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ORIGINAL ARTICLE

# Bounds on the Ultrasensitivity of Biochemical Reaction Cascades

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**Abstract**
The ultrasensitivity of a dose response function can be quantifiably defined using the generalized Hill coefficient of the function. We examined an upper bound for the Hill coefficient of the composition of two functions, namely the product of their individual Hill coefficients. We proved that this upper bound holds for compositions of Hill functions, and that there are instances of counterexamples that exist for more general sigmoidal functions. Additionally, we tested computationally other types of sigmoidal functions, such as the logistic and inverse trigonometric functions, and we provided computational evidence that in these cases the inequality also holds. We show that in large generality there is a limit to how ultrasensitive the composition of two functions can be, which has applications to understanding signaling cascades in biochemical reactions.

**Keywords** Ultrasensitivity · Signal transduction · Hill coefficient · Hill function · Biochemical reactions

## 1 Introduction

The human body is a complex system with a large number of cell types working together to carry out different tasks. In some situations, cells need to be decisive in the sense of ignoring a low level of stimulus, while leading to a significant response when given a larger stimulus. For example consider the case of a wound, in which the skin breaks and bleeds. The surrounding cells immediately send signals to other cells in the skin, essentially telling them to divide quickly. This signal is sent in the form of

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a molecule called epidermal growth factor (EGF) which floats in the neighborhood of the wound, and which binds to a membrane bound receptor called epidermal growth factor receptor or EGFR (Dei Tos and Ellis 2005; Jagodzik 2018). Cells that receive sufficient EGF binding to their receptors will begin to quickly divide. On the other hand, unwanted replication can lead to cancer in contexts other than wound healing. In order to prevent such unwanted cell division, the EGFR dose response is such that a small amount of EGF leads to no response, while a slightly larger input EGF concentration leads to a robust response. This behavior is called *ultrasensitivity*.

In the context of EGFR signaling, the molecules downstream of this receptor are modified by phosphorylation (Ferrell and Ha 2014), the transfer of phosphate molecules mediated by an enzyme. Phosphorylation takes place sequentially over a series of molecules, each molecule phosphorylating (and thereby activating) the next. This particular cascade is known as the Mitogen-Activated Protein Kinase cascade (MAPK), and it is a model signaling pathway in the study of cellular communication (Kholodenko 2000). It is believed that the combination of such multiple steps is in large measure what allows the larger ultrasensitivity of the overall response.

In this paper, we study how connecting a cascade of multiple small reactions with moderate ultrasensitivity can result in a single cascade with significantly larger ultrasensitivity. Specifically, we work to establish an upper bound to the extent of ultrasensitivity in a cascade, in terms of the ultrasensitivity of the individual cascade steps. In the context of the MAPK cascade example, each of the three steps constitutes a smaller set of reactions with its own input–output response, and the overall dose response of the system can be broadly understood as the composition of each of these functions. We therefore ask how ultrasensitive can be the composition of moderately ultrasensitive functions.

In the first instance we use Hill functions to describe the input–output behavior of each cascade step. This function can also be used to quantitatively measure the ultrasensitivity of a response through the so-called Hill coefficient, a component of the function referred to as $n$ in the formula below:

$$f(x) = \frac{cx^n}{K + x^n}. \tag{1}$$

In this case $x$ is the input concentration of ligand (Ryerson and Enciso 2014), and $c$ describes the saturation value of the function for large $x$. The constant $K$ modulates the function horizontally, such that when $x^n = K$ the response is 50% of the maximal output.

Given a positive, increasing, saturating function $f(x)$, the (generalized) Hill coefficient is a number assigned to the function that quantifies how suddenly it increases from a low value to a high, saturating value. Functions with this property are ubiquitous in signal transduction as they implement a Boolean decision given a continuous input. In addition, such functions introduce into the system a strong nonlinearity which can be used to create more complex dynamical behaviors such as limit cycles or switches (Chan et al. 2012; Enciso 2007, 2014, 2013; Haney et al. 2010; Koshland 1987; Legewie et al. 2005; Thattai and Van Oudenaarden 2002; Zhang et al. 2013). To define this quantity for a given function $f(x)$, we consider the numbers

$$EC10 = f^{-1}(0.1 f_{max}), \quad EC90 = f^{-1}(0.9 f_{max}),$$

where $f_{max}$ is the saturation value of the function (Hill 1913; Altszyler 2017). They are the effective concentrations of input that lead to 10% and 90% of the response, respectively. These values can be used to find the Hill coefficient the use of the formula derived by Goldbeter and Koshland (1981):

$$H = \frac{\ln 81}{\ln \frac{EC90}{EC10}} \tag{2}$$

Notice that closer the ratio $EC90/EC10$ becomes to one, the higher the ultrasensitivity of the system, and also the larger the expression above. While the value of $H$ can be calculated for any such sigmoidal function, it also holds that $H = n$ in the case of Hill functions (see Lemma 2 below). This generalized Hill coefficient is therefore helpful to quantify the ultrasensitivity of dose responses in larger generality.

The main conjecture we explore in this paper is inspired by work proposed by Ferrell and Ha (2014); Huang and Ferrell (1996). We propose and prove that the Hill coefficient of the composition of two Hill functions $f(x)$, $g(x)$ satisfies the formula

$$H_{f(g(x))} \leq H_{f(x)} H_{g(x)}. \tag{3}$$

The value of this hypothesis, which we call the Ferrell inequality, is that it provides an upper limit for how ultrasensitive a two-step cascade can be, as a function of the individual steps. We establish here the inequality for two arbitrary Hill functions. We explore computationally the generalization of the inequality to three-step Hill function cascades, as well as for two other families of sigmoidal functions, namely inverse trigonometric functions and logistic functions. We study under what conditions this inequality approaches an equality, and we show that the inequality is not true for any two arbitrary sigmoidal functions, by producing a simple counterexample.

In the work (Huang and Ferrell 1996), Huang and Ferrell show a similar result in terms of the sensitivity of a dose response, rather than the Hill coefficient. The *sensitivity* of a function $f(x)$ is defined as $S(x) = f'(x)x/f(x)$. One can consider sensitivity as the percent change in the output based on a percent change in the input $x$. For example, a sensitivity of 3 means that an increase in the parameter $x$ of 1% will result in an increase of the output by 3%. Huang and Ferrell described a result for compositions of multiple functions, namely that $R = r_1 r_2 r_3$, where R is the sensitivity of the cascade, and $r_1$, $r_2$, and $r_3$ are the sensitivities of each level of the cascade. The proof of this result follows immediately from using the chain rule in the definition of the composition. The sensitivity of a function is also used as a measure of ultrasensitivity, but it has some caveats. For instance, the sensitivity of a Hill function has the somewhat unintuitive property that it is highest at $x = 0$. The Hill coefficient, especially for Hill functions, is a widely followed measure of ultrasensitivity, and it is a natural question to explore the inequality in that context. See also the work by

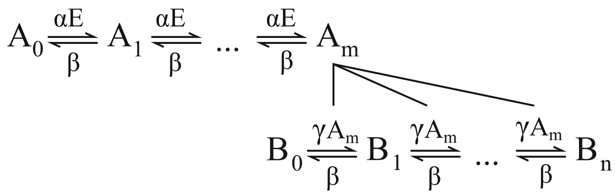


**Fig. 1** Sample biochemical reaction cascade with two steps, input $E$, and output $B_n$

Altszyler (2017), which addresses considerations for the generalized Hill coefficient, but without an inequality estimation such as ours.

