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# Quantitative Analysis of Ultrasensitive Responses with Logarithmic Mean Difference

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**Abstract**

Sigmoidal responses have been observed in various fields of biochemistry such as multi–subunit protein chemistry, enzymology and signal transduction for decades. The input–output curves in these biological phenomena are often well–characterized by the Hill equation, and Koshland and Goldbeter defined them as ultrasensitive when an increase from 10% to 90% maximal activity occurs over less than an 81–fold change in input quantity; i.e. when the effective Hill coefficient related to the $EC_{90}/EC_{10}$ ratio as $n = \ln(81)/\ln(EC_{90}/EC_{10})$ is greater than 1. Contradictorily, by this definition of ultrasensitivity, a non–sigmoidal linear response curve is also ultrasensitive with $n = 2$. Therefore, I present in this report that the logaritmic mean of the $EC_{90}$ and $EC_{10}$ is more suitable for quantitative analysis of ultrasensitive phenomena.

## 1 Introduction

The sigmoidal shape is biologically significant, and it has been recognized in physiology since the work of Hill [1] and Adair [2] at the beginning of the 20th century, and in enzymology since 1960s Monod–Wyman–Changeux [3] and Koshland–Nemethy–Filmer [4] proposed models for accounting the sigmoidal curves. In 1980s Goldbeter and Koshland showed that signal transduction pathways also yield sigmoidal response curves that resemble those of cooperative proteins and alosteric enzymes [5], and they coined a phase ultrasensitivity; i.e. a property of steady–state signal–response relationships that makes them switch–like in character. Quantitative analysis of ultrasensitive responses has been very intesively studied since then [6], and Ferrell and Ha published recently several review papers on various models that describe ultrasensitivity in biochemical systems [7–9]. Although the models differ in number of respects, they share the main feature; i.e. it takes less than 81–fold change in input effective concentrations (EC) to drive the output from 10% to 90% of limiting saturation value. The ratio of effective concentrations $EC_{90}/EC_{10}$ actually equals 81 when steady–state responses follow classical (hyperbolic) saturation or Michaelian–like kinetic behaviour. However, although ultrasensitive responses according to the latter definition should be sigmoidal; i.e. flat at low and high inputs and steep in between, and consequently with a transparent inflexion points, that is not true for gradual linear responses. Therefore, I propose that the logarithmic mean of distinct $EC_{90}$ and $EC_{10}$ values might be a better quantitative measure for ultrasensitivity of biological processes. Otherwise, the logarithmic mean difference between two positive values plays an important role in the study of heat and mass transfer in liquids flowing in pipes, and it has been known among chemical engineers for decades [10]. The logaritmic mean temperature difference is logaritmic average of the temperature differences at the hot (A) and cold (B) ends of coaxial cylindrical bodies; i.e. double pipe exchangers, and it can be used to calculate transfered heat in a heat exchanger. The logatitmic mean concentration difference is also an operationally exact statement for the concentration driving force of mass transfer in a blood dialyzer. Although the logaritmic mean difference is a function in common use in chemical engineering, I believe that it is expedient to illustrate here this function and how to use its relevance for quantitative analysis of ultrasensitive responses that are widely observed in biochemistry. This is the subject of present article, in which examples of specific response curves are given that can be more unambiguously expressed in the logarithmic mean form than in terms of usual Goldbeter–Koshland's definition [5].

## 2 Mathematical properties of the logaritmic mean

The logaritmic mean $L(x_1,x_2)$ (shortly denoted as L) of two unequal positive numbers $x_1$ and $x_2$ is defined as

$$L = L(x_1, x_2) = \frac{x_1 - x_2}{ln(x_1/x_2)} \qquad (1)$$

with the understanding that

$$L = L(x_1, x_1) = \lim_{x_2 \to x_1} L(x_1, x_2) = x_1 \tag{2}$$

After recasting Eq. (1) as

$$\frac{L}{x_2} = \frac{\frac{x_1}{x_2} - 1}{ln(x_1/x_2)} = \frac{\beta - 1}{ln(\beta)} \Rightarrow ln(\beta) = \frac{x_2}{L} \cdot (\beta - 1) \tag{3}$$

where $\beta = x_1/x_2$, Eq. (3) can be finally reformulated as

$$-\frac{x_2 \cdot \beta}{L} \cdot e^{-x_2 \cdot \beta / L} = -\frac{x_2}{L} \cdot e^{-x_2/L} \tag{4}$$

