2019 18th European Control Conference (ECC)
Napoli, Italy, June 25-28, 2019

# Mitigating Biological Signalling Cross-talk with Feedback Control

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*Abstract*— **Biological signalling networks provide decision making and control capabilities for cells as they interact with changing physiological and environmental conditions. Studies of these signalling networks have revealed that they are greatly inter-dependent, which can make isolated analysis of their behaviour challenging. An accurate understanding of the inter-dependence of these systems will thus be of great utility to synthetic biologists as they attempt to engineer control systems within living cells. Cross-talk, which arises when the response of one signalling pathway can directly influence that of another, should either be considered explicitly when designing new signalling networks or controlled to ensure its effects are minimised. In this paper we consider a simple one-kinase two-phosphatase system and investigate potential control schemes that can reduce the impact of cross-talk upon its behaviour, for example by acting to selectively inhibit a kinase enzyme's affinity for one of its target substrates. We demonstrate that these schemes can be used to reduce the impact of cross-talk, and that a closed-loop control architecture provides better performance over a larger range of parameter values when compared to an open-loop equivalent.**

## I. Introduction

All living organisms are host to a complex variety of biological signalling networks, which are responsible for decision making and control of behaviour as environmental and physiological conditions change [1], [2]. A common minimal motif used for information transfer consists of kinase and phosphatase enzymes, and a substrate upon which they act [3]: The kinase phosphorylates the substrate (thereby changing its functionality, for example activating its enzymatic activity), and this is reversed by action of a phosphatase. The relative abundance of substrates in each phosphorylation state is determined by the kinase and phosphatase concentrations and activity, as well as the availability of free phosphate groups (provided by the cellular energy-transfer molecule ATP) [1], [3]. Information is thereby transferred via modulation of kinase or phosphatase activity/concentration, which regulates the phosphorylation state of a substrate serving as an input to downstream systems.

Studies of signalling networks have revealed significant inter-dependency: Some kinase or phosphatase enzymes interact with dozens of different substrates [4], [5]. In some cases inter-dependency can provide useful functionality by transferring information between systems, or between different levels of a single system [6], [7], and may even improve system signalling robustness [8]. However, orthogonality of signalling is often desirable, and natural systems have evolved control capabilities (such as mutual inhibition between adjacent pathways) to achieve this [9], [10]. Inter-dependency can also make analysis or tuning of these processes challenging [11], particularly when synthetic biologists attempt to engineer novel signalling networks within the cellular environment [12]. To this end, one useful biological goal is to be able to manipulate a signalling network such that there is some desired change in particular substrate targets, without introducing unintended changes in the phosphorylation level of other proteins in the network [13], [14]. This motivates the present work in which we examine signalling pathway interdependency in the context of *cross-talk*; this term is used to describe situations where the level of a signal transmitted by one pathway/substrate impacts the signal transmitted by another [15].

A minimal example of a cross-talking system is illustrated in black in Fig. 1. Because the pool of kinase enzyme is shared, if one phosphatase's mean activity is high its corresponding substrate will sequester a large amount of kinase, reducing the amount available to the other substrate. A signal transmitted via modulation of one phosphatase's activity can thereby impact the phosphorylation state of the other substrate (with which the phosphatase does not directly interact). In this paper we analyse synthetic biological control systems which aim to maintain the fidelity of information transfer (in this case via modulation of phosphatase enzyme concentration/activity) by the two sides of this signalling pathway (which share a single kinase enzyme). An ideal control system will allow the value of either signal (phosphatase activity) to be estimated by measurement of its corresponding substrate's phosphorylation state only. We consider two controllers which achieve this via different approaches (summarised in red and blue in Fig. 1). Past work has considered control mechanisms that counter the effects of crosstalk arising from consumption of phosphate groups by other substrate species [16], though this differs from the present work which addresses crosstalk arising from temporary sequestration of kinase enzymes during phosphate group transfer [14].

