

Gains and Optimal Design in Signaling Pathways

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Abstract—Signal amplification and other input-to-output properties of signaling cascades are characterized by the H_∞ gain in the case of linear systems, or by the \mathcal{L}^2 induced norm in the nonlinear case. The effect of the length of the cascade on the output signal is examined. In particular, for a fixed gain, there is an optimal length that generates “sharp” outputs (signals that simultaneously exhibit short duration and high amplitude).

I. INTRODUCTION

The biochemical pathways known as mitogen-activated protein kinase (MAPK) signaling cascades are fundamental mechanisms of cellular signal transduction. Protein kinase cascades are involved in many regulatory processes, including cell cycle regulation, gene expression, and T cell activation. For this reason, the control of kinase cascades by therapeutic intervention has become an attractive area for drug discovery, particularly in the areas of cancer and inflammation [6], [7], [10].

The dynamics of MAPK cascades has been modeled and analysed numerically in several approaches ([2], [3], [4], [8], [9]). In this paper, based on the notion of H_∞ gain of a system, we focus upon the dependence of MAPK cascade signal amplification on the number of kinases in a cascade and the phosphorylation/dephosphorylation rates.

Let R denote the input signal, \tilde{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 β_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 [8]):

$$\frac{dX_1}{dt} = \tilde{\alpha}_1 R \tilde{X}_1 - \beta_1 X_1, \quad \frac{dX_i}{dt} = \tilde{\alpha}_i X_{i-1} \tilde{X}_i - \beta_i X_i, \quad (1)$$

for $i = 2, \dots, n$. Assuming that the total amount of kinase i remains constant, that is, $\tilde{X}_i + X_i = X_{\text{tot},i}$ the differential equations (1) can be rewritten as

$$\begin{aligned} \frac{dX_1}{dt} &= \alpha_1 R \left(1 - \frac{X_1}{X_{\text{tot},1}}\right) - \beta_1 X_1, \\ \frac{dX_i}{dt} &= \alpha_i X_{i-1} \left(1 - \frac{X_i}{X_{\text{tot},i}}\right) - \beta_i X_i, \end{aligned} \quad (2)$$

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for $i = 2, \dots, n$, where $\alpha_i = \tilde{\alpha}_i X_{\text{tot},i}$. For *weakly activated pathways*, there is a low level of kinase phosphorylation, and

$$X_i \ll X_{\text{tot},i} \Rightarrow 1 - \frac{X_i}{X_{\text{tot},i}} \approx 1. \quad (3)$$

In this case the equations (2) are simplified to a linear system of the form:

$$\frac{dX_1}{dt} = \alpha_1 R - \beta_1 X_1, \quad \frac{dX_i}{dt} = \alpha_i X_{i-1} - \beta_i X_i, \quad (4)$$

for $i = 2, \dots, n$. Section II includes remarks regarding the easily computed transfer function and gain for the linear system, and then in Section III we will define a set of measures for the output signal, which closely follow those discussed in [8]. In Section IV we prove our main result, namely, that the most efficient cascade design, for generating sharp signals, has equal on rates and a finite length depending only on the cascade’s gain. While our results are formulated only for weakly activated pathways, they may be extended to the general (nonlinear) case (2). Indeed, in Section V, we sketch a proof of the fact that the \mathcal{L}^2 induced norm for (2) coincides with the H_∞ gain for (3).

II. TRANSFER FUNCTION AND H_∞ GAIN

We will consider the signaling cascade (4) 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,

$$\frac{dX}{dt}(t) = AX(t) + BR(t), \quad Y(t) = CX(t), \quad (5)$$

where $X = (X_1, X_2, \dots, X_n, X_{n+1})'$ 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

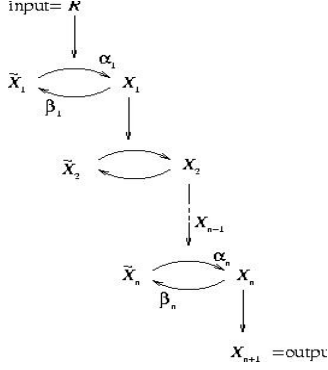


Fig. 1. A model of a MAPK cascade.

