

Use of DNA-Gold Nanoparticle Complexes to Synthetically Model Biological Nucleosomes

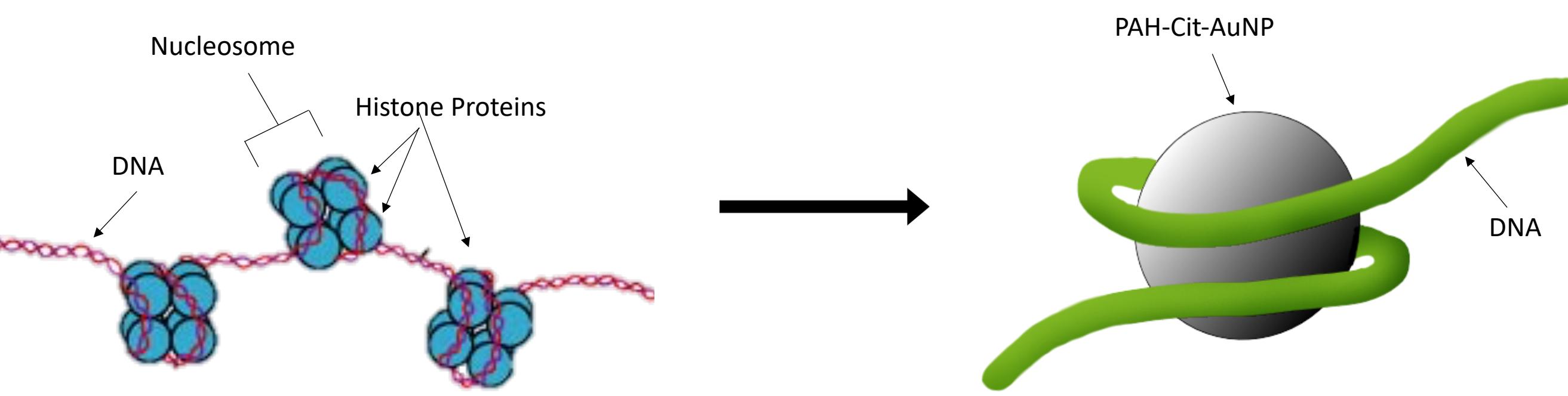
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Abstract

This research focused mainly on the optimal synthesis and Layer-By-Layer preparation of DNA-AuNP complexes, achieved by the wrapping of citrate gold nanoparticles with poly-allylamine hydrochloride (PAH) and sheared calf-thymus DNA. The tight packing of DNA into chromatin with histones has been studied extensively, and the goal of this research was to accurately replicate this through the creation of synthetic histones, modeled by DNA-AuNP complexes. Spherical gold nanoparticles have many biomedical applications including drug and nucleic acid delivery. Sheared DNA may be used in order to introduce the gold nanoparticles to a biological system, but for this to be possible, the interaction between the two must be studied. Further research may be undertaken later in order to study the electrostatic forces between the histone systems and compare them to real histones.

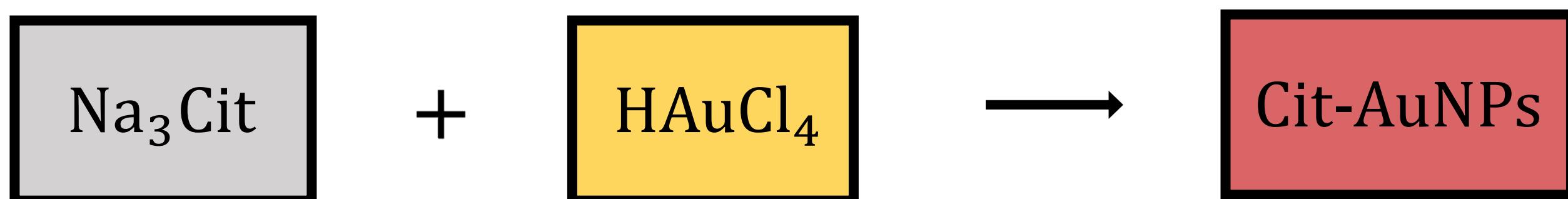
Introduction

- Nucleosomes, an element of DNA packing in eukaryotes, are comprised of eight histones bonded together, with linking DNA conjoining them to other histone groups. They are crucial to packing eukaryotic genomes into the nuclei of cells. The goal of this research was to model this by using gold nanoparticles as a substitute for the clump of eight histones wrapped by DNA.
- The LBL process described in this research includes three layers, each building on the previous. Each adds diameter to the particles and flips their surface charge. The first step in creating synthetic nucleosomes is to create the nanoparticles using gold salt solution and coating with trisodium citrate, the first layer, which has a negative charge. These are then coated with the second layer, poly-allylamine hydrochloride, in order to create a positive surface charge to bond with the DNA. This also stabilizes the particles. The third and final “layer” is to add the sheared DNA, creating the final DNA-Nanoparticle structures.

