Hydration shell dynamics of proteins and ions couple with the dissipative potential of H-bonds within water cluster

Alfred Bennun[1, 2]
[1]Full Professor - Emeritus - Rutgers University, USA
[2]CONICET, AR

ABSTRACT

A homotropic system (crystals) was used by Perutz to show that the breaking of salt-links could trigger a one-way tense (T) to relax (R) change of Hb. The tendency to reach equilibrium by mass-action of reactants characterizes close systems, in which a single peak for activation energy *Ea*, allows microscopic reversibility. However, an open system is required to support a steady state conformational turnover of the protein. The hemoglobin system a T into R and R into T integrates both senses into a cycle, without involving a direct reversal like T<-->R. Open thermodynamics allows the dissipative potential of water cluster $(H_2O)n$ to interact with the hydrophilic asymmetries of Hb, to restrict the kinetic sense randomness of a single peak for activation energy (*Ea*). Instead the conformational dynamics of hydration shells could sequence an enhanced *Ea* into several peaks, to sequentially activate intermediate transitions states. Hence, Δμ (dipole states), Δsliding, ΔpKa, Δn-H-bonds, etc., could became concatenated for vectoriality. $(H_2O)n$ by the loss of hydrogen-bonds (H-bonds) couple with to the hydration turnover of proteins and ions to result in incomplete water cluster $(H_2O)n^*$, with a lower "n". $(H_2O)n^*$ became a carrier of heat/entropy into the cerebrospinal fluid (CSF) which has to be replaced 3.7 times per day. Lung exhales water as vapor which had lost H-bonds present in water cluster a latent form of heat dissipation at homeostatic temperature. Open biological systems have adapted the latent heat of water vaporization to dissipate heat from the inside into the outside of the system to prevent the increase of internal entropy.

Keywords: microscopic reversibility, water cluster, CSF, homeostatic, H-bonds, symmetry breaking, nano-molecular mechanics.

INTRODUCTION

Photophosphorylation studies did not allow detection of reversibility in the absence of un-couplers [1] [2], indicating the presence of a restriction to microscopic reversibility. Reconstitution of the architecture of lipid-protein regions within a membrane was shown to be required for the binding of CF1-ATPase [3].

The dynamics of H-bonds on the hydration shells of ATPase were shown capable to modify the catalytic activity of the enzyme [4]. Energy transduction was shown to be mediated by high energy conformational intermediate coupling across coordinative bonding [5]. These were characterized as delocalizing activation energy (*Ea*) for a vectorial sequencing of transition states [1] [2] [3].

Prigogine (1947) [6] proposed that a system with a minimum entropy production, could steadily go down to a minimum were it stays, supporting for a time a steady state. This non-linear probable state refers to perturbations of thermodynamic forces, too small to invalidate a principle of maximum entropy production [7].

A system is closed when a boundary, allows passage of work and heat. The system is open, when matter and energy can pass across the boundary. Phase boundaries could characterize an organismal relationship with its environment. The uptake of metabolite is mediated by an air phase boundary, allowing uptake of O_2 and release of CO_2 and Heat. The latter, became a carrier of entropy to the outside of the organismal system.

Large hadron colliders have produced a rather large number of high energy particles, which could be plotted within a range from 10^{-25} to 10^{-7} seconds, according to their half-life decay, which became an enthalpy contribution to the arrow of time [8]. These dissipative events generate neutrinos and antineutrinos which scape the open system by lack of return reactivity [8].

In linear systems entropy production is the possibility of cosmic evolution to support self-organization in other regimes of local dynamics [9] [10] [11] [12].

Microscopic reversibility of dynamics implies that the matrix for events is symmetric [13] and generates a single *Ea* peak for either the forward or reverse sense of the reactions.

Prigogine's premise: "Dynamics and thermodynamics limit each other" [14] [15] indicates that these processes could be differentiated.

