# SARS-CoV-2 phoenix: Empirical formulas and thermodynamic properties (enthalpy, entropy and Gibbs energy) of nucleocapsid, virus particle and biosynthesis of BA.2.86 Pirola variant

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*Article*

# SARS-CoV-2 Phoenix: Empirical Formulas and Thermodynamic Properties (Enthalpy, Entropy and Gibbs Energy) of Nucleocapsid, Virus Particle and Biosynthesis of BA.2.86 Pirola Variant

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**Abstract:** Similarly to a phoenix, SARS-CoV-2 has appeared periodically in waves. The new variants that appeared through mutations have during the 4 years of the pandemic suppressed earlier variants, causing new waves of the pandemic. The Omicron BA.2.86 Pirola variant is the latest in the sequence of SARS-CoV-2 variants, which appeared in 2023. The BA.2.86 variant has started to spread rapidly and we are witnesses of a new epidemic wave. In this short period, an increased infectivity was noticed, which results in rapid spreading and decreased pathogenicity, which results in a lower number of severe cases. However, in the public there is a fear of further development of the epidemic. This analysis was made with the goal to assess the risks in the period of late 2023. Mutations that were developed by the BA.2.86 variant have led to a change in empirical formula and thermodynamic properties. It seems that there is no ground for fear of an extensive spreading of severe forms, but there are reasons for caution and monitoring of the spreading of the epidemic and potential appearance of new mutations.

**Keywords:** biothermodynamics; virus-host interaction; COVID-19; pandemic; variant under monitoring; pathogenicity; pathogen; August 2023

## Introduction

Phoenix is an immortal bird that cyclically regenerates. Like a phoenix, SARS-CoV-2 has cyclically regenerated several dozen times through mutations from Hu-1 to the newest Omicron BA.2.86 Pirola variant. With every new mutation and new variant, SARS-CoV-2 has obtained a new life appearing slightly different from its predecessor. Some of the variants have caused pandemic waves of high amplitude [Campi et al., 2022; Dutta, 2022; Nasir et al., 2023; Thakur et al., 2022; Amin et al., 2022]. Differently from the mythological phoenix, the SARS-CoV-2 phoenix has disappeared and reborn in front of our eyes during the three years of the pandemic. Thus, SARS-CoV-2 has appeared in late-2019 in Wuhan and was labeled as the Hu-1 wild type [Holmes et al., 2021; Hu et al., 2021; WHO, 2021; Andersen et al., 2020; Chan et al., 2020]. Mutations have occurred mostly in the part of the genome that encodes the spike glycoprotein [Magazine et al., 2022; Souza et al., 2022; Kumar et al., 2023; Harvey et al., 2021; Rahbar et al., 2021; Gobeil et al., 2021]. However, mutations have occurred in other viral proteins as well [Senthilazhagan et al., 2023; Ichikawa et al., 2022]. Evolution of viruses and formation of new variants has been described in the literature [ECDC, 2023a; CDC, 2023b; WHO, 2023b; Aleem et al., 2023; NCBI, 2023a; Carabelli et al., 2023; Chen et al., 2022; Rahman et al., 2022; Dubey et al., 2021; Singh et al., 2022; Ramesh et al., 2021; Popovic, 2023a, 2022b; Popovic et al., 2023a, 2023b].

BA.2.86 Pirola is the latest variant of SARS-CoV-2, which is characterized by many mutations [CDC, 2023a]. The number of mutations in BA.2.86 variant compared to the XBB.1.5 variant is similar to the difference between the first Omicron variant and its predecessor Delta variant [CDC, 2023a]. This might give the BA.2.86 variant the ability to infect people who have previously had COVID-19

or who have received COVID-19 vaccines [CDC, 2023a], which has raised concerns in the public [EuroNews, 2023; CNBC, 2023]. Globally, as of 30 August 2023, there have been 770,085,713 confirmed cases of COVID-19, including 6,956,173 deaths [WHO, 2023a]. Even though there has been a decrease in number of daily infections worldwide since late 2022, with the appearance of the new Omicron BA.2.86 variant, the number of COVID-19 cases has increased since the mid-2023. Due to this situation, it would be good to perform a physicochemical analysis of the BA.2.86 variant to compare its ability to infect host cells with that of the previous variants of SARS-CoV-2.

SARS-CoV-2 belongs to the *Coronaviridae* family [Coronaviridae Study Group of the International Committee on Taxonomy of Viruses, 2020; Yang et al., 2020; Rajagopalan, 2021; Abdelrahman et al., 2020; Zhu et al., 2020]. It is an enveloped virus, with a single stranded positive sense RNA genome [Bartas et al., 2022; Alexandersen et al., 2020; Cao et al., 2021; Brant et al., 2021; Lee et al., 2022; Chai et al., 2021]. SARS-CoV-2 virus particles contain four kinds of structural proteins: nucleocapsid (N), membrane (M), envelope (E) and spike (S) [Troyano-Hernáez et al., 2021; Satarker and Nampoothiri, 2020; Jackson et al., 2022; Schoeman and Fielding, 2019; Dolan et al., 2022; Yao et al., 2020]. The nucleocapsid protein binds to the viral RNA and forms the nucleocapsid [Wu et al., 2023, 2021; Cubuk et al., 2021; Perdikari et al., 2020; Jack et al., 2021; Wang et al., 2022]. The nucleocapsid is enclosed in a lipid bilayer envelope that contains membrane and envelope proteins [Kumar and Saxena, 2021; Ke et al., 2020; Hardenbrook and Zhang, 2022; Motsa and Stahelin, 2021; Mesquita et al., 2021; Mandala et al., 2020]. The spike proteins point out from the surface of the virus particle [Taha et al., 2023; Huang et al., 2020; Kordyukova et al., 2023; Chen et al., 2021; Zeng et al., 2021; Almehdi et al., 2021]. They represent the virus antigens that bind to host cell receptors [Gale, 2022; Popovic, 2023b, 2023c, 2023d; Popovic and Popovic, 2022].

