Non-associative learning in intra-cellular signaling networks

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Nonlinear systems driven by recurrent signals are known to exhibit complex dynamical responses which, in the physiological context, can have important functional consequences. One of the simplest biological systems that is exposed to such repeated stimuli is the intra-cellular signaling network. In this paper we investigate the periodic activation of an evolutionarily conserved motif of this network, viz., the mitogen-activated protein kinase (MAPK) signaling cascade, with a train of pulses. The resulting response of the cascade, which shows integrative capability over several successive pulses, is characterized by complex adaptive behavior. These include aspects of non-associative learning, in particular, habituation and sensitization, which are observed in response to high- and low-frequency stimulation, respectively. In addition, the existence of a response threshold of the cascade, an apparent refractory behavior following stimulation with short inter-pulse interval, and an alternans-like response under certain conditions suggest an analogy with excitable media.

Nonlinear systems can respond to variations in their environment by exhibiting a wide range of complex dynamical patterns\textsuperscript{1,4} that may often be functionally significant\textsuperscript{5,13}. These variations are commonly associated with natural cycles such as the diurnal rhythm. In particular, biological systems are typically subjected to periodic stimuli with frequencies that can vary over a wide range of time scales, viz., from ultradian to infradian rhythms\textsuperscript{14–16}. Examples include the entrainment of the circadian clock to the day-night cycle\textsuperscript{17}, variations in hormonal levels over a period of a month that drive the menstrual cycle\textsuperscript{18} and calcium oscillations at the time-scale of minutes modulating the efficiency and specificity of gene expression\textsuperscript{19}. Of all the biological systems capable of exhibiting complex functionally significant responses when driven by periodic stimuli, perhaps one of the simplest is the intra-cellular signaling network\textsuperscript{20}. In its natural environment, the membrane-bound receptors of a cell may repeatedly be stimulated on encountering ligands, for instance as a consequence of pulsatile variations in hormones\textsuperscript{21}. Cellular functions may also be modulated by internal cues that vary periodically, e.g., oscillations in the concentrations of intracellular messengers such as Ca\textsuperscript{2+}\textsuperscript{22,23} and cyclic AMP\textsuperscript{24,25}. It is therefore important to investigate how key components of the signaling network in the cell respond when subjected to repeated stimuli in the form of pulse trains.

One of the most ubiquitous motifs of this network is the mitogen-activated protein kinase (MAPK) cascade, which is found across all eukaryotic cells\textsuperscript{26,27}. It consists of a sequential arrangement of three types of protein kinase, viz., MAPK, MAPK kinase (MAP2K) and MAPK kinase kinase (MAP3K). The activated kinase in each layer of the cascade functions as an enzyme for phosphorylating (and thus activating) the kinase in the layer immediately downstream. The subsequent deactivation is mediated by the corresponding dephosphorylating enzyme known as phosphatases (P'ase). Despite its structural simplicity this motif is involved in regulating a wide array of vital cellular functions, including proliferation and apoptosis\textsuperscript{27}, stress response\textsuperscript{28} and gene expression\textsuperscript{29}. Activation of the cascade is initiated when extracellular ligands stimulate membrane-bound receptors, or when intracellular cues occur upstream of the cascade, with the information being relayed to MAP3K through a series of intermediaries. The terminal kinase of the motif (MAPK), transmits the signal further downstream by phosphorylating various proteins including transcription regulators\textsuperscript{30}. The behavior of the cascade subjected to sustained stimulation has been extensively investigated, and the existence of several emergent features has been observed. These include ultrasensitivity\textsuperscript{31}, bistability which allows the system to switch between two states corresponding to low and high activity\textsuperscript{32,30} and oscillations\textsuperscript{30,41}. In earlier work, we have shown that the cascade stimulated with a pulse of finite duration responds with a rich variety of transient behavior, including phenomena indicative of the presence of short-term memory\textsuperscript{32}. The complex modulations seen in the response of the cascade are crucially dependent on the interactions between the time-scales of the intrinsic processes and that of the applied stimulus. It is thus intriguing to consider how the system will respond to repeated stimulation.

