 doi:10.20944/preprints202210.0386.v1

*Article*

# Omicron BA.2.75 Sublineage Follows the Expectations of the Evolution Theory: Less Negative Gibbs Energy of Biosynthesis Indicates Decreased Pathogenicity

**Marko Popovic***

School of Life Sciences, Technical University of Munich, Freising, Germany
* Correspondence: marko.popovic@tum.de ; marko.popovic.td@gmail.com

**Abstract:** SARS-CoV-2 belongs to the group of RNA viruses with a pronounced tendency to mutate. Omicron BA.2.75 is a subvariant believed to be able to suppress the currently dominant BA.5. Omicron BA.2.75 is characterized by a greater infectivity compared to earlier Omicron variants. However, Gibbs energy of biosynthesis of virus particles is slightly less negative compared to those of other variants. Thus, the multiplication rate of Omicron BA.2.75 is lower than that of other variants. This leads to slower accumulation of newly formed virions and less damage to host cells, indicating evolution of SARS-CoV-2 towards decreasing pathogenicity.

**Keywords:** COVID-19; SARS-CoV-2; Pandemic; Empirical formula; Growth stoichiometry; Thermodynamic properties of biosynthesis; Multiplication rate; Enthalpy; Entropy; Gibbs energy.

## 1. Introduction

Multicellular organisms represent hosts for many viruses [Riedel et al., 2019]. They interact. Empirical formulas and thermodynamic properties are available in the literature for human host tissues [Popovic and Minceva, 2020c], plant host organisms [Popovic and Minceva, 2021b] and over 30 viruses [Popovic and Popovic, 2022; Popovic, 2022a, 2022b, 2022d, 2022e, 2022f; Popovic and Minceva, 2021a; Şimşek et al., 2021]. These data are necessary for research on biothermodynamic background of virus-host interactions.

All animate matter represents open thermodynamic systems performing growth [von Bertalanffy, 1950; Balmer, 2010; Şimşek et al., 2021; Ozilgen and Sorgüven, 2017; Lucia et al., 2021, 2020a; Lucia, 2015; von Stockar, 2013a, 2013b; Lucia & Grisolia, 2020; Schrödinger, 1944; Morowitz, 1992, 1968; Popovic, 2018]. During growth and biosynthesis, the state of animate matter system changes. Consequently, thermodynamic properties of the organism change during biosynthesis [Popovic, 2019, 2017, 2014a, 2014b]. During evolution of viruses, mutations occur that change not only thermodynamic properties due to change in elemental composition, but also information content [Hansen et al., 2018]. In that way, the virus evolves.

Infection represents a biological interaction of a multicellular host and microorganisms [Riedel et al., 2019]. However, interactions of viruses with their hosts represent also a thermodynamic process and have been studied extensively using the approaches of biothermodynamics and bioenergetics [Gale, 2021, 2020, 2019, 2018; Popovic and Minceva, 2021a; Katen and Zlotnick, 2009; Ceres and Zlotnick, 2002; Casasnovas and Springer, 1995; Mahmoudabadi et al., 2017; Tzlil et al., 2004; Kaniadakis et al., 2020; Lucia et al., 2020b; Guosheng et al., 2003; Maskow et al., 2010b; Head et al., 2022]. Thus, biothermodynamics is used in analysis of interactions of organisms with their environment and with other organisms [von Stockar et all., 2013, 2006; Maskow et al., 2013, 2010a, 2005]. However, virus-host interactions have still not been explored for many viruses, first of all due to lack of data on elemental composition and thermodynamic properties. This is a consequence of a lack of adequate biosafety levels in most biothermodynamics and chemical analysis

 doi:10.20944/preprints202210.0386.v1



laboratories, as well as difficulties with producing virus samples of adequate purity and in sufficient amount [Popovic, 2022c]. Thus, the atom counting method was developed for calculating elemental composition of various viruses [Popovic, 2022c]. The results of the atom counting method are in agreement with experimentally determined virus empirical formulas [Wimmer, 2006; Molla et al., 1991; Popovic, 2022c].

Based on known empirical formulas of viruses and biosynthesis reactions, it is possible to find standard thermodynamic properties through the Battley, Roels and Sandler-Orbey methods. In the Battley method, standard enthalpy of live matter is found through the Patel-Erickson equation [Patel and Erickson, 1981; Battley, 1998], while entropy is found using the Battley equation [Battley, 1999]. Enthalpy and entropy are then combined to find Gibbs energy. On the other hand, the Roels method uses the Roels equation to find Gibbs energy [Roels, 1982; von Stockar and Liu, 1999] and Patel-Erickson [Patel and Erickson, 1981; Battley, 1998] equation to find enthalpy. Combining the Gibbs energy and enthalpy gives entropy. In the Sandler-Orbey method, enthalpy and Gibbs energy are calculated using equations proposed by Sandler and Orbey [Sandler and Orbey, 1991; Sandler, 2017]. These are combined to find entropy.

Virus-host interaction represents a chemical reaction [Du et al., 2016; Popovic, 2022c]. Antigen-receptor interaction is similar to protein-ligand interactions [Du et al., 2016; Popovic, 2022g]. The reactions of replication and translation represent processes of polymerization of nucleotides and amino acids, respectively, catalyzed by enzymes [Dodd et al., 2020; Johansson and Dixon, 2013; Lee et al., 2020]. The driving force for chemical reactions is Gibbs energy [von Stockar, 2013a, 2013b; von Stockar and Liu, 1999; Demirel, 2014]. Thus, it is necessary to know Gibbs energy of biosynthesis. Biosynthesis forms virus building blocks that undergo self-assembly, forming new virions [Buzón et al., 2020; Garmann et al., 2019]. Virions accumulate inside the cell and lead to its damage [Schmid et al., 2014]. Growth of viruses is reflected in the increase in the size of virus population [Popovic, 2022f]. Gibbs energy of biosynthesis, $\Delta_{bs}G$, is proportional to biosynthesis rate, $r_{bs}$, and thereby multiplication rate according to the phenomenological equation

$$r_{bs} = -\frac{L_{bs}}{T}\Delta_{bs}G \tag{1}$$

where $L_{bs}$ is phenomenological coefficient for biosynthesis and $T$ is temperature [Popovic, 2022b, 2022d]. Phenomenological equations belong to the domain of nonequilibrium thermodynamics, which was shown to be an excellent approach for analysis of life processes by Prigogine and coworkers [Prigogine, 1977, 1947; Prigogine and Wiame, 1946; Glansdorff and Prigogine, 1971].

SARS-CoV-2 belongs to RNA viruses, which has entered the human population for the first time in December 2019 in Wuhan [Kumar et al., 2021]. Human to human transmission was reported in January 2020 [Acuti Martellucci, 2020]. Shortly after in March 2020, WHO has declared the COVID-19 a pandemic [Cucinotta and Vanelli, 2020]. Until today, 623 893 894 COVID-19 cases have been reported, 6 553 936 deaths were confirmed and 12 782 955 639 vaccine doses have been administered [WHO, 2022a]. Despite the large number of administered vaccines, the pandemic has not been suppressed, but only its intensity has been decreased [Worldometer, 2022a]. In Germany, in mid-October 2022, over 172 thousand new cases have been recorded daily [Worldometer, 2022b].

