Selective regulation of cellular processes via protein cascades acting as band-pass filters for time-limited oscillations

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Abstract **We show by mathematical modelling that a two-level protein cascade can act as a band-pass filter for time-limited oscillations. The band-pass filters are then combined into a network of three-level signalling cascades that by filtering the frequency of time-limited oscillations selectively switches cellular processes on and off. The physiological relevance for the selective regulation of cellular processes is demonstrated for the case of regulation by time-limited calcium oscillations.**

Keywords: Band-pass filter; Signalling cascade; Bow-tie structure; Calcium oscillations

1. Introduction

Band-pass filters are well known as electrical frequency filters. Also chemical reaction networks can act as band-pass filters [1,2]. All these filters were designed for sustained, theoretically infinitely long, oscillatory signals. In contrast to previous studies, we present a band-pass filter for time-limited oscillations. It is based on an enzyme cascade [3,4] in which each activated enzyme catalyses the activation of the protein on the next level.

We then combine the band-pass filters into a network that enables selective regulation of cellular processes. The striking feature of the network is that several inputs can influence several targets via only one or a few intermediary components. This architecture has been termed "bow-tie" or "hour-glass" structure [5,6] and characterises also several technical systems, for example, power grids or manufacturing [5]. In communication systems this architecture corresponds to multiplexers. Examples of such procedures are code-division multiple access (CDMA) and time-division multiplexing (TDMA) used by the GSM telephone system [7], for example.

The effectiveness of here proposed bow-tie architecture for selective regulation of cellular processes is demonstrated for the case of regulation by time-limited Ca^{2+} oscillations. This seems to be of special physiological importance since Ca^{2+} oscillations play an important role in intra- and intercellular signal transduction by regulating many cellular processes, from egg fertilisation to cell death [8–10]. There exists experimental evidence that the duration of Ca^{2+} signals modulates gene transcription [11] and egg fertilisation [12], for example. For plant cells, it has been shown that the duration and number of Ca^{2+} spikes regulate the aperture of stomatal pores [13,14]. Therefore, we use time-limited Ca^{2+} oscillations for studying the selective regulation of cellular processes. The Ca^{2+} oscillations are simulated by artificially generated square-shaped signals as also used in some experiments [15] and theoretical studies [16,17].

2. Mathematical model

The regulation of cellular processes is modelled by a bow-tie signalling architecture (Fig. 1). Input stimuli evoke Ca^{2+} oscillations which regulate cellular processes via the network of signalling cascades. Our analysis includes kinase-phosphatase cascades that are organised as parallel branches in three levels. The third level is the activation level, and correspondingly, the whole branch containing one of the third-level proteins at the end is called the activation branch. Each activation branch can consist of several primary branches made up of first and second-level kinase-phosphatase cascades. In Fig. 1 two activation branches are presented in detail, each of them consisting of two primary branches.

Time-limited Ca^{2+} oscillations are mimicked by square-shaped signals:

$$x(t) = \begin{cases} x_{\max} & \text{if } ((t \bmod g) \geqslant (g-d) \text{ and } (t < Mg)), \\ x_{\min} & \text{else}, \end{cases} \quad (1)$$

where $x_{\min}$ and $x_{\max}$ are the minimum and maximum of the oscillations, respectively, g denotes the period of oscillations, d is the spike width, and M is the number of Ca^{2+} spikes. In all calculations, we set $d = 0.01$s and $M = 5$.

For the ith primary branch ($i \in [1,\ldots,n]$), the concentrations of activated proteins at the first (z_{i1}) and second (z_{i2}) levels can be modelled as:

$$\frac{dz_{i1}}{dt} = k_{i1}^{+}(z_{i1}^{\text{tot}} - z_{i1})x^4 - k_{i1}^{-}z_{i1}, \quad (2)$$

$$\frac{dz_{i2}}{dt} = k_{i2}^{+}(z_{i2}^{\text{tot}} - z_{i2})z_{i1}^4 - k_{i2}^{-}z_{i2}, \quad (3)$$

respectively. We assume cooperativity in that four active molecules of the previous level must bind to activate a protein.