In this paper, we investigate the dynamics of the MAPK cascade that is stimulated by periodic trains of pulses. Despite the absence of any explicit feedback, under suitable conditions we find that the system displays adaptive behavior including non-associative learning\textsuperscript{43,44}, viz., habituation (desensitization) and sensitization. These allow plasticity in the behavioral repertoire of the intracellular signaling motif by enabling modification of the strength, duration and even the qualitative nature of its response to recurrent stimulation. In addition to these, we report the occurrence of a temporal sequence of strong and weak responses to successive pulses reminiscent of the phenomenon of “alternans” in excitable cells\textsuperscript{45–46}. This, coupled with the existence of a response threshold and an apparent refractory behavior when subjected to high-frequency stimulation...
strongly suggests an analogy with excitable media. While learning is commonly associated with the behavior at the level of organisms, it is intriguing that rudimentary forms of such complex adaptive responses can be seen in a simple network of sub-cellular components. As the MAPK signaling cascade is involved in coordinating diverse processes in all eukaryotic cells, these results point to the potential functional utility of such emergent dynamical phenomena in biological systems.

We have simulated the dynamics of the three layer kinase cascade using the Huang-Ferrell model of the MAPK signaling motif, schematically illustrated in Fig. I(a). This model consists of 10 enzyme-substrate reactions described by 18 coupled differential equations. Each of the several kinase and phosphatase-mediated enzyme-substrate reactions in the cascade consist of (i) a reversible enzyme-substrate complex formation step, and (ii) an irreversible step corresponding to the activation/deactivation of a kinase (see Supplementary Information for details). The ratio of the activation and deactivation rates range over four orders of magnitude, underlining the vast diversity of dynamical time-scales present in the system. The equations are numerically solved without invoking the quasi-steady-state hypothesis. We explicitly ensure that the total concentrations of each of the constituent kinases in the system are conserved. In our simulations, we assume that the cascade is initially in the resting state, where the kinases are completely non-phosphorylated. Following the exposure of the cascade to a train of pulses, we record the resulting response pattern, viz., the MAPK activity.

Investigations into the dynamics of the Huang-Ferrell model have typically focused on the asymptotic response of the cascade to sustained stimulation. In contrast, here we investigate the response of the system when it is subjected to recurrent activation by periodic stimuli. Specifically, we consider a signal comprising a train of pulses, each having amplitude $S$, duration $P$ and separated from each other by an inter-pulse interval $I$ [Fig. I(a)]. The cascade is released from stimulation between two successive pulses, and attempts to relax back to its resting state. On arrival of the next pulse, the cascade is activated once more, albeit before it has completely relaxed. This, coupled with the multiple time-scales of activation and relaxation present in the system, results in non-trivial adaptive temporal response. Selected examples of such behavior are shown in Fig. I(b-d). These different time series of the activated MAPK concentration (normalized with respect to the total MAPK concentration) correspond to the cascade being subjected to pulse trains characterized by different parameter values of $P$ and $I$.

Fig. I(b) displays the response of the system subjected to high-frequency stimulation by short-duration pulses. Here, starting from its resting state value, each subsequent pulse elicits a slightly higher response of $n_K^{\ast}$ until the peak activation suddenly spikes to a value close to its saturation. This behavior can be interpreted as a

