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CONSTRUCTAL LAW AND ION TRANSFER IN NORMAL AND CANCER CELLS

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Abstract. All the living systems waste heat into environment. It is no more than the result of their internal irreversibility. The Constructal law analysis of the irreversibility related to this wasted heat represents a new useful approach to the study of the cells behaviour. This approach allows us to consider the living systems as black boxes and analyze only the inflows and outflows of energy and mass, and their changes in relation to any environmental modification. The consequence is the analysis of the effect of the ions transport through the membrane, and the related cell-environment pH changes, with consideration on the Krebs or Warburg cycle, used for energy conversion, by the normal and cancer cells respectively. Consequently, the entropy generation related to the pH changes can be obtained, and related to mitosis/apoptosis ratio, fundamental to evaluate the probability of evolution of cancer.

Key words: Constructal law, Cancer, Living cell analysis, Membrane transport.

1. INTRODUCTION

Life involves organisational and thermodynamic processes, which tend towards the maximum conversion of available energy [1–8]. The biochemical reactions produce or consume external metabolites, and connect internal metabolites, at constant concentrations in the cells at their steady states. In order to do so, the cell must exchange energy and matter through its membrane. The fundamental phenomena used by cells to reach their optimality consist of a redistributing of the flow patterns through their metabolic network. Many processes such as replication, transcription and translation, require fluxes of ions and molecules which are driven by the endogenous electric fields and accumulate in the nm-thin layer of water [6, 9, 10]. Electrophoresis of positive ions generates hydrodynamic forces, which draw negative ions in the opposite direction [6]. This transportation of ions induces biochemical reactions, so that appropriate external electromagnetic fields are able to facilitate or oppose these spontaneous fluxes, with the consequent possible control of chemical reactions within cells and tissues. Recently, a correlation between the presence of electric gradients and cellular reactions was highlighted in relation to cell migration, adhesion and differentiation [6]. Direct cell migration is fundamental in tissue formation. But, when proliferation and invasion is out of control, a new behaviour occurs: cancer emerges through a series of steps thought to be sequential, as a disease of abnormal growth [11–14] driven by local cellular expansion, adjacent tissue infiltration, and distant metastases. Consequently, one of the fundamental approaches to carcinogenesis consists of investigating the derangement of mitosis and, perhaps more so, of the mitosis/apoptosis ratio, which will lead to such an abnormal large mass [15, 16]. But, all these processes are driven by fluxes of energy and mass, and geometry results fundamental in their analysis [17–20]; indeed, the spatial and the temporal structures in nature are no more than the results of a global process of optimization of fluxes in relation to the local and global constraints [21–24]. So, the evolution of the shape of finite-size systems is determined by the natural principle of providing the easiest access to their internal currents. The consequence of this principle is the allometric law, which is a power-law relation between geometric and functional parameters (flows for us) of living systems.

Here we develop the thermodynamic analysis of cancer, based on these considerations.

