SARS-CoV-2 Spike Mutations and Their Impacts on Protein Stability: A Computational Approach
Olivia Pericak and Ronald Brown

Introduction or Overview
The SARS-CoV-2 spike protein has acquired many mutations within the past year that may increase viral pathogenicity. Through computational ab initio methods, the degree to which the viral protein is impacted by the D614G mutation was studied.

Research Hypothesis/Objectives
The main objective of this research was to determine the degree to which the stability of the spike protein is affected by the mutation D614G. By calculating dissociation energy, we will theoretically quantify the impact of D614G.

Methodology or Approach
The structure used for all analyses was obtained from the protein data bank (PDB ID:6VXX). The original PDB file was imported directly to GaussView, where all manipulations to the protein structure were performed. Due to computational limitations, only amino acids that directly interact with the D614G mutation were included for calculations. The mutation was modelled with GaussView by directly deleting the aspartate residue and inserting a glycine amino acid. To determine the optimal arrangement of these amino acid chains, various calculations were performed. Geometry optimizations using density functional theory (DFT) were performed with GAUSSIAN09 Starting with a lower level of theory, three calculations were run at B3LYP/6-311G with no solvent. Terminal atom of the chains were frozen to provide a level of restraint on the amino acids from moving uncontrollably. These included the carboxyl carbon and the nitrogen from the amino terminus. These atoms were consistently held fixed through all calculations. To determine the change in energy, the S1 and S2 chains needed to be optimized independently. Another optimization was run with both chains present, allowing them to interact and for an optimal geometry to be determined. To determine the dissociation energy, the energy of the combined chain was subtracted from the sum of the independent chains. The same three optimizations, mimicking the conditions as above, were then run for the mutated version of the S1 chain. The mutated version was created by manually substituting in a glycine residue for the aspartate at position 614 of the S1 chain. The results were analyzed, and a change in energy was calculated. For a more realistic approach to how the spike protein behaves in physiological conditions, the molecules were also optimized with water as the solvent. The calculations were then repeated for a comparison to the mutated version. A last round of calculations was performed at a higher level of theory to increase accuracy of the results. The same restrictions were held as stated above, but with 6-311G(d,p) as the basis set. This also included solvent versus no solvent calculations.

Major Outcomes, Results and Conclusion
Data produced from the optimization calculations showed a consistent decrease in dissociation energy of the mutated spike protein when compared to the wild type aspartate. The decreased energy may influence the ability of the S2 subunit to deform from S1 and facilitate viral fusion. The S1 subunits change in conformation is necessary for S2 to fuse the virus’s genome. It is possible that the glycine substitution encourages the open conformation of S1, making the ACE2 receptor accessible to the RBD. Further research to investigate the mechanisms by which this decreased energy enhances the functions of the SARS-CoV-2 spike glycoprotein would be useful.