Biothermodynamics of Viruses

Subjects: Virology
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Biothermodynamics of viruses is among the youngest but most rapidly developing scientific disciplines. During the COVID-19 pandemic, it closely followed the results published by molecular biologists. Empirical formulas were published for 50 viruses and thermodynamic properties for multiple viruses and virus variants, including all variants of concern of SARS-CoV-2, SARS-CoV, MERS-CoV, Ebola virus, Vaccinia and Monkeypox virus. Biothermodynamics of viruses has suggested a physicochemical mechanism of how viruses can hijack host cell metabolism.

Keywords: thermodynamics ; calorimetry ; entropy ; enthalpy

1. From Thermodynamics to Biothermodynamics

There is a common opinion that thermodynamics is a scientific discipline related to machines, engines and devices, dealing mostly with efficiency of energy transformation and utilization. Indeed, Lazarus Carnot [1][2] and his son Sadi Carnot [3] have, through their brilliant research, imposed such a perception into the public for over two centuries [4]. In this way, classical thermodynamics began its development. It is less widely known that, simultaneously with classical thermodynamics, appeared biothermodynamics. Lavoisier and Laplace [5][6] developed the first calorimeter and one of the first samples for calorimetry was an organism—a live guinea pig. Thus, simultaneously with classical thermodynamics, biothermodynamics started its development.

Often, the same researchers worked in the field of classical thermodynamics and biothermodynamics. Indeed, Boltzmann [7], one of the founders of statistical thermodynamics, has written about change in entropy in living organisms. Clausius [8][9][10] has laid the theoretical foundations of classical thermodynamics, with the goal of analyzing machines. However, von Bertalanffy [11] has suggested the theory of open systems in biology. Schrödinger in his famous book “What is Life?” discussed the thermodynamic background of life processes [12]. Morowitz [13][14][15] has discussed potential controversies related to self-assembly in organisms and emergence of life, and the second law of thermodynamics.

Growth is one of the main characteristics of organisms. The answer to the question of what represents the driving force for the growth of organisms was given by von Stockar [16][17][18][19][20]. It seems that biothermodynamics, even though it is less widely known than classical thermodynamics, has existed in the scientific arena for as long, and has given impressive results. Hansen analyzed whether an extended thermodynamic framework can be used to analyze processes in organisms that involve information, such as biological evolution [21][22][23]. Application of thermodynamics to biological evolution was also discussed by Skene [24]. Battley has made a great contribution towards applying the quantitative thermodynamic approach to living organisms and life processes [25][26][27][28][29][30]. Roels [16][31], and Sandler [32][33] have also contributed to quantifying the thermodynamic properties of organisms. Barros has applied thermodynamics to study the growth of microorganisms in soil ecosystems [34][35][36]. Maskow has applied calorimetry and thermodynamic analysis to study the growth of microorganisms in bioreactors [37][38] and ecosystems [39][40], as well as viruses in host cells [41]. Guosheng et al. [42] have also applied calorimetric methods to study the multiplication of bacteriophages inside host cells.

2. Biothermodynamics Intersects with Biochemistry

Thermodynamic characterization of life processes has been a subject of interest for many researchers. Von Stockar et al. [19][43] applied thermodynamics to quantitatively analyze thermodynamic feasibility of complex metabolic pathways, such as glycolysis. Thermodynamic analysis has been used to find accurate Gibbs energy values with activity coefficient corrections for important biological reactions, including Hexokinase reaction [44], Glucose-6-phosphatase reaction and ATP hydrolysis [45], 3-phosphoglycerate kinase reaction [46], Triosephosphate isomerase reaction [47], Enolase reaction [48], and Glyceraldehyde 3-phosphate dehydrogenase reaction [49]. Additionally, thermodynamic analysis was made of cellulose hydrolysis by microorganisms in the aqueous glucose solution [50]. Niebel et al. [51] found that the cellular metabolism is governed by an upper limit in Gibbs energy dissipation, using metabolomics. Ould-Moulaye et al. [52] found Gibbs energy

changes for the reactions in glycolysis and Krebbs cycle. Kümmel et al. [53] discuss applications of thermodynamics in metabolic network models.

The importance of thermodynamic considerations in life sciences is clearly seen from the Gibbs energy being used to define catabolic and anabolic processes [54]. Annamalai used the quantitative thermodynamic approach to study the metabolic processes [55][56] and the aging of organisms [57][58][59][60][61]. Hayflick was among the first who related a thermodynamic property (entropy) to the aging process in a series of papers [62][63][64][65][66][67][68][69].

