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A MATHEMATICAL ANALYSIS OF COOPERATIVITY AND FRACTIONAL SATURATION OF OXYGEN IN HEMOGLOBIN

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Abstract. Hemoglobin (Hb) possesses good properties of cooperative system and it normally executes oxygen and other essential items via erythrocytes in the body. The chemical action of Hb is to combine with oxygen (O_2) in the lungs to form oxyhemoglobin (HbO_2). Binding of oxygen with a hemoglobin is one of the important cooperative mechanism and is an emerging mathematical research area with wide range of applications in biomedical engineering and medical physiology. To this end, a mathematical model is proposed to study the fractional saturation of oxygen in hemoglobin to understand the binding effect and its stability at various stages. The mathematical formulation is based on the system of ordinary differential equations together with rate equations under different association and dissociation rate constants. The five states of the cooperative systems $Hb, HbO_2, Hb(O_2)_2, Hb(O_2)_3$ and $Hb(O_2)_4$ are modelled and the Hill's function has been used to approximate the binding effect and saturation of ligand (O_2) with respect to various rate constants. Also, the Adair equation has been employed to interpret the saturation concentrations of oxygen in hemoglobin.

1. Introduction

In a biological system the interaction and intricate association between macromolecules is an established fact. This meticulous interaction between different ligands with their respective receptor molecules determine the fate of most cellular processes which decides reaction, adjustment and conduct of basic functions in all living organisms [12, 13]. The well known example in biological system is interaction between hemoglobin with its four binding sites for oxygen [2,3,17]. The ligand oxygen binds to the four binding sites of hemoglobin molecule and the interaction can be seen on the overall binding curve [18]. This is a striking example of allosteric

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binding. The binding curve of hemoglobin when associated with oxygen molecules gives sharp sigmoidal curve which indicates influence of oxygen molecules over the functionality of hemoglobin. The inference which could be drawn from the sigmoid binding curve indicates that the extremal states of full and zero saturation are more stable than the intermediate states of partial saturation [2, 17, 31]. A general kinetic model, presages an unanticipated multiplicative boost in affinity as a function of ligand sites. Modeling of this interaction by a foremost depart time approach denotes that the probability of ligand rebinding increases exponentially with the number of sites [30]. A few small single-dentate molecules when bind to a large polydentate molecule such that affinity of oxygen for binding interactions increases, it arises the cooperativity [14, 23]. In cooperative enzymes, low and high affinity substrate binding sites are present, and the cooperative binding of substrate to enzyme can take place. The binding of one substrate molecule induces structural and/or electronic changes that result in altered substance binding affinities in the remaining vacant site. As there is no straightforward relationship between macroscopic and microscopic binding behavior, a mathematical model has been developed to create a bridge between them. The model considered the minimal interaction essential to produce fixed overall binding curve [20]. The whole binding cascade of human hemoglobin corresponds of a series of partly ligated intermediates. The discrete intervening constants cannot be differentiated in O_2 binding curves. The characterization of these O_2 binding constants has shown the Hb cascade to be unbalanced in nature, with binding dependent upon the particular distribution of O_2 among the four heme sites. The kinetic constant noticed for the dissociation of this intervening O_2 binding constant confirms the value for its equilibrium [19]. The rationale behind the current study was primarily to understand the fractional saturation of oxygen in hemoglobin under various rate constants. Moreover, the cooperative property of hemoglobin has been exhaustively discussed using basic mathematical tools.

2. Materials and Methods

Cooperativity is a fundamental specificity of various biochemical systems [24]. It was Archibald Hill [1, 16] who first analysed the cooperativity binding of oxygen by hemoglobin and postulated that several (n) oxygen molecules bind simultaneously to a hemoglobin molecule:

$$Hb_4 + nO_2 \rightleftharpoons Hb_4O_{2n} \tag{1}$$

The expression for the association constant becomes

$$K_a = \frac{[Hb_4O_{2n}]}{[Hb_4][O_2]^n}, \tag{2}$$

and the binding equilibrium from the stand point of the fraction, Y, of oxygen binding sites on the hemoglobin that are occupied by ligand:

$$Y = \frac{[O_2]^n}{[O_2]^n + K_d}. \tag{3}$$

This Hill equation delineates the sigmoidal binding curves for hemoglobin as shown in Figure 1. The value of n is known as the Hill coefficient. The value of n is not always an integer. Among binding sites, for cooperative binding, the Hill coefficients settled provide a fertile measure of the Gibbs free energy of interaction and their values are independent of the free energy of association for empty sites [5]. The values of the Hill equation parameters also depend on hemoglobin concentration and shows that at high concentration of hemoglobin, the visible Hill coefficient, n, decreases and the binding affinity, k, increases [27]. The Hill's equation has been used to approximate binding effect and saturation of oxygen under various rate constants. The utility of Adair equation helped us to illustrate the saturation of oxygen concentration in hemoglobin. Many enzymes are composed of distinct subunits (oligomers), each bearing an equivalent catalytic site. If the sites are identical and dependent of each other, the presence of substrate at one site effects on substrate binding and catalytic properties at other sites, this process is known as cooperative system. [21]. We considered an oligomeric cooperative system, a cooperative tetramer (hemoglobin) in which we have discussed fractional saturation of hemoglobin at various states under variable rate constants.

2.1. **Some Basic Definitions.**

Definition 1. *A ligand is a substance that binds to a target molecule to serve a given purpose.*

Definition 2. *Allosteric enzymes are the enzymes that change their conformational ensemble upon binding of an effector which results in an apparent change in binding affinity at a different ligand binding site.*

Definition 3. *An oligomer is a protein consisting of many sub-units. It may be dimer, trimer, tetramer and so on, according to the number of subunits.*

Definition 4. *The fractional saturation of O_2 is defined as $Y(O_2)$ = number of occupied binding sites/total number of binding sites.*

2.2. **Mathematical Formulation.**

We shall first consider the theory for a hemoglobin molecule consisting of four protomers, each containing one active centre. Active sites are assumed to be independent of each other in their interaction with the molecule of an oxygen (substrate). The individual reactions of oxygen with hemoglobin are as follows,

$$o_2 + c_j \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} c_{j+1}; \quad j = 0, 1, 2, 3. \tag{4}$$

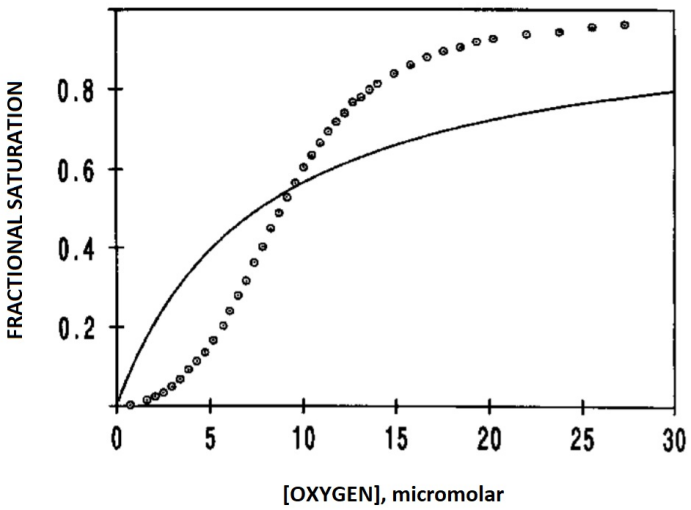

FIGURE 1. Sigmoid- Shaped hemoglobin oxygen- binding curve. These data were measured at pH 7.4 , 21.5^0 , 0.1 M NaCl, 0.1 M Tris, 1.0 mM Na_2EDTA, and a hemoglobin A concentration of 382.5μ M (heme). Also shown is the least-squares estimated hyperbola based on the Hufer model.

where c_j are the complex of the hemoglobin combined with oxygen molecules (j runs from 0 to 4) and the rate constant for binding the oxygen to a particular site of the hemoglobin are denoted by k_{+i} for association and k_{-i} for dissociation, i = 1,2,3,4.

Alternative representation of (4) of the reactions [21], we shall introduce Figure 2 below which has a one - one correspondence with the rate equations for the concentrations of c_j.

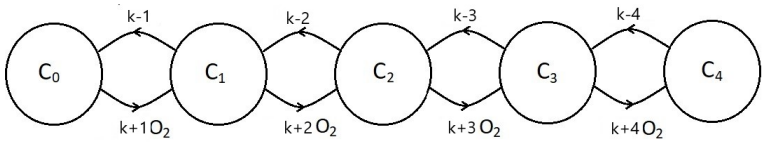

FIGURE 2. Schematic graphical representation of hemoglobin states.