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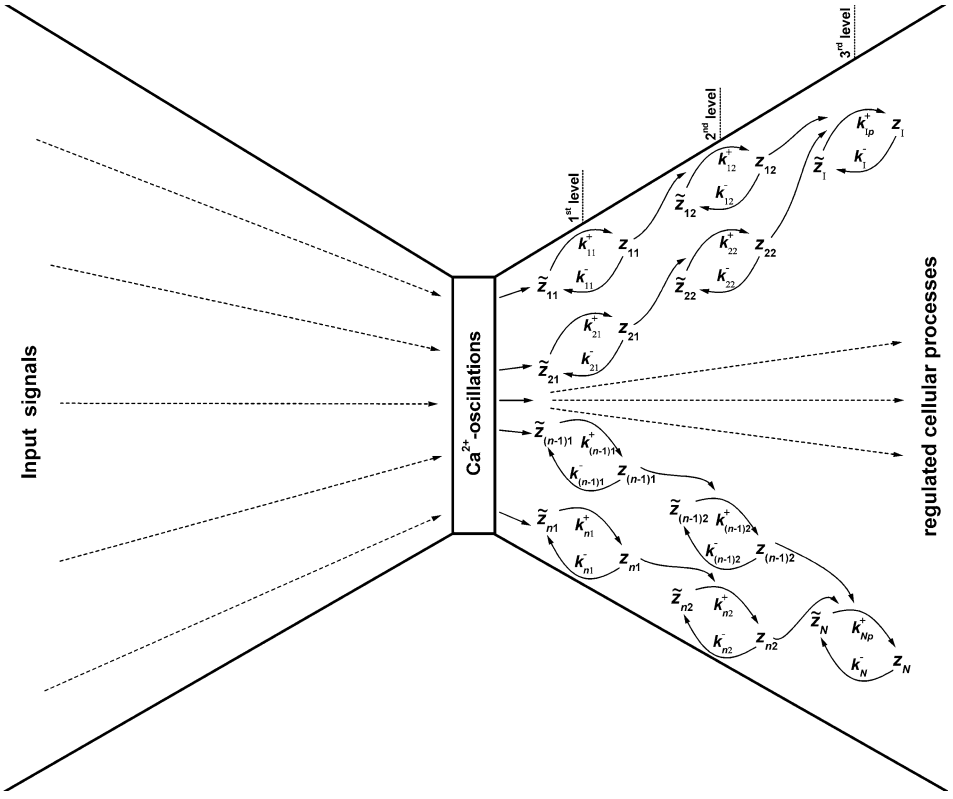

Fig. 1. Bow-tie signalling scheme for selective regulation of cellular processes by Ca^{2+} oscillations. The network of kinase-phosphatase cascades consists of activated (z_{ij}) and inactivated ($\tilde{z}_{ij}$) proteins, where indices $i \in [1,\ldots,n]$ and $j \in [1,2]$ denote the primary branch number and the cascade level, respectively. For the third level of proteins, Roman indices indicate the number of the activation branch. Parameters k_{ij}^{+} and k_{ij}^{-} are the primary branch rate constants for the kinase and phosphatase reactions, respectively. k_{Ap}^{+} and k_{A}^{-} denote the rate constants for each third-level protein, where $A \in [\mathrm{I},\mathrm{II},\ldots,N]$ indicates the number of the activation branch, and p indicates the number of the primary (input) branch (see Eq. (4)). Note that primary branches may partly overlap (see text).

