Optimal Length and Signal Amplification in Weakly Activated Signal Transduction Cascades

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Abstract

Weakly activated signaling cascades can be modeled as linear systems. The input-to-output transfer function and the internal gain of a linear system, provide natural measures for the propagation of the input signal down the cascade and for the characterization of the final outcome. The most efficient design of a cascade for generating sharp signals, is obtained by choosing all the off rates equal, and a “universal” finite optimal length.

1 Introduction

Protein kinase cascades are major functional modules used by cells to translate signals generated by receptor activation into diverse biochemical and physiological responses [16]. Highly conserved throughout evolution and across species, the kinase cascade motif participates in the control of many processes, including cell cycle regulation, gene expression, cellular metabolism, stress responses, and T cell activation. For this reason, control of kinase cascades by therapeutic intervention has become an attractive area for drug discovery, particularly in the areas of cancer and inflammation [7, 9].

Some four mitogen-activated protein kinase (MAPK) signaling cascades have been found in yeast [17] and at least a dozen MAPK cascades have been identified in mammalian cells [12]. The intensive study of MAPK pathways has prompted efforts to characterize these systems theoretically (see, inter alia, [11, 3, 4, 6, 10, 11, 13, 14, 15]). In this paper, we will utilize concepts and methods from the theory of linear control systems to characterize kinase signaling cascades, and in particular the MAPK pathway, in order to understand how the number of kinases in a cascade and their individual enzymatic activities can affect the pathway in its role as a signal transducing module.

Let \( R \) denote the input signal, \( \bar{X}_i \) the inactive (nonphosphorylated) form of kinase \( i \) and \( X_i \) the active (phosphorylated) form of kinase \( i \). The rate constant (or “on” rate) for the \( i \)-th kinase phosphorylation will be denoted by \( \tilde{\alpha}_i \), and the dephosphorylation rate constants (or “off” rate) will be denoted \( \beta_i \). The input signal \( R \) might represent, for example, the concentration of activated receptors, and the dynamics of the signal transduction pathway may be modeled as follows (see [10]):

\[
\frac{dX_1}{dt} = \tilde{\alpha}_1 R \bar{X}_1 - \beta_1 X_1, \quad \frac{dX_i}{dt} = \tilde{\alpha}_i X_{i-1} \bar{X}_i - \beta_i X_i, \quad i = 2, \ldots, n. \tag{1}
\]

Assuming that the total amount of kinase \( i \) remains constant, that is, \( \bar{X}_i + X_i = X_{\text{tot},i} \) the
differential equations (1) can be rewritten as
\[ \frac{dX_1}{dt} = \alpha_1 R \left( 1 - \frac{X_1}{X_{tot,1}} \right) - \beta_1 X_1 \]
and
\[ \frac{dX_i}{dt} = \alpha_i X_{i-1} \left( 1 - \frac{X_i}{X_{tot,i}} \right) - \beta_i X_i, \quad i = 2, \ldots, n. \] (2)
where \( \alpha_i = \tilde{\alpha}_i X_{tot,i} \). Throughout this paper we will focus on the case of weakly activated pathways, by which we mean a low level of kinase phosphorylation, that is
\[ X_i \ll X_{tot,i} \Rightarrow 1 - \frac{X_i}{X_{tot,i}} \approx 1. \] (3)
In this case the equations (2) are simplified to a linear system of the form:
\[ \frac{dX_1}{dt} = \alpha_1 R - \beta_1 X_1, \quad \frac{dX_i}{dt} = \alpha_i X_{i-1} - \beta_i X_i, \quad i = 2, \ldots, n. \] (4)
In Section 2 we will describe how to compute the transfer function and internal gain for this system and then in Section 3 we will define a set of measures for the output signal, which closely follow those discussed in [10]. In Section 4 we prove that the most efficient cascade design, for generating sharp signals, has equal on rates and a finite length depending only on the cascade’s internal gain. In Section 5 positive feedback from the last activated kinase to the first is added to the cascade, and the optimal design is re-examined in this new context. Finally, in Sections 6 and 7 we briefly discuss the effect of delays along the cascade and how to check the cascade’s stability to random small perturbations.

2 The input-to-output transfer function

We will consider the signaling cascade (1) as a system with an input \( R \), and an output which will be some function of the concentration of the last kinase \( X_n \). Specifically, the output will be the “effective” integral of \( X_n \), or in other words, the cascade will be extended one more step to include a “leaky” integrator:
\[ \frac{dX_{n+1}}{dt} = X_n - \ell X_{n+1}, \]
where the output is \( X_{n+1} \). The variable \( X_{n+1} \) expresses the effective concentration of the last kinase (minus losses due to degradation or inactivation of \( X_n \), for instance). Note that the case \( \ell = 0 \) recovers \( X_{n+1} = \int t X_n(t') \, dt' \).

The model for a weakly activated signal transduction cascade may then be written in the more compact form,
\[ \frac{dX}{dt}(t) = AX(t) + BR(t), \quad Y(t) = CX(t), \] (5)
where $X = (X_1, X_2, \ldots, X_n, X_{n+1})'$ is a column vector whose elements are always nonzero, and $A \in \mathbb{R}^{(n+1) \times (n+1)}$, $B \in \mathbb{R}^{(n+1) \times 1}$ and $C \in \mathbb{R}^{1 \times (n+1)}$ are the matrices

$$A = \begin{pmatrix} -\beta_1 & 0 & 0 & \cdots & 0 & 0 & 0 \\ \alpha_2 & -\beta_2 & 0 & \cdots & 0 & 0 & 0 \\ 0 & \alpha_3 & -\beta_3 & \cdots & 0 & 0 & 0 \\ \vdots & \vdots & \vdots & \ddots & \vdots & \vdots & \vdots \\ 0 & 0 & 0 & \cdots & \alpha_n & -\beta_n & 0 \\ 0 & 0 & 0 & \cdots & 0 & 1 & -\ell \end{pmatrix}, \quad B = \begin{pmatrix} \alpha_1 \\ 0 \\ 0 \\ \vdots \\ \vdots \\ \vdots \\ 0 \end{pmatrix},$$

and

$$C = \begin{pmatrix} 0 & \cdots & 0 & 1 \end{pmatrix}.$$  

(6)

It is well known (see [2, 18], or any other book on control systems) that, for a system such as (5), the output can be computed directly as the convolution between the input signal $R$ and the impulse response of the system. The impulse response of the system is the output corresponding to a single input pulse. If we let $G$ denote the impulse response (and assuming that the system starts at rest, with initial condition $X(0) = 0$), then

$$Y(t) = (G * R)(t).$$

The impulse response, $G$, characterizes the action that the internal structure of the system will have on any input, such as the filtering of certain frequency components, and the amplification or dampening of the signal. Biological inputs may take many different forms, such as single pulses, slowly decaying signals, constant stimuli applied for a certain time interval, or oscillatory signals. Thus, it is appropriate to have a model in which the output signal is obtained as a convolution of the input $R$ (which may take many forms) and the transfer function $G$ (which depends only on the intrinsic kinase activity parameters, and needs to be computed only once).
A very convenient way to analyze system (5) is to convert it to the frequency domain, by application of the Laplace transform operator. The Laplace transform of the impulse response is called the *transfer function* of the system, and it provides a simple linear relationship between the Laplace transforms of the input and the output, as well as also providing a measure of amplification/dampening of the input signal. The transfer function is given by a simple formula in terms of the matrices $A$, $B$ and $C$ as summarized in Appendix D. For this cascade system, we will carry out the Laplace transforms in detail so as to gain some insight into the internal structure of the system.

The Laplace transform of $X$ will be denoted by $\hat{X}$, and is defined as

$$\hat{X}_i(s) = \int_{-\infty}^{\infty} e^{-st} X_i(t) dt, \quad \text{and} \quad \hat{R}(s) = \int_{-\infty}^{\infty} e^{-st} R(t) dt,$$

where $s$ is a complex number $s = s_{re} + j\omega$ ($j$ is the imaginary number $\sqrt{-1}$) and takes values in an appropriate region of convergence.

Applying the Laplace transform operator to both sides of equations (4), assuming that $X(0) = 0$, and recalling the properties of the Laplace transform (see Appendix D), we have:

$$s \hat{X}_1(s) = \alpha_1 \hat{R}(s) - \beta_1 \hat{X}_1(s)$$

$$s \hat{X}_i(s) = \alpha_i \hat{X}_{i-1}(s) - \beta_i \hat{X}_i(s), \quad i = 2, \ldots, n$$

$$s \hat{X}_{n+1}(s) = \hat{X}_n(s) - \ell \hat{X}_{n+1}(s),$$

which yields

$$\hat{X}_1(s) = \frac{\alpha_1}{s + \beta_1} \hat{R}(s), \quad \hat{X}_i(s) = \frac{\alpha_i}{s + \beta_i} \hat{X}_{i-1}(s), \quad i = 2, \ldots, n,$$

and

$$\hat{X}_{n+1}(s) = \frac{1}{s + \ell} \hat{X}_n(s).$$

In this way, we may view the cascade as a sequence of $n$ steps, the output of the step $i - 1$ becoming the input to step $i$.