2. THE THERMODYNAMIC APPROACH

Life involves organisational and thermodynamic processes, which tend towards the maximum conversion of available energy in the least time. The biochemical reactions produce or consume external metabolites, and connect internal metabolites, at constant concentrations in the cells at their steady states. In order to do so, the cell must exchange energy and matter through its membrane. The fundamental phenomena used by cells to reach their optimality consist of a redistributing of the flow patterns through their metabolic network. The use of Constructal law [25–30] allows us to describe how different ions have different effects on the use of energy by the cell for growth. From a thermodynamic point of view a cell is a macroscopic system because it contains approximately 10^{14} molecules, with a concentration distribution related to energy and temperature, given by [18] $c_N = c_{N0} \exp(-Ne_N/k_BT)$, where c_N is the concentrations related to the number of molecules, e_N is the energy per molecule, k_B (= 1.38×10^{-23} JK^{-1}) is the Boltzmann constant, R (= 8,314 J mol^{-1} K^{-1}) is the universal gas constant, T is the temperature, c_{N0} is the reference value of c_N at e_N = 0 J molecule^{-1}, and $k_BT \sim 4 \times 10–21$ J molecule^{-1} for ordinary temperature. In such a system biochemical reactions occur involving ions. In this paper we will consider the Ca^{2+} ion, which is responsible for protein folding [32]: its typical concentrations is 1 500 μM extracellular, and 0.1 μM intracellular. But, chemical reactions can occur only if the energy of the molecules is greater than the activation energy per mole e^* of the reaction, so that the rate of reaction r per mole can be obtained by integration the Årrhenius's law [31, 32] for each mole and one direction reaction $\ln r = -e^*/RT$. The evolution of any chemical reaction at constant temperature T and constant pressure p, can be evaluated by using the differential of the Gibbs free energy G, by the condition $dG < 0$ (for spontaneous reaction) where [32] $dG = Vdp - SdT + \Sigma_i \mu_i dN_i$, where V is the volume of the system considered, S is the entropy, μ represents the chemical potential, and N stands for the number of particles. Within cells and across their micro-environment there is always an abundance of water, so atoms and molecules are often ions. Consequently, in relation to the distributions of the different ions there exist electric potential energy differences. In particular, cations (ions with positive charge) accumulate in low electric potential energy regions, while anions (ions with negative charges) present higher concentration at high values of electric potential energy regions [33, 34]. As previously stated, the ion concentrations follow relations $c_N = c_{N0} \exp(-Ne_N/k_BT)$ with $e_N = q\phi$, where q is the ion charge, ϕ is the electric potential, Z is the chemical valence, F (= 96,485.34 A s^{-1}mol^{-1}) is the Faraday constant and, at ordinary temperature, $k_BT/e = RT/F \sim 25$ mV, with e elementary charge ($e = 1.602 \times 10^{-19}$ A s). The electric potential can be evaluated by using the Goldman–Hodgkin–Katz equation [32–34]:

$$\Delta\phi = \frac{RT}{F}\log_{10}\left(\frac{P_{Na^+}[Na^+]_{out} + P_{K^+}[K^+]_{out} + P_{Cl^-}[Cl^-]_{out}}{P_{Na^+}[Na^+]_{in} + P_{K^+}[K^+]_{in} + P_{Cl^-}[Cl^-]_{in}}\right), \tag{1}$$

where P is the permeability of the ion, [A] means concentration of the A-ion, R is the ideal gas constant, T is the temperature, F is the Faraday constant, and *out* stand for outside, while *in* for inside. Relation (1) points out how the membrane potential can be changed by alterations in the conductance of one or more ions. The ion channels and transporters provide different permeability to distinct ions, such as Na^+, K^+, Cl^-, and other ions (H^+, Ca^{2+}, HCO_3^-, Mg^{2+}, etc.). As a consequence of the asymmetry in these ion distributions, a 60–90 mV (negative inside the cell) membrane potential exists between the cytoplasm and the extracellular environment. It is expressed relative to the extracellular environment and a cell depolarizes if the membrane potential is relatively less negative, and vice versa [35]. Any change in the ions concentration changes both the membrane electric potential and the related pH of the cytoplasm, because the concentration of a chemical species follows the law $c_{out} = c_{in} \exp(-\Delta\phi/RT)$ where c_{out} and c_{in} are the concentrations of any ion species outside and inside of the cell membrane; ϕ is the electric field between the two sides of the membrane, R is the universal constant of gasses, T is the temperature, and the concentration is related to the pH variation in any cell. At a cellular level, energy conversion occurs also in biological nano-machines, fundamental natural devices for ion and molecules transport across the cell membrane [34]. These molecular devices consume energy by hydrolysis of ATP, and convert it into mechanical work (rotation of the machine with related transport of the ions) [36, 37]. Any energy conversion process is always accompanied by energy dissipation [37], and by a related entropy generation. Now, considering the Ca^{2+}–ATPase, this molecular motor allows the active transport of the Ca^{2+} ions across the cell membrane by means of its ATPase [44–46]

