Adair-Based Hemoglobin Equilibrium with Oxygen, Carbon Dioxide and Hydrogen Ion Activity*
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*Running title: Adair-based Hb-O₂-pH-CO₂ equilibrium

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ABSTRACT
As has been known for over a century, oxygen binding onto hemoglobin is influenced by the activity of hydrogen ions (H⁺), as well as the concentration of carbon dioxide (CO₂). As is also known, the binding of both CO₂ and H⁺ on terminal valine-1 residues is competitive. One-parametric situations of these hemoglobin equilibria at specific levels of H⁺, O₂ or CO₂ are also well described. However, we think interpolating or extrapolating this knowledge into an "empirical" function of three independent variables has not yet been completely satisfactory. We present a model that integrates three orthogonal views of hemoglobin oxygenation, titration, and carbamination at different temperatures. The model is based only on chemical principles, Adair's oxygenation steps and Van’t Hoff equation of temperature dependences. Our model fits the measurements of the Haldane coefficient and CO₂ hemoglobin saturation. It also fits the oxygen dissociation curve influenced by simultaneous changes in H⁺, CO₂ and O₂, which makes it a strong candidate for integration into more complex models of blood acid-base with gas transport, where any combination of mentioned substances can appear.

Keywords: Acid-Base Equilibrium; Blood Gas Analysis; Carboxyhemoglobin; Hemoglobin A; Oxyhemoglobins
**INTRODUCTION**

Human hemoglobin A is one of the most extensively studied protein macromolecules. The composition and 3D conformation of both α and β chains is known [1, 2] and the binding of O$_2$, H$^+$ and 2,3-DPG has been described [2, 3]. Since the 1930s it has generally been believed that CO$_2$ binding to Hb occurs by carboxylation of the amino-terminus, forming a carboxylate compound [4-6]. This hemoglobin carboxylation was verified by Morrow et al. [7], who used nuclear magnetic resonance of $^{13}$CO$_2$ to find its exact binding sites at valine-1. The shift of titration between oxygenated and deoxygenated forms is also well known. Called the Bohr or Haldane effect [8-10], it is caused by the same valine-1 side and by more than ten other acid-base residues [2, 11].

The most common descriptions of the hemoglobin oxygen dissociation curve (ODC) are the allosteric models [12, 13], the model based on Hill equation [14, 15], and Adair's four-step model [16]. Some of the ODC models [15, 17, 18] also include the effects of varying pH and CO$_2$ concentration. However, these models operate only with interpolation or extrapolation of ODC from normal pH or normal pCO$_2$ and do not take into account the chemical dependences between titration [8] and carboxylation [19]. They fail when both pH and CO$_2$ are not at normal values, especially in the alkaline pH range.

Based on these findings, we propose a hemoglobin binding equilibrium model that starts with a description of hemoglobin-oxygen dissociation using a slightly modified version of Adair’s approach [16]. We continue by describing the relationship between CO$_2$ and H$^+$ as competitive inhibition in the amino group of terminal valine-1 residue on each chain (suffixes z and c), which is in accordance with the known facts [4, 6, 20]. Finally, we lump all other acid-base side chains residues in a hemoglobin subunit into one Bohr proton-binding site of the side chain residues. These are denoted by the suffix $h$ in the article.

**METHODS**

The model is built in Mathematica 9.0 (Wolfram, Champaign, IL) and also in Dymola FD01 2014 (Dassault Systemes, Paris, France) as an example of chemical package in open-source Modelica library Physiolibrary 2.2.0 [21, 22] according to the model structure defined in the following section. The model contains only physiological parameters such as gas solubilities and dissociation coefficients of defined reactions. The unknown parameters are fitted to the data of Siggaard-Andersen [23], Bauer and Schröder [24], Severinghaus [18], Matthew et al. [1] and Reeves [25] using Mathematica function FindFit and also using parameter estimation method suggested by Kulhanek et al. [26].

