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Bohr equation and the lost allosteric Bohr effects in symmetry

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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 allostery, 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

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◀ ***Significance*** ▶

The cooperative hemoglobin is the crowning model of the versatile allosteric enzymes. The failure to model the cooperativity quantitatively since 1904 means the inevitable failures of both the allosteric quest of drugs and the discovery of the allosteric mechanism of disease. The application of the biophysical law of symmetry solved the centennial paradox incurred by the orthosteric Hill coefficient. Hemoglobin turns out to be non-orthosteric and then transforms itself from a descriptive Hill equation into a quantitative Bohr equation. The Monod-Wyman-Changeux (MWC) model erased the allosteric Bohr effects in the misused Hill equation with the prejudiced perceptiveness of cooperative oxygen binding.

[2–4]. 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 (VO_2) [5]. Nevertheless, maximal oxygen consumption (VO_2max) became another unsettled physiology [5]. Illogically, however, the Hill equation is widely used as a dynamic model in pharmacology and the receptor kinetics, where the Hill coefficient (n_H) 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 (n_H) 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 (CO_2) 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

$$K_d = \frac{K_T}{K_R} = K_T \cdot K_A \tag{1}$$

(*The basics of a saturation curve*)
K_d: *the macroscopic dissociation constant*
K_R: *the macroscopic dissociation constants for R state*
K_T: *the macroscopic dissociation constants for T state*
K_A: *the affinity constant,* $K_A = \frac{1}{K_R}$

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 alloseric constants to construct both the homotropic allostery 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 (K_d) 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 ($c = K_R/K_T \leq 1$) and a large, homotropic, allosteric constant ($L = T_0/R_0 \gg 1$). These two conjoined allosteric variables, Lc^n, preserved the spirit of a larger K_d 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 (K_d) and the potentially allosteric Bohr effect degenerated to serve two new constants in the allostery describing the orthosteric (homotropic) allostery (Equation (3)) [2,3].

$$y = \frac{x^n}{K_d + x^n} \left\{ \begin{array}{l} \text{2.1: } \textit{Hill's approximation of Bohr curves,} \\ \textit{unspecified n as a positive real number} \end{array} \right\} \tag{2}$$

$$= \frac{x^n}{K_T \cdot K_A + x^n} \left\{ \begin{array}{l} \text{2.2: } \textit{Wyman's symmetric saturation curve,} \\ \textit{n is a homotropic integer} \end{array} \right\}$$

$$= \frac{\left(\frac{x}{\sqrt[n]{K_A}}\right)^n}{K_T + \left(\frac{x}{\sqrt[n]{K_A}}\right)^n} = \frac{\left(\frac{x}{c}\right)^n}{L + \left(\frac{x}{c}\right)^n}$$

$$\left\{ \begin{array}{l} \text{2.3: } \textit{Introducing two allosteric constants,} \\ \textit{since } c \leq 1, \textit{ then } L \gg 1. \end{array} \right\}$$

$$= \frac{(x)^n}{Lc^n + (x)^n} \left\{ \begin{array}{l} \text{2.4: } \textit{MWC's homotropic allostery} \\ (\textit{page 99 of } [3]) \end{array} \right\}$$

$$= \frac{\left(\frac{x}{\ell \cdot c}\right)^n}{1 + \left(\frac{x}{\ell \cdot c}\right)^n} \equiv \frac{\alpha}{1+\alpha}$$

$$\left\{ \begin{array}{l} \text{2.5: } \textit{The making of a fictional monomer,} \\ n = 1,\ \alpha = \frac{x}{\ell c} \textit{ and } \ell \cdot c = 1 \ (\textit{Fig. } 1) \end{array} \right\}$$

$$\left\{ \begin{array}{l} (\textit{From allosteric (Bohr) } K_d \textit{ to homotropic allostery}) \\ \text{2.6: } K_d = K_T \cdot K_A = L \cdot c^n = (\ell \cdot c)^n \\ \\ \textit{let } L = K_T = \frac{T_0}{R_0} = \ell^n,\ c = \sqrt[n]{K_A} = \sqrt[n]{K_d \cdot \frac{R_0}{T_0}} = \frac{K_r}{K_t}, \\ \\ L, \ell\text{: Large and small "homotropic" allosteric constants,} \\ T_0, R_0, r.t\text{: the macro—and microscopic Two—states} \end{array} \right\}$$

