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A New Model for Thermodynamic Characterization of Hemoglobin

Francesco Farsaci [1], Ester Tellone [2,*], Antonio Galtieri [2] and Silvana Ficarra [2]

[1] Institute for Chemical and Physical Processes (IPCF-C.N.R.), Via Ferdinando Stagno d'Alcontres 37, Faro Superiore, 98158 Messina, Italy

[2] Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Viale Ferdinando Stagno d'Alcontres 31, 98166 Messina, Italy

* Correspondence: etellone@unime.it

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Abstract: In this paper, we formulate a thermodynamic model of hemoglobin that describes, by a physical point of view, phenomena favoring the binding of oxygen to the protein. Our study is based on theoretical methods extrapolated by experimental data. After some remarks on the non-equilibrium thermodynamic theory with internal variables, some thermodynamic functions are determined by the value of the complex dielectric constant. In previous papers, we determined the explicit expression of a dielectric constant as a function of a complex dielectric modulus and frequency. The knowledge of these functions allows a new characterization of the material and leads to the study of new phenomena that has yet to be studied. In detail, we introduce the concept of "hemoglobe", a model that considers the hemoglobin molecule as a plane capacitor, the dielectric of which is almost entirely constituted by the quaternary structure of the protein. This model is suggested by considering a phenomenological coefficient of the non-equilibrium thermodynamic theory related to the displacement polarization current. The comparison of the capacity determined by the mean of this coefficient, and determined by geometrical considerations, gives similar results; although more thermodynamic information is derived by the capacity determined considering the aforementioned coefficient. This was applied to the normal human hemoglobin, homozygous sickle hemoglobin, and sickle cell hemoglobin C disease. Moreover, the energy of the capacitor of the three hemoglobin was determined. Through the identification of displacement currents, the introduction of this model presents new perspectives and helps to explain hemoglobin functionality through a physical point of view.

Keywords: hemoglobin; biological capacitor; non-equilibrium thermodynamics; hemoglobe capacitor; thermodynamic capacitor

1. Introduction

From an evolutionary point of view, the respiratory pigment known as hemoglobin (Hb), appeared very early. Its presence in the symbiotic nitrogen-fixing microorganisms of the *Leguminosae* plant family, where Hb, quickly capturing the oxygen (O_2), plays a "protective" role towards the nitrogenase enzyme complex poisoned by O_2 [1]; in invertebrates, in some bivalve mollusk, such as *Scapharca inaequivalvis*, the role of O_2 transport is played by a more primitive hemocyanin pigment [2]. Once the Hb appears, first in cartilaginous fishes and then in teleosts, it preserves an identical molecular architecture at all levels of the vertebrate evolutionary scale: Amphibians, reptiles, birds, terrestrial, and marine mammals. This unique, extraordinary, and complex respiratory pigment, responsible for the correct and adequate distribution of O_2 at all tissue levels, is a conjugated protein with a tetrameric structure $\alpha_2\beta_2$ that has a fine, and complex functional regulation that defines Hb as the allosteric protein par

excellence [3]. Several modulators (effectors) influence oxygen binding to Hb such as the classic 2,3-bisphosphoglycerate (BPG), the chloride anion, carbon dioxide (CO_2), hydrogen ions responsible for the Bohr effect, temperature (i.e., the enthalpy of oxygenation ΔH), and inositol hexaphosphate and adenosine triphosphate depending on the evolutionary levels. The O_2, Hb preferential ligand, is also responsible for the complex homotropic regulation defined as cooperative, because it develops "mechanically" through a conformational change. This conformational change is due to the O_2 binding to the heme iron of one Hb subunit that in turn causes the sliding of the other subunits on each other. It is truly intriguing and surprising to note that for millions of years Hb has managed to maintain the tertiary and quaternary characteristics that characterize it, although the primary structure of its subunits has considerably varied. This peculiar architecture is basic and indispensable for the proper performance of Hb's delicate function, related to the binding, transport and O_2 release, which are finely and smartly regulated even in very different environments. Even more surprising is the fact that, through contact free of electronic transfers, a small molecule like O_2 can cause such an intramolecular disarrangement in a protein with about 3000 atoms (almost 1500 times larger than O_2). In fact, O_2 binding to the porphyrinic iron, causes a sort of intramolecular transduction that leads to a 15° rotation of the $\alpha_1\beta_1$ protomer with respect to the $\alpha_2\beta_2$. This results in a T-R transition makes the central cavity of Hb smaller by 50% (site of BPG binding, in the T state). In conclusion, the importance of the quaternary structure of Hb, preserved throughout evolution, and the disconcerting structural variation due the O_2 binding to heme suggest the existence of a driving force that is still not well identified and characterized. In detail, the "strength" of breathing work, has arisen from a Hb "intrinsic characteristic" certainly linked to its three-dimensional geometry (strictly maintained). This extraordinary machine, a slightly flattened globe with a central cavity in which the polar charges of the surface amino acid residues are perfectly oriented towards the (polar) medium and helps to stabilize the molecule's architecture, is a unique intrinsically well-designed "tool" to perform its "job" in an almost perfect way (perfect protein?). Moreover, it is a surprising three-dimensional morphology; the distribution and the variation of its charges on the internal and external surfaces that derive surprising thermodynamic responses [4], have led us to consider hemoglobin as an efficient electric machine. An ancestral biological capacitor with variable geometry whose model, in our opinion, needs to be characterized with complex thermodynamic models in order to obtain potential answers which may aid in clarifying that which is still ambiguous.

2. Thermodynamic Considerations

In this section, we remark on some aspects of the Hb capacitor model that justifies our point of view. In particular, we want to show this model as it is related to the non-equilibrium thermodynamics fundamental laws with internal variables and how it is a "consequence" of entropy production and therefore of the phenomenological equations. We observed that entropy is an important function in the thermodynamic description of the phenomenon. Entropy assumes a determinant role in the irreversible processes in which its production is different from zero. In fact, null entropy production is synonymous with reversibility. The choice of the variables on which the entropy depends leads to different developments of non-equilibrium thermodynamics. In all our researches, we chose to use the non-equilibrium thermodynamics with hidden internal variables formulated by Klutenberg in its theoretical aspects [5–9] and further developed by us [10–18]. This choice was made because we think this theory is more suitable in the study of biological systems [12] and it is a more physical–mathematical approach in regard to the classical meaning of this term. Here we consider only electrodynamics phenomena because the experiments on which we based the formulation of our Hb capacitor model was carried out only considering the dielectric measurements at constant temperature T. In this context, we assume the following functional dependence for the specific entropy s:

$$s = s\left(u, \underline{p}, \underline{\Omega}\right) \tag{1}$$

where u is the specific internal energy, p the specific polarization vector, and Ω a hidden vectorial internal variable. It can be shown that the introduction of Ω allows splitting of the polarization p into two parts $p^{(0)}$ and $p^{(1)}$ [7–9], such that:

$$\underline{p}^{(0)} = \underline{p} - \underline{p}^{(1)} \tag{2}$$

After this, the functional dependence in Equation (1) can be rewritten [7–9]:

$$s = s\left(u, p, \underline{p}^{(1)}\right) \tag{3}$$

and the entropy production $\sigma^{(s)}$ is:

$$\sigma^{(s)} = \frac{1}{T}\left[\rho \underline{E}^{(irr)} \frac{d\underline{p}}{dt} + \rho \underline{E}^{(1)} \frac{d\underline{p}^{(1)}}{dt}\right] \tag{4}$$

where by defining the equilibrium electric vector $\underline{E}^{(eq)}$ as:

$$\underline{E}^{(eq)} = -T \frac{\partial s\left(u, \varepsilon_{ik}, p, \underline{p}^{(1)}\right)}{\partial \underline{p}} \tag{5}$$

one has:

$$\underline{E}^{(ir)} = \underline{E} - \underline{E}^{(eq)} \tag{6}$$

$$\underline{E}^{(1)} = -T\rho \frac{\partial s\left(u, \varepsilon_{ik}, p, \underline{p}^{(1)}\right)}{\partial \underline{p}^{(1)}} \tag{7}$$

ρ is the mass density and $\underline{E}$ is the electric field that appears in the Maxwell's equations. Note that Equation (4) is a sum of the inner products of vectors, and therefore, according with the non equilibrium thermodynamics set of linear relations among these quantities can be obtained. For our purpose, we consider only one of these equations (a complete treatment of these questions can be studied in depth in References [7–9]); it is:

$$\rho \frac{dp_i^{(1)}}{dt} = \rho L_{ik}^{(1,0)} \frac{dp_k}{dt} + L_{ik}^{(1,1)} E_k^{(1)} \tag{8}$$

We here adopt the Einstein convection for the index. $L_{ik}^{(1,0)}$ that is a phenomenological tensor that takes into account possible cross effects between the relaxation phenomena described by Equation (8) and other ones described by an equation that we do not consider because it is not necessary for our purpose. $L_{ik}^{(1,1)}$ is the conductivity phenomenological tensor associated to polarization current $dP^{(1)}/dt$. Tensors were assumed as constant in time, but they can depend on a parameter characterizing the perturbation and this is the frequency of perturbation. It is important to emphasize that these tensors are a phenomenological characterization of the medium object of the study. For our purpose, it is enough to limit our consideration to the case of isotropic media neglecting the mentioned cross effects represented by the $L_{ik}^{(1,0)}$ tensor. In this case, one has:

$$L_{ik}^{(1,1)} = L^{(1,1)} \delta_{ik} \tag{9}$$

where δ_{ik} is the Kronecker's tensor. With these specification the Equation (8) can be rewritten:

$$\rho \frac{dp_i^{(1)}}{dt} = L^{(1,1)} \delta_{ik} E_k^{(1)} \tag{10}$$

By putting:

$$P_i^{(i)} = \rho p_i^{(i)} \tag{11}$$

and assuming ρ = constant (in time), Equation (10) becomes:

$$\frac{dP_i^{(1)}}{dt} = L^{(1,1)} \delta_{ik} E_k^{(1)} \tag{12}$$

The position ρ = constant is justified by the short time necessary for the measurement. We are recalling only the concept that we used to introduce our model, especially taking into account the hypothesis under which the dielectric experiment has been carried out. The Hb capacitor model was conceived, starting by Equation (12) and it has been deduced directly by entropy production (4) and in linear approximation, as we will explain in the next section. The experimental apparatus for dielectric measurements on which we based our model allows us to consider only one component of Equation (12) so as to write:

$$\frac{dP^{(1)}}{dt} = L^{(1,1)} E^{(1)} \tag{13}$$

This is a phenomenological equation connecting the affinity electric field $E^{(1)}$ (see Equation (7)) and the time variation of the polarization $p^{(1)}$ that is ascribed to the rotation of polar molecules according the Debye's model. $L^{(1,1)}$ is our keystone to introduce our model, as we will show in the next section.

3. Hemoglobe Model

In our previous paper, we formulated a non equilibrium thermodynamic characterization of the Hb molecule [4]. In particular, we studied the thermodynamic behavior of human hemoglobin (AA) comparing with homozygous sickle hemoglobin (SS) and its heterozygous (SC) originating sickle cell hemoglobin C disease, deducing biochemical properties associated to each one and relative differences. Indeed, we referred to dielectric experimental data reported by Laogun et al. (1997) [19]. Starting from these assumptions, we calculated the thermodynamic functions, which can be considered, so to speak, as the identity card of each Hb in solution. As mentioned in the introduction, the analysis of the so obtained functions, in particular of the $L^{(1,1)}$ coefficient of the $dP^{(1)}/dt$ function and the geometrical aspect of the Hb moleculeallow to evaluate the possibility of consider the Hb as a capacitor with quaternary structure as dielectric. Thus, we consider the Hb as a capacitor (see Figure 1) two walls of which are schematized as capacitors plates and whose dielectric is constituted by almost all the quaternary structure α_1, β_1, α_2, and β_2. Moreover, the three dimensionality of the Hb and a good capacitor functionality suggest the distance between the two plates as the lesser among the three dimension provided by literature. For our purpose, Hb will be only considered has a dipole moment and it is not necessary to evaluate the dipole moment or how data were obtained [20–22]. We indicate the total dipole moment as the vectorial sum of the four dipole moments of each subunits and calculate these last. The model that describes the hemoglobe can answer in an efficient way to three essential requirements of the T-R transition; they are: (1) How is the binding of the first oxygen facilitated? (2) How is the cooperativity generated? (3) How is the energy transmitted during the T-R transition?