matrices

$$\begin{aligned}
 A &= \begin{pmatrix} -\beta_1 & 0 & \cdots & 0 & 0 & 0 \\ \alpha_2 & -\beta_2 & \cdots & 0 & 0 & 0 \\ 0 & \alpha_3 & \cdots & 0 & 0 & 0 \\ \vdots & & \ddots & & & \vdots \\ 0 & 0 & \cdots & \alpha_n & -\beta_n & 0 \\ 0 & 0 & \cdots & 0 & 1 & -\ell \end{pmatrix}, \\
 B &= (\alpha_1 \ 0 \ \cdots \ 0)', \\
 C &= (0 \ \cdots \ 0 \ 1).
 \end{aligned} \tag{6}$$

It is easy to see that the transfer function for the total system is the product of the transfer functions at each step:

$$\hat{G}(s) = \frac{1}{s + \ell} \frac{\alpha_1}{(s + \beta_1)} \cdots \frac{\alpha_n}{(s + \beta_n)}. \tag{7}$$

As usual, the 2-norms of the function Y and its Laplace transform \hat{Y} are given by $\|Y\|_2 := \left[\int_{-\infty}^{+\infty} |Y(t)|^2 dt \right]^{\frac{1}{2}}$ and $\|\hat{Y}\|_2 := \left[\frac{1}{2\pi} \int_{-\infty}^{+\infty} |\hat{Y}(j\omega)|^2 d\omega \right]^{\frac{1}{2}}$. (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 gain of the system is given by

$$\|\hat{G}\|_\infty := \sup_{\omega \in \mathbb{R}} |\hat{G}(j\omega)| = \frac{1}{\ell} \frac{\alpha_1 \cdots \alpha_n}{\beta_1 \cdots \beta_n} \tag{8}$$

and since

$$\|Y\|_2 \leq \|\hat{G}\|_\infty \|R\|_2, \tag{9}$$

a necessary condition for amplification of the signal to occur is that $\|\hat{G}\|_\infty > 1$. Moreover, since ℓ 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

$$\alpha_1 \cdots \alpha_n > \beta_1 \cdots \beta_n. \tag{10}$$

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 $\tilde{\alpha}_i < \beta_i$, $i = 1, \dots, n$, as should be the case for a weakly activated pathway.

Note that, in the case where $\ell = 0$, the gain $\|\hat{G}\|_\infty$ is infinite — meaning that, in at least one step ($X_n \rightarrow X_{n+1}$) 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 still have an estimate for the “strength” of the signal X_n , since $\|X_n\|_2 \leq \frac{\alpha_1 \cdots \alpha_n}{\beta_1 \cdots \beta_n} \|R\|_2$.

III. 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 [8], 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:

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

where it is assumed that $n \in \mathbb{N}$ and α_i, β_i are positive real numbers, for $i = 1, \dots, n$. 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*, τ , and the *output signal duration*, σ , are given by

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

To understand the significance of these definitions, recall the properties of the Laplace transform and compute (with $Y(t) = 0$ for $t \leq 0$): $\hat{Y}(0) = \int_0^\infty Y(t) dt$, $d\hat{Y}/ds(0) = -\int_0^\infty tY(t) dt$, $d^2\hat{Y}/ds^2(0) = \int_0^\infty t^2 Y(t) dt$ and thus we recover expressions (4) and (5) of reference [8]

$$\begin{aligned}
 \tau &= \frac{\int_0^\infty tY(t) dt}{\int_0^\infty Y(t) dt}, \\
 \sigma^2 &= \frac{\int_0^\infty t^2 Y(t) dt}{\int_0^\infty Y(t) dt} - \left(\frac{\int_0^\infty tY(t) dt}{\int_0^\infty Y(t) dt} \right)^2,
 \end{aligned}$$

where τ can be regarded as the expected value (of the time to traverse the pathway), and σ as the corresponding variance.

An estimate of the amplitude of the output signal, as given in equation (6) of reference [8], 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 (9), that takes advantage of the easily computed 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

$$\mathcal{A}(C, \ell, R) := \frac{\|\hat{G}_C\|_\infty}{\sigma(C, \ell, R)} \|R\|_2, \quad (11)$$

where \hat{G}_C is the transfer function (7).

\mathcal{A} may also be regarded as the amplitude of a constant signal of duration σ , but Definition 3.2 differs from the definition of amplitude given in [8] in essentially three points:

1. the meaningful quantity for measuring the amplitude is the area under the curve ($\int Y(t) dt$), 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 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 that $\|\hat{G}\|_\infty$ is the least factor that satisfies the inequality $\|Y\|_2 \leq \kappa \|R\|_2$.