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* ($= T_0/R_0 = \ell^n$) and a small *c* ($= K_R/K_T$ 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]. Franakly, 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 reciprocity (a hyperbolic symmetry of dynamic in Fig. 1) between allosteric effect and affinity, namely, $K_d=K_T\cdot K_A$ or $\ell\cdot c=1$ (Equations (1) & (2)). The dynamic symmetry that Wyman preferred is $\ell=c$ and thus $K_R=K_T$. Furthermore, the cooperativity of hemoglobin can only be orthosteric because the K_R is the dissociation and association constant simultaneously in the name of orthosteric symmetry, and $\alpha=F/K_R$ rather than $\alpha=x/(\ell\cdot c)$. 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].

$$\widehat{Y_F}=\frac{Lc\alpha(1+c\alpha)^{n-1}+\alpha(1+\alpha)^{n-1}}{L(1+c\alpha)^n+(1+\alpha)^n}\equiv\frac{\alpha}{1+\alpha}=\frac{F}{K_R+F} \qquad (3)$$

(*the saturation curve in MWC model*)

$$c=\frac{K_R}{K_T}\le 1,\ L=\frac{T_0}{R_0}\gg 1;\ \alpha=\frac{F}{K_R},$$

$\widehat{Y_F}$: *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* α^4 *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 ($L=\ell^n$), which is separated from the concept of *affinity* (the fifth statement).

Apparently, 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, $1/K_R$, and the dissociation constant, K_T in *a group of* Bohr curves (Equation (1)) by conjoining them into Lc^n 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.

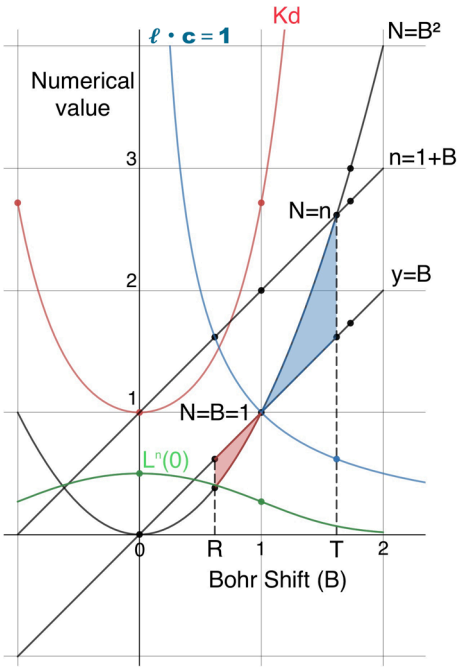


Figure 1 Crick's concern and Parabolic model of allosteric hemoglobin: orthosteric symmetry, allosteric symmetry and reversible cooperativity. The principle of reciprocity is what Crick's concerned about homotropic allostery. Allosteric activity and affinity should follows $(\ell\cdot c)=1$, a hyperbolic hyperbola (the blue curve). This demands a heterotropic definition of allosteric activity and creates this parabolic model of K_d. The K_d (red line) is a symmetric exponential function of the Bohr effect (B) and K_d defines the sigmoid shape in the saturation curve. Bohr shifts swings itself symmetrically on the hyperbolic $(\ell\cdot c)=1$ between T and R states. This proves cooperativity is symmetric. The parabolic nature of cooperativity, $N=B^2$, displays the orthosteric symmetry for $n=1$, the allosteric symmetry for $n=2$, the positively cooperative Bohr effect for $[2<n<3]$ ($N>B>1$, the blue area), a reduction in the allosteric (Hill) number for $[3<n<4]$ ($N>n$), and the negatively cooperative Bohr effect for $[1<n<2]$ ($N<B<1$, the red area). The equivalence of N and n ($N=n=2.618$) occurs at the homeostatic point with $B=1.618$ ($PCO_2=41.5$, pH=7.382). The equivalence of N and B ($N=B=1$) at the allosteric symmetry measures no cooperativity at $n=2$ (for a sigmoid S^2). The range of hemoglobin's allosteric activity (N) is bounded by n in $[0.382<n<2.618]$. The present allosteric studies could only observed the allosteric range in $[1.618<n<2.618]$ (or $[1.7<n<3.2]$ by Hill and Bohr and Q by Crick [2], the blue area) with negation of the negative allostery, ((1-Q) by Crick [2], the red area). Note that the orthosteric symmetry at n^1 ($B=0$) is different from the allosteric symmetry at n^2 ($B=1$). Theoretically, there should be another negative Bohr effect resides in the range of orthosteric symmetry $[0.382<n<1]$ to present the allosteric resistance to receptor inhibition. (ℓ: allosteric constant of monomer, c: affinity defined in Equation (B); $L^n(0)$: the measurement of sigmoidicity as a value deviated from 0.5; K_d: dissociation constant, $((e^{\frac{N}{n}})^n)$; $P_{50}=e^{\frac{N}{n}}$; N: cooperativity number; B: Bohr shift; n: Bohr coefficient; $T=1.618$ as a solution of $B^2=1+B$, $R=0.618$; T, R also present T-state and R-state; the x-axis represents the Bohr shift, B; the y-axis represents a numerical value.)

