The silver bullet: A safe and efficient attenuated vaccine for viral diseases based on biothermodynamics

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Abstract: Live attenuated vaccines have through history proved themselves as safe and efficient. Pasteur has developed a vaccine against rabies, through a long process of passage of the virus wild type through rabbits. The result was one of the most efficient attenuated live-virus vaccines. This paper suggests a method based on calculations of biothermodynamic properties of potential tissues for vaccine application, predilected host tissue and virus wild type. Gibbs energy of biosynthesis represents the thermodynamic driving force for virus multiplication. The attenuated strain of the virus should possess Gibbs energy of biosynthesis, which is less negative than the predilected target tissue, but more negative than the vaccine portal tissue. In that way, the attenuated virus strain should be able to multiply in the vaccine portal of entry tissue and cause an immune response, making it efficient. On the other hand, the attenuated strain cannot multiply in the predilected host tissue, making it safe. The attenuation is achieved by adding a gene to the virus, which encodes a ballast protein. Production of the ballast protein would make virus multiplication less favorable, since it would require energy, but be useless to the virus.

Keywords: COVID-19; SARS-CoV-2; Vaccine; Attenuation; Biothermodynamics; Gibbs energy; Permissiveness; Biosynthesis; Multiplication

"*With infectious disease, without vaccines, there's no safety in numbers*."
Seth Berkley

1. Introduction

Epidemiologic measures have played for a long time an important role in suppressing infectious diseases, dating back to Hippocrates [CDC, 2012; Bartlett and Judge, 1997]. However, vaccination is the most effective method of prevention of serious viral infections [Riedel et al., 2019]. Immune response to viral infections develops against specific viral antigens, which are located on the surface of virus particles or virus-infected cells [Riedel et al., 2019]. In the case of enveloped viruses, the important antigens are surface glycoproteins, for example the spike glycoprotein of SARS-CoV-2 [Riedel et al., 2019; Duan et al., 2020]. However, for some viruses, development of vaccines is very difficult [Riedel et al., 2019]. Examples are viruses with many serotypes (e.g. rhinovirus), viruses with large numbers of antigen variants in host reservoirs (e.g. influenza), viruses that integrate their DNA into host chromosomal DNA (retroviruses) and viruses that attack immune cells (HIV) [Riedel et al., 2019; Lim et al., 2021].

Until 2020, two main types of vaccines have been widely available: inactivated killed-virus vaccines and attenuated live-virus vaccines. Inactivated vaccines are made by cultivating and purifying virus particles, which are then inactivated using methods that do minimum damage to viral structural proteins [Riedel et al., 2019]. Inactivated vaccines are made when attenuated vaccines are not available

[Riedel et al., 2019]. However, they are usually less effective than attenuated vaccines [Riedel et al., 2019].

The second type are attenuated live-virus vaccines. The genetic basis for the attenuation of most viral vaccines is not known because they were selected empirically by serial passages in animals or cell cultures (usually from a species different from the natural host) [Riedel et al., 2019]. Attenuated vaccines can be designed in the laboratory [Riedel et al., 2019]. The advantage of attenuated vaccines is that they better simulate natural infection than inactivated vaccines, especially regarding their effect on immunity [Riedel et al., 2019]. Attenuated vaccines are able to multiply inside host tissues and thereby induce a strong cell-mediated immune response, as well as production of antibodies and resistance at the portal of entry [Riedel et al., 2019].

In 1885, Rabies was a huge problem in France and rest of the world, but the microorganism (virus) causing Rabies could not be identified. However, empirically, by passing the virus through rabbits, Pasteur made the virus less dangerous to human hosts (less virulent), while still retaining enough information for the organism to recognize the wild strain antigens and develop immune response to the wild version of the virus [Hicks *et al.*, 2012]. By serial passage of a virus through a different species, the virus becomes more adapted (Pasteur used the word "*fixed*") to that species (i.e. rabbit), and less adapted to its original host (i.e. Human), decreasing virulence with respect to the original host.

The application of attenuated vaccines is highly effective at preventing the development of rabies, and very few failures have been recorded [Hicks *et al.*, 2012]. Furthermore, oral and parenteral vaccination (various portals of entry) is possible for wildlife using inactivated tissue culture-derived virus [Hicks *et al.,* 2012]. Pasteur actually has developed the "attenuated" virus strain characterized by decreased virulence for humans. Thus, the word attenuated corresponds to decrease of virulence. Virulence represents a microbe's ability to infect or damage a host. Virulence allows a virus to enter its host, replicate, modify host defenses and spread within a host [Flint et al., 2009]. Thus, the change in virulence implies change in susceptibility or permissiveness.

Susceptibility and permissiveness have their thermodynamic mechanisms [Gale, 2020; Casasnovas & Springer, 1995; Popovic & Minceva, 2020a, 2020b]. Pasteur has passed the virus through rabbits multiple times and changed susceptibility. Virus has changed the antigen responsible for virus-host receptor interaction from "human adapted" to "rabbit adapted" during the passage. However, this procedure took a long time. This was not important for a disease like Rabies, characterized by a low basic reproduction number R_0, the average number of secondary infections generated by each infected person. Facing the COVID-19 pandemic there is an urgent need for a safe and efficient vaccine, because SARS-CoV-2 has a high basic reproduction number, between 1.0011 and 2.7936 [Al-Raeei, 2020], or even more [Rahman et al., 2020].

After more than 655 million registered cases and more than 6 million casualties, there is a need for efficient and safe immunization against SARS-CoV-2. Moreover, during the period from 2019 to late-2022, several dozen SARS-CoV-2 variants have appeared [Popovic and Popovic, 2022; Popovic, 2022a, 2022b, 2022c, 2022d, 2022e, 2022f, 2022g, 2022h], some of which have obtained an ability of immune evasion [Cao et al., 2022; Wu et al., 2021]. Thus, even if there are several approved vaccines, due to immune evasion, there is a constant need for development of new vaccines that would immunize against new SARS-CoV-2 variants. The available vaccines are based on the Hu-1 variant of SARS-CoV-2. However, the newer Omicron BA.2.75, BQ.1 and XBB variants are capable of avoiding immune response. This is why

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design of new vaccines is important, which would protect against the newer SARS-CoV-2 variants, and be safe and efficient.

Viral infections occur as a consequence of biological, chemical and thermodynamic interaction of viruses and their human host. Currently, we face the COVID-19 pandemic, but we have relatively few antiviral medicaments. The epidemiological measures have showed themselves to be ineffective in controlling the COVID-19 pandemic. Since December 2020, over 12.7 billion doses of various COVID-19 vaccines have been administered [Our World in Data, 2022]. However, the pandemic is still not finished, mostly due to immune evasion by new variants. Thus, we ultimately need new vaccines efficient against new variant of SARS-CoV-2.

Among the vaccine technologies under evaluation are whole virus vaccines, recombinant protein subunit vaccines, and nucleic acid vaccines [Chen et al, 2020]. Molecular biology, immunology and virology combined are looking for novel approaches to vaccine design [Riedel *et al.*, 2019]. One of them is administration of vaccine locally to stimulate antibody at the portal of entry [Riedel *et al.*, 2019]. Live-attenuated vaccines have demonstrated success in treating infections such as smallpox and poliomyelitis [Minor, 2015]. Three SARS-CoV-2 live-attenuated vaccines that utilize a weakened virus as the antigen are under preclinical evaluation [Dong et al, 2020]. Seo et al. [2020] described a cold-adapted live-attenuated vaccine (SARS-CoV-2/human/Korea/CNUHV03-CA22 °C/2020) developed by gradually adapting the growth (biosynthesis) of SARS-CoV-2 from 37 °C to 22 °C in Vero cells. The one-dose vaccinated mice were completely protected from SARS-CoV-2 infection and did not show body weight loss, death, or the presence of virus in tissues, such as the nasal turbinates, brain, lungs, and kidneys [Seo et al, 2020]. Seo et al. [2020] attempt to change susceptibility.