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), but in fact they are valid for any linear system of the form (5). (For example, the case when there is positive feedback from the last to the first kinase is analyzed in [5].) We next explicitly compute these quantities for the special case when A , B and C are of the form (6), and $\ell \neq 0$:

$$\tau(C, \ell, R) = \left. \frac{1}{\ell} + \sum_{i=1}^n \frac{1}{\beta_i} + \frac{d \ln \hat{R}}{ds} \right|_{s=0} \quad (12)$$

$$\sigma(C, \ell, R) = \sqrt{\frac{1}{\ell^2} + \sum_{i=1}^n \frac{1}{\beta_i^2} + q(R)}, \quad (13)$$

where $q(R) = \left. d^2 \ln \hat{R} / ds^2 \right|_{s=0}$, and

$$\mathcal{A}(C, \ell, R) = \frac{\alpha_1 \cdots \alpha_n}{\ell \beta_1 \cdots \beta_n} \frac{\|R\|_2}{\sqrt{\frac{1}{\ell^2} + \sum_{i=1}^n \frac{1}{\beta_i^2} + q(R)}}. \quad (14)$$

In the case $\ell = 0$, the quantities τ , σ and \mathcal{A} may be computed for $Y \equiv X_n$. The expressions are very similar, except that all the terms in ℓ vanish.

IV. CASCADE DESIGN OPTIMIZATION

From the analysis of the quantities τ , σ and \mathcal{A} , defined in Section III, 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 [8] 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 a starting point, we may think of the family of cascades that have the same 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 gain, $\|\hat{G}\|_\infty = K$, find the optimal combination of the on/off rates and the length of the cascade that maximizes the signal amplitude, \mathcal{A} , for any input R .

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

$$\mathbf{C}_{K, \ell} := \{C : \frac{\alpha_1 \cdots \alpha_n}{\ell \beta_1 \cdots \beta_n} = K\},$$

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

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

Then define the function

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

and observe that it satisfies

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

Finally, define the set of cascades that minimize σ_0 over the family $\mathbf{C}_{K, \ell}$:

$$\mathbf{C}_*(\ell, R) := \{C \in \mathbf{C}_{K, \ell} : \sigma_0(n, \beta_1, \dots, \beta_n) \leq \sigma_0(n', \beta'_1, \dots, \beta'_n), \forall C' \in \mathbf{C}_{K, \ell}\}.$$

Our first result states that in fact the sets $\mathbf{C}_*(\ell, R)$ and $\mathbf{C}_{\max}(\ell, R)$ 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, $\mathbf{C}_{\max}(\ell, R) = \mathbf{C}_*(\ell, R)$, for all inputs $R \in \mathcal{U}$ and leak factors $\ell > 0$.

Proof: Fix any $\ell > 0$, and any $R \in \mathcal{U}$. Recall the notation $C = (n, \alpha_1, \dots, \alpha_n, \beta_1, \dots, \beta_n)$. Given any $C, C' \in \mathbf{C}_{K, \ell}$, the following equivalences are immediate from the definitions of σ and \mathcal{A} :

$$\sigma_0(n, \beta_1, \dots, \beta_n) \leq \sigma_0(n', \beta'_1, \dots, \beta'_n) \Leftrightarrow \sigma(C, \ell, R) \leq \sigma(C', \ell, R) \quad (15)$$

$$\sigma(C, \ell, R) \leq \sigma(C', \ell, R) \Leftrightarrow \mathcal{A}(C, \ell, R) \geq \mathcal{A}(C', \ell, R). \quad (16)$$

Combining (15) and (16) proves the Lemma. ■

An immediate conclusion from Lemma 4.1 is that,

$$\begin{aligned} & \text{maximize } \mathcal{A}(C, \ell, R) \text{ over } \mathbf{C}_{K, \ell} \\ \Leftrightarrow & \text{minimize } \sigma_0(n, \beta_1, \dots, \beta_n) \text{ over } \mathbf{C}_{K, \ell}, \end{aligned}$$

so that, for any fixed 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” ($\mathcal{A} \approx \infty$), which is, of course, not biologically viable. A realistic solution to problem (P) does exist, and is stated in Theorem 1.