## 2 Background

### 2.1 Reaction Cascades

As an example of a reaction cascade, consider the simple system described in Fig. 1. A substrate $A$ is first sequentially modified by an enzyme $E$. The fully modified substrate $A_m$ is the output of the first step in the cascade, and it acts as the input of the second step of the cascade, by sequentially modifying the substrate $B$. The fully modified protein $B_n$ is the output of the second step of the cascade, and also the output of the full reaction system. This is a fairly typical form of reaction cascade, for instance the well known MAP kinase cascade consists of a sequence of proteins, each of which is modified twice before it becomes active.

Regarding the mathematical analysis of the system, we can think of the input and output of each reaction as follows. As we assign dynamical equations to each chemical, we can calculate

$$A_0' = -\alpha A_0 E + \beta A_1.$$

At steady state this means that $A_1 = (\alpha/\beta) E A_0$. For the next protein we calculate

$$A_1' = \alpha A_0 E - \beta A_1 - \alpha A_1 E + \beta A_2.$$

At steady state we use the previous result $0 = -\alpha A_0 E + \beta A_1$, and conclude $0 = -\alpha A_1 E + \beta A_2$, that is, $A_2 = (\alpha/\beta) E A_1$. We can write at steady state $A_k = [(\alpha/\beta)E]^k A_0$, for $k = 1 \cdots m$.

At the same time we have the mass conservation relation $A_t = A_0 + A_1 + \cdots + A_m$, where $A_t$ is the total amount of protein $A$. Setting $x = (\alpha/\beta)E$ to be the (rescaled) enzyme input, we calculate at steady state

$$A_t = A_0(1 + x + \cdots + x^m),$$

and

$$A_0 = \frac{A_t}{1 + x + \cdots + x^m}, \quad A_m = A_t \frac{x^m}{1 + x + \cdots + x^m} =: g(x).$$

In this way the output $A_m$ of the cascade is a function of the input enzyme $E$. We think of the function $A_m = g(x) = g((\alpha/\beta)E)$ as the *dose response* function of the cascade step.

One can similarly calculate the dose response function for the second cascade step, by setting $y = (\gamma/\delta)A_m$. In this case

$$B_m = B_t \frac{y^n}{1 + y + \cdots + y^n} =: f(y).$$

Importantly, the overall dose response of the cascade can be seen as a composition of the two dose response functions:

$$B_m = f(y) = f\left(\frac{\gamma}{\delta} A_m\right) = f\left(\frac{\gamma}{\delta}\, g(x)\right),$$
$$B_m = f\left(\frac{\gamma}{\delta}\, g\left(\frac{\alpha}{\beta}\, E\right)\right).$$

The shape of the individual dose responses will generally be affected by the details in each cascade, for instance in Gunawardena (2005) the reactions are more detailed and include Michaelis–Menten components, but the overall dose response functions are identical when properly annotated.

In what follows below we use instead of the above functions the function $f(x) = cx^n/(K + x^n)$. This simplified function is for instance the dose response function for the system in the classic work by Hill,

$$nO_2 + H \rightleftarrows C,$$

where $n$ molecules of oxygen $O_2$ bind to a molecule of hemoglobin $H$, to form a bound complex $C$ ( Hill (1913), see also Enciso (2013)). Here oxygen acts as input, and bound hemoglobin as output. Hill suggested this system decades before hemoglobin was proved to have multiple oxygen binding sites, and this function is known as Hill function in honor of this example. Of course, this is now one of the most important functions in mathematical biology, and it is in wide use in many other contexts.

The above approach can be carried out in large generality with other reaction cascades. Perhaps the most important assumption here is that the system doesn't have significant *retroactivity*, that is, the output of the first step is not significantly used up or tied up in the reactions of the second step. See for instance (Gyorgy and Del Vecchio 2012; Shah and Del Vecchio 2017) for a more complete discussion of retroactivity in chemical reaction cascades. Another way to think about this is in terms of *sequestration*, that an output protein from one tier should not be significantly sequestered by molecules in lower tiers. We can thus make a general assumption here that such sequestration effect is minimal or negligible in the cascades that we consider.

### 2.2 Logarithmic Sampling

In order to ensure that the trials produced by our algorithm were properly randomized, we utilized logarithmic sampling. Suppose a lower bound and an upper bound are given for a parameter $p$, $0 < a \leq p \leq b$. Since $b$ could be orders of magnitude larger than $a$, we want to ensure that the samples are selected from each order of magnitude with similar likelihood. To do this we choose a number $x$ between $\log_{10} a$ and $\log_{10} b$ using a uniform distribution. The output of the logarithmic sampling algorithm is $p = 10^x$, which must lie between $a$ and $b$.

For instance, suppose that $a = 1$ and $b = 1000$ describe a plausible range for the parameter value of a given biochemical constant. If a number is sampled uniformly from 1 to 1000, most of the numbers sampled will be larger than 100. Using logarithmic sampling, one would choose a number $x$ from 0 to 3, and the sampled number would be $10^x$. In this way, the sample has the same likelihood of being on the intervals [1,10], [10,100], and [100,1000].

## 3 Results

### 3.1 Hill Function Database

We begin with a computational analysis of the Ferrell inequality for Hill functions

$$f(x) = \frac{cx^n}{K + x^n}.$$

We consider two Hill functions, $f(x)$ and $g(x)$, and randomize each of their parameters using logarithmic sampling. The parameters are defined with $n$ from 1 to 10, and each of $c$ and $K$ from 0.1 to 100.

The program randomized the parameters within the defined ranges and tested to see if the Ferrell inequality holds. For each simulation run, in case that either $EC10$ or $EC90$ is not a positive number, then the parameters were discarded and a new simulation was started.

The Hill coefficient for $f(x)$ and $g(x)$ is given by the parameter $n$ as proven by Goldbeter and Koshland. For the composition, the generalized Hill coefficient is calculated by inverting the function, calculating the $EC10$ and $EC90$ algebraically, and applying formula (2). A total of 5000 simulations were performed under the described procedure. The results of this simulation are illustrated in Table 1.

Every case that arose from the 5000 randomized trails in our database was consistent with the Ferrell inequality (3), providing computational evidence that an analytical proof can be pursued in the case of Hill functions.

