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*Essay*

# Biothermodynamic Key Opens the Door of Life Sciences: Bridging the Gap between Biology and Thermodynamics

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> "*Thermodynamics is the only theory of universal content concerning which I am convinced that within the framework of applicability of its basic concepts, it will never be overthrown*"
>
> Albert Einstein, A Stubbornly Persistent Illusion, 2007

**Abstract:** During the last 50 years, an interdisciplinary approach in research in various scientific fields has become widely spread. This process significantly deepens and widens our scientific knowledge, but on the other hand makes more difficult choosing a discipline to be used by students and young researchers. The dynamic development of science makes this choice even harder. There is a widely spread opinion that thermodynamics uses a rigorous mathematical apparatus. This paper describes that knowledge of the fundamental principles of thermodynamics is required for deep understanding of crucial biological processes. Thus the effort put into learning classical and nonequilibrium thermodynamics does not make harder, but easier gaining a better knowledge of biological processes. This essay is an attempt to convey personal experience in using thermodynamics to describe biological systems and processes they perform.

**Keywords:** biology; thermodynamics; biothermodynamics; entropy; Gibbs energy

## 1. Introduction

This essay has the goal to bring closer two at the first glance completely different disciplines – biology and thermodynamics, to life science students and wider scientific audience. Many relate thermodynamics with machines, thermodynamic cycles, abstract terms like activity coefficients, enthalpy, entropy and Gibbs free energy. However, very often in science the concept of entropy is used [Popovic, 2017]. Entropy is in its essence a thermodynamic concept [Müller, 2010], suggested by Rudolph Clausius (father of classical thermodynamics) [1976, 1867, 1870] and Boltzmann (father of statistical thermodynamics) [1974, 1995], which is however used in many other scientific disciplines: statistical mechanics, chemistry, physics, biophysics, cosmology, informatics, social sciences and biology [Popovic, 2017].

Obviously the gap between biology and thermodynamics might not be as deep as it seems at the first glance. The bonds between thermodynamics and biology do not end with the entropy concept. People intuitively conceive living organisms as different from inanimate objects. Such understanding of the world has gone as far as the vitalistic theory [Allen, 2005]. It took several thousand years for the vitalistic theory to be rejected [Allen, 2005]. During the 1950s, Ludwig von Bertalanffy has made a theory of open systems in biology, stating that biology and physics are united by organisms being open thermodynamic systems [von Bertalanffy, 1950; Popovic, 2018a]. An open thermodynamic system is a part of the universe separated from its surroundings by a boundary, exchanging matter and energy with its environment [Atkins and de Paula, 2011, 2014]. Indeed, from the smallest organism of subcellular structure through unicellular organisms, all the way to multicellular organisms, all consist of a certain amount of highly ordered matter, bordered from the surroundings, which exchanges energy and matter with the environment, performing various biological, (bio)chemical and thermodynamic processes obeying the biological, physical, chemical and thermodynamic laws [Popovic, 2017; Popovic, 2018c].

## 2. Methods and Results

Biothermodynamics is among the youngest scientific disciplines. The biothermodynamic methodology has been developed during the last 100 years. Biothermodynamic analysis uses phenomenological equation and nonequilibrium thermodynamics. Moreover, biocalorimetry for determining thermodynamic properties of organisms has been used for a long time [Hansen et al., 2019, 2009, 2007; Barros et al., 2020, 2007; Barros Pena, 2018; Maskow, 2013; Maskow et al., 2010a].

Theoretical methods in biothermodynamic analysis are based on the works of Battley [1999a], Patel and Erickson [Patel and Erickson, 1981; Battley, 1998], Roels [Roels, 1983; von Stockar and Liu, 1999], Sandler and Orbey [1991], Hurst and Harrison [Hurst and Harrison, 1992; Ozilgen and Sorgüven, 2017] and others. They developed methods to find thermodynamic properties of live matter, including enthalpy, entropy, Gibbs energy and heat capacity.

Until now, thermodynamic analysis has been performed for more than 50 bacteria, fungi and algae species [Battley et al., 1997; Duboc et al., 1999; Battley, 1992; Wang et al., 1976], as well as for about 30 viruses [Popovic, 2022b, 2022c; Popovic and Minceva, 2020a, 2020b]. Moreover, thermodynamic characterization has been made for multicellular organisms, like plants [2021b] and human tissues [Popovic and Minceva, 2020c].