Since the signal duration depends only on the cascade length and the “off” rates β_i (besides the input term), we expect the “on” rates, α_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 α_i : either (a) all the α_i have an equal, fixed value, α ; or (b) the product of the α_i is known, at some fixed α_P . We will also assume that the “leak” factor ℓ 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 $f : (1, \infty) \rightarrow (0, \infty)$ by

$$f(k) = k^2 \left[\left(1 + \frac{1}{k}\right) \ln \left(1 + \frac{1}{k}\right) - \frac{1}{k} \right]. \quad (17)$$

It is easy to check that this function is strictly increasing and bounded (namely, $2 \ln 2 - 1 \leq f(k) < 1/2$).

For any real number $M \geq 1$, define the “floor” and “ceiling” functions: $\lfloor M \rfloor =$ largest integer less than or equal to M , and $\lceil M \rceil =$ least integer greater than M . Observing that any real number $M \geq 1$, can be written as the sum of its integral and fractional parts: $M = \lfloor M \rfloor + \delta_M$, where $\delta_M \in [0, 1)$, define the function $\Psi : (-\infty, \infty) \rightarrow \mathbb{N}$ (see Figure 2) by

$$\Psi(M) = \begin{cases} 1, & M \leq 1 \\ \lfloor M \rfloor, & M > 1, \text{ and } \delta_M \leq f(\lfloor M \rfloor) \\ \lceil M \rceil, & M > 1, \text{ and } \delta_M > f(\lfloor M \rfloor). \end{cases}$$

This is a step function where the “jump” discontinuity depends on the fractional part of the number M .

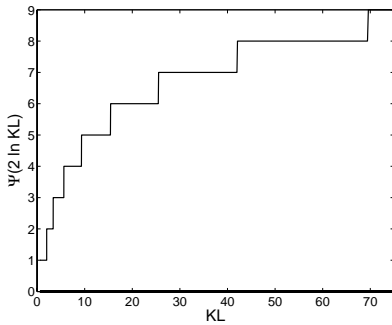


Fig. 2. The function $\Psi(2 \ln K\ell)$. Note that, for a given gain K and leak factor ℓ , the optimal length is given by the integer platform corresponding to the product $K\ell$.

Theorem 1: Let $K > 0$ and $\ell > 0$ be fixed real numbers. Let $\mathbf{C}_{K,\ell}$ be the set of all cascades (5) with gain K , as defined above. Then

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

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

- 2(a). Any element $\mathcal{C} \in \mathbf{C}_*(\ell, R)$ of the form $\mathcal{C} = (n, \alpha, \dots, \alpha, \beta_1, \dots, \beta_n)$ 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 $\mathcal{C} \in \mathbf{C}_*(\ell, R)$ of the form $\mathcal{C} = (n, \alpha_1, \dots, \alpha_n, \beta_1, \dots, \beta_n) \in \mathbf{C}_*(\ell, R)$ with $\alpha_1 \cdots \alpha_n = \alpha_P$ satisfies

$$n = \Psi \left(2 \ln \frac{K\ell}{\alpha_P} \right) \quad \text{and} \quad \beta_i = \beta = \left(\frac{\alpha_P}{K\ell} \right)^{\frac{1}{n}}.$$

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 [8]).

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 [8], and our Lemma 4.1 and Theorem 1 characterize the conditions for achieving this optimization. For each gain K and leak factor ℓ , this optimal length is easily read out from Figure 2. For instance, a cascade with a 6 to 9-fold gain (and $\ell = 1$), is seen to have an optimal length of 4 steps.

Theorem 1 can be proved by successively solving the two optimization problems:

- (P1) For each fixed n , minimize σ_0 , over all possible choices of $\beta_1, \dots, \beta_n \in (0, \infty)$, subject to $\|\hat{G}\|_\infty = K$.
- (P2) Minimize σ_0 , over all possible choices of $n \in \mathbb{N}$ and $\beta_1, \dots, \beta_n \in (0, \infty)$, subject to $\|\hat{G}\|_\infty = K$.

Recall that we are assuming that either (a) all the α_i have an equal, fixed value, α ; or (b) the product of the α_i is known, at some fixed α_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).