### 3.2 Proving the Ferrell Inequality for Hill Functions

In this section we provide a rigorous mathematical proof for the Ferrell inequality in the case of two Hill functions. We start this proof by establishing the notation for the

**Table 1** Sample runs of a computational analysis for randomly chosen Hill function cascade steps

| Trial | $f(x)$ | $g(x)$ | $H_f$ | $H_g$ | $H_{f(g)}$ | $H_f \cdot H_g$ |
|---|---|---|---|---|---|---|
| 1 | $\frac{11.7x^{1.9}}{33.0+x^{1.9}}$ | $\frac{6.2x^{2.5}}{4.1+x^{2.5}}$ | 1.9 | 2.5 | 3.2 | 4.7 |
| 2 | $\frac{0.1x^{6.6}}{4.5+x^{6.6}}$ | $\frac{23.6x^{1.2}}{0.1+x^{1.2}}$ | 6.6 | 1.2 | 7.2 | 7.9 |
| 3 | $\frac{39.2x^{1.4}}{0.1+x^{1.4}}$ | $\frac{1.1x^{4.1}}{0.6+x^{4.1}}$ | 1.4 | 4.1 | 5.1 | 5.7 |
| 4 | $\frac{6.0x^{3.5}}{2.9+x^{3.5}}$ | $\frac{1.2x^{9.1}}{1.9+x^{9.1}}$ | 3.5 | 9.1 | 12.4 | 32 |
| 5 | $\frac{14.1x^{1.5}}{5.2+x^{1.5}}$ | $\frac{5.4x^{2.0}}{1.8+x^{2.0}}$ | 1.5 | 2.0 | 2.5 | 3.0 |
| 6 | $\frac{0.3x^{1.1}}{1.0+x^{1.1}}$ | $\frac{0.2x^{3.7}}{0.3+x^{3.7}}$ | 1.1 | 3.7 | 3.8 | 4.1 |

three functions $f(x)$, $g(x)$, and $h(x) = f(g(x))$,

$$f(x) = \frac{c_1 x^n}{K_1 + x^n}, \quad g(x) = \frac{c_2 x^m}{K_2 + x^m}, \quad h(x) = f(g(x)) = \frac{c_1 (\frac{c_2 x^m}{K_2 + x^m})^n}{K_1 + (\frac{c_2 x^m}{K_2 + x^m})^n}. \tag{4}$$

Recall that we want to prove the Ferrell inequality

$$H_{f(g(x))} \leq H_f H_g. \tag{5}$$

We start by proving some preliminary results.

**Lemma 1** *For a Hill function $y = cx^n/(K + x^n)$, the corresponding inverse function has the formula $y^{-1}(x) = \left(\frac{Kx}{c-x}\right)^{\frac{1}{n}}$.*

***Proof*** Beginning with the formula for the function, we can take the following steps to solve for $x$:

$$\begin{aligned}
(K + x^n)y &= cx^n \\
Ky + x^n y &= cx^n \\
Ky &= x^n(c - y) \\
\frac{Ky}{(c - y)} &= x^n \\
x &= \left(\frac{Ky}{c - y}\right)^{\frac{1}{n}}.
\end{aligned}$$

□

**Lemma 2** *For a Hill function $y = cx^n/(K + x^n)$, the corresponding Hill coefficient is $H = n$.*

***Proof*** We calculate using the previous lemma:

$$v = EC90 = y^{-1}(0.9c) = \left(\frac{K0.9c}{0.1c}\right)^{1/n} = (9K)^{1/n}.$$

Similarly $u = EC10 = y^{-1}(0.1c) = (K/9)^{1/n}$. Then

$$H = \frac{\ln 81}{\ln(v/u)} = \frac{\ln 81}{1/n \ln 81} = n.$$

□

**Lemma 3** *Suppose that the Ferrell inequality (5) holds in the special case $c_1 = c_2 = 1$. Then it must also hold in the general case of arbitrary positive $c_1, c_2$.*

***Proof*** Notice first that $c_1$ only re-scales $f(x)$ and $h(x)$ vertically. It does not affect the Hill coefficients involved, since rescaling a function vertically does not change its Hill coefficient.

We now consider the case where both $c_1$ and $c_2$ are arbitrary positive numbers. We calculate the composition

$$h(x) = f(g(x)) = \frac{c_1 g(x)^n}{K_1 + g(x)^n} = \frac{c_1(\frac{c_2 x^m}{K_2+x^m})^n}{K_1 + (\frac{c_2 x^m}{K_2+x^m})^n} = \frac{c_1(\frac{x^m}{K_2+x^m})^n}{\frac{K_1}{c_2^n} + (\frac{x^m}{K_2+x^m})^n}.$$

Notice that the composition function $h(x)$ is identical to that of the case $c_2 = 1$, in which the coefficient $K_1$ has been replaced by $K_1/c_2^n$. Since by assumption the Ferrell inequality holds for $c_2 = 1$, we have $H_h \leq mn$ using Lemma 2. This implies that the inequality $H_h \leq mn$ is also satisfied for the original arbitrary parameters. □

In the rest of this section, we will use Lemma 3 and assume without loss of generality that $c_1 = c_2 = 1$.

In order to prove the inequality, we will compute an explicit formula for the ultrasensitivity $H_h$ of the composition function. We will first find the saturation value of the composition function, then use the inverse of the composition function to solve for the EC10 and EC90 values. We find the ratio of the EC90 to EC10 values, and then insert that ratio into equation 2.

**Lemma 4** *The composition function $h(x)$ has the saturation value $h_{max} = 1/(K_1+1)$.*

***Proof*** For the composition of two Hill functions, the exterior function evaluated at the saturation point of the interior function will produce the maximum $h$ value:

$$h_{max} = \lim_{x\to\infty} h(x) = \lim_{x\to\infty} f(g(x)) = f(\lim_{x\to\infty} g(x)) = f(g_{max}) = f(c_2).$$

Recall we assumed $c_2 = 1$ without loss of generality. Evaluating $f(c_2)$ results in

$$h_{max} = f(1) = \frac{1}{K_1 + 1}.$$

□

Now that we have the saturation value of the composition function $h(x)$, we can solve for the EC10 and EC90 values.

**Lemma 5** *The composition function $h(x)$ has EC10 and EC90 values*

$$EC10_h = \left(\frac{K_2\alpha}{1-\alpha}\right)^{1/m}, \quad EC90_h = \left(\frac{K_2\beta}{1-\beta}\right)^{1/m},$$

*where*

$$\alpha = \left(\frac{K_1}{10K_1+9}\right)^{\frac{1}{n}}, \quad \beta = \left(\frac{9K_1}{10K_1+1}\right)^{\frac{1}{n}}.$$

***Proof*** Starting with the EC90 value, we need to solve for $x$ in the equation

$$f(g(x)) = 0.9h_{max}.$$

Evaluating the inverse functions on both sides we get

$$\begin{aligned} g(x) &= f^{-1}(0.9h_{max}) \\ EC90 = x &= g^{-1}(f^{-1}(0.9h_{max})). \end{aligned}$$

Using the inverse of a Hill function from Lemma 1, we can apply it to the formula above. This yields

$$EC90 = \left(\frac{K_2 f^{-1}(0.9h_{max})}{1-f^{-1}(0.9h_{max})}\right)^{\frac{1}{m}}. \quad (6)$$

We can follow the same process as above to solve for the EC10 value derived from the equation $EC10 = g^{-1}(f^{-1}(0.1y_{max}))$, which results in

$$EC10 = \left(\frac{K_2 f^{-1}(0.1h_{max})}{1-f^{-1}(0.1h_{max})}\right)^{\frac{1}{m}}. \quad (7)$$