3. From Biothermodynamics to Virothermodynamics

Viruses are the most abundant organisms: there could be more viruses than stars in the universe [70]. There are 9,110 named species listed by the International Committee on Taxonomy of Viruses (ICTV) [71]. Until 2019, despite the wide variety of viruses, they have been the subject of research of microbiology, virology, biology and medicine. However, inside host cells, viruses represent growing open chemical and thermodynamic systems [72][73][74][75]. Until 2019, elemental composition was known only for the poliovirus [76][77]. This is a consequence of the fact that analytical laboratories rarely have biosafety levels required for work with most viruses, as well as the fact that viruses are difficult to isolate in sufficient amounts and purity [78]. Until recently, viruses were not a subject of thermodynamic research. The thermodynamic properties of virus particles and nucleocapsids were unknown.

With the appearance of the COVID-19 pandemic, various scientific disciplines attempted to contribute, in the shortest time possible, to the fight against the pandemic. Molecular biology has played an important role with the reading of genetic sequences of SARS-CoV-2. Thermodynamics has joined the fight and in 2020, thermodynamic properties have been published for multiple viruses [79]. An analysis was made of virus–host interactions in the cytoplasm (virus multiplication) [79]. The first empirical formula and thermodynamic properties of the Hu-1 variant of SARS-CoV-2, as well as SARS-CoV and MERS-CoV were published in 2020 [80]. In 2020, in parallel with the COVID-19 pandemic, an epidemic caused by the rhinovirus occurred, while the influenza epidemic did not occur that year. An explanation of coinfection by rhinovirus and SARS-CoV-2, and interference between influenza and SARS-CoV-2 has been published in [81]. SARS-CoV-2 belongs to the group of RNA viruses, which exhibit a great tendency to mutate [82]. Thus, during the 2.5 years of the pandemic, the virus has mutated several times [83][84][85][86]. The mutants suppressed the older variants and caused new waves of infection during the pandemic. The elemental composition and thermodynamic properties of SARS-CoV-2 variants from Hu-1 to Omicron BA.2.75 have been published in [80][86][87][88][89][90][91][92][93]. The biothermodynamic characterization of viruses was continued for Monkeypox, Vaccinia and Ebola viruses [94][95].

Infectivity and pathogenicity are terms mostly used in microbiology, biology and medicine. These terms have their physical basis and driving forces in biothermodynamics. The basis of the infectivity of viruses is susceptibility and permissiveness (binding affinity and multiplication rate, respectively). Antigen–receptor binding represents a chemical reaction, similar to protein–ligand interactions [96]. The driving force for antigen–receptor binding is the Gibbs energy of binding [86][88][91][97][98][99][100][101]. Thus, biothermodynamic consideration and determination of Gibbs energy of binding is very important for infection spreading [102][103]. More negative Gibbs energy of binding of new variants gave an advantage to new strains during entry over older ones, which led to faster spreading of the virus and shorter incubation period. Gibbs energies of binding and binding affinities of viruses have been reported in the literature for various viruses [86][87][88][89][90][91][95][97][98][99][100][101][104].

To explore the interaction between a virus and its human host, it was necessary to find thermodynamic properties for host organisms. Thermodynamic properties have been reported for human tissues [95][105] since virus–human interactions have been of particular importance. Thermodynamic properties of plant host organisms are reported in [106]. Phage–bacteria interactions are often used as a model in the research of virus–host interactions. Thus, thermodynamic properties have been determined for a large number of bacteria [29][107][108][109][110] and bacteriophages [41][42][79].

The second virus–host interaction is in the cytoplasm. In papers [79][80], a biothermodynamic mechanism was suggested for virus hijacking of host cell metabolism. The permissiveness represents the ability of a virus to multiply inside the host [111]. The multiplication of a virus represents a chemical reaction of polymerization of nucleotides into nucleic acids, and amino acids into structural and functional proteins of the virus [95]. The driving force for these reactions is the Gibbs energy of biosynthesis [112]. After their biosynthesis, the virus components undergo self-assembly into a new virus particle [113][114]. During biosynthesis and self-assembly, viruses change their thermodynamic properties [115][116]. Thus, the virus life cycle represents a biological, chemical and thermodynamic process that should be analyzed using a nonequilibrium thermodynamic apparatus [117].