RNA viruses exhibit a high tendency to mutate [Duffy, 2018]. In 2019, the wild type SARS-CoV-2 has been identified, later labeled Hu-1 variant. The Hu-1 variant has mutated several dozen times, which led to appearance of new variants: Alpha, Beta, Gamma, Delta… and Omicron, with several subvariants [Wang et al., 2021; Barton et al., 2021; Callaway, 2020]. The new subvariant BA.2.75 has appeared in India and spread to over 15 countries throughout the world, but has not yet become the dominant variant [Sheward et al., 2022]. It seems that the sublineage BA.2.75 has the ability to evade the immune answer [Sheward et al., 2022; Takashita et al., 2022]. Genetic sequence data for the Omicron BA.2.75 variant is available at GISAID, the global data science initiative [Khare et al., 2021;

 doi:10.20944/preprints202210.0386.v1



Elbe and Buckland-Merrett, 2017; Shu & McCauley, 2017]. To determine the biological potential of the BA.2.75 subvariant for spreading through the population and pathogenicity, it is necessary to estimate the susceptibility and permissiveness for this variant. In the literature, it has been reported that susceptibility for BA.2.75 is greater, since the antigen-receptor binding reaction is characterized by a lower Gibbs energy of binding [Popovic, 2022g]. The permissiveness that influences the virus multiplication rate is the subject of analysis in this paper.

The Omicron BA.2.75 subvariant is characterized by a more negative Gibbs energy of binding than the competing BA.2 and BA.5 subvariants [Popovic, 2022g]. This leads to faster virus entry into host cells and more rapid spreading through the population, which is in accordance with the observations made in India [Callaway, 2022; Vogel, 2022].

The aim of this paper is to find empirical formulas, molar masses, as well as thermodynamic properties of live matter and biosynthesis for the Omicron BA.2.75 subvariant. Based on this data, a biothermodynamic and bioenergetic analysis of evolution of SARS-CoV-2 will be made from Hu-1, through Delta, to Omicron BA.2.75 subvariant.

## 2. Methods

The methods section begins with discussing sources from which starting data for this research were obtained. Next, the atom counting method is discussed, which was used for finding elemental compositions and molar masses of virus nucleocapsids. Then predictive thermodynamic models are presented, which were used to find standard thermodynamic properties of nucleocapsid live matter. Finally, biosynthesis reactions are introduced and equations used to find standard thermodynamic properties of biosynthesis of virus nucleocapsids.

### *2.1. Data sources*

Genetic sequence data for the Omicron BA.2.75 variant has been taken from GISAID, the global data science initiative [Khare et al., 2021; Elbe and Buckland-Merrett, 2017; Shu & McCauley, 2017]. The genetic sequence of the Omicron BA.2.75 isolate from Germany can be found under the accession number EPI_ISL_13378924. It is labeled hCoV-19/Germany/BW-RKI-I-863813/2022 and has been isolated in the state of Baden-Wurttemberg on June 3, 2022. The genetic sequence of the Omicron BA.2.75 isolate from India can be found under the accession number EPI_ISL_13804325. It is labeled hCoV-19/India/TN-CDFD-E130377/2022 and has been isolated in the city of Vellore, state of Tamil Nadu, on January 7, 2022. The sequence was submitted by CDFD-INSACOG on July 13, 2022. The genetic sequence of the Omicron BA.2.75 isolate from USA can be found under the accession number EPI_ISL_15421780. It is labeled hCoV-19/USA/OR-UW-22091225964/2022 and was isolated in the state of Oregon on September 12, 2022. Thus, the findings of this study are based on metadata associated with 3 sequences available on GISAID up to October 22, 2022, and accessible at https://doi.org/10.55876/gis8.221022gh (GISAID Identifier: EPI_SET_221022gh). More information about the genetic sequence data can be found in the Supplementary Material.

Each of the analyzed BA.2.75 sequences contained a small unknown part. For the sequence originating from Germany, the unknown part occupied the positions from 21985 to 22160. For the sequence originating from India, the unknown part occupied the positions from 26932 to 27153. For the sequence originating from USA, the unknown part occupied the positions from 26747 to 26958. The unknown sequences were filled using complementary sequences of the Delta variant of SARS-CoV-2. The genetic sequence of the Delta variant was taken from the NCBI database [National Center for Biotechnology Information, 2022], under the accession number OM471068.1. The genetic sequence of the Delta variant was aligned with each of the analyzed sequences of the Omicron BA.2.75 variant. The alignment was done using the Needleman-Wunsch algorithm [Needleman and Wunsch, 1970]. For all three Omicron BA.2.75 sequences, the alignment in the area

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 25 October 2022 doi:10.20944/preprints202210.0386.v1



surrounding the unknown parts was good. Then, the unknown parts of the Omicron BA.2.75 sequences were filled with the analogous parts of the Delta variant sequence, as described in [Popovic, 2022d].

The nucleocapsid phosphoprotein sequence was taken from the NCBI database [National Center for Biotechnology Information, 2022]. The accession number of the nucleocapsid phosphoprotein is UKQ14424.1. The number of copies of the nucleocapsid phosphoprotein in virus particle was taken from [Neuman and Buchmeier, 2016; Neuman et al., 2011; Neuman et al., 2006].

### *2.2. Elemental composition*

The virus genetic and protein sequences were used to find the elemental composition of the virus nucleocapsid, using the atom counting method [Popovic, 2022c]. The atom counting method was used to find nucleocapsid empirical formula, molar mass of the nucleocapsid empirical formula and molar mass of the entire nucleocapsid. The atom counting method has been described in [Popovic, 2022]. It calculates elemental composition of virus particles using widely available data on genetic sequences, protein sequences, protein copy numbers and virus size. The atom counting method is implemented using a computer program, which runs along nucleic acids and protein sequences and adds atoms coming from every residue. The contributions of proteins are multiplied by their copy numbers in the virus particle. Finally, the contributions of the nucleic acid and the proteins are summed to find the elemental composition of the virus particle. The atom counting method was found to give results in good agreement with experimentally determined elemental composition of viruses [Wimmer, 2006; Molla et al., 1991; Popovic, 2022c].

### *2.3. Thermodynamic properties of live matter*

Nucleocapsid elemental compositions of the analyzed Omicron BA.2.75 isolates were used to find standard thermodynamic properties of their nucleocapsids. This was done using predictive biothermodynamic models, including the Patel-Erickson equation [Patel and Erickson, 1981; Battley, 1998] and Battley equation [Battley, 1999].

Standard enthalpy of formation was calculated in two steps. First the Patel-Ericson equation [Patel and Erickson, 1981; Battley, 1998] was used to find standard enthalpy of combustion, which was then converted into standard enthalpy of formation using the Hess's law [Atkins and de Paula, 2011, 2014]. The Patel-Erickson equation gives standard enthalpy of combustion of live matter, $\Delta_C H^0(bio)$, from the number of electrons transferred to oxygen during combustion, $E$ [Patel and Erickson, 1981; Battley, 1998].

$$\Delta_C H^0(bio) = -111.14 \frac{kJ}{C-mol} \cdot E \tag{1}$$

$E$ is calculated from the elemental composition, using the equation

$$E = 4n_C + n_H - 2n_O - 0n_N + 5n_P + 6n_S \tag{2}$$

where $n_J$ is the number of atoms of element $J$ in the empirical formula of live matter [Patel and Erickson, 1981; Battley, 1998]. $\Delta_C H^0(bio)$ can be converted into standard enthalpy of formation of live matter, $\Delta_f H^0(bio)$, using the equation [Popovic, 2022b]

$$\begin{aligned}\Delta_f H^0(bio) = n_C\, \Delta_f H^0(CO_2) + \frac{n_H}{2}\, \Delta_f H^0(H_2O) + \frac{n_P}{4}\, \Delta_f H^0(P_4O_{10}) \\ + n_S\, \Delta_f H^0(SO_3) - \Delta_C H^0(bio)\end{aligned} \tag{3}$$