$Ca^{2+}(in) \rightarrow Ca^{2+}(out)$ and $H^{+}(out) \rightarrow H^{+}(in)$ where in means inside and out means outside the cell, and the counter-transport of H^+ is necessary to maintain electroneutrality [37, 38] with the rate of transport reaching 8×10^{-5} mol $s^{-1}kg^{-1}$. While the energy required for the ATP hydrolysis is around 56–57 kJ mol^{-1}, the total process activation energy is approximately 80–90 kJ mol^{-1} due to conformational changes in the enzymes required by the transport [37, 43]. It follows that the biochemical reaction modulates pH due to the change of concentration of H^+ ions, and, it changes also the membrane potential $\Delta\phi = \Delta G_{H^+} + 2.3RT\Delta\text{pH}/F$, where G is the Gibbs' potential, F is the Faradys' constant, and 2.3 ΔpH is the physiological concentration gradient. The presence of the electric energy allows us to consider the electrochemical potential, $\tilde{\mu} = \mu + Ze\phi$ in place of the chemical potential. In a cell, the cytoplasm has a lower electric potential than the cell external environment, hence the Cl^- concentration is lower in the cytoplasm than in the extracellular space. On the contrary the concentrations of positive ions (Ca^{2+}, Na^+, K^+, Mg^{2+}, etc.) are greater in the cytoplasm than in the cell environment. We note that the negative lower electric potential present in the cytoplasm is maintained by pumps, i.e. the molecular systems which use energy to generate fluxes of specific ions in particular directions [31, 44]. For the Ca^{2+}–ATPase motor inside the cell membrane, the entropy generation rate can be evaluated as:

$$\dot{S}_{g,Ca} = -\frac{\Delta G}{T}\exp\left(-\frac{e^*_{Ca}}{RT}\right) - \dot{N}_{out,Ca}\left(\frac{\mu_{Ca,out}}{T_0} - \frac{\mu_{Ca,in}}{T}\right) + \dot{Q}_{Ca,in\rightarrow out}\left(\frac{1}{T_0} - \frac{1}{T}\right). \tag{2}$$

Cells are able to maintain a definite range of variability of the chemical-physical parameters useful for their life (homeostasis). The fuel of cell life is just the ATP, so we analyse the reactions in which ATP is involved. The principal reactions for ATP in a normal cell (hydrolysis) and in a cancer cell (glycolysis), and we will evaluate the useful work as [45–47] (the exergy of ATP results 299 kJ mol^{-1}):

1. For the hydrolysis reaction $ATP + H_2O \rightarrow ADP + P$, the useful work results 57 kJ mol^{-1}, the wasted exergy is 97 kJ mol^{-1}, with total efficiency of 81%;
2. For the glycolysis reaction $C_6H_{12}O_6 + 6O_2 + 30P \rightarrow 6CO_2 + 6H_2O + 30P_{ATP}$, the useful work results in 1,707 kJ mol^{-1}, the wasted exergy is 1,248 kJ mol^{-1}, with a total efficiency 58%.

If a cell employs glycolysis, as in the case of cancer, it follows that it increases its entropy generation rate. But, in order to live, the cell must decrease its entropy through the aforementioned heat transfer. Moreover, as a consequence of the low efficiency of the biochemical reaction, the cell needs a greater quantity of nutrients than the normal cell, which wastes less exergy, maintains its entropy at low level and uses a more efficient life cycle. Glycolysis is regulated by many allosteric factors [48], highlighting the fundamental role of the allosteric properties of the biochemical molecules in cancer. Now, after having evaluated the entropy of a cell, for a biological research and for any medical application it is fundamental to extend the results to human tissues and organs, and consider the consequences of the previous considerations between normal and cancer cells. So, it is possible to evaluate the entropy change in an organ of the human body by the heat transfer to the border of the organ, which we name organ surface layer or capsule, with a consequent variation of this capsule temperature T_S. To do so, we consider our thermodynamic system in equilibrium with the environment so that no changes in the heat power $\dot{Q}$ transfer between capsule and its environment occur. Moreover, we consider the system as a closed system, so that the mechanical power $\dot{W}$ produced by vascularisation or blood is considered as a mechanical power of a hypothetical internal technical device, related to changes in the heart rate nhr, i.e. number of beats per second, that we name the mechanical power of blood $\dot{W}_b$. The first law of thermodynamics for this system yields $dU = -\delta W_b$ where U is the internal energy of the system. The mechanical power of blood $\dot{W}_b$ can be evaluated by considering the mechanical power related to the change in the heart rate, and by considering a mean blood pressure pb inside the organ constant, so that it follows $\dot{W}_b = p_b \dot{v}_{sv} \mathrm{d}n_{hr}/\mathrm{d}t$, where $\dot{v}_{sv}$ is the stroke volume such that the cardiac outflow $\dot{V}_b$, i.e. the volume of blood flow which is ejected by the heart at each beat, results $\dot{V}_b = n_{hr}\dot{v}_{sv}$. Now, introducing the Helmoltz potential $\dot{F}_H = \dot{U} - T_S\Delta\dot{S}_{bp}$ [49, 50] where $\Delta\dot{S}_{bp}$ is the entropy generation rate due to blood pressure, and considering the Maxwell relations [49], it follows that $\Delta\dot{S}_{bp} = p_b\dot{v}_{sv}\mathrm{d}n_{hr}/\mathrm{d}T_S = p_b\mathrm{d}\dot{V}_b/\mathrm{d}T_S$. The changes in the entropy generation rate can be positive or negative in relationo the physiological needs of the tissue or of the organ. From this relation, it follows that any

