Spatial control of biochemical modification cascades and pathways: Supplementary material

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In this document, we present analysis of a number of aspects of topics discussed in the main text. We present, in turn: (i) The models of the modification cascades (ii) Analysis of different aspects of enzymatic cascades, multisite modifications and phosphotransfer mechanisms (iii) Spatial aspects of open signalling cascades. (iv)Analysis of a single tier of a modification cascade with multiple diffusible entities. (v) Response of a two-tier enzymatic modification cascade to spatial gradients (vi) Multisite modification with separated kinase and phosphatase

1 Models

1.1 Cascade

We present the various models which we will study. The primary focus of the paper was to study the effects of compartmentalization on different kinds of cascades. We present the relevant model equations for these different cascades. At the end of each model, we discuss how different variants of each case we have studied are implemented in the model. Fig. S1 depicts a schematic of some of these modification cascades depicting the reactions in the cascade and the compartment in which they occur. The common species of the two compartments diffuses from one compartment through the intervening space (this is not depicted) and into the second compartment.

We first start with an enzymatic 2-step cascade. We first describe a general spatial model of a 2-step cascade. We then discuss how we adapt this to describe a 2-step cascade with spatial localization, as depicted in Fig. S1.

A general spatial model of a 2-stage enzymatic cascade (in one spatial dimension, with periodic boundary conditions, with the spatial variable being denoted by θ) is described by the following equations:

$$
\begin{aligned}
\frac{\partial[X]}{\partial t} &= -k_1[X][K1] + k_{-1}[XK1] + k_4[X^*P1] + D_X\frac{\partial^2[X]}{\partial\theta^2} \\
\frac{\partial[X^*]}{\partial t} &= -k_3[X^*][P1] + k_{-3}[X^*P1] + k_2[XK1] - k_5[Y][X^*] + k_{-5}[X^*Y] + k_6[X^*Y] + D_{X^*}\frac{\partial^2[X^*]}{\partial\theta^2} \\
\frac{\partial[K1]}{\partial t} &= -k_1[X][K1] + k_{-1}[XK1] + k_2[XK1] + D_{K1}\frac{\partial^2[K1]}{\partial\theta^2} \\
\frac{\partial[P1]}{\partial t} &= -k_3[X^*][P1] + k_{-3}[X^*P1] + k_4[X^*P1] + D_{P1}\frac{\partial^2[P1]}{\partial\theta^2} \\
\frac{\partial[XK1]}{\partial t} &= k_1[X][K1] - k_{-1}[XK1] - k_2[XK1] + D_{XK1}\frac{\partial^2[XK]}{\partial\theta^2} \\
\frac{\partial[X^*P1]}{\partial t} &= k_3[X^*][P1] - k_{-3}[X^*P1] - k_4[X^*P1] + D_{X^*P1}\frac{\partial^2[X^*P1]}{\partial\theta^2} \\
\frac{\partial[Y]}{\partial t} &= -k_5[Y][X^*] + k_{-5}[X^*Y] + k_8[Y^*P2] + D_Y\frac{\partial^2[Y]}{\partial\theta^2} \\
\frac{\partial[Y^*]}{\partial t} &= -k_7[Y^*][P2] + k_{-7}[Y^*P2] + k_6[X^*Y] + D_{Y^*}\frac{\partial^2[Y^*]}{\partial\theta^2} \\
\frac{\partial[P2]}{\partial t} &= -k_7[Y^*][P2] + k_{-7}[Y^*P2] + k_8[Y^*P2] + D_{P2}\frac{\partial^2[P2]}{\partial\theta^2} \\
\frac{\partial[X^*Y]}{\partial t} &= k_5[Y][X^*] - k_{-5}[X^*Y] - k_6[X^*Y] + D_{X^*Y}\frac{\partial^2[X^*Y]}{\partial\theta^2} \\
\frac{\partial[Y^*P2]}{\partial t} &= k_7[Y^*][P2] - k_{-7}[Y^*P2] - k_8[Y^*P2] + D_{Y^*P2}\frac{\partial^2[Y^*P2]}{\partial\theta^2}
\end{aligned}
\tag{1}
$$

The above model is simply a depiction of all the kinetic steps in this cascade, described in a standard way, along with the diffusion of each species. The substrate species are X, X^* (and corresponding complexes XK and X^*P1) in the first level of the cascade and in the second level of the cascade, the substrate species (and relevant complexes) are Y, X^*Y, Y^* and Y^*P2. The enzymes for the first stage of the cascade are K (kinase) and P1 (phosphatase). X^* acts as a kinase for the second level of the cascade and $P2$ is the phosphatase. The model encapsulates a fairly standard and broadly used description of the catalytic conversion of substrate by enzyme, explicitly incorporating enzyme-substrate binding/unbinding and irreversible conversion. This kinetic description of the cascade has been used in multiple studies of signalling cascades.

The rate constants of binding of enzyme to substrate are k_1 (K to X), k_3 (P1 to X^*), k_5 (X^* to Y) and k_7 (P2 to Y^*), while the corresponding unbinding constants are denoted by k_{-1}, k_{-3}, k_{-5} and k_{-7} respectively. The relevant catalytic rate constants for these reactions are denoted by k_2, k_4, k_6 and k_8 respectively. The diffusion coefficients are $D_X, D_{K1}, D_{XK1}, D_{X^*}, D_{P1}, D_{X^*P1}, D_Y, D_{X^*Y}, D_{Y^*}, D_{P2}$ and D_{Y^*P1}, where the subscript denotes the species under consideration.

While this is a general spatial model of a 2 step cascade, we now discuss how we employ this in the context of our results.

Response to a gradient. When we aim to study the response of the model to a spatial input gradient, (see section 2.5 below) we incorporate an explicit description of the input, which is the kinase K. There are different ways in which this can be incorporated. One way is to impose a particular free kinase concentration, described by an equation of the form

$$\frac{\partial [K1]}{\partial t} = -k_1[X][K1] + k_{-1}[XK1] + k_2[XK1] + k_{f1}S(\theta) - k_{b1}[K1] + D_{K1}\frac{\partial^2 [K1]}{\partial \theta^2} \tag{2}$$

This describes a production of K1 by an external signal S and removal of K1 (rate constants k_{f1} and k_{b1}) in addition to the other reactions it is involved in. A similar input has been used in (1).

Localization. The primary focus of the paper is to study the effects of localization (and separation) of different steps of the cascade. Thus, we describe localization in the two step cascade as follows. For simplicity, we will assume that all species are essentially non-diffusible apart from the species conveying the information from one location to the other. Other variants such as those where all localized species also diffuse in the individual compartments (but are confined there) can also be employed but we will not require these additional details for the purposes of our investigations here. We thus have K1, P1 and X in location 1, while Y, Y^* and P2 are in location 2. All relevant enzyme substrate complexes are in the respective locations, and these are assumed non-diffusible. X^* is the diffusible species and thus it is present everywhere in the domain. Therefore in our model we employ suitable initial conditions consistent with this scenario, and make all variables non-diffusible except the communicating variable. This then results in the model of the 2-step cascade with compartmentalization.

We employ the model in a periodic domain with the two locations (of equal size) symmetric and diametrically opposite to one another (see Fig. 1). This also exactly corresponds to no-flux boundary conditions in a domain of half the size.

When we study localization, we thus localize $K1$ in the first location (and the relevant complex is contained here as well). The total concentration of kinase (free + complex) is automatically conserved. The total concentration of $K1$ (free +complex) can be regarded as the input to the cascade. Before the input is applied, there is no modified form X^* present (no K1 present before stimulus).

Three Step Cascade. A three step cascade may be described in an analogous manner. The only difference here is that there is more than one way to spatially partition a three step cascade. For instance,

one possibility is that the first two steps are localized in location 1 and the last step is localized in location 2. Alternatively it is possible that the first step is in location 1 (just like the two step cascade above) and the next two steps is in location 2. In each case the only diffusing species is the communicating species, and the relevant models can be easily described by a simple expansion of the model considered above. We therefore do not explicitly describe these equations. A third case also considered is when the first and third steps are in location 1 and the second step is in location 2. This involves two communicating species. Again, this is described in an analogous manner.

Cases studied: We have examined a separated two step cascade, implemented as described above: the first stage is in one spatial location and the second stage is in the second spatial location, with the modified species at stage 1 being diffusible in the entire domain. We also study special cases of this scenario when the first stage operates in the ultrasensitive enzymatic regime, and the second stage operates in the mass-action kinetic regime, and this is implemented simply by an appropriate choice of kinetic parameters. In the case of the three stage cascade, a similar model of a cascade with 3 stages is studied. Here we consider different spatial designs as discussed in the text (see Fig. 3). This corresponds to localizing the entities involved in the stages in either of the compartments (implemented exactly as above) with the communicating species diffusing in the spatial domain.

Multiple Modification of Substrate. We now describe a spatial model of a 2-site ordered modification of substrate X. Here the modification of X to X^* is mediated by the kinase K1 and phosphatase P1, and the modification of X^* to X^{**} is mediated by the kinase K2 and the phosphatase P2. Note that, in an ordered multisite modification, there is a specific order to the modifications, in contrast to a random modification mechanism.

We will present a model which depicts the localization/compartmentalization of the two stages of this modification sequence, a scenario depicted in Fig. S1. In order to discuss this model, we first present a general spatial model of this two ordered site modification system. This is described by the equations:

$$
\begin{aligned}
\frac{\partial[X]}{\partial t} &= -k_1[X][K1] + k_{-1}[XK1] + k_4[X^*P1] + D_X\frac{\partial^2[X]}{\partial\theta^2} \\
\frac{\partial[X^*]}{\partial t} &= -k_3[X^*][P1] + k_{-3}[X^*P1] + k_2[XK1] - k_5[X^*][K2] + k_{-5}[X^*K2] + k_8[X^{**}P2] + D_{X^*}\frac{\partial^2[X^*]}{\partial\theta^2} \\
\frac{\partial[X^{**}]}{\partial t} &= k_7[X^{**}][P2] - k_{-7}[X^{**}P2] + k_6[X^*K2] + D_{X^{**}}\frac{\partial^2[X^{**}]}{\partial\theta^2} \\
\frac{\partial[K1]}{\partial t} &= -k_1[X][K1] + k_{-1}[XK1] + k_2[XK1] + D_{K1}\frac{\partial^2[K1]}{\partial\theta^2} \\
\frac{\partial[P1]}{\partial t} &= -k_3[X^*][P1] + k_{-3}[X^*P1] + k_4[X^*P1] + D_{P1}\frac{\partial^2[P1]}{\partial\theta^2} \\
\frac{\partial[K2]}{\partial t} &= -k_5[X^*][K2] + k_{-5}[X^*K2] + k_6[X^*K2] + D_{K2}\frac{\partial^2[K2]}{\partial\theta^2} \\
\frac{\partial[P2]}{\partial t} &= -k_7[X^{**}][P2] + k_{-7}[X^{**}P2] + k_8[X^{**}P2] + D_{P2}\frac{\partial^2[P2]}{\partial\theta^2} \\
\frac{\partial[XK1]}{\partial t} &= k_1[X][K1] - k_{-1}[XK1] - k_2[XK1] + D_{XK1}\frac{\partial^2[XK1]}{\partial\theta^2} \\
\frac{\partial[X^*P1]}{\partial t} &= k_3[X^*][P1] - k_{-3}[X^*P1] - k_4[X^*P1] + D_{X^*P1}\frac{\partial^2[X^*P1]}{\partial\theta^2} \\
\frac{\partial[X^*K2]}{\partial t} &= k_5[X^*][K2] - k_{-5}[X^*K2] - k_6[X^*K2] + D_{X^*K2}\frac{\partial^2[X^*K2]}{\partial\theta^2} \\
\frac{\partial[X^{**}P2]}{\partial t} &= k_7[X^{**}][P2] - k_{-7}[X^{**}P2] - k_8[X^{**}P2] + D_{X^{**}P2}\frac{\partial^2[X^{**}P2]}{\partial\theta^2}
\end{aligned}
\tag{3}
$$

The above model is simply a description of all the kinetic steps involved in the modification sequence, incorporating the diffusion of each species. The substrate species are X, X^*, X^{**}, while the relevant complexes are XK1, X^*P1, X^*K2 and $X^{**}P2$. The enzymes are K1 and P1 (first modification step) and K2 and P2 (second modification step). The forward association rate constants of the relevant enzyme to substrate are k_1, k_3, k_5 and k_7, the dissociation rate constants of the relevant enzyme substrate complexes are k_{-1}, k_{-3}, k_{-5} and k_{-7} and the corresponding catalytic constants are k_2, k_4, k_6 and k_8. The diffusion coefficients are $D_X, D_{K1}, D_{XK1}, D_{X^*}, D_{P1}, D_{X^*P1}, D_{X^*K2}, D_{X^{**}}, D_{K2}, D_{X^{**}P2}$ and D_{P2}, where the subscript denotes the species under consideration.