## II. A One Kinase-Two Phosphatase System

We build upon the modelling and analysis approach of Rowland *et al.* [14] to investigate a simplified system consisting of a single kinase and two phosphatases (Fig. 1). The kinase ($K$) enzyme catalyses the phosphorylation reaction for two substrates ($S_1$ and $S_2$), transforming them into their phosphorylated states ($S_1^P$ and $S_2^P$). Each substrate

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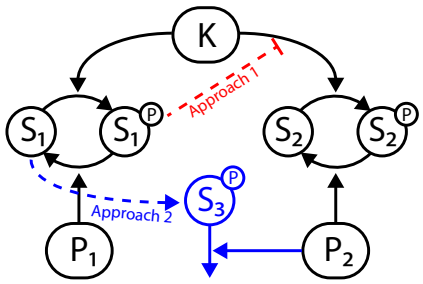


Fig. 1: The one-kinase two-phosphatase system architecture (black) as investigated in [14]. The kinase ($K$) enzyme catalyses the phosphorylation reaction for two substrates ($S_1$ and $S_2$), whilst each substrate has its own specific phosphatase ($P_1$ and $P_2$) to catalyse de-phosphorylation. Control approach 1 (the kinase inhibitor described in Section III) is in red, and control approach 2 (the phosphatase sink described in Section V) is in blue.

has a specific phosphatase ($P_1$ and $P_2$) to catalyse de-phosphorylation. Due to the symmetry of parameter values used in our model, results derived from analysis of this architecture can be translated to the similar system where a single shared phosphatase works against two specific Kinases. We model this system using biochemical reactions of the form:

$$S_i + K \underset{k_{-,K,i}}{\overset{k_{+,K,i}}{\rightleftharpoons}} KS_i \xrightarrow{k_{cat,K,i}} S_i^P + K \tag{1a}$$

$$S_i^P + P_i \underset{k_{-,P,i}}{\overset{k_{+,P,i}}{\rightleftharpoons}} P_i S_i^P \xrightarrow{k_{cat,S,i}} S_i + P_i \tag{1b}$$

with $i = 1, 2$. (1) accounts for reversible binding between the kinase/phosphatase and the $i$th substrate, with these complexes denoted $KS_i$ or $P_iS_i^P$ respectively. These complexes then catalyse phosphorylation/dephosphorylation at rate $k_{cat}$. To simplify the modelling of this system we can define conservation relations for the total quantity of each species:

$$K_0 = K + KS_1 + KS_2 \tag{2a}$$

$$P_{i,0} = P_i + P_iS_i^P \tag{2b}$$

$$S_{i,0} = S_i + S_i^P + KS_i + P_iS_i^P \tag{2c}$$

with $i = 1, 2$. Subscript "0" indicates the total (conserved) concentration of each species in the system. Using these conservation relationships we reduce the number of differential equations required to describe the system to the following:

$$\begin{aligned}
\frac{dS_i^P}{dt} &= -k_{+,P,i} \cdot P_i \cdot S_i^P + k_{-,P,i} \cdot P_iS_i^P + k_{cat,K,i} \cdot KS_i \\
\frac{dKS_i}{dt} &= -(k_{-,K,i} + k_{cat,K,i}) \cdot KS_i + k_{+,K,i} \cdot K \cdot S_i \\
\frac{dP_iS_i^P}{dt} &= -(k_{-,P,i} + k_{cat,P,i}) \cdot P_iS_i^P + k_{+,P,i} \cdot P_i \cdot S_i^P
\end{aligned} \tag{3}$$

with $i = 1, 2$. Using (2) we eliminate variables so that (3) can be expressed only in terms of $(S_i^P, KS_i, P_iS_i^P)$ for $i = 1, 2$. We solve the ODE system (3) at steady state, selecting the solution in which all species concentrations are non-negative, using dimensionless parameter values summarised in Table I. For the intial species concentrations we set $K_0 = 1$ and vary substrate concentrations with $S_{1,0} = S_{2,0}$ between simulations to investigate different operational regimes of the signalling network. $P_{1,0}$ and $P_{2,0}$ are the variables to which we examine the system's signalling sensitivity, we vary their values over the range $[0, 1]$.

| Parameter | Value |
|---|---|
| $k_{+,K,i}$, $k_{+,P,i}$ | 0.1 |
| $k_{-,K,i}$, $k_{-,P,i}$ | 0.01 |
| $k_{cat,K,i}$, $k_{cat,P,i}$ | 1 |

TABLE I: Nominal parameter values used throughout, with $i = 1, 2$. Catalysis ($k_{cat}$) is assumed to occur rapidly once a substrate-enzyme complex forms, and is thus substantially more likely than un-binding prior to catalysis ($k_-$).