change in tissue's entropy rate can be related to the variation of the blood flow with temperature, which is in agreement with experimental results [51]. So, it follows that the entropy generation rate by the life cycle of the cell is no more than the entropy generation rate of the fluxes (inflow and outflow) of blood (into and from the tissue or the organ), because it is the only transporter of molecules:

$$N_{cell}\dot{S}_g = \Delta\dot{S}_{bp} \Rightarrow \frac{p_b}{N_{cell}}\frac{\mathrm{d}\dot{V}_b}{\mathrm{d}T_S} = -\frac{\Delta G}{T}\exp\left(-\frac{e^*_{Ca}}{RT}\right) - \dot{N}_{out,Ca}\left(\frac{\mu_{Ca,out}}{T_0} - \frac{\mu_{Ca,in}}{T}\right) + \dot{Q}_{Ca,in\to out}\left(\frac{1}{T_0} - \frac{1}{T}\right), \quad (3)$$

where N_{cell} is the number of cells in the volume of the tissue or of the organ considered, it follows that, in the case of cancer, the patho-physiological request for an outflow of Ca^{2+} to support accelerated tumor growth, triggers angiogenesis, the process of blood vessel expansion, to satisfy the demand for higher blood flow. The optimization principle adopted by the cell systems can be easily obtained just by using the entropy generation principle. By searching the optimization of the Ca^{2+} fluxes we must consider the maximum value of the entropy generation evaluated by the environment [49], obtaining that:

$$\mathrm{d}\dot{S}_{g,Ca} = 0 \Rightarrow \frac{\mathrm{d}\dot{Q}_{Ca,in\to out}}{\mathrm{d}\dot{N}_{out,Ca}} = \left(\frac{\mu_{Ca,out}}{T_0} - \frac{\mu_{Ca,in}}{T}\right)\left(\frac{1}{T_0} - \frac{1}{T}\right)^{-1}, \quad (4)$$

which considering that a cell, a tissue and an organ exchange heat preferentially by convection, so that $\dot{Q} = \alpha A(T - T_0)$, where α is the laminar coefficient, and A is the external surface of the living system considered, we can obtain:

$$\frac{\mathrm{d}A}{\mathrm{d}\dot{N}_{out,Ca}} = \left(\frac{\mu_{Ca,out}}{T_0} - \frac{\mu_{Ca,in}}{T}\right)\left[\alpha(T - T_0)\left(\frac{1}{T_0} - \frac{1}{T}\right)\right]^{-1} \approx -\frac{1}{2\alpha}\mu_{Ca}\left(\frac{1}{T_0} - \frac{1}{T}\right). \quad (5)$$

This result link the external surface growth of the system considered to the ion fluxes (in this case Ca^{2+}), to the temperature difference between the internal of the system and its environment and to the chemical potential of the ion species. Moreover, this relation allows us to state that the living system will adopt the shape such that its surface will growth towards the maximum possible area in relation to the mechanical and biological constraints in its surrounding and the biological effect of the process. In our example the surface will tend to decrease when the Ca^{2+} ions outflow, so the heat exchange for convection decrease and the cell must uses this energy in other ways (proteins formation, for example). If the Ca^{2+} inflows into the cell, the sign will change and the cell will be able to outflow heat without doing more chemical work. The consequence is that Ca^{2+} inflow should prevent the cancer develop because it decreases the chemicals useful for proliferation. Entropy generation due to such chemical reactions was obtained as a function of the cell reproduction rate, χ_1, and the cell death rate χ_2 (with χ_1 and χ_2 considered constant), defined as $P_m = \chi_1 n^{1/d}$ and $P_a = \chi_2 (1 + F_a) n^{1/d}$ [16], where n is the number of cells, P is the probability per unit time, m means mitosis, a stands for apoptosis, F_a is a dimensionless correction term which represents the relation between the cancer mass radius and a characteristic length of volume; it takes into account the finite size of the host organ or tissue, and d is a constant related to the geometric dimensions of the system considered ($d = 3$ for normal cells, $d = d_f$ fractal dimension for cancer cells such that $2 < d_f < 3$ [51]). So the entropy generation due to affinity was evaluated as [16]:

$$S_{g,cr} \approx k\left(P_m - \frac{P_a}{1 - F_a}\right)\left[\ln\left(\frac{P_m}{P_a}\right) + \ln(1 + F_a)\right] = K\tau_4\left(\dot{\xi}_f - \dot{\xi}_b\right)\ln\left(\frac{\dot{\xi}_f}{\dot{\xi}_b}\right), \quad (6)$$

where $\dot{\xi}_f$ is the forward reaction rate and $\dot{\xi}_b$ the backward reaction rate. It then follows that $P_m = Kn^{1/d}\tau_{cr}\dot{\xi}_f / k$ and $P_a = Kn^{1/d}\tau_{cr}(1 + F_a)\dot{\xi}_b / k$ where τ_{cr} is the time in which the considered chemical reaction occurs [43]: it follows the direct relation between the probability and the time of the reaction, while the ratio between the two probabilities highlights the fundamental role of the chemical reaction rate in the dynamics of tumor growth, but also the critical role of the geometric factor ($1 + F_a$) [51]. Geometry is fundamental in the heat and mass transfer; indeed, the spatial and the temporal structures in nature are no more than the results of a global process of optimization of fluxes in relation to the local and global constraints [19].

3. CONCLUSIONS

The analysis of the Ca^{2+} flux has been developed in order to point out the role of this ion in the decreasing of the cancer growth. We can highlight that it requires energy in relation to its electric charge and the membrane electric field; indeed, the positive charge of the Ca^{2+} ion decrease the cell energy. It is due to the electrochemical work required by the ions to cross the membrane cell. Cancer growth is related to the energy management of cells, and the process of malignant transformation is no more than a difference in energy lost to the microenvironment. Cancer must increase its energy dissipation to reduce its entropy [4–6, 43]. So, the cancer cell must increase the coefficient of convection, through exchange with blood and fluids, so it is poised to induce blood vessel growth towards the tumor, *i.e*. angiogenesis, because it needs to increase this convection coefficient, and the aforementioned metabolite flows, and to sustain the nourishment demands of on-site growth. Moreover, such sprouting vessels should lead to a deterioration of tissue consistency, thus reduce mechanical confinement which consequently supports continued on-site expansion, and serves migrating cells as a path to move along. But, even if dissipation is improved by an increase of convection, it occurs with body fluids at 37°C, insufficient to provide the required relief through facilitated energy outflow. As a consequence, cancer cells attempt to increase the flow of H^+ and other ions to consume ATP and to increase energy dissipation; this induces both a pH variation and a change in membrane potential. Any variation in pH generates a variation in the behavior of the cell; if the environment turns acidic, further carcinogenesis towards more aggressive phenotypes becomes more likely. From these analytical results, it is possible to argue that cell functions are regulated by membrane proteins that are sensitive to the electric field. Changes in the membrane's electric field are then transduced into a conformational change of the biological molecules, and in turn, this allosteric effect triggers the function of membrane proteins, with consequences for the regulation of cell functions or even entire phenotypes. We argue that based on the role of the electrostatic potential in regulating normal cell differentiation, conceivably its control, or rather loss of control, is fundamental for the development of cancer: the voltage-responsive transduction mechanisms on the cell membrane allow bioelectric signals to regulate the polarization of cell molecules. The biochemical reactions that enable cell life produce or consume external metabolites, and connect with internal metabolites. Cancer needs to dissipate energy, which leads to heat storage in the environment, and pH acidification, in a vicious cycle.

