Bohr equation and the lost allosteric Bohr effects in symmetry

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Received August 30, 2019; accepted November 15, 2019

Bohr, Hasselbalch and Krogh demonstrated a group of sigmoid curves under various carbon dioxide contents in 1904. Hill fitted these curves in 1910 with Hill equation without the physical meanings of Hill coefficient and dissociation constant. In 1965, Monod-Wyman-Changeux model (MWC) popularized the word “allostery” with 81 words of symmetry to define an orthosteric nature of cooperativity in a single and symmetric sigmoid curve. Paradoxically the MWC model didn’t quantify the homotropic Hill coefficient and confusingly described the symmetry of sigmoid shapes with three allosteric variables. A heterotropic Bohr equation, by clarifying the biophysical symmetry in allosteric, suggests the solution of allosteric coefficients with only one Bohr variable. We reveal that the mathematical need of a fictional monomer by MWC model justify a symmetric logistic curve with a parabolic kernel of dissociation constant to model the 1904 sigmoid curves. The logistic-derived Bohr equation and its half-saturated $P_{50}$ equation successfully used the embedded $P_{50}$ values in the 1904 sigmoidal curves to quantify their hyperbolic conformational shifts and Hill coefficients (n) pending for a century. Both are the logarithmic functions of carbon dioxide. This truly quantitative Bohr equation digitizes the allosteric regulation of the orthosteric affinity by precisely cloning the original group of dissociation/association curves published in 1904. The Bohr equation honestly suggests that nature should have chosen the allosteric Bohr effects to modify hemoglobin to cope with the swift dynamic of gas exchange. The discovery of the Bohr function in Bohr equation challenges the feasibility of the orthosteric cooperativity of hemoglobin.

Key words: allosteric, Bohr effect, Hill equation, Monod-Wyman-Changeux (MWC) model, symmetry

Nature chose phosphate to modify proteins for the chemical versatility of phosphate [1]. By considering the universal and allosteric posttranslational modifications (PTMs), why would nature choose orthosteric oxygen to modify the most dynamic and ever switching hemoglobin by deleting the allosteric PTMs? The failure to quantify the cooperativity is a paradox to this orthosteric choice of nature [2,3]. Is our interpretation of the allosteric nature insufficient as the hemoglobin is concerned?

“Fractional saturation is not a direct measurement of conformational change.” This is a famous quotation of Wyman regarding when he explored the unexplained difference between the number of ligand binding sites and the Hill coefficient, $n_H$ [2]. The modeling of allosteric cooperativity originated from the sigmoid Hill equation [2,3]. The maximal Hill coefficient or the quantitative cooperativity of the four-heme hemoglobin is well known to be only near 3
Paradoxically, the Hill equation did not provide a quantitative fact, which is still unsolved as a centennial problem [4]. Neither Hill provided a direct measurement of oxygen uptake during exercise but a reverse exponential equation for approximating oxygen uptake (VO2) [5]. Nevertheless, maximal oxygen consumption (VO2max) became another unsettled physiology [5]. Ilogically, however, the Hill equation is widely used as a dynamic model in pharmacology and the receptor kinetics, where the Hill coefficient (nH) is used as a descriptive number of cooperativity, the sigmoid shape and could be derived from the Hill plot [2,6]. Yet “there is still no mathematical description that could describe quantitatively the action of agonists on G-protein-coupled receptors (GPCRs)” [6]. However, why is the Hill coefficient (nH) of hemoglobin orthosteric rather than allosteric? In 1904, Bohr showed a group of sigmoidal curves by oxygen bindings to demonstrate the Bohr effect as the carbon dioxide (CO2) content of fresh canine blood was increased [7]. Why was the mathematical description of these Bohr effects lost in the orthosteric Hill equation since 1910 [2-4,8]?

This first-ever quantitative and heterotropic model of hemoglobin explores the ambiguously allosteric regulation residing in the 1904 hemoglobin dissociation curves. To prove this model, one needs to answer two questions. Is the saturation curve symmetric or asymmetric? And why Wyman described allostery in a single and symmetric sigmoid curve while Bohr presented a group of asymmetric sigmoid curves?

Methods

The analysis of orthosteric symmetry traces the sigmoidal origin of Hill equation

The creation of orthosteric symmetry in MWC model

“Jeffries Wyman had noted several years earlier that the symmetry of the saturation curves of hemoglobin by oxygen seemed to suggest the existence of a structural symmetry within the protein molecule itself; this idea was brilliantly confirmed by the work of Perutz.”

Jacques Monod, Nobel Lecture, December 11, 1965

The ABC of a homotropic MWC model and the dynamic symmetry

\[ K_d = \frac{K_T}{K_R} = K_T K_d \]

(The basics of a saturation curve)

- \( K_T \): the macroscopic dissociation constant
- \( K_R \): the macroscopic dissociation constants for R state
- \( K_T \): the macroscopic dissociation constants for T state
- \( K_i \): the affinity constant, \( K_i = \frac{1}{K_R} \)

Following the basics of a saturation curve (Equation (1)), Wyman described the dynamic symmetry of “one” saturation curve by transforming the macroscopic dissociation constant in Hill equation into two allosteric constants to construct both the homotropic allosteroy of MWC model and the “mathematical fiction of monomer” (Equation (2)) [3]. In 1904, Bohr actually demonstrated the Bohr effect with a larger dissociation constant (Kd) toward T state favoring the decrease of oxygen affinity in a group of sigmoid curves [2,7]. For the creation of MWC model, Wyman improperly erased this prominent Bohr effect by assuming a small microscopic dissociation constant (c=Kd/Ka≤1) and a large, homotropic, allosteric constant (L=T/R≥1). These two conjoined allosteric variables, Lc, preserved the spirit of a larger Kd to construct the upper and lower part of “one” sigmoid, to which Wyman assigned “symmetry” and “the homotropic cooperativity” (Equation (2)). Consequently, the larger dissociation constant (Kd) and the potentially allosteric Bohr effect degenerated to serve two new constants in the allosteroy describing the orthosteric (homotropic) allosteroy (Equation (3)) [2,3].

\[
y = \frac{x^n}{K_d + x^n} \quad \text{2.1: Hill’s approximation of Bohr curves, unspecified } n \text{ as a positive real number} \]

\[
y = \frac{x^n}{K_T K_d + x^n} \quad \text{2.2: Wyman’s symmetric saturation curve, } n \text{ is a homotropic integer} \]

\[
y = \left( \frac{x}{\sqrt{K_T}} \right)^n \quad \text{2.3: Introducing two allosteric constants, since } c \leq 1, \text{then } L \gg 1. \]

\[
y = \frac{(x)^n}{L c^n + (x)^n} \quad \text{2.4: MWC’s homotropic allosteroy} \]

\[
y = \frac{x}{\left( c + \frac{1}{c} \right)^n} = \frac{\alpha}{1 + \alpha} \quad \text{2.5: The making of a fictional monomer, } n=1, \alpha = \frac{x}{\ell c} \text{ and } \ell c = 1 \text{ (Fig. 1)}. \]

(From allosteroy (Bohr) Kd to homotropic allosteroy)

\[
2.6: K_d = K_T / K_R = L \cdot c^x = (1 + \frac{1}{c})^n \]