![FIG. 1: Non-associative learning in a MAPK cascade stimulated by a pulse train. (a) Schematic representation of a linear three-layer MAPK cascade whose component kinases are activated/deactivated by the addition/removal of phosphate groups through phosphorylation/dephosphorylation respectively. Signaling is initiated when MAPK kinase kinase (MAP3K) is activated by a periodic signal comprising a series of pulses having amplitude $S$ and duration $P$, separated by inter-pulse interval $I$. For the cases investigated here, the cascade receives no stimulus between two successive pulses. The response of the cascade to the signal is measured in terms of MAPK activity, viz., the normalized concentration $n_K^{\ast}$ of doubly phosphorylated MAPK. (b-d) Time series representing qualitatively different adaptive responses of the cascade to pulse trains characterized by a range of $S$, $P$, and $I$. The shaded bars correspond to the intervals during which MAP3K is stimulated. (b) Desensitization behavior of the cascade corresponding to an attenuated response on persistent exposure to the periodic stimulus. (c) Sensitization of the cascade characterized by a low level of MAPK activity on initial exposure followed by stronger responses upon repeated stimulation. (d) Alternating high and low levels of MAPK activity ("alternans") in response to successive pulses. (e) Threshold-like response to the pulse duration $P$ of the maximum MAPK activity for a fixed set of values of the signal strength $S$ and inter-pulse duration $I$ of the pulse train. (f) Nonlinear dependence of the MAPK cascade response on the inter-pulse interval for a pair of pulses (shaded bars). For details of system and signal parameter values used see SI.}
form of signal integration, and may be repeated multiple times as the pulse train is continued. However, for an appropriate range of $P$ and $I$ (as in the figure), after a given number of pulses we observe behavior analogous to desensitization when the system no longer shows spiking activity even for sustained periodic stimulation. Thus, following an initial large amplitude response, the subsequent activity of the system is attenuated even though the nature of the received signal remained unchanged. When the cascade is stimulated instead by low-frequency pulse trains having relatively longer pulse durations, we observe a phenomenon analogous to sensitization. Here the cascade exhibits low-level activity on receiving the initial pulse but switches to high-amplitude spiking in response to all subsequent pulses [Fig. 1 (c)]. Thus, the initial low-level activity effectively “primes” the cascade to reach response levels close to saturation. This occurs because of the existence of long relaxation time-scales in certain components of the cascade, allowing for response accumulation over successive stimulations. Decreasing $P$ by a small amount gives rise to a qualitatively distinct phenomenon characterized by alternating low and high peaks of MAPK activity, reminiscent of alternans [46].

As alternans is a phenomenon that is associated with excitable media, it is intriguing to consider whether the periodically stimulated cascade exhibits other characteristics of such systems, in particular, the existence of a response threshold [47]. As seen in Fig. 1 (e), there is indeed a large discontinuous change in the peak activation $n_K^\text{max}$ of MAPK when the pulse duration $P$ crosses a specific value $P_c$ that depends on the choice of $S$ and $I$. Extending the analogy with excitable media, we find that the cascade also exhibits a nonlinear relation between its response to successive pulses and the inter-pulse interval. This can be seen from the behavior displayed in Fig. 1 (f) where the cascade is stimulated by a pair of pulses separated by an interval $I$. When $I$ is reduced, the response duration resulting from the second pulse increases in comparison to the duration of the response caused by the first. As an aside, we note that for the parameter regime considered here, the system exhibits post-stimulus reverberatory activity [42].

Fig. 2 (a-f) depicts representative time-series showing the activity of the cascade on either side of the response threshold, obtained for different choices of the periodic stimulation parameters. We note that in all of the cases shown here, the system shows a gradual build-up of activity over multiple pulses before reaching asymptotic peak activity levels. This corresponds to signal integration (mentioned earlier) where the response of the system to successive stimuli is modulated by the preceding stimuli. Fig. 2 (a) shows a typical subthreshold response (sub), where the peak MAPK activity is highly attenuated ($< 5\%$ of the saturation response value). Note that the nature of the response (i.e., whether it is sub- or supra-threshold) is a function of all three stimulation parameters $S$, $P$, and $I$. For instance, for the same signal strength $S$ considered in panel (a), the steady-state response of the cascade would have been close to satu-
ration if the stimulation had been applied in a sustained fashion (i.e., $I \to 0$).

Panels (b-f) of Fig. 2 depict a variety of suprathreshold temporal behavior, the simplest of which is characterized by a $1 : 1$ spiking response to the periodically applied pulses [sup, Fig. 2(b)]. On varying the different stimulation parameters we observe other types of suprathreshold activity. For example, on increasing $P$ alone (or alternatively, $S$ alone), the system exhibits oscillations of the peak activity close to saturation [pro, Fig. 2(c)]. For high-frequency stimulation (i.e., low $I$) after a transient period we observe suprathreshold peak responses only after every $N$ pulses for values of $P$ and $S$ that lie between those giving rise to sub and sup responses [see the lowest plane of Fig. 2(g)]. This response behavior, which corresponds to the coexistence (cox) of peak activity levels having different amplitudes (ranging from values just above zero to near-saturation) is shown in Fig. 2(d). For lower frequency stimuli, the cox regime corresponds to $M : 1$ response where multiple peaks in MAPK activity, whose amplitudes can again vary widely, are observed in response to each pulse [not shown]. Apart from these, we also observe behavior corresponding to non-associative learning, viz., desensitization [des, Fig. 2(e)] and sensitization [sen, Fig. 2(f)], as described earlier. Specifically, at the interface of the cox and sub regions in the stimulation parameter space, the des response regime is observed for high-frequency pulse trains [Fig. 2(h)] while for low-frequency stimulation we obtain sen [Fig. 2(i)]. We note that for higher values of $S$, the transition from cox to sub gets sharper thereby reducing the range of $P$ over which the des and sen phenomena are observed. An overview of the stimulation parameter space is given in Fig. 2(g) indicating the conditions for which each of the responses described above can be obtained.