REFERENCES

1. Y. DEMIREL, S.I. SANDLER, *Thermodynamics and bioenergetics*, Biophysical Chemistry, **97**, pp. 87–111, 2002.
2. O. TOUSSAINT, E.D. SCHNEIDER, *The thermodynamic and evolution of complexity in biological systems*, Comparative Biochemical Physiology A, **120**, pp. 3–9, 1998.
3. S.R. CAPLAN, A. ESSIG, *Bioenergetics and Linear Nonequilibrium Thermodynamics, The Steady State*, Harvard University Press, Cambridge, 1983.
4. U. LUCIA, *Entropy generation approach to cell systems*, Physica A, **406**, pp. 1–11, 2014.
5. U. LUCIA, *Bioengineering thermodynamics: an engineering science for thermodynamics of biosystems*, I. Jo.T., **18**, pp. 254–265, 2015.
6. U. LUCIA, *Bioengineering thermodynamics of biological cells*, Theor. Biol. Med. Model., **12**, p. 29, 2015.
7. A. KATCHALSKY, P.F. CURRAN, *Nonequilibrium Thermodynamics in Biophysics*, Harvard University Press, Cambridge, 1967.
8. U. LUCIA, *Irreversibility in biophysical and biochemical engineering*, Physica A, **391**, pp. 5997–6007, 2012.
9. C. BUSTAMANTE, Y.R. CHEMLA, N.R. FORDE, D. IZHAKY, *Mechanical Processes in Biochemistry*, Annual Review of Biochemistry, **73**, pp. 705–748, 2004.
10. U. LUCIA, *Thermodynamics and cancer stationary states*, Physica A, **392**, pp. 3648–3653, 2013.
11. J. HARRIS, M. MORROW, L. NORTON, *Malignant tumors of the breast*, In De Vita, V.T. Jr, HELLMAN, S., ROSENBERG, S.A., Eds., *Cancer: Principles and Practice of Oncology*, Fifth Edition, Lippincott-Raven, Philadelphia, 1997.
12. L. NORTON, *Conceptual and Practical Implications of Breast Tissue Geometry: Toward a More Effective, Less Toxic Therapy*, The Oncologist, **10**, pp. 370–381, 2005.
13. E. IZQUIERDO-KULICH, E. ALONSO-BECERRA, J.M. NIETO-VILLAR, *Entropy Production Rate for Avascular Tumor Growth*, Journal of Modern Physics, **2**, pp. 615–620, 2011.
14. J.D. RUPA, A.P. de BRUINE, A.J. GERBERS, M.P. LEERS, M. NAP, A.G. KESSELS, B. SCHUTTE, J.W. ARENDS, *Simultaneous detection of apoptosis and proliferation in colorectal carcinoma by multiparameter flow cytometry allows separation of high and low-turnover tumors with distinct clinical outcome*, Cancer, **97**, pp. 2404–2411, 2003.
15. E.A. COMEN, L. NORTON, J. MASSAGUE, *Breast Cancer Tumor Size, Nodal Status, and Prognosis: Biology Trumps Anatomy*, Journal of Clinical Oncology, **29**, pp. 1–3, 2011.