In this paper an attenuated vaccine design is suggested, based on biothermodynamic properties of predilected target tissues of SARS-CoV-2, vaccine portal of entry tissue and SARS-CoV-2 wild strain, for local administration that attempts to change permissiveness. Fortunately, there is the second, time saving option for attenuation - to try to change permissiveness. Permissiveness is the result of chemical reactions – replication, transcription, translation and self-assembly. They occur at a certain reaction rate. The reaction rate depends on kinetic and thermodynamic factors. Kinetic factors can be neglected because the involved enzymes are the same for the host and virus [Popovic and Minceva 2020a, 2020b]. Thermodynamic factors are summarized by the Gibbs energy of biosynthesis [Westerhoff *et al.*,1982; Hellingwerf *et al.*, 1982; Demirel, 2014; von Stockar, 2013a, 2013b; Popovic and Popovic, 2022; Popovic, 2022a, 2022b, 2022c, 2022d, 2022e, 2022f, 2022g]. Thus, decreasing the Gibbs energy of biosynthesis could result in a decrease in biosynthesis rate (replication, transcription, translation) and self-assembly, leading to decrease in virulence and pathogenicity [Popovic, 2022h]. A strain characterized by low permissiveness and subsequently low virulence (and pathogenicity), could represent an attenuated strain. An attenuated virus strain can potentially be used as an attenuated vaccine.

A virus particle, just as any other organism, represents a highly organized amount of substance, containing nucleic acid, proteins, lipids and carbohydrates, characterized by specific elemental composition (empirical formula) [Molla *et al.*, 1991; Wimmer, 2006; Popovic and Minceva 2020a, 2020b; Degueldre, 2021; Şimşek et al., 2021] and thermodynamic properties (enthalpy, entropy and Gibbs energy) [Popovic and Minceva 2020a, 2020b]. Thus, a virus represents a well-defined open biothermodynamic system that performs biological and chemical processes, governed by natural laws [Popovic, 2022h, 2018a; von Bertalanffy, 1950, 1971]. Virus multiplication is a result of chemical reactions [Popovic, 2022h]. It depends on the kinetic and thermodynamic factors [von Stockar, 2014; Popovic and

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Minceva, 2020a, 2020b]. By artificially changing some of them it is possible to influence the multiplication rate and virulence.

The research on viral infections has been made mostly from the perspective of biological and medical disciplines. Viruses are morphologically well characterized [Riedel *et al*., 2019]. The pathogeneses of most viral diseases has been described [Riedel *et al.*, 2019; Baron *et al.*, 1996]. Viruses and cells/organisms represent open biothermodynamic systems of various levels of organization. Thus, nonequilibrium thermodynamics should be used to analyze processes which they perform [von Stockar, 2014; Demirel, 2014; Popovic & Minceva, 2020a, 2020b; Balmer, 2010; Popovic, 2018b]. The biothermodynamic research on virus-host interactions is still in progress. Katen and Zlotnick [2009] analyzed the thermodynamics of capsid assembly, considering it as a polymerization (chemical reaction). Ceres and Zlotnick [2002] used thermodynamics to analyze hepatitis B virus capsid assembly and found that it has a negative Gibbs energy change. Casasnovas & Springer [1995] studied the kinetics and thermodynamics of rhinovirus interaction with its receptor, determining the enthalpy and Gibbs energy of their association. Gale [2021, 2020, 2019, 2018] analyzed thermodynamics of virus binding to host cell receptors and proposed new *directions for designing antiviral therapies*. Mahmoudabadi *et al*. [2017] developed a quantitative description of viral infection energetics and made predictions about viral evolution. In addition to studies of viral component synthesis and self-assembly, Tzlil *et al.* [2004] made a statistical thermodynamic description of viral budding. Jones *et al.* [2015] developed a model of the viral life cycle. Thermodynamic research on virus particles and processes performed by viruses is in progress [Popovic, 2022h]. Indeed, thermodynamics has a great potential for explaining the mechanisms of processes performed by microorganisms [von Stockar, 2013a, 2013b], especially the mechanisms of interactions of viruses and their host cells [Popovic and Minceva, 2020a; Popovic, 2022L, 2022m, 2022n; Mahmoudabadi *et al*., 2017; Tzlil *et al.* 2004].

Except for their biological nature, viruses have a chemical nature [Stanley, 1941, Beard, 1951, Wimmer, 2006]. Empirical formulas (elemental composition) have been determined for a small number of viruses [Molla *et al.*, 1991; Wimmer, 2006; Popovic, 2022b, 2022c, 2022g, 2022i, 2022j; Popovic and Minceva, 2020a, 2020b, 2021a; Degueldre, 2021; Şimşek et al., 2021]. Thermodynamic characterization has been made for some viruses [Popovic, 2022b, 2022c, 2022g, 2022i, 2022j; Popovic and Minceva, 2020a, 2020b, 2021a; Gale, 2021, 2020, 2019, 2018; Şimşek et al., 2021]. The human host organism, also, has a chemical nature. Various human tissues have been characterized chemically [Mitchell *et al.*, 1945; Widdowson *et al.*, 1951; Hendry, 1962; Snyder *et al.*, 1974, p. 290; Woodard and White, 1986; Wang *et al.*, 1993 and Morowitz, 1976] and thermodynamically [Popovic and Minceva 2020c; Mitchell *et al.*, 1945; Duck, 1990; McIntosh and Anderson, 2010; Van den Berg *et al.*, 1983; Colins *et al.*, 2004; Van Leeuwen *et al.*, 1999; Bernardi *et al.*, 2003].

Viral infection appears as the result of interaction of viruses and their hosts. To make an infection, viruses must have a host cell that is susceptible and permissive [Duponchel and Fischer, 2019]. Both susceptibility [Gale, 2020; Casasnovas and Springer, 1995] and permissiveness [Popovic, 2022b, 2022g, 2022h; Popovic and Minceva, 2020a, 2020b] can be quantified through thermodynamics. Thus, the initial steps have been made towards describing susceptibility and permissiveness. High permissiveness of a wild type virus allows efficient multiplication. It is a chemical process, involving replication of viral nucleic acid,

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transcription, translation and self-assembly of components into an enormous number of new virions. Through metabolic processes, the host cell regenerates damaged molecules and grows. These processes are also chemical. Processes performed by viruses and host cells are competitive, since they use the same building blocks [Popovic, 2022b, 2022g, 2022h; Popovic and Minceva, 2020a, 2020b].

Having in mind the current COVID-19 pandemic, it would be good to have a better understanding of susceptibility and permissiveness properties of SARS-CoV-2. These properties are key factors for virus attenuation, leading to development of a vaccine that could be both efficient and safe [Popovic, 2022h]. Hypothetically, attenuation of a virus is possible through change in susceptibility or permissiveness. Pasteur made attenuation through change in susceptibility. However, through change in chemical and thermodynamic properties, starting from the virus wild type, an attenuated strain can be designed, characterized by desired thermodynamic properties, which would decrease permissiveness. Alternatively, selection of appropriate tissue for the portal of vaccine application, characterized by desirable thermodynamic properties of the tissue can be viewed as a method of "attenuation". Attenuated variant could be used as an attenuated vaccine. A vaccine should possess two properties, important for interaction with the host organism: efficiency and safety.

The goal of this paper is to introduce a new method developed for attenuation of SARS-CoV-2 variants, based on biothermodynamics. Biothermodynamic analysis allows to predict susceptibility and permissiveness for SARS-CoV-2 and other viruses. This fact can be used to create a new attenuated strain, which can represent an attenuated vaccine. Moreover, biothermodynamic analysis allows us to select the portal and method of application for the attenuated live-virus vaccine.

2. Methods

This section begins with specifying the sources of data that were used as a starting point for this research (Section 2.1). Next the design of SARS-CoV-2 variants is discussed, which can be used as attenuated live-virus vaccines (Section 2.2). Then, the methods used for calculating elemental compositions of virus particles and formulating biosynthesis reactions are discussed (Section 2.3). Finally, predictive biothermodynamic models used to find standard thermodynamic properties of virus live matter and biosynthesis are presented (Section 2.4).

2.1. Data sources

The genetic sequences of the Hu-1, Delta and Omicron BA.1 variants of SARS-CoV-2 were taken from the NCBI database [Sayers et al., 2022; NCBI, 2022]. The genetic sequence of the Hu-1 variant can be found under the accession number NC_045512. The genetic sequence of the Delta variant can be found under the accession number OM471068.1. The genetic sequence of the Omicron BA.1 variant van be found under the accession number OL869974.1.