We focus on the effects of localization in the cascade. This is described in the model as follows. All species relevant to the first modification (and demodification) are in the first location, while those relevant to the second modification (and demodification) are present in the second location (see Fig. S1). All these species are regarded as non-diffusible. The initial conditions reflect the spatial localization of these components. Since these species are non-diffusible they remain localized in the relevant compartments. The only species which is diffusible is X^*. Thus the model describes a situation which is an analogue of

the two step cascade considered above: the spatial separation of two stages of this sequence with the common species diffusing from one location to the other to effect the communication. The only difference with the 2-step cascade is that the modified species acts as a substrate in the second stage.

Cases studied: The basic case studied involved unmodified substrate, K1 and P1 in the first compartment, while the enzymes K2 and P2 are in the second compartment. The modified form X^* diffuses everywhere in the spatial domain. The unmodified and doubly modified forms X and X^{**} are non-diffusible, and thus X^{**} remains in the second compartment. Some additional scenarios were also studied. In one case, we examined the case where the phosphatase is common for both steps. Thus in this case, we have exactly the same set up as above, except that the phosphatase in the second compartment is also P1 (at a possibly different concentration). Note that the P1 in each compartment remains in the relevant compartment. Then, we considered a scenario where all modifications occur in one compartment but X^{**} can diffuse out. This amounts to localizing all elements in the first compartment, but allowing X^{**} to be diffusible. A final case which is briefly studied in section 2.6 below involves the scenario where the same kinase K effects both modifications and the same phosphatase P effects reverse modifications. Here K (and corresponding kinase complexes) is localized in compartment 1, P (and corresponding phosphatase complexes) is localized in compartment 2 and the unmodified substrate X and fully modified substrate X^{**} diffuse in the spatial domain.

Phosphorelay. We now consider a different kind of modification sequence: a phosphorelay. We describe a spatial model of 4 step phosphorelay with phosphatases at each step. We first present a general model which allows the output of any of the stages to be the communicating species. All other species are assumed non-diffusible. The 4 step phosphorelay is described by the following equations:

$$
\begin{aligned}
\frac{\partial[X1]}{\partial t} &= -k_s[X1][K1] + k_2[X1^*X2] + k_{p2}[X1^*P1] \\
\frac{\partial[X1^*]}{\partial t} &= k_s[X1][K1] - k_1[X1^*][X2] + k_{-1}[X1^*X2] - k_{p1}[X1^*][P1] + k_{-p1}[X1^*P1] + D_{X1^*}\frac{\partial^2[X1^*]}{\partial\theta^2} \\
\frac{\partial[X2]}{\partial t} &= -k_1[X1^*][X2] + k_{-1}[X1^*X2] + k_4[X2^*X3] + k_{p4}[X2^*P2] \\
\frac{\partial[X2^*]}{\partial t} &= -k_3[X2^*][X3] + k_{-3}[X2^*X3] - k_{p3}[X2^*][P2] + k_{-p3}[X2^*P2] + k_2[X1^*X2] + D_{X2^*}\frac{\partial^2[X2^*]}{\partial\theta^2} \\
\frac{\partial[X3]}{\partial t} &= -k_3[X2^*][X3] + k_{-3}[X2^*X3] + k_{p6}[X3^*P3] + k_6[X3^*X4] \\
\frac{\partial[X3^*]}{\partial t} &= -k_5[X3^*][X4] + k_{-5}[X3^*X4] + k_4[X2^*X3] - k_{p5}[X3^*][P3] + k_{-p5}[X3^*P3] \\
&\quad + D_{X3^*}\frac{\partial^2[X3^*]}{\partial\theta^2} \\
\frac{\partial[X4]}{\partial t} &= -k_5[X3^*][X4] + k_{-5}[X3^*X4] + k_{p8}[X4^*P4] \\
\frac{\partial[X4^*]}{\partial t} &= -k_{p7}[X4^*][P4] + k_{-p7}[X4^*P4] + k_6[X3^*X4] + D_{X4^*}\frac{\partial^2[X4^*]}{\partial\theta^2} \\
\frac{\partial[X1^*X2]}{\partial t} &= k_1[X1^*][X2] - (k_{-1} + k_2)[X1^*X2] \\
\frac{\partial[X2^*X3]}{\partial t} &= k_3[X2^*][X3] - (k_{-3} + k_4)[X2^*X3] \\
\frac{\partial[X3^*X4]}{\partial t} &= k_5[X3^*][X4] - (k_{-5} + k_6)[X3^*X4] \\
\frac{\partial[X1^*P1]}{\partial t} &= k_{p1}[X1^*][P1] - (k_{-p1} + k_{p2})[X1^*P1] \\
\frac{\partial[X2^*P2]}{\partial t} &= k_{p3}[X2^*][P2] - (k_{-p3} + k_{p4})[X2^*P2] \\
\frac{\partial[X3^*P3]}{\partial t} &= k_{p5}[X3^*][P3] - (k_{-p5} + k_{p6})[X3^*P3] \\
\frac{\partial[X4^*P4]}{\partial t} &= k_{p7}[X4^*][P4] - (k_{-p7} + k_{p8})[X4^*P4] \\
\frac{\partial[P1]}{\partial t} &= -k_{p1}[X1^*][P1] + (k_{-p1} + k_{p2})[X1^*P1] \\
\frac{\partial[P2]}{\partial t} &= -k_{p3}[X2^*][P2] + (k_{-p3} + k_{p4})[X2^*P2] \\
\frac{\partial[P3]}{\partial t} &= -k_{p5}[X3^*][P3] + (k_{-p5} + k_{p6})[X3^*P3] \\
\frac{\partial[P4]}{\partial t} &= -k_{p7}[X4^*][P4] + (k_{-p7} + k_{p8})[X4^*P4]
\end{aligned}
\tag{4}
$$

The above model is simply a detailed depiction of all the elementary kinetic steps of a 4-stage phosphorelay, incorporating potential diffusion of the output of each stage of the relay (a 2-stage phosphorelay is depicted in Fig. S1). In the above model, the substrate species are X1, X2, X3, X4,

$X1^*, X2^*, X3^*$ and $X4^*$. The substrate complexes are $X1^*X2, X2^*X3$ and $X3^*X4$. The phosphatase substrate complexes are $X1^*P1, X2^*P2, X3^*P3$ and $X4^*P4$. The enzymes are K1 (the kinase for the first stage), and the phosphatases for the four stages are P1, P2, P3 and P4 respectively. The forward binding rate constants are k_s, k_1, k_3, k_5 (for the relevant forward modifications of each stage) and $k_{p1}, k_{p3}, k_{p5}, k_{p7}$ (for the reverse modifications of each stage). The dissociation/unbinding rate constants are $k_{-1}, k_{-3}, k_{-5}, k_{-p1}, k_{-p3}, k_{-p5}, k_{-p7}$ and the catalytic rate constants are $k_2, k_4, k_6, k_{p2}, k_{p4}, k_{p6}$ and k_{p8}. The diffusion coefficients of the substrate species are $D_{X1^*}, D_{X2^*}, D_{X3^*}$ and D_{X4^*}, where the subscript denotes the species under consideration. The first step of the phosphorelay is assumed to occur through mass action kinetics.

The above model is a simple model of a phosphorelay, where each elementary step is modelled by mass action kinetics; the binding/unbinding of the species is described explicitly. The main difference between this model and that of the cascade arises in the fact when the output at one stage (say $X1^*$) transfers a phosphate group to a species in the next stage (say $X2$), it gets converted back (to $X1$).

As before, we will consider different scenarios where some steps are localized in one location and other steps are localized in the other location. For instance a scenario where the first two steps are in one spatial location and the last two are in a second location, is described in the model by having $X2^*$ be the only diffusible species, and all other components be non-diffusible and initially localized in their respective compartments. The relevant communicating species is the sole diffusing species in such cases and all other species remain localized in their respective compartments. Thus such a model describes a phosphotransfer mechanism with spatial localization.

A two step phosphotransfer model is obtained by considering only the first two steps in the model above. Making $X1^*$ the only diffusible species and localizing the first stage in one location and the second stage in another location results in the two step phosphotransfer model with compartmentalization (see Fig. S1).

Cases studied: The basic cases we studied were two-stage and four-stage phosphorelays, with compartmentalization. This was done by localizing the elements of the corresponding stages in the appropriate compartments, and having the communicating species diffuse in the spatial domain. In the case of the two stage phosphorelay, the first stage entities are in compartment 1 and the second stage entities are in compartment 2, with the species $X1^*$ diffusing in the spatial domain. In the case of a four stage phosphorelay, there are multiple ways to partition the cascade between the two compartments, but again this is implemented in exactly the same way: the first part of the cascade is in location 1, the second part of

the cascade is in location 2, with the communicating species diffusing everywhere in the spatial domain. We also consider some variants of this basic scenario: one was where the phosphatase of the last stage could act as a kinase of the first stage. This phosphatase was always present only in compartment 2, and the model was refined to allow it to modify $X1$ to $X1^*$ at this location if $X1$ was present here. This was studied for both the two stage and four stage phosphorelay.

1.2 Open cascade

For completeness, we also consider open models of modification sequences. For instance, we consider a 3 step modification sequence (assumed irreversible, for simplicity: reversible analogues have also been studied). Here $X1$ is modified to $X2$ which is modified to $X3$. The only difference here is that there is a constant production of $X1$ and a removal of $X3$, proportional to its concentration. This is described by the model

$$\begin{aligned}
\frac{\partial[X1]}{\partial t} &= k_o - k_s[S][X1] \\
\frac{\partial[X2]}{\partial t} &= k_s[S][X1] - k_2[X2] + D_{X2}\frac{\partial^2[X2]}{\partial\theta^2} \\
\frac{\partial[X3]}{\partial t} &= k_2[X2] - k_d[X3]
\end{aligned} \tag{5}$$

The signal S enters the chain of reactions between X1 and X2, catalyzing the conversion of $X1$ to $X2$. k_o is the generation term for X1 (and is non-zero only in the first compartment) and k_s is the rate constant associated with the signal converting X1 to X2. k_2 is the rate constant associated with the conversion of X2 to X3 (this occurs only in the second compartment) and k_d is the degradation constant for X3. We will assume that $X2$ is the communicating species and that $X1$ is in the first location and $X3$ is in the second location. D_{X2} is the diffusion coefficient for X2. It should be noted that by making $X2$ non-diffusible and by localizing all species in the same location, we recover the ODE model of this modification sequence (with input and removal). All reactions are described by mass-action kinetics for simplicity.

One can consider a minor variation of the above structure, where the signal is associated with the

conversion from $X2$ to $X3$. This is described by the model

$$\begin{aligned}\frac{\partial[X1]}{\partial t} &= k_o - k_1[X1] \\ \frac{\partial[X2]}{\partial t} &= k_1[X1] - k_s[S][X2] + D_{X2}\frac{\partial^2[X2]}{\partial\theta^2} \\ \frac{\partial[X3]}{\partial t} &= k_s[S][X2] - k_d[X3]\end{aligned} \tag{6}$$

Here, k_o is the generation term for X1 (present only in compartment 1) and k_1 is the rate constant associated with the conversion of X1 or production of X2. k_s is the rate constant for X2 being converted to X3 by the signal (this happens only in compartment 2) and k_d is the degradation constant for X3. D_{X2} is the diffusion coefficient for X2.

Cases studied: The cases we have studied involve $X1$ in compartment 1, $X3$ in compartment 2, and $X2$ diffusing everywhere. In one case, the signal is associated with the conversion of $X1$ to $X2$ (this happens only in compartment 1) and in the other case, the signal is associated with the conversion of $X2$ to $X3$ (this happens only in compartment 2).

2 Analysis of models

2.1 Cascades

We first consider a two step enzymatic cascade for concreteness. Note that here X^* is the communicating species between the two compartments and also the only diffusible species. By adding all the equations of all the species, we find that all kinetic terms cancel out leaving, at steady state,

$$\frac{\partial^2[X^*]}{\partial\theta^2} = 0$$

This shows that this species has a spatially uniform profile. We now deduce some facts based on this.