SARS-CoV-2 belongs to RNA viruses [V'kovski et al., 2021; Khan et al., 2021; Zhang et al., 2021]. RNA viruses exhibit a great tendency to mutate [Duffy, 2018; Villa et al., 2021; Drake and Holland, 1999; Sanjuán and Domingo-Calap, 2016; Domingo et al., 2021; Elena et al., 2000; Dolan et al., 2018; Schulte et al., 2015; Popovic, 2022c]. Mutations lead to change in information content of the viral genome, chemical changes in elemental composition, as well as thermodynamic properties (enthalpy, entropy and Gibbs energy of formation and biosynthesis) [Popovic, 2022d, 2022e, 2022f]. Mutation as a biological phenomenon, except through sequencing, can be detected through the atom counting method, which allows detection of changes in elemental composition that appear as a consequence of mutations [Popovic, 2022g]. Furthermore, changes in elemental composition lead to changes in thermodynamic properties [Battley, 2013, 1999a, 1998; Battley and Stone, 2000; Patel and Erickson, 1981; Ozilgen and Sorgüven, 2017; Hurst and Harrison, 1992; Popovic, 2019; Popovic et al., 2021].

Since 2019, in the literature, elemental composition and thermodynamic properties have been reported for several virus species: Ebola [Popovic, 2022h], Monkeypox [Popovic, 2022a], SARS-CoV-2 [Şimşek et al., 2021; Degueldre, 2021; Gale, 2022; Popovic and Popovic, 2022; Popovic, 2022e, 2022d; Popovic et al., 2023a, 2023b; Popovic and Minceva, 2020b], MERS-CoV [Popovic and Minceva, 2020b], SARS-CoV [Popovic and Minceva, 2020b], HIV [Gale, 2020], arboviruses [Gale, 2020, 2019, 2018] and bacteriophages [Maskow et al., 2010; Guosheng et al., 2003; Popovic, 2023e]. Biothermodynamic mechanisms that influence infectivity and pathogenicity of different variants and the consequences on epidemiology and mechanisms of spreading of SARS-CoV-2 are available in the literature [Lucia et al., 2021, 2020a, 2020b; Kaniadakis et al., 2020; Head et al., 2022; Özilgen and Yilmaz, 2021; Pateras et al., 2022; Yilmaz et al., 2020; Trancossi et al., 2021].

The aim of this paper is to explore changes in empirical formula, molar mass, biosynthesis reactions, and thermodynamic properties (enthalpy, entropy, Gibbs energy) of formation and biosynthesis of the BA.2.86 Pirola variant. Based on the obtained results, the goal is to perform an assessment of the risk of spreading of an epidemic/pandemic of the BA.2.86 variant in late 2023. Moreover, the pathogenicity of the BA.2.86 variant will be compared to those of the earlier variants of SARS-CoV-2.

## Methods

*Data sources*

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The genetic sequence of the Omicron BA.2.86 Pirola variant of SARS-CoV-2 was taken from GISAID, the global data science initiative [Khare et al., 2021; Elbe and Buckland-Merrett, 2017; Shu and McCauley, 2017]. It can be found under the accession number EPI_ISL_18138566 and is labeled hCoV-19/USA/OH-ODH-SC3032044/2023. It was isolated on July 29, 2023 in Cuyahoga County, Ohio. Thus, the findings of this study are based on metadata associated with one sequence available on GISAID up to September 24, 2023, and accessible at https://doi.org/10.55876/gis8.230924yd (please see the Supplementary Material for more details).

The sequence of the nucleocapsid phosphoprotein of SARS-CoV-2 was obtained from the NCBI database [Sayers et al., 2022; NCBI, 2023b], under the accession number QIK50455.1 [NCBI, 2023c]. The sequence of the membrane protein of SARS-CoV-2 was obtained from the NCBI database [Sayers et al., 2022; NCBI, 2023a], under the accession number QHR63293.1 [NCBI, 2023d]. The sequence of the spike glycoprotein of SARS-CoV-2 was obtained from the NCBI database [Sayers et al., 2022; NCBI, 2023a], under the accession number QHR63290.2 [NCBI, 2023e]. The number of protein copies in the virus particle was taken from [Neuman and Buchmeier, 2016; Neuman et al., 2011; Neuman et al., 2006]. In a SARS-CoV-2 particle, there are 2368 copies of the nucleocapsid phosphoprotein, 1184 copies of the membrane protein and 222 copies of the spike glycoprotein [Neuman and Buchmeier, 2016; Neuman et al., 2011; Neuman et al., 2006].

