Optimization and Characterization of Au Nanoparticle-DNA Conjugate Devices

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Abstract:

In this project, spherical gold nanoparticles (AuNP) were loaded with deoxyribonucleic acid (DNA) oligonucleotides to make a series of single- and double-stranded conjugates, which were characterized via various established methods to optimize loading efficiency and functionality. Characterization methods used include ultraviolet-visible spectrophotometry (UV-Vis) to measure absorbance and estimate loading efficiency; dynamic light scattering (DLS) and zeta potential (ZP) measurements to estimate conjugate size and extent of aggregation; and gel electrophoresis to separate and analyze conjugates based on size and charge. Toehold-mediated strand displacement DNA fluorescent gates were tested in order to relate characterization to device performance. Salt aging to 100 mM NaCl provided high loading (131 ± 7 strands per particle) with low particle aggregation and relatively high hybridization efficiency (58 ± 6%), while maintaining strand displacement logic gate device capability.

Introduction:

AuNPs have been broadly studied as platforms for small molecule, antibody, and aptamer delivery to cells because of the variety of functional groups that can be bound to the AuNP surface [1]. However, many reports of AuNP applications in biomedical diagnostics and therapeutics lack sufficient characterization to fully understand and optimize their loading [2,3].

This project aimed to utilize the multitude of characterization techniques at our disposal in order to provide a more holistic understanding of AuNP loading. Optimizing our AuNP-DNA conjugates while balancing device performance can provide greater functionality for applications such as high payload drug delivery agents.

Experimental Procedure:

We began with approximately 14 nm diameter, citrate-capped AuNPs synthesized via the citrate reduction method [4]. We made a series of conjugates at varying NaCl concentrations (50, 100, 200 mM) to establish a trend between DNA loading and salt concentration. The single-strand DNA covered AuNPs (ssAuNPs) were synthesized by adding the initial DNA oligo capture strand to the AuNP solution in a molar ratio of 195 DNA/AuNP, then left to shake for approximately 16 hours [5]. The conjugates were then salt-aged to the desired NaCl concentrations over a period of six hours to minimize aggregation, then centrifuged for 30 minutes at 10,500 RCF.

Supernatant was collected in separate tubes and the conjugates were resuspended in the same volume and concentration NaCl. This was done three times total. Residual DNA in the supernatant was quantified via ultraviolet visible spectrophotometry (UV-Vis) absorbance measurements to estimate DNA loading on the AuNP surface.

The double-stranded DNA covered AuNPs (dsAuNPs) were synthesized by adding the complementary oligonucleotide to the ssAuNP solution in a 1:1 molar ratio based on the single-stranded loading estimate. They were then left to shake for approximately two hours before quantifying DNA hybridization with UV-Vis as before. Dynamic light scattering (DLS) and zeta potential (ZP) measurements of the conjugates were taken before conducting a second trial at the same concentration (100 mM) to test repeatability. Gel electrophoresis (1.5% agarose gel) was also performed on all samples from each trial.

Single- and double-stranded conjugates were synthesized with a new set of oligos capable of efficient strand displacement [6]. For this trial, the hybridizing strand had a ROX fluorophore modification that upon hybridization would be close to the AuNP surface. Toe-hold mediated strand displacement reactions were performed on this batch of conjugates in order to test device performance. A fluorometer (2× Gain) was used to monitor fluorescence during hybridization and strand displacement (two hours each).

Results:

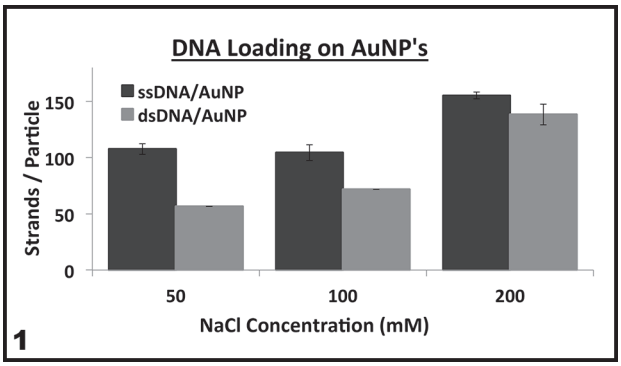
By varying the salt concentration of the conjugate solution, we found hybridization efficiency increased with increasing NaCl concentration (Figure 1). DLS measurements gave us an indication of the relative extent of aggregation for each salt concentration. While conjugates synthesized in 200 mM NaCl showed the highest hybridization efficiency, they also showed the most aggregation (Figure 2).

