IL NUOVO CIMENTO Vol. 31 C, N. 1 Gennaio-Febbraio 2008
DOI 10.1393/ncc/i2008-10286-4

VBL: Virtual Biophysics Lab

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(ricevuto l' 11 Giugno 2008; pubblicato online il 19 Settembre 2008)

Summary. — VBL (Virtual Biophysics Lab) is a computational project to develop a basic numerical model of tumor spheroids. This paper is a status report that describes the structure of the code that implements the model, and the progress made up to February 2008, and also some recent results in modeling the effects of radiations on cells in a bioreactor.

PACS 07.05.Tp – Computer modeling and simulation.
PACS 87.17.Aa – Modeling, computer simulation of cell processes.
PACS 87.17.Ee – Growth and division.
PACS 87.53.-j – Effects of ionizing radiation on biological systems.

1. – Introduction

The speed and the versatility of today's computers open up new opportunities to analyze complex biological systems, and suggest that in the future we shall be able to simulate the behavior of large cell populations *ab initio*, starting from individual molecular reactions in single cells and climbing the ladder of complexity up to the behavior of whole multicellular organisms.

Because of the extreme complexity of the problem many existing numerical studies are limited to rather small subnets of molecular circuits within a single cell, or to the global mechanical properties of cell clusters. There are also more ambitious attempts that try to capture at least some essential biochemical and biomechanical features, and [1] is a recent, comprehensive review (see also the review [2] and references therein; an incomplete list of recent references is [3-18]).

Modeling cell biochemistry is by far the most complex task, and in the last few years we have followed an original approach that ensures the feasability of a comprehensive numerical simulation that includes both a reliable description of metabolism and the mechanical evolution of cell clusters, by giving up, at least temporarily, the detailed description of many biochemical and biophysical processes [19]. We proceed in a partly phenomenological way that leads to simple parameterizations: in exchange, we achieve a huge reduction in computational complexity and a considerable reduction of the space-time scale problems that affect simulations aimed at calculating the properties of macroscopic objects starting from microscopic models. We are in an advanced phase of development of a program that simulates cell metabolism, growth and proliferation and the extracellular environment. The 3D part part of the program is not yet complete, however we can already (February 2008) simulate large populations of dispersed cells, like those in the culture wells used for *in vitro* growth, and we have produced numerical estimates that are in excellent qualitative agreement, and in good quantitative agreement, with experimental data [20, 21].

Although the actual simulation of a living organism is still a faraway goal, with our program we can perform virtual experiments in settings that are difficult to realize or to control *in vitro*, and thus the program is a sort of *in silico* laboratory.

The main application of the numerical model shall be the simulation of populations of tumor cells, to gain insight on the development of small, not yet vascularized tumors. This will be an extremely interesting application, since it is well known that the main problem when managing tumors in the clinical practice does not lie in the treatment of large tumor masses, which are usually removed by surgery, but rather in the control of small masses which are near or below the limits of imaging diagnostics (about $1\,\mathrm{mm}^3$). These small tumor aggregates may escape conventional treatment and, in time, may lead to a recurrence of the primary pathology, often with a different phenotype (*e.g.*, acquired resistance to chemotherapic drugs, acquired ability to grow on different tissue substrates and ability to metastasize).

It is very difficult to study these micromasses also in animal models, because their small size is below the imaging limit and it is not possibile to measure their biological parameters. Alternatively, cells can be grown *in vitro*: in this case cells adhere either directly or indirectly to plastic supports and form a two-dimensional monolayer. In this way all the information associated to the three-dimensional structure of cell clusters is lost. The three-dimensional topology of actual cell clusters effectively determines many important biological features, like the expression of some genes, a slowed-down diffusion of nutrients and waste, and also the expression of new phenotypes like the resistance to radiotherapy. Multicellular tumor spheroids represent a valid and effective experimental cell culture technique [22] (see fig. 1). These cell clusters are obtained preventing cell adhesion to the plastic surface of standard culture flasks. In this case cells adhere to one another, and form small aggregates of few cells which can be handled with standard micromanipulation methods and seeded individually into the wells of culture microplates for growth assays. As cells proliferate these clusters grow to a size of about $1\,\mathrm{mm}^3$ (about one million cells). Since they preserve the three-dimensional topology, multicell tumor spheroids display many interesting biological properties that cannot be observed in monolayer cell cultures. Among them we find:

1) heterogeneous expression of adhesion molecules on the cell membrane; these molecules are important for cell-cell and cell-intercellular matrix interactions, and act as target for specific antitumor drugs;

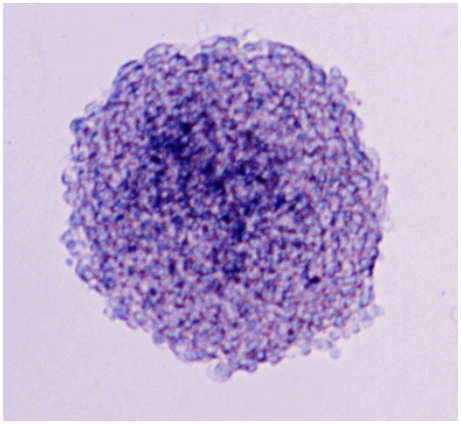

Fig. 1. – Photo a small tumor spheroid: the layered structure is partly visible as a variable opacity (photo by R. Chignola).

2) production of an intercellular matrix (important for cell aggregation and for the penetration of cells of the immune system);

3) heterogeneous distribution of nutrients and oxygen that lead to the formation of a necrotic core and to a gradient of cell proliferation;

4) appearance of resistance phenomena and/or heterogeneous response to antitumor therapies (especially radiotherapy);

5) growth kinetics very similar to those observed *in vivo* [23].

Multicell tumor spheroids are thus intermediate between traditional cell cultures and tumors *in vivo*, and at the same time they are accessible to experimental measurements: they provide many data that can be used to test and validate models of solid tumor growth in the prevascular phase (see, *e.g.*, [23], and references cited therein).

The simulation program shall soon provide an additional tool to study tumor spheroids, it shall be a virtual biological workbench to test and measure *in silico* tumor spheroids. Obviously the experiments that can actually be performed with the simulator depend on the biophysical details in the numerical model: at the time of writing we are completing the geometrical description of the structure of cell clusters and their biomechanical interactions.

Our numerical model has an incremental nature: at the moment it contains a basic description of metabolism, growth and proliferation of cells, and of the extracellular environment. However we shall soon take further important steps with the inclusion of cellular signaling mechanisms, and of a repair-misrepair model of DNA, and we shall perform new studies of the effects of ionizing radiations both on dispersed cell populations and on tumor spheroids. This model holds the promise to lead to a better understanding of tumor kinetics, which is essential to plan new and better therapeutic strategies [24], and we believe that the simulation of irradiated spheroids will eventually hint at new ways to optimize a radiotherapeutic strategy in clinical oncology, in ways impossible to achieve in direct experiments, either *in vivo* or *in vitro*.

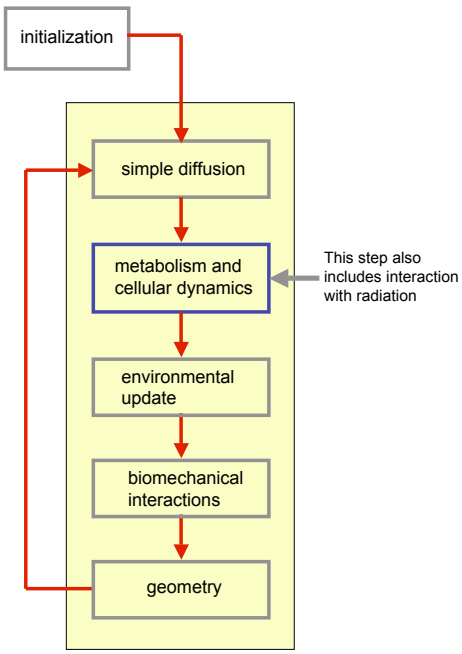

Fig. 2. – Schematic flow diagram of the simulation program: the different modules are described in the main text. The step that involves metabolism and cellular dynamics is the most complex to date, and is further described in [20] and [21].

2. – Structure of simulation program

In the past we have already presented the structure of the simulation program [19], however this structure has slightly changed as our experience with this kind of simulations has grown. The present structure of the simulation program is shown in fig. 2.