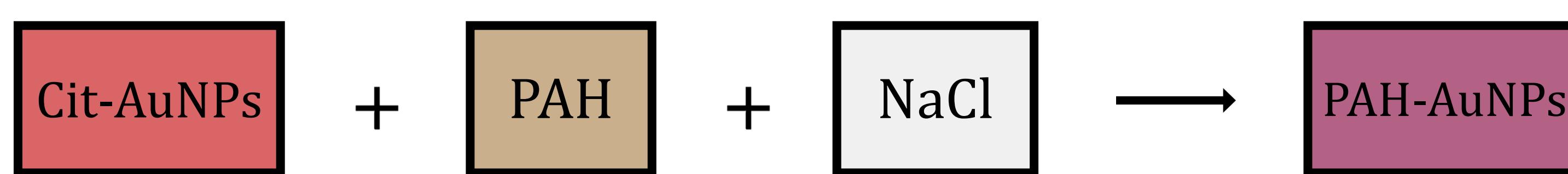


Methods

- Citrate gold particles with a molarity of 3.5nM were synthesized through the addition of trisodium citrate to a gold salt solution in a ratio of 3:100 and thermally mixed until turning a wine-red color.



- The newly made Cit-AuNPs were then coated with a solution of PAH and NaCl by mixing in a ratio of 10:2:1, respectively, and then cleaned them of excess PAH using a centrifuge. This stabilized the particles, ensuring no further aggregation. This step is crucial to allow the DNA to stick to the particles through electrostatic interaction.



- The nanoparticles' concentration was increased tenfold through pellet centrifugation, allowing the dense particles to sink to the bottom and removing supernatant from the top. The pelleted particles were then combined with newly sheared calf-thymus DNA in a ratio of 9:1 in order to create the DNA-AuNP complexes.



- The DNA-AuNP complexes were then cleaned with a centrifuge in order to remove excess floating particles that may tamper with final measurements.

Analysis

- UV-Vis Spectroscopy was used to measure the absorbance spectrum of the particles and ensure proper stabilization and absence of aggregation.