The reversed engineering of allostery 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].

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

$$Hb_n + nO_2 \overset{?}{\leftrightarrow} Hb_nO_{2n} \ \{n: \textit{unknown integer in } 1910\},$$

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

$$\left\{\begin{array}{l} \textit{The original Hill equation},\\ n\textit{: real number reported by Hill} \ [8],\\ K\textit{: aggregation constant},\\ x\textit{: } PaO_2 mmHg \end{array}\right\}$$

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 effect**s** demonstrated in 1904 (the swinging K_d on the hyperbola, $(\ell \cdot c)=1$). 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^n) and the hemoglobin saturation curve (S^n) needs further clarification. The focus is their related transformations through the dissociation constant, K_d. (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]:

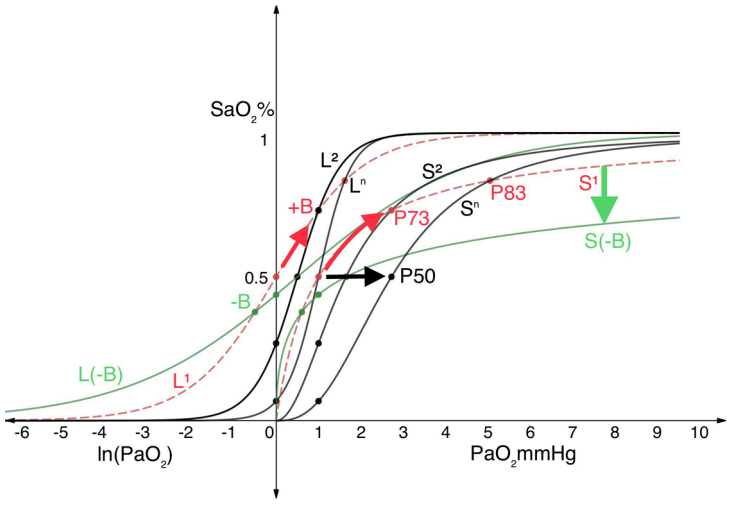


Figure 2 The sigmoidal transformations of the Bohr shifts: positive cooperativity as a sigmoid and negative cooperativity as a hyperbola. Oxygen saturation curves (S^1, S^2, S^n: n=2.618) are the exponentially invariant transformations of the logistic sigmoids (L^1, L^2, L^n). There is loss of the sigmoidal information from L^1 to hyperbolic S^1. A logistic Bohr shift (B) on L^1 would project into a hyperbolic Bohr shift point (P_B) on S^1 (the two red arrows). The P_B of S^1 (the red dot overlapping with the P_{50} of S^1) walks upward along the hyperbolic S^1curve to P_{73} and P_{83} to simultaneously exaggerate the saturation plateau and the dissociation sigmoid of the logistic curve and thus creates the S^2 and S^n. The displacement of P_{50} (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_B). In homeostasis, we have a homeostatic Bohr coefficient of $n^{2.618}$ (black sigmoidal line) with P_{50} 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_B decrease**s** hyperbolically to P_{38} (the lowest green dot on S^1) without sigmoidicity. Note that S^2 is the allosteric translation of the orthosteric S^1, and both the hyperbolic S^1 and sigmoid S^2 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_{73}: P_B of n^2; P_{83}: P_B of $n^{2.618}$.)

$$S^{n_b} = \frac{x^{n_b}}{K_d + x^{n_b}} \tag{4}$$

$$\left\{ \begin{array}{l} S\text{: } saturatin\ sigmoid\text{; } x\text{: } PaO_2\text{; } K_d = (P_{50})^{n_b}, \\ n_b \in positive\ integer\ (number\ of\ oxygen\ binding\ sites), \\ P_{50} = the\ PaO_2\ at\ 50\%\ O_2\ saturation. \end{array} \right\}$$