Free dissolved concentrations of [O$_2$] and [CO$_2$] in red blood cells are calculated from gas partial pressures using Henry’s law ([O$_2$]=αO$_2$*pO$_2$ and [CO$_2$]=αCO$_2$*pCO$_2$) with solubility coefficients αO$_2$=1.005*10$^{-5}$ mol.m$^{-3}$.Pa$^{-1}$ measured at 38 °C by Sendroy et al. [27], and αCO$_2$=2.3*10$^{-4}$ mol.m$^{-3}$.Pa$^{-1}$ at 37 °C, as proposed by Maas et al. [28]. These solubilities are slightly different from solubilities in pure water or in plasma because of the effects of salts and proteins inside erythrocyte [27].

**MODEL STRUCTURE**

The model is built upon several simplifying assumptions. Firstly, each of four hemoglobin tetramer subunits is treated as identical, even though (slight) differences are known to exist between α and β subunits. Secondly, CO$_2$ or H$^+$ binding is supposed not to affect the CO$_2$ or H$^+$ affinities in the other three subunits. Thus, the interaction between the subunits is modeled purely
through the varying affinities of oxygen in each oxygenation step. The third simplifying assumption has already been mentioned; it is lumping all Bohr proton binding sites of subunit into two (first for the valine-1 amino-terminus and second for the side chains residues), a simplification first suggested by Antonini [29].

Model structure of hemoglobin subunit

The three reactions that participate in the Bohr and Haldane effect of each subunit are as follows:

- **Reaction z**: $\text{Hb}_u\text{NH}_3^+ \leftrightarrow \text{Hb}_u\text{NH}_2 + \text{H}^+$
- **Reaction c**: $\text{Hb}_u\text{NH}_2+\text{CO}_2 \leftrightarrow \text{Hb}_u\text{COO}^- + \text{H}^+$
- **Reaction h**: $\text{Hb}_u\text{AH} \leftrightarrow \text{Hb}_u\text{A}^- + \text{H}^+$

where the reactions z and c are competitive on the valine-1 amino-terminus, and the reaction h is independent of z or c.

The chemical equilibrium equations of these three reactions are Eq.1-3, where $K_x$ is the equilibrium dissociation coefficients of the reaction x (i.e., z, c or h).
Reaction z: \[ K_z = \frac{[Hb_uNH_2] \cdot aH^+}{[Hb_uNH_3^+]} \] (1)

Reaction c: \[ K_c = \frac{[Hb_uCOO^-] \cdot aH^+}{[CO_2][Hb_uNH_2]} \] (2)

Reaction h: \[ K_h = \frac{[Hb_uA^-] \cdot aH^+}{[Hb_uAH]} \] (3)

These dissociation coefficients are different between oxy and deoxy subunits, which are distinguished by the subscripts O and D in the following text. They can be also written in their logarithmic form, where \( pK_x \) means \( -\log_{10}(K_x) \). Thus, for instance, \( pK_{zO} \) denotes the equilibrium coefficient of the reaction z for the oxy form of the hemoglobin subunit. Similar notation is used for describing the activity of hydrogen ions (acidity), where pH=\( -\log_{10} (aH^+) \).

Using Eq.1-3 it is possible to express fractions for deoxy and oxy subunits. We label these fractions as follows: Hb\(_u\)NH\(_2\) fractions are called \( f_{zcD} \) \((f_{zcD})\), Hb\(_u\)A\(^-\) fractions \( f_{hD} \) \((f_{hD})\), Hb\(_u\)A\(^-\)NH\(_2\) fractions \( f_{nD} \) \((f_{nD})\) and Hb\(_u\)COO\(^-\) fractions \( sCO_{2D} \) \((sCO_{2D})\) as in Eq. 4-7. The selection of form Hb\(_u\)A\(^-\)NH\(_2\) from the division into Hb\(_u\)NH\(_2\) and Hb\(_u\)A\(^-\) is also illustrated in Fig. 1A, B.