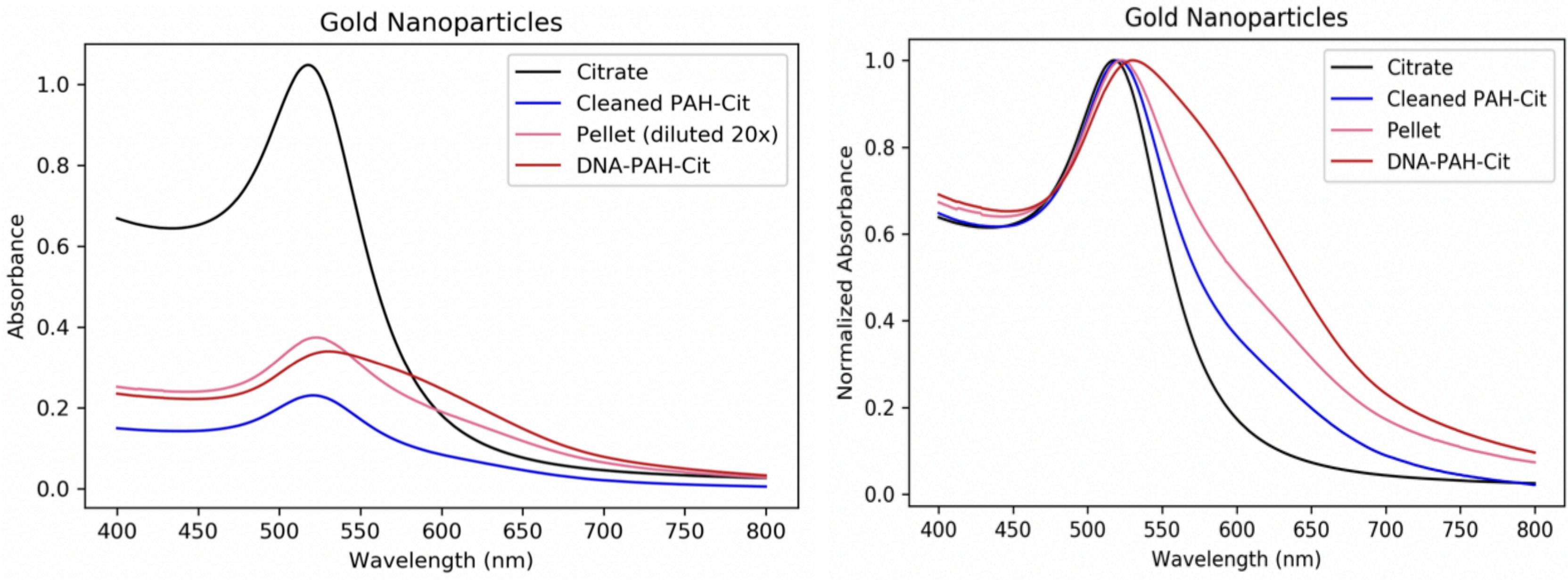


Figure 1. UV-Vis absorbance spectra (left) and its normalized counterpart (right) for 15nm diameter unwrapped citrate AuNPs (black), ~25nm PAH-Cit AuNPs (grey), PAH-Cit AuNPs (cleaned 2x) (blue), Pelleted PAH-Cit AuNPs (diluted 20x) (pink), as well as the final ~35nm DNA-AuNP histone systems (red).

- As shown by Fig. 1, there is a significant red-shift occurring throughout the LBL process, as expected from a size increase. There is also minimal aggregation present during the end of the process, shown by the shoulder near the high end of the spectrum. This signifies that some polydispersity has occurred, most likely due to centrifugation. DLS and Zeta Potential measurements were used to determine the size of the particles and their surface charge, respectively, and ensure that no significant polydispersity had occurred from each step.