After a rather lengthy initialization phase that performs many simple but important chores, the program enters a loop that carries out the following steps:

1) Simple diffusion. The diffusion of chemicals in a cell cluster proceeds either by normal diffusion or by facilitated diffusion across cell membranes. Facilitated diffusion is mostly a biochemical process that has a weaker dependence on concentration differences. This first step includes normal diffusion only, while facilitated diffusion is part of step 2. This step utilizes the proximity relationships provided by a Delaunay triangulation [25] (see [26] for more details).

2) Metabolism and cellular dynamics. This is a complex step that computes the metabolic parameters and regulates growth and proliferation (see fig. 3). For each cell the program starts from the uptake of glucose and glutamine and the availability of molecular oxygen, then it computes the yield of ATP, the protein and DNA synthesis rate, and it also outputs lactic acid, which the cell then excretes in the environment. The program uses an elaborate thresholding mechanism based on multisite phosphorylation [27, 28] to determine when a cell steps beyond the restriction point that separates the initial phase G1 from the start of DNA synthesis. The availability of ATP is phenomenologically connected to the number of

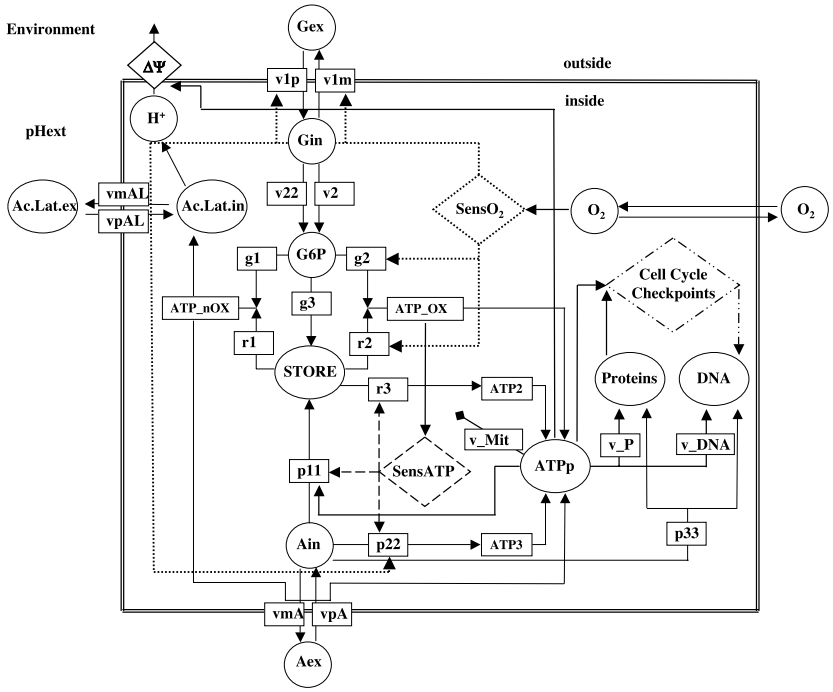

Fig. 3. – Metabolic network implemented in the simulation program. A complete description of this network can be found in [21].

mitochondria and to cell volume. Finally this program module handles cell death and cell proliferation. We wish to remark that this module tries to capture at least part of the stochastic nature of the biochemical events in the cell, although still in a partly phenomenological way. A more complete description of the metabolic step can be found in [20, 21]).

3) Environmental update. The enviroment is included in the simulation, and is modified by the metabolic activity of individual cells. In addition it receives other environmental signals, like the slow flushing of the environmental fluid because of a continuous inflow of nutrients and outflow of exhausted culture medium. This part of the program handles all the external environmental signals and updates the environment.

4) Biomechanical interaction. Cells interact mechanically as well as biochemically: this part of the program is essentially a simple integrator like those found in dissipative dynamics, and is very similar to that implemented in [10]. Cells are approximated by spheres that move in a highly viscous environment (see also [19]).

5) Geometry. The geometry module computes the proximity relationships between cells. The nearest neighbors are defined by the links in a Delaunay triangulation [25] and they are computed by the triangulation methods in the computational geometry package CGAL [29]. In this way all the computational complexity of binary interactions is reduced from a potential $O(N^2)$ to a much more manageable $O(N)$.