In order to further simplify the EC90 and EC10 expressions, we can write $f^{-1}(0.1y_{max})$ and $f^{-1}(0.9y_{max})$ in terms of $K_1$ and $n$, and relabel them as $\alpha$ and $\beta$, respectively:

$$\begin{aligned} f^{-1}(0.1h_{max}) &= \alpha = \left(\frac{\frac{0.1K_1}{K_1+1}}{1-\frac{0.1}{K_1+1}}\right)^{\frac{1}{n}} \\ f^{-1}(0.9h_{max}) &= \beta = \left(\frac{\frac{0.9K_1}{K_1+1}}{1-\frac{0.9}{K_1+1}}\right)^{\frac{1}{n}}. \end{aligned}$$

We can further simplify $\alpha$ and $\beta$:

$$\alpha = \left(\frac{\frac{0.1K_1}{K_1+1}}{1-\frac{0.1}{K_1+1}}\right)^{\frac{1}{n}} = \left(\frac{\frac{0.1K_1}{K_1+1}}{\frac{K_1+1}{K_1+1}-\frac{0.1}{K_1+1}}\right)^{\frac{1}{n}} = \left(\frac{\frac{0.1K_1}{K_1+1}}{\frac{K_1+0.9}{K_1+1}}\right)^{\frac{1}{n}}.$$

Cancelling fractions and further simplifying we get

$$\alpha = \left(\frac{0.1K_1}{K_1+0.9}\right)^{\frac{1}{n}} = \left(\frac{K_1}{10K_1+9}\right)^{\frac{1}{n}}.$$

Following the same process we did for $\alpha$ will allow us to reduce $\beta$ to

$$\beta = \left(\frac{9K_1}{10K_1+1}\right)^{\frac{1}{n}}.$$

□

Notice that $0 < \alpha < \beta < 1$, which will be important below. Now that we have calculated both the EC10 and EC90 values in equations (6), (7) and simplified them in terms of $\alpha$ and $\beta$, we can form the EC90 to EC10 ratio,

$$\frac{EC90}{EC10} = \left(\left(\frac{K_2\beta}{K_2\alpha}\right)\left(\frac{1-\alpha}{1-\beta}\right)\right)^{\frac{1}{m}} = \left(\left(\frac{\beta}{\alpha}\right)\left(\frac{1-\alpha}{1-\beta}\right)\right)^{\frac{1}{m}}.$$

This gives us an explicit formula for the Hill coefficient of the composition using the above expressions for $\alpha$ and $\beta$:

$$H_{f(g(x))} = \frac{\ln 81}{\ln\left(\left(\frac{\beta}{\alpha}\right)\left(\frac{1-\alpha}{1-\beta}\right)\right)^{\frac{1}{m}}}.$$

Now that we have determined the ultrasensitivity for all three functions, $f(x)$, $g(x)$, and $f(g(x))$, we can insert them into our hypothesis, the Ferrell inequality:

$$\frac{\ln 81}{\ln\left(\left(\frac{\beta}{\alpha}\right)\left(\frac{1-\alpha}{1-\beta}\right)\right)^{\frac{1}{m}}} \le mn.$$

In order to simplify this inequality, we can multiply both sides by the natural logarithm expression and apply the properties of logarithms to cancel out $m$,

$$n \ln\left(\left(\frac{\beta}{\alpha}\right)\left(\frac{1-\alpha}{1-\beta}\right)\right) \ge \ln 81$$
$$\leftrightarrow \quad \left(\left(\frac{\beta}{\alpha}\right)\left(\frac{1-\alpha}{1-\beta}\right)\right)^{n} \ge 81$$

$$\leftrightarrow \quad \left(\frac{\beta}{\alpha}\right)^n \left(\frac{1-\alpha}{1-\beta}\right)^n \geq 81. \tag{8}$$

Once again notice that this inequality holds if and only if the Ferrell inequality holds for the given parameter values. In the inequalities above we used the fact that $\beta/\alpha > 1$ and $(1-\alpha)/(1-\beta) > 1$ so that the logarithm in the numerator is positive. Notice also that this expression, which is equivalent to the Ferrell inequality for Hill functions, only depends at this point on the parameters $n$ and $K_1$. Since $\alpha > 0$ and $1-\beta > 0$, this expression is equivalent to

$$\begin{aligned}
&\beta(1-\alpha) \geq 81^{\frac{1}{n}}\alpha(1-\beta)\\
&\leftrightarrow \quad \beta - \beta\alpha \geq 81^{\frac{1}{n}}\alpha - 81^{\frac{1}{n}}\alpha\beta\\
&\leftrightarrow \quad \beta - 81^{\frac{1}{n}}\alpha \geq (1-81^{\frac{1}{n}})\alpha\beta
\end{aligned}$$

$$\leftrightarrow \quad \frac{1}{\alpha} - \frac{81^{\frac{1}{n}}}{\beta} \geq 1 - 81^{\frac{1}{n}}. \tag{9}$$

In order to prove this expression, we first consider the case for $n = 2$:

$$\sqrt{\frac{10K_1+9}{K_1}} - \sqrt{81}\sqrt{\frac{10K_1+1}{9K_1}} \geq 1-9$$

$$\sqrt{10+\frac{9}{K_1}} - \sqrt{90+\frac{9}{K_1}} \geq 1-9$$

We multiply by the conjugate

$$\left(\sqrt{10+\frac{9}{K_1}} + \sqrt{90+\frac{9}{K_1}}\right)\left(\sqrt{10+\frac{9}{K_1}} - \sqrt{90+\frac{9}{K_1}}\right) \geq \left(\sqrt{10+\frac{9}{K_1}} + \sqrt{90+\frac{9}{K_1}}\right)(1-9)$$

$$10-90 \geq \left(\sqrt{10+\frac{9}{K_1}} + \sqrt{90+\frac{9}{K_1}}\right)(-8)$$

$$80 \leq 8\left(\sqrt{10+\frac{9}{K_1}} + \sqrt{90+\frac{9}{K_1}}\right).$$

Since the right hand side is decreasing as a function of $K_1$, it is sufficient to evaluate this inequality as $K_1$ approaches $\infty$,

$$10 \leq \sqrt{10} + \sqrt{90} \approx 12.6.$$

This inequality is satisfied, thus proving the Ferrell inequality for the case $n = 2$.

We now consider the case where $n$ is an arbitrary positive integer. We start by applying the following algebraic identity:

$$(A^{n-1} + A^{n-2}B + \cdots + AB^{n-2} + B^{n-1})(A - B) = A^n - B^n.$$

We define $\Lambda$ as the generalized conjugate expression from the case $n = 2$,

$$\Lambda = \sum_{i=0}^{n-1} \left(\frac{1}{\alpha}\right)^{n-1-i} \left(\frac{81^{\frac{1}{n}}}{\beta}\right)^{i}$$

By multiplying the inequality (9) on both sides by $\Lambda$ we get

$$\begin{aligned} &\left(\frac{1}{\alpha}\right)^{n} - \left(\frac{81^{\frac{1}{n}}}{\beta}\right)^{n} \geq \Lambda(1 - 81^{\frac{1}{n}}) \\ \leftrightarrow \quad &\frac{10K_1 + 9}{K_1} - 81\frac{10K_1 + 1}{9K_1} \geq \Lambda(1 - 81^{\frac{1}{n}}) \\ \leftrightarrow \quad &10 + \frac{9}{K_1} - 90 - \frac{9}{K_1} \geq \Lambda(1 - 81^{\frac{1}{n}}) \end{aligned}$$