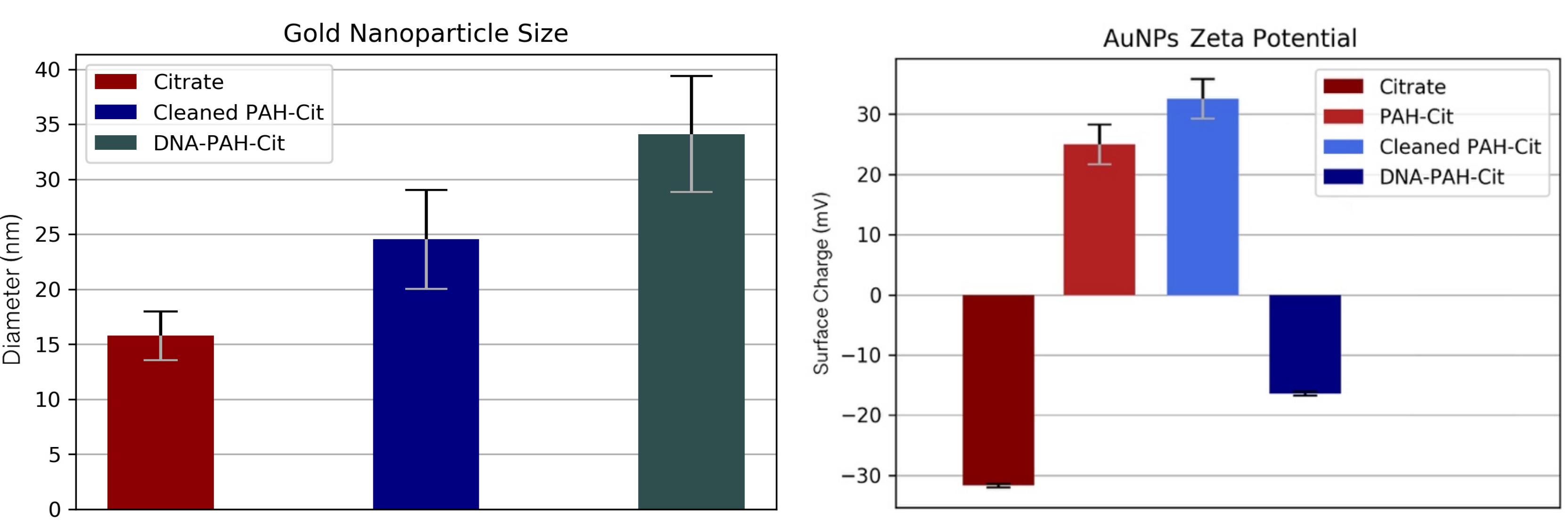


Figure 2. DLS size measurements for Citrate AuNPs (red), Cleaned PAH-Cit AuNPs (blue), and the final DNA-AuNP complexes (green). Error bars represent standard deviation.

- As shown by Fig. 2, there is a significant size increase throughout the LBL process, as expected. Each layer adds on a new coating over the nanoparticles. Each of these layers also flips the surface charge of the particles, as shown in Fig. 4.
- AFM measurements were used to look at DNA on a mica slide using a spin-coating technique. The negatively-charged mica was coated with Poly-L-Lysine and then spin-coated with DNA solution and washed with water. This allowed us to ensure DNA was the appropriate length for bonding with the coated gold nanoparticles.
- The DNA-AuNPs did not stick to the mica well enough for AFM measurements to accurately detect them to a significant extent that allowed for imaging.