Viruses represent the smallest organisms, but also belong to the most contagious and deadly microorganisms. They spread very rapidly, often causing epidemics and pandemics, which result in large numbers of casualties. Furthermore, there are very few antiviral medicines. Thus, the fight against epidemics and pandemics is directed towards epidemiological measures and the application of vaccines. However, vaccine production, especially in the case of new viruses, requires a lot of time and resources. For example, the vaccines against SARS-CoV-2 were awaited for a year. The ability of some viruses to develop mutations fast leads to the need for new vaccines. Some of the available novel vaccines have proved themselves effective for the Hu-1, Alpha, Beta, Gamma and Delta variants. However, these vaccines are much less effective for the newer Omicron variants due to their ability to evade the immune response. This has imposed a need for the production of polyvalent vaccines, which also takes time and long-term testing. Knowing the thermodynamic properties of the host and virus, as well as the application of a mechanistic model of interactions on the cell membrane and in the cytoplasm, could, in the future, contribute to designing new vaccines and antiviral medicines. Moreover, such knowledge could aid in finding places and methods for vaccine application. For example, every human tissue is characterized by a specific value of Gibbs energy of biosynthesis of its building blocks. On the other hand, every virus variant is characterized with its own specific Gibbs energy of biosynthesis. The ratio of these two values is the permissiveness coefficient, which is different for various virus–host cell pairs. The result of this is that some viruses can be synthetized in one type of cell, while in others their multiplication is significantly slower. By choosing a tissue for vaccine application where virus growth is slower, it is possible to give enough time to the immune system to respond to a low virus concentration. Such a vaccine would be attenuated (live), capable of inducing an immune response but, due to the low permissiveness coefficient, unable to cause a disease in a more severe clinical form. The attenuation process of a vaccine based on biothermodynamic properties would not be performed through a long passage that requires great resources and time, but through choosing a place of application where the virus can multiply very slowly. Thus, one of the potential applications of biothermodynamics in virology would be in vaccinology. Such a vaccine would not be based on empirical data but on engineering, using biothermodynamic tools, which would help to significantly save time and resources in the design and production of vaccines.

References

1. Carnot, L. Essai sur les Machines en Général; English translation: “Essay on machines in general”; De l’Imprimerie de Defay: Dijon, France, 1786; ISBN 978-1147666625.
2. Carnot, L. Principes Fondamentaux de l’Equilibre et du Movement; English translation: “Fundamental principles of equilibrium and movement”; De l’Imprimerie de Crapelet: Paris, France, 1803; ISBN 2016170190.
3. Carnot, S. Réflexions sur la Puissance Motrice du Feu et sur les Machines Propres à Développer Cette Puissance; English translation: “Reflections on the motive power of fire and on machines fitted to develop that power”; Bachelier: Paris, France, 1824; ISBN 978-0486446417.
4. Müller, I. A History of Thermodynamics: The Doctrine of Energy and Entropy; Springer: Berlin/Heidelberg, Germany, 2010; ISBN 978-3642079641.
5. Lavoisier, A.L.; Marquis de Laplace, P.S. Mémoire sur la Chaleur: Lû à l’Académie Royale des Sciences, le 28 Juin 1783; English translation: “Memoir on Heat Read to the Royal Academy of Sciences, 28 June 1783”; De l’Imprimerie Royale: Paris, France, 1783.
6. Lavoisier, A.L.; DeLaplace, P.S. Memoir on heat read to the royal academy of sciences, 28 June 1783. Obes. Res. 1994, 2, 189–202, (Modern Translation in English).
7. Boltzmann, L. The second law of thermodynamics. In Theoretical Physics and Philosophical Problems; Translation of the Original Version Published in 1886; McGuinnes, B., Ed.; D. Riedel Publishing Company, LLC: Boston, MA, USA, 1974; ISBN 978-90-277-0250-0.
8. Clausius, R. The Mechanical Theory of Heat—With its Applications to the Steam Engine and to Physical Properties of Bodies; John van Voorst: London, UK, 1867.
9. Clausius, R. On a Mechanical Theorem Applicable to Heat. Philos. Mag. Ser. 1870, 40, 122–127.
10. Clausius, R. On different forms of the fundamental equations of the mechanical theory of heat and their convenience for application. In The Second Law of Thermodynamics; Kestin, J., Ed.; Dowen, Hutchingson and Ross, Inc.: Stroudsburg, PA, USA, 1976; ISBN 978-0470989449.
11. Von Bertalanffy, L. The theory of open systems in physics and biology. Science 1950, 111, 23–29.
12. Schrödinger, E. What is Life? The Physical Aspect of the Living Cell; Cambridge University Press: Cambridge, UK, 1944; ISBN 0-521-42708-8.