For each *single step* in the cascade, the input is $\hat{X}_{i-1}$ and the output is $\hat{X}_i$, and they are related by a multiplicative factor, which is in fact the *transfer function for the step $i$*:

$$\hat{G}_i(s) = \frac{\alpha_i}{s + \beta_i}, \quad i = 2, \ldots, n.$$

For the whole cascade, the input is $R$ and the output is $\hat{X}_{n+1}$, and it is easy to see that the transfer function for the total system is the product of the transfer functions at each step:

$$\hat{G}(s) = \hat{G}_1(s) \cdots \hat{G}_{n+1}(s) = \frac{1}{s + \ell} \frac{\alpha_1 \cdots \alpha_n}{(s + \beta_1) \cdots (s + \beta_n)}.$$  \hspace{1cm} (8)
Therefore
\[ \hat{Y}(s) = \hat{X}_{n+1}(s) = \frac{1}{s + \ell (s + \beta_1) \cdots (s + \beta_n)} \hat{R}(s), \]
and the actual output may now be obtained by the inverse Laplace transform. Alternatively, even without knowing the exact form of the output, that is, the function \( Y(t) \), it is still possible to further characterize the properties of the system, through the 2-norm of the functions \( \hat{Y} \) and \( \hat{R} \). Define the 2-norm of the function \( \hat{Y} \) and the 2-norm of the Laplace transform \( \hat{Y} \) by
\[ \| \hat{Y} \|_2 := \left[ \int_{-\infty}^{+\infty} |Y(t)|^2 \, dt \right]^{\frac{1}{2}}, \]
and similar expressions hold for \( R \) and \( \hat{R} \). (Note: from now on we will assume that the signals are defined only for positive times, that is, \( Y(t) = 0 \) for \( t < 0 \)). The 2-norm \( \| \hat{Y} \|_2 \) provides a measure of the strength of the signals (in analogy to the energy of a mechanical system). Indeed, these norms provide a very convenient way to relate the input and output because, from Parseval’s Theorem, the 2-norm of a function, equals the 2-norm of its Laplace transform, and therefore
\[ \| \hat{Y} \|_2 = \| Y \|_2, \quad \| \hat{R} \|_2 = \| R \|_2, \]
without the need to compute inverse transforms (a very helpful fact, since in general the inverse transforms may not be simple to compute).

Another useful measure is the infinity norm of the transfer function, that selects the least upper bound of the absolute value of \( \hat{G} \),
\[ \| \hat{G} \|_\infty := \sup_{\omega \in \mathbb{R}} |\hat{G}(j\omega)|. \]
As shown in Appendix D a very useful estimate for characterizing the relative strength of the input and output signals is:
\[ \| Y \|_2 \leq \| \hat{G} \|_\infty \| R \|_2, \]
(9)
where it is immediately apparent that the infinity norm of the transfer function gives an upper bound for the amplification of the input signal throughout the cascade. Moreover, the infinity norm \( \| \hat{G} \|_\infty \) is in fact the smallest number that satisfies \( 1 \), for all input/output pairs (that is, pairs \( (R, Y) \), where \( Y \) is the output corresponding to the input \( R \)).
To compute the infinity norm of the transfer function for the whole cascade, note that
\[ |\hat{G}(j\omega)|^2 = \frac{|\alpha_i|^2}{|j\omega + \beta_i|^2} = \frac{\alpha_i^2}{\omega^2 + \beta_i^2} \leq \frac{\alpha_i^2}{\beta_i^2}, \quad \text{for all } \omega \in (-\infty, \infty), \]
and the equality holds for \( \omega = 0 \). Therefore
\[ \| \hat{G} \|_\infty = \frac{1}{\ell} \frac{\alpha_1 \cdots \alpha_n}{\beta_1 \cdots \beta_n}. \]
(10)
A necessary condition for amplification of the signal to occur is that \( \| \hat{G} \|_\infty > 1 \). Moreover, since \( \ell \) is essentially an independent parameter, introduced for the purpose of defining a reasonable measure of the output, we can say that amplification of the input signal occurs only if
\[ \alpha_1 \cdots \alpha_n > \beta_1 \cdots \beta_n. \]
(11)
Recall that $\alpha_i \equiv \tilde{\alpha}_i X_{\text{tot},i}$, where $X_{\text{tot},i}$ is the total concentration of the $i$th kinase and $\tilde{\alpha}_i$ is the (true) rate of phosphorylation. Therefore, we still expect that $\tilde{\alpha}_i < \beta_i$, $i = 1, \ldots, n$, as should be the case for a weakly activated pathway.

The norm $\|G\|_{\infty}$ is often called the internal gain of the system which, through expression (9), provides a useful and easy way to compute the input-to-output strength relation. For example, if a MAPK cascade has a “5-fold amplification”, then its internal gain is $\|G\|_{\infty} = 5$.

Note that, in the case where $\ell = 0$, the internal gain $\|G\|_{\infty}$ is infinite — meaning that, in at least one step ($X_n \to X_{n+1}$) there is no degradation term. Then the estimate (9) contains no useful information. However, for $\ell = 0$, we have

$$Y(t) = X_{n+1}(t) = \int_0^t X_n(t') \, dt',$$

and we also have an estimate for the “strength” of the signal $X_n$, since

$$\|X_n\|_2 \leq \frac{\alpha_1 \cdots \alpha_n}{\beta_1 \cdots \beta_n} \|R\|_2.$$

### 3 Signaling time, signal duration and signal amplitude

Some basic quantities which serve to characterize a signal transduction system are: the overall amplification from the input to the output; the duration of the output signal; and the time it takes the input signal to traverse the cascade. There are several possible definitions and estimates of these quantities: here we extend the definitions given by [10], embedding them in the context of frequency-domain analysis, and generalizing them to arbitrary inputs.

To be concise, let us identify the cascade (5) by its parameters, and associate with it the following $(2n+1)$-tuple:

$$\mathcal{C} := (n, \alpha_1, \ldots, \alpha_n, \beta_1, \ldots, \beta_n),$$

where it is assumed that $n \in \mathbb{N}$ and $\alpha_i, \beta_i$ are positive real numbers, for $i = 1, \ldots, n$. We will also introduce the notation $\mathcal{U}$ for denoting the set of inputs.

**Definition 3.1** For system (5), with parameters $\mathcal{C}$ and a leak factor $\ell > 0$, for each input $R$, the signaling time, $\tau$, and the output signal duration, $\sigma$, are given by

$$\tau(\mathcal{C}, \ell, R) := -\left. \frac{d \ln \hat{Y}}{ds}(s) \right|_{s=0}, \quad \sigma(\mathcal{C}, \ell, R) := \left. \sqrt{\frac{d^2 \ln \hat{Y}}{ds^2}(s)} \right|_{s=0}.$$

The signaling time to step $i$ and the signal duration at step $i$, $i \leq n$ are given by:

$$\tau_i(\mathcal{C}, R) := -\left. \frac{d \ln \hat{X}_i}{ds}(s) \right|_{s=0}, \quad \sigma_i(\mathcal{C}, R) := \left. \sqrt{\frac{d^2 \ln \hat{X}_i}{ds^2}(s)} \right|_{s=0}.$$

To understand the significance of these definitions, recall the properties of the Laplace transform and compute (with $Y(t) = 0$ for $t \leq 0$):

$$\hat{Y}(0) = \int_0^\infty Y(t)dt, \quad \frac{d\hat{Y}}{ds}(0) = -\int_0^\infty tY(t)dt, \quad \frac{d^2\hat{Y}}{ds^2}(0) = \int_0^\infty t^2Y(t)dt.$$
and thus we recover expressions (4) and (5) of reference [10]
\[ \tau = \int_0^\infty tY(t)dt, \quad \sigma^2 = \int_0^\infty t^2Y(t)dt - \left( \int_0^\infty tY(t)dt \right)^2, \]
where \( \tau \) can be regarded as the expected value (of the time to traverse the pathway), and \( \sigma^2 \) as the corresponding variance.

An estimate of the amplitude of the output signal, as given in equation (6) of reference [10], is the value \( S \), such that \( S \times 2\sigma = \int_0^\infty Y(t)dt \). Again we propose a more generalized notion, suggested by the input-to-output estimate [8], that takes advantage of the easily computed internal gain of the system, and also incorporates the strength of the signal.

**Definition 3.2** For system (5), with parameters \( C \) and a leak factor \( \ell > 0 \), for each input \( R \), the **signal amplitude** is given by
\[ A(C, \ell, R) := \frac{\|\hat{G}_C\|_\infty}{\sigma(C, \ell, R)} \|R\|_2, \]
where \( \hat{G}_C \) is the transfer function [3].

\( A \) may also be regarded as the amplitude of a constant signal of duration \( \sigma \), but Definition 3.2 differs from the definition of amplitude given in [10] in essentially three points:

1. the meaningful quantity for measuring the amplitude is not the integral \( \int Y(t)dt \) (which computes the area under the curve \( Y(t) \)), but rather the 2-norm \( \sqrt{\int |Y(t)|^2 dt} \), which computes the strength of the signal;

2. the amplitude is proportional to the product of the internal gain of the system, and the 2-norm of the input. This simplifies calculations since, for each cascade, the \( \|\hat{G}\| \) is computed only once and \( \|R\|_2 \) is computed for each input signal;

3. the product \( \|\hat{G}\|_\infty \|R\|_2 \) is used as an estimate for \( \|Y\|_2 \), but we know (see Appendix) that \( \|\hat{G}\|_\infty \) is the least factor that satisfies the inequality \( \|Y\|_2 \leq \kappa\|R\|_2 \). In fact, [8] shows how to construct examples of inputs for which the equality is approximated. For instance, for any \( \epsilon > 0 \), the input depicted in Figure [3]
\[ R(t) = 2\frac{r}{\pi t} \sin \epsilon t, \quad \text{with} \quad r = \sqrt{\pi/\epsilon}, \quad \text{for} \quad t \geq 0, \]
has unit norm, i.e., \( \|R\|_2 = 1 \), and satisfies \( \|Y\|_2 \approx \|\hat{G}\|_\infty \), for \( \epsilon \) small enough, as shown in the Appendix.