This model derives a logistic sigmoid equation (L^n) that could be symmetric when $n=1$:

$$L^n = \frac{e^{nx}}{(e^B)^{(n-1)} + e^{nx}} \tag{5}$$

$$\left\{ \begin{array}{l} Logistic\ sigmoid, \\ K_d = (K)^{(n-1)},\ K = e^B, \\ n\text{: } Bohr\ coefficient,\ B\text{: } Bohr\ shift,\ x\text{: } ln(PaO_2) \end{array} \right\}$$

$$For\ n=1,\ L^1 = \frac{e^x}{1+e^x}\ vs.\ Hill\ L^1 = \frac{e^x}{K_R + e^x} \tag{6}$$

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

In this logistic sigmoid equation L^n (Equation (5)), K_d 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_R [5,8]. This demanded MWC model to reconstruct a symmetrical dynamic by introducing "a mathematical fiction of equivalent monomer" (Equations (2) & (3)) [3,10].

$$S^1 = \frac{x}{1+x}\ vs.\ Hill\ S^1 = \frac{x}{K_R + x}$$

$$vs.\ the\ fictional\ symmetry\ S^\alpha = \frac{\alpha}{1+\alpha} \tag{7}$$

$$\left\{ \alpha = \frac{x}{K_R} \right\}$$

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^α) 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^α, 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^n 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_d 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_d). By following Wyman's trial to build a symmetric kernel in K_d ($K_R = K_T$ by Wyman) to describe *the symmetry of a saturation curve* (L^1 or S^α), let us outsource the allostery to the deleted Bohr effect reclaimed in the symmetric sigmoid, L^n (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:

$$\ell \cdot c = 1 \tag{8}$$

$$\{Crick's\ concern\ about\ allostery\ [2]\}$$

Logically, the definition of allosteric activities 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.

$$B = n - 1 \tag{9}$$

$$B(n-1) = B^2 = N \tag{10}$$

$$K_d = e^{B^2} = e^N = \left(e^{\frac{N}{n}}\right)^n = (P_{50})^n \tag{11}$$

$$\left\{\begin{array}{l} \textit{Parabolic model of } K_d. \\ n\textit{: Bohr coefficient}, 0<n<3, \\ B\textit{: Bohr shift}, -1<B<2, \\ N\textit{: cooperativity number}, 0<N<4, \\ B \in \textit{real numbers} \end{array}\right\}$$

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_R=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 undividable symmetry of cooperativity (K_d), 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).

$$K_d = e^N = \left(e^{\frac{N}{n}}\right)^n = \left(e^{\frac{N_1+N_2}{n}}\right)^n = \left(e^{N_1}\right)\left(e^{\frac{N_2}{n}}\right)^n = L \cdot c^n \tag{12}$$

$$\left\{\begin{array}{l} \textit{Parabolic model explains the MWC model.} \\ n\textit{: positive integer}, L = e^{N_1}, c = e^{\frac{N_2}{n}}, \\ N = N_1 + N_2, \\ N, N_1, N_2 \in \textit{indefinable real numbers} \end{array}\right\}$$

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

$$S^n = \frac{x^n}{e^{B(n-1)}+x^n} = \frac{x^n}{e^{B^2}+x^n} = \frac{x^n}{e^N+x^n} = \frac{x^n}{\left(e^{\frac{N}{n}}\right)^n+x^n} \tag{13}$$

{*Saturation Sigmoid*: *Bohr equation*}

$$L^n = \frac{e^{nx}}{(e^B)^{(n-1)}+e^{nx}} = \frac{e^{nx}}{e^N+e^{nx}}$$

{*Logistic Sigmoid*}

This Bohr equation (S^n) 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:

$$\textit{Bohr shift} = \left(B, \frac{e^B}{1+e^B}\right) \textit{ on the symmetric monomer},$$
$$S^\alpha = \frac{\alpha}{1+\alpha} \tag{14}$$

{*Bohr shift on the logistic L^1 curve*; $-1<B<2$}

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

$$P_B = \left(e^B, \frac{e^B}{1+e^B}\right) \textit{ on the symmetric monomer},$$
$$S^\alpha = \frac{\alpha}{1+\alpha} \tag{15}$$

{*Bohr shift departs from the P_{50} on the hyperbolic S^1 curve.*}

This P_B of S^n moves from P_{50} of n^1 through P_{73} of n^2 to P_{83} of $n^{2.618}$ on the hyperbolic S^1-curve (Fig. 2). Thus, these Bohr shifts walk hyperbolically upward on S^1 to create their specific sigmoidicity (Equation (11)) and the allosteric conformation. And the saturation plateau of S^n exaggerates the upper sigmoid of the logistic curve, L^n, exponentially and visually. Therefore, on the symmetric monomer (S^1, L^1, and in general, S^α) two equilibriums coexist at the orthosteric symmetry. One is the P_{50} and the other is the lost Bohr shift point (P_B). For an allosteric action of CO_2, the P_{50} exhibits a rightward shift (the bold-black arrow in Fig. 2) while P_B 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}.