Spatial separation can result in reduction of the output of the cascade:

We start by noting that the steady state of the cascade corresponds to the standard kinetic equations (i.e. those resulting from the ODEs) in each compartment, along with the conservation condition. The conservation of total substrate implies that $L_1([X] + [XK1] + [X^*P1] + [X^*Y]) + L[X^*] = L_1X_{tot}$. Note that X_{tot} corresponds to the total concentration of X species present initially and that L_1X_{tot} hence

corresponds to the total amount of this species in the system. Similarly, $[Y] + [X^*Y] + [Y^*] + [Y^*P2] = Y_{tot}$. Y_{tot} corresponds to the total concentration of Y species present initially and that $L_1 Y_{tot}$ hence corresponds to the total amount of this species in the system. The Y species remains localized in the second compartment, and hence the sum of concentrations of all the species involving Y is constant. In the above, L_1 corresponds to the size of the compartments (assumed equal) and L corresponds to the size of the overall domain. Clearly $L > 2L_1$ if the two patches are disjoint and separated (the case we study of spatial cascades). $L = L_1$ corresponds to the situation where the two patches are coincident. Writing $L = L_1 + L_e$ we see from the conservation equation above, that in effect the available total amount of X species in compartment 1 is reduced by a factor $L_e[X^*]$. For simplicity to start with, we will assume that dephosphorylation in the second step occurs via mass action kinetics.

We will approach this in two stages. Suppose there was no retroactivity (i.e. the phosphorylation in the second stage occurred via mass action kinetics). Then, we see that the conservation equation for species X results in $L_1([X] + [XK1] + [X^*P1]) + L[X^*] = L_1 X_{tot}$. In other words $[X] + [XK1] + [X^*] + [X^*P1] \leq X_{tot}$: the total concentration of X species in the first compartment is reduced due to its spreading in the domain.

Now note that the steady state of the first stage is determined by the steady state of the ODE model for this stage (note that at steady state $[X^*]$ is uniform, and the flux out of the compartment is zero) with an appropriate reduced effective total amount of X species in compartment 1 (i.e. a reduced effective X_{tot})), accounting for the leakage of X^*. We now show that at steady state in the ODE kinetic model $d[X^*]/dX_{tot} > 0$. Showing this amounts to showing that the steady state of X^* which is obtained when it leaks out is less than when all the reactions occur in the same compartment. We focus on the ODE kinetic model. Clearly this is the case for small X_{tot}, so if this condition were violated, we must require $d[X^*]/dX_{tot} = 0$ for some value of parameters. Now analyzing the model of the first level of the cascade, we see from conservation of enzymes that $[XK1] = \alpha_1 K_{tot}[X]/(1 + \alpha[X])$, $[X^*P1] = \beta_1 P1_{tot}[X^*]/(1 + \beta[X^*])$ for suitable constants $\alpha, \alpha_1, \beta, \beta_1$. Furthermore the concentrations of these complexes are proportional to each other at steady state. Now if $d[X^*]/dX_{tot} = 0$, then it follows immediately that $d[X^*P1]/dX_{tot} = 0$ and from above that $d[XK1]/dX_{tot} = 0$ (proportionality of complexes). It then follows that $d[X]/dX_{tot} = 0$. These conditions violate the conservation of species, and hence we conclude for a single covalent modification cycle $d[X^*]/dX_{tot} > 0$. Now if we consider this in light of a spatial cascade with no retroactivity, we find that the spreading of X^* in the domain reduces the total X species in compartment 1. This immediately means that X^* is reduced as a result, and since the

kinetics in the second cycle is mass action, it means that $[Y^*]$ is also reduced (it being an increasing function of the concentration of $[X^*]$).

We now examine a situation where the phosphorylation in the second cycle is not necessarily mass action (the dephosphorylation is still assumed to occur via mass action kinetics). Here we employ conservation of species Y to impose $[Y] + [Y^*] + [X^*Y] = Y_{tot}$. Now the steady state for the second cycle means $\gamma_1[Y^*] = \gamma_2[X^*Y] = \gamma_3([X^*])([Y])$ for suitable constants $\gamma_1, \gamma_2, \gamma_3$. From this and the fact that the steady state concentration of the complex X^*Y is proportional to the product of concentrations of X^* and Y, we can infer that at steady state, the functional relationship between $[Y^*]$ and $[X^*]$ is of the form

$$\begin{aligned}[Y^*] &= \frac{Y_{tot}[X^*]}{a_1 + b_1[X^*]} \\ [X^*Y] &= \frac{aY_{tot}[X^*]}{a_1 + b_1[X^*]}\end{aligned} \tag{7}$$

Here a, a_1, b_1 are constants.

Now in this case, the steady state for $[X^*]$ is governed by the same kinetic equations in the first location, along with the modified conservation condition
$L_1([X] + [XK1] + [X^*P1] + [X^*Y]) + L[X^*] = L1X_{tot}$.

We now reason as follows. In the ODE model of the two step cascade, we see (under the conditions above) that $d[X^*]/dX_{tot} > 0$. Clearly this is the case for small X_{tot} so if this condition were violated, we must require $d[X^*]/dX_{tot} = 0$ for some value of parameters. We show that this is not possible. To do this we follow the exact same procedure above. We note that in the conservation relationship for species X, there is an extra term corresponding to the concentration of the complex $[X^*Y]$, which as noted above is related to X^* in the manner described. Thus if $d[X^*]/dX_{tot} = 0$, then it automatically follows that the derivative of this term with respect to X_{tot} is also zero. Thus the above argument carries through exactly. It is impossible to satisfy the conservation condition if $d[X^*]/dX_{tot} = 0$ at steady state. Thus $d[X^*]/dX_{tot} > 0$.

Now for the distributed system at steady state, the species of tier-1 satisfies the same steady state equation as the ODEs with a reduced X_{tot} (corresponding the extra X^* in the medium: compare the conservation conditions of the co-localized cascade, and the distributed cascade). Therefore at steady state $[X^*]$ is reduced when compared to the situation where all species are localized together. From above it immediately follows that the same is true for $[Y^*]$. This shows how the separation of steps leads to a reduction in the output of the cascade.

Retroactivity. The above analysis can be used to examine the amount of X species contained in the

downstream complex X^*Y which is a measure of the retroactivity. We see from the above analysis that the $[X^*Y]$ is related to the $[X^*]$ via a monotonic function. Since the separation leads to a reduction in $[X^*]$ (relative to the ODEs), we find that the $[X^*Y]$ concentration is also reduced. In other words, the separation leads to a reduction in the retroactive effect.

The case where dephosphorylation of the second stage does not occur in the mass action regime. In the above analysis we considered the case where the dephosphorylation of the second stage occurred via mass action kinetics. We now relax that assumption. In this case, we start our analysis of the ODE model from the second stage of our cascade. We show that at steady state $d[Y^*]/dX_{tot} > 0$. Now clearly this is the case for small X_{tot}, and so if this condition were violated, then at some value of X_{tot}, $d[Y^*]/dX_{tot} = 0$. Now at steady state in the second cycle, we have

$$\begin{aligned} [Y^*P2] &= \frac{P2_{tot}[Y^*]}{c_1 + d_1[Y^*]} \\ e_1[X^*Y] &= f_1[Y^*P2] \end{aligned} \tag{8}$$

and $[X^*Y] = \alpha_1([X^*])([Y])$ where $\alpha_1, c_1, d_1, e_1, f_1$ are all positive constants. Now suppose $d[Y^*]/dX_{tot} = 0$, it immediately follows that $d[Y^*P2]/dX_{tot} = 0$ and hence that $d[X^*Y]/dX_{tot} = 0$ from above. Now since $[Y] + [Y^*] + [X^*Y] + [Y^*P2] = Y_{tot}$, it follows that $d[Y]/dX_{tot} = 0$. This, along with the fact that $d[X^*Y]/dX_{tot} = 0$, shows that $d[X^*]/dX_{tot} = 0$. The rest of the argument is identical to the cases above, indicating that this is an impossibility. Therefore we have $d[Y^*]/dX_{tot} > 0$, and from above $d[X^*Y]/dX_{tot} > 0$. Now exactly as before, we note that the the steady state of the distributed cascade satisfies the same equations as the ODEs but with a reduced X_{tot}. This implies both a reduction in output, as well as a reduction in the retroactivity, just as before.

Buffering against dilution. The above analysis also provides insights into how the dilution effects can be buffered against in cascades. We see that the dilution effect occurs through X^* spreading in the medium. There are different ways to reduce this effect. One way is to reduce the length of the medium. A second way, also seen from above, is to increase phosphatase $P1$ concentration. This leads to a reduction in $[X^*]$. Now a low $[X^*]$ will also result in a lower amount of dilution in the medium with the result that the effective total amount of X in location 1 will be modified only slightly. Thus the characteristics of the cascade are affected in only a minor way. While this of course involves operating the cascade in a regime of relatively low $[X^*]$, Table 1 shows how the effect of dilution in both absolute and relative terms is buffered against.

To complement this, we perform some basic analytical calculations to illustrate the main points. We

will assume negligible retroactivity, for simplicity, and focus on a single stage of the cascade. We note that we will be examine scenarios of high $P1$ concentration: this means that the free $P1$ concentration practically equals the total $P1$ concentration. For simplicity, suppose the first step of the cascade occurs through mass action kinetics. Then the functional relationship between the variables at steady state are given by

$$\begin{aligned} a_1[X][K1] &= b_1[X^*][P1] \\ L_1([X]+[X^*])+L_e[X^*] &= L_1 X_{tot} \end{aligned}$$

where $L = L_1 + L_e$, a_1, b_1 are constants. Here $K1, P1$ equal $K1_{tot}, P1_{tot}$ respectively. This results in a steady state

$$[X^*] = \frac{X_{tot}}{1 + L_e/L_1 + b_1 P1_{tot}/a_1 K1_{tot}} \tag{9}$$

The dilution effect is contained in the term L_e/L_1. We see in the above equation that when the last term in the denominator is large, it can dwarf the second term. The ratio of the steady state in the distributed cascade to that of the colocalized cascade ($L_e = 0$) is given by

$$R = \frac{1 + b_1 P1_{tot}/a_1 K1_{tot}}{1 + L_e/L_1 + b_1 P1_{tot}/a_1 K1_{tot}} \tag{10}$$

and this ratio approaches 1 as $P1_{tot}$ is increased. This explains the assertion that it is possible to buffer against dilution, even in relative terms.

We can extend this analysis even when the kinetics is not in the mass action regime. If we focus on tthe functional relationships between the variables, we have at steady state

$$\begin{aligned} [X^*P1] &= \gamma_1 P1_{tot}[X^*] \\ \gamma_2[XK1] &= \gamma_3[X^*P1] \\ [XK1] &= \gamma_4 K1_{tot}[X]/(1+\gamma_4[X]) \\ L_1 X_{tot} &= L1([X]+[XK1]+[X^*P1]+[X^*])+L_e[X^*] \end{aligned} \tag{11}$$

where all the γ terms are constants. Eliminating $[X^*]$ and writing the equation in terms of $[X]$, results in an equation of the form

$$[X] + \gamma_4 \frac{K1_{tot}[X]}{(1+\gamma_4[X])} + \beta_2 \frac{K1_{tot}[X]}{P1_{tot}(1+\gamma_4[X])} + \beta_2 \frac{L_e}{L_1} \frac{K1_{tot}[X]}{P1_{tot}(1+\gamma_4[X])} = X_{tot} \tag{12}$$

where the various β terms are constants. Even without solving this equation we see that the dilution effect (contained in the term L_e/L_1) is actually reduced by the presence of large $P1_{tot}$. X^* can be obtained from X via an equation independent of L_e, and is also hence buffered against.

Cascade with one step in the Goldbeter-Koshland regime. In the text we mentioned that one way to propagate the effects of a Goldbeter-Koshland switch spatially, is to keep that step localized, and have that propagated by a communicating step involving species I and I^* with the output (X^*) of the Goldbeter-Koshland module regulating the conversion of I to I^* through mass-action kinetics. The kinetics for the species I, I^* can be written as

$$\begin{aligned} \frac{\partial [I]}{\partial t} &= -k_{f1}[X^*][I] + k_{r1}P_0[I^*] \\ \frac{\partial [I^*]}{\partial t} &= k_{f1}[X^*][I] - k_{r1}P_0[I^*] + D_{I^*}\frac{\partial^2 [I^*]}{\partial \theta^2} \end{aligned} \tag{13}$$

Here I is localized in region 1 and so is the phosphatase for this stage P_0. In this case, X^* is not taken up in the downstream reaction and so it exhibits a switch like response to its input, since the behaviour of X^* is determined by ODEs for that layer of the cascade. $[X^*]$ is a parameter in the equations above. Further, the communicating layer (I, I^*) can be regarded as the first step of a cascade similar to what we have studied in the paper, and the switch behaviour is already present in the input to this layer. Thus, the switch effect is propagated in the cascade, though dilution via the diffusing species reduces the amplitude, relative to the situation where all steps are co-localized. Overall, a switch behaviour is seen in the spatially distributed cascade.