We remark that these definitions are valid not only for the special case when \( A, B \) and \( C \) are of the form specified in equations (6), (7), but in fact they are valid for any linear system of the form (5). For instance, in Section 5 we compute these quantities for the case when there is positive feedback from the last to the first kinase. We next explicitly compute these quantities for the special case when \( A, B \) and \( C \) are of the form (6) and (7), and \( \ell = 0 \):
Figure 3: An input that satisfies $\|R\|_2 = 1$ and $\|Y\|_2 \approx \|\hat{G}\|_\infty$, with $\varepsilon = 0.2$.

$$\sigma(C, \ell, R) = \sqrt{\frac{1}{\ell^2} + \sum_{i=1}^{n} \frac{1}{\beta_i^2} + q(R)}, \quad \text{where} \quad q(R) = \frac{d^2 \ln \hat{R}}{ds^2} \bigg|_{s=0}$$

(15)

$$A(C, \ell, R) = \sqrt{\frac{1}{\ell^2} + \sum_{i=1}^{n} \frac{1}{\beta_i^2} + q(R)} \frac{\alpha_1 \cdots \alpha_n}{\ell \beta_1 \cdots \beta_n} \|R\|_2.$$  

(16)

In the case $\ell = 0$, the quantities $\tau$, $\sigma$ and $A$ may be computed for $Y \equiv X_n$. The expressions are very similar, except that all the terms in $\ell$ vanish.

**Example 3.3** A typical input is a decaying exponential $R(t) = R_0 e^{-\lambda t}$, with

$$\|R\|_2 = \frac{R_0}{2\lambda}, \quad \hat{R}(s) = \frac{R_0}{s + \lambda}, \quad \frac{d \ln \hat{R}}{ds} \bigg|_{0} = -\frac{1}{\lambda}, \quad q(R) = \frac{1}{\lambda^2}.$$  

A “peak”-like input may be represented by $R(t) = R_0 te^{-\lambda t}$, with

$$\|R\|_2 = \frac{R_0}{4\lambda^3}, \quad \hat{R}(s) = \frac{R_0}{(s + \lambda)^2}, \quad \frac{d \ln \hat{R}}{ds} \bigg|_{0} = -\frac{2}{\lambda}, \quad q(R) = \frac{2}{\lambda^2}.$$  

For a constant signal, of magnitude $R_0$, applied for an interval of time $T_0$, we have:

$$\|R\|_2 = R_0 \sqrt{T_0}, \quad \hat{R}(s) = R_0 \frac{1 - e^{-sT_0}}{s}, \quad \frac{d \ln \hat{R}}{ds} \bigg|_{0} = -\frac{T_0}{2}, \quad q(R) = \frac{T_0^2}{12}.$$  

**4 Cascade design optimization**

From the analysis of the quantities $\tau$, $\sigma$ and $A$, defined in Section 3, we can explore the signaling efficiency of kinase cascades. The definition of an “efficient” response may depend on the particular biological context, but it typically involves the relationship between the length of the cascade, the amplitude of the signal and its duration. A question posed in [10] is whether cascades can respond with sharp signals, i.e., simultaneously of short duration and high amplitude. Our model provides a definite answer to this question.

As we have seen, our linear model has a gain that depends on the length of the cascade and the values of the on/off rate constants, but doesn’t depend on the input. As a starting
point, we may think of the family of cascades that have the same value for the internal gain, say $K$, and examine their length, the distribution of the “on/off” rates and signal amplitude and duration. The problem we would like to study is then:

(P) For each fixed internal gain, $\|G\|_\infty = K$, find the optimal combination of the on/off rates and the length of the cascade that maximizes the signal amplitude, $A$, for any input $R$.

To formulate this problem, first define the family of cascades that have the same internal gain $K$:

$$C_{K, \ell} := \{C = (n, \alpha_1, \ldots, \alpha_n, \beta_1, \ldots, \beta_n) : \frac{\alpha_1 \cdots \alpha_n}{\ell \beta_1 \cdots \beta_n} = K\}.$$ 

For each input $R$, and each leak factor $\ell$, define the set of “optimal” cascades, that is, those cascades which exhibit maximal signal amplitude:

$$C_{\text{max}}(\ell, R) := \{C \in C_{K, \ell} : A(C, \ell, R) \geq A(C', \ell, R), \text{ for all } C' \in C_{K, \ell}\}.$$ 

Then define the function

$$\sigma_0(n, \beta_1, \ldots, \beta_n) := \sum_{i=1}^{n} \frac{1}{\beta_i^2}$$

and observe that it satisfies

$$\sigma(C, \ell, R) = \sqrt{\frac{1}{\ell^2} + \sigma_0(n, \beta_1, \ldots, \beta_n) + q(R)}.$$ 

Finally, define the set of cascades that minimize $\sigma_0$ over the family $C_{K, \ell}$:

$$C_*(\ell, R) := \{C \in C_{K, \ell} : \sigma_0(n, \beta_1, \ldots, \beta_n) \leq \sigma_0(n', \beta_1', \ldots, \beta_n'), \text{ for all } C' \in C_{K, \ell}\}.$$ 

Our first result states that in fact the sets $C_{\text{max}}(\ell, R)$ and $C_*(\ell, R)$ are equal, or in other words, that an optimal cascade will simultaneously maximize the signal amplitude and minimize the signal duration.

**Lemma 4.1** In the notation defined above, $C_{\text{max}}(\ell, R) = C_*(\ell, R)$, for all inputs $R \in U$ and leak factors $\ell > 0$.

*Proof.* Fix any $\ell > 0$, and any $R \in U$. Recall the notation $C = (n, \alpha_1, \ldots, \alpha_n, \beta_1, \ldots, \beta_n)$. Given any $C, C' \in C_{K, \ell}$, the following equivalences hold:

$$\sigma_0(n, \beta_1, \ldots, \beta_n) \leq \sigma_0(n', \beta_1', \ldots, \beta_n'),$$

$$\Rightarrow \sqrt{\frac{1}{\ell^2} + \sigma_0(n, \beta_1, \ldots, \beta_n) + q(R)} \leq \sqrt{\frac{1}{\ell^2} + \sigma_0(n', \beta_1', \ldots, \beta_n') + q(R)},$$

$$\Rightarrow \sigma(C, \ell, R) \leq \sigma(C', \ell, R)$$

and also

$$\sigma(C, \ell, R) \leq \sigma(C', \ell, R) \iff \frac{K \|R\|_2}{\sigma(C, \ell, R)} \geq \frac{K \|R\|_2}{\sigma(C', \ell, R)} \iff A(C, \ell, R) \geq A(C', \ell, R).$$
Therefore, (17) and (18) imply that, for any two cascades \( \mathcal{C}, \mathcal{C}' \in \mathcal{C}_{K,\ell} \),
\[
\sigma_0(n, \beta_1, \ldots, \beta_n) \leq \sigma_0(n', \beta'_1, \ldots, \beta'_n) \quad \iff \quad A(\mathcal{C}, \ell, R) \geq A(\mathcal{C}', \ell, R). \tag{19}
\]
To show that \( \mathcal{C}_*(\ell, R) \) is contained in \( \mathcal{C}_{\max}(\ell, R) \), pick any \( \mathcal{C} \in \mathcal{C}_*(\ell, R) \). Then
\[
\sigma_0(n, \beta_1, \ldots, \beta_n) \leq \sigma_0(n', \beta'_1, \ldots, \beta'_n), \quad \text{for all } \mathcal{C}' \in \mathcal{C}_{K,\ell}.
\]
By (19), this is equivalent to \( A(\mathcal{C}, \ell, R) \geq A(\mathcal{C}', \ell, R) \), for all \( \mathcal{C}' \in \mathcal{C}_{K,\ell} \), and so \( \mathcal{C} \in \mathcal{C}_{\max}(\ell, R) \).

Conversely, we need to show that \( \mathcal{C}_{\max}(\ell, R) \) is contained in \( \mathcal{C}_*(\ell, R) \). So, pick any \( \mathcal{C} \in \mathcal{C}_{\max}(\ell, R) \). It satisfies:
\[
A(\mathcal{C}, \ell, R) \geq A(\mathcal{C}', \ell, R), \quad \text{for all } \mathcal{C}' \in \mathcal{C}_{K,\ell}.
\]
Again by (19), this is equivalent to \( \sigma_0(n, \beta_1, \ldots, \beta_n) \leq \sigma_0(n', \beta'_1, \ldots, \beta'_n) \) for all \( \mathcal{C}' \in \mathcal{C}_{K,\ell} \). We conclude that \( \mathcal{C} \in \mathcal{C}_*(\ell, R) \), as we wanted to show.