Like for $\Delta_f H^0(bio)$, entropy of live matter can also be calculate using predictive biothermodynamic models. Elemental composition can also be used to find its standard molar entropy of live matter, $S^0_m(bio)$, using the Battley equation

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 25 October 2022 doi:10.20944/preprints202210.0386.v1



$$S_m^0(bio) = 0.187 \sum_J \frac{S_m^0(J)}{a_J} n_J \quad (4)$$

Where $S^0{}_m(J)$ and $a_J$ are the standard molar entropy and number of atoms of element $J$ per formula unit in its standard state elemental form [Battley, 1999]. For example, the standard state elemental form of carbon is graphite represented by C, meaning that $S^0{}_m(C)$ = 5.740 J/mol K and $a_C$ = 1 [Atkins and de Paula, 2011, 2014]. On the other hand, the standard state of hydrogen is gaseous $H_2$, meaning that $S^0{}_m(H_2)$ = 130.684 and $a_H$ = 2 [Atkins and de Paula, 2011, 2014]. The Battley equation can be rearranged to give standard entropy of formation of live matter, $\Delta_f S^0(bio)$, by replacing the constant in from of the sum term with -0.813 [Battley, 1999].

$$\Delta_f S^0(bio) = -0.813 \sum_J \frac{S_m^0(J)}{a_J} n_J \quad (5)$$

The changed constant from +0.187 to -0.817 comes from using a different reference state for measuring entropy [Battley, 1999]. The calculated $\Delta_f S^0(bio)$ and $\Delta_f H^0(bio)$ values are combined to find standard Gibbs energy of formation of live matter, $\Delta_f G^0(bio)$.

$$\Delta_f G^0(bio) = \Delta_f H^0(bio) - T\Delta_f S^0(bio) \quad (6)$$

Where $T$ is temperature [Atkins and de Paula, 2011, 2014].

*2.4. Biosynthesis reactions and thermodynamic properties of biosynthesis*

Elemental composition of live matter can be used to construct macrochemical reactions describing production of live matter from nutrients, known as biosynthesis reactions. Stoichiometric coefficients in biosynthesis reactions depend on live matter elemental composition, since the more of an element is present in live matter, the more nutrient that contains it will be needed for biosynthesis. Biosynthesis reactions of viruses can be described by the general reaction

$$AA + O_2 + HPO_4^{2-} + HCO_3^- \rightarrow (Bio) + SO_4^{2-} + H_2O + H_2CO_3 \quad (7)$$

Where *AA* denotes a mixture of amino acids and (*bio*) newly produced nucleocapsid live matter [Popovic, 2022b]. Standard thermodynamic properties of biosynthesis are found by applying the Hess's law to biosynthesis reactions, through the equations

$$\Delta_{bs}H^0 = \sum_{products} \nu\, \Delta_f H^0 - \sum_{reactants} \nu\, \Delta_f H^0 \quad (8)$$

$$\Delta_{bs}S^0 = \sum_{products} \nu\, S_m^o - \sum_{reactants} \nu\, S_m^o \quad (9)$$

$$\Delta_{bs}G^0 = \sum_{products} \nu\, \Delta_f G^0 - \sum_{reactants} \nu\, \Delta_f G^0 \quad (10)$$

where $\Delta_{bs}H^0$, $\Delta_{bs}S^0$ and $\Delta_{bs}G^0$ represent standard enthalpy, entropy and Gibbs energy of biosynthesis, respectively [Popovic, 2022b; Atkins and de Paula, 2011, 2014].

## 3. Results

The empirical formulas of the Omicron BA.2.75 nucleocapsids have been determined for the first time. They are presented in Table 1. For the Omicron BA.2.75 isolate from Germany the nucleocapsid empirical formula was found to be $CH_{1.5736}O_{0.3426}N_{0.3124}P_{0.00601}S_{0.00336}$. The empirical formula of the Omicron BA.2.75 nucleocapsid for the isolate from India is $CH_{1.5735}O_{0.3427}N_{0.3124}P_{0.00603}S_{0.00336}$. The empirical formula of the Omicron BA.2.75 nucleocapsid for the isolate from USA is

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 25 October 2022 doi:10.20944/preprints202210.0386.v1



$CH_{1.5737}O_{0.3425}N_{0.3123}P_{0.00598}S_{0.00336}$. Moreover, Table 1 gives molar mass (molar weight) data for the nucleocapsids for the three isolates, reported in two forms: for unit carbon formulas and for entire nucleocapsids. Molar masses of empirical formulas are 23.75 g/C-mol for the isolates from Germany and India, and 23.74 g/C-mol for the isolate from USA. The molar mass of entire nucleocapsid for the Omicron BA.2.75 isolates from Germany and USA are 117.2 MDa, while that of the isolate from India is 117.1 MDa.

**Table 1: Empirical formulas and molar masses of SARS-CoV-2 Omicron BA.2**.75 nucleocapsids. Empirical formulas have the general form $C_{nC}H_{nH}O_{nO}N_{nN}P_{nP}S_{nS}$. Molar masses are reported in two forms. The first form is the molar mass of the empirical formula of the virus particle, *Mr*, with the units in g/C-mol (Da). The second is molar mass of the entire nucleocapsid, *Mr*(*nc*), expressed in MDa. .

| Origin | $n_C$ | $n_H$ | $n_O$ | $n_N$ | $n_P$ | $n_S$ | Mr (g/C-mol) | Mr(nc) (MDa) |
|---|---|---|---|---|---|---|---|---|
| Germany | 1 | 1.5736 | 0.3426 | 0.3124 | 0.00601 | 0.00336 | 23.75 | 117.2 |
| India | 1 | 1.5735 | 0.3427 | 0.3124 | 0.00603 | 0.00336 | 23.75 | 117.2 |
| USA | 1 | 1.5737 | 0.3425 | 0.3123 | 0.00598 | 0.00336 | 23.74 | 117.1 |

Table 2 shows stoichiometry of biosynthesis for BA.2.75 subvariant, for all three isolates. Table 3 gives standard thermodynamic properties of formation for the nucleocapsids of Omicron BA.2.75 subvariant. Table 4 presents data on standard thermodynamic properties of biosynthesis for the nucleocapsids of Omicron BA.2.75 subvariant, for all three isolates. Thermodynamic properties of biosynthesis refer to production of live matter from nutrients. Gibbs energies of biosynthesis for the nucleocapsids of the isolates from Germany, India and USA are -221.18 kJ/C-mol, -221.24 kJ/C-mol and -221.12 kJ/C-mol, respectively.