Transient response of cascades: effect of pulse duration: In the text we examined the response of three step cascades to pulse inputs, examining the effects of diffusivity of the communicating species, as well as the patch size. We briefly examine the effect of pulse duration here, for fixed diffusivity of communicating species. Increasing pulse duration increases the transient peak concentration and results in a clear plateau in the transient response of the output (Fig. S3(b)). In general, the sensitivity to pulse duration depends on the pulse duration relative to kinetic time scales of the cascade and the time scale of diffusion of the communicating species.

2.2 Multisite modification and phosphotransfer

Multisite Modification. We briefly examine aspects of the multisite modification. Again, since X^* is the

only species which is diffusing, we find by adding all the equations that at steady state

$$\frac{\partial^2[X^*]}{\partial\theta^2} = 0$$

In other words the concentration of X^* is uniform in the domain. The conservation condition for substrate is altered by the fact that X^* spreads in the medium. The equation becomes
$L_1([X] + [XK1] + [X^*P1] + [X^*K2] + [X^{**}] + [X^{**}P2]) + L[X^*] = L_1X_{tot}$.

We will show that in the ODE model of the two site modification $[dX^{**}]/dX_{tot} > 0$. This can be seen by examining the two cycles sequentially. Firstly we see that by applying the conservation conditions for all the enzymes we have $[P1] = P1_{tot}/(1 + \gamma_1[X^*])$ $[K1] = K1_{tot}/(1 + \gamma_2[X])$,
$K2 = K2_{tot}/(1 + \gamma_3[X^*])$, $P2 = P2_{tot}/(1 + \gamma_4[X^{**}])$, where γ_i are constant. Furthermore, all complex concentrations are simply proportional to the product of those of the corresponding free enzyme and substrate.

We note that $d[X^{**}]/dX_{tot} > 0$, for small X_{tot}. Suppose the inequality does not hold, we must require that $d[X^{**}]/dX_{tot} = 0$ at some parameter value. From an analysis of the second covalent modification cycle, since the concentrations of the two complexes are proportional at steady state, and the fact that each complex is related to the substrate concentration as a function of the form $a[S]/(1 + b[S])$, we immediately see that $d[X^{**}P2]/dX_{tot} = 0$, $d[X^*K2]/dX_{tot} = 0$. From this it follows that $d[X^*]/dX_{tot} = 0$.

Now, we use this and repeat the same analysis in the first cycle, to find that the derivative of concentrations of all substrate and complex species with respect to X_{tot} is zero. This leads to a contradiction since this violates the conservation condition for substrate species. This shows therefore that $d[X^{**}]/dX_{tot} > 0$. Now if we contrast the spatially segregated model of the multisite modification model to that where all modifications occur together, we see that at steady state the formal kinetic equations satisfied in both cases is the same. The only difference arises in the conservation conditions. The steady state for the spatially distributed model corresponds to the steady state of a model with co-localized modifications (i.e. an ODE model) with a reduced X_{tot}. Since $d[X^{**}]/dX_{tot} > 0$, we find that the spatially distributed modification results in reduced $[X^{**}]$.

The variation of concentration and total amount of X^* with domain size: basic case We now turn to a different aspect of multisite modification. We examine both the concentration of X^* and the total amount of this species in the situation when all modifications occur in the same location (patch of size L_1) and when the modifications are separated as examined above, also studying the effect of varying the overall

domain size. For simplicity, and to get some intuition, we will assume that all modifications occur via mass action kinetics (i.e. effectively very large catalytic constants for all modifications). In this case we have at steady state $[X^*]/[X] = \alpha K1_{tot}/P1_{tot}$ and $[X^{**}]/[X^*] = \beta K2_{tot}/P2_{tot}$, where α, β are the equilibrium constants for the two reactions. Now the conservation condition reads
$L_1([X] + [X^{**}]) + L[X^*] = L_1 X_{tot}$. From this it simply follows that

$$[X^*] = \frac{L_1 X_{tot}}{L_1(P1_{tot}/(\alpha K1_{tot}) + \beta K2_{tot}/P2_{tot}) + L}$$
$$[X^{**}] = \frac{\beta L_1 X_{tot} K2_{tot}/P2_{tot}}{L_1(P1_{tot}/(\alpha K1_{tot}) + \beta K2_{tot}/P2_{tot}) + L}$$

It is clear from above that steady state concentrations of X^* and X^{**} are decreasing functions of L. Hence the concentration of these species in the separated case is also less than the co-localized case ($L = L_1$), as can also be expected from the previous discussion. Now if we consider the total amount of X^* species in the domain, that is given by $L[X^*]$, we find that this is a function which actually increases with L. We thus see that separated modification and increased separation does indeed decrease the doubly modified phosphoform concentration but in fact increases the total amount of phosphoform species X^* in the domain.

The effect of domain size on the total amount of X^*: the general case. We will now show the behaviour of the total amount of X^* in the domain increasing with domain length L, occurs even when the kinetics is far from mass action. To do this we note

(1) All relevant enzyme-substrate complex concentrations, can be related to their substrate in the form $[ES] = a[S]/(1 + b[S])$ where a,b are constants related to kinetic parameters. Note that the enzymes in the different modification stages are different.

(2) The steady state for the distributed cascade, involves the steady state for each set of modifications at their relevant locations. Considering the two stages, we have equations of the form
$a_1[X^*]/(1 + b_1[X^*]) = a_2[X^{**}]/(1 + b_2[X^{**}])$ and $a_3[X]/(1 + b_3[X]) = a_4[X^*]/(1 + b_4[X^*])$.

(3) By differentiating these equations with respect to L (or inverting them and differentiating them), we see that the derivative of X, X^*, X^{**} all have the same sign, and noting point (1), so do the derivatives of all relevant complexes.

(4) Now the conservation condition states that
$L_1([X] + [XK1] + [X^*P1] + [X^*K2] + [X^{**}] + [X^{**}P2]) + L[X^*] = L_1 X_{tot}$. Suppose that the derivative of all the relevant concentrations with respect to L were positive. Then we find that the conservation condition would be impossible to satisfy. Thus we must have that the derivative of all these

concentrations must be negative. Then, by differentiating the conservation condition with respect to L, we have $d/dL(L[X^*]) > 0$, which demonstrates the point.

Common Phosphatase. It is clear to see in the case of multisite modification with shared (i.e. common) phosphatase, that a situation of separated modifications will result in all the X species in the second domain to be converted to the unmodified form X. Further, at steady state the concentration of X^* (which is uniform in the domain) is 0. This is simply seen by considering the second location. Firstly we know from above that the concentration of X^* is spatially uniform. Now in the second location, by examining the kinetics of conversion we see that all available X^* is converted to X. In other words, by examining the steady state condition for X, we see that $[X^*] = 0$ (since there is nothing to remove X and what is produced is produced via X^*). From this it follows that $[X^{**}] = 0$ and in fact the only species in location 2 is X. In fact all the substrate species ends up as X in the second location. This is true irrespective of the model parameters. In such a situation, an extra mechanism would be needed to transfer the X back to the original location.

Phosphorelay. We briefly discuss a couple of aspects of the phosphorelay mechanism. We asserted that a 2 step separated model of the phosphorelay would result in 0 concentration of the output at steady state. We therefore consider a two step phosphorelay mechanism. Again we find similar to before that the spatial concentration profile of the communicating species $X1^*$ is uniform at steady state. This is obtained by adding all the equations of the species. Now, we notice that owing to the phosphotransfer mechanism, $X1^*$ is converted to $X1$ at the second location. By examining the equation for $X1$ at the second equation at steady state, we find that $[X1^*] = 0$ at steady state. This is because $X1$ is produced by $X1^*$ and not removed by any other mechanism. Therefore $[X1^*] = 0$ at steady state, from which it follows that $[X2^*] = 0$. Naturally $[X2^*]$ may transiently increase from 0. We see a reasoning very similar to the situation above. Again, this conclusion does not depend on model parameters.

Finally, we briefly examine a 4 step phosphorelay, where the phosphatase of the final step is a bifunctional kinase (capable of triggering the phosphorelay). We see that if the phosphorelay is separated after the first step (i.e. the communicating species is $X2^*$ or $X3^*$) then by exactly the same reasoning as above, we find that $[X4^*]$ at steady state is 0. Since the phosphatase of the final stage is the second location, it cannot trigger the conversion of $X1$ to $X1^*$. If however the communicating species is $X1^*$ then this is not necessarily the case: this is because the bifunctional kinase can effect a conversion from $X1$ (created in the second location) back to $X1^*$. Naturally, this pre-supposes the fact that the bifunctional kinase is capable of functioning as a kinase in the second location. For the same reason, if we have a two

stage phosphorelay, with $X1^*$ being the communicating species, it is possible to have a non-zero steady state of $X1^*$ and $X2^*$. This justifies what we mentioned in the text.

2.3 Irreversible cascade with inflow and outflow

We now examine the 3 species irreversible cascade with inflow and outflow. By placing the entire cascade in one location, we find that at steady state, by adding all the equations of species, $[X3] = k_o/k_d$. This shows how at steady state the output of the cascade recovers to pre-stimulus values (it is independent of S), exhibiting an adaptive response. We now consider spatial models of such a cascade. For specificity, we consider a situation where $X2$ is the diffusing species, and the signal mediates the conversion of $X2$ to $X3$. Now by adding all the equations and integrating over the full domain, we find that $k_o L_1 = k_d \int_0^{L_1} [X3] d\theta$. This shows that the spatially averaged concentration of the output of the cascade, X3 is in fact constant at a level independent of the stimulus, at steady state.

In order to obtain the steady state for $[X3]$, we need to find the steady state for $[X2]$. The steady state for $[X2]$ is governed by

$$\begin{aligned} k_o + D_{X2}\frac{\partial^2[X2]}{\partial\theta^2} &= 0, \quad 0 \le \theta < L_1/2 \\ D_{X2}\frac{\partial^2[X2]}{\partial\theta^2} &= 0, \quad L_1/2 \le \theta < (L-L_1)/2 \\ -k_s S[X2] + D_{X2}\frac{\partial^2[X2]}{\partial\theta^2} &= 0, \quad (L-L_1)/2 \le \theta < L/2 \end{aligned} \tag{14}$$

The first equation has been written, eliminating $[X1]$. This is supplemented by no-flux boundary conditions at the two ends (for simplicity the problem is analytically solved in half the domain, applying no flux boundary conditions).

The solution to these equations can be obtained by solving them in a piece-wise manner and matching them at the two interfaces. Thus the solution is obtained as

$$\begin{aligned} &[X2] = -(k_o/2D_{X2})\theta^2 + C_1\theta + C_2, \quad 0 \le \theta < L_1/2 \\ &[X2] = c_3\theta + c_4, \quad L_1/2 \le \theta < (L-L_1)/2 \\ &[X2] = c_5 exp(-\sqrt{(}k_s S/D_{X2})x) + c_6 exp(\sqrt{(}k_s S/D_{X2})x, \quad (L-L_1)/2 \le \theta < L/2 \end{aligned} \tag{15}$$

The net steady state solution can be obtained by matching the concentration and flux at the two interfaces. We will not present the detailed expression here, as the main observations can be obtained from the

expression above. We note that the overall profile (in each of the locations) depends on all the parameters in the above equation (through the matching conditions).

We see here that $[X3]$ can be easily obtained from the $[X2]$ concentration in the last subdomain, and is in fact proportional to it, with a proportionality factor depending on S. The point to note is (i) The solution is dependent on the absolute value of S and (ii) The solution also depends on the absolute value of the diffusion coefficient. We can therefore say that even though the average concentration of X3 exhibits an adaptive response, the local concentration of X3 at any specified location in the second location does not, in fact, adapt. In fact many characteristics of $[X3]$, such as the slope of the profile, do depend on S. Secondly, if the diffusion coefficient of X2 is very high, then the profile of X2 equilibrates at a level equal to $k_o/(k_s[S])$. This can be seen from the original equation in the limit of high D_{X2} by using a perturbation expansion in $1/D_{X2}$. In this case the steady state profile of $X3$ is spatially uniform at a level k_o/k_d. We therefore see how spatial separation can distort the local adaptive response, and how a high diffusion of communicating species can make the response close to adaptive.