Let \( L = K_T / R_0 = \ell c, c^n = \sqrt{K_T}, K_i = \frac{R_0}{K_T}, K_i = L, \ell: \text{ Large and small “homotropic” allosteroy constants, } R_0, R_0, \text{r.t.: the macro—and microscopic Two—states} \]

Or we could view the homotropic MWC model as an integrated model of a group of Bohr curves in one symmetric sigmoid. Unfortunately this conjoined existence of a large, homotropic L (=T/R=R or c) and a small c (=Kd/Ka by Wyman) in Equation (3) creates what Crick described, “the price of
allostery” and “the conflicting needs” of an allosteric protein [2]. The decreasing affinity of oxygen for the lower sigmoid and the more than needed increasing affinity for the upper sigmoid with the increasing orthosteric interaction were revealed by Crick [2,9]. Frankly, a homotropic ℓ(L) compromised the lower Bohr effect to secure needlessly the upper R state affinity only in the name of symmetry [2]. This design of allosteric protein violated the orthosteric (a hyperbolic symmetry of dynamic in Fig. 1) between allosteric effect and affinity, namely, \( K_x = K_T \), \( K_y \), or \( ℓ \cdot c=1 \) (Equations (1) & (2)). The dynamic symmetry that Wyman preferred is \( ℓ=c \) and thus \( K_x = K_T \). Furthermore, the cooperativity of hemoglobin can only be orthosteric because the \( K_y \) is the dissociation and association constant simultaneously in the name of orthosteric symmetry, and \( a=K/Kx \). This is the creation of the orthosteric allostery of hemoglobin (Equation (3)). Consequently Wyman changed the dynamic nature of symmetry in allostery by embracing a homotropic or orthosteric nature of allostery [3].

\[
\frac{Lcα(1+αy)^{(1+αy)}+α(1+α)^{(1+αy)}}{L(1+αy)^{(1+αy)}+(1+α)^{(1+αy)}} = \frac{α}{1+α} = \frac{F}{K_x+F} \quad (3)
\]

\( \bar{Y}_F \): saturation function; \( F \): substrate concentration, The rightward hyperbolic equation of the fictional monomer is derived with \( c=1 \) and an insignificant \( L \). \((L=9054, c=0.014, for \ a \ a^2 \ sigmoid \ fitting) \) [3]

According to the six statements of the orthosteric MWC model in its 1965 publication [3], the protein is an oligomer consisting of \( n \) identical protomers, arranged symmetrically (the first statement) [2]. The hemoglobin tetramer and its \( n \) identical protomers shift between two and only two conformational states (the fourth statement) [2]. R-state is for the relaxed, high-affinity state and T-state is for the tensed, low-affinity state. The equilibrium between the two states determines the “homotropic” allosteric constant \( (L=ℓ) \), which is separated from the concept of affinity (the fifth statement).

Apparantly, Wyman constructed the allostery-detached orthosteric symmetry by introducing two-homotropic allosteric constant and the “heterotropic effects would be due exclusively to displacements of the spontaneous equilibrium between the R and T states of the protein” [3]. Thus the heterotropic effects only changed \( L \) without effects on \( c, K_r \) and \( K_T \) [3]. Furthermore, the MWC model risked losing the separate measurement of the oxygen affinity, \( 1/K_y \), and the dissociation constant, \( K_y \) in a group of Bohr curves (Equation (1)) by conjoining them into \( Lcα \) to describe “one” symmetric saturation curve (Equation (2)). And only by these descriptive words (“The homotropic interactions are independent of absolute affinities.”) [3], the Hill numbers were left alone as an integer without an allosteric definition.

The reversed engineering of allosterity from the sigmoidal Hill equation by Wyman might be due to the stereotypical coexistence of the discrete oxygen-binding equations, also
known as the mass action equation, and the Hill equation listed by Hill since 1910 (below). In fact, Hill had clarified his object was to see whether his equation could satisfy the sigmoidal observations rather than “to base any direct physical meaning on $n$ and $K$” [8].

$$Hb + O_2 \leftrightarrow HbO_2 \{?: unknown allosteric mechanism\},$$

$$Hb_{n} + nO_2 \leftrightarrow Hb_{n}O_{2n} \{n: unknown integer in 1910\},$$

$$y = 100 \frac{Kx^n}{1+Kx^n}$$

The original Hill equation, $n$: real number reported by Hill [8], $K$: aggregation constant, $x$: $PaO_2$; $mmHg$

It is clear in Equation (2) that the symmetry of “$a$” saturation curve fitted by the MWC model in Equation (3) actually has all intrinsic asymmetries that belong to all the lost Bohr effects demonstrated in 1904 (the swinging $K_o$ on the hyperbola, $(L\cdot c)=1$). In plain words, Wyman mistook the sum of heterotropic allosteric effects as “one” homotropic allostery (Another important proof of this error is Equation (18)). Furthermore, the high mixed venous saturation around 75% observed in physiology [5] and the enthusiastically pharmacological exploitations of Hill equation [6] reinforced the pseudo-symmetry of the orthosteric MWC model presented in Equation (3). This manipulation of Hill equation incurred the consequences of inevitable frustrations in the agonistic modeling for other allosteric proteins [6] and a flip-flopped physiology of respiration [5,9]. Thus a thorough understanding of the biophysical symmetry in terms of the hemoglobin saturation curve is inevitable to construct a truly quantitative Hill equation for the Bohr sigmoid curves.

The fictional monomer of MWC model with $n=1$ and the structural symmetry

With a relative view in structure between the allosteric site and orthosteric one, one should ask: “Why is the cooperativity of the oxygen binding curve asymmetric as a sigmoid shape?” To reveal the asymmetric nature of the original Hill equation, the sigmoidal relationship between the logistic curve ($L^*$) and the hemoglobin saturation curve ($S^*$) needs further clarification. The focus is their related transformations through the dissociation constant, $K_o$. (This is the pharmacological approach to the semi-logarithm presentation of substrate-receptor saturation curve. The physiological scaling of Hill equations will be added later for the reproduction of the 1904 sigmoid curves in Results and Discussion: To clone the old 1904 Bohr sigmoid curves is to prove the new Bohr equation.)

From the descriptive Hill equation (Equation (4), Fig. 2) [6,8]:

![Diagram](https://example.com/diagram.png)