An immediate conclusion from Lemma 4.1 is that,
\[
\max A(\mathcal{C}, \ell, R) \quad \text{over } \mathcal{C}_{K,\ell} \quad \iff \quad \min \sigma_0(n, \beta_1, \ldots, \beta_n) \quad \text{over } \mathcal{C}_{K,\ell},
\]
so that, for any fixed internal gain, maximal amplitude is achieved simultaneously with minimal signal duration. This is consistent with the notion that the most efficient cascade would respond with sharp (high-peaked and fast) output signals. In the limit, this notion can be regarded as an “instantaneous response” (\( \sigma \approx 0 \)) coupled with “infinite signal amplitude” (\( A \approx \infty \)), which is, of course, not biologically viable. A realistic solution to problem (P) does exist, and is stated in Theorem 11.

Since the signal duration depends only on the cascade length and the “off” rates, \( \beta_i \) (besides the input term), we expect the “on” rates, \( \alpha_i \), to play a small role in maximizing the efficiency of the output response. So, for addressing the problem (P), we will consider two different assumptions on the available knowledge on the \( \alpha_i \): either (a) all the \( \alpha_i \) have an equal, fixed value, \( \alpha \); or (b) the product of the \( \alpha_i \) is known, at some fixed \( \alpha_P \). We will also assume that the “leak” factor \( \ell \) is fixed, since this parameter was added artificially and may be adjusted independently.

Before stating the main Theorem, we need to introduce some notation. Define the function
\[
f(k) = k^2 \left[ \left( 1 + \frac{1}{k} \right) \ln \left( 1 + \frac{1}{k} \right) - \frac{1}{k} \right].
\]
Some properties of this function are stated in Appendix A. For any real number \( M \geq 1 \), define
\[
[M] = \text{largest integer less than or equal to } M,
\]
\[
[M'] = \text{least integer greater than } M,
\]
which are also known as, respectively, the “floor” and “ceiling” functions of \( M \). Observing that any real number \( M \geq 1 \), can be written as the sum of its integral and fractional parts:
\[
M = [M] + \delta_M,
\]
where \( \delta_M \in [0, 1) \), define the function \( \Psi : (-\infty, \infty) \rightarrow \mathbb{N} \), which is plotted in Figure 2
\[
\Psi(M) = \begin{cases} 
1, & M \leq 1 \\
[M], & M > 1, \text{ and } \delta_M \leq f([M]) \\
[M], & M > 1, \text{ and } \delta_M > f([M]).
\end{cases}
\]
This is a step function where the “jump” between steps always occurs in an interval between two integers, say $k$ and $k+1$, at a point that depends on the number $k$. In particular, since the function $f$ is strictly increasing and takes values in the interval $(2\ln 2 - 1, 1/2)$ (see Appendix A), it follows that in some cases only the fractional part of the number $M$ affects the location of the “jump” discontinuity:

\[
0 \leq \delta_M < 2\ln 2 - 1, \quad \Psi(M) = \lfloor M \rfloor, \\
\frac{1}{2} < \delta_M < 1, \quad \Psi(M) = \lceil M \rceil,
\]

while for the other cases, $2\ln 2 - 1 < \delta_M < 0.5$, the choice depends also on the integral part of $M$.

![Figure 4: Left: the function $\Psi(M)$. Right: the function $\Psi(2\ln KL)$. Note that, for a given gain $K$ and leak factor $\ell$, the optimal length is given by the integer platform corresponding to the product $K\ell$.](image)

**Theorem 1** Let $K > 0$ and $\ell > 0$ be fixed real numbers. Let $C_{K,\ell}$ be the set of all cascades with internal gain $K$, as defined above. Then

1. For each fixed $n = N \in \mathbb{N}$, the elements $C = (N, \alpha_1, \ldots, \alpha_N, \beta_1, \ldots, \beta_N) \in C_s(\ell, R)$ satisfy $\beta_i = \beta$, for all $i = 1, \ldots, N$, where

\[
\beta = \left( \frac{\alpha_1 \cdots \alpha_N}{K\ell} \right)^{\frac{1}{N}};
\]

2(a). Any element $C \in C_s(\ell, R)$ of the form $C = (n, \alpha, \ldots, \alpha, \beta, \ldots, \beta)$ satisfies

\[
n = \Psi(2\ln K\ell) \quad \text{and} \quad \beta_i = \beta = \alpha \left( \frac{1}{K\ell} \right)^{\frac{1}{n}}
\]

2(b). Any element $C \in C_s(\ell, R)$ of the form $C = (n, \alpha_1, \ldots, \alpha_n, \beta_1, \ldots, \beta_n) \in C_s(\ell, R)$ with $\alpha_1 \cdots \alpha_n = \alpha_P$ satisfies

\[
n = \Psi \left( \frac{2\ln K\ell}{\alpha_P} \right) \quad \text{and} \quad \beta_i = \beta = \left( \frac{\alpha_P}{K\ell} \right)^{\frac{1}{n}}.
\]
Before presenting the proof of the Theorem, some remarks on the interpretation of points 1 and 2(a), 2(b). The first part of the result is consistent with the observation that the ordering of the amplification or dampening single steps within the cascade does not influence the final output signal (also observed in [10]).

The second part of the Theorem shows that indefinitely increasing the cascade’s length will not increase amplification. In fact, there is an optimal length for the cascade that provides both maximum signal amplitude and duration. A similar observation was mentioned in [10], and our Lemma [□] and Theorem [□] characterize the conditions for achieving this optimization. For each gain \( K \) and leak factor \( \ell \), this optimal length is easily read out from Figure 4. For instance, a cascade with a 6 to 9-fold gain (and \( \ell = 1 \)), is seen to have an optimal length of 4 steps. Figure 5 illustrates Theorem [□] for an 8-fold cascade gain. The Figure shows the results of two simulations of system (5), both with input \( R(t) = 5te^{-2t} \), but different lengths of the cascade. The various curves represent \( R \), the concentrations of each kinase \( X_i, i = 1, \ldots, n \), and the output \( X_{n+1} \). It is clear that, for the non-optimal \( n = 7 \), the output’s amplitude decreases and the signal duration increases. Note that the output curve \( X_8 \) is more spread out across time and its maximum value is lower, than for the optimal case.

![Figure 5: Signal transduction cascade with \( R(t) = 5te^{-2t} \), with \( K = 8 \), \( \ell = 1 \), \( \alpha_i = 1.2 \). The horizontal lines represent \( A \). Left (optimal case): \( n = 4 \), \( \beta_i \approx 0.714, i = 1, \ldots, n \), \( A = 0.409 \), and \( \sigma_0 = 3.059 \). Right: \( n = 7 \), \( \beta_i \approx 0.892, i = 1, \ldots, n \), \( A = 0.389 \), and \( \sigma_0 = 3.210 \).](image)

Theorem [□] can be proved by successively solving the two optimization problems:

(P1) For each fixed \( n \), minimize \( \sigma_0 \), over all possible choices of \( \beta_1, \ldots, \beta_n \in (0, \infty) \), subject to \( \| \hat{G} \|_\infty = K \).

(P2) Minimize \( \sigma_0 \), over all possible choices of \( n \in \mathbb{N} \) and \( \beta_1, \ldots, \beta_n \in (0, \infty) \), subject to \( \| \hat{G} \|_\infty = K \).

Recall that we are assuming that either (a) all the \( \alpha_i \) have an equal, fixed value, \( \alpha \); or (b) the product of the \( \alpha_i \) is known, at some fixed \( \alpha P \). The solution of (P1) is equal for both cases, but the solution of (P2) is slightly different for (a) or (b). Thus, problem (P1) is part 1 and (P2) is the part 2 of the Theorem. As we will see, the solution of (P1) greatly simplifies the proof of (P2).
4.1 Solving (P1): proof of part 1 of Theorem 1

Given a cascade of length \( n \), this problem consists of finding a set of \( n \) parameters \( \bar{\beta}_1, \ldots, \bar{\beta}_n \) such that the function \( \sigma_0 \) attains a minimum value at \( \bar{\beta}_i \), \( i = 1, \ldots, n \), i.e.,

\[
\frac{1}{\bar{\beta}_1^2} + \frac{1}{\bar{\beta}_2^2} + \cdots + \frac{1}{\bar{\beta}_{n-1}^2} \leq \frac{1}{\beta_1^2} + \frac{1}{\beta_2^2} + \cdots + \frac{1}{\beta_{n-1}^2}
\]

for every \( \beta_1, \ldots, \beta_n \) such that \( \| \hat{G} \|_\infty = K \):

\[
\| \hat{G} \|_\infty = \frac{1}{\ell} \beta_1 \cdots \beta_n = K \iff K \ell \beta_1 \cdots \beta_n - \alpha_1 \cdots \alpha_n = 0.
\]

For simplicity, rescale the values to \( B_i = 1/\beta_i^2 \), and observe that

\[
\frac{1}{B_1 \cdots B_n} = (\beta_1 \cdots \beta_n)^2 = \left( \frac{K \ell \alpha_1 \cdots \alpha_n}{\ell} \right)^2
\]

Then, the problem consists of minimizing the function:

\[
F(B_1, \ldots, B_{n-1}) = B_1 + \cdots + B_{n-1} + \frac{Q}{B_1 \cdots B_{n-1}}
\]

over all possible choices of \( B_i > 0, i = 1, \ldots, n-1 \), where

\[
Q = \left( \frac{K \ell}{\alpha_1 \cdots \alpha_n} \right)^2.
\]

In Appendix we show that the solution to this optimization problem is

\[
B_i = Q^{\frac{1}{n}}, \quad i = 1, \ldots, n-1,
\]

which also implies:

\[
B_n = \frac{Q}{Q^{\frac{1}{n}}} = Q^{\frac{1}{n}}.
\]

So, the choice of the “off” rate constants that minimizes \( \sigma_0 \) is to have \( \beta_1 = \beta_2 = \cdots = \beta_n = \bar{\beta} \), with

\[
\bar{\beta} = \frac{1}{\sqrt{B_n}} = \left( \frac{\alpha_1 \cdots \alpha_n}{K \ell} \right)^{\frac{1}{n}},
\]

as we wanted to show.