The hemoglobe like a capacitor has a set of charges on each plate (the density of which is described by the induction vector $\underline{D}$). A set of opposite polarization charges correspond to these set of charges on adjacent dielectric represented by a side of the four subunities. Somehow there is a strong external mechanical obstruction that is an obstacle for the entry of external agents. Therefore, in order to carry out the T-R transition, it is necessary to use certain energy to overcome the aforementioned obstruction. During the journey in the bloodstream, this energy may be supplied to Hb by the different environments between tissues and lungs. These differences are especially characterized by a shift in: pH, temperature, BPG concentrations bound to Hb, and levels of oxygen and CO_2. For simplicity the explanation of our model will consider only the difference in pH and pO_2 (further differences of BPG bound to Hb and temperature confirm the model). In detail, pH variation (between tissues

and lungs) causes a charging change on the external plate of the hemoglobe. This leads to a change on the internal plate charges and consequently a rearrangement of the structure of the four subunits (dielectric). This change is induced by displacement current processes deactivating ionic pairs, which were a cause of stabilization of the T state. This weakening and the influence of oxygen pressure, for example in the lungs, is approximately in the order of 100 mmHg, push the oxygen inside the Hb. The charges variation on the external plates due to pH changes, is similar to a voltage variation on the capacitor in an electric circuit. In more detail, on the capacitor plates, the change of charges causes the polarization of the dielectric in which displacement current appears. Additionally, this allows for a correlation of the energy propagation inside the Hb to a polarization displacement current. Since the change in pH is of small entity (7.4 in the lungs and 7.2 in tissues), it causes a small variation of charges on the hemoglobe plates. However, in the lungs (as in tissues where the conditions are simply opposite, but of equal entity), this small variation, accompanied by the high pO_2, allow the binds of the first oxygen to Hb. In turn, the oxygen binding causes the chemical rearrangement of the molecule that, by displacement current effects, influences the internal polarization charges allowing for facilitation of the binding of the other oxygen molecules. Further and so on until the T-R transition is complete. It is easy to notice that our model fits perfectly with the sequential model that describes the Hb cooperative behavior and somehow hemoglobe theory provides further explanations on the dynamics of oxygen binding to Hb. In fact, the hemoglobe model gives a physical description of some aspects of the T-R transition based on thermodynamic considerations so we may define it as a "Physical Mathematical Model of Hb". All this is supported by mathematical calculations in Sections 2 and 4.

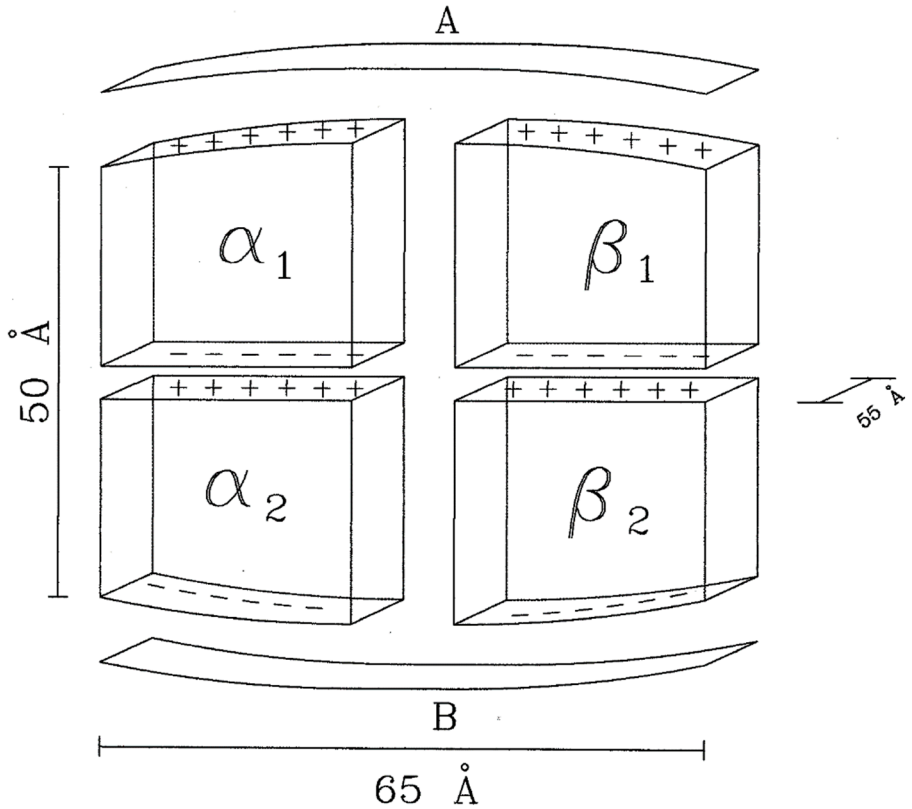

Figure 1. Hemoglobe: schematic model of the four subunits of the Hb assembled to form a capacitor with two faces A and B and surface S (armatures) with relative charges and characteristic dielectric (great part of the internal mass is the quaternary structure).

4. Physical-Mathematics Deductions

Based on the geometrical aspect of the Hb molecule and on the idea of Hb as a capacitor in which the quaternary structure is the dielectric, an important role is assigned to $L^{(1,1)}$ coefficient that

has the dimension of a conductivity. From now on, $L^{(1,1)}$ will be called Displacement Equivalent Conductivity Coefficient (DECC) since it is related to $dP^{(1)}/dt$. $dP(1)/dt$ has the dimension of a current, due to orientation polarization $P^{(1)}$, so we call it: Polarization Displacement Equivalent Current (PDEC). The term "equivalent" is justified by observing that a change of the polarization state implies a movement of charges and so it is equivalent to a current. The connection between PDEC and DECC is represented by Equation (13). The PDEC appearing in Equation (13) is only defined inside the matter (unlike the term $d\underline{D}/dt$, where $\underline{D}$ is the displacement vector) and in the Debye's model inside the matter with polar molecules. Recall that, in an electric circuit RLC [23] (Resistance, Inductance, and Capacitance) subjected to harmonic perturbation of frequency ω we introduce a quantity, called impedance, which is analogous to the Ohmic resistance, in the case of constant perturbation; this results from the sum of three terms, one Ohmic, one inductive, and one capacitive. The capacitance component of the impedance is:

$$Z_c = \frac{1}{i\omega C}$$

and therefore, it has the dimensions of a resistance linked to capacitive factors. Since the reciprocal of a resistance is a conductivity, it follows that ωC has the conductivity dimensions per unit of length. It was written from:

$$i\omega C$$

From this, it follows that ωC_e (conductivity for unitary length) in the MKSQ system has the following dimension:

$$[\omega c] = \frac{1}{s}\frac{Q^2 s^2}{Kg\ m^2} = \frac{Q^2 s}{Kg\ m^2} \tag{14}$$

