Analysis of the wild type SARS-CoV-2 spike protein-mRNA secondary structure stability to predict viral fitness.

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The SARS-CoV-2 has created a pandemic (COVID-19) in 2019 across the globe. It was predicted that mutant viral strains would emerge over time that eventually gain viral fitness and may contribute to the failure of vaccines. However, so far only a few (less than 10) variants of SARS-CoV-2 emerged out of which 4 strains are of health concern. SARS-CoV-2 spike protein binds to ACE-2 (Angiotensin converting enzyme 2) receptors in the human lungs and causes the infection. Mutations in the part of the viral genome coding the spike protein cause changes in the mRNA sequence of the spike protein. These mutations produced multiple viral strains having the ability to infect. Mutations in mRNA sequence change the mRNA stability which influences the production of spike protein. Predicting the mRNA stability of the mutants can help us to understand the infecting capacity of the mutant strain. The data from this analysis can be used for the development of better therapeutics for COVID-19 treatment.

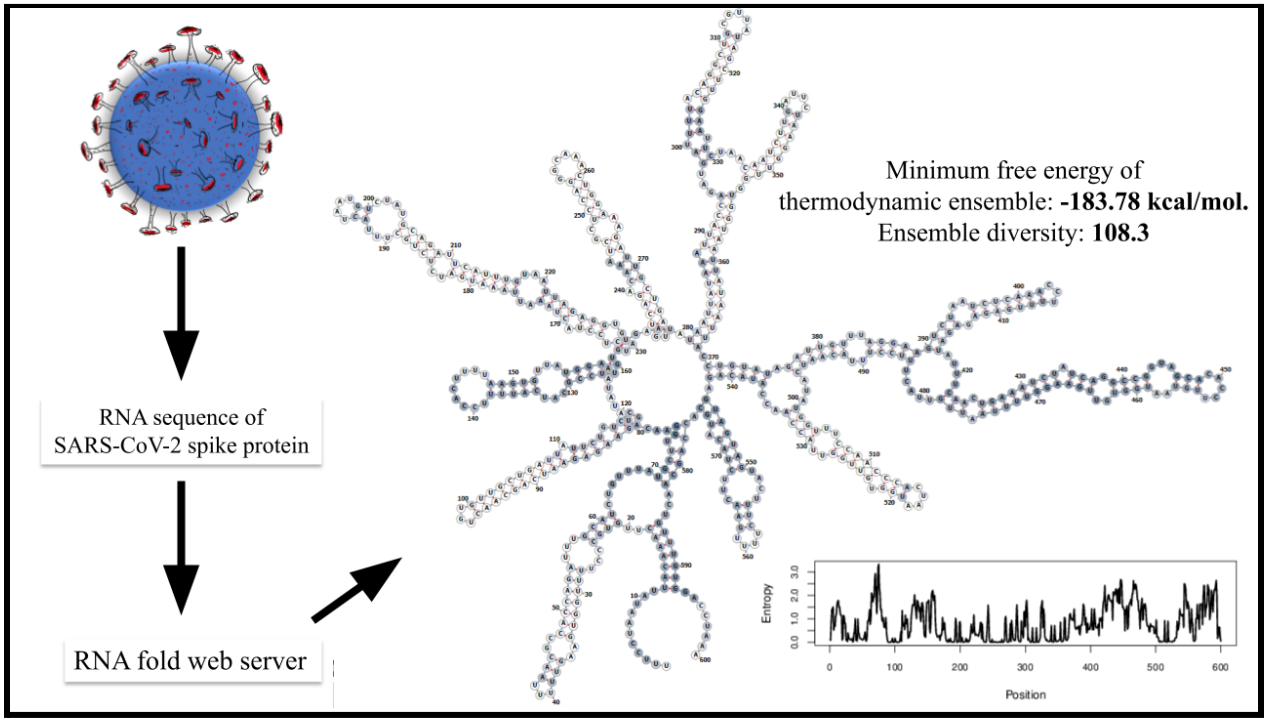

Figure 1. Overall process of calculating the entropy-based thermodynamic viral mRNA secondary structure stability.

SARS-CoV-2, Severe acute respiratory syndrome coronavirus-2 is the causative virus for the Coronavirus Disease-2019 (COVID-19).

COVID-19 was reported to be spread through bats (1). This will transmit human to human mainly through saliva droplets due to close contact.

People with chronic respiratory disease are at very high risk compared to normal people, these are highly transmissible and pathogenic (2). According to the world health organization (WHO), this has spread rapidly across the world and becomes an international public health emergency, declared to be a pandemic now. Globally, as of October 2021, there have been 235,673,032 confirmed cases of COVID-19, including 4,814,651 deaths, reported to WHO (3). It enters the human cells binding to angiotensin-converting enzyme 2 (ACE-2), a membrane protein that regulates the renin-angiotensin system. In some cases, people affected by SARS-CoV-2 can be asymptomatic, which makes it more difficult to control the transmission. It is believed to have a zoonotic origin and has close genetic similarities to bats (4).

According to the central dogma of Molecular Biology, DNA for one gene can be transcribed into an mRNA molecule which will end up making a specific protein (5). The RNAfold web server will predict secondary structures of single-stranded RNA or DNA sequences (6). This helps to access RNA and DNA servers, predicts free energy of ensembles of structures and base-pair probability from the input RNA or DNA sequence. One can simply paste and upload the sequence which follows the results page displaying thermodynamic minimum free energy, thermodynamic ensemble prediction, Graphical output, etc. (6). The stability of the mRNA is predicted using this RNA fold server. The part of the genomic sequence that codes for spike protein is accessed from the National Centre for Biotechnology Information (NCBI) using the accession number >NC_045512.2. This sequence was copied and pasted in the search box on the RNAfold web server, without changing any default parameters provided for the search. The results include the minimum free energy, thermodynamic ensemble, and the graphical output of the mRNA secondary structure. The minimum free energy (MFE) secondary structure is shown in Figure 1. The thermodynamic free energy for the secondary structure of mRNA was found to be -183.78 kcal/mol, which indicates that the structure is stable.

The viral fitness (i.e, viral proliferation and infectivity) of the SARS-CoV-2 mutant strains is potentially dependent on the mRNA stability of the spike protein. Only when the mRNA is stable, it can translate into a stable functional protein. Even if there is a possibility to get many types of viral mutants, according to the clinical data, there are very few mutant forms which are capable of causing an infection. If the mRNA is not stable, it gets degraded by the host cell nucleases and hence the mutant strain of the virus is not viable. This implies that only few mutant forms of the viral strain have stable mRNA secondary structure and thus have the ability to proliferate and cause infection. Analyzing the stability of the mRNA structure of the mutants can help us to study the connection between the mRNA stability and the viability of the mutant.

The data from the analysis of the mRNA secondary structure stability can be used to predict the relation between mRNA stability and viral fitness. This analysis can be used to develop better therapeutics for the treatment of COVID-19. Currently, we are in the process of analyzing more than 500 possible variants of SARS-CoV-2 mRNA molecules with possible mutations incorporated from different strains that have been identified and recorded by the World Health Organization. The ongoing and upcoming research on mRNA stability will be published in the future issues of TCABSE-J.

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Conflict of interest: The Analysis Report presented here is an ongoing project currently at TCABS-E, Rajahmundry, India. The authors invite collaborations without any conflict of interest.