In general we find that step changes in S result in overadaptive responses for $[X3]$ in some parts of the second domain (for instance in the middle, as shown in Fig. S4) and in underadaptive responses in others.

Now if we examine an analogous situation to the one above, but where the signal mediates the modification from $X1$ to $X2$, we find that $[X2]$ attains a profile independent of the signal, irrespective of the diffusion coefficient. Therefore, we find that $[X3]$ actually adapts to a step change in S, even though it does not have a uniform profile in the second compartment. This is seen by noting that the steady state profile of the diffusing species is independent of S. This is seen immediately by inspecting the equations for $[X2]$ in this case:

$$\begin{aligned} k_o + D_{X2}\frac{\partial^2[X2]}{\partial\theta^2} &= 0, \quad 0 \le \theta < L_1/2 \\ D_{X2}\frac{\partial^2[X2]}{\partial\theta^2} &= 0, \quad L_1/2 \le \theta < (L-L_1)/2 \\ -k_2[X2] + D_{X2}\frac{\partial^2[X2]}{\partial\theta^2} &= 0, \quad (L-L_1)/2 \le \theta < L/2 \end{aligned} \tag{16}$$

Note that $[X1]$ has been eliminated in the first equation. We see that this equation is independent of S and hence so is $X2$, even though the profile is not homogeneous. Therefore step changes in S result in locally adaptive responses for $X3$.

Overall, we see how, depending on the position of the signal in an open spatial cascade, one can maintain adaptive responses in some cases, and distort it in others. In contrast, exact adaptive responses result when the entire cascade is spatially localized in one location, irrespective of the position of the signal in the cascade. The diffusivity of $X2$ can affect the amplitude of the transient response of $X3$. If the diffusivity is lowered, the amplitude is lowered. This is because a low diffusivity means that a change in signal causes a practically exact adaptive response of $X2$ in location 1 (its dynamics there essentially governed by the kinetics), and any weak residual effect is slowly communicated to $X3$ through diffusion. Numerical results for the open irreversible cascade are shown in Fig. S4.

2.4 Communicating layer of cascade with multiple diffusing entities

While examining spatially separated model of cascades, we examined situations where the output of one level of the cascade acted as the communicating species. We now examine a variant of this scenario where both the modified and unmodified form are diffusible. In this case the two segments of the cascade may be regarded as being connected via a global layer.

We therefore examine the dynamics of this global layer. For simplicity we will assume that all relevant reactions involving the interconversion of these species act via mass action kinetics, and there is negligible retroactivity in the downstream reaction. We will refer to the species as X and X^* and assume both have the same diffusion coefficient D. At steady state we have

$$\begin{aligned} -k_f K(\theta)[X] + k_r P(\theta)[X^*] + D\frac{\partial^2[X]}{\partial\theta^2} &= 0, \\ k_f K(\theta)[X] - k_r P(\theta)[X^*] + D\frac{\partial^2[X^*]}{\partial\theta^2} &= 0 \end{aligned} \tag{17}$$

Here $K(\theta), P(\theta)$ refer to the kinase and phosphatase profiles. When we consider cascades of the kind examined earlier, $K(\theta)$ will be non-zero only in the first location.

By adding the above equations we find that at steady state $X + X^*$ is a constant, uniform in space. We call this constant X_T. Therefore the equation simplifies to

$$k_f K(\theta)(X_T - [X^*]) - k_r P(\theta)[X^*] + D\frac{\partial^2[X^*]}{\partial\theta^2} = 0 \tag{18}$$

We will focus on some specific aspects of the profile of X^*. In general, in such a scenario, we may expect different possibilities for where the phosphatase is located. It may be located everywhere in the

domain, it may be located in the second location, or it may be located in the first location.

In the case where the phosphatase is present everywhere in the domain, the above steady state can be obtained, and the resulting profile depends on the diffusion coefficient (see Fig. S5). Similarly when the phosphatase is in the second location only, the profile obtained, depends on all parameters, and depends on the diffusion coefficient. In both cases, when the diffusion coefficient becomes high, X^* attains a profile which is uniform.

In the case where the phosphatase is co-localized with the kinase, in the first location, we notice something different. When the kinase and phosphatase profile is uniform in this location, we have

$$[X^*] \quad = \quad X_T \frac{K/P}{k_r/k_f + K/P} \tag{19}$$

The point to note is that this concentration profile is uniform in space, and this is independent of the diffusion coefficient value. Thus we find that co-localizing the kinase and phosphatase of a global communicating layer can insulate the cascade from the effects of diffusivity at steady state. This points to another aspect of design and spatial organization of cascades. We mention one further point in this regard. If we compare this cascade, with a completely co-localized cascade, with the same total amount of X species in the medium (and of course all other factors the same), we find that the spatially distributed cascade involves a dilution effect. This is because the factor X_T is reduced (relative to the co-localized case) due to the fact that the X species is present in the entire medium.

2.5 Response of a modification cascade to spatial gradients

As mentioned in the paper, our framework allows us to also examine aspects of spatial signal transduction in cascades, without localization. We consider one such aspect here.

Diffusion and retroactivity in cascades. Although most of our focus in the paper is on effects of localization, there are other aspects to spatial signal transduction in cascades. One aspect which we briefly discuss is the response of a two tier cascade to spatial gradients. In this case all species are present everywhere in the spatial domain (i.e. no localization). Thus, we consider the same model of the cascade, with all components present everywhere in the domain. The input is provided in the form of a spatial gradient. When none of the signalling components diffuse, then the signal transduction is purely local, and can be studied using ODEs. On the other hand, as we have shown previously (1), diffusion of individual components can significantly alter and distort signal transduction. When we examine signal transduction in

such a cascade, the effects of diffusion of multiple components can be studied, some which can be simply understood from an equivalent study of a single covalent modification cycle (undertaken in (1) and others which rely on the interplay of the two cycles. We discuss one example of the latter.

The effects of diffusion perturbing the first modification cycle can largely be understood by the consideration of a single covalent modification cycle. When we examine the effects of diffusion of species in the downstream covalent modification cycle, some subtle features arise. When the species in the second step Y and X^*Y (the unmodified substrate at the second tier and the relevant complex) diffuse while all other species are weakly diffusible, (Figure S6), we find that the spatial profile of Y^* weakens. Further, the profiles of the species in the upstream cycle change- X becomes sharper, X^* becomes flatter and profiles of complexes in the first cycle are also affected. The underlying reason for the change in profiles of species upstream is that X^* is sequestered in the complex X^*Y. Thus in this case diffusion affects the profile of X^* having a ripple effect on the remaining species in the first step.

This demonstrates how diffusion of species in a signalling cascade can significantly affect species upstream and illustrates one example of the spatial dimension to retroactivity. While the effect of retroactivity has been much studied in kinetic terms, this example shows how diffusion of species in a cascade can have backpropagating effects. In the above example the reference case chosen was one where all other species were weakly diffusible, but the broader conclusion of the back propagating effects of species diffusion remains valid in other scenarios as well. This highlights one aspect of the effect of diffusion in signalling cascades.

2.6 Multisite modification with localization of kinase and phosphatase

The primary focus in the paper has been on the effects of localization in cascades and pathways. When we considered multisite modification, we examined the case where different enzyme pairs were localized in different locations. Here we briefly discuss the situation where there is double site modification by a common enzyme pair, with the kinase and phosphatase localized in different locations. The case of a single site modification with localized kinase and phosphatase in different locations has already been studied in (1).

We briefly consider a two site ordered mechanism of multisite modification by the same kinase and phosphatase pair (See Supplementary Figure S7). We examine two cases. If the kinase is colocalized with the unmodified substrate and the doubly modified substrate is the only substrate species diffusing, then it diffuses to the location of the phosphatase and is completely converted back to the unmodified substrate. If

both unmodified and doubly modified substrates diffuse (and the singly modified substrate does not), then the cycle can be completed. This is an example of spatial localization in multisite phosphorylation different from the ones considered above and is a simple multisite analogue of spatially separated kinase phosphatase pairs in covalent modification cycles seen in bacteria. We find here, that even though the cycle is complete, the behaviour is not a simple analogue of that situation. In fact we observe that singly modified substrate accumulates along with the kinase as well as phosphatase and both modified and double modified substrates exhibit weakly graded profiles. This feature is broadly seen in various parameter ranges, and simply relies on the fact that the partial phosphoform, being non-diffusible, is present at the sites of production, which correspond to the presence of kinase (which produces this phosphoform from unphosphorylated substrate and further modifies it) or phosphatase (which produces this from maximally phosphorylated substrate, and further dephosphorylates it). This can be seen transparently analytically when the various reactions occur via mass-action kinetics, but of course does not rely on this assumption. If all modified and unmodified substrates diffuse, then this is no longer the case. This points to another facet of the interplay between spatial control, localization and chemical modification sequences.

3 Parameter Values

Parameters and Additional Information about the Models:

In this section details about parameter values used in individual figures are presented. All equations are non-dimensionalized, and the appropriate parameter values are dimensionless. Most of the essential trends seen here are seen for other parameter values (diffusion coefficients, kinetic parameters, where applicable). This has been demonstrated analytically. The various parameters are presented in the Models section. Fig S1 depicts a subset of these models with parameters

Two Step Cascade (Figure 2):

$k_1 = k_3 = k_5 = k_7 = 0.1, k_{-1} = k_{-3} = k_{-5} = k_{-7} = 1.0, k_2 = k_6 = 0.05, k_4 = k_8 = 0.1$; Total Substrate = 2.0; Total Kinase= 1.0, Total Phosphatase P1=0.3, Total Phosphatase P2=0.5. A range of diffusion coefficients were tested from low $D_{X*} = 0.01.$ to intermediate $= 0.1, 1.0$ to high 10.0 for all figures. A: width of localized patches is 1/5th of domain length.

Three Step Cascade (Figure 3): The three step enzymatic cascade is an extension of the earlier studied two-step enzymatic cascade with an additional third stage (equations not shown). The parameter denominations for the first two stages are the same as in the two step cascade. In the third stage, the

substrate species are Z, Z^*, ZY^* and Z^*P3 and P3 is the phosphatase of the last step. The association rate constants (for phosphorylation/dephosphorylation) are k_9 and k_{11}, the dissociation rate constants are k_{-9} and k_{-11}, and the catalytic constants are k_{10} and k_{12}. The diffusion coefficients of the species in the last stage are $D_Z, D_{Y*Z}, D_{Z*}, D_{P3}$ and D_{Z*P3}. Parameter values are based on Huang and Ferrell. The association rate constants for step 1 (X and X^*) of the cascade are: $k_1 = k_3 = 1000$; for step 2 (Y to Y^*) are $k_5 = k_7 = 1000$; and for step 3 (Z to Z^*) are $k_9 = k_{11} = 1000$. The dissociation rate constants for each step are $k_{-1} = k_{-3} = 150$ (step 1); $k_{-5} = k_{-7} = 150$ (step 2) and $k_{-9} = k_{-11} = 150$ (step 3), and the catalytic constants are (step 1) $k_2 = k_4 = 150$; (step 2) $k_6 = k_8 = 150$ and (step 3) $k_{10} = k_{12} = 150$. Total Substrates $X^* = 0.003, Y^* = Z^* = 1.2$, Total Phosphatases $P1 = P2 = 0.0003, P3 = 0.12$. A: width of localized patches is 1/5 th of of domain length. B: width of patches is 1/50th of domain length.

Three Step Cascade with a transient input (Figure 3C and D): For this analysis, the first reaction in the first step (X is converted to X*) in the three step cascade model is modified. For simplicity, the reaction is in the mass action regime. This allowed us to directly regulate the free/total Kinase concentration [K] (which is the transient input). $[K] = 0.0001$, forward rate constant associated with K, $k_1 = 1000$. Spatial design III was studied and a pulse duration of t=10 was applied. In Fig. 3C, the diffusivity of X^* was varied and in Fig. 3D the spatial width of the patch was varied (as a fraction of domain length) . The rest of the parameters are same as in Figure 3

Multisite phosphorylation (Figure 4):

(A,B): $k_1 = k_3 = k_5 = k_7 = 0.1, k_{-1} = k_{-3} = k_{-5} = k_{-7} = 1.0, k_2 = k_6 = 0.05, k_4 = k_8 = 0.1$; Total Substrate = 2.0; Total Kinase K1= 1.0, Total Phosphatase P1=0.3, Total Kinase K2= 1.0; Total Phosphatase P2=0.5.