Calculation of thermodynamic properties is based on elemental composition (empirical formulas) of microorganisms and organisms [Popovic, 2022c]. Based on data on elemental composition, it is possible to find thermodynamic properties of formation. These can be combined with growth reactions to find thermodynamic properties of multiplication (enthalpy, entropy, Gibbs energy). Kinetic parameters, like growth rate, can be calculated using phenomenological equations, relating growth rate with its driving force – Gibbs energy of growth [Demirel, 2014; von Stockar, 2013a].

Interactions of microorganism with their host can also be analyzed using biothermodynamic methodology. Since microorganism-host interactions are competitive, this means that interaction rate depends on its Gibbs energy. Thus, by comparing Gibbs energies of interactions and interaction rates, it is possible to predict which interaction will be realized during competition. In that way, it is possible to predict whether there will be inhibition of growth or parallel growth of two microorganisms – interference or coinfection.

## 3. Discussion

Every organism has a characteristic elemental composition, just like inanimate matter. Everyone knows that water (inanimate matter) can be represented using the chemical formula $H_2O$. But, it is less widely known that the human organism can be represented by the chemical formula $CH_{1.7296}O_{0.2591}N_{0.1112}P_{0.0134}S_{0.0030}Na_{0.0027}K_{0.0031}\ldots$ [Popovic and Minceva, 2020c]. Moreover, various human tissues are characterized by their own empirical formula [Popovic and Minceva, 2020c]. Bacteria can also be represented by an empirical formula (unit carbon formula) – for example Escherichia coli has the formula $CH_{1.74}O_{0.34}N_{0.22}$ [Popovic, 2019; Popovic et al., 2021]. Even the modern “horrible” adversary of the humanity, the SARS-CoV-2 virus, can be represented by the empirical formula $CH_{1.6390}O_{0.2851}N_{0.2301}P_{0.0065}S_{0.0038}$ [Popovic and Minceva, 2020b; Popovic, 2022f]. Moreover, every coronavirus strain, from Hu-1 to Omicron BA.5, can be represented by empirical formulas, which are different for every strain [Popovic and Popovic, 2022; Popovic, 2022a, 2022b, 2022c, 2022d, 2022e; 2022f; Degueldre, 2021; Şimşek et al., 2021; Popovic and Minceva, 2021a, 2020a, 2020b]. Differences in chemical composition are a consequence of various mutation characteristics for various strains [Harvey et al., 2021]. Plants can also be represented by empirical formulas [Popovic and Minceva, 2021b].

Since organisms can be considered as open thermodynamic systems, they can be characterized using extensive (mass, volume, number of moles, enthalpy, entropy…) and intensive thermodynamic properties (temperature, pressure, specific enthalpy, specific entropy…). In general, all biological processes occur at approximately constant temperature.

All open thermodynamic systems with the property of growth, including organisms, perform interactions with their environment. The environment can be inanimate matter, which is the case for unicellular microorganisms. A unicellular microorganism exchanges matter and energy with its environment, performing growth. However, cells in a multicellular organism interact with neighboring cells of same or different kinds, as well as with their environment, exchanging matter, energy and information [Hansen et al., 2018, Popovic 2014a, 2014b]. Finally, multicellular organisms interact with other multicellular organisms, forming communities and ecosystems. All these interactions have their biological, chemical and thermodynamic aspects. Thus, the perspectives of chemistry, biology and thermodynamics are perspectives on the same system or process, but from different angles. No perspective alone is sufficient to fully understand the interaction process performed by the organism as a system.

Growth is one of the fundamental characteristics of living organisms. Growth can be performed through physical increase in the volume of a single cell or by multiplication of cells comprising an organism. According to the first law of thermodynamics, growth occurs through accumulation of matter. In open systems such as organisms, matter and energy are exchanged with the environment through import and export. Growth occurs through the net import being greater than the net export. Growth of microorganisms is led by a driving force. The driving force for growth of microorganisms is Gibbs energy [von Stockar, 2013a, 2013b; von Stockar and Liu, 1999]. The biological property of growth can be related to the thermodynamic property of Gibbs energy of growth, through chemical kinetics and nonequilibrium thermodynamics [Demirel, 2014]. Namely, growth rate is proportional to Gibbs energy of growth, according to the phenomenological equation, which belongs to the domain of nonequilibrium thermodynamics [Demirel, 2014]. At the molecular level, a cell imports from its environment the building blocks (e.g. amino acids, nucleotides, monosaccharides etc.).

Inside cells, macromolecules are synthetized and accumulated, enabling their growth and function. During this process, thermodynamic properties of cells change, including enthalpy, entropy and Gibbs energy. Thus, growth represents a biological, as well as chemical and thermodynamic process.