Table 1
Parameter values

Level	Activation branch I		Activation branch II
	1st primary branch	2nd primary branch (common branch)	3rd primary branch
1st level	$k_{11}^{+} = 100\mu M^{-4}s^{-1}$ $k_{11}^{-} = 1.0s^{-1}$ $z_{11}^{tot} = 1.0\mu M$	$k_{21}^{+} = 1.0\mu M^{-4}s^{-1}$ $k_{21}^{-} = 0.01s^{-1}$ $z_{21}^{tot} = 10\mu M$	$k_{31}^{+} = 0.1\mu M^{-4}s^{-1}$ $k_{31}^{-} = 0.001s^{-1}$ $z_{31}^{tot} = 90\mu M$
2nd level	$k_{12}^{+} = 10\mu M^{-4}s^{-1}$ $k_{12}^{-} = 0.1s^{-1}$ $z_{12}^{tot} = 1.0\mu M$	$k_{22}^{+} = 0.1\mu M^{-4}s^{-1}$ $k_{22}^{-} = 0.001s^{-1}$ $z_{22}^{tot} = 60\mu M$	$k_{32}^{+} = 0.01\mu M^{-4}s^{-1}$ $k_{32}^{-} = 0.0001s^{-1}$ $z_{32}^{tot} = 85\mu M$
3rd level	$k_{\mathrm{I}1}^{+} = k_{\mathrm{I}2}^{+} = 1.0\mu M^{-4}s^{-1}$ $k_{\mathrm{I}}^{-} = 0.5s^{-1}$ $z_{\mathrm{I}}^{tot} = 1.0\mu M$		$k_{\mathrm{II}2}^{+} = k_{\mathrm{II}3}^{+} = 0.8\mu M^{-4}s^{-1}$ $k_{\mathrm{II}}^{-} = 0.3s^{-1}$ $z_{\mathrm{II}}^{tot} = 0.7\mu M$

Moreover, in Eqs. (2) and (3) the conservation relation for each protein cycle has been considered.

At the third level of the cascade network, the proteins are activated by one or several activated complexes from the second level. In general, the third level of the Ath activation branch ($A \in [\mathrm{I},\mathrm{II},\ldots,N]$) can be described as

$$\frac{dz_A}{dt} = k^+_{Ap}(z^{tot}_A - z_A)z^4_{p2} + \cdots + k^+_{Ar}(z^{tot}_A - z_A)z^4_{r2} - k^-_A z_A, \quad (4)$$

where $p,\ldots,r \in [1,\ldots,n]$ denote primary branches pertaining to the second level complexes by which the third-level protein of activation branch A is activated. In Section 3, we demonstrate the effectiveness of this architecture on a very simple network consisting of only two activation branches:

$$\frac{dz_{\mathrm{I}}}{dt} = k^+_{\mathrm{I}1}(z^{tot}_{\mathrm{I}} - z_{\mathrm{I}})z^4_{12} + k^+_{\mathrm{I}2}(z^{tot}_{\mathrm{I}} - z_{\mathrm{I}})z^4_{22} - k^-_{\mathrm{I}} z_{\mathrm{I}}, \quad (5)$$

$$\frac{dz_{\mathrm{II}}}{dt} = k^+_{\mathrm{II}2}(z^{tot}_{\mathrm{II}} - z_{\mathrm{II}})z^4_{22} + k^+_{\mathrm{II}3}(z^{tot}_{\mathrm{II}} - z_{\mathrm{II}})z^4_{32} - k^-_{\mathrm{II}} z_{\mathrm{II}}. \quad (6)$$

The parameter values used throughout are listed in Table 1.

3. Results

For the cascade network (Fig. 1), the protein activation as a response to time-limited Ca^{2+} oscillations (Eq. (1)) is analysed in dependence on the oscillation frequency. The results for activation branch I (Eqs. (2), (3), and (5), where $i = 1, 2$) are presented in Fig. 2. Since the concentrations of the activated proteins increase in time in an oscillatory manner, the average protein activation during the last (5th) period is presented.

Fig. 2 shows that at the first level the proteins are activated in a sigmoidal manner. Since the frequency of Ca^{2+} oscillations is changed so that the spike width remains constant (physiologically relevant), and the number of spikes is constant, the time for protein activation is the same for all frequencies of Ca^{2+} oscillations. On the other hand, by increasing the oscillation frequency, the time between Ca^{2+} spikes and, hence, the time for protein deactivation between spikes is shortened. Therefore, the average activation during the 5th period $\langle z_{i1}\rangle$ monotonically increases with the frequency of Ca^{2+} oscillations and becomes saturated at higher frequencies.