Figure 2  The sigmoidal transformations of the Bohr shifts: positive cooperativity as a sigmoid and negative cooperativity as a hyperbola. Oxygen saturation curves ($S'\cdot L', S': n=2.618$) are the exponentially invariant transformations of the logistic sigmoid ($L^*, L^0, L^0$). There is loss of the sigmoidal information from $L^*$ to hyperbolic $S'$. A logistic Bohr shift ($B$) on $L^*$ would project into a hyperbolic Bohr shift point ($P_B$) on $S'$ (the two red arrows). The $P_S$ of $S'$ (the red dot overlapping with the $P_o$ of $S'$) walks upward along the hyperbolic $S'$ curve to $P_{50}$ and $P_{83}$ to simultaneously exaggerate the saturation plateau and the dissociation sigmoid of the logistic curve and thus creates the $S'$ and $S^*$. The displacement of $P_o$ (Black arrow) is an exponential function of the Bohr shift. This finding clarifies that the cooperative origin of the sigmoid shape is the Bohr shift ($B$ or $P_o$). In homeostasis, we have a homeostatic Bohr coefficient of $n^{2.618}$ (black sigmoidal line) with $P_o$ of approximately 27 mmHg that Hill equation took it as $n=N=2.7$. A theoretical and negative Bohr effect saturation curve (green line, $n=0.5$) is shown. The negative and leftward Bohr shift ($-B$) transforms a flattened logistic sigmoid into a downward shifted hyperbolic saturation curve to maintain a relatively high-affinity plateau under suppressed $n$. In addition, $P_o$ decreases hyperbolically to $P_{50}$ (the lowest green dot on $S'$) without sigmoidicity. Note that $S^*$ is the allosteric translation of the orthosteric $S'$, and both the hyperbolic $S'$ and sigmoid $S^*$ are not allosteric cooperative because both are the symmetry of cooperativity. ($L^*$: Logistic curve; $S$: saturation curve; $S'$: $B$: saturation curve of a negative Bohr effect; $SaO_2$: oxygen saturation; $PaO_2$: oxygen partial pressure; $ln(PaO_2)$: natural logarithm of $PaO_2$; $P_50$: $PaO_2$ at 50% saturation; $P_{75}$: $P_B$ of $n^2$; $P_{83}$: $P_B$ of $n^{2.618}$.)
This model derives a logistic sigmoid equation ($L^n$) that could be symmetric when $n=1$:

$$S^n = \frac{x^n}{K_x x^n} \quad (4)$$

S: saturating sigmoid; $x$: PaO$_2$; $K_x = (P_o)^n$, 
$n_o \in \text{positive integer (number of oxygen binding sites)}$

$P_o$ is the PaO$_2$ at 50% $O_2$ saturation.

In this logistic sigmoid equation $L^n$ (Equation 5), $K_x$ is 1 by the initial condition of $n=1$. This logistic sigmoid with $n=1$ ($L^1$), which transforms from the hyperbolic monomer proposed by MWC model, has the simplest symmetry of a dynamic system (Equation 6, Fig. 2). Then the allosteric transition, for $n>1$, is asymmetric because it translates from the orthosteric symmetry of this logistic sigmoid ($L^1$). Note that the hyperbolic $S^1$ saturation curve to which “we assign no cooperativity” transforms itself to this sigmoid $L^1$ (Fig. 2). Thus, the initial logistic modeling of $L^1$ as the orthosteric sigmoid of the saturation curves ($S^n$) is valid. Therefore, the three orthosteric, hyperbolic equations in Equation (7) present symmetry, asymmetry and fictional symmetry respectively in terms of the dissociation constant when $n=1$. Regrettably the high mixed venous PvO$_2$ that Hill observed in the exercise oxygen consumption studies warps this sigmoidal symmetry of the Hill equation with a deviated $K_a$ [5,8]. This demanded MWC model to reconstruct a symmetrical dynamic by introducing “a mathematical fiction of equivalent monomer” (Equations (2) & (3)) [3,10].

$$S^1 = \frac{x}{1+x} \quad \text{vs. Hill } S^1 = \frac{x}{K_x + x} \quad (6)$$

$\{L^1(0) = 0.5 \text{ vs. Hill } L^1(ln(K_R)) = 0.5\}$

In fact, the loss of the lower sigmoidal information during the orthosteric transformation from the logistic $L^1$ to the hyperbolic $S^1$ suggests the hyperbolic nature of the orthosteric bindings rather than the sigmoidal association of the orthosteric affinities, which the MWC model enthusiastically adopted into the allosteric concept as homotropic cooperativity (orthosteric cooperativity [2–4,6]). Additionally, the hyperbolic equation ($S^n$) of the fictional monomer (Equations (3) & (7)) also supports this hyperbolic assumption of the orthosteric symmetry before the allosteric interaction. Therefore, both the hyperbolic $S^1$ and sigmoid $L^1$ should be the proper symmetry of the fictional monomer as a structural symmetry that was proposed by Wyman [3] and plausibly both represent the orthosteric symmetry. This model will describe this structural symmetry of a fictional monomer as the symmetric monomer, $S^1$, which in essence of symmetry equals $L^1$. The Equations (5) & (6) and the need of a mathematical fiction of equivalent monomer by the MWC model to reconstruct symmetry in allostery differentiates the allosteric Bohr equation from the pharmacological Hill equation which describes the law of mass actions [6].

After revealing the structural symmetry as a primitive logistic curve and delineating the compromising symmetry of a homotropic dynamic proposed by MWC model yet concerned by Crick (Equations (3) & (7)), we then ask: “What is the mathematical connection between the hyperbolic $S^1$ curve and the sigmoid Bohr $S^1$ curves?” and in allosteric sense, “How the allosteric transition shifts the fictional orthosteric symmetry to the real allosteric symmetry?” The answer is in the problematic $K_a$ shown in the ABC of MWC model.

**Results and Discussion**

**Rediscover the lost Bohr shift (B) in the symmetric monomer**

**Crick and the heterotropic definition of allostery**

It deserves to repeat that Hill did not base any physical meaning of $n$ and $K$ for any Hill approximation of the Bohr sigmoid curve [6,8]. The indefinable orthosteric Hill coefficients by the MWC model could neither quantify the 1904 sigmoid curves nor numerate the allosteric interaction coefficients [2,3] despite its claim that curve fitting was not necessary to verify its model. And Crick’s definition of allosteric coefficients still depended on a quantitative saturation equation that did not exist (Equation (3)) [2,6]. Therefore, this heterotropic model drops the two fictional constants ($L$ and $c$) and returns to the basic, the dissociation constant ($K_b$). By following Wyman’s trial to build a symmetric kernel in $K_a$ ($K_a = K_b$ by Wyman) to describe the symmetry of a saturation curve ($L^1$ or $S^n$), let us outsource the allostery to the deleted Bohr effect reclamed in the symmetric sigmoid, $L^1$ (Equation 5) and then verify this assumption with the 1904 sigmoid curves. Firstly, the mathematical symmetry of reciprocity (Equation (8)) should be preserved during the making of the fictional monomer and the symmetric monomer in Equations (2) & (3).

For an allosteric hemoglobin, it should conform to:

$$L \cdot c = 1 \quad (8)$$

$\{\text{Crick’s concern about allostery [2]}\}$
The allosteric symmetry for \( n > 1 \) in Equation (9) should be "heterotropic, not homotropic," to conform to this Crick's concern about allostery [2]. Secondly, if the metabolic goal of the allosteric Bohr effect is principally to maximize the CO\(_2\) uptake level of RBCs under metabolic stresses and synchronously to regulate oxygen delivery in a feedback mechanism, then we can define the allosteric Bohr regulation of hemoglobin function in Equations (9) & (10).