*Empirical formulas*

The empirical formulas and molar masses of the virus particle and nucleocapsid of the Omicron BA.2.86 Pirola variant of SARS-CoV-2 were determined through the atom counting method [Popovic, 2022g]. They were determined based on the genetic sequence, protein sequences and virus morphology.

The atom counting method is a computational approach for determination of empirical formulas, chemical formulas and molar masses of macromolecules and macromolecular assemblies [Popovic, 2022g; Popovic et al., 2023c]. The atom counting method can analyze a wide range of macromolecules, including be double-stranded DNA, single-stranded DNA, single-stranded RNA, double-stranded RNA, proteins, polypeptides, oligopeptides etc. [Popovic, 2022g; Popovic et al., 2023c]. Furthermore, the atom counting method can be used to analyze macromolecular assemblies, such as virus particles, virus nucleocapsids, protein complexes, complexes of nucleic acids and proteins etc. [Popovic, 2022g; Popovic et al., 2023c].

The atom counting method is implemented with a computer program [Popovic, 2022g]. The input of the program are genetic sequences, protein sequences and morphological data [Popovic, 2022g]. The morphological data include protein copy numbers in macromolecular assemblies, size of the macromolecular assembly, whether the macromolecular assembly possesses lipids and carbohydrates etc. [Popovic, 2022g]. The output of the atom counting method are chemical formulas of macromolecules, empirical formulas of macromolecules, chemical formulas of macromolecular assemblies, empirical formulas of macromolecular assemblies, molar masses of empirical formulas of macromolecules, molar masses of empirical formulas of macromolecular assemblies, molar masses of macromolecules and molar masses of macromolecular assemblies [Popovic, 2022g].

The program that implements the atom counting method goes along the sequences of macromolecules (e.g. nucleic acids or proteins), which consist of residues [Popovic, 2022g]. Every residue has a well-defined chemical formula [Popovic, 2022g]. Thus, the program adds the atoms of different elements that come from every residue [Popovic, 2022g]. This gives the chemical formula of the macromolecule [Popovic, 2022g]. In case of macromolecular assemblies, the numbers of atoms of different elements are multiplied by the numbers of copies of the macromolecule in the macromolecular assembly [Popovic, 2022g]. If a macromolecular assembly contains lipids, the atoms coming from lipids are taken into account based on morphological data [Popovic, 2022g]. Then atoms coming from all the macromolecules are added to find the numbers of atoms of different elements in the macromolecular assembly [Popovic, 2022g]. These are used to find the empirical formula through the equation

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$$n_J = \frac{N_J}{N_C} \quad (1)$$

where $n_J$ is the number of atoms of element $J$ in the empirical formula, $N_J$ is the total number of atoms of element $J$ in the molecule or macromolecular assembly, and $N_C$ is the total number of carbon atoms in the molecule or macromolecular assembly [Popovic, 2022g].

*Thermodynamic properties of live matter*

Thermodynamic properties of virus particle and nucleocapsid of the Omicron BA.2.86 variant were determined with the Patel-Erickson model [Patel and Erickson, 1981; Battley, 1998] and Battley model [Battley, 1999a; Battley and Stone, 2000]. They were determined based on empirical formulas. The Patel-Erickson model was used to find enthalpy [Patel and Erickson, 1981; Battley, 1998] and the Battley model was used to find entropy [Battley, 1999a; Battley and Stone, 2000], which were then combined to find Gibbs energy.

To find enthalpy of live matter (i.e. virus particle or nucleocapsid) with the Patel-Erickson model, the empirical formula is used to find the number of electrons transferred to oxygen during complete oxidation, $E$, with the equation [Patel and Erickson, 1981; Battley, 1998]

$$E = 4n_C + n_H - 2n_O - 0\,n_N + 5n_P + 6n_S \quad (2)$$

$E$ is then used to find standard enthalpy of combustion of live matter, $\Delta_C H^0$, with the equation

$$\Delta_C H^0(bio) = -111.14\ \frac{kJ}{C-mol} \cdot E \quad (3)$$

The Patel-Erickson model is based on Thornton's theory of combustion, sometimes called Thornton's rule [Thornton, 1917]. Thornton's rule states that the process that releases energy during combustion is acceptance of electrons by oxygen which is highly electronegative [Thornton, 1917]. $\Delta_C H^0$ is then used to calculate standard enthalpy of formation of live matter, $\Delta_f H^0$, with the equation [Battley, 1998, 1999b, 1992]

$$\Delta_f H^0(bio) = n_C\ \Delta_f H^0(CO_2) + \frac{n_H}{2}\ \Delta_f H^0(H_2O) + \frac{n_P}{4}\ \Delta_f H^0(P_4O_{10}) + n_S\ \Delta_f H^0(SO_3) - \Delta_C H^0 \quad (4)$$