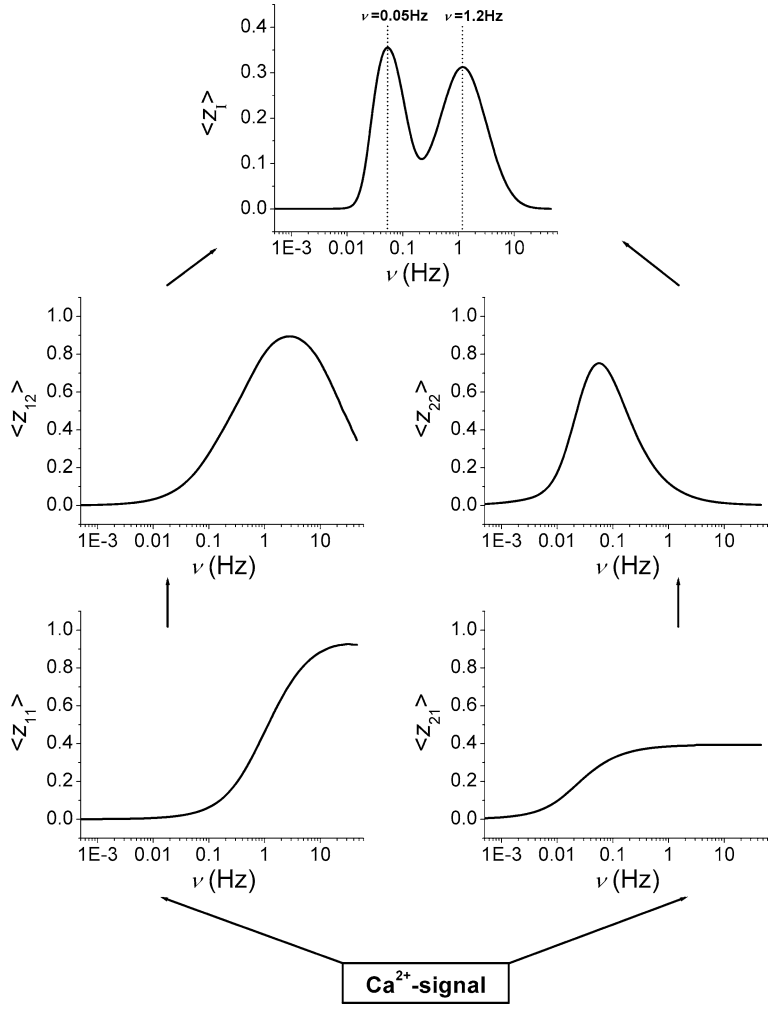

Fig. 2. Average activation of proteins in the activation branch I during the 5th oscillation period. At the third level, the activation curve has two maxima corresponding to the two frequencies of Ca^{2+} oscillations at which the protein is most efficiently activated. This is due to the superposition of the two activation curves from the second level.

At the second level, the average protein activation during the 5th period ($\langle z_{i2} \rangle$) shows a resonant dependence on the frequency of Ca^{2+} oscillations (see Fig. 2). This band-pass filter property is a consequence of the cascade structure of two chain-linked protein-binding reactions (Eqs. (2) and (3)). The non-zero concentrations of z_{i1} during the interspike intervals can increase the protein activation z_{i2} in the time between Ca^{2+} spikes. This is always the case when the oscillation frequency is high enough. However, by increasing the frequency, the total time of Ca^{2+} stimulation decreases and, hence, the time for the protein activation at the second level is reduced. Thus, for an optimal resonant protein activation z_{i2}, two conditions have to be fulfilled: (i) the frequency of Ca^{2+} oscillations should be high enough in order to have non-zero concentrations of z_{i1} during the interspike intervals (the period should be close to (or shorter than) the characteristic time $\tau_{i1} = 1/k_{i1}^{-}$ of Ca^{2+} dissociation from z_{i1}); (ii) the total time of Ca^{2+} signal should be long enough to allow a maximal protein activation z_{i2}.

