Investigation of the Temperature Dependent Affinity of SARS-CoV-2 Spike Protein to Gold Nanoparticles

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BACKGROUND

Based on a previous study on the Beta Amyloid peptide, it was found that the conformation of the peptide (protein) was able to change with external conditions such as pH. We decided to test the conformational changes with pH hopping of the spike protein of SARS-CoV-2. The spike protein is the surface protein of SARS-CoV-2 which initiates an immune response and binds to the receptors on the cell membrane. At a pH of 10, which is a basic condition, the receptor on the spike protein would be facing downwards and not be able to form any gold aggregations. In a pH of 3, which is an acidic condition, we would expect either the "parallel in opposite direction" or "head to head dimer" which would both form gold aggregation.

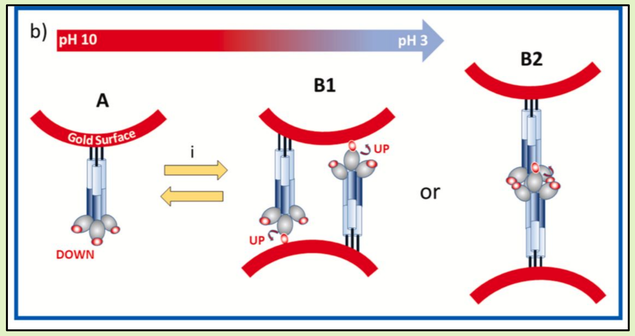

Figure 1. Conformational changes of the spike protein between the pH of 3 and 10

Proteins are invisible to the eye and under a microscope so it is essential that they are able to be observed in some sense. We do this by attaching the protein to gold nanoparticles. Proteins will embed a small portion of themselves into the particles and stick out the rest of their form outside of the particle. This allows us to visualize the protein interaction under a transmission electron microscope (TEM). When the proteins are unfolded they will interact with the other proteins attached to other particles and cluster the gold together making a dark clump visible on a TEM image. Nano materials tend to have different properties based on their size so it is essential to test for this variable. We make different samples to test using gold particles ranging from 10nm to 100nm.

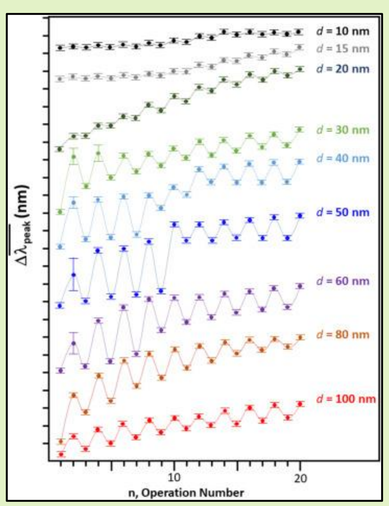

Figure 2. The increase in absorbed wavelengths as a function of operation number and nano particle size

METHODOLOGY

We had nine different sizes of the gold nanoparticles that were prepared and used to observe the shift in the peak position wavelength that was due to external factors such as pH. We changed the pH by adding HCl, which is a strong acid, and NaOH, which is a strong base maintaining it at a pH of 3 and of 10 for every other sample up to 20 samples. Observing the position of the maximum wavelength on the UV-Vis spectrophotometer, we were able to interpret the reversibility of the protein.

RESULTS

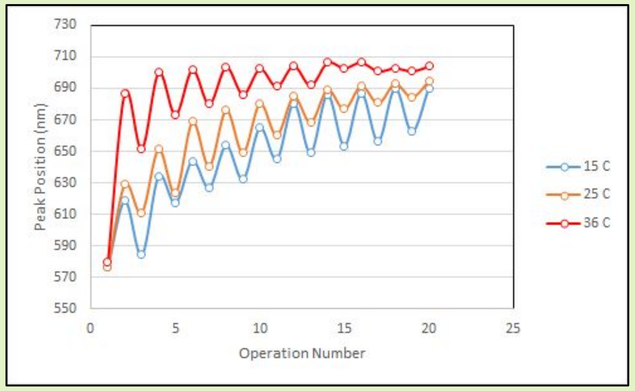

Figure 3. Au 80 nm & Spike Protein - representation of varying red shifts at different temperatures and pH levels