In plain words, the Bohr shift creates the allosteric activity that reciprocally regulates the affinity of oxygen (Equation (9)). Thus the allosteric definition in Equation (9) is solid both physically and physiologically.

\[
B = n - 1 \quad (9)
\]
\[
B(n - 1) = B^2 = N \quad (10)
\]
\[
K_d = e^B = e^N = (e^{2N}) = (P_{50})^n \quad (11)
\]

From this heterotropic definition of allostery, the emerging parabolic model of a heterotropic \( K_d \) reestablishes the fundamental symmetry of cooperativity (Equations (10) & (11), Fig. 1) that was violated by the "conjoined Bohr effects", the homotropic allosteric constants, and the indiscriminate \( n \) as a positive integer (Equations (2) & (12)). Actually the three allosteric variables, \( L \), \( c \), and the integer \( n \), reduced the potentially quantitative Hill equation to a qualitative one. For the sake of the definition of \( K_d = K_t \) in the fictional monomer, the MWC model took the price of allostery by assigning homotropic cooperativity to take over the heterotropic effect [2,3]. The nonlinear and undivisible symmetry of cooperativity (\( K_c \)), which most allosteric studies linearly divided into two fictional constants (\( L \) & \( c \)) in Equation (12), quantifies the allostery with only one allosteric variable, the Bohr shift (\( B \)) (Fig. 1).

\[
K_d = e^N = (e^{2N}) = (e^{N+cN}) = (e^N)(e^{cN}) = L \cdot e^c \quad (12)
\]

The walks of the Bohr shifts on the orthosteric symmetries: the modeling of conformational shifts

How does the Bohr shift restore the lost, lower sigmoid shape of the \( L^1 \)-logistic curve from the orthosteric, hyperbolic \( S^1 \)?

The saturation equation, \( S^s \), and the logistic equation, \( L^s \), are a pair of functions that allosterically couple the gas exchange inside the RBCs (Equation (13)).

\[
S^s = \frac{x^n}{e^B x + x^n} = \frac{x^n}{e^{N+X^n}} = \frac{x^n}{e^{N^s + X^n}} \quad (13)
\]

\{Saturation Sigmoid: Bohr equation\}

\[
L^s = \frac{e^{x^n}}{(e^B(x^n)) + e^{x^n}} = \frac{e^{x^n}}{e^{N^s} + e^{x^n}} \quad (14)
\]

\{Logistic Sigmoid\}

This Bohr equation (\( S^s \)) approximates the hemoglobin dissociation curve better than the orthosteric Hill equation because the lost Bohr shift is included with the corrected orthosteric symmetry and the lower part of sigmoid shape is a function of the lost Bohr shift (Fig. 2). The positive Bohr shift (+\( B \)) moves the orthosteric symmetry (0, 0.5) on the logistic \( L^1 \) curve upward and along this logistic sigmoid to a Bohr-shifted point representing the positive allosteric action of CO\(_2\). This Bohr shift on the logistic \( L^1 \) would be as follows:

\[
\text{Bohr shift} = (B, \frac{e^B}{1+e^B}) \text{ on the symmetric monomer,}
\]

\[
S^s = \frac{\alpha}{1+\alpha} \quad (14)
\]

\{Bohr shift on the logistic \( L^1 \) curve; \(-1 < B < 2\)\}

On the Bohr \( S^s \)-curve, the corresponding point would be the hyperbolic Bohr shift, \( P_{50} \) (Equation (15)), tracing on the \( S^s \) (the bold-red arrow in Fig. 1). Without allosteric action or \( B \) as zero, \( P_{50} \) remains unmoved as a symmetric \( P_{50} \) in \( S^s \) (Equation (15)).

\[
P_{50} = \left( e^B, \frac{e^B}{1+e^B} \right) \text{ on the symmetric monomer,}
\]

\[
S^s = \frac{\alpha}{1+\alpha} \quad (15)
\]

\{Bohr shift departs from the \( P_{50} \) on the hyperbolic \( S^s \) curve.\}

This \( P_{50} \) of \( S^s \) moves from \( P_{50} \) of \( n^1 \) through \( P_{75} \) of \( n^2 \) to \( P_{43} \) of \( n^{2.615} \) on the hyperbolic \( S^s \)-curve (Fig. 2). Thus, these Bohr shifts walk hyperbolically upward on \( S^s \) to create their specific sigmoidicity (Equation (11)) and the allosteric conformation. And the saturation plateau of \( S^s \) exaggerates the upper sigmoid of the logistic curve, \( L^s \), exponentially and visually. Therefore, on the symmetric monomer (\( S^1 \), \( L^1 \), and in general, \( S^s \)) two equilibriums coexist at the orthosteric symmetry. One is the \( P_{50} \) and the other is the lost Bohr shift point (\( P_{50} \)). For an allosteric action of CO\(_2\), the \( P_{50} \) exhibits a rightward shift (the bold-black arrow in Fig. 2) while \( P_{50} \) moves hyperbolically upward (the bold-red arrow in Fig. 2) to reset the allosteric equilibrium between the two conformational states depending on the Bohr shift (\( B \) or the
allosteric coefficient \((n=1+B)\) [2,10].

The horizontal shift of \(P_{50}\) provides a precise ruler-equation because the three allosteric variables \(B, N\) and \(n\) are calculable from the constant Equation with a symmetric value, 0.5 (Equation (16)) with any measured value of \(P_{50}\)

\[
P_{50} = \left( e^{2}, 0.5 \right)
\]

\[
\{ \text{The quantitative Bohr effect}, \}
\]

\[
\{ \text{The quantitative Bohr effect}, \}
\]

\[
\{ \text{The quantitative Bohr effect}, \}
\]

The orthosteric Hill equation by MWC model divided this allosteric \(P_{50}\) and used part of it as the fixed, orthosteric \(K_a\) (Equation (11)) [11]. Consequently, the Bohr effect has been unfavorably marginalized in the allosteric researches [2–4,9,10], despite the failure of the original Hill equation and MWC model to provide a symmetric allosteric equation like the allosteric \(P_a\) and \(P_{50}\) in Equations (15) & (16) [2–4,8,10,11]. The divided and fictionally fixed \(K_a\) in MWC model (Equation (11)) generated the most confusing hypothesis that in the absence of ligand there were several coexistent conformations (at least two) with up to eight equilibrium constants in the reversible two states of a single protein [3,4,10] and lost the significant linkage between the Bohr shift and \(P_{50}\) in Equation (16). The structural mechanism within this linkage deserves further explorations. The positive hydrogen ion (H+) or \(CO_2\) should be the absent ligand in the allosteric sense because metabolism (with a specific output of \(CO_2\)) determines the specific need of oxygen as a common sense. The very original concept of allostery is the feedback regulation of enzymes, which was decoupled and lost since the introduction of MWC model [3,6].

**Identity and inverse: the allosteric symmetry and the negative allosteric (\(B<1\)) effect**

By the parabolic model of heterotropic dissociation constant, the orthosteric symmetry \(S^1\) (\(B=0 \& N=0\)) translates to the allosteric symmetry \(S^2\) (\(B=1 \& N=0\)) under the heterotropic (Bohr) modification (Fig. 1). The identity of the group of hemoglobin then transforms to the real allosteric symmetry from the fictional orthosteric symmetry (\(S^1 \rightarrow S^2\), Fig. 2). From this allosteric identity (\(n=2, B=1\) and by the parabolic (symmetric) nature of the cooperativity (Equation (10); Fig. 1), the allosteric transitions differentiate into the positive cooperativity (if \(B>1\), then \(N>B\), Bohr effect, \(n>2\)) and the negative cooperativity (if \(B<1\), then \(N<B\), and \(1<n<2\)). Thus symmetric Bohr effects should exist naturally [12,13]. This should be the result of hemoglobin modification by Bohr protons, a particular PTM. Theoretically another negative Bohr effect could develop under receptor inhibition (defined as \(n<1\)); then, for example, the reasonable sigmoid curve for an allosteric receptor inhibition of \(n=0.5\) could be presented in Figure 2. Logically this Bohr effect would show a negative allosteric mechanism that counters the receptor inhibition with a hyperbola under pressure. This negative allosteric transition sitting in the orthosteric symmetry could explain the allosteric resistance to receptor inhibition or a quantitative mechanism of drug resistance [5]. The Bohr equation, rather than the orthosteric Hill equation, quantitatively explains both the positive allosteric transition (with \(B>1\)) and the negative allosteric transition (with \(B<1\)) by its mathematical precision in modeling the switching mechanism of allosteric regulation (1). Most of laboratory observations with the orthosteric concept of allostery had difficulties in quantifying these microscopic transformations of cooperativity from sigmoid to hyperbola and reciprocally [9,10,12–15]. Paradoxically the MWC model, a reversed explanation of the allostery, introduced more unquantifiable variables for the insignificant \(c\) as unity and the indiscriminate orthosteric cooperativity, for example, \(n=4\) (Equation (17), the original equation 7 and Fig. 2 in reference [3]). The pharmacological adoption of the MWC model (Equation (17)) should be careful [6].