The organization of molecules within defined membrane structures imposes physicochemical constraints, which are not especially subject to a random distribution of *Ea* [16]. By the contrary in a lipid-enzyme membrane the inter- and intra-molecular kinetic energy could be represented by coupling between several peaks driving specific transitions states, along the vectorial progress of an energy transduction process [16].

Intra-molecular asymmetry could be confers by water dynamics capacity to differentiate a hydrophilic space, or region from a hydrophobic one [4]. This allows that the hydration shell of proteins and ions could confer turnover to water architectures when couple with the dissipative potentials of water cluster $(H_2O)n$ [4]. Hence, the H-bonds formed by a $-\Delta G$ of restructuring hydration shells enhances the energy requirements to reach transition state.

METHODS

The quaternary structure of Hb show specific regions that could be characterized in terms of variable function dynamics. This localizes events according to hydrophilic versus hydrophobic regions and dynamics of R-groups reactivity during the transition from oxy to deoxyHb.

The four Heme groups of Hb show a 2-fold symmetric axis [17] [18] [19] leading to the idea that any one Heme site, would interact equally over the 24Å inter Heme to Heme distances for cooperative O_2-ligation [20].

The two states concerted MWC model (Monod-Wyman-Changeux model) [21] proposed an allosteric mechanism which in stereochemical basis was assumed to implicate that the relative ratio of the equilibrium between low vs. high O_2 affinity forms of Hb, could represent conformational forms, a tense (T) versus a relax (R) [22]. Protein dynamics explain the allosteric behaviors of hemoglobin. The study of deoxyHb revealed a presence of a central cavity for 2,3-DPG binding [23] [24] and the tendency of oxygenation to induces the association of Mg^{2+} [25] [26] or Zn^{2+} [27] to Hb.

RESULTS

1. Structure and Function

Hb structure shows hydrophobic regions at $\beta_1\alpha_1$ and $\beta_2\alpha_2$ intradimer interfaces and hydrophilic polar R-groups at the $\beta_2\alpha_1$ and $\beta_1\alpha_2$ interfaces [31] [32].

During oxygenation the hydrophilic asymmetry of Hb allows a fully hydrated Mg^{2+}/Zn^{2+} ($[Mg(H_2O)_6](H_2O)_{12}$) to enter first into the $\beta_2\alpha_1$ interface for specific sequential chelation of R-groups. The process leads the hydrated metal to an exergonic binding with Hb compensated by the endergonic loss of most of the divalent metal hydration shell [33].

Human-RBC hemolysates show an increment of $^{14}CO_2$ released from the consumption of [1-^{14}C]-glucose, within the hexose monophosphate (HMP) pathway by the Mn^{2+} stimulation of the redox recycling of NADPH (nicotine adenine dinucleotide phosphate), which contribute to homeostasis of pH, because otherwise could favor lactic acid formation [34].

The level of glucose at the red cell could act as an integrated sensor of the brain needs, because in the Rapapport-Luebering scheme the 2,3-DPG phosphor-mutase, which is inhibited by low pH, becomes maxi-mally activated at pH=7.4. Since cerebrospinal fluid (CSF) is slightly alkaline could signal the red cell to increase the erythrocyte level of 2,3-DPG [35] to form 2,3-DPG-deoxyHb-$(H_2O)_T$ (Figure 1) and release of O_2 to match glucose uptake and maintain aerobic glycolysis in brain generating the ATP required to operate Na^+-pump [36]. OxyHb in transition to deoxy releases a $[Mg(H_2O)inc]^{2+}$ in CSF, which by binding proteins to the membrane could protect the tendency of ATP^{4-} to subtract Mg^{2+} from the protein lipid structure [3] of neuron.