The “learning” behavior associated with the periodically stimulated cascade is seen in the vicinity of the response threshold mentioned earlier corresponding to the boundary of the sub regime [Fig. 2(g)]. Hence, we examine the dependence of the threshold on the stimulation parameters in Fig. 2(j). The reciprocal relation between the signal strength $S$ and the critical pulse duration $P_c$ necessary for suprathreshold response seen over a wide range of $S$ suggests that the total signal intensity of a pulse, measured as the product of $S$ and $P$, determines the threshold. Deviation from this simple relation is observed for sufficiently low signal strength. This implies that a minimal value of $S$ is required to observe suprathreshold response, regardless of the duration for which the pulse is maintained. We note that in the limit of $I \to 0$, this minimal signal strength corresponds to the lower critical value required to observe a transition from low level of MAPK activity to high-amplitude oscillations when the cascade is subjected to sustained stimulation [36, 42]. As $I$ is increased, we observe that the response threshold (measured in terms of the critical pulse duration $P_c$) increases, which suggests that the excitability of the system reduces as the frequency of the periodic stimulus decreases.

The phenomena reported here are robust with respect to variations in the model parameters around the values used in this paper, including the kinetic rate constants and the molecular concentrations of the constituent kinases and phosphatases. We have also observed similar behavior with cascades having branched architecture, e.g., MAP3K activating two different types of MAP2K [55]. While we have assumed that the same phosphatase acts on both the singly and doubly phosphorylated forms of the kinase in a particular layer of the cascade (as in the canonical Huang-Ferrell model), we have explicitly verified that our results are not sensitively dependent on this.

A mechanistic understanding of the phenomena reported here is made difficult by the large number of coupled dynamical variables in the model that operate across different time-scales. This complexity may be untangled by using the framework of excitable systems. As alluded to earlier, many of the characteristic features associated with excitability are present in the system investigated here. These include the existence of two qualitatively distinct states of activation separated by a threshold [Fig. 1(e)], nonlinear response to repeated stimulation [Fig. 1(f)], an apparently refractory behavior as seen most prominently during desensitization [Fig. 1(b)] and phenomena analogous to alternans [Fig. 1(d)]. This appealing analogy provides a means by which a phenomenological understanding of the emergent behavior of this complex system might be achieved. We note that the excitability paradigm has been invoked earlier to explain aspects of cellular activity in the context of antigen recognition by T cells [56, 57]. Our results show that the emergent dynamics of MAPK cascade, which is known to mediate immune response [55], provides an explicit mechanistic basis for such a theoretical framework for explaining the adaptive response of the immune system to its microenvironment.

Among the functionally significant dynamical phenomena reported here, “learning” is perhaps the most intriguing. It confers on the system the ability to modify its behavior in response to information, which is critical for adapting to a changing environment. The capability to learn often presupposes the existence of a feedback that allows bidirectional communication between the components associated with receiving a signal and those that initiate a corresponding response [59]. In the kinase cascade investigated here, an explicit feedback is absent as each layer activates the one immediately downstream. However, an implicit feedback results from the inherent features of kinase activation, viz., sequestration and multi-site phosphorylation [33, 34, 36, 55]. This can have non-trivial consequences, such as the appearance of short-term memory, even when the MAPK cascade is subjected to a single pulse [42].