$$P_{50} = \left(e^{\frac{N}{n}}, 0.5\right) \tag{16}$$

$$\left\{ \begin{array}{c} \textit{The quantitative Bohr effect,} \\ n = 1 + B, N = B^2 \end{array} \right\}$$

The orthosteric Hill equation by MWC model divided this allosteric P_{50} and used *part of it* as the fixed, orthosteric K_R (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_B and P_{50} in Equations (15) & (16) [2–4,8,10,11]. The divided and fictionally fixed K_R 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 Equatioin (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 allostric (|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=B) 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 [6]. 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].

$$\bar{Y}_s = \frac{\alpha(1+\alpha)^{n-1}}{L\frac{(1+\beta)^n}{(1+\gamma)^n} + (1+\alpha)^n} \tag{17}$$

$$\left\{ \begin{array}{l} \bar{Y}_s\textit{: saturation function,} \\ \alpha = \dfrac{F}{K_R}, \beta = \dfrac{I}{K_I}, \gamma = \dfrac{A}{K_A}, \boldsymbol{c = 1}, n = 4, L = 10^{(n-1)}, \\ K_1 \textit{ and } K_A\textit{: the microscopic dissociation constant} \\ \textit{of inhibitor } (I) \textit{ and activator } (A) \textit{ with the two states} \end{array} \right\}$$

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_R 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.

$$S^2 = \frac{x^2}{\left(e^{\frac{1}{2}}\right)^2 + x^2} \equiv \frac{\alpha}{1+\alpha}\left(\neq \frac{F}{K_R + F}\ (\textit{Equation } (3))\right) \tag{18}$$

$$\left\{\alpha = \left(\frac{x}{\sqrt{e}}\right)^2 = \left(\frac{x}{P_{50}}\right)^2 \neq \frac{F}{K_R}, K_d = \frac{K_T}{K_R} = e\right\}$$

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

not be used as a "real" monomer with an allosteic constant, L and a homotropic 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} under the heterotropic 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 consistenly 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^1. 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)).

$$S^n = \frac{x^n}{K_d + x^n} \equiv S^\alpha = \frac{\alpha}{1+\alpha} \equiv \frac{\left(\frac{x}{P_{50}}\right)^n}{1+\left(\frac{x}{P_{50}}\right)^n} \tag{19}$$

$$\left\{ \begin{array}{l} \textit{Bohr equation as a symmetric monomer,} \\ \quad \textit{If } x = P_B = e^B, \\ \quad \textit{then } \left(\frac{x}{P_{50}}\right)^n = \left(\frac{P_B}{P_{50}}\right)^n = \left(e^{\frac{B}{1+B}}\right)^n = \alpha, \\ \quad N = B^2,\ K_d = e^N, \\ \quad n\textit{: Bohr coefficients} \end{array} \right\}$$

Structure-function relation

This Bohr equation with the parabolic kernel of K_d 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 αβ 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_R, the two perpendicular symmetries (the dynamic symmetry and the structural symmetry) intersect and thus both $\ell = c$ and $\ell \cdot c = 1$ are satisfied by a sigmoid without cooperativity like L^1. 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 counterintuitive allostery, 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).

$$P_{50} = \left(10 \cdot e^{\frac{N}{n}}, 0.5\right) \tag{20}$$

{*the allosteric P_{50} equation with physiological scaling*}

For a measured P_{50}: $P_{50} = 10 \cdot e^{\frac{N}{n}} = 10 \cdot e^{\frac{(n-1)^2}{n}}$,
then to obtain n is to solve the quadratic equation below:

$$n^2 - \left(2 + ln\left(\frac{P_{50}}{10}\right)\right)n + 1 = 0 \tag{21}$$

$\{n > 1$ *for the 1904 experiment*$\}$

These original Bohr coefficients (n) ranged from 1.82 to 3.09 with the corresponding PCO_2 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

Table 1 Allosteric Bohr coefficients (*n*, Hill numbers) calculated from the embedded P_{50s} in the second figure of the 1904 Bohr experiment with the original CO_2 concentrations [7]