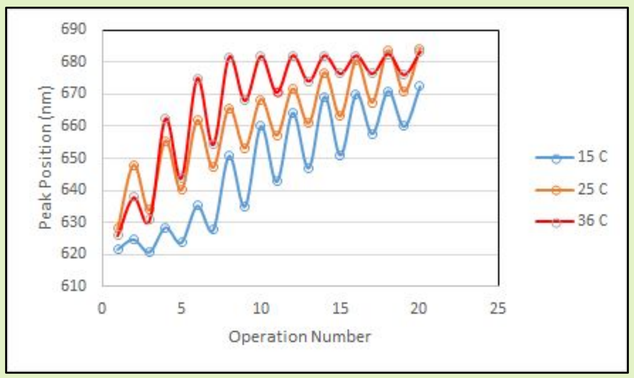

Figure 4. Au 100 nm & Spike Protein - representation of varying red shifts at different temperatures and pH levels

These graphs (Fig. 3 & 4) show that there is a temperature dependence occurring in the change in conformation of the SARS-CoV-2 spike protein. For the gold nanoparticles of 80 and 100 nm the wavelength of peak position of adsorption was generally lower at the lower temperature and increased as temperature increases. Another observation is that at the highest temperature of 36 C, the amplitude of oscillation decreased as operation number (samples) increased. In addition, as the temperature is increased the red shift (shift towards higher wavelengths) is also increased. This is the same for both the 80 and 100 nm gold nanoparticles. The main difference between the 80 nm and the 100 nm aggregates is that the position of peak adsorption occurs in different places; the aggregates with 100 nm show that peak adsorption occurs within a smaller range of wavelengths.

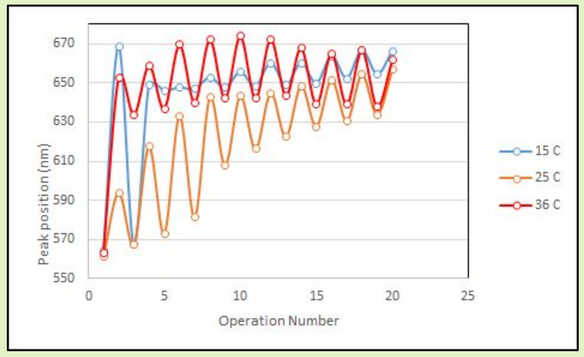

Figure 5. Au 60 nm & Spike Protein - representation of varying red shifts at different temperatures and pH levels

When the spike protein is bound to 60 nm gold nanoparticles (Fig. 5) the trends are different compared to the 80 nm and 100 nm gold nanoparticles aggregates. In the case of 60 nm gold nanoparticles, the red shift does not follow the trend where as temperature increases so does the overall red shit. Instead, the general slope of the 15 C line is higher and more red shifted that the 25 C line. In addition, the amplitude of oscillation seems to remain about the same and does not show a dampening effect like what occurs with the 80 nm and 100 nm gold nanoparticles.

DISCUSSION

The trends shown in Figures 3, 4, & 5 suggest that there is a temperature dependence on the folding conformation of the spike protein. The difference in trends exhibited in the 60 nm gold nanoparticles suggests that the aggregation of the gold nanoparticles and spike protein also depends upon the size of the gold nanoparticles and can be affected by many different factors working together.

The type of oscillation exhibited in each of the graphs means that the spike protein exhibits semi-reversible conformation changes. Generally, at more acidic pHs (even operation numbers) the aggregation of the gold nanoparticles and spike protein is higher.

These results are important because they can be used to implement efficient storage practices of the virus. If the spike protein is tightly bound and aggregated with the gold nanoparticles, then it is not able to be spread. Understanding the optimal conditions under which the spike protein will be bound to other proteins and gold nanoparticles is necessary for this.

Further research should be done to further investigate the temperature dependence at additional temperatures and additional sizes of gold nanoparticles. In addition, these methods can be used to test aggregation of additional virus surface proteins to gold nanoparticles.

REFERENCES

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