From the Figure 2, it is obvious to see that, there are four unoccupied sites in the state c_0 and the rate constant from c_0 to c_1 is k_{+1}. Also from c_1 to c_0 , dissociation rate constant is k_{-1}. Similarly, the rate constant from c_3 to c_4 is k_{+4} and from c_4 to c_3, the dissociation rate constant is k_{-4}.

By deriving the above process, the rate equations (in lower case letters) are defined as:

$$o_2 + c_0 \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} c_1, \tag{5}$$

$$o_2 + c_1 \underset{k_{-2}}{\overset{k_{+2}}{\rightleftharpoons}} c_2, \tag{6}$$

$$o_2 + c_2 \underset{k_{-3}}{\overset{k_{+3}}{\rightleftharpoons}} c_3, \tag{7}$$

$$o_2 + c_3 \underset{k_{-4}}{\overset{k_{+4}}{\rightleftharpoons}} c_4. \tag{8}$$

The differential equations corresponding to the above reactions are as follows:

$$\frac{dc_0}{dt} = -k_{+1}o_2c_0 + k_{-1}c_1, \tag{9}$$

$$\frac{dc_1}{dt} = k_{+1}o_2c_0 - k_{-1}c_1 - k_{+2}o_2c_1 + k_{-2}c_2, \tag{10}$$

$$\frac{dc_2}{dt} = k_{+2}o_2c_1 - k_{-2}c_2 - k_{+3}o_2c_2 + k_{-3}c_3, \tag{11}$$

$$\frac{dc_3}{dt} = k_{+3}o_2c_2 - k_{-3}c_3 - k_{+4}o_2c_3 + k_{-4}c_4, \tag{12}$$

$$\frac{dc_4}{dt} = k_{+4}o_2c_3 - k_{-4}c_4. \tag{13}$$

2.3. **Solution of the Model.**

To estimate the concentration of O_2 at various states and subsequent changes of complexes in different states, it is important to compute the values of Eq.(9) - Eq.(13) at steady state points. Now the steady state values of Eq.(13) are given by

$$\frac{dc_4}{dt} = 0.$$

In the equilibrium model, the substrate-binding step is considered to be rapid comparative to the rate of breakdown of the ES complex. Therefore, the substrate binding reaction is considered to be at equilibrium and depends on rate constants [22, 25]. Similarly, in this case, oxygen binding reaction is assumed to be at equilibrium.

Define the equilibrium constant as $k_{di} = \frac{k_{-i}}{k_{+i}}$, the dissociation constant.

It follows that

$$k_{+4} o_2 c_3 = k_{-4} c_4,$$

$$\Rightarrow c_4 = \frac{k_4}{k_{-4}} o_2 c_3. \tag{14}$$

Note that k_{di} has dimensions of oxygen concentration, so $\frac{o_2}{k_{di}}$ is dimensionless. Going next to (12) and setting $\frac{dc_3}{dt} = 0$, it follows that

$$c_3 = \frac{k_3}{k_{-3}} o_2 c_2. \tag{15}$$

Proceeding in this fashion, we find that

$$c_2 = \frac{k_2}{k_{-2}} o_2 c_1, \tag{16}$$

and

$$c_1 = \frac{k_1}{k_{-1}} o_2 c_0. \tag{17}$$

By combining the equations(14), (15), (16)and (17) we see that all the equilibrium values of $c_j; j \geq 1$ may be expressed in terms of c_0 in a regular fashion.
Thus,

$$c_1 = \frac{1}{k_{d1}} [o_2][c_0], \tag{18}$$

$$c_2 = \frac{1}{k_{d1} k_{d2}} [o_2]^2 [c_0], \tag{19}$$

$$c_3 = \frac{1}{k_{d1} k_{d2} k_{d3}} [o_2]^3 [c_0], \tag{20}$$

$$c_4 = \frac{1}{k_{d1} k_{d2} k_{d3} k_{d4}} [o_2]^4 [c_0]. \tag{21}$$

The complexes c_1, c_2, c_3 and c_4 occupy oxygen sites partially with an ascending behaviour until they fully saturate, therefore the saturation function $Y(o_2)$ can be computed as [21, 25]:

$$Y(o_2) = \frac{c_1 + 2c_2 + 3c_3 + 4c_4}{4(c_0 + c_1 + c_2 + c_3 + c_4)} \tag{22}$$

using the concentration levels obtained in equations (18)-(21) and from Eq.(22), we have