A. 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 $\bar{\beta}_1, \dots, \bar{\beta}_n$ for which the function σ_0 attains a minimum value, i.e.,

$$\frac{1}{\bar{\beta}_1^2} + \frac{1}{\bar{\beta}_2^2} + \cdots + \frac{1}{\bar{\beta}_{n-1}^2} \leq \frac{1}{\bar{\beta}_1^2} + \frac{1}{\bar{\beta}_2^2} + \cdots + \frac{1}{\bar{\beta}_n^2}$$

for every β_1, \dots, β_n such that $\|\hat{G}\|_\infty = K$:

$$\|\hat{G}\|_\infty = K \Leftrightarrow K\ell\beta_1 \cdots \beta_n - \alpha_1 \cdots \alpha_n = 0.$$

For simplicity, rescale the values to $B_i = 1/\beta_i^2$, and

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

Then, the problem consists of minimizing the function:

$$F(B_1, \dots, B_{n-1}) = B_1 + \cdots + B_{n-1} + \frac{Q}{B_1 \cdots B_{n-1}} \quad (18)$$

over all possible choices of $B_i > 0$, $i = 1, \dots, n-1$, where

$Q = \left(\frac{K\ell}{\alpha_1 \cdots \alpha_n} \right)^2$. In [5] we show that the solution to this optimization problem is $B_i = Q^{\frac{1}{n}}$, $i = 1, \dots, n-1$, which also implies $B_n = Q^{\frac{1}{n}}$. So, finally, the choice of the ‘‘off’’ rate constants that minimizes σ_0 is to have $\beta_1 = \beta_2 = \cdots = \beta_n = \bar{\beta}$, with $\bar{\beta} = \frac{1}{\sqrt{B_n}} = \left(\frac{\alpha_1 \cdots \alpha_n}{K\ell} \right)^{\frac{1}{n}}$. ■

B. 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 α (respectively, α_P), suppose that we have found a solution of (P2), i.e., an integer n^* and a set of constants β_i^* , $i = 1, \dots, n^*$ satisfying

$$\sigma_0(n^*, \beta_1^*, \dots, \beta_{n^*}^*) \leq \sigma_0(n, \beta_1, \dots, \beta_n) \quad (19)$$

for any other cascade $\mathcal{C} = (n, \alpha_1, \dots, \alpha_n, \beta_1, \dots, \beta_n)$ with $\alpha_i = \alpha$, $i = 1, \dots, n$ (respectively, $\alpha_1 \cdots \alpha_n = \alpha_P$).

We have already showed that

$$\sigma_0(n^*, \bar{\beta}^*, \dots, \bar{\beta}^*) \leq \sigma_0(n^*, \beta_1^*, \dots, \beta_{n^*}^*) \quad (20)$$

with $\bar{\beta}^* = (\alpha_1 \cdots \alpha_{n^*} / (K\ell))^{\frac{1}{n^*}}$ and we know this choice yields the unique minimum of σ_0 for a fixed length n . So, it follows that the solution of (P2) must also satisfy $\beta_i^* = \bar{\beta}^*$, $i = 1, \dots, n^*$.

This observation allows us to simplify the statement of problem (P2), and look only for solutions where all β_i ’s are equal. Now, from the constraint $\|\hat{G}\|_\infty = K$ we have

$$\text{case 2(a):} \quad \sigma_0(n, \beta(n)) = \frac{1}{\alpha^2} n (K\ell)^{\frac{2}{n}}.$$

$$\text{case 2(b):} \quad \sigma_0(n, \beta(n)) = n \left(\frac{K\ell}{\alpha_P} \right)^{\frac{2}{n}}.$$

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 $n \in \mathbb{N}$, where M is a positive constant with value either $M = 2 \ln K\ell$, for case 2(a); or $M = 2 \ln \frac{K\ell}{\alpha_P}$, for case 2(b).

For a fixed M , the minimizer of $F(n, M)$ over $n \in \mathbb{N}$ is $n^*(M) := \{n \in \mathbb{N} : F(n, M) \leq F(n', M), \forall n' \in \mathbb{N}\}$.

In [5] it is shown that: $n^*(M) = \Psi(M)$. 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 β is given according to part 1. ■

V. THE \mathcal{L}^2 -INDUCED NORM

We analyzed the linearized (‘‘weakly activated’’) form (4) of the original system (2). We now sketch a proof of the fact that the *precise value of the H_∞ gain is obtained in this manner*. In other words, the original nonlinear system (2) has finite induced \mathcal{L}^2 gain, and this gain coincides with that of (4), provided that inputs R are not allowed to take negative values. (This is the case in biological applications, and under such a constraint, nor can states X ever be negative when starting from $X(0) = 0$.) This rather surprising equality of gains follows from arguments involving the concept of monotone i/o system as well as comparison theorems. We state a general theorem for a class of systems which contains our models.