\[
\begin{align*}
\frac{f_{zcD}}{[Hb_u]} & = \frac{[Hb_uNH_2]}{[Hb_u]} = \frac{1}{1 + 10^{pK_{zD} - pH} + [CO_2]10^{pH - pK_{zD}}} \\
\frac{f_{hD}}{[Hb_u]} & = \frac{[Hb_uA^-]}{[Hb_u]} = \frac{1}{10^{pK_{hD} - pH} + 1} \\
\frac{f_{nD}}{[Hb_u]} & = \frac{[Hb_uA^-NH_2]}{[Hb_u]} = f_{zcD} \times f_{hD} \\
\frac{sCO_{2D}}{[Hb_u]} & = \frac{[Hb_uCOO^-]}{[Hb_u]} = 10^{pH - pK_{zD}} f_{zcD}[CO_2]
\end{align*}
\] (4-7)

We define a titration shift as the amount of acid that must be added to achieve the same pH after full deoxygenation of the hemoglobin subunit. This change of subunit charge during deoxygenation (by the Bohr protons) is also called Haldane coefficient \( \Delta H^+_z \). The coefficient can be divided into contributions of the previously mentioned reactions \( \Delta H^+_h \), \( \Delta H^+_z \) and \( \Delta H^+_c \), as is algebraically expressed by Eq. 8-11.

\[
\begin{align*}
\Delta H^+_h & = -\frac{[Hb_uA^-] - [Hb_uA^-]}{[Hb_u]} = -(f_{hD} - f_{hO}) \\
\Delta H^+_z & = \frac{[Hb_uNH_2^+] - [Hb_uNH_2^+]}{[Hb_u]} = \left(10^{pK_{zD}} \frac{f_{zcD}}{10^{pH} f_{zcO}} - 10^{pK_{zO}} f_{zcO}\right)
\end{align*}
\] (8-9)
\[ \Delta H_c^+ = - \frac{[Hb_{uO}COO^-] - [Hb_{uO}COO^-]}{[Hb_u]} = -(sCO_{2D} - sCO_{2O}) \] (10)

\[ \Delta H^+ = \Delta H_c^+ + \Delta H_c^+ + \Delta H_h^+ \] (11)

Model structure of hemoglobin tetramer

The speciation of the hemoglobin tetramer molecule can be considered at various levels of detail; the one chosen as appropriate in our approach is indicated in Fig. 1C. The possible forms include different combinations of oxygenated and deoxygenated hemes, protonated and deprotonated oxygen-linked acid groups, and free or carboxylated amino endings of each chain. Yet because of the law of detailed balance in equilibrium [30], it is not necessary to calculate all reactions between these forms. As a result, only four oxygenation reactions need be selected for the calculation. This is done in accordance with Fig. 1C, by selecting a tetramer form \((O_2)_iHb_{tn}\) composed only of four subunits in the form \(Hb_u\) with \(NH_2\) \((H^+ and CO_2 free)\), and modeling oxygen binding to these forms with Adair-type coefficients of the following reactions:

\[
\begin{align*}
O_2Hb_{tn} & \leftrightarrow Hb_{tn} + O_2 \\
(O_2)_2Hb_{tn} & \leftrightarrow O_2Hb_{tn} + O_2 \\
(O_2)_3Hb_{tn} & \leftrightarrow (O_2)_2Hb_{tn} + O_2 \\
(O_2)_4Hb_{tn} & \leftrightarrow (O_2)_3Hb_{tn} + O_2
\end{align*}
\]

where dissociation coefficients are defined by Eq.12, which are also represented by vertical arrows at Fig. 1C.

\[ K_i = \frac{[(O_2)_{i-1}Hb_{tn}] \cdot [O_2]}{[(O_2)_iHb_{tn}]} \] (12)

For the next calculation, all forms can be expressed as a fraction of the deoxy-tetramer form \((O_2)_0Hb_{tn}\), as shown in Eq. 13.

\[ [(O_2)_iHb_{tn}] = \frac{[(O_2)_0Hb_{tn}] \cdot [O_2]^i}{\prod_{j=1}^i K_j} \] (13)