$$Y(o_2) = \frac{\frac{[o_2]}{k_{d1}} + 2\frac{[o_2]^2}{k_{d1}k_{d2}} + 3\frac{[o_2]^3}{k_{d1}k_{d2}k_{d3}} + 4\frac{[o_2]^4}{k_{d1}k_{d2}k_{d3}k_{d4}}}{4(1 + \frac{[o_2]}{k_{d1}} + \frac{[o_2]^2}{k_{d1}k_{d2}} + \frac{[o_2]^3}{k_{d1}k_{d2}k_{d3}} + \frac{[o_2]^4}{k_{d1}k_{d2}k_{d3}k_{d4}})} \tag{23}$$

Eq.(23) is known as Adair equation for 4 sites.

The graphs of Y with respect to O_2 gives a sharp sigmoidal curve (see Fig.3) which indicates influence of oxygen molecules over the functionality of hemoglobin i.e; it increases the affinity for oxygen molecules. When the affinities of the later binding events are fundamentally greater than those of the previous events. This is called as positive cooperativity. For positive cooperativity ($k_4 \ll k_3, k_2, k_1$), the concentration of c_1, c_2, c_3 are small compared to the concentration of c_4. Thus, if these terms are omitted from Eq.(22), so Eq.(23) becomes,

$$Y(o_2) = \frac{4\frac{[o_2]^4}{k_{d1}k_{d2}k_{d3}k_{d4}}}{4(1 + \frac{[o_2]^4}{k_{d1}k_{d2}k_{d3}k_{d4}})} \tag{24}$$

$$= \frac{[o_2]^4}{\alpha^4 + [o_2]^4} \tag{25}$$

where $\alpha^4 = k_{d1}k_{d2}k_{d3}k_{d4}$

The Eq.(25) is formalised in the Hill function as in Eq.(3),

$$Y = \frac{[o_2]^n}{\alpha^n + [o_2]^n} \tag{26}$$

Eq.(26) is utilized to depict measures that include multiple near-simultaneous binding events. The constant α is the half-saturating concentration of ligand, and thus can be interpreted as an averaged dissociation constant.

It is trivial that in hemoglobin, the partial pressure of oxygen, pO_2 is the concentration of free oxygen $[O_2]$. Then $\alpha^4 = (p_{50})^4$, where p_{50} is the value of po_2 when half of the oxygen-binding sites are occupied. Making these replacements and taking logarithms, Eq.(25) may be revised to yield,

$$\log \frac{Y(o_2)}{1 - Y(o_2)} = 4\log[po_2] - 4\log p_{50}. \tag{27}$$

So, if hemoglobin bound all four oxygen molecules in a single step, then a plot of $\log \frac{Y}{1-Y}$ versus $\log[po_2]$ would be a straight line with a slope of n = 4 (called a Hill plot) and an intercept on the $\log[po_2]$ axis of $\log p_{50}$ (see Figure 4). We can also write, if $n = 1$ in Eq. (26), then it becomes the same result, if the protein is a monomer and Hill function reduces to hyperbolic function [4]. There are few

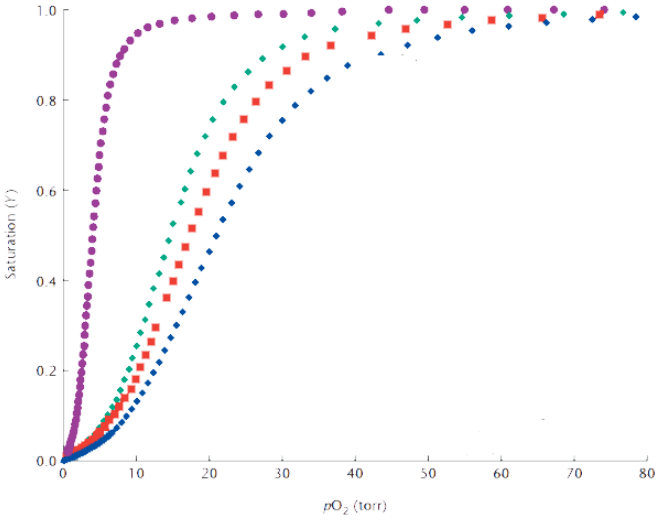

FIGURE 3. Relationship between the partial pressure of oxygen (pO_2) and percentage saturation of the hemoglobin with oxygen.