To conclude, in this paper we have shown that a rich repertoire of responses can be obtained when the system is exposed to a train of pulses. This results from the im-
plicit feedback, which orchestrates an interplay between the periodic stimulus and the diverse activation and relaxation time-scales of the signaling components. In particular, the system can exhibit sensitization and desensitization, which are examples of non-associative learning. These may play an important role in the cell’s ability to function in its natural environment, where it is continually exposed to signals of varying intensity and duration. This necessitates an ability to respond selectively to the received stimuli. Such adaptive mechanisms allow the cell to ignore persistent background stimuli through habituation (desensitization) but respond strongly to signals to which it has been primed through earlier exposure (sensitization). Given that a single linear cascade exhibits such complex adaptive behavior, it is intriguing to speculate about the potential capabilities inherent in the coordinated action of multiple subcellular processes. The mechanism through which learning at the sub-cellular scale can impact adaptive behavior in an organism at cellular and possibly higher scales remains an intriguing question.

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SUPPLEMENTARY INFORMATION

for

“Non-associative learning in intra-cellular signaling networks”

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I. THE MODEL EQUATIONS

TABLE S1: Components of the MAPK Cascade

| Component                                             | Notation | Symbol |
|-------------------------------------------------------|----------|--------|
| Mitogen-activated Protein Kinase Kinase Kinase         | MAP3K    | 3K     |
| Singly Phosphorylated Mitogen-activated Protein Kinase Kinase | MAP3K*   | 3K*    |
| Mitogen-activated Protein Kinase                       | MAP2K    | 2K     |
| Singly Phosphorylated Mitogen-activated Protein Kinase | MAP2K*   | 2K*    |
| Doubly Phosphorylated Mitogen-activated Protein Kinase | MAP2K**  | 2K**   |
| Mitogen-activated Protein Kinase                       | MAPK     | K      |
| Singly Phosphorylated Mitogen-activated Protein Kinase | MAPK*    | K*     |
| Doubly Phosphorylated Mitogen-activated Protein Kinase | MAPK**   | K**    |
| MAP3K-Phosphatase                                       | 3K P'ase | P\textsubscript{3K} |
| MAP2K-Phosphatase                                       | 2K P'ase | P\textsubscript{2K} |
| MAPK-Phosphatase                                        | K P'ase  | P\textsubscript{K} |

The three layer MAPK cascade comprises the following enzyme-substrate reactions:

\[ S + 3K \xrightleftharpoons[k_{-1}]{k_1} S.3K \xrightarrow{k_2} S + 3K^* \]

\[ P_{3K} + 3K^* \xrightleftharpoons[k_{p_{-1}}]{k_{p_1}} 3K^*.P_{3K} \xrightarrow{k_{p_2}} P_{3K} + 3K \]

\[ 3K^* + 2K \xrightleftharpoons[k_{-3}]{k_3} 3K^*.2K \xrightarrow[k_4]{k_3} 3K^* + 2K^* \]

\[ P_{2K} + 2K^* \xrightleftharpoons[k_{p_{-3}}]{k_{p_3}} 2K^*.P_{2K} \xrightarrow[k_{p_4}]{k_p} P_{2K} + 2K \]
\[ 3K^* + 2K^* \xrightarrow{k_5} 3K^*.2K^* \xrightarrow{k_6} 3K^* + 2K^{**} \]

\[ P_{2K} + 2K^{**} \xrightarrow{k_{p5}} 2K^{**}.P_{2K} \xrightarrow{k_{p8}} P_{2K} + 2K^* \]

\[ 2K^{**} + K \xrightarrow{k_7} 2K^{**}.K \xrightarrow{k_8} 2K^{**} + K^* \]

\[ P_K + K^* \xrightarrow{k_{p7}} K^*.P_K \xrightarrow{k_{p8}} P_K + K \]

\[ 2K^{**} + K^* \xrightarrow{k_9} 2K^{**}.K^* \xrightarrow{k_{10}} 2K^{**} + K^{**} \]

\[ P_K + K^{**} \xrightarrow{k_{p9}} K^{**}.P_K \xrightarrow{k_{p10}} P_K + K^* \]