Let us move the attention from specific forms of \(Hb_{tn}\) to the description of the equilibrium within the whole group of Hbt. Looking at Fig. 1C, one can see that for each oxygenation step we can calculate the equilibrium in each horizontal line using Eq. 14.

\[ [(O_2)_iHb_{tn}] \cdot f n_D^{4-i} \cdot f n_0^i = [(O_2)_iHb_{tn}] \] (14)

The CO₂- and pH-dependent oxygen saturation equation in the Adair style (Eq. 15) is algebraically derived from Eq. 13-14, where \(a_i = 1/(\prod_{j=1}^i K_j)\) and \(x = (f n_D(pH, [CO_2])/f n_0(pH, [CO_2])) * [O_2].\)
\[ sO_2 = \frac{a_1 x + 2 a_2 x^2 + 3 a_3 x^3 + 4 a_4 x^4}{4 + 4 a_1 x + 4 a_2 x^2 + 4 a_3 x^3 + 4 a_4 x^4} \]  

(15)

Hemoglobin saturation with CO\(_2\) (sCO\(_2\)) is calculated separately in oxygenated and deoxygenated subunits forms (sCO\(_2\)O and sCO\(_2\)D) using Eq. 16.

\[ sCO_2 = sO_2 \cdot sCO_{20} + (1 - sO_2) \cdot sCO_{2D} \]  

(16)

Finally, the shift of titration after deoxygenation and decarbamation of the hemoglobin subunit can be expressed by Eq. 17.

\[ dTH = sO_2 \cdot \Delta H^+ + sCO_{2D} + \frac{sCO_{2D}}{1 + 10^{pH-pKzD}} \]  

(17)

**Temperature dependences**

The temperature dependences are integrated using the Van’t Hoff equation (Eq. 18), where R=8.314 J.K\(^{-1}\).mol\(^{-1}\) is gas constant, \(\Delta H^\theta\) is the standard enthalpy change as an amount of heat consumed by a reaction changing one mole of substrates to products, and K2 and K1 are Henry’s coefficients of solution or the dissociation coefficient of the chemical reaction at temperature T2 and T1 (expressed in Kelvin).

\[ \ln \left( \frac{K_2}{K_1} \right) = \frac{-\Delta H^\theta}{R} \left( \frac{1}{T_2} - \frac{1}{T_1} \right) \]  

(18)

**RESULTS**

The Adair’s coefficients are fitted to the collection of ODC measurements by Severinghaus [18] at pCO\(_2\)=0 Pa and pH\(_p\)=7.4, see Fig. 2A and Table I. The dissociation coefficients of carboxylation are determined in close agreement with Bauer and Schröder using their data [24]; the resulting fit can be seen in Fig. 2B, the coefficients are in Table II. The lumped acid dissociation coefficients for the side-chains pK\(_{HD}\) and pK\(_{HO}\) are estimated by optimization of Siggaard-Andersen’s data [23] at DPG/Hbt=0.84, pH=6.5 to 8.0 and 37°C; the resulting fit can be seen in Fig. 2C, and the coefficients complete Table II.

**Table I**

| Estimated form-specific Adair's coefficients [mol.m\(^{-3}\)] at 37°C |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| \(K_1\)         | \(K_2\)         | \(K_3\)         | \(K_4\)         |
| 0.0121          | 0.0117          | 0.0871          | 0.000386        |
Figure 2. Model curves and measured data points