16. U. LUCIA, A. PONZETTO, T.S. DEISBOECK, *A thermodynamic approach to the 'mitosis/apoptosis' ratio in cancer*, Physica A, **436**, pp. 246–255, 2015.
17. A. BEJAN, *Heat Transfer*, John Wiley & Sons, Hoboken, 1993.
18. A. BEJAN, *Convection Heat Transfer*, John Wiley & Sons, Hoboken, 2013.
19. A. BEJAN, *Shape and Structure, from Engineering to Nature*, Cambridge University Press, Cambridge, 2000.
20. A. BEJAN, S. LORENTE, *Design with Constructal Theory*, John Wiley & Sons, Hoboken, 2008.
21. A. BEJAN, S. LORENTE, *The constructal law and the evolution of design in nature*, Phil. Trans. B, **365**, p. 1545, 2010.
22. A.H. REIS, *Constructal theory: from engineering to physics, and how flow systems develop shape and structure*, Appl. Mech. Rev., **59**, pp. 269–282, 2006.
23. A.F. MIGUEL, *The physics principle of the generation of flow configuration*, Phys. Life Rev., **8**, pp. 243–244, 2011.
24. A.F. MIGUEL, A. BEJAN, *The principle that generates dissimilar patterns inside aggregates of organisms*, Physica A, **388**, pp. 727–731, 2009.
25. A.H. REIS, *Design in nature, and the laws of physics*, Phys. Life Rev., **8**, pp. 255–256, 2011.
26. A. BEJAN, *The Constructal Law of Organization in Nature: Tree-shaped flows and body size*, J. Exp. Biol. **208**, pp. 1677–1686, 2005.
27. A. BEJAN, S. LORENTE, *Design with Constructal Theory*, Wiley, Hoboken, 2008.
28. A. BEJAN, *Theory of Organization in Nature: Pulsating Physiological Processes*, Int. J. Heat Mass Trans., **40**, pp. 2097–2104, 1997.
29. A. BEJAN, *The Physics of Life: The Evolution of Everything*, St. Martin's Press, New York, 2016.
30. A.H. REIS, *Use and validity of principles of extremum of entropy production in the study of complex systems*, Ann. Phys. **346**, pp. 22–27, 2014.
31. J. WOLFE, *Cellular thermodynamics: the molecular and macroscopic point of views*, eLS-John Wiley & Sons, Chichester, 2015.
32. P. ATKINS, J. De PAULA, *Physical Chemistry for Life Sciences*, Oxford University Press, New York, 2006.
33. M. ASHRAFUZZAMAN, J.A. TUSZYNSKI, *Membrane Biophysics*, Springer, Berlin, 2013.
34. S. KJELSRUP, J.M. RUBI, D. BEDEAUX, *A Thermodynamic Description of Active Transport*, in Franzese, G., Rubi, J.M. (Eds.), *Aspects of Physical Biology: Biological Water, Protein, Solutions, Transport and Replication*, Springer, Berlin, 2008.
35. D.E. GOLDMAN, *Potential, impedance, and rectification in membranes*, J. Gen. Physiol., **27**, pp. 37–60, 1943.
36. P. MITCHELL, *Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type mechanism*, Nature, **191**, p. 144, 1961.
37. J.M. RUBI, M. NASPREDA, S. KJELSTRUP, D. BEDEAUX, *Energy transduction in biological systems: a mesoscopic non-equilibrium thermodynamics perspective*, J. Non-Equil. Thermodyn., **32**, pp. 351–378, 2007.
38. M.C. BERMAN, *Slippage and uncoupling in P-type cation pumps; implication for energy transduction mechanism and regulation of metabolism*, Biochem. Biophys. Acta, **1513**, pp. 95–121, 2001.
39. L. de MEIS, *Energy Interconversion in Transport ATPase*, in Franzese, G., Rubi, J.M. (Eds.), *Aspects of Physical Biology: Biological Water, Protein, Solutions, Transport and Replication*, Springer, Berlin, 2008.
40. U. LUCIA, *Electromagnetic waves and living cells: A kinetic thermodynamic approach*, Physica A, **461**, pp. 577–585, 2016.
41. U. LUCIA, *Thermodynamic approach to nano-properties of cell membrane*, Physica A, **407**, pp. 185–191, 2014.
42. U. LUCIA, *Entropy generation and cell growth with comments for a thermodynamic anticancer approach*, Physica A, **406**, pp. 107–118, 2014.
43. U. LUCIA, *Different chemical reaction times between normal and solid cancer cells*, Medical Hypotheses, **81**, pp. 58–61, 2013.
44. R.K. HOBBIE, *Intermediate Physics for Medicine and Biology*, Springer-Verlag, New York, 1997.
45. J. SZARGUT, D.R. MORRIS, F.R. STEWARD, *Exergy analysis of thermal, chemical, and metallurgical processes*, Hemisphere Publishing Co., London, 1988.
46. S. LEMS, H.J. van der KOOI, J. de SWAAN ARONS, *Thermodynamic analysis of the living cell: design of an exergy-based method*, Int. J. Exergy, **4**, pp. 339–356, 2007.
47. S. LEMS, H.J. van der KOOI, J. de SWAAN ARONS, *The second-law implications of biochemical energy conversion: exergy analysis of glucose and fatty-acid breakdown in the living cell*, Int. J. Exergy, **6**, pp. 228–248, 2009.
48. E. RACKER, *Warburg effect revisited*, Science, **213**, pp. 1313, 2008.
49. A. BEJAN, *Advanced Engineering Thermodynamics*, John Wiley, New York, 2006.
50. S. BOREGOWDA, R. HANDY, D. SLEETH, A. MERRYWEATHER, *Measuring Entropy Change in a Human Physiological System*, J. Thermodyn., **2016**, pp. 1–6, 2016.
51. L. NORTON, *Conceptual and Practical Implications of Breast Tissue Geometry: Toward a More Effective, Less Toxic Therapy*, The Oncologist, **10**, pp. 370–381, 2005.