In Fig. 2 the system is solved at steady state over this range of parameters, demonstrating the impact of cross-talk as $S_{i,0}$ changes: For small values of $S_{1,0}$, $S_{2,0}$ (Fig. 2a,b) the fraction of each substrate that is phosphorylated depends only on the concentration of its corresponding phosphatase (e.g. the proportion of phosphorylated $S_1$ in Fig. 2a is independent of $P_{2,0}$). Thus, in this situation crosstalk is minimal, and by measuring the fraction of a substrate $i$ in its phosphorylated state, $P_{i,0}$ can be inferred. In Fig. 2c,d ($S_{1,0} = S_{2,0} = 50$) we observe that the phosphorylation state of either substrate is strongly dependent on both $P_{1,0}$ and $P_{2,0}$, and as such the system exhibits substantial cross-talk. This arises because there is a large quantity of substrate $i$ in its de-phosphorylated state ($S_i$) when $P_{i,0}$ is high, which then depletes the pool of $K$ available to bind to and phosphorylate $S_j$, thus dramatically reducing the levels of $S_j^P$. This cross-talk is undesirable because it greatly decreases the network's sensitivity to information transmitted through one signalling pathway, which may compromise the function of important downstream regulatory processes to which it connects [1].

We now consider two control approaches to reducing the impact of cross-talk on this system's behaviour. These control schemes are one-sided, aiming to reduce the influence that $P_{1,0}$ has on $S_2^p$, though a similar (symmetric) system could be implemented to make the system two-sided [16]. In Section III we analyse an architecture that uses selective inhibition of the interaction between the kinase enzyme and $S_2$ (illustrated in red in Fig. 1) to increase the pool of available $K$ that can phosphorylate $S_1$. In Section V we analyse a phosphatase sink architecture (illustrated in blue in Fig. 1) that provides a secondary target for $P_2$, thereby increasing the fraction of $S_2$ in its phosphorylated state (reducing the amount of $K$ it sequesters).

## III. Control Approach 1 - Kinase Inhibitor

The first control (Fig. 1, red) system utilises a selective kinase inhibitor to reduce the affinity of the kinase enzyme $K$ for substrate $S_2$, without substantially impacting its interaction with $S_1$ [14]. This will decrease the pool of $K$ available to phosphorylate $S_2$ when $S_1^P$ is high, which will serve to balance the decreasing availablility of $K$ when

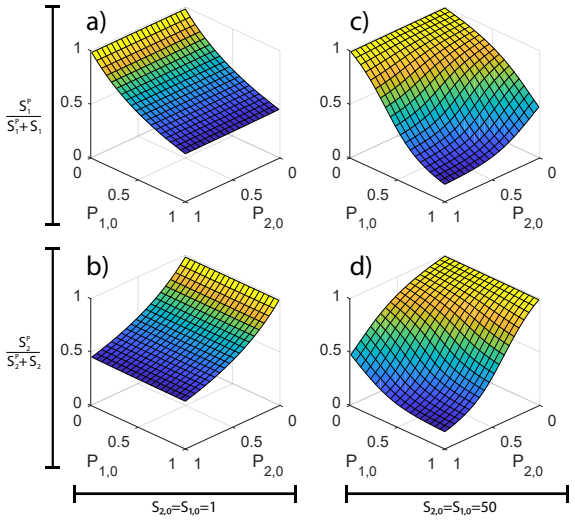


Fig. 2: Simulated behaviour of the basic system (black in Fig. 1) with parameters as in Table I. (a,c) The proportion of substrate 1 and (b,d) the proportion of substrate 2 that is in its phosphorylated state as a function of total phosphatase concentrations ($P_{1,0}$ and $P_{2,0}$). In (a,b) we have $S_{1,0} = S_{2,0} = 1$ and in (c,d) $S_{1,0} = S_{2,0} = 50$.

$P_{1,0}$ increases. This will ideally reduce the impact of $S_1$ concentration on the phosphorylation state of $S_2$, which is the undersirable cross-talk we aim to avoid. The exact biological mechanism of kinase inhibition is not investigated here, but we note that this mechanism must leave inhibited kinase free to bind and catalyse phosphorylation of $S_1$. We model the control input as a changed value of $k_{+,K,2}$, which we denote $\hat{k}_{+,K,2}$ and then substitute into (3) when solving for steady state species concentrations. For the closed loop system dependence upon the phosphorylation state of $S_1$ is introduced as:

$$\hat{k}_{+,K,2} = \frac{\alpha}{\alpha + S_1^P} \cdot k_{+,K,2} \tag{4}$$

This expression takes the form of a Hill equation with Hill coefficient 1, which might arise if $S_1^P$ acted as a transcriptional activator for production of a peptide inhibitor that can specifically disrupt the kinase – substrate 2 interaction [17].