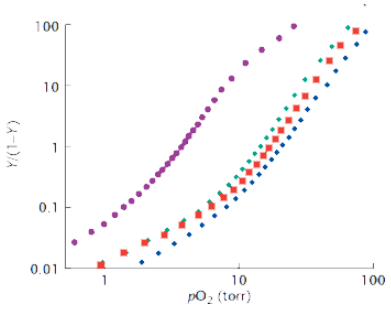

FIGURE 4. Lineweaver Burk plot of Hemoglobin.

examples like Ribonuclease, hexokinase, glucokinase in which a single monomeric enzyme show sigmoidal behavior, but cooperativity of these enzymes could not have been generated by the interaction of subunits. This mechanism is known as Kinetic cooperativity [15]. However, the Hill plot exhibits a sigmoidal shape (as shown in Fig.3), indicating that binding occurs in stages, such that the oxygen affinity of hemoglobin depends on the number of subunits in the tetramer that are already oxygenated.

3. Discussion and Conclusion

A mathematical model has been established that describes the interaction between an oxygen molecule and the hemoglobin molecule. Hemoglobin is a tetrameric protein and shows cooperativity i.e; consisting of four subunits and each subunit binds with one oxygen molecule. Cooperativity is a fundamental specificity of various biochemical systems [24]. Different biochemical mechanisms can create ultra sensitivity, including zero-order kinetics, second- and higher-order dependence on enzyme concentration, positive feedback and protein translocation [9, 10]. In this work, we have more focused on the role of mathematics on fractional saturation of oxygen in hemoglobin. A number of simplifying hypothesis are available to explore binding processes. In this case, we have introduced steady state hypothesis and then setting time derivatives (eq.13) equal to zero and solve for the steady state process. Although, this model provides a basis for understanding the S-shaped or sigmoidal character binding curve but it also confirms that the nature of individual binding sites (five binding states discussed above) does not account for sigmoid behaviour. In Figure 3 plots of the free oxygen concentration (partial pressure of oxygen) versus saturation (Y) exhibit a sigmoidal character and indicates that the affinity of hemoglobin for the first oxygen molecule is less than the subsequent ones. Figure 4 represents the Lineweaver Burk plot of hemoglobin. Furthermore, our theoretical results are a step towards understanding the role of differential equations in cooperativity in relation with empirical models. A simple, straightforward and a new method of estimating the fractional saturation of oxygen in haemoglobin is derived in this paper. The role of mathematical tools have made the estimation of oxygen transition from one state to another more realistic and reasonable. The proposed model can be further extended by incorporating other parameters like temperature dependent rate constants, flux of diffusion at various binding sites etc.

Author Contribution Statements Both the authors contributed equally to formulate, evaluate and interpret the model and its analysis proposed in this paper. All authors read and approved the final manuscript.

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References

[1] Barcroft, J., Hill, A. V., The nature of oxyhemoglobin, with a note on its molecular weight, *J. Physiol.,* 39 (1910). doi: 10.1113/jphysiol.1910.sp001350

[2] Barcroft, J., The combinations of hemoglobin with oxygen and with carbon monoxide. *II. Biochem J.,* 7(5) (1913), 481-491. doi: 10.1042/bj0070481