The protein sequences of the Hu-1, Delta and Omicron BA.1 variants were obtained from the NCBI database [Sayers et al., 2022; NCBI, 2022]. For the Hu-1 variant, the nucleoprotein (N-protein) was found under the accession number QIK50455.1, the membrane protein (M-protein) was found under the accession number QHR63293.1, and the spike glycoprotein (S-protein or SGP) was found under the accession number QHR63290.2. For the Delta variant, the nucleoprotein was found under the accession

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number UIO52968.1, the membrane protein was found under the accession number QUX81285.1, and the spike glycoprotein was found under the accession number UKA47839.1. For the Omicron BA.1 variant, the nucleoprotein was found under the accession number UGY75362.1, the membrane protein was found under the accession number UFO69282.1, and the spike glycoprotein was found under the accession number UGY75354.1. Protein copy numbers and virus morphology data were taken from [Neuman and Buchmeier, 2016; Neumann et al., 2011, 2006].

Standard Gibbs energies of biosynthesis, $\Delta_{bs}G^o$, of the nucleic acid and all structural proteins of the Hu-1 wild type and the Omicron BA.2.75 variant were taken from [Popovic, 2022g]. Standard Gibbs energy of biosynthesis for the nucleic acid and all structural proteins of the Hu-1 variant is -172.84 kJ/C-mol, while that of the Omicron BA.2.75 variant is -173.57 kJ/C-mol [Popovic, 2022g].

Elemental compositions and standard thermodynamic properties of live matter for the human host tissues were obtained from [Popovic and Minceva, 2020c]. These include standard enthalpy of formation, $\Delta_f H^o$, standard molar entropy, S^o_m, and standard Gibbs energy of formation, $\Delta_f G^o$, of live matter.

2.2. Attenuated live-virus vaccine variants

Biothermodynamics gives an insight into energetics of virus-host interactions at the membrane (antigen-receptor binding) and in the cytoplasm (biosynthesis of virus particles). Every virus particle is characterized by enthalpy, entropy and Gibbs energy of biosynthesis. Every variant has its specific thermodynamic properties [Popovic and Popovic, 2022; Popovic, 2022a, 2022b, 2022c, 2022d, 2022e, 2022f, 2022g, 2022h, 2022j]. Thus, there are SARS-CoV-2 variants with various values of thermodynamic properties. The variant that has the least negative Gibbs energy of biosynthesis will multiply the slowest [Popovic, 2022b, 2022g, 2022h].

Multiplication rate depends on the type of host tissue. The rate of biosynthesis of cell building blocks cannot be modified. However, it is possible to modify virus particles to make its Gibbs energy of biosynthesis approximately equal to the rate of biosynthesis of the building blocks of host cells, through genetic engineering. The rate of biosynthesis of the wild type virus is much greater than that of host cell building blocks. This is why the virus can hijack the host cell's metabolic machinery. However, the modified variant that would have a Gibbs energy of biosynthesis approximately equal to that of the host cell, would multiply more slowly, since the permissiveness coefficient would decrease. This would lead to decrease in virulence and pathogenicity of the virus, enabling the virus to be produced in small amount, locally in the tissue of application of the attenuated strain, which would make it recognizable to the immune system. This could lead to an immune response. An attenuated strain applied locally to chosen tissues would cause an immune response. In a subsequent infection with the wild type or a more virulent variant that appeared through the evolution of the virus, the immune system would be able to give an adequate immune response.

The attenuation method of the virus implies adding ballast genetic material, with the goal of making Gibbs energy of biosythesis of the virus particle less negative, making it equal to the Gibbs energy of biosynthesis of the components of the host cell. This greatly decreases the driving force – Gibbs energy of biosynthesis of the virus. In that way, the virus has a lower ability to hijack the host cell's metabolism

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and lower biosynthesis (and multiplication) rate. This can theoretically be achieved through replacement of the domain encoding the envelope (E) protein [Neuman and Buchmeier, 2016]. Manipulations on domains that encode the spike glycoprotein, membrane protein and nucleoprotein result in inviable virus particles [Neuman and Buchmeier, 2016]. Attenuation of viruses could be conducted on the genome, by replacement of GC with AU segments. The added genes would encode ballast proteins, which would require energy for synthesis, but serve no purpose to the virus. The additional energy expenditure would make virus multiplication even less thermodynamically favorable.

The attenuated variants of SARS-CoV-2 were made by replacing the sequence encoding the envelope protein in the Hu-1 wild type, with ballast sequences. The Ile450 variant was made by adding a genetic sequence that encodes a 450 amino acid long chain consisting of isoleucine, to the genome of the Hu-1 variant, instead of the envelope protein gene. Also, one ballast protein consisting of 450 isoleucine residues was added for each spike glycoprotein in the virus particle.

The AU450 variant was made by replacing the envelope protein gene in Hu-1 with a gene that encodes a ballast protein consisting of isoleucine, lysine, phenylalanine, tyrosine, asparagine and leucine in the ratio 2:1:1:1:1:1. The ballast protein is 450 amino acids long. One copy of the ballast protein was added for each spike glycoprotein in the virus particle.

The AU901 variant made by replacing the envelope protein gene from the Hu-1 variant with a gene encoding a ballast protein consisting of isoleucine, lysine, phenylalanine, tyrosine, asparagine and leucine, in the ratio 2:1:1:1:1:1. The ballast protein is 901 amino acids long. One copy of the ballast protein was added for every spike glycoprotein in the virus particle.

The resulting modified Ile450, AU450 and AU901 variants have a less negative the Gibbs energy of biosynthesis. This leads to a lower driving force for biosynthesis, lower rate of biosynthesis of viral components, lower multiplication rate and lower damage to host tissues, as will be discussed below.

2.3. Elemental composition and biosynthesis reactions

Genetic and protein sequences were used to find empirical formulas of nucleic acids and all proteins of the Delta, Omicron BA.1, Ile450, AU450 and AU901 variants of SARS-CoV-2. This was done using the atom counting method [Popovic, 2022i]. The atom counting method is implemented using a computer program [Popovic, 2022i]. The input are genetic and protein sequences of the virus of interest, as well as the number of copies of proteins in the virus particle and the virus particle size [Popovic, 2022i]. The program goes along the nucleic acid and protein sequences and adds atoms coming from each residue in the sequence, to find the number of atoms contributed by that macromolecule to the virus particle [Popovic, 2022i]. The contributions of viral proteins are multiplied by their copy numbers, since proteins are present in multiple copies in virus particles [Popovic, 2022i]. The output of the program is elemental composition of virus particles, in the form of empirical formulas, and molar masses of virus particles [Popovic, 2022i]. The advantage of the atom counting method is that it can provide the empirical formulas of virus particles, based on widely available data on genetic and protein sequences [Popovic, 2022i]. The

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atom counting method was shown to give results in good agreement with experimental results [Popovic, 2022i].

The empirical formulas of virus particles were used to construct biosynthesis reactions, summarizing conversion of nutrients into new live matter [von Stockar, 2013a, 2013b; Battley, 1998]. The biosynthesis reaction for virus particles has the general form

$$(\text{Amino acid}) + O_2 + HPO_4^{2-} + HCO_3^- \longrightarrow (\text{Bio}) + SO_4^{2-} + H_2O + H_2CO_3 \quad (1)$$

where (Bio) represents new live matter, described by an empirical formula given by the atom counting method [Popovic, 2022b, 2022g]. (Amino acid) represents a mixture of amino acids with the empirical formula $CH_{1.798}O_{0.4831}N_{0.2247}S_{0.022472}$ (expressed per mole of carbon), representing the source of energy, carbon, nitrogen and sulfur [Popovic, 2022b, 2022g]. O_2 is the electron acceptor [Popovic, 2022b, 2022g]. HPO_4^{2-} is the source of phosphorus [Popovic, 2022b, 2022g]. HCO_3^- is a part of the bicarbonate buffer that takes excess H^+ ions that are generated during biosynthesis [Popovic, 2022b, 2022g]. SO_4^{2-} is an additional metabolic product that takes excess sulfur atoms [Popovic, 2022b, 2022g]. H_2CO_3 takes the oxidized carbon atoms and is also a part of the bicarbonate buffer [Popovic, 2022b, 2022g].