C: $k_1 = k_3 = k_7 = 1.0 k_5 = 0.2, k_{-1} = k_{-3} = k_{-5} = k_{-7} = 1.0, k_2 = k_6 = 0.05, k_4 = k_8 = 0.1$; Total Substrate = 2.0; Total Kinase K1= 0.5, Total Phosphatase P=0.3, Total Kinase K2= 0.5; C: width of localized patches is 1/5th of domain length. Diffusion coefficient of diffusing species is 0.01.

Phosphorelay (Figure 5):

4-step relay (Fig. 5 C): $k_s = 1.0 = k_1 = k_3 = k_5 = k_{p1} = k_{p3} = k_{p5} = k_{p7} = 0.1$, the dissociation rate constants are $k_{-1} = k_{-3} = k_{-5} = k_{-p1} = k_{-p3} = k_{-p5} = k_{-p7} = 1.0$ and the catalytic rate constants are $k_2 = k_4 = k_6 = 0.05, k_{p2} = k_{p4} = k_{p6} = k_{p8} = 0.1$. Total Substrates $X1 = X2 = X3 = X4 = 2.0$; Input signal $K = 1.0$, Total Phosphatase $P1 = P3 = P4 = 0.3$, Total Phosphatases $P2 = 0.5$.

In a 2 step relay (Fig. 5 A,B) the parameters associated with steps 3 and 4 are zero and the remaining parameters are the same as Fig. 5C: Thus $k_3, k_5, k_{p5}, k_{p7}, k_{-3}, k_{-5}, k_{-p5}, k_{-p7}, k_4, k_6, k_{p6}, k_{p8}$. are all 0.

A, B and C: width of localized patches is 3/10th of domain length.

Supplementary Figures

Figure S2: $k_1 = k_3 = 10.0, k_{-1} = k_{-3} = 1.0, k_2 = k_4 = 0.1$ Total Substrate $= 2.0$, Total Phosphatase $P = 0.03$. Width of the localized patch is 1/5th of domain length. A range of diffusion coefficients were tested from low $D_{X*} = 0.01$ to intermediate $= 0.1 and 1.0$ to high 10.0. The diffusion coefficient shown here is D$= 1.0$.

Fig. S3. The kinetic parameters used are those in Fig. 3 C, D. In (b) the duration of the pulse is varied.

Figure S4: Top row: $k_o = 0.1; S = 0.2; k_s = 1.0; k_2 = 0.5, k_d = 1.0$. Row 2: $k_o = 0.1; S = 0.2; k_1 = 0.5; k_s = 1.0; k_d = 1.0$. RHS plots: $D_{X2} = 0.01$ (solid line) and $D_{X2} = 10.0$ (dashed line) Width of localized patches is 1/5th of domain length. The basal level of the signal was $S = 0.1$.

Figure S5: (A and B) $k_1 = k_3 = k_7 = k_{11} = 0.1, k_5 = 0.2$ and $k_9 = 0.3$, the dissociation rate constants are $k_{-1} = k_{-3} = k_{-5} = k_{-7} = k_{-9} = k_{-11} = 1.0$, and the catalytic constants are $k_2 = k_6 = k_{10} = 0.05, k_4 = k_8 = k_{12} = 0.1$. Total Substrates $X^* = 2.0, Y^* = 1.0; Z^* = 1.2$, Total Phosphatases $P1 = 0.5; P2 = 1.0, P3 = 0.5$, Total Kinase $K = 1.0$. The diffusion coefficients of Y and Y* are equal and are D=0.01 (solid line), D=0.1 (dashed line), D=1.0 (crosses) and D=10.0 (solid line with circles). Width of localized patches is 1/5th of domain length.

Figure S6:

$k_1 = k_3 = k_7 = 0.1, k_5 = 0.2, k_{-1} = k_{-3} = k_{-5} = k_{-7} = 1.0, k_2 = k_6 = 0.05, k_4 = k_8 = 0.1$; Total Substrate $X = 2.0$; Total Kinase $K = 1.0 + 0.3 cos\theta$, Total Substrate $Y = 1.0$; Total Phosphatase $P1 = P2 = 1 + 0.2Cos(\pi + \theta)$. All upstream X species are weakly diffusing at $D_{X*} = 0.001$ and Y and YX* diffuse at $D = 1.0$.

Figure S7:

$k_1 = k_3 = k_7 = 0.1, k_5 = 0.2, k_{-1} = k_{-3} = k_{-5} = k_{-7} = 1.0, k_2 = k_6 = 0.05, k_4 = k_8 = 0.1$; Total Substrate $= 2.0$; Total Kinase K$= 0.5$, Total Phosphatase P$= 0.5$. The width of localized patches is 1/5 th of domain length. A range of diffusion coefficients were tested from low $D_{X*} = 0.01$ to intermediate $= 0.1 and 1.0$ to high 10.0. The diffusion coefficient shown here is D$= 0.01$.

Figure Legends

Figure S1

The depiction of the basic kinetic steps involved in (A) enzymatic cascades (B) multiple modifications of the substrate and (C) phosphorelays. Also depicted is that fact that these modifications may happen in two different compartments. The communicating species in each case diffuses from one compartment to the other,connecting the two stages of the cascade. Hence, it is present in both compartments.

Figure S2

A 2-step modification cascade where the first step is in the ultrasensitive regime, and the second step occurs via mass action kinetics is considered. We focus on the first step. The steady state input/output curve of [X^*] is shown. When none of the species diffuse (solid line), the module shows an ultrasensitive response. If X^* itself diffuses (solid line with triangle markers) the sensitivity of the input-output curve is greatly reduced. Therefore if X^* is the communicating species in a spatially separated cascade, the switch-like effect is severely attenuated.

Figure S3

Transient behaviour in 3 step cascades. (a) The effect of the patch width on the transient behaviour in spatial design IV is presented showing non-monotonic behaviour, similar to the case seen in the text. (b) The effect of variation of pulse duration shown for design II: as pulse duration increases the output acquires a clear plateau.

Figure S4

Open cascade. The transient behaviour of X3 is shown in response to a step change in signal. The first row shows the case where the signal S enters the cascade between X1 and X2 and the second row shows the case where S enters the cascade between X2 and X3. The column on the left shows the case when all the species are localized together; X3 shows an adaptive response. The plots in the right column show the case where X1 and X3 are in two different compartments. [X3] in the middle of the second domain is shown. (Top RHS plot) X3 does exhibit an adaptive response and its amplitude is higher for higher diffusivities of X2 (dashed line) and low when X2 diffusion is weak (solid line). In contrast, in the second case, for low

diffusion coefficients (solid line) X3 does not perfectly adapt while it essentially does for high diffusion coefficients (dashed line).

Figure S5

A three step cascade with a global second step, i.e. Y and Y^* both diffuse: the spatial concentration profiles of Y^* (second step) and Z^* (last step) are shown for different scenarios. (A) P2 is uniform and present everywhere in the domain. In the bottom LHS plot Z^* is in location 1 and in the bottom RHS plot Z^* is in the opposite location. The profile of the Z^* is determined by the diffusion coefficient values of Y and Y^* and its own position in the domain. The arrow denotes the direction of increasing diffusion coefficient for Y and Y^*. (B) P2 is in the second location and the resultant profile of Y^* is shown. The spatial profile of Z^* behaves in a similar fashion as in (A). Increasing the diffusion coefficient flattens the profile of Y^*. In these plots the $D = 1$ and $D = 10$ curves are practically indistinguishable.

Figure S6

Two step covalent modification cascade subject to a gradient: The enzymes are graded and of the form a+bCos(θ). The case depicted here is for when the phosphatase enzymes are counter-aligned with the input kinase profile. Spatial concentration profiles of species are shown for two cases- 1) All the species weakly diffuse (solid line) and 2) Y and YX^* both diffuse strongly (dashed line) (other species are weakly diffusing). In the latter case, the spatial concentration profiles of all species, including the species in the upstream step are modified. The spatial profile of X becomes enhanced and that of X^* becomes weaker. The spatial profile of Y^* becomes weaker as well. The effect of diffusion propagates upstream causing a modification of spatial profiles both upstream and downstream.

Figure S7

Double site modification with a single enzyme pair that are localized in opposite patches in the domain (K is localized on the left in the domain and P is on the right). All substrate is initially localized alongside the kinase.The spatial concentration profiles of species X, X^* and X^{**} are shown. When there is no diffusion (solid line), X^{**} profile is present where K is localized, X and X^* are zero at steady state. If both X and X^{**} diffuse (line with dots) then both spatial profiles are spread in the domain and X^* is localized in both locations.

References

1. Alam-Nazki, A., J. Krishnan, 2013. Covalent Modification Cycles through the Spatial Prism. *Biophys. J.*, 105:1720-1731.

Figure S1

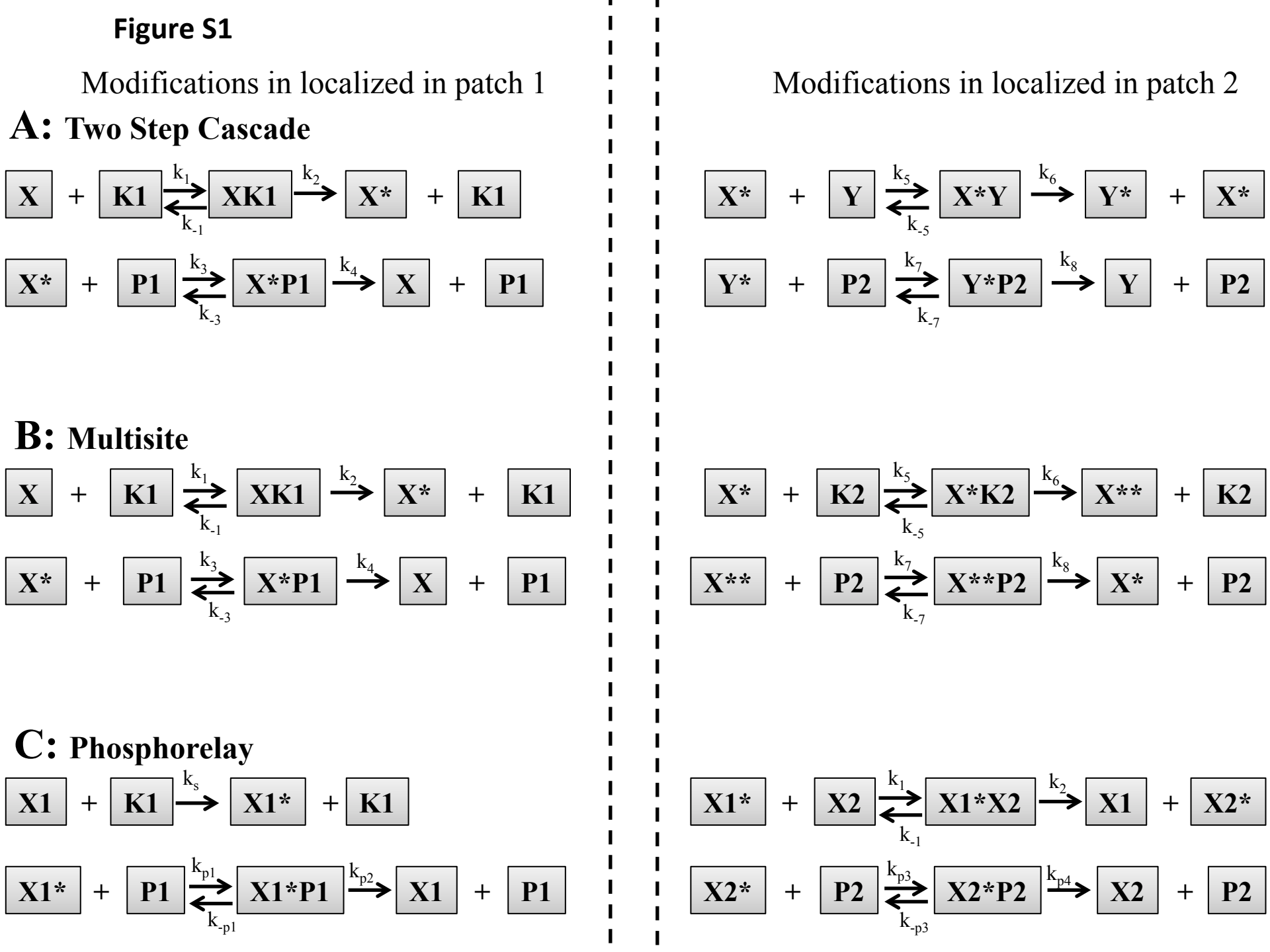

Modifications in localized in patch 1
Modifications in localized in patch 2
A: Two Step Cascade
X + K1 ⇄ XK1 → X* + K1
X* + P1 ⇄ X*P1 → X + P1
X* + Y ⇄ X*Y → Y* + X*
Y* + P2 ⇄ Y*P2 → Y + P2
B: Multisite
X + K1 ⇄ XK1 → X* + K1
X* + P1 ⇄ X*P1 → X + P1
X* + K2 ⇄ X*K2 → X** + K2
X** + P2 ⇄ X**P2 → X* + P2
C: Phosphorelay
X1 + K1 → X1* + K1
X1* + P1 ⇄ X1*P1 → X1 + P1
X1* + X2 ⇄ X1*X2 → X1 + X2*
X2* + P2 ⇄ X2*P2 → X2 + P2