13. Morowitz, H.J. Beginnings of Cellular Life: Metabolism Recapitulates Biogenesis; Yale University Press: New Haven, CT, USA, 1992; ISBN 978-0300102109.
14. Morowitz, H.J. Energy Flow in Biology: Biological Organization as a Problem in Thermal Physics; Academic Press: New York, NY, USA, 1968. Available online: https://lccn.loc.gov/67031041 (accessed on 7 December 2022).
15. Morowitz, H.J. Some order-disorder considerations in living systems. Bull. Math. Biophys. 1955, 17, 81–86.
16. Von Stockar, U.; Liu, J.-S. Does microbial life always feed on negative entropy? Thermodynamic analysis of microbial growth. Biochim. Biophys. Acta (BBA)—Bioenerg. 1999, 1412, 191–211.
17. Von Stockar, U. Live cells as open non-equilibrium systems. In Biothermodynamics: The Role of Thermodynamics in Biochemical Engineering; von Stockar, U., Ed.; EPFL Press: Lausanne, Switzerland, 2013; pp. 399–421.
18. Von Stockar, U. Biothermodynamics of live cells: Energy dissipation and heat generation in cellular structures. In Biothermodynamics: The Role of Thermodynamics in Biochemical Engineering; von Stockar, U., Ed.; EPFL Press: Lausanne, Switzerland, 2013; pp. 475–534.
19. Von Stockar, U.; Maskow, T.; Liu, J.; Marison, I.W.; Patiño, R. Thermodynamics of microbial growth and metabolism: An analysis of the current situation. J. Biotechnol. 2006, 121, 517–533.
20. Patiño, R.; Janssen, M.; von Stockar, U. A study of the growth for the microalgaChlorella vulgaris by photo-bio-calorimetry and other on-line and off-line techniques. Biotechnol. Bioeng. 2006, 96, 757–767.
21. Hansen, L.D.; Tolley, H.D.; Woodfield, B.F. Transformation of matter in living organisms during growth and evolution. Biophys. Chem. 2021, 271, 106550.
22. Hansen, L.D.; Popovic, M.; Tolley, H.D.; Woodfield, B.F. Laws of evolution parallel the laws of thermodynamics. J. Chem. Thermodyn. 2018, 124, 141–148.
23. Hansen, L.D.; Criddle, R.S.; Battley, E.H. Biological calorimetry and the thermodynamics of the origination and evolution of life. Pure Appl. Chem. 2009, 81, 1843–1855.
24. Skene, K.R. Life's a Gas: A Thermodynamic Theory of Biological Evolution. Entropy 2015, 17, 5522–5548.
25. Battley, E.H. A Theoretical Study of the Thermodynamics of Microbial Growth UsingSaccharomyces cerevisiae and a Different Free Energy Equation. Q. Rev. Biol. 2013, 88, 69–96.
26. Battley, E.H. An empirical method for estimating the entropy of formation and the absolute entropy of dried microbial biomass for use in studies on the thermodynamics of microbial growth. Thermochim. Acta 1999, 326, 7–15.
27. Battley, E.H. The thermodynamics of microbial growth. In Handbook of Thermal Analysis and Calorimetry, vol. 4: From Macromolecules to Man; Kemp, E.B., Ed.; Elsevier: Amsterdam, NX, USA, 1999; pp. 219–235.
28. Battley, E.H. The development of direct and indirect methods for the study of the thermodynamics of microbial growth. Thermochim. Acta 1998, 309, 17–37.
29. Battley, E.H.; Putnam, R.L.; Boerio-Goates, J. Heat capacity measurements from 10 to 300 K and derived thermodynamic functions of lyophilized cells of Saccharomyces cerevisiae including the absolute entropy and the entropy of formation at 298.15 K. Thermochim. Acta 1997, 298, 37–46.
30. Battley, E.H. On the enthalpy of formation ofEscherichia coli K-12 cells. Biotechnol. Bioeng. 1992, 39, 5–12.
31. Roels, J.A. Energetics and Kinetics in Biotechnology; Elsevier: Amsterdam, NX, USA, 1983; ISBN 978-0-444-80442-6.
32. Sandler, S.I.; Orbey, H. On the thermodynamics of microbial growth processes. Biotechnol. Bioeng. 1991, 38, 697–718.
33. Sandler, S.I. Chemical, Biochemical, and Engineering Thermodynamics, 5th ed.; Wiley: Hoboken, NJ, USA, 2017; ISBN 978-1-119-32128-6.
34. Barros, N. Thermodynamics of Soil Microbial Metabolism: Applications and Functions. Appl. Sci. 2021, 11, 4962.
35. Barros, N.; Fernandez, I.; Byrne, K.A.; Jovani-Sancho, A.J.; Ros-Mangriñan, E.; Hansen, L.D. Thermodynamics of soil organic matter decomposition in semi-natural oak (Quercus) woodland in southwest Ireland. Oikos 2020, 129, 1632–1644.
36. Barros, N.; Hansen, L.; Piñeiro, V.; Pérez-Cruzado, C.; Villanueva, M.; Proupín, J.; Rodríguez-Añón, J. Factors influencing the calorespirometric ratios of soil microbial metabolism. Soil Biol. Biochem. 2016, 92, 221–229.
37. Maskow, T. Miniaturization of calorimetry: Strengths and weaknesses for bioprocess monitoring. In Biothermodynamics: The Role of Thermodynamics in Biochemical Engineering; von Stockar, U., Ed.; EPFL Press: Lausanne, Switzerland, 2013; pp. 423–442.
38. Maskow, T.; Harms, H. Real Time Insights into Bioprocesses Using Calorimetry: State of the Art and Potential. Eng. Life Sci. 2006, 6, 266–277.