Biosynthesis reactions for the host tissues have the general form

$$(\text{Amino acid}) + CH_2O + O_2 + HPO_4^{2-} + HCO_3^- + Na^+ + K^+ + Cl^- \longrightarrow (\text{Bio}) + SO_4^{2-} + H_2O + H_2CO_3 \quad (2)$$

where CH_2O represents a carbohydrate molecule and (Bio) represents the empirical formula of live matter of the human host tissue [Popovic, 2022k]. The ions Na^+, K^+ and Cl^- represent the sources of sodium, potassium and chlorine, respectively [Popovic, 2022k].

2.4. Standard thermodynamic properties of live matter and biosynthesis

Empirical formulas of virus nucleocapsids were used to find standard thermodynamic properties of their live matter, using predictive biothermodynamic models: the Patel-Erickson and Battley equations [Patel and Erickson, 1981; Battley, 1999, 1998, 1992]. The Patel-Erickson equation was used to find enthalpy of live matter, based on its elemental composition. The Patel-Erickson equation gives standard enthalpy of combustion, $\Delta_C H^o$, of live matter

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

where E is number of electrons transferred to oxygen during combustion [Patel and Erickson, 1981; Battley, 1998, 1992; Popovic, 2019]. E can be calculated from the empirical formula of live matter

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

where n_C, n_H, n_O, n_N, n_P and n_S represent the number of C, H, O, N, P and S atoms in the live matter empirical formula, respectively [Patel and Erickson, 1981; Battley, 1998, 1992; Popovic, 2019]. Once calculated using the Patel-Erickson equation, $\Delta_C H^o$ can be converted into standard entropy of formation, $\Delta_f H^o$, of live matter. $\Delta_C H^o$ is the enthalpy change of the reaction of complete combustion of live matter.

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$C_{nC}H_{nH}O_{nO}N_{nN}P_{nP}S_{nS} + (n_C + ¼\ n_H + 1¼\ n_P + 1½\ n_S - ½\ n_O)\ O_2 \rightarrow n_C\ CO_2 + ½\ n_H\ H_2O + ½\ n_N\ N_2 + ¼\ n_P\ P_4O_{10} + n_S\ SO_3$ (5)

This means that $\Delta_C H^o$ can be used to find $\Delta_f H^o$ of live matter using the equation, using the equation [Popovic, 2022b; Atkins and de Paula, 2011, 2014; Popovic, 2019]

$$\Delta_f H^0(bio) = n_C\ \Delta_f H^0(CO_2) + \frac{n_H}{2}\ \Delta_f H^0(H_2O) + \frac{n_P}{4}\ \Delta_f H^0(P_4O_{10}) + n_S\ \Delta_f H^0(SO_3) - \Delta_C H^0 \quad (6)$$

The Battley equation gives standard molar entropy of live matter, S^o_m, based on its empirical formula

$$S^0_m(bio) = 0.187\ \sum_J \frac{S^0_m(J)}{a_J} n_J \quad (7)$$

where n_J is the number of atoms of element J in the empirical formula of live matter [Battley, 1999; Battley and Stone, 2000; Popovic, 2019]. S^o_m and a_J are standard molar entropy and number of atoms per formula unit of element J in its standard state elemental form [Battley, 1999; Battley and Stone, 2000; Popovic, 2019]. The Battley equation can be modified to give standard entropy of formation, $\Delta_f S^o$, of live matter [Battley, 1999; Battley and Stone, 2000; Popovic, 2019]

$$S^0_m(bio) = -0.813\ \sum_J \frac{S^0_m(J)}{a_J} n_J \quad (8)$$

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

$$\Delta_f G^0(bio) = \Delta_f H^0(bio) - T\Delta_f S^0(bio) \quad (9)$$

Once live matter is characterized by finding its $\Delta_f H^o$, S^o_m and $\Delta_f G^o$, these properties can be combined with biosynthesis reactions to find standard thermodynamic properties of biosynthesis. Standard thermodynamic properties of biosynthesis include standard enthalpy of biosynthesis, $\Delta_{bs} H^o$, standard entropy of biosynthesis, $\Delta_{bs} S^o$, and standard Gibbs energy of biosynthesis, $\Delta_{bs} G^o$. These properties are found by applying the Hess's law to biosynthesis reactions

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

$$\Delta_{bs} S^0 = \sum_{products} \nu\ S^o_m - \sum_{reactants} \nu\ \ S^o_m \quad (11)$$

$$\Delta_{bs} G^0 = \sum_{products} \nu\ \Delta_f G^0 - \sum_{reactants} \nu\ \Delta_f G^0 \quad (12)$$

where ν represents a stoichiometric coefficient [Popovic, 2022b; Atkins and de Paula, 2011, 2014; von Stockar, 2013a, 2013b; Battley, 1998]. The most important of these three properties is standard Gibbs energy of biosynthesis, which represents the thermodynamic driving force for growth of all organisms [von Stockar, 2013a, 2013b; von Stockar and Liu, 1999], including viruses [Popovic, 2022b].

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3. Results

Elemental composition, in the form of empirical formulas, was calculated for nucleic acids and all structural proteins of SARS-CoV-2 variants, including Delta, Omicron BA.1, Ile450, AU450 and AU901. They are reported in Table 1. The empirical formula of nucleic acid and all structural proteins of the Delta variant is $CH_{1.5637}O_{0.3203}N_{0.2930}P_{0.003839}S_{0.005528}$. The empirical formula of the nucleic acid and all structural proteins of the Omicron BA.1 variant is $CH_{1.5665}O_{0.3201}N_{0.2935}P_{0.003834}S_{0.004904}$. The empirical formula of the nucleic acid and all structural proteins of the Ile450 variant is $CH_{1.6074}O_{0.3226}N_{0.2848}P_{0.003693}S_{0.004557}$. The empirical formula of the nucleic acid and all structural proteins of the AU450 variant is $CH_{1.5543}O_{0.2861}N_{0.2579}P_{0.003133}S_{0.003062}$. The empirical formula of the nucleic acid and all structural proteins of the AU901 variant is $CH_{1.5496}O_{0.2703}N_{0.2417}P_{0.002827}S_{0.002223}$.

Table 1: Elemental compositions of SARS-CoV-2 variants. This table shows elemental composition in form of empirical formulas of nucleic acid and all structural proteins for Delta, Omicron BA.1, Ile450, AU450 and AU901 variants of SARS-CoV-2. All the empirical formulas are expressed per mole of carbon. They have the general form $C_{nC}H_{nH}O_{nO}N_{nN}P_{nP}S_{nS}$.

Variant	C	H	O	N	P	S
Delta	1	1.5637	0.3203	0.2930	0.003839	0.005528
Omicron BA.1	1	1.5665	0.3201	0.2935	0.003834	0.004909
Ile450	1	1.6074	0.3226	0.2848	0.003693	0.004557
AU450	1	1.5543	0.2861	0.2579	0.003133	0.003062
AU901	1	1.5496	0.2703	0.2417	0.002827	0.002223

Based on the empirical formulas, biosynthesis reactions were constructed for nucleic acids and all structural proteins of SARS-CoV-2 variants, including Delta, Omicron BA.1, Ile450, AU450 and AU901. They are reported in Table 2.

Table 2: Stoichiometry of biosynthesis of SARS-CoV-2 variants. This table shows biosynthesis reactions of nucleic acid and all structural proteins for Delta, Omicron BA.1, Ile450, AU450 and AU901 variants of SARS-CoV-2. (Bio) denotes the empirical formula of live matter from Table 1.