A) Points are from Severinghaus's oxygen dissociation curve collection [18] at pCO₂=0 Pa, erythrocyte intracellular pH=7.2 and temperature 37°C. Lines are sO₂ defined by Eq. 15. B) Carboxylation of hemoglobin measured and estimated by Bauer and Schröder [24]. The lines (Eq. 7) are sCO₂D and sCO₂O at pCO₂=5.33 kPa. C) Bohr protons released during oxygenation of one hemoglobin subunit. Dots are data measured by Siggaard-Andersen [23] in erythrolysate at 37°C, DPG/Hbt = 0.84. Lines are calculated from Eq. 11 using data from Table II. From top to bottom data are plotted for different pCO₂=0 kPa, 1.47 kPa, 4.53 kPa, 10.67 kPa and 26.66 kPa. D) Naeraa et al.'s [29] oxygen saturation data comparison. The two groups of lines (Eq. 15) are determined mainly by pO₂ around 6.7 kPa and 11.7 kPa. The small nuances in each group are caused by different pCO₂ changed from 0 kPa to 12 kPa. E) Matthew et al.'s [1] carbamination data measured at 30°C with constant content of all carbonates 55 mmol.L⁻¹. Fitted dissociation constants, which determine the lines, are used to estimate enthalpies of their reactions to be consistent with Bauer and Schröder [24]. F) Reeves [32] oxygen saturation data at different temperatures. Enthalpies of reaction h and specific Adair oxygenation step enthalpy is estimated (lines) to fit the data (circles).
We concluded that the model also describes the ODC shifts by comparing with Naerea et al.’s [31] oxygen saturation measurements at different plasma pH and CO₂ levels, see Fig. 2D. The recalculation of data from plasma pH to intracellular erythrocyte pH uses the equation pH=7.2464+0.796(pHₚ-7.4), as presented by Siggaard-Andersen and Salling [32].

<table>
<thead>
<tr>
<th>reaction z</th>
<th>reaction c</th>
<th>reaction h</th>
</tr>
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<tbody>
<tr>
<td>pKᵥD=7.73</td>
<td>pKᵥO=7.25</td>
<td>pKᵥD=7.52</td>
</tr>
<tr>
<td>pKᵥO=7.25</td>
<td>pKᵥO=5.35</td>
<td>pKᵥO=6.89</td>
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The gas solubility as Henry’s coefficient at different temperatures can be recalculated using the enthalpy of gas solution (-14 kJ.mol⁻¹ for O₂, and -20 kJ.mol⁻¹ for CO₂ [33]). If we assume that the examined hemoglobin of Matthew [1] is at compatible conditions, but at a temperature of 30°C with pKᵥD=7.53, pKᵥO=7.28, pKᵥD=4.77 and pKᵥO=5.20 as plotted in Fig. 2E, then the enthalpies of these reactions are -51, 8, 59 and -41 kJ.mol⁻¹. The heat of oxygenation unaffected by oxygen heat of solution, by carbon dioxide and by Bohr protons was measured by Atha and Ackers [34] as 59 kJ.mol⁻¹. Using this value for each Adair oxygenation step it is possible to optimize other enthalpies to fit the Reeves data [25], measured at 19-43°C. Resulting as the enthalpies 59 kJ.mol⁻¹ of deoxy and -127 kJ.mol⁻¹ of oxy version of reaction h to reach ODCs curves plotted in Fig. 2F.

**DISCUSSION**

Having a precise quantitative description of hemoglobin behavior is a crucial aspect in describing the behavior of whole blood, which has numerous uses in medicine today. It is important in areas such as blood gas analysis [15, 35], the building of complex models [36], simulation for teaching purposes [37], or rebreathing-based methods for cardiac output estimation, which represent a more specific use [38]. For instance, the precision of the latter methods is crucially dependent on the exact calculation of the total amount of carbon dioxide in blood for various (abnormal) patient conditions, as has been recently pointed out [39].

This model offers a precise description of various phenomena that take place with hemoglobin, based on relatively simple starting points, such as competitive binding of H⁺ and CO₂ at valine-1 amino terminus or the law of detailed balance [30]. Even with a simple structure, it offers a remarkably good fit to the data of hemoglobin oxygenation, titration, and carbamination at different temperatures, as shown in Fig. 2A-E. These can be calculated with any combination of oxygen, carbon dioxide and hydrogen ions defined as open system (lungs), where the partial pressures are equilibrated, and also in a closed system (tissues), where mass conservation laws and the total amount of substances take place, as was also modeled by Rees and Andreassen [40], among others.