39. Maskow, T.; Paufler, S. What does calorimetry and thermodynamics of living cells tell us? Methods 2015, 76, 3–10.
40. Maskow, T.; Kemp, R.B.; Buchholz, F.; Schubert, T.; Kiesel, B.; Harms, H. What heat is telling us about microbial conversions in nature and technology: From chip- to megacalorimetry. Microb. Biotechnol. 2009, 3, 269–284.
41. Maskow, T.; Kiesel, B.; Schubert, T.; Yong, Z.; Harms, H.; Yao, J. Calorimetric real time monitoring of lambda prophage induction. J. Virol. Methods 2010, 168, 126–132.
42. Guosheng, L.; Yi, L.; Xiangdong, C.; Peng, L.; Ping, S.; Songsheng, Q. Study on interaction between T4 phage and Escherichia coli B by microcalorimetric method. J. Virol. Methods 2003, 112, 137–143.
43. Von Stockar, U.; Maskow, T.; Vojinovic, V. Thermodynamic analysis of metabolic pathways. In Biothermodynamics: The Role of Thermodynamics in Biochemical Engineering; von Stockar, U., Ed.; EPFL Press: Lausanne, Switzerland, 2013; pp. 581–604.
44. Meurer, F.; Bobrownik, M.; Sadowski, G.; Held, C. Standard Gibbs Energy of Metabolic Reactions: I. Hexokinase Reaction. Biochemistry 2016, 55, 5665–5674.
45. Meurer, F.; Do, H.T.; Sadowski, G.; Held, C. Standard Gibbs energy of metabolic reactions: II. Glucose-6-phosphatase reaction and ATP hydrolysis. Biophys. Chem. 2017, 223, 30–38.
46. Wangler, A.; Schmidt, C.; Sadowski, G.; Held, C. Standard Gibbs Energy of Metabolic Reactions: III The 3-Phosphoglycerate Kinase Reaction. ACS Omega 2018, 3, 1783–1790.
47. Greinert, T.; Baumhove, K.; Sadowski, G.; Held, C. Standard Gibbs energy of metabolic reactions: IV. Triosephosphate isomerase reaction. Biophys. Chem. 2020, 258, 106330.
48. Greinert, T.; Vogel, K.; Seifert, A.I.; Siewert, R.; Andreeva, I.V.; Verevkin, S.P.; Maskow, T.; Sadowski, G.; Held, C. Standard Gibbs energy of metabolic reactions: V. Enolase reaction. Biochim. et Biophys. Acta (BBA)—Proteins Proteom. 2020, 1868, 140365.
49. Greinert, T.; Vogel, K.; Mühlenweg, J.-K.; Sadowski, G.; Maskow, T.; Held, C. Standard Gibbs energy of metabolic reactions: VI. Glyceraldehyde 3-phosphate dehydrogenase reaction. Fluid Phase Equilibria 2020, 517, 112597.
50. Popovic, M.; Woodfield, B.F.; Hansen, L.D. Thermodynamics of hydrolysis of cellulose to glucose from 0 to 100 °C: Cellulosic biofuel applications and climate change implications. J. Chem. Thermodyn. 2018, 128, 244–250.
51. Niebel, B.; Leupold, S.; Heinemann, M. An upper limit on Gibbs energy dissipation governs cellular metabolism. Nat. Metab. 2019, 1, 125–132.
52. Ould-Moulaye, C.; Dussap, C.; Gros, J. Estimation of Gibbs energy changes of central metabolism reactions. Biotechnol. Tech. 1999, 13, 187–193.
53. Kümmel, A.; Panke, S.; Heinemann, M. Systematic assignment of thermodynamic constraints in metabolic network models. BMC Bioinform. 2006, 7, 512.
54. Berg, J.M.; Tymoczko, J.L.; Stryer, L. Biochemistry, 5th ed.; W H Freeman: New York, NY, USA, 2002; ISBN 978-0716746843.
55. Annamalai, K. Oxygen Deficient (OD) Combustion and Metabolism: Allometric Laws of Organs and Kleiber's Law from OD Metabolism? Systems 2021, 9, 54.
56. Annamalai, K.; Miller, J.A. Link between O2 Deficient Metabolism in Organs and Group Combustion in Engineering. In Proceedings of the 10th US National Combustion Meeting, College Park, MD, USA, 23–24 April 2017; pp. 23–26.
57. Annamalai, K.; Nanda, A. Biological Aging and Life Span Based on Entropy Stress via Organ and Mitochondrial Metabolic Loading. Entropy 2017, 19, 566.
58. Annamalai, K.; Silva, C. Entropy Stress and Scaling of Vital Organs over Life Span Based on Allometric Laws. Entropy 2012, 14, 2550–2577.
59. Silva, C.A.; Annamalai, K. Entropy Generation and Human Aging: Lifespan Entropy and Effect of Diet Composition and Caloric Restriction Diets. J. Thermodyn. 2009, 2009, 186723.
60. Silva, C.; Annamalai, K. Entropy Generation and Human Aging: Lifespan Entropy and Effect of Physical Activity Level. Entropy 2008, 10, 100–123.
61. Silva, C.; Annamalai, K. 1st Law, Metabolism; 2nd Law and Entropy Generaion: Secret to Longevity in Lifespan? In Proceedings of the 9th AIAA/ASME Joint Thermophysics and Heat Transfer Conference, San Francisco, CA, USA, 5–8 June 2006; p. 2940.
62. Hayflick, L. Unlike the Stochastic Events That Determine Ageing, Sex Determines Longevity. In Cellular Ageing and Replicative Senescence. Healthy Ageing and Longevity; Rattan, S., Hayflick, L., Eds.; Springer: Cham, Switzerland, 2016.