Variant	Reactants				→	Products			
	Amino acid	O_2	HPO_4^{2-}	HCO_3^-		Bio	SO_4^{2-}	H_2O	H_2CO_3
Delta	1.3037	0.3748	0.0038	0.0399	→	1	0.0238	0.0683	0.3436
Omicron BA.1	1.3060	0.3778	0.0038	0.0412	→	1	0.0244	0.0660	0.3473
Ile450	1.2676	0.3217	0.0037	0.0405	→	1	0.0239	0.0497	0.3080
AU450	1.1479	0.1711	0.0031	0.0392	→	1	0.0227	0.0887	0.1871
AU901	1.0758	0.0765	0.0028	0.0382	→	1	0.0220	0.0987	0.1140

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Table 3 gives standard thermodynamic properties of live matter for the nucleic acids and all structural proteins of SARS-CoV-2 variants, including Delta, Omicron BA.1, Ile450, AU450 and AU901. These include standard enthalpy of formation, $\Delta_f H^o$, standard molar entropy, S^o_m, and standard Gibbs energy of formation, $\Delta_f G^o$. For the Delta variant, standard enthalpy of formation is -69.08 kJ/C-mol, standard molar entropy is 31.59 J/C-mol K, and standard Gibbs energy of formation is -28.13 kJ/C-mol. For the Omicron BA.1 variant, standard enthalpy of formation is -69.29 kJ/C-mol, standard molar entropy is 31.63 J/C-mol K, and standard Gibbs energy of formation is -28.29 kJ/C-mol. For the Ile450 variant, standard enthalpy of formation is -71.21 kJ/C-mol, standard molar entropy is 32.02 J/C-mol K, and standard Gibbs energy of formation is -29.71 kJ/C-mol. For the AU450 variant, standard enthalpy of formation is -61.72 kJ/C-mol, standard molar entropy is 30.17 J/C-mol K, and standard Gibbs energy of formation is -22.61 kJ/C-mol. For the AU901 variant, standard enthalpy of formation is -58.23 kJ/C-mol, standard molar entropy is 29.52 J/C-mol K, and standard Gibbs energy of formation is -19.97 kJ/C-mol.

Table 3: Standard thermodynamic properties of live matter of SARS-CoV-2 variants. This table shows standard enthalpy of formation, $\Delta_f H^o$, standard molar entropy, S^o_m, and standard Gibbs energy of formation, $\Delta_f G^o$. Thermodynamic properties are reported for nucleic acid and all structural proteins (nucleoprotein, membrane protein and spike glycoprotein) for Delta, Omicron BA.1, Ile450, AU450 and AU901 variants of SARS-CoV-2.

Variant	$\Delta_f H^o$ (kJ/C-mol)	S^o_m (J/C-mol K)	$\Delta_f G^o$ (kJ/C-mol)
Delta	-69.08	31.59	-28.13
Omicron BA.1	-69.29	31.63	-28.29
Ile450	-71.21	32.02	-29.71
AU450	-61.72	30.17	-22.61
AU901	-58.23	29.52	-19.97

Table 4 gives standard thermodynamic properties of biosynthesis for nucleic acids and all structural proteins of SARS-CoV-2 variants, including Delta, Omicron BA.1, Ile450, AU450 and AU901. These include standard enthalpy of biosynthesis, $\Delta_{bs} H^o$, standard entropy of biosynthesis, $\Delta_{bs} S^o$, and standard Gibbs energy of biosynthesis, $\Delta_{bs} G^o$. For the Delta variant, standard enthalpy of biosynthesis is -178.07 kJ/C-mol, standard entropy of biosynthesis is -26.81 J/C-mol K, and standard Gibbs energy of biosynthesis is -170.10 kJ/C-mol. For the Omicron BA.1 variant, standard enthalpy of biosynthesis is -179.62 kJ/C-mol, standard entropy of biosynthesis is -27.06 J/C-mol K, and standard Gibbs energy of biosynthesis is -171.57 kJ/C-mol. For the Ile450 variant, standard enthalpy of biosynthesis is -153.82 kJ/C-mol, standard entropy of biosynthesis is -22.20 J/C-mol K, and standard Gibbs energy of biosynthesis is -147.22 kJ/C-mol. For the AU450 variant, standard enthalpy of biosynthesis is -84.20 kJ/C-mol, standard entropy of biosynthesis is -8.63 J/C-mol K, and standard Gibbs energy of biosynthesis is -81.66 kJ/C-mol. For the AU901 variant, standard enthalpy of biosynthesis is -40.54 kJ/C-mol, standard entropy of biosynthesis is -0.17 J/C-mol K, and standard Gibbs energy of biosynthesis is -40.52 kJ/C-mol.

Table 4: Standard thermodynamic properties of biosynthesis of SARS-CoV-2 variants. This table shows standard enthalpy of biosynthesis, $\Delta_{bs} H^o$, standard entropy of biosynthesis, $\Delta_{bs} S^o$, and standard Gibbs

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energy of biosynthesis, $\Delta_{bs}G^o$. Thermodynamic properties are reported for nucleic acid and all structural proteins (nucleoprotein, membrane protein and spike glycoprotein) for Delta, Omicron BA.1, Ile450, AU450 and AU901 variants of SARS-CoV-2.

Variant	$\Delta_{bs}H^o$ (kJ/C-mol)	$\Delta_{bs}S^o$ (J/C-mol K)	$\Delta_{bs}G^o$ (kJ/C-mol)
Delta	-178.07	-26.81	-170.10
Omicron BA.1	-179.62	-27.06	-171.57
Ile450	-153.82	-22.20	-147.22
AU450	-84.20	-8.63	-81.66
AU901	-40.54	-0.17	-40.52

Based on elemental composition of human host tissues given in Table 5, biosynthesis reactions were formulated and are presented in Table 6. The results from Table 6 were combined with thermodynamic properties of live matter from Table 7, to find standard thermodynamic properties of biosynthesis of human host tissues. These are reported in Table 8 and include standard enthalpies of biosynthesis, $\Delta_{bs}H^o$, standard entropies of biosynthesis, $\Delta_{bs}S^o$, and standard Gibbs energies of biosynthesis, $\Delta_{bs}G^o$. For the adipose tissue, standard enthalpy of biosynthesis is -37.48 kJ/C-mol, standard entropy of biosynthesis is 52.61 J/C-mol K, and standard Gibbs energy of biosynthesis is -51.88 kJ/C-mol. For the skeletal muscle tissue, standard enthalpy of biosynthesis is -6.60 kJ/C-mol, standard entropy of biosynthesis is 12.37 J/C-mol K, and standard Gibbs energy of biosynthesis is -10.18 kJ/C-mol. For the skin tissue, standard enthalpy of biosynthesis is -15.06 kJ/C-mol, standard entropy of biosynthesis is 22.33 J/C-mol K, and standard Gibbs energy of biosynthesis is -21.29 kJ/C-mol. For the tissue of the lower respiratory pathways, standard enthalpy of biosynthesis is -50.51 kJ/C-mol, standard entropy of biosynthesis is -2.80 J/C-mol K, and standard Gibbs energy of biosynthesis is -49.76 kJ/C-mol.

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Table 5: Elemental composition of human host tissues. This table shows elemental composition of human host tissues, in the form of empirical formulas. The empirical formulas have the general form $C_{nC}H_{nH}O_{nO}N_{nN}P_{nP}S_{nS}Na_{nNa}K_{nK}Cl_{nCl}$. Data taken from [Popovic and Minceva, 2020c].

Tissue	Live matter composition								
	C	H	O	N	P	S	Na	K	Cl
Adipose	1	1.8005	0.1218	0.0216	0.000000	0.000725	0.001011	0.000000	0.000655
Skeletal muscle	1	1.6390	0.2695	0.2039	0.005423	0.007858	0.003653	0.008593	0.002369
Skin	1	1.6631	0.2195	0.1578	0.001551	0.004495	0.004179	0.001229	0.004065
Lower respiratory pathways	1	1.6268	0.2836	0.2532	0.007386	0.010702	0.009951	0.005851	0.009679

Table 6: Biosynthesis reactions of human host tissues. (Bio) denotes newly synthetized live matter, represented by an empirical formula from table 5.