Both sides of Eq. (4) are of the following form

$$y \cdot e^y = z \tag{5}$$

where y can be expressed as the inverse solution to Eq. (5) in terms of the Lambert W function [13]

$$y = W(z) = W(y \cdot e^y) \tag{6}$$

Thus, taking the Lambert W function of both sides of Eq. (4) gives

$$-\frac{x_2}{L} \cdot \beta = W\left(-\frac{x_2}{L} \cdot e^{-x_2/L}\right) \Rightarrow \beta = -\frac{W\left(-\frac{x_2}{L} \cdot e^{-x_2/L}\right)}{(x_2/L)} \tag{7}$$

In Eq. (7), if one number $x_2$ and optional value of logaritmic mean L are known, then unknown number $x_1$ which defines L in composition with $x_2$ can be determined from the evaluated β value.

Because the argument $-x_2/L \cdot \exp(-x_2/L)$ of W is negative for any positive real number $x_2$, there are two roots for the solution to Eq. (7) as shown in Fig. 1. Although the root from the

$W_0^-$ branch is 'trivial' solution with $\beta = 1$; i.e. $x_1 = x_2$, the root from the $W_{-1}$ branch gives 'real' solution for a system with $\beta \neq 1$, and thus $x_1 \neq x_2$.

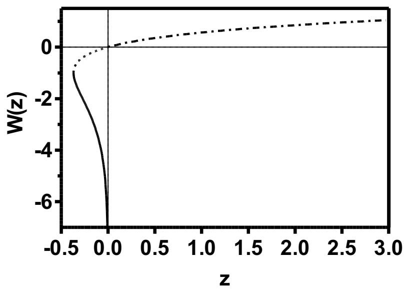


**Figure 1.** The three branches of the Lambert W(x) function for the corresponding values of argument z. Region 1 (dashed) $z > 0$, region 2 (dotted) $-\exp(-1) < z < 0$ and $0 > W(z) > -1$; region 3 (solid) $-\exp(-1) < z < 0$ and $W(z) < -1$. Regions 1 and 2 divide the principal $W_0(z)$ branch as $W_0^+(z)$ and $W_0^-(z)$, respectively, while region 3 represents the lower $W_{-1}(z)$ branch.

Although the exact values of both roots may be computed using advanced mathematical software such as Wolfram Mathematica, MathWorks Matlab or Maple from Maplesoft, the Lambert W function is still not available in the standard mathematical software libraries that are widely used among life scientists. However, there are simple analytical functions that can be incorporated into standard computer programs that can accurately approximate W for all three branches [13,14]. As application of solving β in Eq. (7) is confined to finding solution from the lower branch of the Lambert W function, analytical function that can approximate $W_{-1}$ with a maximum relative error of only 0.025% is also provided as:

$$W_{-1}(z) \approx \ln(-z) - 5.9506 \cdot \left( 1 - \frac{1}{\left( 1 + \frac{0.3361 \cdot \sqrt{-(1+\ln(-z))/2}}{\left(1 + 0.0042 \cdot (1+\ln(-z)) \cdot \exp\left(-0.0201 \cdot \sqrt{-(1+\ln(-z))}\right)\right)} \right)} \right) \quad (8)$$

# 3 Ultrasensitivity and Hill equation

An ultrasensitive response is often described with the sigmoidal curve which can be usually well–approximated by the Hill equation as

$$output = \frac{input^n}{K_{0.5}^n + input^n} \quad (9)$$

where n represents effective Hill coefficient which is real number greater than 1. If n–values are high ($n > 10$), then the steeply sigmoidal input–output curves are similar to switch–like (i.e. Heaviside step) responses. Although the $K_{0.5}$ parameter in Eq. (9) is actually the $EC_{50}$ value, i.e. the effective concentration of an input stimulus required for half–maximal response, this parameter is usually denoted as $K_{0.5}$.