63. Hayflick, L. Unlike ageing, longevity is sexually transmitted. Médecine Longévité 2010, 2, 114–128.
64. Hayflick, L. Entropy Explains Aging, Genetic Determinism Explains Longevity, and Undefined Terminology Explains Misunderstanding Both. PLoS Genet. 2007, 3, e220.
65. Hayflick, L. Biological Aging Is No Longer an Unsolved Problem. Ann. N. York Acad. Sci. 2007, 1100, 1–13.
66. Hayflick, L. Modulating aging, longevity determination and the diseases of old age. In Modulating Aging and Longevity; Rattan, S., Ed.; Springer: Berlin/Heidelberg, Germany, 2003; pp. 1–16. ISBN 978-1402013690.
67. Hayflick, L. The Quest for Immortality: Science at the Frontiers of Aging. Radiat. Res. 2001, 156, 334–336.
68. Hayflick, L. How and why we age. Exp. Gerontol. 1998, 33, 639–653.
69. Hayflick, L. Theories of biological aging. Exp. Gerontol. 1985, 20, 145–159.
70. Wu, K.J. There Are More Viruses than Stars in the Universe. Why Do Only Some Infect Us? National Geographic Magazine, 15 April 2020. Available online: https://www.nationalgeographic.com/science/article/factors-allow-viruses-infect-humans-coronavirus(accessed on 7 December 2022).
71. Dance, A. Beyond coronavirus: The virus discoveries transforming biology. Nature 2021, 595, 22–25.
72. Popovic, M. Biothermodynamic Key Opens the Door of Life Sciences: Bridging the Gap between Biology and Thermodynamics. Preprints 2022, 2022100326.
73. Popovic, M. Living organisms from Prigogine's perspective: An opportunity to introduce students to biological entropy balance. J. Biol. Educ. 2017, 52, 294–300.
74. Popovic, M.E. Research in entropy wonterland: A review of the entropy concept. Therm. Sci. 2018, 22, 1163–1178.
75. Popovic, M. Researchers in an Entropy Wonderland: A Review of the Entropy Concept. Therm. Sci. 2018, 22, 1163–1178.
76. Wimmer, E. The test-tube synthesis of a chemical called poliovirus. EMBO Rep. 2006, 7, S3–S9.
77. Molla, A.; Paul, A.V.; Wimmer, E. Cell-Free, De Novo Synthesis of Poliovirus. Science 1991, 254, 1647–1651.
78. Popovic, M. Atom counting method for determining elemental composition of viruses and its applications in biothermodynamics and environmental science. Comput. Biol. Chem. 2022, 96, 107621.
79. Popovic, M.; Minceva, M. A thermodynamic insight into viral infections: Do viruses in a lytic cycle hijack cell metabolism due to their low Gibbs energy? Heliyon 2020, 6, e03933.
80. Popovic, M.; Minceva, M. Thermodynamic insight into viral infections 2: Empirical formulas, molecular compositions and thermodynamic properties of SARS, MERS and SARS-CoV-2 (COVID-19) viruses. Heliyon 2020, 6, e04943.
81. Popovic, M.; Minceva, M. Coinfection and Interference Phenomena Are the Results of Multiple Thermodynamic Competitive Interactions. Microorganisms 2021, 9, 2060.
82. Duffy, S. Why are RNA virus mutation rates so damn high? PLoS Biol. 2018, 16, e3000003.
83. Callaway, E. The coronavirus is mutating—Does it matter? Nature 2020, 585, 174–177.
84. Barton, M.I.; MacGowan, S.A.; Kutuzov, M.A.; Dushek, O.; Barton, G.J.; van der Merwe, P.A. Effects of common mutations in the SARS-CoV-2 Spike RBD and its ligand, the human ACE2 receptor on binding affinity and kinetics. eLife 2021, 10, e70658.
85. Wang, R.; Chen, J.; Gao, K.; Hozumi, Y.; Yin, C.; Wei, G.-W. Analysis of SARS-CoV-2 mutations in the United States suggests presence of four substrains and novel variants. Commun. Biol. 2021, 4, 228.
86. Popovic, M.; Popovic, M. Strain Wars: Competitive interactions between SARS-CoV-2 strains are explained by Gibbs energy of antigen-receptor binding. Microb. Risk Anal. 2022, 21, 100202.
87. Popovic, M. Strain wars 2: Binding constants, enthalpies, entropies, Gibbs energies and rates of binding of SARS-CoV-2 variants. Virology 2022, 570, 35–44.
88. Popovic, M. Strain wars 3: Differences in infectivity and pathogenicity between Delta and Omicron strains of SARS-CoV-2 can be explained by thermodynamic and kinetic parameters of binding and growth. Microb. Risk Anal. 2022, 22, 100217.
89. Popovic, M. Strain wars 4—Darwinian evolution through Gibbs' glasses: Gibbs energies of binding and growth explain evolution of SARS-CoV-2 from Hu-1 to BA.2. Virology 2022, 575, 36–42.
90. Popovic, M. Strain wars 5: Gibbs energies of binding of BA.1 through BA.4 variants of SARS-CoV-2. Microb. Risk Anal. 2022, 22, 100231.