Figure S2

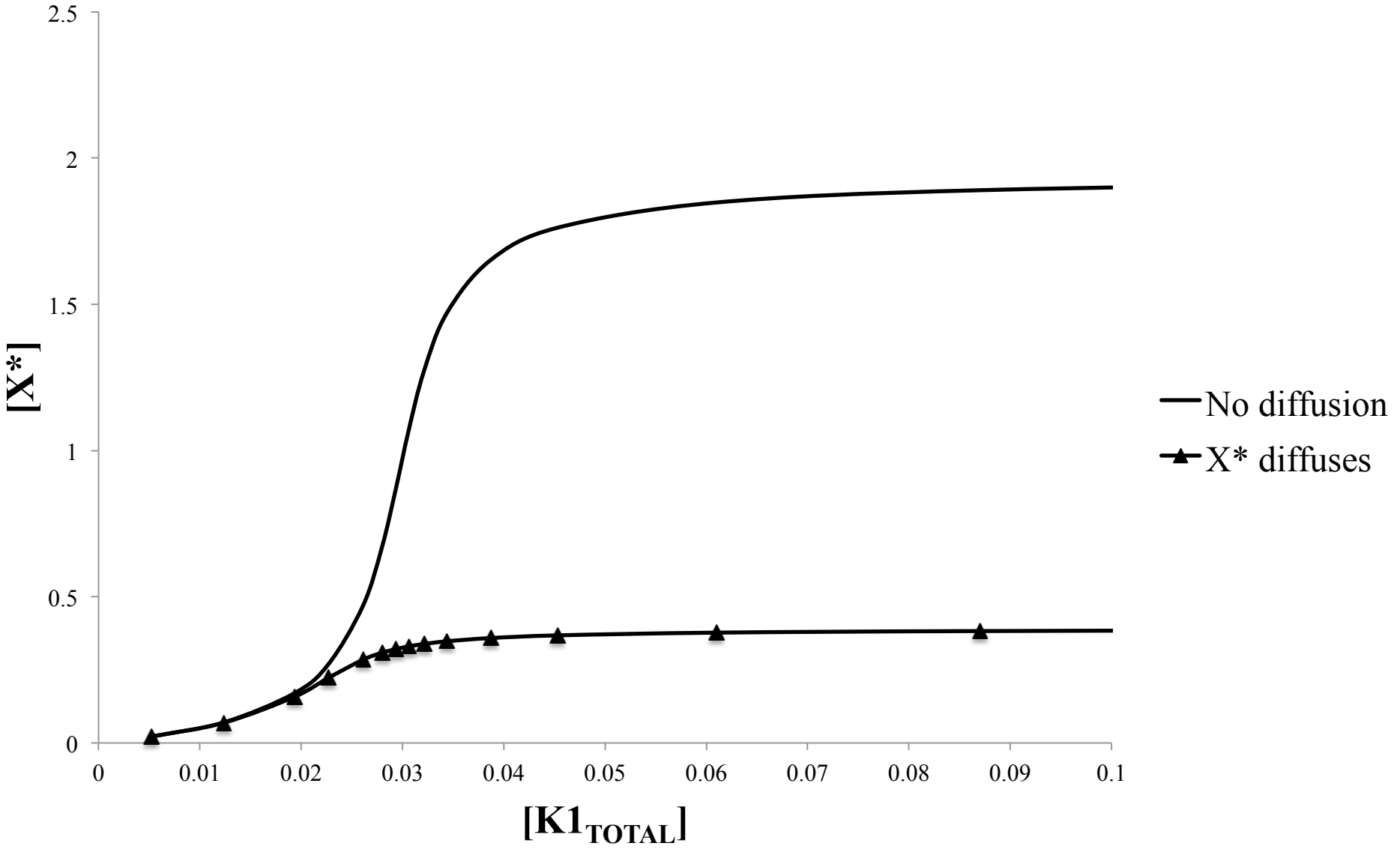
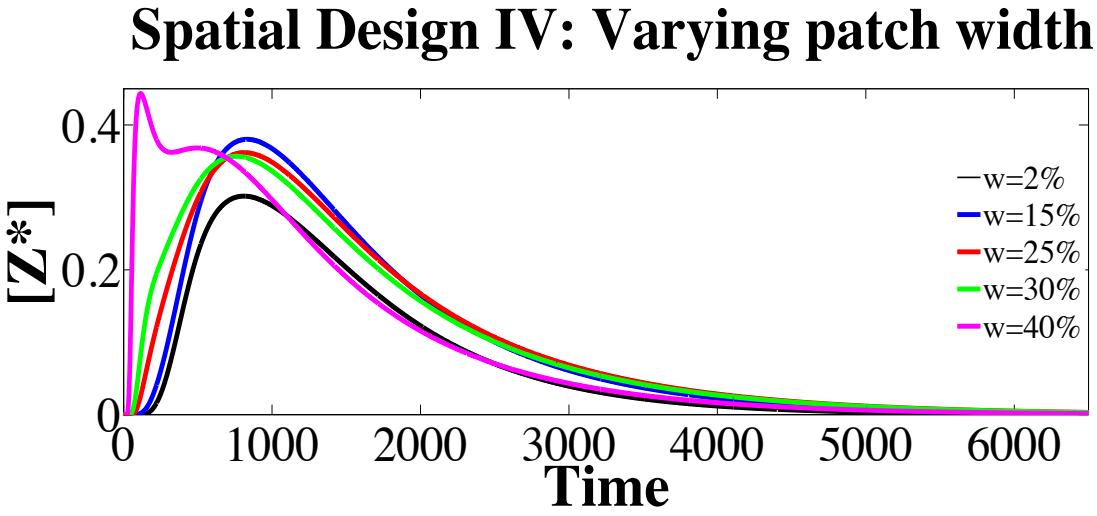

2.5
2
1.5
1
0.5
0
[X*]
0
0.01
0.02
0.03
0.04
0.05
0.06
0.07
0.08
0.09
0.1
[K1TOTAL]
No diffusion
X* diffuses

Figure S3

A


Spatial Design IV: Varying patch width
[Z*]
0.4
0.2
0
0
1000
2000
3000
4000
5000
6000
Time
w=2%
w=15%
w=25%
w=30%
w=40%

B

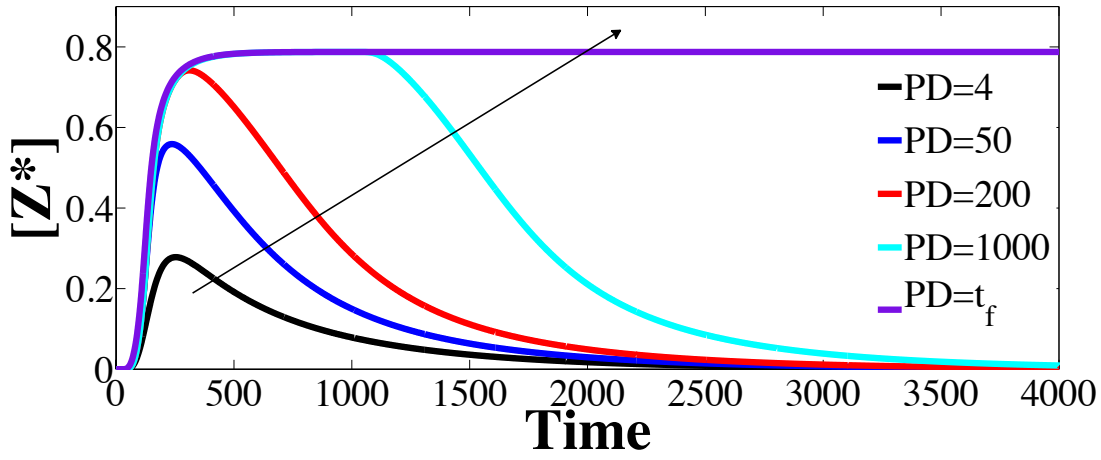

Spatial Design II: Varying pulse duration
[Z*]
0.8
0.6
0.4
0.2
0
0
500
1000
1500
2000
2500
3000
3500
4000
Time
PD=4
PD=50
PD=200
PD=1000
PD=t_f

Figure S4

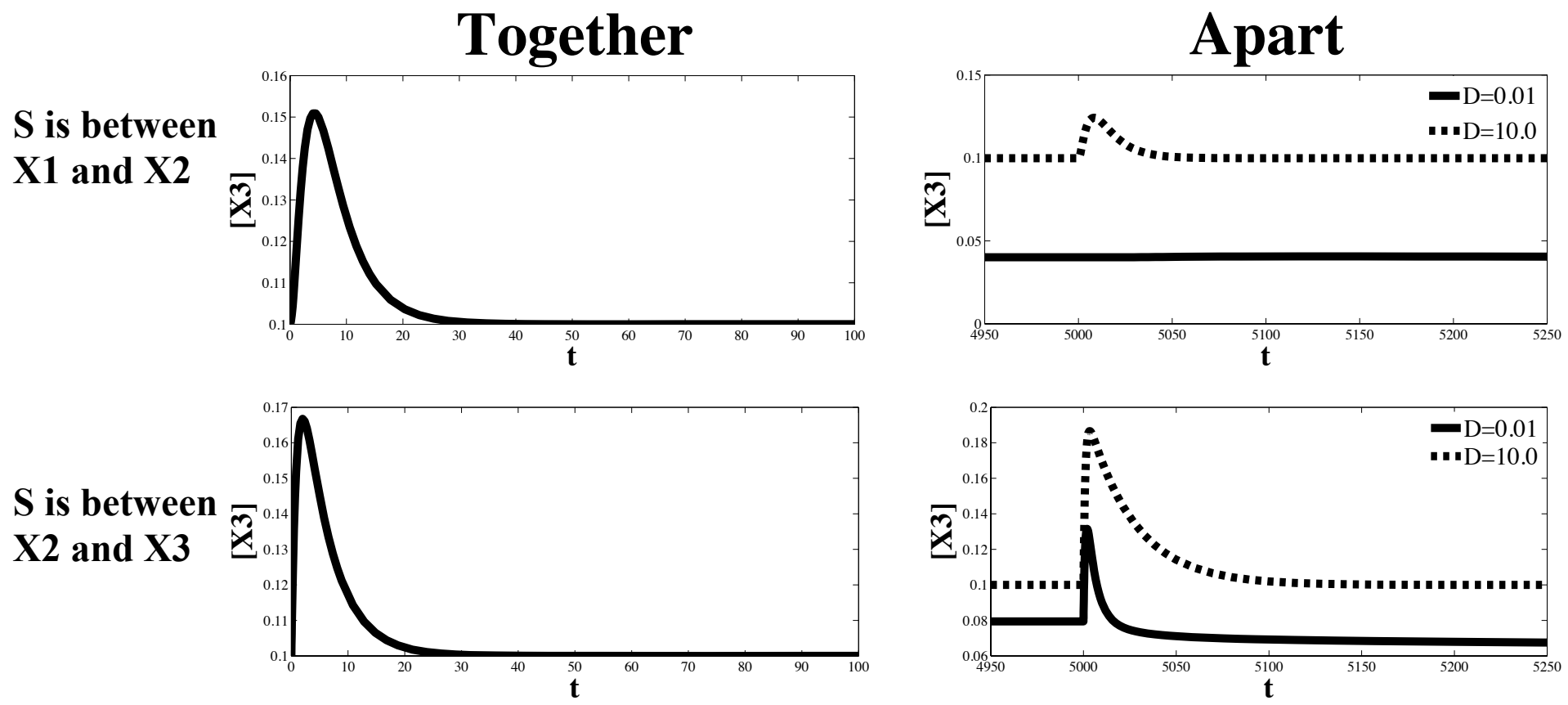

Together
Apart
S is between X1 and X2
S is between X2 and X3
[X3]
t
D=0.01
D=10.0