The above enzyme-substrate reactions can be expressed in terms of the following coupled ordinary differential equations (ODEs):

\[
\begin{align*}
\frac{d[3K]}{dt} &= k_{-1}.[S.3K] + k_{p2}.[3K^*.P_{3K}] - k_1.[S].[3K], \\
\frac{d[S.3K]}{dt} &= k_1.[S].[3K] - (k_{-1} + k_2).[S.3K], \\
\frac{d[3K^*.P_{3K}]}{dt} &= k_{p1}.[P_{3K}^f].[3K^*] - (k_{p2} + k_{p-1}).[3K^*.P_{3K}], \\
\frac{d[3K^*]}{dt} &= k_2.[S.3K] + k_{p-1}.[3K^*.P_{3K}] - k_{p1}.[P_{3K}^f].[3K^*] \\
&\quad + (k_{-3} + k_4).[3K^*.2K] - k_3.[3K^*].[2K] \\
&\quad + (k_{-5} + k_6).[3K^*.2K^*] - k_5.[3K^*].[2K^*], \\
\frac{d[2K]}{dt} &= k_{-3}.[3K^*.2K] + k_{p4}.[2K^*.P_{2K}] - k_3.[3K^*].[2K], \\
\frac{d[3K^*.2K]}{dt} &= k_3.[3K^*].[2K] - (k_{-3} + k_4).[3K^*.2K], \\
\frac{d[2K^*.P_{2K}]}{dt} &= k_{p3}.[P_{2K}^f].[2K^*] - (k_{p4} + k_{p-3}).[2K^*.P_{2K}], \\
\frac{d[2K^*]}{dt} &= k_4.[3K^*.2K] + k_{p-3}.[2K^*.P_{2K}] - k_{p3}.[P_{2K}^f].[2K^*] \\
&\quad + k_{-5}.[3K^*.2K^*] - k_5.[3K^*].[2K^*] + k_{p6}.[2K^{**}.P_{2K}].
\end{align*}
\]
\[
\frac{d[3K^*.2K^*]}{dt} = k_3[3K^*.2K^*] - (k_6 + k_{-5})[3K^*.2K^*], \\
\frac{d[2K^{**}.P_{2K}]}{dt} = kp_5[P_{2K}^{f}].[2K^{**}] - (kp_6 + kp_{-5}).[2K^{**}.P_{2K}], \\
\frac{d[2K^{**}]}{dt} = k_6.[3K^*.2K^*] + kp_{-5}.[2K^{**}.P_{2K}] - kp_5.[P_{2K}^{f}].[2K^{**}] \\
+ (k_{-7} + ks).[2K^{**}.K] - k_7.[2K^{**}].[K] \\
+ (k_{-9} + k_{10}).[2K^{**}.K^*] - k_9.[2K^{**}].[K^*], \\
\frac{d[K]}{dt} = k_{-7}.[2K^{**}.K] + kp_8.[K^*.P_{K}] - k_7.[2K^{**}].[K], \\
\frac{d[2K^{**}.K]}{dt} = k_7.[2K^{**}].[K] - (k_8 + k_{-7}).[2K^{**}].K], \\
\frac{d[K^*.P_{K}]}{dt} = kp_7.[P_{K}^{f}].[K^*] - (kp_{-7} + kp_8).[K^*.P_{K}], \\
\frac{d[K^*]}{dt} = k_8.[2K^{**}.K] + kp_{-7}.[K^*.P_{K}] - kp_7.[P_{K}^{f}].[K^*] \\
+ k_{-9}.[2K^{**}.K^*] - k_9.[2K^{**}].[K^*] + kp_{10}.[K^{**}.P_{K}], \\
\frac{d[2K^{**}.K^*]}{dt} = k_9.[2K^{**}].[K^*] - (k_{-9} + k_{10}).[2K^{**}.K^*], \\
\frac{d[K^{**}.P_{K}]}{dt} = kp_9.[P_{K}^{f}].[K^{**}] - (kp_{-9} + kp_{10}).[K^{**}.P_{K}], \\
\frac{d[K^{**}]}{dt} = k_{10}.[2K^{**}.K^*] + kp_{-9}.[K^{**}.P_{K}] - kp_9.[P_{K}^{f}].[K^{**}].
\]

where

\[
[S] = [S]_{tot} - [S.3K], \\
[P_{3K}^{f}] = [P_{3K}] - [3K^*.P_{3K}], \\
[P_{2K}^{f}] = [P_{2K}] - [2K^*.P_{2K}] - [2K^{**}.P_{2K}], \\
[P_{K}^{f}] = [P_{K}] - [K^*.P_{K}] - [K^{**}.P_{K}].
\]

It is explicitly ensured that the total concentrations of all individual kinases and phosphatases are conserved at all times. The concentrations of the different molecular species can vary over several orders of magnitudes. We have therefore numerically solved the equations using low relative and absolute tolerances in order to ensure the accuracy of the resulting time-series.
II. SYSTEM PARAMETERS