Tissue	Reactants								→	Products			
	Amino acid	CH_2O	O_2	HPO_4^{2-}	HCO_3^-	Na^+	K^+	Cl^-		Bio	SO_4^{2-}	H_2O	H_2CO_3
Adipose	0.0960	1.2713	0.0000	0.0000	0.0032	0.0010	0.0000	0.0007	→	1	0.0014	0.0884	0.3704
Skeletal muscle	0.9073	0.1696	0.0000	0.0054	0.0241	0.0037	0.0086	0.0024	→	1	0.0125	0.0794	0.1010
Skin	0.7021	0.4433	0.0000	0.0016	0.0208	0.0042	0.0012	0.0041	→	1	0.0113	0.0878	0.1662
Lower respiratory pathways	1.1266	0.0000	0.1070	0.0074	0.0206	0.0100	0.0059	0.0097	→	1	0.0146	0.0661	0.1472

Table 7: Standard thermodynamic properties of live matter of human host tissues. This table shows standard enthalpies of formation, $\Delta_f H^o$, standard molar entropies, S^o_m, and standard Gibbs energies of formation, $\Delta_f G^o$. Data taken from [Popovic and Minceva, 2020c].

Tissue	$\Delta_f H^o$ (kJ/C-mol)	S^o_m (J/C-mol K)	$\Delta_f G^o$ (kJ/C-mol)
Adipose	-33.27	25.78	0.15
Skeletal muscle	-62.25	30.16	-23.16
Skin	-50.88	28.57	-13.85
Lower respiratory pathways	-65.61	31.37	-24.94

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Table 8: Standard thermodynamic properties of biosynthesis of human host tissues. This table shows standard enthalpies of biosynthesis, $\Delta_{bs}H^o$, standard entropies of biosynthesis, $\Delta_{bs}S^o$, and standard Gibbs energies of biosynthesis, $\Delta_{bs}G^o$.

Tissue	$\Delta_{bs}H^o$ (kJ/C-mol)	$\Delta_{bs}S^o$ (J/C-mol K)	$\Delta_{bs}G^o$ (kJ/C-mol)
Adipose	-37.48	52.61	-51.88
Skeletal muscle	-6.60	12.37	-10.18
Skin	-15.06	22.33	-21.29
Lower respiratory pathways	-50.51	-2.80	-49.76

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4. Discussion

The results of the research are used to develop a new methodology for designing attenuated live-virus vaccines. The elemental compositions and thermodynamic properties of live matter of viruses and their host cells are first compared (Section 4.1). Then, thermodynamic properties of biosynthesis of the viruses are discussed (Section 4.2). This is then used to develop a new method for attenuated vaccine design (Section 4.3).

4.1. Chemical and thermodynamic properties of live matter

Tables 1 and 5 show elemental composition of SARS-CoV-2 strains and human host tissues, respectively, in the form of empirical formulas. The C:H ratios of the SARS-CoV-2 strains are lower than those of the human tissues. On the other hand, the C:O and C:N ratios of the SARS-CoV-2 strains are higher than those of the human host tissues. The C:P ratios of the SARS-CoV-2 strains and the human tissues are not very different. The C:S ratio of SARS-CoV-2 strains is slightly greater than for the human tissues.

Tables 3 and 7 show standard thermodynamic properties of live matter of the SARS-CoV-2 strains and human host tissues, respectively. Standard enthalpies and Gibbs energies of formation of SARS-CoV-2 live matter are more negative than those of the host tissues. Standard molar entropies of SARS-CoV-2 live matter are slightly greater than those of the host tissues.

4.2. *Thermodynamic properties of biosynthesis*

Tables 4 and 8 show standard thermodynamic properties of biosynthesis of the SARS-CoV-2 strains and human host tissues. Standard enthalpies of the natural Delta and Omicron BA.1 variants are much more negative than those of the human tissues. This means that biosynthesis of the virus particles is much more exothermic, making them thermodynamically more favorable. Standard entropies of biosynthesis of the Delta and Omicron BA.1 particles are negative, while those of host tissues are positive. This means that the entropic $-T\Delta S$ component for biosynthesis of the Delta and Omicron variants is not thermodynamically favorable. However, the much more negative enthalpies of biosynthesis overweigh the unfavorable entropic component, resulting in much more negative Gibbs energies of biosynthesis of the Delta and Omicron variants than those of the host tissues. Thus, production of viral components has a much greater thermodynamic driving force than production of the host cell building blocks.

Except for allowing for the biosynthesis process to occur, the thermodynamic driving force is important since it determines the biosynthesis rate. Gibbs energy of biosynthesis, $\Delta_{bs}G$, is proportional to the biosynthesis rate, r_{bs}, according to the biosynthesis phenomenological equation

$$r_{bs} = -\frac{L_{bs}}{T}\Delta_{bs}G \tag{13}$$

where T is temperature and L_{bs} is the biosynthesis phenomenological coefficient [Popovic, 2022b, 2022c, 2022g]. Thus, the more negative Gibbs energy of biosynthesis of the Delta and Omicron variants will make their biosynthesis proceed faster than those of the host cell building blocks [Popovic, 2022b, 2022c, 2022g]. Thus, practically only viral components will be produced and the virus will hijack the host cell's

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metabolism [Popovic, 2022b, 2022c, 2022g]. Since the Delta and Omicron variants have a much more negative Gibbs energy of biosynthesis (Table 4) than the four human host tissues (Table 8), they will be able to multiply in all four human tissues.

Adding ballast sequences to the SARS-CoV-2 virus makes their production thermodynamically less favorable. The Ile450 variant was made by adding, for each of spike glycoprotein, a 450 amino acid long sequence consisting of Ile residues. This sequence does not encode for a useful protein for the virus, but adds a metabolic burden to the biosynthesis of the virus particles, since additional Ile polypeptide chains need to be produced, as well as the nucleic acid encoding for the ballast polypeptide.

The additional metabolic burden of the Ile450 variant made the enthalpy of biosynthesis less negative than those of the Delta and Omicron variants (Table 4) and closer to those of the host tissues (Table 8). The entropy of biosynthesis of the Ile450 is slightly more favorable than those of the Delta and Omicron variants. However, this cannot overweigh the less favorable enthalpy, making Gibbs energy of biosynthesis of the Ile450 variant less negative than those of the Delta and Omicron variants. Thus, adding a ballast sequence to the SARS-CoV-2 virus has made its multiplication less thermodynamically favorable, due to the energy cost of producing the ballast sequence. The less favorable driving force will result in slower virus multiplication, according to the biosynthesis phenomenological equation (13). The Ile450 variant will thus cause less damage to the host tissue than the Delta and Omicron variants.

The ballast effect described above is even more pronounced in the AU450 and AU901 variants, which were made by adding even longer ballast sequences to the viral nucleic acid. These sequences encode proteins that are useless to the virus, but require energy to be produced. This waste of energy makes the virus biosynthesis less thermodynamically favorable and thus slower, according to the biosynthesis phenomenological equation (13). The slower biosynthesis rate will make it more difficult for the viruses to hijack the host cell metabolism.

4.3. Designing an attenuated vaccine

Experience learns us that a goal can be achieved in several ways. If the goal is to produce a safe and efficient vaccine, then 4 types of vaccines has been developed in 4 different ways. First, whole virus vaccines belong to the inactivated vaccines. They are safe, but require two doses. Its portal of application is intramuscular. Second, RNA or mRNA vaccines are also administered intramuscularly and require two or more doses. Third, nonreplicating viral vector vaccines require two doses and their portal of application is also intramuscular. Fourth, protein subunit requires two or more doses and their portal of entry is intramuscular. Notice that the portal of application for all the available vaccines is the muscle tissue. However, this paper proposes a fifth type of vaccine – live attenuated vaccine. The idea is based on biothermodynamic properties – Gibbs energies of biosynthesis of the host tissue and virus, and permissiveness.