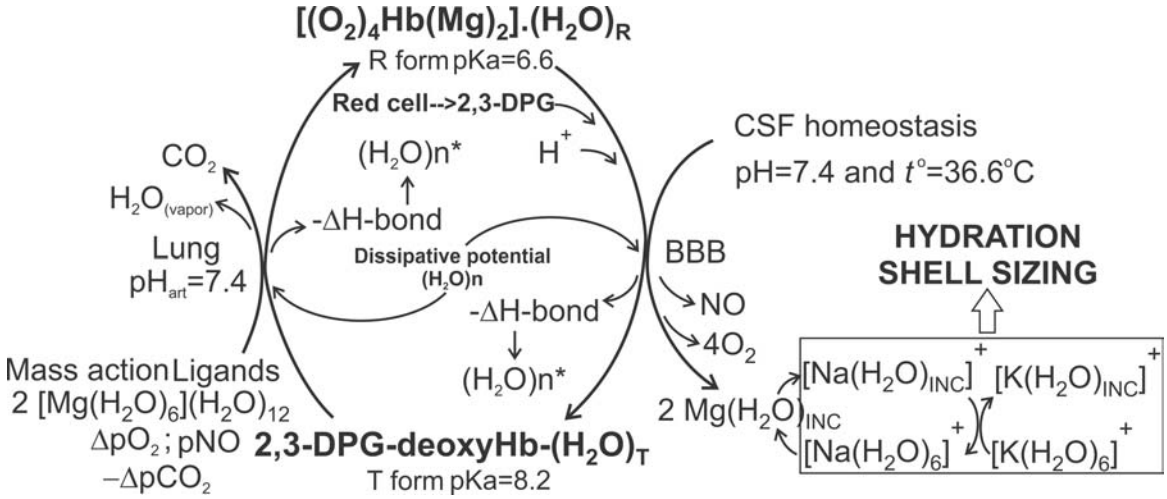

Figure 1: Configuration of turnover from oxy- into deoxy- and return to oxy-Hb. An open system supports in steady state the reactivity of water by its H-bond dissipative potential from incoming cluster-rich $(H_2O)n$, n=12-14, versus its exit as water cluster-poor $(H_2O)n^*$, n=5-6. The sub-indexing is use to indicate that the number of water molecules in their hydration shells allows to differentiate the "R" for relax and the "T" for tense forms of Hb. Interconversion of the deoxy- to oxy- depends on sliding shifting α Pro 44 allowing entrance at the two hydrophilic interfaces βα of $2[Mg(H_2O)_6](H_2O)_{12}$. The Mg^{2+} ion losses most of hydration shell when binding Hb and during deoxygenation is released with an incomplete hydrated shell $[Mg(H_2O)inc]^{2+}$ of greater effective charge. Mg^{2+} increases permeability of the blood-brain barrier (BBB). Its smaller size allows to be discharged at CSF and interact for ion shell sizing decreasing the hydration shell of Na^+: $[Na(H_2O)inc]^+$ which enters into its channel at the Na^+-pump to take H_2O-out of the hydration shell of K^+: $[K(H_2O)_6]^+$.

Figure 1 shows that the dissipative potential of $(H_2O)n$ can be maintained in steady state when an open system allows its input in the reaction media to be balanced by the output of exhausted water cluster (H2O)n* [36] [37] [38] [39].

During deoxygenation H^+-uptake became associated to the amphoteric response of protonating the imidazole of His residues. The NH^+ in the rings attracts H_2O molecules for an exergonic H-bonding within deoxyHb, balancing the break (endergonic) of the coordinative bonds between Mg^{2+} and Hb. Thus, releasing from chelating state, the $[Mg(H2O)inc]^{2+}$ with a high spontaneous tendency to subtract H_2O from the hydration shells of protein an ions, allowing sizing for the fitting of ions into their respective gates. This may provide the $-\Delta G$ input required to build hydration shells even if CSF maintains a thermal-homeostatic [40].

CSF is produced in clusters at the thin walled capillaries called chorideplexes that line the walls of the ventricles. Its high water turnover maintains allostasis of cerebral water with cluster sizes of about 12 molecules [41].

The increase in the internal vibrational state of molecules in an incomplete H-bonded network, could contribute within CSF, to maintain its homeostatic temperature.