We can now cancel out $\frac{9}{K_1}$ from the left hand side and multiply the equation by $-1$, to get the equivalent inequality

$$80 \leq \Lambda(81^{\frac{1}{n}} - 1) \tag{10}$$

Recalling the expression $\Lambda$, we calculate

$$\Lambda = \sum_{i=0}^{n-1} \left(10 + \frac{9}{K_1}\right)^{\frac{n-1-i}{n}} \left(\frac{10K_1 + 1}{9K_1}\right)^{\frac{i}{n}} 81^{\frac{i}{n}} = \sum_{i=0}^{n-1} \left(10 + \frac{9}{K_1}\right)^{\frac{n-1-i}{n}} \left(90 + \frac{9}{K_1}\right)^{\frac{i}{n}}$$

Since $\Lambda$ is a decreasing function of $K_1$, to prove inequality 10, it is sufficient to show it in the case where $K_1$ approaches $\infty$:

$$\begin{aligned} &80 \leq (81^{\frac{1}{n}} - 1) \sum_{i=0}^{n-1} 10^{\frac{n-1-i}{n}} 90^{\frac{i}{n}} \\ \leftrightarrow \quad &80 \leq (81^{\frac{1}{n}} - 1) \sum_{i=0}^{n-1} 10^{\frac{n-1}{n}} 9^{\frac{i}{n}}. \end{aligned}$$

In order to evaluate the series recall that

$$A + AB + AB^2 + \cdots + AB^{n-1} = A\frac{B^n - 1}{B - 1}.$$

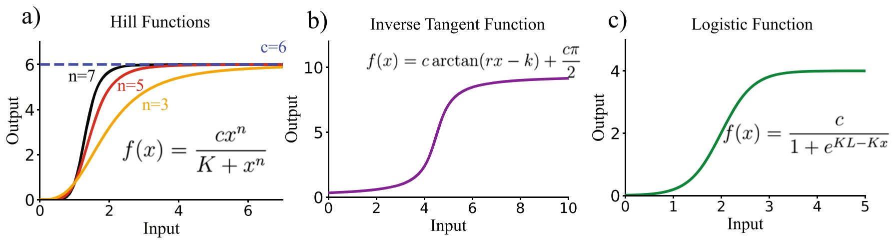


**Fig. 2** **a** Hill function graphs, each with a different value of $n$ to illustrate how the Hill coefficient affects the properties of each function. All functions on the graph were evaluated at $c = 6$ and $K = 7$. **b** The graph of an inverse tangent function evaluated at $c = 3$, $r = 2$, and $K = 9$. **c** The graph of a logistic function evaluated at $c = 4$, $K = 3$, and $L = 2$ (color figure online)

If we evaluate the last inequality by applying the above identity, where $A = 10^{\frac{n-1}{n}}$ and $B = 9^{\frac{1}{n}}$, we get that it is equivalent to

$$\begin{aligned} & 80 \leq (81^{\frac{1}{n}} - 1)10^{\frac{n-1}{n}} \frac{9^{\frac{n}{n}} - 1}{9^{\frac{1}{n}} - 1} \\ \leftrightarrow \quad & 10 \leq (9^{\frac{2}{n}} - 1)10^{\frac{n-1}{n}} \frac{1}{9^{\frac{1}{n}} - 1} \\ \leftrightarrow \quad & 10 \leq 10^{\frac{n-1}{n}} (9^{\frac{1}{n}} + 1) \\ \leftrightarrow \quad & 10^{\frac{1}{n}} \leq 9^{\frac{1}{n}} + 1 \end{aligned}$$

$$\leftrightarrow \quad 1 \leq \sqrt[n]{\frac{9}{10}} + \sqrt[n]{\frac{1}{10}}. \tag{11}$$

Now, notice that

$$\frac{9}{10} \leq \sqrt[n]{\frac{9}{10}}, \quad \frac{1}{10} \leq \sqrt[n]{\frac{1}{10}}.$$

Inequality (11) is shown by adding both of these inequalities,

$$\frac{9}{10} + \frac{1}{10} \leq \sqrt[n]{\frac{9}{10}} + \sqrt[n]{\frac{1}{10}}.$$

Since the truth value of inequality (11) is equivalent to that of the Ferrell inequality, we have proved the following result.

**Theorem 1** *For any positive real values of $c_1, c_2, K_1, K_2$, and any positive integer values of $n, m$, it holds for the general Hill functions $f(x)$, $g(x)$ and for their composition $h(x) = f(g(x))$ that $H_h \leq H_f \cdot H_g$.*

### 3.3 Sharp Behavior of the Inequality

One question regarding the Ferrell inequality for Hill functions is when the inequality approaches an equality, i.e. whether this inequality is conservative or whether in certain limiting cases it becomes an equality

$$H_{f(g(x))} = H_f H_g.$$

We show in this short section that this inequality becomes equality in two cases: for the special case $n = 1$, and as $K_1$ approaches zero.

First, consider the case $n = 1$. In this case $\alpha = K_1/(10K_1+9)$, $\beta = 9K_1/(10K_1+1)$, and a simple calculation shows that

$$1-\alpha = 9\frac{K_1+1}{10K_1+9}, \quad 1-\beta = \frac{K_1+1}{10K_1+1}.$$

Then the left expression in (8) becomes

$$\frac{\beta}{\alpha}\frac{1-\alpha}{1-\beta} = \frac{9K_1}{10K_1+1}\cdot\frac{10K_1+9}{K_1}\cdot 9\frac{K_1+1}{10K_1+9}\cdot\frac{10K_1+1}{K_1+1} = 81.$$

In particular (8) becomes an equality, which means that the Ferrell inequality itself is also an equality.

Next, consider the case as $K_1 \to 0$ for arbitrary $n$. In that case, $\alpha \to 0$, $\beta \to 0$, and

$$\frac{\beta}{\alpha} = \left[\frac{9K_1}{10K_1+1}\cdot\frac{10K_1+9}{K_1}\right]^{1/n} = \left[9\frac{10K_1+9}{10K_1+1}\right]^{1/n} \to 81^{1/n}.$$

Once again the left hand side in (8) can be calculated as

$$\left(\frac{\beta}{\alpha}\right)^n\left(\frac{1-\alpha}{1-\beta}\right)^n \to 81\cdot\frac{1}{1} = 81,$$

and the Ferrell inequality holds as an equality.

One can also ask the question of whether there is a *lower* bound to the inequality, such as in the case where the two functions have a high Hill coefficient. It is easy to see that if the two functions don't match in their ultrasensitive regions, the corresponding composition doesn't need to be ultrasensitive, or even sigmoidal. For this reason, no such lower bound may exist.

