to the

retroactivity phenomenon in a significant extent. We suggest that further studies be taken up on the biological interpretation of our findings and develop wet lab strategies to achieve spectral correction so that retroactivity in synthetic biology networks can be attenuated.

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