\[
\tilde{Y}_s = \frac{\alpha(1+\gamma)^{n-1}}{L(1+\gamma)^n + (1+\alpha)^n}
\]

\[
\tilde{Y}_r: \text{saturation function,}
\]

\[
\alpha = \frac{F}{K_s}, \quad \beta = \frac{I}{K_r}, \quad \gamma = \frac{A}{K_s}, \quad c = 1, n = 4, L = 10^{n+1},
\]

\[
K_a \text{ and } K_r: \text{the microscopic dissociation constant of inhibitor (I) and activator (A) with the two states}
\]

**The allosteric symmetry as a symmetric monomer**

The allosteric symmetry, \(S^2\), shows no allosteric cooperativity because both \(N\) and \(B\) are equally unity despite its sigmoid shape. This non-cooperative allosteric symmetry (\(S^2\) in Fig. 2; \(N=B=1\) in Fig. 1) between the symmetric Bohr effects shown in the group of Bohr curves in 1904 is the fictional symmetry of fictional monomer between two states; namely, the fixed \(K_a\) in a single symmetric saturation curve created by the MWC model (Equations (2) & (3)). Thus the symmetric monomer (with the fictional coefficient, \(n=1\)) conceived by the MWC model should be this non-cooperative \(S^2\) (with allosteric number, \(n=2\), Equation (18)) rather than a non-cooperative hyperbolic one with Hill coefficient \(n=1\). Equation (18) is another proof that MWC model interpreted the heterotropic allostery as the homotropic allostery.

\[
S^2 = \frac{x^2}{(c^2)^2 + x^2} \equiv \frac{a}{1 + a} \left( \frac{F}{K_s + F} \right) \quad (\text{Equation (3)})
\]

\[
\left\{ a = \left( \frac{x}{P_{50}} \right)^2 = \left( \frac{x}{P_{50}} \right)^2 \frac{F}{K_s}, K_a = \frac{K_s}{K_r} = e \right\}
\]

Then Wyman’s proposal of the fictional monomer should be able to reduce the Bohr equation abstractly “as a symmetric monomer” (Equation (19)). The fictional monomer should
Lee: The allosteric symmetry

not be used as a “real” monomer with an allosteic constant, \( L \) and a homotrophic integer \( n \) of Hill number (Equations (3) & (17)). It abstracts the conformational shifts into the relative and symmetric relation of Bohr shift versus \( P_{50} \). This relation has a symmetric relation of Bohr shift versus \( P_{50} \) under the heterotrophic allosteric interactions, \( n \) (In Equations (14) & (15), the two Bohr shifts in the separate logistic and hyperbolic curves walk on the same symmetric monomer.). The increasing \( n \) corresponds to the increasing tightness in the fictional hemoglobin monomer. By fixing on the allosteric symmetry [16] and in the opposite direction, “there are always two states reversibly accessible to the allosteric oligomers” [3]. Then any allosteric oligomer could consistently reduce itself as a symmetric monomer by following the law of symmetry and by this reasoning, the symmetry of cooperativity always preserves (the sixth statement of MWC model) [2,3,16]. In plain words, the asymmetric saturation curve in appearance is symmetric in the allosteric sense of a symmetric monomer, \( L \). By comparative studies of the Equations (18) & (19) versus Equation (3), the errors MWC model made are clearly corresponding to those mentioned in Methods. Thus the one and only one symmetric sigmoid that MWC model had been after is the symmetric monomer of Bohr equation (Equation (19)).

\[
S^n = \frac{x^n}{K_x + x^n} = \frac{a}{1 + \alpha} = \frac{\left(x/P_{50}\right)^n}{1 + \left(x/P_{50}\right)^n}
\]

(19)

**Bohr equation as a symmetric monomer,**

If \( x = P_{50} = e^{B}\),

\[
\left(\frac{x}{P_{50}}\right)^n = \left(\frac{P_{50}}{P_{50}}\right)^n = \left(e^{B}\right)^n = a,
\]

\[
N = B; \quad K_x = e^{B};
\]

\( n \): Bohr coefficients

**Structure-function relation**

This Bohr equation with the parabolic kernel of \( K_x \) clearly demonstrates the physical law of symmetry that resides in the hemoglobin structurally as a symmetric monomer and dynamically as the fictional monomer [17]. Interestingly and confusingly, Wyman interpreted this fictional monomer inversely with the increasing discrete bindings by oxygen [the contemporary science]. If we follow how Wyman extends the concept of symmetry from the saturation function into the molecular structure, then the tetramer of hemoglobin might operate allosterically as a symmetric dimer of \( \alpha \beta \) dimer (Equation (18)) that Ackers *et al.* had suggested [16]. Because at the very symmetric point of the non-cooperative allosteric symmetry, which Wyman interpreted as a fixed \( K_x \), the two perpendicular symmetries (the dynamic symmetry and the structural symmetry) intersect and thus both \( \ell = c \) and \( \ell = c = 1 \) are satisfied by a sigmoid without cooperativity like \( L \). By transforming a group of Bohr sigmoid curves into one single symmetric monomer, MWC model lost one yet essential allosteric information, the heterotropic effect and unavoidably produced a lot of paradoxical information.

**The conformational constrains**

Bohr equation fulfills most statements of MWC model with this simplest concept of a symmetric monomer because allostery conforms to the physical law of symmetry that is universal despite MWC model’s improper interpretations of three allosteric constants. The confusing concept of conformational constraints is exactly the Bohr modifications that impose upon the identity hemoglobin, \( S^2 \) [3,12]. Consistently, the Bohr shift (and the hydrogen bond), not oxygen, creates the symmetric cooperativity of hemoglobin within the asymmetric Bohr curves (Figs. 1, 2). Thus a sigmoid shape does not always equal cooperativity and a hyperbolic curve could show cooperativity in the name of symmetry. To prove this counterviative allosteric, we should verify this model with the original Bohr experiment in 1904 [7].