Figure 1 shows that the turnover process breaks the electrical bonding of H_2O molecules in $(H_2O)n$ from n=12-14 to about n=5-6 [41] [42]. Thus, $(H_2O)n^*$ by de-creasing the number of H-bonds within the cluster traps a heat-attenuated carrier of the increment in entropy, to be released-out of the reaction boundary. However, outside the body in contact with lower temperature, the dipole tendency of H_2O [43] slowly but spontaneously will allows reconstitution of $(H_2O)n$ state.

The steady state of available cluster rich water demands a 3.7 times of the 160ml volume of CSF/24hs about 600ml of CSF, matching the brain requirement of 20% of total body metabolic activity.

DISCUSSION

Physiological integration allows the Red cell-Hb-CSF to function as a sensor adapting response to Hb heterotropic equilibriums. At the lungs the mutual inclusion of O_2 and Mg^{2+}, each one increasing affinity for the other, stabilizes the relax (R) form in a $[(O_2)_4Hb(Mg)_2].(H_2O)_R$ complex. At tissue level, the inclusion of H^+ and 2,3-DPG couples for exclusion of O_2 and Mg^{2+} to stabilize the tense (T) form in a 2,3-DPG-deoxyHb-$(H_2O)_T$ complex.

OxyHb formation involves sliding-down of $\beta_2\alpha_1$ versus $\beta_1\alpha_2$ chains, to shift Pro 44 in α_1 and α_2 into allowing the entrance of a fully hydrated $[Mg.(H_2O)_6](H_2O)_{12\text{-}14}{}^{2+}$ (or Zn^{2+}) into the hydrophilic $\beta_2\alpha_1$ and $\beta_1\alpha_2$ interfaces. OxyHb pKa of 6.4 leads to H^+-dissociation increasing negative charge of R-groups. This at $\beta_2\alpha_1$ sequence two tetradentate chelates, first a Mg^{2+}, enters into coordinative bonding with β_2 His 92 and a second Mg^{2+} with α_1 His 87, to cooperatively release hindrance. The interconversion of oxy-to-deoxyHb, pKa=8, leads to the amphoteric imidazole to became positively charged and proximal histidines return into hindrance position, releasing incompletely hydrated $[Mg(H_2O)inc]^{2+}$ and O_2 into CSF. The protonated β_1 and β_2 His 143 are released from the chelates to salt-link with 2,3-DPG.

Rotation and sliding during oxygenation pulled the C-termini of β chains away from contact with α chains and β His 97 shifted in between α Thr 41 and α Thr 38 [20]. Hence, in oxyHb down-sliding unlocks α Pro 44 shifting it into to a position in which cannot longer sieve the access of fully hydrated $[Mg.(H_2O)_6](H_2O)_{12\text{-}14}{}^{2+}$ into the hydrophilic interface. Mg^{2+} is required to activate adenylyl cyclase (AC) [42] [43], its product cAMP [44] and/or cGMP [45] could be transported into human erythrocyte (Human-RBC), against significantly large differentials of the intracellular-extracellular concentrations [46] [47], and therefore could be involved in feedback modulation.

CONCLUSIONS

At room temperature thermal processes affect R-groups translational, vibrational, and rotational kinetics at the level of 0.8kcal/mol. The breakdown of H-bonds in $(H_2O)n$ could be evaluated for the bond O-H…:O as 5kcal/mol and for HO-H…:OH_3^+ as 4kcal/mol. The decrease in "n" within $(H_2O)n$ could couple with the increase (exergonic) in the number of H-bonds within a protein or an incomplete hydrated ion. These events increase tendency to drive the progress of transition states with a vector-sense. Therefore, coupling *Ea* with water dynamics provide an energy level, which can overcome thermic randomness. Moreover, allows the increment in the H-bonds within a molecule to retain a coupling potential for a longer time that allowed by the heat dissipation of *Ea*.

Hence, the need to discern for vectorial systems a mechanism, which could prevents microscopic reversibility from entangling the directionality of processes lead to analyze Hb in function of multiple-equilibrium including $(H_2O)n$. Open systems couple the continuous entrance of S and its P exit, with a dissipative potential $-\Delta G$ to operate at far from the system final thermodynamic equilibrium. A thermodynamic dissipative potential of O_2 and metabolites uptake with CO_2 releases out of the system for entropy exclusion.