### 3.4 Inverse Tangent Function Database

While constructing and analyzing the Hill function database, we recognized that we could computationally test our hypothesis with additional sigmoidal functions. We

**Table 2** Computational simulations for randomly chosen inverse tangent function cascade steps

| $f(x)$ | $g(x)$ | $H_f$ | $H_g$ | $H_{f(g)}$ | $H_f \cdot H_g$ |
|---|---|---|---|---|---|
| $0.4\tan^{-1}(19.4x - 7.6) + \frac{0.4\pi}{2}$ | $0.5\tan^{-1}(5.7x - 9.5) + 0.5\frac{\pi}{2}$ | 5.1 | 6.5 | 24.8 | 33.1 |
| $1.5\tan^{-1}(0.4x - 4.4) + \frac{1.5\pi}{2}$ | $2.7\tan^{-1}(28.1x - 18.5) + \frac{2.7\pi}{2}$ | 2.5 | 13.1 | 15.4 | 32.8 |
| $4.6\tan^{-1}(6.9x - 10.5) + \frac{4.6\pi}{2}$ | $2.5\tan^{-1}(0.2x - 8.7) + \frac{2.5\pi}{2}$ | 7.3 | 6.1 | 26.7 | 44.5 |
| $1.9\tan^{-1}(28.6x - 34.6) + \frac{1.9\pi}{2}$ | $0.5\tan^{-1}(92.7x - 3.4) + \frac{0.5\pi}{2}$ | 24.6 | 1.5 | 22.7 | 36.9 |
| $\tan^{-1}(14.6x - 12) + \frac{\pi}{2}$ | $0.1\tan^{-1}(17.9x - 7.6) + \frac{0.1\pi}{2}$ | 8.4 | 5.1 | 17.4 | 42.8 |
| $1.3\tan^{-1}(0.8x - 4.6) + \frac{1.3\pi}{2}$ | $2.4\tan^{-1}(0.9x - 19.1) + \frac{2.4\pi}{2}$ | 2.7 | 13.6 | 14.9 | 36.7 |
| $2.9\tan^{-1}(0.5x - 6.9) + \frac{2.9\pi}{2}$ | $2.4\tan^{-1}(0.5x - 4.4) + \frac{2.4\pi}{2}$ | 4.6 | 2.5 | 5.2 | 11.5 |

discuss our results using the inverse tangent function,

$$f(x) = c\arctan(rx - K) + \frac{c\pi}{2}.$$

An example of the inverse tangent function can be found in Fig. 1b. The parameters $c$, $r$, and $K$ were randomized for the various trial cases using the logarithmic sampling method described in Sect. 2.1. We analyzed over 5000 trial cases for our hypothesis using inverse tangent functions. The parameters used for the inverse tangent database were from 0.1 to 10 for $r$ and $k$, while the parameter $c$ was any real number from 0.1 to 100. A portion of the database created by our algorithm is shown below in Table 2 for illustrative purposes. The results of the simulation indicate that all 5000 randomized rows were consistent with the Ferrell inequality.

The general Hill coefficient for the inverse tangent function can be derived using equation 2. The inverse tangent function saturates at $\frac{\pi}{2}$ as $x \to \infty$. After adding $\frac{\pi}{2}$ and multiplying by $c$, we can see that the function $f(x)$ as defined in this section saturates towards $c\pi$. In order to find the $EC90$ value, we set $f(x) = 0.9c\pi$ and solve the equation for x:

$$\begin{aligned}
0.9c\pi &= c\arctan(rx - K) + \frac{c\pi}{2} \\
0.9\pi - \frac{\pi}{2} &= \arctan(rx - K) \\
\tan(0.4\pi) &= rx - K \\
EC90 = x &= \frac{K + \tan(0.4\pi)}{r}
\end{aligned}$$

Similarly,

$$EC10 = \frac{K - \tan(0.4\pi)}{r}.$$

**Table 3** Sample simulations of logistic function cascade steps, and the ultrasensitivity of their compositions

| $f(x)$ | $g(x)$ | $H_f$ | $H_g$ | $H_{f(g)}$ | $H_f \cdot H_g$ |
|---|---|---|---|---|---|
| $\frac{2.8}{1+e^{-1.9(x-2.8)}}$ | $\frac{7.5}{1+e^{-4.1(x-2.2)}}$ | 5 | 8.8 | 26.8 | 44 |
| $\frac{0.1}{1+e^{-2.3(x-2.8)}}$ | $\frac{9}{1+e^{-0.7(x-5.8)}}$ | 6.2 | 3.6 | 13.7 | 22.3 |
| $\frac{2.2}{1+e^{-6.5(x-0.7)}}$ | $\frac{2.6}{1+e^{-1.1(x-4.5)}}$ | 4.2 | 4.6 | 11.8 | 19.3 |
| $\frac{1.7}{1+e^{-6.9(x-0.9)}}$ | $\frac{8.5}{1+e^{-6.5(x-1.7)}}$ | 5.9 | 10.9 | 47.2 | 64.3 |
| $\frac{0.2}{1+e^{-1.1(x-4.6)}}$ | $\frac{8.1}{1+e^{-4.4(x-0.6)}}$ | 4.7 | 1.8 | 5.9 | 8.5 |
| $\frac{0.7}{1+e^{-(x-3.3)}}$ | $\frac{6.7}{1+e^{-2.3(x-3.2)}}$ | 2.7 | 7.1 | 10.6 | 19.2 |
| $\frac{0.3}{1+e^{-2.6(x-2.6)}}$ | $\frac{7.6}{1+e^{-0.3(x-8.1)}}$ | 6.5 | 1.5 | 7.3 | 9.8 |

Placing these values into equation (2) yields the Hill coefficient for the inverse tangent function

$$H = \frac{\ln 81}{\ln \frac{\frac{K+\tan(0.4\pi)}{r}}{\frac{K-\tan(0.4\pi)}{r}}} = \frac{\ln 81}{\ln \frac{K+\tan(0.4\pi)}{K-\tan(0.4\pi)}}.$$

Notice in particular that the Hill coefficient of a single inverse tangent function is determined solely by the parameter $K$. This is of course not the case for compositions, and a proof of the result evidenced in our simulations is beyond the scope of this work.

### 3.5 Logistic Function Database

The logistic function is another type of sigmoidal function that we used to computationally analyze our hypothesis. The logistic function is defined as

$$f(x) = \frac{c}{1 + e^{-K(x-L)}}.$$

An example of the logistic function can be found in Fig. 1c. It could itself be the result of certain types of signal transduction cascade steps, see for instance (Enciso et al. 2014). The parameters used for the logistic function in our simulation were defined as $K$ and $L$ from 0.1 to 10, and $c$ from 1 to 1000. We computationally analyzed over 5000 trial cases for our hypothesis using the logistic function, and we found no exceptions to the Ferrell inequality. A random selection of the database created by our algorithm can be seen below in Table 3 for illustrative purposes.