We analyse this control architecture at steady state over the same parameter range as Fig. 2; results are presented in Fig. 3. When $S_{1,0} = S_{2,0} = 1$ (where crosstalk is minimal) the results are similar to those in Fig. 2. For $S_{1,0} = S_{2,0} = 50$ we observe that $S_2^P$ is largely independent of $P_{1,0}$, though the system's sensitivity has been shifted to regions of lower $P_{2,0}$. Furthermore, $S_1^P$ is less dependent upon $P_{2,0}$ (compare Fig. 3c to Fig. 2c). In Fig. 3e-h we analyse the dependence of the controlled system's behaviour on the parameter $\alpha$ in (4): When $\alpha = \infty$ the system is in its open loop state (as in Fig. 2), and for $\alpha = 0$ the interaction between the kinase and substrate 2 is completely blocked (meaning both $S_1^P$ and $S_2^P$ are independent of $P_{2,0}$).

## IV. Stability Analysis

To analyse the stability properties of the controller proposed in Section III we now provide an algorithm to test if a set lies in the region of attraction of the system's equilibrium for a particular set of parameters. This allows estimation of the region of attraction of the equilibrium. The algorithm is based on the monotone systems theory [18] and specifically on the results from [19]. First, consider the following compact set

$$\mathcal{S} = \Big\{ S_i^P, P_i S_i^P, KS_i \in \mathbb{R}_{\geq 0} \Big| P_i S_i^P \leq P_{i,0} \\ S_i^P + P_i S_i^P + KS_i \leq S_{i,0}, KS_1 + KS_2 \leq K_0, \Big\}.$$

Since the vector field is pointing into the interior the set $\mathcal{S}$ on its boundary $\partial\mathcal{S}$, and there are no equilibria on $\partial S$, it can be shown that $\mathcal{S}$ is forward-invariant under our system. Therefore, we will consider only equilibria in $\mathcal{S}$, and we will say that the system is globally stable in $\mathcal{S}$ if the stability definition is valid for all initial conditions in $\mathcal{S}$.

Our stability analysis is based on a decomposition of the system into a (negative feedback) interconnection of a monotone system and a static function. Note that our decomposition is not "natural", meaning that we "break" the newly introduced feedback from $S_1^P$ to $K$ as well as the interconnections from $KS_i$ to $S_i^P$. This is done in order to obtain a monotone system. In particular, we update (3) with:

$$\begin{aligned}
\frac{dS_i^P}{dt} &= -k_{+,P,i} \cdot P_i \cdot S_i^P + k_{-,P,i} \cdot P_i S_i^P + k_{\text{cat},K,i} \cdot u_i, \\
\frac{dP_i S_i^P}{dt} &= -(k_{\text{cat},P,i} + k_{-,P,i}) P_i S_i^P + k_{+,P,i} \cdot P_i \cdot S_i^P, \\
\frac{dKS_1}{dt} &= -(k_{\text{cat},K,1} + k_{-,K,1}) \cdot KS_1 + k_{+,K,1} \cdot K \cdot S_1 \\
\frac{dKS_2}{dt} &= -(k_{\text{cat},K,2} + k_{-,K,2}) \cdot KS_2 + \hat{k}_{+,K,2} \cdot K \cdot S_2,
\end{aligned} \tag{5}$$

where $\hat{k}_{+,K,2} = \alpha/(\alpha + u_3) k_{+,K,2}$ and $u_i$ are considered to be inputs. We form the state vector as $\begin{pmatrix} S_1^P & P_1 S_1^P & S_2^P & P_2 S_2^P & KS_1 & KS_2 \end{pmatrix}^T$ and compute the Jacobian of the vector field as follows:

$$J = \begin{pmatrix} J_{11} & 0 & 0 \\ 0 & J_{22} & 0 \\ -k_{+,K,1} \cdot K \cdot A_{31} & -\hat{k}_{+,K,2} \cdot K \cdot A_{32} & J_{33} \end{pmatrix}$$