Indeed, the division of metabolism into catabolism and anabolism is based on energy usable to the cell - Gibbs energy [Berg et al., 2002]. Those reactions that transform fuels into Gibbs energy usable to cells are called catabolic reactions or, more generally, catabolism [Berg et al., 2002]. On the other hand, those reactions that consume Gibbs energy such as the synthesis of glucose, fats, or DNA are called anabolic reactions or anabolism [Berg et al., 2002]. Thus, anabolic reactions use Gibbs energy released by catabolism to produce complex structures from simple ones or convert energy poor states into energy rich ones [Berg et al., 2002]. Both catabolism and anabolism are driven by the same driving force – Gibbs energy.

A good example of the necessity of knowing thermodynamic properties of biological systems and processes they perform are virus-host interactions. It is known that a virus, after binding to and entering a susceptible host cell, decomposes and releases its nucleic acid into the cytoplasm. After that, the virus hijacks the cell metabolic machinery, and through replication, transcription, translation and self-assembly multiplies inside the cell, through catalyzed reactions. The process certainly proceeds qualitatively in that way. However, the viral life cycle in the host cell has its quantitative side. A question is raised of why the virus has the ability to hijack the cell machinery and building blocks.

Obviously, a virus possesses a favoring mechanism enabling it to hijack the metabolic machinery. The mechanistic model used in thermodynamic analysis names the driving force – the force that gives the virus an advantage in the competition for dominance over the cell metabolic machinery and resources [Popovic and Minceva, 2020a]. Von Stockar has identified the driving force of growth of microorganisms to be Gibbs energy of growth [von Stockar, 2013a, 2013b; von Stockar and Liu, 1999]. Indeed, the Gibbs energy of growth of the SARS-CoV-2 nucleocapsid is -222.2 kJ/C-mol [Popovic and Minceva, 2020b]. Gibbs energy of the susceptible tissue for SARS-CoV-2, the respiratory pathways, is -50 kJ/C-mol [Popovic and Minceva, 2020c]. According to the phenomenological equation, the rate of synthesis of the virus components is 4 times greater than that of the host cell.

$$r = \frac{L}{T} \Delta_{bs} G \tag{1}$$

The phenomenological equation relates rate of biosynthesis, $r$, to its thermodynamic driving force (Gibbs energy), $\Delta_{bs}G$. The constant of proportionality is the phenomenological coefficient $L$, while $T$ is temperature. Indeed, the virus will multiply in the respiratory mucosa, while the cell building blocks required to repair the damage made to the host cell will not be synthetized, which will contribute to the damage done to the cell.

Another interesting example is phenomenon of interference. During a simultaneous infection of an organism with two different viruses, two outcomes are possible: coinfection or interference [Popovic and Minceva, 2021a]. Sometimes, both viruses simultaneously multiply in the same host, resulting in coinfection [Popovic and Minceva, 2021a]. It is also possible that infection by one virus inhibits the multiplication of another virus, a phenomenon known as interference [Popovic and Minceva, 2021a]. These phenomena cannot be explained without using the thermodynamic terms of driving force of growth and Gibbs energy, as well as the kinetic term - reaction rate. A mechanistic model of simultaneous infection with two different viruses predicts that if the multiplication rates (and thereby Gibbs energies of growth) are equal or similar, which is the case of influenza and parainfluenza viruses, the result is coinfection – simultaneous multiplication of both viruses in the same host. On the other hand, if two viruses have a great difference in Gibbs energies of growth and growth rates, the virus with the more negative Gibbs energy of growth will multiply. This will lead to the phenomenon of interference. Indeed, this is what happened with influenza and SARS-CoV-2, when in 2020 and 2021 the influenza epidemics did not occur. On the other hand, the rhinovirus has a very similar Gibbs energy of growth to SARS-CoV-2. Thus, the epidemic caused by the rhinovirus occurred in parallel with the COVID-19 pandemic.

Another widely known phenomenon can be explained using a thermodynamic model. Aging is an obvious process, which occurs in live and inanimate matter. The changes that occur during aging which can be seen the easiest are in the appearance and functionality of the organism. Changes in these two parameters are a consequences of changes in chemical composition and thermodynamic properties of tissues [Popovic, 2018b]. The problem of aging is a complex process. During aging, changes occur in extensive and intensive thermodynamic properties. Mass, volume, enthalpy, entropy etc. change, as well as chemical composition. Substances like lipofuscin accumulate [Gray and Woulfe, 2005; Höhn and Grune, 2013] and structures like telomeres are lost [Vaiserman and Krasnienkov, 2021; Whittemore et al., 2019]. ROS as a potential cause of aging could also lead to changes in chemical composition and thermodynamic properties [Buffenstein et al., 2008]. Moreover, Hayflick has underlined the role of entropy change in the aging process [Hayflick, 2007, 1985].