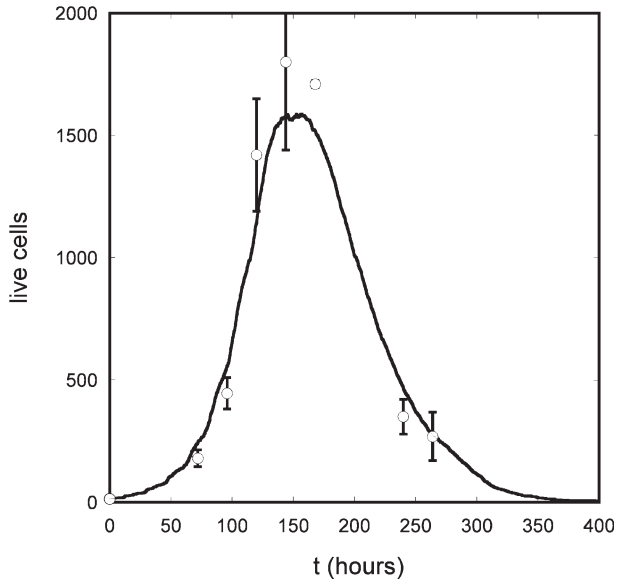

Fig. 4. – Cells in a small culture well, simulation (black line) *vs.* experiment (disks). Here the experimental data have been obtained with Raji leukemic cells, while the simulation was performed with the standard parameters (listed in [20, 21]). The black line is not a fit, but the result of a single simulation: the only adjustment is a modest scaling of time (by a factor 0.83) because of different cell cycle duration in the two cases (see also [21]).

Here we wish to mention that Delaunay triangulations and the associated Voronoi diagrams have a powerful and striking visual similarity with biological structures, and for this reason they have a long story of use in numerical biophysics (see, *e.g.*, [10, 30-33]).

3. – Summary of recent results

Differences in the biochemical behavior of different cell lines are much less marked than could naively be expected: at least from a biochemical point of view they behave in a strikingly uniform manner (see, *e.g.*, [34] and [35]). This consideration justifies at least partly the approach that we have taken in the construction of the metabolic network, where we have assembled a mechanism that is very similar to those found in most cells. The actual biochemical parameters have been obtained in a few cases by direct experiment, and otherwise with a painstaking search through the existing literature: there is no uniform database and the parameters of the metabolic network are derived from many different cell lines, so that the simulated cells resemble some sort of "average" human cell. This kind of approach has been thus far validated by some remarkable quantitative results. Here we show just one such result (see also [20]), a comparison between experimental data with Raji leukemic cells, and a corresponding simulation with an environment that closely corresponds to the actual culture wells (see fig. 4).

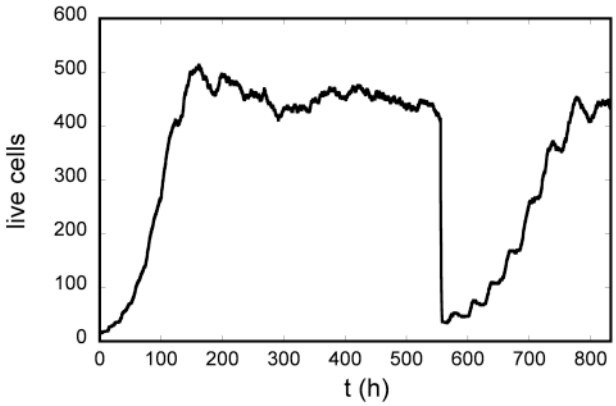

Fig. 5. – Live cells in a small bioreactor. A bioreactor is a standard tool of biology laboratories where cells are cultivated in an environment that is constantly flushed by a small flow of clean nutrient solution. The figure shows the number of live cells *vs.* time: in this simulation the initial equilibration phase is followed by a sharp pulse of radiation (10 Gy in 10000 s) and by a renewed growth phase.

4. – VBL and radiobiology

The interaction of radiation with living matter produces a vast array of different effects, and the most evident is cell death. Although the exact mechanisms are still poorly understood, many observations indicate that the cell survival fraction of irradiated cells follows a rather simple law, "the linear-quadratic law" [36]

$$P(D) = \exp\left[- \alpha D - \beta D^2 \right], \tag{1}$$

where D is the radiation dose, and where α and β are numerical coefficients that are estimated from experimental data. This phenomenological, probabilistic description of radiation-induced death can easily be incorporated in the program module that manages metabolism, growth, death and proliferation (see fig. 2). In particular, the structure of the program, with the progression of the cell cycle through all the different phases, allows also for the incorporation of phase-dependent α and β coefficients, and thus we can model the experimentally observed radiosensitivities of different cell phases.