Entropy of live matter is calculated with the Battley model, based on its elemental composition. Standard molar entropy of live matter, $S^0_m$, is given by the equation

$$S^0_m(bio) = 0.187\ \sum_J \frac{S^0_m(J)}{a_J} n_J \quad (5)$$

where $S^0_m(J)$ is standard molar entropy of element $J$, $a_J$ number of atoms of element $J$ in its standard state elemental form, and $n_J$ the number of atoms of element $J$ in the empirical formula of live matter [Battley, 1999a; Battley and Stone, 2000]. The summation is over all elements $J$ of which the live matter consists [Battley, 1999a; Battley and Stone, 2000]. The changed environment of the atoms of elements in live matter is taken into account by the constant 0.187. The Battley model can also be used to find standard entropy of formation of live matter, $\Delta_f S^0$, if the constant 0.187 is changed to -0.813 [Battley, 1999a; Battley and Stone, 2000]

$$\Delta_f S^0(bio) = -0.813\ \sum_J \frac{S^0_m(J)}{a_J} n_J \quad (6)$$

Finally, $\Delta_f S^0$ and $\Delta_f H^0$ are combined to find standard Gibbs energy of formation, $\Delta_f G^0$, of live matter

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

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

*Biosynthesis reactions*

Biosynthesis reactions of the virus particle and nucleocapsid of the Omicron BA.2.86 variant were formulated based on their empirical formulas. Biosynthesis reactions are macrochemical

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equations of conversion of nutrients into new live matter in metabolism [Assael et al., 2022; von Stockar, 2013a, 2013b; Battley, 2013, 1999b]. The general biosynthesis reaction for viruses has the form

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

where (Amino acid) represents a mixture of amino acids, which has the empirical formula $CH_{1.798}O_{0.4831}N_{0.2247}S_{0.022472}$ [Popovic et al., 2023a, 2023b; Popovic, 2023a, 2023b, 2022c]. Newly synthetized live matter, (Bio), is represented with its empirical formula [Popovic et al., 2023a, 2023b; Popovic, 2023a, 2023b, 2022c]. The source of energy, carbon, nitrogen and sulfur for biosynthesis are the amino acids [Popovic et al., 2023a, 2023b; Popovic, 2023a, 2023b, 2022c]. The electron acceptor is $O_2$ [Popovic et al., 2023a, 2023b; Popovic, 2023a, 2023b, 2022c; Annamalai, 2021]. The source of phosphorus is $HPO_4^{2-}$ [Popovic et al., 2023a, 2023b; Popovic, 2023a, 2023b, 2022c]. Excess $H^+$ ions generated during biosynthesis are absorbed by the $HCO_3^-$ ion, which is a part of the bicarbonate buffer [Popovic et al., 2023a, 2023b; Popovic, 2023a, 2023b, 2022c]. Excess sulfur atoms are released in the form of the $SO_4^{2-}$ ion, which is an additional metabolic product [Popovic et al., 2023a, 2023b; Popovic, 2023a, 2023b, 2022c]. The oxidized carbon atoms are released in the form of $H_2CO_3$, which is also a part of the bicarbonate buffer [Popovic et al., 2023a, 2023b; Popovic, 2023a, 2023b, 2022c].

*Thermodynamic properties of biosynthesis*

Thermodynamic properties of biosynthesis of the virus particle and nucleocapsid of the Omicron BA.2.86 variant of SARS-CoV-2 were calculated with the Hess's law. They were found based on the biosynthesis reactions and thermodynamic properties of live matter. Thermodynamic properties of biosynthesis include standard enthalpy of biosynthesis, $\Delta_{bs}H^0$, standard entropy of biosynthesis, $\Delta_{bs}S^0$, and standard Gibbs energy of biosynthesis, $\Delta_{bs}G^0$ [von Stockar, 2013a, 2013b]. They can be found by application of the Hess's law to the biosynthesis reactions

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

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

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

where $\nu$ represents a stoichiometric coefficient [Popovic et al., 2023a, 2023b; Popovic, 2023a, 2023b, 2022c; Atkins and de Paula, 2011, 2014; von Stockar, 2013b; Battley, 1998]. Of particular importance among these properties is $\Delta_{bs}G^0$, since it represents the physical driving force for the process of multiplication of microorganisms [von Stockar, 2013a, 2013b; von Stockar and Liu, 1999], including viruses [Popovic et al., 2023a, 2023b; Popovic, 2023a, 2023b, 2022c].

**Results**

Empirical formulas and molar masses were determined for the first time for the virus particle and nucleocapsid of the Omicron BA.2.86 Pirola variant of SARS-CoV-2. They are shown in Table 1. The empirical formulas were determined through the atom counting method [Popovic, 2022g], based on the genetic sequence, protein sequences and morphology of the virus. The empirical formula of the virus particle of the Omicron BA.2.86 variant is $CH_{1.639023}O_{0.284130}N_{0.230031}P_{0.006440}S_{0.003765}$ and has a molar mass of 21.75 g/C-mol. The molar mass of the entire virus particle of the Omicron BA.2.86 variant is 219.7 MDa. The empirical formula of the nucleocapsid of the Omicron BA.2.86 variant is $CH_{1.570946}O_{0.343118}N_{0.312432}P_{0.006007}S_{0.003349}$ and has a molar mass of 23.75 g/C-mol. The molar mass of the entire nucleocapsid of the Omicron BA.2.86 variant is 117.6 MDa.

