Quantifying nucleic acid association to nanoparticles

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Introduction:

The goal of this project is to investigate if nanoplastics and gold nanoparticles (AuNPs) will sorb nucleic acids such as DNA. Nanoplastic wastes potentially originate from consumer products that are common to households, such as face washes, body washes, and cosmetic products. These nanoscale plastic particles potentially interact with their surrounding environment as they are discharged to wastewater treatment facilities and ultimately to water bodies. In wastewater treatment facilities, there is potential for the creation of recombinant genetic material to potentially propagate antimicrobial resistance (AMR). We hypothesize that nanoplastic wastes could alter AMR propagation by sorbing and later releasing AMR promoting genetic materials.

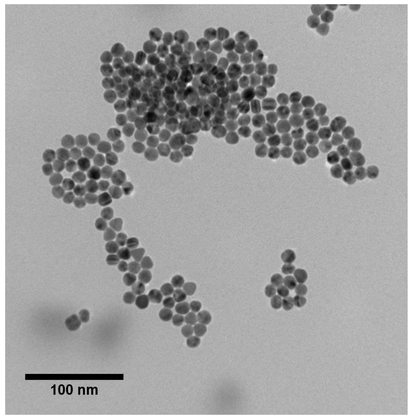


Figure 1. Citrate Gold Nanoparticles (13 nm) synthesized in-house

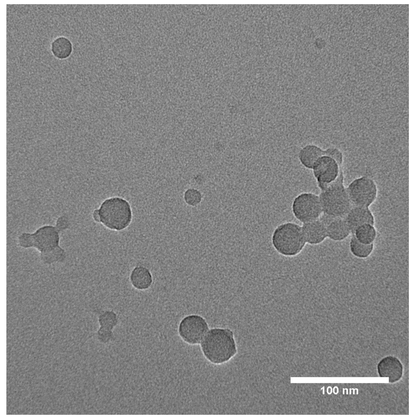


Figure 2. Latex Nanoparticles (28 nm) purchased from Invitrogen

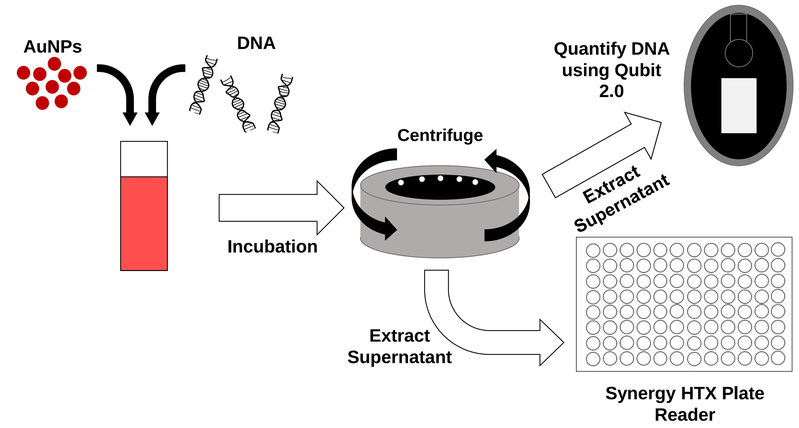
Characterization:

Gold and latex nanoparticles were characterized via UV-Vis spectroscopy, dynamic light scattering, and TEM microscopy. This is some of the most important information on the particles because size, zeta potential, and pH all play a role in the stability of the particles and how they could potentially react in their environment. This preliminary knowledge can allow for better educated hypotheses on how the particles will interact with the nucleic acids and better explain the results.

Nanoparticle Name	Citrate Gold NP	Carboxyl Latex Nanoparticles	L-Cysteine Gold NP
Diameter (TEM, nm)	12 ± 1.3	26 ± 6.3	4.8 ± 1.8
Z_{ave} (nm)	18 ± 0.18	40 ± 0.28	50 ± 2.1
Particle Concentration (particles/mL)	3.0 x 10^{12}*	5.0 ± 1.97 x 10^{16}	TBD
UV-Vis Wavelength (nm)	518	N/A	518
pH	6.1 ± 0.002	6.8 ± 0.002	2.8 ± 0.002
Mobility (µm·cm/V·s)	-2.3 ± 0.14	-4.0 ± 2.0	2.2 ± 1.5
Zeta Potential (mV)	-30 ± 1.8	-35 ± 1.7	28 ± 1.5