while

$$\left[L^{(1,1)}\right] = \frac{Q^2 s}{Kg\ m^3} \tag{15}$$

This dimensional analysis and by observing that $L^{(1,1)}$ refers to Hb, now considered as a capacitor of defined size, suggest to us the following equality (in agreement with Somerfeld point of view on dimensional analysis) [23]:

$$L^{(1,1)}(meter) = \omega C_e \tag{16}$$

From which:

$$C_e = \frac{L^{(1,1)}(meter)}{\omega} \tag{17}$$

Since, from Equation (17), C_e is connected to DECC, we call it Displacement Equivalent Capacity (DEC). Obviously it results in:

$$C_e = C_e(w) \tag{18}$$

The position (16) is legitimate if we consider capacitor with polar dielectric (Debye). Figure 1 shows Hb like a parallelepiped with curved faces, since the protein is a permanent dipole, it can be considered like a plane capacitor of A and B plates, and a great part of the quaternary structure as dielectric. We are conscious that A and B sides are not plane, but we can deduce that they do not have an important curvature. Indeed, the Hb molecule is not a well defined figure, but it certainly is a permanent dipole with a dipole moment [20,21]. The non-angular shape allows us to coin a new term, well representing a geometrical image of the Hb model introduced by us. Thus, we call Hemoglobe the plane capacitor with A and B plates of area S placed to a distance d.

It will be remembered a capacitor is made up of two plates placed at a very small distance, so as to have distribution of charges, whose density is σ. The field near a plate is (see Somerfeld) [23]:

$$E = \frac{\sigma}{\varepsilon} \tag{19}$$

and capacity C (i.e., the possibility of the system to assume charges) is:

$$C = \frac{S}{d}\varepsilon \tag{20}$$

being S the surface area of the plate and d the distance between the two plates; ε is the dielectric constant of the medium interposed between the frames. If the charges on the surface remain constant over time, then C will be constant because ε is constant too; this occurs when the capacitor is subjected to a constant perturbation. If the perturbation is not constant and of the harmonic type like the perturbation used in the paper [23], then dispersive phenomena can be observed and a complex dielectric function is introduced that will vary with the perturbation frequency. It will be written [23]:

$$\varepsilon = \varepsilon_1 - i\varepsilon_2 \tag{21}$$

where ε_1 is linked to storage phenomena and ε_2 to loss phenomena. Replacing Equation (21) in (20) one has:

$$C = \frac{S}{d}\varepsilon_1 - i\frac{S}{d}\varepsilon_2 \tag{22}$$

therefore, the capacity can be considered as a complex function depending on the perturbation frequency ω and the real part of C is introduced:

$$C_1 = \frac{S}{d}\varepsilon_1 \tag{23}$$

and the imaginary one is:

$$C_2 = \frac{S}{d}\varepsilon_2 \tag{24}$$

so Equation (4) is:

$$C(\omega) = C_1(\omega) - iC_2(\omega) \tag{25}$$

The imaginary part of C is related to dissipation phenomena since this occur for ε_2. Taking into account that a Hb molecule has the following dimension: 64Å*55Å*50Å, we have S = 64*55Å and d = 50Å [22]. If $\varepsilon(\omega)$ is the dielectric loss of the Hb one has:

$$C_2 = \varepsilon_2(w) \times (64 \times 55)/50\text{Å} = \varepsilon_2(w)70\text{Å} \tag{26}$$

C_2 is the capacity of the hemoglobe. Both Equations (17) and (26) are two capacities expressed as functions of the perturbation frequency ω. While Equation (17) is obtained by thermodynamic considerations (we use the coefficient $L^{(1,1)}$), Equation (26) is the geometrical definitions of capacity for a plane capacitor. We note that Equations (17) and (26) should give similar values. This is true as can be seen in the Figures 2–4, that will be commented in the next section.

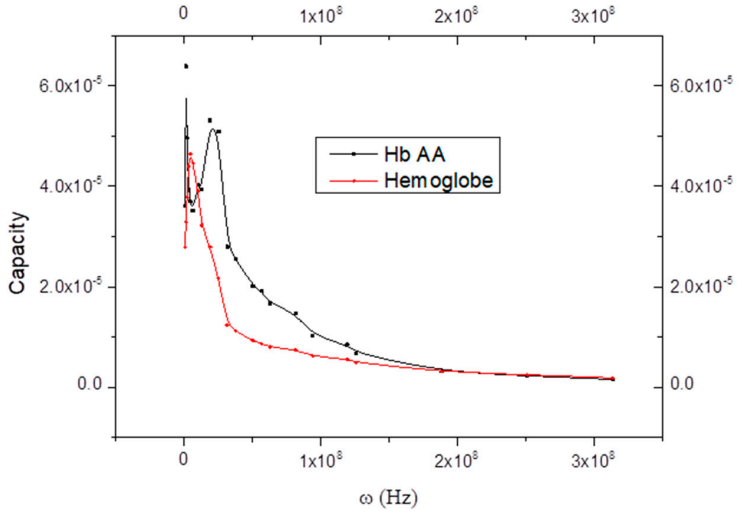

Figure 2. Comparison between HbAA (black line) and Hemoglobe (red line) capacity expressed in p-Farad (pF) calculated by Equations (30) and (31), respectively, as a function of frequency.

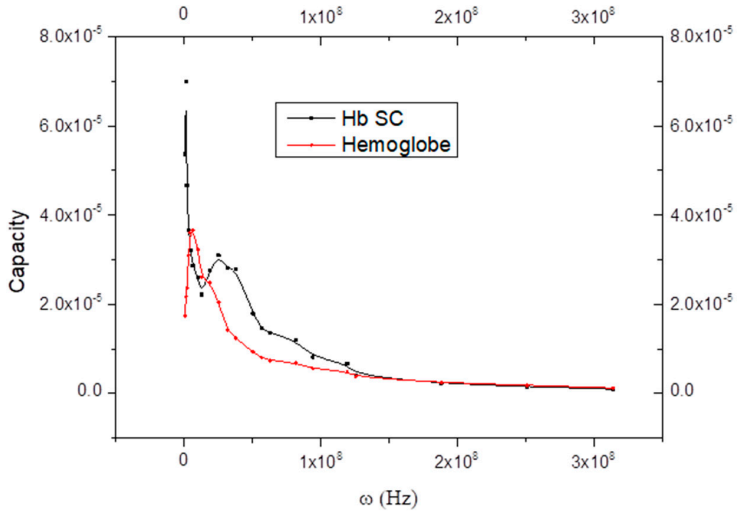

Figure 3. Comparison between HbSC (black line) and Hemoglobe (red line) capacity expressed in p-Farad (pF) calculated by Equations (30) and (31), respectively, as a function of frequency.