Lab. CO_2	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*[b]	0.67	1.39	2.10	3.13	4.36
$PCO_2(B)$*[c]	6.6	15.1	28.2	58.9	123
Lab. Error*[d]	(+32%)	(+51%)	(+41%)	(+47%)	(+54%)
$Log_{10}[CO_2]_{Lab}$	0.70	1	1.30	1.60	1.90

*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. $PCO_2(B)=10^B$ by reversing the Equation (25). These were the working PCO_2 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[4] = $[(PCO_2(B) - \text{Lab. } CO_2)/(\text{Lab. } CO_2)]\%$.
(Lab. CO_2: the experimental settings of PCO_2 in 1904; $PCO_2(B)$: the calculated PCO_2 corresponding to the measured P_{50} (by Equation 25); B: Bohr shift, n: Bohr coefficient, N: cooperativity number.)

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 orthosteric interaction coefficient [2–4,6]. And inversely, the oxyhemoglobin ($Hb(O_2)_4$) has a smaller Hill number (n=1.618) than the one of deoxyhemoglobin (Hb) (n=2.618) under a PCO_2 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.

$$\textit{Bohr equation}: S^n = 100 \times \frac{(0.1x)^n}{\left(e^{\frac{N}{n}}\right)^n + (0.1x)^n} = 100 \times \frac{x^n}{\left(P_{50}\right)^n + x^n} \quad (22)$$

The explicit logarithmic Bohr function of CO_2 in the 1904 *S*-curves

The central question of allosteric quantification remains unanswered. What is the physical entity of the Bohr shift defined as Bohr coefficient minus one ($B=n-1$)? Or what is the quantitative relationship between the Bohr shift (B) and the CO_2 content used by Bohr in 1904? This model further investigated the simple linear regression between the logarithm of the contents of CO_2 set by Bohr in 1904 and the Bohr shift (B) listed in Table 1. The Equation (23) shows the statistically significant correlation, the lost result of the 1904 Bohr experiment ($p<0.0001$, Statistical details are shown in the legend of Fig. 3b).

$$B = 0.11 + 1.04 \cdot log_{10}\,[CO_2] \quad (23)$$

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 Equation (24) for the modeling of simplicity.