where

$$J_{11} = \begin{pmatrix} -k_{+,P,1} \cdot P_1 & k_{-,P,1} + k_{+,P,1} \cdot S_1^P \\ k_{+,P,1} \cdot P_1 & -k_{-,P,1} - k_{\text{cat},P,1} - k_{+,P,1} \cdot S_1^P \end{pmatrix},$$

$$J_{22} = \begin{pmatrix} -k_{+,P,2} \cdot P_2 & k_{-,P,2} + k_{+,P,2} \cdot S_2^P \\ k_{+,P,2} \cdot P_2 & -k_{-,P,2} - k_{\text{cat},P,2} - k_{+,P,2} \cdot S_2^P \end{pmatrix},$$

$$J_{33} = \begin{pmatrix} -a_1 & -k_{+,K,1} \cdot S_1 \\ -\hat{k}_{+,K,2} \cdot S_2 & -a_2 \end{pmatrix},$$

$$a_1 = k_{\text{cat},K,1} + k_{-,K,1} + k_{+,K,1} \cdot (K + S_1),$$

$$a_2 = k_{\text{cat},K,2} + k_{-,K,2} + \hat{k}_{+,K,2} \cdot (K + S_2),$$

$$A_{31} = \begin{pmatrix} 1 & 1 \\ 0 & 0 \end{pmatrix}, \; A_{32} = \begin{pmatrix} 0 & 0 \\ 1 & 1 \end{pmatrix}.$$

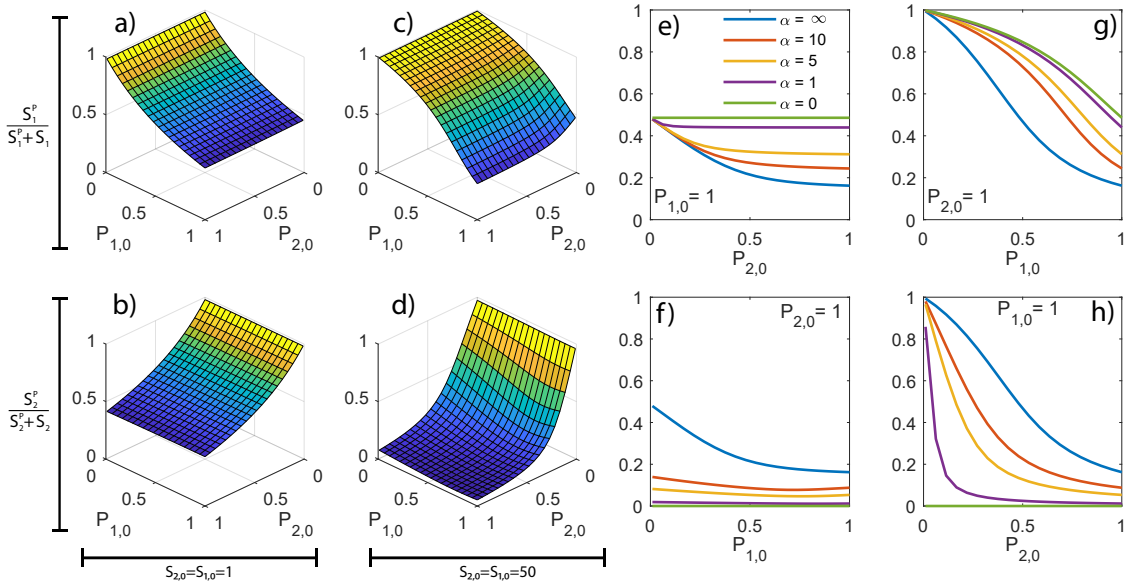


Fig. 3: Simulated behaviour of the kinase inhibitor control system (red in Fig. 1) discussed in Section III. (a,b,c,d) are similar to Fig. 2, and (e,f,g,h) are are cross sections (with fixed $P_{i,0}$) of panels c,d (with $S_{1,0} = S_{2,0} = 50$) and varying feedback parameter $\alpha$ in (4).