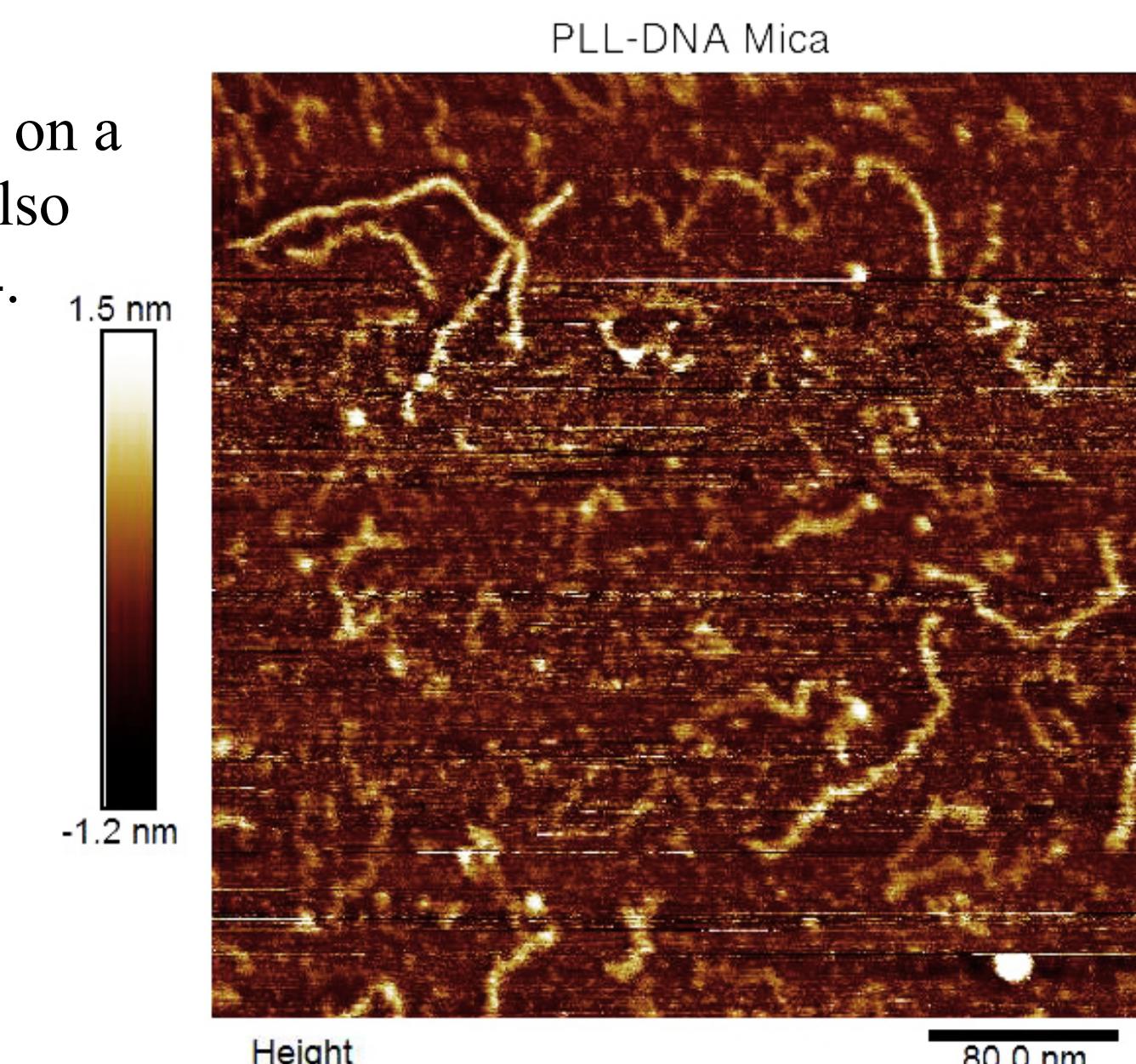


Figure 4. Height AFM measurements of DNA on mica slides at a zoom of 500nm.

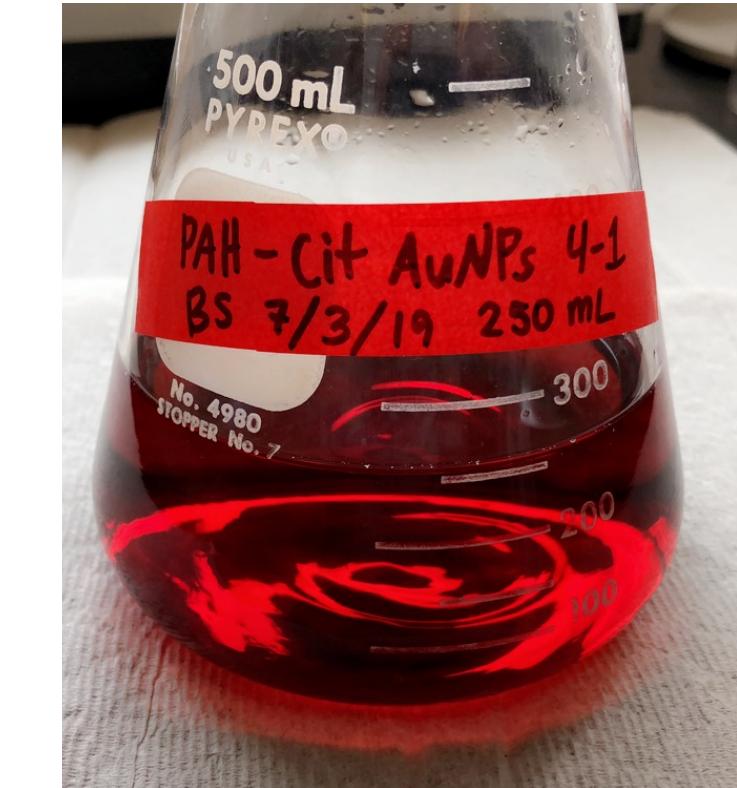


Figure 5. An example of clean, non-polydisperse nanoparticles. These are in the second step of the LBL process and have been coated in PAH. They were used for data collection, due to their pristine condition.



Figure 6. Pelleted particles that have been coated with PAH and cleaned. This solution has a much higher concentration of nanoparticles compared to Fig. 5.



Figure 7. An example of contaminated, polydisperse nanoparticles that have aggregated to the extent of no longer being usable (right), and clean nanoparticles (left).

Conclusion and Future Work

- In this research, the synthesis and preparation of DNA-Gold nanoparticle complexes has been developed using a LBL method. This research has been mostly successful, however multiple improvements are yet to be explored, as discussed below.
- Investigate interaction between mica and nanoparticle complexes in order to achieve a clear AFM image.
- Achieve better DNA wrapping with a higher surface charge and lower amount of particle aggregation.
- Develop a method, if possible, to clean nanoparticles without causing mass aggregation.
- Investigate the electrostatic interaction between AuNPs and DNA.
- Compare synthetic nanoparticle systems to a biological system.
- Discover other potential uses of nanoparticles and DNA.

References

- <http://faculty.ccbcmd.edu/~gkaiser/biotutorials/dna/fg10.html>
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