4.2 Solving (P2): proof of part 2 of Theorem 1

To solve the more general problem, we first show how its statement can be simplified. Given the value of \( \alpha \) (respectively, \( \alpha_P \)), suppose that we have found a solution of (P2), i.e., an integer \( n^* \) and a set of constants \( \beta_i^*, i = 1, \ldots, n^* \) satisfying

\[
\sigma_0(n^*, \beta_1^*, \ldots, \beta_{n^*}) \leq \sigma_0(n, \beta_1, \ldots, \beta_n)
\]

for any other cascade \( C = (n, \alpha_1, \ldots, \alpha_n, \beta_1, \ldots, \beta_n) \) with \( \alpha_i = \alpha, i = 1, \ldots, n \) (respectively, \( \alpha_1 \cdots \alpha_n = \alpha_P \)).
We have already showed that
\[ \sigma_0(n^*, \bar{\beta}^*, \ldots, \bar{\beta}^*) \leq \sigma_0(n^*, \beta_1^*, \ldots, \beta_n^*) \] (21)
with
\[ \bar{\beta}^* = \left( \frac{\alpha_1 \cdots \alpha_n^*}{K\ell} \right)^{\frac{1}{n^*}} \]
and we know this choice yields the unique minimum of \( \sigma_0 \) for a fixed length \( n \). So, it follows that the solution of (P2) must also satisfy
\[ \beta_i^* = \bar{\beta}^*, \quad i = 1, \ldots, n^*. \]
This observation allows us to simplify the statement of problem (P2), and look only for solutions where all \( \beta_i \)'s are equal:

(P2)' Minimize \( \sigma_0(n, \beta, \ldots, \beta) = n/\beta^2 \), over \( n \in \mathbb{N} \) and \( \beta \in (0, \infty) \), subject to \( (\alpha/\beta)n = K\ell \).

From the constraint \( \| \hat{G} \|_\infty = K \) we have

\[
\text{case 2(a):} \quad \left( \frac{\alpha}{\beta} \right)^n = K\ell \quad \Leftrightarrow \quad \beta = \alpha \left( \frac{1}{K\ell} \right)^{\frac{1}{n}} \quad \Rightarrow \quad \sigma_0(n, \beta(n)) = \frac{1}{\alpha^2} n (K\ell)^{\frac{2}{n}}.
\]

\[
\text{case 2(b):} \quad \frac{\alpha P}{\beta^n} = K\ell \quad \Leftrightarrow \quad \beta = \left( \frac{\alpha P}{K\ell} \right)^{\frac{1}{n}} \quad \Rightarrow \quad \sigma_0(n, \beta(n)) = n \left( \frac{K\ell}{\alpha P} \right)^{\frac{2}{n}}.
\]

In either case, to solve the problem, it is enough to minimize the function \( \ln[\sigma_0(n, \beta(n))] \):
\[
F(n, M) = \ln n + \frac{1}{n} M
\over n \in \mathbb{N}, \text{ where } M \text{ is a positive constant with value either}

\]
\[
M = 2 \ln K\ell, \quad \text{for case 2(a) (22)}
\]
\[
M = 2 \ln \frac{K\ell}{\alpha P}, \quad \text{for case 2(b) (23)}.
\]

For a fixed \( M \), let the minimizer of \( F(n, M) \) over \( n \in \mathbb{N} \) be
\[
n^*(M) := \{ n \in \mathbb{N} : F(n, M) \leq F(n', M), \text{ for every } n' \in \mathbb{N} \},
\]
which is given by Lemma C.1 (Appendix C):
\[
n^*(M) = \Psi(M).
\]

Thus, for part 2(a) of the theorem we have \( n = n^*(2 \ln K\ell) = \Psi(2 \ln K\ell) \), and for part 2(b) we have \( n = \Psi(2 \ln K\ell/\alpha P) \). The value \( \beta \) is given according to part 1.

As shown in the example of Figure 6, evaluation of \( \sigma_0 \) at \( n^*(M) \) yields a value which is actually quite close to the “true” \( \sigma_0(M, \beta(M)) \).
5 Cascades with positive feedback

In this Section we investigate the behavior of cascades under positive feedback. Assume that the last kinase, \( X_n \), also contributes to the activation of the first kinase: then the differential equation for \( X_1 \) includes one more term and becomes

\[
\frac{dX_1}{dt} = \alpha_1 R(t) + \varepsilon X_n - \beta_1 X_1.
\]

We will assume that \( \varepsilon \) is small enough:

\[
\beta_1 \cdots \beta_n > \varepsilon \alpha_2 \cdots \alpha_n.
\]

This guarantees that the cascade is stable with respect to small perturbations (that is, all the eigenvalues of the system’s matrix \( A \) have negative real parts, see Section 7).

We can compute the transfer function for the system with feedback \( (\varepsilon > 0) \), just as we did in Section 2 for a given cascade \( C = (n, \alpha_1, \ldots, \alpha_n, \beta_1, \ldots, \beta_n) \) and any input \( R \), and leak factor \( \ell > 0 \). We obtain:

\[
\hat{G}(s) = \frac{1}{s + \ell} \frac{\alpha_1 \cdots \alpha_n}{(s + \beta_1) \cdots (s + \beta_n) - \varepsilon \alpha_2 \cdots \alpha_n}.
\]  

(24)

The infinity norm is again obtained for the case \( \omega = 0 \) \( (s = j\omega) \) (see below, at the end of this Section):

\[
\|\hat{G}\|_{\infty} = \frac{1}{\ell} \frac{\alpha_1 \cdots \alpha_n}{\beta_1 \cdots \beta_n - \varepsilon \alpha_2 \cdots \alpha_n}.
\]

(25)

Computing the signaling time \( (\tau) \), and the signal duration \( (\sigma) \) and amplitude \( (A) \), we have

\[
\tau_{fb}(C, \ell, R) = \frac{1}{\ell} + \frac{\beta_1 \cdots \beta_n}{\beta_1 \cdots \beta_n - \varepsilon \alpha_2 \cdots \alpha_n} + \left. \frac{d \ln \hat{R}}{ds} \right|_{s=0}
\]

(25)

\[
\sigma_{fb}(C, \ell, R) = \sqrt{\frac{1}{\ell^2} + \frac{(\beta_1 \cdots \beta_n)^2}{(\beta_1 \cdots \beta_n - \varepsilon \alpha_2 \cdots \alpha_n)^2} + q(R)}.
\]  

(26)
\[
A_{fb}(C, \ell, R) = \frac{1}{\sigma(C, \ell, R)} \frac{\alpha_1 \cdots \alpha_n}{\beta_1 \cdots \beta_n - \varepsilon \alpha_2 \cdots \alpha_n} \|R\|_2,
\]

Comparison of these quantities for the models with and without feedback leads to the following conclusions:

1. the system with feedback exhibits higher internal gain;

2. the system with feedback exhibits larger signaling time and signal duration \(\tau_{fb} > \tau\) and \(\sigma_{fb} > \sigma\).

So, for an arbitrary cascade, the existence of a positive feedback leads to a less sharp output signal: the signal transduction down the cascade takes a longer time, and the output signal has greater duration.

On the other hand, the existence of feedback may be used to great advantage in the design of an optimal cascade: positive feedback (at a constant rate \(\varepsilon\)) allows the cascade to be of shorter length and still have the same maximal amplitude/minimal duration. The results in Theorem 1 are valid just as before, with suitable adjustments to some of the constants. Thus, we now have

\[
\|\hat{G}\|_{\infty} = K \Leftrightarrow \beta_1 \cdots \beta_n = \frac{(\alpha_1 + \varepsilon K \ell)\alpha_2 \cdots \alpha_n}{K \ell},
\]

and now, similarly to the proof in Section 4.1, we set

\[
Q_{fb} = \left(\frac{K \ell}{(\alpha_1 + \varepsilon K \ell)\alpha_2 \cdots \alpha_n}\right)^2,
\]

which leads to the optimal value for \(\beta_i = \beta_{fb}, \ i = 1, \ldots, n\)

\[
\beta_{fb} = \left(\frac{(\alpha_1 + \varepsilon K \ell)\alpha_2 \cdots \alpha_n}{K \ell}\right)^{\frac{1}{n}}.
\]

To find the optimal length of the cascade with feedback, note that

\[
\sigma_0(n, \beta(n)) = n M_{fb}^{\frac{1}{n}}, \quad \text{with} \ M_{fb} = 2 \ln \left(\frac{K \ell}{(\alpha_1 + \varepsilon K \ell)\alpha_2 \cdots \alpha_n}\right)^{\frac{1}{n}}.
\]

Since \(M_{fb} \leq M\), then also \(n_{fb}^*(M_{fb}) \leq n^*(M)\). Therefore, we conclude that, for the cascade with feedback,

3. for each fixed \(n\), the value of the off rates that maximizes \(A\) (minimizes \(\sigma_0\)) over \(C_*(\ell, R)\) is larger, \(\beta_{fb} > \beta\);

4. the length of the cascade that maximizes \(A\) (minimizes \(\sigma_0\)) over \(C_*(\ell, R)\) is smaller, \(n_{fb}^* < n^*\).