**To clone the old 1904 Bohr sigmoid curves is to prove the new Bohr equation**

How to realize the 1904 Bohr shifts (\( B \)) and the allosteric coefficients quantitatively? I probed the illustrated \( P_{50} \) values in the 1904 sigmoidal curves of the underestimated Bohr experiment [7] by a ruler and placed them into the simplest \( P_{50} \) equation (Equations (20) & (21)) to determine their corresponding Bohr coefficients, \( n \) (Table 1, Fig. 3a).

\[
P_{50} = \left(10 \cdot e^x, 0.5\right)
\]

(20)

{the allosteric \( P_{50} \) equation with physiological scaling}

For a measured \( P_{50} \): \( P_{50} = 10 \cdot e^x = 10 \cdot e^{(x-1/2)} \),

then to obtain \( n \) is to solve the quadratic equation below:

\[
n^2 \left(2 + \ln\left(\frac{P_{50}}{10}\right)\right) n + 1 = 0
\]

(21)

{\( n > 1 \) for the 1904 experiment}

These original Bohr coefficients (\( n \)) ranged from 1.82 to 3.09 with the corresponding PCO values ranging from 5 mmHg to 80 mmHg (Table 1). Compatibly, Hill reported the range of Hill coefficients of hemoglobin to be from 1.67 to 3.19 in 1910 [2,3,8]. Furthermore, with these re-discovered allosteric Bohr coefficients, the Bohr equation (Equation (22)) can easily clone the original 1904 sigmoidal curves (Fig. 3a) and their Bohr shifts were algebraically quantified for the first time (Table 1). Thus, the centennial 1904 Bohr experiment justifies this allosteric Bohr equation with their embedded, symmetric \( P_{50} \) values. For its inherited symmetry, \( P_{50} \) is a precise ruler to measure the allosteric coefficients, the Hill numbers. Please note the pseudo-Hill equation of Equation (22), with \( PaO_2 \) as an orthosteric variable in the allosteric power of Bohr coefficients, is the quantitative description
of allosteric regulation that MWC model missed. Unquestionably, the physical meaning of the Bohr coefficient (n) is neither a number of oxygen binding sites nor an ortho-
steric interaction coefficient [2–4,6]. And inversely, the oxy-
hemoglobin (HbO₂) has a smaller Hill number (n=1.618) than the one of deoxyhemoglobin (Hb) (n=2.618) under a 
PCO₂ of 41.5 mmHg and a pH of 7.38 in the homeostatic 
blood (see below: Bohr function). Because the larger Bohr 
coefficient (n) means a larger dissociation constant due to a 
positive allosteric effect that favors T state and the lower 
affinity.

Bohr equation: \[ S^n = 100 \times \frac{(0.1x)^n}{(e^x + 0.1x)^n} = 100 \times \frac{x^n}{(P_{50})^n + x^n} \] (22)

The explicit logarithmic Bohr function of CO₂ in the 1904 S-curves

The central question of allosteric quantification remains unanswered. What is the physical entity of the Bohr shift
content used by Bohr in 1904? This model further
investigated the simple linear regression between the loga-
thm shifted in the 1904 sigmoid curves. Bohr admitted in the published report, “However, from a quantitative point
of view, the results were only reproducible with a relatively large error, which may be due to
great variability of the hemoglobin molecule.” [7]

And, by considering the Bohr’s outspoken statement of the
“large” laboratory errors in his 1904 experiment [7], we
could reduce Equation (23) to Equation (25) through Equa-
tion (24) for the modeling of simplicity.

\[ B = \frac{10.11}{1.04} = \log_{10}(\text{CO}_2), \] (24)

\[ B = \log_{10}(\text{CO}_2). \] (25)

\[ \text{And, } n = 1 + \log_{10}(\text{CO}_2) \] (26)

Equations (25) & (26) could be the covered gems of the 1904 Bohr experiment. Equation (25), the Bohr function, translates
the quantitative connection between the \(P_{50}\) and the CO₂ content shown in the original sigmoidal curves (Equation (16)).
Thus, the Bohr function in Bohr equation consolidates the non-orthosteric nature of the alloster in hemoglobin that is
demonstrated by the 1904 Bohr experiment [7]. This Bohr function, a natural function unappreciated by most con-
temporary scientists, should be the major contribution of the coauthor in the 1904 Bohr experiment, Karl Albert
Hasselbalch (12), who envisioned the Anderson-Hasselbalch
pH equation [9,10,12] that provided the connection between
the Bohr function and pH.

With the Bohr function (Equation (25)), the parabolic
Bohr kernel in the dissociation constant transforms into a
logarithmic Bohr model. The allosteric analysis thus changes
from a real number of Bohr shifts to a function of the CO₂
content. Next, we can justify using the Bohr function to an-
alyze the allosteric Bohr coefficient (n) by simply extends the
mathematical properties of logarithm.

### Table 1

| Lab. CO₂ | 5   | 10  | 20  | 40  | 80  |
|---------|-----|-----|-----|-----|-----|
| P_{50}^{*a} | 14.5 | 19  | 23.5| 31  | 41  |
| n^{*b}   | 1.82 | 2.18| 2.45| 2.77| 3.09|
| B^{*b}   | 0.82 | 1.18| 1.45| 1.77| 2.09|
| N^{*c}   | 0.67 | 1.39| 2.10| 3.13| 4.36|
| PCO₂(B)^{*c} | 6.6  | 15.1| 28.2| 58.9| 123 |
| Lab. Error^{*d} | (+32%) | (+51%)| (+41%)| (+47%)| (+54%)|

* a. A ruler retrieved \(P_{50}\) values directly from the sigmoid curves illustrated by Bohr in 1904 [7].
* b. B, n, and N are calculated values from the \(P_{50}\) equation (Equations (9), (10) & (20)).
* c. PCO₂(B)=10^n by reversing the Equation (25). These were the working PCO₂ that created the 1904 sigmoid curves. Bohr admitted in the published report, “However, from a quantitative point of view, the results were only reproducible with a relatively large error, which may be due to great variability of the hemoglobin molecule.” [7]
* d. Laboratory error of measurement^*=[(PCO₂(B)−Lab. CO₂)/(Lab. CO₂)]%

(Lab. CO₂: the experimental settings of PCO₂ in 1904; PCO₂(B): the calculated PCO₂ corresponding to the measured \(P_{50}\) by Equation 25; B: Bohr shift, n: Bohr coefficient, N: cooperativity number.)

![File not found](https://example.com/pcob.png)
Figure 3  (a) Mathematical restoration of all 1904 Bohr sigmoidal curves confirms the Bohr equation as the allosteric modeling of hemoglobin. The Bohr-drawn hemoglobin saturation/dissociation curves are cloned from the original $P_{50}$ values in the 1904 sigmoidal curves provided by Bohr (All allosteric numbers are listed in Table 1). Initially, we calculated $n$ with the $P_{50}$ equation and the measured $P_{50}$ values (Equation (21)). With the calculated $n$ and Bohr equation, we recreated the 1904 Bohr curves (Equation (22)). Bohr equation is perfect in curve fittings. These recreated Bohr curves are illustrated as the 1904 format with their specific Hill numbers in parallel with the CO$_2$ contents. Acceptable operational errors in a laboratory in 1904 resulted in overshooting the cooperativity number $N$ ($4.36>4$). The physiological Bohr (Hill) coefficient (under $P_{aCO_2}=40$ mmHg) coded in the 1904 experiment was 2.77 ($>2.618$) with $N$ as 3.13 ($>2.618$), and $n\neq N$. The red-dotted line represents the orthosteric symmetry, the symmetric monomer of $n=1$. It is a hyperbola corresponding to the symmetry of cooperativity and not cooperative. In vivo we should focus on the allosteric range of the Bohr shifts spanning along this $n^1$ hyperbola. The different shapes of the sigmoid and the hyperbola, which correlate to the relatively different affinities of oxygen, prove in this graph to be functionally attributed to the Bohr equation with the various Bohr coefficients calculable from the measurements of $P_{50}$. (b) Linear regression of the relation between Bohr shift ($B$) and $\log_{10}$[CO$_2$]. ([CO$_2$]: the laboratory settings of the Bohr experiment in 1904; y: Bohr shift ($B$); x: $\log_{10}$[CO$_2$]; $m=1.040\pm0.02261$ and $b=0.1092\pm0.03095$ for a linear relation: $y=mx+b$. Correlation coefficient: $r=0.99929$, Coefficient of determination: $R^2=0.9986$, Standard error=0.012, and $p<0.0001$. Statistical drawing software: Desmos® graphing calculator)
**Logarithm identity as the allosteric symmetry**