Gibbs energy of biosynthesis is the driving force for virus multiplication and synthesis of building blocks of the host cell. These two chemical reactions are competitive [Popovic and Minceva, 2020a; Popovic, 2022g]. According to the biosynthesis phenomenological equation, which belong to nonequilibrium thermodynamics, chemical reaction rate (multiplication of viruses and biosynthesis of the

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building blocks of the host cell) are proportional to Gibbs energy. Since the reactions are competitive, the reaction that is characterized by more negative Gibbs energy will occur [Popovic, 2022h]. There are three possible outcomes of the competition. (a) The Gibbs energy of biosynthesis of the virus is much more negative than the Gibbs energy of biosynthesis of the host cell building blocks. In this case, reactions of biosynthesis of virus components, self-assembly and cell damage will occur. The outcome of this is clinical manifestation of COVID-19. (b) On the other hand, if Gibbs energy of biosynthesis of the host cell building blocks is much more negative than Gibbs energy of biosynthesis of viral components, then the virus will not be able to multiply at all. In that case, COVID-19 will not appear. (c) The third possibility is that Gibbs energy of biosynthesis of the virus is more negative than Gibbs energy of biosynthesis of the tissue where the attenuated strain is applied, but less negative than Gibbs energy of biosynthesis of the predilected tissue for the wild type virus (i.e. respiratory mucosa). In that case, the attenuated strain will be able to multiply slowly only in the tissue where the vaccine is applied, but will not be able to multiply in the predilected tissue of the wild type virus and cause COVID-19.

To successfully develop a vaccine based on the biothermodynamic background, it is necessary to know biothermodynamic properties of biosynthesis for various human tissues, especially for the predilected tissue (i.e. respiratory mucosa) and potential tissues for vaccine application (i.e. muscles, skin, adipose tissues etc.). Thermodynamic properties of biosynthesis for various human tissues are given in [Popovic, 2022k; Popovic and Minceva, 2020c]. It is also necessary to know thermodynamic properties for the virus wild type and for the attenuated strain. They are given in Figure 1. The orange line denotes the Gibbs energy of biosynthesis of the predilected tissue of SARS-CoV-2: lower respiratory pathways. Figure 1 also shows calculated thermodynamic properties for several variants of the attenuated virus.

The AU901 is characterized by a Gibbs energy of biosynthesis that is less negative than Gibbs energy of biosynthesis of the predilected tissue – lower respiratory pathways. The ratio of Gibbs energies of biosynthesis of the virus and its host tissue is the permissiveness coefficient for the virus, *P*. The permissiveness coefficient is equal to the ratio of the biosynthesis rates [Popovic, 2022k].

$$P = \frac{r(virus)}{r(host)} = \frac{\Delta_r G^0(virus)}{\Delta_r G^0(host)} \quad (14)$$

If the permissiveness coefficient is greater than 1, viral components will be produced faster than those of the host cell and the virus will hijack the host cell's metabolism [Popovic, 2022k]. If the permissiveness coefficient is less than 1, the viral components will not be produced at a sufficient rate for the virus to hijack the host cell's metabolism and there will be no infection [Popovic, 2022k]. Thus, the AU901 variant can enter the lower respiratory system, but the permissiveness coefficient for the interaction of the AU901 with the lower respiratory system is less than 1. This means that the AU901 variant cannot multiply in the lower respiratory pathways, damage them nor cause COVID-19. On the other hand, Gibbs energy of biosynthesis of muscle cell building blocks was found to be -10.18 kJ/C-mol. This means that the AU901 variant can enter the susceptible muscle host tissue and multiply within it, performing its life cycle, cause small local damage and expose virions to immune system.

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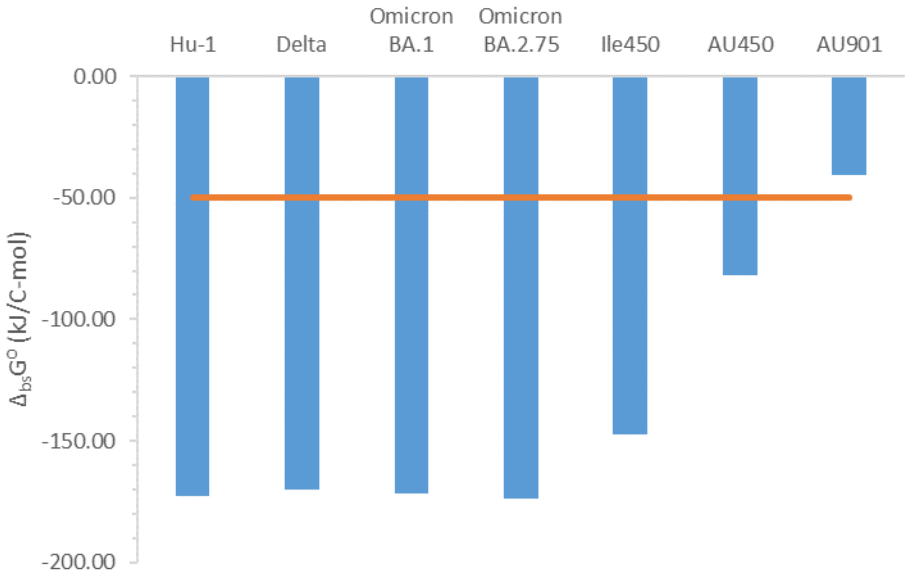

Figure 1: Gibbs energies of biosynthesis of natural and artificial SARS-CoV-2 variants. The blue columns (■) show standard Gibbs energies of biosynthesis, $\Delta_{bs}G^{o}$, of SARS-CoV-2 variants. The natural variants include the Hu-1 (Wild type), Delta, Omicron BA.1 and Omicron BA.2.75 variants. The data for the Hu-1 and Omicron BA.2.75 variants were taken from [Popovic, 2022g]. The artificial variants include Ile450, AU450 and AU901. The orange line (—) represents Gibbs energy of biosynthesis of the predilected tissue – lower respiratory pathways.

Figure 2 shows Gibbs energies of biosynthesis of the Wild type (Hu-1) and attenuated strains (Ile450, AU450 and AU901), as well as Gibbs energies of biosynthesis of potential tissues for vaccine application. The columns represent Gibbs energies of biosynthesis of SARS-CoV-2 strains. The columns show that as ballast sequence is prolonged, Gibbs energy of biosynthesis becomes less and less negative. The horizontal lines represent Gibbs energies of biosynthesis of human host tissues, including the predilected tissue of the Hu-1 wild type (lower respiratory pathways) and potential tissues for vaccine application (adipose, skeletal muscle and skin).

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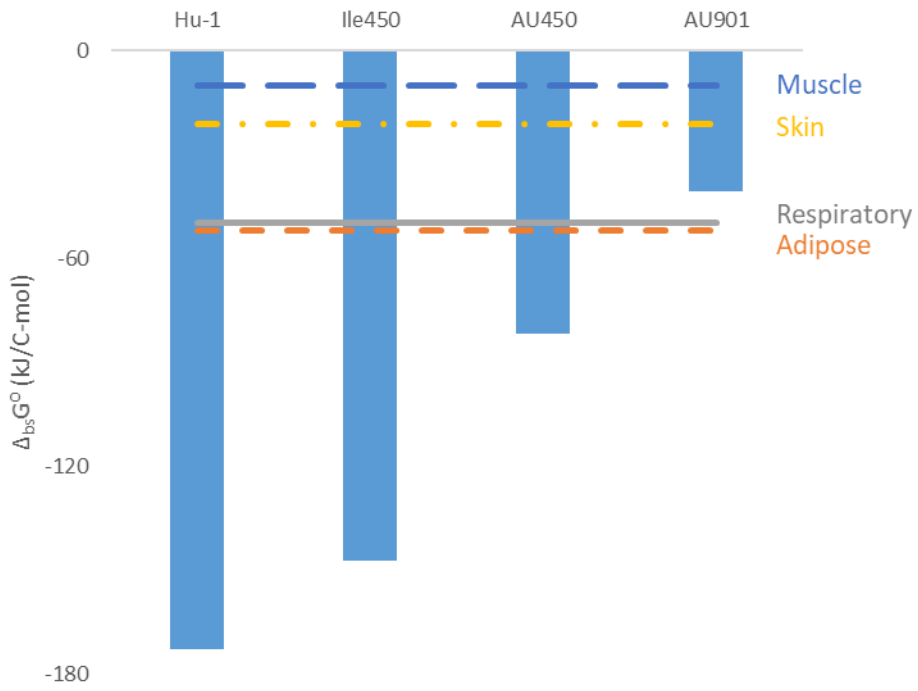

Figure 2: Gibbs energies of biosynthesis of SARS-CoV-2 variants and human host tissues. This figure shows standard Gibbs energies of biosynthesis, $\Delta_{bs}G^{o}$. The light blue columns (■) represent $\Delta_{bs}G^{o}$ values of SARS-CoV-2 variants, including the wild type (Hu-1) and modified variants (Ile450, AU450 and AU901). The lines represent $\Delta_{bs}G^{o}$ values of human host tissues. The more-dashed orange line (- - - -) represents the adipose tissue. The full gray line (—) represents the tissue of the lower respiratory pathways. The dot-and-dash yellow line (- · -) represents the skin tissue. The less-dashed dark blue line (— —) represents the skeletal muscle tissue.