Now using Corollary III.3 from [18] it is straightforward to verify, that for any nonnegative inputs $u_1$, $u_2$, $u_3$ the system (5) is monotone with respect to the orthants $\mathrm{diag}\left\{\begin{pmatrix}-1 & -1 & 1 & 1 & 1 & -1\end{pmatrix}\right\}\mathbb{R}^6_{\geq 0}$, $\mathrm{diag}\left\{\begin{pmatrix}1 & -1 & -1\end{pmatrix}\right\}\mathbb{R}^3_{\geq 0}$ on the set $\mathcal{S}$.

The system (5) has a unique equilibrium in $\mathcal{S}$, which is globally (in $\mathcal{S}$) asymptotically stable, provided that the inputs are constant and chosen such that the trajectories stay in $\mathcal{S}$. Indeed, consider three subsystems: $G_1$ with the states $S_1^P$, $P_1S_1^P$, $G_2$ with the states $S_2^P$, $P_2S_2^P$, and $G_3$ with the states $KS_1$ and $KS_2$. The Jacobian of $G_1$ is equal to $J_{11}$. For any constant input (such that the concentrations of all species lie in $\mathcal{S}$) the trace of $J_{11}$ is negative (for positive $P_1$), while its determinant is positive. Note that we can rule out the case with $P_1 = 0$ in the equilibrium since $S_1^P$ will grow exponentially and the trajectory of the system will leave $\mathcal{S}$. Therefore, in our setting $J_{11}$ is Hurwitz, which implies that there exists a unique locally asymptotically stable equilibrium in $\mathcal{S}$ for $G_1$ according to [20], [21]. In our case, this equilibrium is also globally (in $\mathcal{S}$) asymptotically stable since we can rule out limit cycles using monotonicity theory. Similarly we can show that $G_2$, $G_3$ are globally (in $\mathcal{S}$) asymptotically stable for any constant inputs $u_1$, $u_2$, $u_3$ keeping the trajectories in $\mathcal{S}$. Since the open loop system is a cascade of three globally asymptotically stable systems for constant inputs, the cascade itself is globally asymptotically stable for $u_1$, $u_2$ and $u_3$ provided that the trajectories do not leave $\mathcal{S}$.

Now we can use Proposition 1 from [19] to test if a set lies in the basin of attraction of the equilibrium of the system. In order to use this result we need to choose the space of control signals $\mathcal{U}$ so that (i) $\mathcal{U}$ is a box (Cartesian product of intervals), (ii) verify that the space of inputs $\mathcal{Y}$ is such that $\mathcal{Y} \subseteq \mathcal{U}$, and (iii) the trajectories of the system (5) do not leave $\mathcal{S}$. We set $S_{1,0} = S_{2,0} = 50$, $P_{1,0} = P_{2,0} = 2$, and $\mathcal{U} = \{u \in \mathbb{R}^3_{\geq 0} \,|\, u_1 \leq 0.4, u_2 \leq 0.6, u_3 \leq 50\}$ for which we have $\mathcal{Y} \subseteq \mathcal{U}$ and the species stay in $\mathcal{S}$. The equilibrium of the closed loop system lies at $\begin{pmatrix}14.5039 & 1.1528 & 0.2531 & 0.0228 & 0.2531 & 0.0228\end{pmatrix}$. Application of Proposition 1 from [19] establishes that "the control signals $u$" (which are $u_1 = KS_1$, $u_2 = KS_2$ and $u_3 = S_1^P$) converge to the point $\begin{pmatrix}0.2531 & 0.0228 & 14.5039\end{pmatrix}$. This means that the closed loop system has a unique attractive equilibrium in the set, where $u_1$, $u_2$, $u_3$ lie in $\mathcal{U}$ and the concentrations of the other species lie in $\mathcal{S}$. This implies that the closed loop system has a unique attractive equilibrium in $\mathcal{U}$ and $\mathcal{U}$ lies in the region of attraction of this equilibrium.

## V. Control Approach 2 - Phosphatase Sink

Our second control approach employs a phosphatase sink to reduce the concentration of free phosphatase enzyme ($P_2$), thereby shifting the equilibrium of the $S_2$ phosphorylation process towards its phosphorylated state. This counteracts the effect of a reduced free kinase concentration when $P_{1,0}$ is large, which is the crosstalk that we aim to avoid. A phosphatase sink can be introduced using a substrate ($S_3$ in Fig. 1) to which a phosphatase reversibly binds, which could (for example) be a mutated kinase [22]. The precise biological mechanism employed to achieve this is not of critical importance for our analysis, it will suffice to assume that we are able to introduce a new phosphorylated substrate $S_3^P$, and that its concentration can be regulated by the concentration of $S_1$.