The Bohr coefficient, \( n \), by the definition of the Bohr function and the parabolic model, should reflect that cooperativity \((N)\) should cover the *limited* allosteric range \((\Delta n)\) of unity (Fig. 2). This allosteric \( N \) establishes a cooperative connection between two Bohr coefficients \((n \text{ and } n-1, B)\). Thus, we need two coupled 1904 sigmoid curves of different Bohr coefficients to describe the symmetric positive and negative allosteric transitions in RBCs in vivo because the natural compartmentalisation of the arterial and venous circulations (Fig. 4). Following the discovery of Bohr function (Equation (25)), the logarithm identity realizes physically the “two-states” of the MWC model.

\[
1 = \log_{10} 10 = \log_{10} \left( \frac{PCO_2}{(PCO_2)} \right) \left( \frac{x_a}{10} \right) \left( \frac{x_a}{a} \right)
\]

\( x_a \): the allosteric ligand

As the homeostatic example, let us consider a dissociation curve \((S^0)\) with a high Bohr coefficient of 2.618 and a Bohr shift of 1.618 \((B > 1)\). Then, the saturation curve \((S^{n-1})\) would have a low Bohr coefficient of 1.618 and a Bohr shift of 0.618 \((B < 1)\). The allosteric range between the coupled allosteric Bohr activities is exactly unity according to Equation (27). Moreover, the cooperativity \((N)\) connects these two-states sigmoid curves in a reciprocal continuum of a tenfold concentration gradient of carbon dioxide within this allosteric range in RBCs (from 4.15 mmHg-PCO\(_2\) to 41.5 mmHg-PCO\(_2\) in this physiological example with a Bohr coefficient of 2.618). Then the hemoglobin becomes an oscillator under various metabolic rates to meet the need of oxygen (Equation (28)).

\[
1 = \Delta n = \log_{10} [10] = \log_{10} \left( \frac{26.0}{2.60} \right) = \log_{10} \left( \frac{41.5}{4.15} \right)
\]

\[
= \log_{10} \left( \frac{54.0}{5.40} \right) = \log_{10} \left( \frac{58.3}{5.83} \right)
\]  

(28)

---

**Figure 4** Implicit allosteric range, allosteric constant, reversible allosteric transition and respiratory exchange ratio in the “two-states” sigmoid curves (The group of hemoglobin saturation curves shown in 1904). The Bohr effect modified the orthosteric symmetry, \(S^1 (n=1)\), to the allosteric symmetry, \(S^2 (n=2)\) and both curves are non-cooperative. The coupled Bohr coefficients circumscribe the allosteric range of hemoglobin between the negatively cooperative \(S^{n-1}\) and positively cooperative \(S^n (n=2.618)\). The allosteric range is consistently unity. The circle dots are the \(P_B\) (Bohr shift). The black dots are the \(P_{50}\). The non-cooperative \(S^2\) with Bohr coefficient of two, or the allosteric switching point at the logarithmic identity, subserves the reversible allosteric Bohr effects between \(S^{n-1}\) and \(S^n\). Note \(P_{O_2}\) is 105 mmHg-PaO\(_2\) at the position of the alveoli. This value is below the allosteric range of the Bohr coefficient at 2.732 (the black arrow), where the maximal PaO\(_2\) needs to be 117 mmHg-PaO\(_2\) (the allosteric constant shifted rightward). This finding suggests why we need the Root effect and negative Bohr effect under stress (relative hypoxia) that was demonstrated in fish [12,13]. The peripheral tissue cells non-cooperatively use 74.7 mmHg-PO\(_2\) to produce two fractions of 37.35 mmHg-PCO\(_2\) (41.5 mmHg minus 4.15 mmHg), which RBCs rebreathe cooperatively to deliver 93.6 mmHg-PaO\(_2\). This 125% bio-efficiency is inversely downgraded to an orthosteric respiratory-exchange-ratio (RER) of 79.8%. Allostery supersedes the law of mass action by a parabolic rule that is moderated by a logarithmic function. This 93.6 mmHg-PaO\(_2\) (PaO\(_2\).ac) is the dynamic equilibrium point of the allosteric constant, described *inversely* in MWC model with a value near 100, which is the ratio of \(K_T/K_R\). Here, \(K_T\neq K_R\). \((S^{n-1}, \text{saturation curve with } K_T; S^n, \text{dissociation curve with } K_R, \text{PaO}_2\).ac: PaO\(_2\) mmHg at allosteric constant)
2.618 = \log_{[4.15]} + \log_{[41.5]}

\equiv [\text{Capacity}] + B \quad (29)

{\text{The homeostatic Bohr coefficient with } \text{PaCO}_2 = 41.5 \text{ mm}}

In Equation (28), the corresponding allostery Bohr coefficients are 2.414, 2.618, 2.732 and 2.766, respectively. The buffering capacity of hemoglobin expands and telescopes, and vice versa. This explains the Root effect existing as a logarithmic variant of Bohr effect, which Runmer et al. suggested [2, 13]. By this identity of logarithm, the allostery range is constantly unity [2–4] that is already shown in the suggested [12,13]. By this identity of logarithm, the allostery logarithmic variant of Bohr effect, which Rummer et al. documented the inappropriateness of extending the orthosteric MWC model into the allostery operative. These words documented the inappropriateness of the MWC model missed the allostery Bohr effect.

In the original manuscript on the nature of allostery transition [3], there was a paragraph describing why the MWC model missed the allostery Bohr effect. “Since, again, the homotropic interactions are independent of absolute affinities, certain conditions or agents may modify the affinity of an allostery ligand without altering its interaction coefficient. This is apparently the case for the Bohr effect shown by hemoglobin: as is well known, the oxygen saturation curves obtained at different values of pH can all be superimposed by a simple, adequately chosen, change of the abscissa scale. In terms of the model, this would mean that the binding of the ‘Bohr protons’ does not alter the equilibrium between the two hypothetical states of the protein. Hence also the Bohr protons themselves would not be allostery ligand, and their own binding is not expected to be cooperative.” These words documented the inappropriateness of extending the orthosteric MWC model into the allostery search of medicines or allostery pharmacology [6,11,14,15], which compounded the large and fictional allostery constants with the small ratio of microscopic dissociation constants without an allostery definition of Hill numbers (Equation (17)) [2–4,6,10,14,15]. Thus MWC model struggled in calculating the numerical difference between the number of orthosteric binding sites and the Hill number [2,4,10]. The difference is outside the allostery range of cooperativity according to the parabolic model of cooperativity (Fig. 2) [2].

