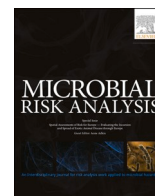




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# Strain wars 5: Gibbs energies of binding of BA.1 through BA.4 variants of SARS-CoV-2

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## ABSTRACT

This paper reports, for the first time, standard Gibbs energies of binding of the BA.1, BA.2, BA.3, BA.2.13, BA.2.12.1 and BA.4 Omicron variants of SARS-CoV-2, to the Human ACE2 receptor. Variants BA.1 through BA.3 exhibit a trend of decreasing standard Gibbs energy of binding and hence increased infectivity. The BA.4 variant exhibits a less negative standard Gibbs energy of binding, but also more efficient evasion of the immune response. Therefore, it was concluded that all the analyzed strains evolve in accordance with expectations of the theory of evolution, albeit using different strategies.

"The virus is mankind's ultimate predator."

R. Cook, Formula for death, 1995

## 1. Introduction

Living organisms represent open thermodynamic systems with the property of growth (von Bertalanffy, 1950; Popovic, 2019; Popovic et al., 2021, Popovic, 2018, 2017). SARS-CoV-2, except for being a biological system, also represents a chemical system, characterized by an empirical formula (Popovic and Minceva, 2020b; Degueldre, 2021; Popovic, 2022c). Moreover, SARS-CoV-2 represents an open thermodynamic system, with the property of growth (Popovic and Minceva, 2020a). Thus, thermodynamics has proved itself to be a powerful tool in analysis of infections caused by SARS-CoV-2 (Lucia et al., 2021, 2020a). It was shown that thermodynamic property - entropy can be used in analysis and prediction of development of the COVID-19 pandemic (Lucia et al., 2020b; Kaniadakis et al., 2020). Moreover, thermodynamics can be used in deeper analysis of fundamental life functions (Lucia and Grisolia, 2020, Lucia, 2015).

SARS-CoV-2 has evolved into a great number of variants, each characterized by specific chemical and thermodynamic properties (Popovic and Popovic, 2022; Popovic, 2022a, 2022b). Newer variants differ from earlier ones in genetic sequence and information content. Change in information acquired through mutations can be analyzed using equations similar to those of thermodynamics (Hansen et al., 2018) and leads to change of elemental composition and thermodynamic properties. The host organism (human) represents an open thermodynamic system, characterized by an empirical formula and

thermodynamic properties (Popovic and Minceva, 2020c). Various strains of SARS-CoV-2 compete with each other in the human population, leading to domination of new mutants, from Hu-1 to BA.5 (Popovic and Minceva, 2021a).

The first step in SARS-CoV-2 infection is antigen-receptor binding. The antigen of SARS-CoV-2 is the spike glycoprotein trimer (SGP), while the host cell receptor is the human angiotensin-converting enzyme 2 (hACE2) (Scialo et al., 2020; Cao et al., 2022). SGP-hACE2 binding represents a chemical equilibrium, characterized by a dissociation constant, binding constant, as well as standard thermodynamic properties: standard reaction enthalpy, entropy and Gibbs energy (Gale 2022, 2020, 2019, 2018). Thermodynamic properties play an important role in research on SGP-hACE2 interaction (Head et al., 2022). The energetics of virus-host interactions are important for understanding the infection process (Şimşek et al., 2021). All chemical processes have a thermodynamic driving force in the form of Gibbs energy (Demirel, 2014; Atkins and de Paula, 2011, 2014). Gibbs energy of growth represents the driving force for multiplication of microorganisms (von Stockar, 2013a, 2013b, 2010; Demirel, 2014; Hellingwerf et al., 1982; Westerhoff et al., 1982), including viruses (Popovic and Minceva, 2022a).

Virus-host interactions occur at two levels: at the membrane and in the cytoplasm (Popovic and Minceva, 2021a). The interaction at the membrane represents the antigen-receptor binding. The interaction in the cytoplasm represents virus multiplication. Both processes are driven by Gibbs energy (Popovic and Minceva, 2021a). The goal of this paper is to, starting from dissociation constant of various variants of SARS-CoV-2, calculate binding constants and Gibbs energies of binding

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**Table 1**

Standard thermodynamic properties of SGP-hACE2 binding for the variants BA.1 through BA.4 of SARS-CoV-2. The  $K_D$  data were taken from (Cao et al., 2022). The  $K_B$  and  $\Delta_B G$  values were calculated, as described in the Methods section.