**Table 1.** Empirical formulas and molar masses of the Omicron BA.2.86 Pirola variant of SARS-CoV-2. Empirical formulas have the general form $C_{nC}H_{nH}O_{nO}N_{nN}P_{nP}S_{nS}$, where $n_C$, $n_H$, $n_O$, $n_N$, $n_P$ and $n_S$ are numbers of C, H, O, N, P and S atoms in the empirical formula, respectively. Molar masses were reported in two forms: molar mass of the empirical formula, *Mr*, and total molar mass of the macromolecular assembly (entire virus particle or entire nucleocapsid), *Mr*(*tot*).

| Name | $n_C$ | $n_H$ | $n_O$ | $n_N$ | $n_P$ | $n_S$ | Mr (g/C-mol) | Mr(tot) (MDa) |
|---|---|---|---|---|---|---|---|---|
| BA.2.86 virus particle | 1 | 1.639023 | 0.284130 | 0.230031 | 0.006440 | 0.003765 | 21.75 | 219.7 |

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| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| BA.2.86 nucleocapsid | 1 | 1.570946 | 0.343118 | 0.312432 | 0.006007 | 0.003349 | 23.75 | 117.6 |

Table 2 shows thermodynamic properties of the virus particle and nucleocapsid of the Omicron BA.2.86 variant. They were determined through the Patel-Ericson model [Patel and Erickson, 1981; Battley, 1998] and Battley model [Battley, 1999a; Battley and Stone, 2000], based on the empirical formulas (Table 1). They include standard enthalpy of formation, $\Delta_f H^0$, standard molar entropy, $S_m^0$, and standard Gibbs energy of formation, $\Delta_f G^0$. For the virus particle of the Omicron BA.2.86 variant, standard enthalpy of formation is -64.43 kJ/C-mol, standard molar entropy is 30.70 J/C-mol K and standard Gibbs energy of formation is -24.64 kJ/C-mol. For the nucleocapsid of the Omicron BA.2.86 variant, standard enthalpy of formation is -75.41 kJ/C-mol, standard molar entropy is 32.47 J/C-mol K and standard Gibbs energy of formation is -33.32 kJ/C-mol.

**Table 2.** Thermodynamic properties of live matter of the Omicron BA.2.86 variant of SARS-CoV-2: standard enthalpy of formation, $\Delta_f H^0$, standard molar entropy, $S_m^0$, and standard Gibbs energy of formation, $\Delta_f G^0$.

| Name | $\Delta_f H^0$ (kJ/C-mol) | $S_m^0$ (J/C-mol K) | $\Delta_f G^0$ (kJ/C-mol) |
|---|---|---|---|
| BA.2.86 virus particle | -64.43 | 30.70 | -24.64 |
| BA.2.86 nucleocapsid | -75.41 | 32.47 | -33.32 |

Table 3 shows the biosynthesis stoichiometry of the virus particle and nucleocapsid of the Omicron BA.2.86 variant. They were formulated based on the empirical formulas (Table 1). The general biosynthesis reaction has the form (Amino acid) + $CH_2O$ + $O_2$ + $HPO_4^{2-}$ + $HCO_3^-$ → (Bio) + $SO_2^{2-}$ + $H_2O$ + $H_2CO_3$, where (Amino acid) represents a mixture of amino acids with the formula $CH_{1.798}O_{0.4831}N_{0.2247}S_{0.022472}$ and (Bio) represents the empirical formula of live matter.

**Table 3.** Biosynthesis stoichiometry for the Omicron BA.2.86 variant of SARS-CoV-2. The general biosynthesis reaction has the form (Amino acid) + $CH_2O$ + $O_2$ + $HPO_4^{2-}$ + $HCO_3^-$ → (Bio) + $SO_2^{2-}$ + $H_2O$ + $H_2CO_3$. "Amino acid" represents a mixture of amino acids with the formula $CH_{1.798}O_{0.4831}N_{0.2247}S_{0.022472}$. "Bio" represents the empirical formula of live matter from Table 1.

| Name | Reactants | | | | | | Products | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Amino acid | $CH_2O$ | $O_2$ | $HPO_4^{2-}$ | $HCO_3^-$ | → | Bio | $SO_4^{2-}$ | $H_2O$ | $H_2CO_3$ |
| BA.2.86 virus particle | 1.023637 | 0.010469 | 0.000000 | 0.006440 | 0.025596 | → | 1 | 0.019238 | 0.067397 | 0.059701 |
| BA.2.86 nucleocapsid | 1.390323 | 0.000000 | 0.492478 | 0.006007 | 0.043774 | → | 1 | 0.027894 | 0.055049 | 0.434097 |

Table 4 gives thermodynamic properties of biosynthesis of the virus particle and nucleocapsid of the Omicron BA.2.86 variant of SARS-CoV-2. They were calculated with the Hess's law [Atkins and de Paula, 2011, 2014; von Stockar, 2013a, 2013b], based on the biosynthesis stoichiometry (Table 3) and thermodynamic properties of live matter (Table 2). They include standard enthalpy of biosynthesis, $\Delta_{bs}H^0$, standard entropy of biosynthesis, $\Delta_{bs}S^0$, and standard Gibbs energy of biosynthesis, $\Delta_{bs}G^0$. For the virus particle of the Omicron BA.2.86 variant, standard enthalpy of biosynthesis is -4.80 kJ/C-mol, standard entropy of biosynthesis is 6.94 J/C-mol K and standard Gibbs energy of biosynthesis is -6.94 kJ/C-mol. For the nucleocapsid of the Omicron BA.2.86 variant, standard enthalpy of biosynthesis is -232.88 kJ/C-mol, standard entropy of biosynthesis is -37.48 J/C-mol K and standard Gibbs energy of biosynthesis is -221.75 kJ/C-mol.