**Table 2: Stoichiometric coefficients for the biosynthesis reactions of the nucleocapsids of the Omicron BA.2**.75 subvariant. (Bio) denotes the empirical formula of the nucleocapsid live matter (from Table 1).

| Name | Reactants | | | | | Products | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Amino acid | $O_2$ | $HPO_4^{2-}$ | $HCO_3^-$ | → | Bio | $SO_4^{2-}$ | $H_2O$ | $H_2CO_3$ |
| Germany | 1.3900 | 0.4911 | 0.0060 | 0.0437 | → | 1 | 0.0279 | 0.0538 | 0.4337 |
| India | 1.3901 | 0.4913 | 0.0060 | 0.0437 | → | 1 | 0.0279 | 0.0538 | 0.4338 |
| USA | 1.3899 | 0.4910 | 0.0060 | 0.0438 | → | 1 | 0.0279 | 0.0537 | 0.4337 |

**Table 3: Standard thermodynamic properties of nucleocapsid live matter of the Omicron BA.2**.75 subvariant. The table contains data on standard enthalpy of formation, $\Delta_f H^0$, standard molar entropy, $S^0_m$, and standard Gibbs energy of formation, $\Delta_f G^0$. .

| Name | $\Delta_f H^o$ (kJ/C-mol) | $S^o_m$ (J/C-mol K) | $\Delta_f G^o$ (kJ/C-mol) |
|---|---|---|---|
| Germany | -75.37 | 32.49 | -33.26 |
| India | -75.40 | 32.49 | -33.28 |
| USA | -75.35 | 32.49 | -33.23 |

**Table 4: Standard thermodynamic properties of biosynthesis of the Omicron BA.2**.75 subvariant. The table gives data on standard enthalpy of biosynthesis, $\Delta_{bs}H^0$, standard entropy of biosynthesis, $\Delta_{bs}S^0$, and standard Gibbs energy of biosynthesis, $\Delta_{bs}G^0$.

| Name | $\Delta_{bs}H^o$ (kJ/C-mol) | $\Delta_{bs}S^o$ (J/C-mol K) | $\Delta_{bs}G^o$ (kJ/C-mol) |
|---|---|---|---|
| Germany | -232.26 | -37.33 | -221.18 |
| India | -232.34 | -37.34 | -221.24 |
| USA | -232.20 | -37.31 | -221.12 |

## 4. Discussion

In 2019, the year when COVID-19 appeared and SARS-CoV-2 was identified as the cause of the disease, the entire empirical formula of only one virus was known – the poliovirus [Wimmer, 2006; Molla et al., 1991], as well as partial formulas of some bacteriophages [Jover, 2014]. Thermodynamic properties of human viruses were not available in the literature. Enthalpy change during the multiplication of the T4 phage in *E. coli* cells

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 25 October 2022 doi:10.20944/preprints202210.0386.v1



was measured by Guosheng et al. [2003] and the transition from lysogenic into lytic cycle of the Lambda phage was studied by Maskow et al. [2010b] using calorimetry. This situation has made a need to determine the empirical formula and thermodynamic properties for SARS-CoV-2 and other viruses. The obstacle to determining the empirical formula of viruses is the fact that viruses are hard to obtain in sufficiently pure state and in amount required for analysis, as well as the fact that most thermodynamic laboratories do not possess an adequate biosafety level. Determining the empirical formula has become possible after development of the atom counting method [Popovic, 2022c]. Through application of this method, elemental compositions of the poliovirus and some other viruses were determined [Popovic and Minceva, 2020a] and compared with the values obtained experimentally [Wimmer, 2006; Molla et al., 1991]. It was found that the calculated results obtained using the atom counting method are in good agreement with the experimentally determined values [Popovic, 2022c]. The empirical formula of SARS-CoV-2 wild type (Hu-1) has been reported in [Popovic and Minceva, 2020b]. Degueldre [2021] has suggested a modified empirical formula of SARS-CoV-2 and an experimental method for accurate measurement of virus elemental composition using mass spectrometry. Şimşek et al. [2021] have computationally found the formula of the Hu-1 variant of SARS-CoV-2.

The empirical formula of Hu-1 variant of SARS-CoV-2 nucleocapsid [Popovic and Minceva, 2020b] is $CH_{1.5708}O_{0.3452}N_{0.3125}P_{0.0060}S_{0.0033}$. SARS-CoV-2 has during the last 3 years mutated multiple times. As a consequence of mutations, there were changes in elemental composition and thermodynamic properties. One of the goals of this paper is to find the empirical formula of the BA.2.75 subvariant. Using the atom counting method, the empirical formula of Omicron BA.2.75 was calculated and are reported in Table 1. The empirical formula of the Omicron BA.2.75 nucleocapsid isolated in Germany is $CH_{1.5736}O_{0.3426}N_{0.3124}P_{0.00601}S_{0.00336}$. The empirical formula of the Omicron BA.2.75 nucleocapsid isolated in India is $CH_{1.5735}O_{0.3427}N_{0.3124}P_{0.00603}S_{0.00336}$. The empirical formula of the Omicron BA.2.75 nucleocapsid isolated in USA is $CH_{1.5737}O_{0.3425}N_{0.3123}P_{0.00598}S_{0.00336}$. As expected, since all three formulas are for the same BA.2.75 subvariant, the empirical formulas are almost identical for all three samples taken from different continents. Also, molar masses were determined, which are given in Table 1. The molar masses have been reported on two bases. First as the molar mass of empirical formula of the nucleocapsid in daltons (g/C-mol), *Mr*, while the second is the molar mass of the entire nucleocapsid in megadaltons, *Mr*(*nc*). Dividing *Mr*(*nc*) with *Mr* gives the number of empirical formulas (C-atoms) in the entire nucleocapsid. The molar masses are as expected almost identical for the three BA.2.75 isolates. The molar mass of the entire nucleocapsid for the Omicron BA.2.75 isolates from Germany and USA are 117.2 MDa, while that of the isolate from India is 117.1 MDa. However, if we compare the empirical formula of BA.2.75 with that of Hu-1, we can notice significant differences. The differences are the greatest in H and O content.

Table 2 gives stoichiometric coefficients for the biosynthesis reactions of the nucleocapsids of BA.2.75 subvariant. For the Hu-1 wild type, the biosynthesis reaction is

$$1.3905\ AA + 0.4937\ O_2 + 0.006\ HPO_4^{2-} + 0.0437\ HCO_3^- \rightarrow (Bio) + 0.0279\ SO_4^{2-} + 0.0551\ H_2O + 0.4342\ H_2CO_3 \quad (X)$$

Where AA denotes amino acids and (bio) denotes the empirical formula of the Hu-1 variant ($CH_{1.5708}O_{0.3452}N_{0.3125}P_{0.0060}S_{0.0033}$) [Popovic and Minceva, 2020b]. The stoichiometric coefficients are very similar to those of all three samples of the BA.2.75 subvariant (Table 2).

Table 3 gives standard thermodynamic properties of nucleocapsids of the 3 isolates of Omicron BA.2.75 subvariant. Standard enthalpies and Gibbs energies of formation of the 3 isolates are only slightly different. Standard entropies of formation are identical for all three samples. Thus, standard thermodynamic properties of formation are very similar for all 3 samples, since they belong to the same Omicron BA.2.75 subvariant.

Table 4 shows standard thermodynamic properties of biosynthesis of nucleocapsids of Omicron BA.2.75 subvariant samples from Germany, India and USA. Standard thermodynamic properties of biosynthesis refer to production of nucleocapsid live matter

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 25 October 2022 doi:10.20944/preprints202210.0386.v1



from nutrients [Popovic, 2022b]. Their values are very similar for all three BA.2.75 samples. However, for the Hu-1 variant $\Delta_{bs}H^0$ = -233.4 kJ/C-mol, $\Delta_{bs}S^0$ = -37.7 kJ/C-mol and $\Delta_{bs}G^0$ = -222.2 kJ/C-mol. It can immediately be noticed that Gibbs energy of biosynthesis of the Omicron BA.2.75 subvariant (all three samples) is less negative than that of the Hu-1 variant. According to the evolution theory, it is expected that the SARS-CoV-2 virus evolves towards an increased infectivity and decreased or constant pathogenicity. Indeed, Gibbs energy of antigen-receptor binding, of the Hu-1 variant is -43.4 kJ/mol [Popovic, 2022f], while that of the Omicron BA.2.75 variant is -49.41 kJ/mol [Popovic, 2022g]. Gibbs energy of antigen-receptor binding is proportional to binding rate, according to the binding phenomenological equation [Popovic, 2022g]. This leads to the conclusion that the binding rate and thereby infectivity have increased during evolution from Hu-1 to BA.2.75. However, the expected decrease in pathogenicity would have to be caused by decreased rates of multiplication and biosynthesis of virus structural elements. The rates of multiplication and biosynthesis are proportional to Gibbs energy of biosynthesis. In that case, Gibbs energy of biosynthesis would have to be the same or less negative for BA.2.75 compared to Hu-1. Indeed, Gibbs energy of biosynthesis of the nucleocapsid for BA.2.75 is -221.2 kJ/C-mol, while that of Hu-1 is -222.2 kJ/C-mol. Thus, Gibbs energy of biosynthesis of BA.2.75 is slightly less negative, implying a lower rate of biosynthesis, multiplication, accumulation of viruses in host cells and damage to host cells. Thus, it seems that BA.2.75 subvariant has evolved exactly as expected by the theory of evolution, regarding the decrease in pathogenicity.