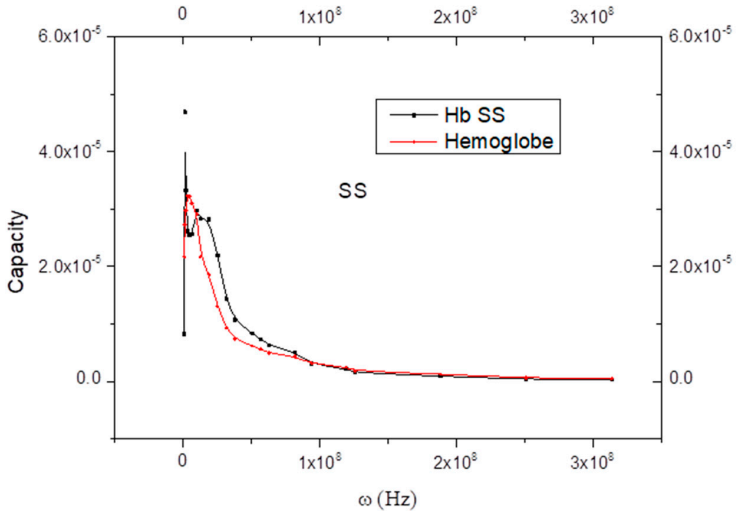

Figure 4. Comparison between HbSS (black line) and Hemoglobe (red line) capacity expressed in p-Farad (pF) calculated by Equations (30) and (31), respectively, as a function of frequency.

5. Hemoglobin as a Capacitor

From the non-equilibrium thermodynamics point of view, why is Hb regarded as a capacitor? The answer lies in the comparison between the following positions:

$$L^{(1,1)}(meter) = \omega C_e \tag{27}$$

from which it derives:

$$C_e = \frac{L^{(1,1)}(meter)}{\omega} \tag{28}$$

and comparing with capacity:

$$C_2 = \frac{S}{d}\varepsilon_2 \tag{29}$$

it is possible to deduct that both positions (28 and 29) have a similar behavior with different values for frequency of about 2.5×10^7 Hz. The comparison between these two curves (3 and 4) is not a fit. It must be considered as only a guideline to the concept that a flat capacitor with Hb structure as dielectric has a capacity with orders of magnitude similar to the capacity calculated using the equality (27). Furthermore, as an experimental support in a "Hypotheses non fingo" perspective we neglect the edge effects for the shape of the Hemoglobe. In a frequency range between 2.5×10^7 Hz and 10^8 Hz, the difference between the two curves (28) and (29) is due to a type of phenomena emerging when the thermodynamic coefficient $L^{(1,1)}$ is used. In line with our studies [4,5,10–18], the introduced coefficients (not only $L^{(1,1)}$) show phenomena not detectable with ε, because ε is the sum of many phenomena. The above on $L^{(1,1)}$ and ε is reported in the previous paper [4]. In this range of frequency, $L^{(1,1)}$ shows a fluctuating trend while ε shows a "regular" trend. The question arises, which of the calculated capacities is the correct one. Hb is a polar molecule, so the orientation polarization $P^{(1)}$ is predominant and only the displacement conductivity $L^{(1,1)}$ associated with $P^{(1)}$ will be considered. The correct Hb conductivity, or rather the most reliable, is that calculated with (28), where only displacement phenomena are considered as it should be done in a dielectric. In the ε_2 value, which appears only in Equation (29), a series of phenomena are present, because ε_2 is not selective as the $L^{(1,1)}$. Hb is not a perfect dielectric, because it presents dispersive phenomena. Then, from a capacitive point of view, it is very important to select displacement phenomena. In these circumstances, Equation (29) is not very suitable to calculate the capacity; position (29) is only considered because it can give an idea of the order of magnitude of the capacity. It should be remembered that these statements arise from the desire to study dielectric relaxation phenomena resulting from a harmonic perturbation. In the physiological reality, this does not happen and therefore these affirmations have the role to support the idea that Hb may be considered as a capacitor when perturbed by a harmonic action. Now, some considerations, further justifying what has been said so far, will be made. Let us start by pointing out that our deductions are founded on experimental data reported by Laogun et al. (1997) [19]. The analysis of the experiments described in this paper allow us to state that each point of the solutions of Hb AA, SC, and SS is subjected to the same perturbation and therefore each Hb molecule is subjected to the same field generated by the surface change on the plates [19]. Let us not forget that we are in linear approximation. Moreover, Equation (17) implies that conductivity phenomena have a capacitive character, therefore not inductive and not resistive. This statement is reasonable if the experiment is analyzed and especially the range of frequencies investigated. Even if $L^{(1,1)}$ refers to the solution, Equation (13) induces us to write position (16) so that DECC has a capacitive character, peculiar of the displacement current in a capacitor. In the range of frequency under investigation, the only elements of the solution irrefutably influencing $L^{(1,1)}$ are the Hb molecules, whence the approach is to a capacitor. Obviously, the "Hemoglobe" is the first approach to a capacitor model: a curve is substituted with a plane side, even if the difference is small; moreover, we only study the dielectric relaxation of the very complex phenomena occurring in the Hb molecule. However, we think that, by the development of

this approach, it can be possible to conceive the model more and more near the reality. After these clarifications, for a Hb molecule, Equation (17) is:

$$C_e = (L^{(1,1)}/\omega) \times 56.3\text{Å}$$

from which, in the MKSQ system, this becomes:

$$C_e = (L^{(1,1)}/\omega) \times 56.3 \times 10^2\text{pF} \tag{30}$$

where 56.3 is the medium value of the three Hb dimensions. Equation (26) become:

$$C_2 = \varepsilon_2(\omega) \times 70\text{Å} = 8.856 \times 10^{-12} \times 70 \times 10^{-10}\ \varepsilon_{2r}{}'(w)10^{12}\text{pF} \tag{31}$$

where $\varepsilon_{2r}(\omega)$ is the relative dielectric constant [19]. By substituting, respectively, $L^{(1,1)}$ calculated in our previous paper [4] in Equation (30) and $\varepsilon_{2r}(\omega)$ measured by Laogun et al. (1997) [19] in Equation (31), we obtain two curves as a function of the frequencies for the Hb AA, SC, and SS plotted in Figures 2–4. The capacity (26) shows characteristics only obtained by taking into account $\varepsilon_{2r}(\omega)$, while the capacity (17) shows more details that are deductible by the theory developed by us, as is shown in the Figures 2–4. This is to confirm that the developed approach is able to evidence and detect some phenomena not considered by all other approaches. Extending this idea to the real physiological case, in the next paragraph, some aspects of the hemoglobin T-R transition will be described considering the protein as a "plane capacitor" not subjected to harmonic actions, but to a variation of surface charges induced by physiological phenomena.