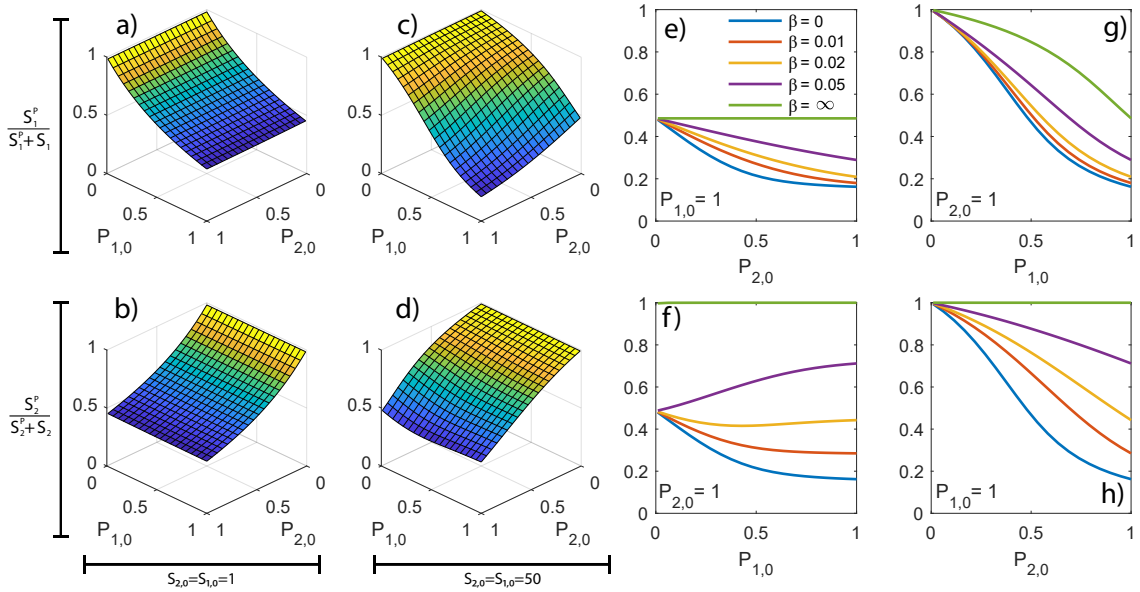


Fig. 4: Simulated behaviour of the phosphatase sink control system (blue in Fig. 1) discussed in Section V. (a,b,c,d) are similar to Fig. 2, and (e,f,g,h) are cross sections (with fixed $P_{i,0}$) of panels c,d (with $S_{1,0} = S_{2,0} = 50$) and varying feedback parameter $\beta$ in (9).

To model this system we include the new reversible reaction:

$$S_3^P + P_2 \underset{k_{-,P,3}}{\overset{k_{+,P,3}}{\rightleftharpoons}} P_2 S_3^P \tag{6a}$$

which introduces a new differential equation:

$$\frac{dP_2S_3^P}{dt} = -k_{-,P,3} \cdot P_2 S_3^P + k_{+,P,3} \cdot P_2 \cdot S_3^P \tag{7}$$

here we set the parameters $k_{+,P,3}$ and $k_{-,P,3}$ equal to the corresponding values in Table I. We include a new conservation law, and must also update the conservation relation in (2b) for $i = 2$ to reflect the new binding state:

$$P_{2,0} = P_2 + P_2 S_2^P + P_2 S_3^P \tag{8a}$$
$$S_{3,0} = S_3^P + P_2 S_3^P \tag{8b}$$

The value of $S_{3,0}$, the total concentration of the third substrate, is the actuating parameter used to control our system. In the closed-loop system the concentration of $S_{3,0}$ is dependent upon the concentration of dephosphorylated $S_1$, and takes the form:

$$S_{3,0} = \beta S_1 \tag{9}$$

The expression (9) introduces a linear dependence of $S_{3,0}$ on $S_1$ which might (for example) arise if $S_1$ acted as a transcriptional activator for the expression of $S_3^P$.