Figure 1 shows standard Gibbs energies of biosynthesis of nucleocapsids and their dates of appearance of SARS-CoV-2 variants, including Hu-1 (Wild type), Delta B.1.617.2, Omicron B.1.1.529, Omicron BA.2 and Omicron BA.2.75. From the graph, it is possible to see the evolution of SARS-CoV-2 related to multiplication inside the host cells. The trend of evolution is given by the dotted line. The values for Omicron B.1.1.529, Omicron BA.2 and Omicron BA.2.75 variants are very similar. This implies that the mutations have mostly occurred in the part of the nucleic acid related to the binding domain, rather than multiplication inside the host cell.

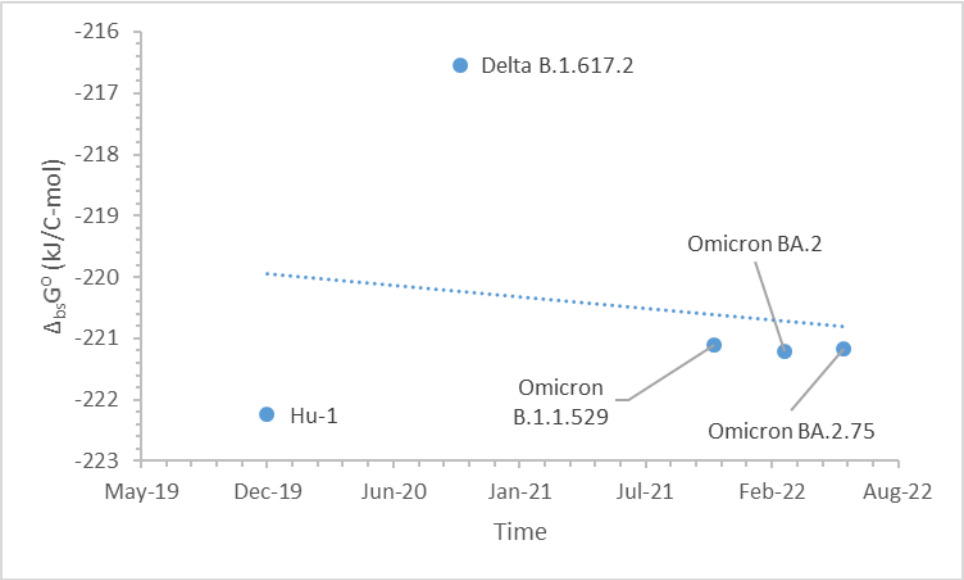


**Figure 1: Standard Gibbs energy of biosynthesis during evolution of SARS-CoV-2 variants.** The graph shows standard Gibbs energy of biosynthesis, $\Delta_{bs}G^0$, of SARS-CoV-2 variants versus the time they appeared. The dotted line shows the trend of evolution in multiplication rate of SARS-CoV-2 variants.

## 5. Conclusions

 doi:10.20944/preprints202210.0386.v1



Empirical formulas of BA.2.75 subvariant nucleocapsid have been determined for three isolates from Germany, India and USA. The empirical formulas and thermodynamic properties are very similar for the three isolates. The empirical formula of the isolate from Germany is $CH_{1.5736}O_{0.3426}N_{0.3124}P_{0.00601}S_{0.00336}$. For the isolate from India it is $CH_{1.5735}O_{0.3427}N_{0.3124}P_{0.00603}S_{0.00336}$. Finally, for the isolate from USA it is $CH_{1.5737}O_{0.3425}N_{0.3123}P_{0.00598}S_{0.00336}$. The similar empirical formulas can be explained by the three isolates belonging to the same BA.2.75 subvariant.

Molar masses have been determined for the Omicron BA.2.75 subvariant nucleocapsids. Molar masses were reported on two bases, molar masses of empirical formulas and molar masses of entire nucleocapsids. Molar masses of empirical formulas are 23.75 g/C-mol for the isolates from Germany and India, and 23.74 g/C-mol for the isolate from USA. The molar masses of the entire nucleocapsids for the isolates from Germany and India are 117.2 MDa, while that of the isolate from USA is 117.1 MDa. Therefore, the molar masses are very similar for all three isolates of Omicron BA.2.75 subvariant.

Gibbs energy of biosynthesis has been calculated for nucleocapsids of Omicron BA.2.75 subvariant. Gibbs energies of biosynthesis for the nucleocapsids of the isolates from Germany, India and USA are -221.18 kJ/C-mol, -221.24 kJ/C-mol and -221.12 kJ/C-mol, respectively. The three values are very similar to each other. However, they are all slightly less negative compared to that of the Hu-1 wild type, which is -222.2 kJ/C-mol. Thus, even though there is a great homogeneity in elemental composition and thermodynamic properties within the Omicron BA.2.75 subvariant, there is a difference compared to the Hu-1 wild type.

Due to less negative Gibbs energy of nucleocapsid biosynthesis, the pathogenicity of the Omicron BA.2.75 subvariant should be slightly lower than that of Hu-1. This is in accordance with the predictions of the evolution theory. Mutations that appeared during evolution from Hu-1 to BA.2.75 variant have led to decrease in Gibbs energy of biosynthesis and thereby decrease in rates of virion multiplication, virion accumulation inside the host cells and damage to host cells.

**Acknowledgements**

The author gratefully acknowledges all data contributors, i.e., the Authors and their Originating laboratories responsible for obtaining the specimens, and their Submitting laboratories for generating the genetic sequence and metadata and sharing via the GISAID Initiative, on which this research is based.

**References**

Acuti Martellucci, C., Flacco, M. E., Cappadona, R., Bravi, F., Mantovani, L., & Manzoli, L. (2020). SARS-CoV-2 pandemic: An overview. *Advances in biological regulation*, *77*, 100736. https://doi.org/10.1016/j.jbior.2020.100736

Atkins, P. W., & de Paula, J. (2011). *Physical Chemistry for the Life Sciences (2nd edition)*, W. H. Freeman and Company.

Atkins, P.W. & de Paula, J. (2014). *Physical Chemistry: Thermodynamics, Structure, and Change, 10th Edition*. New York: W. H. Freeman and Company.