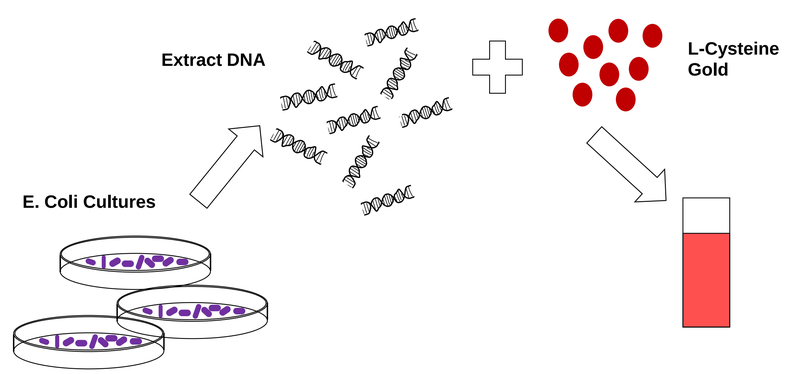
*Calculated based off of the gold nanoparticles absorbance at 450 nm from Haiss, W. et al. *Anal. Chem.* **2007**

Methodology:

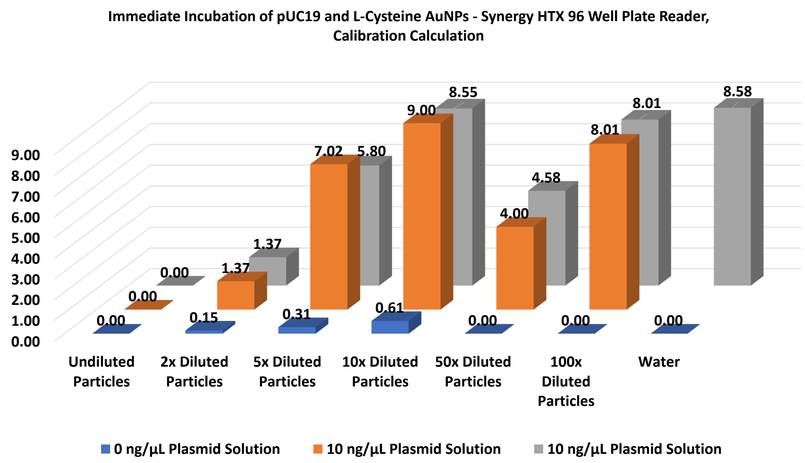


In this study, gold nanoparticles of 13 and 4 nm in diameter were used as model nanomaterials. DNA samples were exposed to these nanoparticles, incubated for a fixed period of time, and then the nanoparticles were removed via centrifugation and the DNA that remained in the supernatant was quantified using a Qubit 2.0 fluorometer and Synergy HTX 96 Well Plate Reader.

Some percentage of the DNA will be sorbed onto the surface of the nanoparticles, and therefore the amount of DNA quantified in the supernatant will be less than the total amount of DNA present. The citrate gold nanoparticles did not exhibit a definitive trend in DNA sorption. Now we have been able to control the DNA concentration by growing, extracting and amplifying our own DNA.



Results:



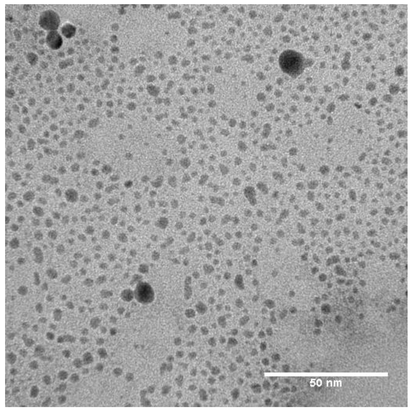


Figure 3. L-Cysteine Gold Nanoparticles (4 nm) synthesized in house

The new results are promising in that there is a trend of the DNA being completely removed from the sample with undiluted particles. The concentration of DNA increases until most of the DNA is not sorbed to the L-Cysteine AuNPs. The 50x diluted particles exhibited a strange decrease in DNA that was not expected, as both the 10x and 100x have nearly all the DNA remaining in solution.

Conclusion:

We now understand that DNA is able to sorb onto the surface of the L-Cysteine (positively charged) AuNPs more predictably than onto the citrate (negatively charged) AuNPs. However, as these are preliminary results the trials must still be shown to be reproducible.

Future Work:

We will move forward by performing more experiments with latex nanoparticles and developing a Langmuir Isotherm to describe the affinity between DNA and the positively charged surface.

Acknowledgments: This material is based upon work supported by the National Science Foundation (NSF) and the Environmental Protection Agency (EPA) under NSF Cooperative Agreement EF-0830093, Center for the Environmental Implications of NanoTechnology (CEINT). Any opinions, findings, conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the NSF or the EPA. This work has not been subjected to EPA review and no official endorsement should be inferred. Environmental DNA samples provided by Dr. Emily Garner at Virginia Tech.