$$\left(\frac{B-0.11}{1.04}\right) = log_{10}[CO_2], \quad (24)$$

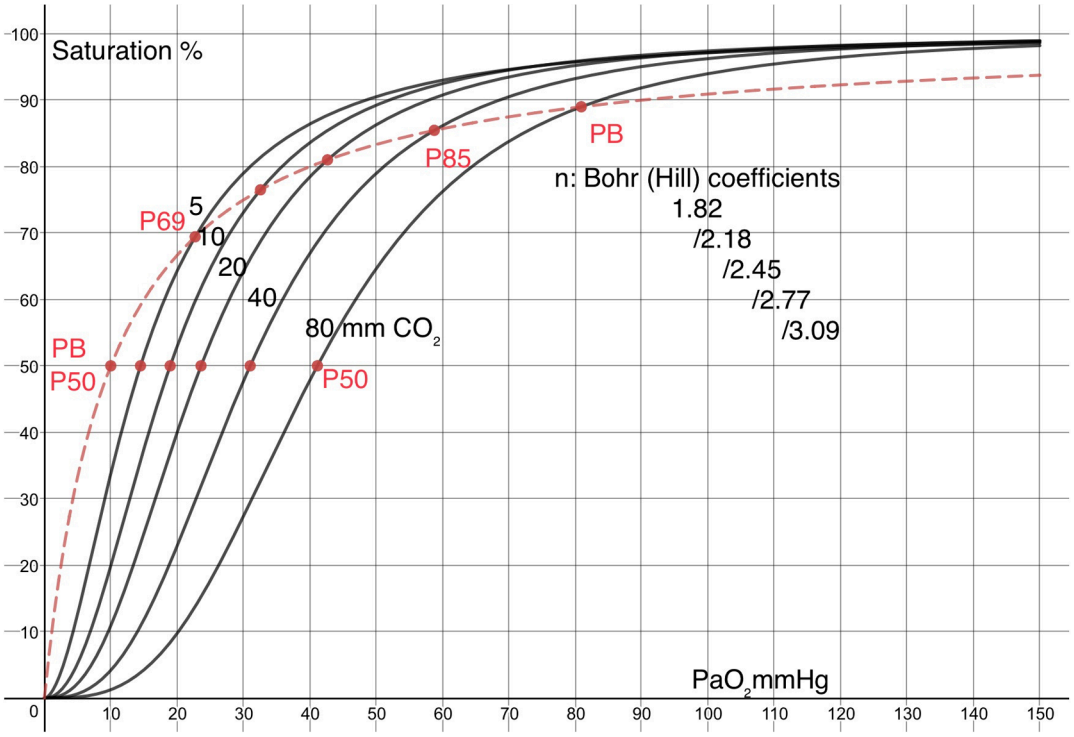
$$B = log_{10}[CO_2], \quad (25)$$

$$\textit{And, } n = 1 + log_{10}[CO_2] \quad (26)$$

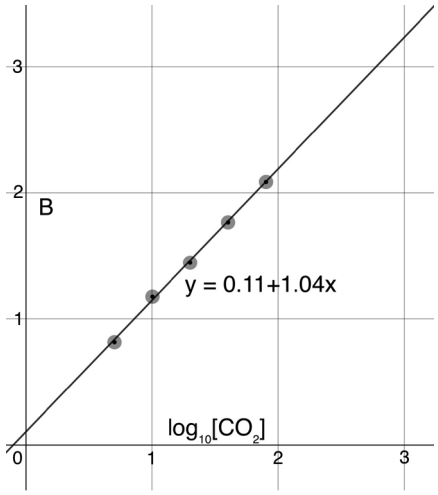
$$\left\{ \begin{array}{l} [1\ mmHg < [CO_2] < 100\ mmHg], \\ B\textit{: Bohr shift}, 0 < B < 2, \\ n\textit{: Bohr coefficient or Hill number.} \end{array} \right\}$$

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 CO_2 content shown in the original sigmoidal curves (Equation (16)). Thus, the Bohr function in Bohr equation consolidates the non-orthosteric nature of the allostery in hemoglobin that is demonstrated by the 1904 Bohr experiment [7]. This Bohr function, a natural function unappreciated by most contemporary 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 CO_2 content. Next, we can justify using the Bohr function to analyze the allosteric Bohr coefficient (n) by simply extends the mathematical properties of logarithm.



(a)



(b)

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 $PaCO_2$=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±0.02261 and b=0.1092±0.03095 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 (Δn) of unity (Fig. 2). This allosteric N establishes a cooperative connection between two Bohr coefficients (n 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 compartmentalisations 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.

$$1 = \log_{10}10 = \log_{10}\frac{PCO_2}{\left(\frac{PCO_2}{10}\right)}\left(= \log_a \frac{[x_a]}{\left(\frac{[x_a]}{a}\right)}\right) \quad (27)$$

$\{x_a$: *the allosteric ligand*$\}$

As the homeostatic example, let us consider a dissociation curve (S^n) 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)).

$$1 = \Delta n = \log_{10}[10] = \log_{10}\frac{[26.0]}{[2.60]} = \log_{10}\frac{[41.5]}{[4.15]}$$
$$= \log_{10}\frac{[54.0]}{[5.40]} = \log_{10}\frac{[58.3]}{[5.83]} \quad (28)$$