For this control architecture (Fig. 4) we find (as in Fig. 3) that $S_2^P$ is largely independent of $P_{1,0}$ (Fig. 4c), though in this case the system's sensitivity is not shifted to low $P_{2,0}$ values. Again the control system provides some reduction in the dependence of $S_1^P$ on $P_{2,0}$. Fig. 4e-h illustrates that the trade-off for varying $\beta$ values is the opposite of that in Fig. 3: Now, when $\beta$ is large the sensitivity of the system is shifted to very large $P_{2,0}$ values, and for sufficiently large $\beta$ all of the substrate is in its phosphorylated form ($S_2^P$). Since no free phosphatase remains to dephosphorylate $S_2$, the interaction between the kinase and substrate 2 never occurs. In this regard this control strategy may be superior to the Kinase inhibitor method, since when it is in place the response of $S_2^P$ is similar to that expected if Substrate 1 was not present (equivalent to the $\beta = \infty$ case in Fig. 4e,g or the $\alpha = 0$ case in Fig. 3e,g).

## VI. Discussion

Both control schemes considered demonstrate an ability to reduce cross-talk, but with fundamentally different outcomes: The first (selective kinase inhibition) decreases the range of $P_{2,0}$ values over which $S_2^P$ varies, and by reducing the rate at which $S_2$ binds $K$ (thereby reducing the total quantity of Kinase enzyme sequestered in state $KS_2$) it also significantly attenuates the dependence of $S_1^P$ on $P_{2,0}$. In the second control scheme (the phosphatase sink) the response of $S_2^P$ to changing $P_{2,0}$ is similar to that expect in the absence of the first substrate, though this approach does less to attenuate the dependence of $S_1^P$ on $P_{2,0}$. The selection of a particular control scheme in practice will be dictated in part by which of these outcomes is more favourable given the system's purpose. Other important considerations would be the ease (and reliablity) with which each control scheme could be implemented biologically, as well as consideration of any secondary effects that the specific implementation may have.

Depending on the particular control goals for our system it is possible that alternate control architectures may provide superior performance to those considered in this work. For example, if only signal transmission via $P_1$ and $S_1$ is of

interest then an open loop kinase inhibitory architecture could achieve this goal: By completely blocking the kinase from interacting with the second signalling pathway no cross-talk would take place, maximising the fidelity of signalling through $P_1$ and $S_1$ (illustrated when $\alpha = 0$ in Fig. 3). Repression of $S_2$ or $P_2$ production would produce a similar effect by shutting down activity in the second pathway.

In the present work we have focused on a simplified one-kinase two-phosphatase system, however, it is known that in natural systems each of these enzymes may be associated with a large number of substrate targets [11]. This can sequester a large proportion of the free kinase (or phosphatase), without requiring any individual substrate to be present at a particularly high concentration [14]. Systems that involve a large number of interactions may limit the feasibility of some of the considered control schemes: For example, if a large number of phosphatase enzymes play a part in the system's behaviour, expressing a sink for each one would require a complex and burdensome synthetic circuit. To avoid this intelligent control design will therefore require an in-depth understanding of these systems' extended architectures, as well as their possible interactions with other cellular processes.

Future work will include exploration of these control systems' dynamic behaviour [23], as well as more complex models of the phosphorylation process. For example, consumption of phosphate groups is not currently considered in our model. This was justified by our assumption that the kinase concentration would be small, making free kinase the limiting factor (and hence primary means via which cross-talk is introduced). This may prove to be a weakness for the phosphatase sink architecture, because depending upon the implementation chosen this control system may consume additional phosphate groups (during the phosphorylation of $S_3$), thereby reducing the quantity available to other processes in the cell.

## VII. Conclusion

In this paper we examined the cross-talk behaviour of a one-kinase two-phosphatase cellular signalling pathway. We observe that in certain operational regimes crosstalk due to kinase sharing can introduce a strong dependence of a substrate's phosphorylation state on the activity of a phosphatase with which it does not directly interact. Two control approaches for mitigation of cross-talk are proposed; the first produces a sink for one of the system's phosphatase enzymes, and the other selectively inhibits the kinase enzyme's affinity for one substrate. Both control schemes are able to reduce the impact of cross talk, though trade-offs exist in their differing approaches to re-shaping the system's response. This work demonstrates the challenges inherent in the engineering of biological signalling networks, and the control approaches proposed may aid synthetic biologists as they attempt to design such systems.

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