**Table 4.** Thermodynamic properties of biosynthesis for the Omicron BA.2.86 variant of SARS-CoV-2: 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 | $\Delta_{bs}H^0$ (kJ/C-mol) | $\Delta_{bs}S^0$ (J/C-mol K) | $\Delta_{bs}G^0$ (kJ/C-mol) |
|---|---|---|---|
| BA.2.86 virus particle | -4.80 | 6.94 | -6.94 |

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| | | | |
|---|---|---|---|
| BA.2.86 nucleocapsid | -232.88 | -37.48 | -221.75 |

**Discussion**

*Empirical formula and thermodynamic properties of live matter*

The empirical formula of the virus particle of the Omicron BA.2.86 variant of SARS-CoV-2 is reported for the first time: $CH_{1.639023}O_{0.284130}N_{0.230031}P_{0.006440}S_{0.003765}$ (Table 1). Empirical formulas have been reported in the literature for other SARS-CoV-2 variants. The empirical formula of the virus particle of the Hu-1 wild type of SARS-CoV-2 is $CH_{1.6390}O_{0.2851}N_{0.2301}P_{0.0065}S_{0.0038}$ [Popovic and Minceva, 2020b]. The empirical formula of the virus particle of the Delta variant of SARS-CoV-2 is $CH_{1.6383}O_{0.2844}N_{0.2294}P_{0.0064}S_{0.0042}$ [Popovic, 2022e]. The virus particle of the Omicron BA.1 variant of SARS-CoV-2 is characterized by the empirical formula $CH_{1.6404}O_{0.2842}N_{0.2299}P_{0.0064}S_{0.0038}$ [Popovic, 2022e]. The empirical formula of the virus particle of the BA.2 variant of SARS-CoV-2 is $CH_{1.6403}O_{0.2838}N_{0.2298}P_{0.0064}S_{0.0038}$ [Popovic, 2022f]. Moreover, empirical formulas of other virus species have been reported in the literature. The empirical formula of a *Poxviridae* virus particle is $CH_{1.5876}O_{0.3008}N_{0.2538}S_{0.00223}P_{0.00554}$ [Popovic, 2022a]. A *Vaccinia* virus particle is characterized by the empirical formula $CH_{1.5877}O_{0.3232}N_{0.2531}P_{0.00371}S_{0.00540}$ [Popovic, 2022a]. Therefore, every virus species and variant is characterized by a different empirical formula. Based on the empirical formula, it is possible to identify the virus. This provides a rapid method for virus identification through single particle inductively coupled plasma mass spectroscopy, as described by Degueldre [Degueldre, 2021].

Empirical formulas have been reported in the literature for various species of cellular organisms. The empirical formula of *Escherichia coli* (bacteria) is $CH_{1.918}O_{0.528}N_{0.257}P_{1.76\times10^{-2}}S_{5.54\times10^{-3}}K_{5.87\times10^{-3}}Mg_{2.07\times10^{-3}}Ca_{8.36\times10^{-4}}Mn_{9.89\times10^{-6}}Fe_{7.82\times10^{-5}}Cu_{1.62\times10^{-6}}Zn_{2.41\times10^{-5}}$ [Popovic et al., 2021]. The empirical formula of *Penicillium chrysogenum* (mold fungi) is $CH_{2.026}O_{0.511}N_{0.185}P_{9.15\times10^{-3}}S_{4.17\times10^{-3}}K_{3.45\times10^{-3}}Mg_{1.47\times10^{-3}}Ca_{3.69\times10^{-4}}Mn_{1.08\times10^{-5}}Fe_{9.51\times10^{-5}}Cu_{1.24\times10^{-6}}Zn_{2.15\times10^{-5}}$ [Popovic et al., 2021]. *Saccharomyces cerevisiae* (yeast fungi) is characterized by an empirical formula $CH_{1.613}O_{0.557}N_{0.158}P_{0.012}S_{0.003}K_{0.022}Mg_{0.003}Ca_{0.001}$ [Battley, 1999a]. The empirical formula of the human organism is $CH_{1.7296}O_{0.2591}N_{0.1112}P_{0.0134}S_{0.003}Na_{0.0027}K_{0.0031}Ca_{0.0173}Cl_{0.0018}$ [Popovic and Minceva, 2020c]. The empirical formula of the virus particle of the Omicron BA.2.86 variant of SARS-CoV-2 is $CH_{1.639023}O_{0.284130}N_{0.230031}P_{0.006440}S_{0.003765}$ (Table 1). Therefore, every class of organisms is characterized by a unique empirical formula different than those of other organisms.

Except for its empirical formula, the Omicron BA.2.86 variant of SARS-CoV-2 has its characteristic thermodynamic properties of live matter (enthalpy, entropy, Gibbs energy), which were determined in this research (Table 2). Gibbs energy of formation of the Omicron BA.2.86 virus particle is -24.64 kJ/C-mol, while that of the BA.2.86 nucleocapsid is -33.32 kJ/C-mol (Table 2). Therefore, the virus particle has a greater (less negative) Gibbs energy than the nucleocapsid. This means that the virus particle has a greater usable energy content. The reason for this are the lipids in the viral envelope. The SARS-CoV-2 virus particle contains a lipid envelope [Riedel et al., 2019]. The lipids in the envelope have a high energy content [Balmer, 2010]. Therefore, the usable energy content of the virus particle is greater than that of the nucleocapsid.