These results agree with what would be expected from a signaling pathway: indeed, the existence of positive feedback enhances the activation at each step, so a larger amount of the phosphorylated kinase will be produced; to keep this amount at a “weak” level, the phosphatases should increase their activity. On the other hand, since the amount of phosphorylated kinases increased, a smaller number of steps is required to produce the same signal amplitude as in the cascade with no feedback.
To compute the infinity norm $\|\hat{G}\|_\infty$, we first note that the denominator of $\hat{G}(j\omega)$, which we will denote by $\text{den}(\hat{G}(j\omega))$, satisfies (by the triangle inequality):

$$|\text{den}(\hat{G}(j\omega))| \geq |j\omega + \ell| |j\omega + \beta_1| \cdots |j\omega + \beta_n| - \varepsilon \alpha_2 \cdots \alpha_n.$$ 

Also

$$|j\omega + \beta_n| \cdots |j\omega + \beta_n| = \sqrt{(\omega^2 + \beta_1^2) \cdots (\omega^2 + \beta_n^2)} \geq \beta_1 \cdots \beta_n,$$

for every $\omega \in \mathbb{R}$, where the equality holds if and only if $\omega = 0$. Thus

$$|\text{den}(\hat{G}(j\omega))| \geq \ell |\beta_1 \cdots \beta_n - \varepsilon \alpha_2 \cdots \alpha_n| = \text{den}(\hat{G}(0)) > 0,$$

where the last inequality follows from the assumption $\beta_1 \cdots \beta_n > \varepsilon \alpha_2 \cdots \alpha_n$. Therefore, if the expression $|\text{den}(\hat{G}(j\omega))|$ is minimized at $\omega = 0$, then the function $|\hat{G}(j\omega)|$ is maximized at $\omega = 0$, as we wanted to show.

6 Signal delay

The frequency domain approach for linear systems also provides an answer to certain problems involving delays and stability within a signaling cascade. For instance, if there is delay in transmitting the signal at any step along the cascade, then both the amplitude and the signal duration are not affected. Suppose that, at each step, there is a delay $\delta_i$ in the transmission of the signal. The differential equation becomes

$$\frac{d}{dt} \begin{pmatrix} X_1(t) \\ X_2(t) \\ \vdots \\ X_{n+1}(t) \end{pmatrix} = A \begin{pmatrix} X_1(t-\delta_1) \\ X_2(t-\delta_2) \\ \vdots \\ X_{n+1}(t-\delta_{n+1}) \end{pmatrix} + B R.$$

The Laplace transform of $X_i(t-\delta_i)$ is, from the properties listed in the Appendix, $e^{-s\delta_i} \hat{X}_i$ so that

$$\hat{X}_{i+1} = \frac{\alpha_{i+1}}{s + \beta_{i+1}} e^{-s\delta_i} \hat{X}_i.$$

The transfer function becomes:

$$\hat{G}(s) = \frac{1}{s + \ell} \frac{\alpha_1 \cdots \alpha_n}{(s + \beta_1) \cdots (s + \beta_n)} e^{-s\delta_1} \cdots e^{-s\delta_{n+1}}.$$

But, for an imaginary number $j\omega$, $|e^{-j\omega\delta}| = 1$, so the norm $||\hat{G}||_\infty$ is unchanged; and since $e^{-s\delta_i} = 1$ when evaluated at $s = 0$, the signal duration and amplitude are also unchanged. This is not surprising, because in a linear system, delay simply causes a temporal translation of the signal, by a fixed amount, without affecting amplitudes.
7 Stability of cascades

A signaling pathway is considered *stable* (see [10]) if small and random perturbations (those that do not consist of biologically relevant inputs) are not amplified. So, in the presence of small perturbations, the amount of phosphorylated kinases should not be allowed to grow very large, and should return to the stable state, with \( X_i \approx 0 \), for all \( i = 1, \ldots, n \). Thus, the behavior of a signaling pathway in the absence of a relevant input always satisfies expression (3), that is, \( X_i \ll X_{\text{tot},i} \) for each \( i = 1, \ldots, n \), and hence its stability may be established by analysis of the model (5).

In the absence of an input \( (R(t) \equiv 0) \), the point \( (X_1, X_2, \ldots, X_{n+1}) = (0, 0, \ldots, 0) := 0 \) is an equilibrium point of system (5), and the stability of this equilibrium determines the stability of the pathway. The equilibrium point \( 0 \) is stable if all the eigenvalues of the matrix \( A \) have negative real parts. This is indeed the case for the system described by equations (5). We know that, after a perturbation, the system will always return to \( 0 \). Moreover, we can estimate that a small perturbation will also generate a small response, since:

\[
\|Y_{\text{pert}}\|_2 \leq \kappa \|R_{\text{pert}}\|_2,
\]

where \( \kappa \) is a constant, equal to \( \|\hat{G}\|_\infty \).

For signaling cascades which exhibit a lower degree of kinase specificity, the problem of stability of the cascade (see [10]) becomes significant. If a kinase \( X_i \) affects both the downstream kinases and some upstream kinase, then the eigenvalues of \( A \) change, and stability is not guaranteed. Allowing for kinase non-specificity, a resulting matrix \( A \) could be of the form:

\[
A_\varepsilon = \begin{pmatrix}
-\beta_1 & \varepsilon_{12} & 0 & \cdots & 0 & \varepsilon_{1n} & 0 \\
\alpha_2 & -\beta_2 & 0 & \cdots & 0 & 0 & 0 \\
\varepsilon & \alpha_3 & -\beta_3 & \cdots & 0 & 0 & 0 \\
\varepsilon & \varepsilon & \alpha_4 & \cdots & 0 & 0 & 0 \\
\vdots & \vdots & \vdots & \ddots & \vdots & \vdots & \vdots \\
\varepsilon & \varepsilon & \varepsilon & \cdots & \alpha_n & -\beta_n & 0 \\
0 & 0 & 0 & \cdots & 0 & 1 & \ell
\end{pmatrix},
\]

and the signaling pathway is stable if all the eigenvalues of \( A_\varepsilon \) have negative real parts.

Some relevant easy-to-compute examples are:

- suppose that each kinase \( i \) is only allowed to activate its downstream kinases (\( \varepsilon_{1n} = 0, \varepsilon_{12} = 0 \) and \( \varepsilon \neq 0 \)); in this case it is not surprising that stability is not affected at all, because this situation corresponds to a lower triangular matrix, again with eigenvalues \(-\beta_i \);

- suppose that there exists feedback from the last activated kinase to the first kinase (\( \varepsilon_{1n} = \varepsilon_0, \varepsilon_{12} = 0 \) and \( \varepsilon = 0 \)); in this case, if \( \varepsilon_0 \) satisfies

\[
\beta_1 \cdots \beta_n > \varepsilon_0 \alpha_2 \cdots \alpha_n \quad \Leftrightarrow \quad \varepsilon_0 < \frac{\alpha_2 \cdots \alpha_n}{\beta_1 \cdots \beta_n},
\]

then the eigenvalues of the matrix \( A_\varepsilon \) all have negative real parts and the new cascade is stable. To prove this, suppose that there exists an eigenvalue of \( A_\varepsilon \) with positive real part, that is, a complex number \( \lambda \) such that

\[
\lambda = \lambda_re + j\lambda_im, \quad \lambda_re \geq 0,
\]

(28)
and

\[ \det(A - \lambda I) = (-1)^n \ell[(\lambda + \beta_1) \cdots (\lambda + \beta_n) - \varepsilon_0 \alpha_2 \cdots \alpha_n] = 0. \] (29)

Then (28) implies \(|\lambda + \beta_i| \geq \beta_i\), for \(i = 1, \ldots, n\) and so

\[
\begin{align*}
|\lambda + \beta_1| \cdots (\lambda + \beta_n) - \varepsilon_0 \alpha_2 \cdots \alpha_n | & \geq |(\lambda + \beta_1) \cdots (\lambda + \beta_n)| - \varepsilon_0 \alpha_2 \cdots \alpha_n \\
& \geq \beta_1 \cdots \beta_n - \varepsilon_0 \alpha_2 \cdots \alpha_n > 0,
\end{align*}
\]

which contradicts equation (29).

8 Conclusions

By modeling weakly activated signal transduction cascades as linear systems and applying techniques from control systems theory one can identify the cascade’s input-to-output transfer function and internal gain. Based on these properties, the concepts of signal duration, signaling time and signal amplitude may be defined in an intuitive and general form, for any input signal.