Does the orthosteric cooperativity exist in reciprocity?

Following the elucidation of the allostery symmetry, the cooperative Bohr equation in the form of a symmetric monomer is:

\[ S^* = 100 \times \frac{x^*}{K_T+x^*} = 100 \times \frac{x^*}{(P_{50})^n+x^*} \]

\[ = 100 \times \left( \frac{x}{L-c} \right)^n \equiv 100 \times \frac{\alpha}{1+\alpha} \quad (30) \]

This allostery Bohr equation (Equation (30)) is frankly different from the pharmacological Hill equation (Equation (4)) despite that equations both are sigmoidal and even describe the same homeostatic dissociation curve when \( N \) equals \( n \) at 2.618. The former has an allostery Bohr coefficient, and the latter has a homotropic Hill numbers. This is a “rabbit-duck illusion” in science. Naturally, the \( K_a \) has a duality in the ligand affinity of orthosteric receptor under the allostery regulation by the principle of reciprocity (Equation (8)). The MWC model actually reintroduced the deleted, positive Bohr effect. Within the hyperbolic symmetric monomer of Bohr equation, the methodologically lost affinity-information in the lower sigmoid of the logistic monomer, \( L^1 \), \((c=1 \text{ in Equation (30) [14]})\) trespassed on the symmetric \( P_{50} \) by disintegrating the allostery symmetry into one large allostery constant, one insignificant microscopic dissociation constant, \( K_a \), and the stray \( K_c \). Consequently, the unnatural MWC model inversely elaborated the positive Bohr effect as the positive cooperativity of oxygen binding in the allostery range of \([Q: 2<n<3]\) [3] and then lost most biophysical and physiological correlations. For example, the lost allostery range as the negative allostery \([(1-Q): 1<n<2]\) [2,4], the Equations (16) & (18) & (19) and the constant fingertip saturation with the pulse oximeter corresponds to a PaO2 that is the fixed allostery constant (\( K_a \)) that MWC model described in Equation (3) (Fig. 4). The price of allostery in pharmacology is even bigger for the unsettling issues of affinity and efficacy, the entangling effects of ligand binding and effects of conformational changes and the interpretations of mutant effect on receptor (by paraphrasing the review essay of Colquhoun [18]).

The Bohr equation is the simplest, symmetric and quantitative model showing how the feedback mechanism couples the allostery [18,19]. The magnificent trial of MWC model as a symmetric Group theory had foreseen the communicated network of allostery groups of more than twenty enzymes coupled in the glycolysis and the Krebs cycle in addition to the crowning hemoglobin, carbonic anhydrase complex and anion exchanger [12]. According to the preserved “allostery organization” proposed by Changeux [12,19], then we could treat the membrane receptors, for example GPCRs, as the allostery organization of hemoglobin linked-carbonic anhydrase-anion exchanger receptors (“HbCARs”) by following the universal physical law of symmetry in the symmetric monomer model of allostery cooperativity [6,12,19,20].
Conclusion

The allosteric symmetry, the symmetry of a hemoglobin saturation curve, could be a symmetric monomer, a sigmoid, a logistic, a hyperbola, a rectangular hyperbola, a parabola, a point and the logarithm identity in this heterotropic model of allostery. Bohr equation bases its allosteric interpretations on the physical law of symmetry to quantify the 1904 Bohr sigmoid curves [7,16]. Therefore, fractional saturation is a direct measurement of conformational change (the Bohr shift) by interpreting the allosteric hemoglobin and Bohr equation as a symmetric monomer. The allostery of hemoglobin presents the cooperative increase or decrease of orthosteric affinity as a reciprocal inverse of the reversible cooperativity that is moderated by the Bohr coefficients, a logarithm function of carbon dioxide. An allosteric force that depends cooperatively on CO₂ at a finite distance away (the carbonic anhydrase-Anion exchanger complexes) modifies hemoglobin [12,16] to change its oxygen affinity between two symmetric conformations. In vivo, the heterotropic Bohr effect determines these two conformational states. The non-cooperative allosteric symmetry, both as a dimer of dimer [17] and the identity of a logarithm function, is the identity hemoglobin that supports the reversible allosteric transition by preserving the symmetry of cooperativity within the allosteric range of unity. The orthosteric MWC model failed the biophysical symmetry by deleting the Bohr effect [3,16,21] and misused a pseudo-symmetry to replace the allosteric symmetry, the sigmoid \( S^2 \) [11,16,21]. Most of us, like Wyman, prejudiced an intuitive and orthosteric definition of Hill numbers and denied the physical meanings of \( n \) and \( K \) as the functions of heterotropic Bohr shifts. If the PTMs and allosteric modulation were indispensable for all functional proteins then the lost allosteric Bohr effect could be a reductionist evolution of the ubiquitous PTMs in RBCs for the feedback regulation of gas exchange [1,18,19]. The Bohr equation, or Hill equation with the allosteric Bohr coefficient, fulfills the cooperative mechanism within the allosteric organization of hemoglobin and carbonic anhydrase-anion exchanger complexes inside the red blood cells. By following the comparative principles laid out by Krogh, the other coauthor of the 1904 Bohr experiment, the crowning Hemoglobin again provides the quantitative model of allostery for the other allosteric proteins and generally is a universal model describing the feedback mechanism [16,18,19].

In the simplest conclusion, an allosteric hemoglobin is a mathematical fiction of symmetry monomer that swings itself reciprocally on a hyperbolic hyperbola, \( t \cdot e = 1 \), by keeping its balance at \( \log_{t}(x_0/((x_0)/a)) = 1 \).

Glossary

Allosteric: This term describes the action from a finite distant site. Synonym: Heterotropic

Bohr coefficient (\( n \)): When the allosteric Bohr equation was equal to the orthosteric Hill equation at \( N = n_{10} \), we could not differentiate whether the allosteric number was a Bohr coefficient or a Hill coefficient. This is a “rabbit-duck illusion” proposed by Wittgenstein and Kuhn [22]. Thus, I used the Bohr coefficient (\( n \)) to emphasize the allosteric difference due to the Bohr effect from the orthosteric Hill coefficient (\( n_{10} \)). Synonym: Hill number

Orthosteric: This term describes the action at the substrate site. Synonym: Homotropic

Root effect: the logarithmic variant of Bohr effect

Symmetry: A group has a set and a binary operation, *. A group conforms to the four axioms:
1. Closure. For all \( a, b \in \mathbb{G} \), \( a \cdot b \in \mathbb{G} \).
2. Associativity. For all \( a, b, c \in \mathbb{G} \), \( a \cdot (b \cdot c) = (a \cdot b) \cdot c \).
3. Identity. There exists one and only one identity, \( e \in \mathbb{G} \), such that for all \( a \in \mathbb{G} \), \( e \cdot a = a \) \( e \cdot a = a \).
4. Inverse. For all \( a \in \mathbb{G} \), there exists one and only one \( a^{-1} \in \mathbb{G} \), such that \( a \cdot a^{-1} = e \).

Conflict of Interest

The author declares no financial competing interest or non-financial competing interests.

Author Contributions

Lee Lihsin is the only author of this manuscript as an independent physician of anesthesia presently.

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