91. Popovic, M. Omicron BA.2.75 Subvariant of SARS-CoV-2 Is Expected to Have the Greatest Infectivity Compared with the Competing BA.2 and BA.5, Due to Most Negative Gibbs Energy of Binding. BioTech 2022, 11, 45.
92. Şimşek, B.; Özilgen, M.; Utku, F. How much energy is stored in SARS-CoV-2 and its structural elements? Energy Storage 2021, 4, e298.
93. Degueldre, C. Single virus inductively coupled plasma mass spectroscopy analysis: A comprehensive study. Talanta 2021, 228, 122211.
94. Popovic, M. Formulas for death and life: Chemical composition and biothermodynamic properties of Monkeypox (MPV, MPXV, HMPXV) and Vaccinia (VACV) viruses. Therm. Sci. 2022, 26, 4855–4868.
95. Popovic, M. Why doesn't Ebola virus cause pandemics like SARS-CoV-2? Microb. Risk Anal. 2022, 22, 100236.
96. Du, X.; Li, Y.; Xia, Y.-L.; Ai, S.-M.; Liang, J.; Sang, P.; Ji, X.-L.; Liu, S.-Q. Insights into Protein–Ligand Interactions: Mechanisms, Models, and Methods. Int. J. Mol. Sci. 2016, 17, 144.
97. Popovic, M. Standard Gibbs Energy of Binding of the gp120 Antigen of HIV-1 to the CD4 Receptor. Preprints 2022, 2022110482.
98. Gale, P. Using thermodynamic equilibrium models to predict the effect of antiviral agents on infectivity: Theoretical application to SARS-CoV-2 and other viruses. Microb. Risk Anal. 2021, 21, 100198.
99. Gale, P. How virus size and attachment parameters affect the temperature sensitivity of virus binding to host cells: Predictions of a thermodynamic model for arboviruses and HIV. Microb. Risk Anal. 2020, 15, 100104.
100. Gale, P. Towards a thermodynamic mechanistic model for the effect of temperature on arthropod vector competence for transmission of arboviruses. Microb. Risk Anal. 2019, 12, 27–43.
101. Gale, P. Using thermodynamic parameters to calibrate a mechanistic dose-response for infection of a host by a virus. Microb. Risk Anal. 2018, 8, 1–13.
102. Lucia, U.; Grisolia, G.; Deisboeck, T.S. Seebeck-like effect in SARS-CoV-2 Bio-thermodynamics. Atti Accad. Peloritana Pericolanti 2020, 98, 6.
103. Lucia, U.; Deisboeck, T.S.; Grisolia, G. Entropy-Based Pandemics Forecasting. Front. Phys. 2020, 8, 274.
104. Casasnovas, J.M.; Springer, T.A. Kinetics and Thermodynamics of Virus Binding to Receptor. J. Biol. Chem. 1995, 270, 13216–13224.