Variant	$K_D$ (M)	$K_B$ ( $M^{-1}$ )	$\Delta_B G$ (kJ/mol)
BA.1	3.05E-09	3.28E+08	-48.60
BA.2	1.56E-09	6.41E+08	-50.27
BA.3	2.62E-09	3.82E+08	-48.98
BA.2.13	1.61E-09	6.21E+08	-50.19
BA.2.12.1	1.77E-09	5.65E+08	-49.95
BA.4	1.67E-08	5.99E+07	-44.39

for Omicron variants BA.1 through BA.4, using thermodynamic methodology.

## 2. Methods

The calculation starts from dissociation constants,  $K_D$ , for SGP of the BA.1, BA.2, BA.3, BA.2.13, BA.2.12.1 and BA.4 Omicron variants of SARS-CoV-2, to the Human ACE2 receptor. The  $K_D$  values were taken from (Cao et al., 2022) and are shown in Table 1. They have been measured using surface plasmon resonance, at room temperature (Cao et al., 2022).

Antigen-receptor binding represents a chemical process. The rate of this process is given by the phenomenological equation

$$r_B = -\frac{L_B}{T} \Delta_B G \quad (1)$$

Where  $r_B$  is the rate of SGP-hACE2 binding,  $L_B$  a constant (known as binding phenomenological coefficient),  $T$  temperature and  $\Delta_B G$  Gibbs energy of SGP-hACE2 binding (Popovic and Popovic, 2022; Popovic, 2022a, 2022b von Stockar, 2013a; Demirel, 2014). The rate of binding is proportional to the absolute value of the Gibbs energy of binding.

Eq. (1) considers binding of one hACE2 receptor to one virus SGP antigen. However, Gale (2022) suggested a more realistic model that considers virus-cell binding and takes into account the fact that a single virus can bind to multiple receptors. The equation has the form Gale (2022)

$$F_B = \frac{1}{1 + \frac{1}{K_{a,virus,T} C_{free}} \left(1 + \frac{[I]}{K_{VI}}\right)} \quad (2)$$

Where  $F_B$  is the fraction of viruses bound to host cells,  $K_{a,virus,T}$  association constant for binding of the virus to host cells at temperature  $T$ ,  $C_{free}$  total number of cells in the host tissue that have hACE2 receptors and can bind to SARS-CoV-2 but have no bound virus,  $[I]$  concentration of a virus inhibitor (if present),  $K_{VI}$  dissociation constant of virus-inhibitor complex (if an inhibitor is present) Gale (2022).

The process of binding of the virus to its receptor can be analyzed at two levels. The first is the binding process of a single virus antigen (SGP) to a single receptor (hACE2), described by a dissociation constant. However, in reality, a virus attaches to the host cell using multiple receptors. This process is characterized by its own specific dissociation constant. Eqs. (1) and (2) are complementary. However, the first is based on a mechanistic model that assumes antigen-receptor binding, while the second is more realistic and describes virus-cell binding.

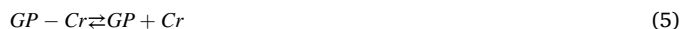
It is well documented that mutations lead to change in Gibbs energy of binding (Barton et al., 2021). The ability of coronaviruses to infect humans is related to their binding strengths to human receptor proteins (Zou et al., 2020). Mutation may induce significant conformational transitions in the spike glycoprotein (Istifli et al., 2021). Natural selection promotes mutations that increase the SGP-hACE2 binding affinity (Istifli et al., 2021). Binding affinity is quantified by Gibbs energy of binding (Gale 2022, 2020, 2019, 2018). Gibbs energy of binding can be calculated from dissociation constants, through the equation

$$\Delta_B G^0 = -R_g T \ln(K_B) \quad (3)$$

Where  $R_g$  is the universal gas constant and  $T$  temperature (Popovic and Popovic, 2022; Du et al., 2016).  $K_B$  represents the binding constant, which is the reciprocal of the dissociation constant  $K_D$  (Du et al., 2016).