[3] Eaton, W. A., Henry, E. R., Hofrichter, J., Mozzarelli, A., Is cooperative oxygen binding by Hemoglobin really understood, *Arch. Physiol.,* 16 (1904). doi: 10.1038/7586
[4] Brian, I., Mathematical Modeling in Systems Biology: An Introduction, 2012.
[5] Cattoni, D. I., Chara, O., Kaufman, S. B., Flecha, F. L., Cooperativity in binding processes: new insights from phenomenological modeling, *Plos One,* 10(12) (2015) e0146043. doi: 1371/journal.pone.014604-3
[6] Chien, HO. Y., Hemoglobin: Cooperativity in Protein - Ligand Interactions. In: Encyclopedia of Life Sciences (ELS), John Wiley Sons, Ltd: Chichester, 2010. doi: 10.1002/9780470015902.a0001345.pub2
[7] David, L. N., Cox, M. M., Lehninger, Principles of Biochemistry, 4th edition, W.H. Freeman and Company New York, 2005.
[8] Edsall, J. T., Blood and hemoglobin: The evolution of knowledge of functional adaptation in a biochemical system, part I: The adaptation of chemical structure to function in hemoglobin, *J. Hist. Biol.*, 5 (1972). doi: 10.1007/BF00346659
[9] Ferrell, J. E., Tripping the switch fantastic: how a protein kinase cascade can convert graded inputs into switch-like outputs, *Trends Biochem. Sci.,* 21 (1996). doi.org/10.1016/S0968-0004(96)20026-X
[10] Ferrell, J. E., Self-perpetuating states in signal transduction: positive feedback, double - negative feedback and bistability, *Curr. Opin. Cell Biol.,* 14 (2002). doi: 10.1016/s0955-0674(02)00314-9
[11] Francisco, L. O., Herrera, A., A dynamical model for a cooperative enzyme, *J. Theor. Biol.,* 154 (1992). doi: 10.1016/s0022-5193(05)80402-3
[12] Garbett, N. C., Chaires, J. B., Binding: a polemic and rough guide, *Methods Cell Biol.,* 84 (2008).
[13] Gutfreund, H., Kinetics for the Life Sciences: Receptors, Transmitters and Catalystd, Cambridge: Cambridge University Press, 1995. doi.org/10.1017/CBO9780511626203
[14] Hammes, G. G., Thermodynamics and Kinetics for the Biological Sciences, John Wiley and Sons, New York, 2000.
[15] Bisswanger, H., Enzyme Kinetics, Principles and Methods, Wiley-Vch, 2002.
[16] Hill, A. V., The possible effects of the aggregation of the molecules of hemoglobin on its dissociation curves, *J. Physiol.,* 40 (1910).
[17] Hill, A. V., The combinations of hemoglobin with oxygen and with carbon monoxide. I., *Biochem. J.* 7(5) (1913), 471. doi: 10.1042/bj0070471
[18] Hill, T. L., Cooperativity Theory in Biochemistry: Steady-State and Equilibrium Systems, Springer Series in Molecular and Cell Biology, Springer, New York, 1985.
[19] Holt, JO. M., Ackers, G. K., Kinetic trapping of a key hemoglobin intermediate, *Methods in Molecular Biology*, 796, Allostery: Methods and Protocols, Springer, 2012. doi: 10.1007/978-1-61779-334-9_2
[20] Kaihnsa, N., Safey El Din, M., Maritine, J. W. R., Cooperativity, absolute interaction, and algebraic optimization, *Journal of Mathematical Biology*, 81 (2020), 1169-1191. doi: 10.1007/s00285-020-01540-8
[21] Khanday, M. A., Introduction to Modeling and Biomathematics, Dilpreet Publishing House, 2016.
[22] Khanday, M. A., Bhat, Roohi., Transformation of glucokinase under variable rate constants and thermal conditions: a mathematical model, *Applications and Applied Mathematics: An International Journal*, 16 (2021), 953-964.
[23] Klotz, I. M., Ligand - Receptor Energetics, John Wiley and Sons, New York, 1997.
[24] Mammen, M., Choi, S. K., Whitesides, G. M., Polyvalent interactions in biological systems: implications for design and use of multivalent ligands and inhibitors, *Angew. Chem.,* 37 (1998). doi: 10.1002/(SICI)1521-3773(19981102)37:20<2754::AID-ANIE2754>3.0.CO;2-3

[25] Marangoni, A. G., Enzyme Kinetics, A Modern Approach, Wiley John and Sons, Inc., Hoboken, New Jersey, 2003.
[26] Johnson, M. L., Mathematical modeling of cooperative interactions in hemoglobin, *Methods in Enzymology*, 323 (2000). doi.org/10.1016/S0076-6879(00)23364-8
[27] Mills, F. C., Biochemistry, 1976.
[28] Monod, J., Wyman, J., Changeux, J. P., On the nature of allosteric transition: A plausible model, *Journal of Molecular Biology,* 12 (1965). doi.org/10.1016/S0022-2836(65)80285-6
[29] Pertuz, M., Stereochemistry of cooperativity effects in hemoglobin, *Nature,* 228 (1970).
[30] Klein, P., Pawson, T., Tyers, M., Mathematical modeling suggests cooperative interactions between a disordered polyvalent ligand and a single receptor site, *Current Biology*, 13 (2003). doi: 10.1016/j.cub. 2003.09.027
[31] Stefan, M. I., Novera, N. L., Cooperative binding, *Plos Comput. Biol.,* 9(6) (2013). doi.org/10.1371/journal.pcbi.1003106