However, the overcoming of microscopic reversibility requires the integration of thermal and water dynamics, acting over the asymmetric reactive tendencies of Hb, under low vs. high pO_2. In a asymmetrically structured system an energy dissipative potential spread by coupling, between several transitions states could restrict reversibility. In deoxyHb, the sliding of the α- vs β-chains move α Pro 44 into blocking the entrance of fully hydrated $[Mg(H_2O)_6](H_2O)_{12}$, but allows the exclusion of $[Mg(H_2O)inc]^{2+}$ and $(H_2O)n$*. The mechanism could differentiate between two forms of the same molecule by its size and the energy contained in "n" H-bonds. Maxwell implicated the capability to decrease entropy as a mechanism separating molecules by their difference in energy contained, between their heated vs cooled states.

After *Ea* of sliding dissipates the molecule of Hb could no longer acquire the transition states capable to allow microscopic reversibility, because the R-groups which were previously in a reactive proximity shift into a more distant no longer reactive position. The separated of unidirectionality formed 2,3-DPG-deoxyHb-$(H_2O)_T$ to $[(O_2)_4Hb(Mg)_2].(H_2O)_R$ complex and viceverse functions in both senses as a exergonic ligand-sequence by coupling to H-bonds dissipative potential of $(H_2O)n$.

Not only the mass-action of ligand at the lungs vs CSF separate oxygenation from deoxygenation but also the dissipative clustering potential of $(H_2O)n$ into $(H_2O)n^*$, which allows structural dynamics, with the capability to transfer free energy for the turnover of hydrated molecular architectures, well surpassing a simplistic radiator role.

Hence, CSF became replaced at a rate of three and a half times its volume per 24 hours helping to dissolve and eliminate toxins and $(H_2O)n^*$. H_2O enters in CSF with a larger cluster size than the one eliminated, to function as a carrier of entropy to the outside of the boundaries of the dynamics phases of the reactive R-groups in the system.

References

[1] Bennun A. and Avron M.. Light-dependent and light-triggered adenosine triphosphatase in chloroplasts. *Biochim. Biophys.* Acta, 79, 646-648 (1964).

[2] Bennun A. and Avron M. The relation of the light-dependent and light triggered adenosine triphosphatases to photophosphorylation. *Biochim, Biophys*. Acta, 109, 117-127 (1965).

[3] Bennun A. and Racker E. Partial Resolution of the Enzymes Catalyzing photophosphorylation. *The Journal of Biological Chemistry* Vol. 244, No. 5, 1325-1331 (1969).

[4] Bennun A. The dynamics of H-bonds of the hydration shells of ions, ATPase and NE-activated adenylyl cyclase on the coupling of energy and signal transduction. arXiv:1208.5673v1 [q-bio.OT] (2012)

[5] Bennun A. Hypothesis for coupling energy transduction with ATP synthesis or ATP hydrolysis, *Nature New Biology*, 233 (35), 5-8 (1971).

[6] Prigogine I. Etude thermodynamique des phénomenes irréversibles. *Acad. Roy. Belg. Bull. Cl. Sc.* 31, 600 (1945).

[7] Martyushev L.M., Seleznev V.D.. Maximum entropy production principle in physics, chemistry and biology. *Physics Reports* 426: 1-45 (2006).

[8] Bennun A. Primordial Open-System Thermodynamics and the Origin of a Biophysics Selection Principle. *Open Journal of Biophysics*. 2, 72-79 (2012).

[9] Prigogine I., Lefever R., Goldbeter A. and Herschkowitz-Kaufman. Symmetry breaking instabilities in biological systems. *Nature* 223, 913-6 (1969).

[10] Glansdorff P., Prigogine I. Thermodynamics Theory of Structure, Stability and Fluctuations. Wiley-Interscience, London (1971).

[11] Prigogine, I. Introduction to thermodynamics of irreversible processes. New York 3th ed. John Wiley & Sons (1976).