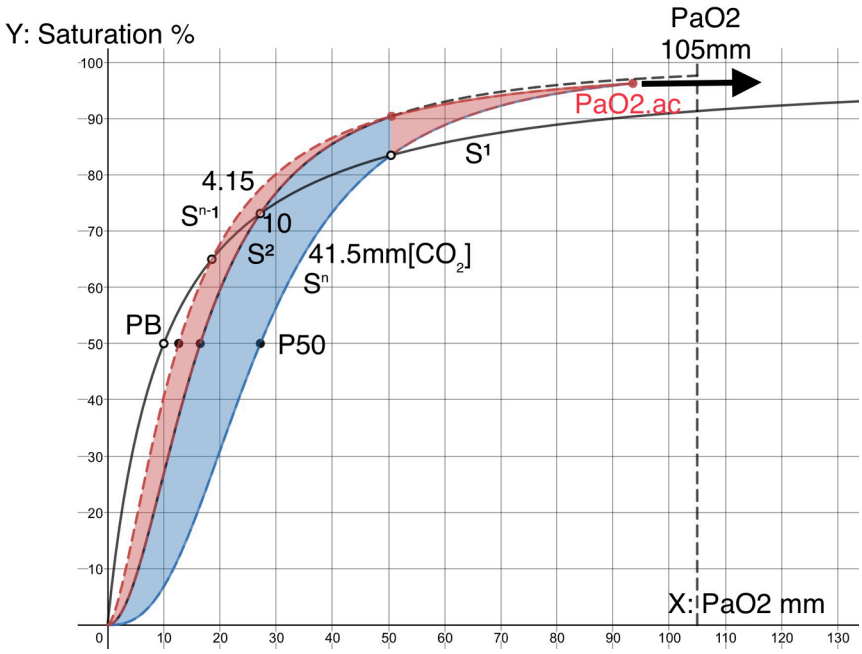


Figure 4 Implicit allosteric range, allosteric constant, reversible allosteric transition and respiratory exchange ratio in the "two-states" sigmoid curves (The group of hemoglobin saturation curves shown in 1904). The Bohr effect modified the orthosteric symmetry, S^1 (n=1), to the allosteric symmetry, 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_AO_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 (PaO2.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. K_T/K_R is larger than 1. And $K_T \neq K_R$. (S^{n-1}, saturation curve with K_R; S^n, dissociation curve with K_T, PaO2.ac: PaO_2 mmHg at allosteric constant)

$$2.618 = \log_{10}\frac{[41.5]}{[4.15]} + \log_{10}[41.5]$$
$$\equiv [Capacity] + B \quad (29)$$

{*The homeostatic Bohr coefficient with* $PaCO_2$ = 41.5 *mm*}

In Equation (28), the corresponding allosteric 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 Rummer *et al.* suggested [12,13]. By this identity of logarithm, the allosteric range is constantly unity [2–4] that is already shown in the parabolic model of dissociation constant (Fig. 2). Crick coined and quantified the term "allosteric range" (Q) in his footnote on allostery [2]. The Equations (28) & (29) imply that the allosteric symmetry is the logarithm identity. And there are numerous two-states corresponding to different metabolic rates. As for a specific metabolic rate, there exists one and only one symmetric two-states (Fig. 4).

The Bohr effect lost in the MWC model

In the original manuscript on the nature of allosteric transition [3], there was a paragraph describing why the MWC model missed the allosteric Bohr effect. "Since, again, the homotropic interactions are independent of absolute affinities, certain conditions or agents may modify the affinity of an allosteric 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 *p*H 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 allosteric ligand, and their own binding is not expected to be cooperative." These words documented the inappropriateness of extending the orthosteric MWC model into the allosteric search of medicines or allosteric pharmacology [6,11,14,15], which compounded the large and fictional allosteric constants with the small ratio of microscopic dissociation constants without an allosteric 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 allosteric 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 allosteric symmetry, the *cooperative* Bohr equation in the form of a symmetric monomer is:

$$S^n = 100 \times \frac{x^n}{K_d + x^n} = 100 \times \frac{x^n}{(P_{50})^n + x^n}$$
$$= 100 \times \frac{\left(\frac{x}{\ell \cdot c}\right)^n}{1 + \left(\frac{x}{\ell \cdot c}\right)^n} \equiv 100 \times \frac{\alpha}{1+\alpha} \quad (30)$$

This allosteric 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 allosteric Bohr coefficient, and the latter has a homotropic Hill numbers. This is a "rabbit-duck illusion" in science. Naturally, the K_d has a duality in the ligand affinity of orthosteric receptor under the allosteric 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 in Equation (30) [14]) trespassed on the symmetric P_{50} by disintegrating the allosteric symmetry into one large allosteric constant, one insignificant microscopic dissociation constant, K_T, and the stray K_R. Consequently, the unnatural MWC model inversely elaborated the positive Bohr effect as the positive cooperativity of oxygen binding in the allosteric range of [Q: $2<n<3$] [3] and then lost most biophysical and physiological correlations. For example, the lost allosteric 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 oxymeter corresponds to a PaO_2 that is the fixed allosteric constant (K_R) 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 allosteric 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 "allosteric organization" proposed by Changeux [12,19], then we could treat the membrane receptors, for example GPCRs, as the allosteric organization of *hemoglobin linked-carbonic anhydrase-anion exchanger receptors* ("HbCARs") by following the universal physical law of symmetry in the symmetric monomer model of allosteric 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 CO_2 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 transitions 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, $\ell \cdot c=1$, by keeping its balance at $log_a([x_a]/([x_a]/a))=1$.

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 $N=n_H$, 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_H). 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∈G, a*b∈G.***
2. Associativity. ***For all a, b, c∈G, a*(b*c)=(a*b)*c.***
3. Identity. ***There exists one and only one identity, e∈G, such that for all a∈G, e*a=a*e=a***
4. Inverse. ***For all a∈G,there exists one and only one a^{-1}∈G, such that $a*a^{-1}=e=a^{-1}*a$***

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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