Using ‘‘ u ’’ instead of R for inputs, and x_i for state coordinates, we deal with systems of the following form:

$$\dot{x}(t) = A(x(t))x(t) + B(x(t))u(t), \quad x(0) = 0 \quad (21)$$

where $x(t) \in \mathbb{R}_{\geq 0}^n$ and $u(t) \in \mathbb{R}_{\geq 0}^m$ for all $t \geq 0$, and $A : \mathbb{R}_{\geq 0}^n \rightarrow \mathbb{R}^{n \times n}$, $B : \mathbb{R}_{\geq 0}^n \rightarrow \mathbb{R}^{n \times m}$. We also have an output $y(t) = h(x(t)) = C(x(t))x(t) \in \mathbb{R}^p$, for some integer p , where $C : \mathbb{R}_{\geq 0}^n \rightarrow \mathbb{R}^{p \times n}$. We make several assumptions concerning the matrix functions A , B , and C , as follows.

Stability: The matrix $A(0)$ is Hurwitz, that is, all eigenvalues of $A(0)$ have negative real parts.

Maximization at $\xi = 0$: For each $\xi \in \mathbb{R}_{\geq 0}^n$, $A(\xi) \leq A(0)$, $B(\xi) \leq B(0)$, and $C(\xi) \leq C(0)$, meaning that $A(\xi)_{ij} \leq A(0)_{ij}$ for each i, j and similarly for B, C .

Positivity of system: For each $\xi \in \mathbb{R}_{\geq 0}^n$ and each $i \in \{1, \dots, n\}$ such that $\xi_i = 0$, it holds that: $A(\xi)_{ij} \geq 0$ for all $j \neq i$ and $B(\xi)_{ij} \geq 0$ for all j . Also, for every $\xi \in \mathbb{R}_{\geq 0}^n$, $C_{ij}(\xi) \geq 0$ for all i, j .

Local Lipschitz assumption: The matrix functions $A(\xi)$, $B(\xi)$, and $C(\xi)$ are locally Lipschitz in ξ .

The special ‘‘state dependent linear form’’ form is in itself not very restrictive, as for any affine in controls system $\dot{x} = F(x) + B(x)u$ we can write $F(x) = A(x)x$, provided only that F be a continuously differentiable vector field and $F(0) = 0$. Of course, the difficulty is in satisfying the above assumptions, but the systems considered in this paper do satisfy them. It is also worth pointing out that the assumed structure is preserved under cascading (serial connections), which allows building up larger systems which satisfy our hypotheses, by interconnecting smaller subsystems which do. In addition, an even more general class may be obtained by considering other orders in the state space different from the coordinatewise order, similarly to what is done in [1].

Redefining constants, the systems considered in this paper are as follows, with n arbitrary and $m = 1$: $\dot{x}_1 = \alpha_1 u(c_1 - x_1) - \beta_1 x_1$, $\dot{x}_i = \alpha_i x_{i-1}(c_i - x_i) - \beta_i x_i$, $i = 2, \dots, n$, and output $y = x_n$, and the $\alpha_i, \beta_i, c_i > 0$ for all i (Adding a leaky integrator at the output does not change the conclusions.) We represent this system in the above form using: $A(\xi)_{1,1} = -\beta_1$, $A(\xi)_{i,i-1} = \alpha_i c_i$ for $i = 2, \dots, n$, $A(\xi)_{i,i} = -\alpha_i \xi_{i-1} - \beta_i$ for $i = 2, \dots, n$,

$B(\xi)_{1,1} = \alpha_1 c_1 - \alpha_1 \xi_1$, and the other entries zero. All properties hold for this example. Note that $A(\xi) \leq A(0)$ and $B(\xi) \leq B(0)$, for all $\xi \in \mathbb{R}_{\geq 0}^n$, because $-\alpha_i \xi_i \leq 0$ for all i . The matrix $A(0)$ is lower triangular with negative diagonals, and hence is Hurwitz. Positivity holds as well: if $i = 1$ and ξ is such that $\xi_1 = 0$, then $A(\xi)_{1j} = 0$ for all $j \neq 1$ and $B(\xi)_{11} = \alpha_1 c_1 > 0$; if instead $i > 1$ and ξ is such that $\xi_i = 0$, then $A(\xi)_{ij} = 0$ for all $j \notin \{i-1, i\}$, $A(\xi)_{i,i-1} = \alpha_i c_i > 0$, and $B(\xi)_{i1} = 0$.