The resonant response of protein activation at the second cascade level enables efficient selective regulation of cellular responses in dependence on the oscillation frequency. The third level of protein cascades then enables a superposition and allows a better resonance, i.e., a narrower maximum. It combines the second-level resonant frequencies at which the protein is activated. Indeed, Fig. 2 shows that the activation curve of the protein at the third level has two maxima corresponding to the two frequencies of Ca^{2+} oscillations at which the protein is most efficiently activated.

Now we consider activation branch II (Eqs. (2), (3), and (6), where $i = 2, 3$), which has the second primary branch in common with activation branch I. The results for branches I and II are qualitatively the same. However, because of different kinetics (see Table 1) the proteins respond optimally to other frequencies. Fig. 3 shows the activation curves of the third-level proteins in activation branches I and II together. Each of the curves has two maxima corresponding to the two frequencies of oscillations at which the proteins are most efficiently activated. However, the maxima are shifted; the cellular processes activated via branch I are most efficiently initiated with Ca^{2+} oscillation frequencies $\nu = 0.05$ Hz and $\nu = 1.2$ Hz, whereas the processes activated via branch II are most efficiently initiated with frequencies $\nu = 0.005$ Hz and $\nu = 0.05$ Hz.

Since, in our example, the two activation branches (I and II) have one common primary branch ($i = 2$), the activation curves of the two activation branches in Fig. 3 have one maximum at the same frequency of Ca^{2+} oscillations ($\nu = 0.05$ Hz) and two branch-specific maxima ($\nu = 0.005$ Hz and $\nu = 1.2$ Hz). Accordingly, in dependence on the frequency of Ca^{2+} oscillations, two cellular processes can be switched on independently of each other or simultaneously.

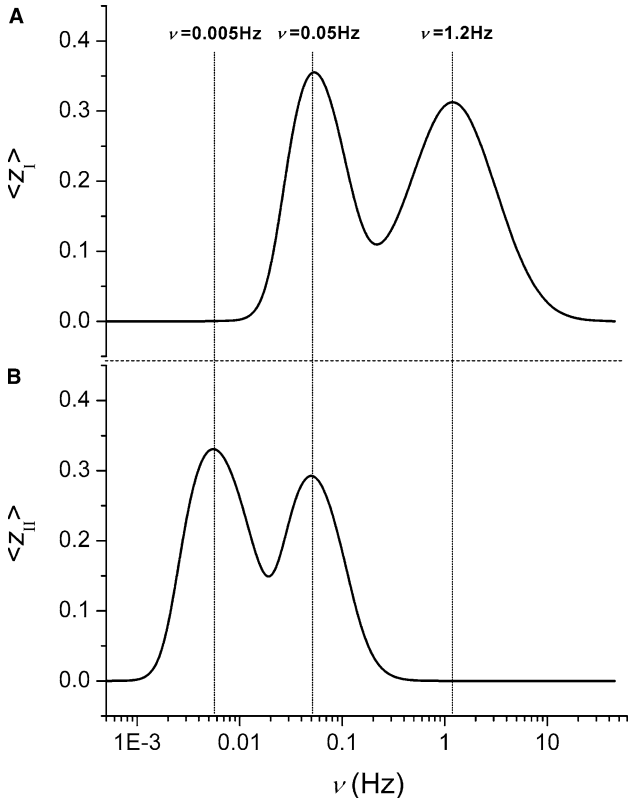

Fig. 3. Selective regulation of cellular processes: (A) activation of the third-level protein in process I with Ca^{2+} oscillation frequencies $\nu = 0.05$ Hz and $\nu = 1.2$ Hz, (B) activation of the third-level protein in process II with Ca^{2+} oscillation frequencies $\nu = 0.005$ Hz and $\nu = 0.05$ Hz. Ca^{2+} oscillations with the frequency $\nu = 0.05$ Hz activate both processes simultaneously.