5.1. Observations

The in vitro measurements do not give any information about the Hb functions when the protein is inside its biological place (inside the blood). Thus, all the processes that occur in human body cannot be studied with a Hb solution in vitro. Nevertheless, our theoretical deductions, based on a Hb solution perturbed with an electric field (dielectric measurements), allows to hazard the hypothesis that somehow processes occur because it was stimulated by the field. It is as to partially stimulate the Hb molecule to work as an electrical machine. Physical considerations induced us to consider this machine as a capacitor and to think that this behavior can be preserved even in the human body. Better yet, can this model be used to explain some energetic phenomena? In other words: If the Hb shows behaviors similar to a capacitor in vitro, why can it not work as a capacitor even in vivo, inside the human body? This will explain, qualitatively (but not quantitatively because we have not experimental data), some energetic balance processes occurring in vivo; this is in agreement with a characteristic of the capacitor: To accumulate energy and release it at the appropriate time and with a certain frequency.

5.2. Capacitor Model

The variation of the surface charge density of the Hb during its travel from the tissues to the lungs occurs in a chaotic way, we would say randomly, because it is due to turbulence of the components inside the Hb and to the influence of the blood flow that carries it. This disorder can be considered as a background sensor to the phenomenon that somehow directs this randomness towards a well defined causal order: The T-R transition. The phenomenon to which we refer is linked to the potential binding of an oxygen molecule (and then the other two) to iron by penetrating the surface barrier of Hb. We believe that the oxygen binding to Hb may be favored by the generation of displacement currents through the breaking of chemical bonds inside the Hb. Now let us see how the introduced model takes into account the generation of these displacement currents. Comparing the Hb to a capacitor, we assume that a randomly variable charge is formed on its walls, during the journey in the blood stream. For the reasons mentioned above, we consider the contribution of this charge to the generation of displacement currents irrelevant, which will be random and with random directions too. We cannot

quantify the magnitude of this current, but we believe that it is slight because it was caused by factors internal to the Hb. When Hb reaches the pulmonary environment, the influence of charges generated on the walls of the hemoglobin capacitor is different. Here, the different environmental conditions cause the generation of polarization charges in a very short time. Given the rapidity with which this occurs, we think that these charges can be represented with an exponential function, which we indicate with σ.

$$\sigma = \sigma_0 e^{\pm Rt} \tag{32}$$

where σ_0 is a constant, whose dimensions are of a superficial charge density and R is a constant, whose dimensions are t^{-1} and it is characteristic of Hb. The sign ± indicates an increase or a decrease in surface charges, what is important is the charge variation because this is the cause of the generation of displacement currents occurring in this time frame. In other words, the variation of s over time (very short) generates the displacement currents. Only the variations in occurring when the Hb reaches the lungs, will be taken into account like favoring factor to the binding of the first oxygen molecule to Hb. Therefore, the function (32) is defined in a time interval T indicated with $\Delta t = t_1 - t_0$ and that will also be written as:

$$t_0 < t < t_1$$

being t_0 and t_1 the instants in which the Hb arrives in the lungs and the oxygen binds to the iron, respectively. A change of σ on the "Hb wall" generates an electric field E on the "inner wall", given by:

$$E = \frac{\sigma}{\varepsilon}$$

i.e.,:

$$E = \frac{\sigma_0 e^{\pm Rt}}{\varepsilon} \tag{33}$$

where ε is the dielectric constant of the quaternary structure of the Hb. ε will be a function of time as the displacement currents act by breaking the chemical bonds. Therefore, there will be orientation polarization currents identified by the function $P^{(1)}$; the deformation polarization will be neglected because Hb is a polar molecule so the effects due to permanent dipoles prevail. Neglecting the deformation polarization, the field $E^{(1)}$ related to the orientation polarization $P^{(1)}$ is [4]:

$$E^{(1)} = \left(a^{(0,0)} - a^{(1,1)}\right) P^{(1)} \tag{34}$$

this field is equal to the one expressed by (33) for what was said before:

$$E^{(1)} = \frac{\sigma_0 e^{\pm Rt}}{\varepsilon} \tag{35}$$

The displacement currents $dP^{(1)}/dt$ due to orientation polarization phenomena are expressed by [4]:

$$\frac{dP^{(1)}}{dt} = L^{(1,1)} E^{(1)}$$

in which, substituting Equation (35):

$$\frac{dP^{(1)}}{dt} = L^{(1,1)} \frac{\sigma_0}{\varepsilon} e^{\pm Rt} \tag{36}$$

The integration of this differential equation allows for calculation of the orientation polarization in the function of the time. Unfortunately we do not know the expression of $\varepsilon(t)$ because there are no experimental data, so we cannot integrate it. From Equation (36):

$$P^{(1)}(t) = \int L^{(1,1)} \frac{\sigma_0}{\varepsilon} e^{\pm Rt} dt + c \tag{37}$$

C is an integration constant. From the Equation (32), σ_0 is the density of charge for $t = 0$, so assuming $t_0 = 0$ as the time in which the Hb reaches the lungs, σ_0 will be the value of the surface charge density on the Hb wall at the moment the protein reaches the lungs.