Figure 5 shows one simulation run for cells in a bioreactor, which is one of the environments that can be simulated by the program: the bioreactor is seeded with a small number of cells, which experience an initial exponential growth phase, and at the same time the environment becomes toxic because of the accumulation of metabolites. An equilibrium is reached as the death rate associated with this environmental toxicity balances the proliferation rate. The bioreactor in equilibrium is irradiated with a rather large dose of X-rays (a flat pulse of 10 Gy in 10000 s), and the number of live cells decreases sharply. Afterwards the system recovers and cells reach again the plateau level which is determined by the bioreactor volume and flow, and by the cell phenotype.

Unlike real experiments, all variables are readily accessible in the simulation program, and we can plot the fraction of cells in the S-phase in the experiment of fig. 5. This is shown in fig. 6, where we notice first a dying oscillation (a remnant of the initial cell synchronization), then, as soon as the irradiation starts, most cells are killed and the sur-

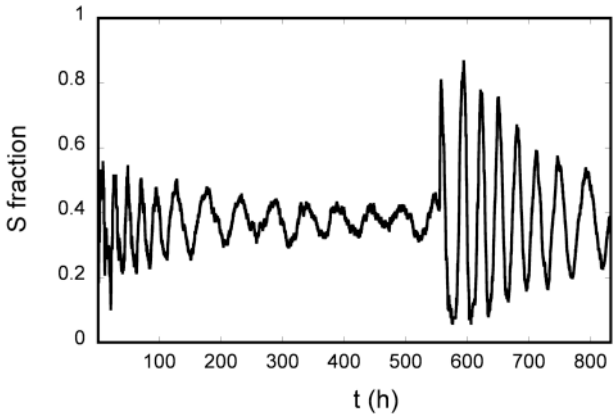

Fig. 6. – Fraction of cells in the S-phase *vs.* time in the virtual experiment of fig. 5. The cell population becomes synchronized because of the different radiosensitivity of cell phases. The figure suggests how one could attack solid tumors combining radiotherapy with chemotherapy.

viving fraction is concentrated in the S-phase (because of the higher radiation-resistance of cells in the S-phase), so that cells are again well synchronized. This effect could be exploited in a combined radiotherapic-chemotherapic attack against solid tumors using chemicals that kill cells in the S-phase after a preliminary irradiation: this would minimize the adverse effects of such drugs on other fast-cycling cells like those of the bone marrow.

5. – Future perspectives

The final goal of this effort is the development, step by step, of a numerical model to simulate tumor spheroids. Tumor spheroids have much in common with unvascularized solid tumors, and thus we shall eventually be able to simulate the initial growth phase of solid tumors. This kind of numerical simulation has several important implications:

- it is possible to perform virtual experiments *in silico* that complement *in vitro* measurements, where many parameters are not directly accessible, and also *in vivo* observations, where accessibility problems are even greater, and where one often meets difficult human and ethical problems;
- the simulation focuses the modeling effort on the important details of cellular biophysics and spawns new ideas, both theoretical and experimental;
- the numerical model includes many complex non-linear interactions between different parts of the cell, and thus it has interesting predictive properties (the knowledge of individual phenomena does not simply add up in a nonlinear model, that is why we need a numerical model).

In the near future the program shall incorporate the following features:

1) a full-fledged 3D structure of the cell cluster;
2) a repair-misrepair model of DNA synthesis, damage and repair;
3) a signaling network based on TNF (Tumor Necrosis Factor), associated with the induction of cell apoptosis or cell stimulation.

The last two parts are closely related, because experimental observations indicate that cells with seriously damaged DNA communicate with neighboring cells: these molecular signals seem to involve cytokines, and to induce apopototic death in the vicinity of the damaged cell: this is called "bystander effect". The program will thus simulate some effects of ionizing radiations on whole cell populations. The inclusion of the TNF cellular signaling is the first step of a broader study (like, *e.g.*, the signals between cells of the immune system and tumor cells).

If all goes as planned we shall eventually be able to carry out sophisticated virtual radiobiological experiments. We anticipate that the virtues of the simulation program will be really outstanding in the field of radiobiology, where experiments *in vivo* are very complex and often not feasible, and those *in vitro* sometimes yield ambiguous results. Obviously the validity of the whole framework depends on the correctness of the simulation of cellular processes and for this reason we shall check both the correctness and the robustness of the program using standard software engineering practices. This procedure will validate the simulation procedure and the biophysical models, but most of all it shall put to the test the model robustness, which is an essential feature of living organisms, and must be shared by any valid model of cellular processes.