The effective Hill coefficient is related to the effective concentrations $EC_{90}$ and $EC_{10}$. Putting these values into Eq. (9)

$$0.1 = \frac{EC_{10}^n}{K_{0.5}^n + EC_{10}^n}, \qquad 0.9 = \frac{EC_{90}^n}{K_{0.5}^n + EC_{90}^n} \tag{10}$$

yields the following relationships

$$EC_{10} = \sqrt[n]{1/9} \cdot K_{0.5}, \qquad EC_{90} = \sqrt[n]{9} \cdot K_{0.5} \tag{11}$$

The effective Hill coefficient can be expressed with the $EC_{90}/EC_{10}$ ratio according to the Goldbeter and Koshland definition [5] using Eq. (12)

$$n = \frac{ln(81)}{ln(EC_{90}/EC_{10})} = \frac{ln(81)}{ln(\sqrt[n]{81})} \tag{12}$$

Thus, an input–output response system shows ultrasensitivity (and sigmoidality) only when $n > 1$, because the functional form of Eq. (9) is the same as that of the Michaelis–Menten equation for $n = 1$, and in that case $EC_{90}/EC_{10} = 81$.

However, there is a problem with the Goldbeter and Koshland definition of ultrasensitivity (and sigmoidality) when the response curve approaches a straight line that does not bend over until the response is nearly maximal (see Fig. 2). The increasing input–output part of such curve is described by simple linear equation with the slope k as shown in Eq. (13)

$$output = k \cdot input; \quad k = \frac{1}{2 \cdot K_{0.5}} \tag{13}$$

Putting the $EC_{90}$ and $EC_{10}$ values into Eq. (13) gives

$$EC_{10} = \frac{0.1}{k} = 0.2 \cdot K_{0.5}, \qquad EC_{90} = \frac{0.9}{k} = 1.8 \cdot K_{0.5} \tag{14}$$

and results with the effective Hill coefficient n = 2 according to the Goldbeter and Koshland definition

$$\frac{\ln(81)}{ln(EC_{90}/EC_{10})} = \frac{ln(81)}{ln(9)} = 2 \tag{15}$$

Hence, in this way linear curve shows the same ultrasensitivity as sigmoidal Hill curve with n = 2 (see Fig. 2), although nowhere on the linear curve can be found inflexion point, and consequently at least local sensitivity. Therefore, a replacement for the definition is proposed in this paper.

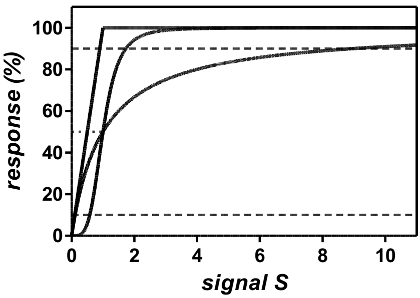


**Figure 2.** The three various response–curves with different effective Hill coefficients evaluated by Eq. (12) according to the Goldbeter–Koshland definition: Michaelian hypebolic–like in shape with n = 1, ultrasensitive sigmoid–like in shape with n = 2, and linear in shape with n = 2. The horizontal dashed lines intersect with response–curves at effective $EC_{10}$ and $EC_{90}$ concentrations.

# 4 Ultrasensitivity and the logaritmic mean of the $EC_{90}$ and $EC_{10}$

The logarithmic mean of the $EC_{90}$ and $EC_{10}$ that are evaluated from Hill equation; i.e. Eq. (10), and are written by Eq. (11), is defined as

$$L = \frac{EC_{90} - EC_{10}}{ln(EC_{90}/EC_{10})} = \frac{\left(\sqrt[n]{9} - \sqrt[n]{1/9}\right)}{ln\left(\sqrt[n]{81}\right)} \cdot K_{0.5} = \frac{\left(9^{1/n} - 9^{-1/n}\right)}{2 \cdot ln\left(9^{1/n}\right)} \cdot K_{0.5} \tag{16}$$

Eq. (16) may be recasted as

$$L = \frac{(e^{z} - e^{-z})}{2 \cdot ln(e^{z})} \cdot K_{0.5} = \frac{sinh(z)}{z} \cdot K_{0.5} \tag{17}$$

where z = ln(9)/n. For clasical hyperbolic or Michaelian–like response when n = 1, the logaritmic mean of the $EC_{90}$ and $EC_{10}$ equals L = sinh(ln(9))/ln(9)·$K_{0.5}$ ≈ 2.023·$K_{0.5}$. If n increases and sigmoidal curve becomes similar in shape to switch–like response, then the logarithmic mean of the $EC_{90}$ and $EC_{10}$ approaches to $K_{0.5}$ according to the limit expression given by

$$L = \lim_{n \to \infty} \frac{(9^{1/n} - 9^{-1/n})}{2 \cdot ln(9^{1/n})} \cdot K_{0.5} = \lim_{z \to 0} \frac{sinh(z)}{z} \cdot K_{0.5} = K_{0.5} \tag{18}$$