Recalling Equations (32) and (37), we have:

$$\sigma = \sigma_0 e^{\pm Rt} \tag{38}$$

$$P^{(1)}(t) = \int L^{(1,1)} \frac{\sigma_0}{\varepsilon} e^{\pm Rt} dt + c \tag{39}$$

since σ_0 and $L^{(1,1)}$ are temporal constants, Equation (39) can be written as:

$$P^{(1)}(t) = L^{(1,1)} \sigma_0 \int \frac{e^{\pm Rt}}{\varepsilon} dt + c \tag{40}$$

The problem to calculate this integral arises from the lack of analytical knowledge of $\varepsilon(t)$. ε derives from the dielectric phenomena occurring inside the Hb and these are affected by the displacement currents generated at $t = 0$ (due to the pulmonary environment) so ε may also vary exponentially, but with a time lag with respect to density expressed by Equation (38). Therefore, the dielectric function can be expressed in the following form:

$$\varepsilon = \varepsilon_0 e^{st+\phi} \tag{41}$$

s is a constant whose dimensions are t^{-1} and ϕ is the time delay with respect to the variation of σ. Substituting Equation (41) in (40) is:

$$P^{(1)}(t) = \frac{L^{(1,1)} \sigma_0}{\varepsilon_0} \int e^{\pm Rt} e^{-st-\phi} dt + c$$

$$P^{(1)}(t) = \frac{L^{(1,1)} \sigma_0 e^{-\phi}}{\varepsilon_0} \int e^{(\pm R-s)t} dt + c \tag{42}$$

$$P^{(1)}(t) = \frac{L^{(1,1)} \sigma_0 e^{-\phi}}{\varepsilon_0 (\pm R - s)} e^{(\pm R-s)t} + c \tag{43}$$

This is the expression of orientation polarization. Only the variations of the phenomena for $t > 0$ (i.e., from when the Hb reaches the lungs) were considered, so $P^{(1)}$ expressed by Equation (43) will be null for $t = 0$ and therefore:

$$P^{(1)}(t) = \frac{L^{(1,1)} \sigma_0 e^{-\phi}}{\varepsilon_0 (\pm R - s)} + c = 0$$

i.e.,:

$$c = -\frac{L^{(1,1)} \sigma_0 e^{-\phi}}{\varepsilon_0 (\pm R - s)} \tag{44}$$

and Equation (43) is:

$$P^{(1)}(t) = \frac{L^{(1,1)} \sigma_0 e^{-\phi}}{\varepsilon_0 (\pm R - s)} \left[e^{(\pm R-s)t} - 1 \right] \tag{45}$$

this is the final expression of $P^{(1)}(t)$.

5.3. Hemoglobe Energy In Vitro

It is well known that all capacitors have an intrinsic energy given by:

$$U = \frac{1}{2} \times Q^2 / C_0 \tag{46}$$

where Q is the charge in a side of the Hb and C_0 is its capacity. Now Q is calculated. We remember that:

$$E = E^{(eq)} + E^{(ir)} \tag{47}$$

and

$$Q = D \times S \tag{48}$$

where D is the dielectric displacement from equation:

$$D = \varepsilon E \tag{49}$$

and from Equation (48), it follows:

$$U = \frac{1}{2} \times E^2 S^2 \times \left(E^{(eq)} + E^{(ir)}\right)^2 / C_0 \tag{50}$$

By substituting Equations (17), (26), and (27) become, respectively:

$$U_G = \frac{1}{2} \times ESd \times \left(E^{(eq)} + E^{(ir)}\right)^2 \tag{51}$$

$$U_T = \frac{1}{2} \times E^2 S^2 \left(E^{(eq)} + E^{(ir)}\right)^2 / L^{(1,1)} \tag{52}$$

By considering the explicit expression of $E^{(eq)}$ and $E^{(ir)}$ [13], Equations (51) and (52) become:

$$U_G = \frac{1}{2} \varepsilon s d P_0^2 \left(\Gamma_1 \sin \omega t + L^{(0,0)} \omega \cos \omega t\right)^2 \tag{53}$$

$$U_T = \frac{1}{2} \frac{\varepsilon^2 s^2 P_0^2 \left(\Gamma_1 \sin \omega t + L^{(0,0)} \omega \cos \omega t\right)^2 \omega}{L^{(1,1)}(meter)} \tag{54}$$

where we remember that $P = P^{(0)}$ sen ωt, and $P = P^{(0)} + P^{(1)}$.

In both Equations (53) and (54) P_0 is an unknown value since we do not know the field values applied in the experiment. From Equation (53), one obtains:

$$P_0 = \sqrt{\frac{2U_g}{\varepsilon s d}} \left(\Gamma_1 \sin \omega t + L^{(0,0)} \omega \cos \omega t\right) \tag{55}$$

and from Equation (54):

$$P_0 = \sqrt{\frac{L^{(1,1)}(meter)}{\omega}} \frac{2U_T}{\varepsilon s \left(\Gamma_1 \sin \omega t + L^{(0,0)} \omega \cos \omega t\right)} \tag{56}$$

These two expressions are able to assign the values of P_0 if we know the energy U_G and U_T, that in this case are the same; they are calculated with other techniques. Other terms are known.

5.4. Hemoglobe Energy In Vivo

It is well known that all capacitors have an intrinsic energy given by

$$U = \frac{1}{2} \times Q^2/C \tag{57}$$

where Q, taking into account Equation (32), is:

$$Q = s \times S = S\sigma_0 e^{\pm Rt} \tag{58}$$

Taking into account Equations (20) and (57) become:

$$U = \frac{1}{2} \frac{Sd\sigma_0^2 e^{\pm 2Rt}}{\varepsilon} \tag{59}$$

This is the in vivo energy of Hb considered as a capacitor.

6. Curve Description

Figures 2–4 show the capacity of hemoglobe evaluated by the mean of Equation (31); it is possible to note a sufficiently regular course of the capacity and an asymptotically decreasing trend for the three Hbs. By observing Equation (30) and the $L^{(1,1)}$ trend, in this range, we say that they are in agreement, as can be noted by the $L^{(1,1)}$ curve shown in Farsaci et al. (2019) [4]. Apart of this, the curves evaluated by Equations (30) and (31) show a sufficient similarity. It is important to emphasize that, by Equation (30), results were obtained that are not shown by Equation (31). This means that the "thermodynamic" evaluation of the capacity (by Equation (30)) gives information that cannot be obtained in another way. Figures 5 and 6 show a comparison of the three Hbs, on the trend of thermodynamic capacity and energy, evaluated by Equations (30) and (54), respectively. More careful analysis of all curves reveals significant differences, certainly attributed to primary structural substitutions on β6 residues in pathological HbSS (homozygous) and HbSC (heterozygous) with respect to HbA. In detail, on the shape of the curves the substitutions with loss of charge (as in HbSS, where two negative charges are replaced by two residues without charge) have more significant effects than those with an opposite charge (as in HbSC, where two negative charges are replaced with two positive). All this strongly supports the idea of the capacitor, which electrical and thermodynamic functionality is due to electric charges and is expressed through them.