[12] Nicolis G. and Prigogine I. Self-Organization in Nonequilibrium Systems. New York: John Wiley and Sons. (1977).

[13] Rubin H. and Sitgreaves R. Tech. Rep. N^o 19A. Appl. Math. Statist. Lab. Stanford University. Stanford, Cal (1954).

[14] Prigogine I. Time, structure and fluctuations. *Science, New Series*. Vol. 201, Issue 4358, 777-785 (1978).

[15] Bennun A. Hypothesis on the role of liganded states of proteins in energy transducing systems. *BioSytems*, *7*, 230-244 (1975).

[16] Bennun A. The unitary hypothesis on the coupling of energy transduction and its relevance to the modeling of mechanisms. Annals of the New York Academy of Sciences, 227, 116-145 (1974)

[17] Gibson J.F., Ingram D.J. and Perutz M.F. Orientation of the four haem groups in haemoglobin. *Nature*. 178(4539), 906-908 (1956).

[18] Perutz M.F., Rossmann M. G., Cullis A.F., Muirhead H., WILL G. and North A.C.T.. Structure of Hæmoglobin: A Three-Dimensional Fourier Synthesis at 5.5-Å. Resolution, Obtained by X-Ray Analysis. *Nature*. 185, 416 - 422 (1960)

[19] Perutz M.F., Muirhead H., Cox J.M. and Goaman L.C.G. Three-dimensional Fourier Synthesis of Horse Oxyhaemoglobin at 2.8 Å Resolution: The Atomic Model, *Nature*. 219, 131 - 139 (1968).

[20] Fermi G. and Perutz M.F. Atlas of molecular structures in Biology, 2 Haemoglobin & Myoglobin D.C., Phillips and Richards F.M. (Eds.) Clarendon Press, Oxford (1982).

[21] Monod J, Wyman J, Changeux JP. On the nature of allosteric transitions: a plausible model. J. Mol. Biol. 12:88-118 (1965)

[22] Perutz MF. Stereochemistry of cooperative effects in haemoglobin. *Nature*. 228(5273), 726-39 (1970).

[23] Benesch R. and Benesch R. E. Intracellular organic phosphates as regulators of oxygen release by haemoglobin. *Nature*, 221(5181), 618-622 (1969).

[24] Arnone A. X-ray diffraction study of binding of 2,3-diphosphoglycerate to human deoxyhaemoglobin. *Nature*, 237, 146-9 (1972).

[25] Gerber, G., Berger, H., Janig, G. R. and Rapoport, S. M. Interaction of haemoglobin with ions. Quantitative description of the state of magnesium, adenosine 5'-triphosphate, 2,3-bisphosphoglycerate, and human haemoglobin under simulated intracellular conditions. *Eur. J. Biochem.* 38(3), 563-571 (1973).

[26] Raftos J.E., Lew V.L. and Flatman P.W. Refinement and evaluation of a model of Mg^{2+} buffering in human red cells. *Eur. J. Biochem*. 263(3), 635-45 (1999).

[27] Rifkind J. M. and Heim J. M. Interaction of zinc and hemoglobin: binding of zinc and the oxygen affinity. *Biochemistry* 16(20), 4438-4443 (1977).

[28] Perutz M.F., Wilkinson A.J., Paoli M. and Dodson G.G. The stereochemical mechanism of the cooperative effects in hemoglobin revisited. *Annu. Rev. Biophys Biomol Struct*. 27, 1-34 (1998).

[29] Bennun A., Seidler N. and DeBari V.A. A model for the regulation of haemoglobin affinity for oxygen. *Biochemical Society Transactions*, 13, 364-366 (1985).

[30] Bennun A., Seidler N. and DeBari V.A. Divalent Metals in the Regulation of Hemoglobin Affinity for Oxygen. *Ann. N.Y. Acad. Sci*., 463, 76-79 (1986).

[31] Bennun A.. A coupling mechanism to inter-relate regulatory with haem-haem interactions of haemoglobin. *Biomed. Biochim.*, 46, 314-319 (1987).