Thus, the L values for sigmoidal curves are roughly between $K_{0.5}$ (for n >> 1) and 2·$K_{0.5}$ (for n ≈ 1). Contrary, the logarithmic mean of the $EC_{90}$ and $EC_{10}$ for linear response curve results in

$$L = \frac{EC_{90} - EC_{10}}{ln(EC_{90}/EC_{10})} = \frac{(0.9 - 0.1)}{ln(9)} \cdot 2 \cdot K_{0.5} = \frac{1.6}{ln(9)} \cdot K_{0.5} \approx 0.728 \cdot K_{0.5} \tag{19}$$

This is where the usefulness of the logaritmic mean comes in. The L values for response curves that show no ultrasensitivity in respect of sigmoidal shape are clearly outside the ($K_{0.5}$, 2·$K_{0.5}$) boundaries. Thus, the controversy about ultrasensitivity based on effective Hill coefficient which is evaluated by Eq. (12) can be avoided with the replacement of Goldbeter–Koshland definition.

However, the effective Hill coefficient is still well accepted parameter for sigmoidal curves, and therefore, it is reasonable to determine n–value from curve–determined parameters; i.e. logaritmic mean L and effective concentration $K_{0.5}$. The effective Hill coefficient can be easily evaluated (n = ln(9)/z) when the solution z to Eq. (17) is determined. Although the exact explicit solution to Eq. (17) does not exist, the solution can be easily numerically calculated. Eq. (17) can be reformulated as

$$F(z) = \sinh(z) - \frac{K_{0.5}}{L} \cdot z = 0 \tag{20}$$

The latter equation has one trivial solution z = 0; i.e. when n (= ln(9)/z) approaches infinity. Another solution can be computed either by using appropriate professional mathematical software or simply by Newton's iteration method where each further approximation root can be evaluated as

$$z_{n+1} = z_n - \frac{F(z_n)}{F'(z_n)} = z_n - \frac{\left(\sinh(z_n) - \frac{K_{0.5}}{L} \cdot z_n\right)}{\left(\cosh(z_n) - \frac{K_{0.5}}{L}\right)} \qquad (21)$$

Because of the characteristics of function F(z) this numerical approach for calculating the nontrivial solution to Eq. (20) is very efficient, and it needs only a few steps as approximation roots strongly converge to the exact solution.

It should be remembered that difficulties may arise when accurate calculations of the logarithmic mean must be performed with original Eq. (1) in case of a switch–like response curve with high n–value as $EC_{90}$ approaches $EC_{10}$. Although L reduces theoretically to $K_{0.5}$ according to definition (see Eq. (2)) in such cases, the numerical evaluation of L using Eq. (1) might cause inconvenience for switch–like curves with n >> 1. Hence, various approximations for the logarithmic mean have been proposed to chemical engineering programmers [15–17] for decades, but the most widely used equation attributed to Chen [16]

$$L = L(x_1, x_2) = \frac{x_1 - x_2}{ln(x_1/x_2)} \approx \sqrt[3]{x_1 \cdot x_2 \cdot \left(\frac{x_1 + x_2}{2}\right)} \qquad (22)$$

Approximation Eq. (22) avoids numerical problems when $x_1$ approaches $x_2$, and simultaneously provides limit value $L = x_1$ when $x_2$ equals $x_1$ according to definition (see Eq. (2)). I believe that approximation Eq. (22) can be reasonably used for quantitative analysis of ultrasensitive responses in such cases.

# 5 Conclusion

This report has described the quantitative analysis of ultrasensitivity within the sigmoidal Hill framework, to extract the effective Hill coefficient from the logaritmic mean L of the $EC_{90}$ and $EC_{10}$ instead of the $EC_{90}/EC_{10}$ ratio relationship which was proposed by Goldbeter and Koshland. The primary improvement of this work is the use of well–known logarithmic mean L among chemical engineers, and thereby permitting parameter L for characterization of sigmoid–like in shape curves. The latter parameter actually improves the clarity of definition of ultrasensitivity for several biochemical phenomena. Thus, I conclude that the above–

described logarithmic mean of the $EC_{90}$ and $EC_{10}$ for the quantitative analysis of ultrasensitivity can be considered as useful alternative to the Goldbeter–Koshland's definition.

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