Figure 2 shows that Gibbs energy of biosynthesis of the Hu-1 wild type (-172.84 kJ/C-mol) is much more negative than those of all four human tissues. Gibbs energy of biosynthesis of the Ile450 strain (-147.22 kJ/C-mol) is a little less negative than that of Hu-1, but still much more negative than those of human tissues. Thus, Ile450 is able to multiply in all four human tissues. This makes the Ile450 strain unsuitable for an attenuated vaccine. Even smaller is Gibbs energy of biosynthesis of the AU450 strain (-81.66 kJ/C-mol), which is about 2 times less negative than that of the Hu-1 wild type. This means that the AU450 strain will multiply much slower in host tissues, according to the biosynthesis phenomenological equation (13). However, Gibbs energy of biosynthesis of the AU450 strain is still more negative than those of human host tissues, meaning that it will still be able to multiply in them, although more slowly than the Hu-1 wild type. Therefore, the AU450 strain is also not suitable for an attenuated vaccine.

The AU901 strain has the least negative Gibbs energy of biosynthesis (-40.52 kJ/C-mol) of all the considered SARS-CoV-2 strains (Figure 2). It is even lower than those of the adipose and lower respiratory pathway tissues. This means that the AU901 strain cannot multiply in these tissues. Since AU901 cannot multiply in the lower respiratory pathway tissue, it is unable to damage them and cause the symptoms of COVID-19. However, Gibbs energy of biosynthesis of the AU901 strain is slightly more negative than those of skin and skeletal muscle tissues. Thus, according to the biosynthesis phenomenological equation (13), the AU901 strain can multiply in these tissues very slowly. Therefore, the AU901 variant is a good choice

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for an attenuated vaccine, since it cannot multiply in the predilected tissue of the Hu-1 wild type, but can multiply slowly in other tissues. The ideal tissues for application of the AU901 attenuated strain are the skin and skeletal muscle tissues. The adipose tissue is not a suitable tissue for application, since Gibbs energy of biosynthesis of the AU901 strain is not negative enough for it to multiply in the adipose tissue.

In summary, Figure 2 shows that the Ile450 and AU450 strains are not suitable for use as an attenuated vaccine because their Gibbs energy of biosynthesis is more negative than predilected tissue (i.e. respiratory mucosa). Thus, they are able to multiply, damage host cells and cause COVID-19. The AU901 strain can be used as an attenuated vaccine, since its Gibbs energy of biosynthesis is less negative than that of the predilected tissue of the wild type, but more negative than the vaccine application tissue. The adipose tissue is characterized by more negative Gibbs energy of biosynthesis than the respiratory mucosa. Thus, AU901 strain cannot multiply in both respiratory mucosa and adipose tissue. This makes the adipose tissue an inappropriate portal for vaccine application. However, skin and muscles are characterized by less negative Gibbs energy of biosynthesis allowing multiplication of AU901. This makes them suitable portals of application for the attenuated vaccine.

This method can be used to computationally design attenuated strains for SARS-CoV-2 (i.e. AU901) and for other viruses. Certain parts of RNA of SARS-CoV-2 shouldn't be manipulated, to preserve the antigenic properties of the virus. The spike glycoprotein can't be manipulated, since it is detected by the immune system as an antigen. The spike glycoprotein is the major target for COVID-19 vaccine development [Wu, 2020]. The membrane protein can't be replaced because it binds the viral capsid to the envelope. The nucleoprotein sequence can't be changed because it forms the virus nucleocapsid. However, an artificial decrease in the GC content in the viral nucleic acid part encoding the envelope protein should be feasible, since the virions can function without the envelope protein [Neuman and Buchmeier, 2016; DeDiego*et al.*, 2007; Kuo and Masters, 2010]. Notice that the virus keeps its biological vitality, even without the envelope protein. By losing the envelope (E) protein it is still able to multiply, although at a lower rate and virulence. By artificial manipulation that removes the E-encoding segment and replaces it with an artificial segment with a low GC content, we are adding ballast to virus multiplication, in order to make its Gibbs energy of biosynthesis less negative and replication rate lower.

If the inserted AU rich segment into AU901 strain is prolonged to encode the polypeptide (Ile-Lys-Phe-Tyr-Asn-Leu-Ile)$_{901}$, the Gibbs energy of biosynthesis becomes -40.5 kJ/C-mol. The empirical formula of such a virus is $CH_{1.550}O_{0.270}N_{0.242}P_{0.003}S_{0.002}$. To compare, the SARS-CoV-2 wild type Hu-1 has the empirical formula $CH_{1.565}O_{0.321}N_{0.294}P_{0.004}S_{0.005}$ [Popovic and Minceva, 2020b].

It seems that the biothermodynamic method and calculations could find an application in designing vaccines for various viruses. Based on the known nucleic acid and protein sequences, virus morphology and protein copy numbers, it is possible to use the atom counting method to find the empirical formula for various viruses [Popovic, 2022i]. Based on the empirical formula, it is possible to find the driving force for virus multiplication – Gibbs energy of biosynthesis [Popovic, 2022h]. For example, thermodynamic properties of the new BQ.1, BQ.1.1, XBB and XBB.1 variants of SARS-CoV-2 are available in [Popovic, 2022j]. Gibbs energy of biosynthesis of the attenuated strain should be between the Gibbs energy of biosynthesis of the planned tissue of application and the natural predilected tissue. In that way, the attenuated strain will be able to multiply in the vaccine application tissue, but will not be able to multiply in the predilected tissue. Such a vaccine would be safe. On the other hand, having in mind that

the starting point for production of vaccines can be any SARS-CoV-2 variant, to which ballast replacement for the gene encoding the E protein, such a vaccine should be 100% efficient.

5. Conclusions

A new systematic method is suggested for designing attenuated live-virus vaccines, which are both efficient and safe. The new method is based on biothermodynamics, using thermodynamic properties of the virus wild type, its predilected target tissue and potential vaccine portal of entry tissues.

The first step is to make thermodynamic characterization of the virus wild type (e.g. SARS-CoV-2), its predilected target tissue (e.g. lower respiratory pathways) and potential vaccine portal of entry tissues (e.g. adipose, skin, skeletal muscle, respiratory mucosa etc.). The most important biothermodynamic property is Gibbs energy of biosynthesis, which represents the thermodynamic driving force for biosynthesis and determines the multiplication rate.

The second step is to find a suitable vaccine portal of entry tissue, using Gibbs energy of biosynthesis. Gibbs energy of biosynthesis of the portal of entry tissue should be less negative than that of the predilected tissue of the virus wild type.

The third step is to design the attenuated virus strain. Gibbs energy of biosynthesis of the attenuated strain should be between Gibbs energies of biosynthesis of the predilected target tissue and the vaccine portal of entry tissue. Gibbs energy of the virus is modified by adding ballast sequences to the virus nucleic acid, which serve no purpose, but require energy for the proteins they encode to be produced. This energy cost makes virus multiplication less thermodynamically favorable. Multiplication of the virus should be hindered to make it have less negative Gibbs energy of biosynthesis than the predilected host tissue, but more negative than the vaccine portal of entry tissue.

The result should be an attenuated live-virus vaccine that is able to multiply in the vaccine portal of entry tissue, but unable to multiply in the predilected target tissue. Such a vaccine should be both efficient and safe. The vaccine should be efficient since the ballast sequences can be added to any virus species or variant, resulting in a highly specific vaccine. It should also be safe, since the virus will not be able to multiply in its predilected tissue, due to less negative Gibbs energy of biosynthesis.

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