Gibbs energies of formation have been reported in the literature for other virus species and variants. The virus particle of the Hu-1 wild type of SARS-CoV-2 is characterized by a Gibbs energy of formation -24.8 kJ/C-mol [Popovic and Minceva, 2020b]. Gibbs energy of formation of the virus particle of the Omicron BA.2.86 variant of SARS-CoV-2 is -24.64 kJ/C-mol (Table 2). Thus, Gibbs energy of formation of the BA.2.86 variant is different than that of the Hu-1 wild type. Moreover, Gibbs energy of a *Poxviridae* virus particle is -25.3 kJ/C-mol [Popovic, 2022a], while that of a *Vaccinia* virus particle is -30.0 kJ/C-mol [Popovic, 2022a]. Thus, the virus particle of the Omicron BA.2.86 variant of SARS-CoV-2 has a different Gibbs energy of formation than those of the *Vaccinia* and *Poxviridae* virus particles. Therefore, every virus species and variant has a characteristic Gibbs energy of formation.

Gibbs energies of formation of cellular microorganisms can also be found in the literature. Gibbs energy of formation of some cellular microorganisms are: -66.98 kJ/C-mol for *Escherichia coli* bacteria,

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-87.07 kJ/C-mol for *Saccharomyces cerevisiae* yeast fungi and -18.99 kJ/C-mol for *Penicillium chrysogenum* mold fungi [Popovic, 2019]. Thus, Gibbs energies of these cellular microorganisms are different than that of the Omicron BA.2.86 variant of SARS-CoV-2 (-24.64 kJ/C-mol). Furthermore, Gibbs energy of formation of the human organism is -37.54 kJ/C-mol [Popovic and Minceva, 2020c], which is different than that of the Omicron BA.2.86 variant of SARS-CoV-2. This means that every class of organisms should have a characteristic Gibbs energy of formation, summarizing the usable energy content in its life matter.

*Biosynthesis reaction and thermodynamic properties of biosynthesis*

Based on the empirical formulas of the virus particle and nucleocapsid of the Omicron BA.2.86 Pirola variant of SARS-CoV-2, biosynthesis reactions were formulated. The biosynthesis reaction of the virus particle of the Omicron BA.2.86 variant is

$$1.023637\ CH_{1.798}O_{0.4831}N_{0.2247}S_{0.022472} + 0.010469\ CH_2O + 0.006440\ HPO_4^{2-} + 0.025596\ HCO_3^- \rightarrow CH_{1.639023}O_{0.284130}N_{0.230031}P_{0.006440}S_{0.003765} + 0.019238\ SO_2^{2-} + 0.067397\ H_2O + 0.059701\ H_2CO_3 \quad (12)$$

where $CH_{1.798}O_{0.4831}N_{0.2247}S_{0.022472}$ is the empirical formula of amino acids and $CH_{1.639023}O_{0.284130}N_{0.230031}P_{0.006440}S_{0.003765}$ is the empirical formula of the BA.2.86 virus particle (Table 1). The biosynthesis reaction of the nucleocapsid of the Omicron BA.2.86 variant is

$$1.390323\ CH_{1.798}O_{0.4831}N_{0.2247}S_{0.022472} + 0.492478\ O_2 + 0.006007\ HPO_4^{2-} + 0.043774\ HCO_3^- \rightarrow CH_{1.570946}O_{0.343118}N_{0.312432}P_{0.006007}S_{0.003349} + 0.027894\ SO_2^{2-} + 0.055049\ H_2O + 0.434097\ H_2CO_3 \quad (14)$$

where $CH_{1.570946}O_{0.343118}N_{0.312432}P_{0.006007}S_{0.003349}$ is the empirical formula of the BA.2.86 nucleocapsid (Table 1). The biosynthesis reaction of the BA.2.86 virus particle contains both amino acids and carbohydrates as an energy source, while that of the BA.2.86 nucleocapsid contains only amino acids. This means that biosynthesis of the BA.2.86 virus particle takes more energy than biosynthesis of the nucleocapsid alone. The reason for this is the higher energy content in the virus particle, due to the lipids in the viral envelope, as discussed above. The lipids in the viral envelope have a high energy content [Balmer, 2010]. This means that the virus particle that contains the lipid envelope takes more energy for biosynthesis than the nucleocapsid which doesn't contain lipids. This energy comes from the carbohydrates in the biosynthesis reaction. The biosynthesis reaction of the BA.2.86 virus particle requires more hydrogen phosphate ion than that of the nucleocapsid. The $HPO_4^{2-}$ ion is the phosphorus source for biosynthesis. The higher amount of $HPO_4^{2-}$ in the biosynthesis reaction is due to phospholipids in the envelope of the virus particle.

Based on the biosynthesis reactions, thermodynamic properties of biosynthesis of the BA.2.86 variant were determined for the first time. Enthalpy of biosynthesis of the BA.2.86 variant nucleocapsid is -232.88 kJ/C-mol (Table 4). This means that the enthalpy of biosynthesis contributes favorably to the biosynthesis process. Entropy of biosynthesis of the BA.2.86 nucleocapsid -37.48 kJ/C-mol (Table 4). The negative entropy change is unfavorable for the biosynthesis reaction. Gibbs energy of biosynthesis of the BA.2.86 variant is -221.75 kJ/C-mol. The negative Gibbs energy, which is due to the negative enthalpy of biosynthesis, means that the biosynthesis process is thermodynamically favorable.