The general Hill coefficient of the logistic function can be derived using equation 2. It is easy to see that this function saturates towards $c$ as $x \to \infty$. In order to find the $EC90$ value, we set $f(x) = 0.9c$ and solve the equation for $x$:

$$
\begin{aligned}
0.9c &= \frac{c}{1+e^{-K(x-L)}} \\
0.9(1+e^{-K(x-L)}) &= 1 \\
(1+e^{-K(x-L)}) &= \frac{1}{0.9} \\
e^{-K(x-L)} &= \frac{0.1}{0.9} \\
-K(x-L) &= \ln\frac{1}{9} \\
-Kx &= \ln\frac{1}{9} - KL \\
EC90 = x &= \frac{KL - \ln\frac{1}{9}}{K}.
\end{aligned}
$$

Similarly,

$$EC10 = \frac{KL - \ln 9}{K}.$$

Placing these values into equation (2) yields the Hill coefficient for the logistic function

$$H = \frac{\ln 81}{\ln \frac{\frac{KL+\ln 9}{K}}{\frac{KL-\ln 9}{K}}} = \frac{\ln 81}{\ln \frac{KL+\ln 9}{KL-\ln 9}}.$$

### 3.6 Counterexample to the Ferrell Inequality

As seen through our results thus far, we have analytically proven that the Ferrell inequality holds for all Hill functions. There is also significant evidence through our computational work that the inequality holds for other sigmoidal functions. However, there are counterexamples showing that the result is not true in general for sigmoidal functions. Recall our hypothesis,

$$H_{f(g(x))} \leq H_{f(x)} H_{g(x)}.$$

Define the following functions:

$$f(x) = \begin{cases} ax & \text{if } x \leq b \\ ab & \text{if } x > b \end{cases}$$

$$g(x) = \begin{cases} 0 & \text{if } x < c \\ \dfrac{e(x-c)}{d+x-c} & \text{if } x \geq c. \end{cases}$$

See Fig. 2 for a graph of these two functions. The function $f(x)$ is a diagonal function that saturates, while the function $g(x)$ is a Hill function with $n = 1$ that has been shifted to the right. As before, we define $h(x) = f(g(x))$.

In order to better understand the composition function, we assume that as $x$ grows, $g(x)$ eventually becomes larger than $b$, which is the saturation value for $f(x)$. This is satisfied only if $b < e$, which we assume throughout our analysis. For $x \le c$, $g(x) = 0$ so $h(x) = 0$. For $x > c$, the value of $h(x)$ depends on whether $g(x) < b$:

$$g(x) < b \ \leftrightarrows \ x < g^{-1}(b) \ \leftrightarrows x < \frac{bd - bc + ec}{e - b}$$

We denote the right hand side of the last inequality by $p$. It follows that if $c \le x < p$, then $h(x) = ag(x)$. If $x > p$, then $h(x) = ab$. The full composition function is then defined as

$$h(x) = f(g(x)) = \begin{cases} 0 & \text{if } x < c \\ ag(x) & \text{if } c \le x < p \\ ab & \text{if } p \le x. \end{cases}$$

We can calculate the $\frac{EC90}{EC10}$ ratio and use it to solve for the Hill coefficient of all three functions. In the case of $f(x)$ we know that the maximum y-value is $ab$. By the definition of $f(x)$,

$$f(EC90) = 0.9ab$$
$$f(EC10) = 0.1ab$$

With this information we find the corresponding EC90 and EC10 values of the function, which are $0.9b$ and $0.1b$. Then, by using equation 2,

$$H_f = \frac{\ln 81}{\ln \frac{EC90}{EC10}} = \frac{\ln 81}{\ln \frac{0.9b}{0.1b}} = \frac{\ln 81}{\ln 9} = 2. \tag{12}$$

The Hill coefficient of $f(x)$ is 2 regardless of the values for $a$ and $b$.

Similarly, we can determine the Hill coefficient of $g(x)$. The maximum value for $g(x)$ is $e$ as $x$ becomes increasingly large. We calculate the $EC90$ value as follows,

$$0.9e = \frac{e(x - c)}{x + d - c}$$
$$0.9(x + d - c) = (x - c)$$
$$0.9x + 0.9d - 0.9c = x - c$$

Simplifying and multiplying by 10 on both sides we get

$$EC90_g = x = 9d + c$$

Similarly, we can calculate the $EC10$ value,

$$EC10_g = \frac{d+9c}{9}$$

We can plug both of these values into the Goldbeter and Koshland formula

$$H_g = \frac{\ln 81}{\ln \frac{9d+c}{\frac{d+9c}{9}}} = \frac{\ln 81}{\ln \frac{81d+9c}{d+9c}}.$$

Notice that when $c = 0$ the Hill coefficient is one, while $H$ increases arbitrarily as $c$ grows.

In order to calculate the Hill coefficient of the composition, notice that by construction the composition consists of truncating the function $g(x)$ at the value $ab$. Recall that we assume $e > b$, in such a way that for large enough $x$, $g(x) > b$ and the composition reaches saturation at $ab$.

$$h(EC90_h) = 0.9ab$$
$$h(EC10_h) = 0.1ab$$

We can do a similar calculation as for the function $g(x)$, noticing that at the point $x = EC90_h$ saturation has not yet been reached and therefore $h(x) = ag(x)$:

$$\begin{aligned}
&0.9ab = h(x) = ag(x) = \frac{ae(x-c)}{x+d-c}\\
&0.9b(x+d-c) = e(x-c)\\
&9bx + 9bd - 9bc = 10ex - 10ec\\
&9bx - 10ex = 9bc - 9bd - 10ec\\
&EC90_h = x = \frac{9bc - 9bd - 10ec}{9b - 10e}
\end{aligned}$$

We can apply a similar method to find the EC10,

$$EC10_h = \frac{bc - bd - 10ec}{b - 10e}$$

We use for concreteness use the parameters $a = 1$, $b = 2$, $c = 3$, $d = 4$ and $e = 25$. Then

$$H_g = \frac{\ln 81}{\ln \frac{81d+9c}{d+9c}} \approx 1.8$$

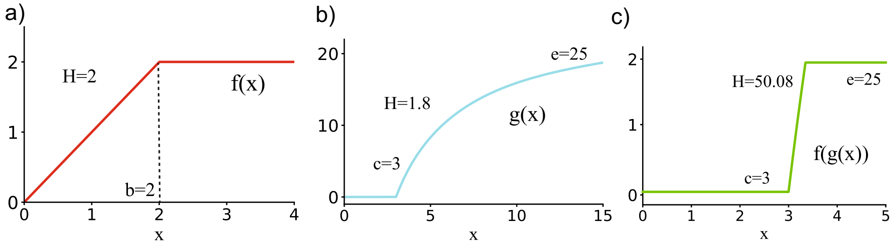


**Fig. 3** Two functions with small Hill coefficients, $f(x)$ and $g(x)$, can produce a composition function, $f(g(x))$, with a significantly higher Hill coefficient. This example shows that there are instances of sigmoidal functions where the Ferrell inequality does not hold (color figure online)

The product of $H_f$ and $H_g$ for these specific parameters is 3.62. However

$$EC10_h = \frac{(2 \cdot 3) - (2 \cdot 4) - 10(25 \cdot 3)}{(2) - (10 \cdot 25)} = \frac{-752}{-248} \approx 3.03,$$
$$EC90_h = \frac{0.9(2 \cdot 3) - 0.9(2 \cdot 4) - (25 \cdot 3)}{0.9(1 \cdot 2) - 25} = \frac{-76.8}{-23.2} \approx 3.31.$$

We calculate

$$H_h = \frac{\ln 81}{\ln \frac{3.31}{3.03}} \approx 50.08.$$

Notice that neither $f(x)$ nor $g(x)$ are particularly ultrasensitive, with Hill coefficients of 2 and 1.8, respectively. However, the Hill coefficient of the composition is 50.08. Figure 3 illustrates each of the functions and shows the stark difference in the ultrasensitivity of the composition.