REFERENCES

[1] Roose T., Chapman S. J. and Maini P. K., *SIAM Rev.*, **49** (2007) 179.
[2] Araujo R. P. and McElwain D. L. S., *Bull. Math. Biol.*, **66** (2004) 1039.
[3] Beyer T. and Meyer-Hermann M., *Phys. Rev. E*, **76** (2007) 021929.
[4] Byrne H. *et al.*, *Appl. Math. Lett.*, **16** (2003) 567.
[5] Drasdo D. and Hoehme S., *Math. Comp. Modelling*, **37** (2003) 1163.
[6] Drasdo D., Hoehme S. and Block M., *J. Stat. Phys.*, **128** (2007) 287.
[7] Galle J. *et al.*, *Cytometry*, **69A** (2006) 704.
[8] Galle J., Loeffler M. and Drasdo D., *Biophys. J.*, **88** (2005) 62.
[9] Jiang Y., Pjesivac-Grbovic J., Cantrell C. and Freyer J. P., *Biophys. J.*, **89** (2005) 3884.
[10] Kansal A. R. *et al.*, *J. Theor. Biol.*, **203** (2000) 367.
[11] Meinecke F., Potten C. S. and Loeffler M., *Cell Prolif.*, **34** (2001) 253.
[12] Palsson E., *FGCS*, **17** (2001) 835.
[13] Patel A. A., Gawlinski E. T., Lemieux S. K. and Gatenby R. A., *J. Theor. Biol.*, **213** (2001) 315.
[14] Schaller G. and Meyer-Hermann M., *Phys. Rev. E*, **71** (2005) 051910.
[15] Sherratt J. A. and Chaplain M. A. J., *J. Math. Biol.*, **43** (2001) 291.
[16] Stamatakos G. S. *et al.*, *IEEE Trans. Inf. Tech. Biomed.*, **5** (2001) 279.
[17] Turner S. and Sherratt J. A., *J. Theor. Biol.*, **216** (2002) 85.
[18] Zhang L., Athale C. A. and Deisboeck T. S., *J. Theor. Biol.*, **244** (2007) 96.
[19] Chignola R. and Milotti E., *Physica A*, **338** (2004) 261.
[20] Chignola R. and Milotti E., *Phys. Biol.*, **2** (2005) 8.
[21] Chignola R., Del Fabbro A., Dalla Pellegrina C. and Milotti E., *Phys. Biol.*, **4** (2007) 114.
[22] Sutherland R. M., *Science*, **240** (1988) 177.
[23] Chignola R. *et al.*, *Cell Prolif.*, **33** (2000) 219.
[24] Norton L., *Semin. Oncol.*, **26** (1999) 11.
[25] de Berg M., van Kreveld M., Overmars M. and Schwarzkopf O., *Computational Geometry: Algorithms and Applications* (Springer-Verlag, New York) 2000, p. 183 and following.
[26] Del Fabbro A., Chignola R. and Milotti E., arXiv:physics/0603001.

[27] Chignola R., Del Fabbro A., Dalla Pellegrina C. and Milotti E., *Physica A*, **371** (2006) 463.
[28] Milotti E., Del Fabbro A., Dalla Pellegrina C. and Chignola R., *Physica A*, **379** (2007) 133.
[29] Website: `http://www.cgal.org`.
[30] Gerstein M., Tsai J. and Levitt M., *J. Mol. Biol.*, **249** (1995) 955.
[31] Honda H., Tanemura M. and Yoshida A., *Acta Biotheor.*, **48** (2000) 121.
[32] Brodland G. W. and Veldhuis J. H., *J. Biomech.*, **35** (2002) 673.
[33] Schaller G. and Meyer-Hermann M., *Phys. Rev. E*, **71** (2005) 051910.
[34] Cornish-Bowden A., *The Pursuit of Perfection: Aspects of Biochemical Evolution* (Oxford University Press, New York) 2004, p. 2.
[35] Harold F. M., *The Way of the Cell: Molecules, Organisms and the Order of Life* (Oxford University Press, New York) 2001, p. 56.
[36] Fowler J. F., *Phys. Med. Biol.*, **51** (2006) R263.