Balmer, R.T. (2010). *Modern Engineering Thermodynamics*, Cambridge, MA: Academic Press. https://doi.org/10.1016/C2009-0-20199-1

Barton, M. I., MacGowan, S. A., Kutuzov, M. A., Dushek, O., Barton, G. J., & van der Merwe, P. A. (2021). Effects of common mutations in the SARS-CoV-2 Spike RBD and its ligand, the human ACE2 receptor on binding affinity and kinetics. eLife, 10, e70658. https://doi.org/10.7554/eLife.70658

Battley, E.H. (1999). 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. *Thermochimica Acta*, 326(1-2), 7-15. https://doi.org/10.1016/S0040-6031(98)00584-X

Battley, E. H. (1998). The development of direct and indirect methods for the study of the thermodynamics of microbial growth. *Thermochimica Acta*, *309*(1-2), 17-37. https://doi.org/10.1016/S0040-6031(97)00357-2

Buzón, P., Maity, S., & Roos, W. H. (2020). Physical virology: From virus self-assembly to particle mechanics. *Wiley interdisciplinary reviews. Nanomedicine and nanobiotechnology*, *12*(4), e1613. https://doi.org/10.1002/wnan.1613

Callaway E. (2022). Will 'Centaurus' be the next global coronavirus variant? Indian cases offers clues. *Nature*, *608*(7923), 462–463. https://doi.org/10.1038/d41586-022-02154-4

Callaway E. (2020). The coronavirus is mutating - does it matter?. *Nature*, *585*(7824), 174–177. https://doi.org/10.1038/d41586-020-02544-6

Casasnovas, J.M. and Springer, T.A. (1995). Kinetics and thermodynamics of virus binding to receptor: Studies with rhinovirus, intercellular adhesion molecule-1 (ICAM-1), and surface plasmon resonance. *Journal of Biological Chemistry*, 270(22), 13216-13224. https://www.doi.org/10.1074/jbc.270.22.13216

Ceres, P. and Zlotnick, A. (2002). Weak protein-protein interactions are sufficient to drive assembly of hepatitis B virus capsids. *Biochemistry*, 41(39), 11525-11531. https://doi.org/10.1021/bi0261645

Cucinotta, D., & Vanelli, M. (2020). WHO Declares COVID-19 a Pandemic. *Acta bio-medica : Atenei Parmensis*, *91*(1), 157–160. https://doi.org/10.23750/abm.v91i1.9397

Degueldre C. (2021). Single virus inductively coupled plasma mass spectroscopy analysis: A comprehensive study. *Talanta*, *228*, 122211. https://doi.org/10.1016/j.talanta.2021.122211

Demirel, Y. (2014). *Nonequilibrium Thermodynamics: Transport and Rate Processes in Physical, Chemical and Biological Systems, 3rd ed*. Amsterdam: Elsevier.

Dodd, T., Botto, M., Paul, F. et al. (2020). Polymerization and editing modes of a high-fidelity DNA polymerase are linked by a well-defined path. *Nat Commun* 11, 5379. https://doi.org/10.1038/s41467-020-19165-2

Du, X., Li, Y., Xia, Y. L., Ai, S. M., Liang, J., Sang, P., ... & Liu, S. Q. (2016). Insights into protein–ligand interactions: mechanisms, models, and methods. *International journal of molecular sciences*, *17*(2), 144. https://doi.org/10.3390/ijms17020144

Duffy S. (2018). Why are RNA virus mutation rates so damn high?. *PLoS biology*, *16*(8), e3000003. https://doi.org/10.1371/journal.pbio.3000003

Elbe, S., & Buckland-Merrett, G. (2017). Data, disease and diplomacy: GISAID's innovative contribution to global health. *Global challenges (Hoboken, NJ)*, *1*(1), 33–46. https://doi.org/10.1002/gch2.1018

Gale P. (2021). Using thermodynamic equilibrium models to predict the effect of antiviral agents on infectivity: Theoretical application to SARS-CoV-2 and other viruses. Microbial risk analysis, 100198. Advance online publication. https://doi.org/10.1016/j.mran.2021.100198

Gale P. (2020). 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. *Microbial risk analysis*, *15*, 100104. https://doi.org/10.1016/j.mran.2020.100104

Gale P. (2019). Towards a thermodynamic mechanistic model for the effect of temperature on arthropod vector competence for transmission of arboviruses. *Microbial risk analysis*, *12*, 27–43. https://doi.org/10.1016/j.mran.2019.03.001

Gale P. (2018). Using thermodynamic parameters to calibrate a mechanistic dose-response for infection of a host by a virus. *Microbial risk analysis*, *8*, 1–13. https://doi.org/10.1016/j.mran.2018.01.002

Garmann, R. F., Goldfain, A. M., & Manoharan, V. N. (2019). Measurements of the self-assembly kinetics of individual viral capsids around their RNA genome. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(45), 22485–22490. https://doi.org/10.1073/pnas.1909223116

Glansdorff, P. and Prigogine, I. (1971). *Thermodynamic Theory of Structure, Stability and Fluctuations.* Hoboken, NJ: Wiley.

Guosheng, L., Yi, L., Xiangdong, C., Peng, L., Ping, S., & Songsheng, Q. (2003). Study on interaction between T4 phage and Escherichia coli B by microcalorimetric method. *Journal of virological methods*, *112*(1-2), 137–143. https://doi.org/10.1016/s0166-0934(03)00214-3

Hansen, L.D., Popovic, M., Tolley, H.D. and Woodfield, B.F. (2018). Laws of evolution parallel the laws of thermodynamics. *Journal of Chemical Thermodynamics*, 124, 141-148. https://doi.org/10.1016/j.jct.2018.05.005

Head, R. J., Lumbers, E. R., Jarrott, B., Tretter, F., Smith, G., Pringle, K. G., Islam, S., & Martin, J. H. (2022). Systems analysis shows that thermodynamic physiological and pharmacological fundamentals drive COVID-19 and response to treatment. *Pharmacology research & perspectives*, *10*(1), e00922. https://doi.org/10.1002/prp2.922

Johansson, E., & Dixon, N. (2013). Replicative DNA polymerases. *Cold Spring Harbor perspectives in biology*, *5*(6), a012799. https://doi.org/10.1101/cshperspect.a012799

Jover, L. F., Effler, T. C., Buchan, A., Wilhelm, S. W., & Weitz, J. S. (2014). The elemental composition of virus particles: implications for marine biogeochemical cycles. *Nature reviews. Microbiology*, *12*(7), 519–528. https://doi.org/10.1038/nrmicro3289

Katen, S. and Zlotnick, A. (2009). The thermodynamics of virus capsid assembly. In: M.L. Johnson, J.M. Holt, G.K. Ackers, eds., *Methods in Enzymology, Volume 455*, Cambridge, MA: Academic Press, pp. 395-417. https://doi.org/10.1016/S0076-6879(08)04214-6

Kaniadakis, G., Baldi, M. M., Deisboeck, T. S., Grisolia, G., Hristopulos, D. T., Scarfone, A. M., Sparavigna, A., Wada, T., & Lucia, U. (2020). The κ-statistics approach to epidemiology. Scientific reports, 10(1), 19949. https://doi.org/10.1038/s41598-020-76673-3

Khare, S., Gurry, C., Freitas, L., Schultz, M. B., Bach, G., Diallo, A., Akite, N., Ho, J., Lee, R. T., Yeo, W., Curation Team, G. C., & Maurer-Stroh, S. (2021). GISAID's Role in Pandemic Response. *China CDC weekly*, *3*(49), 1049–1051. https://doi.org/10.46234/ccdcw2021.255

Kumar, A., Singh, R., Kaur, J., Pandey, S., Sharma, V., Thakur, L., Sati, S., Mani, S., Asthana, S., Sharma, T. K., Chaudhuri, S., Bhattacharyya, S., & Kumar, N. (2021). Wuhan to World: The COVID-19 Pandemic. *Frontiers in cellular and infection microbiology*, *11*, 596201. https://doi.org/10.3389/fcimb.2021.596201