4. Discussion

Our results show that a two-level protein cascade can act as a band-pass filter for time-limited oscillations. This implies a new possible mechanism for band-pass filtering of cellular signals that differs from those normally used in electrical circuit design, where the band-pass filtering of sustained, theoretically infinitely long, oscillations is of primary interest. The proposed band-pass filter for time-limited cellular signals, which in some cases consist of only several spikes, is likely to be of high physiological importance. For Ca^{2+}-calmodulin kinase II, for example, it has been shown experimentally that the kinase can be selectively activated by band-pass filtering of time-limited Ca^{2+} spike trains [15]. The underlying mechanism has been analysed theoretically by a four-step binding scheme [18]. Since in biological systems larger motifs are often combinations of smaller motifs [19], our model for the band-pass filter, based on a two-level protein cascade, can be seen as a basic motif providing a selective regulation of cellular processes.

In biological systems, several motifs and modules are known, acting as switches, amplifiers, filters, etc. [20]. In prokaryotes, for example, simple one- and two-component systems link external signals with cellular responses [21,22]. For frequency filters, a simple motif can act as a low-pass filter, never as a high-pass one, and only under special constraints as a band-pass filter [1,2]. Here, we provide evidence that a relatively simple motif, which would expectably act as a low-pass filter, behaves as a band-pass filter if the input oscillations are time-limited. In addition to the simplicity of the proposed band-pass filter, it is also much more robust to the choice of parameter values than those proposed previously. As stated by Arkin [1], the studied chemical systems acting as band-pass filters are admittedly artificial, since the parameter constraints are not likely to be met in biological systems.

We further show that the proposed band-pass filters can be simply combined into a network providing selective regulation of cellular processes. The number of activation branches in the network limits the total number of regulated processes, whereas the number of common primary branches at the first two levels of every activation branch determines the flexibility in a concomitant response to a given Ca^{2+} signal, i.e., the number of cellular processes that can be activated simultaneously.

The effectiveness of the proposed regulatory network to provide modularity in terms of enabling selective regulation of numerous cellular processes depends significantly on the variability of network proteins and their kinetics. A characteristic property of the regulatory proteins at the last cascade level is that maxima in their activation curves are slightly, though significantly shifted with respect to each other (Fig. 3). To accomplish this, the kinetic properties of proteins involved in the network have to be different, as is the case for matched pairs of proteins in bacterial two-component regulatory systems [21,22], for example. In plant and animal cells, it has been found experimentally that a superfamily of Ca^{2+}-dependent protein kinases or calmodulin-like domain protein kinases (CDPK) exists [23]. Some authors have predicted that differences in isoforms of CDPK (in particular their sensitivity to Ca^{2+}) suggest that each isoform is prone to respond only to a specific set of Ca^{2+} signals that, in dependence on the external stimulus [24], differ in their frequency of oscillations, as well as magnitude and duration. However, the difficult problem of how to test for differential activities of specific isoforms of CDPK in vivo remains unsolved [25].

Little is known about the kinetic properties of proteins that constitute intracellular signalling networks [3,26,27], and also the computational function of many of the signalling networks is poorly understood [28]. However, it is clear that complex networks of signalling cascades are able to process vast amounts of external inputs, including those transmitted by various hormones and neurotransmitters, as well as signals from neighbouring cells, and selectively convert them into precise intracellular actions that regulate different processes, ranging from egg fertilisation to cell death [8,25]. Our study provides novel insights into how such a complex array of coherently functioning elements can be modelled mathematically with mass-action kinetics, simple spike-like oscillations, and a bow-tie architecture. This provides a generally applicable scheme that can easily be upgraded and extended to more complex and specific problems. In the future, however, additional experiments are necessary to verify model predictions and indicate ways for their improvements.

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