$$K_B = \frac{1}{K_D} \quad (4)$$

The dissociation constant is the equilibrium constant of the dissociation reaction of the antigen-receptor complex into the free receptor and antigen



where  $Cr$  represents the host cell receptor (hACE2),  $GP$  the virus antigen (SGP) and  $GP-Cr$  the antigen-receptor complex (Popovic and Popovic, 2022; Du et al., 2016). Thus,  $K_D$  is defined as the ratio of concentrations of the free receptor  $[Cr]$  and antigen  $[GP]$  to the receptor antigen complex  $[GP-Cr]$  (Popovic and Popovic, 2022; Du et al., 2016)

$$K_D = \frac{[GP][Cr]}{[GP-Cr]} \quad (6)$$

Therefore, Gibbs energy of binding is the driving force for the antigen-receptor binding process.

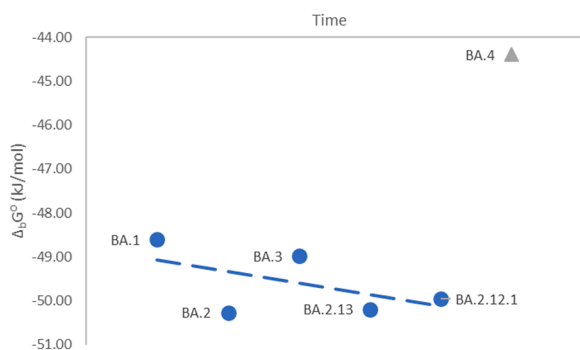
## 3. Results

Starting from SGP-hACE2 dissociation constants for the BA.1, BA.2, BA.3, BA.2.13, BA.2.12.1 and BA.4 variants of SARS-CoV-2, published in (Cao et al., 2022), binding constants and standard Gibbs energies of SGP-hACE2 binding were calculated, as described in the Methods section. They are given in Table 1. Standard Gibbs energies of binding for BA.1 and BA.3 are very similar (-48.60 kJ/mol and -48.98 kJ/mol, respectively). On the other hand, standard Gibbs energies of binding of BA.2, BA.2.13 and BA.2.12.1 also have similar values (-50.27 kJ/mol, -50.19 kJ/mol and -49.95 kJ/mol, respectively). The BA.4 strain is characterized by the least negative standard Gibbs energy of binding (-44.39 kJ/mol). A similar trend can be seen with the binding constants. BA.1 and BA.3 have similar binding constant values ( $3.28 \cdot 10^8 M^{-1}$  and  $3.82 \cdot 10^8 M^{-1}$ , respectively). The variants BA.2, BA.2.13 and BA.2.12.1 are also characterized by similar binding constant values ( $6.41 \cdot 10^8 M^{-1}$ ,  $6.21 \cdot 10^8 M^{-1}$  and  $5.65 \cdot 10^8 M^{-1}$ , respectively). The BA.4 variant has a much lower binding constant compared to the other variants ( $5.99 \cdot 10^7 M^{-1}$ ).

## 4. Discussion

SARS-CoV-2 has evolved many times since 2019, through acquisition of mutations in various locations, and many variants were registered (Sun et al., 2022, Magazine et al., 2022). The latest variants have been recorded in 2022 (Cao et al., 2022). Dissociation constants have been reported for binding of spike glycoprotein trimer (SGP) to human angiotensin-converting enzyme 2 (hACE2), for practically all major SARS-CoV-2 variants (Cao et al., 2022; Wu et al., 2022; Han et al., 2022; Barton et al., 2021; Laffeber et al., 2021; Augusto et al., 2021). Using the SGP-hACE2 dissociation constants, in this paper, binding constants and standard Gibbs energies of binding have been determined (Table 1). For comparison, the binding constant for the Hu-1 variant (wild type) was found to be  $5.88 \cdot 10^7 M^{-1}$ , which gives a Gibbs energy of binding of -44.35 kJ/mol (Popovic, 2022a). Notice that the virus has evolved towards a more negative Gibbs energy of binding. More negative Gibbs energy of binding influences, according to Eq. (1), the antigen-receptor binding rate. The more negative Gibbs energy of binding leads to greater binding rate. The greater binding rate leads to increased infectivity and decreased incubation period.