[32] Gary K. Ackers and Jo M. Holt. Asymmetric Cooperativity in a Symmetric Tetramer: Human Hemoglobin. Published. *Journal Biological Chemistry*, Vol. 281, No 17, 11441–11443, (2006)

[33] M. Pavlov, P. Siegbahn and M. Sandstron. Hydration of Beryllium, Magnesium, calcium, and zinc ions using density functional. *J. Phys. Chem. A*. 102, 219-228 (1998)

[34] Bennun A., Needle M.A. and DeBari V.A. Stimulation of the hexose monophosphate pathway in the human erythrocyte by Mn^{2+}: Evidence for a Mn^{2+}-dependent NADPH peroxidase activity. *Biochemical Medicine*. 33, 17-21 (1985).

[35] Deuticke B., Duhm J. and Dierkesmann R. Maximal elevation of 2,3-diphosphoglycerate concentrations in human erythrocytes: Influence on glycolytic metabolism and intracellular Ph. *Pflugers Archiv European Journal of Physiology*, 326 (1), 15-34 (1971).

[36] Bennun A. Molecular Mechanisms Integrating Adenylyl Cyclase Responsiveness to Metabolic Control on Long-Term Emotional Memory and Associated Disorders. Horizons in Neuroscience Research., Nova Science Publishers, Inc. Vol. 10, pp. 41-74 (2013).

[37] Steinhoff H.J., Kramm B., Hess G., Owerdieck C. and Redhardt A. Rotational and translational water diffusion in the hemoglobin hydration shell: dielectric and proton nuclear relaxation measurements. *Biophys J.* 65(4), 1486–1495 (1993).

[38] Salvay A.G., Grigera J.R. and Colombo M.F. The role of hydration on the mechanism of allosteric regulation: in situ measurements of the oxygen-linked kinetics of water binding to hemoglobin. *Biophys J*. 84(1), 564-70 (2003).

[39] Colombo M.F., Rau D.C. and Parsegian V.A. Protein solvation in allosteric regulation: a water effect on hemoglobin. *Science*. Vol. 256, No 5057, 655-659 (1992).

[40] Goldbeck R.A., Paquette S.J. and Kliger D.S. The effect of water on the rate of conformational change in protein allostery. *Biophys J*. 81(5), 2919-34 (2001).

[41] Maheshwary S., Patel N., Sathyamurthy N., Kulkarni A.D. and Gadre S.R. Structure and Stability of Water Clusters $(H_2O)_n$, n = 8-20: An *Ab Initio* Investigation. *J. Phys. Chem.* 105 (46), 10525 (2001).

[42] Bennun A. Characterization of the norepinephrine-activation of adenylate cyclase suggests a role in memory affirmation pathways. Overexposure to epinephrine inactivates adenylate cyclase, a casual pathway for stress-pathologies. *BioSystems*, 100, 87-93(2010).

[43] Brydon-Golz S., Ohanian H. and Bennun A. Effects of noradrenaline on the activation and the stability of brain adenylate cyclase. *Biochem. J.*, 166, 473-483 (1977).

[44] Thomas E.L., King L.E. and Morrison M. The uptake of cyclic AMP by human erythrocytes and its effect of membrane phosphorylation. *Archs. Biochem. Biophys*. 196, 459-464 (1979).

[45] DeBari V.A. and Bennun A. Cyclic GMP in the human erythrocyte. Intracellular levels and transport in normal subjects and chronic hemodialysis patients. *Clin Biochem*., 15, 219-221 (1982).

[46] DeBari V.A., Novak N.A. and Bennun A. Cyclic Nucleotide Metabolism in the Human Erythrocyte. *Clin. Physiol. Biochem*., 2, 227-238 (1984).

[47] Novembre P., Nicotra J., DeBari V.A., Needle M.A. and Bennun A. Erythrocyte transport of cyclic nucleotides. *Annals of the New York Academy of Sciences*. 435, 190-194 (1984).