Theory of evolution is generally accepted in the scientific community. However, even today there is resistance, which is minor, but pretty aggressive. I believe that mechanistic models used by thermodynamics could contribute to removing the doubts in the skeptics. For example, a mechanistic model used for evolution of the SARS-CoV-2 virus, starting from Hu-1 to Omicron BA.5 during 3 years, has showed the tendency towards decreasing Gibbs energy of multiplication (biosynthesis) and Gibbs energy of binding. The trend in decrease in Gibbs energy of binding is very sharp, while that of biosynthesis is mild. This is in accordance with the predictions of the evolution theory. The organism is evolving towards becoming better adapted to its environment. In this case, SARS-CoV-2 is evolving towards increase in infectivity, which is followed by a stagnation in pathogenicity. Indeed, the thermodynamic driving force for these two processes (binding and growth) confirms the predictions of theory of evolution [Popovic and Popovic, 2022; Popovic, 2022b].

It seems that thermodynamics, even though it was primarily developed for the needs of industry and improvement of steam engine efficiency, due to its universal approach can significantly contribute to deeper understanding of biological processes.

Thermodynamic methods have been applied many times in life sciences. Thermodynamic analysis of organisms is as old as thermodynamics itself. The founders of thermodynamics, Lavoisier and Laplace were the first to measure heat released by a mouse using a calorimeter [Lavoisier and marquis de Laplace, 1783; Müller, 2010]. The first theoretical consideration was made by Boltzmann, who considered the role of entropy in energy transformations performed by organisms [Boltzmann, 1974; Popovic, 2017]. The next was the founder of biothermodynamics – Schrödinger [1944], who used a thermodynamic concept – entropy – to define life [von Stockar and Liu, 1999, Skene, 2015]. Moreover, organisms were identified as open thermodynamic systems by von Bertalanffy [1950]. Analysis of organisms became much easier due to the work of Prigogine [Glansdorff and Prigogine, 1971; Prigogine and Wiame, 1946; Prigogine, 1947, 1977], who developed nonequilibrium thermodynamics as a tool that proved very useful in analysis of life processes. Moreover, a great contribution to the field was given by Morowitz [1992; 1968, 1955], who discussed the role of thermodynamics in life processes and the origin of life. He discussed numerous applications of thermodynamics in biology [Morrowitz, 1968] and proposed a scenario of the origin of life on Earth based on biothermodynamic and bioenergetic considerations [Morowitz, 1992]. Moreover, Morowitz [1955] was the first to calculate the total information content of an entire cell, relating it to orderliness and entropy of organisms. The first experimental determination of entropy of organisms was made 42 years later, by Battley et al. [1997]. Battley [1999a, 199b, 1998, 1992] has made a great contribution to quantifying thermodynamic properties of organisms. More recently, von Stockar analyzed the thermodynamic driving force for life processes, identifying Gibbs energy as a convenient parameter for quantitative analysis of microbial multiplication [von Stockar and Liu, 1999; von Stockar, 2013a, 2013b; von Stockar et al., 2006]. Hansen discussed change in information content and entropy in organisms during life processes, including evolution [Hansen et al., 2009, 2018]. The importance of entropy and Gibbs energy in virology has been discussed by Lucia [Lucia et al., 2021, 2020a, 2020b]. Calorimetric measurements on viruses and other microorganisms have been performed by Maskow et al. [2010a, 2010b, 2013]. Therefore, thermodynamics has been in use in biological research for over 200 years and has given many interesting results [Berg et al., 2002].

## 5. Conclusion

Isaac Newton once wrote *"If I have seen further it is by standing on the shoulders of Giants"*. [Chen, 2003]. Indeed, the names of scientists mentioned in the above paragraph have significantly contributed to deepening our knowledge on the deep background of biological processes. However, there are many biological processes, many more than could be studied. In the future, it will be necessary to perform thermodynamic characterization of many cellular and subcellular organisms, as well as to elucidate the thermodynamic background of interactions of these organisms between themselves and with their environment and biochemical/physiological processes they perform. The author hopes that this short essay will turn the attention of students and young researchers, who could through their hard work significantly enrich our knowledge on the fundamental principles of quantitative biology.

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