We include some insights regarding the choice of functions for the counterexample, specifically the function $g(x)$. Surprisingly, it is in a sense the limit of a function that appears in our first example of a cascade in Sect. 2.1.

**Lemma 6** *The following limit holds for the cascade step functions described in Sect. 2.1:*

$$\lim_{m\to\infty} \frac{x^m}{1 + x + \cdots + x^m} = \begin{cases} 0 & \text{if } 0 \le x < 1, \\ \dfrac{(x-1)}{1+(x-1)} & \text{if } x > 1. \end{cases}$$

***Proof*** Notice first that

$$\frac{x^m}{1 + x + \cdots + x^m} = \frac{x^m(x-1)}{x^{m+1} - 1} = \frac{x^{m+1} - x^m}{x^{m+1} - 1}.$$

For $0 < x < 1$, this last expression converges to $0/(-1) = 0$ as $m \to \infty$. For $x > 1$,

$$\lim_{m\to\infty} \frac{x^{m+1} - x^m}{x^{m+1} - 1} = \lim_{m\to\infty} \frac{x - 1}{x - 1/x^m} = \frac{x - 1}{x} = \frac{x - 1}{1 + (x - 1)}.$$

□

This limit was first pointed out by Gunawardena (2005), thus establishing a relation between simple signal transduction cascades and horizontal translations of Hill functions. It also helps to resolve a related question, namely whether a counterexample can be built using sigmoidal functions that are not translations. Using a sufficiently large value of $m$, the function $g(x)$ can be replaced with a rescaling of the function $x^m/(1 + x + \cdots + x^m)$, which is not itself a translation, and in this way violate the Ferrell inequality.

We finish with another question, namely whether compositions from mixed families of functions above could potentially be counterexamples. We carried out 5000 computational simulations where one of the two functions (either $f(x)$ or $g(x)$) is a Hill function and the other an inverse trigonometric function, and we always found that the Ferrell inequality was satisfied. When combining Hill functions and logistic functions, while most of the time the inequality was satisfied, we found some examples where it was not, such as for

$$f(x) = 234\frac{x^2}{8 + x^2}, \quad g(x) = \frac{26}{973 + e^{-5x+15}}.$$

In that case $H_f = 2$, $H_g = 1.47$, $H_f H_g = 2.94$, but $H_{f\circ g} = 9.35$, violating the result.

### 3.7 Three Hill Function Dataset

In order to better understand the reach of our results, we carried out a computational analysis in the case of cascades of three Hill functions. We use the generalized inequality

$$H_{f(g(q(x)))} \leq H_f \cdot H_g \cdot H_q$$

along with logarithmic sampling with the same parameter ranges as in the case of two-step cascades of Hill functions. We generated a database of 5000 rows and found that in all cases the results were consistent with the hypothesis. We display some of the simulations in Table 4 for illustration purposes. This analysis suggests that the Ferrell inequality holds in a natural way for cascades of more than two steps.

## 4 Discussion

An analysis of the Ferrell inequality indicates that it holds for the composition of any two Hill functions, but not all sigmoidal functions. We have shown that in large

**Table 4** Simulations of Hill function cascades with three steps, including the ultrasensitivity of each step as well as that of their overall composition

| $f(x)$ | $g(x)$ | $q(x)$ | $H_f$ | $H_g$ | $H_q$ | $H_{f \circ g \circ q}$ | $H_f \cdot H_g \cdot H_q$ |
|---|---|---|---|---|---|---|---|
| $\frac{1.38x^{4.18}}{568.01+x^{4.18}}$ | $\frac{1.03x^{3.51}}{271.54+x^{3.51}}$ | $\frac{63.74x^2}{29.63+x^2}$ | 4.2 | 3.5 | 2 | 7.9 | 29.4 |
| $\frac{26.63^{2.03}}{16.51+x^{2.03}}$ | $\frac{82.13x^{1.33}}{453.6+x^{1.33}}$ | $\frac{86.34x^2}{4.01+x^2}$ | 2 | 1.3 | 2 | 4.4 | 5.2 |
| $\frac{17.36x^{1.75}}{35.05+x^{1.75}}$ | $\frac{2.41x^{4.44}}{416.02+x^{4.44}}$ | $\frac{16.51x^{10}}{12.18+x^{10}}$ | 1.8 | 4.4 | 10 | 36.9 | 79.2 |
| $\frac{6.58x^{4.28}}{21.07+x^{4.28}}$ | $\frac{2.44x^{1.1}}{9.61+x^{1.1}}$ | $\frac{16.03x^8}{95.26+x^8}$ | 4.3 | 1.1 | 8 | 11.3 | 37.8 |
| $\frac{4.52x^{2.69}}{946.46+x^{2.69}}$ | $\frac{9.81x^{3.95}}{79.37+x^{3.95}}$ | $\frac{5.2x^4}{129.04+x^4}$ | 2.7 | 4 | 4 | 7.9 | 43.2 |
| $\frac{93.78x^{4.87}}{17.44+x^{4.87}}$ | $\frac{3.94x^{1.25}}{3.6+x^{1.25}}$ | $\frac{99.76x^2}{1.81+x^2}$ | 4.9 | 1.3 | 2 | 6.3 | 11.8 |
| $\frac{17.15x^{5.36}}{1.45+x^{5.36}}$ | $\frac{1.71x^{3.19}}{21.28+x^{3.19}}$ | $\frac{23.81x^2}{1.33+x^2}$ | 5.4 | 3.2 | 2 | 11.7 | 34.6 |

generality there is a limit to how ultrasensitive the composition of two functions can be. From these results it follows that when using Hill functions to model biochemical dose responses, two functions with low ultrasensitivity cannot be combined in a clever way to produce a composition function with high ultrasensitivity. Instead, functions with lower ultrasensitivity must be combined so that their composition has a higher degree of switch-like behavior. It is also important to note, however, that sigmoidal functions other than the Hill function can be used to analyze and represent biological systems. Since the Ferrell inequality does not hold in all cases, we cannot predict with certainty the characteristics of ultrasensitivity in these biological systems in the same way we can for just Hill function-based systems.

The functions proposed in the counterexample can potentially be produced using standard biochemical reactions. For instance, the function $g(x)$ which is a simple Michaelis–Menten response that has been shifted to the right, has been described in work by Gunawardena (2005) as a threshold rather a switch, and calculated as the result of multisite phosphorylation. The current work shows that this threshold can be converted into a switch by adding a further downstream layer into that proposed system.

Other mechanisms for high ultrasensitivity involve the creation of bistability and hysteretic switches. Such a switch could be arbitrarily ultrasensitive and involves a feedback loop from a downstream molecule back to an upstream component of the circuit. It also requires a certain amount of nonlinear behavior, which can be obtained from mechanisms such as multisite phosphorylation.

Since many reaction cascades in biochemistry involve more than two steps, such as the MAPK cascade, we provide computational evidence that this result also holds for such longer cascades, at least in the case of Hill functions. A proof for such a general case is out of the scope of this manuscript, but it could be attempted in future work.

It remains unclear whether realistic cascades are sufficiently close to their Hill function approximation that the inequality holds in practice. One possibility is that a biochemical system could try to 'game the system' by purposefully building non-Hill function responses in such a way that they combine to create high Hill coefficient compositions.

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