Let us write $\mathcal{L}_k^2 = \mathcal{L}^2([0, \infty), \mathbb{R}_{\geq 0}^k)$ for any positive integer k . For any system (21), and any input $u \in \mathcal{L}_p^2$, we define $x = Tu$ as the unique solution of the initial value problem (21). In principle, this solution is only defined on some maximal interval $[0, T)$, where $T > 0$ depends on u , but it turns out that $T = +\infty$ and that x is again square integrable (and nonnegative), so we may view x as an element of \mathcal{L}_n^2 and T as an (nonlinear) operator

$$T : \mathcal{L}_m^2 \rightarrow \mathcal{L}_n^2.$$

We will write $|\cdot|$ for Euclidean norm, and use $\|\cdot\|$ to denote \mathcal{L}^2 norm: $\|u\|^2 = \int_0^\infty |u|^2 dt$. For the operator T , we consider the usual induced operator norm:

$$\|T\| := \sup_{u \neq 0} \frac{\|Tu\|}{\|u\|}.$$

We also consider the linear system

$$\dot{z} = A(0)z + B(0)u, \quad z(0) = 0 \quad (22)$$

with output $v = \ell(z) = C(0)z$, and its associated operator $L : \mathcal{L}_m^2 \rightarrow \mathcal{L}_n^2 : u \mapsto z$. Since $A(0)$ is a Hurwitz matrix, $z(t)$ is defined for all $t \geq 0$, and L indeed maps \mathcal{L}^2 into \mathcal{L}^2 . Furthermore, its induced norm $\|L\|$, the H_∞ gain of the system with output $y = z$, is finite. Moreover, the $\mathcal{L}^2 \rightarrow \mathcal{L}^\infty$ (or “ H_2 ”) induced gain is also finite. Our object of study are the compositions with the output maps, i.e. the input/output operators:

$$T_o : \mathcal{L}_m^2 \rightarrow \mathcal{L}_p^2 : u \mapsto y = C(x)x = C(Tu)Tu$$

$$L_o : \mathcal{L}_m^2 \rightarrow \mathcal{L}_p^2 : u \mapsto v = C(0)z = C(0)Lu$$

and their corresponding induced norms. Our main result in [12] is as follows:

Theorem 2: The norm of T_o is finite, and $\|T_o\| = \|L_o\|$.

The proof is based upon comparison principles for the two systems, and use the theory of monotone i/o systems.

Note that the H_∞ gain is defined, in principle, for arbitrary-valued inputs $u \in \mathcal{L}^2([0, \infty), \mathbb{R}^m)$, not necessarily nonnegative. However, the same H_∞ norm obtains whether using positive or arbitrary controls, for a positive system such as (22): simply decompose any input u as $u^+ - u^-$, where u^+ and u^- are nonnegative and orthogonal, and remark that $L_o u$ is majorized by $L_o v$, where $v := u^+ + u^-$ has the same norm as u . (An alternative is to look at the self-adjoint compact operator $M = L_o^* L_o$, and use spectral theory together with the Krein-Rutman Theorem to show that the norm of L_o (its largest singular value) corresponds to a positive eigenvector of M .)

VI. CONCLUSIONS

The concepts of signal duration, signaling time and signal amplitude may be defined in an intuitive and general form, for any input signal, based on the transfer function and H_∞ gain of the (linear) weakly activated system. The concept of signal amplitude may be further extended to general nonlinear MAPK cascades of the form (2), since the \mathcal{L}^2 induced norm of this system coincides with the H_∞ gain.

Our analysis shows that 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 β .

We also show that, for each fixed gain K , 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 step function Ψ . The off constants should all have the same optimal value β , which depends on the gain and the length of the system.

Finally, other issues, such as delay at each phosphorylation step, the effect of a positive feedback term on the cascade (that enhances the optimal design, as shown in [5]) 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.

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