Lee, J., Schwarz, K. J., Kim, D. S., Moore, J. S., & Jewett, M. C. (2020). Ribosome-mediated polymerization of long chain carbon and cyclic amino acids into peptides in vitro. *Nature communications*, *11*(1), 4304. https://doi.org/10.1038/s41467-020-18001-x

Lucia, U., Grisolia, G., & Deisboeck, T. S. (2021). Thermodynamics and SARS-CoV-2: neurological effects in post-Covid 19 syndrome. *Atti della Accademia Peloritana dei Pericolanti*, 99(2), A3. https://doi.org/10.1478/AAPP.992A3

Lucia, U., Grisolia, G., & Deisboeck, T. S. (2020a). Seebeck-like effect in SARS-CoV-2 bio-thermodynamics. *Atti della Accademia Peloritana dei Pericolanti-Classe di Scienze Fisiche, Matematiche e Naturali*, *98*(2), 6. https://doi.org/10.1478/AAPP.982A6

Lucia, U., Deisboeck, T. S., & Grisolia, G. (2020b). Entropy-based pandemics forecasting. Frontiers in Physics, 8, 274. https://doi.org/10.3389/fphy.2020.00274

Lucia, U., & Grisolia, G. (2020). How life works—A continuous Seebeck-Peltier transition in cell membrane?. *Entropy*, *22*(9), 960. https://doi.org/10.3390/e22090960

Lucia U. (2015). Bioengineering thermodynamics of biological cells. *Theoretical biology & medical modelling*, *12*, 29. https://doi.org/10.1186/s12976-015-0024-z

Mahmoudabadi, G., Milo, R. and Phillips, R. (2017). Energetic cost of building a virus. *PNAS,* 114(22), E4324-E4333. https://doi.org/10.1073/pnas.1701670114

Maskow, T. (2013). Miniaturization of calorimetry: Strengths and weaknesses for bioprocess monitoring. In: *Biothermodynamics: the role of thermodynamics in Biochemical Engineering*, von Stockar, U., ed., Lausanne: EPFL Press, pp. 423-442.

Maskow, T., Kemp, R., Buchholz, F., Schubert, T., Kiesel, B., & Harms, H. (2010a). What heat is telling us about microbial conversions in nature and technology: from chip- to megacalorimetry. *Microbial biotechnology*, *3*(3), 269–284. https://doi.org/10.1111/j.1751-7915.2009.00121.x

Maskow, T., Kiesel, B., Schubert, T., Yong, Z., Harms, H., & Yao, J. (2010b). Calorimetric real time monitoring of lambda prophage induction. *Journal of virological methods*, *168*(1-2), 126–132. https://doi.org/10.1016/j.jviromet.2010.05.002

Maskow, T., & von Stockar, U. (2005). How reliable are thermodynamic feasibility statements of biochemical pathways?. *Biotechnology and bioengineering*, *92*(2), 223–230. https://doi.org/10.1002/bit.20572

Molla, A., Paul, A. V., & Wimmer, E. (1991). Cell-free, de novo synthesis of poliovirus. *Science (New York, N.Y.)*, *254*(5038), 1647–1651. https://doi.org/10.1126/science.1661029

Morowitz, H.J. (1992). *Beginnings of Cellular Life: Metabolism Recapitulates Biogenesis*. New Haven, CT: Yale University Press.

Morowitz, H.J. (1968). *Energy Flow in Biology: Biological Organization as a Problem in Thermal Physics*. New York: Academic Press.

National Center for Biotechnology Information (2022). NCBI database [online] https://www.ncbi.nlm.nih.gov/ (Accessed on October 22, 2022)

Needleman, S. B., & Wunsch, C. D. (1970). A general method applicable to the search for similarities in the amino acid sequence of two proteins. *Journal of molecular biology*, *48*(3), 443–453. https://doi.org/10.1016/0022-2836(70)90057-4

Neuman, B.W. and Buchmeier, M.J. (2016). Supramolecular architecture of the coronavirus particle. *Advances in Virus Research*, 96, 1-27. https://doi.org/10.1016/bs.aivir.2016.08.005

Neuman, B. W., Kiss, G., Kunding, A. H., Bhella, D., Baksh, M. F., Connelly, S., Droese, B., Klaus, J. P., Makino, S., Sawicki, S. G., Siddell, S. G., Stamou, D. G., Wilson, I. A., Kuhn, P., & Buchmeier, M. J. (2011). A structural analysis of M protein in coronavirus assembly and morphology. *Journal of structural biology*, *174*(1), 11–22. https://doi.org/10.1016/j.jsb.2010.11.021

Neuman, B. W., Adair, B. D., Yoshioka, C., Quispe, J. D., Orca, G., Kuhn, P., Milligan, R. A., Yeager, M., & Buchmeier, M. J. (2006). Supramolecular architecture of severe acute respiratory syndrome coronavirus revealed by electron cryomicroscopy. *Journal of virology*, *80*(16), 7918–7928. https://doi.org/10.1128/JVI.00645-06

Ozilgen, M. and Sorgüven, E. (2017). *Biothermodynamics: Principles and Applications.* Boca Raton: CRC Press.

Patel, S.A. and Erickson, L.E. (1981). Estimation of heats of combustion of biomass from elemental analysis using available electron concepts. *Biotechnology and Bioengineering,* 23, 2051-2067. https://doi.org/10.1002/bit.260230910

Popovic, M. and Popovic, M. (2022). Strain Wars: Competitive interactions between SARS-CoV-2 strains are explained by Gibbs energy of antigen-receptor binding. *Microbial Risk Analysis*. https://doi.org/10.1016/j.mran.2022.100202

Popovic, M. (2022a). Strain wars 2: Binding constants, enthalpies, entropies, Gibbs energies and rates of binding of SARS-CoV-2 variants. *Virology*, 570, 35-44. https://doi.org/10.1016/j.virol.2022.03.008

Popovic M. (2022b). 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. *Microbial risk analysis*, 100217. Advance online publication. https://doi.org/10.1016/j.mran.2022.100217

Popovic M. (2022c). Atom counting method for determining elemental composition of viruses and its applications in biothermodynamics and environmental science. *Computational biology and chemistry*, *96*, 107621. https://doi.org/10.1016/j.compbiolchem.2022.107621

Popovic, M. (2022d). 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.* https://doi.org/10.1016/j.virol.2022.08.009

Popovic, M. (2022e). Strain Wars 5: Gibbs energies of binding of BA.1 through BA.4 variants of SARS-CoV-2. *Microbial Risk Analysis*. https://doi.org/10.1016/j.mran.2022.100231

Popovic M. (2022f). Beyond COVID-19: Do biothermodynamic properties allow predicting the future evolution of SARS-CoV-2 variants? *Microbial Risk Analysis*. https://doi.org/10.1016/j.mran.2022.100232

Popovic, M. (2022g). 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*, *11*(4), 45. MDPI AG. Retrieved from http://dx.doi.org/10.3390/biotech11040045

Popovic, M., & Minceva, M. (2021a). Coinfection and Interference Phenomena Are the Results of Multiple Thermodynamic Competitive Interactions. *Microorganisms*, *9*(10), 2060. https://doi.org/10.3390/microorganisms9102060

Popovic, M., & Minceva, M. (2021b). Standard Thermodynamic Properties, Biosynthesis Rates, and the Driving Force of Growth of Five Agricultural Plants. *Frontiers in plant science*, *12*, 671868. https://doi.org/10.3389/fpls.2021.671868