105. Popovic, M.E.; Minceva, M. Thermodynamic properties of human tissues. Therm. Sci. 2020, 24 Pt B, 4115–4133.
106. Popovic, M.; Minceva, M. Standard Thermodynamic Properties, Biosynthesis Rates, and the Driving Force of Growth of Five Agricultural Plants. Front. Plant Sci. 2021, 12, 671868.
107. Popovic, M. Thermodynamic properties of microorganisms: Determination and analysis of enthalpy, entropy, and Gibbs free energy of biomass, cells and colonies of 32 microorganism species. Heliyon 2019, 5, e01950.
108. Popovic, M.; Stenning, G.B.; Göttlein, A.; Minceva, M. Elemental composition, heat capacity from 2 to 300 K and derived thermodynamic functions of 5 microorganism species. J. Biotechnol. 2021, 331, 99–107.
109. Duboc, P.; Marison, I.; Von Stockar, U. Quantitative calorimetry and biochemical engineering. In Handbook of Thermal Analysis and Calorimetry, vol. 4: From Macromolecules to Man; Kemp, E.B., Ed.; Elsevier: Amsterdam, NX, USA, 1999; pp. 267–365.
110. Wang, H.Y.; Mou, D.-G.; Swartz, J.R. Thermodynamic evaluation of microbial growth. Biotechnol. Bioeng. 1976, 18, 1811–1814.
111. Hou, W.; Armstrong, N.; Obwolo, L.A.; Thomas, M.; Pang, X.; Jones, K.S.; Tang, Q. Determination of the Cell Permissiveness Spectrum, Mode of RNA Replication, and RNA-Protein Interaction of Zika Virus. BMC Infect. Dis. 2017, 17, 239.
112. Popovic, M. Omicron BA.2.75 Sublineage (Centaurus) Follows the Expectations of the Evolution Theory: Less Negative Gibbs Energy of Biosynthesis Indicates Decreased Pathogenicity. Microbiol. Res. 2022, 13, 937–952.
113. Buzón, P.; Maity, S.; Roos, W.H. Physical virology: From virus self-assembly to particle mechanics. WIREs Nanomed. Nanobiotechnology 2020, 12, e1613.
114. Garmann, R.F.; Goldfain, A.M.; Manoharan, V.N. Measurements of the self-assembly kinetics of individual viral capsids around their RNA genome. Proc. Natl. Acad. Sci. USA 2019, 116, 22485–22490.
115. Popovic, M. Comparative study of entropy and information change in closed and open thermodynamic systems. Thermochim. Acta 2014, 598, 77–81.

116. Popovic, M. Entropy change of open thermodynamic systems in self-organizing processes. Therm. Sci. 2014, 18, 1425–1432.
117. Popovic, M.E.; Minceva, M. Comment on: “A critical review on heat and mass transfer modelling of viral infection and virion evolution: The case of SARS-COV2”. Therm. Sci. 2021, 25, 4823–4825.