Our analysis shows that, for linear cascades, signal amplitude and duration are, respectively, maximized and minimized simultaneously. So, a cascade can respond with signals that are both fast and exhibit high amplification. To achieve the highest amplification and the shortest duration response, the cascade should have all off rates equal to some value \(\beta\).

We also show that, for each fixed internal gain, there are finite values for the length of the cascade and the off constants that simultaneously maximize (resp., minimize) the signal amplitude (resp., signal duration). To achieve these optimal conditions, the optimal length should be given by the well defined step function \(\Psi\). This function \(\Psi\) depends only on, and increases logarithmically with, the internal gain of the system. The off constants should all have the same value \(\beta\). This optimal value \(\beta\) depends on the internal gain and the length of the system.

In addition, our analysis shows that a positive feedback term on the cascade enhances the optimal design, by allowing the same signal amplitude and duration to be achieved with a shorter length and higher off rates.

Finally, other issues, such as delay at each phosphorylation step, and the stability of the signaling pathway when there is a high degree of non-specificity among the kinases, are also naturally examined within this framework. The stability of the zero steady-state of the cascade with respect to small perturbations is established by checking that the eigenvalues of the matrix \(A\) all have negative real parts.

A Properties of function \(f(k)\)

The function \(f : (1, \infty) \rightarrow (0, \infty)\)

\[ f(k) = k^2 \left[ \left(1 + \frac{1}{k}\right) \ln \left(1 + \frac{1}{k}\right) - \frac{1}{k} \right] \]

has the following properties:

1. \(f\) is strictly increasing;
2. \(f(1) = 2 \ln 2 - 1 \approx 0.386\) and \(\lim_{k \to \infty} f(k) = 1/2\).
To prove property 1, notice that another expression for \( f \) is \( f(k) = k[(k + 1) \ln(k+1)/k - 1] \), and compute the first and second derivatives:

\[
\frac{df}{dk} = (2k + 1) \ln\frac{k+1}{k} - 2
\]
\[
\frac{d^2 f}{dk^2} = (2k + 1) \ln\frac{k+1}{k} + \frac{2k + 1}{k(k+1)}.
\]

It is clear that the second derivative is always positive, and hence the first derivative is strictly increasing. Since \( df/dk(1) = 3 \ln 2 - 1 > 0 \), it follows that the first derivative is also always positive and therefore the function \( f \) is strictly increasing.

To prove property 2, the value \( f(1) \) is straightforward, and for the limit as \( k \to \infty \), it is easier to consider \( x = 1/k \) and compute:

\[
\lim_{k \to \infty} f(k) = \lim_{x \to 0} f(1/x) = \lim_{x \to 0} \frac{(1 + x) \ln(1 + x) - x}{x^2} = 0.
\]

This indeterminacy can be solved by twice applying L'Hôpital's rule:

...first time: \( \frac{\ln(1 + x) + (1 + x)\frac{1}{1+x} - 1}{2x} \to 0 \), as \( x \to 0 \)

...second time: \( \frac{1}{2} \to \frac{1}{2} \), as \( x \to 0 \).

![Figure 7: The function \( f(k) \).](image)

**B Minimization of \( \sigma_0 \)**

Let \( Q \) be a positive real number and \( n > 2 \) an integer. Consider the function \( F : (0, \infty)^{n-1} \to (0, \infty) \) given by

\[
F(B_1, \ldots, B_{n-1}) = B_1 + \cdots + B_{n-1} + \frac{Q}{B_1 \cdots B_{n-1}}.
\]

**Lemma B.1** The choice of \( B_i > 0, i = 1, \ldots, n-1 \) that minimizes the function \( F \) is: \( B_i = Q^{1/n}, i = 1, \ldots, n-1 \).
Proof. First, we claim that the search for a point \((B_1, \ldots, B_{n-1})\) where \(F\) is minimized can be constrained to the compact set:

\[
\left[ \frac{1}{n^{n-1}} Q^{1/n}, \ n Q^{1/n} \right]^{n-1}.
\] (30)

To justify the upper bound of the interval, observe that

\[
F(Q^{1/n}, \ldots, Q^{1/n}) = n Q^{1/n}
\] (31)

and that, for any \(j = 1, \ldots, n-1,\)

\[
B_j \geq n Q^{1/n} \Rightarrow F(B_1, \ldots, B_{n-1}) > n Q^{1/n}.
\] (32)

So, it is enough to look for a minimum of \(F\) in the region \(B_i < n Q^{1/n}, i = 1, \ldots, n-1\) (because inside this region there is at least one point – equation (31) – where \(F\) has a lower value than anywhere outside of this region).

To justify the lower bound, suppose that \(B_j \leq \frac{1}{n^{n-1}} Q^{1/n},\) for some \(j = 1, \ldots, n-1.\) Then using the already established upper bounds

\[
F(B_1, \ldots, B_{n-1}) > \frac{Q}{B_1 \cdots B_j \cdots B_{n-1}} \geq \frac{1}{n^{n-1} Q^{1/n}} = n Q^{1/n},
\] (33)

and similarly we conclude that it is enough to look for a minimum of \(F\) in the region \(B_i > \frac{1}{n^{n-1}} Q^{1/n},\) for \(i = 1, \ldots, n-1.\)

The function \(F\) is continuous, in fact differentiable, in the compact set (30), and so \(F\) has (absolute) maximum and minimum values in this set. The maximum and minimum may be attained either at a critical point of \(F,\) or at the boundary points of (30). Equations (32) and (33) show that the minimum is not attained at any of the boundary points. So the minimum will be attained at an interior point of the set (30), which must also be a critical point of \(F.\) The critical points of \(F\) are given by:

\[
\frac{dF}{dB_j} = 0 \iff 1 - \frac{1}{B_j B_1 \cdots B_{n-1}} Q = 0, \ j = 1, \ldots, n-1,
\]

or, equivalently,

\[
B_j = \frac{Q}{B_1 \cdots B_{n-1}} = B_s, \ j = 1, \ldots, n-1,
\]

where \(B_s\) satisfies

\[
1 - \frac{1}{B_s B_{n-1}^{n-1}} = 0 \iff B_s = Q^{1/n}.
\]

Thus, there exists a unique critical point of \(F, (B_s, \ldots, B_s),\) which indeed belongs to the compact set (30). By the discussion above, this point must be the minimizer of \(F,\) as we wanted to show. \(\blacksquare\)
C Minimization of $F(n, M)$

For a fixed $M$, let the minimizer of $F(n, M)$ over $n \in \mathbb{N}$ be

$$n^*(M) := \{ n \in \mathbb{N} : F(n, M) \leq F(n', M), \text{ for every } n' \in \mathbb{N} \},$$

Lemma C.1 Let $M$ be any fixed real number. Then $n^*(M) = \Psi(M)$.

Proof. Since $n^*(M)$ is the minimizer of $F(n, M)$ over the (positive) natural numbers, we start by computing the derivative of $F(n, M)$:

$$\frac{dF}{dn}(n, M) = \frac{1}{n} - \frac{1}{n^2}M = \frac{1}{n^2}[n - M].$$

We consider two distinct cases:

- Case $M \leq 0$

  $$\frac{dF}{dn}(n, M) > 0, \text{ for all } n \geq 1,$$

  so $F(\cdot, M)$ is a strictly increasing function and thus its minimizer over $\mathbb{N}$ is the smallest natural number, i.e., $n^*(M) = 1$.

- Case $M > 0$

  $$\frac{dF}{dn}(n, M) = 0 \iff n = M,$$

  and the derivative is negative for $n < M$ and positive for $n > M$: in other words, the function $F$ has indeed a minimum at $n = M$. However, in general, $M$ is not an integer, so it cannot be a solution to our minimization problem. We should choose

$$n^*(M) = \begin{cases} 1, & M \leq 1 \\ \lfloor M \rfloor, & M > 1, \text{ and } F(\lfloor M \rfloor, M) \geq F(\lceil M \rceil, M) \\ \lceil M \rceil, & M > 1, \text{ and } F(\lfloor M \rfloor, M) < F(\lceil M \rceil, M). \end{cases}$$

Note that we pick $n^* = 1$ whenever $M \leq 1$, since a cascade of length zero is meaningless.

To further analyze this condition, observe that we can write, for $M > 1$,

$$M = k + \delta, \quad \lfloor M \rfloor = k, \quad \lceil M \rceil = k + 1$$

where $k \geq 1$ is the integral part of $M$ and $\delta \in [0, 1)$ is the fractional part of $M$. Now, the point $\delta$ for which $n^*$ “jumps” from $\lfloor M \rfloor$ to $\lceil M \rceil$ can be found by setting

$$0 = F(\lfloor M \rfloor, M) - F(\lfloor M \rfloor, M) = F(k + 1, k + \delta) - F(k, k + \delta)$$

$$= \ln(k + 1) + \frac{1}{k + 1}(k + \delta) - \ln k - \frac{1}{k}(k + \delta).$$

Simplifying this equation we obtain:

$$\ln \frac{k + 1}{k} - \frac{k + \delta}{k(k + 1)} = 0 \iff \delta = k(k + 1) \ln \frac{k + 1}{k} - k$$

$$\iff \delta = k^2 \left[ \frac{k + 1}{k} \ln \frac{k + 1}{k} - \frac{1}{k} \right]$$

$$\iff \delta = k^2 \left[ \left( 1 + \frac{1}{k} \right) \ln \left( 1 + \frac{1}{k} \right) - \frac{1}{k} \right] = f(k).$$
Analysis of this function (see Appendix A), shows that \( f \) is positive and strictly increasing, so we have

\[
F([M], M) - F([M], M) \geq 0 \iff f([M]) - \delta \geq 0.
\]

Therefore, we should choose

\[
n^*(M) = \begin{cases} 
1, & M \leq 1 \\
[M], & M > 1, \text{ and } \delta \leq f([M]) \\
[\lceil M \rceil], & M > 1, \text{ and } \delta > f([M]). 
\end{cases}
\]

This proves the Lemma.

\[\blacksquare\]

D  Dictionary: Laplace transforms and transfer functions

For further details about these topics see, for instance, [5] and [2], [8], [18].