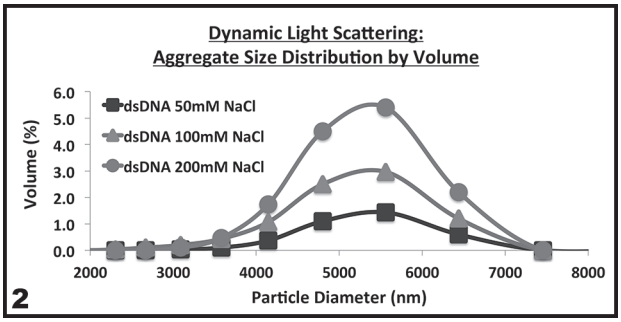
ZP measurements of the conjugates were distinctly different than that of the bare AuNP control, varying by an average of about 20 mV. The conjugates had ZP values around -40 mV, indicating particle stability.

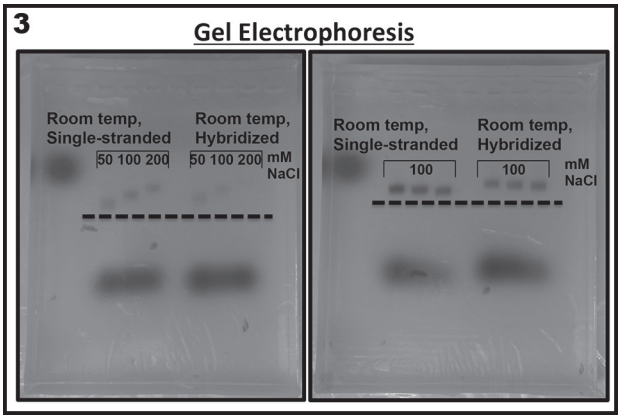
We used 100 mM NaCl for our second trial testing repeatability. The conjugates synthesized at this concentration showed slightly reduced hybridization efficiency than the higher salt concentration, but also showed less aggregation (about 3% by volume).

Slight mobility differences between the ssAuNPs and dsAuNPs in gel electrophoresis (Figure 3) indicated hybridization had occurred in both trials. The gel for the 100 mM NaCl conjugates also showed our process to be repeatable.

Our final round of conjugates employing new oligos tested device performance via strand displacement (Figure 4). The hybridizing strand had a fluorescent dye modification (ROX) that quenched in proximity to the AuNP, as seen in the fluorescence decrease during hybridization in Figure 4. As the invading strand displaced the fluorescent oligo, fluorescence increased again as expected. This successful strand displacement proved the capability of our device and assay.







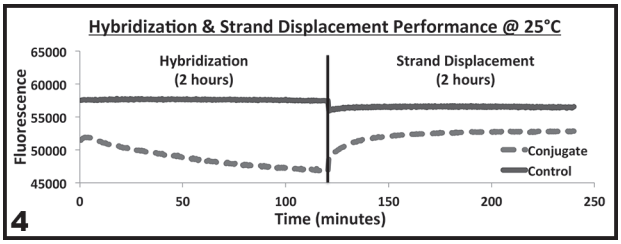


Figure 1: *Single- and double-stranded DNA loading on AuNP surface, estimated from UV-Vis absorbance measurements.* ***Figure 2:*** *DLS measurements in aggregate size range; dsDNA refers to hybridized DNA on conjugate surface.* ***Figure 3:*** *Gel electrophoresis for varying salt concentration trial (left) and same salt concentration trial (right).* ***Figure 4:*** *Fluorometer measurements during hybridization and strand displacement.*

Conclusions:

Our results show promising possibilities for attaining optimal DNA loading efficiency on spherical gold nanoparticles. Our data suggests salt-aging to 100 mM NaCl could be a good starting point from which to continue studying characterization and performance of nucleic acid devices and their relation to DNA loading. In the future we would like to pursue different DNA tethers, vary the length of the carbon linker chain, and incorporate aptamers onto the device to study the full extent of its performance. Further quantification of device performance is possible based on the tested strand displacement assay.

Acknowledgments:

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