The results of our model (Fig. 2A-E) show strong nonlinear dependences between variables, which is in agreement with the known data [1, 23-25, 41]. Looking at Fig. A, one can compare sets of ODCs, where each color represents a different pH. Various curves of each color represent
ODCs for various levels of pCO₂ for a given pH. As can be seen, the effect of pCO₂ on the ODC is stronger at high (alkaline) pH, which is in agreement with the data [23]. Similarly, one can compare the curves within the sets of solid or dashed lines, where each set represents dissociation curves for various values of pH at a given level of pCO₂. The variation between the curves of each line type represents the Bohr effect for the given level of CO₂, this effect can also be appreciated from data of Fig. 2C, which shows the average amount of released H⁺ upon oxygenation of one hemoglobin subunit.

The model uses enthalpies to calculate temperature dependences of hemoglobin behavior (Fig. 2F,E), which allows examination of the heat transfers during single chemical processes. For instance, binding of aqueous oxygen onto hemoglobin produces 30-40 kJ/mol of heat [42-45], and the same amount of heat is consumed by deoxygenation process in metabolically active tissues, thus helping to cool them down. When the hemoglobin model is used in the standard conditions of a large-scale HumMod model [36], the resulting heat transfer due to the exothermy of the hemoglobin oxygen reaction is 4-7% of the total heat produced by muscle, which is in agreement with experimental results [42, 44, 45]. It is interesting to note that this heat transfer occurs without any increase in blood temperature.

The integration of chemical processes in a macromolecule requires a precise view into their underlying principles. Some physiologists use the elementary chemical equations [40], but do not implement the principle of detailed balance [30]. Other physiologists make empirically-based equations with a raw linear gradient approximation of possible combinations of model values [15]. We feel that it is almost impossible to see the problem as this type of black-box function with more than two inputs. Instead, it is better to have an integrated model of oxygenation, titration and carbamation.

Today, allosteric hemoglobin oxygenation models do exist that seem more in agreement with the structural knowledge of hemoglobin [12, 46-48]. These models take into account two or more structurally different forms: relaxed and tensed. However, these models have so far been limited to hemoglobin oxygenation only. Our Adair-based model can explain not only oxygen and carbon dioxide saturation, but also their cooperation with acid-base buffering properties of hemoglobin. All three of these connected phenomena fit to measured data in physiological ranges. The limitation of our model is that it does not contain the tetramer space conformations. In future the model could be extended with dependences on electrolytes such as chloride, 2,3-bisphosphoglycerate or other organic phosphates and their binding reactions, as many research studies show these interactions [49-52]. The oxygenation could also be improved for the allosteric models [12, 48], assuming that oxy-reactions are almost in relaxed forms and deoxy-reactions are in tensed forms. The next extension of this model could be performed by the integration of an intracellular red cell environment to calculate with phosphate acid-base buffers, and finally the membrane changes with blood plasma, where the chloride shift reaches a Gibbs-Donnan equilibrium and establishes chloride, bicarbonate and hydrogen ion activity ratios. Having an integrated model of blood gases and acid-base is crucial if we want the precise computational algorithms of the current state of a patient. These calculations could be used, for example, inside the next generation of medical devices to estimate not only blood properties, but also the connected properties of circulation [38] or metabolic functions [40].

In this article, we present a hemoglobin model that integrates O₂, CO₂ and H⁺ binding. The model is not just empirical, but is based on sound theoretical principles, such as the competitive binding of CO₂ and H⁺ on the valine-1 NH₂ terminus, the Bohr and Haldane effect [9, 53] and on the principle of detailed balance. The principle of detailed balance is used for the first time in the
Adair type of model, offering mass and energy conservation principles, and accumulates substances and heat inside hemoglobin forms, which is very useful for integration in higher-scale dynamic models.