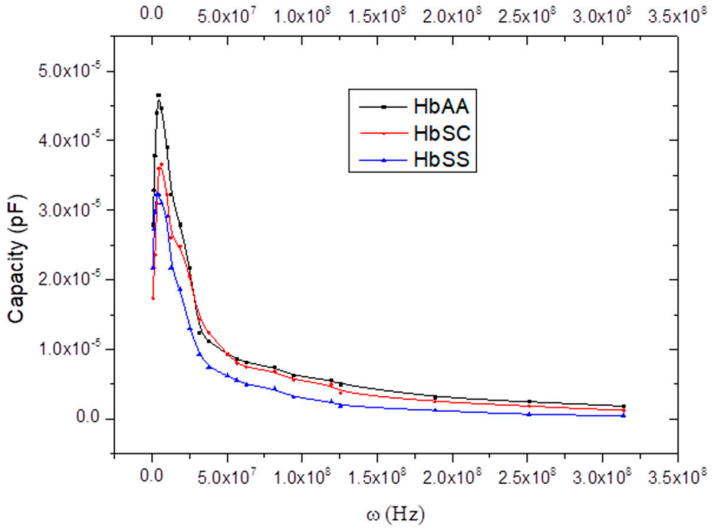

Figure 5. Comparison between HbAA (black line), HbSC (red line) and HbSS (blue line) capacity expressed in p-Farad (pF) calculated by Equation (30), as a function of frequency.

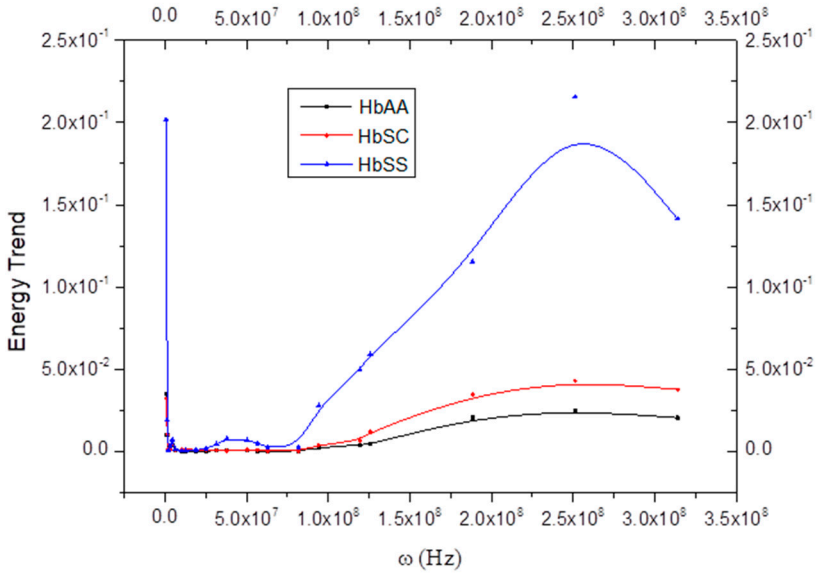

Figure 6. Comparison between HbAA (black line), HbSC (red line) and HbSS (blue line) Energy trend calculated by Equation (54), as a function of frequency.

7. Conclusions

The characterization of the Hb, trough the non equilibrium thermodynamic with internal variables allowed for reaching the goal of our study: The formulation of a physical mathematical model of Hb, the "Hemoglobe" a plane capacitor "with non-plane faces" and with dielectric characters. This simple model, which we (etymologically) call hemoglobe, reports the Hb as a geometric figure with the dimensions of 64 Å × 55 Å × 50 Å, very close to its complex biological reality. In a comparative context with the pathological hemoglobins HbSS and HbSC, the capacity that is a function of the angular frequency and the energy of the hemoglobe have been calculated. The phenomenon of conductivity, represented by the term $L^{(1,1)}$, has been interesting and determinative, because it revealed a capacitive character, therefore neither inductive nor Ohmic and peculiar of the displacement currents in a capacitor. Furthermore, at the molecular level and at the frequencies considered, the experimental logics described, allowed for the evaluation of the Hb molecules as the only "agents" of the solution that could influence $L^{(1,1)}$, from which the association almost unquestionable to a plane capacitor. The introduction of the hemoglobe discloses a new vision of the T-R transition of the Hb, which does not contrast with the existing theories but rather strengthens them. Because, our thermodynamic results provide useful information, or at least points of correlation, between mechanical movements of a certain entity, such as the rotation of Hb protomers, and structures where production and storage of the energy, required for the T-R transition, occurs. Through the physical mathematical model of hemoglobe, we can explain some aspects of the T-R transition and cooperativity of the Hb in terms of capacitor functionality, looking at the oxygenation-deoxygenation cycle of the protein in the blood circle as a voltage variation on the capacitor in an electric circuit. In other words, the theory of "hemoglobe" reinforces the importance of the peculiar quaternary structure of the protein whose molecular architecture is preserved at all levels of the vertebrate evolutionary scale. The protein appears constructed in such a way to full maximize its functionality as an oxygen carrier, so that to do this, the molecule does not only exploit the chemistry of its bonds, but also the mechanics of its structure and the thermodynamics of its charges. We can state that, during the vertebrate evolution, Hb is perhaps the only molecule, that although significantly changes its primary structure manages to maintain its functionality, almost ignoring the dogma that correlates each structural variation to the functional one. An architecture of quaternary structure ables of generating and accumulating energy and of being able to release appropriately it at the moment of the respiratory transition of the protein. Concluding the capacitor (hemoglobe) from simple and elementary electric machine becomes

a complex and unusual, but effective electric machine. In fact, as thermodynamically highlighted, the protein is able not only to properly store and release energy but also to strategically produce it for a "mechanical consequence" induced by the O_2 binding. In fact, the breaking of the salt bridges that stabilize the structure T, generates new electric charges on the capacitor and these provide new capacity and new energy charge that are stored. An oxy-deoxy cycle of the Hb is carried out through the T-R and R-T transitions that produce and conserve energy, so Hb is able to generate energy (under the oxygen mechanical thrust) and at the same time, being a capacitor, it can conserve and release energy.

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Abbreviations

Hemoglobin (Hb); Oxygen (O_2); 2,3-bisphosphoglycerate (BPG); carbon dioxide (CO_2); Human Hemoglobin (HbAA); Homozygous Sickle Hemoglobin (HbSS); Sickle Cell Hemoglobin C Disease (HbSC); Displacement Equivalent Conductivity Coefficient (DECC); Polarization Displacement Equivalent Current (PDEC); Displacement Equivalent Capacity (DEC).

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