Popovic, M. E., & Minceva, M. (2020c). Thermodynamic properties of human tissues. *Thermal Science*, *24*(6 Part B), 4115-4133. https://doi.org/10.2298/TSCI200109151P

Popovic, M. (2019). Thermodynamic properties of microorganisms: determination and analysis of enthalpy, entropy, and Gibbs free energy of biomass, cells and colonies of 32 microorganism species. *Helyon*, 5(6), e01950. https://doi.org/10.1016/j.heliyon.2019.e01950

Popovic, M. (2018). Research in entropy wonderland: A review of the entropy concept, *Thermal Science*, 22(2), 1163-1178. https://doi.org/10.2298/TSCI180115012P

Popovic, M. (2017). Living organisms from Prigogine's perspective: an opportunity to introduce students to biological entropy balance. *Journal of Biological Education*, 52(3), 294-300. https://doi.org/10.1080/00219266.2017.1357649

Popovic, M. (2014a). Comparative study of entropy and information change in closed and open thermodynamic systems. *Thermochimica Acta*, 598, 77-81. https://doi.org/10.1016/j.tca.2014.11.002

Popovic, M. (2014b). Entropy change of open thermodynamic systems in self-organizing processes. *Thermal Science*, 18(4), 1425-1432. https://doi.org/10.2298/TSCI140424065P

Prigogine, I. and Wiame, J.M. (1946). Biologie et thermodynamique des phénomènes irréversibles. *Experientia*, 2, 451–453. https://doi.org/10.1007/BF02153597

Prigogine, I. (1977). Nobel lecture: Time, Structure and Fluctuations. [Online] Available at: https://www.nobelprize.org/prizes/chemistry/1977/prigogine/lecture/ (Accessed on: July 17, 2022)

Prigogine I. (1947). *Etude thermodynamique des phénomènes irréversibles.* Paris: Dunod.

Riedel, S., Morse, S., Mietzner, T. & Miller, S. (2019). *Jawetz, Melnick & Adelbergs Medical Microbiology, 28th ed.*, New York: McGraw-Hill Education.

Roels, J.A. (1983). *Energetics and Kinetics in Biotechnology*, Amsterdam: Elsevier.

Sandler, S. I., & Orbey, H. (1991). On the thermodynamics of microbial growth processes. *Biotechnology and bioengineering*, *38*(7), 697–718. https://doi.org/10.1002/bit.260380704

Sandler, S.I. (2017). Chemical, Biochemical, and Engineering Thermodynamics, 5th ed. Hoboken, NJ: Wiley. ISBN: 978-1-119-32128-6

Schmid, M., Speiseder, T., Dobner, T., & Gonzalez, R. A. (2014). DNA virus replication compartments. *Journal of virology*, *88*(3), 1404–1420. https://doi.org/10.1128/JVI.02046-13

Schrödinger, E. (1944). *What Is Life? The Physical Aspect of the Living Cell*, Cambridge, UK: Cambridge University Press. ISBN: 0-521-42708-8

Sheward, D. J., Kim, C., Fischbach, J., Sato, K., Muschiol, S., Ehling, R. A., Björkström, N. K., Hedestam, G., Reddy, S. T., Albert, J., Peacock, T. P., & Murrell, B. (2022). Omicron sublineage BA.2.75.2 exhibits extensive escape from neutralising antibodies. *The Lancet. Infectious diseases*, S1473-3099(22)00663-6. Advance online publication. https://doi.org/10.1016/S1473-3099(22)00663-6

Shu, Y., & McCauley, J. (2017). GISAID: Global initiative on sharing all influenza data - from vision to reality. *Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin*, *22*(13), 30494. https://doi.org/10.2807/1560-7917.ES.2017.22.13.30494

Şimşek, B., Özilgen, M., & Utku, F. Ş. (2021). How much energy is stored in SARS-CoV-2 and its structural elements?. *Energy Storage*, e298. https://doi.org/10.1002/est2.298

Takashita, E., Yamayoshi, S., Fukushi, S., Suzuki, T., Maeda, K., Sakai-Tagawa, Y., Ito, M., Uraki, R., Halfmann, P., Watanabe, S., Takeda, M., Hasegawa, H., Imai, M., & Kawaoka, Y. (2022). Efficacy of Antiviral Agents against the Omicron Subvariant BA.2.75. *The New England journal of medicine*, *387*(13), 1236–1238. https://doi.org/10.1056/NEJMc2209952

Tzlil, S., Deserno, M., Gelbart, W.M. and Ben-Shaul, A. (2004). A statistical-thermodynamic model of viral budding. *Biophysical Journal*, 86(4), 2037-2048. https://doi.org/10.1016/S0006-3495(04)74265-4

Vogel G. (2022). New Omicron strains may portend big COVID-19 waves. *Science (New York, N.Y.)*, *377*(6614), 1479. https://doi.org/10.1126/science.adf0777

Von Bertalanffy, L. (1950). The theory of open systems in physics and biology. *Science*, 111(2872), 23-29. https://doi.org/10.1126/science.111.2872.23

Von Stockar, U. (2013a). Live cells as open non-equilibrium systems. In Urs von Stockar, ed., *Biothermodynamics: The Role of Thermodynamics in Biochemical Engineering*, Lausanne: EPFL Press, 475-534.

Von Stockar, U. (2013b). 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., Lausanne: EPFL Press, pp. 475-534.

Von Stockar, U., Maskow, T. and Vojinovic, V. (2013). Thermodynamic analysis of metabolic pathways. In: *Biothermodynamics: the role of thermodynamics in Biochemical Engineering*, von Stockar, U., ed., Lausanne: EPFL Press, pp. 581-604.

Von Stockar, U., Maskow, T., Liu, J., Marison, I. W., & Patino, R. (2006). Thermodynamics of microbial growth and metabolism: an analysis of the current situation. *Journal of Biotechnology*, *121*(4), 517-533. https://doi.org/10.1016/j.jbiotec.2005.08.012

Von Stockar, U. and Liu, J.-S. (1999). Does microbial life always feed on negative entropy? Thermodynamic analysis of microbial growth. *Biochimica et Biophysica Acta (BBA) – Bioenergetics*, 1412(3), 191-211. https://doi.org/10.1016/S0005-2728(99)00065-1

Wang, R., Chen, J., Gao, K., Hozumi, Y., Yin, C., & Wei, G. W. (2021). Analysis of SARS-CoV-2 mutations in the United States suggests presence of four substrains and novel variants. *Communications biology*, *4*(1), 228. https://doi.org/10.1038/s42003-021-01754-6

 doi:10.20944/preprints202210.0386.v1



WHO (2022a). Coronavirus disease (COVID-19) pandemic [Online] World Health Organization. Available at: https://www.who.int/emergencies/diseases/novel-coronavirus-2019 (Accessed on October 23, 2022).

Worldometer (2022a). COVID-19 Coronavirus Pandemic [Online] Available at: https://www.worldometers.info/coronavirus/ (Accessed on October 23, 2022).

Worldometer (2022b). WORLD / COUNTRIES / GERMANY [Online] Available at: https://www.worldometers.info/coronavirus/country/germany/ (Accessed on October 23, 2022).

Wimmer E. (2006). The test-tube synthesis of a chemical called poliovirus. The simple synthesis of a virus has far-reaching societal implications. *EMBO reports*, *7 Spec No*(Spec No), S3–S9. https://doi.org/10.1038/sj.embor.7400728