**LIST OF ABBREVIATIONS**

ODC  
- hemoglobin oxygen dissociation curve

2,3-DPG  
- 2,3-diphosphoglycerate

[X]  
- molar concentration of X in mol.m⁻³

aH⁺  
- activity of hydrogen ions, where pH=⁻log₁₀(aH⁺)

αO₂  
- O₂ solubility in mol.m⁻³.Pa⁻¹

αCO₂  
- CO₂ solubility in mol.m⁻³.Pa⁻¹

pO₂  
- partial pressure of O₂ in Pa

pCO₂  
- partial pressure of CO₂ in Pa

Hbₐ  
- hemoglobin alpha or beta subunit

HbₐD  
- deoxygenated Hbₐ

HbₐO  
- oxygenated Hbₐ

HbₐNH₃⁺  
- Hbₐ with protonated N-terminus

HbₐDNH₃⁺  
- HbₐD with protonated N-terminus

HbₐO NH₃⁺  
- HbₐO with protonated N-terminus

HbₐNH₂  
- Hbₐ with –NH₂ form of N-terminus

HbₐD NH₂  
- HbₐD with –NH₂ form of N-terminus

HbₐO NH₂  
- HbₐO with –NH₂ form of N-terminus

HbₐCOO⁻  
- Hbₐ with carboxylated N-terminus

HbₐD COO⁻  
- HbₐD with carboxylated N-terminus

HbₐO COO⁻  
- HbₐO with carboxylated N-terminus

HbₐAH  
- Hbₐ with protonated side-chains

HbₐD AH  
- HbₐD with protonated side-chains

HbₐO AH  
- HbₐO with protonated side-chains

HbₐA⁻  
- Hbₐ with deprotonated side-chains

HbₐD A⁻  
- HbₐD with deprotonated side-chains

HbₐO A⁻  
- HbₐO with deprotonated side-chains

Hbₐ A⁻NH₂  
- selected normalized form of Hbₐ with deprotonated side-chains and -NH₂ form of N-terminus

HbₐD A⁻NH₂  
- deoxygenated form of Hbₐ A⁻NH₂

HbₐO A⁻NH₂  
- oxygenated form of Hbₐ A⁻NH₂

fᵣD  
- fraction of HbₐD A⁻NH₂ from HbₐD

fᵣO  
- fraction of HbₐO A⁻NH₂ from HbₐO

fₓD  
- fraction of HbₐD NH₂ form HbₐD

fₓO  
- fraction of HbₐO NH₂ form HbₐO

fₙD  
- fraction of HbₐD A⁻ form HbₐD

fₙO  
- fraction of HbₐO A⁻ form HbₐO

ΔH⁺ₗ  
- change of valence (charge) on side-chains during deoxygenation per one Hbₐ

ΔH⁺ₗ  
- protonation of –NH₂ form of N-terminus during deoxygenation per one Hbₐ

ΔH⁺ᵣ  
- decarboxylation of carboxylated N-terminus during deoxygenation per one Hbₐ

ΔH⁺  
- Haldane coefficient per hemoglobin subunit

Hbₜ  
- hemoglobin tetramer without bound O₂ molecules

(O₂)ᵢHbₜ  
- hemoglobin tetramer with the number of i bound O₂ molecules
(O₂)₅Hb₅n hemoglobin tetramer composed only of Hb₅A NH₂ subunit forms with the number of i bound O₂ molecules
sO₂ O₂ saturation of hemoglobin
sCO₂ CO₂ saturation of deoxyhemoglobin
sCO₂O CO₂ saturation of oxyhemoglobin
sCO₂O₂ CO₂ saturation of hemoglobin
tCO₂O total concentration of CO₂ = free dissolved CO₂ + HCO₃⁻ + CO₃²⁻ + Hb₅COO⁻
dTH shift of titration curve, which equals how many mols of titrant must be added to one mol of hemoglobin subunit to reach the same pH of the base (O₂- and CO₂-free) titration curve
pH acidity/basicity of hemoglobin solution (e.g. inside erythrocytes)
pHₚ pH in plasma (e.g. acidity/basicity of blood)

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