Figure S5

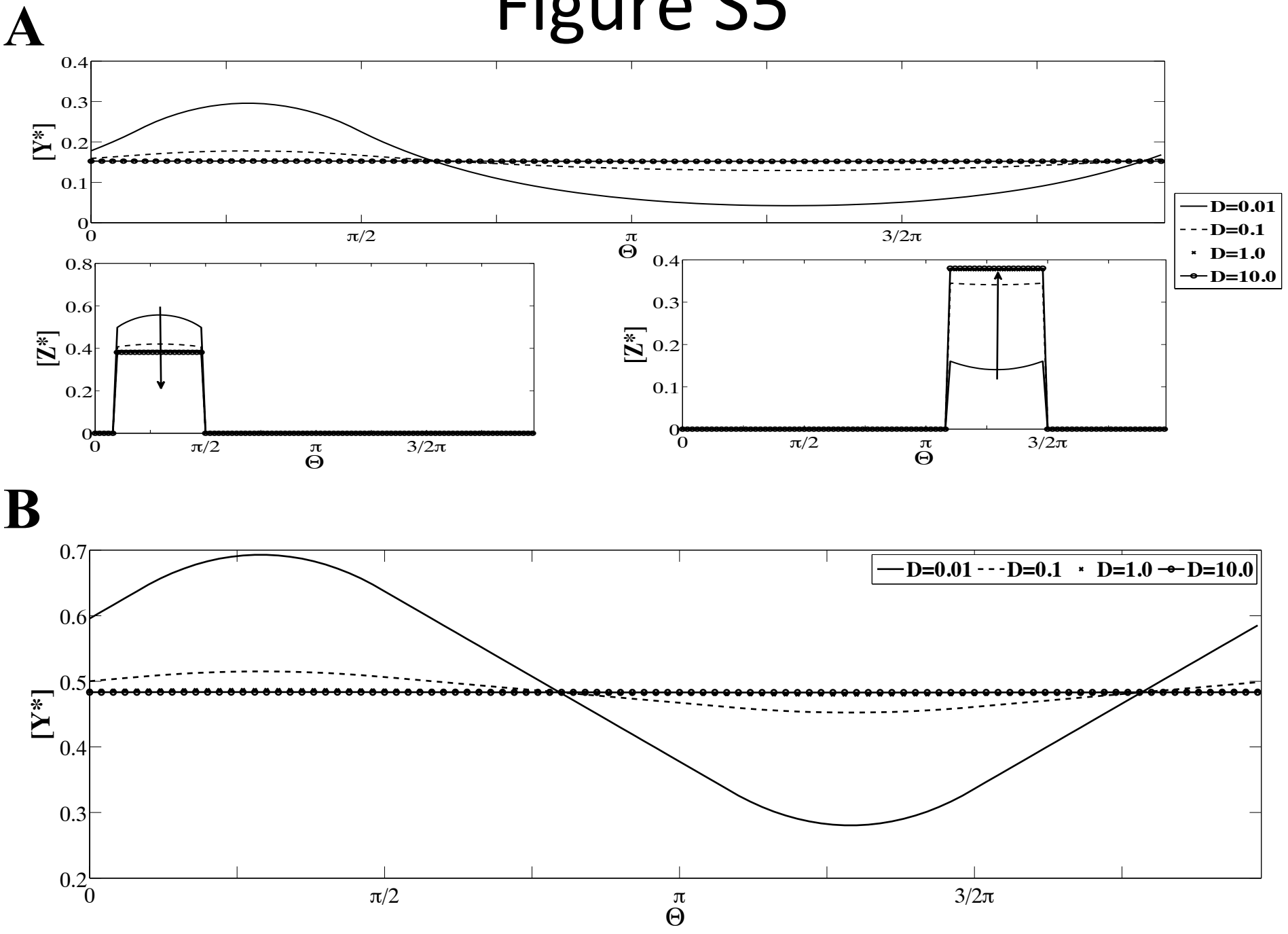


Figure S6

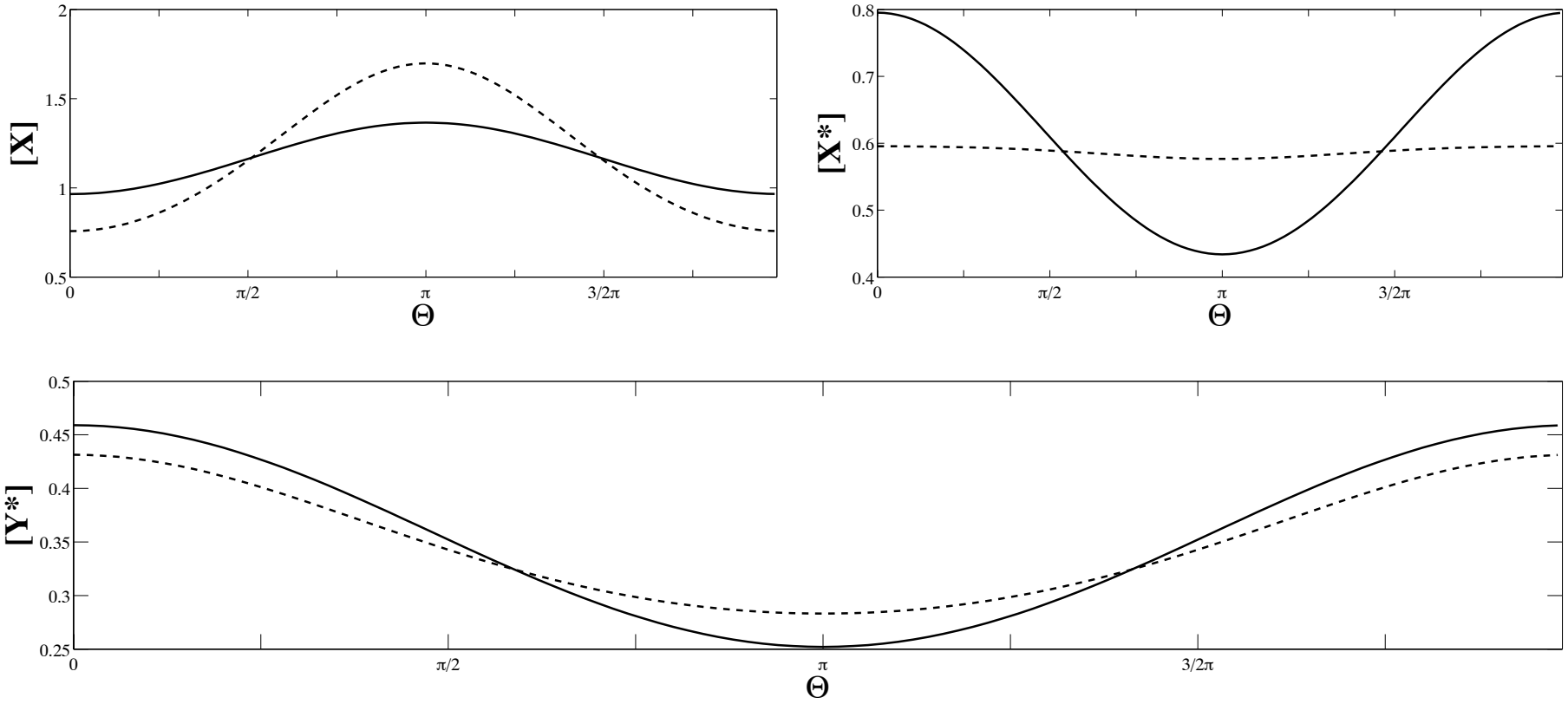
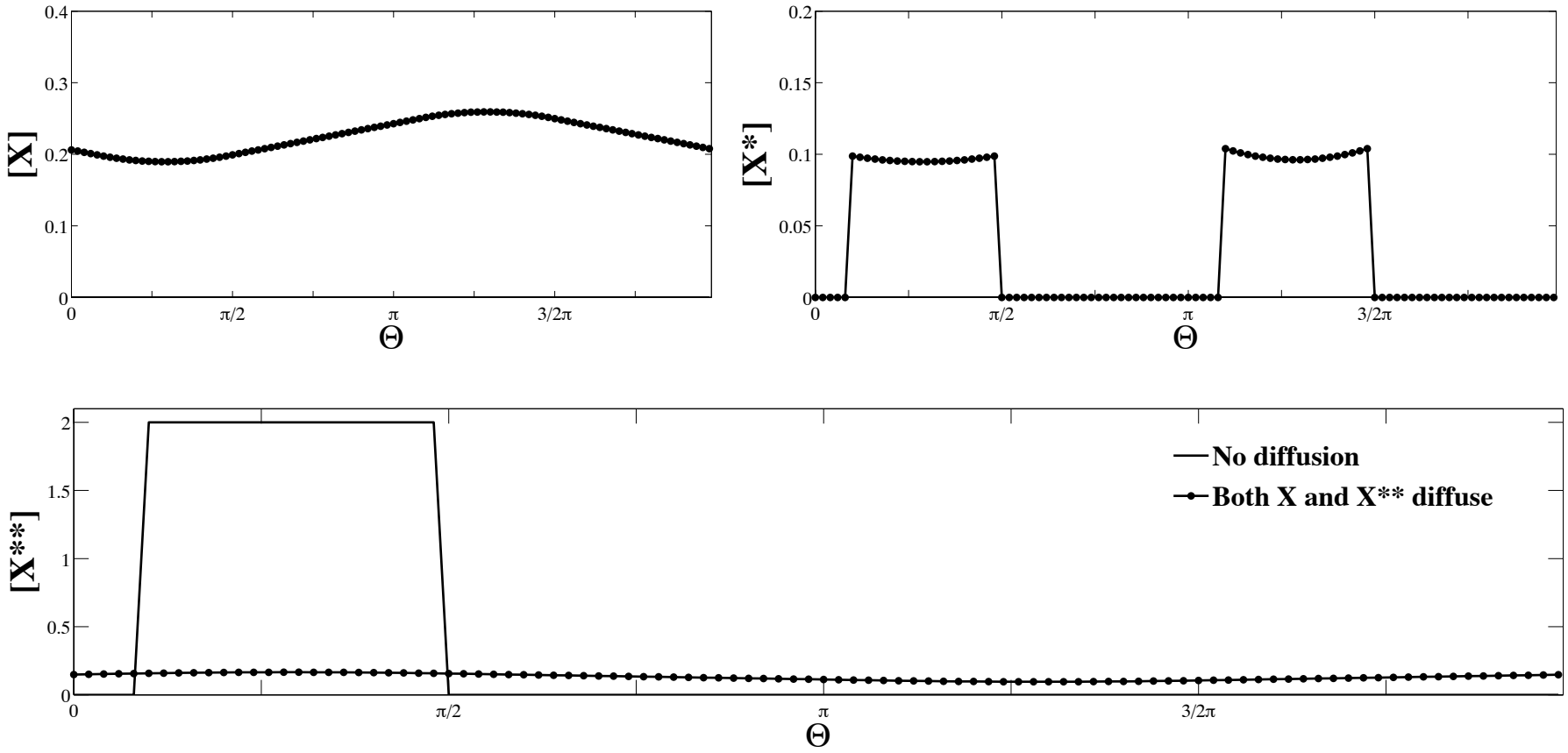

[X]
2
1.5
1
0.5
0
π/2
π
3/2π
Θ
[X*]
0.8
0.7
0.6
0.5
0.4
0
π/2
π
3/2π
Θ
[Y*]
0.5
0.45
0.4
0.35
0.3
0.25
0
π/2
π
3/2π
Θ

Figure S7


[X]
0.4
0.3
0.2
0.1
0
0
π/2
π
3/2π
Θ
[X*]
0.2
0.15
0.1
0.05
0
0
π/2
π
3/2π
Θ
[X**]
2
1.5
1
0.5
0
0
π/2
π
3/2π
Θ
No diffusion
Both X and X** diffuse

[P1]	Dx*=0 (Together)		Dx*=0.1 (Apart)	
	X*	Y*	X*	Y*
0.3	1.177	1.074	0.3461	0.5318
0.7	0.7548	0.8702	0.2996	0.478
1	0.5908	0.7578	0.2715	0.4434
1.5	0.4324	0.6214	0.2342	0.3948
2	0.3406	0.5256	0.2055	0.3551
3	0.2389	0.401	0.1645	0.2949
10	0.07719	0.15	0.06778	0.1329

Table S1: The effect of varying the concentration of P1 phosphatase on X* and Y* when the modifications are together and apart for a two-step cascade is shown. The effect of varying P1 concentrations in a two step spatial cascade is compared between the cases when both steps are localized in the same location (columns 2 and 3) and when they are separated (columns 4 and 5). Increasing P1 concentrations results in the steady state output of the separated cascade, becoming close to that of the completely localized cascade, both in absolute and relative terms.