The BA.2 variant is characterized by a dissociation constant of  $1.56 \cdot$



**Fig. 1.** Standard Gibbs energies of SGP-hACE2 binding of SARS-CoV-2 variants, isolated from November 2021 to January 2022.

$10^{-9}$  M (Cao et al., 2022). This gives a binding constant of  $6.41 \cdot 10^8$   $M^{-1}$  and a Gibbs energy of binding of  $-50.27$  kJ/mol. The trend of decreasing Gibbs energy of binding during evolution of SARS-CoV-2 variants continues in the case of BA.2 (Fig. 1).

The SGP-hACE2 binding constant for BA.3 was calculated to be  $3.82 \cdot 10^8$   $M^{-1}$ , which gives a standard Gibbs energy of binding of  $-48.98$  kJ/mol. The SGP-hACE2 binding constant of BA.2.13 was found to be  $6.21 \cdot 10^8$   $M^{-1}$ , while its standard Gibbs energy of binding was found to be  $-50.19$  kJ/mol. Variant BA.2.12.1 was found to have a SGP-hACE2 binding constant of  $5.65 \cdot 10^8$   $M^{-1}$  and standard Gibbs energy of binding of  $-49.95$  kJ/mol. The calculated values for the variants BA.1 through BA.3 exhibit a decreasing trend in standard Gibbs energy of SGP-hACE2 binding, as can be seen from Fig. 1. This means that binding rate and infectivity of the mentioned variants have increased through evolution, in the period from November 2021 to January 2022. This is in agreement with the predictions of the theory of evolution. Virus evolution is expected to proceed towards better adaptation, meaning an increased infectivity. A possible reason for the increase in infectivity is faster entrance of the virus into the host cell. This could be a consequence of a greater SGP-hACE2 binding rate. The increase in SGP-hACE2 binding rate is, according to Eq. (1), a consequence of a more negative Gibbs energy of binding.

The BA.4 variant is characterized by a SGP-hACE2 binding constant of  $5.99 \cdot 10^7$   $M^{-1}$  and standard Gibbs energy of binding of  $-44.39$  kJ/mol. It is represented by the gray triangle in Fig. 1. It can be noticed that BA.4 is characterized by a less negative Gibbs energy of binding. This implies that BA.4 has a lower SGP-hACE2 binding rate, which should lead to a decreased infectivity. Indeed, (Cao et al., 2022) reported that BA.4 and BA.5 variants have a decreased binding affinity. At the first glance, it seems that evolution of BA.4 and BA.5 opposes the expectations of the theory of evolution and observed trends. However, mutations on BA.4 that led to less negative Gibbs energy of binding and consequent decreased binding affinity, have also led to more efficient evasion of immune response (Barton et al., 2021; Cao et al., 2022). More effective evasion of immune response (Khan et al., 2022; Tuekprakhon et al., 2022) allows better accommodation of the virus to its environment (Barton et al., 2021; Cao et al., 2022). All this can finally result in increased infectivity. It seems that infectivity is a complex phenomenon, which depends on Gibbs energy of binding, binding rate and evading immune response (Harvey et al., 2021).

## 5. Conclusions

Evolution of SARS-CoV-2 variants from BA.1 to BA.3 exhibits a tendency towards more negative Gibbs energy of SGP-hACE2 binding and hence increased SGP-hACE2 binding rate. The goal of this process is in agreement with the expectations of the theory of evolution, in the sense of increased virus infectivity.

BA.4 variant deviates from the trend, in the sense of evolution

towards less negative Gibbs energy of SGP-hACE2 binding, which is followed by more effective evasion of the immune response.

When predictions of the direction of viral evolution are made, it is necessary to have in mind changes in Gibbs energy of binding, binding affinity and evasion of the immune system.

## CRedit authorship contribution statement

**Marko Popovic:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization.

## Declaration of Competing Interest

The author declares no conflict of interest.

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