Laplace transforms

For a function \( X : (-\infty, \infty) \to \mathbb{R}^n \) (with \( |X(t)| \leq ce^{kt} \), for all \( t \), for some positive constants \( c \), \( k \)), the Laplace transform is another function \( \tilde{X} : \mathcal{R} \to \mathbb{C}^n \) defined as

\[
\tilde{X}(s) := \int_{-\infty}^{\infty} e^{-st}X(t)dt
\]

where \( \mathcal{R} \subset \mathbb{C} \) is the region of convergence of the integral. For example, if \( X(t) = e^{-3t} \), for \( t \geq 0 \) and \( X(t) = 0 \) otherwise, then \( \tilde{X}(s) = 1/(s+3) \), and \( \mathcal{R} = \{ s = s_{re} + js_{im} : s_{re} > -3 \} \) (\( j \) is the imaginary number \( \sqrt{-1} \)).

Some of its properties are:

1. For any constant matrix \( A \in \mathbb{R}^{n \times n} \)

\[
\tilde{A\tilde{X}}(s) = A \tilde{X}(s);
\]

2. The Laplace transform of the derivative of \( X \) is

\[
\frac{d\tilde{X}}{dt}(s) = X(0) + s \int_{-\infty}^{\infty} e^{-st}X(t)dt = X(0) + s \tilde{X}(s);
\]

3. If \( X(t+\delta) =: W(t) \) is a translation of \( X \), then

\[
\tilde{W}(s) = e^{-s\delta} \tilde{X}(s);
\]

4. The inverse Laplace transform is

\[
X(t) = \frac{1}{2\pi j} \int_{s_{re}-j\infty}^{s_{re}+j\infty} e^{st} \tilde{X}(s)ds
\]

with \( s = s_{re} + js_{im} \), where \( s_{re} \) is chosen so that \( s_{re} + js_{im} \) is in the region of convergence \( \mathcal{R} \).
**Transfer function**

Let $A \in \mathbb{R}^{n \times n}$, $B \in \mathbb{R}^{n \times m}$ and $C \in \mathbb{R}^{p \times n}$ be matrices, and let $X \in \mathbb{R}^{n}$, $Y \in \mathbb{R}^{m}$, $R \in \mathbb{R}^{p}$, and consider the $n$-dimensional linear system with $m$ inputs and $p$ outputs:

\[
\begin{align*}
\frac{dX}{dt} &= AX + BR, \\
Y &= CX.
\end{align*}
\]

Applying the Laplace transform operator on both sides of the linear system (34)-(35) yields an algebraic equation relating the new functions $\hat{X}(s)$, $\hat{Y}(s)$ and $\hat{R}(s)$:

\[
\begin{align*}
s\hat{X}(s) &= A\hat{X}(s) + B\hat{R}(s) \\
\hat{Y}(s) &= C\hat{X}(s).
\end{align*}
\]

Moreover, for every $s$ for which the matrix $sI - A$ is invertible ($I$ is the identity matrix),

\[(sI - A) \hat{X}(s) = B\hat{R}(s) \Rightarrow \hat{X}(s) = (sI - A)^{-1}B\hat{R}(s)\]

and thus, one can solve immediately for the output

\[
\hat{Y}(s) = C(sI - A)^{-1}B\hat{R}(s).
\]

The transfer function of the system 331 is

\[
\hat{G}(s) := C(sI - A)^{-1}B,
\]

and *depends only on the internal structure of the system* (i.e., $A$, $B$ and $C$).

**Impulse response**

A useful case is that of the impulse response:

\[
R(t) = \delta(t), \quad \Rightarrow \quad \hat{R}(s) \equiv 1
\]

and therefore:

\[
\hat{Y}(s) \equiv \hat{G}(s) \quad \Leftrightarrow \quad Y(t) \equiv G(t),
\]

so that the transfer function of the system is the output corresponding to a single pulse of input.

**The gain $\|\hat{G}\|_\infty$**

We have

\[
\|\hat{Y}\|^2 = \frac{1}{2\pi} \int_{-\infty}^{\infty} |\hat{G}(j\omega)\hat{R}(j\omega)|^2 d\omega \leq \frac{1}{2\pi} \sup_{\omega} |\hat{G}(j\omega)|^2 \int_{-\infty}^{\infty} |\hat{R}(j\omega)|^2 d\omega
\]

which is equivalent to

\[
\|\hat{Y}\|_2 \leq \|\hat{G}\|_\infty \|\hat{R}\|_2 \quad \Leftrightarrow \quad \|Y\|_2 \leq \|\hat{G}\|_\infty \|R\|_2.
\]
So, the infinity norm of the transfer function is an upper bound on the strength of the output.

To see that it is indeed the least upper bound, see for instance [8]: we can always choose a frequency $\omega_0$ so that

$$\|\hat{G}\|_\infty = |\hat{G}(j\omega_0)|.$$  

In our case, this is $\omega_0 = 0$. Then choose a control such that

$$|\hat{R}(j\omega)| = \begin{cases} r, & \text{if } |\omega| < \varepsilon \\ 0, & \text{otherwise,} \end{cases}$$  \hspace{1cm} (37)$$

where $\varepsilon > 0$ and $r$ should be such that $\hat{R}$ has unit 2-norm, for instance $r = \sqrt{\frac{\pi}{\varepsilon}}$. For very small $\varepsilon > 0$, $|\hat{R}(j\omega)|$ is zero, except on a very small neighborhood of $\omega_0 = 0$ and we may approximate:

$$\frac{1}{2\pi} \int_{-\infty}^{\infty} |\hat{G}(j\omega)|^2 |\hat{R}(j\omega)|^2 \, d\omega \approx \frac{1}{2\pi} \int_{-\varepsilon}^{\varepsilon} r^2 |\hat{G}(j\omega_0)|^2 \, d\omega$$

$$= \frac{1}{2\pi} |\hat{G}(j\omega_0)|^2 \int_{-\varepsilon}^{\varepsilon} r^2 \, d\omega$$

$$= \|\hat{G}\|^2_\infty$$

where the last equality follows from the definitions of $\omega_0$ and $r$. Therefore

$$\|Y\|_2 = \left[ \frac{1}{2\pi} \int_{-\infty}^{\infty} |\hat{G}(j\omega)|^2 |\hat{R}(j\omega)|^2 \, d\omega \right]^{\frac{1}{2}} \approx \|\hat{G}\|_\infty.$$  

As an example of an input that (approximately) satisfies (37), consider $R(t) = 2\frac{\pi}{\varepsilon} \sin \varepsilon t$ (for $t \geq 0$), the input plotted in Figure 3. 

Computation of the Laplace transform yields $\hat{R}(s) = \frac{r}{\pi} \left[ \pi - \text{Arctan} \left( \frac{s}{\varepsilon} \right) \right]$, where the function Arctan is the principal branch of the complex inverse tangent function. It can be shown that, for sufficiently small $\varepsilon$, the function $\hat{R}$ approximately satisfies condition (37), except at the discontinuity points $\omega = \pm \varepsilon$.

More generally, in a system with $m$ inputs and $p$ outputs, one defines the internal gain of the system $\|\hat{G}\|_\infty$ as the induced $L^2$ operator norm of the map from the inputs to the outputs. 

It is possible to prove that

$$\|\hat{G}\|_\infty = \sup_{\omega \in \mathbb{R}} \bar{\theta}(\hat{G}(j\omega))$$

where $\bar{\theta}$ denotes the largest singular value of the matrix $\hat{G}(j\omega)$.

**Stability of the transfer function**

As remarked above, expression (36) is valid if and only if the matrix $sI - A$ is invertible, or equivalently

$$s \neq \lambda, \quad \text{for every eigenvalue, } \lambda, \text{ of } A.$$  

If $\lambda_m = \max\{\text{Re}(\lambda) : \lambda \text{ is an eigenvalue of } A\}$, then the region of definition of the transfer function is included in the set $\mathcal{R} = \{s = s_{re} + js_{im} : s_{re} > \lambda_m\}$.

If all the eigenvalues of the matrix $A$ have negative real parts, then the transfer function is said to be stable. This is case for the matrix of the signaling cascade (5), whose eigenvalues are: $-\beta_1, \ldots, -\beta_n$, so the transfer function $\hat{G}(s)$ is stable and well defined on $\mathcal{R} = \{s = s_{re} + js_{im} : s_{re} > -\min \beta_i\}$. 25
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