*Virus-host and virus-virus interactions*

Gibbs energy of biosynthesis represents the driving force for the biosynthesis process [Assael et al., 2022; von Stockar, 2013a, 2013b; von Stockar and Liu, 1999; Westerhoff et al., 1982; Hellingwerf et al., 1982; Demirel, 2014]. A more negative Gibbs energy of biosynthesis, $\Delta_{bs}G$, implies a greater biosynthesis rate, $r_{bs}$, according to the biosynthesis phenomenological equation

$$r_{bs} = -\frac{L_{bs}}{T}\Delta_{bs}G \quad (X)$$

where $L_{bs}$ is the biosynthesis phenomenological coefficient and $T$ is temperature [Popovic, 2022c; Popovic, 2022e; Popovic et al., 2023a]. Gibbs energy of the biosynthesis of the nucleocapsid of the BA.2.86 Pirola variant of SARS-CoV-2 is -221.75 kJ/C-mol (Table 4). On the other hand, Gibbs energy

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of biosynthesis for the lung tissue is -49.76 kJ/C-mol [Popovic, 2022h]. Therefore, the BA.2.86 variant has a much more negative Gibbs energy of biosynthesis. This means that, according to the biosynthesis phenomenological equation, the biosynthesis rate of the BA.2.86 variant will be much greater than that of its host tissue. Due to this, the infected host cells will produce virus particles at a much greater rate than their own building blocks. This allows the hijacking of the host cell metabolism by the virus. The virus and its host cell compete for the cellular metabolic machinery and resources. The competition occurs in the host cell cytoplasm, at the ribosomes. The virus has a much greater driving force of biosynthesis, in the form of negative Gibbs energy. This means that the virus will have a much greater biosynthesis rate, which will allow it to hijack the host cell metabolism.

Gibbs energy of biosynthesis is proportional to the biosynthesis rate of a virus, according to the biosynthesis phenomenological equation. In case that several virus species or virus variants are simultaneously in circulation in the population, the virus with the most negative Gibbs energy of biosynthesis will have a competitive advantage [Popovic and Minceva, 2021; Popovic, 2023c]. The virus characterized by a more negative Gibbs energy of biosynthesis will have a greater biosynthesis rate [Popovic and Minceva, 2021; Popovic, 2023c]. This will allow it to dominate over other viruses circulating in the population [Popovic and Minceva, 2021; Popovic, 2023c]. Gibbs energy of biosynthesis of the nucleocapsid of the BA.2.86 Pirola variant of SARS-CoV-2 is -221.75 kJ/C-mol (Table 4). Gibbs energies of biosynthesis of nucleocapsids of other variants under monitoring are -221.21 for the Omicron CH.1.1 variant [Popovic, 2023a] and -221.19 kJ/C-mol for the Omicron XBB.1.16 variant [Popovic et al., 2023a]. Therefore, Gibbs energies of biosynthesis of the BA.2.86, CH.1.1 and XBB.1.16 variants are very similar. This means that in case these SARS-CoV-2 variants appear in a population, they will have very similar biosynthesis rates. This means that no variant will have an advantage in the competition. As a result, all three variants should circulate in the population during a pandemic wave and no variant should be able to suppress the other variants.

The concern expressed in the social media, concerning the greater pathogenicity of the new BA.2.86 variant seems not to be reasonable, since its Gibbs energy of biosynthesis is only slightly different than that of the other variants. The epidemiological measures that were undertaken in the fight against the other variants that caused the pandemic should result in an adequate response against the spreading of the BA.2.86 variant. However, the data related to kinetics of binding of the new variant to the host cell receptors are still not available. Therefore, in this work, it is not possible to predict with certainty the potential changes in infectivity of the new BA.2.86 variant compared to the other variants of SARS-CoV-2.

## Conclusions

This research reports for the first time the empirical formula, molar mass, biosynthesis reactions and thermodynamic properties (enthalpy, entropy and Gibbs energy) of formation and biosynthesis of the Omicron BA.2.86 Pirola variant of SARS-CoV-2. The empirical formula of the BA.2.86 virus particle is $CH_{1.639023}O_{0.284130}N_{0.230031}P_{0.006440}S_{0.003765}$, which has a molar mass of 21.75 g/C-mol. The empirical formula of the BA.2.86 variant is different than the empirical formulas of other SARS-CoV-2 variants, virus species and cellular organisms.

The nucleocapsid of the BA.2.86 variant is characterized by a Gibbs energy of biosynthesis of -221.75 kJ/C-mol. This is very similar to Gibbs energies of biosynthesis of the other variants under monitoring: CH.1.1 and XBB.1.16. Gibbs energy of biosynthesis represents the driving force for biosynthesis of virus particles and is proportional to their biosynthesis rate. Since the BA.2.86, CH.1.1 and XBB.1.16 variants have similar Gibbs energies of biosynthesis, they will have similar biosynthesis rates. This means that they will have very similar pathogenicity.

**Supplementary Materials:**

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