The numerical values for the reaction rates used in all our simulations are obtained from Ref. [55], and are listed in Table S2. Please note that these values of kinetic rate constants are very close to that of Huang-Ferrell base values [31].

| Rate constant | Our base value | Huang-Ferrell value | Units       |
|---------------|----------------|---------------------|-------------|
| $k_1$         | 1002           | 1000                | $(\mu M \cdot \text{min})^{-1}$ |
| $k_2$         | 150            | 150                 | $\text{min}^{-1}$ |
| $k_3$         | 150            | 150                 | $\text{min}^{-1}$ |
| $kp_1$        | 1002           | 1000                | $(\mu M \cdot \text{min})^{-1}$ |
| $kp_2$        | 150            | 150                 | $\text{min}^{-1}$ |
| $k_3$         | 1002           | 1000                | $(\mu M \cdot \text{min})^{-1}$ |
| $k_4$         | 30             | 150                 | $\text{min}^{-1}$ |
| $k_5$         | 30             | 150                 | $\text{min}^{-1}$ |
| $kp_3$        | 1002           | 1000                | $(\mu M \cdot \text{min})^{-1}$ |
| $kp_4$        | 150            | 150                 | $\text{min}^{-1}$ |
| $k_5$         | 1002           | 1000                | $(\mu M \cdot \text{min})^{-1}$ |
| $k_6$         | 30             | 150                 | $\text{min}^{-1}$ |
| $k_6$         | 30             | 150                 | $\text{min}^{-1}$ |
| $kp_5$        | 1002           | 1000                | $(\mu M \cdot \text{min})^{-1}$ |
| $kp_6$        | 150            | 150                 | $\text{min}^{-1}$ |
| $k_7$         | 1002           | 1000                | $(\mu M \cdot \text{min})^{-1}$ |
| $k_7$         | 30             | 150                 | $\text{min}^{-1}$ |
| $k_8$         | 30             | 150                 | $\text{min}^{-1}$ |
| $kp_7$        | 1002           | 1000                | $(\mu M \cdot \text{min})^{-1}$ |
| $kp_8$        | 150            | 150                 | $\text{min}^{-1}$ |
| $k_9$         | 1002           | 1000                | $(\mu M \cdot \text{min})^{-1}$ |
| $k_9$         | 150            | 150                 | $\text{min}^{-1}$ |
| $k_{10}$      | 150            | 150                 | $\text{min}^{-1}$ |
| $kp_{10}$     | 1002           | 1000                | $(\mu M \cdot \text{min})^{-1}$ |
| $kp_{10}$     | 150            | 150                 | $\text{min}^{-1}$ |
The signal parameters used to generate representative time-series in Fig. 1–2 following the introduction of a signal are listed in Table S3 and Table S4 respectively.

### TABLE S3: Signal parameters for the panels in Fig. 1

| Parameter | (b) | (c) | (d) | (e) | (f) | Units  |
|-----------|-----|-----|-----|-----|-----|--------|
| S         | 1.2 | 1.2 | 1.2 | 1.2 | 3   | $10^{-6}$µM |
| P         | 71  | 372 | 371 | 60-80| 300 | mins   |
| I         | 106.75 | 2000 | 2000 | 105 | 100-700 | mins |

### TABLE S4: Signal parameters for the panels in Fig. 2

| Parameter | (a) | (b) | (c) | (d) | (e) | (f) | Units |
|-----------|-----|-----|-----|-----|-----|-----|-------|
| S         | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | $10^{-6}$µM |
| P         | 49  | 100 | 300 | 50  | 49.5| 287 | mins  |
| I         | 105 | 105 | 105 | 105 | 105 | 2000 | mins  |

### TABLE S5: Total concentration (in µM) of the kinases and phosphatase proteins for Figs. 1–2

| Protein                      | Value   |
|------------------------------|---------|
| $[K_{tot}]$                  | 4.8     |
| $[2K_{tot}]$                 | 1.2     |
| $[3K_{tot}]$                 | 0.0030  |
| MAP3K-Phosphatase            | $1 \times 10^{-4}$ |
| MAP2K-Phosphatase            | $3 \times 10^{-4}$ |
| MAPK-Phosphatase             | 0.05    |